SYNTHESIS OF SOME POTENTIAL ANTIMICROBIAL CARBOCYCLIC COMPOUNDS

by

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ABSTRACT

Some naturally occurring compounds containing the naphtho [2,3 - *c*] pyran ring system have been found to be useful antibiotic agents and thus the laboratory synthesis of these compounds and their derivatives are of importance in the medical field. Their antibiotic and even antineoplastic activities are attributed to their potential to act as alkylating agents, *via* a bioreductive process.

This thesis deals with studies directed towards the synthesis of some benzo [*c*] pyrans as these compounds also possess the correct structural configuration to undergo the bioreductive process, and act as alkylating agents to cellular nucleic acids.

Chapter Two describes the synthesis of 3,4 dihydro - 1,3 - dimethyl - 1H - benzo - [*c*] pyran - 5,8 - quinone (13) by employing a base induced cyclization with potassium *tertiary* butoxide. This compound was proven to be biologically active against both Gram positive and Gram negative organisms.

(±) (1R, 3R, 4R) - 3,4 - Dihydro - 4 - hyroxy -1,3 - dimethyl - 1H - benzo [*c*] pyran - 5,8 - quinone (17) and its 4 S diastereomer (18) were synthesized with cerium (IV) ammonium nitrate as the cyclizing reagent. Antimicrobial evaluation of compound (17) displayed inhibitory activity against both Gram positive and Gram negative organism growth. This is discussed in Chapter Three.

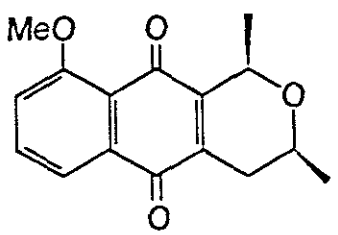
In Chapter Four, the synthesis of the benzo analogue of the naturally occurring naphthopyran antibiotic, hongconin, is discussed. The synthetic route used for this synthesis could well be applied to synthesise hongconin.

INTRODUCTION

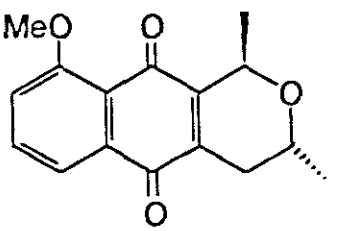
Several naturally occurring pyranquinones containing the [2,3-*c*] pyran ring system have been shown to possess antimicrobial and antineoplastic activity. Thus laboratory synthesis of these compounds or their derivatives have become increasingly important.

Many of these compounds are found as naturally occurring compounds in the plant and animal kingdom[1].

Eleutherin (1) and isoeleutherin (2) were first isolated from the tubers of *Eleutherine bulbosa* by Schmid and co-workers[2-4].

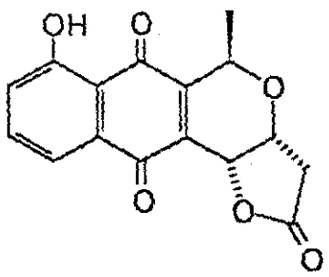


(1)



(2)

Other naturally occurring pyranquinones include kalafungin[5] (3), the nanaomycins[6,7] A (4), B (5), C (6), D (7) and naphthocyclinone[8] (8).



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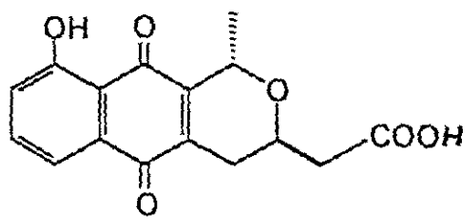

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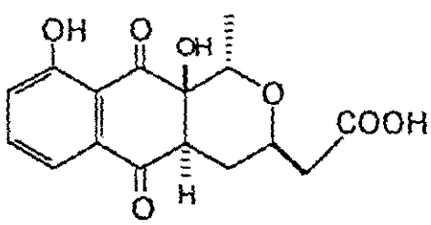

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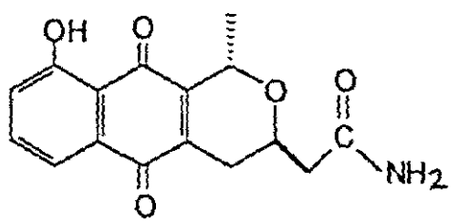

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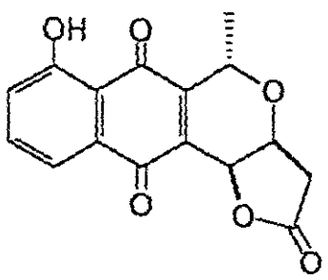

