CHEMICAL PROFILE OF WALNUTS (*JUGLANS REGIA* L.) AND SYNTHESIS OF STILBENES FROM *ARFORMOSIA ELATA.*

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by

MOLAHLEHI SAMUEL SONOPO

Supervisor: Dr B. I. Kamara
Co-supervisor: Prof B.C.B. Bezuidenhoudt

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Key words: Isolation, structural elucidation, Walnuts, polyphenols, hydrolysable tannins *Juglans regia* L. synthesis, stilbenes, monomers, dimers, Grubbs catalyst, Heck reaction.

Firstly, this study presents an in-depth investigation on Walnuts (the nuts of *Juglans regia L.*). Walnuts (*Juglans regia L.*) are members of the relatively small *Juglandaceae* family, which have shown positive results in humans, in the treatment of metabolic syndrome. Besides the very high content of unsaturated fatty acids (60-70%) in Walnuts (*Juglans regia L.*), previous investigations have revealed tannins as the only phenolics present. Generally, plants have had their biological activities attributed to the presence phenolics, specifically the flavonoids, which are the most abundant polyphenols in nature. Since Walnuts leave behind an astringent taste in the mouth after ingestion, a characteristic associated with presence of phenolics, especially tannins, it was reasonable to assume that Walnuts may also contain flavonoids. Besides having well-established biological activities such as, antioxidant, anticancer, and anti-inflammatory properties, flavonoids are believed to augment the ability of Walnuts to act as a possible candidate for treatment of metabolic syndrome. In the previous studies, isolation of flavonoids has not been reported. Therefore, in this study we carried out an in-depth investigation to establish the presence of flavonoids in the Walnuts *Juglans regia* L.

Pure compounds were obtained after repeated column and preparative thin layer chromatography and characterized by extensive NMR spectroscopic methods. The phenolics isolated in this study as peracetate and permethyl derivatives from the Walnuts *Juglans regia* L. are: catechin, gallocatechin, penta-*O*-acetyl-*O*-β-D-xylopyranosylellagic acid, gallic acid, methyl gallate, pedunculagin, casuarinin, hexaacetoxy-4-O-β-D-glucopyranosylnapthalene and 2,3-*O*-*(S)*-heptamethoxy-β-D-glucopyranosyldiphenoyl. Tetra-*O*-acetyl-9-β-D-glucopyranosylmegastigmen-3-one, tetraacetoxy-3-*O*-β-D-glucopyranosylsitosterol, glucose and sucrose were isolated as non-phenolics.

Secondly, the study exploits methods to synthesize stilbene monomers and dimers isolated from *Afrormosia elata*. *Afrormosia elata* (*Pericopsis elata*) Harms, *Fabaceae,* is a tree native to the Guinean equatorial forests of West and Central Africa. The bark of this tree is used as a treatment for cancer by the local population. Stilbenes are a class of polyphenols with very

limited taxonomic distribution and varied biological activities which include, blood sugar reduction, antibacterial, antifungal, antioxidant, anti-HIV and anti-inflammatory. They posses COX-1 and COX-2 inhibitory effects, affect lipid peroxidation, LDL oxidation, function as phytoalexins, and have chemopreventative effects on cancer. The reported biological activities of stilbenes highlight the importance of stilbenoids as a resource for development of new drugs and pesticides. Since the occurrence of these stilbenoids in plants is in extremely low concentrations, we attempted synthesis of dimeric stilbenes with the aim of developing methods which may yield qualitative amounts. Syntheses of the monomeric stilbenes preceded that of the dimers. The classic Wittig reaction and the most recently developed metathesis reactions were the routes used to synthesize the monomers, while the route *via* the Heck coupling was considered for synthesis of the dimeric stilbenes.

CHAPTER 1

1. Introduction

This study presents two separate investigations with a unified goal of identifying and synthesizing biologically active compounds from natural plant products which may in a way contribute towards the discovery of new drugs. The first part of the study focuses on isolation of secondary metabolites from the Walnuts (*Juglans regia* L.), which may be important in the treatment of metabolic syndromes. The second part deals with synthesis of monomeric and dimeric stilbenes, from *Afrormosia elata*, which have been found to be biologically active, for example, in treatment of cancer as well as being potential anti HIV drugs.

1.1. **Part A**: Isolation of metabolites from Walnuts (*Juglans regia* L.).

The use of herbal or natural medicines for the treatment of various disorders has a long and extensive history. Many of these herbal medicines are finding their way onto the world market as alternatives to prescribed drugs currently available to treat various disorders/ailments. Clinical trials of Walnuts (*Juglans regia* L.), members of the relatively small *Juglandaceae* family, have shown positive results in humans, in the treatment of metabolic syndromes. The syndrome, also known as ‘‘*Syndrome X*’’, can be defined by a cluster of abnormalities including obesity, impaired glucose tolerance and type 2 diabetes, atherogenic dyslipidaemia, hypertension and coagulopathy.[79,80]

Walnuts comprise of about 60 species, of which 21 are placed in the genus juglans.[81,82] The nuts from all species are edible and rich in flavour. The walnut tree (*Juglans regia* L, Juglandaceae) is native in southeastern Europe, Asia Minor, India and China. Some species of the walnut tree is now cultivated throughout Europe, North America, North Africa and East Asia. Walnuts

[79] Tenenbaum, A., Fisman E. Z., Motro, M. *Cardiovasc. Diabetol.* **2003**, *2*, 4.
[80] Meigs, J. B. *Amer.J. Manag. Care*, **2002**, *8*, 283.
[81] McGranahan G., Leslie C. *Walnuts (Juglans). In: Genetic resources of temperate fruit and nut crops*. Eds. Moore J. N., Ballington J. R. Int Soc Hortic Sci, Wageningen, **1990**, *2*, 907 and 951.
[82] McGranahan, G., Leslie C. A., Driver, J. A. *HortScience.* **1988**, *23*, 220.

(*Juglans regia* L.) are harvested from large deciduous trees with the height of about 25-35 m. The tree start to produce at about six years of age, mature at 20-40 years and can continue to produce fruit until the age of 70. The fruits mature in autumn into husks containing large brown corrugated nuts with relatively thin shells (**Figure 1**). These fiber nuts are rich in oil, have bioactive compounds and are excellent source of omega-3 polyunsaturated fatty acids, which reduce cholesterol level and heart disorders.[83,84] The amount of the unsaturated fatty acids is very high (85.0%) while the percentage of the saturated fatty acids was found to be 15.0%.[85]

Figure 1: Harvested husks and nuts of Walnuts *Juglans regia* L.

Although previous investigations on Walnuts (*Juglans regia* L.) revealed presence of hydrolysable tannins,[86] isolation of flavonoids has not been reported. In this study we carried out an in-depth investigation to establish the presence of flavonoids which in a way may augment the already existing ability of walnuts to treat metabolic syndrome.

The taxodermic classification of Walnuts (*Juglans regia L.*)

Kingdom : Plants

[83] Patel, G. *J. Am. Diet Assoc.*, **2005**, *105*, 1096.
[84] Stevens, L. J., Zentall, S. S., Abate, M., L. *Physiol. Behav*., **1996**, *59*, 915.
[85] Zhao, G., Etherton, T. D., Martin, K. R., West, S. G., Gillies, P. J., Kris-Etherton, P. M. *J. Nutr*., **2004**, *134*, 299
[86] Fukuda, T., Ito, H., Yoshida, T. *Phytochemistry*. **2003**, *63*, 795.

Subkingdom :Tracheobionta
Superdivision :Spermartophyta
Division :Magnoliophyta
Class :Magnoliopsida
Subclass :Hamamelidae
Order :Julandales
Family :Juglandaceae
Genus :Juglans L.
Species :Juglans regia L.

1.2. **Part B:** Syntheses of monomeric and dimeric stilbenes from *Afrormosia elata*

Afrormosia elata (*Pericopsis elata*) Harms, *Fabaceae,* a tree native to the Guinean equatorial forests of West and Central Africa, is an economically important timber producing species. The bark is used as a treatment for cancer by the local population. Phytochemical studies of *Afrormosia elata* resulted in isolation of monomeric and dimeric stilbenes, and various flavonoids.[87,88] Stilbenes, mainly present in grapes and wines, are a class of polyphenols with very limited taxonomic distribution. In general, reported biological activities of stilbenes include, blood sugar reduction,[89] antimalarial, antibacterial, antifungal,[90] antioxidant,[91] anti-HIV and anti-inflammatory as well as potent antimitotic activity.[92] They posses COX-1 and COX-2 inhibitory effects,[93] affect lipid peroxidation, LDL oxidation,[94] function as phytoalexins,[95] and have chemopreventative effects on cancer.[96] The monomer resveratrol and its glycosides are widely reported to be beneficial to human health, and are used in treatment of a variety of diseases including dermatitis, gonorrhea, fever, hyperlipidemia, arteriosclerosis, cancer and

[87] Swanepoel, A. Masters Thesis, University of the Free State, Bloemfontein. **1987.**
[88] Litedu, E. M., PhD Thesis: *Polyphenols from Pericopsis elata and synthesis of selected stilbenes*. Department of Chemistry, University of the Free State, Bloemfontein, South Africa. **2005.**
[89] Huang, K. S., Li, R. L., Wang, Y. H., Lin, M. *Planta Med*., **2001**, *67*, 61.
[90] Ferreira, R. B., Monteiro, S. S., Picarra-Pereira, M. A., Teixeira, A. R. *Trends Biotech*., **2004**, *22*, 68.
[91] Lee, H. J., Seo, J. W., Lee, B. H., Chung, K-H., Chi, D. Y. *Bioorg. Med. Chem. Lett.*, **2004**, *14*, 463.
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[93] Cichewicz, R. H., Kouzi, S. A., Hamann, M, T. *J. Nat. Prod*., **2000**, *63*, 29.
[94] Iliya, I., Ali, Z., Tanaka, T., Iinuma, M., Furusawa, M., Nakaya, K., Murata, J., Ubukata, M. *Phytochemistry.* **2003,** *62*, 601.
[95] Serazetdinova, L., Oldach, K. H., Lorz, H., *J. Plant Physiol.*, **2005**, *162*, 985.
[96] Stewart, J. R., Artime, M. C., O'Brian, C. A. *J. Nutr.*, **2003**, *133*, 2440.

inflammation.[97,98] Phytoalexins on the other hand are natural antibiotic compounds proposed as fungicides or templates for production of new pesticides.[99] Recently, the search for novel antifungal compounds has received special attention because of an enhanced microbial resistance to current pesticides. The reported biological activities of stilbenes highlight the importance of stilbenoids as a resource for development of new drugs and pesticides. Since occurrence of these stilbenoids in plants is in extremely low concentrations, we attempted the synthesis of dimeric stilbenes with the aim of developing methods which will allow generation of substantial amounts of these biologically important compounds. Syntheses of the monomeric stilbenes proceeded *via* the classic Wittig reaction,[100] and the most recently developed methathesis reactions.[101] The route considered for synthesis of the dimeric stilbenes was conceived to proceed *via* the Heck coupling,[102] where the key step involves coupling of the halogenated aromatic halide with the olefinic stilbene monomer to form a C-C bond.

[97] Adrian, M., Jeandet, P., Veneau, J., Weston, L. A., Bessis, R. *J. Chem. Ecol.*, **1997,** *23*, 1689.
[98] Delmas, D., Lançon, A., Colin, D., Jannin, B., Latruffe, N., *Curr. Drug Targets.* **2006**, *7*, 423.
[99] Saigne-Soulard, C., Tristan-Richard, T., Mérillon, J-M., Monti, J-P., *Analy. Chim. Act*a. **2006**, *563*, 137.
[100] Rao, V. P., Jen, A. K., Wong, K. Y., Drost, K. J. *Tetrahedron Lett*., **1993**, *34*, 1747.
[101] Ferré-Filmon, K., Delaude, L., Demonceau, A., Noels, A., F. *Eur. J. Org. Chem.*, **2005**, 3319.
[102] Guiso, M., Marra, C., Farina, A. *Tetrahedron lett*., **2002**, *43*, 597.

CHAPTER 2

2. Polyphenols

2.1. Introduction

Plant polyphenols, also denoted as phenolic compounds, are secondary metabolites[25,26] with structures characterized by the presence of one or more aromatic rings bearing hydroxyl substituent(s). They are widely distributed in many foods from plant origin including, fruits, vegetables, nuts, coffee, tea, wine and chocolate.[27,28,29] Synthetic compounds belonging to the same class with diverse hydroxylation patterns broaden the range of these phenolic compounds. Flavonoids are the most studied compounds in this class.

Structure related biological properties of the polyphenols including, anticancer, antioxidant[30,31] and antibacterial have been reported. In plants, phenolic compounds are involved in several processes, such as acting as phytoalexins and promotion of plant growth.[25]

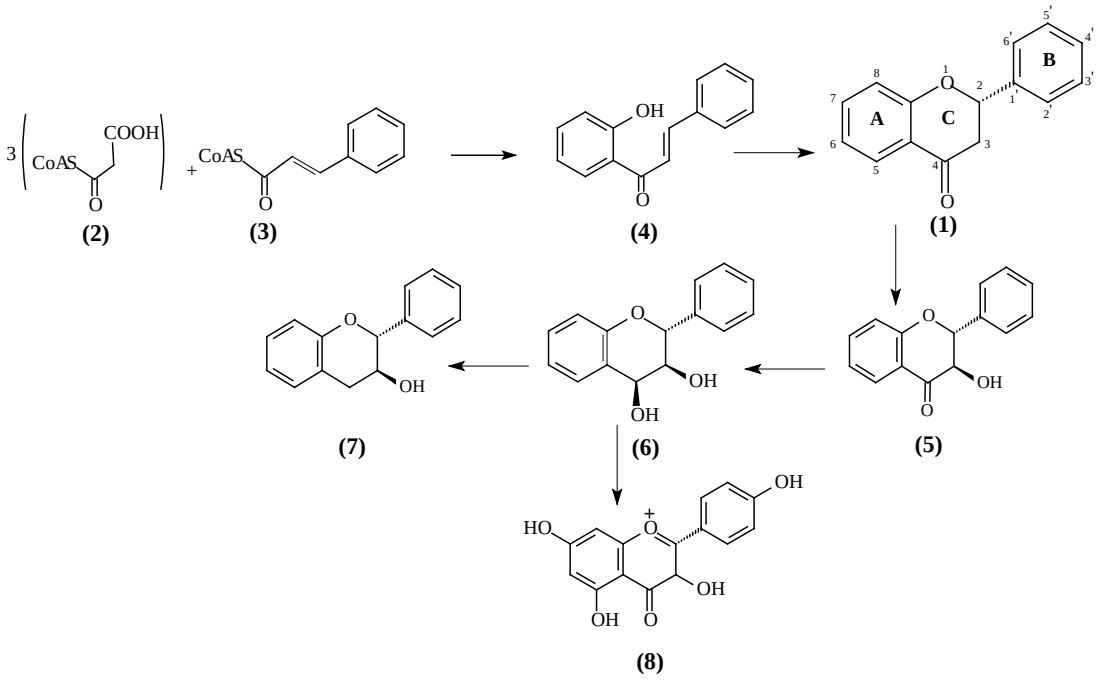
Biosynthesis of the phenolic compounds follows either the shikimate or the polyketide pathways,[32] giving rise to phenolic compounds which are divided into five groups: 1) the C_6 group comprising the simple phenols and benzoquinones, 2) the C_6C_n group, which includes phenolic acid derivatives and hydroxycinnamic acid derivatives, 3) the C_6-C_n-C_6 group, for example, the flavonoids (C_6-C_3-C_6), 4) the (C_6-C_3)$_n$, group consisting of lignans and lignins and 5) the tannin group, which is divided into hydrolysable tannins and condensed tannins.[32]

[25] Parr, A. J., Bolwell, G. P. *J. Sci. Food Agric.*, **2000**, *80*, 985.
[26] Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P., Glover, W. *Food Chem*., **1999**, *66*, 401.
[27] Lakenbrink, C., Lapcynski, S., Maiwald, B., Engelhardt, U. H. *J. Agric. Food Chem.*, **2000**, *48*, 2848.
[28] Clifford, M. N. *J. Sci. Food Agric.*, **1999**, *79*, 362.
[29] Arts, I. C. W., Hollman, P. C. H., Kromhout, D. *The Lancet.* **1999**, *354*, 488.
[30] Zheng, W., Wang, S. Y. *J. Agric. Food Chem.*, **2001**, *49*, 5165.
[31] Cai, Y. Z., Sun, M., Corke, H. *J. Agric. Food Chem.*, **2003**, *8*, 2288.
[32] O'Connell, J. E., Fox, P. F. *Int. Dairy J.*, **2001**, *11*, 103.

2.2. Flavonoids

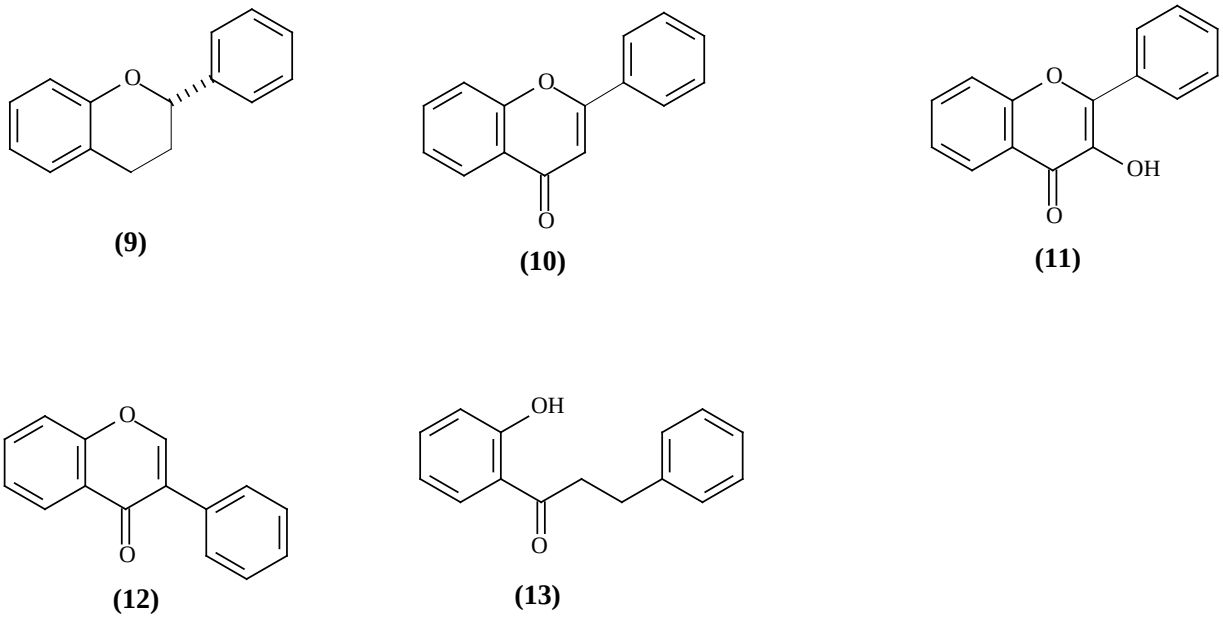
2.2.1. Introduction

Flavonoids are representatives of naturally occurring secondary plant metabolites, which have been found to possess positive biological effects on human health as well as important effects in plant biochemistry and physiology including acting as antioxidants, enzyme inhibitors, precursors of toxic substances, and pigment and light screens.[33,34,35] All flavonoids derive their 15 carbon skeletons **(1)** from two basic metabolites, malonyl-CoA **(2)** and *p*-coumaroyl CoA **(3)**,[36] which are enzymatically maneuvered into common phenyl benzopyran structure ($C_6C_3C_6$) **(1)**, **Scheme 1**. The broad family of flavonoids is categorized into a number of structural classes according to both the position of the B-ring and the oxidation state on the C_3 unit as, flavanones **(1)**, chalcones **(4),** dihydroflavonols **(5)**, flavan-3,4-diols **(6)**, flavan-3-ols **(7)**, flavans **(9),** flavones **(10)**, flavonols **(11)**, isoflavones **(12)**, and dihydrochalcones **(13)** among others (**Figure 2**).[36]



Scheme 1: Biosynthesis of the flavan-3-ols.

[33] Heim, K. E., Tagliaferro, A. R., Bobilya, D. J. *J. Nutri. Biochem.*, **2002**, *13*, 572.
[34] Harbone, J. B., Baxter, H. *Handbook of natural Flavonoids*, John Wiley and Sons. Chichester **1999**, *2*.
[35] Harbone, J. B., Willams, C. A. *Phytochemistry.* **2002**, *55*, 481.
[36] Geisman, T. A., Hinreiner, E. *Bot. Rev.*, **1952**, *18*, 77.



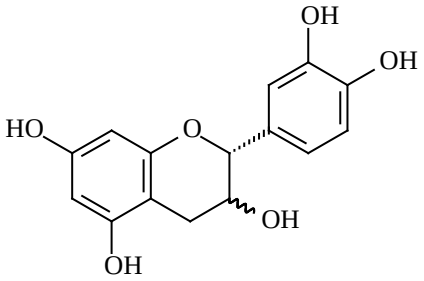
(9) **(10)** **(11)** **(12)** **(13)**

Figure 2: Skeleton structures of flavonoids

2.2.2. Flavan-3-ols

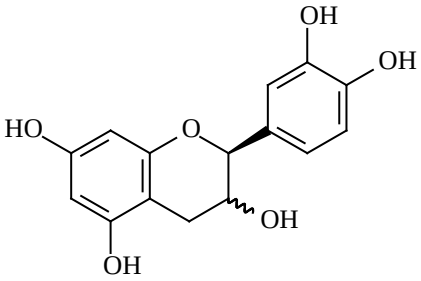
Flavan-3-ols **(7)** are the largest class of naturally occurring $C_6C_3C_6$ monomeric flavonoids.[37] Catechin **(14)** and epicatechin **(15)** are the most common flavonoids in this class. In contrast to this, ent-catechin **(16)** and ent-epicatechin **(17)** are fairly rare in nature. According to the biogenetic studies in plants, flavan-3-ols **(7)** are biosynthesized from flavan-3,4-diols **(6)** by reductase enzyme.[38] These compounds serve as building block monomers for the formation of proanthocyanidins **(18)** and are biologically essential when complexed with other biopolymers for example proteins, carbohydrates or metal ions.

[37] Green B. S., Heller, L. *J. Org. Chem.*, **1974,** *39*, 196.
[38] Haslam, E., *Flavonoids*, Ed. Harbone, J. B., and Mabry, H.Chapman and hall, New York, **1975**, 551.



14; ~ = ▬

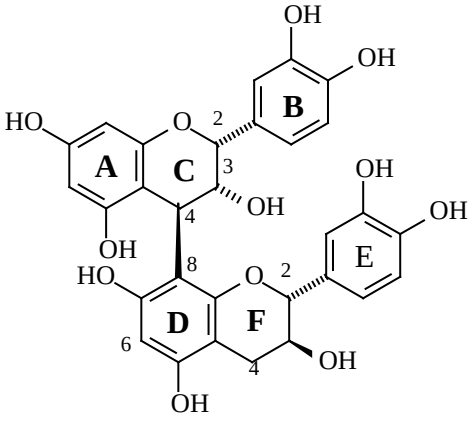
15; ~ = ┈┈



16; ~ = ▬

17; ~ = ┈┈

Flavan-3-ols are believed to have abilities to protect plants from insects, diseases and herbivores.[39,40] Flavan-3-ols also serve as important intermediates in the biogenesis of different types of tannins since they are nucleophiles that terminate the polymerization process.[41]



(18)

2.2.2.1. Nomenclature and structure

Trivial names for the flavans, **Table 2,** were adopted to define flavan-3-ols according to the different stereochemistry at C-2 and C-3 where they all exist with (2*R*,3*S*) configurations e.g.

[39] Harbone, J. B. *in Natural Products of woody plants I*, Springer-Verlag, Berlin Heidelberg, New York, **1989**, 586.
[40] Hemingway, R. W., Laks, P. E., Branham, S. J. *Plant Polyphenols: Synthesis, Properties, Significance*, Plenum Press, New York, **1992**.
[41] Stafford, H. A. *in Chemistry and Significance Condensed Tannins*, Ed. Hemingway, R. W., and Karchesy, J. J, Plenum, New York, **1988**, 301.

(14). Flavan-3-ols with configurations of (2*R*,3*R)* are prefixed with 'epi' **(15)** and those with a 2*S* configuraration distinguished by the enantio (ent) prefix **(16** and **17)**.

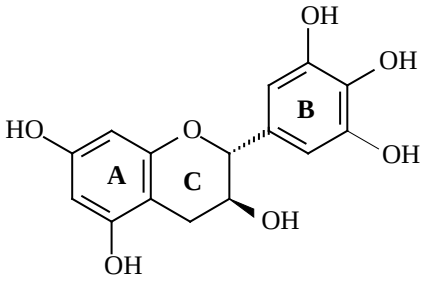
Monomer	5	7	8	3′	4′	5′	Configuration	
Afzelechin	OH	OH	H	H	OH	H	*R*	*S*
Ent-epicatechin	OH	OH	H	OH	OH	H	*S*	*S*
Catechin	OH	OH	H	OH	OH	H	*R*	*S*
Epicatechin	OH	OH	H	OH	OH	H	*R*	*R*
Gallocatechin	OH	OH	H	OH	OH	OH	*R*	*S*
Epigallocatechin	OH	OH	H	OH	OH	OH	*R*	*R*

Table 2: Examples of monomeric flavan-3-ols with different configurations at C-2 and C-3.

2.2.2.2. Distribution

The flavan-3-ols, catechin and epicatechin are widely distributed in the leaves, woody parts and fruits of plants. Analogues carrying a pyrogallol B-ring, gallocatechin **(19)** and epigallocatechin (**Table 2**) are dominant in primitive plants (*Coniferrae* being the outstanding). Although a number of flavan-3-ols with 2*S* configuration are known, their distribution is quite restricted. Ent-epicatechin and ent-epiafzelechin are present in the *Palmae* species.[42] The hydroxyflavan-3-ols, elephantorrhizol (5,6,7,8,3′,4′-hexahydroxyflavan-3-ol) identified in *Elephantorrhiza goetzei* and guiboutinidol (4,7-dihydroxyflavan-3-ol) isolated from the natural source (*Cassia abbreviate*), both with the absolute stereochemistry of 2*R*,3*S*,[43] have been reported.

[42] Waterman, P. G., Faulkner, D. F. *Plant Med.*, **1979**, *37*, 178.
[43] Nel, R. J. J., Mthembu, M., Coetzee, J. C., van Rensburg, H., Malan, E., Ferreira, D. *Phytochemistry*. **1999**, *52*, 1152.



