DEVELOPMENT OF A VIABLE PROTOCOL AIMED AT THE SYNTHESIS OF A SELECTED NATURAL PRODUCT WITH POSSIBLE APPLICATION IN THE INDUSTRY

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by

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This is not just an MSc dissertation, but is truly a dream…

When I walked into Prof Steenkamp's office in 2001, we never thought that the search to combine cosmetics and Chemistry would end up to be this exciting. I placed my trust in God and He is the only reason that this dream is a reality.

I want to express my sincere gratitude to Prof Steenkamp, not only for the guidance but also for the trust and commitment he has shown me over the past few years.

Thank you to Charlene Marais, whose passion for the academic and rooibos has inspired her to share her knowledge in order to guide me.

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Inus, thank you for keeping me company during long hours of TLC monitoring and for inspiring and motivating me,

And my family, especially my parents, thank you for always believing in me and for supporting me in any venture I intend to take on!

Lizette Jordaan

AIM OF PROJECT

This project is aimed at the establishment of different synthetic routes, towards a natural polyphenolic biflavonoid which is characterized by health-related biological activity. It is also envisaged that analogues of this natural product will be synthesized to ascertain their activity compared to the parent compound.

These desired compounds have the potential to act as active ingredients in commercial products, hence the synthetic route which is the most attractive in terms of economic viability and "green" strategies, is to be established in this research project.

Challenging in this project is not only to discover the most appropriate synthetic protocol, but also find routes that will minimize the utilization of protecting groups for those precursors containing closely related functionalities.

ABSTRACT

Since the introduction of synthetic analogues in both the health-related and cosmetic industry, a new generation has emerged in search of beneficial bioactivity compounds. This generation of "natural and green" focuses mainly on natural compounds and their health relating application. This research project focused on the natural polyphenolic compounds, Flavonoids.

Flavonoids are known to be strong antioxidants, these are molecules that quenches reactive oxygen species (ROS). This generation of free radicals in the stratum corneum is the main factor in the development of skin damage and premature ageing. The two main sources of antioxidants are our body's own in-house antioxidants or dietary antioxidants. Vitamins E and C were briefly discussed as antioxidants, but the main focus was the antioxidant activity of flavonoids. Through this study were unraveled the reaction pathways of natural antioxidants and their synthetic analogues, in chemical and biological systems. Emphasis was placed on their structure-activity relationship and correlated to their chemical and biological activities.

Rooibos extract, known locally and overseas, was pursued not only for its bioactivity but rather its strong radical scavenging abilities. It is known that rooibos is not only unique to South Africa, but is hitherto the only natural source of the dihydrochalcone aspalathin (proven to be a very strong antioxidant). The uniqueness of this dihydrochalcone prompted the establishment of a viable synthetic route towards the construction of those crucial bonds in this target molecule, aspalathin.

The first step would be the construction of the dihydrochalcone, 3,4,2',4',6'-pentahydroxy dihydrochalcone, which proved to be a challenging array of chemical reactivity. With acylations like Friedel-Craft and Fries, that is known to be very successful, it was decided to commence with the construction of the dihydrochalcone

via an appropriate acylation step. Acylation of phenols can either occur *via* *C*-acylation (Friedel-Crafts reaction) or *O*-acylation (esterfication). This regioselectivity is governed by a set of principles incorporated in a theoretical premise, conveniently named as hard and soft acids and bases (HSAB). A new group of water tolerant Lewis acids, namely the lanthanide triflates have been introduced, and also the use of $BF_3 \cdot (C_2H_5)_2O$ has proven success as catalyst in *C*-acylation.

Simple phenolic substrates were used in the acylation process to assist the eventual establishment of a viable protocol. With these we were able to synthesize 1-hydroxy-2-acetonaphthone and 3-(3,4-dihydroxy-phenyl)-1-(1-hydroxy-naphthalen-2-yl)-propan-1-one successfully, but in unsatisfactory yields (36 %). Despite many experiments under different conditions, starting with different model compounds, we were unable to improve the reaction yields. Within these reactions resorcinol produced the *O*-acylation product, 3'-*O*-hydroxy-phenyl 3-phenyl-propanoate and the *C*-acylation product, 2',4'-dihydroxydihydrochalcone, whereas phloroglucinol only produced the *O*-acylated product, 3',5'-dihydroxy-phenyl 3-phenyl-propanoate. From this analysis the conclusion can be made that, first occurring is the *O*-acylation followed by a Fries rearrangement in some cases. The neighboring hydroxy functionalities of phloroglucinol for example, posed a significant steric challenge for incoming electrophiles

From the commencement of the project, replacement of the carboxylic acid group with the related, but with different chemical characteristics, nitrile groups was a necessary alternative. The Hoesch reaction was a good example of the HSAB principle, where in acid medium the nitrogen of the cyano group is protonated to afford the reactive electrophilic intermediate, the carbon of which is clearly a "*softer*" acidic site according to the HSAB theory. The *C*-acylated product, 2',4',6'-trihydroxy dihydrochalcone was produced in an impressive yield (73 %). During this reaction, an interesting result was also obtained, where the phenolic oxygen ("*hard*" base) as well as the aromatic ring ("*soft*" base) reacted with the nitrile to produce the product, 3',5'-dihydroxy-4'-phenyl-propionic acid 1'-3-phenyl-propanoate.

It is noteworthy to mention the fact that phloroglucinol was by far the most potent *C*- and *O*-nucleophile in a 'normal' series of model phenolic entries (phenol, resorcinol, catechol etc.) and resulted in the formation of the biphenyl, 3,5-dihydroxy-phenyl-2',4',6'-trihydroxy-phenylether. Since the formation of a biphenyl ether is a rare occurrence, extensive methylation was employed to confirm the structure.

Another part of this study includes the investigation and comparison of similar reactions under the influence of microwaves. Microwave reactions are known for their very short reaction times, higher product yields, less solvent utilized and more cost-effective energy consumption, but it was proved that selectivity was not increased. $BF_3{\cdot}(C_2H_5)_2O$ was the catalyst of choice for the selective *C*-acylation of phloroglucinol, rather than the water soluble $Hf(OTf)_4$ Lewis acid. Different carboxylic acids were reacted with resorcinol and phloroglucinol with both Lewis acids as catalyst. In the one reaction between resorcinol and 3-phenylpropanoic acid with $Hf(OTf)_4$ as catalyst, a reaction mixture was produced. The reaction mixture was acetylated to give both the *O*- and *C*-acetylated products, and from this result it was indicated that $Hf(OTf)_4$ can act as both a Brønsted and Lewis acid in a catalytic cycle.

The use of protecting groups was not only to optimize the yields obtained but also to understand $BF_3{\cdot}(C_2H_5)_2O$ and $Hf(OTf)_4$ as catalysts. The low yields for the synthesis of the unprotected dihydrochalcones can be ascribed to: the formation of 3,5-dihydroxy-phenyl-2',4',6'-trihydroxy-phenylether, and the formation of 6,7-dimethoxy-indan-1-one and 5,6-dihydroxy-indan-1-one (intramolecular cyclization).

At last the *C*-glycosylated flavonoid, aspalathin was synthesized. The best reaction result of phloroglucinol and 3,4-dihydroxyhydrocinnamic acid was catalyzed by $BF_3{\cdot}(C_2H_5)_2O$ to produce 3,4,2',4',6'-pentahydroxy dihydrochalcone, which resulted in a 20 % yield. A reliable method for the direct *C*-glycosylation of 3,4,2',4',6'-pentahydroxy dihydrochalcone with an unprotected sugar, D-glucose in aqueous media was used and yielded synthetic aspalathin (10.7%). Not only was this reported

as the first 2 step synthesis of aspalathin, but was distinguished as the first complete free phenolic synthesis of a *C*-glycosylated flavonoid being reported.

Combining this unique synthesis with a global industry such as cosmetics was possible. A study was conducted by Miao-Juei Huang and according to their results it was confirmed that aspalathin would be ideal for the use in topically applied cosmetic products, due to the accumulation of aspalathin in the stratum corneum. This causes a barrier on the skin with strong antioxidant properties, which protects the skin from harmful UV rays, reduce reactive oxygen species and slow down the aging process. Finally the potential of the desired compound to act as an active ingredient in commercial products was confirmed.

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LITERATURE SURVEY

NATURAL PRODUCTS IN THE EVOLUTION OF THE HEALTH-RELATED AND COSMETIC INDUSTRIES

1.1 INTRODUCTION

Throughout the recorded history of man, plant extracts have been used in the possible treatment of illnesses or the relief to ailments. It is also known that natural extracts have played a significant role in the beautification of the body (i.e. the cradle of the current cosmetic industry) over several centuries. Notable is the truism that the ancient Egyptians were well versed in the use of eye and face paints, body oils, and ointments. Alexander the Great (356-323 B.C.) reported the use of unguents, incense, and other cosmetics by countries of the Indo-Sumerian civilization. It is also claimed that Cleopatra bathed in milk not realizing that the benefits observed were due to the effect of alpha hydroxy acids present as lactic acid in the milk. Victorian ladies kept out of the rays of the sun by protecting themselves with garments and parasols for they had learned from experience that the rays of the sun causes premature ageing of the skin, and men in the 17th century unashamedly powdered their wigs, painted beauty spots on their faces and lavished perfume all over their bodies because at that time it was fashionable to do so.[1] The 20th century has been characterized by the introduction of synthetic analogues in both the health-related and cosmetic industry. As the knowledge of the structural array of natural molecules and also their synthesis has grown, the search for beneficial bioactivity has matured to such an extent that it is envisaged that the pharmaceutical and also the cosmetic industries will grow to levels

[1] Milstein. S. R., Bailey. J. E., Halper. A. R., "Handbook of Cosmetic Science and Technology", Marcel Dekker Inc., New York & Basel, **1**, 5, (2001).

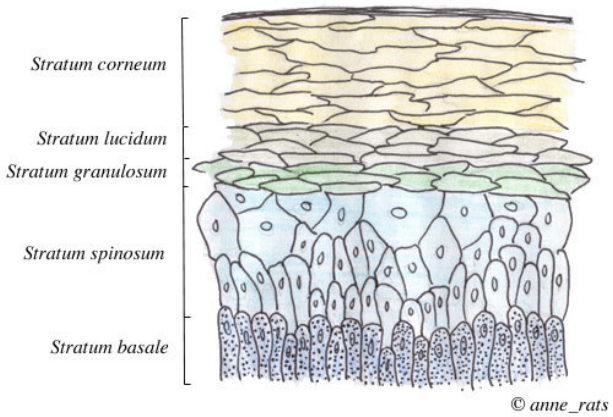
of understanding and application which are currently incomprehensible. The financial incentive in these endeavors has unfortunately led to effects which are not in harmony with sound health and an agreeable physical appearance.

It is, therefore, not a surprise that informed societies have become very suspicious of the claims presented in these fields. This understandable skepticism has given credence to a new philosophy of "natural and green", which in turn has created an awareness of natural compounds and their health relating application. For the purpose of this research project, focus will be placed on the natural polyphenolic compounds (flavonoids) – refer to paragraph 1.3.

1.2 THE SKIN

The skin is the largest organ of the body, comprising approximately 6% of the total body mass. The function of the skin is to protect and maintain the body while it synthesizes, secretes and absorbs substances. The skin consists of three layers, i.e. the epidermis, dermis and hypodermis.[2]

The **Epidermis** is divided into 5 layers (Figure 1):



[2] Society of Cosmetic Chemists South Africa (COSCHEM), Diploma Course Module 1, Unit 6, "Skin Care", Part 1, 4-11, (2003).

Figure 1: The five layers of the epidermis

- *Stratum corneum*

  The stratum corneum is also called the horny layer and consists of stacked layers of flattened dead epidermal cells. This membrane becomes dissociated and ready for shedding.

- *Stratum lucidium*

  The stratum lucidium is the barrier layer, consists of transparent cells and is where the degeneration of the cell nucleus occurs. Degeneration of the cell nucleus involves water loss and intercellular cementing takes place.

- *Stratum granulosum*

  The stratum granulosum is the granular layer. Cells move towards the surface of the skin and are filled with granules, keratohyalin,[3] which is required for keratin formation.

- *Stratum spinosum*

  The stratum spinosum is the prickle cell layer. The cells start losing their shape, flatten and intercellular lipids are formed.

- *Stratum basale*

  The stratum basale is the basal cell layer. It is the division between the epidermis and dermis. Again, cell division occurs and this is where the skin pigment, melanin is produced.

The **Dermis** provides a support for the epidermis as well as for the sensory nervous system.

[3] Thibodeau. G. A., Patton. K. T., *Anatomy & Physiology*, Mosby, St. Louis, 129-152, (1993).

The **Hypodermis** consists of blood vessels for transport of nutrients, and fat, which insulates the body against excessive heat loss.

The epidermis is of most importance to the cosmetic chemist as this is the exposed part of the skin.[2] Hence contact with the epidermis is attained at the interface between the external and internal environments.

1.2.1 SKIN DAMAGE AND PREMATURE AGEING

The generation of free radicals is one of the most significant factors in the development of skin damage and premature ageing. These radicals cause skin irritation, depolymerisation of the skin's natural polymers such as collagen and hyaluronic acid, and possibly even skin cancer.[4] Collagen fibres are found in the dermis and deeper subcutaneous layer of the skin and determine the relative mobility of the skin, whereas the stratum corneum, the most superficial layer of the epidermis, acts as a barrier to water loss and many environmental threats. Once the stratum corneum is damaged, contaminants can easily pass through to the lower layers of the cellular epidermis.[3]

Reactive oxygen species (ROS) are constantly being generated either deliberately (in for example phagocytes, fibroblasts and vascular endothelial cells) or "accidentally" (e.g. "leakage" of electrons from mitochondrial electron transport chains onto O_2) in living cells. [5] Furthermore, various exogenous sources contribute towards hazardous reactive oxygen species, e.g. chemicals, herbicides, ozone, radiation, cigarette smoke and other sources of pollution.[5]

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[4] Aerosol Manufacturer's Association, *Pharmaceutical & Cosmetic Review*, 11-17, (1997).
[5] Halliwell. B., Murcia. M. A., Aruoma. O. I., *Critical Reviews in Food Science and Nutrition*, **35**, 7-20, (1995).

1.2.2 OXIDATION OF BIOLOGICAL CONSTITUENTS BY REACTIVE OXYGEN SPECIES

When produced in excess, attack of these ROS on DNA, proteins and lipids, leads invariably to the destruction or disablement of cells and enzyme systems.[6] Table 1 depicts some examples of the involvement of ROS in the damage of cellular substrates and the corresponding repair processes of the body.

Table 1. Oxidative damage by ROS and *in vivo* repair systems[5]

Substrate of damage	**Repair system**
DNA Singlet O_2 attacks guanine preferentially. All components of DNA can be attacked by ·OH. H_2O_2 and $O_2^{\cdot-}$ do not attack DNA.	A wide range of enzymes recognize abnormalities in DNA and repair it by excision, resynthesis and rejoining of DNA strands.
Proteins -SH groups can be oxidized by many ROS. Various amino acid residues are attacked by ·OH. Proteins often bind transition metal ions, which renders them susceptible to site-specific ·OH generation.	Methionine sulfoxide reductase repairs oxidized methionine residues; Cellular proteases recognize other damaged proteins and preferentially destroy it.

[6] Woodford. F. P., Whitehead. T. P., *Ann. Clin. Biochem.*, **35**, 48-56, (1998).

Lipids Lipid peroxidation can be initiated by some ROS [·OH, RO· (lipid alkoxy radical) and ROO· (lipid peroxy radical), but not $O_2^{\cdot-}$ and H_2O_2]	Antioxidants (especially α-tocopherol) quench peroxy radicals. Phospholipid hydroperoxide glutathione peroxidase and also some phosphor lipases are capable of converting peroxides in membranes to stable molecules. Normal membrane turnover can release damaged lipids.

Since the stratum corneum of the skin serves as a barrier to oxidative insults[7] and consists of keratinocytes that is characterized by thickened cell membranes (consisting of a double layer of phospholipids with cholesterol and protein molecules embedded therein) and the replacement of the cytoplasm by keratin (a water-repellent protein), it is conceivable that damage to the stratum corneum is mainly due to oxidation of lipids.[3] Based on these considerations, the remainder will mainly focus on lipid peroxidation.

1.2.3 LIPID PEROXIDATION

Lipid peroxidation (LPO) is a result of an overabundance of free radicals in the cell membrane. This can result in pathological conditions that may include atherosclerosis and cancer.[8] The LPO proceeds in three stages:

Initiation stage (I): $RH + ROS \rightarrow R\bullet$

[7] Traber. M. G., Rallis. M., Podda. M., Weber. C., Maibach. H. I., Packer. L., *Lipids*, **33**, 87-91, (1998).
[8] Cook. N. C., Samman. S., *Nutritional Biochemistry: Review*, **7**, 66-76, (1996).

Lipid peroxidation is initiated by ROS (·OH, RO· and ROO·, but not $O_2^{\cdot-}$ or H_2O_2).[5] The initiation stage is when free radicals abstract a hydrogen from polyunsaturated fatty acids (R) to form the lipid radical (R·).

Propagation stage (II):	R• + O_2	→	ROO•
	ROO• + RH	→	ROOH + R•

These lipid radicals react with molecular oxygen to form lipid peroxy radicals (ROO·) which react with other molecules to generate more free radicals in a chain extending mode.

Termination stage (III):	ROO• + ROO•	→	inert product
	R• + R•	→	inert product
	ROO• + R•	→	inert product

1.2.4 ANTIOXIDANT DEFENSE SYSTEMS

There are two main sources of antioxidants: either our body's own in-house antioxidants or dietary antioxidants. The body stores antioxidants, which can be mobilized to neutralize dangerous excesses of ROS within cells, in cell membranes, or in extracellular fluids.[6] The antioxidant defense system of the body include, amongst others, antioxidant enzymes, reduced glutathione (GSH) and thiols as well as exogenous antioxidants.[9]

Exogenous antioxidants represent those substrates that are not synthesized in the body and hence need to be supplemented by the diet. Among these, vitamin E (found in cell membranes and carried by lipoproteins in the blood plasma)[6] is the most important lipid soluble, chain-breaking antioxidant, delaying lipid peroxidation by reacting faster with peroxyl radicals than these radicals can react with proteins or fatty

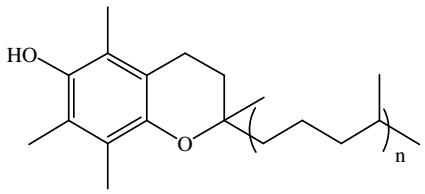
[9] Ji. L. L., *The American Journal of Sports Medicine*, **24**, S-20-S-24, (1996).

acid side chains.[5] Vitamin C is claimed to be an important antioxidant *in vivo* as the semidehydroascorbate radical formed is much less reactive than the many radicals that can be scavenged by ascorbate. Furthermore, it is involved in the regeneration of α-tocopherol.[5] Research on the antioxidant activity of the flavonoids, which occurs naturally in fruit, vegetables and beverages such as tea and wine, has escalated since the discovery of the French paradox, i.e. the low cardiovascular mortality rate observed in Mediterranean populations associated with large amounts of red wine consumption and high saturated fat intake.[10]

This study will focus on exogenous sources of antioxidants relevant to cosmetic vehicles. Vitamins E and C as antioxidants will be briefly discussed, followed by an in-depth inquiry into the antioxidant activity of the flavonoids.

1.2.5 VITAMIN E

Vitamin E **1** is the major lipophilic antioxidant in the skin and is the most common natural antioxidant used in topical formulations. Vitamin E is a term used to describe a family of tocopherols of which α-tocopherol is the most abundant and important member.[11]



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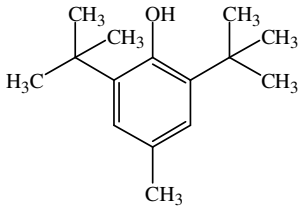
[10] Nijveldt. R. J., Van Nood. E., Van Hoorn. D. E. C., Boelens. P. G., Van Norren. K., Van Leeuwen. P. A. M., *Am. J. Clin. Nutr.*, **74**, 418-425, (2001).
[11] Kretz. A., Moser. U., "Handbook of Cosmetic Science and Technology", Marcel Dekker Inc., New York & Basel, **1**, 463-472, (2001).
[12] Shahidi. F., Janitha. P. K., *Crit. Rev. Food Sci. Nutr.* **32**, 67, (1992).

All forms of tocopherols consist of a chromanol nucleus that carries the redox-active phenolic hydroxyl group and a lipophilic alkyl chain which can be partly saturated or fully unsaturated. The side chain is anchored in the lipid membranes while the nucleus is located at the lipid / aqueous interphase. Notable in the structure of Vitamin E is the presence of three methyl groups attached to the phenolic ring, two of which are flanking the phenol functionality. The latter structural feature is reminiscent of the hydroxyl group flanked by two bulky *t*-butyl groups in the commercially available antioxidant, 2,6-di-*tert*-butyl-*p*-cresol (BHT) **2**.



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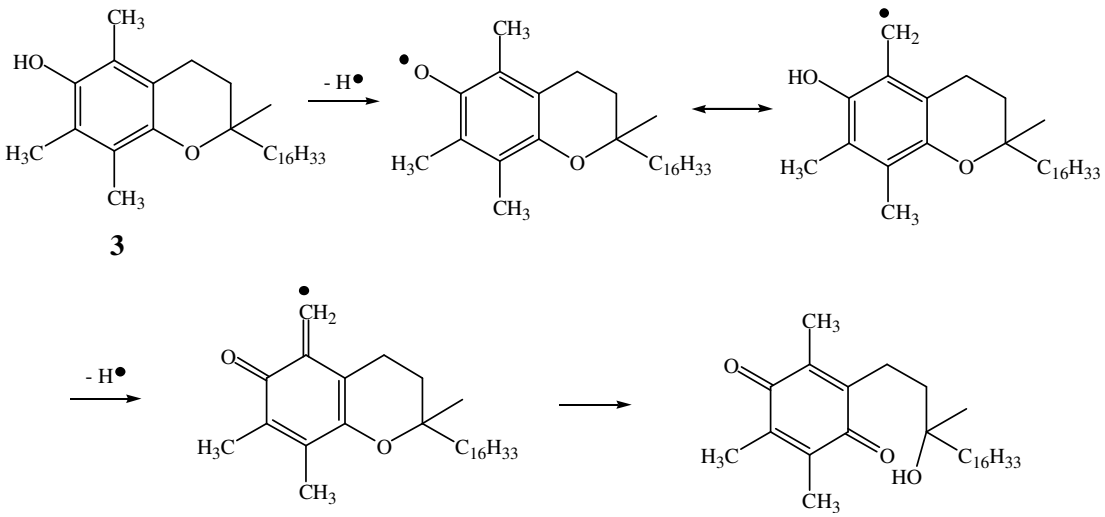
The relevance of the alkyl groups in Vitamin E and BHT is most likely explicable in terms of their ability to create a steric shield over the formed free radical oxygens by their spatial bulkiness. In addition, this radical is stabilized by delocalisation over the aromatic system.[12] The scavenging of lipid peroxyl free radicals *via* the donation of H• by α-tocopherol **3** is explained in the mechanism below (Scheme 1):



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Scheme 1: The scavenging of lipid peroxyl free radicals

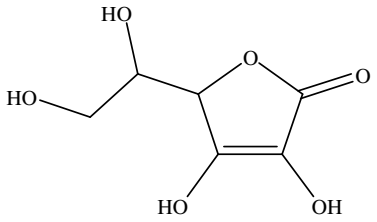
Vitamin E is often used in sunscreens and other skin cosmetics.

Traber *et al.*[7] demonstrated the rapid penetration of γ-tocotrienol, α-tocotrienol and α-tocopherol into murine skin with large increases in the stratum corneum and the subcutaneous concentrations of these vitamin E homologs. It was unclear whether the penetration was into skin cells (keratinocytes or fibroblasts), around the cells in the skin lipids, or down the hair follicles into the deepest layers. Vitamin E applied topically to the skin also had a protective effect to oxidative stress applied to the skin. In the stratum corneum the α-tocopherol concentrations were significantly higher than those of either γ- or α-tocotrienol, whereas there were no significant differences in the concentrations of the vitamin E forms in the other skin layers.

1.2.6 VITAMIN C

Vitamin C **4** (L-ascorbic acid) is one of the most important water soluble antioxidants and are present in high amounts in the skin. Numerous studies, with varying

outcomes, have been undertaken to investigate the effects of vitamin C.[13] It was revealed that ascorbic acid is capable of effectively scavenging many types of radicals, including hydroxyl- (OH•), superoxide- (O_2•$^-$), and peroxyl- (ROO•) radicals as well as singlet oxygen ($^1O_2$) – shown in Scheme 2[14] below.



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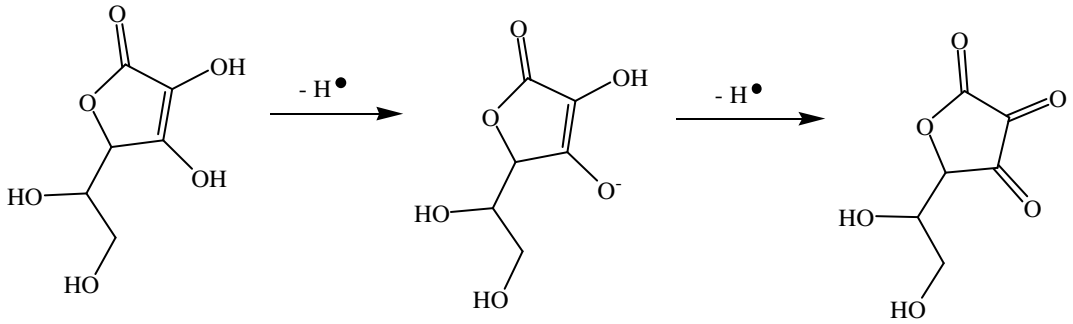

Scheme 2: Effective scavenging capability of ascorbic acid

Vitamin C helps inhibit UV damage when it is applied to the skin[11] and helps to regenerate tocopheryl radicals formed during the course of the inhibition of lipid peroxidation. Based on these characteristics, it is tentatively suggested that a combination of vitamin C and E may constitute an attractive mixture to probe as a possible enrichment to the skin-care products.

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[13] Rietjens. I. M. C. M., Boersma. M. G., De Haan. L., Spenkelink. B., Awad. H. M., Cnubben. N. H. P., Van Zanden. J. J., Van der Woude. H., Alink. G. M., Koeman. J. H., *Environ. Toxicol. Pharmacol.*, **11**, 321-333, (2002).

[14] Marais. C., "Struktuur en Sintese van Metaboliete uit Rooibostee (*Aspalathus linearis*). Fisiologiese aktiwiteit en biomimetiese model vir die fermentasieproses", Ph.D-dissertation, University of the Free State, (1995).

1.3 THE FLAVONOIDS

Flavonoids are an important group of polyphenolic compounds[15] with a plethora of structural features giving rise to an almost overwhelming number of compounds. Not only is the number of polyphenolic metabolites remarkable, but they are also characterized by an impressive array of biological activities.[16] Biological effects of the flavonoids include amongst others antibacterial[17], antiviral[18], anti–inflammatory[19], anti-allergic[20], iron-chelation[21] and anticancer acitivities[8] as well as vasodilator actions[22]. The past few years have been characterized by an impressive growth in the isolation and evaluation of the biological profile of flavonoids, the impetus based on a justifiable realization by informed societies that these compounds, which constitute such an integral part of daily intakes, have a vivid potential to act as health protectors, e.g. antioxidants. It is fair to say that antioxidants, naturally and synthetically, have become a health-relating icon as research into the mechanism of the role of the antioxidant has revealed that the obvious and destructive role of radicals in the biological environment may be linked to many ailments.

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[15] Harborne. J. B., Baxter. H., "The Handbook of Natural Flavonoids", John Wiley & Sons, New York, **2**, (1999).
[16] Handique. J. G., Baruah. J. B., *Reactive & Functional Polymers*, **52**, 163-188, (2002).
[17] Scalbert. A., *Phytochemistry*, **30**, 3875, (1991).
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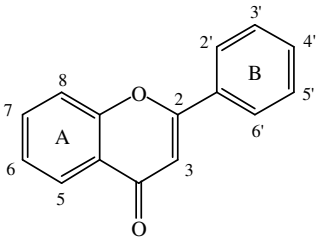
[20] Hope. W. C., Welton. A. F., Fielder-Nagy. C., Batula-Bernardo. C., Coffey. J. W., *Biochem. Pharmacol.*, **32**, 367-371, (1983).
[21] Van Acker. F. A. A., Hulshof. J. W., Haenen. G. R. M. M., Menge. W. M. P. B., Van der Vugh. W. J. F., Bast. A., *Free Rad. Biol. Med.*, **31**, 31-37, (2001).
[22] Duarte. J., Vizcaino. F. P., Utrilla. P., Jimenez. J., Tamargo. J., Zarzuelo. A., *Biochem. Pharmacol.*, **24**, 857-862, (1993).

1.3.1 CLASSIFICATION OF FLAVONOIDS

Flavonoids are characterized by a C_6-C_3-C_6 backbone and are grouped into different classes, amongst others the flavones **5**, flavanones **6**, flavonols **7**, dihydroflavonols **8**, chalcones **9**, dihydrochalcones **10**, flavan-3-ols **11**, the flavan-3,4-diols **12**, anthocyanidins **13**, the different isoflavonoids and a plethora of related polyphenolics. These classes are typified by varying structural features, including different hydroxylation patterns of the A– and B-rings (e.g., resorcinol **14**, catechol **15**, phloroglucinol **16**, and pyrogallol **17** types).[23] The heterocyclic C-ring is discerned as the structural facet containing the stereogenic centres (maximum of 3) giving rise to numerous stereochemical isomers. Over the time numerous different naturally occurring flavonoids have been described and the list is still growing.[24]



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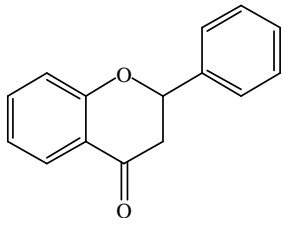

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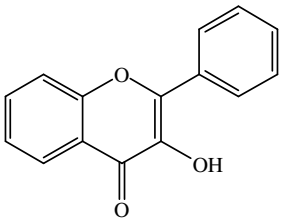

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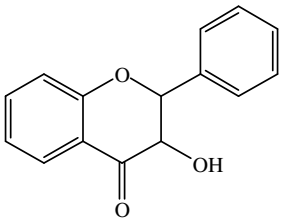

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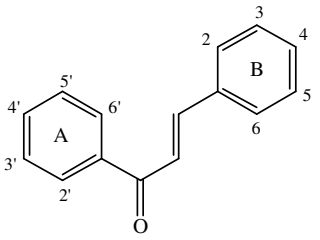
[23] Harborne. J. B., "The Flavonoids: Advances in Research since 1986", Chapman & Hall, London, UK, (1993).
[24] Harborne. J. B., Baxter. H., "The Handbook to Flavonoid Pigments", John Wiley & Sons, New York, (July 16, 1999).



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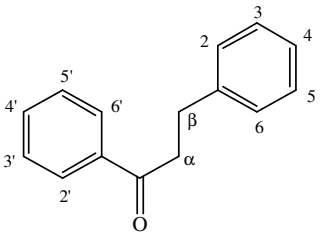

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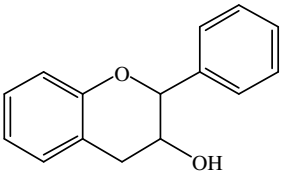

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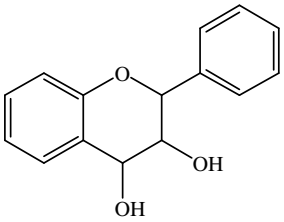

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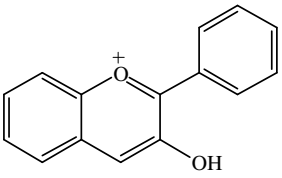

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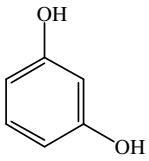

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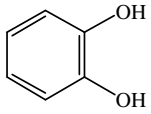

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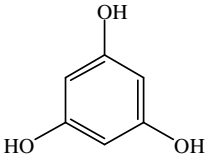

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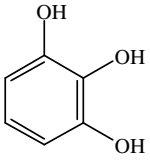

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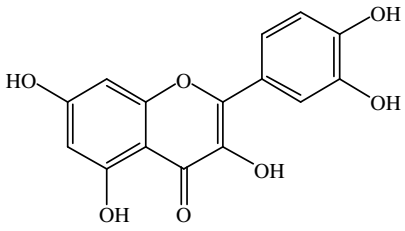
1.3.2 ANTIOXIDANT ACTIVITY OF FLAVONOIDS

For a polyphenol to be defined as an antioxidant it must satisfy two basic conditions, *viz.* in low concentration relative to the substrate to be oxidized, it must be able to delay, retard, or prevent autoxidation or free radical-mediated oxidation and secondly, the formed radical must be stabilized *via* intramolecular hydrogen bonding and other contributing factors, to prevent it from taking part in further oxidation.[25]

Quercetin **18** (a flavonol), which occurs widely in plants, is known for its notable antioxidant activity. The molecule is characterized by a double bond between C-2 and C-3 on the central C-ring, a hydroxyl on C-3, a carbonyl at C-4 of the C-ring and a

[25] Rice-Evans. C. A., Miller. N. J., Paganga. G., *Free Rad. Biol. Med.: Review*, **20**, 933-956, (1996).

number of hydroxyl groups on the B-ring. This structural array of **18** complies with a structure/antioxidant relationship[8], which conclusively confirms that the flavonol **18** is a potent antioxidant which is, in general, superior to other flavanols.[26]



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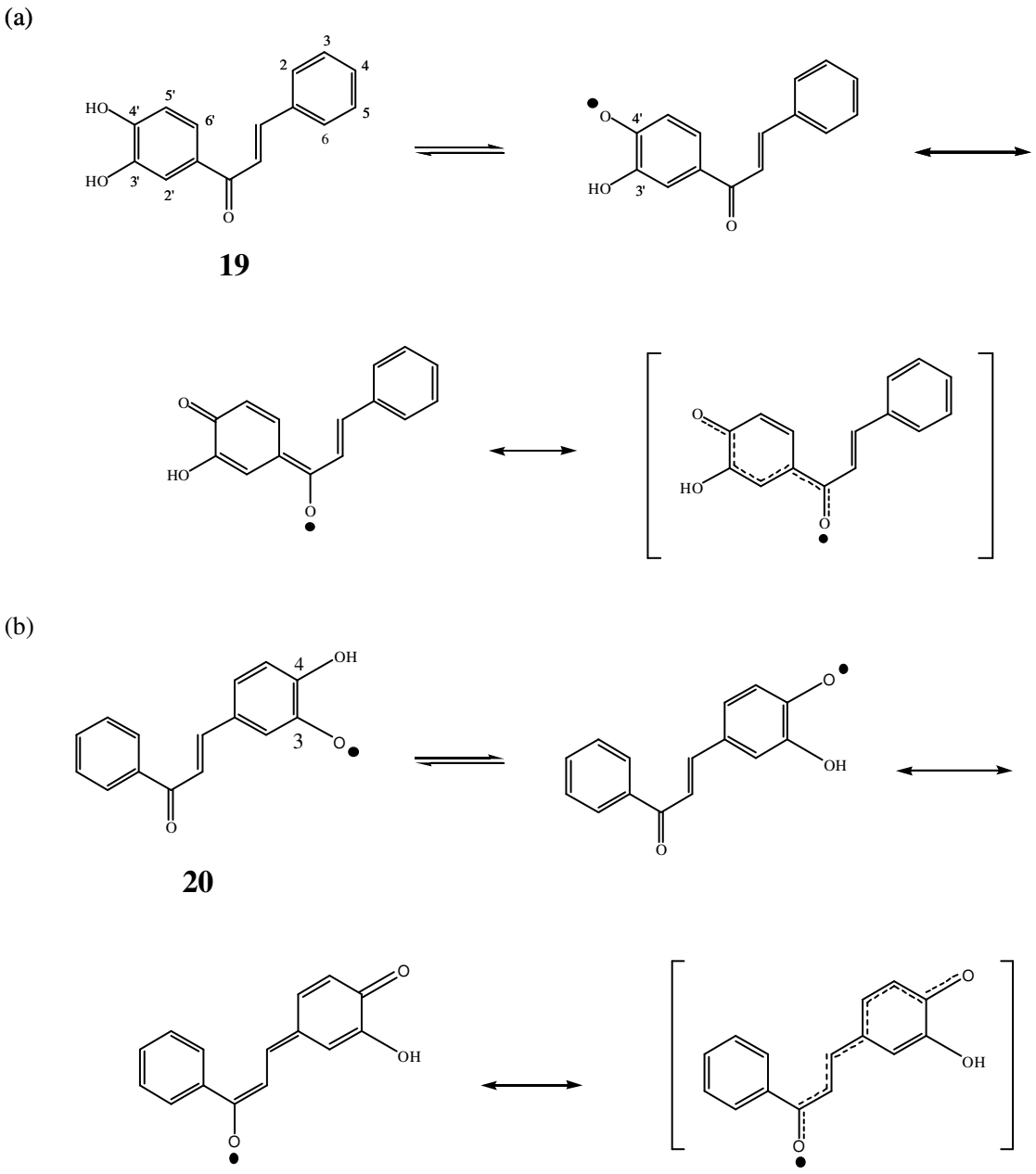
The influence of different hydroxylation patterns of the aromatic rings on antioxidant activity of two different chalcones, are conveniently illustrated in Scheme 3. The more effectively the formed radical is delocalized as depicted by various resonance structures, the more stable it is. The very useful antioxidant activity of 3,4-chalcone **19**(b) compared to the 3',4'-chalcone **20**(a)[27], is expediently rationalized in Scheme 3 in terms of resonance-stabilized radicals:

[26] Miller. A. L., *Alternative Medicine Review*, **1**, 103-111, (1996).
[27] Marais. C., "Struktuur en Sintese van Fenoliese Metaboliete uit Rooibostee (*Aspalathus linearis)*", M.Sc-dissertation, University of the Free State, (1992).