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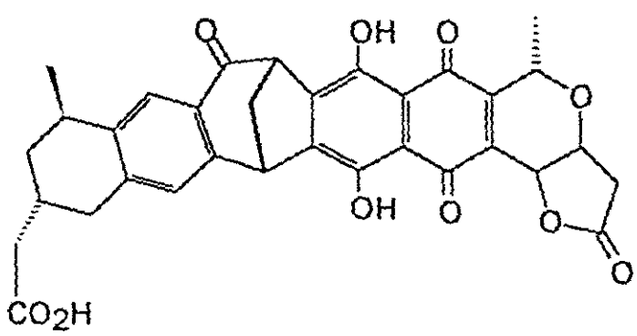
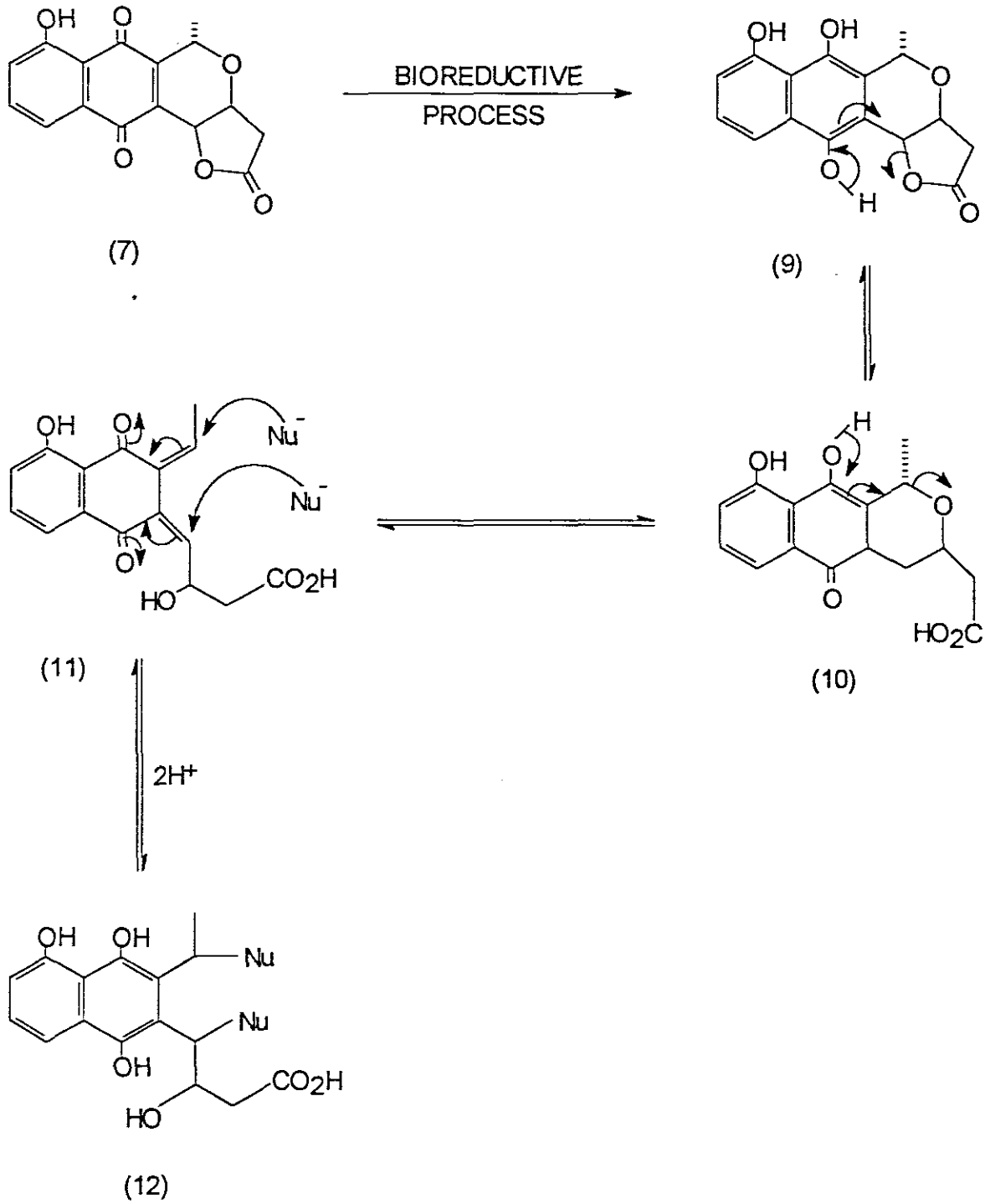


(8)

Moore[3] has reviewed several compounds which by virtue of their structures would be expected (and several have been shown) to possess significant antineoplastic activity by acting as bioreductive alkylating agents. A compound such as nanaomycin D (7) which contains the fused pyrano - δ - lactone moiety is biologically inactive but can be transformed by reduction *in vivo* to an active hydroquinone which functions as a bis-alkylating agent.

BIOREDUCTIVE PROCESS : SCHEME A



The quinone (7) is reduced *in vivo* to the corresponding hydroquinone (9) which then undergoes two ring opening steps as in Scheme A.

