Photochemistry of Pentoxifylline- A Xanthine Derivative

Thesis submitted in fulfilment of the requirement for the degree

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By

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Co-supervisor: Dr S.L.Bonnet

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SUMMARY

Key words

1. Pentoxifylline
2. Photochemistry
3. Internal standard
4. Synthetic chemistry
5. Xanthine derivatives
6. Medicinal chemistry
7. Cyclobutanol
8. Aromatic radical substitution
9. 1-Allyl-3,7-dimethyl-1-(5-oxohexyl)-3, 7-dihydro-1*H*-purine-2, 6- dione
10. Diastereoisomer
11. Stereoselectivity

Pentoxifylline [1-(5'-oxohexyl)-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione], sold under the trade name Trental®, is a methylxanthine derivative used in treatment of peripheral and cerebrovascular diseases and poor regional microcirculation (intermittent claudication). It has recently been investigated as an antitumor agent. It improves tumor perfusion and influences cytokine –mediated inflammation.

Our objectives were to synthesise 1-(3-oxobutyl)-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione and some of its derivatives for use as internal standards in the determination of biological fluids by liquid chromatography and for pharmaceutical/
biological screening as enzyme inhibitors. These efforts were hampered by the low reactivity of the N-1 position on the theobromine towards alkylation with electrophiles.

As an alternative method to achieve the aforementioned goals, we investigated the photochemistry of pentoxifylline. Of particular interest was the fact that pentoxyphylline has two chromophores, i.e. carbonyl and xanthine, separated by a linear butyl alkyl chain.

We now report a series of photochemical reactions of pentoxifylline and reaction conditions that were used to synthesise novel analogues.
The carbonyl moiety reacted predictably to yield three products in toluene. Norrish II fission yielded 1-allyl-3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione (**A**) in yields of up to 40%, and Yang cyclisation yielded (R^*, R^*,)-(±)-1-{[2-Hydroxy-2-methylcyclobutyl]methyl}-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione/(**B**) (10% yield). The ratio of these two products was always 4:1.

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The yield of the reduction product (lisophylline) was not improved by the addition of tri-butyltin hydride (TBTH). This observation indicated that the pentoxifylline carbonyl group reacted via singlet-excited states and yielded products **A**, **B** and **C**. The improvement of the yield from 32 to 48% with naphtalene and the decrease in the yield with benzophenone supports a singlet intermediate in the Norrish II type reaction of the carbonyl moiety in pentoxifylline.

The tri N-substituted xanthine moiety coupled photochemically with isopropanol to yield 8-(1'-hydroxy-1-methyl)ethyl pentoxifylline (**D**). This reaction involves substitution of the aromatic 8-hydrogen with an isopropyl group, probably via radical initiated aromatic substitution. The highest yield of this product (55%) was obtained in the presence of 50% acetone. This supports a triplet mechanism for the excited xanthine chromophore.

Several unknown products were isolated in low yields from the 2-propanol, EtOH/acetone photochemical reaction mixtures where further purification and structure elucidation will be performed. These are likely products derived from some new rearrangements of 8-substituted products.

We have developed methods to expand the range of derivatives of pentoxifylline that can be synthesised in reasonable yields. These products will be used as internal standards for bio-analytical purposes and in our biological assays. Conditions have been established that selectively encourage reactions at the carbonyl moiety (toluene, triplet quencher) or the xanthine moiety (protic solvents, photosensitiser or radical initiator).

OPSOMMING

Pentoksifelien (1-(5'-oksoheksiel)-3,7-dimetielxantien), word as Trental® bemark. Dit is 'n metielxantien derivaat wat gebruik word vir priferale en cerebrovaskulere siektes en swak mikrosirkulasie. Dit word ook ondersoek as 'n potensiële antikankermiddel. Dit verbeter tumor perfusie en het 'n effek op sitokien gekoppelde inflammasie.

Ons het probeer om pentoksifelien en derivate daarvan te maak vir gebruik as interne standaarde vir die kwantifisering daarvan in ligaamsvloeistowwe met vloeistofchromatografie en om dit te toets vir biologiese aktiwiteit as ensieminhibeerders. Ons pogings het misluk weens die lae reaktiwiteit van die N-1 posisie van xantien teen alkilering met elektrofiele.

Ons het gevolglik fotochemiese metodes ondersoek om derivate van pentoksifelien te maak. Dit is nog nooit vantevore gedoen nie. Ons het veral belang gestel in die feit dat pentoksifelien twee chromofore het (karboniel en xantien gedeelte) wat deur 'n lineêre $-(CH_2)_4-$ alkielketting geskei word. Ons rapporteer nou 'n reeks fotochemiese reaksies van pentoxifelien en die gepaardgaande reaksietoestande wat ons gebruik het om nuwe derivate te maak.

Die karbonielgedeelte reageer soos verwag om drie produkte te lewer. Norrish II splyting lewer 1-alliel-3,7-dimetielxantien in opbrengste van tot 50%. Yang siklisering lewer 1-[(2-hidroksie-2-metelsiklobutiel)metiel]-3,7-dimetielxantien (12.5% opbrengs). Die verhouding tussen hierdie twee produkte was altyd 4:1. Ons isoleer ook die verwagte lisofelien (10% opbrengs) weens die reduksie van die karbonielgroep na 'n alkohol.

Volgens dunlaagchromatografie het dit gelyk of byvoeging van tributieltinhidried tot verhoogde opbrengste gelei het. Hoëdrukvloeistofchromatografie het egter getoon dat dit nie die geval was nie en ook die 4:1 verhouding onder verskillende reaksietoestande bevestig.

In benseen is geen lisofelien verkry nie. Dit dui aan dat tolueen as waterstofdonor optree in die fotochemiese reduksie van die karbonielgroep. Tributieltinhidried het nie as waterstof donor opgetree nie.

Byvoeging van naftaleen (singulet sensitiseerder en triplet blusser) verhoog die opbrengs van 1-alliel-3,7-dimetielxantien van 26 tot 40% en bensofenoon (triplet sensitiseerder) verlaag die opbrengs. Dit dui op ’n singulet tussenproduk in die Norrish tipe II eliminasie. Die opbrengsverhoging van 10 na 46% in metanol wanneer die stikstofatmosfeer met ’n lugatmosfeer vervang word ondersteun ook ’n singuletmeganisme omdat triplet grondtoestandsuurstof die triplet xantienchromofoor blus. Die xantiengedeelte tree as ’n interne tripletblusser op wat voorkom dat die karbonielchromofoor vanuit die triplettoestand reageer.

Die xantiengedeelte van pentoksifelien koppel fotochemies met isopropanol in die 8-posisie om 8-isopropielgesubstitueerde pentoksifelien te lewer. Die reaksie behels verplasing van die aromatiese 8-waterstof met ’n isopropanol groep waarskynlik via ’n radikaalmeganisme. Die hoogste opbrengs (55%) word in 50% asetoon verkry. Dit dui op ’n tripletmeganisme vir die fotochemie van die xantiengedeelte. Met di-tert-butielperoksied as radikaalinisieerder kon ons ook 8-hidroksiemetiel en 8-hidroksietiel gesubstitueerde pentoksifelien in goeie opbrengste onder fotolitiese toestande isoleer.

Ons het kondisies onwikkel om ’n reeks van nuwe derivate van pentoksifelien in redelike opbrengste te maak. Ons benodig hierdie derivate as interne standaarde vir bio-analises en vir biologiese proewe. Ons het toestande ontwikkel om reaksies op die karbonielgroep te laat plaasvind (toluene, triplet blusser) of op die xantiengedeelte te laat plaasvind (polere oplosmiddels, triplet sensitiseerders of radikaalinisieerders).

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CHAPTER 1: LITERATURE SURVEY

This literature survey covers the following:

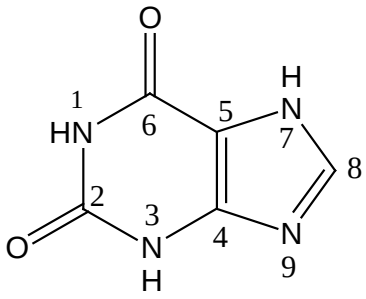
1. The pharmacology of pentoxifylline.
2. The chemistry of the xanthine moiety of pentoxifylline.
3. The photochemistry of the xanthine moiety of pentoxifylline.
4. The photochemistry of the aliphatic carbonyl moiety of pentoxifylline.
5. The chemistry of pentoxifylline.

The chemistry of the carbonyl moiety is trivial and will not be reviewed, while the photochemistry of pentoxifylline has not been studied previously.

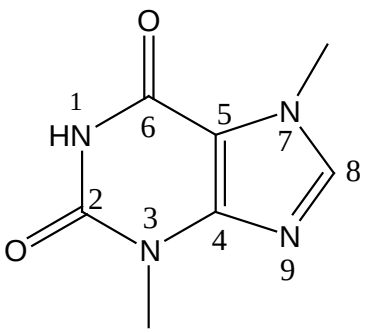
1. Pharmacology of pentoxifylline

Xanthine (3,7-dihydro-1*H*-purine-2,6-dione)/**(1)** and its methylated derivatives such as theobromine **(2)** theophylline **(3)** and caffeine **(4)** are an important group of alkaloids (**Figure1)** that exhibit a variety of pharmacological activities including anti-asthmatic, diuretic, respiratory-, central nervous-, cardiac stimulatory and analgesic adjuvant activities. Such activities reflect blockage of A_1- and A_2 - adenosine receptors[1]. The inhibition of adenosine receptors stimulates adenylcyclase and increases intracellular cyclic adenosine monophosphate (AMP)[2].

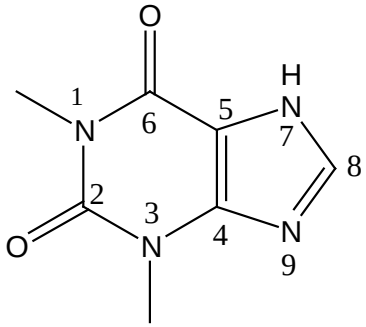
Figure 1



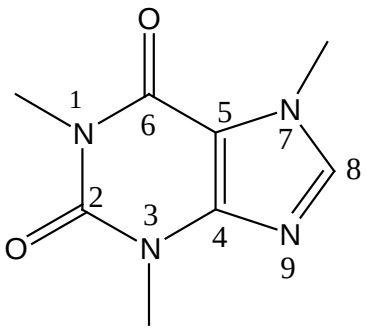
1: Xanthine



2: Theobromine



3: Theophylline

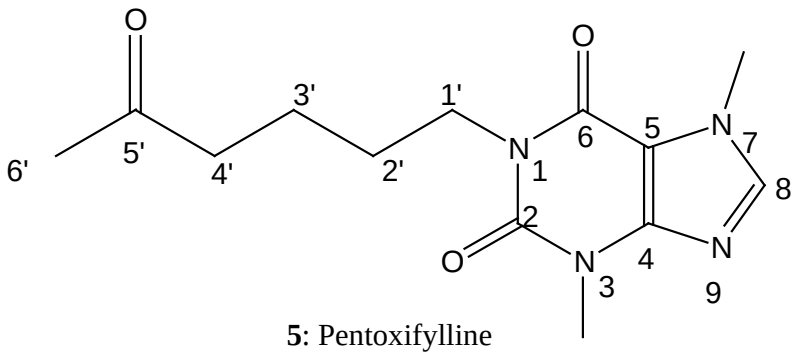


4: Caffeine

Caffeine, theophylline, theobromine and most of the other xanthines exhibit limited selectivity between A_1 and A_2 receptors. Structural modification of caffeine and theophylline has the potential for the development of clinical agents and research tools. Replacement of the 1-methyl moiety of caffeine with n-propyl, allyl, or propargyl increases affinity at A_1 only slightly while causing a marked increase in activity at A_2[3].

Pentoxifylline(**5**)/[1-(5'-oxohexyl)-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione] (**Figure 2**), sold under the trade name Trental®, is an hamorheologic agent used in treatment of peripheral and cerebrovascular diseases and poor regional microcirculation[5]. It can be considered as a derivative of theobromine and caffeine with a 5'-oxohexyl substituent in the 1-position. Pentoxifylline and its metabolites improve the flow properties of blood by decreasing its viscosity.

Figure 2



5: Pentoxifylline

The precise mode of action of pentoxifylline is still uncertain. Pentoxifylline administration has been shown to improve peripheral circulation and increase tissue oxygen levels by increasing erythrocyte deformability, inhibiting platelet aggregation[6], reducing blood viscosity[7], and diminishing fibrinogen concentration[8].

Development of new xanthine derived clinical agents related to pentoxifylline are impeded by the limited availability of synthetic methods to broaden the scope of derivatives for testing. Efforts to prepare 1-allyl-3,7-dimethylxanthine via the reaction of theobromine with allylbromide at the 1-position failed[4].

Pentoxifylline has also been investigated as an antitumor agent. It improves tumor perfusion and influences cytokine –mediated inflammation[9].

2. Chemistry of xanthine and its N-substituted derivatives

The chemistry of xanthine (**1**) can be classified as follows:

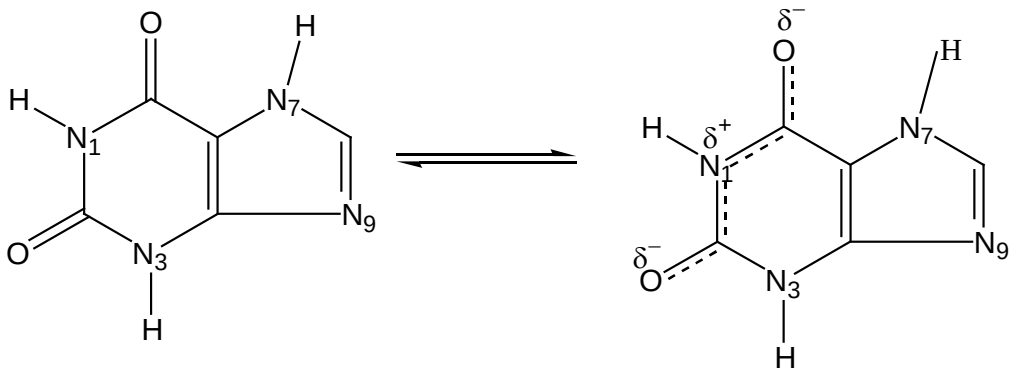
1. N-alkylation (at N-1, N-3 and N-7)
2. C-alkylation (at C-8)
3. C-amination (at C-8)
4. C-oxidation (at C-8)

2.1 N-alkylation

Various alkylating agents, such as dialkyl sulfate, alkyl p-toluenesulfonate, and alkyl halide have been used for the N-alkylation of xanthine and its derivatives[10].

N-alkylation takes place under alkaline conditions[4]. The reactivity of the three nitrogens, i.e. N-1, N-3 and N-7, depends on the acidity of the nitrogeneous proton. N-3 is the most reactive, N-7 has intermediate reactivity and N-1 is the least reactive. Yamauchi[4] states that the low reactivity of the N-1 towards alkylation may be attributed to steric hindrance around the N-1 position towards attacking alkylating agents by the C-2 and C-6 carbonyl groups. We, however, believe that the low reactivity of N-1 to alkylation has more to do with the notoriously low nucleophilicity of the double amide-type nitrogen function. (**Figure 3**)

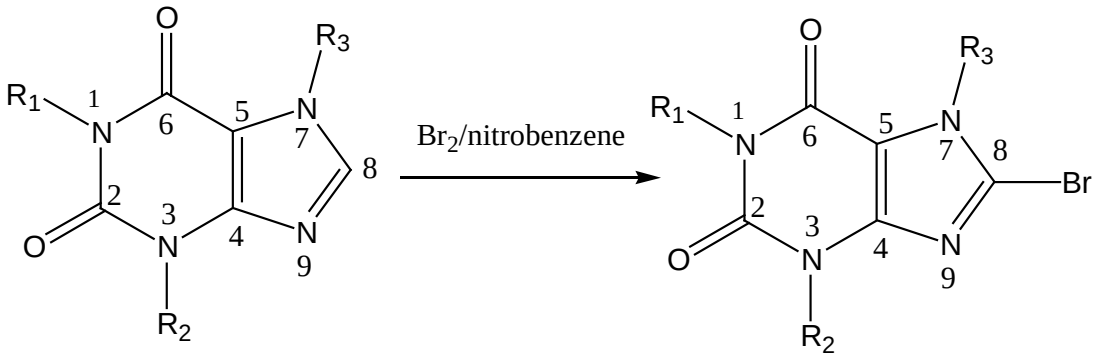
Figure 3



2.2 C-8 alkylation

Alkylation of xanthines under basic conditions[4] with alkyl halides gives exclusively N-alkylation. Xanthines can however be brominated in the 8 position to yield 8-bromoxanthines (**Scheme 1**). Alkylation of these halides under conditions[4] that yield N-alkylation results in 8-alkyl substituted xanthines[11].