(19)

2.2.2.3. Biosynthesis

Flavan-3-ols **(7)** form part of the pathway of anthocyanidins **(8)** biosynthesis **Scheme 1**. Enzymological studies have shown that 2*R*,3*S*-*trans*-flavan-3-ols **(7)** are derived from (+)-dihydroflavonols **(6)** by the sequential action of two classes of NADPH-dependent reductase.[44,45,46] These early studies established the enzymatic basis for the formation of the 2,3-*trans*-catechin derived series of flavan-3-ols, which was presumed to involve the consecutive action of a dihydroflavanol reductase, and a leucoanthocyanidin reductase to yield leucoanthocyanidin (**Scheme 1**).

2.3. Tannins

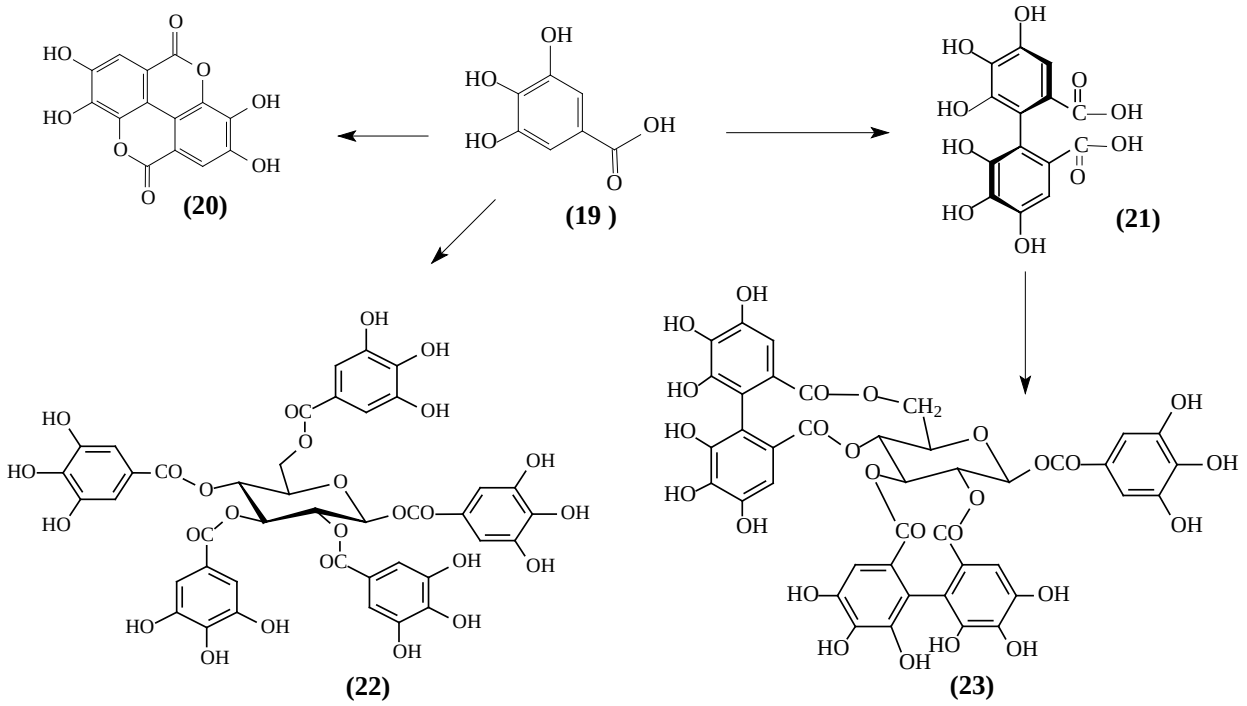
2.3.1. Introduction

In general, the term tannin refers to the source of tannins used in tanning animal hides into leather. This term is applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups to form strong complexes with proteins and other macromolecules. Tannins have molecular weights ranging from 500 to over 20,000. Generally,

[44] Platt, V. P., Opie, C. T., Haslam, E. *Phytochemistry*. **1984**, *23*, 2211.
[45] Singh, S., McCallum, J., Gruber, M. Y., Towers, G. H. N., Muir, A. D., Bohm, B. A. Koupai-Abyazani, M. R., Glass, A. D. M. *Phytochemistry*. **1997**, *44*, 425.
[46] Xie, D-Y., Dixon, R. A. *Phytochemistry*. **2005**, *66*, 2127.

tannins form a large group of polyphenolic plant constituents which differ from most other natural phenolic compounds in their ability to precipitate proteins from solutions.[47,48]

Plant tannins can be divided into two broad structural themes: a) the hydrolysable tannins, HTs [(ie gallotannins, GTs **(22)** and ellagitannins, ETs **(23**)][49] with the galloyl and hexadiphenoyl esters and their derivatives and b) the condensed tannins **18** in which the fundamental unit is the phenolic flavan-3-ols (catechin) nucleus.[23] Mixed tannins,[23] with the structural composition of both HTs and GTs, are a minor group which also occurs in nature.



Scheme 2: Tannins with gallic acid as precursor.

2.3.2. Hydrolysable tannins

HTs are characterized by a central polyol moiety (most often β-D-glucose) which is esterified with gallic acid **(19)** or hexahydroxydiphenic acid **(21)** moieties to form gallotannins, GTs **(22)**

[47] Salminem, J-P., Ossipov, V., Haukija, E., Pihlja, K. *Phytochemistry*, **2001**, *57*, 15.
[48] Niehaus, J. U., Gross, G. G. *Phytochemistry*. **1997**, *45*, 1555.
[49] Gross, G. G., *Biosynthesis of hydrolyzable tannins. In: Comprehensive Natural Products Chemistry*. Ed. Pinto, B.M. Elsevier, Amsterdam, **1999**, *3*, 799.

or ellagitannins, ETs **(23)**, respectively,[50] **Scheme 2.** These metabolites are almost invariably found as multiple esters of 3,4,5-trihydroxybenzoic (gallic) acid.

Gallic acid is most frequently encountered in plants in the form of esters. The derivatives of gallic (trihydroxybenzoic) acid **(19)** include ellagic acid **(20)**, hexahydroxydiphenic acid **(21)** and hydrolysable tannins (HTs) [ie gallotannins, GTs (**22)** and ellagitannins, ETs (**23**)], **Scheme 2**. Ellagic acid is a polyphenol antioxidant found in numerous fruits and vegetables including raspberries, strawberries, cranberries, walnuts, pecans and pomegranates. Gallic acid is an indispensable precursor in the synthesis of hydrolysable tannins which are generally distributed in higher plants. Plants produce ellagic acid and glucose that combine to form ellagitannins. Ellagitannins are water-soluble compounds that animals absorb easily in their diets. Gallotannins **(22)** and ellagitanins **(23)** constitute the two major classes of hydrolysable tannins.[51]

2.3.2.1. Occurrence and structure

A wide variety of plants and trees synthesize hydrolysable tannins,[52,53] which are usually found in the heartwood, bark, leaves and fruits.[54,55] Because of the highly hydroxylated phenolic rings in their structures, HTs show different nutritional, ecological and medicinal effects.[56,57] Complexity of the extracts and similarity in most of their structures renders isolation and structural elucidation very difficult. The most applied methods in isolation and structural elucidation of the HTs is repeated chromatography and extensive NMR spectroscopy. Owing to the presence of the depside bonds for example in compound **24** (**Scheme 3**) in their structures, HTs are highly susceptible to hydrolysis on addition of methanol, pH 6.0 at room temperature to yield the gallic acid derivatives **25** and **26**, **Scheme 3**. Therefore, it is important to carefully monitor the conditions during the extraction process. Large and complex tannins are easily degraded into smaller tannins by water or dilute acids.

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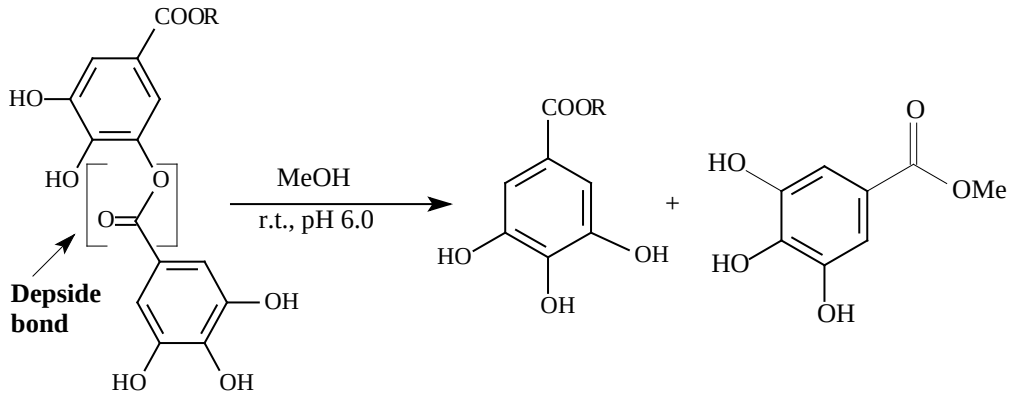
[50]Helm, R. F., Ranatunga, T. D., Chandra, M. *J. Agric. food Chem.*, **1997**, *45*, 3100.
[51] Khanbabaee, K., van Ree, T. *Nat. Prod. Rep.*, **2001**, *18*, 641.
[52] Muller-Haervey, I. *Anim. Feed. Sci. Technol.*, **2001**, *91*, 3.
[53] Okuda, T., Yoshida, T., HatanO,T. *Heterocyles.* **1990,** *30*, 1195.
[54] Feng, S., Tang, A., Jiang, J., Fan, J. *Anal. Chim. Acta*., **2002**, *455*, 187.
[55] Yoshida, T., Maruyama, T., Nitta, A., Okuda, T. *Phytochemistry*. **1996**, *42*, 1171.
[56] Okuda, T. *Phytochemistry.* **2005,** *66***,** 2012.
[57] Monteiro, J. M., Albuquerque, U.P., Araújo, E.L. *Quim. Nova*., **2005**, *28*, 892.

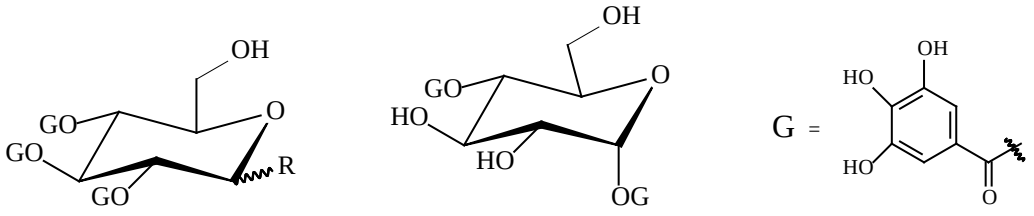


(24) **(25)** **(26)**

Scheme 3: Methanolysis of depside bond.

2.3.2.2. Gallotannins

Gallotannins are the simplest hydrolysable tannins, containing both the polyphenolic and the polyol residues. Although tannins with a variety of polyol residues are possible, mostly gallotannins e.g. casuarictin (**23**) containing one polyol residue (derived from D-glucose), where the hydroxy functions of the polyol are partly or fully substituted with the galloyl units have been isolated from plants. In the case of partial substitution with the galloyl moieties the remaining hydroxyl groups may either be unsubstituted or substituted with different other residues.[58] Gallotannins, 2,3,4,6-*tetra*-*O*-*g*alloyl-D-*g*lucopyranose (TGG) (**27**) and 1,2,3,4,6-*penta*-*O*-*g*alloyl-β-D-*g*lucopyranose (β-PGG) (**28**), found in many plant families, are key intermediates in the biosynthesis of nearly all hydrolysable plant polyphenols.[59,60]



(27); R = α or β-OH **(29)**

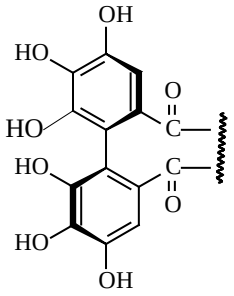
[58] Haslam, E. *J. Nat. prod.*, **1996**, *59*, 205.
[59] Gross, G. G. *Acta. Hortic.*, **1994**, *74*, 384.
[60] Rausch, H., Gross, G. G. *Z. Naturforsch, C, Biosci.*, **1996**, *51*, 473.

(28); R = β-OG

Most of the gallotannins substituted with a galloyl unit at the anomeric centre of their D-glucosyl unit have the β-configuration at the anomeric centre e.g. **(27** and **28)**, although gallotannins such as, 1,4-di-*O*-galloyl-α-D-glucopyranose (**29**) with the α-configuration have also been isolated.[61,62]

2.3.2.3. Ellagitannins

Ellagitannins e.g. **(23)** constitute members of a large class of polyphenolic natural products from higher plants, that are formed from the gallotannins by the oxidative coupling of at least two galloyl units, yielding, for example, the chiral hexahydroxydiphenylphenoyl (HHDP) unit (**30** or **31**) and a tergalloyl **(32)**. The chirality is caused, by the atropisomerism due to the inhibition of free rotation around the axis caused by the bulky *ortho* substituents, arising from esterification of the polyol moiety (usually D-glucopyranose).[63,64,65]



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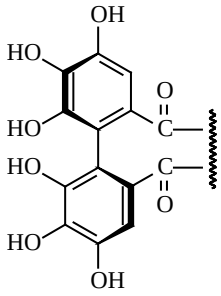
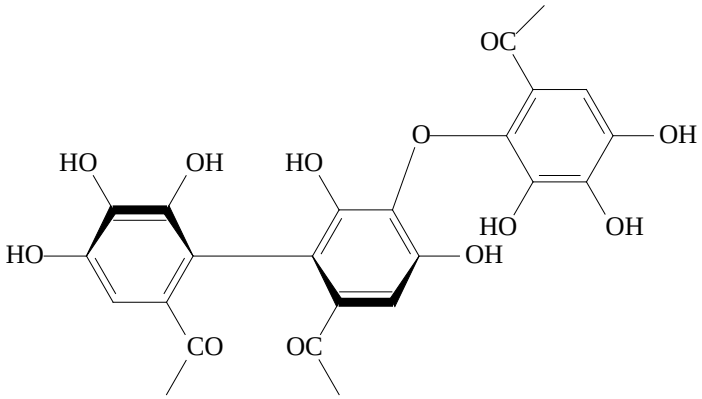


(31)

[61] Kashiwada, Y., Nonaka, G-I., Nishioka, I., Ballas, L M., Jiang, J. B., Janzen, W. P., Lee, K-H. *Bioorg. Med. Chem. Lett.*, **1992**, *2*, 239.
[62] Kashiwada, Y., Nonaka., G-I., Nishioka, I., Chang J-J., Lee, K-H. *J. Nat. Prod.*, **1999**, *55*, 1033.
[63] Krow, G., *Top. Stereochem.*, **1970**, *5*, 59.
[64] Cahn, R. S., Ingold, C., Prelog, V. *Angew. Chem.*, **1966**, *78*, 413.
[65] Oki, M. *Top. Stereochem.*, **1983**, *14*, 1.



(32)

2.3.4. Biological importance of ellagic acid and hydrolysable tannins

Several phenolic hydroxyl groups located on the surface of the tannin molecule are believed to participate strongly in the properties and biological activity of the tannins. Tannins at relatively high concentrations usually inhibit the activity of enzymes, but at low concentrations they often stimulate enzyme activity[66]. Tannins have been found to have long lasting inhibitory effects,[67] a factor which is attributed to the presence of many phenolic hydroxyl groups which produce stable free radicals one after another. Several oligomeric hydrolysable tannins have revealed strong antitumor activity.[68] Tannins have been found to inhibit the growth of HIV and herpes simplex virus.[44] Owing to their astringency, hydrolysable tannins play an important role in plants as protectors against infection, insects, or animal herbivory. For medicinal purposes tannin-containing plant extracts are used as diuretics,[69,70] antiseptic, against stomach and duodenal tumours,[71] in treatment of diarrhea,[72] antiinflamatory, and haemostatic

[66] Maxson, E. D., Rooney, L. W., Lewis, R. W., Clark, L. E., Johnson, J. W. *Nutr. Reports International,* **1973,** *8,* 145.
[67] Feeney, P. P. *Phytochemistry,* **1969,** *8,* 2119.Maxson, E. D., Rooney, L. W., Lewis, R. W., Clark, L. E., Johnson, J. W. *Nutr. Reports International,* **1973,** *8,* 145.
[68] Okuda. T., Yoshida, T., Hatano T. Chemistry and Biological Activity of Tannins in Medicinal Plants. In *Economic and Medicinal Plant Research,* **1991**, *5,* 129.
[69] Okuda, T., Hatano, T., Yazaki, K. *Chem. Pharm. Bull.*, **1983**, *31,* 333.
[70] T. Hatano, T., Yazaki, K., Okonogi, A., Okuda, T. *Chem. Pharm.Bull.*, **1991**, *39,* 1689.
[71] Saijo, R. , Nonaka, G-I., Nishioka, I. *Chem. Pharm. Bull.*, **1989**, *37,* 2063.
[72] T. Yoshida, T., Ohbayashi, H., Ishihara, K., Ohwashi, W., Haba, K., Okano, Y., Shingu, T., Okuda, T. *Chem. Pharm. Bull.*, **1991**, *39,* 2233.

pharmaceuticals.[73] Ellagic acid is a very strong anti-oxidant, a potent anti-carcinogen, has antibacterial and anti-viral properties and the ability to inhibit mutations within a cell's DNA as well as reduce LDL cholesterol.[74,75,76] Ellagic acid not only helps protect healthy cells from free radical damage, but also helps detoxify potential cancer-causing substances and helps prevent cancer cells from replicating.

[73] Haslam, E. *Plant Polyphenols– Vegetable Tannins Revisited-Chemistry and Pharmacology of Natural Products*, Cambridge University Press, Cambridge, **1989,** 165.
[74]Daniel, E. M., Stoner., G., D. *Cancer Lett.,* **1991**, *56*, 117.
[75]Mandal, S., Stoner, G., D. *Carcinogenesis*. **1990,** *11*, 55**.**
[76]Tanaka, T., Iwata, H., Niwa, K., Mori, Y., Mori, H. *J. Cancer Res.*, **1988**, *79*, 1297.

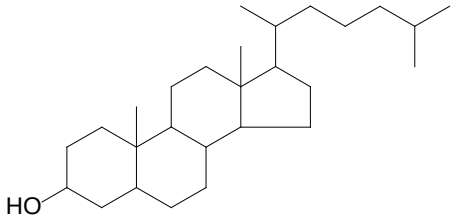
3. Non-phenolics

3.1. Introduction

Compounds whose structures have no aromatic rings are classified as non-phenolics. These compounds comprise the steroids, carbohydrates, lipids, etc. They are widely distributed in both higher and lower plants. While carbohydrates are the major natural source of energy, steroids can act as antioxidants and reduce serum as well as LDL cholesterol levels.

3.2. Sterols

Sterols **(33)** are amphipathic lipids synthesised from Acetyl CoA and are part of the vast family of isoprenoids.[80] They are important for the physiology of eukaryotic organisms either synthesized *de novo* or taken up from the environment. They form part of the cellular membrane where they modulate their fluidity and function and participate as secondary messengers in developmental signaling. Plant sterols have been extensively studied in past years with a major focus on biosynthetic and biochemical aspects.[81]

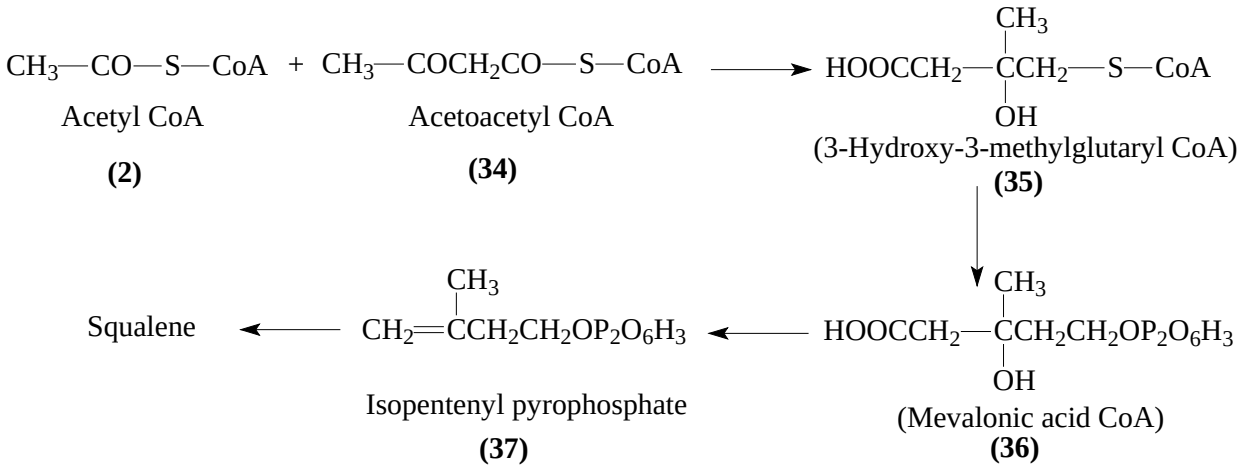


(33)

[80] Rahier A., Benveniste, P. *Target sites of sterol biosynthesis inhibitors: In: Target sites of fungicides action.* CRC Press; **1992**, 207.
[81] Benveniste, P. *Ann. Rev. Plant Physiol.,* **1986,** *37*, 275.

3.2.1. Biosynthesis

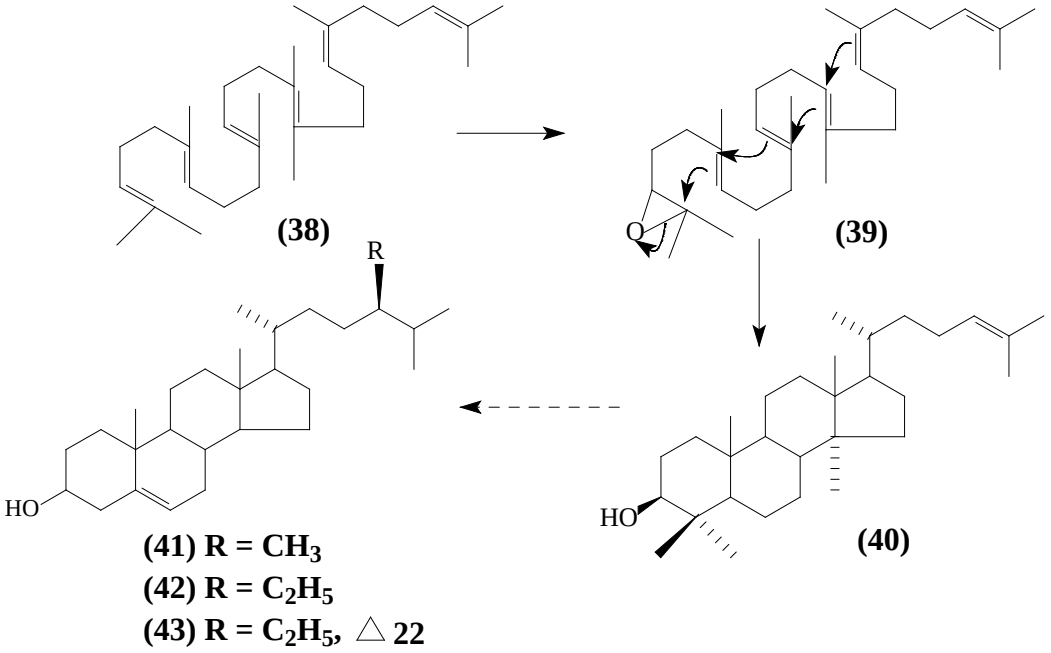
Sterol biosynthesis is one of the few areas of difference in primary metabolism between animals and plants (including the fungi and protozoa). Unlike animals that synthesize the C-27 cholestane-based members of the steroid family, plants synthesize C-28 and C-29 compounds (hereafter phytosterols) in which an extra methyl or ethyl group is added to carbon-24 of the sterol side chain.[82] Plant cells have two interesting peculiarities when compared to animals or fungi with respect to biosynthesis of sterols. The first part of the synthesis, similar to cholesterol biosynthesis, follows the mevalonate pathway[83]. In this pathway the biosynthesis involves the first steps of isopentenoids, where acetoacetyl Co A **(34)** condenses with acetyl Co A **(2)** to form mevalonic-5-pyrophosphate **(36)** *via* a C-6 intermediate (β-hydroxy-β-methylglutaric acid **(35)**. This is followed by phosphorylation of mevalonic acid to mevalonate-5-phosphate **(36)**, and subsequentially the formation of isopentenyl pyrophosphate **(37),** the precursor of the C_5 isoprene unit, **Scheme 4**. Tail-to-tail condensation of two C_{15} units produces the acyclic C_{30} triterpene squalene **(38)**.[84,85]



Scheme 4: Biosynthesis of sterols; Part 1

[82] Schaller, H., Bouwer-Nave, P. *Plant physiol.*, **1998,** *118*, 461.
[83] Dubey, V. S. *Current Sci.,* **2002,** *83*, 685.
[84] Benveniste, P., Hirth, L., Ourisson, G. *C. R. Acad. Sci.*, **1967**, *265*, 1749.
[85] Benveniste, P., Ourisson, G., Hirth, L. *Phytochemistry*. **1970**, *9*, 1073.

Secondly, in the first committed step of sterol biosynthesis, squalene epoxide **(39)** is cyclized to give cycloartenol **(40)**, which is transformed into end product sterols in a series of enzyme catalyzed methylations, demethylations, and desaturations. The major plant sterol end products are campesterol **(41)**, sitosterol **(42)** and stigmasterol **(43)**, (**Scheme 5)**.



Scheme 5. Biosynthesis of sterols; Part 2

3.2.2. Sterol glucosides

Stigmasterol, β-sitosterol and campesterol glucosides are well-known sterol glucoside components found in plants. They are synthesized by an UDP-glucose sterol glucosyl transferase (SGT) associated with the plasma membrane[86,87] where they are thought to be involved in a fine tuning of the free sterol concentration.[88] These sterol derivatives are present in small amounts in most of the plants including the model species *Arabidopsis*

[86] Hartmann-Bouillon, M.A., Benveniste, P. *Phytochemistry*. **1978**, *17*, 1037.
[87] Mudd, J. B. *Biochem. Plants.* **1980**, *4*, 509.
[88] Ullmann, P., Ury, A., Rimmele, D., Benveniste, P., Bouvier-Navé, P. *Biochimie*., **1993,** *75***,** 713.

from which a cDNA encoding SGT was cloned.[89]

3.2.4. Biological importance

Plant sterols and their hydrogenated forms, stanols, have attracted much attention because of their benefits to human health. They reduce serum and LDL cholesterol levels, with vegetable oil processing being their major source in several food products. As plant components, phytosterols may offer protection against cancer in ways which include, inhibiting cell division, stimulating tumor cell death and modifying some of the hormones that are essential to tumor growth. Many oxysterols have been found to be potent inhibitors of cholesterol biosynthesis.[90] Oxysterols also inhibit cell replication and have cytotoxic properties effects which suggest that these sterols may participate in the regulation of cell proliferation and may be potentially useful as therapeutic agents for cancer. Furthermore, there is considerable evidence that oxysterols may be involved in the pathogenesis of atherosclerosis.