Scheme 3: The influence of different hydroxylation patterns of the aromatic rings on antioxidant activity of two different chalcones

In the burgeoning field aimed at the establishment of reliable relationships between the structures of flavonoids and their antioxidant activity, it has emerged that the number and positions occupied by the phenolic groups play a vital role. Despite a notable progress in this regard, it remains a formidable challenge to present a model which is infallible for the variety of biological effects and the complexity of bio-

systems. Hence, a protocol has been established to investigate the relate antioxidant activity with flavonoid structures within a chemically homogeneous group.

1.3.3 PROPERTIES OF FLAVONOIDS (POLYPHENOLS)

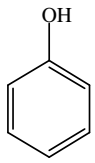
1.3.3.1 BOND DISSOCIATION ENTHALPY

Free radical scavenging activity of polyphenols is characterized by its hydrogen atom donating ability to scavenge the radicals.

$$ArO\text{-}H + ROO\cdot \rightarrow ROO\text{-}H + ArO\cdot$$

The ability to donate a hydrogen atom is mainly governed by the O-H bond dissociation enthalpy (BDE). The weaker the O-H bond, the smaller the BDE and the greater the free radical scavenging ability of the antioxidants.

In a recent publication of Thavasi *et al*.[28] the BDE was used to elucidate the effect of the OH groups of phenol **21**, catechol **15**, resorcinol **14**, hydroquinone **22**, pyrogallol **17**, phloroglucinol **16**, 1,2,4-benzenetriol **23**, and 5-hydroxypyrogallol **24**.



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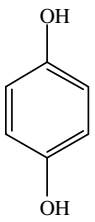

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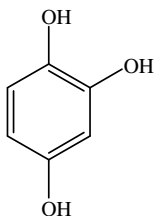

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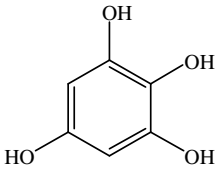

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The different effects the positioning of the OH groups have (*ortho*-, *meta*- and *para*-effect, and the combination of *ortho* and *para* effect) on the aromatic ring were investigated and it was concluded that the position of the OH's, but not the number of

[28] Thavasi. V., Leong. L. P., Bettens. R. P. A., *J. Phys. Chem. A*,**110**, 4918-4923, (2006).

OH's, are very important for a lower bond dissociation enthalpy value. The *ortho* arrangement of the OH groups leads to a development of an intramolecular hydrogen bond (IHB). Increasing the number of OH's in the *ortho* position (more IHB's), decreases the BDE, but increasing the number of OH's in the *meta* position has little impact compared to phenol **21**. An OH in the *para* position lowers the BDE, and hence the largest radical scavenging activity is expected for 5-hydroxyprogallol **24**.

1.3.3.2 HAMMETT σ-VALUE AND PEROXYNITRATE SCAVENGING

Peroxynitrate ($ONOO^-$) forms in the reaction of $NO\cdot$ with $O_2^{-}\cdot$ and plays an important role in various diseases, e.g. cardiovascular, neurological and airway diseases. The known antioxidant ability of flavonols prompted Heijnen *et al.*[29] to study the influence of substituents on the reactivity of those relevant substrates.

Their results revealed that the aromatic OH groups are the reactive centers in the peroxynitrate scavenging activity of flavonols. The activity of these aromatic OH groups can be enhanced by electron donating effects of other substituents when there is an even number of C-atoms between the active and stimulating group. The 3-OH group is stimulated by the presence of the 5- or 7-OH groups and the double bonded oxygen at position 4 and the ring oxygen at position 1. The OH groups at position 5 and 7 are only stimulated by the 3-OH group (Figure 2).[20]

[29] Heijnen. C. G. M., Haenen. G. R. M. M., Vekemans. J. A. J. M., Bast. A., *Environ. Toxicol. Pharmacol.*, **10**, 199-206, (2001).

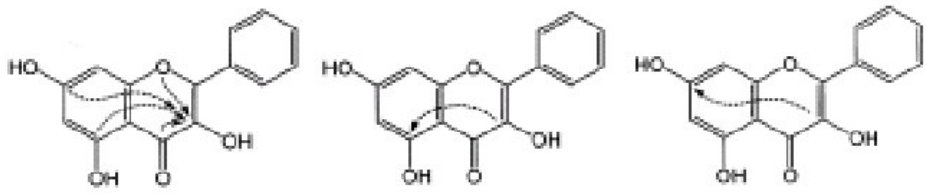


Figure 2[20]: The enhancement of the peroxynitrate scavenging activity of flavonols by an even number of C-atoms at the aromatic OH groups

The electronic effect of substituents is best explained with the Hammett σ-value. An OH group in a conjugated system has an electron donating effect to the ring. This electron donation from the substituent to the oxygen of the active OH group weakens the O-H bond making it easier to release an H·. The Hammett σ-value gives the electron donating (negative value) or the withdrawing effect (positive value) of a substituent.[30] The Hammett σ-value of the OH group depends on the relative position of the OH substitution at the ring compared to the active centre (Figure 3).[29]

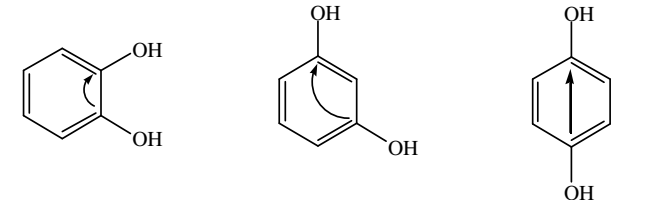


Figure 3: The Hammett σ-value of the OH group depends on the relative position of the OH substitution

The maximal electron donating effect, i.e. the most negative σ-value, is observed when the OH is at the *ortho* or *para* position. An electron withdrawing effect is seen at the *meta* position. This agrees with the order of rank of activity observed for the catechol **15**, resorcinol **14** and hydroquinone **22**.

[30] Hansh. C., Leo. A., *(Eds.)*, "Exploring QSAR. Fundamentals and applications in chemistry and biology", Washington DC: American Chemical society, 1995.

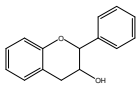
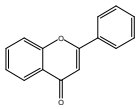
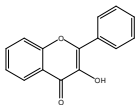
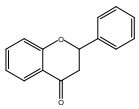
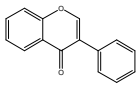
1.3.4 FREE RADICAL SCAVENGING ABILITY OF DIFFERENT CLASSES OF FLAVONOIDS

In the classification of flavonoids there consist lines of evidence to support specific structural elements as central determinants of their free radical scavenging ability. Heim *et al.*[31] recently published a review article (Table 2) in which the major flavonoid subclasses are classified, a general structure is given and their Trolox equivalent antioxidant activities (TEAC)[32] are summarized.

A higher TEAC value reflects a greater antioxidant capability. This confirms that a free 3-hydroxyl group and 3',4'-catechol (dihydroxy) **15** structure, a 2-3 double bond, a 4-oxo group endow the flavonoid with activity superior to isoforms lacking these features. Glycosidic substitution decreases the TEAC value.[25,33]

[31] Heim. K. E., Tagliaferro. A. R., Bobilya. D., *J. Nutrit. Biochem.*, **13**, 572-584, (2002).
[32] The TEAC is defined as the concentration of Trolox solution with equivalent antioxidant potential to a 1mM concentration of the compound under investigation. The TEAC reflects the ability of hydrogen-donating antioxidants to scavenge the $ABTS^{\cdot+}$ radical cation, absorbing in the near infra red-region at 645, 734 and 815 nm, compared with that of Trolox (water soluble vitamin E analog).
[33] Rice-Evans. C. A., Miller. N. J., Bolwell. P. G., Bramley. P. M., Pridham. J. B., *Free Radic. Res.*, **22**, 375-383, (1995).

Table 2. Radical scavenging ability of selected flavonoids in terms of TEAC values[31]

Class + General Structure	Flavonoid	Substitution Pattern	TEAC (mM)
Flavanol 	(+)-catechin **25**	5,7,3',4' - OH	2.40
	(-)-epicatechin **26**	5,7,3'.4' - OH	2.50
	Epigallocatechin gallate **27**	5,7,3',4',5' - OH	
		3-gallate	4.75
Flavone 	Chysin **28**	5,7 - OH	1.43
	Apigenin **29**	5,7,4' - OH	1.45
	Rutin **30**	5,7,3',4' - OH	
		3-rutinose	2.40
	Luteolin **31**	5,7,3',4' - OH	2.10
	Luteolin glucosides **32**	5,7,3' - OH	
		4'-glucose	1.74
	33	5,4' - OH	
		4',7 - glucose	0.79
Flavonol 	Kaempferol **34**	5,7,4' - OH	1.34
	Quercetin **18**	5,7,3',4' - OH	4.70
	Myricetin **35**	5,7,3',4',5' - OH	3.10
	Tamarixetin **36**	5,7,3' - OH	
		4-OMe	
Flavanone (dihydroflavone) 	Naringin **37**	5,4' - OH	
		7-rhamnoglucose	0.24
	Naringenin **38**	5,7,4' - OH	1.53
	Taxifolin **39**	3,5,7,3',4' - OH	1.90
	Eriodictyol **40**	5,7,3',4' - OH	1.80
	Hesperidin **41**	3,5,3' - OH	
		4'-OMe	
		7-rutinose	1.08
Isoflavone 	Genistin **42**	5,4' - OH	
		7-glucose	1.24
	Genistein **43**	5,7,4' - OH	2.90
	Daidzin **44**	4' - OH	
		7-glucose	1.15
	Daidzein **45**	7,4' - OH	1.25
Anthocyanidin	Apigenidin **46**	5,7,4' - OH	2.35
	Cyanidin **47**	3,5,7,4' - OH	
		3,5 - OMe	4.42

1.3.5 STRUCTURE-ANTIOXIDANT ACTIVITY RELATIONSHIPS OF FLAVONOIDS

The unique structure of each flavonoid determines its antioxidant activity. Montoro *et al*.[34] studied the quercetin **18** derivatives, kaempferol **34** and myricetin **35** in order to compare their antioxidant activity. From these results the diversion was made that quercetin **18** and its derivatives are of most interest in the comparison of the structure activity relationship (SAR) of flavonoids. The following paragraphs outline a summary of their results.

1.3.5.1 HYDROXYL GROUPS

The superiority of quercetin **18** in inhibiting metal- and nonmetal-induced oxidative damage is partially ascribed to its free 3-OH substituent, which is thought to increase the stability of the flavonoid radical.[31]

Quercetin **18** (flavonol), exhibits a TEAC of approximately 4.7, whereas luteolin **31** (flavone) has a value of 2.1, supporting the role of the 3-OH group in free radical scavenging. The torsion angle of the B-ring with respect to the rest of the molecule strongly influences free radical scavenging ability. Flavonols and flavanols with a 3-OH are planar, while the flavones and flavanones, lacking this feature, are slightly twisted.[35] Planarity permits conjugation, electron delocalization, and a corresponding increase in flavonoid phenoxyl radical stability. Removal of a 3-OH abrogates coplanarity and conjugation, thereby compromising scavenging ability. From a cosmetic point of view, it is also important that these antioxidants are able to access the biological structures they need to protect against oxidation. A planar structural conformation (e.g., flavonols and anthocyanidins) will promote incorporation of

[34] Montoro. P., Braca. A., Pizza. C., De Tommasi. N., *Food Chemistry*, 1-7, (2004).
[35] Van Acker. S. A. B. E., De Groot. M. J., Van den Berg. D., Tromp. M. N. J. L., Donne-Op den Kelder. G., Van der Vijgh. W. J. F., Bast. A., *Chem. Res. Toxicol.*, **9**, 1305-1312, (1996).

molecules into the biological membrane, whereas a twisted conformation (e.g., flavanones) or substituents out of the plane of the flavone skeleton (e.g., tartaric acid esters or glycoside derivatives of flavonols and anthocyanidins) will obstruct incorporation of molecules into the membrane.[36] It is thus envisaged that flavonols like quercetin **18** are structurally suitable to protect phospholipids in cell membranes against lipid peroxidation.

It is postulated that B-ring hydroxyl groups form hydrogen bonds with the 3-OH, aligning the B-ring with the heterocycle and A-ring. Eliminating this hydrogen bond effects a minor twist of the B-ring, compromising electron delocalization capacity.[35] Due to this intramolecular hydrogen bond, the influence of a 3-OH is potentiated by the presence of a 3',4'-catechol **15**, explaining the potent antioxidant activity of flavan-3-ols and flavon-3-ols that possess the latter feature.

The anthocyanidins **13** represents a group of flavonoids with exceptionally good scavenging activities. They do not only have a very low first oxidation potential, but also show more than one oxidation wave. Depending on the conditions, this low oxidation potential renders them into either pro-oxidants by redox-cycling, or very good antioxidants. Their high activity is most likely to be caused by their peculiar structure, namely the O^+ (oxonium ion) in ring C. Similar to quercetin **18**, these flavonoids are completely conjugated, which gives very stable radical products due to the delocalization possibilities.[37]

1.3.5.2 TAUTOMERISM

Heijnen *et al*.[38] highlighted an interesting fact related to the hydroxyl groups of the A-ring. The reactivity of the 3-OH group is influenced by a 5-OH or a 7-OH group,

[36] De Beer. D., Joubert. E., Gelderblom. W. C. A., Manley. M., *Food Chem.*, **90**, 569-577, (2005).
[37] Van Acker. S. A. B. E., van den Berg. D., Tromp. M. N. J. L., Griffioen. D. H., Van Bennekom. W. P., Van der Vijgh W. J. F., Bast. A., *Free Radic. Biol. Med.*, **20**, 331-342, (1996).
[38] Heijnen. C. G. M., Haenen. G. R. M. M., Van Acker. F. A. A., Van der Vijgh W. J. F., Bast. A., *Toxicology in Vitro*, **15**, 3-6, (2001).

which explains the high activity of kaempferol **34** and galangin **48** during peroxynitrite scavenging. The elevated activity is rationalized by an intramolecular rearrangement which may take place when the 5-OH group is present, giving a catechol-like structure in ring C (Figure 4). This feature is known as tautomerism and is not just known for flavonol structures but are also common in dihydrochalcones.[14]

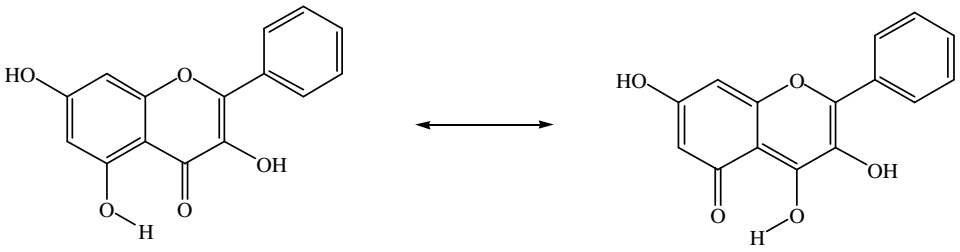


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Figure 4: Tautomers of galangin

On the other hand, the positive influence on the activity ascribed to the presence of the 7-OH, is credibly attributed to the *p*-directing electronic effect of the phenolic group which is likely to enhance the electron density of the C-4 carbonyl functionality and hence the scavenging capability.[38]

1.3.5.3 *O*-METHYLATION

The differences in antioxidant activity of polyhydroxylated and polymethoxylated flavonoids are most likely due to the differences in both hydrophobicity and molecular planarity, although there are exceptions. An example of this exception is quercetin **18**, which is a potent peroxyl radical scavenger, followed by its *O*-methylated and *O*-glycosylated derivatives.[39] Although the ratio of methoxy to hydroxyl substituents does not necessarily predict the scavenging ability of a flavonoid, the B-ring is particularly sensitive to the position of the methoxy group.

[39] Dugas. A. J. Jr., Castaneda-Acosta. J., Bonin. G. C., Price. K. L., Fischer. N. H., Winston. G. W., *J. Nat. Products*, 327-331, (2000).

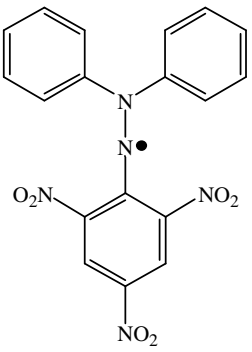
Suppression of antioxidant activity by *O*-methylation may reflect steric effects that perturb planarity. Steric obstruction of the 3',4'-catechol **15** structure by 4'-*O*-methylation significantly compromises antioxidant capability. For example, 4'-*O*-methylation of quercetin **18** to tamarixetin **36** decreases percentage inhibition of ferrous sulfate-induced lipid peroxidation from 98.0 % to -2.6 %[39] and kaempferol-3',4'-dimethylether exhibits approximately half the peroxyl scavenging activity of kaempferol **34**.[40] This information suggests that isorhamnetin, the 3-methoxy metabolite of quercetin **18** detected in humans, is a less effective antioxidant than the parent compound.

1.3.5.4 THE 2-3 DOUBLE BOND AND 4 OXO FUNCTION

A distinguishing feature among the general flavonoid structural classes is the presence or absence of an unsaturated 2-3 double bond in conjugation with a 4-oxo function.

Experiments of catechins **15** and anthocyanidins **13** suggest that these may be dispensable provided that other structural criteria are fulfilled. For example, the TEAC of quercetin **18** (4.7) and cyanidin **47** (4.44) differ by a narrow margin of 0.26 Trolox equivalents.[31] In a systematic study of 33 flavonoids, Burda *et al*.[41] identified no consistent correlation between 2-3 unsaturation and antioxidant activity in a methanol solution of DPPH· (1,2-diphenyl-2-picrylhydrazyl) **49**. However, comparison of quercetin **18** with taxifolin **39** suggests that in flavonoids fulfilling other structural criteria, the 2-3 double bond conjugated with the 4-oxo group distinguishes the better antioxidant.

[40] Cao. G., Sofic. E., Prior. R. L., *Free Radic. Biol. Med.*, **22**, 749-760, (1997).
[41] Burda. S., Oleszek. W., *J. Agric. Food Chem.*, **49**, 2774-2779, (2001).



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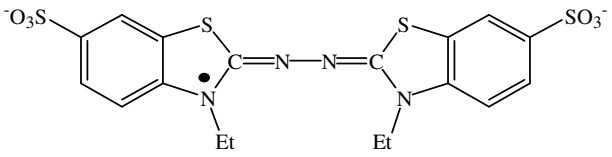
The premise that the selected flavonols are more effective free radical scavengers than selected flavones may be ascribed to the greater number of hydroxyl groups and also the presence of the 3-OH in the former. The profound influence of discernible structural features is strikingly illustrated by the TEAC of quercetin **18** (4.7) which is almost twice that of (+)-catechin **25** (2.4), which accentuates the significance of both the 2-3 double bond and a carbonyl at position 4. Apigenin **29**, TEAC value of 1.45 and naringenin **38**, TEAC value of 1.53, exhibit a much smaller difference in $ABTS^{\bullet+}$ [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate)] radical **50** scavenging, thus a 2-3 unsaturation may be less important than the 4-oxo itself. Although the structural elements must essentially be considered, free radical scavenging by flavonoids is variably enhanced by the presence of both elements.



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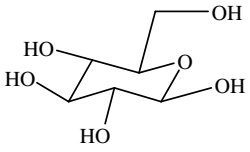
1.3.5.5 CARBOHYDRATE MOIETIES

Plumb *et al*.[42] reported that the antioxidant properties of flavonol glycosides from tea decreased as the number of glycosidic moieties increased. Aside from mere presence and total number, the position and structure of the sugar play an important role.

Luteolin **31** and quercetin **18** aglycones significantly exceeded their 3'-, 4'- and 7-*O*-glucosides in retarding the accumulation of hydroperoxides in membrane layers, but a 4'-sugar was more suppressive than 3- or 7-substitution. Since *C*-glucosylation in the A-ring also decreases activity, this negative effect may stem from the properties of the sugar itself, rather than displacement of a free OH. Like methylation, *O*-glycosylation interferes with the coplanarity of the B–ring and the rest of the flavonoid and the ability to delocalize electrons.[35] Glycosides are usually weaker antioxidants than aglycones, e.g. the addition of rutinose to quercetin **18** to form rutin **30** decrease the antioxidant ability, the TEAC of quercetin **18** (4.7) is nearly twice that of rutin **30** (2.4).[31] Whether the sugar moiety is glucose **51**, rhamnose **52**, or rutinose **53** is also relevant. For example, compared to rutinose **53**, a rhamnose **52** moiety on quercetin **18** significantly reduces scavenging of radicals generated by stimulated human neutrophils.



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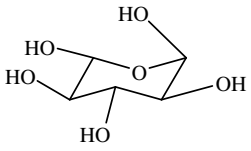

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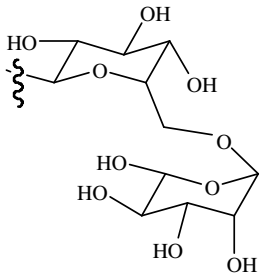

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Aside from occupying free OH groups necessary for hydrogen abstraction and radical scavenging, any sugar substituent is capable of (i) diminishing coplanarity of the B-

[42] Plumb. G. W., Price. K. R., Williamson. G., *Redox. Rep.*, **4**, 13-16, (1999).

ring relative to the rest of the flavonoid, and/or (ii) lending hydrophilicity and altering access to lipid peroxyl and alkoxyl radicals during the propagation stage of LPO in membranes.

1.3.5.6 POLYMERIZATION

Tannins (polyphenolic compounds comprising monomeric flavonoids linked together.) constitute a substantial fraction of daily flavonoid intake among Western cultures in the form of black tea, red wine and cocoa.[31] For example, grapeseed extract consists primarily of oligomeric and dimeric tannins, but due to the relative complexity and diversity of tannins, less is known regarding structure-activity relationships.

Procyanidin dimers and trimers are more effective than monomeric flavonoids against superoxide anions, but the activities of dimers and trimers differ little. Tetramers exhibit greater activity against peroxynitrite- and superoxide-mediated oxidation than trimers. Heptamers and hexamers demonstrate significantly greater superoxide scavenging properties than trimers and tetramers. [43]

It appears that to a point, increasing the degree of polymerization enhances the effectiveness of procyanidins against a variety of radical species. Extensive conjugation between 3-OH and B-ring catechol groups, together with abundant $\beta_{4\text{-}8}$ linkages, endow a polymer with significant radical scavenging properties by increasing the stability of its radical.[44] A reproducible hierarchy of structure activity relationships of procyanidins and other tannins has yet to materialize.

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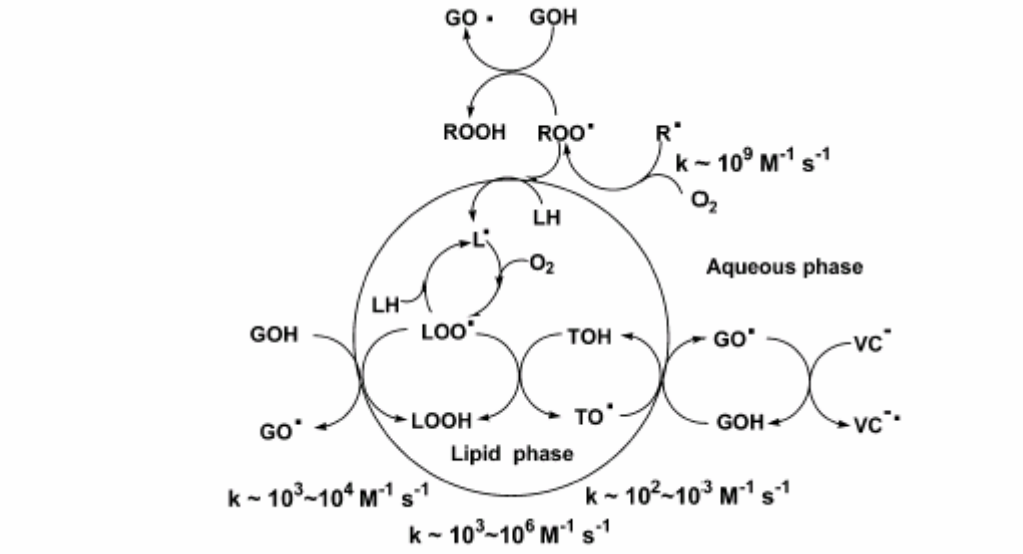
[43] Vennat. B., Bos. M. A., Pourrat. A., Bastide. P., *Biol. Pharm. Bull.*, **17**, 1613-1615, (1994).
[44] Castillo. J., Benavente-Garcia. O., Lorente. J., Alcaraz. M., Redondo. A., Ortuno. A., Del Rio. J. A., *J. Agric. Food Chem.*, **48**, 1738-1745, (2000).

1.4 BIOACTIVITY OF NATURAL ANTIOXIDANTS

The mechanisms to rationalize the actions of antioxidants *in vitro* and especially on the intricate *in vivo* level, remain still clouded in uncertainty, hence creating opportunities for a constructive contribution. Recently, Zhou *et al*.[45] outlined the results of an impressive study which was aimed at unraveling the reaction pathways of natural antioxidants and their synthetic analogues, in chemical and biological systems. Emphasis was placed on their structure-activity relationship and to correlate their chemical and biological activities.

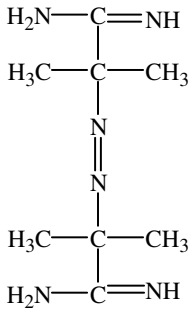
The authors (*vide supra*) of this highly informative article, focused their research on polyphenols (conveniently abbreviated as GOH's) found in green tea. These GOH's are known to act as effective natural antioxidants and it is also known that they are associated with the lower incidence of human cancer. Their results revealed that these GOH's could only decrease the rate of initiation in homogeneous solutions, while they could produce a clear inhibition period in micelles, in human LDL and in human erythrocyte ghosts. These facts demonstrated that GOH's can only trap the initiating radicals in a homogeneous solution, while they can also trap the propagating lipid peroxyl radicals (LOO·) in micelles and lipid peroxyl radicals (ROO·) in biomembranes. The antioxidant synergism of a natural antioxidant polyphenol (GOH), vitamin E (TOH) and vitamin C (VC) is exemplified in Scheme 5 below:

[45] Zhou. B., Liu. Z., *Pure and Applied Chemistry*, **77**, 1887-1903, (2005).



Scheme 5[45]: The antioxidant synergism of a natural antioxidant

It is clear from Scheme 5 that TOH is the only lipophilic antioxidant in the system capable to react with the lipid peroxyl radicals (LOO·) in the interior of the micelle to furnish the corresponding α-tocopheroloxyl radical (TO·), which is subsequently reduced by a natural polyphenolic antioxidant (GOH) to regenerate the vitamin E analogue (TOH) hence prolonging the inhibition period. The natural GOH's can also react with ROO· on the surface of the micelle, while vitamin C (VC) can reduce GO· in the aqueous phase to regenerate GOH. The role of GOH is also against the water soluble free radical initiator 2,2'-azobis(2-methylpropionamide)dihydrochloride (APPH) **54** initiated lipid (LH) peroxidation in micelles and membranes, which might involve trapping the initiating radicals (R•) in the bulk water phase, trapping the propagating ROO• in the water-membrane interface and reducing TO• to regenerate vitamin E.



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It is also evident from the mechanistic pathways mapped out (Scheme 5) that there is a correlation between the structure of a natural antioxidant and its bioactivity. Each natural antioxidant plays a vital role depending on its lipo- or hydrophilicity. It is fair to claim that this interdependence of structure and bioactivity must also hold true for the flavonoids.

ROOIBOS: THE PLANT, CHEMICAL PROFILE AND HEALTH-RELATED CHARACTERISTICS

2.1 ROOIBOS (*ASPALATHUS LINEARIS*)

2.1.1 CULTIVATION HISTORY

Rooibos, meaning "red bush", is a South African plant and a member of the legume family. The shrub *Aspalathus linearis* has bright green needle-like leaves that turn red upon processing. Rooibos has a very limited growing area, *viz.* only the Cederberg Mountain region of the Western Cape in South Africa (Figure 5).[46]

The mountain-dwelling people of the Khoi tribe (also called "bushmen") who lived in this area were the first to develop a method for making tea from rooibos since 1772.[47] Today the beverage, rooibos tea, is gaining popularity on international markets, not only because of its unique taste, but also due to its reputation as being caffeine-free, its proven antioxidant properties, and its therapeutic and physiological advantages.[48]

The process to prepare the tea leaves for brewing has become more automated in recent years, although the steps remain the same as before: the leaves (and sometimes twigs) are picked (Figure 6), bruised, fermented ("sweated"), and then sun-dried. It is the bruising step, in which the leaves are hammered or crushed, that allows the

[46] Montego, *Rooibos Herbal tea*, Retrieved 31 January 2007 from www.itmonline.org/jintu
[47] Morton. J. F., *Economic Botany*, **37**, 164-173, (1983).
[48] Jaganyi. D., Wheeler. P. J., *Food Chemistry*, **83**, 121-126, (2003).

material to develop its distinctive red color during the sweating process. Recently, "green rooibos" has entered the market, the difference between rooibos and green rooibos being the bruising and fermentation steps. Green rooibos is picked, but instead of the fermentation step, it is directly freeze-dried in order to reduce oxidation.[49]

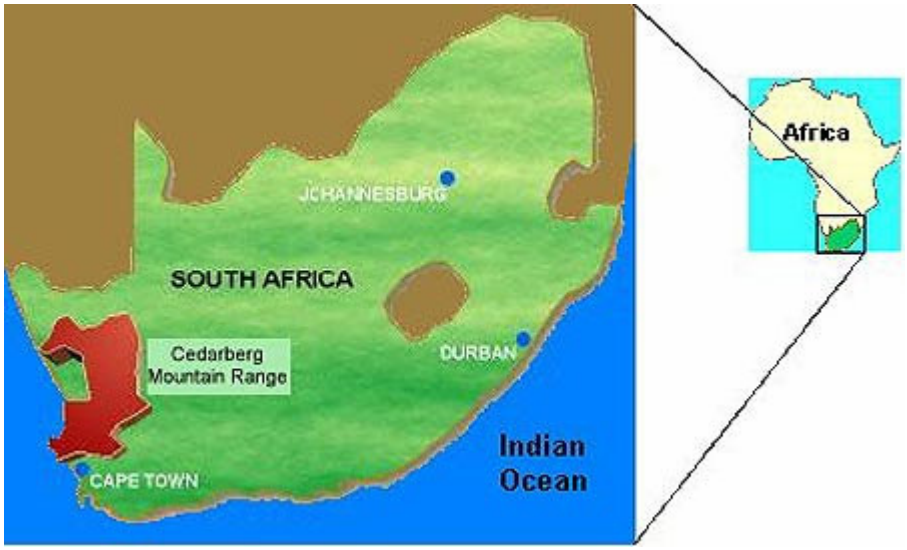


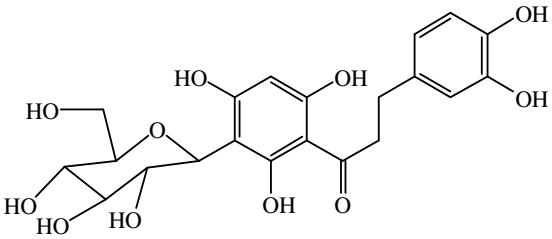
Figure 5: The natural habitat of the *Aspalathus linearis*, in the Cederberg Mountain region (marked in red)

[49] De Beer. S. W., Joubert. E., Preparation of tea-like beverages, South African Patent No. 2002/2808, (2002).

Figure 6: Local workers picking rooibos

2.1.2 RESEARCH ON AND BIOACTIVITY OF *ASPALATHUS LINEARIS*

Koepen *et al*.[50] have been some of the first scientists to characterize the structural composition of rooibos tea. They have also identified aspalathin **55** as the principle monomeric flavonoid in unfermented rooibos.[50] Since then, other phenolic compounds have been identified. They include the flavones orientin **56** and iso-orientin **57**[51], vitexin **58** and iso-vitexin **59**[27], chrysoeriol **60**[52], luteolin **31**[53] and flavoneglucoside, luteolin-7-*O*-glucoside **61**[27]. The flavonols include rutin **30**, the flavonolglucosides isoquercitrin **62**[50] and quercetin **18**[53], while the phenolic acids comprise of benzoic and cinnamic acids[52]. Some non-volatile compounds were identified by Marais *et al*.[27] which include uridine **63** and (+)-pinitol **64**, 5,7-dihydroxy-6-β-D-glucopyranosylchromone **65** and the tannin initiator, catechin **66** with tannins, procyanidin B_3 **67** and *bis*-fisetinidol-(4β,6:4β,8)-catechin **68**. Marais *et al*. went further to also identitify a new pair of flavanone *C*-glucosides, (*S*)- and (*R*)-eriodictyol-6-*C*-β-D-glucopyranoside **69**[54] and the propenoic acid, (Z)-3-phenyl-2-β-D-glucopyranosyloxypropenoic acid **70**[55]



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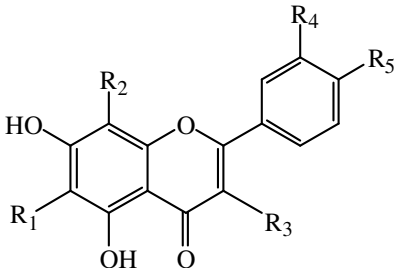
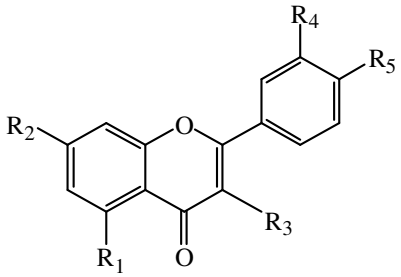
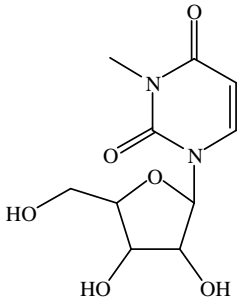
[50] Koeppen. B. H., Smit. C. J. B., Roux. D. G., *Biochem. J.*, **83**, 507-511, (1962).
[51] Koeppen. B. H., Roux. D. G., *Biochem. J.*, **97**, 444-448, (1965).
[52] Rabe. C., Steenkamp. J. A., Joubert. E., Burger. J. F. W., Ferreira. D., *Phytochemistry*, **35**, 1559-1565, (1994).
[53] Snyckers. F. O., Salemi. G., *J. S. A. Chem. Inst.*, **27**, 5-7, (1974).
[54] Marais. C., Janse van Rensburg. W., Ferreira. D., Steenkamp. J. A., *Phytochemistry*, **55**, 43-49, (2000).
[55] Marais. C., Steenkamp. J. A., Ferreira. D., *Tetrahedron Letters*, **37**, 5763-5764, (1996).



56 R_1 = H, R_2 = C-C β-D-glucopyranosyl, R_3 = H, R_4 and R_5 = OH
57 R_1 = C-C β-D-glucopyranosyl, R_2 and R_3 = H, R_4 and R_5 = OH
58 R_1 = H, R_2 = C-C β-D-glucopyranosyl, R_3 and R_4 = H, R_5 = OH
59 R_1 = C-C β-D-glucopyranosyl, R_2, R_3 and R_4 = H, R_5 = OH
60 R_1, R_2 and R_3 = H, R_4 = OMe, R_5 = OH
62 R_1 and R_2 = H, R_3 = C-O β-D-glucopyranosyl, R_4 and R_5 = OH



61 R_1 = OH, R_2 = C-O β-D-glucopyranosyl, R_3 = H, R_4 and R_5 = OH



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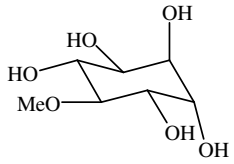

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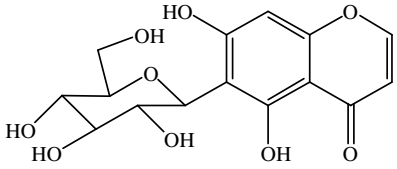

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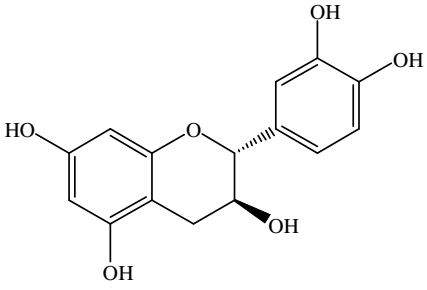

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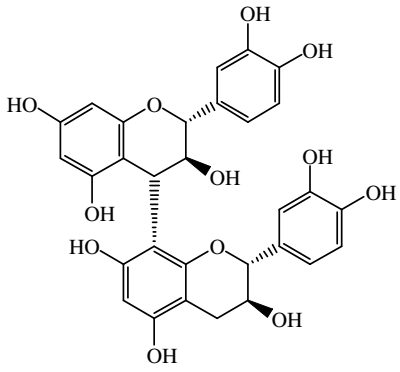

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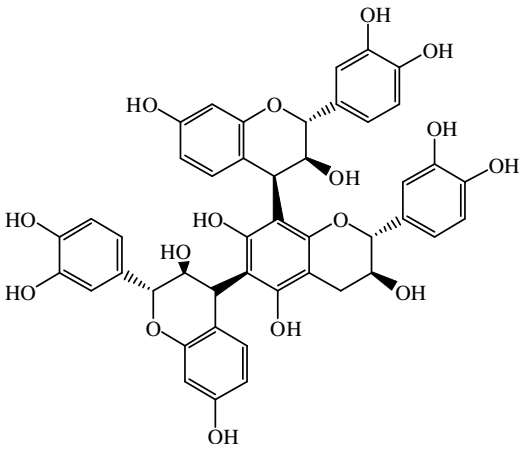

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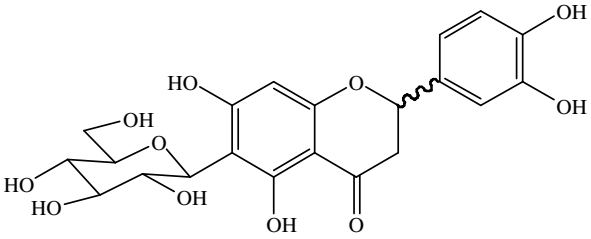

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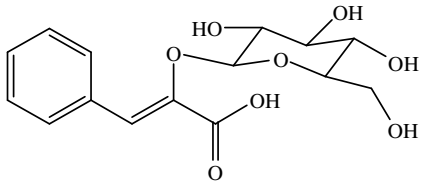

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The publication of these scientists' results in scientific journals, as well as in the more popular magazines, has unexpectedly, but to great satisfaction, expanded rooibos production and especially its application to a significant degree (refer to paragraph 2.1.3 below). More importantly, the cosmetic industry (locally and overseas) was, and still is, the principal beneficiaries to utilize the scientific knowledge of the health-relating properties of the phenolic compounds in their formulations. Furthermore, new methods have been developed for the extraction process and other methodologies to get this wonderful extract in its refined form to the consumer.[56] It is, therefore, no surprise that the search for more positive bioactivity in the rooibos extract is being pursued in many countries.[57]

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[56] Von Gadow. A., Joubert. E., Hansmann. C. F., *J. Agric. Food Chem.*, **45**, 1370-1374, (1997).