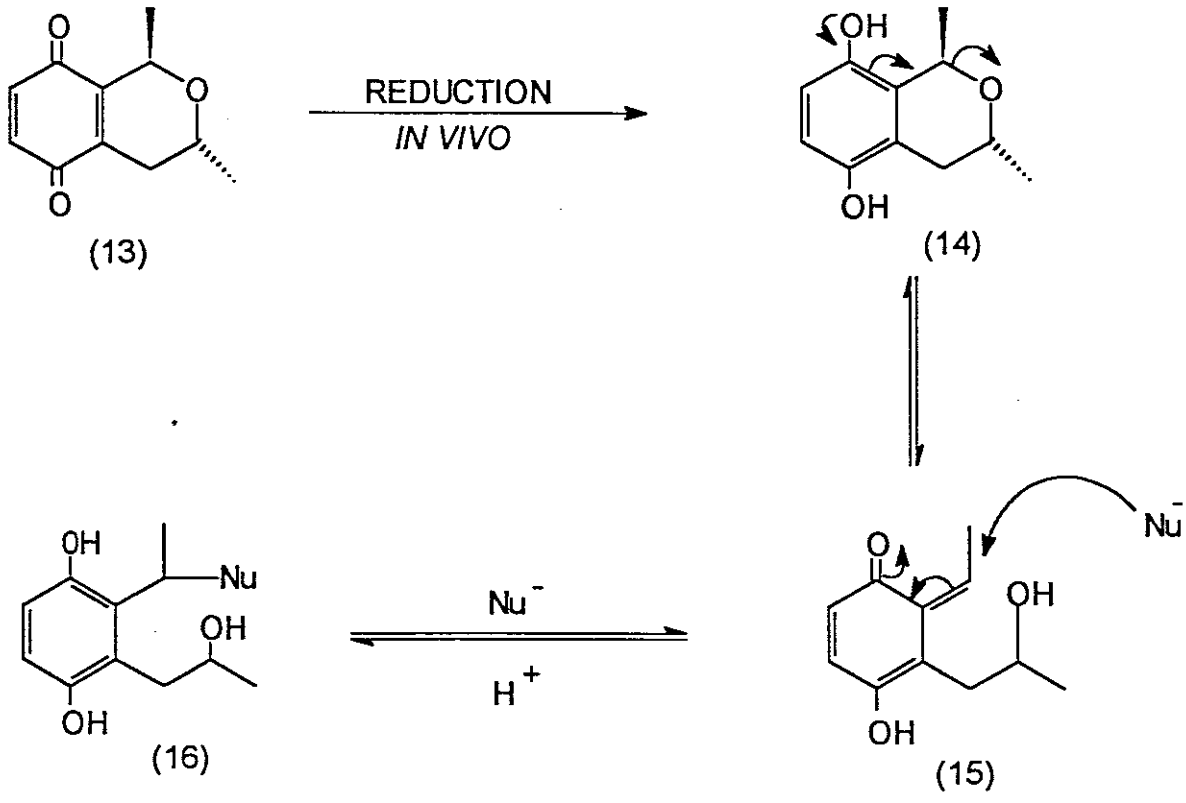
The quinone methide (11) that results could then act as a Michael-type acceptor to nucleophiles (Nu^-) which may be certain nucleophilic centres in the D.N.A. molecule thereby binding the nucleic acid as in (12), preventing replication and thus cell growth.

Much research[10] has shown that several compounds containing the naphtho[2,3-c]pyran ring system could well be biologically active as a result of the described bioreductive alkylation.

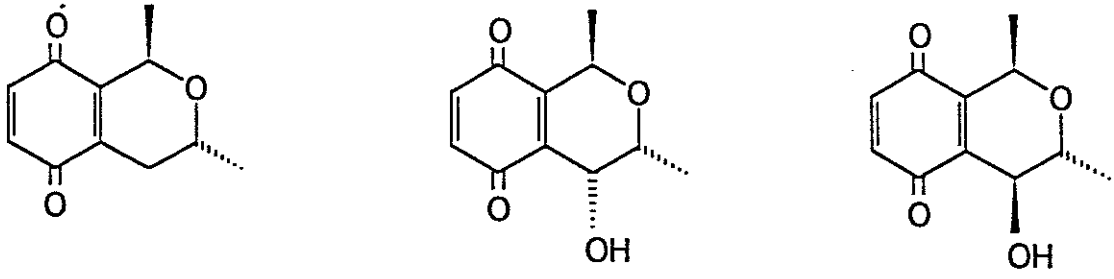
In view of the above, the possibility was considered that benzo analogues also containing the [c] pyran ring system may show activity by means of the same bioreductive alkylation mechanism.

Benzo[c] pyranquinones may thus behave in the same way as their naphthalene analogues and also be able to act as bioreductive alkylating agents by the mechanism of quinone methide formation proposed in Scheme B.

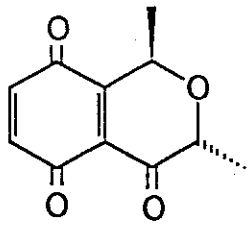
BIOREDUCTIVE PROCESS : SCHEME B



The objective of this work was to synthesise the benzo[c]pyranquinones (13), (17), (18), and (19) for antimicrobial evaluation. Comparison with the activities of their naphthalene analogues could give further insight into a structure-activity relationship.



(13) (17 (18)



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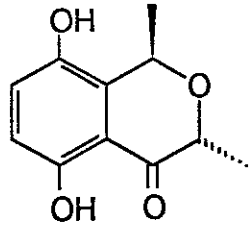
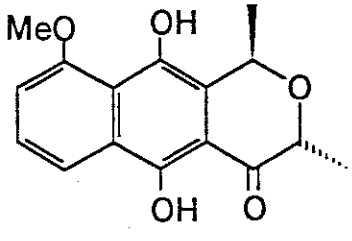


(20)

Another objective of this study was to devise a model synthetic route for the synthesis of the naturally occurring [2,3-c]pyran, hongconin (21). Hongconin was isolated from the rhizome of *Eleutherine americana* Merr. et Heyne (Iridaceae) (Chinese name; Hong-Cong) along with three known naphthalene derivatives, eleutherol (22), eleutherin (1) and isoeleutherin (2)[2].