3.3. CARBOHYDRATES

3.3.1. Introduction

Carbohydrates are primary metabolites, namely polyhydroxyaldehydes or ketone, alcohols and their polymer derivatives serve as building blocks for fats and nucleic acids and are broken down by both plants and animals to release energy. They are identified as the most important class of naturally occurring chemical compounds e.g. cellulose, hemi-cellulose and starch that give structure to plants, flowers, vegetables and trees. Carbohydrates are synthesized by plants as products of photosynthesis, an

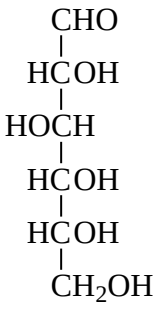
[89] Warnecke, D. C., Baltrusch, M., Buck, F., Wolter, F. P., Heinz, E. *Plant Mol. Biol.*, **1997,** *35*, 597.
[90] Hwang, P. L. *BioEssays.* **1991**, *13*, 583.

endothermic reductive condensation of carbon dioxide which requires light energy and the pigment chlorophyll.

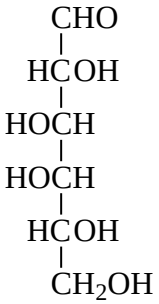
Carbohydrates are classified as simple carbohydrates e.g. monosaccharide (which cannot be converted into smaller sugars by hydrolysis) and complex carbohydrates such as disaccharide, oligosaccharide and polysaccharide (made of long chains of simple sugars). Identification of monosaccharides is based mainly on chromatographic techniques as well as ^{1}H and ^{13}C NMR spectroscopy. FAB-MS and NOESY spectroscopy are regarded as standard techniques for determining the linkages in the oligosaccharides

3.3.2. Monosaccharides

The majority of monosaccharides contain four to six carbon atoms with cyclic structures, usually in the expected pyranose form although occasionally the less stable furanose forms have been reported. Compounds that form monosacharides are known as aldoses (the aldehydes) and ketoses (the ketones). The most common aldoses are D-glucose **(44)**, D-galactose **(45)**, and D-ribose **(46)** while fructose **(47)**, a pentahydroxylketone, is the most common ketose.



(44)



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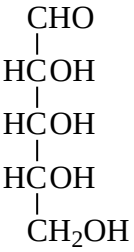
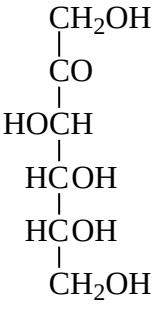


(46)



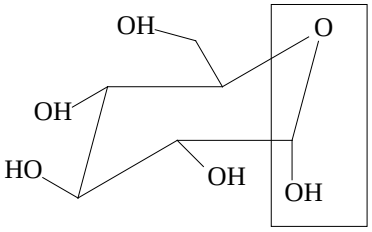
(47)

Monosaccharides are optically active molecules classified according to their molecular configuration at carbon 2, as dextrorotatory (D) or levorotatory (L). Monosaccharides serve as the building blocks for larger carbohydrates including

disaccharides, trisaccharides and polysaccharides. Besides the first and last carbon atoms, the four carbon atoms supporting, each supporting a hydroxyl group in the monosaccharides is chiral, a property which gives rise to a number of isomeric forms such as galactose and glucose which are aldohexoses with different chemical and physical properties.

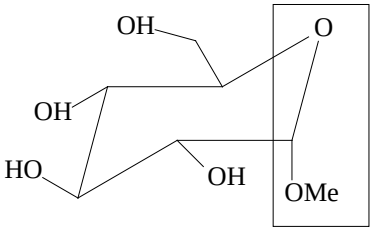
3.3.3. Oligosaccharides

Oligosaccharides are formed from simple monosaccharides units denoted by (n) and are coupled together by the elimination of n-1 molecules of water.[91] The coupling is specified as α or β where oxygen atom forms part of the acetyl or ketal group.[92] They are classified as reducing **(48)** and non-reducing sugars **(49)** e.g glycosyl aldoses (or glucosyl ketoses) and glycosyl aldosides (or glycosyl ketosides) respectively, where the latter involves the elimination of water molecule between the original reducing carbon atoms of two monosaccharides.



Hemiacetal-reducing

(47)



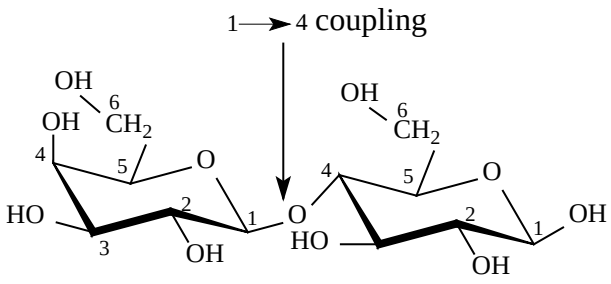
Acetal-nonreducing sugar

(49)

3.3.3.1 Disaccharides

Disaccharides are composed of two monosaccharides units joined together either by α or β linkages to form either the 1→4 [e.g Lactose **(50)**] or 1→6 linkage.

[91] Bailey, R.,W. *Oligosaccharides*. Pergamon Press: London, **1989.**
[92] Binkley, R.,W. *Modern carbohydrate chemistry*. Marcel Dekker, Inc: New York, **1988**, 1.



(50)

On hydrolysis, disaccharides gives two monosaccharides units, which are either the same or different as in maltose and sucrose. Although other combinations have been established, most disaccharides are dihexoses with the molecular formular $C_{12}H_{22}O_{11}$. The fundametal unit for naming disaccharides is starting from their right hand residue e.g Rutinose (α-L-rhamnosyl-(1→6)-D-glucose), which is the most widespread in plants. Reducing sugars (e.g maltose) are hydrolysable from starch by enzymes, while non-reducing sugars e.g sucrose can be hydrolyzed by both acids and enzymes to yield D-glucose and D-fructose in equal amount. The glycosidic linkage is easily recognizable from ^{1}H NMR spectra where the α linkage resonates at *ca* δ 5.6 (J = 2-3 Hz) and the β-linkage at *ca* δ 4-5 (J = 7-8 Hz).

3.3.3.2. Trisaccharides

Trisaccharides are classified as branched and linear, where the former occurr rarely in nature. Most of trisaccharides are mainly identified by FAB-MS, ^{13}C NMR and NOESY spectroscopy. The trisaccharide β-D-oleandropyranosyl-(1→4)-O-β-D-cymaropyranosyl-(1→4)-β-D-oleandropyranose, isolated from *Marsdenia roylei* (*Asclepiadaceae*), is one of the rare deoxy sugars occurring in free from in nature.[93] Example of the branched trisaccharides include apiosyl-(1→2)-[-rhamnosyl-(1→6)-

[93] Kumar, A., Khare, A., Khare, N. K. *Phytochemistry.* **1999**, *52*, 675.

galactose] linked to the 3-position of kaempferol[94,95] and glucosyl-(1→6)-[apiosyl-(1→2)-glucose] attached *via* the 3-hydroxy of palutein.[96]

3.3.3.3. Tetrasaccharides

Although no linear tetrasaccharides have been reported so far, branched tetrasaccharides, α-D-xylopyranosyl-(1→3)-α-L-galactopyranosyl-(1→2)-β-D-xylopyranosyl-(1→2)-L-arabinofuranoside and α-D-galactopyranosyl-(1→3)-α-L-galactopyranosyl-(1→2)-β-D-xylopyranosyl-(1→2)-L-arabinofuranoside are reported as the most complex heteroxylan side-chains that have been isolated from maize bran. Characterisation of tetrasaccharides sugars is mainly by UV, 1D/2D NMR spectroscopy, and HPLC-MS. The absolute configuration is determined by chiral GC after acidic hydrolysis.

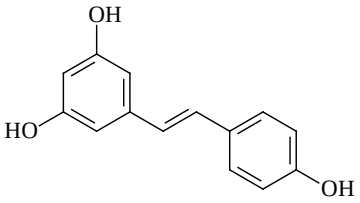
[94] De Simone, F., Dini, A., Pizza, C., Saturnino, P., Schettino, O. *Phytochemistry.* **1990**, 3690.
[95] Bashir, A., Hamburger, M., Gupta, M. P., Solis, P. N., Hostettmann, K. *Phytochemistry.* **1991**, *30*, 3781.
[96] Aritomi, M., Komori, T., Kawasaki, T. *Phytochemistry.* **1986**, *25,* 231.

CHAPTER 4

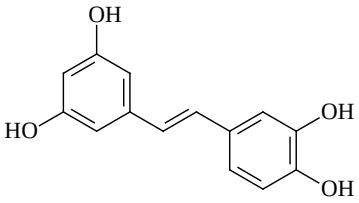
4. Synthesis

4.1. Introduction

Stilbenes are a class of phenolic compounds which occur in various families of plants.[97,98,99] The most common monomer, resveratrol **(51)**, is well distributed in grapes and wines.[100] Although stilbene dimers occur infrequently in nature, they have been isolated from several plant families including, *Cyperaceae*, *Dipterocarpaceae*, *Gnetaceae*, *leguminosae and Vitaceae*, and the common oxygenation pattern of the 3,5-dioxy substitution has resveratrol as the major building monomer. Monomeric stilbenes [e.g. resveratrol, piceatannol **(52)**] and their accompanying oligomers [e.g. ε-veniferin **(53)** and balanocarpo **(54)**] display a variety of biological activities[101] including, anti-cancer, anti-microbial[102,103] and antivirals (such as anti-HIV drugs), and they act as phytoalexins[104] in plants. These properties implicate the importance of synthetic methodologies for development of stilbene-based drugs.

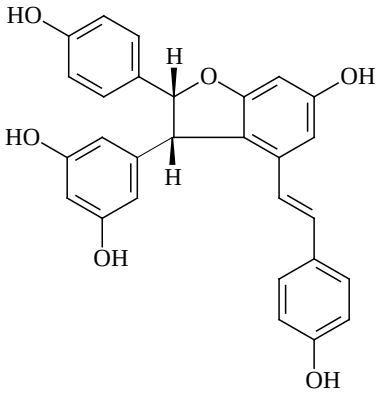


(51)



(52)

[97] Cuendet, M., Potterat, O., Salvi, A., Testa, B., Hostettmann, K. *Phytochemistry*. **2000**, *54,* 871.
[98] Pacher, T., Seger, C., Engelmeie, D., Vajrodaya, S., Hoferand, O., Greger, H. *J. Nat. Prod.*, **2002**, *65*, 820.
[99] Su, B. N., Cuendet, M., Hawthorne, M. E., Kardono, L. B. S., Riswan, S., H. Fong, H. S., Metha, R. G., Pezzuto, J. M., Kinghorn, A. D. *J. Nat. Prod.*, **2002**, *65*, 163.
[100] Burns, J., Yokota, T., Ashihara, H., Leanand, M. E. J., Crozier. A. *J. Agric. Food Chem.*, **2002**, *50*, 337.
[101] Williams, R. L., Elliot, M., Perry, R., Greaves, B. K. *Polyphenols Communications 96, Bordeaux Franceb*, **1996**, 210 and 489.
[102] Chan, M. M. *Biochem. Pharmacol.*, **2002**, *63*, 99.
[103] Dotcherty, J. J., Fu, M. M., Tsai, M. *J. Antimicrob. Chemotherapy*. **2001,** *47*, 871.
[104] Shimuzi, K., Kondo, R., Sakai, K. *Planta Med.*, **2000**, *66*, 11.



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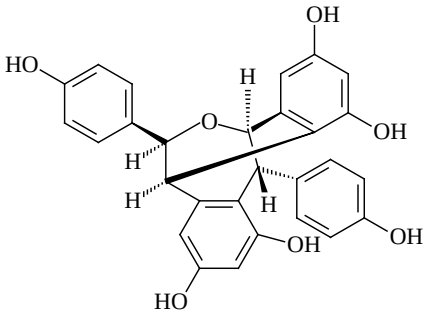


(54)

4.2. Synthesis of stilbenes

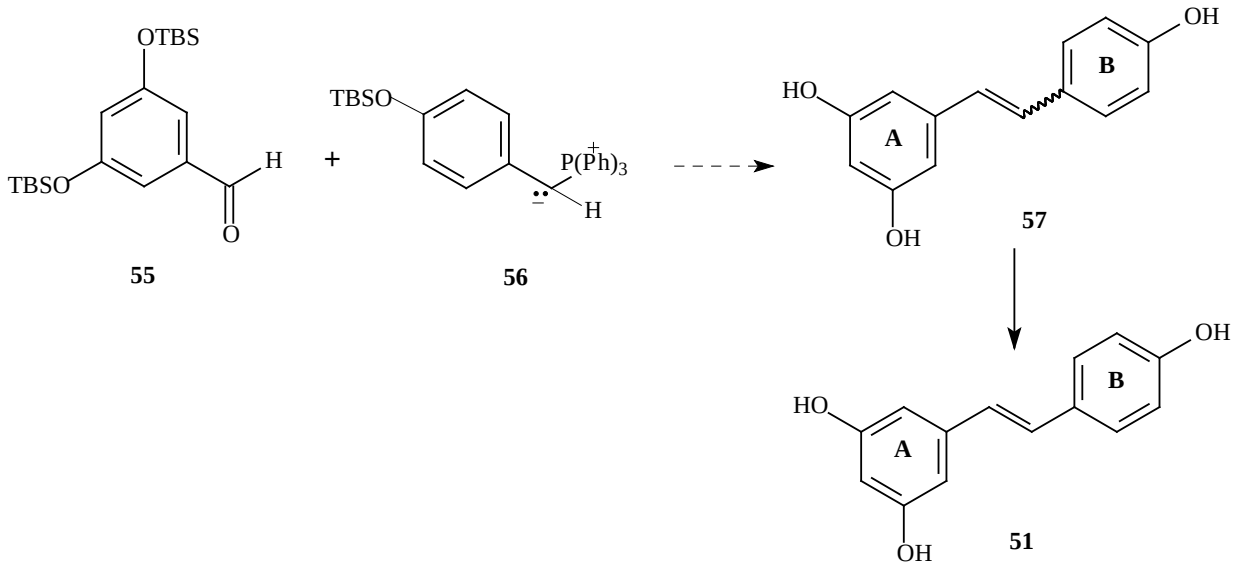
In the synthesis of the monomeric stilbenes methods such as, the classical Wittig reaction[105] have been utilized extensively. Oligomeric stilbenes have been synthesized by oxidative coupling reactions.[106] Drawbacks, including low yields and lack of stereoselectivity (i.e. generation of *E* and *Z* isomers), make the classic methods unappealing.

4.2.1. Wittig reaction

Among the various methods used to synthesize unsymmetrical stilbenes e.g. resveratrol **(51)**, is the most commonly used Wittig reaction **(Scheme 6**). The prototype Wittig reaction is an important, simple, efficient and versatile reaction in organic chemistry for synthesizing alkenes with unambiguous positioning of the double bond.[107] Wittig condensation of the protected aldehyde **55** with a phosphonium ylide **56** (generated by the reactions of phosphonium salts and bases e.g., sodium hexamethyldisila-zide NaHMDS, LiHMDS, PhLi, BuLi or NaNH) to afford a stilbene **57**, as a mixture of (E) and (Z)-geometrical isomers in the ratio of 2:1 (**Scheme 6**). This E/Z mixture is efficiently converted to (E)-geometric isomer **51** through heating with the catalytic amount of I_2 with heptane as solvent and refluxing for 12 hours. Most of the studies of

[105] Rao, V. P., Jen, A. K., Wong, K.Y., Drost, K. J. *Tetrahedron Lett.*, **1993**, *34*, 1747.
[106]Thomas, N. F., Lee, K. C., Paraidathathu, T., Weber, J. F. F., Awang, K., Rondeau, D., Richomme, P. *Tetrahedron.* **2002**, *58*, 7201.
[107]Hwang, J-J., Lin, R-L., Shieh, R-L., Jwo, J-J. *J. of Molecular Catalysis A: Chemical.* **1999**, *142*, 125.

the Wittig reactions are carried out homogeneously in organic solvents e.g., THF, CH_2Cl_2, DMF, and MeOH.

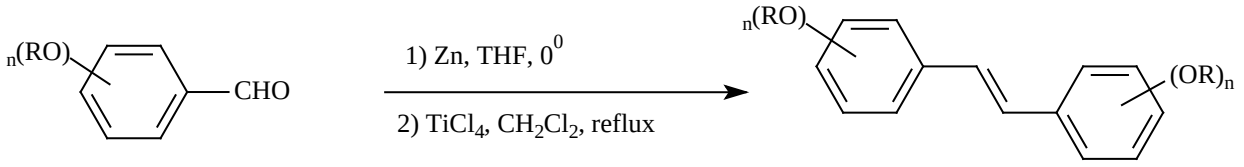


Scheme 6: Synthesis of stilbenes *via* the Wittig reaction

4.2.2. McMurry coupling of aldehydes and ketones

Aldehydes and ketones undergo reductive dimerization to yield alkenes upon treatment with low-valent titanium reagents. Such a carbonyl coupling reaction is usually referred to as the McMurry coupling.[108] It plays a significant role in the synthesis of phytoalexins, because it provides ready access to the formation of symmetrical stilbenes from protected aldehydes **58** or ketones.[109] Thus, symmetrical polyalkoxy- and polysilyloxystilbenes **59** are obtained by reductive coupling of alkoxy- and silyloxybenzaldehydes, respectively, with zinc and titanium tetrachloride as catalysts (**Scheme 7**). All the products isolated under these conditions possessed a *trans* double bond.

[108]Ali, M. A., Kondo, K., Tsuda, Y. *Chem. Pharm. Bull.* **1992**, *40***,** 1130.
[109]McMurry, J. E. *Chem. Rev.*, **1989**, *89*, 1513.



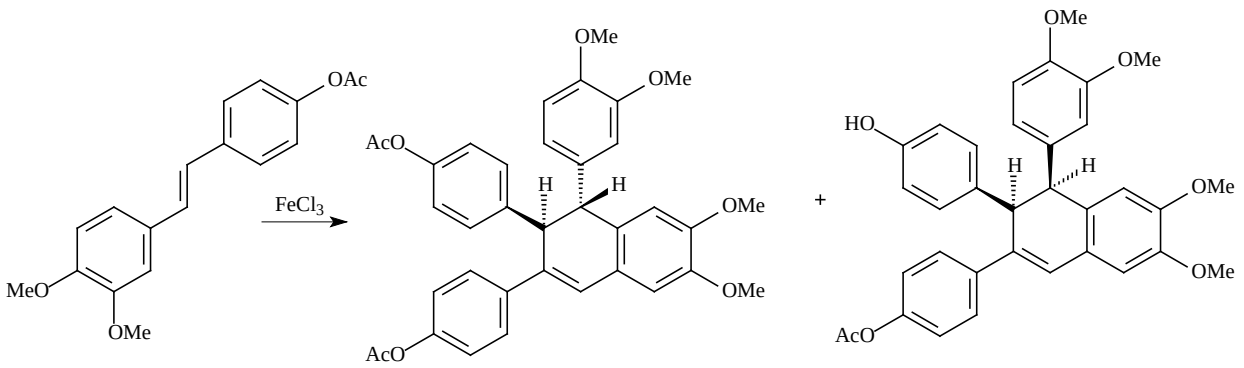
(58) R= Me or iPr **(59)**

Scheme 7: Synthesis of the stilbenes *via* the McMurry coupling

Although the McMurry coupling reaction usually gives almost exclusively *E*-stilbenes, the *Z*-isomers may be predominantly obtained if geometric constraints control the orientation of the two carbonyl moieties.[110]

4.2.3. Oxidative coupling of stilbenes

The biochemistry of the stilbenoids is very rich but it is still largely unexplored in its synthetic aspects. The most widely distributed oligostilbenoid polyphenols have the monomers resveratrol and piceatannol as precursors. Biosynthetically, it is invisaged that these oligomers are generated by oxidative phenolic coupling of two monomeric stilbenes. To date, only a few stilbenoid dimers have been prepared in the laboratory by oxidative coupling with oxidants such as, $FeCl_3$ and AgOAc (**Scheme 8**). Oxidative coupling of compound **60** yields diastereomers **61** and **62**[106]



(60) **(61)** **(62)**

Scheme 8: Synthesis of the dimeric stilbenes by oxidative coupling

[110] Sukwattanasinitt, M., Rojanathanes, R., Tuntulani, T., Sritana-Anant, Y., Ruangpornvisuti, V. *Tetrahedron Lett.* **2001,** *42*, 5291.

DISCUSSION

5. Polyphenols

5.1. Introduction

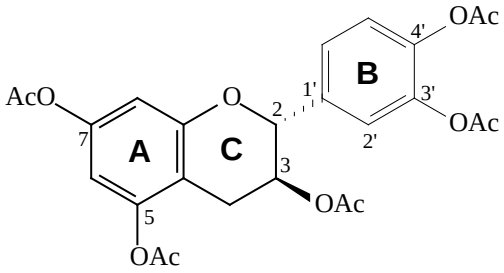
Polyphenols for example flavonoids, stilbenes, and tannins are classified as secondary metabolites. Secondary metabolites are not necessary for the growth and reproduction of a plant, but they may serve some role in herbivore deterrence due to the astringent taste they leave behind on ingestion or they may act as phytoalexins. They are well utilized by humanity because they have biological activities such as anticancer, antioxidant and antimicrobial. These properties are influenced by factors such as, the hydroxylation patterns on the rings, prenylation, presence of double bonds and methoxy groups in the molecule. The methanol extract of the pulverized nuts of Walnuts (*Juglans regia* L.) afforded a complex mixture of mainly sugars along with extremely low concentrations of phenolic compounds which were resolvable only after extensive enrichment and fractionation procedures. Polyphenols isolated from Walnuts (*Juglans regia* L) include catechin, gallocatechin, gallic acid, methylgallate, ellagic acid, casuarinin and peduncalagin. Structural elucidation was by extensive ^{1}H NMR, namely, COSY, NOESY, HMQC, DEPT, HMBC.

5.2. Flavonoids

5.2.1. Penta-*O*-acetylcatechin

The ^{1}H NMR spectrum (**Plate 1**) of compound **63,** isolated as a peracetate derivative, displays the ABX and AB spin-systems attributed to the *meta*-doublets on the A-ring and the *para*-substituted B-ring, respectively (**Table 5.1**). The ^{1}H NMR spectrum also displays the flavan-3-ol typical protons at δ 5.17 (H-2), 5.27 (H-3), and 2.68 and 2.54 (2H-4). The large coupling constant (J = 7.0 Hz) of H-2 confirms the 2,3-*trans* relative configuration in the C-ring. The coupling constants (J = 5.1 and 5.5 Hz) between H-3 and

the non-equivalent 4-CH_2 further serve as confirmation of *trans*-configuration. Compound **63** is identified as catechin and its ^{1}H NMR data is identical to that of the authentic sample in our department. This is the first time a catechin is isolated from Walnuts (*Juglans regia L*).

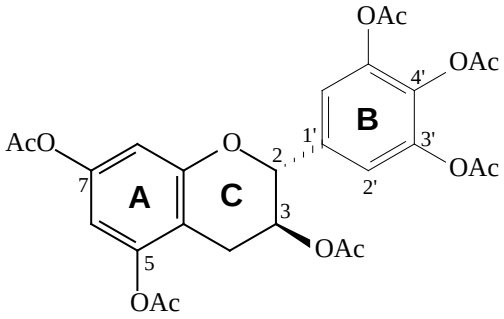


(63)

Ring	**proton**	**$CDCl_3$, 298K**
A	H-6	6.61 (d, J 2.0 Hz)
	H-8	6.67 (d, J 2.0 Hz)
B	H-2'	7.18 (d, J 2.0 Hz)
	H-6'	7.27 (dd, J 8.0 Hz and 2.0 Hz)
	H-5'	7.21 (d, J 8.0 Hz)
C	H-2	5.17(d, J 7.0 Hz)
	H-3	5.27 (m)
	H-4	2.68 (dd, J 16.8 and 5.1 Hz)
	H-4	2.54 (dd, J 16.8 and 6.0 Hz)
	OAc	2.0-2.31 (x5) (s)

Table 5.1: ^{1}H NMR data of penta-*O*-acetylcatechin **(63)**

5.2.2. Hexa-*O*-acetylgallocatechin



(64)

Ring	**proton**	**$CDCl_3$, 298K**
A	H-6	6.67 (d, J 2.2 Hz)
	H-8	6.62 (d, J 2.2 Hz)
B	H-2'/6'	7.14 (s)
C	H-2	5.13(d, J 7.0 Hz)
	H-3	5.22 (ddd, J 7.0, 6.0 and 5.3 Hz)
	H-4	2.93 (dd, J 16.8 and 5.1 Hz)
	H-4	2.68 (dd, J 16.8 and 6.9 Hz)
	OAc	2.0-2.31 (x 6) (s)

Table 5.2: ^{1}H NMR data of hexa-*O*-acetylgallocatechin **(64)**

Compound **64**, isolated as a peracetate derivative, displays the flavan-3-ols characteristic protons at δ 5.17 (H-2), 5.27 (H-3), and 2.68 (2H-4) in the ^{1}H NMR spectrum (**Plate 2, Table 5.2**). The deshielded two-proton singlet at δ_H 7.14, typical of the pyrogallol B-ring, and the two *meta*-doublets on the A-ring in the ^{1}H NMR spectrum confirm

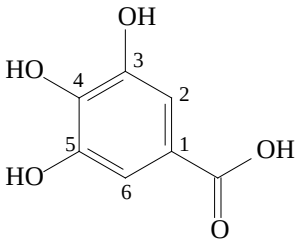
compound **64** to be the peracetate derivative of gallocatechin.[1] This is the first time gallocatechin has been isolated from Walnuts *Juglans regia* L.