[57] [a]Kucharska. J., Ulicna. O., Gvozdjakova. A., Sumbalova. Z., Vancova. O., Bozek. P., Nakano. M., Greksak. M., *Physiol. Res.*, **53**, 515-521, (2004); [b]Weiss. J. F., Landauer. M. R., *Toxicology*, **189**, 1-20, (2003); [c]Marnewick. J. L., Gelderblom. W. C. A., Joubert. E., *Mutation Research*, **471**, 157-166, (2000); [d]Edenharder. R., Sager. J. W., Glatt. H., Muckel. E., Platt. K. L., *Mutation Research*, **521**, 57-72, (2002); [e]Shimoi. K., Masuda. S., Shen. B., Furugori. M., Kinae. N., *Mutation Research*, **350**, 153-161, (1996).

Noteworthy in this regard is the results of Von Gadow *et al*.[58] who, amongst others, compared the antioxidant activity of rooibos tea to green, oolong and black tea. Their results revealed that the antioxidant activity (β-carotene bleaching method) follows the sequence: green tea > black tea > oolong tea > fermented rooibos > unfermented rooibos > semifermented rooibos. On the other hand, scavenging of the DPPH radical **49**, brought another sequence to light: green tea > unfermented rooibos > fermented rooibos > semifermented rooibos > black tea > oolong tea. These results support the notion that rooibos has strong radical scavenging abilities and can compete favorably with well known international herbal teas in this regard.

It is known that rooibos is not only unique to South Africa, but is hitherto the only natural source of the dihydrochalcone aspalathin **55**.[51] This solitary feature of rooibos has, understandably, precipitated a more than normal interest in the rooibos extract by scientists across the globe. According to the HPLC quantifications of Joubert[59] and Bramati *et al*.[60], the main compound determined in *Aspalathus linearis* is aspalathin **55**. It was also established that the content of **55** is higher in unfermented rooibos ("green rooibos") compared to the fermented rooibos.[57] Marais *et al*.[59] demonstrated convincingly that aspalathin **55** is oxidized during the fermentation process. The chemical transformation is rationalized in Scheme 6. Aspalathin **55** in the ground state might be excited to the unstable singlet state **71** on absorption of light, giving rise to the triplet excited state **72** *via* intersystem crossing (ISC) energy release. This triplet state **72** presumably permits hydrogen abstraction from the ground state aspalathin **55** to give a phenoxyl radical **73** and subsequently a quinone methide **76**, *via* *o*-quinone-quinone methide tautorism (**75**,**76**).[54] The electrophilic exo-cyclic carbon of **76** is then trapped in an exo-trig cyclization *via* a phenolic oxygen of the phloroglucinol ring. The excellent hydrogen atom donating ability of aspalathin **55** supports the proposed mechanism.[54]

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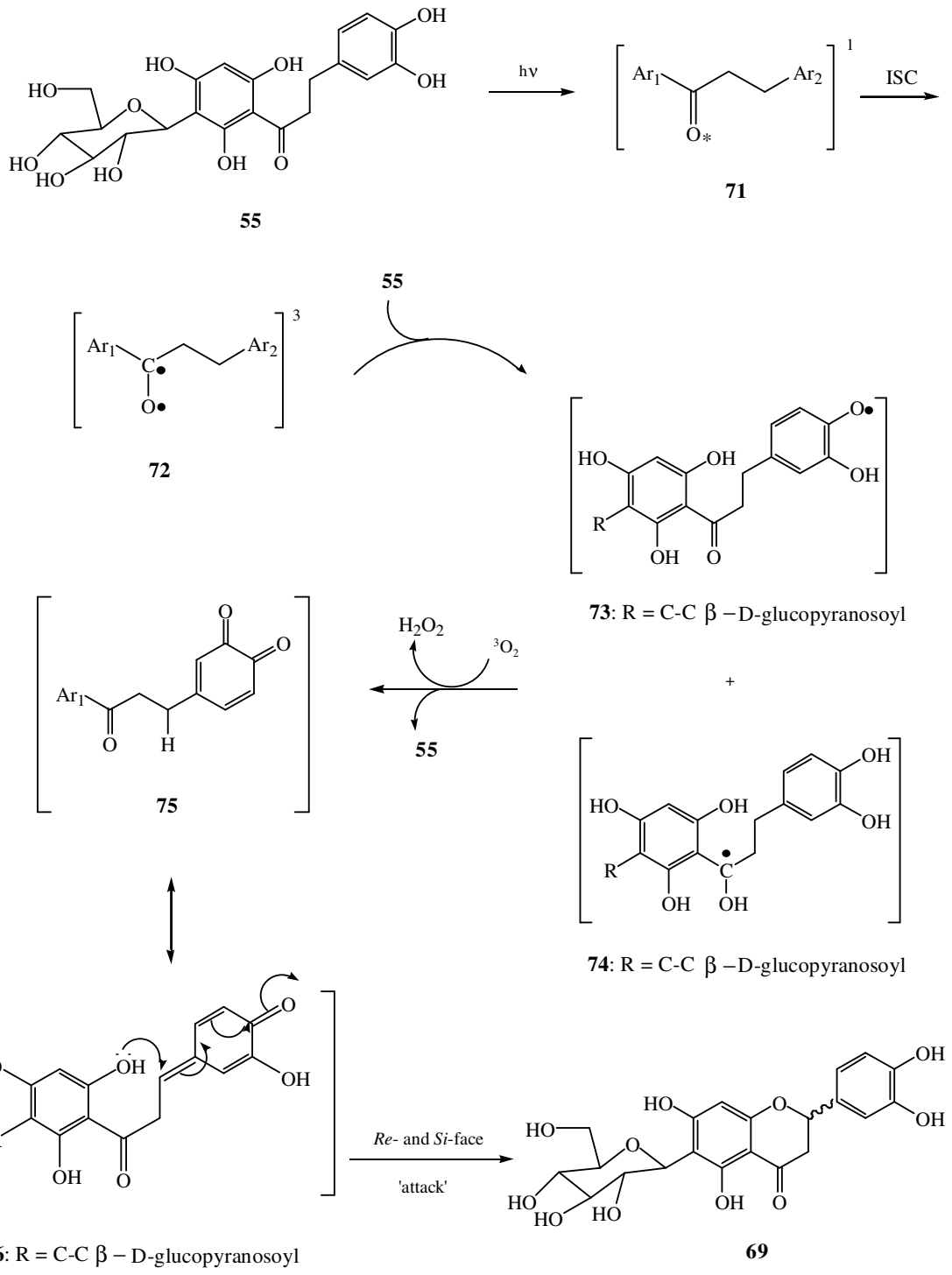
[58] Von Gadow. A., Joubert. E., Hansmann. C. F., *Food Chemistry*, **60**, 73-77, (1997).
[59] Joubert. E., *Food Chemistry*, **55**, 403-411, (1996).
[60] Bramati. L., Aquilano. F., Pietta. P., *J. Agric. Food Chem.*, **51**, 7472-7474, (2003).



Scheme 6[54]: Proposed mechanism for the oxidation of aspalathin

2.1.3 ECONOMIC SUMMARY OF ROOIBOS

In the 1980's, the Rooibos Tea Board marketed rooibos in the RSA as a substitute for tea or coffee.[61] In overseas countries, however, the rooibos extract has mainly been consumed as an herbal tea. The results of Marais *et al*.[14,27] relating those isolated phenolic compounds with their health-promoting characteristics, spearheaded a resurgence in rooibos affiliated activities.

The market share of rooibos in the South African tea market grew from 12% in 1984 to 18% in 1999.[61] From 1995 to 1999, the value of total domestic and export sales increased from 17 million to 65-70 million South African rands (R).[61] In table 3 is a list of the top 10 importing countries of rooibos tea in 1999. Exports of rooibos tea increased with 742% from 1993 to 2003.[62]

Table 3.[61] Top 10 countries importing rooibos tea in 1999

Country	Volume (kg)	Percentage of Total exports
Germany	987,560	53.61
Japan	309,285	16.79
Netherlands	199,061	10.8
United Kingdom	95,482	5.18
Malaysia	54,120	2.93
South Korea	40,060	2.17
Poland	34,000	1.85
United States	22,016	1.19
Colombia	12,792	0.69
China	11,960	0.65

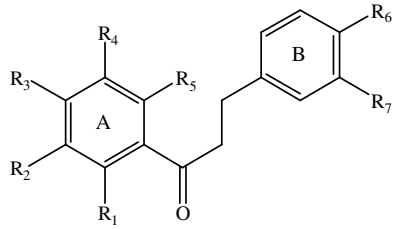
The Rooibos Tea Board, that had handled about two-thirds of the rooibos market in South Africa, became a private firm, Rooibos Ltd. in 1993.[61] Other smaller firms have been established to also gain from this illuminated rooibos plant. It is obvious that the rooibos tea industry has shown an important growth over the last few years. Perusal of the "World Wide Web" revealed that not only have new cosmetic and other health-

[61] Wilson. N. L. W., *Review of Agricultural Economics*, **27**, 1-10, (2005).
[62] Bonthuys, J., *Die Burger*, 11, (6 January 2007).

related products been developed, but that existing and associated formulations have been enriched by the rooibos “magic”.

2.1.4 OCCURENCE AND SYNTHESIS OF DIHYDROCHALCONES

Dihydrochalcones **77** are the open-chain hydrogenated analogues of chalcones **9**, both being represented by the C_6-C_3-C_6 flavonoid skeleton. The A- and B-rings display the full complement of all the possible hydroxylated patterns characteristic to the flavonoid family (e.g. phloroglucinol **A**-ring and catechol **B**-ring):



77

This structural array is amenable to a variety of useful transformations, *viz*. by the utilization of the reactivity of the α-carbon while the benzylic β-carbon is known for a variety of radical and heterogeneous reactions. In addition, the aromaticity of the A- and B-ring allows many electrophilic substitutions, while the chemical versatility of phenolic groups merits a chapter on its own.

While dihydrochalcones constitute a salient group in the flavonoid family, only a few naturally occurring dihydrochalcones are known and the ones with unique features are depicted in Table 4: (Refer to structure of dihydrochalcone **77**).

Table 4. Naturally occurring dihydrochalcones

Dihydrochalcone	Substitution Pattern
Dihydrochalcone **78**	$R_{1\text{-}7}$ = H
79	R_1 = OH, R_3 = OMe, R_5 = OH
Davidin **80**	R_1 = C-O Glu[a], R_3 = OH, R_7 = OH
Nothofagin **81**	R_1 = OH, R_3 = OH, R_5 = OH, R_7 = OH, C-*C*(3')-Glu[a]
Aspalathin **55**	R_1 = OH, R_2 = Glu[a], R_3 = OH, R_5 = OH, R_6 = OH, R_7 = OH

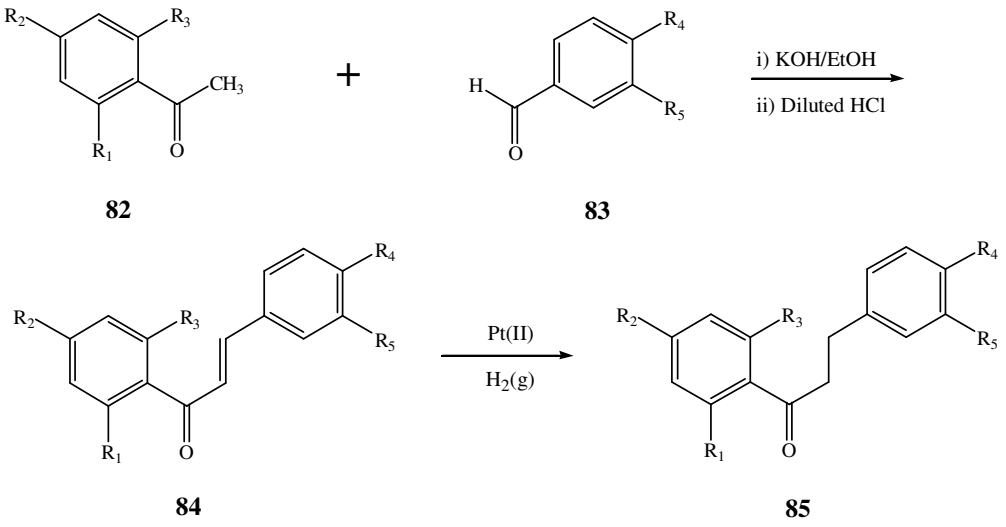
[a]Glu = Glucosyl

While one of these compounds **(78)** is characterized by no phenolic sustituents**,** the remaining dihydrochalcones are all hydroxylated.[63] In addition, there is only one dihydrochalcone that has no B-ring hydroxylation **79** and the only dihydrochalcone with a resorcinol-based A-ring, is davidin **80**. Moreover, some of the dihydrochacones are glycosylated at the *O*-atoms of the hydroxyl groups, but more importantly is the occurrence of only two known dihydrochalcones with a *C*-glycosyl function on the A-ring, *viz.* nothofagin **81** and aspalathin **55**. The latter is characterized by the presence of the prominent phloroglucinol and catechol aromatic rings, one of them with a very rare *C*-glycosyl linkage.

2.1.4.1 SYNTHESIS OF DIHYDROCHALCONES

The best known methodology known for the synthesis of dihydrochalcones **77,** is the aldol condensation between **82** and **83**[64] followed by the reduction of the chalcone **84** in order to produce the dihydrochalcone **85** (Scheme 7).

[63] Bohm. B. A., "The Flavonoids", (J. B. Harborne, T. J. Mabry, H. Mabry, ed.), Chapman & Hall, London, UK, 442-504, (1975).
[64] Wagner. H., Farkas. L., "The Flavonoids", (J. B. Harborne, T. J. Mabry, H. Mabry, ed.), Chapman & Hall, London, UK, 131, (1975).



Scheme 7: Aldol condensation in order to produce dihydrochalcone

Some other methods also include Friedel-Crafts acylation, the Fries-[65]and Hoesch reactions[66]. These reaction methodologies (*vide supra*) were crucial to the eventual establishment of a successful synthesis of aspalathin **55** and will be discussed in detail in the following chapter 3.

2.1.5 ASPALATHIN

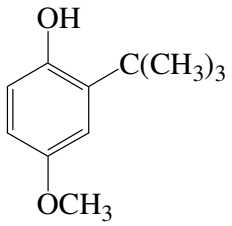
The uniqueness of the structure and occurrence of aspalathin **55** has been demonstrated in previous paragraphs. More importantly, are the impressive bioactivity displayed by aspalathin **55**, for example the inhibition of tumour promotion in mouse skin.[67]

In a recent publication by Joubert *et al*.[68], it was revealed that the major flavonoids of the unfermented rooibos are aspalathin **55** and its dehydroxy analogue, nothofagin **81** - they comprise in some instances as much as 9.3 and 1.03 % of the plant material

[65] Blatt. A. H., *Chem. Rev.*, **27**, 413-429, (1940).
[66] Ruske. W., in Olah, *Review*, **3**, 383, (1964).
[67] Marnewick. J., Joubert. E., Joseph. S., Swanevelder. S., Swart. P., Gelderblom. W., *Cancer Letters*, **224**, 193-202, (2005).
[68] Joubert. E., Winterton. P., Gelderblom. W. C. A., *J. Agric. Food Chem.*, **53**, 10260-10267, (2005).

(dry basis), respectively.[67,68] In the aqueous extract of unfermented rooibos between 35 and 68% of the total polyphenols comprised of aspalathin **55**, which contributes 22-57% of the total antioxidant activity subjected to the $ABTS^{\cdot+}$ **50** scavenging method.[69]

The valuable and sought-after antioxidant activity of aspalathin **55** has been compared with known antioxidants, such as BHT (butylated hydroxytoluene) **2**, BHA (butylated hydroxyanisole) **86**, and α-tocopherol **3**, and in all instances aspalathin **55** showed a very acceptable degree of DPPH radical **49** scavenging. In the case of the scavenging ability towards the superoxide anion ($O_2^{-\bullet}$), quercetin **18** and aspalathin **55** showed the highest capacity compared to the other rooibos flavonoids and tannins.[70]



86

This admirable antioxidant activity of aspalathin **55** is convincingly ascribed to the presence of the 3,4-dihydroxyl arrangement on the B-ring (catechol moiety), the 2',4',6'-trihydroxy-substituted A-ring (phloroglucinol moiety)[71] and keto-enol tautomerism (**55** and **87**) that effectively supports the stabilization of the formed radical after hydrogen abstraction[72].

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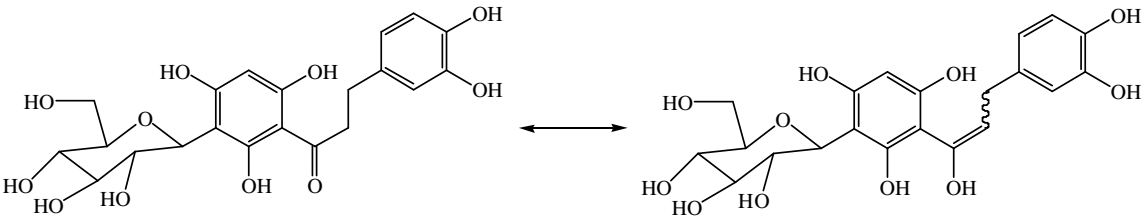
[69] Schulz. H., Joubert. E., Schutze. W., *Eur. Food Res. Technol.*, **216**, 539-543, (2003).
[70] Joubert. E., Winterton. P., Britz. T. J., Ferreira. D., *Food Research International*, **37**, 133-138, (2004).
[71] Nakamura. Y., Watanabe. S., Miyake. N., Kohno. H., Osawa. T., *J. Agric. Food Chem.*, **51**, 3309-3312, (2003).
[72] Rezk. B. M., Haenen. G. R. M. M., Van der Vijgh. W. J. F., Bast. A., *Biochem. Biophys. Res. Commun.*, **295**, 9-13, (2002).



55 **87**

Clearly, there is a demand for this special and unique natural flavonoid. The very low percentage of aspalathin **55** in the rooibos extract[14,27], similarly prevalent for many natural sources yielding minute quantities of precious compounds, prompted a search for a viable synthetic protocol for **55**, details of which will be discussed in chapter 3 (p. 46).

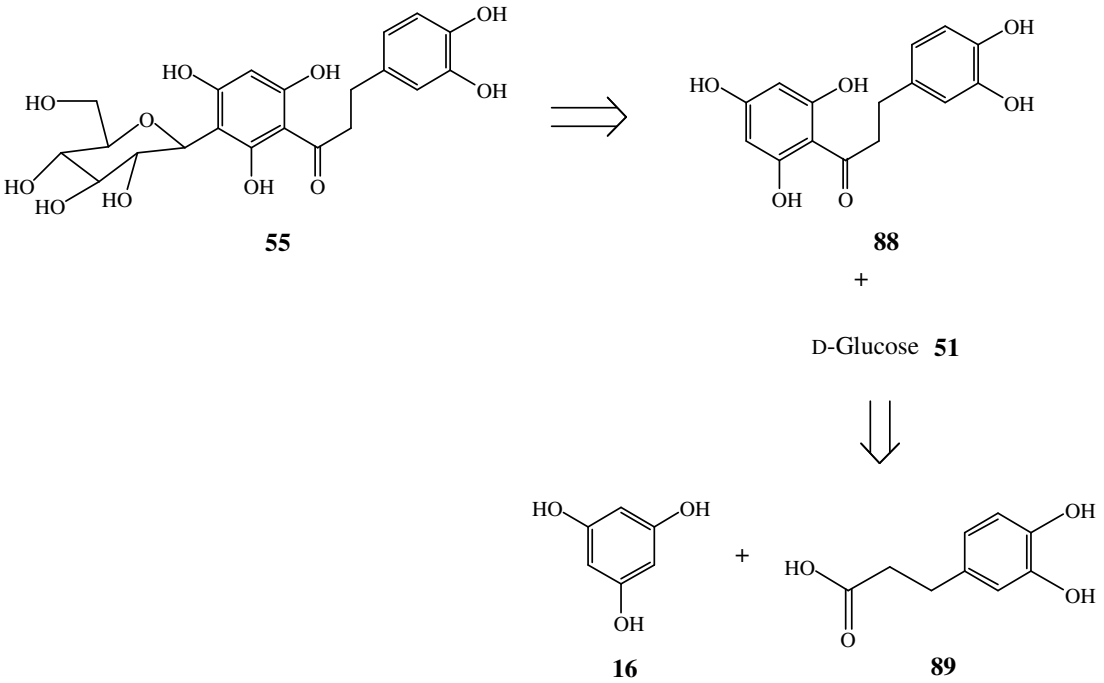
DISCUSSION

STRATEGIES TOWARDS THE SYNTHESIS OF ASPALATHIN, A NATURAL FLAVONOID

3.1 INTRODUCTION

Since no free phenolic synthesis of aspalathin **55** was known when this investigation was launched, a project aimed at the firm establishment of viable synthetic routes towards the construction of those crucial bonds in the target molecule, aspalathin **55**, in an intriguing environment of highly sensitive functionalities, was initiated. In addition, there was a firm commitment to also include economic and 'green' considerations in the envisaged synthetic route.

A retro synthesis revealed a 2-step sequence depicted in scheme 8:



Scheme 8: Retrosynthesis of aspalathin

Scrutiny of the unique structure of aspalathin **55** revealed an impressive, but also challenging, array of chemical reactivity, *viz.* carbon and oxygen nucleophilic sites, aromaticity, carbonyl functionality, keto-enol tautomerism and the jewel of them all, the 1,2-dihydroxybenzene ring (i.e. the catechol functionality) capable to function as a potent antioxidant moiety.

Furthermore, it is clear from a first order analysis that the C_3-C_6 fragment (the dihydrochalcone) is feasible by the acylation of phloroglucinol **16** by an appropriate C_6–acyl unit. The successful construction of the desired dihydrochalcone will be followed by a highly selective glycosylation of the latter. Appraisal of the reactivity of the proposed building blocks revealed not only the presence of a variety of reactive functionalities, but also illuminated a formidable challenge to achieve the goal to minimize protection of the variety of related and reactive functionalities.

It was decided to commence with the construction of the dihydrochalcone **88**, *via* an appropriate acylation step. A literature survey had revealed the existence of a few methodologies or modifications thereof which seemed to have the potential to achieve

the construction of the desired target molecules. Clearly, the envisaged acylation of the phenolic substrate (ploroglucinol, **16**) had to involve the effective interaction of the activated electrophilic carbonyl carbon and a nucleophilic site on the phloroglucinol **16**, *viz.* an oxygen atom of one of the hydroxyl groups or one of the carbon atoms. Such a regioselectivity is governed by a set of principles incorporated in a theoretical premise, conveniently named as hard and soft acids and bases (HSAB).

3.2 HARD AND SOFT ACIDS AND BASES

According to this HSAB theory, hard acids prefer hard bases while soft acids coordinate to soft bases.

The different types of acids and bases are classified according to the following criteria:[73]

soft base – donor atom of high polarizability, low electronegativity, easily oxidized, and associated with empty, low-lying orbitals, e.g. CN^-, H^-, Br^-

hard base – donor atom is of low polarizability, high electronegativity, hard to oxidize, and associated with empty orbitals of high energy and which is almost inaccessible, e.g. H_2O, OH^-, ClO_4^-

soft acid – the acceptor atom is of low positive charge, large size, and has several easily excitable outer electrons, e.g. Hg^+, HO^+, Br_2

hard acid – acceptor atom is of high positive charge, small size, and does not have easily excitable outer electrons, e.g. BF_3, H^+, $B(OR)_3$

A base is known as an electron donor and an acid as an electron acceptor. Pearson[74] classified "*hard*" acceptors and donors as class A type and "*soft*" acceptors and donors as class B type.

[73] Pearson. R. G., Songstad. J., *J. Am. Chem. Soc.*, **89**, 1827-1836, (1967).
[74] Pearson. R. G., *Journal of Chemical Education*, **45**, 581-587, (1968).

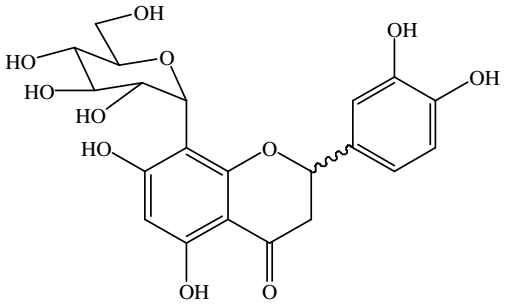
3.3 REACTIONS OF ASPALATHIN

A wide range of and sometimes contrasting, chemical reactivity of aspalathin **55**, posing a formidable challenge in the design of a viable synthesis thereof, is illustrated by the following *in vitro* reactions:

3.3.1 ENZYMATIC OXIDATIVE CYCLIZATION

Marais *et al.*[27] illustrated the *in vitro* oxidation of aspalathin **55** during the fermentation process (refer to paragraph 2.1.2, p. 34). During these experiments, **55** was exposed to light, heat, oxygen and pH. The influence of these elements was investigated in order to identify natural catalysts.

Their results demonstrated that **55** is oxidized in the presence of light and heat to produce the flavones (2*R*)- and (2*S*)-2,3-dihydro-iso-orientin **69**, and with further oxidation the flavones, iso-orientin **57** and orientin **56**. Koeppen and Roux[51] already demonstrated in 1965 that **55** is oxidized in an ethanol solution in the presence of sunlight to produce (2*R*)- and (2*S*)-2,3-dihydro-orientin **90**.

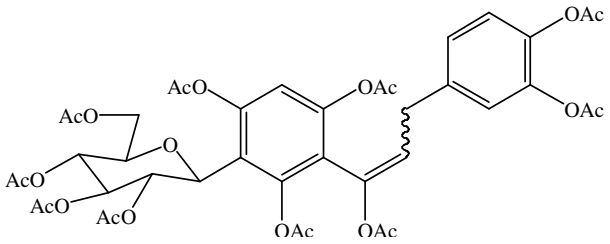


90

The enzyme laccase is known for its characteristic ability to oxidize *p*- and *o*-diphenolic aromatic substrates to the corresponding quinones. Therefore the influence of an enzyme on the oxidation of **55**, as reported in scheme 6 (paragraph 2.1.2, p. 39), was explored. The *in vitro* experiment of laccase as catalyst resulted in the following: the exposure of **55** to laccase at pH 5 and at 30°C resulted in a 14% formation of **69**. Different carbohydrates were also investigated *in vitro* as catalysts in the oxidative cyclization of **55**. I was concluded that a phenolic radical is formed and that a radical mechanism is responsible for the oxidative cyclization of **55** in the presence of oxygen and that heat and ultraviolet light accelerates the oxidation process.

3.3.2 ACETYLATION CONFIRMS THE KETO-ENOL TAUTORISM OF ASPALATHIN

The ^{1}H NMR data of the aspalathin **55** sample in the isolation experiments of rooibos by Marais *et al.*[27] was reminiscent of a mixture of closely related compounds. Subsequent acetylation of these samples in the presence of a catalytic amount of triethylamine, followed by separation, afforded the enolacetate **91** of **55**. (refer to paragraph 2.1.5, p. 43).

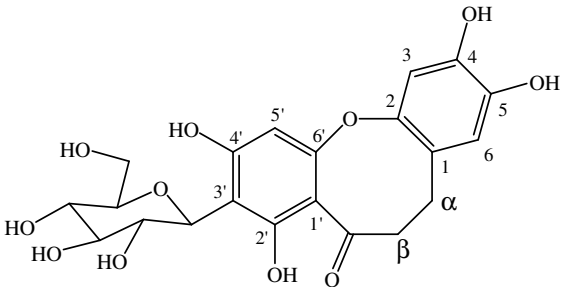


91

While the formation of the enol-acetate **(91)** illustrates a viable route under basic catalysis, the excistance of the corresponding deprotected enol tautomer **91** is likely to play an important role in the biosynthetic elaboration of **91**. Thus under acid catalysis, the carbonyl oxygen atom (a Lewis base) is protonated to give an intermediate cation that can lose H^{+} from its α carbon to yield the enol tautomer.

3.3.3 OXIDATIVE COUPLING OF THE HYDROXYL (ON A-RING) TO C-6 OF THE B-RING, I.E. A 1,4 ADDITION ON THE FORMED QUINONE

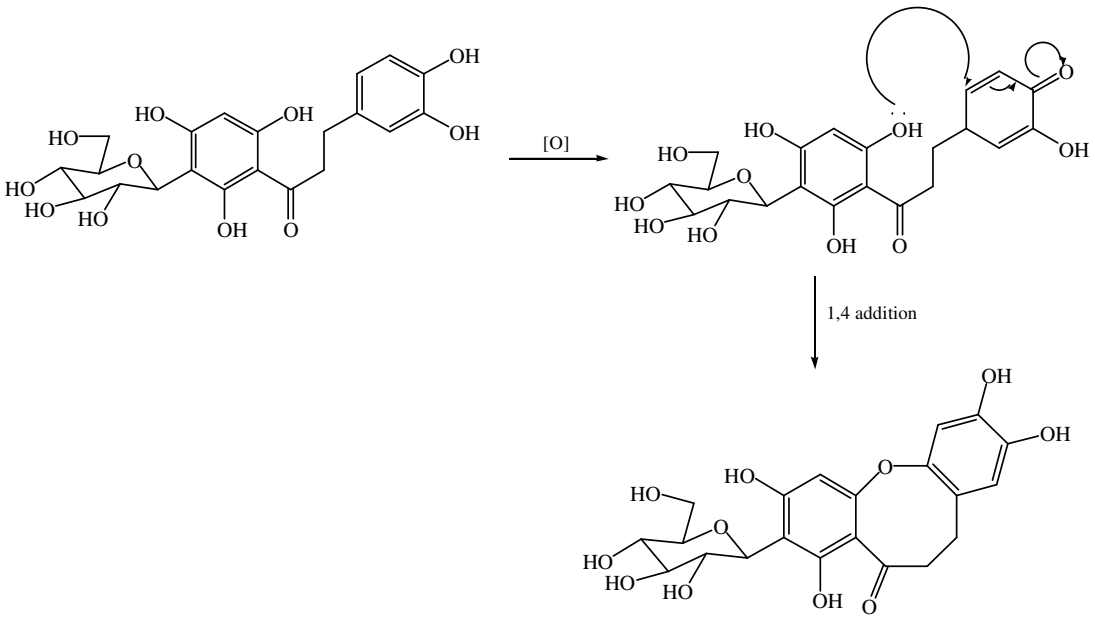
Recently Shimamura *et al.*[75] isolated a new compound from the leaves of *Aspalathus linearis* (rooibos), known as aspalalinin **92**.



92

This compound **92** is not only conspicuous similar to aspalathin **55**, but is indeed a metabolite derived from aspalathin **55**. Based on the known reactivity of aspalathin, a mechanistic rationale is conveniently proposed to account for the formation of the novel aspalalinin **92** (Scheme 9).

[75] Shimamura. N., Miyase. T., Umehara. K., Warashina. T., Fujii. S., *Biol. Pharm. Bull.*, **29**, 1271-1274, (2006).



Scheme 9: Mechanistic proposed formation of aspalalinin (92)

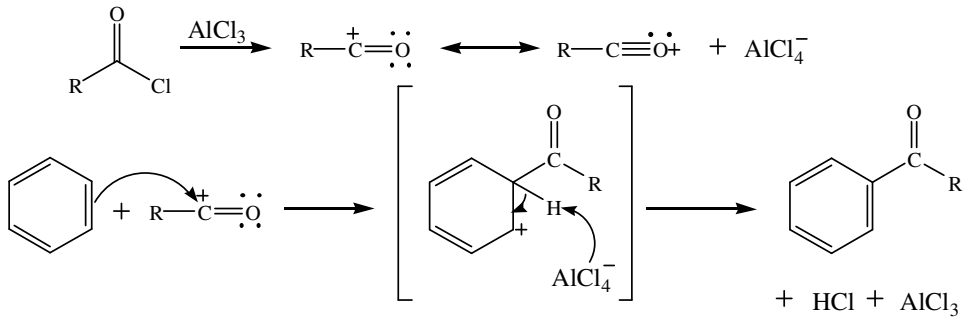
The following paragraphs will deal with a summary of the theory underpinning the selected reaction methodologies.

3.4 ACYLATION OF PHENOLIC SUBSTRATES

3.4.1 FRIEDEL-CRAFTS ACYLATION AND FRIES REARRANGEMENT

The Friedel-Crafts alkylation reaction is one of the most useful electrophilic aromatic substitution reactions known.[76] The mechanism of the Friedel-Crafts acylation is similar to that of Friedel-Crafts alkylation as indicated in Scheme 10.

[76] McMurry. J., "Organic Chemistry", (5th Edition), BROOKS/COLE, USA, 600-605, (2000).