Scheme 1



$R_{1:}$ Me, Et, *n*-Prop, *n*-Bu
$R_{2:}$ Me, Et, *n*-Prop, *n*-Bu
$R_{3:}$ Me, Et, *n*-Prop, *n*-Bu

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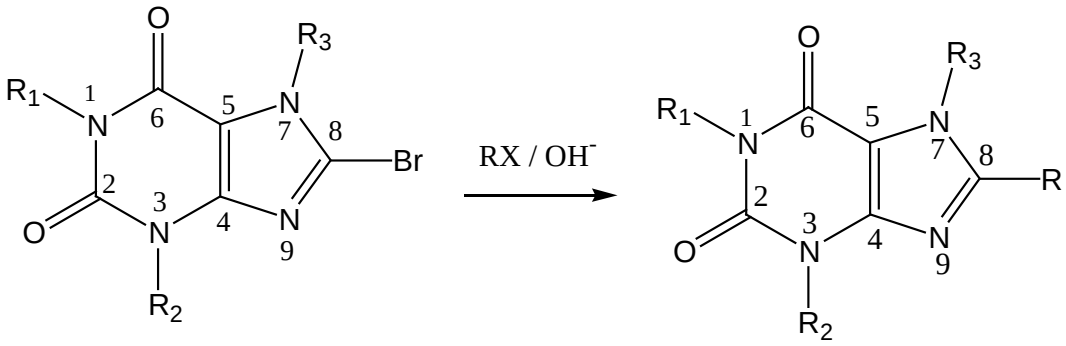


$R_{1:}$ Me, Et, *n*-Prop, *n*-Bu
$R_{2:}$ Me, Et, *n*-Prop, *n*-Bu
$R_{3:}$ Me, Et, *n*-Prop, *n*-Bu

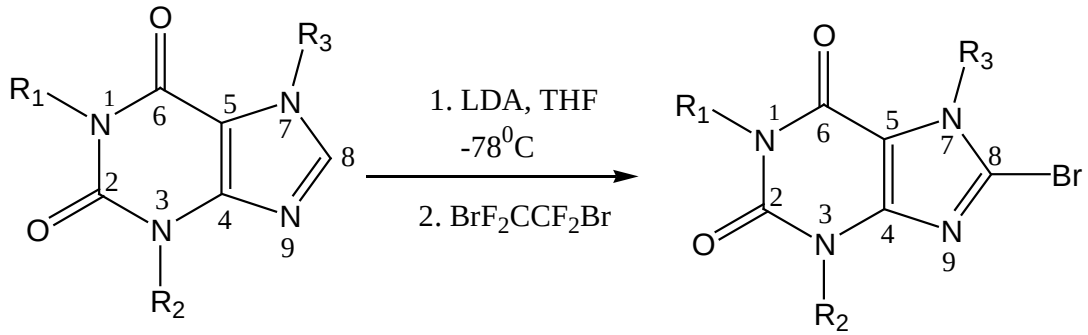
R: alkyl
X: halides

$R_{1:}$ Me, Et, *n*-Prop, *n*-Bu
$R_{2:}$ Me, Et, *n*-Prop, *n*-Bu
$R_{3:}$ Me, Et, *n*-Prop, *n*-Bu

2.3 C-8 amination

The 8-bromoxanthines utilised to alkylate the 8-position can also be aminated (**Scheme 2**) by an alkylamino group to yield 8-alkylaminoxanthines[12, 13].

Scheme 2



R_1: Me, Et, *n*-Prop, *n*-Bu
R_2: Me, Et, *n*-Prop, *n*-Bu
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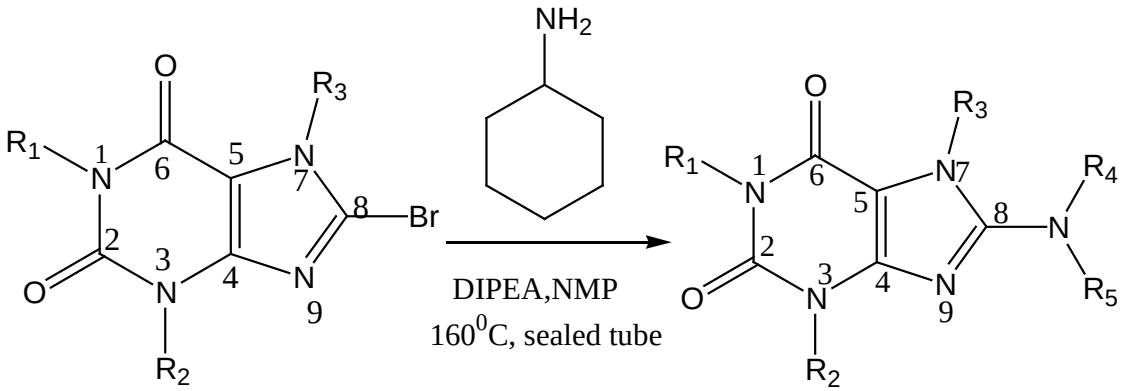

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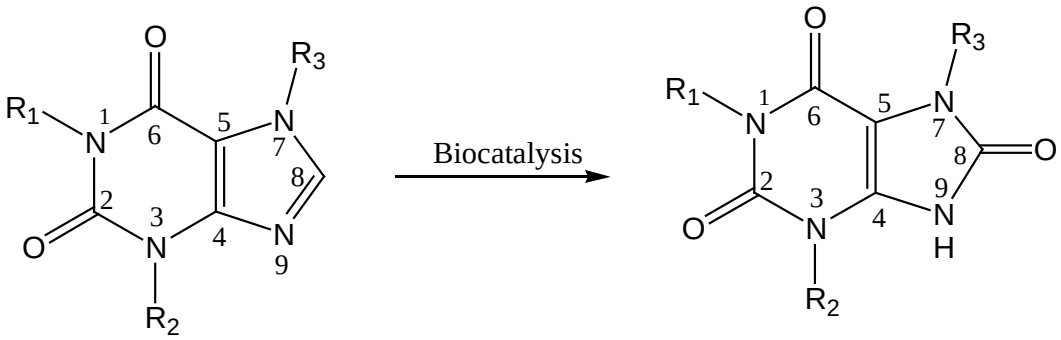
R_3: Me, Et, *n*-Prop, *n*-Bu

R_1: Me, Et, *n*-Prop, *n*-Bu
R_2: Me, Et, *n*-Prop, *n*-Bu
R_3: Me, Et, *n*-Prop, *n*-Bu
R_4: Me, Et, $-(CH_2)_2Cl$, C_6H_5, $-(CH_2)_2OH$
R_5: Me, Et, $-(CH_2)_2Cl$, C_6H_5, $-(CH_2)_2OH$

2.4 C-oxidation

1,3,7-Trisubstituted xanthines can be oxidised enzymatically (**Scheme 3**) to 8-oxo compounds (methylated uric acid).[14]

Scheme 3



$R_{1:}$ Me, Et, *n*-Prop, *n*-Bu
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$R_{2:}$ Me, Et, *n*-Prop, *n*-Bu
$R_{3:}$ Me, Et, *n*-Prop, *n*-Bu

3. Photochemistry of xanthine derivatives

The few published photochemical reactions of xanthines can be summarized as follows:

1. Oxidation at C-8, C-4 and C-5.
2. Substitution at C-8.
3. Photodealkylation at C-8.

Most of these reactions can be envisaged as being the result of radicals generated by photolysis.

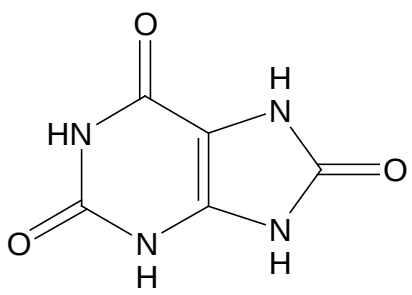
3.1. Oxidation reactions

It is well known that singlet oxygen radicals generated photolytically can act as oxidising agents. An example of a product isolated includes uric acid[15] (**6**) in (**Figure 4**).

Some authors have reported the oxidation of xanthines with hydroxyl radicals, either generated conventionally or photochemically. An example of a product isolated includes 5, 6-dihydroxy compounds[16] of type (**7**) in (**Figure 4**).

These reactions are important in the investigation of the mechanism of radical damage to DNA.

Figure 4



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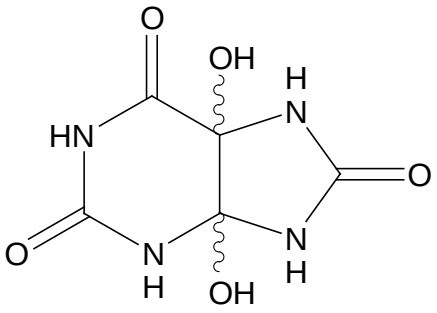

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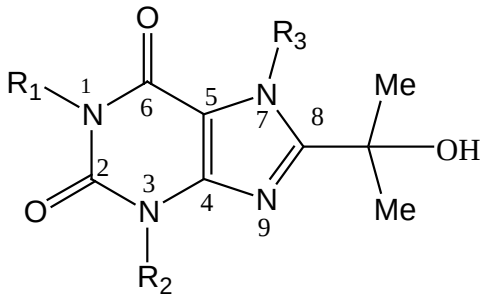
3.2. Substitution reactions

In the absence of oxygen, radicals derived photolytically from alcohols, amines and ethers, including cyclic ethers, can substitute the C-8 hydrogen to yield xanthines with α-hydroxyalkyl[17] (**8**), α-aminoalkyl[17](**9**) or α-alkoxyalkyl[18] (**10**) substituents at C-8. Trace amounts of 8-alkylsubstituted products[19] (**11**) were sometimes observed. (**Figure 5**)

Irradiation of caffeine with 2-propanol also yielded moderate amounts (ca.14- 25%) of a product from alkylation at C-8 with a 1-hydroxy-1-methylethyl group (**12**)[17]. The free radical nature of these reactions is indicated by the increase in yields of up to 65% by addition of di-tert-butyl peroxide.

Figure 5

8:



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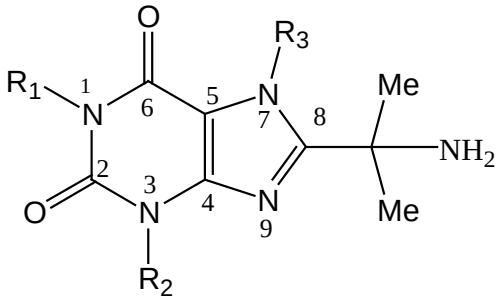

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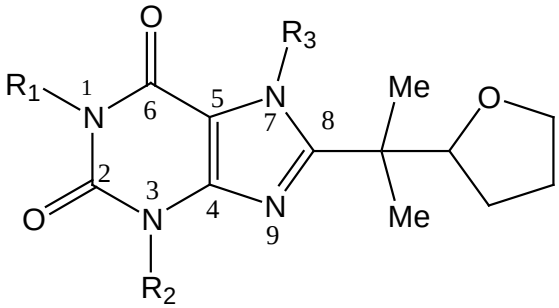
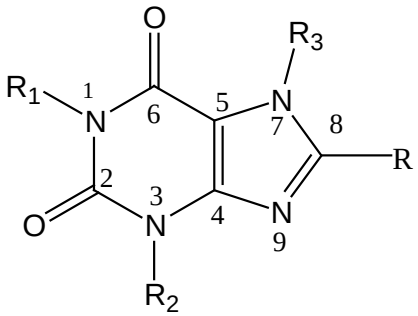
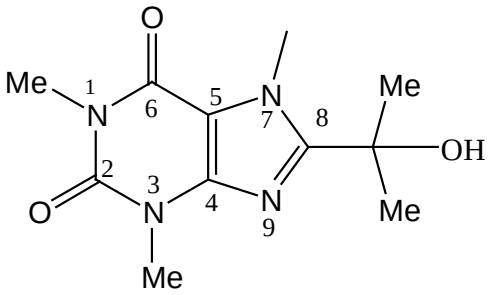


11:



R: Ethyl, *n*-Propyl, *iso*-Propyl

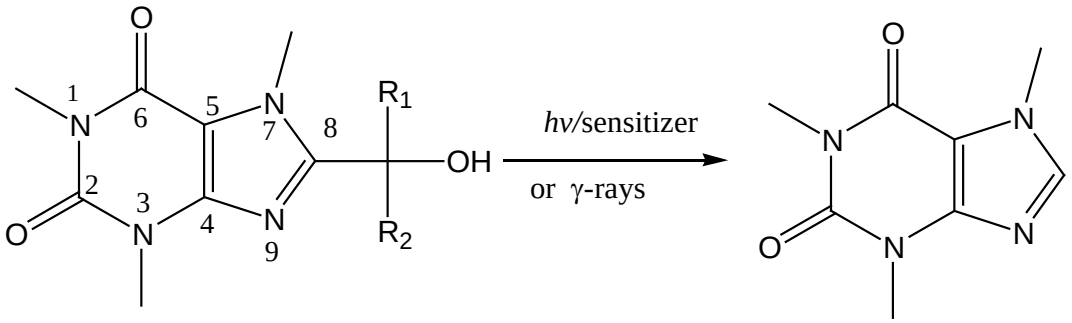
12:



3.3 Photo-dealkylation

8-(1-Hydroxylisopropyl) caffeine and other xanthines with a 1-hydroxylalkyl group at C-8 can be dealkylated photolytically (**Scheme 4**) to yield the 8-unsubstituted xanthines[22]. These reactions are of importance in the re-activation and repair of photo- and γ-ray induced lesions in the purine moieties of nucleic acids. The efficiency of the reaction increases with enhanced stability of the "released" C-8 side chain free radicals, i.e. $^{\cdot}CH_2OH$ < $^{\cdot}CHMeOH$ < $^{\cdot}CMe_2OH$. 8-Alkylpurines were stable under the reaction conditions and no dealkylation was observed[22].

Scheme 4



$R_{1:}$ Me, Et, *n*-Prop, *n*-Bu
$R_{2:}$ Me, Et, *n*-Prop, *n*-Bu

$R_{1:}$ Me, Et, *n*-Prop, *n*-Bu
$R_{2:}$ Me, Et, *n*-Prop, *n*-Bu

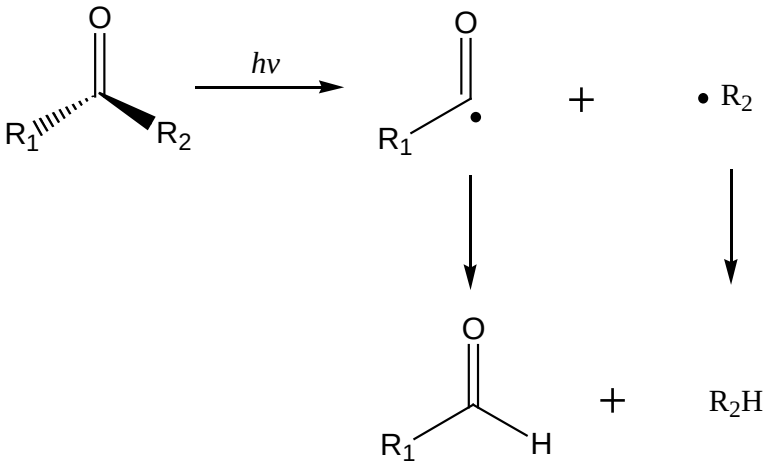
4. Photochemistry of aliphatic carbonyl compounds

Aliphatic carbonyl reactions can undergo the following photolytical transformations[24].