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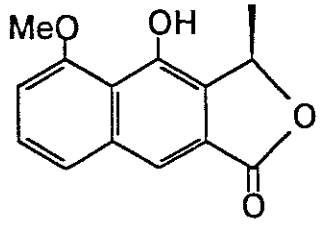


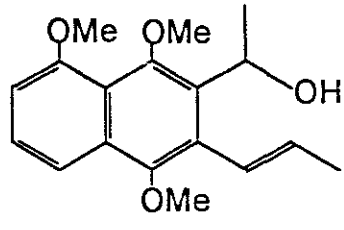
(22)

Eleutherine americana Merr. et Heyne (Iridaceae) is a herbal plant cultivated in Hainan Island of South China[11]. The rhizome of this plant was used as a folk medicine for the treatment of

coronary disorders[30]. Pharmacological testing confirmed the biological activity of Hong-Cong using an isolated guinea pig heart.

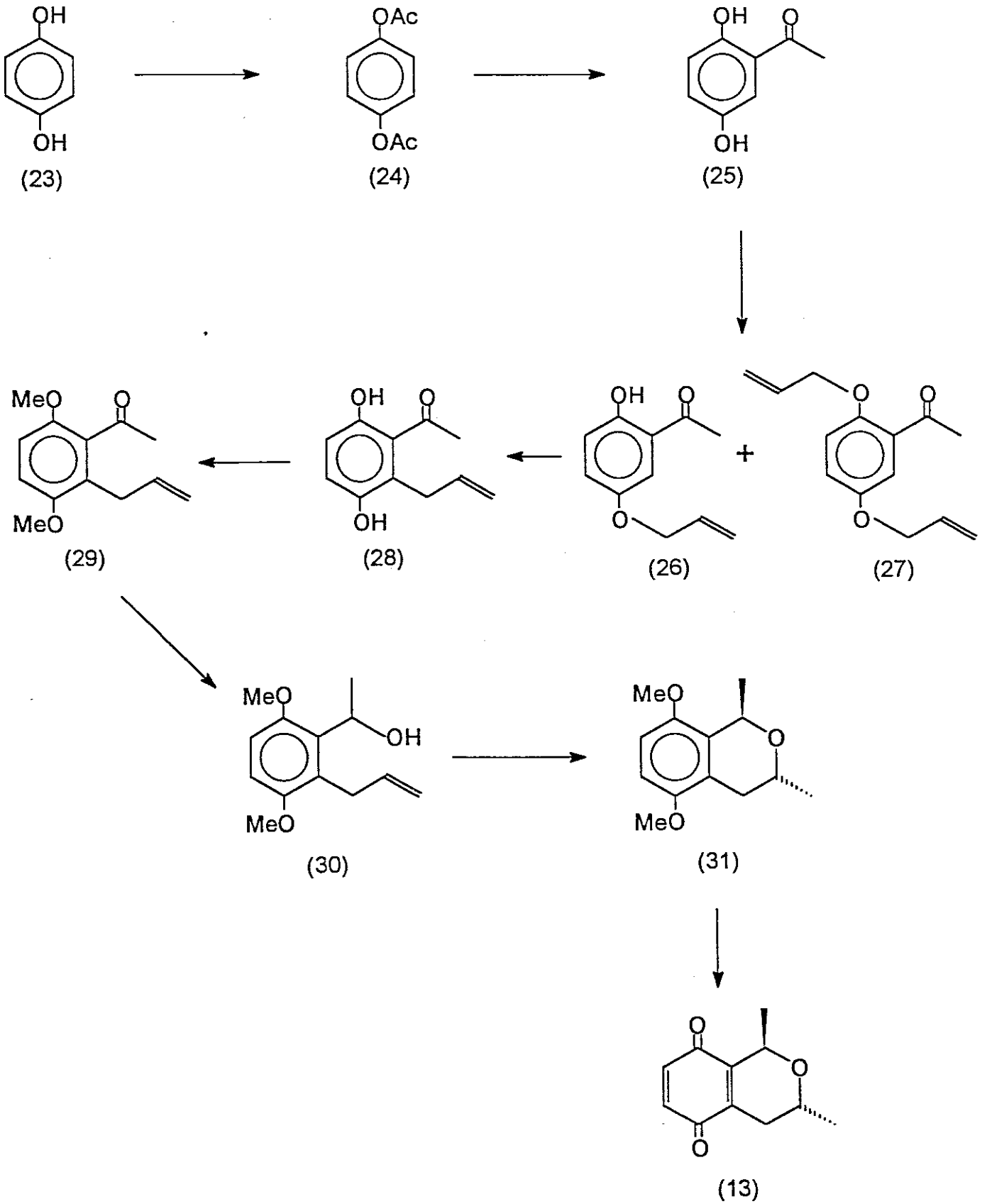
Hong-Cong was also shown to be effective to angina pectoris in a preliminary clinical trial. The four naphthalene derivatives; eleutherol (22), eleutherin (1), Isoeleutherin (2) and hongconin (21) have showed the effect of increasing coronary flow on isolated guinea pig heart[31].

Compound (20), one of the target compounds of this work, can be said to be the benzo-analogue of the hongconin (21). The synthetic route envisaged (Scheme C) to *inter alia* compound (20) can thus later be applied as a model route for the synthesis of hongconin (21), by employing the known[10]naphthalene alcohol (34) as starting material in place of (33).