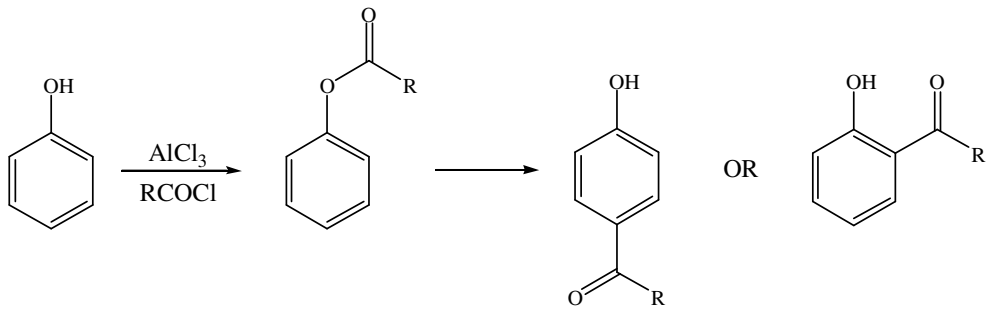


Scheme 10: Proposed general mechanism of Friedel-Crafts acylation

The reactive electrophile is a resonance stabilized acyl cation, which is stabilized by the overlap of the vacant orbital on the carbon with the orbital containing the lone pair of electrons on the neighboring oxygen. The acyl cation does not rearrange but attacks the aromatic ring to give an unrearranged substitution product. The Friedel-Craft acylation reaction does not allow multiple substitutions on the ring, because the acylated product is less reactive than the starting material.[76]

Since the introduction of a protocol (known as the Fries rearrangement) in the early 20th century to convert a phenolic ester to an *o*- or *p*-hydroxyketone, or both, *via* treatment with aluminium chloride, scientists have utilized the Fries rearrangement (Scheme 11), or modifications thereof, with impressive success. Intensive research has revealed that the acylation of phenols can either occur *via C*-acylation (Friedel-Crafts reaction) or *O*-acylation (esterfication) and that this regioselectivity is governed by factors like reaction conditions, reactants and mole ratio.[77] The relative ratio of the resulting products of *C*-acylation and *O*-acylation, is predictable by the HSAB theory (*vide supra*). Accordingly, the more stable *C*-acylated product predominates under conditions of thermodynamic control, whereas the product of *O*-acylation forms faster and predominates under conditions of kinetic control.[78]

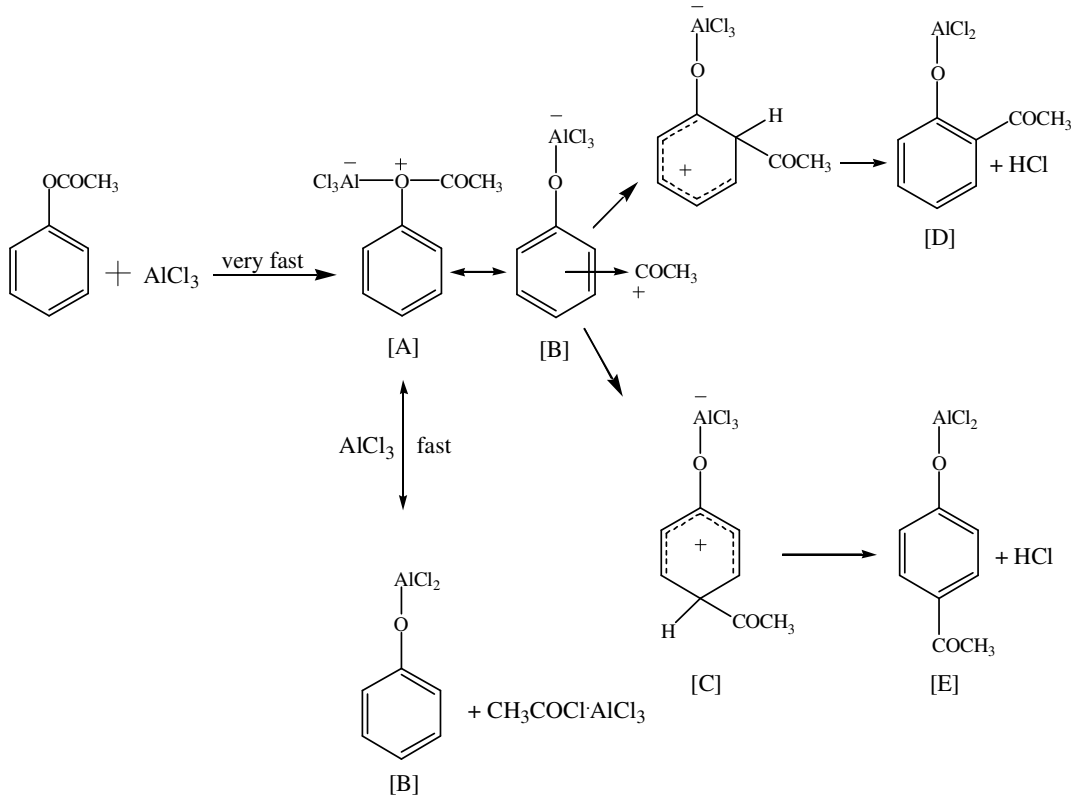
[77] [a]Blatt. A. H., *Organic Reactions*, **1**, 342, (1942); [b] Ogata. Y., *Chem. Rev.*, **9**, 199, (1943).
[78] Acylation, From *Wikipedia* (the free encyclopedia), Retrieved 31 January 2007 from http://en.wikipedia.org/wiki/Acylation



Scheme 11: Fries rearrangement

The Fries reaction is preferred to the Friedel-Crafts synthesis for the preparation of phenolic ketones as better yields are obtained. In addition, it is also common knowledge that an elaborate modification of the experimental procedure applicable to a variety of esters is rarely obligatory.[79] The mechanism of the Fries reaction has been studied by several investigators[77], and there seems to be no conclusive evidence to suggest either the intermolecular[80] or intramolecular[81] migration of the acyl group, or both. It is generally accepted that the most likely route involves a comparatively fast ester interchange followed by an intramolecular acyl migration, probably *via* a π-complex as is depicted in Scheme 12 below.[77b]

[79] Blatt. A. H., *Chem. Rev.*, **27**, 413-429, (1940).
[80] Skraup. S., Poller. K., *Ber. Dtsch. Chem. Ges.*, **57**, 2033, (1924).
[81] Rosenmund. K. W., Schnurr. W., *Liebig's Ann.*, **460**, 56, (1928).



Scheme 12: Proposed mechanism of intramolecular acetyl migration *via* a π-complex

It is known that the coordination complex [A] forms very rapidly. The step from [A] to the π-complex [C] is probably reversible, while the steps towards the hydroxyacetophenones [D] and [E] are irreversible, since the ketones cannot be converted into the ester. The mechanism mapped out in Scheme 12 is highly suggestive of the existence of a common intermediate in the Fries rearrangement and the acylation of a phenol, i.e. a complex of phenyl acetate with aluminium chloride.

It was clear from the literature survey that the synthetic route whereby the envisaged unprotected phenolic substrate may react with a suitable carboxylic acid under conditions outlined by Kobayashi *et al.*,[82] had the potential to comply with the aims of this multistep synthesis of aspalathin **55**.

[82] Kobayashi. S., Moriwaki. M., Hachiya. I., *Tet. Lett.*, **37**, 4183-4186, (1996).

Classic Lewis acids, which are hygroscopic, have posed a big challenge for researchers who have utilized them. To dry relevant solvents, to store them, and to transfer those under dry conditions are time consuming, expensive and the disposal of the used solvents is environmentally problematic. Recently, an alternative to the classical Lewis acid, $AlCl_3$, was introduced in the form of a new generation of water-tolerant Lewis acids such as metal triflates.[83] The benefits of this type of Lewis acids were recently outlined in a review article by Fringuelli *et al*.[84] The obvious advantages of water as a solvent for reactions must have been known and dreamed of for decades, but it was only demonstrated recently[84] that there indeed exist Lewis acids which function effectively in *aqueous* media.

Hence, the introduction of this new group of Lewis acids, *viz*. the lanthanide triflates (trifluoromethanesulfonates, $Ln(OTf)_3$, Ln = La, Ce, Pr, Nd, Sm, Eu, Gd, Dy, Yb, Lu), together with $Bi(OTf)_3$, $Sc(OTf)_3$ and $Y(OTf)_3$, has been an extremely important addition to the arsenal of chemical reactions at the disposal of researchers.[84] One of these, hafnium trifluoromethanesulfonate (hafnium triflate, $Hf(OTf)_4$), was demonstrated to be a most effective catalyst in the Friedel-Crafts acylation reactions.[85]

3.4.2 HAFNIUM TRIFLATE AS CATALYST

Kobayashi *et al*. reported the intramolecular Fries rearrangement of acyloxybenzenes and naphthalenes[86] as well as the direct C-acylation of phenol and naphthol derivatives with acid chlorides, using $Sc(OTf)_3$ as catalyst. In a more recent publication[83], carboxylic acids were used as acylating reagents and $Hf(OTf)_4$ as a more efficient Lewis acid. The same authors reported the acylation of phenols with carboxylic acids in the presence of the catalyst $Hf(OTf)_4$ in a lithium perchlorate-

[83] Kobayashi. S., Nagayama. S., Busujima. T., *J. Am. Chem. Soc.*, **120**, 8287-8288, (1998).
[84] Fringuelli. F., Piermatti. O., Pizzo. F., Vaccaro. L., *Eur. J. Org. Chem.*, 439-455, (2001).
[85] Hachiya. I., Moriwaki. M., Kobayashi. S., *Tet. Lett.*, **36**, 409-412, (1995).
[86] Kobayashi. S., Moriwaki. M., Hachiya. I., *J. Chem. Soc. Chem. Comm.*, 1527-1528, (1995).

nitromethane ($LiClO_4$-$MeNO_2$) system.[85] The ClO_4^- anion seems to have the following roles:

- react with $Hf(OTf)_4$ to form a cationic hafnium complex[$^+Hf(OTf)_3$ ClO_4^-] with enhanced activity[87],
- regenerate the catalyst[88] and increase the turnover of the catalyst[89] .

The combined metal triflate ($Hf(OTf)_4$) and trifluoromethanesulfonic acid (TfOH) catalytic system was found to be more potent than either of the reagents alone.[90a] The proposed mechanism for this synergistic effect of the Friedel-Crafts Acylation, rather than the Fries rearrangement[90b] of a phenolic ester is depicted in scheme 13. Three possible pathways are indicated in this scheme, namely:
(i) *via* pure Brønsted catalysis (path a), since these rearrangements are known to be proton-catalyzed[91],
(ii) *via* a ligand exchange between trifluoromethanesulfonic acid and the metal triflate (path b)[90a], or
(iii) *via* a Lewis acid catalysis resulting in the activation of the ester (path c)[91].

A Brønsted acid is a substance that donates a hydrogen ion (path a), and a Lewis acid is a substance that accepts an electron pair (path c).

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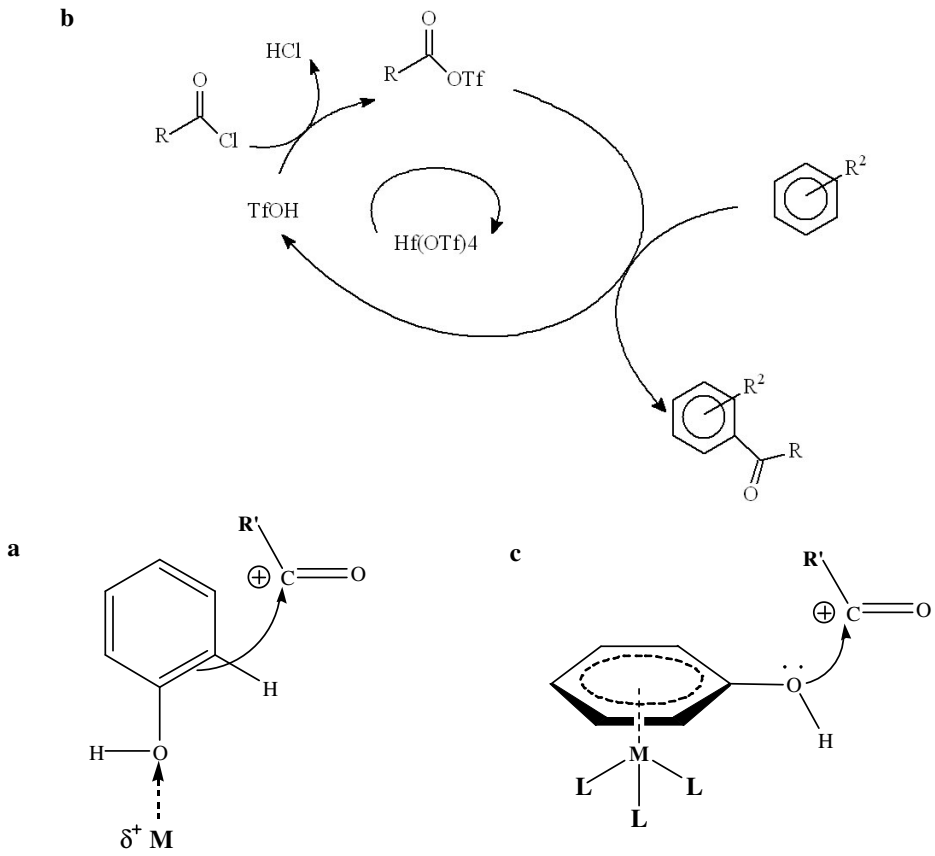
[87] Kawada. A., Mitamura. S., Kobayashi. S., *Chem. Comm.*, 183-184, (1996).
[88] Arai. S., Sudo. Y., Nishida. A., *Tetrahedron*, **61**, 4693-4642, (2005).
[89] Kobayashi. S., Iwamoto. S., *Tetrahedron*, **56**, 6463-6465, (2000).
[90] [a]Kobayashi. S., Iwamoto. S., *Tet. Lett.*, **39**, 4697-4700, (1998), [b]Noji. M., Ohno. T., Futaba. N., Tajima. H., Ishii. K., *J. Org. Chem.*, **68**, 9340-9347, (2003).
[91] [a]Gerecs. A., "Friedel-Crafts and Related Reactions", (Olah. G. A., ed.), Wiley-Interscience, New York, **3**, 499-533, (1964), [b]Martin. R., *Org. Prep. Proc. Int.*, **24**, 369-435, (1992), [c]Bensari. A., Zaveri. N. T., *Synthesis*, 267-271, (2003).



Scheme 13: A mechanistic illustration for the catalytic cycle

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Recently, photochemical rearrangements (Photo–Fries rearrangement) of phenyl benzoate were published.[92] Despite the positive results reported, it was decided not to entertain the idea to engage the Photo–Fries rearrangement in this investigation due to time constraints.

The method of Kobayashi *et al.*[82] was considered as a viable option for the *C*-acylation of phloroglucinol **16** in the first step towards the synthesis of aspalathin **55**. Pilot reactions were thus undertaken to establish a reliable acylation step of the appropriate phenolic substrates.

It is noteworthy to mention the fact that phloroglucinol **16** is by far the most potent *C*- and *O*-nucleophile in a 'normal' series of model phenolic entries (phenol, resorcinol,

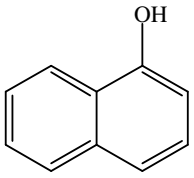
[92] [a]Stenberg. V. I., *Org. Photochem.*, **1**, 127, (1967), [b] Sriraghavan. K., Ramakrishnan. V. T., *Tetrahedron*, **59**, 1791-1796, (2003).

catachol etc.). Furthermore, it is obvious that the neighboring hydroxy functionalities of phloroglucinol pose a significant steric challenge for incoming electrophiles. It has, therefore, become commonplace in the synthetic flavonoid research endeavors, to commence with simple phenolic substrates to assist the eventual establishment of a viable protocol.

3.4.3 MODEL ACYLATION REACTIONS

3.4.3.1 1-NAPHTHOL

Reimplementation of the reported reaction of 1-naphthol **93** with acetic acid in toluene:CH_3NO_2 (6.7:1, v/v) in the presence of $Hf(OTf)_4$[82] afforded the desired acylated product, 1-hydroxy-2-acetonaphthone (**94**, 85%), and the starting material, 1-naphthol (**93**, 23%). Evident in the ^1H NMR spectrum (Acetone-d_6) of **94** was a shift of the aromatic protons towards the low field region, which was caused by the aromatic system conjugated with the carbonyl. When the ^1H NMR spectrum of **94** was compared to that of **93**, not only the shift of the aromatic protons to the low field region was noted but also the disappearance of the doublet assigned to H-2. This confirmed *C*-acylation[77] on the naphthol ring. Acylation was furthermore confirmed by the appearance of the methyl signal at δ 2.75.



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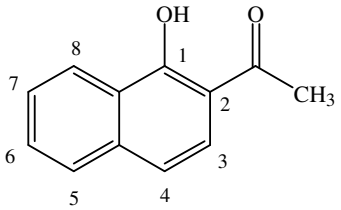
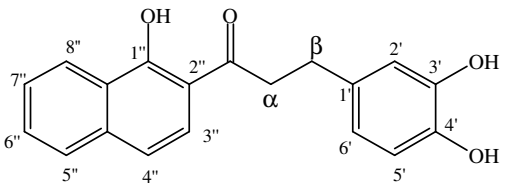


94

Acetic acid was subsequently replaced by 3,4-dihydroxyhydrocinnamic acid **89**, the synthon towards the synthesis of aspalathin **55**. Under the same conditions,[82] **93** was allowed to react with **89** to yield the desired 3-(3,4-dihydroxy-phenyl)-1-(1-hydroxy-

naphthalen-2-yl)-propan-1-one (**95**, 35.7%). Evident from the ^{1}H NMR spectrum (Acetone-d_6) of **95**, is the shift of the aromatic proton signals, accounting for H-3"-8" of the naphthol moiety, to the low field region (δ 8.42-7.40). Acylation is confirmed by the appearance of signals associated with the 3-(3,4-dihydroxyphenyl)-propionyl moiety, namely the α-protons at δ 3.46 (t, CH_2), the β-protons at δ 2.97 (t, *J* 7.63 Hz, CH_2) and the ABX-system at δ 6.82 (d, *J* 2.17 Hz, H-2'), δ 6.75 (d, *J* 8.01 Hz, H-5') and δ 6.66 (dd, *J* 7.98, 2.07 Hz, H-6'). The chemical shift of the α-proton is influenced by the electron withdrawing effect of the *oxygen of the carbonyl* which causes the α-proton to resonate in the low field region (reported in ^{1}H chemical shift tables for δ 3.7-4.1), and the *benzylic positioning* of the β-proton resonates in the low field region (reported in ^{1}H chemical shift tables for δ 2.2-3). In addition, *C*-acylation of 1-naphthol **93** is confirmed by the disappearance of the doublet at δ 7.40 (d, *J* 8.87 Hz, H-2), and the distinct shift of H-3' at δ 7.32 in the spectrum of **93** to the low field region at δ 7.93 (d, *J* 8.89 Hz, H-3") in the spectrum of **95**. The position of the substitution (C-2") was determined *via* the identification of two low field doublets at δ 8.42 and δ 7.93, which was conveniently correlated by a TOCSY spectrum to H-8" and H-3", respectively. These shifts to lower field are a reflection of the electron withdrawing effect of the carbonyl (**96,** the resonance structure of **95**) and thus corroborate the suggested *ortho* substitution position.



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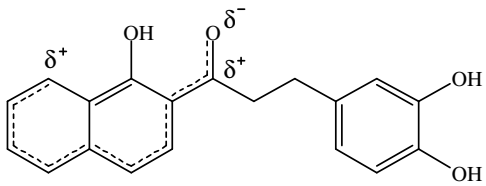


96

The positive result of *C*-acylation confirmed the catalytic reactivity of $Hf(OTf)_4$. This indicated that the acylation between a free phenolic substrate and a carboxylic acid, containing the oxygen sensitive free phenolic catechol moiety, is possible, and was an encouraging indicator towards the successful synthesis of aspalathin **55**.

3.4.3.2 PHLOROGLUCINOL

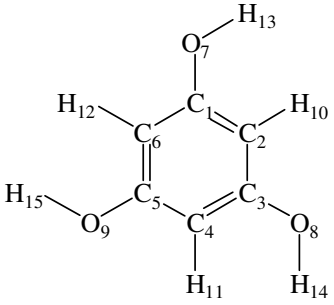
These reaction conditions were subsequently elaborated to include phloroglucinol **16** (the pinnacle of the phenolic building blocks of flavonoids in terms of nucleophilicity and steric constraints), which was exposed to acetic acid in the presence of $Hf(OTf)_4$[82] to afford, after 6 hours of reaction time and short interval monitoring (TLC), unreacted starting material **16** and an intractable mixture of compounds of which the characteristics were reminiscent of the known polymeric flavonoids (also known as tannins). Noteworthy is the observation that the highly hydrophilic phloroglucinol **16** produced a non- homogeneous reaction mixture in toluene:CH_3NO_2 (6.7:1, v/v), which prompted a search for alternative and suitable solvents and the use of ultrasonic waves in order to form a homogeneous reaction mixture. Despite various experimental attempts, there was no significant improvement in the outcome. These unsatisfactory results could be attributed either to the highly sterically congested nature of **16**, or the affinity of the "hard" carbonyl carbon of the carboxylic acid to the "soft" aromatic carbons of the phenolic substrate **16**.

The vaguely articulated steric constraints posed by phloroglucinol towards incoming electrophilic species, was illuminated in a recent paper by Rudyk *et al*.[93] wherein the experimental dipole moment of **16** in ethanol was explained and the formation of solute-solvent association complexes was proposed. This means that intermolecular hydrogen bonds (IHBs) lead to the formation of complexes, which involves one molecule of **16** and several molecules of ethanol. Two molecular conformations were suggested for **16** (Figure 8), namely conformers **PG1** and **PG2**, both of which have a 3:1 stoichiometry, i.e. they are formed by three molecules of ethanol and one of **16**. A conformational equilibrium was proposed between **PG1** and **PG2**, which are characterized by the conformational equilibrium constant, K_c. It was concluded that

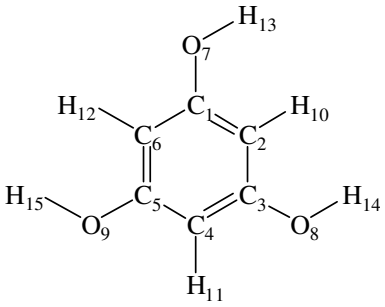
[93] Rudyk. R., Molina. M. A. A., Yurquina. A., Gomez. M. I., Blanco. S. E., Ferretti. F. H., *J. Mol. Struct. (Theochem)*, **673**, 231-238, (2004).

the dipole moment of phloroglucinol **16** in ethanol is due to a contribution of 21.7% of $(EtOH)_3$-**PG1** complex and 78.3% of $(EtOH)_3$-**PG2** complex, with a K_c value of 3.61. From the data obtained, the indication was clear that both conformers (**PG1** and **PG2**) possess planar structures. A scarce number of theoretical studies about the molecular and electronic structures of phloroglucinol **16** in gaseous phase have been reported[94]. From the results found for the diluted solution of **16** in ethanol, it was determined that **PG2** exhibits slight or moderate variations in the values of molecular properties in vacuum as opposed to those calculated in ethanol. This means that the structure of **PG2** in the gaseous phase differs from that in alcoholic solutions, implicating that the polarity of the solvent has an influence on the dipole moment and stability of the conformer.

Microwave irradiation (details of which are conveyed in the following paragraphs) provides an alternative option to circumvent the sometimes negative effect of the solvent.



PG 1



PG 2

Figure 8: Structures of conformers of phloroglucinol

[94] [a]Mandix. K., Colding. A., Elming. K., Sunesen. L., Shim. I., *Int. J. Quant. Chem.*, **46**, 159-170, (1993); [b]Spoliti. M., Bencivenni. L., Quirante. J., Ramondo. F., *J. Mol. Struct. (Theochem.)*, **390**, 139-147, (1997).

3.4.4 MICROWAVE REACTIONS

Since the pioneering works of Gedye *et al.*[95] and Giguere *et al.*[96], there has been a significant growth in the application of microwave irradiation as is illustrated by the increasing numbers of publications in this field since 1986.[97] The incentive for this impressive growth is strongly linked to the growing awareness of the desirable thrust towards 'green' chemistry. Notable in microwave reactions are the very short reaction times (normally minutes)[98], higher product yields, less solvent (or frequently no solvent) utilized and more cost-effective energy consumption.

Microwaves are a form of electromagnetic energy[99], with most appliances operating at a fixed frequency of 2.45 GHz. Contrary to conventional heating, microwave-accelerated heating entails microwaves that interact directly with the molecules of the entire reaction mixture, which leads to a rapid rise in temperature.[100] Microwave irradiation will directly activate most molecules that either possess dipole moments or are ionic. This prevents liquids without dipole moments from being directly heated by microwaves. Gas molecules, on the other hand, are not heated due to the large distances between these molecules, which cause a lack of microwave conductivity.

Most of the commercially available microwave reactors feature built-in magnetic stirrers, direct temperature control of the reaction mixture with the aid of fiber-optic probes, shielded thermocouples or IR sensors, and software that enables on-line temperature/pressure control by regulation of microwave power output. In general, regardless of the specific microwave reactor employed, microwave-enhanced processes can be carried out either under sealed vessel or open vessel (atmospheric pressure) conditions.[100]

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[95] Gedye. R., Smith. F., Westaway. K. C., Ali. H., Baldisera. L., Laberge. L., Roussel. J., *Tet. Lett.*, **27**, 279, (1986).
[96] Giguere. R. J., Bray. T. L., Duncan. S. M., Majetich. G., *Tet. Lett.*, **27**, 4945, (1986).
[97] Loupy. A., *C. R. Chimie*, **7**, 103-112, (2004).
[98] Lidstrom. P., Tierney. J., Wathey. B., Westman. J., *Tetrahedron*, **57**, 9225-9283, (2001).
[99] Stadler. A., Pichler. S., Horeis. G., Kappe. C. O., *Tetrahedron*, **58**, 3177-3183, (2002).
[100] Hayes. B., "Microwave Synthesis: Chemistry at the Speed of Light", CEM Publishing: Matthews, NC, (2002).

3.4.4.1 NON-POLAR SOLVENTS, SOLVENTLESS CONDITIONS AND IONIC LIQUIDS

The most suitable conditions for the observation of certain microwave effects necessarily involve non-polar solvents ("transparent" to microwave irradiation, and consequently allowing specific microwave interactions with reagents only). These, and other relevant topics related to solvents in microwave reactions, were the contents of a review article by Loupy *et al*.[101] In the case of solvent-free microwave reactions, it is imperative that one of the reagents melts in order to produce a homogeneous mixture, thus obtaining a uniform heating pattern.

Similar to the resurgence of microwave-assisted reactions, the utilization of ionic liquids has become a very attractive alternative to the "classical" solvents. In addition to other benefits, they are environmentally friendly, because of their recyclable characteristics.[102] It is also noteworthy to highlight the excellent dielectric properties of ionic liquids which serve to positively benefit a vast number of reactions. They also absorb microwave irradiation in a very efficient manner and, in addition, exhibit a very low vapour pressure, thereby enhancing the suitability thereof for microwave heating even further. Despite the fact that ionic liquids are salts, they dissolve to an appreciable extent in a wide range of organic solvents, contrary to the "normal" behavior of salts, which are mostly only soluble in water and alcohols.[98]

3.4.4.2 THE USE OF A CATALYST IN MICROWAVE REACTIONS

Similar to their well-known classical counterparts, catalysts have become an integral part of the burgeoning field of microwave reactions. The reader is referred to

[101] Loupy. A., Petit. A., Hamelin. J., Texier-Boullet. F., Jacquault. P. Mathe. D., *Synthesis*, 1213-1234, (1998).
[102] [a]Seddon. K. R., *Kinet. Katal.*, **37**, 743-748, (1996); [b]Welton. T., *Chem. Rev.*, **99**, 2071-2083, (1999).

informative publications [103,104,105,106,107] for more information. A literature survey[108] revealed that the use of $BF_3{\cdot}(C_2H_5)_2O$ as catalyst in the acylation of phenolic substrates e.g. phenol **21** and naphthol **93** with aliphatic acids under optimal microwave conditions, presented an ideal synthetic protocol with the potential to fulfill most of the aims set out for the preparation of aspalathin **55**.

3.4.4.3 DOMESTIC MICROWAVE REACTIONS

To probe the applicability of this synthetic protocol (*vide supra*), reported for the relative simple substrates like phenol **21** and 1-naphthol **93**,[108] a few pilot reactions were performed in a domestic microwave oven with known specifications.

Indeed, 1-naphthol reacted smoothly with acetic acid in the presence of $BF_3{\cdot}(C_2H_5)_2O$ as catalyst and under solvent-free conditions when irradiated with microwaves for 2 minutes at 650 Watt to furnish 1-hydroxy-2-acetonaphthone (**94**, 80%) and recovered starting material **93** (13.7 %). The ^{1}H NMR data of **94** is completely consistent with those previously reported.[108]

Phloroglucinol **16** is polar due to the presence of a dipole moment and can therefore be directly heated by microwave irradiation. The use of solvent-free conditions and the melting of **16** under the applied conditions resulted in a homogeneous reaction mixture. Exposure of **16** to acetic acid and $BF_3.(C_2H_5)_2O$ under the same reaction

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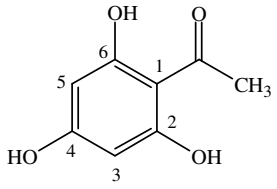
[103] Gronnow. M. J., Macquarrie. D. J., Clark. J. H., Ravenscroft. P., *Journal of Molecular Catalysis A: Chemical*, **231**, 47-51, (2005).
[104] Laporte. C., Marquie. J., Laporterie. A., Desmurs. J., Dubac. J., *C. R. Acad. Sci. Paris*, **2**, 455-465, (1999).
[105] [a]Noto. T., Babushok. V., Burgess. D. R., Iiamins. A., Isang. W., Miziolek. A., "International Twenty-sixth Symposium on combustion/the combustion in Situe", 1337, (1996); [b]Babushok. V. Noto. T., Burgess. D. R., Hamins. A., Tsang. W., *Combust. Flame.*, **107**, 351, (1996); [c]Linteris. G. T., Truett. L., *combust. Flame*, **105**, 15, (1996).
[106] [a]Potter. S. C., Tildesley. D. J., Burgess. A. N., Rogers. S. C., *Mol. Phys.*, **92**, 825, (1998); [b]Mclaughlin. A. J., Bonar. J. R., Jubber. M. G., Marques. D. V. S., Hicks. S. E., Wilkinson. C. D. W., *J. Vac. Sci. Technol. B*, **16**, 1860, (1998).
[107] Smith. B. K., Sniegowski. J. J., Lavigne. G., Brown. C., *Sens. Actuators A*, **70**, 1590, (1998).
[108] Naeimi. H., Moradi. L., *Bull. Chem. Soc. Jpn.*, **78**, 284-287, (2005).

conditions as mentioned above, gave the sought after product, 2,4,6-trihydroxyacetophenone (**97**, 20%). The ^{1}H NMR data of **97** was consistent with that of the corresponding commercial material.



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The disappointing low yield of **97,** although not a complete surprise (see comments about the extraordinary reactivity of phloroglucinol **16**, p. 60), can be rationalized by either the incongruity of the "hard" carbonyl carbon of the carboxylic acid and the "soft" aromatic carbons of the phenolic substrate **16** (boron trifluoride would act as a Lewis acid and would rather react with the phenolic hydroxyl group, which is a Lewis base to produce a Lewis acid-base product), while it is also conceivable that the watt input may have impacted negatively. The latter obstacle my be overcome conveniently by the utilization of a microwave reactor whereof the power can be controlled, whereas the parameter of hard and soft acids and bases (HSAB)[73] will be discussed in-depth in paragraph 3.4.4.6 (p. 67).

As the impressive applications of microwave-assisted reactions have become progressively prominent, the demand for more sophisticated microwave apparatus has grown and this in turn has assisted the normal trend of reducing the prices. It was the privilege of L Jordaan to use the sophisticated microwave reactor, CEM Discover at the Chemistry Department, University of the Witwatersrand (WITS), for extended periods. This microwave reactor was used to assess the influence of the watt input on the reaction outcome. It featured a built-in magnetic stirrer and direct temperature/pressure control by regulation of microwave power output. Two settings were available, *viz*. standard mode or power mode, whereas it also gave the option of operation under either open or closed vessel reaction conditions.

3.4.4.4 COMMERCIAL MICROWAVE REACTOR REACTIONS

Commercial microwave reactors are known to be used for Enhanced Microwave Synthesis (EMS). It provides the ability to cool a reaction vessel externally while simultaneously administering microwave irradiation, resulting in energy to be applied directly to only the reagents. A higher microwave power input results in a substantial enhancement of yields and cleaner chemistries while maintaining a desired bulk temperature.[109] EMS was applied to investigate the influence of watt input and to compare the reactivities of phloroglucinol **16** and resorcinol **14**.

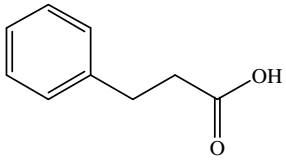
3.4.4.5 STANDARD OR POWER MODE

Standard mode only uses a specified watt input in order to obtain the required temperature and gradually increases the *temperature* over a period of time intervals. The power mode, on the other hand, is specifically designed to increase the *watt input* over a period of time intervals, thus increasing power. A maximum temperature and pressure for the reaction procedure need to be entered in order to protect the microwave reactor. During these reactions the use of simultaneous cooling (by means of nitrogen) is important in order not to exceed the maximum temperature, but still obtain high watt inputs. Various reactions between either resorcinol **14** or phloroglucinol **16** and carboxylic acids **98** catalyzed by $Hf(OTf)_4$ or $BF_3{\cdot}(C_2H_5)_2O$ were attempted in order to obtain the desired reaction conditions for the standard and power modes. These results produced the basis for a comparison of **14** with **16**.

[109] Hayes. B. L., *Aldrichimica ACTA*, **37**, 66-76, (2004).

3.4.4.6 PHLOROGLUCINOL AND RESORCINOL

The phloroglucinol **16** entity is ubiquitous in most natural flavonoid structures, but interestingly enough, the selective *C*-acylation of phloroglucinol **16** has not been reported up to 2005. The only applicable examples that were found in the literature up to then concerned reactions with protected versions of phloroglucinol **16**, as it contains six nucleophilic sites[110]. For example, the cinnamylation of phloroglucinol **16**[111] and the reaction of **16** with β-ketoesters[112] both examples contained protected substrates. The selective *C*-acylation of resorcinol **14** on the other hand has been reported in good yield under microwave irradiation conditions.[108] Three different carboxylic acids were chosen to react with phloroglucinol **16** and resorcinol **14**, namely 3-phenylpropionic acid **98**, 3-(4-hydroxyphenyl)-propionic acid **99** and 3,4-dihydroxyhydrocinnamic acid **89**.



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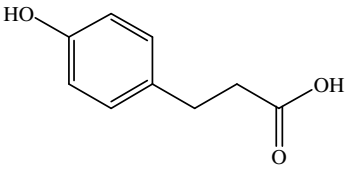

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The reactivities of **14** and **16** towards acylation with the said carboxylic acids in the presence of $Hf(OTf)_4$ or $BF_3{\cdot}(C_2H_5)_2O$, were thus compared. After various reactions, the optimum standard (Figure 9) and power (Figure 10) mode reaction conditions for each reactant and Lewis acid were obtained. From these reactions, it seemed that standard mode proved to be the preferred mode for reactions catalyzed by the Lewis acid $Hf(OTf)_4$ due to the mild reaction conditions which allows the temperature to be controlled, whereas power mode is preferred for $BF_3{\cdot}(C_2H_5)_2O$ catalyzed reactions due to harsh reaction conditions allowing the watt input to be increased.

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[110] Vay. B., Collet. M., Lebon. M., Cheze. C., Vercauteren. J., *Tetrahedon Lett.*, **43**, 2675-2678, (2002).
[111] Gissot. A., Wagner. A., Mioskowski. C., *Tetrahedron*, **60**, 6807-6812, (2004).
[112] Seijas. J. A., Vazquez-Tato. M. P., Carballido-Reboredo. R., *J. Org. Chem.*, **70**, 2855-2858, (2005).

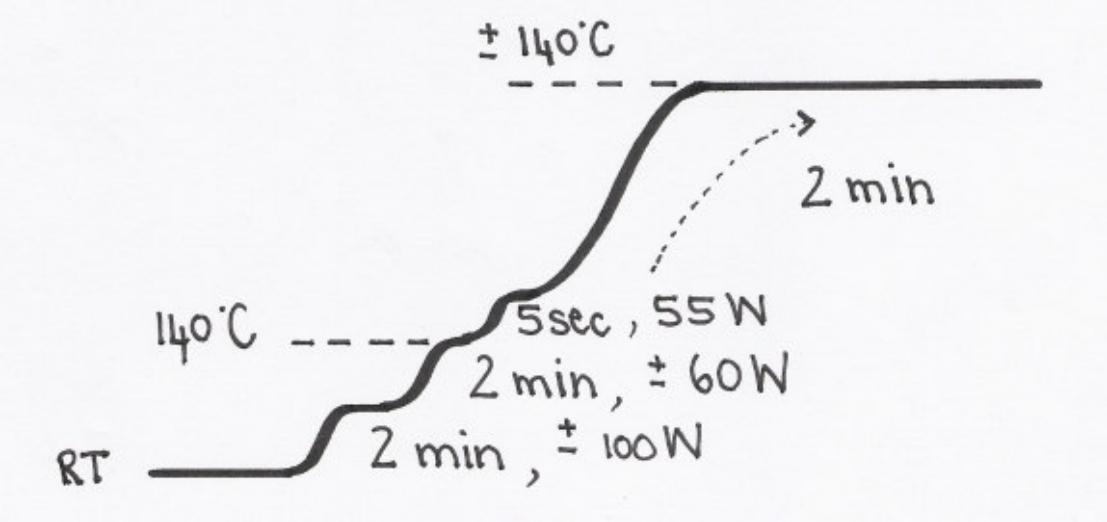


Figure 9: Standard mode reaction conditions for both reagents

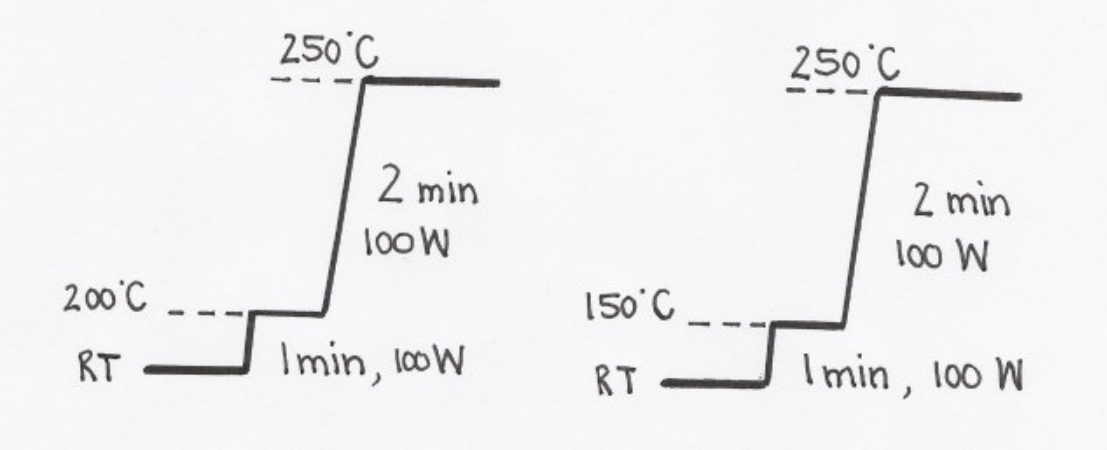


Figure 10: Power mode reaction conditions for resorcinol (14) and phloroglucinol (16) respectively

The results are depicted in table 5:

Table 5. Yields obtained for *C*-acylated products from microwave reactor reactions

A			B		
Phenol	**Acid**	**Yield**	**Phenol**	**Acid**	**Yield**
14	98	81.70% (**100**)	14	98	41.40%(**100**)
16	98	30.20% (**101**)	16	98	
14	99	70% (**102**)	14	99	50.10%(**102**)
16	99	29.80% (**103**)	16	99	15.90%(**103**)
14	89	38.87%*(**104**)	14	89	14.58%*(**104**)
16	89	4.56%*(**88**)	16	89	7.62%*(**88**)

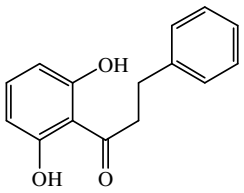
A = reactions with $BF_3{\cdot}(C_2H_5)_2O$ as catalyst under the specified power mode and **B** = reactions with $Hf(OTf)_4$ as catalyst under standard mode. No result means that an intractable mixture of highly polymerized products was formed.
***** Yields obtained with HPLC analysis

It is clear from table 5 that:

- The yields obtained for the acylation of resorcinol **14** exceeded that of phloroglucinol **16** in all the reactions, which was a clear indication of the highly sterically congested nature of **16**
- The use of $BF_3{\cdot}(C_2H_5)_2O$ **(A)** as Lewis acid improved the reactivity of **16** in most of the reactions
- The addition of OH groups to the phenolic ring of the carboxylic acid had a negative effect in the yields of the reactions, which is likely the result of more steric congestion (caused by π-stacking) as one goes from resorcinol **14** to phloroglucinol **16** and the reaction thereof with different carboxylic acids[113]

[113] Singh. R.P., Kamble. R. M., Chandra. K. L., Saravanan. P., Singh. V. K., *Tetrahedron*, **57**, 241-247, (2001).