4.1 The Norrish I reaction / α-cleavage

The Norrish I reaction[27] (**Scheme 5**) involves cleavage of the carbon bond next to the carbonyl group followed by subsequent rearrangements.

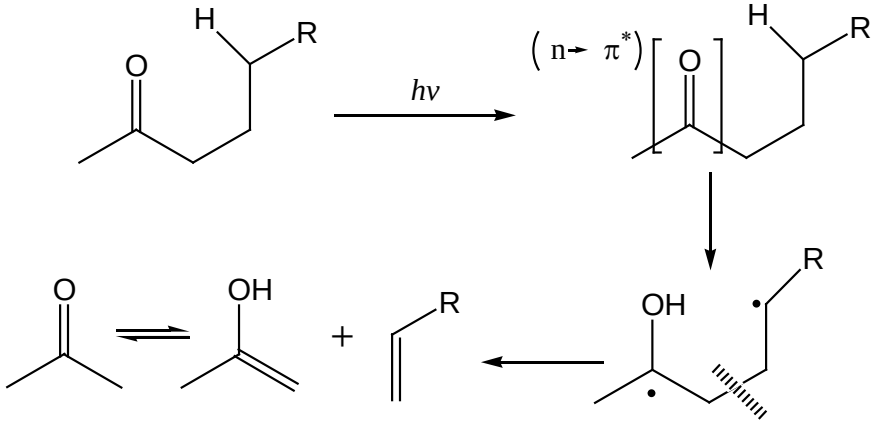
Scheme 5



4.2 The Norrish II / β-cleavage reaction

The Norrish II or β-cleavage reaction[28] (**Scheme 6**) involves hydrogen abstraction from a γ-carbon (if available) by the exited carbonyl group. Then the biradical intermediate undergoes cleavage of the β-bond leading to elimination products.

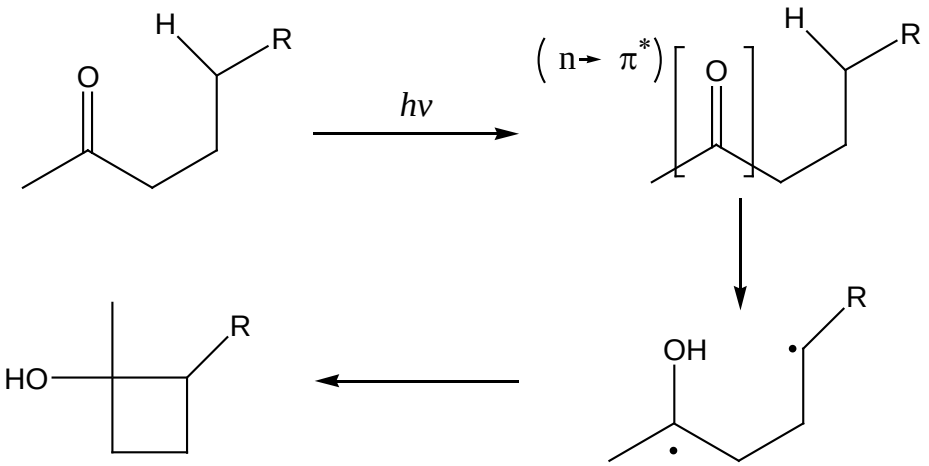
Scheme 6



4.3 The Yang cyclisation reaction

The Yang cyclisation reaction[24] (**Scheme 7**) also involves a biradical intermediate as the result of the Norrish II type γ-hydrogen abstraction. The biradical, however, does not undergo β-cleavage but instead cyclises to form α-substituted cyclobutanol.

Scheme 7

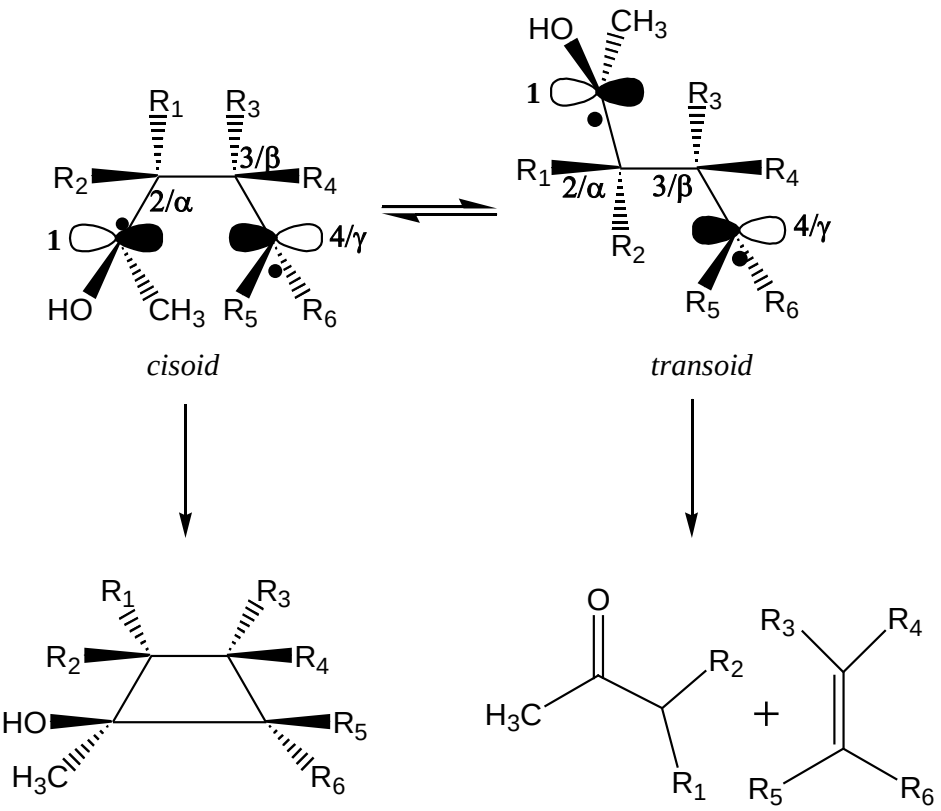


4.4 Stereochemistry of Yang cyclisation and Norrish II reaction

Triplet exited state ketones pass through chair-like cyclic transition states to allow γ-hydrogen abstraction and formation of intermediate 1,4-biradicals. These biradical intermediates may then either cleave between the C-2–C-3 bond to form Norrish II type alkenes and enols or combine at C-1 and C-4 to afford Yang cyclisation products.

If the most stable conformation of the intermediate 1,4-biradical is *cisoid*, then we would expect predominantly Yang cyclisation products. If steric repulsion between C-2 and C-3 substituents on the 1,4-biradical destabilises the *cisoid* conformation to the extent that the *transoid* conformation becomes more stable, then Norrish II type elimination products would be expected. (**Scheme 8**)

Scheme 8



Moorthy and co-workers[25] demonstrated that the conformational stability of the 1, 4-diradicals, determined by substituents on the α-, β- and γ- positions relative to the carbonyl group, determines partitioning between these two alternative pathways.

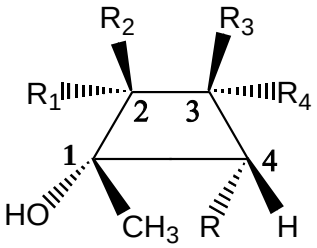
The configurational variation in the reaction products, i.e., SS, RR, RS, and SR diastereoisomers, is explicable in terms of the geometries of the ketones. The triplet excited state that reacts via a *cisoid* transition state has a lifetime of 290ns while the triplet excited state that reacts via a *transoid* transition state has a lifetime of 460ns[25].

Yang cyclisation occurs with a remarkable high degree of stereoselectivity. The existence of the *cisoid* conformation in the transition state (**scheme 8**) is necessary for the formation of cyclobutanol derivatives. This is controlled by nonbonding and steric interactions as the two ends (C-1 and C-4) of the biradical begin to bond.

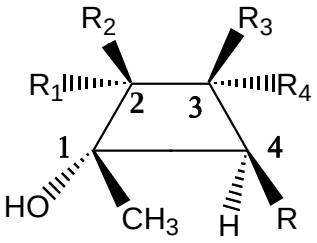
The strain associated with ring formation may also play a role. Moorthy and Mal[26] pointed out that the solvation of the hydroxy group increases its steric bulk. The

major product (**cis-CB1**) having *cis*-configured OH and R groups (**figure 6**) in nonpolar solvents such as benzene becomes the minor product in a polar solvent such as acetonitrile or methanol. The product (**cis-CB2**) having *trans*-configured OH and R groups becomes the major product in polar solvents.

Figure 6



cis-CB1,
the favourite form in non-polar solvent
R: Me, Et, *n*-Prop, *n*-Bu

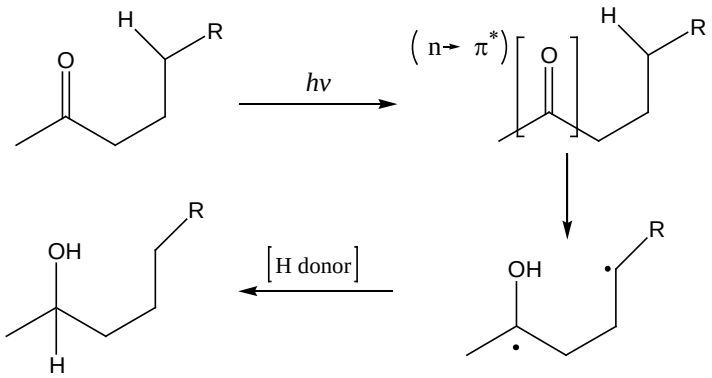


cis-CB2 ,
the favourite form in polar solvent
R: Me, Et, *n*-Prop, *n*-Bu

4.5 Photoreduction

Photoreduction of the excited carbonyl chromophore to a secondary alcohol may take place in the presence of hydrogen donors. (**Scheme 9**)

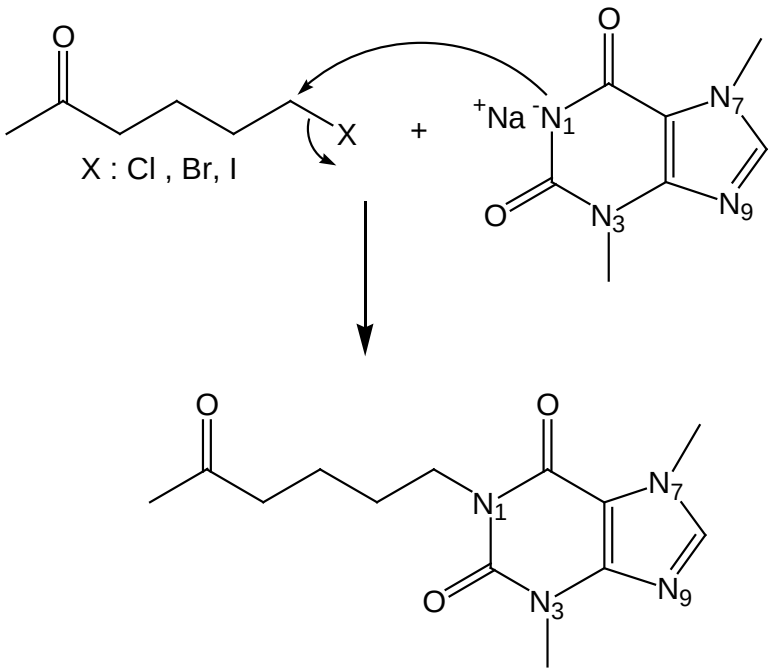
Scheme 9



5. Chemistry of pentoxifylline

The key reaction in the patented synthesis of pentoxifylline[23] is the reaction of 1-halohexan-5-one with an alkali metal salt of theobromine (**Scheme 10**). Apart from this patent we could not find any related references to the chemistry of pentoxifylline.

Scheme 10



Yields are low because of the low reactivity of the N-1 position on the theobromine towards alkylation with electrophiles[4].

Under alkaline conditions, alkylation of xanthine takes place in the order of decreasing acidity of the appropriate proton i.e. N_3-H, N_7-H, and then N_1-H. The low reactivity of N-1 position towards alkylation[4] maybe attributed to the decreased nuceophilicity of the N-1 nitrogen and the steric hindrance of the N-1 position by the adjacent carbonyl groups[4].

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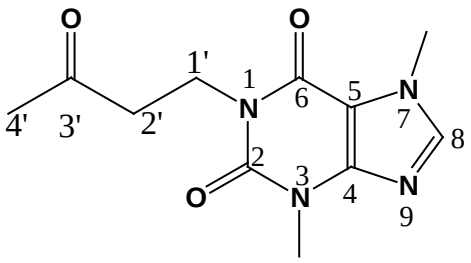
CHAPTER 2: RESULTS AND DISCUSSIONS

1. Biological assays

Development of new xanthine derived clinical agents related to pentoxifylline are impeded by the limited availability of synthetic methods to increase the scope of derivatives availability for testing. This is particularly important in view of the fact that xanthine derivatives are considered privileged structures[1] with a higher than average probability of demonstrating bio-activity. It has been demonstrated that structural modifications at the 1-position of caffeine leads to selective inhibition of A_1 and A_2 adenosine receptors.[2,3] Adenosine receptors are ubiquitous through the human body and has been demonstrated to be involved in regulating brain function, myocardial oxygen consumption and human melanoma cell growth[4] amongst other things.

Our efforts to synthesise 1-(3'-oxobutyl)-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (**Figure 7**) for biological assay and as an internal standard of biological drug and some of its derivatives, were hampered by the low reactivity of the N-1 position of theobromine towards alkylation with electrophiles. The low reactivity of this position is not only attributed to steric hindrance[5] from the two adjacent carbonyl groups at the 2 and 6 positions but also to the low intrinsic nucleophilicity of the double amide-type nitrogen function. Commercially available pentoxifylline is presumably manufactured by reacting 1-halohexan-5-one with an alkali metal salt of theobromine as was patented[6]. The patent has however not been followed by a publication in a peer reviewed journal and the exact details of the synthetic procedure remain uncertain.

Figure 7



Because of our failure to allylate the N-1 position of theobromine, we investigated the potential of photochemistry to transfer the commercially available pentoxifylline into internal standards and novel pharmaceutical agents.

2. Photochemical transformations

Pentoxyfylline is an ambident chromophore with two different functional groups separated by a linear saturated butane moiety. The two groups that can absorb ultraviolet light are:

1. The aliphatic carbonyl group
2. The heterocyclic aromatic xanthine group that contains two carbonyls and an imine group embedded in the aromatic system.

This ambident nature gives rise to the following questions:

1. Can we isolate products from photochemistry of the carbonyl group only?
2. Can we isolate products from photochemistry of the aromatic heterocyclic group only?
3. Is there interaction between the two chromophores? This interaction will have to be through space as electronic interaction through five σ-bonds is improbable.
4. Can we manipulate reaction conditions to obtain reaction at one chromophore at the expense of the other chromophore?