5.3. Tannins

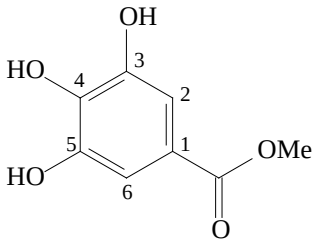
5.3.1. Gallic acid and methyl gallate

Compounds **19** and **26** were isolated as free phenolics from the methanol extract. The ^{1}H NMR spectrum (**Plate 3**) of compound **19** displays only one singlet at δ 7.14 in the aromatic region. Compound **26** on the other hand displays two singlets at δ_H 7.12 and δ_H 3.78 in the ^{1}H NMR spectrum (**Plate 4**). Analysis of the carbon spectrum (**Plate 3a**) of compound **19** reveals five resonances at δ_C 109.5, 121.2, 137.9 and 145.2 in the aromatic region whose intensities are representative of symmetry in an aromatic ring. The deshielded peak at δ_C 166.8 could belong to the carbonyl of either an acid or an ester group in the molecule. The possibility of the carbonyl carbon belonging to the ester functionality was eliminated on the grounds that the ester functionality would probably belong to a symmetrical ellagic acid **(20)** molecule. The carbon spectrum of ellagic acid would then display seven resonances and not six as in the ^{13}C NMR of compound **19**. Since there are no aliphatic carbons in the ^{13}C NMR spectrum, compound **19** is proposed to be gallic acid. Further analysis of the ^{13}C NMR spectrum (**Plate 3a**) allowed assignment of the four aromatic quaternary carbons to the equivalent oxygenate C-3 and -5 (δ_C 145.2), the oxygenated C-4 (δ_C 137.9) and C-1 (δ_C 121.2). The remaining shielded signal at δ_C 109.5 is assigned to the proton-bearing C-2 and -6. The ^{1}H NMR of compound **19** is identical to that of the authentic gallic acid. Gallic acid is a well known for its powerful antioxidant properties as well as acting as an anticancer agent.[51]

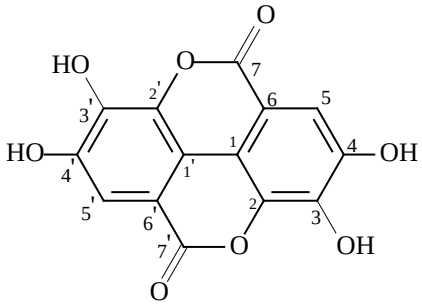
[1] Li, H. J., Deinzer, M. L. *J Agric. Food Chem.*, **2006,** *54*, 4048.



(19)



(26)

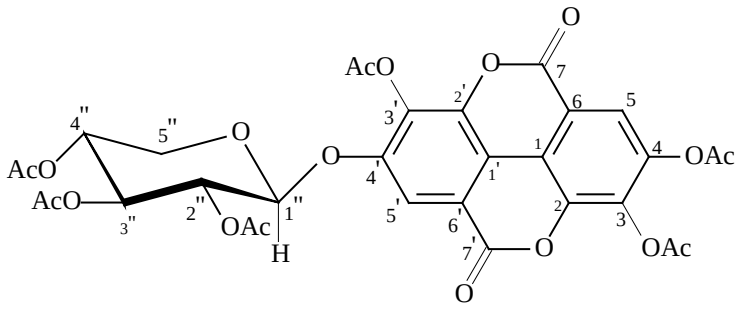


(20)

The two singlets resonating at δ_H 7.12 and 3.78 in the ^{1}H NMR spectrum (**Plate 4**) of compound **26** are in a ratio of 2:3 suggesting the presence of two aromatic protons and a methyl group, respectively, in the molecule. Except for an additional signal at δ_C 51.7, the resonances in the ^{13}C NMR (**Plate 4a**) at δ_C 167.3, 146.1, 138.6, 121.8, and 109.6 are very similar to those of compound **19** indicating that compound **26** is a methyl ester derivative of **19**. HMBC correlations (**Plate 4b**) of O*C*H_3 to carbonyl carbon (C-7) and a strong NOESY association of the methyl protons with H-2/6 confirm the methoxy group to be that of an ester and not a substituent on the ring. Compound **26** is methyl gallate[2]**.** Besides the astringency taste methyl gallate leaves behind in the mouth on ingestion, it is a medicinally important compound that has been found to have biological activities such as, acting as a powerful antioxidant, and it protects mammalian and bacterial cells from cytotoxicity induced by hydroperoxides.[85]

[2] Chaubal, R., Deshpande V. H., Deshpande, N. R. *J. Agric. Food Chem.*, **2005**, *4*, 956.

5.3.2. Hexa-*O*-acetyl-*O*-β-D-xylopyranosylellagic acid (65)



(65)

The ^{1}H NMR spectrum of compound **65 (Plate 5)** displays a very simple spin system of two aromatic singlets (δ 7.89 and 8.07) along with six oxomethines protons in the aliphatic region, suggesting the presence of an aromatic glycoside. Since only six aliphatic protons attached to oxygen-bearing carbons are present in the spectrum, the possibility of having a pyranoside sugar moiety with seven protons e.g. glucose was eliminated. The sugar moiety could then be either a pyranoside with five carbons or a furanoside. The ^{13}C NMR spectrum **(Plate 5a), Figure 5.1** of compound **65** displays 31 carbons, which from the HMQC, and HMBC (**Plate 5b**) experiments are revealed to be 12 aromatic protons, four methines, one methylene group, two ester carbonyl carbons and six acetoxy groups, **Table 5.3**. Analysis of the chemical shifts and coupling constants in the ^{1}H-^{1}H COSY spectrum, reveals that the methylene protons, H-5" (δ 3.74 and 4.35) are coupled to H-4" which is sequentially coupled to H-3", H-2" and H-1" indicating the presence of a xylose unit. Presence of only three aliphatic acetoxy groups confirms that the methylene group has no acetyl group and that it is not part of an aliphatic chain, but it is in a heterocyclic ring. Strong NOESY associations observed between H-2", and H-4", as well as H-1" with H-3" confirm the sugar moiety to be xylose. The 1,3-diaxial NOESY association (**Plate 5c**, **Figure 5.2**) establishes the β-configuration.

PLC separation of the acetylated methanol extract yielded the *O*-acetyl derivative compound **65**

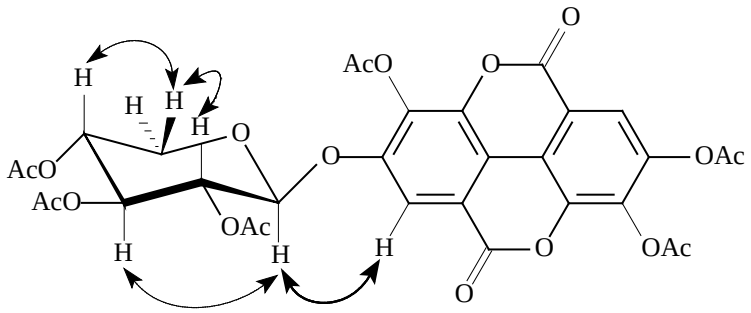


Figure 5.1: Relevant NOESY associations in hexa-*O*-acetyl-*O*-β-D-xylopyranosylellagic acid **(65)**

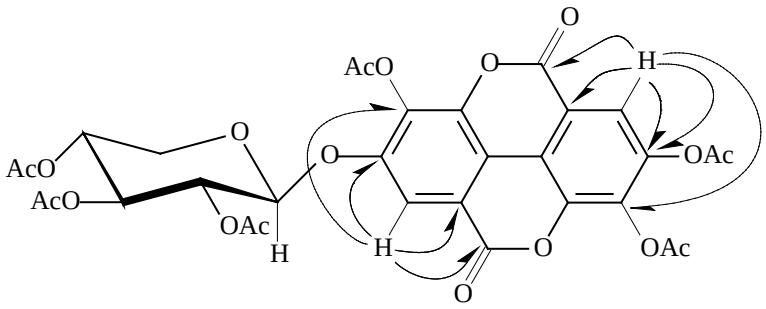


Figure 5.2: Relevant HMBC correlations in hexa-*O*-acetyl-*O*-β-D-xylopyranosylellagic acid **(65)**

Moeties	**Protons**	**δ_{H} (ppm)**	**Carbon**	**δ_{C} (ppm)**	**^{2} J and ^{3}J (^{1}H→^{13}C)**
Aglycone	1	-	1	113.7	
	2	-	2	142.5	
	3	-	3	152.1	
	4	-	4	135.8	
	5	7.89	5	111.9	C-1,-3,-4,-6,-7
	6	-	6	116.3	
	7	-	7	157.6	
	1'	-	1'	110.8	
	2'	-	2'	142.9	
	3'	-	3'	145.4	
	4'	-	4'	133.4	
	5'	8.07	5'	121.1	C-3',-4',-6',-7'
	6'	-	6'	116.9	
	7'	-	7'	157.7	
Xylose	1"	5.35	1"	99.6	
	2"	Overlapping	2"	69.12	
	3"	Overlapping	3"	71.4	
	4"	5.1	4"	70.2	
	5"	α 3.74 β 4.35	5"	63.3	
Aromatic	COC$\underline{H_3}$	2.41 (s), 2.51 (s), 2.54 (s)	CO$\underline{C}$H$_3$	19.0-21.0	
			$\underline{CO}$CH$_3$	169.4-170.0	
Aliphatic	COC$\underline{H_3}$	2.09-2.12 (s)	CO$\underline{C}$H$_3$	19.0-21.0	
			$\underline{CO}$CH$_3$	166.2-167.6	

Table 5.3: NMR data of penta-*O*-acetyl-*O*-β-D-xylopyranosylellagic acid **(65)**

The aromatic aglycone of compound **65** is established by the almost duplicated ^{1}H→^{13}C, ^{2}J and ^{3}J correlations **(Table 5.3, Figure 5.1)** observed in the HMBC spectrum **(Plate 5b).** The HMBC correlations confirm the aglycone to be the ellagic acid moiety. Linkage of the xylose moiety to the aglycone is confirmed by a NOESY association of H-1" with H-5'. Compound **65** is the *O*-acetyl derivative of *O*-β-D-xylopyranoside ellagic acid. Among the established biological activities of the remarkable ellagic acid are its

abilities to act as a strong antioxidant, anti-inflamatory, anticancer, and it is a naturally-occurring phytochemical pesticide.[3]

5.4. Hydrolysable tannins

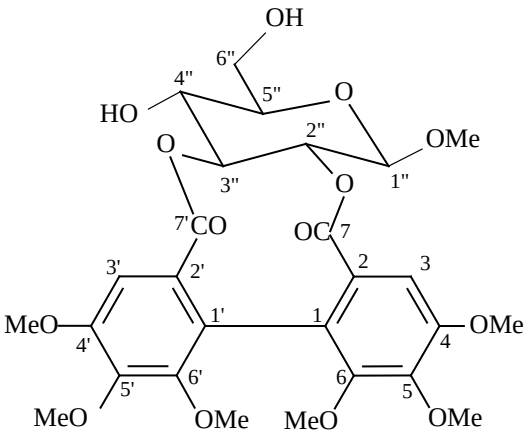
Ellagitannin metabolites fall into two broad categories namely the monomeric species formed by intramolecular C-C oxidative coupling and the oligomeric species formed by intermolecular C-O coupling. Numerous intramolecular C-C linked ester groups have been located in the monomers and similarly various intermolecular C-O linking ester groups have been defined in the formation of the oligomeric structures.

5.4.1. 2,3-*O*-*(S)*-Heptamethoxy-β-D-glucopyranosyldiphenoyl ester

The ^{1}H NMR spectrum (**Plate 6**) of the *O*-methyl derivative **66**, from the methanol extract, exhibits two one proton-singlets in the aromatic region, and seven aliphatic protons on oxygen-bearing carbons resonating from δ_H 3.40 to 5.25 (**Table 5.4)**. The ^{13}C NMR spectrum (**Plate 6a**) displays 12 aromatic carbons, two ester carbonyl carbons, seven oxygen-bearing aliphatic carbons and seven methoxy groups. These resonances suggest the presence of the diphenoyl and glucosyl moieties in the molecule. The resonance at δ_H 6.80 in the ^{1}H NMR spectrum is assigned to H-3 of the hexamethoxydiphenoyl (HMDP) and confirmed by correlations of H-3 to C-1, 2, 4 and 5 **Figure 5.3** in the HMBC spectrum (**Plates 6b i and ii**). Similarly, the signal at δ_H 6.79 is allocated to H-3' of the HMDP moiety, and confirmation is by HMBC correlations of H-3' with C-1', 2', 4' and 5' **Figure 5.3**. The β-configuration in the sugar moiety is deduced from the coupling constants (J = 7.0 Hz) of the anomeric proton and confirmation by 1,3-diaxial NOESY association between H-1" and H-3". Sequential ^{1}H-^{1}H COSY correlations of H-1'" to H-6'" in the COSY spectrum allow allocation of the sugar protons. The connectives of the O-2 and O-3 of the glucose moiety to the HMDP unit are confirmed by HMBC correlations of H-2" and H-3" of the glucose moiety to the C-7 and

[3] Lee, J-H., Johnson, J. V., Talcott, S. T. *J. Agric. Food Chem.,* **2005**, *53,* 6003.

7' (carbonyls), respectively, in the ester linkages (**Figure 5.3, Table 5.4**). Further confirmation is by ^{3}J correlations of the aromatic H-3 and H-3' to the respective carbonyls. (**Figure 5.3, Table 5.4**).



(66)

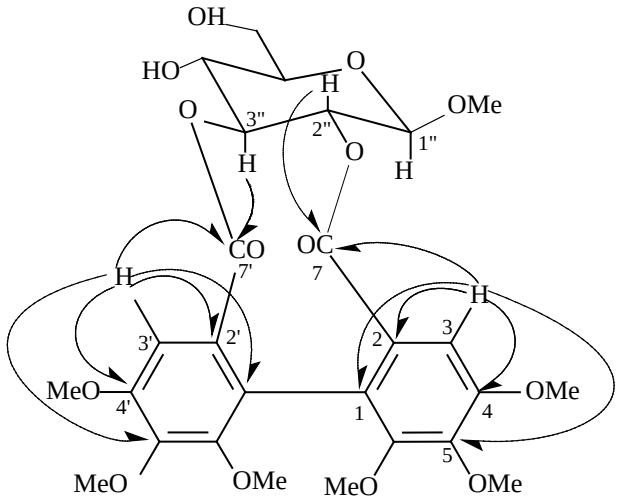


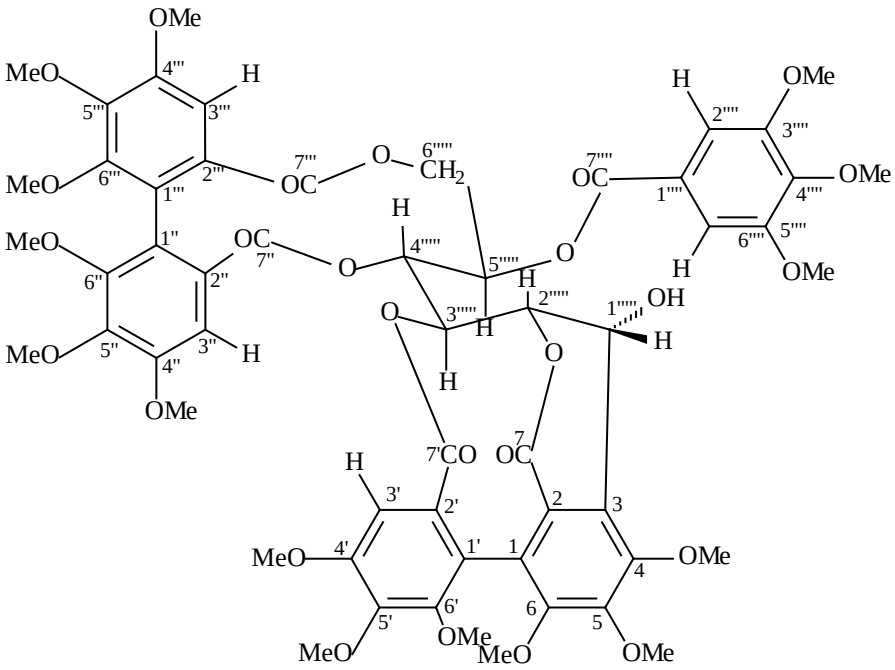
Figure 5.3: Relevant HMBC correlations in heptamethoxy-2,3-β-D-glucopyranosyldiphenoyl ester **(66)**.

Moeties	**Protons**	**δ_H (ppm)**	**Carbon**	**δ_C (ppm)**	**^2J and ^3J (H→C)**
HMDP	1		1	121.6	
	2		2	129.1	
	3	6.80	3	105.0	C-1,-2,-4,-5,-7
	4		4	153.8	
	5		5	144.8	
	6		6	156.0	
	7		7	169.4	
	1		1	121.5	
	2		2	129.2	
	3	6.79	3	105.0	C-1',-2',-4',-5',-7'
	4		4	153.8	
	5		5	144.7	
	6		6	156.0	
	7		7	168.3	
	O*CH*$_3$	3.68-3.99 (x 6)	O*C*H$_3$	55-58 (x 6)	
Glucose	1'''	4.68	1''	101.3	
	2'''	4.96	2''	75.7	
	3'''	5.18	3''	80.2	
	4'''	4.02	4''	68.7	
	5'''	3.51	5''	76.4	
	6'''	3.69	6''	62.7	
	6'''	3.98	6''	62.7	

Table 5.3: NMR data for heptamethoxy-2,3-β-D-glucopyranosyldiphenoyl ester **(66)**.

5.4.2. Pentadeca-*O*-methylcasuarinin (67)

Following column chromatography of the methanol extract on the Sephadex LH-20, fraction A1 was methylated, fractionated on silica gel and purified by repeated PLC to yield the methoxy derivative **67** as clear oil. The ^{1}H NMR spectrum **(Plate 8)** of compound **67** exhibits a simple spin system of three protons, intergrating for one proton each, at δ_H, 7.13, 6.75 and 6.54 and a two-proton singlet (δ_H 7.33) in the aromatic region. The signal at δ_H 7.33 is typical of a galloyl group. ^{1}H→^{13}C correlations in the HMBC spectrum (**Plate 7b**), ^{13}C NMR (**Plate 7a**) and HMQC assisted the allocations of all the carbons in the structure. Absence of the characteristic hemiacetal carbon (δ_C 90.0-100.0) and presence of the five ester carbonyls (**Table 5.4**) in the ^{13}C NMR spectrum (**Plate 7a**) confirms presence of an open chain form of the glucose moiety. The β-linkage of the glucosyl moiety in (**67**) was deduced from the coupling constant (J = 7.0 Hz) in the ^{1}H NMR spectrum. Compound **67** is pentadeca-*O*-methylcasuarinin.[4]



(67)

[4] Okuda, T., Yoshida, T., Ashida, M., Yakazi, K. *J. Chem. Soc. Perkin Trans.* 1. **1983**, 1765.

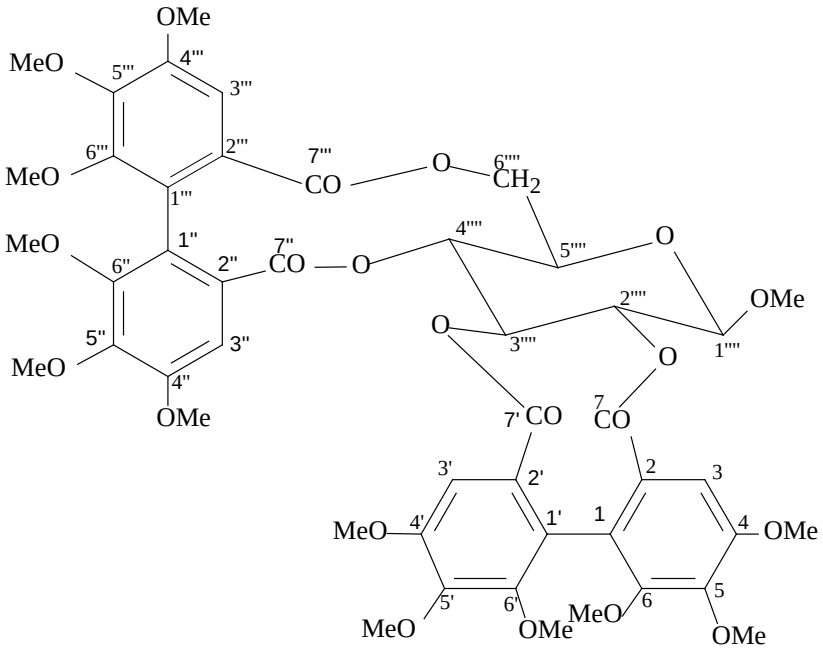
Moeties	**Protons**	**$\delta_{H\ (ppm)}$**	**Carbon**	**$\delta_{C\ (ppm)}$**	**^{2}J and ^{3}J ($^{1}H \rightarrow ^{13}C$)**
HMDPA	1		1	121.7	
	2		2	129.4	
	3		3	106.0	
	4		4	153.6	
	5		5	144.3	
	6		6	152.9	
	7		7	167.6	
B	1'		1'	121.6	
	2'		2'	128.2	
	3'	6.54	3'	103.9	C-1',-2',-4',-5'
	4'		4'	154.6	
	5'		5'	143.7	
	6'		6'	153.0	
	7'		7'	168.5	
C	1"		1"	122.3	
	2"		2"	128.7	
	3"	6.75	3"	105.1	C-1",-2",-4",-5"
	4"		4"	154.0	
	5"		5"	144.6	
	6"		6"	152.6	
	7"		7"	168.7	
D	1'"		1'"	123.0	
	2'"		2'"	128.3	
	3'"	7.13	3'"	107.3	C-1'",-2'",-4'",-5'"
	4'"		4'"	154.1	
	5'"		5'"	146.5	
	6'"		6'"	153.4	
	7'"		7'"	168.4	
Galloyl E	1""		1""	124.8	

	2""	7.33	2""	107.0	C-1"",-3"",-4""
	3""		3""	153.9	
	4""		4""	143.4	
	5""		5""	153.9	
	6""	7.33	6""	107.0	C-1"",-5"",-4""
	7""		7""	165.5	
Glucose F	1"''	4.48	1"''	66.3	
	2"''	4.96	2"''	75.4	
	3"''	5.34	3"''	69.2	
	4"''	5.41	4"''	70.6	
	5"''	5.73	5"''	72.8	
	6"''	5.02	6"''	64.3	
	7"''	4.26	7"''	64.3	

Table 5.4: NMR data for pentadeca-*O*-methylcasuarinin **(67)**

5.4.3. Trideca-*O*-methylpedunculagin (68)

Following the procedure used for isolation of pentadeca-*O*-methylcasuarinin **(67)**, compound **68** was obtained from the same fraction as a methoxy derivative.



(68)

In the ^{1}H NMR spectrum (**Plate 8, Table 5.5**), the simple spin system of four one-proton singlets (δ_H 6.86, 6.80, 6.79, 6.65) displayed in the aromatic region, accompanied with 13 methoxy groups suggest compound **68** to be an ellagitannin. Using ^{13}C NMR (**Plate 8a**), HMQC, and HMBC (**Plate 8b, Figure 5.4**), the relevant carbons were assigned. The two aromatic proton signals at δ_H 6.54 and 6.75 in the ^{1}H NMR spectrum are attributed to H-3 and -3' of the hexahydroxydiphenoyl (HMDP) moiety. The six protons attached to the oxygen-bearing carbons resonating in the aliphatic region are assigned to the sugar moiety *via* sequential coupling from the ^{1}H-^{1}H COSY experiment, with the anomeric proton appearing at δ 4.71. The ^{13}C NMR spectrum exhibits a signal at δ_C 101.5 ppm, indicative of the anomeric carbon of a hemiacetal in a closed glucose moiety. The four carbonyls resonating at δ_C 169.3, 168.1, 167.8, and 167.5 confirm presence of the expected four ester groups in the molecule. The linkage at the acyl groups of HMDP units to the glucose core is evident from the ^{3}J ^{1}H$\rightarrow$^{13}C, correlations of H-3, 3', 3" and 3"' to the respective carbonyl carbons indicated in (**Plate 8b, Figure 5.4**). The β-configuration of the glucosyl moiety was deduced from the large coupling constant (J = 7.0 Hz) of the anomeric carbon in the ^{1}H NMR spectrum. The structure was deduced to be that of trideca-*O*-methylpedunculagin.[110]

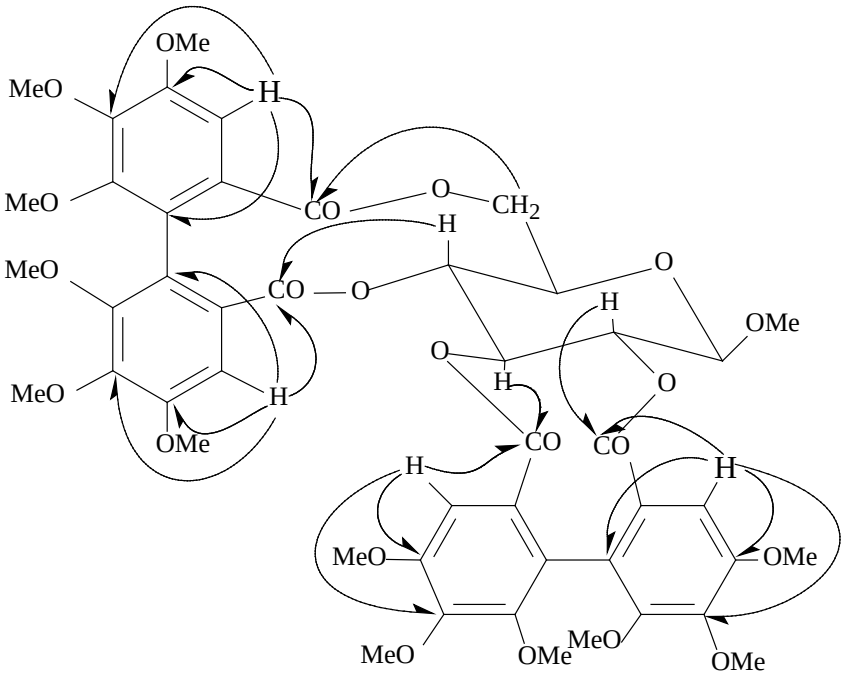


Figure 5.4: Relevant HMBC correlations for trideca-*O*-methylpedunculagin **(68)**

Moeties	**Protons**	**δ_H (ppm)**	**Carbon**	**δ_C (ppm)**	**^2J and ^3J (H→C)**
HMDP	1		1	121.3	
	2		2	130.0	
	3	6.63	3	105.9	C-1,-2,-4,-5
	4		4	145.0	
	5		5	153.7	
	6		6	145.5	
	7		7	169.3	

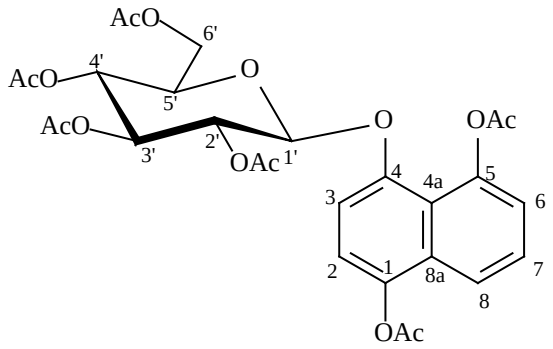
HMDP	1'		1'	122.9	
	2'		2'	127.0	
	3'	6.74	3'	106.0	C-1',-2',-4',-5'
	4'		4'	145.1	
	5'		5'	153.7	
	6'		6'	145.5	
	7'		7'	167.5	
	1"		1"	121.7	
	2"		2"	127.9	
	3"	6.78	3"	105.4	C-1",-2",-4",-5"
	4"		4"	144.7	
	5"		5"	153.3	
	6"		6"	152.8	
	7"		7"	167.8	
	1'"		1'"	123.2	
	2'"		2'"	127.3	
	3'"	6.84	3'"	106.6	C-1'",-2'",-4'",-5'"
	4'"		4'"	145.3	
	5'"		5'"	153.9	
	6'"		6'"	153.0	
	7'"		7'"	168.1	
Glucose	1'"	4.64	1'"	100.1	
	2'"	5.06	2'"	75.9	
	3'"	5.29	3'"	76.7	
	4'"	5.16	4'"	69.4	
	5'"	3.98	5'"	72.5	
	6'"	5.33	6'"	62.6	
	6'"	4.09	7'"	62.5	

Table 5.5: NMR data for trideca-*O*-methylpedunculagin **(68)**

5.4.4. Hexaacetoxynapthalene-4-*O*-β-D-glucopyranoside (69)

Following acetylation and PLC purification, compound **69** was obtained as a peracetate derivative. The ^{1}H NMR spectrum (**Plate 9**) displays five aromatic and seven aliphatic protons (which indicate the presence of a sugar moiety), two aromatic and four aliphatic acetoxy groups. The ^{13}C NMR **(Plate 9a**) of this compound reveals the presence of 28

carbons of which 10 carbons are in the aromatic region indicative of a naphthalene ring, the six oxygen linked carbons are in the aliphatic region as well as the ones for six acetoxy groups. The proton-bearing ^{13}C resonances were assigned with the HMQC experiment (**Table 5.5**), while the rest of the carbons **(Plate 9a)** were assigned from the HMBC experiment **(Plate 9b)**. The NMR data of compound **69** is presented in **Table 5.5.**



(69)

The protons δ_H 7.09, H-2, J = 8.0 Hz and δ_H 7.21, H-3, J = 8.0 Hz, are typical of the *ortho* doublets in an aromatic ring. The remaining three protons are observed as two doublets H-8 at δ_H 7.76, H-6 at δ_H 7.15 and a triple H-7 at δ_H 7.55, all resonating as a system. The assignment was confirmed by ^1H-^1H COSY spectrum.