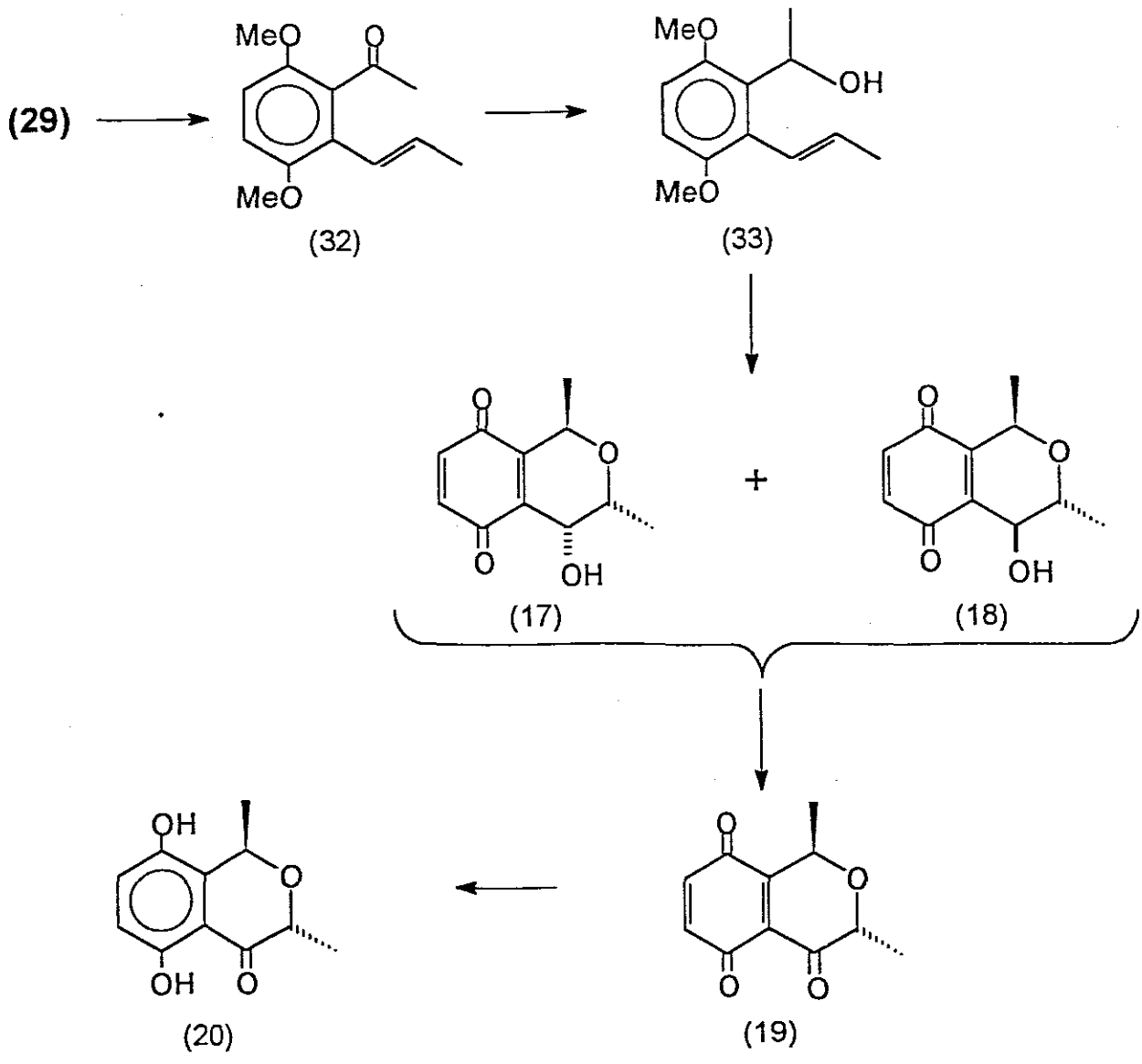


(34)

SCHEME C


OH
OH
(23)
OAc
OAc
(24)
OH
O
OH
(25)
OH
O
O
O
+
(26)
(27)
OH
O
OH
(28)
MeO
O
MeO
(29)
MeO
OH
MeO
(30)
MeO
O
MeO
(31)
O
O
O
(13)

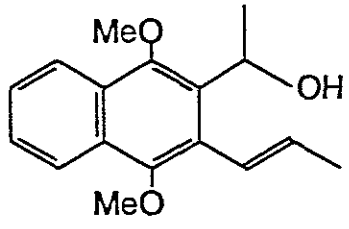
SCHEME C continued



CHAPTER 1

LITERATURE METHODS ON THE CONSTRUCTION OF THE [2,3-c] PYRAN RING SYSTEM

Giles et al[12] studied the synthesis of naturally occurring quinones of the eleutherin type by treating alcohol (35) with argentic oxide. The intention of this reaction was to cyclise the alcohol (35) to form the pyran (37) *via* the quinone (36).