2.1 Photochemistry of the carbonyl moiety of pentoxifylline

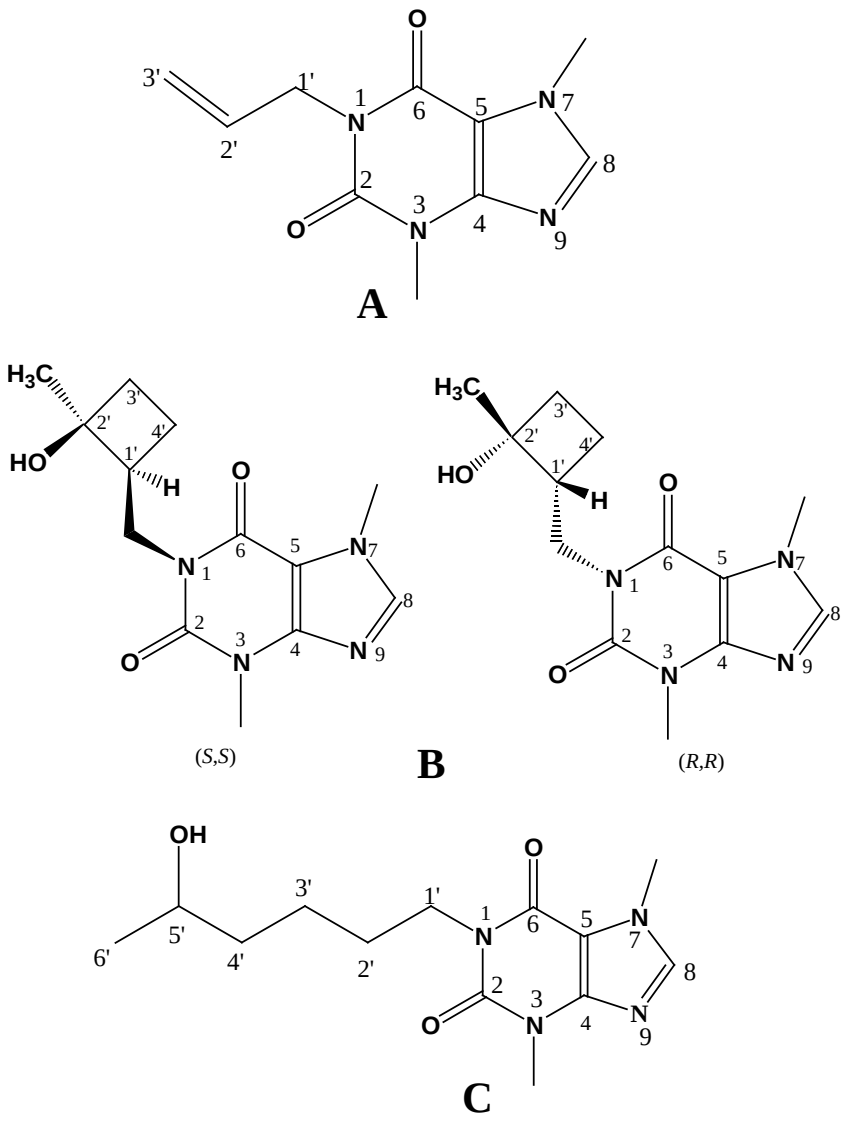
In the absence of a photosensitizer, the photolysis of pentoxifylline in non-polar solvent (toluene) at 300 nm led to three products (**Figure 8**), related to reaction at the carbonyl moiety:

A: 1-Allyl-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione

B: (R^*, R^*,)-(±)-1-{[2-Hydroxy-2-methylcyclobutyl]methyl}-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione

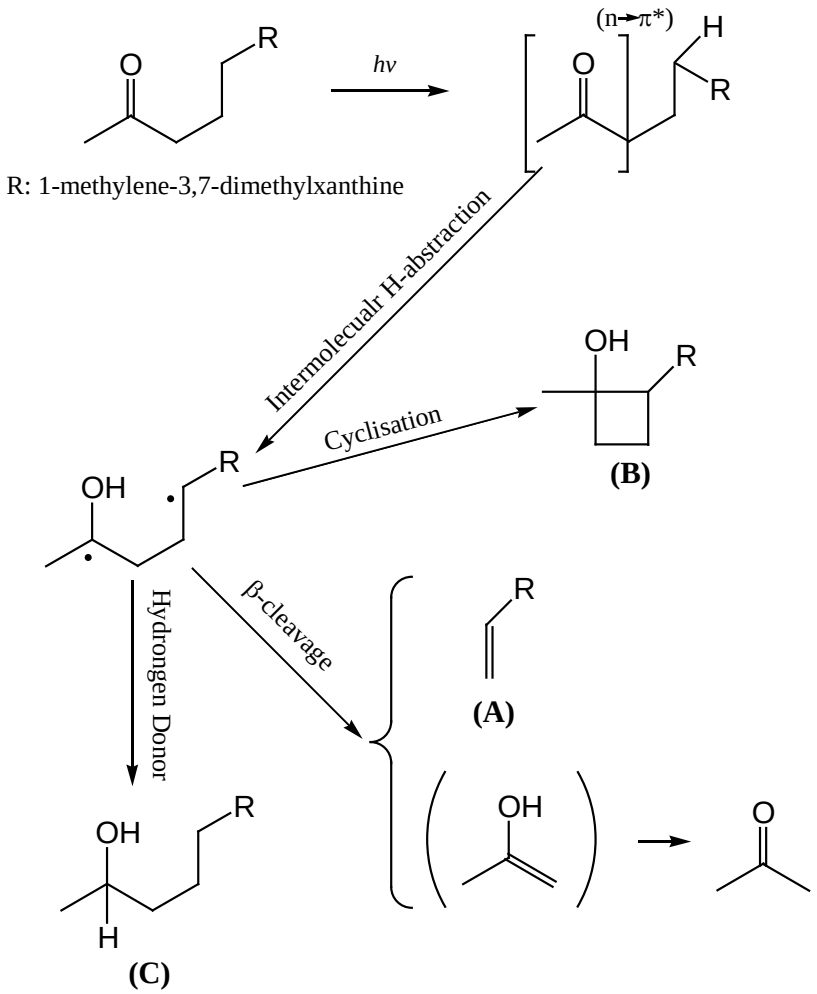
C: 1-(5-Hydroxy-hexyl)-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione

Figure 8



The formation of these products can be explained by photochemical excitation of the carbonyl group to form a 1,4-diradical intermediate. (**Scheme 11**)

Scheme 11



To obtain a better understanding of the mechanism and factors controlling this reaction and in an effort to improve yields, we experimented with a variety of conditions. (**Table 1**)

Table 1

Conditions	**(Product) yield %**			
	(**A**)	(**B**)	(**C**)	Time (hr)
	H-donor solvent *vs* **non-H donor solvent under irradiation at 300 nm**			
Benzene	40	10	none	24
Toluene	32	8.2	6.5	24
	Addition of TBTH *vs* **absence of TBTH under irradiation at 300 nm**			
Toluene	32	8.2	6.5	24
Toluene & TBTH	28	7.3	6.0	24
	Addition of triplet quencher *vs* **absence of triplet quencher under irradiation at 300nm**			
Toluene	32	8.2	6.5	24
Toluene & Naphtalene	48	12	9.0	24
	Addition of triplet sensitizer *vs* **absence of triplet sensitizer under irradiation at 300nm**			
Toluene 300nm	32	8.2	6.5	24
Toluene & Benzophenone	7.5	1.8	1.2	24
Toluene& Acetophenone	9.5	2.3	1.5	24
	Aprotic solvent *vs* **polar protic solvents under irradiation at 300nm**			
Benzene	40	10	none	24
H_2O	16	3.7	2.4	48

(TBTH : Tributyltin hydride ; PGN : Phloroglucinol)

From **Table 1**, the following salient observations regarding the photochemical reaction of the carbonyl moiety could be made:

1. At 350nm, no photochemical products are formed. This can be explained by the fact that the carbonyl chromophore does not absorb ultraviolet light at this wavelength.

2. At 250nm, the yields of photochemical reactions of carbonyl moiety are poor. TLC indicates a large number of products in low yields.

3. The optimum irradiation conditions for product **A** (40% yield) and **B** (10%) is at 300nm wavelength in benzene.

4. The ratio between product **A** and **B** is always 4:1, irrespective of the yield.

5. In toluene, we also obtained product **C**, probably because the excited carbonyl group can abstract a hydrogen radical from the toluene methyl group.

6. Addition of tributyltin hydride as hydrogen donor does not improve the yield of the product **C**. This is confirmed by HPLC experiments. (**Tables 2** & **3**) and ("**HPLC spectra I** & **II**" in the APPENDIX).

Table 2

(**[a]HPLC plate I**)

peak	retention time R_T (minutes)	% area	compound
1	8.871	32.8	**A**
2	9.777	8.2	**B**
3	8.266	1.8	**C**
4	9.499	53.2	**P**

[a] The crude reaction product was injected into the HPLC column after 24 hours irridation of pentoxifylline. The compounds in crude product were identified according to their individual retention times.

Table 3

[b]HPLC plate II

peak	retention time/R_T(minutes)	% area	compound
1	8.928	30.9	**A**
2	9.816	8.0	**B**
3	8.303	1.9	**C**
4	9.551	53.9	**P**

[b] The crude reaction product was injected into the HPLC column after 24 hours irridation of pentoxifylline with 1equvilent of tributyltin hydride. The compounds in crude product were identified according to their individual retention times.

A: 1-Allyl-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione

B: (R^*, R^*,)-(±)-1-{[2-Hydroxy-2-methylcyclobutyl]methyl}-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione

C: 1-(5-Hydroxyhexyl)-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione

P: Pentoxifylline

7. Addition of triplet sensitisers (benzophenone and acetophenone) does not enhance the carbonyl photochemical reaction yields as expected but indeed inhibits the reaction.

8. Addition of a triplet quencher (naphthalene) unexpectedly enhances the yield in toluene.

9. The reaction does take place in water but at a lower yield and at a reduced rate (48 hours).

10. No product arising from α-cleavage/Norrish I was observed.

2.2 Photochemistry of the heterocyclic aromatic group/xanthine

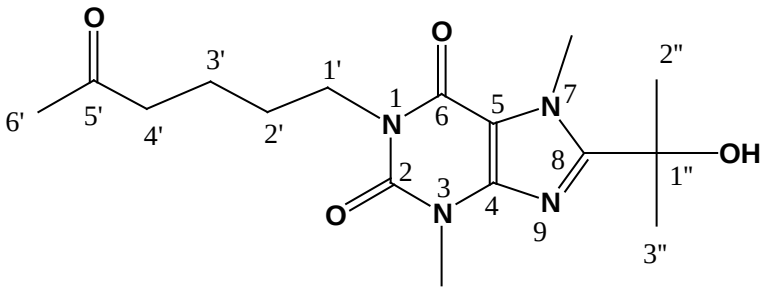
In solvents with an α-hydroxyalkyl hydrogen we obtained the following 8-substituted pentoxifylline derivatives (**Figure 9**)

D: 8-(1-Hydroxy-1-methylethyl)-3,7-dimethyl-1-(5-oxohexyl) -3,7-dihydro-1*H*-purine- 2,6-dione

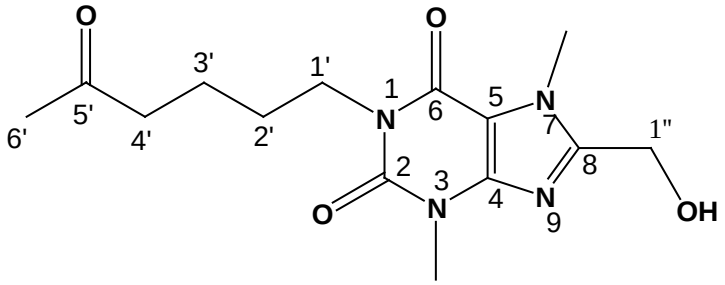
E: 8-(1-Hydroxymethyl)-3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione

F: 8-(1-Hydroxyethyl)-3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione

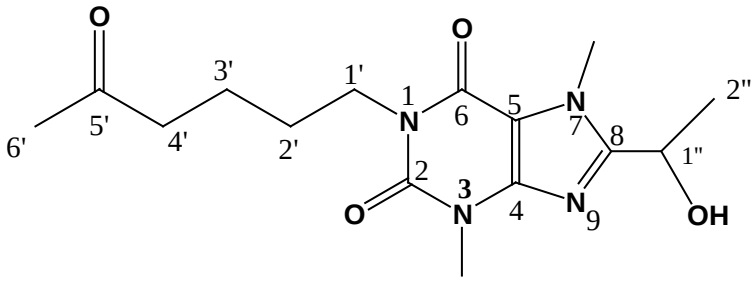
Figure 9



D



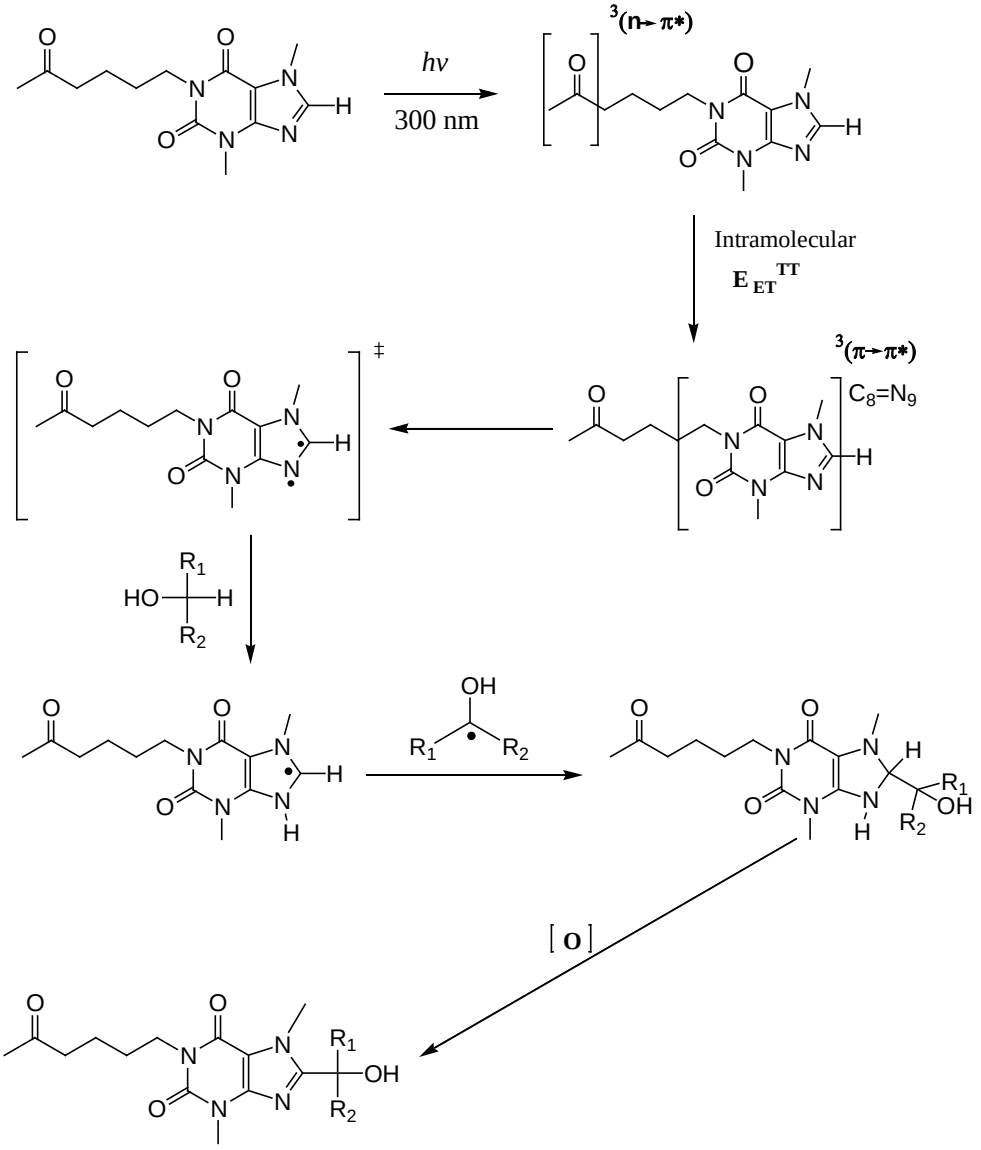
E



F

These products were obtained by photochemical excitation of the aromatic group according to the following mechanism. (**Scheme 12)**

Scheme 12



To obtain a better understanding of the mechanism and factors controlling this reaction, and in an effort to improve yields, we experimented with a variety of conditions. (**Tables 4, 5 and 6**)

Table 4

	Product (yield %)

Conditions	**A** (%)	**B** (%)	**C** (%)	**D** (%)	**E** (%)	Time (hr)
	MeOH *vs* MeOH*					
MeOH 300nm	10	2.5	2	19	10	24
MeOH * 300nm	46	11	9	none	none	24
MeOH *vs* MeOH/ acetone (v/v:1/1)						
MeOH 300nm	10	2.5	2	19	10	24
MeOH/Acetone 300nm	8	2	2	28	6	24
	MeOH *vs* MeOH/ DTBP					
MeOH 300nm	10	2.5	2	19	10	24
MeOH/DTBP 300nm	7	1.8	1.4	none	48	24
	MeOH *vs* MeOH/H_2O (v/v:1/1)					
MeOH 300nm	10	2.5	2	19	10	24
MeOH/H_2O 300nm	11	3	2.5	none	none	48
	MeOH *vs* acetone (v/v:1/1)					
MeOH 300nm	10	2.5	2	16	13	24
Acetone 300nm	10	2.5	none	6.7	none	24