The deshielded carbons at δ_C 142.6 and 151.8 are assigned to the oxygen bonded carbons (C1 and C4, respectively) in the HMQC spectrum and the quarternary carbon signal at δ_C 130.02 corresponds to C8a. HMBC correlations of both H-2 and -3 to C1 and C-4 (**Figure 5.5**) confirm the *ortho* coupling of H-2 and H-3. HMBC correlations ^3J (strong) of H-8 to C4a and ^2J (weak) of H-8 to C-1 confirm the allocation of H-8, while, the correlations of H-6 to C4a and C-5 support the assignment of H-6 (**Figure 5.6**).

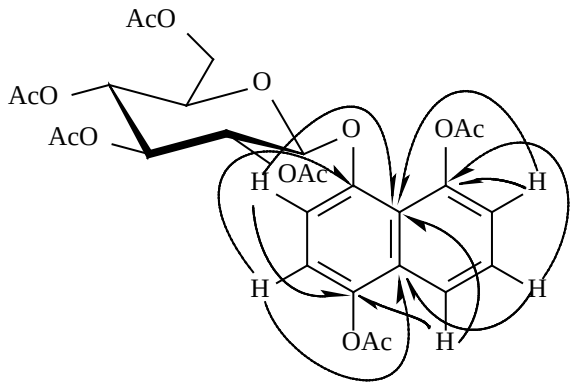


Figure 5.5: Relevant HMBC correlations for hexaacetoxynapthalene-4-*O*-β-D-glucopyranoside **(69)**

In the NOESY spectra **Plate 9c**, the strong association observed between the anomeric proton H-1' of the sugar and H-3 in the aromatic region confirms the 1'→4 glucosidic linkage of the glucosyl moiety to the aglycone (**Figure 5.6**). Protons of the glucosyl moiety are allocated by sequential coupling of H-1' to H-6'. The resonance of H-1' at δ_H 5.25 with large coupling constant (J = 8.0 Hz) confirms the β-configuration of the glucose moiety.

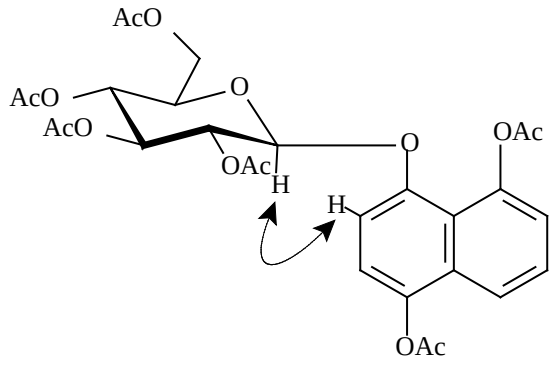


Figure 5.6: Relevant NOESY correlations for hexaacetoxynapthalene-4-*O*-β-D-glucopyranoside **(69)**

Proton	**δC, ppm**	**Carbons**	**δC, ppm**	**^{2}J and ^{3}J (H→C)**
1	-	1	142.6	-
2	7.20 (d, J 8.5 Hz)	2	118.6	C-1, -4, -8a
3	7.09 (d, J 8.5 Hz)	3	111.1	C-1, -4, -4a
4	-	4	151.8	-
4a	-	4a	121.5	-
5	-	5	146.9	-
6	7.17 (d, J 8.5 Hz)	6	121.3	C-4a, -5
7	7.53 (t, J 8.5 Hz)	7	127.4	C-5, -8a
8	7.75 (d, J 8.5 Hz)	8	112.0	C-1, -4a
8a	-	8a	130.0	-
1'	5.25 (d, J 7.0 Hz)	1'	101.4	
2'	overlapped	2'	72.65	
3'	overlapped	3'	73.65	
4'	overlapped	4'	68.35	
5'		5'	72.06	
6'		6'	62.31	
6		6		

Table 5.6: NMR data for hexaacetoxynapthalene-4-*O*-β-D-glucopyranoside **(69)**

6. Non-phenolics

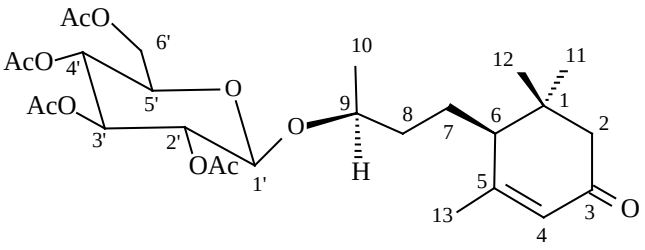
6.1. Introduction

Although numerous biological activities useful to mankind have been attributed to most phenolic compounds, not all uses of non-phenolics compounds have been established. Among those established are those of the carbohydrates, which are essential nutrients that readily provide energy to animals and plants. Sitosterols on the other hand are not important components of our atherogenic diet, the concentrations of 3-OH-sterols reflect concentrations of cholesterol. A bisnorsesquiterpen, sitosterols and sugars are the non-phenolics which have been isolated from the methanol extract of Walnuts *Juglans regia* L.

6.2. Bisnorsesquiterpene

6.2.1. Tetra-*O*-acetyl-9-β-D-glucopyranosylmegastigmen-3-one

PLC purification of the acetylated methanol extract afforded the *O*-methyl derivatives **70** as colourless oil. The ^{1}H NMR spectrum (**Plate 10**) exhibits a dieshielded methyl singlet at δ_H 2.02 two angular methyl singlets (δ_H 1.01, 1.03), a methyl doublet at δ_H 1.17, five aliphatic protons (δ_H 1.3-1.9), six oxomethines and four methylene groups (**Table 6.1**). The ^{13}C spectrum (**Plate 10a**) of compound **70** shows 19 carbon signals, identified by DEPT experiment (**Plate 10b**) as carbons belonging to four methyls, four methylenes, seven aliphatic methines, one olefinic, three quaternaries and a carbonyl carbon of a ketone (δ_C 199.9), (**Table 6.1**).



(70)

The broadened singlet at δ_H 5.85 (δ_C 125.5) typical of a tri-substituted double bond strongly correlates *via* HMBC to the methine C-6 (δ_C 51.3) and C-13 in the HMBC spectrum (**Plate 10c), (Figure 6.1)**. Strong NOESY association (**Plate 10d, Figure 6.2**) of the methyl singlet H-13 to H-4 confirms the presence of a tri-substituted double in the molecule. HMBC correlations of H-6 (δ_C 50.9), to a quaternary C-1 and to C-7 of a methylene group confirm the suggested arrangement. Further confirmation is by strong ^{1}H-^{1}H COSY correlations of H-7 to both H-6 and -8 (**Plate 10e**). ^{1}H-^{1}H COSY correlation of H-8 to H-9 confirms the chain progression. Strong HMBC correlations from the deshielded oxomethine, H-9 to C-7 and C-10 confirm arrangement.

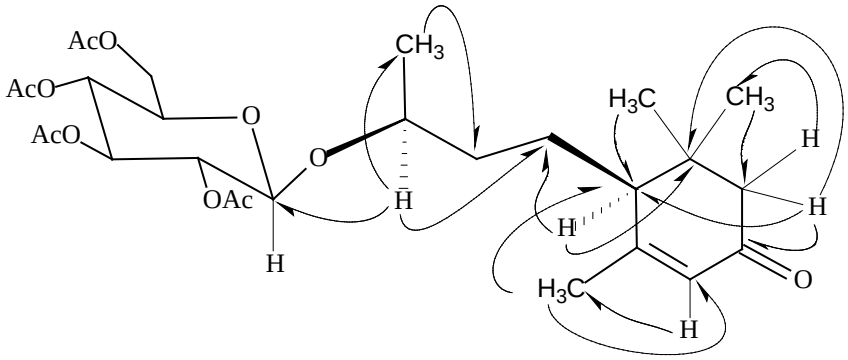


Figure 6.1: Selected HMBC correlations of tetra-*O*-acetyl-9-β-D-glucopyranosylstigmen-3-one **(70).**

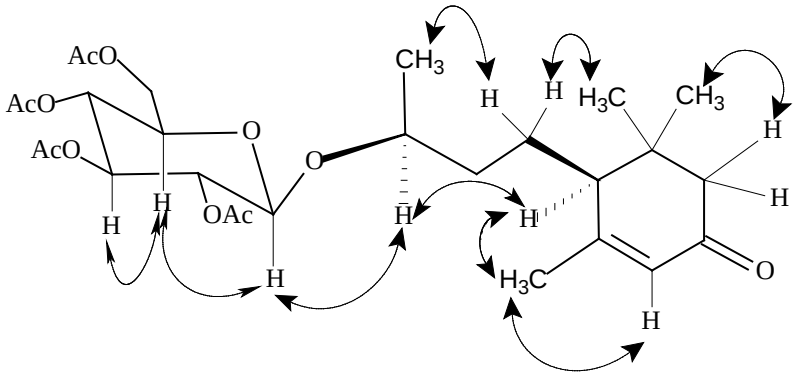


Figure 6.2: Selected NOESY associations of tetra-*O*-acetyl-9-β-D-glucopyranosylstigmen-3-one **(70).**

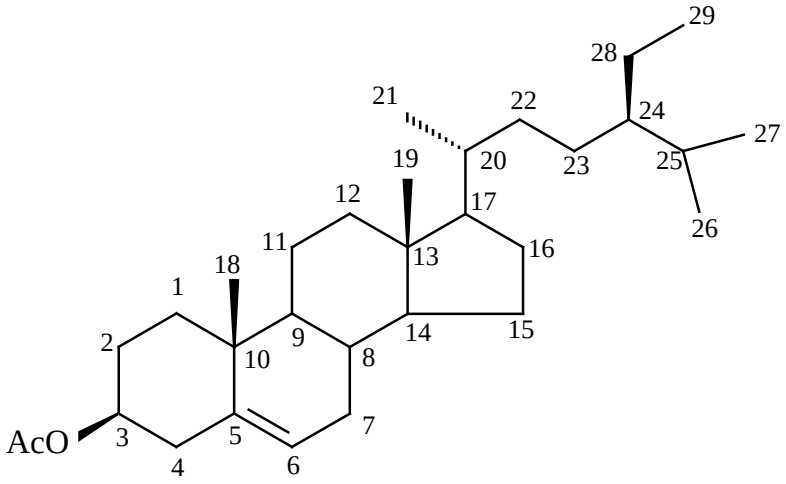
The remaining methylene group H-2 (δ_H 2.03 and 2.30) is characteristic of protons alpha to the carbonyl functionality. HMBC correlations of H-2 to C-1, -6 and-11 unambiguously confirm the substituted cyclohexanone skeleton in the molecule. The sugar moiety is established from the COSY spectrum (**Plate 10e, Table 6.1**) as a glucosyl. The coupling constant J = 7.0 Hz of the anomeric proton and the 1,3,5-triaxial NOESY correlations (**Figure 6.2**) confirm the β-configuration. Connectivity of the glucose unit to the aglycone is established from the HMBC correlation of H-9 to C-1' and confirmed by NOESY correlation of H-9 with H-1'. The relative configuration at C-6 and C-9 is established from NOESY associations of H-6 to H-9 and H-13. Compound **70** showed a HREIMS with a molecular ion at m/z 544.1525 consistent with a molecular formula of $C_{15}H_{22}O_4$.

Protons	**δ_H (ppm)**	**Carbon**	**δ_C (ppm)**	**^2J and ^3J (H→C)**
1		1	38.0	
2	2.36 (d, J 18.0 Hz)	2	47.7	C-1, -3, -6, -11, -12
	2.03 (d, J 18.0 Hz)			
3		3	199.9	
4	5.85 (bs)	4	125.5	C-6, -13
5		5	163.5	
6	1.88s	6	51.3	
7	1.38 (m)	7	26.1	C-1, -6
	1.47 (m)			
8	1.57 (m)	8	36.6	
9	3.70 (m)	9	76.1	C-7, -10
10 (C*H*$_3$)	1.17 (s)	10 (*C*H$_3$)	20.6	C-9, -10
11 (C*H*$_3$)	1.01 (s)	11 (*C*H$_3$)	29.3	C-1, -2, -6, -12
12 (C*H*$_3$)	1.03 (s)	12 (*C*H$_3$)	27.7	C-1, -2, -6, -11
13 (C*H*$_3$)	2.02 (s)	13 (*C*H$_3$)	25.2	C-4, -6
1'	4.56 d (J 8.0 Hz)	1'	99.9	
2'	4.98 dd (J 10, 8.0 Hz)	2'	72.1	
3'	5.25 t (J 10 Hz)	3'	73.3	
4'	5.11 t (J 10 Hz)	4'	68.9	
5'	3.7 m	5'	71.8	
6'	4.26 dd (J 12.5, 4.5 Hz)	6'	62.4	
6'	4.18 dd (J 12.5, 2.5 Hz)	6'	62.4	

Table 6.1: NMR data of tetra-*O*-acetyl-9-glucopyranosylmegastigmen-3-one **(70).**

6.3. Sitosterols

6.3.1. 3-*O*-Acetoxysitosterol



(71)

Compound 71 was isolated as a monoacetate derivative from the methanol extract. The ^{1}H NMR spectrum of compound 71 (Plate **12**, Table 6.2) shows two tertiary methyls at δ 0.69 (*Me*-18) and 1.01 (*Me*-19), four secondary methyls at δ 0.99 (Me-21), 0.89 (*Me*-26), 0.82 (*Me*-27) and 0.82 (*Me*-29), and a carbinol proton at 4.72 (H-3). The same spectrum shows a broadened singlet at δ_H 5.44 (H-6) characteristic of tri-substituted olefinic proton, and only one acetoxy group at δ 2.0. The ^{13}C NMR spectrum (**Plate 11a**) reveals 31 carbon signals whose degree of protonation was determined by DEPT (**Plate 11b**) and HMQC experiments. The DEPT spectrum shows six methyl, 11 methylene and eight methine carbon signals. The olefinic resonance at δ_C 123.1 ppm corresponds to the C-6 olefinic methine while the signal at δ_C 140.1 corresponds to the quartenary carbon (C-5). Compound **71** is an *O*-acetyl derivative of a known β-sitosterol.[1]

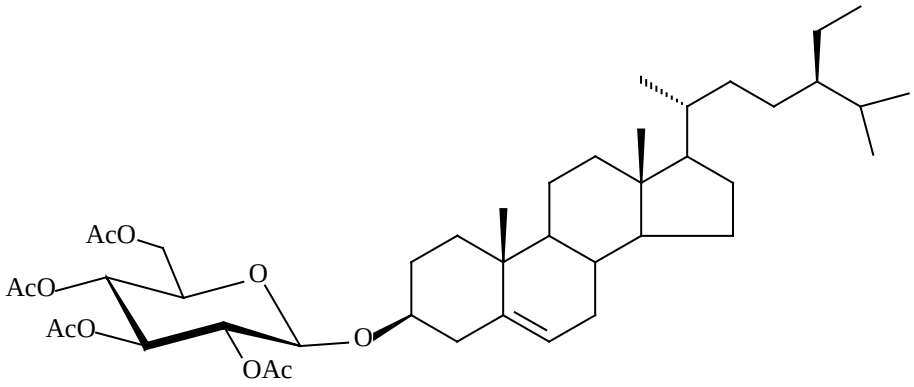
[1] Rowe, J. W. *Natural products of woody plants II.* Ed. Rowe, J. W., London, **1989**, 808.

Proton	**Compound 71** δ_H	**Compound 72** δ_H
Me-18	0.69	0.70
Me-19	1.01	1.04
Me-21	0.99	0.97
Me-26	0.89	0.86
Me-27	0.82	0.85
Me-29	0.82	0.55
H-3	4.72	3.70
H-6	5.44	5.38
H-1'	-	4.61
H-2'	-	4.78
H-3'	-	5.21
H-4'	-	5.09
H-5'	-	3.51
H-6'	-	4.28
H-6'	-	4.12

Table 6.2: NMR spectra of compounds **71** and **72**

6.3.2 Tetra-*O*-acetoxysitosterol-3-*O*-β-D-glucopyranoside (72)

Purification of the acetylated methanol extract yielded compound **72** as clear oil. In comparison to compound **71**, the ^{1}H NMR spectrum (**Plate 12**) of compound **72** displays protons with similar chemical shifts and seven additional protons attached to oxygen-bearing carbons resonating between δ_H 5.43 and 4.8 (are accompanied by four aliphatic acetoxy groups). The additional seven oxomethine protons (**Table 6.2**) of the glucose moiety are sequentially assigned from H-1' to -6' using the COSY experiment. The resonances at δ_C 122.7 and δ_C 140.7 in the ^{13}C NMR spectrum (**Plate 12a**) correspond to the olefinic C-6 and the quaternary C-5, respectively, of the sitosterol moiety. All the carbons in the molecule are assigned using data from the DEPT (**Plate 12b**), HMQC, and HMBC experiments. The glycosidic linkage of the glucosyl to the aglycone is confirmed by NOESY association of H-1' to H-2, -3 and -4, **Figure 6.3**.



(72)

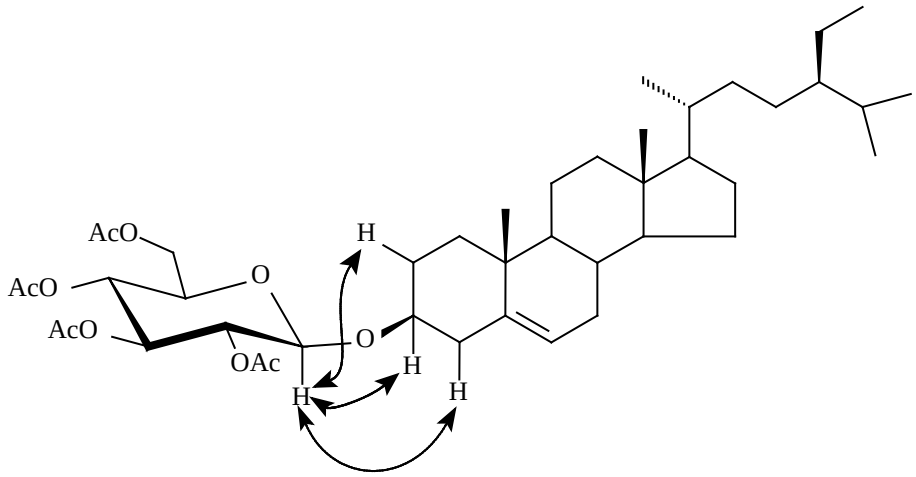


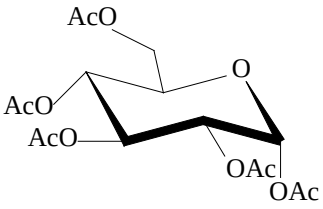
Figure 6.3: Relevant NOESY associations showing the connectivity of the glucosyl to the aglycone in compound **72**.

6.4. Carbohydrates

Due to limited time we were able to isolate only monomeric and dimeric carbohydrates from the complex column B2 whose TLC indicated the presence of sugars.

6.4.1. Monosaccharides

6.4.1.1. Penta-*O*-acetyl-α-D-glucopyranoside



(73)

The ^{1}H NMR spectrum of penta-*O*-acetyl-α-D-glucopyranoside (**Plate 13, Table 6.3**) displayed seven aliphatic protons assignable to H-1 to -7 resonating between δ 4.0 and 6.4. These are accompanied by five aliphatic acetoxy groups. The small coupling constant (J = 4.0 Hz) of H-1 indicates the α-configuration. The 1,2,4-triaxial NOESY correlations between H-1, H-2 and H-4 confirm the α-configuration.

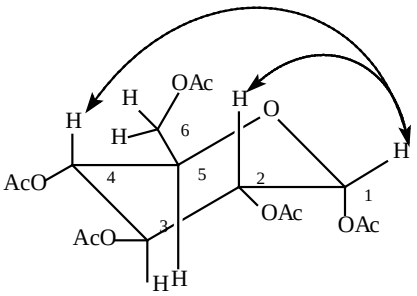


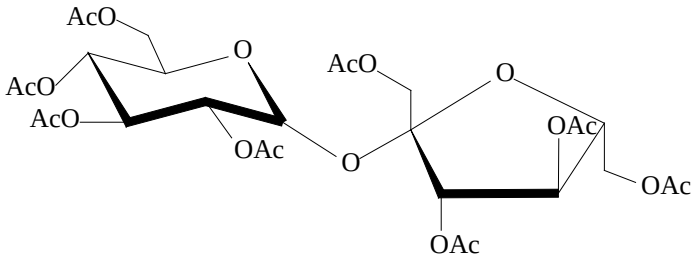
Figure 6: NOESY associations of penta-*O*-acetyl-α-D-glucopyranoside **(73)**

Proton	**Compound 73 $CDCl_3$, 298 K**
1-H	6.35 (d, *J* 4.0 Hz)
2-H	5.16 (t, *J* 10.0 Hz)
3-H	5.49 (t, *J* 10.0 Hz)
4-H	5.12 (dd, *J* 10.0, 4.0 Hz)
5-H	4.15 (m)
6-H (x 2)	4.30 (dd, *J* 13.0, 5.0 Hz)
	4.16 (dd, *J* 13.0, 2.0 Hz)
5 (x OAc)	2.12 x 2, 2.06, 2.05, 2.04 (s)

Table 6.3 : ^{1}H NMR data of penta-*O*-acetyl-α-D-glucopyranoside **73**.

6.4.2. Disaccharides

6.4.2.1. Octa-*O*-acetyl-α-D-glucopyranosyl-β-D-fructofuranoside



(74)

The ^{1}H NMR spectrum **(Plate 14)** of the acetate derivative of compound **74** displays eight oxomethine protons and three methylene groups attached to oxygen-bearing carbons. The ^{1}H NMR spectral data of **74** was found to correspond with that of the acetylated sucrose authentic sample. The α-configuration of the glucose moiety was assigned from the coupling constant (J = 3.0 Hz) of the anomeric proton H-1'' and the connectivity of the two moieties were confirmed by n.O.e association of H-1 with H-4" in the NOESY experiment.