- The use of 3,4-dihydroxyhydrocinnamic acid **89** as carboxylic acid required the use of HPLC analysis for product separation and quantification due to the low yields obtained



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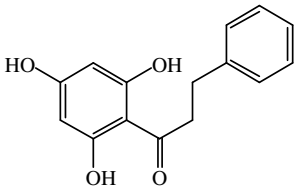

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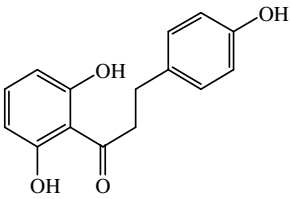

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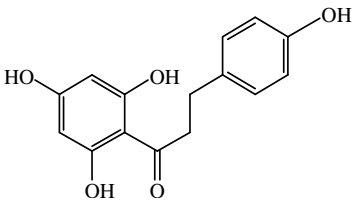

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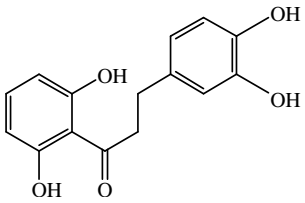

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From these observations the conclusion was made that $BF_3{\cdot}(C_2H_5)_2O$ was the better Lewis acid catalyst in the reactions of phloroglucinol **16** under the suggested power mode condition, and that the hydroxyl arrangement on the phenolic ring for the different carboxylic acids played a vital role in the selectivity of *C*-acylation. After it was established that $BF_3{\cdot}(C_2H_5)_2O$ was the catalyst of choice for the selective *C*-acylation of **16**, a strategy to improve the compatibility of the reacting species by changing the prevailing electrophilic intermediate (the "*hard*" acid) to a "*softer*" equivalent, was decided upon.

This HSAB set of principles can be extended to include Lewis acids such as BF_3 and BH_3. The HSAB theory categorizes BF_3 as a "*hard*" Lewis acid and BH_3 as a "*soft*"

acid. This difference is rationalized in terms of the characteristics of the bonded fluorine atoms in the one case compared to the hydrogen atoms in the BH_3. The boron in BF_3 is bonded to "*hard*" fluorine atoms and thus significantly electropositive, allowing it to form complexes with N, O, and F donors, or with hard bases,[74] and is thus ionic to a significant degree. The "*soft*" hydrogen atoms in BH_3, on the other hand, are not capable of polarizing the bonds to a large extent, hence rendering the boron atom less electropositive in this compound, and therefore, this boron atom is a "*soft*" acid.[74] From HSAB considerations, it is clear that the carbonyl carbon of most acylating agents is functioning as a hard acid in the presence of Lewis acids.[114] Based on the same set of criteria, the hydroxyl *O*-atoms of phloroglucinol **16** must be considered as "*hard*" bases and hence will interact and react with the carbonyl carbon to afford the preferred and kinetically controlled *O*-acylation product. The *C*-atoms of phloroglucinol **16** by contrast, are classified as "soft" bases and will either react with the acylium cation (produced in the presence of TfOH), or the *O*-acylated product (being less stable than its *C*–acylated regioisomer) will isomerise to the thermodynamically more stable *C*-isomer. Results based on this theory have found widespread and successful application. In the cases where outcomes of reactions do not conform to HSAB theory, it is mostly attributed to a wrong classification of the various substrates.

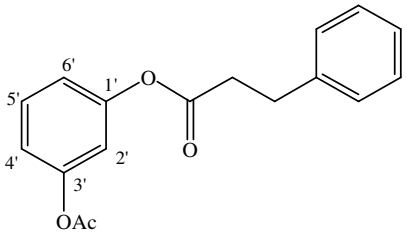
As the incompatibility of the "*hard*" acid (reactive electrophilic intermediate) and the "*soft*" carbon bases of the aromatic substrate proved to be a significant impediment of a highly successful outcome of this desired acylation step based on various classical methodologies, concomitant experiments were conducted aimed at probing the efficacy of "acyl" substrates in which the reactive "acyl" equivalent is considered as a "softer" acid. By surveying potential "acyl" equivalents with the favored "*soft*" character, it became clear that the cyano group in the appropriate nitriles offered the best choice to verify a more successful outcome of the pivotal acylation step. The results of the following reactions display the relative success achieved in this regard.

[114] Ho. T., *Journal of Chemical Education*, **55**, 355-360, (1978).

3.4.4.7 RESORCINOL – SOLVENT CONDITIONS

Due to the impressive yield obtained for the reaction between resorcinol **14** and 3-phenylpropanoic acid **98** with $BF_3{\cdot}(C_2H_5)_2O$ as catalyst (table 5, p.69), the reaction was repeated under the same power mode reaction conditions (Figure 10, p.68), but with $Hf(OTf)_4$ as Lewis acid. Substrates **14** and **98** were dissolved in toluene:CH_3NO_2 (6.7:1, v/v) in the presence of $Hf(OTf)_4$ and exposed to microwave irradiation at 200 watts for 2 min. Thin Layer Chromatography (TLC) revealed only one spot after spraying with H_2SO_4-HCHO (40:1, v/v) - the spot developed a red-brown colour, which was an indication of the presence of phenolic compounds. The ^{1}H NMR (Acetone-d_6) data suggested a mixture of two products, but separation was to no avail after various attempts to change the eluant.

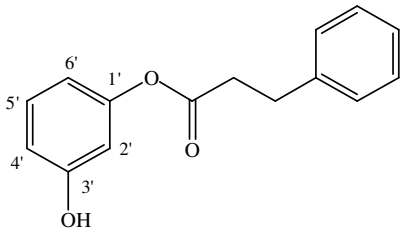
The reaction mixture was thus acetylated to give both the *O*- **(105)** and *C*-acetylated **(108/109)** products. The ^{1}H NMR data (Acetone-d_6) of the *O*-acetylated product, 3'-*O*-acetyl-phenyl 3-phenyl-propanoate (**105**, 21.9 %) clearly indicated an OAc signal at δ 2.31, which accounted for only one OH group being acetylated. The acetylation caused a shift in the resonance of the aromatic proton H-2' to the high field region, thus corroborating the loss of the shielding effect of the hydrogen bonds as explained below after de-acetylation to produce the ^{1}H NMR data of the *O*-acylation product.



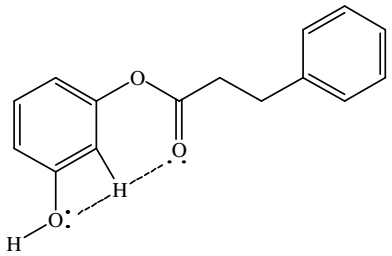
105

Evident from the ^{1}H NMR data (Acetone-d_6) of the *O*-acylation product, 3'-hydroxy-phenyl 3-phenyl-propanoate (**106**, 14.6 %), was the presence of the carboxylic moiety **98** confirmed by the α-H and β-H triplets at δ 3.04 and δ 2.9 respectively. In the low

field region at δ 7.19, a triplet (*J* 7.92 Hz) was observed which was accounted for the aromatic proton H-2'. This shielding of the H-2' proton is caused by neighboring hydrogen bonds **(107)**.

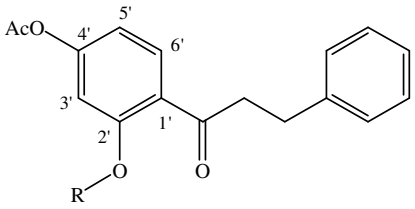


106



107

The ^{1}H NMR data (Acetone-d_6) of the *C*-acetylated product, 2',4'-di-acetoxy dihydrochalcone **109,** was clearly reminiscent of those of **100**. Furthermore, the acetyl signal at δ 2.33 accounted for "one and a half" OH groups acetylated. This could only be rationalized by the formation of the product, 2'-hydroxy-4'-acetoxy dihydrochalcone (**108**, 63%) also, with the hydrogen bonding between OH-2' and the carbonyl.

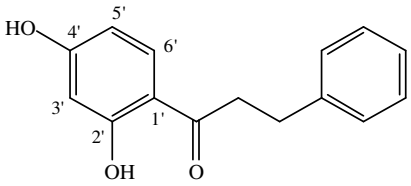


108 R = H

109 R = Ac

After de-acetylation, it was evident from the ^{1}H NMR data (Acetone-d_6) of the *C*-acylation product, 2',4'-dihydroxydihydrochalcone (**100**, 37.2 %), that the aromatic protons of the resorcinol **14** moiety integrated for only three protons, thus indicating *C*-acylation. This was corroborated by the low field doublet at δ 7.83 (d, H-6', *J* 8.84 Hz), the shift to lower field was again the result of the electron withdrawing effect of the carbonyl (*vide supra*). The doublet of doublets at δ 6.44 (*J* 8.81, 2.39 Hz) of H-5'

and the doublet at δ 6.36 (*J* 2.38 Hz) of H-3' also confirmed *C*-acylation at H-1'. The presence of the carboxylic moiety **98** was confirmed by the α-H and β-H triplets at δ 3.31 and δ 3.02 respectively and the aromatic protons at δ 7.3-7.2.



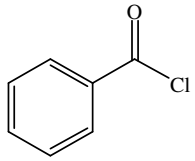
100

These results indicated that $Hf(OTf)_4$ can act as both a Brønsted and Lewis acid in a catalytic cycle. This meant that the TfO^- ion coordinated with the carboxylic acid to produce RCOOTf. In the case of the *C*-acylation product the reaction is *via* Friedel-Crafts Acylation (see Scheme 10 p.52), where an acylium cation is produced (R-+C=O) with the uptake of TfO^- by $^+Hf(OTf)_3$ to regenerate $Hf(OTf)_4$. The *O*-acylation product is the result of complex [A] (see Scheme 12 p.54) that forms. The difference is that there is no rearrangement to produce the *C*-acylation product (*via* Fries rearrangement), the TfO^- takes up H^+ to generate TfOH.[90a] The complex formed with the phenolic ring was the result of a "*soft*" HSAB reaction principle and the result of the weaker electrophilic acylium carbon cation, which resulted in the *C*-acylation product. The complex formed with the oxygen of the phenolic hydroxyl group, was the result of a "*hard*" HSAB reaction principle, which coordinated with the stronger electrophilic carbonyl carbon and resulted in the *O*-acylation product.

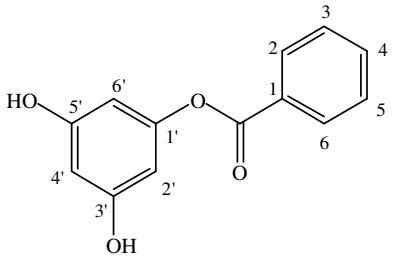
3.4.4.8 PHLOROGLUCINOL – SOLVENTLESS CONDITIONS

The carboxylic acid (softer acid) was replaced with an acid chloride (hard acid) in the following reaction. Benzoyl chloride **110**, **16** and $BF_3{\cdot}(C_2H_5)_2O$ were reacted under solvent-free conditions and microwave irradiation for 2 minutes at 650 Watt. The result was two prominent bands, namely starting material **16** and only the *O*-acylated product, 3',5'-dihydroxy-phenyl 1'-benzoate (**111**, 53.1%). The ^1H NMR data

(Acetone-d_6) of **111** was consistent with the proposed structure, featuring three aromatic protons at δ 6.35 (br. s, H-2', H-4', H-6') and five aromatic protons at δ 8.16, δ 7.71 and δ 7.58.



110



111

The formation of the *O*-acylated product is in agreement with the HSAB principle, the lone pair of :BF_3 ("*hard*" Lewis acid) coordinates with the phenolic hydroxyl oygen, which results in a "*hard*" base hydroxyl oxygen that attacks the stronger electrophilic carbonyl carbon ("*hard*" acid)[104]. The *C*-acylated product is not present, confirming $BF_3 \cdot (C_2H_5)_2O$ to be a preferred "*hard*" Lewis acid catalyst.

3.4.4.9 PHLOROGLUCINOL – IONIC LIQUIDS

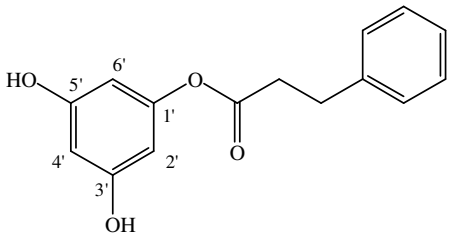
The question towards the role of the solvent in the HSAB principle was considered by Gritzner[115]. Gritzner questioned the HSAB principle of Pearson[74] and discussed enhanced Lewis donor (nucleophilic) properties of solvents. It was shown that the original concept of Lewis, where *one* partner supplies *one* electron pair for a chemical bond and the other partner accepts this electron pair, is limited to "*hard-hard*" interactions. The "*soft-soft*" interactions where both solute and solvent donate and accept electron pairs cannot be accounted for by parameters derived from model substances. Different solvent parameters were studied by Gritzner to interpret the different chemical behavior of "*hard-hard*" and "*soft-soft*" interactions of kinetic and

[115] Gritzner. G., *Journal of Molecular Liquids*, **73**, **74**, 487-500, (1997).

thermodynamic properties. This prompted us to study different solvents and to interpret their chemical behavior.

It was thus decided to repeat the $Hf(OTf)_4$ –catalyzed reaction outlined in table 5 (p. 69) between phloroglucinol **16** and 3-phenylpropionic acid **98** under the same microwave reaction conditions but in the presence of an ionic liquid. After a search for ionic liquids supportive of the acylation of aromatic compounds, a stable ionic liquid, $[bmim]PF_6$,[116] was decided upon ($[bmim]^+$ = 1-butyl-3-methylimidazolium).

Phloroglucinol **16** was thus *O*-acylated to produce 3',5'-dihydroxy-phenyl 3-phenyl-propanoate (**112**, 21.5 %). From the ^1H NMR data (Acetone-d_6) of **112**, it was observed that the free rotation around the C-1'-*O*-bond allowed hydrogen bonds between the carbonyl oxygen either H-2' or H-6'. This caused a splitting into three singlet's in the aromatic region at δ 5.9 (H-4') and 5.7 (H-2' and H-6') which integrate for three aromatic protons belonging to the phloroglucinol **16** moiety. The splitting of the resonance of the aromatic protons into three singlets instead of only two, where H-2' and H-6' are equivalent, can be rationalized by π-stacking. The aromatic ring of the **98** moiety can assume a conformation which allows π-stacking with the phenolic ring, which might prevent free rotation around the C-1'-*O*-bond and thus non-equivalence of H-2' and H-6'. Acylation was confirmed by signals associated with the 3-phenylpropionyl moiety, namely δ 2.78 (t, α-H, *J* 7.77 Hz) and δ 2.48 (t, β-H, *J* 7.64 Hz).



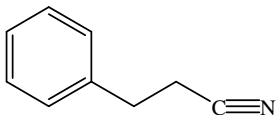
112

[116] [a]Gmouh. S., Yang. H., Vaultier. M., *Org. Lett.*, **5**, 2219-2222, (2003); [b]Kim. M., Choi. M. Y., Lee. J. K., Ahn. Y., *J. Mol. Cat. B*, **26**, 115-118, (2003).

The difference between this result and that discussed in par. 3.4.4.6, confirmed the influence of a solvent with Lewis donor properties on the selectivity of acylation. In paragraph 3.4.3.2 (p. 60) an insight was given into the chemical characteristics of **16**. Those findings together with the results obtained in this paragraph (3.4.4.9, p. 75), indicates that **16** forms a complex with the solvent, thus influencing the nucleophilic properties of **16**. Since the result can not be predicted, the best reaction conditions would be under solventless conditions.

3.4.4.10 PHLOROGLUCINOL – HOESCH REACTION

The Hoesch reaction is well known among scientists, but has not been utilized extensively, most likely due to a lack of suitable and commercially available nitriles. There are, however, a few methodologies known to convert the more available carboxylic acids to their corresponding nitriles.[117] In acid medium the nitrogen of the cyano group is protonated to afford the reactive electrophilic intermediate **114**, the carbon of which is clearly a "*softer*" acidic site according to the HSAB theory. Hence the commercially available nitrile derivative of 3-phenylpropionic acid **98**, hydrocinnamonitrile **113**, was used in a reaction based on the Hoesch reaction[118] and another according to the procedure reported by Mustafa *et al.*[119].



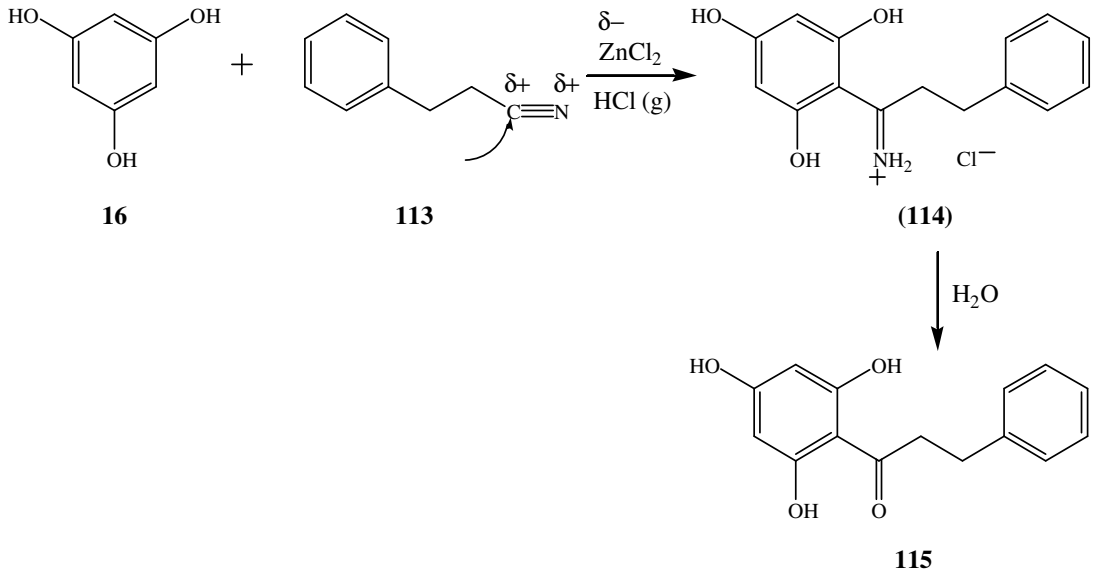
113

[117] [a]Miller. C. S., *Organic Synthesis*, **3**, 646, (1955); [b]Imamoto. T., Takaoka. T., Yokahama. M., *Syn. Comm.*, **12**, 25, (1982); [c] Klein. D. A., *J. Org. Chem.*, **36**, 3050, (1971); [d]Huber. V. J., Bartsch. R. A., *Tetrahedron*, **54**, 9281-9288, (1998).

[118] Furniss. B. S., Hannaford. A. J., Smith. P. W. G., Tatchell. A. R., Vogel, "Textbook of Practical Organic Chemistry" (5th Ed.), Pearson, Prentice Hall, England, p.1017

[119] Mustafa. K'. A., Kjaergaard. H. G., Perry. N. B., Weavers. R. T., *Tetrahedron*, **59**, 6113-6120, (2003).

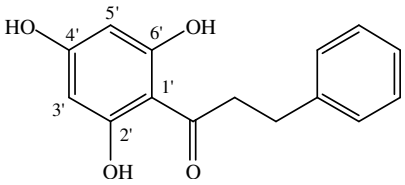
Both reactions involve the reaction of phloroglucinol **16** and the nitrile **113** in a dry ethereal solution in the presence of anhydrous zinc chloride and hydrogen chloride to produce an imine hydrochloride **114**, which was converted into an alkyl trihydroxyphenyl ketone **115** by hydrolysis (Scheme 13).



16 **113** **(114)** **115**

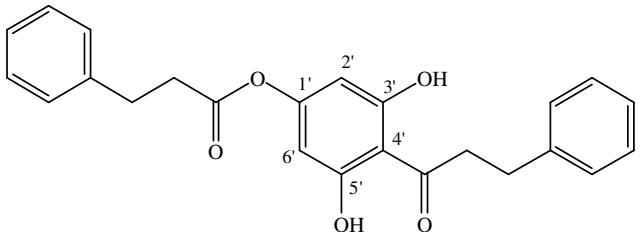
Scheme 13: Reaction procedure based on the Hoesch reaction and the procedure reported by Mustafa *et al.*[119]

The main difference between the reaction conditions of the general Hoesch reaction[118] and the procedure of Mustafa *et al.*[119] was the reaction temperature, which was 10°C and -10 to -20 °C, respectively. The *C*-acylated product, 2',4',6'-trihydroxy dihydrochalcone **101** was obtained by the method of Mustafa *et al.*[119] in an impressive yield of 72.5 %. *C*-acylation was confirmed by the ^{1}H NMR spectrum (Acetone-d_6) of **101**, with a shift of the signals of the α- and β-protons to the low field region at δ 3.42 and δ 3.0, respectively, compared to the corresponding signals in the spectrum of **113**. The aromatic protons of the phloroglucinol moiety **16** integrated for only two protons at δ 5.85 (s, H-3' and H-5'), which confirmed *C*-acylation at C-1'.



101

In the Hoesch reaction[118] an interesting result was obtained, as the phenolic oxygen ("*hard*" base) as well as the aromatic ring ("*soft*" base) reacted with the nitrile to produce the product, 3',5'-dihydroxy-4'-phenyl-propionic acid 1'-3-phenyl-propanoate (**116**, 14.2 %).



116

The ^{1}H NMR data (Acetone-d_6) indicated two sets of α- and β-protons corresponding to two 3-phenylpropionyl moieties, one set at δ 2.92 (t, α-H) and δ 2.62 (t, β-H) corresponding to the *O*-acylated moiety, and the other at δ 3.4 (t, α-H') and δ 2.98 (t, β-H') corresponding to the *C*-acylated moiety. The phenolic protons of the 3-phenylpropionyl moiety also indicated double acylation, whereas the singlet at δ 5.95 (H-2' and H-6') was a further confirmation of a single *C*-acylation.

The reaction conditions of Mustafa *et al.*[119] seemed promising and offered an opportunity to synthesize 3,4,2',4',6'-pentahydroxy dihydrochalcone **88**. Various attempts to convert the carboxylic acids 3-phenylpropionic acid **98**, 3-(4-hydroxyphenyl)-propionic acid **99** and 3,4-dihydroxyhydrocinnamic acid **89** into their corresponding nitriles[117] was to no avail, though.

3.4.5 REVISITING CONVENTIONAL HEATING

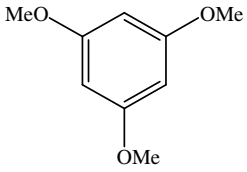
3.4.5.1 HAFNIUM TRIFLATE

Resorcinol **14** and 3-phenylpropionic acid **98** were allowed to react in the presence of $Hf(OTf)_4$ as catalyst and toluene:CH_3NO_2 (6.7:1, v/v) as solvent. The reaction mixture was gradually heated from 40°C to 85°C over a period of *ca.*10 hours. TLC monitoring in one-hour intervals over this period only indicated the presence of the starting materials **14** and **98**. Due to the fact that *C*-acylation is thermodynamically determined, the reaction mixture was left to stir overnight at 95°C, which yielded the *C*-acylation product, 2',4'-dihydroxydihydrochalcone **100** in a 63 % yield. It was confirmed that $Hf(OTf)_4$ prefers to act as a "*soft*" Lewis acid, where the acylium carbon cation ("*soft*" acid) is formed and the regeneration of $Hf(OTf)_4$ results in a Friedel-Crafts Acylation reaction. The ^{1}H NMR data of the product obtained was completely consistent with those previously reported (p. 73).

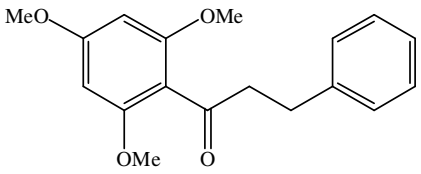
The next step was the reaction of phloroglucinol **16** and 3-phenylpropionic acid **98**. The same reaction conditions were used as described above, but only the *O*-acylated product, 3',5'-dihydroxy-phenyl 3-phenyl-propanoate (**112**, 31.4 %) was reported. The ^{1}H NMR data of the product obtained was completely consistent with those previously reported (p. 75). This result was an indication that the acylium cation did not form and that a complex [A] (see Scheme 12 p. 54) was formed. Therefore, it was decided to protect phloroglucinol **16** by methylation in order to allow only *C*-acylation and to determine whether *C*-acylation with $Hf(OTf)_4$ as Lewis acid occurs ***via*** the Fries rearrangement.

The methylated product of **16**, 1,3,5-trimethoxybenzene **117**, is commercially available. The reaction mixture of **117** and **98** with $Hf(OTf)_4$ as catalyst and toluene: CH_3NO_2 (6.7:1, v/v) as solvent was refluxed at 65°C for 3 hours, where after only the starting materials were observed. The reaction mixture was thus left at 95°C for another 3 hours to yield the *C*-acylated product, 1,3,5-trimethoxy dihydrochalcone

(**118**, 20.5 %). The ^{1}H NMR data of the product was consistent with those reported by Batt *et al*.[120]



117



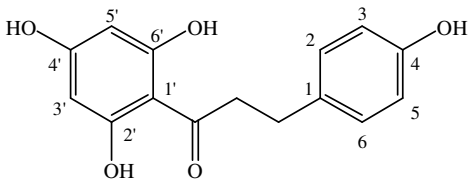
118

Direct Friedel-Crafts acylation thus took place, which means that *C*-acylation of unprotected phenols might either follow this mechanism or esterfication followed by Fries rearrangement. At this stage in our research, Siddaiah *et al*.[121] published an acylation reaction of phloroglucinol **16** with 3-(4-hydroxyphenyl)-propionic acid **99** in the presence of boron trifluoride etherate ($BF_3{\cdot}(C_2H_5)_2O$) under solventless condions (30 % yield).

3.4.5.2 BORON TRIFLUORIDE ETHERATE

Siddaiah *et al*.[121] used the Lewis acid, boron trifluoride etherate ($BF_3{\cdot}(C_2H_5)_2O$), as a catalyst under solventless conditions at 80-90°C for 90 min. Exposure of 3-(4-hydroxyphenyl)-propionic acid **99** and phloroglucinol **16** to these reaction conditions in our hands resulted in the product, 4,2',4',6'-tetrahydroxy dihydrochalcone (**103**, 36.1 %). The ^{1}H NMR spectrum (Acetone-d_6) revealed the presence of a typical AA-BB system for the B-ring protons at δ 7.11 (d, *J* 8.53 Hz, H-2 and H-6) and δ 6.76 (d, *J* 8.51 Hz, H-3 and H-5), the A-ring protons at δ 5.95 (s, H-3' and H-5'), implying *C*-acylation, and the α- and β-proton resonance at δ 3.34 and δ 2.89, respectively - chemical shifts indicating *C*-acylation.

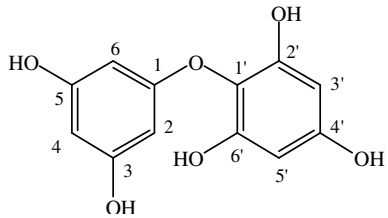
[120] Batt *et al*., *J. Med. Chem.*, **36**, 1434-1442, (1993).
[121] Siddaiah. V., Venkata Rao. C., Venkateswarlu. S., Subbaraju. G. V., *Tetrahedron*, 1-7, (2005).



103

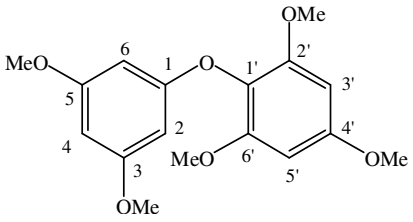
When phloroglucinol **16** and 3,4-dihydroxyhydrocinnamic acid **89** (1:1 eq.) were allowed to react in the presence of $BF_3{\cdot}(C_2H_5)_2O$ (5 eq.) at 80-90°C for 90 min under solventless conditions, resulted in the product 3,4,2',4',6'-pentahydroxy dihydrochalcone **88** to be obtained in 7.9 % yield.

Reaction of **16** and **89** (1:0.5 eq.) catalyzed by $BF_3{\cdot}(C_2H_5)_2O$ (1.2 eq.) at 80-90°C for 90 min, resulted in **88** in 20 % yield and an unknown product. The latter was identified by ^{1}H NMR (Acetone-d_6) as 3,5-dihydroxy-phenyl-2',4',6'-trihydroxy-phenylether (**119**, 34.8 %). Evident from the ^{1}H NMR spectrum was a doublet at δ 6.35 (*J* 2.24 Hz, H-2' and H-6') and a triplet at δ 6.28 (*J* 2.24 Hz, H-4'), and a broad singlet at δ 6.25, integrating for two protons (H-3 and H-5). The structure of **119** was further confirmed with HSQC and HMBC. The HSQC spectra indicated the position of the tertiary carbons, H-4' at 101.3 ppm correlates to C-4', H-2'and H-6' at 109.5 ppm correlates to C-2' and C-6', and H-3 and H-5 at 94.5 ppm correlates to C-3 and C-5. The HMBC spectra was an indication of neighboring hydrogens, C-2' and C-6' correlates with H-4', and H-3, H-5 but C-4', C-3 and C-5 does not correlate to any other hydrogens.



119

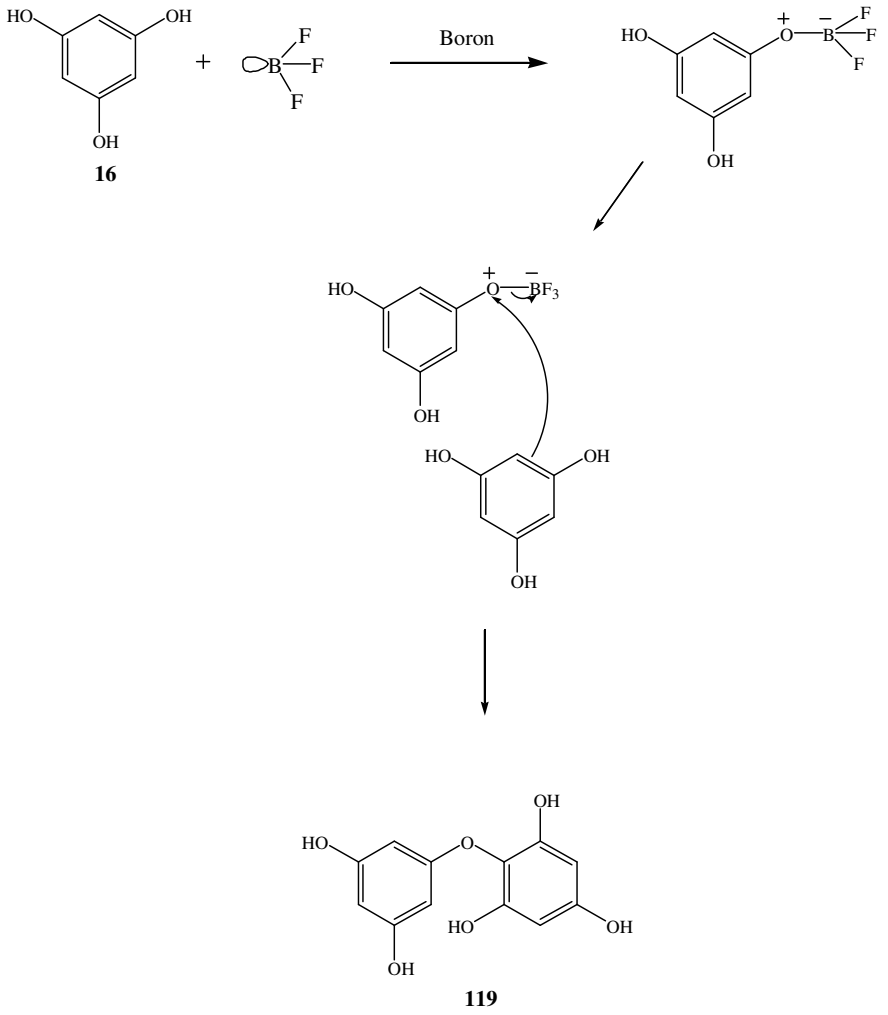
Since the formation of a biphenyl ether **119** is a rare occurrence, extensive methylation was employed ($TMSCHN_2$),[122] to confirm the structure of **119**. The reaction mixture was left to stir for two days to yield 3,5-dimethoxy-phenyl-2',4',6'-trimethoxy-phenylether **120**. The ^1H NMR spectrum of **120** revealed the doublet at δ 6.48 (*J* 2.28 Hz, H-2 and H-6) and the triplet at δ 6.41 (*J* 2.32 Hz, H-4), the singlet at δ 6.21 (H-3' and H-5') and five methoxy resonances.



120

This occurrence of **119** can either be attributed to, the presence of a radical or the coordination of boron trifluoride to the phenolic oxygen (Scheme 14).

[122] Aoyama. T., Terasawa. S., Sudo. K., Shioiri. T., *Chem. Pharm. Bull.*, **32**, 3759-3760, (1984).

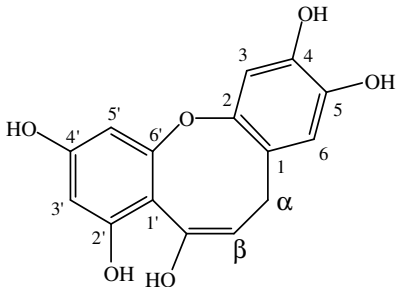


Scheme 14: Proposed mechanism for the coordination of boron trifluoride

3.4.5.3 RADICAL TERMINATOR

A commonly used radical terminator in organic reactions is the natural antioxidant, BHT **2**. It was used to determine the presence of a radical, however the formation of 3,5-dihydroxy-phenyl-2',4',6'-trihydroxy-phenylether (**119**, 27.8 %) still occurred together with the formation of **88** (17.1 %) and another molecule **121** with a structure similar to that of aspalalinin[75], presumably the enol tautomer of **92**, in trace amounts. The ^{1}H NMR spectrum (Acetone-d_6) of **121** consisted of a singlet at δ 6.42, accounting for H-3' and H-5', another singlet at δ 6.17, probably accounting for H-3 and H-6, and the α- and β-protons at δ 3.58 (multiplet) and δ 3.44 (triplet)

respectively. The enolic tautomer is reflected by the chemical shift as well as the splitting pattern of the α- and β-protons. Due to the fact that there were only traces of **121**, the structure could not be confirmed by further studies. The mechanistic rationale proposed for the formation of **92** (as the enol tautomer) in Scheme 9 could also account for the formation of **121**.



121

The formation of **119** in the presence of the radical terminator proved that a radical was not the initiator for the formation of **119**.

3.4.5.4 INFLUENCE OF BORON COORDINATION

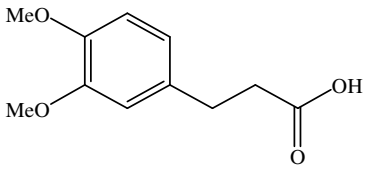
The influence of boron coordination on the formation of **119** was demonstrated by the following two reactions. Firstly, phloroglucinol **16** in dried ether was refluxed for five hours at 40°C, resulting only in the recovery of **16**. In the second reaction, **16**, dried ether and $BF_3 \cdot (C_2H_5)_2O$ were gradually heated from 0°C to reflux over five hours to yield **119**. This outcome confirmed that the coordination of boron to the phenolic oxygen led to the formation of **119** (scheme 14, p. 83).

Different methods of optimization, e.g. specific temperature control or changing the order of the addition of the substrates, were used in order to increase the yield of 3,4,2',4',6'-pentahydroxy dihydrochalcone **88**. The best result was achieved by the

addition of $BF_3{\cdot}(C_2H_5)_2O$ to 3,4-dihydroxyhydrocinnamic acid **89** at 0°C while stirring. The temperature of the reaction mixture was gradually increased until reflux. Phloroglucinol **16** was added and the mixture was left to stir and reflux for an hour and a half to form **88** in 19.7 % yield. As **119** still formed, the only viable route that was left was the protection of **16**.

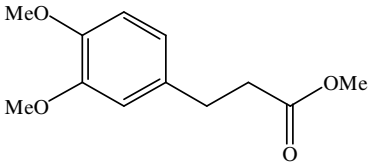
3.4.5.5 PROTECTING GROUPS

Another attempt was made to utilize $Hf(OTf)_4$ as catalyst in the *C*-acylation of **16**, but with the catechol moiety protected as its methyl ether 3-(3,4-dimethoxyphenyl)-propionic acid **122**.



122

3,4-Dihydroxyhydrocinnamic acid **89** was methylated with trimethylsilyldiazomethane ($TMSCHN_2$)[122] in n-tributylamin. Total methylation occurred to yield 3-(3,4-dimethoxy-phenyl)-propionic acid methyl ester **123** in 91.6% yield. This product was subsequently selectively hydrolyzed[123] with LiOH in a $MeOH/H_2O$ (1:1, v/v) solution to produce the commercially available product **122** (85.7 % yield).