(*: Photolysis is under atmospheric conditions)

(DTBP: Di-*tert*-butyl peroxide; note: DTBP was added in catalytic amount)

A: 1-Allyl-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione

B: (R^*, R^*,)-(±)-1-{[2-Hydroxy-2-methylcyclobutyl]methyl}-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione

C: 1-(5-Hydroxyhexyl)-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione

D: 8-(1-Hydroxy-1-methylethyl)-1-(5-oxohexyl)-3,7-dimethyl-3,7-dihydro -1*H*-purine- 2,6-dione

E: 8-(1-Hydroxymethyl)-1-(5-oxohexyl)-3,7-dimethyl-3,7-dihydro -1*H*-purine-2,6-dione

Table 5

	Product (yield%)

Conditions	**A** (%)	**B** (%)	**C** (%)	**D** (%)	**F** (%)	Time (hr)
	EtOH *vs* EtOH *					
EtOH 300nm	10	2.5	2	22	9	24
EtOH * 300nm	50	13	10	none	none	24
	EtOH *vs* EtOH /acetone (v/v:1/1)					
EtOH 300nm	10	2.5	2	22	9	24
EtOH/Acetone 300nm	8	2	2	30	7	24
	EtOH *vs* EtOH/DTBP					
EtOH 300nm	10	2.5	2	22	9	24
EtOH/DTBP 300nm	none	none	none	none	66	24
	EtOH *vs* acetone					
EtOH 300nm	10	2.5	2	22	9	24
Acetone 300nm	10	2.5	none	6.7	none	24
	EtOH *vs* EtOH/H_2O (v/v:1/1)					
EtOH 300nm	10	2.5	2	22	9	24
EtOH/H_2O 300nm	12	3	2.5	none	none	48

(*: Photolysis is under atmospheric conditions)

(DTBP: Di-*tert*-butyl peroxide; note: DTBP was added in catalytic amount)

A: 1-Allyl-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione

B: (R^*, R^*,)-(±)-1-{[2-Hydroxy-2-methylcyclobutyl]methyl}-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione

C: 1-(5-Hydroxyhexyl)-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione

D: 8-(1-Hydroxy-1-methylethyl)-1-(5-oxohexyl)-3,7-dimethyl-3,7-dihydro -1*H*-purine- 2,6-dione

F: 8-(1-Hydroxyethyl)-1-(5-oxohexyl)-3,7-dimethyl-3,7-dihydro -1*H*-purine-2,6-dione

Table 6

Conditions	Product (yield, %)				
	A (%)	**B** (%)	**C** (%)	**D** (%)	Time (hr)
	2-Propanol *vs* 2-propanol *				
2-Propanol 300nm	8	2	2	22	24
2-Propanol * 300nm	30	8	7	none	24
	2-Propanol *vs* 2-propanol/acetone (v/v:1/1)				
2-Propanol 300nm	8	2	2	22	24
2-Propanol/Acetone 300nm	17	4.5	3.5	55	24
	2-Propanol *vs* 2-propanol/DTBP				
2-Propanol 300nm	8	2	2	22	24
2-Propanol/DTBP 300nm	8	2	none	36	24
	2-Propanol *vs* acetone				
2-Propanol 300nm	8	28	2	22	24
Acetone 300nm	10	2.5	none	6.7	24
	2-Propanol *vs* 2-propanol/H_2O (v/v:1/1)				
2-Propanol 300nm	8	28	2	22	24
2-Propanol/H_2O 300nm	12	3	2.5	20	48

(*: Photolysis is under atmospheric conditions)

(DTBP: Di-*tert*-butyl peroxide; note: DTBP was added in catalytic amount)

A: 1-Allyl-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione

B: (R^*, R^*,)-(±)-1-{[2-Hydroxy-2-methylcyclobutyl]methyl}-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione

C: 1-(5-Hydroxyhexyl)-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione

D: 8-(1-Hydroxy-1-methylethyl)- 1-(5-oxohexyl)- 3,7-dimethyl-3,7-dihydro -1*H*-purine- 2,6-dione

From **Tables 4, 5** and **6** the following salient observations may be noted

1. The aromatic chromophore is not photochemically reactive at 350nm.

2. The aromatic chromophore led to many products all with low yields at 250nm. This observation was confirmed by TLC.

3. The optimum irradiation condition for product (**D**) comprises the absence of a radical initiator (55% of yield) at 300 nm with 2-propanol/acetone (1:1) as solvents and the absence of oxygen (purged with nitrogen).

4. Lowered amounts of the C-8 substituted product (**D**) is formed in the presence of 2-propanol only.

5. In the presence of oxygen (no purging with nitrogen) the aromatic chromophore is photochemically inert while the carbonyl moiety is still photo-reactive. The Norrish II cleavage product (**A**) and Yang cyclisation product (**B**) (4:1 ratio) become the major products.

6. The yields of the 8-substituted products **E** and **F** are dramatically increased by the addition of a catalytic amount of di-*tert*-butyl peroxide as the radical initiator.

From **Tables 1, 4, 5,** & **6** and **Schemes 11** & **12**, the following conclusions may be drawn:

For aliphatic carbonyl moiety of pentoxifylline

1. Both singlet and triplet states of aliphatic ketones participate in the Norrish II photoelimination process[7].

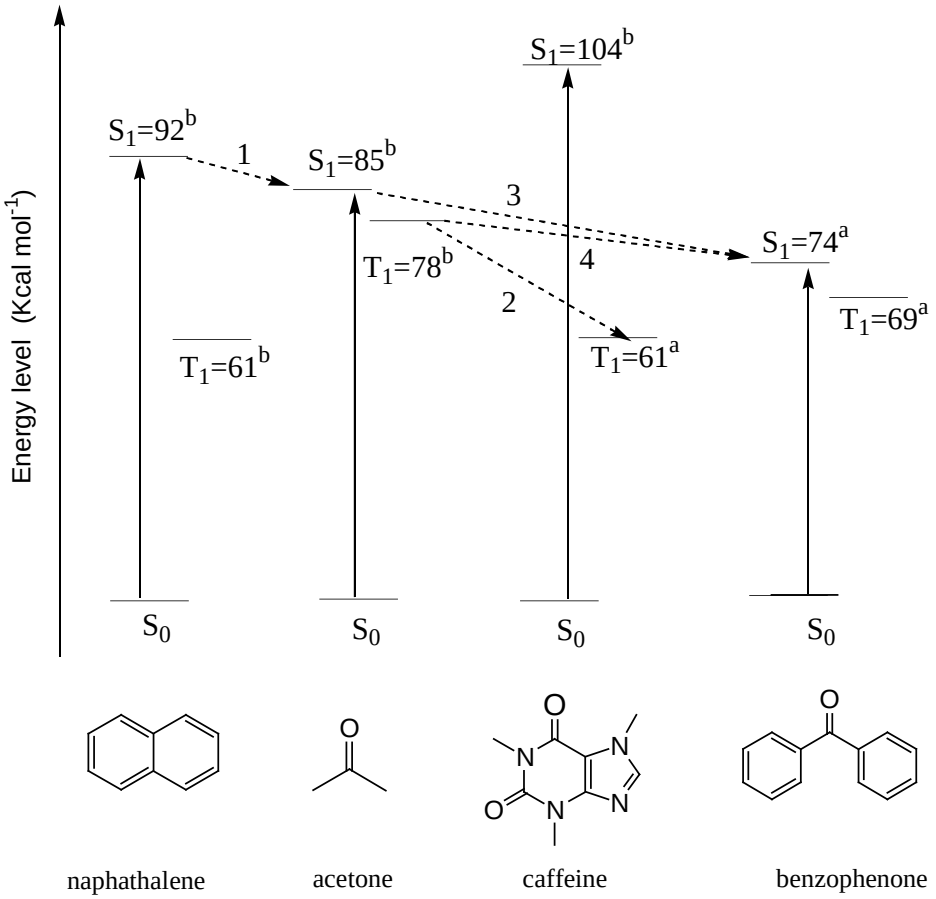
2. Addition of a hydrogen donor (tributyltin hydride) does not increase the yield of photoreductive product (**C**). Tributyltin hydride is known to photoreduce most of the triplet but none of the singlet excited carbonyl[8].

3. Polar solvents do not increase the yields of products **A**, **B**, and **C**. Wagner

reported[9] that the quantum efficiency of type II photo-elimination from triplet aliphatic ketone carbonyl is enhanced appreciably by polar solvents. Yang[10] proved that there is no polar solvent effect on singlet-state carbonyl quantum yields.

4. Unconjugated aliphatic ketones (acetone) have relatively high-energy triplet excited states (T_1)[11]. This means that the carbonyl group of pentoxifylline, which is a hexan-2-one, also possesses a higher triplet exited state (T_1) than both acetophenone (T_1)[11] and benzophenone (T_1)[11]. This energy gap is appropriate (as indicated in **Scheme 13**) for triplet-triplet energy transfer from triplet excited carbonyl of pentoxifylline to ground states of acetophenone and benzophenone. Consequently, the triplet excited carbonyl of pentoxifylline is probably quenched by both acetophenone and benzophenone.

Scheme 13



[a] Values from H. Moustafa , S. H. Shalaby , K.M. El-Sawy , R. Hilal. *Spectrochimica Acta Part A* 58 (2002) 2013-2027

[b] Values from N.J.Turro, "Modern Molecular Photochemistry", 1978 ® The Benjamin/ Cummings Publishing Company, NEW YORK

5. Naphthalene has a higher singlet excited energy (S_1)[11] than that of acetone[11] and this energy difference is appropriate. It has such a big energy difference[11] between its S_1 and T_1 states that the rate of intersystem crossing is very low. Hence, its excited states are mostly singlets. These two conditions allow the energy transfer from singlet excited states of naphthalene to the ground states of the carbonyl moiety of pentoxifylline. As a result, the photochemical reaction is enhanced.

6. Turro[12] and coworkers demonstrated that γ-hydrogen abstraction is over an order

of magnitude faster for singlet than for triplet 2-pentanone. He also demonstrated that α-cleavage takes place predominantly (over two orders of magnitude faster) from the triplet excited state. Competition from Norrish type I /α-cleavage is, however, considerable only when an α-carbon is tertiary or substituted with strong radical stabilising groups[13]. These support our hypothesis that the aliphatic carbonyl group of pentoxifylline reacts from the singlet state and explains the absence of α-cleavage products.

7. Photoreduction is an intermolecular reaction with a hydrogen donor and cannot compete with the intramolecular Norrish II and Yang cyclisation reactions. This explains why a very efficient hydrogen donor such as tributyltin hydride has no enhancing effect on the photoreduction.

8. The 4:1 ratio between product A (from Norrish II / β-cleavage) and product B (from Yang cyclisation) agrees with evidence that singlet excited biradicals mainly cleave in preference to cyclisation[14].

For the aromatic xanthine moiety of pentoxifylline

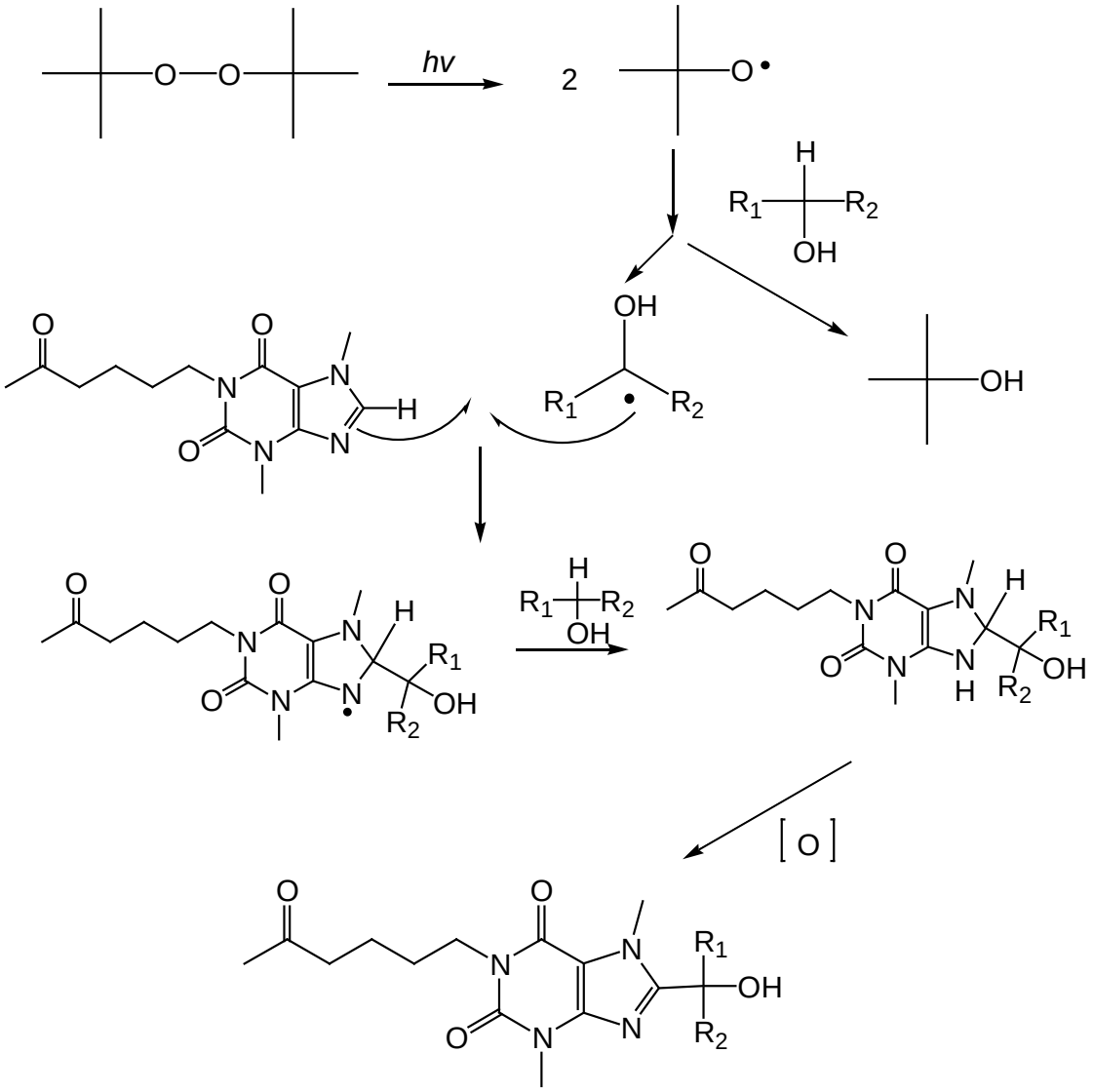
8-Substitution takes place from the triplet excited state of the xanthine moiety:

1. Oxygen, a well-known triplet quencher[11], inhibits the formation of products **D**, **E**, and **F** by quenching the long-lived triplet excited states T_1[15] of the xanthine moiety in pentoxifylline.

2. The presence of acetone, a well-known triplet sensitizer is essential to obtain a good yield (55%) of product **D** but not necessarily the related analogues **E** and **F**. In this case, acetone serves as the light absorbing system and the excited acetone abstracts a hydrogen atom from the hydrogen donor (2-propanol) forming a free radical of the latter. This radical is scavenged by a neighbouring purine molecule which subsequently yields the appropriate photoproduct.