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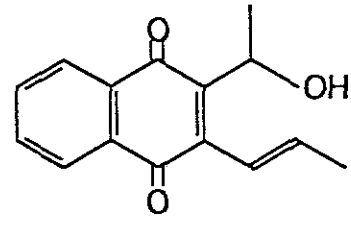

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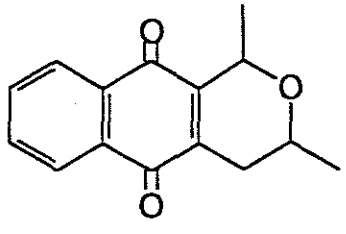
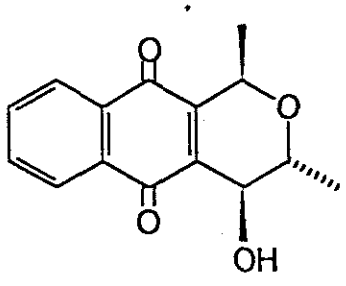


(37)

It was found that the attempted cyclisation of (35) afforded only one product namely, the quinonoid alcohol (36).

This compound (36) exhibited little proclivity to undergo cyclisation in the required manner.

It was further found by Giles[13] that oxidation of dimethyl ether (35) with 4 molar equivalents of cerium (IV) ammonium nitrate (C.A.N.) afforded a mixture of the two naphthopyranquinones (38) and (39) (as their racemates) - the latter being the major product.



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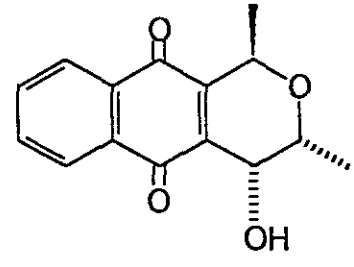
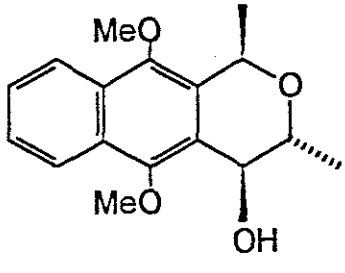
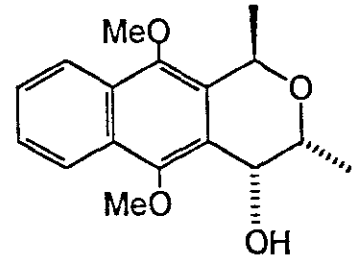


(39)

Dimethyl ether (35) when treated with 2 mole equivalents of C.A.N. underwent cyclisation to give rise to the two racemic naphthopyrans (40) and (41)[13].

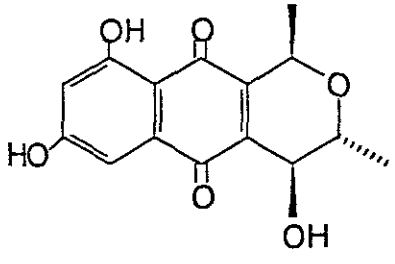


(40)



(41)

Compounds (40) and (41) when separately treated with two mole equivalents of C.A.N. produced the naphthopyranquinones (38) and (39) respectively. This conversion of dimethyl ether (35) to the naphtho[2,3-*c*]pyran-5,10-quinones (38) and (39) in one easy step was of considerable significance since these products are the 7,9-dideoxy derivatives of quinones (42) and (43) respectively, and they possess the correct stereochemistry in each case at C_1, C_3, and C_4.



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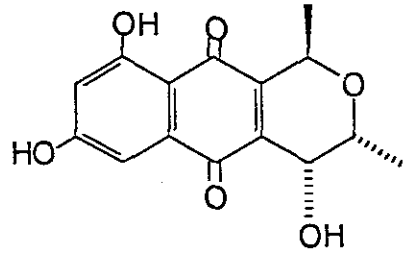
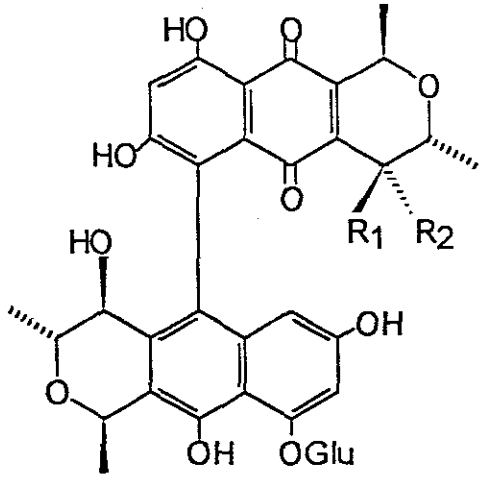


(43)

Compounds (42) and (43) are structurally and stereochemically correct to be used as precursors in the synthesis of the naturally occurring protoaphin-*fb* (44) and protoafin-*sl* (45) respectively[14].



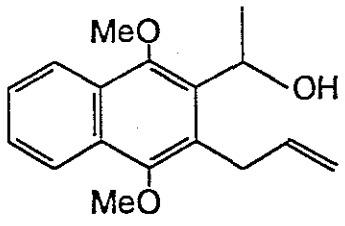
(44) R_1 = OH ; R_2 = H

(45) R_1 = H ; R_2 = OH

Protoaphins are brownish yellow, hygroscopic pigments that are essentially naphthyl derivatives[15]. Protoaphin-*fb* (44) was first isolated from the heamolymph of the broad bean aphid *Aphis fabae Scop*, but has subsequently been found in other species of Aphididae. Protoaphin-*sl* (45), a compound closely related to protoaphin-*fb*, was isolated from *Tuberolachnus Salignus* Gmelin.