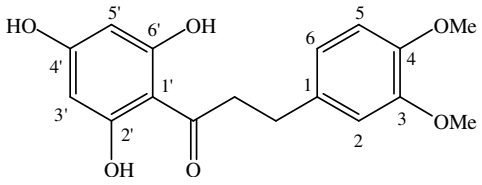


123

[123] Alam. A., Takaguchi. Y., Ito. H., Yoshida. T., Tsuboi. S., *Tetrahedron*, **61**, 1909-1918, (2005).

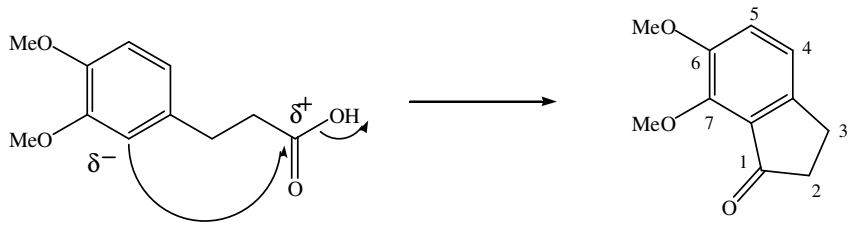
Once **122** was obtained the reaction between **16** and **122** with $Hf(OTf)_4$ as catalyst in dried ether was set up. The reaction mixture was left to stir and reflux for six hours at 80-90°C. After an hour and a half the reaction mixture was monitored with TLC and the result was the formation of 3,5-dihydroxy-phenyl-2',4',6'-trihydroxy-phenylether **119**. The reaction mixture was still left to stir for the remainder of the time, but none of the desired product was obtained. It was thus decided to discard of $Hf(OTf)_4$ as catalyst and to attempt further reactions with $BF_3{\cdot}(C_2H_5)_2O$ as catalyst.

To a mixture of **16** and **122** in dried ether, $BF_3{\cdot}(C_2H_5)_2O$ was added at 0°C where after the reaction mixture was refluxed for 90 min to yield the desired product, 3,4-dimethoxy-2',4',6'-trihydroxy dihydrochalcone (**124**, 7 %) as well as **119** (33.2 % yield). From the ^{1}H NMR data ($CDCl_3$) of **124**, the shift of the α- and β-protons (δ 3.38 and δ2.93 respectively) to the low field region compared to that of **122**, was an indication of *C*-acylation. The spectrum also revealed the presence of a typical ABX system for the B-ring protons at δ 6.91 (d, *J* 1.85 Hz, H-2), δ 6.85 (d, *J* 8.13 Hz, H-5) and δ 6.79 (dd, *J* 8.13, 1.91 Hz, H-6), the A-ring protons at δ 5.95 (s, H-3' and H-5') and two methoxy resonances at δ 3.79 and δ 3.77.



124

Another compound with a higher R_f value (R_f = 0.56) than **124** (R_f = 0.50) but a lower R_f value than **122** (R_f = 0.66), was identified by ^{1}H NMR as 6,7-dimethoxy-indan-1-one (**125**, 62.3 %). The formation of **125** can be ascribed to the intramolecular electrophilic attack of the aromatic carbon C-2' of the 3-phenylpropionyl moiety on the electrophilic carbonyl as depicted in scheme 15.



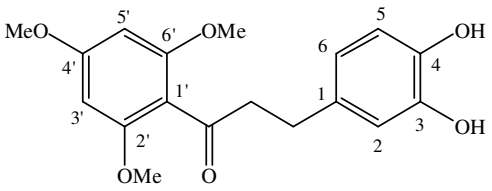
122 **125**

Scheme 15: Formation of 6,7-dimethoxy-indan-1-one (125)

Evident from the ^{1}H NMR data ($CDCl_3$) of **125** was the resonances of the α- and β-protons at δ 3.01 and δ 2.73, and two aromatic protons (br. singlet's) in the low field region at δ 7.2 and δ 6.95, which can be assigned to either H-4 or H-5.

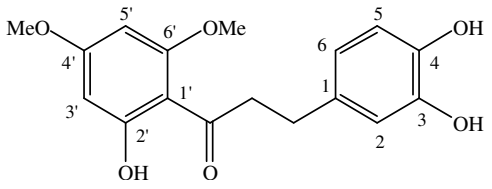
The formation of **119** and the intramolecular cyclization of the carboxylic acid to afford **125** could thus explain the low yield obtained for **124**.

Reaction between protected phloroglucinol **16** (1,3,5-trimethoxybenzene **117**) and 3,4-dihydroxyhydrocinnamic acid **89** under the same reaction conditions as mentioned above, resulted in the formation of the desired product, 3,4-dihydroxy-2',4',6'-trimethoxy dihydrochalcone (**126**, 4.7 %), without the concomitant formation of **119**. The ^{1}H NMR data ($CDCl_3$) of **126** indicated a split of the A-ring proton resonances into two doublets at δ 6.09 and δ 5.94, each integrating for a single proton (d, *J* 2.28 Hz and *J* 2.29 Hz respectively, H-3' and H-5'), thus indicating *C*-acylation. The spectrum also revealed the resonances of the B-ring protons at δ 6.8 (H-2 and H-5) and δ 6.69 (dd, *J* 8.13, 1.70 Hz, H-6), the α- and β-protons at δ 3.28 and δ 2.9, respectively, and only two methoxy resonances. An NMR spectrum obtained from an experiment at increased temperature (45 °C) still displayed only two methoxy resonances. The splitting of the A-ring proton resonances into two singlets rather than one can serve as an indication that the 2'-OMe group has been demethylated to a hydroxyl group capable of forming a hydrogen bond with the carbonyl oxygen and thereby preventing free rotation around the C-1'-CO bond.



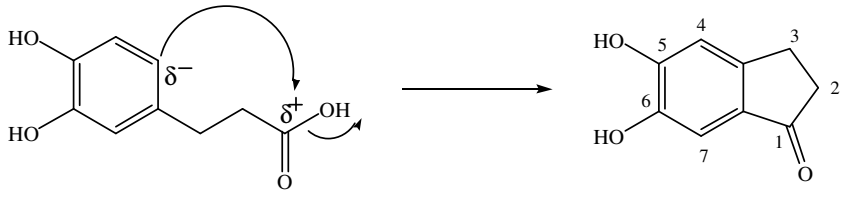
126

It can thus be rationalized that the product 2',3,4-trihydroxy-4',6'-dimethoxy dihydrochalcone **127**, was formed from **126** in the presence of $BF_3{\cdot}(C_2H_5)_2O$. It has been reported that Lewis acids can act as good demethylation agents.[124] They possess a high affinity for oxygen, and activation thus occurs by the acceptance of the lone pair electrons of the oxygen-methyl bond which results in the loss of the methyl group.



127

The product (**128**, 46 %) resulting from intramolecular cyclization was once again identified by ^{1}H NMR analysis.



89 **128**

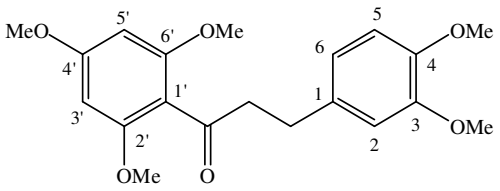
Scheme 16: Formation of 5,6-dihydroxy-indan-1-one (128)

Evident from the ^{1}H NMR data ($CDCl_3$) of **128** was the resonances of the α- and β-protons at δ 2.8 and δ 2.64, respectively, and two aromatic proton resonances

[124] Wilhelm. H., Wessjohann. L. A., *Tetrahedron*, **62**, 6961-6966, (2006).

(singlet's) in the low field region at δ 7.1 and δ 6.7, which can be assigned to either H-4 or H-7.

From a previous paragraph (3.4.5.2, p. 81) it was clear that phloroglucinol **16** needed to be protected in order to reduce the formation of 3,5-dihydroxy-phenyl-2',4',6'-trihydroxy-phenylether **119**, but from these reactions it is also clear that the cyclization of the carboxylic acid can also contribute to the low yields obtained in the attempt to synthesize 3,4,2',4',6'-pentahydroxy dihydrochalcone **88**. The final reaction that was attempted, was the reaction between 1,3,5-trimethoxybenzene **117** and 3-(3,4-dimethoxyphenyl)-propionic acid **122**. The same reaction procedure was used as in the previous paragraphs (p. 85) with $BF_3{\cdot}(C_2H_5)_2O$ as catalyst to yield a reaction mixture consisting of starting material, 6,7-dimethoxy-indan-1-one (**125**, 12.1 %) and the desired product, 3,4,2',4',6'-pentamethoxy dihydrochalcone (**129**, 37 %). No **119** was formed. The ^1H NMR spectrum ($CDCl_3$) of **129** revealed the resonances of the B-ring protons at δ 6.8 (H-2 and H-5) and δ 6.69 (dd, *J* 8.05, 2.01 Hz, H-6), the α- and β-protons at δ 3.28 and δ 2.91, respectively, and methoxy resonances integrating for five –OMe groups. The aromatic protons of the A-ring resonated as two doublets at δ 6.09 (*J* 2.38 Hz) and δ 5.94 (*J* 2.32 Hz) integrating for one proton each and which can be assigned to either H-3' or H-5'.



129

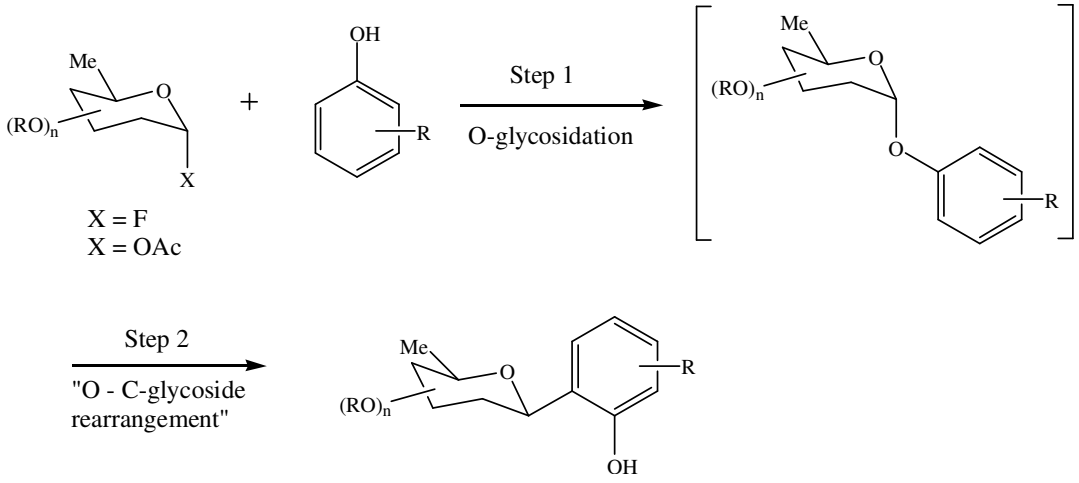
The results obtained up to this stage were a clear indication of the complexity within the synthesis of **88**.

The next step in the retro synthesis of aspalathin as depicted in scheme 8 (p. 47), namely the direct *C*-coupling of D-glucose **51** (glycosylation) to the A-ring of **88**, was thus attempted.

3,4,2',4',6'-pentahydroxy dihydrochalcone **88** obtained from previous reactions were combined and purified by column chromatography (Sephadex LH 20, ethanol as eluent).

3.5 ENVIRONMENTALLY FRIENDLY C-GLYCOSYLATION

C-glycosylflavonoids are present in low concentrations in plant tissue (such as citrus fruit peel)[125] and only a handful has been isolated from microorganisms. The best known synthetic method for preparing *C*-glycosides, is Suzuki's *C*-glycosylation technique[126], which entails the reaction of a selectively protected polyphenol with per-*O*-benzylglycosyl fluoride followed by an *O*→*C* glycoside rearrangement (Scheme 17).



Scheme 17: Suzuki's *C*-glycosylation technique[126b]

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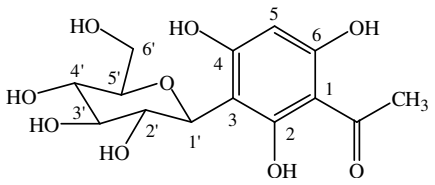
[125] [a]Matsubara. Y., Sawabe. A., *J. Synth. Org. Chem. Jpn.*, **52**, 318-327, (1994); [b] Chopin. J., Dellamonica. G., *The Flavonoids*, (J. B. Harborne, ed.), Chapman & Hall, London, UK, 63-97, (1988).
[126] [a]Matsumoto. T., Katsuki. M., Suzuki. K., *Tet. Lett.*, **30**, 833-836, (1989); [b]Matsumoto. T., Hosoya. T., Suzuki. K., *Tet. Lett.*, **31**, 4629-4632, (1990); [c]Matsumoto. T., Hosoya. T., Suzuki. K., *Synlett.*, 709-711, (1991).

This method results in high yields and high regio- and stereo-selectivities[127], but requires the use of undesirable solvents such as CH_2Cl_2. Toshima *et al.*[128] reported the environmentally benign *C*-glycosylation of selective hydroxyl-protected aryl compounds with unprotected 2-deoxy sugars, but they report that difficulties were encountered with the deprotection of the methoxyl groups.

Sato *et al.*[129] recently reported a reliable method for the direct *C*-glycosylation of unprotected phenols with unprotected sugars, e.g. D-glucose **51,** in aqueous media. They investigated the use of the rare-earth metal triflates (as outlined by Kobayashi *et al.*[82]), which have the ability to function as Lewis acids in aqueous media, as catalysts. The *C*-glycosylation of phloroacetophenone **97** was best achieved with $Sc(OTf)_3$ as catalyst at high temperatures in the presence of an excess of glucose **51**.

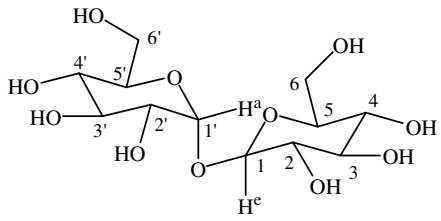
The reaction of 2,4,6-trihydroxyacetophenone **97** with **51** was repeated according to the reaction conditions outlined by Sato *et al.*[129] in the presence of $Sc(OTf)_3$ in ethanol:water (2:1, v/v). The ^{1}H NMR spectrum of the product obtained was not first order and fully resolved, comparison with the most informative signals of the published data of Matsumoto *et al.*[126b] enabled the confirmation of a successful outcome. The final structure was confirmed as that of the desired product, 2,4,6-trihydroxyacetophenone 3-*C*-β-glucopyranoside (**130**, 36.5%). Evident from the ^{1}H NMR data (Acetone-d_6 + D_2O) of **130** was the singlet of the aromatic proton at δ 5.80, the β-coupled anomeric proton at δ 4.84 (d, *J* 9.82 Hz, H-1') and the methyl group at δ 2.57 (s, -CH_3). The integration of the glucoside protons were not clear due to the influence of deuteriated water but the "bumps" at δ 3.4-3.9 do correspond to the multiplets at δ 3.47-3.89 mentioned by Matsumoto *et al.*[126b].

[127] Kumazawa. T., Kimura. T., Matsuba. S., Sato. S., Onodera. J., *Carbohydr. Res.*, **334**, 183-193, (2001).
[128] Toshima. K., Ushiki. Y., Matsuo. G., Matsumura. S., *Tet. Lett.*, **38**, 7375-7378, (1997).
[129] Sato. S., Akiya. T., Suzuki. T., Onodera. J., *Carbohydr. Res.*, **339**, 2611-2614, (2004).



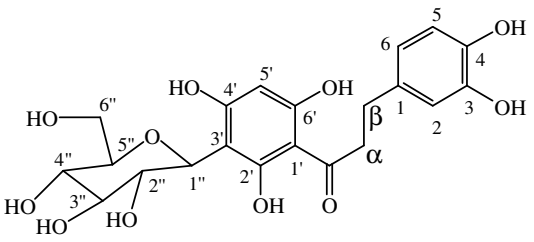
130

The *C*-glycosylation of 3,4,2',4',6'-pentahydroxy dihydrochalcone **88** was thus attempted. At first the reaction was repeated as outlined above, with a three mole equivalence excess of glucose **51**. The ^{1}H NMR spectrum (DMSO, D_2O) of the product revealed doublets at δ 5.75 (d, *J* 3.47 Hz, H-1α) and at δ 5.15 (d, *J* 7.80, H-1' β), which suggested 1-*O*-(β-D-glucopyranosyl)-α-D-glucopyranose **131** to be the product. The integrals of the assigned sugar protons were also consistent with two glucosyl **51** units.



131

Reaction of **51** and **88** (2:1 eq.) under the same conditions yielded synthetic aspalathin **55** for the first time ever, and, furthermore, *via* an unprotected route, albeit in low yield (10.7%). The free phenolic synthetic **55** was analyzed *via* HPLC analysis and confirmed. HPLC-analyses were carried out with a Shimadzu LC-20 SPD-20 AV HPLC and the peaks were monitored at 260 nm. Separation was performed by solvent gradient elution (see Chapter 5 p.110) on a Phenomenex C-18 110 A (5µm) column (250 x 4.60 mm). Solvent A consisted of 2% (v/v) formic acid and solvent B was pure HPLC-grade methanol.



55

After various HPLC separations, samples of **55** were combined for NMR analyses. The ^{1}H NMR spectrum (Acetone-d_6) of **55** revealed a typical ABX system of the B-ring protons at δ 6.76 (d, *J* 1.99 Hz, H-2), δ 6.73 (d, *J* 7.99 Hz, H-5) and δ 6.60 (dd, *J* 8.03, 2.02 Hz, H-6), the A-ring proton resonance at δ 5.94 (s, H-5') and the signals of the α- and β-protons at δ 3.33 and δ 2.83, respectively. The anomeric proton of the glucosyl moiety at δ 4.94 (d, *J* 9.76 Hz, H-1") indicates the β-coupling of **51**, whereas the rest of the carbohydrate protons are assigned for at δ 3.87 (m, H-6"), δ 3.66 (t, *J* 9.35 Hz, H-2" and H-4"), δ 3.55 (br. t, *J* 8.96 Hz, H-3") and δ 3.50 (m, H-5"). This result corresponds to the published data of **55** isolated from rooibos (*Aspalathus linearis*).[27]

The low yield of **55** obtained can be ascribed to the following factors:

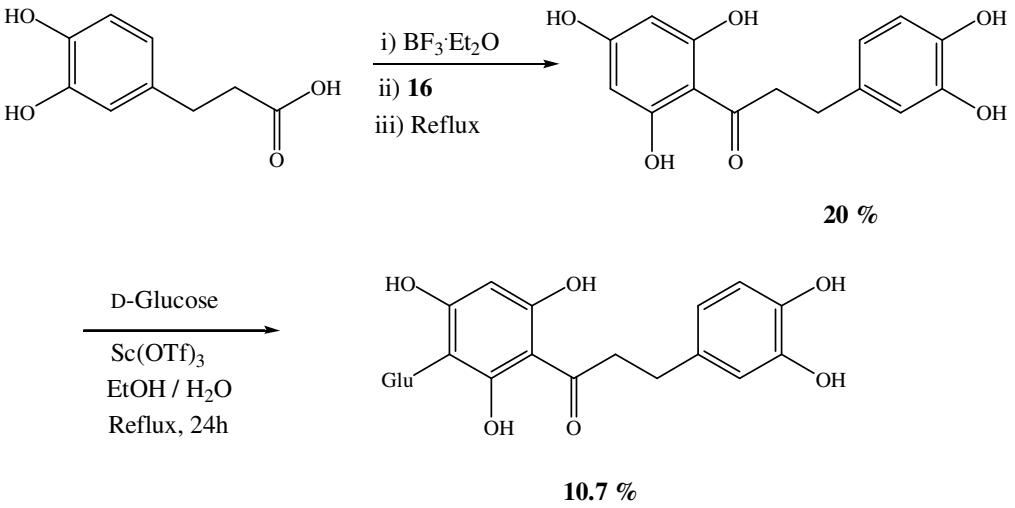
- The small amount of starting material **88** obtained from the previous steps limited the optimization of the reaction procedure.
- An excess of glucose **51** used resulted in the formation of **131**, which reduced *C*-glycosylation.
- π-Stacking of the A- and B-rings of 3,4,2',4',6'-pentahydroxy dihydrochalcone **88** can hamper *C*-glycosylation of the A-ring.

3.6 CONCLUSION

From these discussions it is clear that the synthesis of aspalathin **55** from unprotected phenolic substrates was complicated by the reactivity of these synthons. Among

these were the ability of phloroglucinol **16** to act as both a nucleophile and electrophile, and the intramolecular cyclization of the phenylpropionyl moiety. To the best of our knowledge, a complete free phenolic synthesis of flavonoids has not been reported yet, though Siddaiah *et al.*[121] reported a partly free phenolic synthesis of polyhydroxydihydrochalcones and homoisoflavonoids. Furthermore, no method for the free phenolic synthesis of *C*-glycosylated flavonoids has been reported. Therefore, the synthesis of aspalathin **55** was not only the first but also a distinguished complete free phenolic synthesis of a flavonoid and, more importantly, a *C*-glycosylated flavonoid.

Finally the following protocol (Scheme 18) for the successful synthesis of aspalathin **55** was established by employing modern and sophisticated methodologies. It must be emphasized that the researcher is aware of the relatively low yields, and that a comprehensive optimization project is envisaged.



Scheme 18: Total free phenolic synthesis of aspalathin

From the first step in this synthesis, activation by the Lewis acid posed a problem. The boron coordinates with the phenolic oxygen species of **16**, thus causing two electrophilic sites in the reaction mixture, namely the activated phenol and the carbonyl carbon. In order to reduce the coordination of boron, the reaction was commenced with the addition of the catalyst, $BF_3{\cdot}(C_2H_5)_2O$, to the carboxylic acid at

0ºC. In the final step of the synthesis of **55**, a commercially viable direct recycling route of glucose **51** and 3,4,2',4',6'-pentahydroxy dihydrochalcone **88** could be the best option. This would allow the yield to be optimized with the re-use of the starting materials.

SKIN CARE TECHNOLOGY

SKIN CARE TECHNOLOGY

4.1 INTRODUCTION

According to Sec. 201(i) FD&C Act. Mentioned in the Senate Report No. 493 (USA), cosmetics can be defined in the following way: "A cosmetic is a product, except soap, intended to be applied to the human body for cleansing, beautifying, promoting attractiveness or altering the appearance". In short, one may say that a cosmetic is a product intended to exert a physical, and not a physiological, effect on the human body.

In the previous chapters emphases have been placed on the reactivity of natural products, e.g. polyphenols / flavonoids and specifically their antioxidant activity. In this chapter, the application of these natural products in cosmetic formulations will be discussed. These products are called "cosmeceuticals", and are described as cosmetics containing ingredients that are bioactive, exerting effects on people. Cosmeceuticals are cosmetics with therapeutic, disease-fighting or healing properties, serving as a transition between personal care products and pharmaceuticals and are developed specifically for their medicinal benefits. Some of these products allegedly have promised to smooth wrinkles, strengthen skin, inhibit enzymes, trigger growth in elastin and collagen, manipulate skin colour or to prevent baldness. The research and developer's challenge exists in order to deliver the active ingredient to the skin while maintaining the maximum therapeutic effect. Only a handful of products are successful, but still this is a new generation of scientifically-advanced skin care and cosmetic products. In the following paragraphs a background on formulations and

skin permeation of the flavonoid aspalathin **55**, will be discussed. In addition, these results will be related to the chemical structure of **55** to rationalize the benefits thereof as a "cosmeceutical" in topical formulations.

4.2 SKIN CARE FORMULATIONS

Skin care formulations are aimed at three aspects of skin care, ***viz.*** cleansing, moisturizing and the treatment of the condition of the skin. Different formulations exist, e.g. soap bars (probably the most traditional), solutions, emulsions (lotions and creams), gels etc. These formulations act as vehicles for the active ingredients[130] – the active principle is embedded into a matrix, called the vehicle. The vehicle aids the delivery of the active principle to the application site or to the site where the desired effect need to be achieved. Emulsions are most commonly used as vehicles in skin care formulations.

4.2.1 EMULSIONS

An emulsion can be defined as: "a two-phase system consisting of two immiscible or partially miscible liquids, one being dispersed in the other in the form of very small droplets".[131] The two basic emulsion types are oil in water (o/w) or water in oil (w/o). Figure 11 demonstrates how an oil in water emulsion can be established.

[130] Knowlton. J., Pearce. S., "Handbook of Cosmetic Science and Technology", *Emulsion Technology*, Cotswold Publishing Co., (1997).
[131] Society of Cosmetic Chemists South Africa (COSCEM), Diploma Course Module 1, Unit 14, "Emulsion Technology", Part 1, 5, (2003).

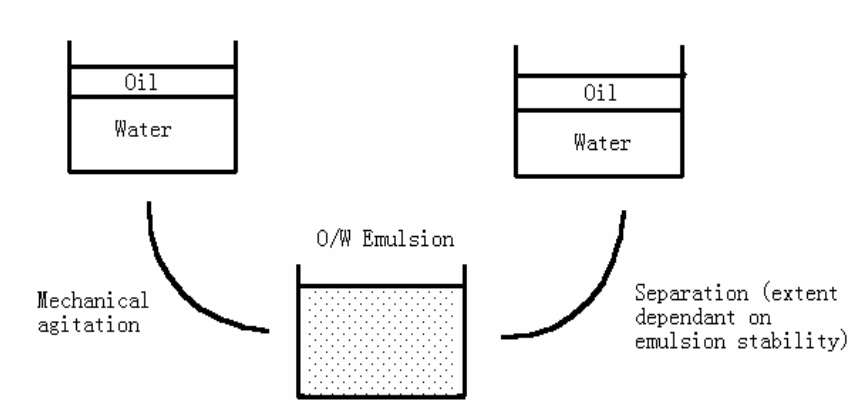
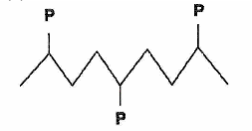


Figure 11[131]: General illustration for the creation of an oil in water emulsion

Emulsions are thermodynamically unstable and therefore they need to be stabilized.[132] This could be achieved either by the addition of (i) polymers, (ii) powders or (iii) nonionic surfactants to the emulsion.

(i) Stabilization of emulsions by the addition of polymers is explained by figure 12:



e.g. Polymer

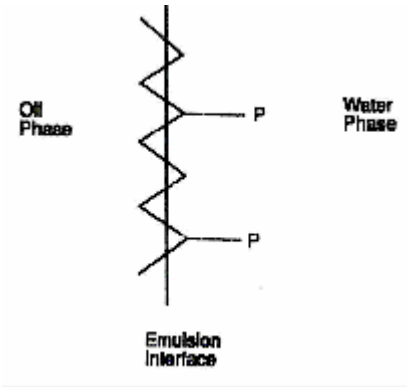


Figure 12[132]: Stabilization of emulsion by a polymer

[132] Society of Cosmetic Chemists South Africa (COSCEM), Diploma Course Module 1, Unit 15, "Emulsion Technology", Part 2, 4-7, (2003).

The polar side groups are orientated towards the aqueous phase, whereas the polymer backbone has an affinity for the lipid/oil phase. This orientation provides a mechanical film strengthening.

(ii) As far as the addition of powders is concerned, the particle size of the powder needs to be smaller than the dispersed phase droplet. If the powder is only partially wetted, migration to the interface will occur in the following way:

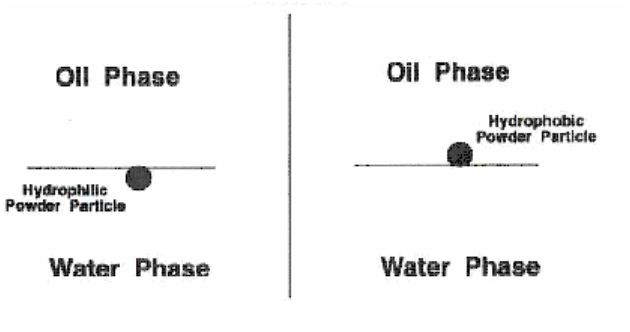


Figure 13[132]: Stabilization of emulsion *via* powder addition

(iii) The effect of the addition of nonionic surfactants is best described in an oil/water system wherein the primary emulsifier is anionic:

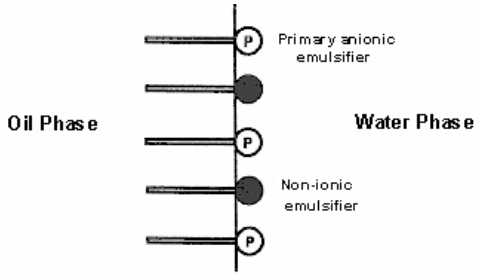


Figure 14[132]: Stabilization of emulsion *via* nonionic surfactant

This feature where the nonionic surfactant slot in between the primary anionic emulsifier, increase the density of the emulsion, providing a more stable emulsion with increased interfacial strength.

4.2.2 APPLICATION OF NATURAL FLAVONOIDS IN SKIN CARE PRODUCTS

The use of terms like "organic" and "natural" is often the top billing on cosmetic labels and advertisements. To the average consumer, the nomenclature of the chemical compounds listed as the ingredients in the cosmetic product are often unpronounceable and therefore, for them, these compounds belong to a totally unfamiliar territory, thus giving rise to unnecessary and negative perceptions. It seems, therefore, obligatory for suppliers to bridge the suspicions by conveying the correct and apprehensible message.

The biological effects of flavonoids have already been discussed in previous paragraphs and it is, therefore, suffice to note that the burgeoning prominence of flavonoids (polyphenols) is best exemplified by statements similar to that of an international company like Symrise, viz. "it is becoming a new efficient ingredient for cosmetic products that satisfies the growing demands of today's sophisticated clientele for valuable new natural and organically grown plant materials with proven efficacy".[133]

In a recent article of Marnewick *et al*.[67] it was reported that the activity of rooibos (*Aspalathus linearis*), towards the inhibition of tumor promotion on the skin of mice was tested and that results are indeed suggestive of the inhibition of TPA-induced tumor promotion in mouse skin by both the unprocessed and processed rooibos. It is noteworthy to mention that aspalathin **55** is the major flavonoid in the unprocessed rooibos ethanol/acetone extract, while iso-orientin **57**, orientin **56** and aspalathin **55** represented the major flavonoids in processed rooibos.

[133] Symrise Research, *Newsletter*, Hamburg, November 22, (2004).

In addition to the application of rooibos, other and sometimes related plant polyphenolics have found their way as active ingredients in skin care formulations, e.g. the use of soybean-germ oil in topical application against UVB-induced skin inflammation, where these results *in vivo*, exceeds that of tocopherol acetate by a factor of two[134]. The plant phenols of the aqueous acetone extract of the leaves of *Malus dounteri A. CHEV. Var. formosana* (Taiwanese indigenous plant), are used as anti-aging cosmetic products[135]. Researchers from South Korea showed that mixtures of plant extracts are beneficial for the treatment of skin inflammation and also for the treatment of chronic skin inflammatory disorders such as atopic dermatitis[136]. These few examples represent a list of compounds and related applications of immense and growing magnitude.

Noteworthy in the cosmetic field, is a modern tendency to associate botanical extracts with vitamins to improve skin conditions by synergism. This protocol seems to result in pronounced antioxidant activity *in vitro*, but the treatment of erythema (inflammation) and the prevention of UV damage were not that profound.[137] These results suggest that individually each compound has a specific effect, but that it does not per se follow that the combination of more than one bioactive compound will only and always lead to a "mixture" that will exert all the positive characteristics of the separate compound. It is worthwhile to quote Wolfgang Goertz, personal care marketing manager of Goldschmidt, in this regard:

"Everyone likes natural products, but they have their limitations. The quality of natural products may vary from year to year depending on the harvest. Plant extracts are often accompanied by unknown or unwanted material. On the other hand, synthetics are generally very pure and safe. Chemists can modify synthetic molecules to develop what can't be found in nature".[138]

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[134] Bonina. F., Puglia. C., Avogadro. M., Baranelli. E., Cravotto. G., *Archiv der Pharmazie*, **338**, 598-601, (2005).
[135] Leu. S. J., Lin. Y. P., Lin. R. D., Wen. C. L., Chen. K. T., Hsu. F. L., Lee. M. H., *Biol. Pharm. Bull.*, **29**, 740-745, (2006).
[136] Lim. H., Son. K. H., Chang. H. W., Kang. S. S., Kom. H. P., *Arch. Pharm. Res.*, **29**, 503-507, (2006).
[137] Campos. P. M. B. G., Gianeti. M. D., Kanashiro. A., Lucisano-Valim. Y. M., Gasper. L. R., *Photochemistry and Photobiology*, **82**, 683-688, (2006).
[138] Reisch. M. S., *C & EN Northeast News Bureau*, Retrieved 8 May 2006 from www.cen-online.org

This philosophical argument is best explained by the results discussed in paragraph 4.2.2.1.

4.2.2.1 SYNTHETIC ANALOGUES OF FLAVONOIDS

Hakamata *et al.*[139] recently reprted on the synthesis of flavonoid analogues with the potential to reduce mitochondrial oxidative damage *via* antioxidative and α-glucosidase inhibitory activities. They furthermore aimed at the development of a strong lipophilic antioxidant (which does not occur in plant extracts), to suppress mitochondrial ROS production and LDL oxidation, as such a compound would have a affinity for lipid particles or the membranes of the mitochondria. (+)-Catechin **132** was thus modified because, firstly, the catechol moiety is known for its strong antioxidant ability and secondly, a planar molecule is needed in order to reduce the risk of steric hindrance. Planar catechin analogues with various lengths of alkyl side chains, were thus synthesized.

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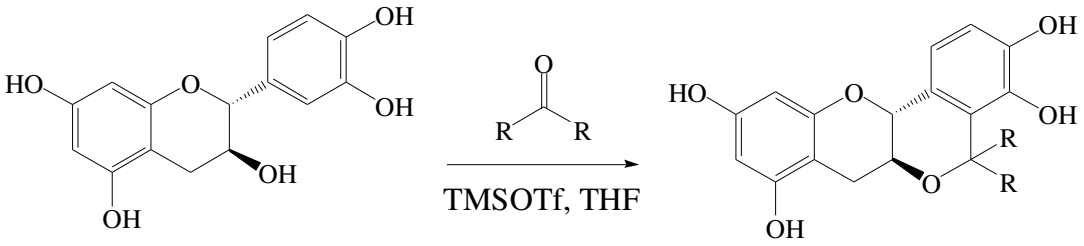
[139] Hakamata. W., Nakanishi. I., Masuda. Y., Shimizu. T., Higuchi. H., Nakamura. Y., Saito. S., Urano. S., Oku. T., Ozawa. T., Ikota. N., Miyata. N., Okuda. H., Fukuhara. K., *J. Am. Chem. Soc.*, **128**, 6524-6525, (2006).



(+)-Catechin (132)

PC_n

PC_1 R = CH_3
PC_2 R = CH_2CH_3
PC_3 R = $(CH_2)_2CH_3$
PC_4 R = $(CH_2)_3CH_3$
PC_5 R = $(CH_2)_4CH_3$
PC_6 R = $(CH_2)_5CH_3$

Scheme 19: Modification of (+)-catechin

The results indicated that the alkyl side chains indeed did affect the antioxidant activity. An increase in the length of the side chain (*n* = 1-3) resulted in an increase of antioxidant activity, but a further increase (*n* = 4-6) seemed to weaken the antioxidant effect. This is consistent with suggestions that longer alkyl side chains result in the formation of amphiphilic micelles in an aqueous solvent.

4.3 SKIN PERMEATION OF ASPALATHIN

An as yet unpublished study was conducted by Miao-Juei Huang, for her Master of Science in Pharmacy at the University of the Witwatersrand (Johannesburg) on both the topical and intestinal absorption of rooibos tea.[140] The absorption of aspalathin **55** in an unfermented (green) rooibos aqueous extract and an aqueous solution of pure aspalathin **55** were studied. The results obtained for the percutaneous permeation experiments were of interest.

[140] Huang. M., "The transport of the Rooibos tea flavonoid aspalathin across the skin and the intestinal epithelium", MPharm-dissertation, University of the Witwatersrand, Johannesburg, (2007).

4.3.1 INTRODUCTION ON SKIN PERMEATION

The term "skin uptake" is commonly used to describe the fate of drugs upon their entry into the skin. It is a broad definition that encompasses retention, permeation, metabolism, degradation and binding to skin components, these are various activities that an active component can undergo upon partitioning into the skin. In case of topical delivery, the objective is to have optimal active component residence in the skin layers i.e. skin retention, whereas in the case of transdermal delivery, an optimal net transport across the skin (skin permeation) becomes an important criterion in determining the efficacy of the active component.[141]

The stratum corneum is the outermost layer of the epidermis. In humans it consists of between 10 and 25 layers of dead, elongated, fully keratinized corneocytes that are embedded in a matrix of lipid bilayers.[142] The stratum corneum consists of ~40% protein of which 80% is keratin. The type and amount of lipid in the stratum corneum depends on the body-site and, currently, it is generally accepted that skin permeability is affected by stratum corneum lipids. Since the stratum corneum has been recognized as the rate controlling membrane in transdermal delivery of active components, drugs and chemicals[143] only this layer of the epidermis will be discussed in further detail.