3. The reaction is radical-like in nature. Our efforts to synthesise the 8-hydroxymethyl and 8-hydroxyethyl derivatives (**E** and **F**) by using acetone presumably as a photosensitizer in methanol and ethanol did not succeed in terms of the low yields (**Table 5** and **6**). By using di-*tert*-butyl peroxide as photo-initiator (catalytic amounts), however, we succeeded to obtain these products in good yields. We were particularly pleased to have developed a good method to synthesise 8-(1-hydroxymethyl-3,7-dimethyl-1-(5-oxo-hexyl)-3,7-dihydro-1H-purine-2,6-dione, which does not form under normal photolytic conditions, as this will be tested for an internal standard. Photolysis of di-*tert*-butyl peroxide at 254nm gives *tert*-butoxy radicals that can abstract the α-hydrogen of alcohols and amines[16]. As expected from radical hydrogen abstraction, the weakest C-H bond (linked to the carbon with the hydroxy or amine groups) is broken to give an α-hydroxyalkyl radical that attacks the 8-position. (**Scheme 14**)

Scheme 14



D:R_1=R_2=CH_3
E:R_1=R_2=H
F:R_1=CH_3;R_2=H

Hilal[15] investigated the electronic absorption spectra of theophylline, caffeine, and derivatives. These compounds exhibit broad absorption spectra with a strong absorption at about 280nm corresponding to an S_1 ($\pi\rightarrow\pi^*$) excited state. An ($n\rightarrow\pi^*$) transition at about 270nm corresponding to the S_2 excited state exhibited a strong solvent dependence (red shift and intensification upon increasing solvent polarity).

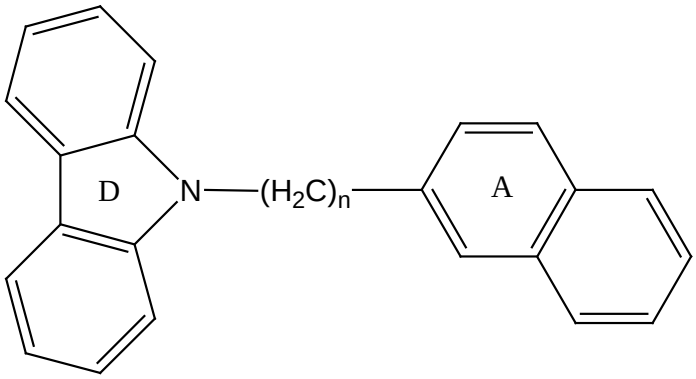
A long-lived low-lying T_1 state that bears π, π^* excited states properties was identified and postulated to underlie the photochemical reactivity of alkylxanthine. It

was measured[15] that the T_1 excited state energy level equals 61*kcal.mol*$^{-1}$ (**Scheme 13**).

Combination of aliphatic carbonyl moiety and aromatic xanthine moiety

Many examples of intramolecular triplet-triplet energy transfer (**figure 10**) have been reported[17]. The carbazole (Cz) group as an energy donor and naphthalene (Np) group as an energy acceptor system shows only naphthalene-like phosphorescence when the carbazole group is selectively excited. Since singlet-singlet energy transfer is energetically forbidden and since control experiments rule intermolecular energy transfer out, it was concluded that triplet-triplet energy transfer is the major pathway. A nearest neighbour collision is not required for intramolecular triplet-triplet transfer. The rate constant was estimated to be close to 10^{10}sec^{-1}.

Figure 10



D: donor
A: acceptor
n = 8-12

The close intramolecular proximity of a high energy triplet exited carbonyl group and a low energy triplet unexcited xanthine group in pentoxifylline implies that triplet energy transfer (process (2) in **Scheme 13**) from the carbonyl group to the xanthine group is extremely efficient and limits the lifetime of the carbonyl triplet state to such an extend that reaction from the triplet state is not possible. The carbonyl group can thus only react from the singlet-state. Because of the short lifetime of the singlet-state

only intramolecular Norrish II and Yang reaction products are possible in competition with deactivation of the singlet state to the triplet state and immediate quenching of this state by energy transfer to the xanthine moiety.

Triplet sensitisers such as benzophenone do not have a positive effect on the reactivity of the carbonyl group of pentoxifylline. This observation is due to the fact that the excited energy levels of benzophone are lower than that of aliphatic ketone carbonyl of pentoxifylline (referred to T_1 of acetone[11]).

Photoreduction is an intermolecular reaction with a hydrogen donor and cannot compete with the intramolecular Norrish II and Yang reactions (or intramolecular triplet energy transfer to the xanthine chromophore). This explains why a very efficient hydrogen donor such as tributyltin hydride has no enhancing effect on the photoreduction.

The 4:1 ratio between product **A** (from Norrish II β-cleavage) and product **B** (from Yang cyclisation) agrees with evidence that singlet excited biradicals mainly cleave in preference to cyclisation.[14]

Although singlet-singlet energy transfer is less common than triplet-triplet energy transfer it is by no means unusual. Naphthalene is a well known triplet quencher but it also absorbs light very efficiently at about 320 nm to sensitise formation of singlet ketones[18]. The increased yields of **A** and **B** are probably simply due to singlet-singlet energy transfer from singlet excited naphthalene to the aliphatic ketone carbonyl moiety of pentoxifylline. (process (1) in **Scheme 13**) The decreased yields of **A** and **B** are probably due to singlet-singlet energy transfer [process (3) in **Scheme 13**] or/and triplet-singlet energy transfer [process (4) in **Scheme 13**] from the excited aliphatic carbonyl moiety of pentoxifylline to benzophenone.

3. Internal standards

An internal standard should be used when performing quantitation with mass spectroscopy. An internal standard should control for variability in extraction, injection and ionisation. An internal standard is added at the beginning of the sample work up at about the same concentration of the analyte to be quantified.

The ideal internal standard is an isotopically labeled form of the molecule that is being quantified. An isotopically labeled internal standard will have a similar extraction recovery, ionisation response in ESI mass spectrometry and a similar chromatographic retention time. Polarity and pKa plays an important role in these parameters. If isotopically labeled internal standards are not available, structural analogues may be used. Of importance is that it must have a slightly different mass (at least three Daltons) and that it co-elutes with the compound to be quantified.
A chlorinated version of the parent molecule often have the same chromatographic retention time and differs sufficiently in mass. Hydroxylated (+16 amu) and de-methylated (-14 amu) versions should be avoided as the human body often manufactures these analogues in unknown quantities as part of its normal metabolic processes from the parent compound.

The limited availability of synthetic methods due to the low reactivity of the 1-position towards nucleophiles that are responsible for the scaresity of derivatives available for bioactivity testing also makes the synthesis of isotopically labeled pentoxifylline difficult.

At least five of the photochemical products from pentoxifylline will be tested for suitability as internal standards, as they have similar R_F values and similar ionisation responses than pentoxifylline. (**Table 7**)

Table 7

Name	M^+	*R_F
1-Allyl-3,7-dimethyl-3,7-dihydro-1*H*-purine -2,6-dione (**A**)	220	0.31
(*R**, *R**,)-(±)-1-{[2-Hydroxy-2-methylcyclobutyl] methyl}-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (**B**)	280	0.26
1-(5-Oxohexyl)-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione/ Pentoxifylline	278	0.22
8-(1-Hydroxymethyl)-1-(5-oxohexyl)-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (**E**)	308	0.19
1-(5-Hydroxyhexyl)-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (**C**)	280	0.14

*: Hexane /EtOAc /acetone /methanol (v / v / v / v: 2.0 / 7.0 / 0.5 / 0.5)

4. Structure elucidation

Comprehensive interpretations of the ^1H and ^{13}C NMR spectra of pentoxifylline and it's photolytic products are listed below:

4.1 Starting material (pentoxifylline)

4.1.1 ^{1}H NMR spectrum (Plate 1A)

The ^{1}H NMR spectrum of pentoxyfylline[19] in $CDCl_3$ is characterised by the following salient features:

1. The aromatic 8-H resonates at δ 7.51 as a singlet.
2. The two N-attached CH_3 groups (N_7-CH_3 and N_3-CH_3) resonate at δ 4.00 and 3.57, respectively, both as singlets.
3. The 6'-CH_3 group is deshielded by the 5'-carbonyl group and resonates at δ 2.14.
4. The 1'-CH_2 protons are deshielded by the N_1 atom of the xanthine moiety and resonate at δ 4.02 as a triplet (J=12 *Hz*) .
5. The 4'-CH_2 protons resonate as a triplet at δ 2.52 (J=7.5 *Hz*).

6. The remaining aliphatic 2'- and 3'- CH_2 protons resonate as overlapping multiplets at δ 1.66.

4.1.2 ^{13}C NMR spectra (Plates 2A and 2B)

The ^{13}C NMR spectrum[19] of pentoxyfylline in $CDCl_3$ is characterised by the following salient features:

1. The aliphatic carbonyl carbon (5'-C) resonates at δ 208.7.
2. The C-8 methine carbon resonates at δ 141.4. This assignment is supported by an inverted absorption in the ATP experiment (**Plate 2B**).
3. The C-6, C-2, C-4 and C-5 quaternary aromatic carbons resonate at δ155.3, 151.5, 148.7 and 107.6, respectively.
4. The nitrogen attached N_1-C and carbonyl attached C-4' resonate at δ 43.2 and 40.8, respectively.
5. The N_3-C, N_7-C and C-6' resonate at δ 33.6, 29.9 and 29.7, respectively. These carbons give inverted resonances in the APT experiment (**Plate 2B**).
6. The aliphatic C-2' and C-3' resonate at δ 27.4 and 21.0, respectively.

4.2 1-Allyl-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (A)

4.2.1 ^{1}H NMR spectrum (Plate 3A)

The ^{1}H NMR spectrum of 1-allyl-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (**A**) in $CDCl_3$ is characterised by the following salient features (in comparison with pentoxifylline):

1. The aromatic 8-H resonance remains at δ 7.51.
2. The N_7-CH_3 and N_3-CH_3 resonances remain at δ 4.00 and 3.57.
3. The 6-membered side chain resonances of pentoxifylline are replaced by an $ABMX_2$ system, typical of a 1-allylic group. The one-proton multiplet at δ 5.94 (J_{BM}=17.2, J_{AM}=10.3, J_{MX}=5.8*Hz*) is assigned to H_M of the allylic group.

The two one-proton doublet of doublets at δ 5.27 (J_{BM}=17.2, J_{AB}=1.3*Hz*) and 5.19 (J_{AM}=10.3, J_{AB}=1.3*Hz*) are assigned to the two terminal alkene-carbon hydrogens H_B and H_A, respectively. H_B is *trans* to H_M while H_A is *cis* to H_M. The two-proton doublet (2× H_X) at δ 4.63 (J_{MX}=5.8*Hz*) corresponds to the N-attached 1'-CH_2 group (2× H_X).

4.2.2 ^{13}C NMR spectrum (Plate 4A)

The ^{13}C NMR spectrum of 1-allyl-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (**A**) in $CDCl_3$ is characterised by the following salient features (in comparison with pentoxifylline):

1. The aliphatic carbonyl carbon resonance (C-5') at δ 208.7 disappears.
2. The four quaternary aromatic C-6, C-2, C-4 and C-5 resonate at δ 154.8, 151.1, 148.7 and 107.6, respectively, almost identical to the pentoxifylline resonances.
3. The C-8 methine resonates at δ 141.6 (δ 141.4 inpentoxifylline).
4. The N_7-C and N_3-C resonances remain at δ 33.4 and 29.5.
5. On the side chain, the nitrogen attached N_1-C resonance remains at δ 43.1.
6. The saturated carbons on the side chain are replaced by the unsaturated C-2' and C-3' at δ 132.1 and 117.3, respectively.

4.3 (*R**, *R**,)-(±)-1-{[2-Hydroxy-2-methylcyclobutyl]methyl}-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (B)

4.3.1 ^{1}H NMR spectra (Plates 5A , 5C and 5D)

The ^{1}H spectrum of (*R**, *R**,)-(±)-1-{[2'-Hydroxy-2'-methylcyclobutyl]methyl}-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (**B**) in $CDCl_3$ is characterised by the following salient features (in comparison with pentoxifylline):

1. The aromatic 8-H singlet resonance remains at δ 7.53.
2. The N_7-CH_3 and N_3-CH_3 resonances remain at δ 4.00 and 3.60.

3. The resonance of N_1-CH_2 is slightly deshielded to δ 4.17 (δ 4.0 in pentoxifylline) and changes from a triplet in pentoxifylline to a doublet of doublets (J=14 and 11*Hz*).
4. The one-proton multiplet at δ 2.42 is attributed to the C-1'-CH in the cyclobutane ring. This assignment is supported by the 2D-COSY experiment (**Plate 5C**) which shows an interaction between C-1'-CH and N_1-CH_2.
5. The four-proton multiplet of pentoxifylline at δ1.66 (C-2'-CH_2 and C-3'-CH_2) is replaced by two two-proton multiplets at δ 1.92 and 1.81 respectively, attributable to the C-3'- and C-4'-CH_2 groups in the cyclobutane ring of product **(B)**. The assignment that C-3'-CH_2 is more deshielded than C-4'-CH_2 is supported by the 2D-COSY experiment (**Plate -5D**) where C-4'-CH_2 correlates with C-1'-CH.
6. The singlet of the C-2'-CH_3 has lost the deshielding of C-5' carbonyl group and shifts to the δ 1.14 (δ 2.14 in pentoxifylline).
7. The C-2'-OH resonates as a one-proton singlet at δ 4.34 and disappears with the addition of D_2O.

4.3.2 ^{13}C NMR spectra (Plates 6A and 6B)

The ^{13}C NMR spectrum of (*R**, *R**,)-(±)-1-{[2'-Hydroxy-2'-methylcyclobutyl]methyl}-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (**B**) in $CDCl_3$ is characterised by the following salient features (in comparison with pentoxifylline):

1. The aliphatic carbonyl resonance at δ 208.7 (C-5' in pentoxifylline) is replaced by a saturated carbon resonance at δ 74.3 (C-2' in product **B**), being deshielded by the C-2'-OH.
2. The N_7-C and N_3-C resonances remain at δ 33.6 and 29.9, respectively.
3. The C-2' attached methyl carbon resonates at δ 29.0.
4. The nitrogen attached N_1-C resonates at δ 40.9.
5. The three remaining cyclobutane carbons (C-1', C-3' and C-4') resonate at 44.7, 32.8 and 19.5, respectively.
6. The four quaternary aromatic carbons, i.e. C-6, C-2, C-4 and C-5, resonances remain at δ 155.3, 151.5, 148.7 and 107.6, respectively.
7. The C-8 resonance remains at δ 141.4.

8. The HMQC spectrum (**Plate 6B**) is in agreement with these assignments.

4.3.3 Nuclear Overhause Effect Experiment (Plates 5E, 5F, and 5G)

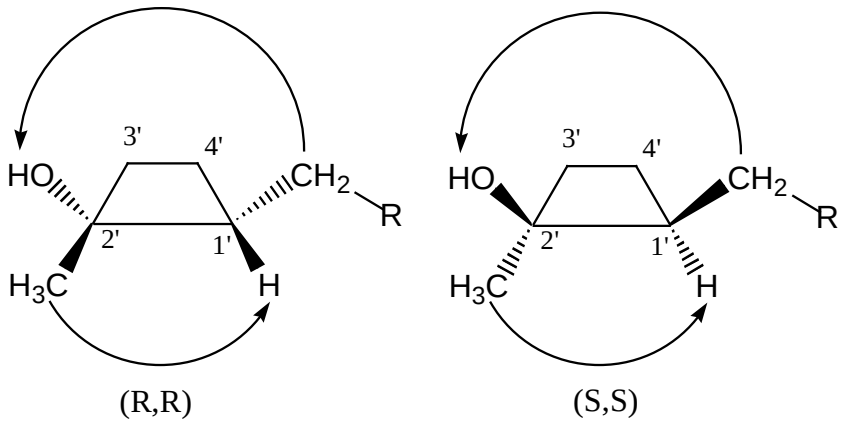
Nuclear Overhause Effect Experiments were done to determine the relative configuration of the cyclobutane moiety in (*R**, *R**,)-(±)-1-{[2-Hydroxy-2-methylcyclobutyl]methyl}-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (**B**).

The selective ge-1D NOESY spectra show the following:

1. Irradiation (selective pulse) at δ 4.34 (C-2'-OH) gives a negative value at δ 4.17 (N_1-CH_2). (see **Plate5E**)
2. Irradiation (selective pulse) at δ 1.14 (C-2'-CH_3) gives a negative value at δ 2.42 (C-1'-CH). (see **Plate 5F**)
3. Irradiation (selective pulse) at δ 2.42 (C-1'-CH_2) gives a negative value at δ 1.14 (C-2'-CH_3). (see **Plate 5G**)

These NOE effects are indicated in **figure 11**.