Another method of constructing the [2,3-*c*] pyran ring system was discovered by Hugo and Giles[16] which entailed the base-induced cyclisation of naphthalene alcohols. This was achieved by using the base, potassium *tertiary* butoxide in N,N-dimethylformamide in a nitrogen atmosphere.

The alkenyl alcohol (46) was subjected to the above conditions and a high yield of the *trans* - 1,3 - dimethyl - naphthopyran (47) was recovered.



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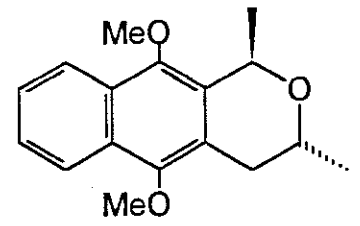


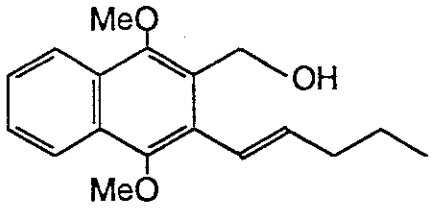
(47)

The conjugated isomer of (46), namely compound (35) cyclised equally well under the same conditions to afford (47) in high yield.

The value of the above result was the stereochemistry of the 1,3 disubsituted pyran ring in (47).

Non-basic cyclisations of (46) on closely related substances were performed by Kometani et al[17] and Maruyama et al[18] but the result was a diastereomeric mixture of compound (47) and its cis isomer.

The ortho pentyl naphthyl alcohol (48) [19] also underwent base induced cyclisation to the pyran (49), followed by oxidative demethylation with cerium (IV) ammonium nitrate affording the corresponding quinone (50).



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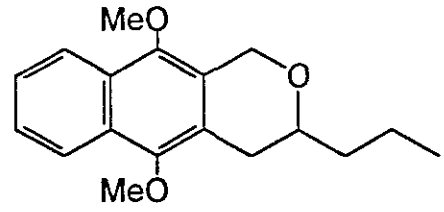

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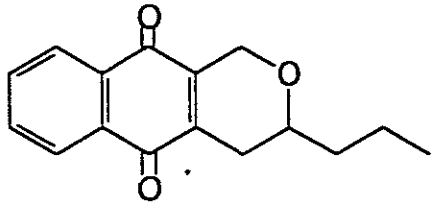


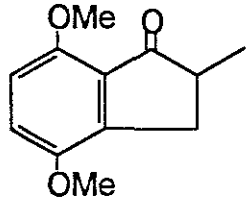
(50)

The two methods described above for constructing naphthopyranquinones which contain the [2,3-*c*] pyran ring have been used in this work as the crucial steps in synthesising the target benzopyranquinones containing the same [*c*] pyran ring system.

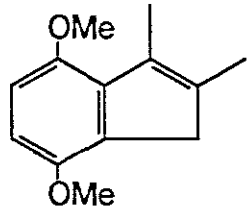
CHAPTER TWO

SYNTHESIS OF 3,4 DIHYDRO-1,3-DIMETHYL-1H-BENZO[C] PYRAN-5,8 QUINONE (13)

Kometani [17] in 1981 prepared the benzopyranquinone (13) using indene (59) [obtained from the reaction of indanone (58) by employing the Grignard reagent methylmagnesium iodide which was subsequently shaken up with dilute hydrochloric acid] as starting material.

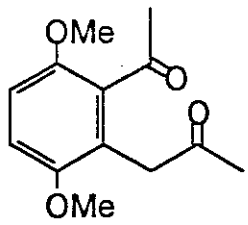


(58)

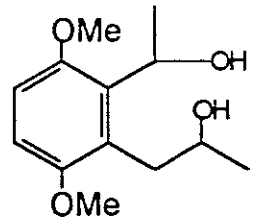


(59)

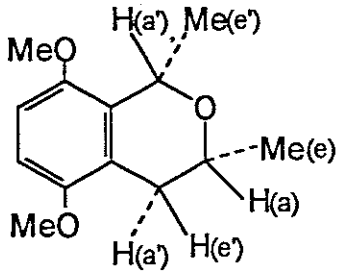
Indene (59) was oxidized by the Lemieux - Johnson method to afford the dione (60). Compound (60) was treated with lithium aluminium hydride to give (61) which was stirred in an etherial solution with hydrochloric acid and subsequently dehydrated to afford 1,3 - isochromans as a 1:2 mixture of the stereoisomers (31a) and (31). The stereoisomers were separated by silica gel chromatography.



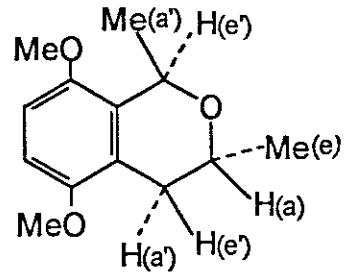
(60)



(61)



(31 a)



(31)

The structures of compounds (31 a) and (31) were supported by their ^{1}H nmr spectra, each showing the 3-Me and 1-Me as doublets at δ 1.34 and 1.54 (each J 7 Hz) for (31 a) and at δ 1.31 and 1.49 (each J 7 Hz) for (31). The stereochemistry of the isomers was assigned by comparing long - range coupling constants ($J_{1,4}$). The minor product (31 a) which exibited larger coupling constants ($J_{1a',4a'}$ 2 Hz; $J_{1a',4a'}$ 1 Hz) was assigned the *cis* - structure, and the major ($J_{1,4}$ < 1 Hz) the *trans* - isomer.