When a topical formulation is placed on the skin, the active ingredient has to penetrate form the stratum corneum into viable tissues. The main limiting factor for this process is the slow diffusion through the stratum corneum, which is known to be a dead layer. There are three primary transport pathways through the skin: (1) intercellular diffusion through the lipid lamellae; (2) transcellular diffusion through

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[141] Behl. C. R., Kuma. S., "Choice of membrane for in vitro skin uptake studies and general experiment techniques"(In B. W. Kemppainnen and W. G. Reifenrath eds. Methods for skin absorption), Baco Raton, Fla: CRC Press Inc., 1-21, (1990).
[142] Degim. I. T., *Drug Discovery Today*, **11**, 517-523, (2006).
[143] Hadgraft. J., Lane. M. E., *International Journal of Pharmaceutics*, **305**, 2-12, (2005).

the corneocytes and lipid lamellae; and (3) diffusion through the skin appendages (hair follicles and sweat ducts).[142] Figure 15 illustrates these proposed pathways.

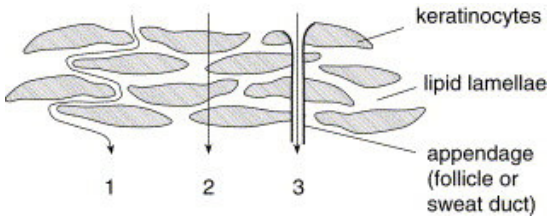


Figure 15: Skin permeation routes

The predominant route of transport is intercellular diffusion through the lipid lamellae.[144] The intercellular spaces consist of structured lipids and diffusing molecules need to cross a variety of lipophilic and hydrophilic domains of the skin to reach the underlying cell layers.[144] In brief[140], active ingredients applied onto the skin undergo passive diffusion within their carrier system (vehicle) to the surface of the skin, followed by partitioning into the stratum corneum. After entering the lipophilic stratum corneum and the sweat glands, an inward diffusion of the active ingredient takes place and continues into the hydrophilic viable epidermis and dermis (local tissue). This creates a concentration gradient across the skin between the surface of the skin and the microcirculation imbedded in the dermal layer. Once the active ingredient reaches the general circulation it is distributed very rapidly and due to reasonable rates of systemic metabolism and elimination, generally no appreciable systemic build-up occurs. This phenomenon, maintaining a near-zero concentration of the active ingredient on the plane of the capillaries of the dermal layer is called a sink condition and is the driving force for the diffusion process.[145]

Key factors that control molecular permeation through the stratum corneum of the skin, in decreasing sequence of importance, are molecular hydrophobicity, size, and

[144] Hadgraft. J., *European Journal of Pharmaceutics and Biopharmaceutics*, **58**, 291-299, (2004).
[145] Flynn. G. L., "Topical drug absorption and topical pharmaceutical systems" (In G. S. Banker and C. T. Rhodes, eds. Modern Pharmaceutics), Marcel Dekker, New York, 263-327, (1979).

the ability to interact with the other molecules, e.g. *via* hydrogen bond formation.[146] Consequently, molecules larger than a few hundred Dalton and/or highly polar compounds that cannot efficiently pass through the skin by simple diffusion may alternatively traverse the skin *via* the hydrophilic porous pathways, i.e. the transport shunts.[146]

Factors that influence the absorption of active ingredients are: physicochemical properties of the permeant, condition and type of skin, other chemicals (e.g. vehicles or enhancers) present with the permeant, and external conditions (e.g. temperature, humidity, and occlusion).[140] Diffusion through the skin can best be described by Fick's law of diffusion, which states, that the flux, J, of a component of concentration, C, across a membrane of unit area, in a predefined plane, is proportional to the concentration differential across that plane, and is expressed by: $J = -D\nabla C$.[144] Flynn interpreted data from the literature in terms of a risk asssessment and concluded that penetration through the skin is related to the octanol/water partition coefficient (K_{oct}, often is expressed as log-K_{oct}).[142]

4.3.2 PERMEATION STUDY OF ASPALATHIN

4.3.2.1 TRANSPORT OF ASPALATHIN ACROSS THE SKIN AND ANALYSIS TECHNIQUES

The percutaneous permeation experiments conducted by Huang[140] were conducted with vertical Franz diffusion cells using human female abdominal skin obtained from patients who underwent cosmetic surgery. The most commonly used device for *in vitro* diffusion work and for determining penetration through the skin is the Franz-type diffusion cell, which consists of a donor compartment (containing the permeant) and a receptor compartment separated by a membrane (excised human skin).[147] The green rooibos extract and pure aspalathin solution buffered in phosphate buffer

[146] Cevc. G., *Advanced Drug Delivery Reviews*, **56**, 675-711, (2004).
[147] Friend. D. R., *J. Cont. Rel.*, **18**, 235-248, (1992).

system (pH 5.5) were applied to the skin for 12 hours. Samples of the permeants were obtained from the receptor fluid phase and from the stratified layers of the skin by using the tape-stripping technique and analyzed by HPLC. Tape stripping is the use of polypropylene adhesive tape (2 cm wide and 5 cm long), which is applied to the stratum corneum of the skin. The tape is smoothed out on the skin to ensure even adhesion, and then it is gently pulled off from the skin with one fluent and decisive movement, to remove some cells of the stratum corneum.

4.3.2.2 RESULTS AND CONCLUSION

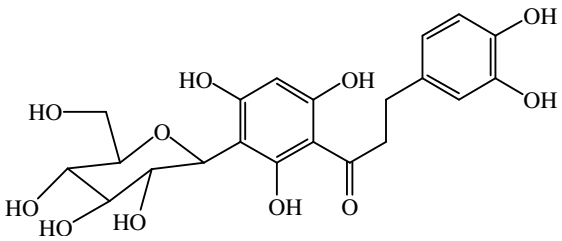
The following results were obtained by Huang *et al.*[140] Degradation of aspalathin occurred in solution and it was found that it was only stable in a phosphate buffer system (buffered between pH 5 and pH 6), which resulted in less than 30% loss over 14 hours. This can be attributed to the oxidation of aspalathin **55** as described in chapter 2 (p. 32). Aspalathin **55** exhibits an octanol/water partition coefficient (K_{oct}) below the value for optimal partition behavior. Two commercial skin care products were analyzed and aspalathin **55** was detected only in one, amounting to less than 0.1% in 1 g of cream. A permeation experiment on the product was thus not considered feasible. In the permeation experiments of green rooibos extract and pure aspalathin **55** solution, **55** accumulated mostly in the stratified layers of the skin, rather than permeating into the receptor fluid. Approximately 80% of the total aspalathin **55** absorbed were distributed in the stratum corneum and less than 0.1% of **55** in the applied doses of green rooibos extract and pure aspalathin **55** solution permeated the skin.

The results obtained from the study of Huang[140] brought the following conclusion about: since antioxidant activities are important in the stratum conrneum[148], aspalathin **55** accumulation in the stratum corneum may be desirable in the topical use of rooibos.

[148] Mavon. A., Raufast. V., Redoules. D., *Journal of Controlled Release*, **100**, 221-231, (2004).

4.3.2.3 STRUCTURE-ANTIOXIDANT ACTIVITY RELATIONSHIP OF ASPALATHIN

The poor permeation of aspalathin **55**, can be rationalized in terms of its structure (as depicted below).



55

- Because aspalathin **55** is a polar compound, possessing as many as nine ionizable hydroxyl groups available for hydrogen-bond formation and interaction with cellular components of the skin, its transport across the skin was hindered.
- Large molecules will tend to diffuse slowly[4], therefore the size of **55** could cause it to diffuse slowly. The 0.1% which did permeate the skin probably diffused through the skin *via* the hydrophilic porous pathways.
- Molecules with good solubility in both oils and water will permeate well.[4] The attached *C*-glycosyl would thus increase the solubility of **55** in water and reduce its solubility in oils, which will decrease permeation.

Therefore aspalathin **55** would be ideal for the use in topically applied cosmetic products. The accumulation of **55** with its strong antioxidant properties (due to the catechol moiety) in the stratum corneum would protect this barrier against oxidation (refer to par. 1.2.2 p. 5) and would thus protect the skin against harmful UV rays, and reactive oxygen species, thereby slowing down the aging process.

EXPERIMENTAL

EXPERIMENTAL

5.1 STANDARD EXPERIMENTAL TECHNIQUES

Unless specified to the contrary, the following techniques were applied throughout the course of this study.

5.1.1 CHROMATOGRAPHY

5.1.1.1 THIN LAYER CHROMATOGRAPHY

Qualitative thin layer chromatography (TLC) was conducted on "Merck TLC-foil sheets: Silica Gel F_{254}" (0.2 mm layer) divided into strips of 3 x 5 cm. R_f values are those observed in these qualitative TLC assessments.

Preparative scale thin layer chromatography (PLC) was conducted on glass plates (20 x 20 cm) coated with a layer (1.0 mm) of unactivated Merck Kieselgel 60 PF_{254} (100g Kieselgel in 230 ml distilled water for every 5 plates) and which were dried overnight at room temperature. After development in the appropriate eluant the plates were dried in a fast stream of air and the bands distinguished either by UV (254 nm) or by the appropriate spray reagent. The bands were eluted with ethyl acetate and the ethyl acetate removed under reduced pressure on a water bath at *ca* 40°C. The plates were generally loaded with 10 – 15 mg of crude product. Small scale separations were conducted on Merck "Pre-coated (0.25 mm) TLC plates silica Gel 60 F_{254}" with each plate loaded with 3 – 5 mg crude product.

5.1.1.2 SEPHADEX LH 20

A glass column of 150 cm^3 was charged with a slurry of pre-washed Sephadex LH 20 (Sigma) in ethanol. Approximately 500mg of crude product was dissolved in a minimum quantity of ethanol and carefully applied to the top of the column. The purified product was recovered by elution under atmospheric pressure with ethanol and collected in 5 ml fractions.

5.1.1.3 COLUMN CHROMATOGRAPHY (CC)

A glass column 50 cm^3 (1 cm diameter) was charged with 30g Merck "Kieselgel 60 (230 – 400 mesh) for column chromatography" for every 100mg of crude product. Air was disposed of elution with the appropriate solvent. The crude product was dissolved in a minimum quantity of the solvent and carefully applied to the top of the column. The purified product was recovered by elution with the appropriate solvent system and collected in 3 ml fractions.

For 1g of crude product a 5 cm diameter column was used and the purified product collected in 15 ml fractions.

5.1.1.4 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

HPLC-analyses were carried out with a Shimadzu LC-20 SPD-20 AV HPLC equipped with a UV/vis detector and variable wavelength detector. Samples were injected with a manual valve using a 0.80 µl loop. Peaks were monitored at 260 nm. Separation was performed by solvent gradient elution (see below) at a flow rate of 0.6 µl min^{-1} on a Phenomenex (Torrance, CA, Gemini) C-18 110 A (5µm) column (250 x 4.60 mm). The column temperature was maintained at 38°C and reconditioning took place at a flow rate of 1.2 µl min^{-1}. Solvent A consisted of 2% (v/v) formic acid

(Merck; 88% m/v) in glass distilled water and solvent B was pure HPLC-grade methanol (BDH HiPerSolv; UV transmission 98% at 260 nm).

Time (min)	Solvent composition (% methanol)
0	20
0	0
5	20
15	30
35	35
45	40
50	50
60	60
70	80
80	60
90	20
95	20

5.1.2 SPRAY REAGENTS

5.1.2.1 FORMALDEHYDE – SULPHURIC ACID

Thin layer chromatographs were gently sprayed with 2% v/v solution of formaldehyde (37 wt. % solution in water) in concentrated sulphuric acid and gently heated to *ca* 120°C to effect maximum development of colour.

5.1.3 CHEMICAL METHODS

5.1.3.1 METHYLATION WITH TRIMETHYLSILYLDIAZOMETHANE ($TMSCHN_2$)[125]

$TMSCHN_2$ (2.2 M hexane solution, 8 mmol) was added to a stirred solution of the phenolic material (< 200mg, 1 mmol) and N,N-diisopropylethylamine (1.4 mmol) in methanol (2 mmol) at room temperature. The mixture was stirred for a certain amount of hours/days at room temperature (the flask covered with foil), and the

excess $TMSCHN_2$ were allowed to evaporate in a fume cupboard with strong ventilation at room temperature.

5.1.3.2 ACETYLATION

To a solution of the crude product dissolved in a minimum of dry pyridine, was added twice the amount of acetic anhydride. After 24 hours at 35°C the reaction was stopped by adding excess amounts of crushed ice. After 1 hour the resulting precipitate was filtered off and washed repeatedly with distilled water to afford a pyridine and acid free product.

5.1.4 SPECTROSCOPICAL AND SPECTROMETRICAL METHODS

5.1.4.1 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (NMR)

NMR-spectroscopy was performed on a Bruker 300 MHz Avance DPX 300 or Bruker 600 MHz Avance^{II} 600 spectrometer at 296 K with deuterated chloroform (δ 7.24), deuterated acetone (δ 2.08) or addition of small drops of deuterated water as interval standards. Chemical shifts are reported in parts per million (ppm), accurate to 2 decimal values on the δ-scale, and coupling constants are given in Hz, accurate to 1 decimal value.

The following abbreviations are used:

s	=	singlet
d	=	doublet
t	=	triplet
dd	=	doublet of doublets
ddd	=	doublet of doublet of doublets
m	=	multiplet
br.	=	broadened

5.1.4.2 MASS SPECTROMETRY (MS)

Compilation of fragmentation spectra and accurate mass estimations were conducted on a TermoFinnigan, Finnigan LCQ Trap mass spectrometer. The molecular ion was generated *via* Electro Spray with a Reodine Injector. The following abbreviation is used:

M^+ = molecular ion

5.1.5 MICROWAVES

5.1.5.1 DOMESTIC MICROWAVE OVEN

The domestic microwave oven used, was a LG, Multiwave, MS-283MC model with settings for 100, 200, 400, 650 or 900 Watts.

5.1.5.2 MICROWAVE REACTOR

The microwave reactor, CEM Discover, was situated at the University of the Witwatersrand. The different parameters that could be set, were temperature, pressure, watt input, and power or standard mode.

5.1.6 DRYING OF REAGENTS

5.1.6.1 AZEOTROPIC DRYING OF PHLOROGLUCINOL

With the use of azeotropic distillation under reduced pressure, phloroglucinol was dried using ethanol:toluene (1:1, v/v) to remove water. This procedure was repeated

four times and then the phloroglucinol was left over night in vacuo in the presence of phosphorous pentoxide.

5.1.6.2 NITROMETHANE

Nitromethane was left over dry $CaCl_2$ (oven dried, 24 hours, 300°C) with a $CaCl_2$ drying tube attach to it for 24 hours. The $CaCl_2$ was filtered off and the solvent subsequently distilled over dried 3Å molecular sieves and stored under N_2.

5.1.6.3 ETHER

Ether was left over sodium wire for 24 hours. The solvent was subsequently distilled off.

5.1.7 ABBREVIATIONS

The following abbreviations are used for solvents in descriptions of developing systems for TLC and Column Chromatography separations:

A	=	Acetone
M	=	Methanol
EtOH	=	Ethanol
C	=	Chloroform
H	=	Hexane
EtOAc	=	Ethyl acetate
Et_2O	=	Ether
AcOH	=	Acetic acid
H	=	Water
T	=	Toluene

5.2 ACYLATION OF PHENOLIC SUBSTRATES

5.2.1 ACYLATION OF PHLOROGLUCINOL WITH CARBOXYLIC ACID

The following experimental procedure was conducted in an inhert atmosphere and anhydrous reagents were used. To $Hf(OTf)_4$ (47.78mg, 0.06168 mmol, 20 mol%) was added phloroglucinol **16** (50mg, 0.3084 mmol) and 3,4-dihydroxyhydrocinnamic acid **89** (56.18mg, 0.3084 mmol) in toluene (1ml) and CH_3NO_2 (0.15ml), the latter two in a 6.7:1 v/v ratio, under argon conditions at room temperature. The mixture was stirred for 6 h at 100°C and was then cooled to room temperature. Water was added to quench the reaction and a water-EtOAc liquid-liquid extraction (3 x 20ml) performed. The EtOAc extracts were combined, washed with saturated NaCl solution (1 x 10ml), dried (Na_2SO_4), filtered and the EtOAc evaporated under reduced pressure (35°C). PLC separation afforded 3 bands: R_f 0.78 (C:A:AcOH = 7:2:1, v/v; unreacted **16**, 32.4mg), R_f 0.95 (unreacted **89**, 4.8mg) and R_f 0.98 (unidentified sticky tar).

5.2.2 REACTION BETWEEN 1-NAPHTHOL AND ACETIC ACID

To $Hf(OTf)_4$ (53.74mg, 0.06936 mmol, 20 mol%) was added 1-naphthol **93** (50mg, 0.3468 mmol) and acetic acid (20.83mg, 0.3468 mmol) in toluene (1ml) and CH_3NO_2 (0.15ml), the latter two in a 6.7:1 v/v ratio, under argon conditions at room temperature. The mixture was stirred for 6 h at 90°C and was then cooled to room temperature. Water was added to quench the reaction and a water-EtOAc liquid-liquid extraction (3 x 20ml) performed. The EtOAc extracts were combined, dried (Na_2SO_4), filtered and the EtOAc evaporated under reduced pressure (35°C). PLC

separation afforded 2 bands: R_f 0.79 (T:A = 9:1:drop of M, v/v; unreacted **93**, 6.9mg, 10.7%) and R_f 0.90 **(94)**.

5.2.2.1 1-Hydroxy-2-acetonaphthone (94)

The fraction with R_f 0.90 afforded a *light yellow amorphous solid* (54.8mg, 85%).

^{1}H NMR 300 MHz [Acetone-d_6, Plate 1]: δ 8.42 (d, *J* 8.35 Hz, H-8), δ 7.88 (t, *J* 8.95 Hz, H-3 and H-5), δ 7.71 and δ 7.60 (m, H-6 or H-7), δ 7.41 (d, *J* 8.87 Hz, H-4), δ 2.75 (br. s, -CH_3).

5.2.3 REACTION BETWEEN 1-NAPHTHOL AND 3,4-DIHYDROXYHYDROCINNAMIC ACID

To $Hf(OTf)_4$ (53.74mg, 0.06936 mmol, 20 mol%) was added 1-naphthol **93** (50mg, 0.3468 mmol) and 3,4-dihydroxyhydrocinnamic acid **89** (63.18mg, 0.3468 mmol) in toluene (2ml) and CH_3NO_2 (0.30ml), the latter two in a 6.7:1 v/v ratio, under argon conditions at room temperature. The mixture was stirred for 8 h at 80°C, and then $Hf(OTf)_4$ (53.74mg) was added again. The reaction mixture was left to stir overnight at 80°C and was then cooled to room temperature. Water was added to quench the reaction and a water-EtOAc liquid-liquid extraction (3 x 20ml) performed. The EtOAc extracts were combined, dried (Na_2SO_4), filtered and the EtOAc evaporated under reduced pressure (35°C). PLC separation afforded 3 bands: R_f 0.15 **(95)** (H:EtOAc:A = 7.5:2:0.5, v/v), R_f 0.56 (unreacted **93**, 58.4mg, 54.6%), R_f 0.88 (unreacted **89**, trace amount).

5.2.3.1 3-(3,4-Dihydroxy-phenyl)-1-(1-hydroxy-naphthalen-2-yl)-propan-1-one (95)

The fraction with R_f 0.15 afforded a *light pink amorphous solid* (38.2mg, 35.7%).

^{1}H NMR 600 MHz [Acetone-d_6, Plate 2a]: δ 8.42 (d, *J* 8.38 Hz, H-8"), δ 7.93 (d, *J* 8.89 Hz, H-3"), 7.89 (d, *J* 8.20 Hz, H-5"), δ 7.70 (m, H-7"), δ 7.60 (t, *J* 7.59 Hz, H-6"), δ 7.40 (d, *J* 8.87 Hz, H-4"), δ 6.82 (d, *J* 2.17 Hz, H-2'), δ 6.75 (d, *J* 8.01 Hz, H-5'), δ 6.66 (dd, *J* 7.98, 2.07 Hz, H-6'), δ 3.46 (t, α-H), δ 2.97 (t, *J* 7.63 Hz, β-H).

^{13}C NMR 600 MHz [Acetone-d_6, Plate 2b]: Ar-C-OH (145, 137, 132.5), Ar-C-H (130, 127.5, 126, 124.9, 123.5, 119.5, 118.5, 115.5, 115), α-C (40.5), β-C (29.5).

TOCSY spectrum 600 MHz [Acetone-d_6, Plate 2c]

MS: *m/z* 307.50 (M^+, 100%)

Found **:** M^+ 307.50. $C_{19}H_{16}O_4$ *requires* M 308.333

5.2.4 REACTION BETWEEN PHLOROGLUCINOL AND ACETIC ACID

To $Hf(OTf)_4$ (47.78mg, 0.06168 mmol, 20 mol%) was added phlroglucinol **16** (50mg, 0.3048 mmol) and acetic acid (18.52mg, 0.3048 mmol) in toluene (2ml) and CH_3NO_2 (0.30ml), the latter two in a 6.7:1 v/v ratio, under argon conditions at room temperature. The mixture was stirred for 8 h at 80°C, and then $Hf(OTf)_4$ (47.78mg) was added again. The reaction mixture was left to stir at 80°C and after 24 hours no product formation could be detected after various TLC analyses.

5.3 MICROWAVE REACTIONS

5.3.1 REACTION BETWEEN 1-NAPHTHOL AND ACETIC ACID

A mixture of 1-naphthol **93** (100mg, 0.7mmol), $BF_3 \cdot (C_2H_5)_2O$ (58mg, 0.41mmol) and acetic acid (72mg, 1.2mmol), reacted together under domestic microwave irradiation (650W), without any solvent for 2 minutes. After cooling to room temperature, the reaction mixture was dissolved in EtOAc (10ml) and H_2O (about 20ml). After a

water-EtOAc liquid-liquid extraction (3 x 25ml), the organic phases were combined and washed with aqueous $NaHCO_3$ (20ml), dried with Na_2SO_4, filtered and the EtOAc evaporated under reduced pressure (35°C) to give a crude product. PLC separation afforded 2 bands: R_f 0.55 (T:A:drops of M = 9:1, v/v; unreacted **93**, 240mg, 18.5%) and R_f 0.78 **(94)**.

5.3.1.1 1-Hydroxy-2-acetonaphthone (94)

The fraction with R_f 0.78 afforded a *light yellow amorphous solid* (1.04g, 80%).

Physical data *cf*: paragraph 5.2.2.1

5.3.2 REACTION BETWEEN PHLOROGLUCINOL AND ACETIC ACID

A mixture of phloroglucinol **16** (162mg, 1mmol), $BF_3 \cdot (C_2H_5)_2O$ (58mg, 0.41mmol) and acetic acid (72mg, 1.2mmol), reacted together under domestic microwave irradiation (650W), without any solvent for 2 minutes. After cooling to room temperature, the reaction mixture was dissolved in EtOAc (10ml) and H_2O (about 20ml). After a water-EtOAc liquid-liquid extraction (3 x 25ml), the organic phases were combined and washed with aqueous $NaHCO_3$ (20ml), dried with Na_2SO_4, filtered and the EtOAc evaporated under reduced pressure (35°C) to give a crude product. PLC separation afforded 3 bands: R_f 0.52 **(97)** (T:A:M = 7:2:1, v/v), R_f 0.63 (unreacted acetic acid, 27.9mg, 15%) and R_f 0.76 (unreacted **16**, 148.8mg, 80%).

5.3.2.1 2,4,6-Trihydroxyacetophenone (97)

The fraction with R_f 0.52 afforded a *yellow amorphous solid* (37.2mg, 20%).

^{1}H NMR 300 MHz [Acetone-d_6, Plate 3]: δ 5.93 (br. s, H-3 and H-5), δ 2.61 (br. s, -CH_3).

5.3.3 REACTION BETWEEN RESORCINOL AND 3-PHENYL-PROPIONIC ACID, 3-(4-HYDROXYPHENYL)-PROPIONIC ACID AND 3,4-DIHYDROXYHYDROCINNAMIC ACID WITH $BF_3 \cdot (C_2H_5)_2O$ AS CATALYST

General procedure under optimized power mode reaction conditions with a microwave reactor: A mixture of resorcinol **14** (50mg, 1mmol), $BF_3 \cdot (C_2H_5)_2O$ (0.098ml, 0.41mmol) and the carboxylic acid (1.2mmol), reacted together under microwave irradiation (100W), without any solvent for a short time. After cooling to room temperature, the reaction mixture was dissolved in EtOAc (10ml) and H_2O (*ca*. 20ml). After a water-EtOAc liquid-liquid extraction (3 x 25ml), the organic phases were combined and washed with aqueous $NaHCO_3$ (20ml), dried with Na_2SO_4, filtered and the EtOAc evaporated under reduced pressure (35°C) to give a crude product. PLC separation afforded the *C*-acylation products (T:A:M = 7:2:1, v/v).

5.3.3.1 2',4'-Dihydroxy dihydrochalcone (100)

The fraction with R_f 0.70 afforded a *yellow amorphous solid* (0.1399g, 81.7%).

^{1}H NMR 300 MHz [Acetone-d_6, Plate 4]: δ 7.83 (d, *J* 8.84 Hz, H-6'), δ 7.3 – 7.2 (H-2-6, A-ring aromatic protons), δ 6.44 (dd, *J* 8.81, 2.39 Hz, H-5'), δ 6.36 (d, *J* 2.38 Hz, H-3'), δ 3.31 (t, *J* 7.63 Hz, α-H), δ 3.02 (t, *J* 7.61 Hz, β –H).

5.3.3.2 4,2',4'-Trihydroxy dihydrochalcone (102)

The fraction with R_f 0.45 afforded a *yellow amorphous solid* (836mg, 70%).

^{1}H NMR 300 MHz [Acetone-d_6, Plate 5]: δ 7.85 (d, *J* 8.86 Hz, H-6'), δ 7.21 (d, *J* 8.50 Hz, H-2 and H-6), δ 6.84 (d, *J* 8.62 Hz, H-3 and H-5), δ 6.43 (dd, *J* 11.11, 2.26 Hz, H-5'), δ 6.34 (d, *J* 2.34 Hz, H-3'), δ 3.28 (t, *J* 7.62 Hz, α-H), δ 2.96 (t, β –H).

5.3.3.3 3,4,2',4'-Tetrahydroxy dihydrochalcone (104)

HPLC analysis afforded the product **104** (area % = 38.87 %).

^{1}H NMR 600 MHz [Acetone-d_6, Plate 6]: δ 7.84 (d, *J* 8.84 Hz, H-6'), δ 6.78 (d, *J* 1.99 Hz, H-2), δ 6.74 (d, *J* 8.02 Hz, H-5), δ 6.62 (dd, *J* 8.02, 2.03 Hz, H-6), δ 6.44 (dd, *J* 8.83, 2.39 Hz, H-5'), δ 6.34 (d, *J* 2.38 Hz, H-3'), δ 3.24 (t, *J* 7.64 Hz, α-H), δ 2.88 (t, *J* 7.49 Hz, β –H).

HPLC: Profile 1

5.3.4 REACTION BETWEEN PHLOROGLUCINOL AND 3-PHENYL-PROPIONIC ACID, 3-(4-HYDROXYPHENYL)-PROPIONIC ACID AND 3,4-DIHYDROXYHYDROCINNAMIC ACID WITH $BF_3 \cdot (C_2H_5)_2O$ AS CATALYST

General procedure under optimized power mode reaction conditions with a microwave reactor: A mixture of phloroglucinol **16** (50mg, 1mmol), $BF_3 \cdot (C_2H_5)_2O$ (0.066ml, 0.41mmol) and the carboxylic acid (1.2mmol), reacted together under microwave irradiation (100W), without any solvent for a short time. After cooling to room temperature, the reaction mixture was dissolved in EtOAc (10ml) and H_2O (*ca*. 20ml). After a water-EtOAc liquid-liquid extraction (3 x 25ml), the organic phases were combined and washed with aqueous $NaHCO_3$ (20ml), dried with Na_2SO_4, filtered and the EtOAc evaporated under reduced pressure (35°C) to give a crude product. PLC separation afforded the *C*-acylation products (T:A:M = 7:2:1, v/v).

5.3.4.1 2',4',6'-Trihydroxy dihydrochalcone (101)

The fraction with R_f 0.42 afforded a *yellow amorphous solid* (42mg, 30.2%).

^{1}H NMR 300 MHz [Acetone-d_6, Plate 7]: δ 7.28 – 7.17 (H-2-6, A-ring aromatic protons), δ 5.98 (br. s, H-3' and H-5'), δ 3.42 (t, α-H), δ 2.99 (t, β –H).

5.3.4.2 4,2',4',6'-Tetrahydroxy dihydrochalcone (103)

The fraction with R_f 0.65 (T:A:M = 6:3:1) afforded a *yellow amorphous solid* (50mg, 29.8%).

^{1}H NMR 300 MHz [Acetone-d_6, Plate 8]: δ 7.11 (d, *J* 8.53 Hz, H-2 and H-6), δ 6.76 (d, *J* 8.51 Hz, H-3 and H-5), δ 5.95 (br. s, H-3' and H-5'), δ 3.35 (t, α-H), δ 2.89 (t, β –H).

5.3.4.3 3,4,2',4',6'-Pentahydroxy dihydrochalcone (88)

HPLC analysis afforded the product **88** (area % = 4.56 %).

^{1}H NMR 600 MHz [Acetone-d_6, Plate 9]: δ 6.78 (d, *J* 2.09 Hz, H-2), δ 6.73 (d, *J* 7.99 Hz, H-5), δ 6.60 (dd, *J* 8.02, 2.05 Hz, H-6), δ 5.94 (s, H-3' and H-5'), δ 3.33 (t, α-H), δ 2.84 (t, β –H).

HPLC: Profile 2

5.3.5 REACTION BETWEEN RESORCINOL AND 3-PHENYL-PROPIONIC ACID, 3-(4-HYDROXYPHENYL)-PROPIONIC ACID AND 3,4-DIHYDROXYHYDROCINNAMIC ACID WITH $Hf(OTf)_4$ AS CATALYST

General procedure under optimized standard mode reaction conditions with a microwave reactor: A mixture of resorcinol **14** (50mg, 1mmol), $Hf(OTf)_4$ (703.5mg, 20mol %) and the carboxylic acid (1.2mmol), reacted together under microwave

irradiation, with toluene:CH_3NO_2 (0.67:1, v/v) as solvent for a short time. After cooling to room temperature, the reaction mixture was extracted with EtOAc (3 x 20ml). After a water-EtOAc liquid-liquid extraction, the organic phases were combined, dried with Na_2SO_4, filtered and the EtOAc evaporated under reduced pressure (35°C) to give a crude product. PLC separation afforded the *C*-acylation products (T:A:M = 7:2:1, v/v).

5.3.5.1 2',4'-Dihydroxy dihydrochalcone (100)

The fraction with R_f 0.70 afforded a *yellow amorphous solid* (494mg, 41.4%).

Physical data *cf*: paragraph 5.3.3.1

5.3.5.2 4,2',4'-Trihydroxy dihydrochalcone (102)

The fraction with R_f 0.45 afforded a *yellow amorphous solid* (614mg, 50.1%).

Physical data *cf*: paragraph 5.3.3.2

5.3.5.3 3,4,2',4'-Tetrahydroxy dihydrochalcone (104)

HPLC analysis afforded the product **104** (area % = 14.58 %).

Physical data *cf*: paragraph 5.3.3.3

HPLC: Profile 3

5.3.6 REACTION BETWEEN PHLOROGLUCINOL AND 3-PHENYL-PROPIONIC ACID, 3-(4-HYDROXYPHENYL)-PROPIONIC ACID AND 3,4-DIHYDROXYHYDROCINNAMIC ACID WITH $Hf(OTf)_4$ AS CATALYST

General procedure under optimized standard mode reaction conditions with a microwave reactor: A mixture of phloroglucinol **16** (50mg, 1mmol), $Hf(OTf)_4$ (47.8mg, 20mol %) and the carboxylic acid (1.2mmol), reacted together under microwave irradiation, with toluene:CH_3NO_2 (0.67:1, v/v) as solvent for a short time. After cooling to room temperature, the reaction mixture was extracted with EtOAc (3 x 20ml). After a water-EtOAc liquid-liquid extraction, the organic phases were combined, dried with Na_2SO_4, filtered and the EtOAc evaporated under reduced pressure (35°C) to give a crude product. PLC separation afforded the *C*-acylation products (T:A:M = 7:2:1, v/v).

5.3.6.1 2',4',6'-Trihydroxy dihydrochalcone (101)

No *C*-acylation was observed, only polymerization.

5.3.6.2 4,2',4',6'-Tetrahydroxy dihydrochalcone (103)

The fraction with R_f 0.65 (T:A:M = 6:3:1) afforded a *yellow amorphous solid* (26mg, 15.9%).

Physical data *cf*: paragraph 5.3.4.2

5.3.6.3 3,4,2',4',6'-Pentahydroxy dihydrochalcone (88)

HPLC analysis afforded the product **88** (area % = 7.62 %).

Physical data *cf*: paragraph 5.3.4.3

HPLC: Profile 4

5.3.7 REACTION BETWEEN RESORCINOL AND 3-PHENYLPROPIONIC ACID

A mixture of resorcinol **14** (50mg, 1mmol), $Hf(OTf)_4$ (70.4mg, 20mol %) and 3-phenylpropionic acid **98** (81.8mg, 1.2mmol), reacted together under microwave irradiation (200W), with toluene:CH_3NO_2 (0.67:1, v/v) as solvent for 2 minutes. After cooling to room temperature, the reaction mixture was extracted with EtOAc (3 x 20ml). After a water-EtOAc liquid-liquid extraction, the organic phases were combined, dried with Na_2SO_4, filtered and the EtOAc evaporated under reduced pressure (35°C) to give a crude product. PLC separation afforded one product (T:A:M = 7:2:1, v/v), but the ^{1}H NMR data was suggestive of a combination of two products. Acetylation of the crude product afforded the *O*- and *C*-acylation products.

5.3.7.1 3'-*O*-Acetyl-phenyl 3-phenyl-propanoate (105)

The fraction with R_f 0.92 afforded a *yellow amorphous solid* (23.3mg, 21.9%).

^{1}H NMR 300 MHz [$CDCl_3$, Plate 10a]: δ 7.35 – 7.28 (H-2-6, aromatic protons from carboxylic moiety), δ 6.99, δ 6.93 and δ 6.87 (ddd, *J* 8.20, 2.15, 0.80 Hz; ddd, *J* 8.14, 2.13, 0.79 Hz; t, *J* 2.17 Hz; H-2',4',5',6'), δ 3.09 (t, *J* 7.60 Hz, α-H), δ 2.90 (t, *J* 7.77 Hz, β-H), δ 2.31 (br. s, -OAc).

5.3.7.2 3'-*O*-Hydroxy-phenyl 3-phenyl-propanoate (106)

De-acetylation of 3'-*O*-acetyl-phenyl 3-phenyl-propanoate **105** afforded **106** (14.6%).

^{1}H NMR 300 MHz [Acetone-d_6, Plate 10b]: δ 7.33 – 7.23 (H-2-6, aromatic protons from carboxylic moiety), δ 7.19 (t, *J* 7.92 Hz, H-2'), δ 6.72 (ddd, *J* 8.21, 2.33, 0.91 Hz, H-6'), δ 6.54 (m, H-4' and H-5'), δ 3.04 (t, *J* 7.19 Hz, α-H), δ 2.90 (t, β-H).

5.3.7.3 2',4'-Di-acetoxy dihydrochalcone (108)

The fraction with R_f 0.86 afforded a *yellow amorphous solid* (67.1mg, 63%).

^{1}H NMR 300 MHz [$CDCl_3$, Plate 11]: δ 7.80 (d, *J* 8.58 Hz, H-6'), δ 7.30 (H-2-6, A-ring aromatic protons), δ 7.10 (dd, *J* 8.59, 2.27 Hz, H-5'), δ 6.98 (d, *J* 2.25 Hz, H-3'), δ 3.22 (t, *J* 7.99 Hz, α-H), δ 3.03 (t, *J* 7.75 Hz, β-H), δ 2.33 (br. s, -OAc).

5.3.7.4 2',4'-Dihydroxy dihydrochalcone (100)

De-acetylation of 2',4'-di-acetoxy dihydrochalcone **108** afforded **100** (37.2%).

Physical data *cf*: paragraph 5.3.3.1

5.3.8 REACTION BETWEEN PHLOROGLUCINOL AND BENZOYL CHLORIDE

A mixture of phloroglucinol **16** (162mg, 1mmol), $BF_3{\cdot}(C_2H_5)_2O$ (58mg, 0.41mmol) and benzoyl chloride **110** (169mg, 1.2mmol), reacted together under domestic microwave irradiation (650W), without any solvent for 2 minutes. After cooling to room temperature, the reaction mixture was dissolved in EtOAc (10ml) and H_2O (*ca*. 20ml). After a water-EtOAc liquid-liquid extraction (3 x 25ml), the organic phases were combined and washed with aqueous $NaHCO_3$ (20ml), dried with Na_2SO_4, filtered and the EtOAc evaporated under reduced pressure (35°C) to give a crude product. PLC separation afforded 3 bands: R_f 0.37 **(111)** (T:EtOAc:M = 7:2:1, v/v), R_f 0.57 (unreacted **16**, 96mg, 30%) and R_f 0.78 (unreacted **110**, 45mg, 14.1%).

5.3.8.1 3',5'-Dihydroxy-phenyl 1'-benzoate (111)

The fraction with R_f 0.37 afforded a *yellow amorphous solid* (170mg, 53.1%).

^{1}H NMR 300 MHz [Acetone-d_6, Plate 12]: δ 8.16 (d, *J* 7.27 Hz, H-2 and H-6), δ 7.71 (t, *J* 7.40 Hz, H-4), δ 7.58 (t, *J* 7.59 Hz, H-3 and H-5), δ 6.36 (br. s, H-2', H-4' and H-6').