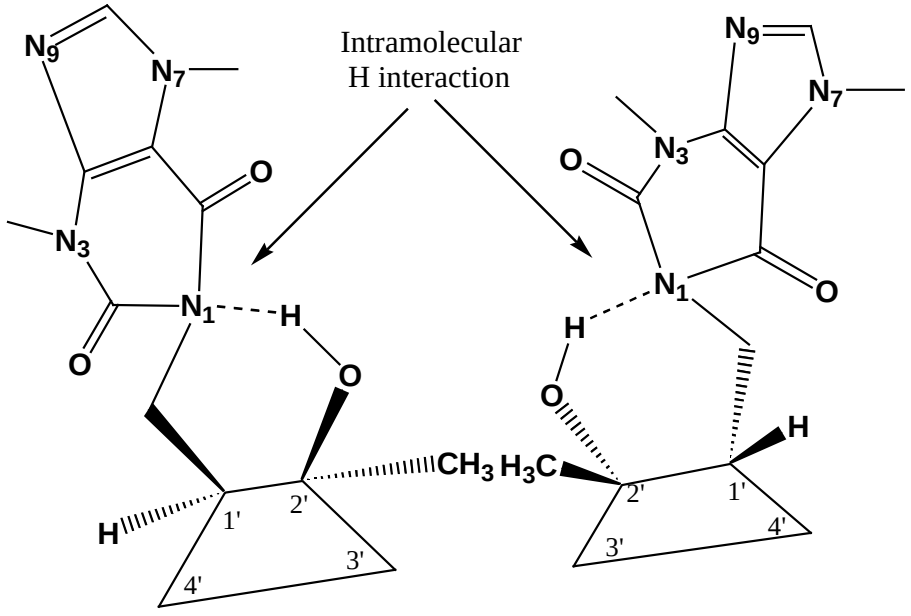
Figure 11



R: 3,7-Dimethyl-3,7-dihydro-1*H*-purine-2,6-dione

This experiment indicates unequivocally that the OH group on the cyclobutane ring (C-2'-OH) is *cis* to the N_1-CH_2 which is linked to the xanthine moiety. The similar **cis-CB1** stereoselectivity of carbonyl photocyclisation was reported by Moorthy and Mal[20] (**Figure 6,** page17). They explained that this **cis-CB1** has the most stable precursor biradical (**cis-BR1)** if there is no significant steric bulk of the OH group caused by salvation effect from aprotic solvents. We observed in **figure 12** that the hydrogen binding/interaction between the nitrogen (N_1) and the hydrogen of C-2'-OH group further favours the stereo-selectivity of the formation of **cis-CB1** cyclobutanol derivatives from pentoxifylline photolysis in aprotic solvents, such as benzene and toluene. While in protic solvents, the salvation effect on the molecule **B** predominate over the intramolecular hydrogen interaction mentioned above. As the result, the **cis-CB2** (see **Figure 6,** page17) becomes stereo-selectively favourable cyclisation product due to the significant OH group steric bulk in the precursor biradical (**cis-BR1)** transition states.

Figure 12



4. 4 1-(5-Hydroxyhexyl)-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (C)

4.4.1 ^{1}H NMR spectrum (Plate 7A)

The ^{1}H NMR spectrum of 1-(5-hydroxyhexyl)-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (**C**) in $CDCl_3$ is characterised by the following salient features (in comparison with pentoxifylline):

1. The aromatic 8-H singlet remains at δ 7.51.
2. The two N-attached CH_3 groups (N_7-CH_3 and N_3-CH_3) resonate at δ 4.00 and 3.57, respectively.
3. The N-attached N_1-CH_2 resonates at δ 4.02 as a triplet (J=12*Hz*).
4. The 2'- and 3'-CH_2 groups appear as a four-proton overlapping multiplet at δ 1.52 (δ 1.66 in pentoxifylline).
5. The 4'-CH_2 triplet at δ 2.50 in pentoxifylline is replaced by a two-proton multiplet at δ 1.69.
6. An additional one-proton multiplet at δ 3.81 is attributed to the 5'-CH (deshielded by the 5'-OH group).
7. The 6'-CH_3 singlet at δ 2.14 of pentoxifylline is replaced by a doublet (J=6.2*Hz*) at δ 1.19.
8. A broad resonance appears at δ 1.64 (5'-OH) and disappears with the addition of D_2O.

4.4.2 ^{13}C NMR spectra (Plates 8A, 8B and 8C)

The ^{13}C NMR spectrum of 1-(5-hydroxyhexyl)-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (**C**) in $CDCl_3$ is characterised by the following salient features (in comparison with pentoxifylline):

1. The aliphatic carbonyl carbon (C-5') resonance at δ 208.7 in pentoxifylline is replaced by a resonance at δ 67.9 (hydroxy attached C-5') with an inverted resonance in the APT experiment (**Plate 8B**).
2. The C-8 methine carbon resonance remains at δ 141.4.
3. The four quarternary aromatic carbons C-6, C-2, C-4 and C-5 resonate at δ

155.4, 151.5, 148.8 and 107.7, respectively. (δ 155.3, 151.5, 148.7 and 107.6, respectively, in pentoxifylline).

4. The N_1- C resonates at δ 43.2 (δ 43.1 in pentoxifylline).
5. The C-6' resonates at δ 23.5 (δ 33.6 in pentoxifylline due to the attached carbonyl group).
6. The N_3-C, N_7-C resonate at δ 33.6, 29.9, respectively, and are inverted in the ATP experiment (**Plate 8B**).
7. The aliphatic carbons (C-2' and C-3') resonate at δ27.9 and 22.9, respectively. (δ 27.4 and 21.0, respectively, in pentoxifylline).
8. The HMQC spectrum (**Plate 8C**) confirms these assignments.

4.5 8-(1-Hydroxy-1-methylethyl)-3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione (D)

4.5.1 ^{1}H NMR spectra (Plates 9A and 9B)

The ^{1}H NMR spectrum of 8-(1-hydroxy-1-methylethyl)-3,7-dimethyl-1-(5-oxo-hexyl)-3,7-dihydro-1*H*-purine-2,6-dione (**D**) in $CDCl_3$ is characterised by the following salient features (in comparison with pentoxifylline):

1. The aromatic one-proton 8-H singlet at δ 7.51 in pentoxifylline is replaced by a six-proton singlet at δ 1.68. This resonance is assigned to the two CH_3 groups (C-2"-CH_3 and C-3"-CH_3) on the C-8 attached isopropyl moiety.
2. The N_7-CH_3 singlet (δ 4.00 in pentoxifylline) shifts slightly downfield to δ 4.16 and the N_7-CH_3 singlet remains at δ 3.52.
3. The 6'-CH_3 singlet remains at δ 2.14.
4. The side chain 1'-CH_2 triplet remains at δ 4.00.
5. The side chain 4'-CH_2 triplet remains at δ 2.51.
6. The four-proton overlapping multiplet (2'-CH_3 and 3'-CH_3) appears at δ 1.62 (δ 1.66 in case of pentoxifylline).
7. The 2D-COSY spectrum (**Plate 9B**) supports these assignments

4.5.2 ^{13}C NMR spectrum (Plate 10A)

The ^{13}C NMR spectrum of 8-(1-hydroxy-1-methylethyl)-3,7-dimethyl-1-(5-oxo-hexyl)-3,7-dihydro-1*H*-purine-2,6-dione (**D**) in $CDCl_3$ is characterised by the following salient features (in comparison with pentoxifylline):

1. One additional quaternary carbon resonance at δ 70.8 is assigned to C-1" of the isopropyl moiety, being deshielded by the C-1"-OH and aromatic xanthine moiety.
2. Two additional aliphatic carbons resonate at δ 29.5 and 29.3. These are assigned to the two methyl carbons (C-2" and C-3") of the C-8 attached 2-propyl moiety.
3. The aliphatic carbonyl C-5' resonance remains at δ 208.9 (δ 208.7 in pentoxifylline).
4. The aromatic C-8 shifts slightly downfield to δ 146.7 (δ 141.4 in pentoxifylline).
5. The four quarternary aromatic carbons C-6, C-2, C-4 and C-5 resonate at δ 157.0, 155.3, 151.4 and 108.5, respectively. (δ 155.3, 151.5, 148.7 and 107.6, respectively, in pentoxifylline).
6. The C-1' and C- 4' resonance remain at the same chemical shifts of δ 43.2 and 40.8, respectively.
7. The N_3-C , N_7-C and C-6' resonate at δ 33.6, 31.4 and 29.9, respectively. (δ 33.6, 29.9 and 29.7, respectively, in pentoxifylline).
8. The two aliphatic C-2' and C-3' resonate at δ 27.4 and 21.0, respectively. (δ 27.4 and 21.0, respectively in pentoxifylline).

4.6 8-(1-Hydroxymethyl)-3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione (E)

4.6.1 ^{1}H NMR spectrum (Plate 11A)

The ^{1}H NMR spectrum of 8-(1-hydroxymethyl)-3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione (**E**) in $CDCl_3$ is characterised by the following salient features (in comparison with pentoxifylline):

1. The aromatic 8-H singlet at δ 7.51 in pentoxifylline disappears and a two-proton singlet appears at δ 4.76. This resonance is attributed to the 1"-CH_2 group, being deshielded by the attached 1"-OH and the xanthine moiety.
2. The N_3-CH_3 and N_7-CH_3 resonate at δ 4.02 and 3.57, respectively. (δ 4.00 and δ 3.57 in case of pentoxifylline).
3. The 6'-CH_3 resonance remains at δ 2.14.
4. The 1'- and 4'-CH_2 triplets remain at δ 4.02 and 2.50, respectively.
5. The four-proton overlapping multiplet (2' and 3'-CH_3) appears at δ 1.65 (δ 1.66 in pentoxifylline).

4.6.2 ^{13}C NMR spectrum (Plate 12A)

The ^{13}C NMR spectrum of 8-(1-hydroxymethyl)-3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione (**E**) in $CDCl_3$ is characterised by the following salient features (in comparison with pentoxifylline):

1. The additional quaternary carbon absorption at δ 56.6 is assigned to C-1" of the hydroxymethyl moiety (deshielded by the 1"-OH group).
2. The aliphatic carbonyl C-5' resonance remains at δ 208.7.
3. The aromatic C-8 shifts downfield to δ 147.4 (δ 141.4 in case of pentoxifylline).
4. The four quarternary aromatic carbons C-6, C-2, C-4 and C-5 resonate at δ 155.2, 151.5, 151.3 and 108.3, respectively.
   (δ 155.3, 151.5, 148.7 and 107.6 respectively in pentoxifylline).
5. The two resonances of C-1' and C- 4' remain at δ 43.16 and 40.78, respectively.
6. The N_3-C, N_7-C and the C-6' resonate at δ 33.6, 31.4 and 29.9, respectively. (δ 33.6, 29.9 and 29.7, respectively, in case of pentoxifylline).
7. The two aliphatic C-2' and C-3' resonate at δ 27.4 and 21.0.
   (δ 27.4 and 21.0 in pentoxifylline).

4.7 8-(1-Hydroxyethyl)-3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione (F)

4.7.1 ^{1}H NMR spectrum (Plate 13A)

The ^{1}H NMR spectrum of 8-(1-hydroxyethyl)-3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione (**F**) in $CDCl_3$ is characterised by the following salient features (in comparison with pentoxifylline):

1. The aromatic 8-H singlet at δ 7.51 is replaced by a one proton quartet (J=6.7 Hz) at δ 4.99. This is assigned to the 1"-CH on the ethyl moiety, being deshielded by the attached 1"-OH and the xanthine moieties.
2. An additional three-proton doublet (J=6.7 Hz) at δ1.63 is assigned to the methyl group (2"-CH_3) of the ethyl moiety.
3. The N_7-CH_3 and N_3-CH_3 resonate at δ 4.00 and 3.57, respectively.
4. The 6'-CH_3 group absorption remains at δ 2.14
5. The side chain 1'-CH_2 and 4'-CH_2 triplets at δ 4.02 and 2.52, respectively, remain.
6. The four-proton multiplet at δ 1.62 attributed to 2'-CH_3 and 3'-CH_3 remains. (δ 1.66 in case of pentoxifylline)
7. A broad resonance at δ 2.95 that disappears with the addition of D_2O is attributed to the 1"-OH of the ethyl moiety.

4.7.2 ^{13}C NMR spectra (Plates 14A, 14B, and 14C)

The ^{13}C NMR spectrum of 8-(1-hydroxyethyl)-3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione (**F**) in $CDCl_3$ is characterised by the following salient features (in comparison with pentoxifylline):

1. An additional CH carbon resonance at δ 62.9 is assigned to the C-1" of the hydroxyethyl moiety (deshielded by the 1"-OH group and xanthine moiety).
2. An additional resonance at δ 22.1 is attributable to the C-2" of the C-8 attached hydroxyethyl moiety.

3. The aliphatic carbonyl C-5' resonates at δ 208.8 (δ 208.7 in pentoxifylline).
4. The aromatic C-8 shifts downfield to δ 147.3. (δ 141.4 in pentoxifylline)
5. The four quarternary aromatic carbons C-6, C-2, C-4 and C-5 resonate at δ 155.3, 154.9, 151.4 and 108.1, respectively.
   (δ 155.3, 151.5, 148.7 and 107.6, respectively, in pentoxifylline).
   These carbons give inverted resonances in the APT experiment (**Plate-14B**).
6. The four aliphatic C-1', C-2', C-3' and C-4' resonate at δ 43.3, 40.8, 27.4 and 20.9, respectively.
   These carbons give inverted resonances in the APT experiment (**Plate-14B**).
7. The N_3-C, N_7-C and C-6' resonate at δ 32.1, 29.9 and 29.7, respectively. (δ 33.6, 29.9 and 29.7, respectively, in case of pentoxifylline).
8. The HMQC spectrum (**Plate 14C**) confirms these assignments.

5. Conclusions

The following were achieved in this research project:

1. We have expanded the range of derivatives of pentoxifylline that can be synthesised in reasonable yields for use in biological assays.

2. We have synthesised four derivatives of pentoxifyline for use as internal standards in the determination of pentoxifyline in body fluids.

3. We have established conditions that selectively encourage reactions at either the carbonyl moiety (toluene, triplet quencher) or the xanthine moiety (protic solvents, photo-sensitisor/radical initiator) of pentoxifylline.

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CHAPTER 3: EXPERIMENTAL

The following standard experimental techniques were used in this study.

1 Chromatographic Methods

1.1 Chromatographic techniques

1.1.1 Thin Layer Chromatography

Qualitative thin layer chromatography was performed on Merck aluminium sheets (silica gel 60 F_{254}, 0.25mm). Preparative thin layer chromatography was perfomed on glass plates (20x20cm), covered with a layer (1.0 mm) Kieselgel PF_{254} (100g Kieselgel in 230mℓ distilled water per 5 plates). The plates were dried at room temperature and used unactivated. The plates were loaded to a maximum of 25mg material per plate. After development the plates were dried at room temperature in a fast stream of air and the different bands were distinguished under UV light (254nm), scraped off and eluted with acetone.

1.1.2 Centrifugal Chromatography

Centrifugal Chromatography is performed in a thin layer of silica gel coated on a circular piece of glass called a rotor. A motor drives the rotor at a constant speed by a shaft passing through a hole in the centre. The compound to be separated is applied as a solution at the centre of the pre-cast rotor by way of a solvent pump or hand held syringe. The chosen solvent mixture is then pumped to the centre. The solvent is forced by centrifugal forces through the adsorbent layer effectively separating the individual components as a result of their different affinities for the layer and solvent mixture. As the individual rings reach the outer rim of the rotor they are spun off of the edge of the glass together with the solvent. A solvent channel collects the elute and brings it to the output tube where the fractions are collected.

Centrifugal chromatography was performed with an Analtech Cyclograph^{TM} with commercially available Analtech rotors (4mm).