Ring	**Protons**	**Compound 74 C_6D_6, 298K**
pyranosyl	1-H	5.94 (d, J 3.0
	2-H	5.14 (dd, J 10.0, 3.0 Hz)
	3-H	5.94 (dd, J 10.0, 9.0 Hz)
	4-H	5.45 (dd, J 10.0, 10.0 Hz)
	5-H	4.63 (m)
	2 × 6-H	4.43-4.57 (m)
furanosyl	C_1-CH_2	4.39 (d, J 12.0 Hz)
		4.38 (d, J 12.0 Hz)
	2'-H	5.82 (d, J 6.0 Hz)
	3'-H	5.65 (dd, J 6.0, 6.0 Hz)
	4'-H	4.30 (dd, J 10.0, 6.0 Hz)
	2 × 5'-H	4.43-4.57 (m)
	8 × -OAc	1.65-2.0 (s)

Table 6.4: ^{1}H NMR data of octa-*O*-acetyl-α-D-glucopyranosyl-β-D-fructofuranoside **(74).**

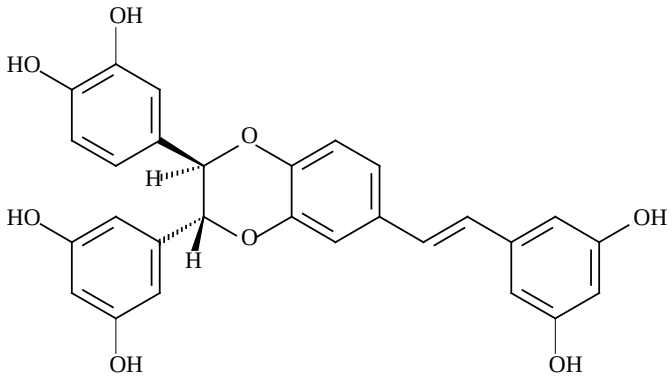
CHAPTER 7

PART B

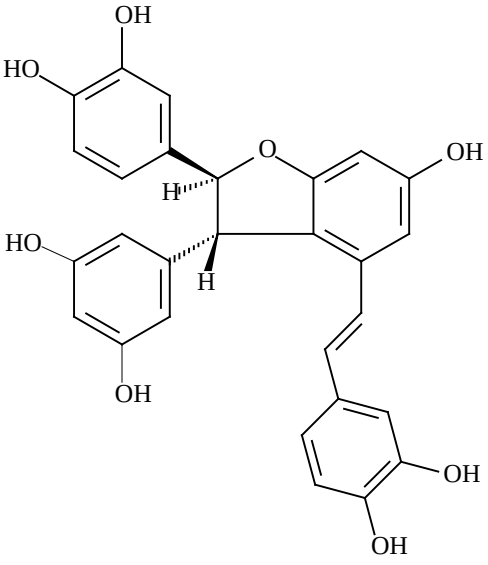
7. Sythesis of the stilbenes

7.1. Introduction

This part of the study addresses synthesis of the monomeric and dimeric stilbenes from *Afrormosia elata*. The people of the Republic of Congo use the bark of *A. elata* to treat cancer. Naturally occurring dimeric stilbenes with varied hydroxylation patterns (e.g. Maackin **(75)** Scirpusin B **(76)**,[113,114] and 3-(3,5-dihydroxyphenyl)-2-(3,4-dihydroxyphenyl)-6-hydroxy-2,3-dihydrobenzofuran-4-carbaldehyde **(77),** which have piceatanol as the precursor monomer are present in the heartwood of *Afrormosia elata*.[10]

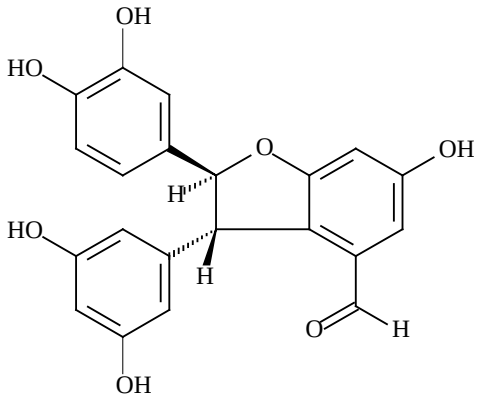


(75)



(76)

[113] Fedoreyev, S. A., Pokushalova, T. V., Veselova, M. V., Glebko, L. I., Kulesh, N. I., Muzarok, T. I., Seletskaya, L. D., Bulgakov, V. P., Zhuravlev, Y. N. *Fitoterapia*, **2000**, *71*, 365.
[114] Yang, G. X., Zhou, J. T., Li, Y. Z., Hu, C. Q. *Planta med*., **2005**, *71*, 596.



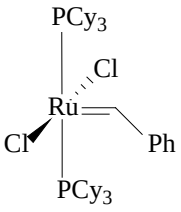
(77)

Generally, stilbenes have been found to exhibit biological activities, such as, chemoprevention of cancer,[115] protein kinase C inhibition,[116] anti-HIV and cytotoxity,[117] anti-fungal,[118] and cyclooxygenase (COX I, COX II) inhibition.[119,120] Natural dimeric stilbenes exhibit potent anti-inflammatory activities, including inhibition of leukotriene (LTB_4, C_4, D_4) and its receptor antagonism, affinity of HL 60, and CEC multiplet in *vitro* and in *vivo* models.[121,122] Scirpusin B **(76)** has been found to be active against HIV, with EC_{50} values of 7μ/mL[114]. Piceatannol (**52**), the major phenolic constituent monomer of A. *elata*, has been identified as the active ingredient of the species used in traditional herbal medicine,[123] and it acts as an anti-inflammatory and anti-proliferative plant-derived stilbene[124]. While valuable to humans, stilbenes like other secondary metabolites are often very difficult to isolate in large quantities, because they are available in such low amounts. Biotechnology has not been very efficient in enabling scientists to up regulate the biosynthesis of many of the valuable secondary metabolites, and as a result, some of the products of secondary metabolism can be very expensive to acquire. The diverse activities and low natural abundance of the dimeric stilbenes from *Afrormosia elata* when coupled with

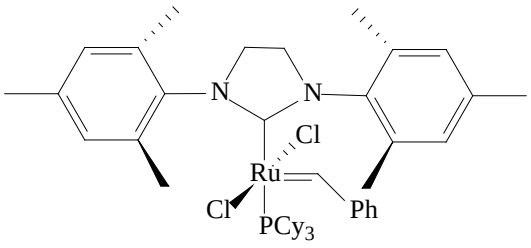
[115]Jang, M., Cai, L., Udeani, G. O., Slowing, K. V., Thomas, C. F., Beecher, C. W. W., Fong, H. H. S., Farnsworth, N. R., Kinghorn, A. D., Mehta, R. G., Moon, R. C., Pezzuto, J. M. *Science*, **1997,** *275*, 218.
[116] Kulanthaivel, P., Janzen, W. P., Ballas, L. M., Jiang, J. B., Hu, C. Q., Darges, J. W., Seldin, J. C., Cofield, D. J., Adamas, L. M. *Planta Med.*, **1995**, *61*, 41.
[117] Dai, J. R., Hallock, Y. F., Cadellina II, J. H., Boyd, M. R. *J. Nat. Prod.,* **1998**, *61*, 351.
[118] Ducrot, P. H., Kollmann, A., Bala, A. E., Majira, A., Kerhoas, L., Delorme, R., Einhorn, J. *Tetrahedron Lett.,* **1998,** *39*, 9655.
[119] Cichewicz, R. H., Kouzi, S. A., Hamann. M. T. *J. Nat. Prod.,* **2000**, *63*, 29.
[120] Chen, J. Master Thesis**,** *Chinese Academy of Medical Sciences and Perking Union Medical College***, 1997**.
[121] Li, Y. T., Zhong, M., Deng, Y. J., Zhu, X. Y., Cheng, G. F. *Acta Pharm. Sin.*, **1999**, *34*, 189.
[122] Hu, Y. N., Zhu, X. Y., Liang, X. L., Cheng, G. F. *Acta Pharm. Sin.*, **2001**, *36*, 81.
[123] Tsururnga, T., Chun, T. T., Ebizuka, Y., Sankawa, U. *Chem. Pharm. Bull.* **1991**, *39*, 3276.
[124] Wung, B. S., Hsu, M. C., Wu, C. C., Hsieh, C. W. *Pharmacological Research.*, **2006**, *53*, 113.

their molecular complexity warrant the development of new and efficient synthetic methods and strategy for the total synthesis of these compounds. This study aims at exploring and establishing synthetic methods towards the synthesis of both the monomeric and dimeric stilbenes.

Drawbacks, including low yields and lack of stereoselectivity (i.e. generation of *E* and *Z* isomers) make the classic methods such as the Wittig reaction unappealing. Owing to the development of transition metal-assisted organic syntheses,[125] several catalytic approaches have been proposed to achieve better selectivities in the preparation of stilbene derivatives.[126] Most strategies employed so far involve palladium-catalyzed reactions e.g. the Heck reaction,[127,128] which stands out for its synthetic versatility and efficiency. Other transition metal-promoted stilbene syntheses rely upon the olefin metathesis based on ruthenium catalysts e.g. Grubbs I **(77)**[129,130] and Grubbs II **(78)**.[131,132,133,134,135] It is worth noting that so far olefin cross metathesis reactions have found little application in the synthesis of stilbenes.



(77)



(78)

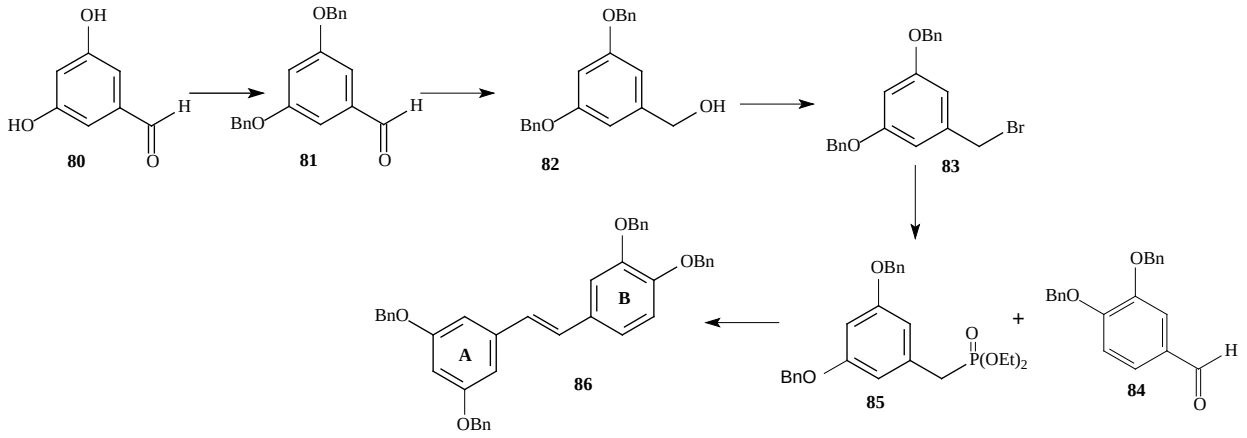
[125] Mori, M., Kuzuba, Y., Kitamura, T., Sato, Y. *Org. Lett.*, **2002,** *4*, 3855.
[126] Ferre-Filmon, K., Delaude, L., Democeau, A., Noels, A. F. *Coord. Chem. Rev.*, **2004**, *248*, 2323.
[127] Thomas, N. F., Lee, K. C., Paraidathathu, T., Weber, J. F. F., Awang, K., Rondeau, D., Richomme, P. *Terahedron.* **2002**, *58*, 7201.
[128] Jeffrey, T., Ferber, B. *Tetrahedron lett.*, **2003**, *44*, 193.
[129] Scholl, S.; Ding, S., Lee, C. W., Grubbs, R. H. *Org. Lett.*, **1999**, *1*, 953.
[130] Sanford, M. S., Love, J. A., Grubbs, R. H. *J. Am. Chem. Soc.* **2001**, *123*, 6543.
[131] Schrock, R. R., Murdzek, J. S., Bazan, G. C., Robbins, J., DiMare, M., O'Regan. *J. Am. Soc.*, **1990**, *112*, 385.
[132] Chang, S., Na, Y., Shin, H. J., Choi, E., Jeong, L. S. *Tetrahedron lett.*, **2002**, *43*, 7445.
[133] Schwab, P., France, M. B., Ziller, J. W., Grubbs, R. H. *Angew. Chem.*, Int. Ed. Engl. **1995**, *34*, 2039.
[134] Schwab, P., Grubbs, R. H., Ziller, J. W. *J. Am. Chem. Soc.*, **1996**, *118*, 100.
[135] Belderrain, T. R., Grubbs, R. H. *Organometallics.* **1997**, *16*, 4001.

7.2. Synthesis of monomeric stilbenes

We aimed at synthesizing free phenolic stilbenes which would then be subjected to bioassays and therefore, verify the claimed biological applications. While the protected stilbene monomers for synthesis of the dimeric compounds were prepared *via* the stoichiometric Wittig reaction, the emphasis for the syntheses of the free phenolic compounds was on utilising the rhuthenium catalyzed methathesis (RCM) reaction. Benzylation was the protection method of choice for the Wittig approach, because the benzyl groups are not very labile in acidic or basic media, making these groups stable in the subsequent reaction. Also debenzylation is relatively a milder process than other deprotection procedures, for example demethylation. Since literature precedents for free phenolic metathesis reactions do exist,[135] no protecting of OH functions were required for this approach.

7.2.1. The Wittig route (Scheme 7.1)

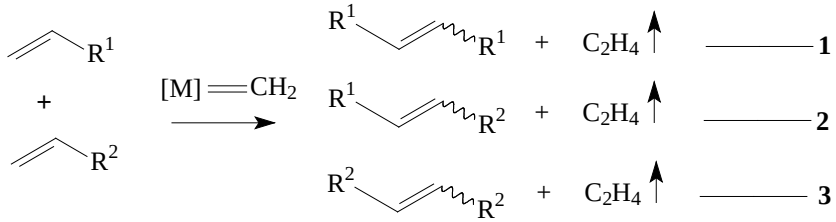
3,5-Dihydroxybenzaldehyde (**80**) was benzylated and the resulting 3,5-dibenzyloxybenzaldehyde **(81) (Plate 16)** was successfully reduced with $NaBH_4$ to 3,5-dibenzyloxybenzylalcohol **(82) (Plate 17)**, which was subsequently brominated using phosphorous tribromide to yield the 3,5-dibenzyloxybenzylbromide **(83)** (80%). The Arbuzov reaction of benzyl bromide with triethylphoshite (**85**) produced the phosphonate in relatively good yields (~70%). Coupling of the phosphonate with 3,4-dibenzyloxybenzyladehyde (**84**) **(Plate 18)** using sodium methoxide as a base in DMF gave the protected stilbene in 70% yield. The Horner-Emmons step using diethyl phosphonate in this case selectively yielded tetra-*O*-benzylpiceatannol (**86) (Plate 19), (Scheme 7.1)**.



Scheme 7.1: Synthesis of tetra-*O*-benzylpiceatannol **(86)**

7.2.2. Cross coupling metathesis route (Scheme 7.2)

Of key importance in the transition metal-catalyzed syntheses, is the efficient formation of the C=C bond. Investigations of the synthesis of olefins *via* cross metathesis (**Scheme 7.2**) (reactions **1, 2** and **3**) includes olefins of several classes, i.e. substituted and functionalized styrenes, secondary allylic alcohols, tertiary allylic alcohols, and olefins with R-quaternary centres.



Scheme 7.2: Olefin cross-metathesis reaction.

In the olefin metathesis reactions, a unique carbon skeleton redistribution in which unsaturated carbon-carbon bonds are rearranged in the presence of metal carbene complexes takes place.[136,137] The uniqueness of the olefin metathesis reaction lies in the utilization of only catalytic amounts of metal carbene and formation of volatile olefins, such as ethylene, as the

[136] Grubbs, R. H., Pine, S. H. in C*omprehensive Organic Synthesis*, Eds. Trost, B. M., Fleming, I., Paquette, L. A., Pergamon: New york, **1991**, *5*, Chapter 9.3.
[137] Shrock, R. R. in *The Strem Chemiker, Strem Chemicals: Newburgpot*, **1992**, *XIV*, 1.

only by-products. The cross metathesis reaction is, however, hampered by the formation of self-metathesis products (reactions **1** and **3** above). Since the formation of self-metathesis products always complicates the cross metathesis reactions, it was decided to investigate the effect of different levels of electron donation on the aromatic ring on the self- *vs.* cross- metathesis product ratio. If electronic effects would have an influence on this ratio, it might be possible to tame the cross metathesis reaction away from the statistical product distribution and focus it towards the desired products. In order to study the effect of the hydroxylation pattern on the CM reaction, a series of OH-substituted styrenes had to be synthesized.

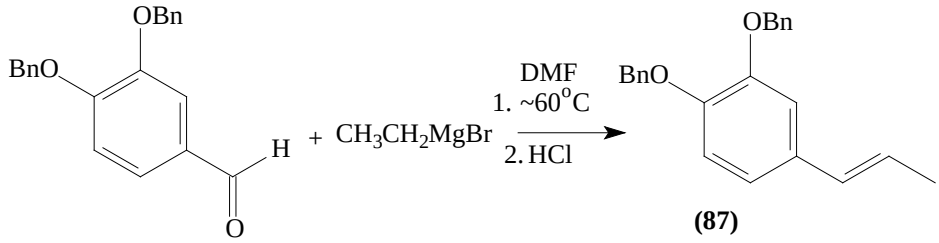
7.3. Synthesis of the styrenes

Considering that styrenes are very unstable compounds, several methods were explored in their synthesis with the aim of optimizing the yields. In all the methods, aldehydes or acetophenones were used as precursors. An interesting observation which eventually was a good indicator of the product formation was appearance of a light purple colour in the reaction mixtures. After repeating the reactions several times, it was observed that a persistent light purple colour in the reaction mixture was an indication that only ethers were present as the products. This colour formed instantly in some of the reactions.

7.3.1. By utilising the Grignard reaction

Since aldehydes of with varied hydroxylation patterns required for flavonoid synthesis are readily available, it was decided to start the styrene synthetic protocol by utilising a Grignard approach for preparing benzyl alcohols, which could subsequently be dehydrated to produce the required styrenes. Methylated-3,4-dihydroxybenzaldehyde was therefore reacted with EtMgBr to form the benzylic alcohol (**Scheme 7.3**) , which on addition of HCl yielded the corresponding styrene. On purification, however, the expected product was obtained in very low yields. The ^{1}H NMR spectrum of compound **87** (**Plate 20**) shows resonances at δ_H 6.12 (m H-β), δ_H 6.41 (d, J = 16 Hz, H-α), H-2, -5 and -6, (δ 6.9-7.1overlapping) and a methyl group at δ_H1.95 (d, J = 7.0Hz). Although the reaction was repeated with 3,4-benzyloxybenzaldehyde, no improvement in yield was observed after purification. Since it was thought that the acid catalyst used in the elimination

of the OH from the alcohol could be responsible for polymer formation during this approach, this method was suspended.

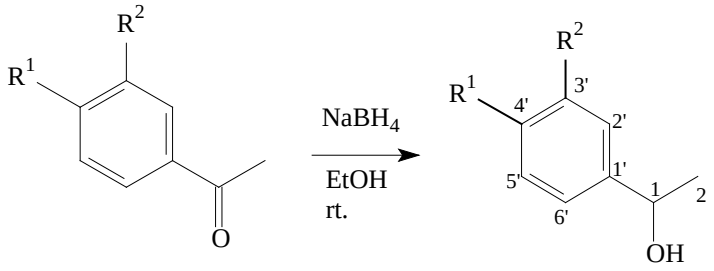


(87)

Scheme 7.3: Synthesis of styrene β-methyl styrenes

7.3.2. Synthesis of styrenes *via* dehydration of the corresponding phenyl alcohols.

As an alternative to starting from the aldehyde it was subsequently decided to utilize acetophenones as starting materials in the styrene synthesis. Since these compounds with the required oxygenation patterns are also readily available and they could easily be reduced to the benzyl alcohol through mild procedures that would not require the addition of acids during work-up, this seemed to be a better approach than the Grignard methodology. The required alcohols were therefore produced in excellent yields (98-100%) according to **Scheme 7.4** from the corresponding acetophenones by $NaBH_4$**.** With the phenyl alcohols in hand, attention was subsequently turned towards the dehydration step. In this regard H_2SO_4, H_3PO4, and anhydrous $CuSO_4$ were evaluated as dehydrating agents.



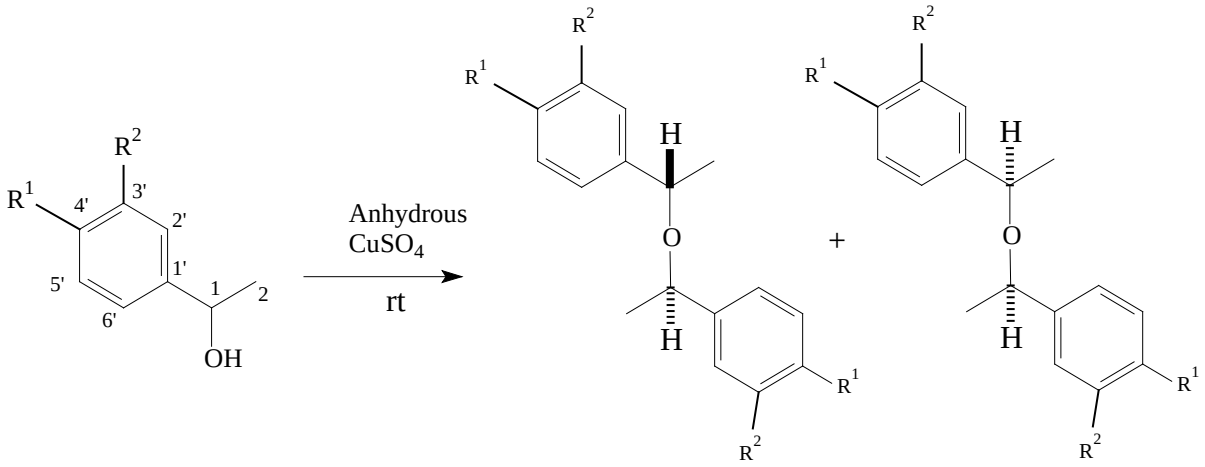
(88) R^2 = H, R^1 = OMe

(89) R^1= R^2 = OMe

(90) R^2 = H, R^1 = OMe

(91) R^2 = H, R^1 = OMe

Scheme 7.4: Preparation of the phenyl alcohols



(90) R^2 = H, R^1=OMe
(91) R^1= R^2 = OMe
(92) R^2 = H, R^1=OMe
(93) R^1= R^2 = OMe
(94) R^2 = H, R^1=OMe
(95) R^1= R^2 = OMe

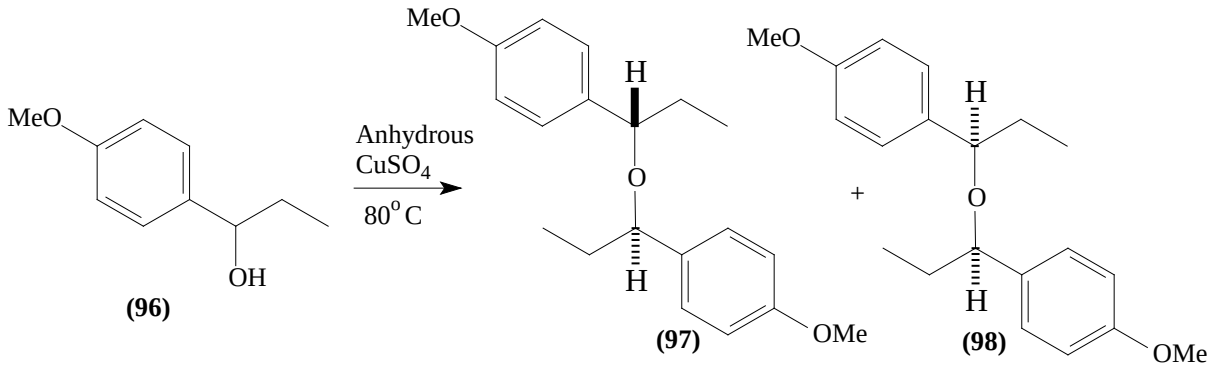
Scheme 7.5: Formation of ethers from the phenyl alcohols

Thus, refluxing of the alcohols **90** and **91** with 85% H_3PO_4 or 50% H_2SO_4 for 100 min as described in literature,[138] gave only polymeric products (analyzed by ^{1}H NMR) which could not be resolved. Repeating the reaction with shorter reaction times (40, 20, and 10 min) led to the same result, while lowering the temperature also had no beneficial effect.

To avoid working with acids and having water in the reaction which could lead to an equilibrium being set-up, anhydrous $CuSO_4$ was subsequently employed as a dehydrating agent. Initially the procedure described in literature,[139] whereby the reaction mixture of the appropriate alcohol (3,4-dimethoxyphenylethanol) anhydrous $CuSO_4$ in hexane was refluxed for ~2 hours was followed (**Scheme 7.5**). While only trace amounts of the starting material could be detected, TLC of the reaction mixture showed two intense spots and a very light one. PLC purification yielded two compounds for each alcohol, which were analyzed by ^{1}H NMR spectroscopy. Analysis of their ^{1}H NMR spectra showed that alcohol **90** (**Plate 21**) yielded the ethers **92** and **94** (**Plates 22** and

[138] Bethell, Newall and Whittaker, *J. Chem. Soc.*, **1971**, 23.
[139] Nishiguchi, T., Machida, N., Yamamoto, E. *Tetrahedron Lett.*, **1988**, 4565.

23, respectively)[140] while alcohol **91** (**Plate 24**) gave the ethers **93** and **95** (**Plates 25** and **26,** respectively). ^{1}H NMR spectra of the alcohols and ethers show almost identical peaks, with the only noticeable difference being the shift in the resonances of the methine protons. In the ^{1}H NMR spectra of the ethers the methine protons are shielded with respect to the methine protons of the alcohols (**Table 7**). Similarly, dehydration of other alcohols, for example 1-(4-methoxyphenyl)-1-propanol **(96) Plate 27** gave similar ethers **97** and **98** (^{1}H NMR spectrum **Plates 28** and **29), Scheme 7.6.**



Scheme 7.6: Formation of ethers from 1-(4-methoxyphenyl)-1-propanol **(90)**

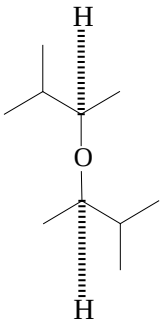
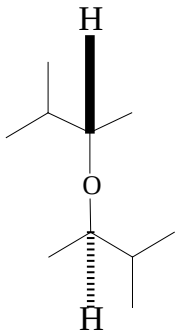
Alcohol	**Chemical Shifts δ_H (ppm)**	**Ether**	**Chemical shift δ_H (ppm)**	**Ether**	**Chemical Shifts δ_H (ppm)**
					
90	5.85	**92**	4.24	**94**	4.52
91	5.85	**93**	4.25	**95**	4.51
96	4.57	**97**	4.00	**98**	4.34

Table 7.1: Chemical shifts of the methine protons of the alcohols and the ethers.

[#] Since two different ethers (δ_H 4.24 and 4.52) were isolated the only explanation could be one to e the " *trans*" and the other one the "*cis*"-isomer. Assigning a structure to each one was not attempted.

The ^{1}H NMR spectra of compounds **97 (Plate 28)** and **98 (Plate 29)** show the same number of resonances which are also similar to those of the respective alcohol starting material. There is an observable change in the chemical shift values of the oxomethine protons for the ethers for example, **97** and **98** at δ_H H-4.0 and H-4.34, respectively, when compared with the methine of the starting material (**96**) resonating at δ_H 4.57. Since NMR resonances similar to the alcohol (starting material) (but with different chemical shift values) are only explicable in terms of invoking a symmetrical ether structure for the unknown compounds, structures **97** and **98** were assigned to the two products. The second aspect of the two products of the unknown structures that was difficult to explain, even with the ether hypothesis, was how two symmetrical ethers could be formed. If the relative stereochemistry of the products is taken into account, however, it can be seen that since two chiral centers are present in the molecule, the formation of a pair of diastereomers is probable the cause of the existence of the two very similar products. Spectrometric analysis of compounds **97** and **98** did not show the expected molecular mass of *m/z* 314. However, a molecular ion peak at 167 corresponding to the mass of the precursor alcohol was observed, which reflects cleavage of the weak ether bond. Although unambiguous structure elucidation for each of the two products are still outstanding, structures **97** and **98** could be assigned with confidence to the two products isolated from the dehydration reaction of the benzylic alcohol with copper sulphate.