5.3.9 REACTION BETWEEN PHLOROGLUCINOL AND 3-PHENYLPROPIONIC ACID

A mixture of phloroglucinol **16** (30mg, 1mmol), $Hf(OTf)_4$ (48mg, 20mol %) and 3-phenyl-propionic acid **98** (33mg, 1.2mmol), reacted together under standard mode microwave irradiation, with toluene (1ml) and [bmim]PF_6 (15drops) as solvent at 15W for 58 min and 25W for 58min (temperature = 110°C). After cooling to room temperature, the reaction mixture was extracted with EtOAc (3 x 20ml). After a water-EtOAc liquid-liquid extraction, the organic phases were combined, dried with Na_2SO_4, filtered and the EtOAc evaporated under reduced pressure (35°C) to give a crude product. PLC separation afforded 3 bands: R_f 0.20 **(112)** (T:A:M = 6:3:1, v/v), R_f 0.34 (unreacted **16**, 49mg, 83.2%) and R_f 0.78 (unreacted **98**, 1.3mg, 2.2%).

5.3.9.1 3',5'-Dihydroxy-phenyl 3-phenyl-propanoate (112)

The fraction with R_f 0.20 afforded a *yellow amorphous solid* (8.6mg, 14.6%).

^{1}H NMR 300 MHz [Acetone-d_6, Plate 13]: δ 7.2 (H-2-6, aromatic protons from carboxylic moiety), δ 6.03 (s, H-4'), δ 5.9 and δ 5.7 (s, H-2' or H-6'), δ 2.78 (t, *J* 7.77 Hz, α-H), δ 2.48 (t, *J* 7.64 Hz, β-H).

5.4 CONVENTIONAL HEATING

5.4.1 REACTION BETWEEN PHLOROGLUCINOL AND HYDROCINNAMONITRILE

Into a mixture of dry phloroglucinol **16** (500mg, 3.08mmol), hydrocinnamonitrile **113** (508mg, 3.88mmol), and $ZnCl_2$ (154mg, 1.129mmol) in dry Et_2O (10ml), was passed dry HCl gas for 3 h, while the reaction mixture was under vigorous stirring and cooled in an ice-methanol bath (-10 to -20°C). The reaction mixture was allowed to stand overnight in a freezer, and again dry HCl gas was passed through it for 3 h. After further standing in a freezer, the Et_2O was decanted and the residual brown viscous syrup was hydrolyzed by refluxing in H_2O (15ml) for 2h. The yellow solid was collected by filtration. A water-EtOAc liquid-liquid extraction (3 x 25ml) was performed. The EtOAc extracts were combined, washed with saturated NaCl solution (1 x 15ml), dried (Na_2SO_4), filtered and the EtOAc evaporated under reduced pressure (35°C). PLC separation afforded 3 bands: R_f 0.48 (T:A:M = 6:3:1, v/v; unreacted **16**, 140.4mg, 22.7%), R_f 0.64 **(101)** and R_f 0.93 (unreacted **113**, trace amount).

5.4.1.1 2',4',6'-Trihydroxy dihydrochalcone (101)

The fraction with R_f 0.64 afforded a *yellow amorphous solid* (477.5mg, 72.5%).

Physical data *cf*: paragraph 5.3.4.1

5.4.2 REACTION BETWEEN PHLOROGLUCINOL AND HYDROCINNAMONITRILE – HOESCH REACTION

Phloroglucinol **16** (500mg, 3.084mmol), hydrocinnamonitrile **113** (809mg, 6.168mmol), dry Et_2O (1.5ml) and $ZnCl_2$ (100mg, 0.7337mmol) was placed in a 50ml

flask fitted with a wide gas inlet tube. The side-arm was protected with a $CaCl_2$ guard tube. The flask was cooled in an ice-salt mixture in the fume cupboard and a rapid stream of dry HCl was passed through the solution for 2 h while stirring with a magnetic stirrer. The reaction mixture was allowed to stand overnight in a freezer, and again dry HCl gas was passed through it for 2 h. After further standing in a freezer for 3 days, the Et_2O was decanted and the yellow solid was washed with EtOAc (3 x 25ml). The EtOAc extracts were combined, washed with saturated NaCl solution (1 x 15ml), dried (Na_2SO_4), filtered and the EtOAc evaporated under reduced pressure (35°C). PLC separation afforded 3 bands: R_f 0.08 **(116)** (T:A:M = 6:3:1, v/v), R_f 0.46 (unreacted **16**, 318.6mg, 40.1%) and R_f 0.75 (unreacted **113**, trace amount).

5.4.2.1 3',5'-Dihydroxy-phenyl-4'-propionic acid 3-phenyl-propanoate (116)

The fraction with R_f 0.08 afforded a *yellow amorphous solid* (112.8mg, 14.2%).

^{1}H NMR 300 MHz [Acetone-d_6, Plate 14]: δ 7.1-7.3 (10 x Aromatic-H), δ 5.95 (br. s, H-2' and H-6'), δ 3.40 (t, α-H'), δ 2.98 (t, β-H'), δ 2.92 (t, α-H), δ 2.62 (t, β-H).

5.4.3 REACTION BETWEEN RESORCINOL AND 3-PHENYLPROPIONIC ACID

To $Hf(OTf)_4$ (68mg, 20 mol%) was added resorcinol **14** (48.3mg, 04388mmol) and 3-phenylpropionic acid **98** (79.1mg, 0.5266mmol) in toluene (2ml) and nitromethane (0.30ml). The substrates were dissolved with the use of an ultrasonic bath. The reaction mixture was refluxed at a temperature starting at 40°C which was increased over a time period of *ca*. 10 h to 85°C. The reaction mixture was left to reflux at 95°C overnight, after which a water-EtOAc liquid-liquid extraction (3 x 20ml) was performed. The EtOAc extracts were combined, washed with saturated NaCl solution

(1 x 10ml), dried (Na_2SO_4), filtered and the EtOAc evaporated under reduced pressure (35°C). PLC separation afforded 3 bands: R_f 0.42 (T:A:M = 7:2:1, v/v; unreacted **14**, 23.3mg, 21.9%), R_f 0.47 (unreacted **98**, trace amount) and R_f 0.65 **(100)**.

5.4.3.1 2',4'-Dihydroxy dihydrochalcone (100)

The fraction with R_f 0.65 afforded a *yellow amorphous solid* (67.1mg, 63%).

Physical data *cf*: paragraph 5.3.3.1

5.4.4 REACTION BETWEEN PHLOROGLUCINOL AND 3-PHENYLPROPIONIC ACID

To $Hf(OTf)_4$ (35.2mg, 20 mol%) was added phloroglucinol **16** (36.8mg, 0.2272mmol) and 3-phenylpropionic acid **98** (40.9mg, 0.2726mmol) in toluene (2ml) and nitromethane (0.30ml). The substrates were dissolved with the use of an ultrasonic bath. The reaction mixture was refluxed at a temperature starting at 40°C which was increased over a time period of *ca*. 10 h to 85°C. The reaction mixture was left to reflux at 95°C overnight, after which water-EtOAc liquid-liquid extraction (3 x 20ml) was performed. The EtOAc extracts were combined, washed with saturated NaCl solution (1 x 10ml), dried (Na_2SO_4), filtered and the EtOAc evaporated under reduced pressure (35°C). PLC separation afforded 3 bands: R_f 0.35 **(112)** (T:A:M = 7:2:1, v/v), R_f 0.46 (unreacted **16**, 9.4mg, 16%) and R_f 0.56 (unreacted **98**, trace amount).

5.4.4.1 3',5'-Dihydroxy-phenyl 3-phenyl-propanoate (112)

The fraction with R_f 0.35 afforded a *yellow amorphous solid* (18.4mg, 31.4%).

Physical data *cf*: paragraph 5.3.9.1

5.4.5 REACTION BETWEEN 1,3,5-TRIMETHOXYBENZENE AND 3-PHENYLPROPIONIC ACID

To $Hf(OTf)_4$ (46.1mg, 20 mol%) was added 1,3,5-trimethoxybenzene **117** (50mg, 0.2973mmol) and 3-phenylpropionic acid **98** (53.6mg, 0.3567mmol) in toluene (2ml) and nitromethane (0.30ml). The substrates were dissolved with the use of an ultrasonic bath. The reaction mixture was refluxed at 65°C for 3 h. The temperature was increased and the reaction mixture was left to reflux for another 3 h at 95°C, after which water-EtOAc liquid-liquid extraction (3 x 20ml) was performed. The EtOAc extracts were combined, washed with saturated NaCl solution (1 x 10ml), dried (Na_2SO_4), filtered and the EtOAc evaporated under reduced pressure (35°C). PLC separation afforded 3 bands: R_f 0.37 (H:EtOAc = 7.5:2.5, v/v; unreacted **98**, trace amount), R_f 0.70 (unreacted **117**, 66.3mg, 65%) and R_f 0.91 **(118)**.

5.4.5.1 1,3,5-Trimethoxy dihydrochalcone (118)

The fraction with R_f 0.91 afforded a *yellow amorphous solid* (20.9mg, 20.5%).

^{1}H NMR data: The data is completely consistent with those reported by Batt *et al.*[123]

5.4.6 REACTION BETWEEN PHLOROGLUCINOL AND 3-(4-HYDROXYPHENYL)-PROPIONIC ACID

A mixture of phloroglucinol **16** (48.6mg, 0.3mmol), 3-(4-hydroxyphenyl)-propionic acid **99** (49.9mg, 0.3mmol) and $BF_3 \cdot (C_2H_5)_2O$ (0.19ml, 1.53mmol) was stirred at 80-90°C for 90 min under N_2. The reaction mixture was poured into 10% aqueous NaOAc solution (10ml) and allowed to stand for 4 h and the solution was extracted with water-EtOAc liquid-liquid (3 x 20ml). The EtOAc extracts were combined, washed with saturated NaCl solution (1 x 10ml), dried (Na_2SO_4), filtered and the

EtOAc evaporated under reduced pressure (35°C). PLC separation afforded 3 bands: R_f 0.37 (T:A:M = 6:3:1, v/v; unreacted **16**, 23.9mg, 47.7%), R_f 0.41 **(103)** and R_f 0.47 (unreacted **99**, trace amount).

5.4.6.1 4,2',4',6'-Tetrahydroxy dihydrochalcone (103)

The fraction with R_f 0.41 afforded a *yellow amorphous solid* (18.1mg, 36.1%).

Physical data *cf*: paragraph 5.3.4.2

5.4.7 REACTION BETWEEN PHLOROGLUCINOL AND 3,4-DIHYDROXYHYDROCINNAMIC ACID

A mixture of phloroglucinol **16** (48.6mg, 0.3mmol), 3,4-dihydroxyhydrocinnamic acid **89** (54.7mg, 0.3mmol) and $BF_3 \cdot (C_2H_5)_2O$ (0.19ml, 1.53mmol) was stirred at 80-90°C for 90 min under N_2. The reaction mixture was poured into 10% aqueous NaOAc solution (10ml) and allowed to stand for 4 h, whereafter the solution was extracted with water-EtOAc liquid-liquid (3 x 20ml). The EtOAc extracts were combined, washed with saturated NaCl solution (1 x 10ml), dried (Na_2SO_4), filtered and the EtOAc evaporated under reduced pressure (35°C). PLC separation afforded 3 main bands: R_f 0.37 (T:A:M = 5:4:1, v/v; unreacted **16**, 38.0mg, 38.8%), R_f 0.44 **(88)** and R_f 0.51 (unreacted **89**, 44.6mg, 45.6%).

5.4.7.1 3,4,2',4',6'-Pentahydroxy dihydrochalcone (88)

Sephadex LH 20 separation afforded the fraction with R_f 0.44, a *light yellow amorphous solid* (7.73mg, 7.9%).

Physical data *cf*: paragraph 5.3.4.3

5.4.8 OPTIMIZED REACTION CONDITIONS FOR THE REACTION BETWEEN PHLOROGLUCINOL AND 3,4-DIHYDROXYHYDROCINNAMIC ACID

$BF_3 \cdot (C_2H_5)_2O$ (0.19ml, 1.53mmol) was added to 3,4-dihydroxyhydrocinnamic acid **89** (56.2mg, 0.3083mmol) at 0°C while stirring. The temperature of the reaction mixture was readily increased to 80°C and phloroglucinol **16** (50mg, 0.3083mmol) was added to the mixture. The mixture was refluxed at 80-90°C for 90 min under N_2. The reaction mixture was poured into 10% aqueous NaOAc solution (10ml) and allowed to stand for 4 h whereafter the solution was extracted with water-EtOAc liquid-liquid (3 x 20ml). The EtOAc extracts were combined, washed with saturated NaCl solution (1 x 10ml), dried (Na_2SO_4), filtered and the EtOAc evaporated under reduced pressure (35°C). PLC separation afforded 3 bands: R_f 0.37 **(119)** (T:A:M = 6:3:1, v/v), R_f 0.48 (unreacted **16**, 47.2mg, 45.1%) and R_f 0.52 **(88)**.

5.4.8.1 3,5-Dihydroxy-phenyl-2',4',6'-trihydroxy-phenylether (119)

The fraction with R_f 0.37 afforded a *light pink amorphous solid* (36.4mg, 34.8%).

^{1}H NMR 600 MHz [Acetone-d_6, Plate 15a]: δ 6.35 (d, *J* 2.24 Hz, H-2' and H-6'), δ 6.28 (t, *J* 2.24 Hz, H-4'), δ 6.02 (br. s, H-3 and H-5).

^{13}C NMR 600 MHz [Acetone-d_6, Plate 15b]: 3 x Ar-C-OH (135.60, 155.05, 157.60), C-4 (158.80), C-2' and C-6' (109.80), C-4' (101.20), C-3, C-5 and Ar-C-OH (94.80).

HSQC spectrum 600 MHz [Acetone-d_6, Plate 15c]

HMBC spectrum 600 MHz [Acetone-d_6, Plate 15d]

5.4.8.2 3,5-Dimethoxy-phenyl-2',4',6'-trimethoxy-phenylether (120)

Methylation of 2,4,6-trihydroxy-3',5'-dihydroxy-phenylether (54.2mg) afforded the pentamethylether (R_f 0.83, T:A:M = 7:2:1, v/v) as *light yellow amorphous solid* (35.5mg, 97.4%).

^{1}H NMR 600 MHz [Acetone-d_6, Plate 16]: δ 6.48 (d, *J* 2.28 Hz, H-2' and H-6'), δ 6.41 (t, *J* 2.32 Hz, H-4'), δ 6.21 (br. s, H-3 and H-5), δ 3.86 (s, -OMe), δ 3.77 (s, -OMe), 3.72 (s, -OMe).

5.4.8.3 3,4,2',4',6'-Pentahydroxy dihydrochalcone (88)

Sephadex LH 20 separation afforded the fraction with R_f 0.52, a *light yellow amorphous solid* (21mg, 20%).

Physical data *cf*: paragraph 5.3.4.3

5.4.9 REACTION BETWEEN PHLOROGLUCINOL AND 3,4-DIHYDROXYHYDROCINNAMIC ACID WITH BHT ACTING AS RADICAL TERMINATOR

To a suspension of phloroglucinol **16** (50mg, 0.3083mmol), 3,4-dihydroxyhydrocinnamic acid **89** (28.1mg, 0.1542mmol) and BHT (13.5mg, 0.0832mmol), $BF_3 \cdot (C_2H_5)_2O$ (0.59ml, 0.4624mmol) was added at 0°C. The reaction mixture was stirred for 90 min and then the temperature was readily increased to 45°C and the reaction mixture left to reflux for 5 h. The reaction mixture was poured into 10% aqueous NaOAc solution (10ml) and allowed to stand for 4 h, whereafter the solution was extracted with water-EtOAc liquid-liquid (3 x 20ml). The EtOAc extracts were combined, washed with saturated NaCl solution (1 x 10ml), dried (Na_2SO_4), filtered and the EtOAc evaporated under reduced pressure (35°C). PLC

separation afforded 3 bands: R_f 0.39 **(119)** (T:A:M = 6:3:1, v/v), R_f 0.49 (unreacted **16**, 46mg, 28.1%) and R_f 0.51 **(88)**.

5.4.9.1 3,5-Dihydroxy-phenyl-2',4',6'-trihydroxy-phenylether (119)

The fraction with R_f 0.39 afforded a *light pink amorphous solid* (65.7mg, 40.1%), with a trace amount of **121**

Physical data *cf*: paragraph 5.4.8.1

5.4.9.2 Enol Tautomer of Aspalalinin (121)

^{1}H NMR 300 MHz [Acetone-d_6, 121]: δ 6.42 (br. s, H-3' and H-5'), δ 6.17 (br. s, H-3 and H-6), δ 3.68 (m, α-H), δ 3.44 (t, β-H).

5.4.9.3 3,4,2',4',6'-Pentahydroxy dihydrochalcone (88)

Sephadex LH 20 afforded the fraction with R_f 0.51, a *light yellow amorphous solid* (28mg, 17.1%).

Physical data *cf*: paragraph 5.3.4.3

5.4.9.4 INFLUENCE OF BORON TRIFLUORIDE COORDINATION

A mixture of phloroglucinol **16** (50mg) and dry ether (1ml) was refluxed for 5 h at 40°C. After water-EtOAc liquid-liquid extraction (3 x 20ml), the crude product was dried (Na_2SO_4), filtered and the EtOAc evaporated under reduced pressure (35°C).

No formation of 3,5-dihydroxy-phenyl-2',4',6'-trihydroxy-phenylether **119** could be detected *via* TLC analysis.

Phloroglucinol **16** (50mg, 0.3083mmol), dry ether (1ml) and $BF_3 \cdot (C_2H_5)_2O$ (0.59ml, 0.4624mmol) were added together at 0°C and then the temperature was increased and the reaction mixture refluxed at 40°C for 5 h. After water-EtOAc liquid-liquid extraction (3 x 20ml), the crude product was dried (Na_2SO_4), filtered and the EtOAc evaporated under reduced pressure (35°C). TLC analysis indicated the formation of 3,5-dihydroxy-phenyl-2',4',6'-trihydroxy-phenylether **119**.

5.5 ACYLATION OF PROTECTED PHENOLIC SUBSTRATES

5.5.1 REACTION BETWEEN PHLOROGLUCINOL AND 3-(3,4-DIMETHOXYPHENYL)-PROPIONIC ACID WITH $Hf(OTf)_4$ AS CATALYST

A mixture of phloroglucinol **16** (50mg, 0.3083mmol) and 3-(3,4-dimethoxyphenyl)-propionic acid **122** (64.8mg, 0.3083mmol) and $Hf(OTf)_4$ (47.7mg, 0.0616mmol) was refluxed for 6 h at 80-90°C. After 90 min no product formation could be detected after various TLC analyses. The reaction mixture was left to reflux for the remainder of the time, but no product formation could be detected after a prolonged time. The formation of **119** was detected with TLC analysis.

5.5.2 REACTION BETWEEN PHLOROGLUCINOL AND 3-(3,4-DIMETHOXYPHENYL)-PROPIONIC ACID WITH $BF_3 \cdot (C_2H_5)_2O$ AS CATALYST

To the mixture of phloroglucinol **16** (50mg, 0.3083mmol) and 3-(3,4-dimethoxyphenyl)-propionic acid **122** (64.8mg, 0.3083mmol), $BF_3 \cdot (C_2H_5)_2O$ (0.19ml, 1.53mmol) was added while stirring and the reaction mixture was refluxed for 90 min

at 80-90°C. The reaction mixture was poured into 10% aqueous NaOAc solution (10ml) and allowed to stand for 4 h, whereafter the solution was extracted with water-EtOAc liquid-liquid (3 x 20ml). The EtOAc extracts were combined, washed with saturated NaCl solution (1 x 10ml), dried (Na_2SO_4), filtered and the EtOAc evaporated under reduced pressure (35°C). PLC separation afforded 5 bands: R_f 0.32 **(119)** (T:A:M = 6:3:1, v/v), R_f 0.50 **(124)**, R_f 0.56 **(125)**, R_f 0.58 (unreacted **16**, 6mg, 0.5%) and R_f 0.68 (unreacted **122**, 14.3mg, 12.5%).

5.5.2.1 3,5-Dihydroxy-phenyl-2',4',6'-trihydroxy-phenylether (119)

The fraction with R_f 0.32 afforded a *light pink amorphous solid* (37.9mg, 33.2%).

Physical data *cf*: paragraph 5.4.8.1

5.5.2.2 3,4-Dimethoxy-2',4',6'-trihydroxy dihydrochalcone (124)

The fraction with R_f 0.50 afforded a *light yellow amorphous solid* (8mg, 7%).

^{1}H NMR 300 MHz [Acetone-d_6, Plate 17]: δ 6.91 (d, *J* 1.85 Hz, H-2), δ 6.85 (d, *J* 8.13 Hz, H-5), δ 6.79 (dd, *J* 8.13, 1.91 Hz, H-6), δ 5.95 (br. s, H-3' and H-5'), δ 3.79 (s, -OMe), δ 3.77 (s, -OMe), δ 3.38 (t, α-H), δ 2.93 (t, β-H).

5.5.2.3 6,7-Dimethoxy-indan-1-one (125)

The fraction with R_f 0.56 afforded a *light yellow amorphous solid* (71mg, 62.3%).

^{1}H NMR 300 MHz [$CDCl_3$, Plate 18]: δ 7.2 and δ 6.95 (s, H-4 or H-5), δ 3.01 (t, α-H), δ 2.73 (t, β-H).

5.5.3 REACTION BETWEEN 1,3,5-TRIMETHOXYBENZENE AND 3,4-DIHYDROXYHYDROCINNAMIC ACID

To the mixture of 1,3,5-trimethoxybenzene **117** (50mg, 0.297mmol) and 3,4-dihydroxyhydrocinnamic acid **89** (54.1mg, 0.297mmol), $BF_3 \cdot (C_2H_5)_2O$ (0.045ml, 0.357mmol) was added while stirring and the reaction mixture was refluxed for 90 min at 80-90°C. The reaction mixture was poured into 10% aqueous NaOAc solution (10ml) and allowed to stand for 4 h, whereafter the solution was extracted with water-EtOAc liquid-liquid (3 x 20ml). The EtOAc extracts were combined, washed with saturated NaCl solution (1 x 10ml), dried (Na_2SO_4), filtered and the EtOAc evaporated under reduced pressure (35°C). PLC separation afforded 4 bands: R_f 0.42 (T:A:M = 6:3:1, v/v; unreacted **89**, 21.2mg, 25%), R_f 0.44 **(128)**, R_f 0.50 **(126)** and R_f 0.86 (unreacted **117**, 12.7mg, 15%).

5.5.3.1 2',3,4-Trihydroxy-4',6'-dimethoxy dihydrochalcone (127)

The fraction with R_f 0.50 afforded a *light yellow amorphous solid* (4mg, 4.7%).

^{1}H NMR 300 MHz [$CDCl_3$, Plate 19]: δ 6.80 (m, H-2 and H-5), δ 6.69 (dd, *J* 8.13, 1.70 Hz, H-6), δ 6.09 and δ 5.94 (d, *J* 2.28 Hz, d, *J* 2.29 Hz, H-3' and H-5'), δ 3.86 (s, -OMe), δ 3.84 (s, -OMe), δ 3.28 (t, α-H), δ 2.90 (t, β-H).

5.5.3.2 5,6-Dihydroxy-indan-1-one (128)

The fraction with R_f 0.44 afforded a *light yellow amorphous solid* (39mg, 46%).

^{1}H NMR 300 MHz [$CDCl_3$, Plate 20]: δ 7.10 and δ 6.70 (s, H-4 or H-7), δ 2.80 (t, α-H), δ 2.64 (t, β-H).

5.5.4 REACTION BETWEEN 1,3,5-TRIMETHOXYBENZENE AND 3-(3,4-DIMETHOXYPHENYL)-PROPIONIC ACID

To the mixture of 1,3,5-trimethoxybenzene **117** (50mg, 0.297mmol) and 3-(3,4-dimethoxyphenyl)-propionic acid **122** (62.5mg, 0.297mmol), $BF_3 \cdot (C_2H_5)_2O$ (0.045ml, 0.357mmol) was added while stirring and the reaction mixture was refluxed for 90 min at 80-90°C. The reaction mixture was poured into 10% aqueous NaOAc solution (10ml) and allowed to stand for 4 h, whereafter the solution was extracted with water-EtOAc liquid-liquid (3 x 20ml). The EtOAc extracts were combined, washed with saturated NaCl solution (1 x 10ml), dried (Na_2SO_4), filtered and the EtOAc evaporated under reduced pressure (35°C). PLC separation afforded 4 bands: R_f 0.51 (T:A:M = 7:2:1, v/v; unreacted **117**, trace amount), R_f 0.62 **(125)**, R_f 0.75 **(129)** and R_f 0.85 (unreacted **122**, 51.7mg, 44.7%).

5.5.4.1 6,7-Dimethoxy-indan-1-one (125)

The fraction with R_f 0.62 afforded a *white amorphous solid* (14mg, 12.1%).

Physical data *cf*: paragraph 5.5.2.3

5.5.4.2 3,4,2',4',6'-Pentamethoxy dihydrochalcone (129)

The fraction with R_f 0.75 afforded a *white amorphous solid* (43mg, 37%).

^{1}H NMR 300 MHz [$CDCl_3$, Plate 21]: δ 6.80 (m, H-2 and H-5), δ 6.69 (dd, *J* 8.05, 2.01 Hz, H-6), δ 6.09 and δ 5.94 (d, *J* 2.38 Hz, d, *J* = 2.32 Hz, H-3' and H-5'), δ 3.86 (s, -OMe), δ 3.90 – 3.84 (s, 5 x -OMe), δ 3.28 (t, α-H), δ 2.91 (t, β-H).

5.6 C-GLYCOSYLATION

5.6.1 REACTION BETWEEN PHLOROGLUCINOL AND D-GLUCOSE

A mixture of phloroglucinol **16** (50mg, 0.3083mmol), D-glucose **51** (166.7mg, 0,9249mmol) and $Sc(OTf)_3$ (30.3mg, 0.0616mmol) were dissolved in EtOH (2ml)/H_2O (1ml) and refluxed for 9 h. The resulted mixture was dried in vacuo and separated by PLC to produce only the recovery of the starting materials.

5.6.2 REACTION BETWEEN CATECHOL AND D-GLUCOSE

A mixture of catechol **15** (50mg, 0.454mmol), D-glucose **51** (245mg, 1.362mmol) and $Sc(OTf)_3$ (44.6mg, 0.0907mmol) were dissolved in EtOH (2ml)/H_2O (1ml) and refluxed for 9 h. The resulted mixture was dried in vacuo and separated by PLC to produce only the recovery of the starting materials.

5.6.3 REACTION BETWEEN 2,4,6-TRIHYDROXYACETOPHENONE AND D-GLUCOSE

A mixture of 2,4,6-trihydroxyacetophenone **97** (10mg, 0.054mmol), D-glucose **51** (19mg, 0.107mmol) and $Sc(OTf)_3$ (5mg, 0.011mmol) were dissolved in EtOH (2ml)/H_2O (1ml) and refluxed for 9 h. The resulted mixture was dried in vacuo and separated by PLC to afford 2 bands: R_f 0.53 **(130)** (A:EtOAc:H:AcOH = 6:3:0.4:0.2, v/v), R_f 0.91 (unreacted **97**, 33.4mg, 32.9%).

5.6.3.1 2,4,6-Trihydroxyacetophenone 3-*C*-β-glucopyranoside **(130)**

The fraction with R_f 0.53 afforded a *white amorphous powder* (37mg, 36.5%).

^{1}H NMR 300 MHz [Acetone-d_6 + D_2O, Plate 22]: δ 5.80 (s, H-5), δ 4.84 (d, *J* 9.82 Hz, H-1'), δ 3.4-3.9 (m's, glucosyl-H's), δ 2.57 (s, -CH_3).

5.6.4 REACTION BETWEEN 3,4,2',4',6'-PENTAHYDROXY DIHYDROCHALCONE AND D-GLUCOSE

A mixture of 3,4,2',4',6'-pentahydroxy dihydrochalcone **88** (125mg, 0.4306mmol), D-glucose **51** (233mg, 1.29mmol) and $Sc(OTf)_3$ (42.4mg, 0.0861mmol) were dissolved in EtOH (3ml)/H_2O (1.5ml) and refluxed for 9 h. The resulted mixture was dried in vacuo and separated by PLC to afford 2 bands: R_f 0.06 (A:EtOAc:H:AcOH = 6:3:0.4:0.2, v/v), R_f 0.88 (unreacted **88**, 118mg, 76.6%).

5.6.4.1 1-*O*-β-D-Glucopyranoside α-D-glucopyranoside (131)

The fraction with R_f 0.06 afforded a *grey amorphous solid* (35.9mg, 23.3%).

^{1}H NMR 300 MHz [D_2O / DMSO (2.71 ppm), Plate 23]: δ 5.75 (d, *J* 3.47 Hz, equatorial-H), δ 5.15 (d, *J* 7.80 Hz, axial-H), δ 3.90 – 3.20 (glucosyl protons).

5.6.5 REACTION BETWEEN 3,4,2',4',6'-PENTAHYDROXY DIHYDROCHALCONE AND D-GLUCOSE

A mixture of 3,4,2',4',6'-pentahydroxy dihydrochalcone **88** (5mg, 0.0172mmol), D-glucose **51** (1.5mg, 0.0085mmol) and $Sc(OTf)_3$ (1.7mg, 0.0034mmol) were dissolved in EtOH (1ml)/H_2O (0.5ml) and refluxed at 80°C. After 4 and a half hours no product formation could be detected with various TLC analyses. D-glucose **51** (2mg) was added and the reaction mixture was refluxed at 90°C, being monitored in 1 h intervals. After 9 h, D-glucose **51** (2mg) and $Sc(OTf)_3$ (2mg) was added and left to reflux 90°C for 24 hours. The resulting mixture was dried in vacuo and TLC analysis afforded 2 bands: R_f 0.10 **(55)** (T:A:M = 6:3:1, v/v) and R_f 0.43 **(88)**.

5.6.5.1 Aspalathin (55)

HPLC analysis identified the fraction with R_f 0.10, **55** (area % = 8.6%, retention time = 0.7) as synthetic aspalathin **55**.

HPLC: Profile 5

5.6.5.2 3,4,2',4',6'-Pentahydroxy dihydrochalcone (88)

The fraction with R_f 0.43 afforded a *light yellow amorphous solid* (21.9mg, 91.4%).

Physical data *cf*: paragraph 5.3.6.3

5.6.6 REACTION BETWEEN 3,4,2',4',6'-PENTAHYDROXY DIHYDROCHALCONE AND D-GLUCOSE

A mixture of 3,4,2',4',6'-pentahydroxy dihydrochalcone **88** (9mg, 0.031mmol), D-glucose **51** (11mg, 0.062mmol) and $Sc(OTf)_3$ (3mg, 0.006mmol) were dissolved in EtOH (0.4ml)/H_2O (0.2ml) and refluxed at 50°C for 24 hours. The resulting mixture was dried in vacuo.

5.6.6.1 Aspalathin (55)

HPLC analysis afforded the product **55** (area % = 10.7%, retention time = 0.26).

^{1}H NMR 600 MHz [Acetone-d_6, Plate 24]: δ 6.76 (d, *J* 1.99 Hz, H-2), δ 6.73 (d, *J* 7.99 Hz, H-5), δ 6.60 (dd, *J* 8.03, 2.02 Hz, H-6), δ 5.94 (br. s, H-5'), δ 4.94 (d, *J* 9.76 Hz, H-1"), δ 3.87 (m, H-6"), δ 3.66 (t, *J* 9.35 Hz, H-2" and H-4"), δ 3.55 (t, *J* 8.96

Hz, H-3”), δ 3.50 (m, H-5”), δ 3.33 (dt, *J* 7.27, 7.14, 1.51 Hz, α-H), δ 2.83 (t, *J* 7.69 Hz, β-H).

HPLC: Profile 6

5.6.6.2 3,4,2’,4’,6’-Pentahydroxy dihydrochalcone (88)

HPLC analysis afforded the product **88** (area % = 27.4%, retention time = 0.75).

Physical data *cf*: paragraph 5.3.6.3

PHYSICAL DATA

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Plate 1 – 24

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

(HPLC)

Profile 1 – 6

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OPSOMMING

Die doel van hierdie studie was om ’n sintetiese roete vir die sintese van aspalatien te ontwikkel. Hierdie unieke molekule het ’n uitdaging na vore gebring met sy reaktiewe posisies van die koolstof en die suurstof, keto-enol tatomerism, en die merkwaardigste van almal, die 1,2-dihidroksiebenzeen ring (die katechol funksionalitiet, ’n sterk anti-oksidant moieteit).

Met asilerings soos Friedel-Craft en Fries herrangskikking wat bekend is vir hul sukses, is besluit op die aanvang van die konstruksie van die dihidrochalkoon, 3,4,2’,4’,6’-pentahidroksie dihidrochalkoon, via die toepaslike asileringsreaksie. Asilering van fenols kan òf via *C*-asilering (Friedel-Crafts reaksie) òf *O*-asilering (esterfisering) geskied. Die regioselektiwiteit word bepaal deur ’n konsep wat saam gevat word in ’n teorie, naamlik “hard and soft acids and bases” (HSAB). ‘n Nuwe groep Lewissure met ‘n water toleransie, naamlik lanthanied triflaat, asook $BF_3{\cdot}(C_2H_5)_2O$ het sukses as kataliste in *C*-asilering bewys.

Daar is gebruik gemaak van eenvoudige fenoliese substrate in die asileringsreaksies om te help met die ontwikkeling van ’n moontlike protokol. Hulle is gebruik in die sintese van 1-hidroksie-2-asetonaphtoon en 3-(3,4-dihidroksie-feniel)-1-(1-hidroksie-naphtaleen-2-yl)-propan-1-oon, maar het lae opbrengste gelewer. Ten spyte van verskeie eksperiment wat onder verskillende kondisies uitgevoer is en telkens ander model komponente gebruik is, kon die opbrengste nie verbeter word nie. Tydens hierdie reaksies het resorsinol die *O*-asilerings produk, 3’-*O*-hidroksie-feniel 3-feniel-propanoaat, en die *C*-asilerings produk, 2’,4’-dihidroksie dihidrochalkoon geproduseer, maar floroglusinol het slegs die *O*-asilerende produk, 3’,5’-dihidroksie-feniel 3-feniel-propanoaat geproduseer. Uit hierdie analise kan die gevolgtrekking gemaak word dat *O*-asilering eerste ontstaan, wat dan gevolg word deur die Fries herrangskikking.

Vanaf die aanvang van die projek is die vervanging van die karboksielsuur groep deur ’n nitrielgroep as ’n nodige alternatief beskou. Die verandering van die reaktiewe karboniel koolstofatoom (wat as “harde” suur beskou word), na die van die karbonerende nitriel (wat as “sagte” suur beskou word), het tot beter resultate gelei om 2’,4’,6’-trihidroksie dihidrochalkoon via die Hoesch reaksie te produseer.

Dit is belangrik om te noem dat floroglusinol beskou word as die mees potente *C*- en *O*-nukleofiel in vergelyking met ’n “normale” reeks van model fenoliese inskrywings (fenol, resorsinol, katechol ens.) en het gelei het tot die vorming van ’n bifeniel, 3,5-dihidroksie-feniel-2’,4’,6’,-trihidroksie-fenieleter. Beskermende groepe is gebruik om die reaksieprosedures van die Lewissure te bevestig en die resultaat was die sintese van verskillende dihidrochalkone. Die lae opbrengste van die onbeskermende dihidrochalkone kan toegeskryf word aan die volgende: die vorming van 3,5-dihidroksie-feniel-2’,4’,6’,-trihidroksie-fenieleter, die vorming van 6,7-dimetoksie-indan-1-oon en 5,6-dihidroksie-indan-1-oon (intramolekulêre siklisering).

Die ander deel van die studie sluit die ondersoek en vergelyking van dieselfde reaksies onder die invloed van mikrogolwe in. Verskillende mikrogolftoestande in verskillende reaksies van resorsinol en floroglusinol met verskeie karboksielsure wat deur $BF_3{\cdot}(C_2H_5)_2O$ en $Hf(OTf)_4$ gekataliseer is, is ondersoek. Die resultate het getoon dat die opbrengste van resorsinol die van floroglusinol oorskry en dat $BF_3{\cdot}(C_2H_5)_2O$ as Lewissuur die reaktiwiteit van floroglusinol in die meeste reaksies bevorder.

Die beste resultaat in die reaksie van floroglusinol en 3,4-dihidroksiehidrosinnamiese suur was die katalitiese reaksie van $BF_3{\cdot}(C_2H_5)_2O$ om 3,4,2’,4’,6’-pentahidroksie dihidrochalkoon te produseer. ’n Betroubare reaksiemetode vir die direkte *C*-glukolisering met ’n onbeskermende suiker, D-glukose in ’n water medium is gebruik om sintetiese aspalatien vir die eerste keer, en deur ’n onbeskermende roete, in ’n lae opbrengs te produseer.

Die gebruik van sintetiese aspalatien in veloppervlakte-formulasies is ideaal vir kosmetiese produkte. Die rede hiervoor is omdat aspalatien in die stratum corneum akkomuleer. Dit bring ’n beskermingslaag met sterk anti-oksidant eienskappe op die vel tot stand. Hierdeur word die vel beskerm teen skadelike UV strale. Die reaktiewe suurstofspesies word verlaag en sodoende word die verouderingsproses vertraag. Ten einde is die potensiaal van die begunstigde komponent om as aktiewe bestandeel in kommersiële produkte gebruik te word, bevestig.