1.1.3 Column Chromatography

Separations on Sephadex LH-20 from Pharmacia and Kieselgel from Merck (Art 773, 170-230 mesh) were performed with various column sizes and at differing flow rates. Fractions were collected in test tubes.

1.1.4 High Performance Liquid Chromatography

A TSK gel® ODS-80TM (15cm × 4.6mm × 5µ) column (Tosch Bioscience, Japan) was used at ambient temperature. Two mobile phase component solution A and B were pumped and mixed by the solvent delivery system (Agilent series 1100 quternary pump) to provide the required mobile phase gradient.
Solution A: 30g tetraethyl ammonium hydroxide (20% solution in water) was diluted with water to 800ml, the pH adjusted to 2.8 by titration with 25% H_3PO_4 and the solution then made up to 1000ml with water. To 900ml of this solution, referred to as the TEAP buffer, 100ml of acetonitrile was added to produce mobile phase solution A.

Solution B: to 1000ml of acetonitrile, 100µl of H_3PO_4 (85%) was added to produce mobile phase solution B.

The mobile phase was pumped at 1ml/min and the gradient used to increase solution B from 0% at time 0 to 90% at 30 minutes. A re-equilibration time of 5 minutes was allowed after each gradient run. 5µl sample injections were made with a Agilent series 1100 autosampler and UV spectra collected with a diode array detector (Agilent series 1100 DAD) from 190nm to 400nm with a resolution of 2nm at the rate of approximately one spectrum per 2 seconds throughout each run, and saved electronically.

The chromatograms were monitored at 254 and 275 nm.

1.1.5 Spraying Reagents

All TLC plates were sprayed with a 2% (v/v) solution of formaldehyde (40%) in concentrated sulphuric acid and subsequently heated to 110^0C for maximum colour development.

2 Spectroscopic Methods

2.1 Magnetic Resonance Spectrocopy

A 600MHz Brucker spectrometer was used to record the ^{1}H NMR, NOE, COSY, HMQC, HMBC (600MHz) and ^{13}C, APT (150MHz) experiments in either $CDCl_3$, acetone-d_6 or benzene-d_6. Chemical shifts are given in parts per million (ppm) on the delta (δ) scale and coupling constants (J) are accurate to 0.01Hz. The abbreviations of **s, d, dd, t, q, m** and **br** are used to denote **singlet, doublet, doublet of doublets, triplet, quarted, multiplet** and **broad**, respectively.

2.2 Mass Spectrometry

High-resolution mass spectra were recorded at 70eV on a VG 70 SEQ mass spectrometer with a MASPEC II data system.

3 Physical Properties Measurement

3.1 Melting Point

Melting points were recorded and are uncorrected on a REICHERT, AUSTRIA, Nr: 351375

4 Photochemical Reactions

All photochemical reactions were carried out inside the photochemical reactor RAYON manufactured by SOUTHERN N. E. ULTRAVIOLET Co. Middletown,

Connecticut, USA, equipped with RAYONET PHOTOCHEMICAL REACTOR lamps CAT. NO. RPR-3000 Å, 3500 Å, 2537 Å respectively.

5 General Procedures for Photolysis of Pentoxifylline

5.1 Extracting pentoxifylline (PTX) from Trental®

Trental® tablets (400mg/each) were crushed, extracted with toluene, concentrated, and crystallized from acetone.

5.2 Solvents preparation

All the solvents being applied for photolysis of PTX were distilled and dried in advance.

5.3 Photolysis of PTX

Pentoxifylline (278mg, 1mmol) was dissolved in the relevant solvent or solvent mixture (**Tables 1, 4, 5,** and **6**). The solution was flushed with nitrogen and irradiated for 24 hours at 300nm under nitrogen. The solvent was removed under vacuum. TLC (hexane-EtOAc-acetone-MeOH 2 : 7 : 0.5 :0.5) yielded the following fractions: R_f 0.53, 0.32, 0.30, 0.26, 0.21, 0.18 and 0.14 (the yield of each fraction depends on the particular solvent system used and the presence of initiators and quenchers. They are given in **Tables 1, 4, 5,** and **6** in the "**Result and Discussion**" section).

(I) *8-(1-Hydroxy-1-methylethyl)-3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1H-purine 2,6-dione* **(D)**

The R_F 0.53 fraction yielded 8-(1-hydroxy-1-methylethyl)-3,7-dimethyl-1-(5-oxo-hexyl)-3,7-dihydro-1*H*-purine-2,6-dione (**D**) as *white needles* from acetone, mp:191-192^{0}C.

Found C (57.1327); H (7.1915%); N (16.6623%) M^+ 336.3864, $C_{16}H_{24}N_4O_4$

Requires C (57.1332); H (7.1921%) N (16.6630%) M^+336.3858,

$\underline{M}^+$ 336 (100), m/e 321 (14.7), 293 (14.1), 279 (67.5), 251 (47.9), 238 (26.6), 223 (70.6), 193 (9.2), 180 (17.6), 82 (9.2), 67 (19.6), 43 (42.1).

^{1}H: δ ($CDCl_3$) 1.62 (m, 2'-CH_2& 3'-CH_2), 1.68 (s, 2"&3"-CH_3), 2.12 (s, 6'-CH_3), 2.48 (t, 4'-CH_2), 3.50 (s, N_3-CH_3), 3.96 (t, 1'-CH_2), 4.15 (s, N_7-CH_3).

^{13}C: δ ($CDCl_3$) 20.9 (C-2'), 27.4 (C-3'), 29.4&29.6 (C-2"& C-3"), 29.9 (C-6'), 31.4 (N_3-C), 34.0 (N_7-C), 40.7 (C-1'), 43.2 (C-4'), 70.8 (C-1"), 108.5 (C-5), 146.7 (C-8), 151.4 (C-4), 155.3 (C-2), 157.0 (C-6), 208.9 (C-5').

[^{1}H NMR: plates 9A and 9B; ^{13}C NMR: plate 10A; Mass: mechanisms V(a) and V(b)].

(II) *1-Allyl-3,7-dimethyl-3,7-dihydro-1H-purine-2,6-dione* **(A)**

The R_F 0.32 fraction yielded 1-allyl-3,7-dimethyl-3,7-dihydro-*1H*-purine-2,6-dione (**A**) as *white needles* from methanol, mp:142-143^{0}C.

Found C (54.5463 %); H (5.4933%); N (25.4417%) $\underline{M}^+$ 220.0967, C_{10} H_{12} N_4 O_2

Requires C (54.5457%); H (4.5927%); N (25.4409%)$\underline{M}^+$ 220.0952,

$\underline{M}^+$ 220 (100), m/e 205 (61.9), 193 (4.5), 180 (5.6), 165 (9.3), 109 (29), 82 (12), 67 (21.4) 55 (14).

^{1}H: δ ($CDCl_3$) 3.59 (s, N_3-CH_3), 4.00 (s, N_7-CH_3), 4.64 (d, 1'-CH_3), 5.25 (dd, 3'-CH_2), 5.94 (m, 2'-CH_2), 7.52 (s, 8-H).

^{13}C: δ ($CDCl_3$) 29.5 (N_3-C), 33.4 (N_7-C), 43.1 (C-1'), 107.4 (C-5), 117.3 (C-3'), 132.1 (C-2'), 141.3 (C-8), 148.7 (C-4), 151.1 (C-2), 154.8 (C-6).

[^{1}H NMR: plate 3A; ^{13}C NMR: plate 4A; Mass: mechanisms II(a) and II(b)].

(III) *8-(1-Hydroxyethyl)-3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1H-purine-2,6-dione* **(F)**

The R_F 0.30 fraction yielded 8-(1-hydroxyethyl)-3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6- dione (**F**) as *white needles* from chloroform, mp:186-187^{0}C.

Found *C* (55.8951%); H (6.8842%*)*; N (17.3836%). $\underline{M}^+$ 322.3624, C_{15} H_{22} N_4 O_4

Requires *C* (55.8944%); H (6.8835%); N (25.4428%). $\underline{M}^+$ 322.3608,

$\underline{M}^+$ 322 (93.4), m/e 307 (12.3), 279 (21.3), 265 (90.6), 237 (64.2), 224 (100), 220 (89.3), 209 (37.2), 180 (62), 132 (22.1), 110 (14.5), 82 (28.9), 67 (36.5) 43 (46.2).

^{1}H: δ ($CDCl_3$) 1.63 (d, 2"-CH_3), 1.66 (m, 2'&3'-CH_3), 2.14 (s, 6'-CH_3), 2.50 (t, 4'-CH_2), 3.52 (s, N_3-CH_3), 4.00 (t, 1'-CH_2), 4.00 (s, N_7-CH_3). 4.99 (q, 1"-CH).
^{13}C: δ ($CDCl_3$) 20.9 (C-2'), 22.1(C-2") 27.4 (C-3'), 29.6 (C-6'), 29.9 (N_3-C), 32.1 (N_7-C), 40.7 (C-1'), 43.2 (C-4'), 62.9 (C-1"), 108.5 (C-5), 146.7 (C-8), 151.4 (C-4), 155.3 (C-2), 157.0 (C-6), 208.9 (C-5').
[^{1}H NMR: plate 13A; ^{13}C NMR: plates 14A, 14B, and 14C; Mass: mechanisms VII(a), VII(b) and VII(c)] .

(V) *(R^*,R^*,)-(±)-1-{[2-Hydroxy-2-methylcyclobutyl]methyl}-3,7-dimethyl-3,7-dihydro-1H-purine-2,6-dione* **(B)**

The R_F 0.26 fraction yielded (*R^**, *R^**,)-(±)-1-{[2-Hydroxy-2methylcyclobutyl] methyl}-3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione(**B**) as *white needles* from methanol, mp 148-149^{0}C.
Found C (56.1067%); H (6.5247%), N (20.1362%) M^+ 278.1367, $C_{13}H_{18}O_3N_4$
Requires C (56.1058%); H (6.5239%), N (20.1353%) M^+ 278.1378,
M^+ 278 (15.8), m/e 250 (12), 221 (100), 205 (18.3), 193, (11.5) 180 (94.3), 137 (10.5), 109 (26.2), 83 (11.4), 69 (19.2), and 55 (24.9).
^{1}H: δ ($CDCl_3$) 1.14 (s, C-2'-CH_3), 1.81 (m, 4'-CH_2), 1.92 (m, 3'-CH_2), 2.42 (m, 1'-CH), 3.60 (s, N_3-CH_3), 4.00 (s, N_7-CH_3), 4.17 (dd, N_1-CH_2), 7.53 (s, 8-H).
^{13}C: δ ($CDCl_3$) 29.0 (C-2'-C), 29.4 (N_3-C) 29.8 (C-3'-C), 32.7 (C-4'-C), 33.6 (N_7-C), 40.8 (C-1'), 44.6 (N_1-C), 74.6 (C-2'), 107.6 (C-5), 141.4 (C-8), 148.7 (C-4), 151.5 (C-2), 155.3 (C-6),
[^{1}H NMR: plates 5A, 5C, 5D, 5E, 5F, and 5G; ^{13}C NMR: plates 6A and 6B;
Mass: mechanisms III(a) and III(b)].

(VI) *3,7-Dimethyl-1-(5-oxohexyl)-3,7-dihydro-1H-purine-2,6-dione* (**Pentoxifylline)**

The R_f 0.22 fraction yielded 3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1H-purine-2,6-dione as *white needles* from chloroform, mp:104-106^{0}C.
Found C (54.7426%); H (6.5433%), N (21.2862%) M^+ 278.1382, $C_{13}H_{18}N_4O_3$
Requires C (54.7417%); H (6.5425%), N (21.2851%) M^+ 278.1379,
M^+ 278 (100), m/e 235 (16.5), 221 (100), 207 (21.2), 193 (75.7) 180 (88.5), 137 (13.2), 109 (29.1), 82 (9.1), 67 (18.3), and 55 (11.1).

^{1}H: δ ($CDCl_3$) 1.66 (m, 2'&3'-CH_3), 2.14 (s, 6'-CH_3), 2.50 (t, 4'-CH_2), 3.57 (s, N_3-CH_3), 3.98 (s, N_7-CH_3), 4.01 (t, 1'-CH_2), 7.51(s, 8-H).
^{13}C: δ ($CDCl_3$) 20.9 (C-3'), 27.4 (C-2'), 29.7 (N_3-C), 29.9 (C-6') 33.6(N_7-C), 40.8 (C-1'), 43.2 (C-4'), 107.6 (C-5), 141.4 (C-8), 148.8 (C-4), 151.5 (C-2), 155.3 (C-6), 208.7 (C-5').
[^{1}H NMR: plate 1A; ^{13}C NMR: plates 2A and 2B; Mass: mechanisms I(a) and I(b)].

(VII) *8-(1-Hydroxymethyl)-3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1H-purine-2,6-dione* **(E)**

The R_F 0.18 fraction yielded 8-(1-hydroxymethyl)-3,7-dimethyl-1-(5-oxohexyl)-3,7-dihydro-1*H*-purine-2,6-dione (**E**) as *white needles* from chloroform, mp:177-178^{0}C.
Found C(54.5427%); H(6.5443%), N (18.1761%) M^+ 308.3336, C_{15} H_{22} N_4 O_4
Requires C(54.5420%); H(6.5436%), N (18.1754%) M^+ 308.3327.
M^+ 308 (91.2), m/e 292 (32.4), 265 (32.1), 251 (96.1), 237 (24.2), 223 (75.2), 223 (75.2), 220 (93.3), 210 (100), 194 (37.2), 176 (30.2), 144 (21.1), 82 (28.1), 67 (44.2), 43 (45.1).
^{1}H: δ ($CDCl_3$) 1.65 (m, 2'&3'-CH_3), 2.14 (s, 6'-CH_3), 2.50 (t, 4'-CH_2), 3.54 (s, N_3-CH_3), 4.00 (t, 1'-CH_2), 4.00 (s, N_7-CH_3), 4.76 (s, 7'-CH_2).
^{13}C: δ ($CDCl_3$) 20.9 (C-2'), 27.4 (C-3'), 29.6 (C-6'), 29.9 (N_3-C), 32.0 (N_7-C), 40.8 (C-1'), 43.2 (C-4'), 56.6 (C-1''), 108.3 (C-5), 147.4 (C-8), 151.3 (C-4), 151.5 (C-2), 155.3 (C-6), 208.8 (C-5'). '
[^{1}H NMR: plate 11A; ^{13}C NMR: plate 12A; Mass: mechanisms VI(a), VI(b) and VI(c)]

(VIII) *1-(5-Hydroxyhexyl)-3,7-dimethyl-3,7-dihydro-1H-purine-2,6-dione* **(C)**

The R_F 0.14 fraction yielded 1-(5-hydroxyhexyl)-3,7-dimethyl-3,7-dihydro-*1H*-purine-2,6-dione (**C**) as *white needles* from chloroform, mp:169-170^{0}C.
Found C (55.7562%); H (7.1942%), N (19.9915%) M^+ 280.1526, C_{13} H_{20} N_4 O_3
Requires C(55.7546%); H(7.1927%), N (19.9904%) M^+ 280.1535.
M^+ 280 (22.4), m/e 265 (6.7), 236 (10.5), 221 (22.5), 193 (29.2) 180 (100), 137 (8.3), 109 (17.6), 82 (5.1), 67 (9.8).

^{1}H: δ ($CDCl_3$) 1.19 (s, 6'-CH_3), 1.52 (m, 2'&3'-CH_3), 1.69 (m, 4'-CH_2), 3.57 (s, N_3-CH_3), 3.81 (m, 5'-CH), 3.99 (s, N_7-CH_3), 4.01 (t, 1'-CH_2), 7.50(s, 8-H).

^{13}C: δ ($CDCl_3$) 22.9 (C-3'), 23.5 (C-6'), 27.9 (C-2'), 29.7 (N_3-C), 33.6 (N_7-C), 38.8 (C-4'), 41.1 (C-1'), 67.9 (C-5'), 107.7 (C-5), 141.4 (C-8), 148.8 (C-4), 151.5 (C-2), 155.4 (C-6).

[^{1}H NMR: plate 7A; ^{13}C NMR: plates 8A, 8B and 8C; Mass: mechanisms IV(a) and IV(b)]