7.3.3. Knoevenagel condensation reaction

Since literature precedent for the synthesis of ortho- or *para*-hydroxystyrenes *via* the Knoevenagel reaction do exist[138] and eventually the metathesis reaction would be applied to the synthesis of stilbenes in the free phenolic form, synthesising of the free phenolic styrenes through application of the Knoevenagel reaction was next attempted. In a one-pot reaction, the aldehyde **99**, **101**, **103** were each reacted with malonic acid (activated methylene) and piperidine in pyridine (**Table 7.1**) to yield the styrenes **100**, **102** and **104**, respectively The solvent and bases were removed in *vacuo* on a rotary evaporator at *ca*~40 ^{o}C in the presence of toluene to yield the desired products (60-90%).

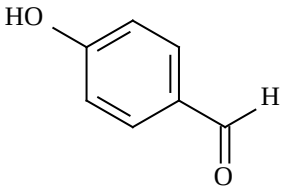
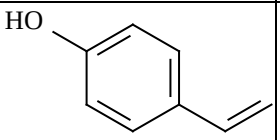
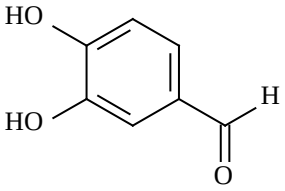
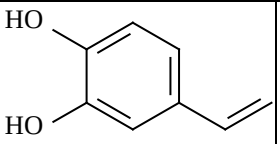
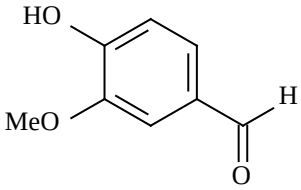
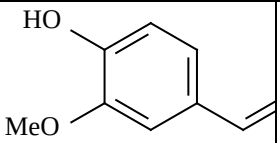
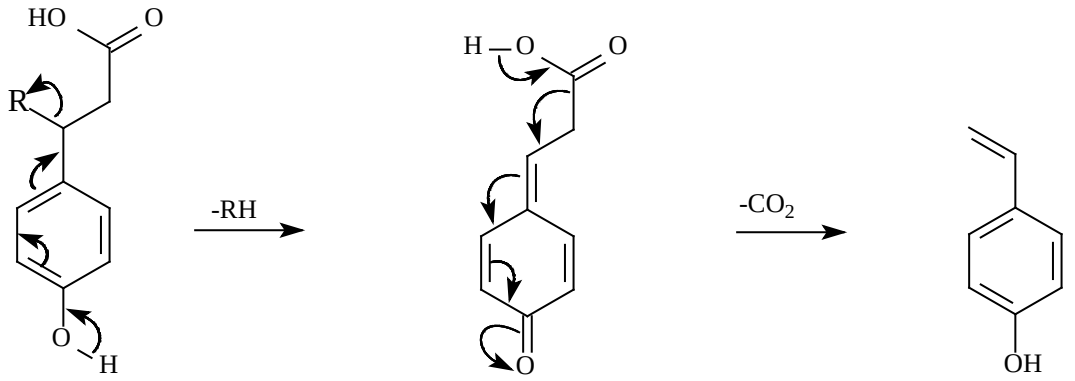
Substrates	Reagents/solvents	Conditions	Products	Yields %
 **99**	1. Malonic acid (1.5 equiv), Piperidine (4 equiv) Pyridine 2. Toluene	Reflux (4 h), evaporate in vacuo (30-40 ^{0}C)	 **100** **(Plate 30)**	90
 **101**	1. Malonic acid (1.5 equiv), Piperidine (4 equiv) Pyridine 2. Toluene	Reflux (4 h), evaporate in *vacuo* (30-40 ^{0}C)	 **102** **(Plate 31)**	80%
 **103**	1. Malonic acid (1.5 equiv), Piperidine (4 equiv) Pyridine 2. Toluene	Reflux (4 h), evaporate in *vacuo* (30-40 ^{0}C)	 **104** **Plate 32**	60%

Table 7.2: Synthesis of styrenes with different hydroxylation patterns.

The mechanism of the reaction is envisaged to follow the participation of a quinone in the double decarboxylation necessary to afford a vinylphenol (**Scheme 7.7**). By following the initial decarboxylation, the phenolic oxygen participates in the elimination of the amine function in the condensation product through the generation of a quinone methide. Decarboxylation then proceeds readily to give the corresponding vinyl phenol either through conjugate addition to give the unstable β-lactone or more likely by direct decarboxylation (**Scheme 7.7**). Instability of the very reactive styrenes, however, hindered the purification process, and hence they were used in the synthesis of the stilbenes while in the impure state.

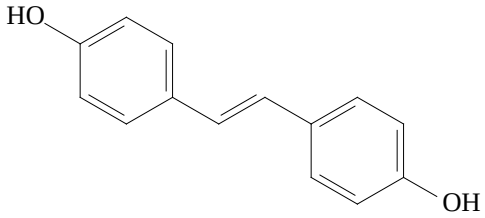


Scheme 7.7 Mechanism for the synthesis of styrenes

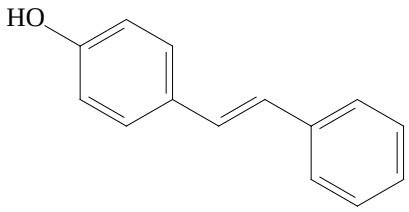
Analysis of the ^{1}H NMR spectra of the styrenes (**100, 102** and **104**) Plates **30, 31** and **32**, respectively) showed the characteristic protons of a vinylic double bond at δ_H 6.64 (H-1), δ_H 5.15 (H-2) and δ_H 5.67 (H-2).

7.4. Synthesis of stilbenes *via* the cross metathesis reactions (CM).

Although the styrenes could not be obtained in purified form, it was decided to have an attempt at the metathesis reaction with starting materials that contained trace amounts of cinnamic acids and bases anyway. Grubbs catalysts were selected because they are unique catalysts which are applicable to a variety of functional groups and they have demonstrated the ability to prepare highly substituted olefins by CM, often in a stereoselective manner. To synthesize the stilbenes, the impure styrenes, 4-hydroxystyrene **(100)** and 3,4-dihydroxystyrene **(102)** (**Table 7.1)**, were coupled *via* the cross metathesis (CM) procedure in the presence the Grubbs catalyst (**79)** in THF and the mixture refluxed for 2 hours at 80 ^{o}C. Following PLC purification of the reaction mixture, a product **105** (**Plate 33**) from the self-coupling was obtained in very low yields (25%) The product from the cross coupling and the second self-coupling were not observed. Instead, compound **106** (^{1}H NMR spectrum **Plate 34)** was obtained as the major product (45%) along with other products whose ^{1}H NMR indicated that dimerization was also taking place in the reaction. The ^{1}H NMR spectrum (**Plate 33**) of compound **105** displays three resonances of a symmetrical stilbene at δ_H 7.40, d, J = 8.5 Hz, δ_H 6.83, d, J = 8.5 Hz and a singlet at δ_H 6.98 assignable to H-2/6 or H-2'/6', H-3/5 or H-3'/5', and α/β, respectively. The proton ratio of 2:2:1 of H-2/6 (H-2'/6'):H-3/5 (H-3'/5'):α/β is in agreement with the proposed structure.



(105)

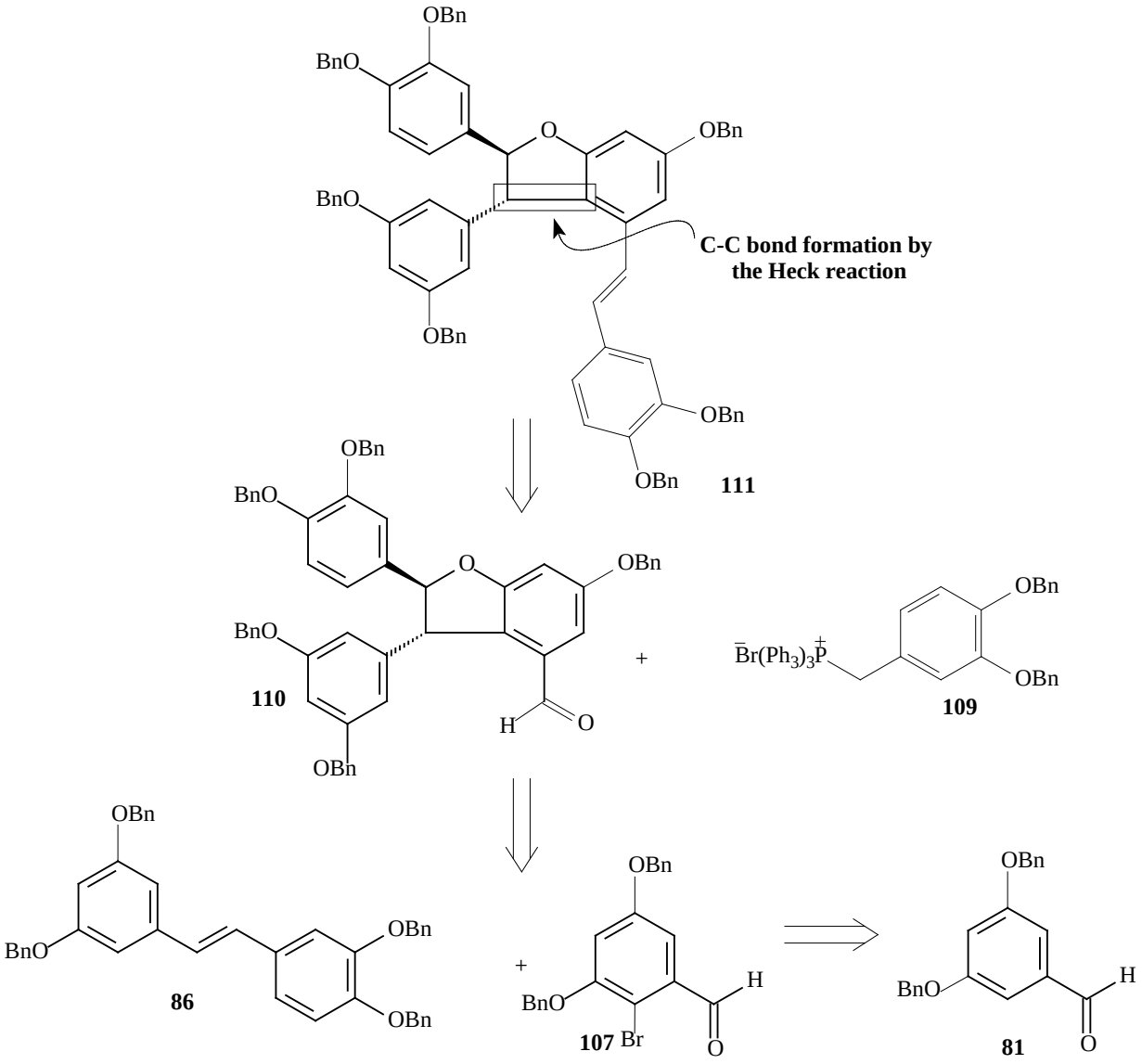


(106)

The ^{1}H NMR spectrum of compound **106** shows an AA'BB' spin system (δ_H 6.89 (d, J = 8.5 Hz, H-2/6, 2H) and δ_H 7.49 (d, J = 8.5 Hz, H-3/5, 2H), two olefinic protons (H-α, δ 7.09 d, J = 16.0 Hz and H-β, δ 7.19, d, J = 16.0 Hz) and three resonances at δ_H 7.58 (d, J = 8.5 Hz, H-2'/6', 2H), δ_H 7.37 (t, J = 8.5, Hz, H-3'/5', 2H) and δ_H 7.24 (t, J = 8.5 Hz, H-4', 1H) typical of a mono-substituted benzene ring. In this reaction, only one (self coupling of the *para*-dihydroxystyrene) of the three expected products was obtained. Failure to obtain the 3,4-dihydroxy self coupling and the cross coupling in the reaction could probably be due to the highly reactive nature of 3,4-dihydroxystyrene, a factor which has been observed during its preparation. Since the styrenes were used in the impure state and that there presence in the reaction mixture were established from resonances of the characteristic olefinic protons, there is a possibility that the styrene in the reaction were not 3,4-dihydroxystyrene. Low yields 30-40 % obtained in this reaction do not reflect the versatility of the reaction. We may attribute the relatively low yields to the instability of the styrenes. Efforts to address the drawbacks such as, purification of the products and stabilizing of the styrenes before and during the reaction were not optimized due to time restrictions. Although stilbenes have been synthesized by Ferre-Filmon, *et, al*. starting from styrenes as the substrates[23], there is no mention of how the styrenes are synthesized. It is therefore, apparent that a method be developed which also addresses factors such as the nature and effects of the substituents on the ring, to synthesize styrenes.

7.5. Synthesis of the dimeric stilbenes

Synthesis of the dimeric stilbenes was conceived to proceed *via* the protocol outlined in the retrosynthetic **Scheme 7.8**.

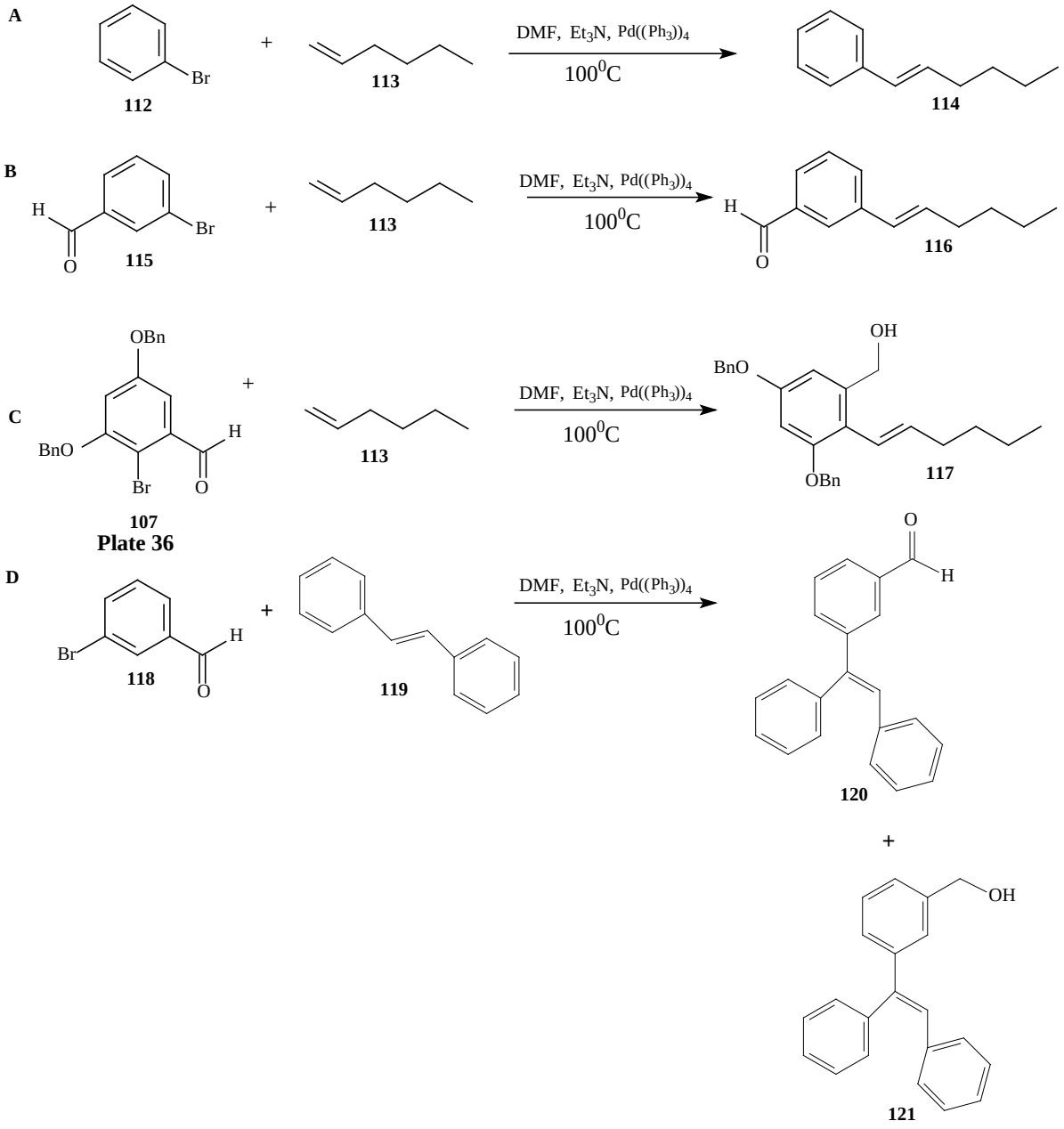


Scheme 7.8: Retrosynthetic pathway of the dimeric stilbenes *via* the Heck reaction

The Heck reaction is one of the most important methods in synthetic organic chemistry for the formation of C-C bonds.[141,142,143] The reaction is normally catalyzed by either Pd^0 or Pd^{II} complexes in the presence of a base. The Heck reaction can be carried out under mild and environmentally friendly conditions which offer excellent regioselectivity of *para*- over *ortho*-substitution, for example in phenyl iodides especially with electron-donating groups.[144]

Normally the Heck coupling of phenolic substrates with only one hydroxyl group e.g. 4-bromophenol and simple olefins give high yields (80%). However, the target molecule **111** we had to synthesize has four highly hydroxylated rings. Generally, the intensity of activation on an aromatic ring increases with increase in the number of hydroxyl groups substituted. Therefore, reactivity of the highly activated aryl halide towards the olefin would be reduced in the reaction. Additionally, the aldehyde group (the second functional group in the starting material **107**) also affects the coupling because it deactivates the ring. Owing to the anticipated challenges such as, curbing the reactivity of the hydroxyl groups, presence of a benzilic double bond as well as hindered C-C bond to be formed in **111** (see boxed part **Scheme 7.8**), we carried out model reactions outlined in **Scheme 7.9**.

[141] Heck, R. F. *Palladium Reagents in Organic Synthesis,* Academic Press, London, **1985**.
[142] Beletskaya, I. P., Cheprakov, A.V. *Chem. Rev.,* **2000,** *100,* 3009.
[143] Biffis, A., Zecca, M., Basato, M. *J. Mol. Catal.,* **2001**, *173,* 249.
[144] Zhang, Z., Zha, Z., Gan C., Pan, C., Zhou, Y., Wang, Z., Zhou, M. M. *J Org. Chem.,* **2006** 71, 4339.



Scheme 7.9: Model Heck reactions

7.5.1. Model Heck reactions

To establish the steric hinderance effect of the substrates on the Heck reaction, aryl halides with different substituents were coupled with either 1-hexene **113** or the stilbene **119** (**Scheme 7.9**) as the olefins, under the same conditions. Results from this investigation would project on the

factors such as, a) steric bulkiness of the protecting groups in the benzylated stilbene **86,** and b) the electronic effects from the substituents on the benzylated alkyl halide (**Scheme 7.9**), which may hinder coupling in the more complex target molecule **111.** The 16 h reactions yielded very interesting results.

7.5.1.1. Heck reaction of bromobenzene and 1-hexene, (Plate 35)

In reaction A (**Scheme 7.9)**, the product was obtained in very low yields, (20%). Since extended periods of time between completion of the reaction and work-up led to even lower yields, and in the light of the results from subsequent reactions, it might be concluded that polymerization of the highly reactive styrene-type derivative could be the cause of the low yields. Due to time constraints and the fact that this was only a model reaction, the actual cause of the low yields was not investigated further. The ^{1}H NMR spectrum (**Plate 35)** of compound **114** displays the conspicuous protons at δ_H 6.52 (H-1, d, J = 16.2 Hz), δ_H 6.35 (H-2, m), which are assigned to the olefinic protons in the structure, while the phenyl protons resonate in the aromatic region between δ_H 7.2-7.8. The aliphatic protons showing as multiplets are assignable to protons in the hexane chain with the most deshielded methylene protons (adjacent to the olefinic bond) appearing at δ_H 2.54.

7.5.1.2. Heck reaction of 3-bromobenzaldehyde and 1-hexene (Plate 36)

The coupling in reaction B (**Scheme 7.9)** afforded compound **116**, albeit in the modest yield (45%), implicating that introduction of an electron withdrawing group (the aldehyde) into the ring may have positive effect on the reaction. The ^{1}H NMR spectrum (**Plate 36**) of product **116** shows aromatic protons between δ_H 7.4 and 8.0 and the olefinic protons at δ_H 6.4 (2H) as a multiplet, which may be considered to be a characteristic feature in coupling of an aryl halide and a terminal aliphatic double bond. The methylene protons resonate at δ_H 2.25 and δ_H 1.14, while the methyl group resonates at δ_H 0.9.

7.5.1.3. Heck reaction of 2-bromo-3,5-dibenzyloxybenzaldehyde and 1-hexene, (Plate 38)

In reaction C (**Scheme 7.9**), a more bulky aryl halide, which is the same substrate needed for the target molecule, was coupled with 1-hexene. This reaction yielded the product in very low yields (~15%) indicating that steric bulkiness on the aryl halide may be affecting the coupling process. The ^{1}H NMR spectrum (**Plate 38**) of product **117** shows aromatic protons between δ_H 6.4 and 6.8 and the olefinic protons at δ_H 6.6 and 6.1. The methylene protons resonate at δ_H 2.70, and δ_H 2.25, while the methyl group resonates at δ_H 0.95. Surprisingly the aldehyde proton was missing in the NMR which indicated a reduction of that functionality to have occurred to give an alcohol.

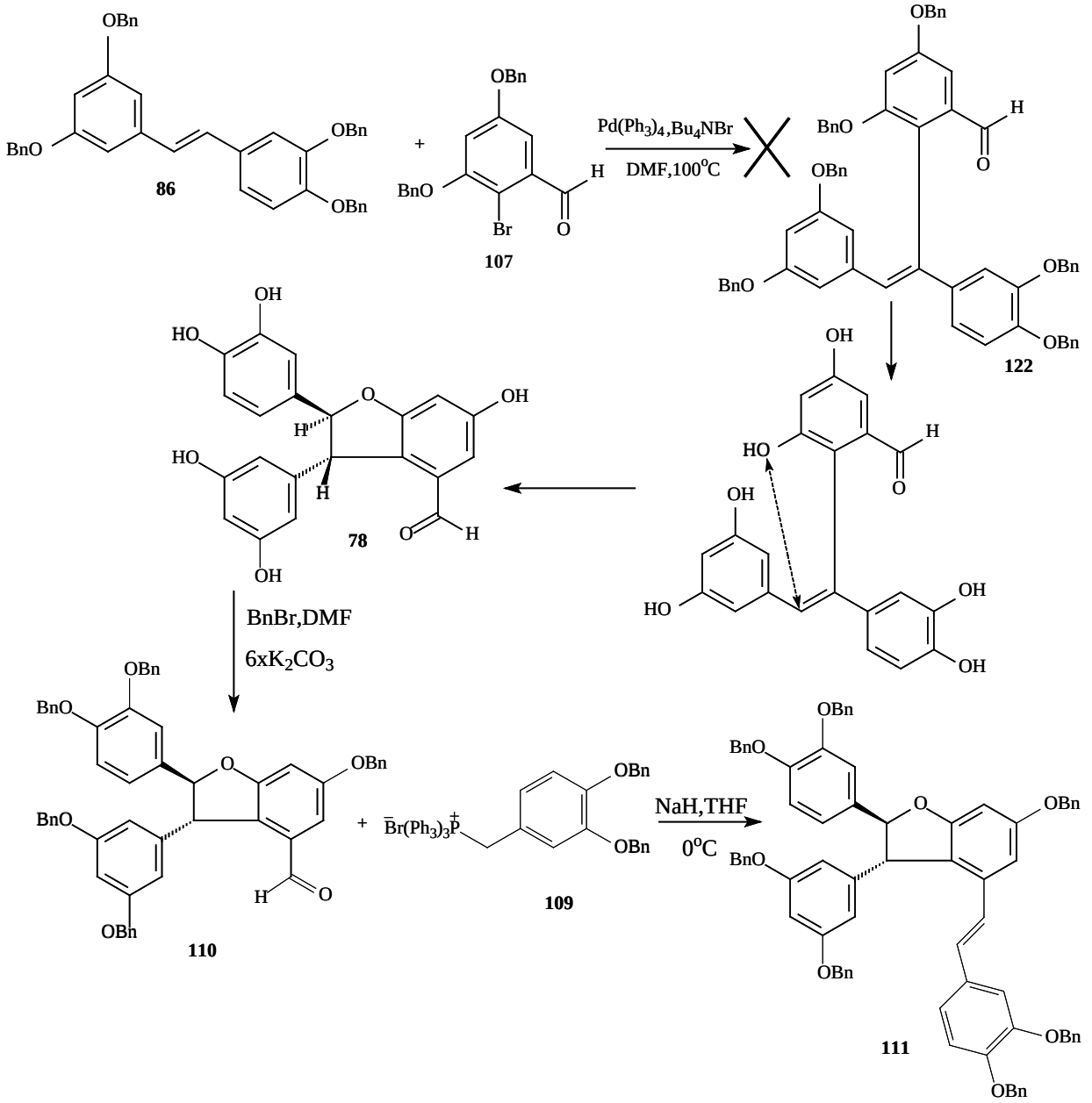
7.5.1.4. Heck reaction of 3-bromobenzaldehyde and the stilbene, (Plate 39).

When the stilbene was reacted with bromobenzene (reaction D, **Scheme 7.9**), compound **120** was afforded in moderate yields of 50%. Ability of the substrates to couple demonstrates that bulkiness of the stilbene does not affect the reaction, provided the aryl halide is not sterically hindered. The ^{1}H NMR of compound **120 (Plate 39)** shows a very dieshielded peak at δ_H 7.25, which can be assigned to the vinylic poton, along with the 13 aromatic protons in the benzene rings. The deshielding effect of the singlet may be attributed to the fact that it is both an allylic and a benzylic proton.

7.5.2. Attempted synthesis of 111 *via* the Heck reaction

Because the model reactions A, B, C and D (**Scheme 7.9)** were successfully accomplished, we applied the same concept towards the synthesis of the target molecule. Bromination of the protected benzaldehyde **81** with N-bromosuccinamide gave bromobenzaldehyde **107** (**Plate 37**) in yields 80%. The procedure of the Heck reaction entailed refluxing a mixture of $Pd(Ph)_3)_4$ (1 mol %), the brominated benzaldehyde **107**, the protected stilbene **86**, Et_3N as base and DMF as solvent. However, we were unable to obtain a product. Since the brominated benzaldehyde **107** was successfully coupled with the simple olefin (1-hexene) (**Scheme 7.9**), the electronic effects which may be caused by this highly substituted aldehyde were eliminated. Failure of this

reaction may be attributed to steric factors of the benzylated stilbene. Although we envisaged the reaction to proceed *via* **Scheme 7.10** compound **122** was not formed probably due to factors discussed above. Therefore, we could not execute the following steps in **Scheme 7.10**. Thus, if compound **122** had been formed, it would be deprotected and subsequently cyclized under mild acidic conditions to yield compound **110**. Subjecting the aldehyde **110** to the Wittig conditions in the presence of the separately prepared phosphate salt **109** would then yield the required dimer **111**.



8. Conclusion

The conclusion is on the two legs of the study which converge to a common goal of developing biologically active compounds originating from natural plant products that are used by man for treatment of various ailments. While one leg focuses on isolation, purification and characterization of phenolics from Walnuts *Juglans regia* L, the other leg is developed around synthesis of biologically active stilbenoids which have been isolated from *Afrormosia elata*.

Phytochemical analysis of Walnuts revealed presence of the flavonoids; catechin and gallocatechin, albeit in very low concentrations, along with the previously isolated tannins. Besides being radical scavengers, catechin polyphenols are considered to invoke a spectrum of cellular mechanisms of action related to their neuroprotective activity. These include pharmacological activities like iron chelation, scavenging of radicals, activation of survival genes and cell signaling pathways, and regulation of mitochondrial function[144]. Catechins may also reduce the risk of ischemic heart disease mortality. The metabolic syndrome appears to do damage to the cardiovascular system *via* the excess production of free radicals. Since flavonoids, the free radical scavengers have been found to augment the ability of Walnuts to treat the metabolic syndrome, it is arguably correct to conclude that catechins may in a way contribute to the treatment of the metabolic syndrome. We have, therefore, successfully isolated flavonoids from Walnuts *Juglans regia* L.

Synthesis of the monomeric stilbenes was executed *via* the Wittig and methathesis reactions. Synthesis of the monomer, piceatannol, *via* the Wittig reaction yielded the required product in 40% yield. On the other hand, the methathesis reactions of 3,4-dihydroxy and 4-hydroxystyrenes with Grubbs II as a catalyst, afforded only the symmetrical 4-dihydroxystilbene from the self coupling reactions. Although early

[144] Mandel, S., Youdim, M. B. Free Radic, Biol, Med., **2004,** *37*, 304.

attempts at influencing the self-vs cross methathesis product ratio was not very successful, this aspect will receive more attention through substrates that show a wider variety of activating and de-activating substituents.

Since formation of a C-C bond *via* the Heck reaction requires an alkyl halide and an alkene, we explored the reaction with varied substrates. Thus, we successfully coupled the simple aryl halides (4-bromobenzene and 3-bromobenzaldehyde) with the 1-hexene and the stilbene. However, coupling of the benzylated 2-bromo-3,5-dihydroxybenzaldehyde with the benzylated 3,4,3'5'-tetrahydroxystilbene was unsuccessful. Failure of this reaction could probably be attributed to the steric hinderance caused by the very bulky benzyl groups. In conclusion, we demonstrated that although coupling of the Heck reaction was successful, bulky starting materials limit the success of the reaction. Due to time constraints, we were unable to explore the reaction with other protecting groups. Future work will, therefore, entail exploration of other protective groups and optimization of conditions for the Heck approach, as well as other methods of synthesizing the dimeric stilbene.

EXPERIMENTAL

9. Standard experimental techniques

Unless otherwise stated the following techniques were applied through the course of this study.

9.1. Chromatographic techniques

9.1.1. Paper chromatography

Two-dimensional paper chromatograms were conducted on Whatman no 1 paper (28.5X 46cm) in two directions, using water-saturated butan-2-ol in the first direction and 2% (v/v) acetic acid in the second direction. After development, the chromatograms were air dried and investigated by UV-light and spraying reagents.

9.1.2. Column chromatography

Separations on Sephadex LH-20 were carried out on various column sizes at differing flow rates as specified. The Sephadex LH-20 was prepared by soaking it in the eluant [ethanol or methanol/water (50/50)] for 24-hours. The crude extract in a minimum amount of the eluant was applied to the packed Sephadex LH-20 column. Column fractions were eluted at a flow rate of 0,5 ml/min, and fractions of 15 ml were collected with an ISCO (model 273) automatic fraction collector.

Merck Kieselgel 60 column chromatography separations were performed on various column sizes at different flow rates under gravity. The glass column was charged with this suspension of Kieselgel 60 and the crude extract, dissolved in a minimum of the appropriate solvents applied to the column. Columns were eluted at a flow rate of approximately 0.5 ml/min and fractions of 15 ml were collected with an ISCO (model 273) automatic fraction collector.

Flash column chromatography (FCC) was performed on a glass column (5 cm diameter) charged with 100g of Merck Kieselgel 60 (230-400 mesh) for every 1 g of the crude material. Air was displaced by elution with the appropriate solvent under N_2-pressure (*ca.* ~ 40kPa). The

crude product was dissolved in a minimum of the appropriate solvent and applied to the column. The purified product was recovered by elution under N_2-pressure in 15 ml fractions.

9.1.3. Thin layer chromatography

Qualitative thin layer chromatography (TLC) was performed on pre-coated Merck plastic sheets (silica gel $PF_{254,}$ 0.25mm). After development, the plates were sprayed with vanillin-H_2SO_4. R_f values reported are those observed in these qualitative TLC assessments.

Preparative scale thin layer chromatography (PLC) was conducted on glass plates (20 X 20cm) coated with Kieselgel PF254 (1.0 mm), which were air-dried overnight at room temperature. The plates (loaded with 10-15 mg of material per plate) were developed in an appropriate eluent and dried in a stream of air. The bands were distinguished by UV (254 nm) light and scraped off. Compounds were eluted from the adsorbent with acetone, which was removed on a rotary evaporator under reduced pressure at 40^{o}C. Small-scale separations were conducted on Merck pre-coated (0.25) TLC plates, silica gel 60 F_{254} with each plate charged with 3-5 mg of the crude product.

9.2. Spraying reagents

9.2.1. Vanillin-sulphuric acid

The TLC plates were sprayed lightly with a solution of vanillin (1 g) in concentrated sulphuric acid (100 ml) and subsequently heated (*ca* 100 ^{o}C) to ensure optimum colour development.

9.2.2. Anisaldehyde

Thin-layer chromatograms were sprayed with a solution of anisaldehyde (5 ml) in concentrated sulphuric acid (5 ml) and ethanol (90 ml) and gently heated to 100 ^{o}C to ensure optimum colour development.

9.2.3. Formaldehyde-sulphuric acid[1]

TLC plates were quickly sprayed with 2% (v/v) solution of formaldehyde (37 wt % solution in water) in concentrated sulphuric acid and heated to 120 ^{o}C to ensure maximum development of colour.

9.2.4. Bis-diazotized benzidine[2]

Benzidine (5 g) dissolved in concentrated hydrochloric acid (14 ml) was added to distilled water (980 ml). The mixture (30 ml) was dissolved in sodium nitrite (10 % m/v, 20 ml). Paper chromatograms were gently sprayed with the freshly prepared mixture of this solution and subsequently washed with for 1 hour under running tap water.

9.3. Chemical methods

9.3.1. Acetylation[3]

Dry phenolic material was dissolved in a minimum volume of pyridine and twice the amount of acetic anhydride was added. After 8-12 hours at ambient temperatures, crushed ice was added to precipitate the acetylated material which was filtered and excess pyridine washed out with cold water.

9.3.2. Methylation with diazomethane[4]

Methylations were performed with an excess of diazomethane prepared by the reaction of a cold (-10 ^{o}C) potassium hydroxide [5g in ethanol (95%, 55 ml, v/v)] with N-methyl-N-nitroso-*p*-toulene sulphonamide (22 g) in cold ether (150 ml) and distilled directly into a solution of dry phenolic material (250 mg) in methanol (5-10 ml) at –10 ^{o}C. After 48 hours at –15 ^{o}C the excess diazomethane and solvents were evaporated at room temperature.

[1] H. M. Saayman and D. G. Roux. *Biochem. J.*, **1965**, *96*, 36.
[2] D. G. Roux and E. A. Maihs. *J. Chromatogr.*, **1960**, *4*, 65.
[3] T. Kametani and S. Kano. *J. Pharmac. Soc. Japan*, **1962**, *82*, 1059.
[4] A. I. Vogel. In *Textbook of Practical Organic Chemistry*, Longmans, London, **1967**, 979.

9.4. Anhydrous solvents and reagents

THF was predried with sodium metal, and was refluxed over sodium/benzophenone under N_2 until a dark blue colour persisted with subsequent fresh distillation under N_2 prior to use.

Acetonitrile was left over 4Å molecular sieves and was refluxed under N_2 for 48 hours with subsequent distillation under N_2 prior to use.

DMF was left over barium oxide for 24 hours. Barium oxide was filtered off and the solvent subsequently refluxed over NaH under N_2 and stored under N_2.

Methanol was left over anhydrous potassium carbonate for 24 hours. The potassium carbonate was filtered off and the solvent subsequently distilled over 3Å molecular sieves and stored under N_2.

9.5. Spectroscopical methods

9.5.1. Nuclear magnetic resonance spectroscopy

NMR spectra were recorded on a Bruker AVANCE DPX_{300} spectrometer with tetramethylsilane an internal standard. The solvents used were deuteriochloroform ($CDCl_3$, δ_H 7.24) and deuterioacetone [$(CD_3)_2CO$/acetone d_6, δ_H 2.04]. Chemical shifts are reported in parts per million (ppm) on the δ-scale and coupling constants were measured in Hz. Abbreviations are used as follows:

Abbreviation	Signal multiplicity	Abbreviation	Signal multiplicity
s	singlet	dd	doublets of doublets
d	doublet	br	broadened
t	triplet	m	multiplet

Table 9.1: Abbreviations used in describing ^1H NMR signal multiplicities.

9.6. Abbreviations

The following abbreviations for solvents are used through out the experimental section:

A	=	acetone
C	=	chloroform
DMF	=	dimethylformamide
E	=	diethyl ether
EA	=	ethyl acetate
Et_3N	=	triethylamine
H	=	hexane
MeOH	=	methanol
THF	=	tetrahydrofuran
T	=	toluene

CHAPTER 10

10. Isolation of compounds from Walnuts (*Juglans regia* L.)

10.1 Enrichment of the extract

The ground walnuts (*Juglans regia* L.) (6 Kg) were subsequently extracted with hexane (7 x 2.5 L) and methanol (3 x 2.5 L) for 48 hours each time at about 40^0C. The solvents were filtered and evaporated under reduced pressure on a rotary evaporator at 40^0C to yield the yellow residual oil (5 L) and a dark brown solid (137.8 g) from the hexane and methanol extracts, respectively.

The methanol extract was partitioned in H_2O: sec BuOH: hexane (5:4:1) in a ten-tube Craig counter current assembly (50 ml of organic and 50ml of aqueous layer per tube), by ten transfers of the top layer. Following the chromatographic analysis of the individual ten tubes, fractions from the Craig were combined into two fractions, MA (tubes 1-5, 6.45 g) and MB (tubes 6-15, 16.67 g).

10.2. Isolation of compounds from fraction MA

Fraction MA (6.45 g) was dissolved in ethanol and subjected to column chromatography on the Sephadex LH-20 (4 x 150 cm column, flow rate of 20 ml / 32 min) in ethanol to give fractions A1- A8 (**Table 9.1**) after analysis with TLC (T:A: EtOAc, 6:3:1, v/v/v).

Fractions	Test-tubes	Mass (g)
A1	1-16	0.6202
A2	17-48	0.5379
A3	49-62	1.6770
A4	63-74	0.6083
A5	75-102	0.3847
A6	103-110	0.1129

Table 10.1: Fractions obtained from the column separation of MA.

Methylation of fraction A1 (0.5 g) followed by PLC purification (T:A, 8:2) yielded bands A1.1 (0.15 g, R_f 0.36) and A1.2 (0.11g R_f 0.28).

Band A1.1 was repeatedly purified by PLC [H:A: EtOAc:CH_2Cl_2:$CHCl_3$, 4:2:2:1:1 (x 4)] to give 2 bands.

10.2.1. 2,3-*O*-*(S)*-Heptamethoxy-β-D-glucopyranosyldiphenoyl ester (66)

Band A1.1.1 afforded the title compound as a yellow armophous solid (R_f 0.67, 5.0 mg).
^{1}H NMR: **Plate 6, Table 5.3**

10.2.2. Pentadeca-*O*-methylcasuarinin (69)

Band A1.1.2 afforded the title compound as a light yellow armophous solid (R_f 0.50, 12 mg).
^{1}H NMR: **Plate 8, Table 5.5**

Repeated PLC of band A1.2 H:A:EtOAc:CH_2Cl_2:$CHCl_3$, 4:2:2:1:1 (x 4) yielded two bands of compounds **68** and **70**.

10.2.3. Trideca-*O*-methylpendunculagin (70)

Band A1.2.1 afforded the title compound as a light yellow armophous solid (R_f 0.41, 3.0 mg)
^{1}H NMR : **Plate 9, Table 5.6**

10.3. Isolation of compounds from fraction MB

The methanol extract (45.0 g) was subjected to column chromatography on Sephadex LH-20 (4 X 150cm column, flow rate 20ml/32 min) with ethanol as the eluent to give 11 fractions (A1-A11) after analysis with TLC (EtOAc:M:H_2O; 7:2:1, v/v/v). Since the research was aimed at isolation of flavonoids from the nuts, only bands B1, B, B2, B3 and B9 which were visible on TLC under UV light (254 nm), indicating presence of the phenolics, were investigated further.

Bands	Test tubes	Mass
B1	1-60	2.5340
B2	61-101	4.0986
B3	102-135	1.7818
B4	136-153	3.6755
B5	153-167	0.6557
B6	168-174	0.3211
B7	175-190	0.6695
B8	191-201	0.2818
B9	202-288	4.6789
B10	289-333	1.5897
B11	334-394	4.1125

Table 10.2: Fractions obtained from the column separation of MB.

Acetylation of fraction B1 (100 mg) followed by PLC (T:A:M; 80:15:5) gave one visible band, R_f 0.4, 50 mg,.

Further PLC (T:A:M, 8:1:1) of B1 afforded two bands, B1.1 (0.9 mg, R_f 0.49) and B1.2, (0.7 mg, R_f 0.44) which were further subjected to PLC to afford two bands.

10.3.1. Penta-*O*-acetylcatechin (63)

Band B1.1 yielded the title compound as an off white powder (R_f 0.49, 0.9 mg)
^{1}H NMR: **Plate 1, Table 5.1**

10.3.2. Hexa-*O*-acetylgallocatechin (64)

Band B1.2 afforded the title compound as an off white powder (R_f 0.49, 0.7 mg)
^{1}H NMR: **Plate 2, Table 5.2**

Acetylation of fraction B9 (100 mg) followed by PLC (T:A:M; 80:15:5) yielded 2 bands, B9.1 (20.0 mg, R_f 0.57) and B9.2 (15.0 mg, $R_{f,}$ 0.34).

Further purication of B9 by PLC (T:A:M; 80:15:5 x 3) gave four bands.

10.3.3. Penta-*O*-acetyl-*O*-β-D-xylopyranosylellagic acid (65)

Band B9.1 afforded the title compound **65** as yellow amorphous powder (R_f 0.54, 10.8 mg).
^{1}H NMR: **Plate 5, Table 5.3**

10.3.4. Methyl-3,4,5 acetoxybenzoate (26)

Fraction B 9.2 afforded the title compound **26** as an off white powder (R_f, 0.45, 9.0 mg).
^{1}H NMR: **Plate 4**

10.3.5. 3,4,5-Tri-*O*-acetylbenzoic acid (19)

Band B9.3 gave the title compound as a yellow amorphous powder. (R_f 0.34, 33.0 mg).
^{1}H NMR: **Plate 3**

Further purification of band B9.2 was by PLC and 3 bands were yielded.

10.3.6. Hexaacetoxy-4-O-β-D-glucopyranosylnapthalene (74)

Band B9.4 afforded title compound as brown amorphous powder. (R_f, 0.47, 3.2 mg)
^{1}H NMR: **Plate 10, Table 5.4**

Acetylation of fraction B2 (100 mg) followed by PLC yielded two visible bands, B2.1 (10.0 mg ,R_f 0.31) and B2.2 (20.5 mg, R_f 0.26)

10.3.7. Penta-*O*-acetyl-α-D-glucopyranose (75)

Bands B2.1 afforded the title compound as colourless oil (R_f 0.31, 10.0 mg, T:M:A, 85:15:5).
^{1}H NMR: **Plate 14, Table 6.3**

10.3.8. Octa-*O*-acetyl-α-D-glucopyranosyl-β-D-fructofuranoside (76)

Band B2.2 afforded the title compound as colourless oil (R_f 0.26, 20 mg, T:M:A, 85:15:5).
^{1}H NMR: **Plate 15, Table 6.4**

Acetylation of fraction B3 (100 mg) followed by PLC yielded three bands.

10.3.9. 3-*O*-Acetoxysitosterol (72)

Repeated purification of B3.1 afforded the title compound as white amorphous powder (R_f 0.57, 8.3 mg, T: M: A, 6:3:1).
^{1}H NMR: **Plate 12, Table 6.2**

10.3.10. Tetra-*O*-acetoxy-3-*O*-β-D-glucopyranosylsitosterol (73)

Band B3.2 afforded the title compound as white amorphous powder (R_f 0.48, 20.5 mg, T:M:A, 6:3:1).
^{1}H NMR: **Plate 13, Table 6.2**

10.3.11. Tetra-*O*-acetyl-9-β-D-glucopyranosylstigmen-3-one (71)

Band B3.3 yielded compound **71** as yellow oil (R_f 0.35, 7mg).
^{1}H NMR: **Plate 11, Table 6.1**

CHAPTER 11

11. Synthesis of the stilbenes

11.1. General benzylation procedure

Benzylbromide (1.01 ml; 1.2 *eq*) was added to a mixture of the appropriate aldehyde or (1g; 0.006 mol) and K_2CO_3 (5.08 g; 0.037 mol; 5eq) in dry DMF (20 ml) and refluxed for 12 hours. The reaction mixture was acidified with HCl (3 M), extracted with EtOAc (3 x 20 ml) and the organic phase was washed with water (25 ml).

11.2. 3,5-dibenzyloxybenzaldehyde (81) and 3,4-dibenzyloxybenzaldehyde (84)

Protection of 3,5-dihydroxybenzaldehyde (**Plate 16**) and 3,4-dihydroxybenzaldehyde (**Plate 18**) were effected following the procedure in 10.1. The products were collected as light yellow amorphous powders in 60-70 % yield.

^{1}H NMR **Plate 16** δ_H; 9.93 (s, C*H*O, 1H), 7.35-7.51 (m, OCH$_2$*Ph*, 10H), 7.15 (d, J 2.5 Hz, H-2/6, 2H), 6.90 (t, J 2.5 Hz, , H-4, 1H), 5.12 (s, OC*H*$_2$Ph, 4H).

^{1}H NMR **Plate 18** δ_H; 9.83 (s, C*H*O, 1H), 7.35-7.61 (m, OCH$_2$*Ph*, 10H), 7.05 (d, J 2.5 Hz, H-2, 1H), 7.35-7.61 (overlapped H-5 and -6), 5.15 (s, OC*H*$_2$Ph, 2H), 5.18 (s, OC*H*$_2$Ph, 2H).

11.3. General procedure for the preparation of alcohols and ethers

To the mixture of the appropriate aldehyde and acetophenone (50 mmol) in ethanol (20 mL), $NaBH_4$ (50 mmol) was added with constant stirring (30 min) at room temperature. To the reaction mixture, cold H_2O and 1.0 M HCl (1.0 ml) were added and the mixture was extracted with EtOAc (3 x 30 ml), dried (Na_2SO_4) and the solvent evaporated to give the products (90-95 %). The alcohols were subsequently treated with anhydrous $CuSO_4$ (excess) in hexane as a solvent to yield the corresponding ethers.

11.4. 2-bromo-3,5-dibenzyloxybenzaldehyde (107)

To a solution of 3,5-dibenzyloxybenzaldehyde (0.52 g, 1.66 mmol) in $CHCl_3$ (16 mL) silica gel (1.98 g) and NBS (0.33 g, 1.85 mmol) were added. The mixture was stirred at room temperature for 20 h in the dark. The insoluble material was filtered off and the filtrate was washed with 10% aqueous solution of NaS_2O_3 (3 x10 mL) and brine (10 ml), dried over $MgSO_4$ and evaporated to dryness in *vacuo* in a 72% yield.
^{1}H NMR **Plate 37** δ_H; 10.58 (s, C*H*O, 1H), 7.30-7.55 (m, OCH_2*Ph*, 10H), 7.15 (d, J 2.5 Hz, H-6, 1H), 6.80 (d, J 2.5 Hz, H-4,1H), 5.05 (s, OC*H*$_2$Ph, 2H), 5.10 (s, OC*H*$_2$Ph, 2H).

11.5. The Wittig reaction

11.5.1. 3,5-dibenzyloxybenzylalcohol (82)[149]

Following the general procedure for the preparation of alcohols in (), the above alcohol was obtained as yellow product in a 95 % yield. ^{1}H NMR **Plate 17** δ_H; 7.30-7.60 (m, OCH_2*Ph*, 10H), 6.68 (d, J 2.5 Hz, H-2/6, 2H), 6.57 (t, J 2.5 Hz, H-4,1H), 5.09 (s, OC*H*$_2$Ph, 4H), 4.60 (s, C*H*$_2$OH, 2H).

11.5.2. 3, 5-Dibenzyloxybenzylbromide (83)[150]

PBr_3 (1.2 eq) was mixed with a solution of 3,5-dibenzyloxybenzylalcohol (0.20 g) in dry THF 10 ml) and the mixture stirred for 1hr at room temperature. The reaction mixture was washed with hexane and the solvent dried under pressure to yield 60% of the product.

[149]Garden, S. J., Correa, M. B., Pinto, A. C. *Tetrahedron lett*., **2003**, *44*, 7617.
[150] Noller, C. R., Dinsmore, R. *Org. Syn. Coll*., **1943**, *2*, 358.

11.5.3 3,5-Dibenzyloxy-diethyl-benzylphosphonate ester[151] (85)

To a solution of 3,5-dibenzyloxybenzyl bromide (0.50 g) in dry acetonitrile, triethyl phosphate (0.3 mL) was added and the reaction mixture refluxed for 1hr. The cooled mixture was filtered and the precipitate dried to give the required product in a 35% yield.

11.5.4. Synthesis of monomeric stilbene. (86)[151]

A mixture of 3,5-dibenzyloxytriethylbenzylphosphonate (1 g, mmol) and dry DMF (20 ml) containing sodium methoxide (0.25 g) was cooled to 0°C for 30 minutes. 3,4-dibenzyloxybenzaldehyde was added to the reaction mixture and refluxed for 2 hours. The reaction mixture was allowed to stand overnight at room temperature. A quantity of H_2O (5 ml) was added, the mixture was extracted with ether (3 x 30 ml) and dried with $MgSO_4$. Evaporation of the solvent followed by PLC (H:EA, 9:1) gave the benzylated *trans*- stilbene (65%).

^{1}H NMR Plate 17 δ_H; 7.30-7.60 (m, OCH_2*Ph*, 20H), 7.0-7.15 (overlapping H-5' and -6'), 1H)**,**6.98 (d, J 16.0 Hz, H-α, 1H)**,** 6.95 (d, J 2.5 Hz, H-2', 1H)**,** 6.80 (d, J 16.0 Hz, H-β, 1H)**,** 6.75 (d, J 2.5 Hz, H-2/6, 2H), 6.57 (t, J 2.5 Hz, H-4, 1H), 5.23 (s, OC*H*$_2$Ph, 2H), 5.22 (s, OC*H*$_2$Ph, 2H), 5.09 (s, OC*H*$_2$Ph, 4H).

11.6. Metathesis

11.6.1. Typical procedure for heterocoupling[133]

A solution of styrene A (1.0 mmol) and styrene B (1 0 mmol) in dry toluene or THF (2 ml) was added to a solution of ruthenium catalyst 1 (0.03 mmol) in dry toluene or THF under inert gas. The mixture was refluxed under N_2 for 1h. It was then cooled to room temperature and filtered through celite. The filtrate was concentrated under vacuum and purified by PLC on silica gel using acetone as eluant. The structure of the stilbene was confirmed by ^{1}H NMR spectroscopy.

[151] Eddarir, S., Abdelhadi, Z., Rolando, C. *Tetrahedron lett*., **2001**, *42*, 9127.

11.6.2. General procedure for palladium catalyzed Heck coupling[141]

To a stirred solution of bromobenzene (6 mmol) in DMF 3 ml $Pd(Ph_3)_4$ (0.21 mol), Bu_4NBr (0.15 mmol) and Et_3N (12.0 mmol) were added. The mixture was stirred for 1h at room temperature. Hexene ($1.4_{eq.}$) was added to the reaction mixture and stirring continued at room temperature for 1hr, after which the temperature was increased to 60ºC and stirring maintained overnight. The mixture was then cooled and poured into water (20 ml) and extracted with EA (3 x 20 ml). The combined organic extracts were washed with 10% $NaHCO_3$ (20 ml) and water (30 ml), dried and concentrated under vacuum. The product was obtained as yellow oil after PLC purification. Decomposition of the products hindered calculation of the yields.

11.6.3. Preparation of styrene by Knoevanagel condensation reaction.

To a solution of aldehyde (5 mmol) and malonic acid (20 mmol) in pyridine (21 mL) was added piperidine (0.75 mL), 7.6 mmol). The stirred reaction solution was heated at reflux (115ºC) for 4 hrs, followed by cooling to room temperature. To the cooled reaction mixture, toluene (40 mL) was added and the solvent volume was evaporated in vacuo at 30-40ºC. Additional toluene (20 mL) was then added and the solvent again removed in vacuo eliminating all traces of pyridine to afford the crude product in 80% yields.