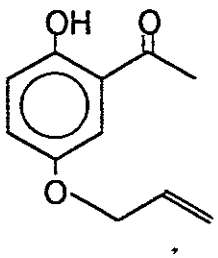
Oxidative demethylation of compound (31) was then carried out with cerium (IV) ammonium nitrate in aqueous acetonitrile to produce the corresponding quinone (13).

In this work hydroquinone diacetate (24) was obtained by dissolving hydroquinone (23) in acetic anhydride[20]. The diacetate was recrystallised and converted to 2,5 dihydroxyacetophenone (25) (60 %) by employing the Fries rearangement reaction.

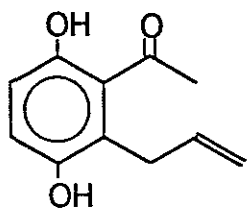
Compound (25) was dissolved in acetone and refluxed with allyl bromide in the presence of potassium carbonate to afford (26) in a yield of 93 % after chromatography. It was found that when the above reactants were in a 1:1:1 mol ratio, the monoallylated product (26) was almost exclusively formed. The diallylated product (27) was not formed under these conditions due to the strong hydrogen bonding which exists between the hydroxyl group at C_2 and the allylic oxygen. Allylation at C_2 is thus prevented under these conditions.

When the molar equivalent of the allyl bromide was increased to 1.2, the diallylated product (27) began to form significantly (5 %) and the monoallylated (26), still being the major product, decreased in yield to 89 %.

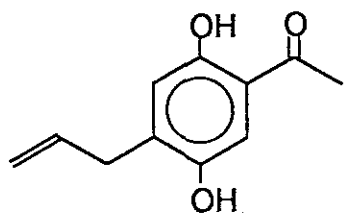
The next step in the synthetic scheme (Scheme C) was to convert (26) to 2,5 - dihydroxy - 6 - allylacetophenone (28). This was achieved *via* a thermal Claisen rearrangement.



(26)



(28)



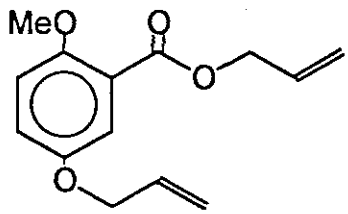
(28 a)

It is theoretically possible for the allyl group to migrate to position 4 or 6 on the benzene ring. It was however found that when compound (26) was heated at 220°C for two hours under nitrogen the allyl group favoured the sterically more hindered position 6 (28). Migration of the allyl group to position 4 as in 28a to afford the alternative isomer was less than 3 %.

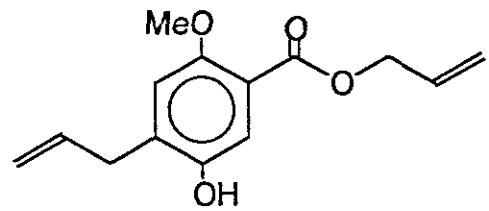
Bruce and Ali (1981)[23] had previously studied this type of thermal Claisen Rearrangement and found that when the group meta to the migrating group is electron accepting [as in the above case (26)] that migration to the *ortho* position nearest to this electron accepting group is favoured.

It was also established [24] that when no hydrogen bonding exists between the electon withdrawing (accepting) group and its

adjacent group [as in compound (51)] migration to the sterically less hindered position 4 is favoured when rearrangement is thermally induced.

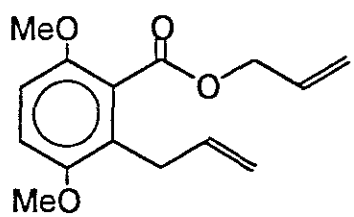


(51)



(52)

The ^{1}H nmr spectrum of (52) showed *inter alia*, a singlet (D_2O-exchangeable) at δ 6.1 corresponding to the phenolic hydroxyl. Two singlets, at δ 6.75 and 7.40 respectively, corresponding to the two aromatic protons para to one another, provided evidence that rearrangement had occurred to the sterically more favourable position. Had rearrangement occured to the less favourable *ortho* position to yield compound (53), one would have expected two *ortho* coupled doublets in the aromatic region of the spectrum.



(53)

The formation of compound (52) could be explained in terms of stereochemical factors. The freely rotating ester group inhibits rearrangement of the allyl group to the position *ortho* to it, thus forcing migration to the sterically less hindered position. When hydrogen bonding does exist [as in the case of 2 - hydroxy - 5 - allyloxyacetophenone (26)], the ester group is not freely rotating and thus migration to the sterically less favoured position is possible.

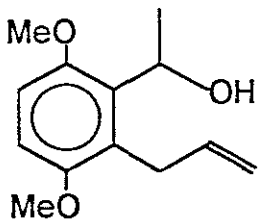
The ^{1}H nmr spectrum of (28) showed *inter alia* two D_2O exchangeable singlets at δ 8.05 and δ 11.80, corresponding to the two phenolic protons and two *ortho* coupled doublets (J 9.5 Hz) at δ 6.60 and δ 6.80, corresponding to the two aromatic protons.

The above provided evidence that compound (28) had formed (62 % yield) and that the migration of the allyl group was to the sterically less favoured position, as was expected with thermal Claisen rearrangements of compounds of the type (26).

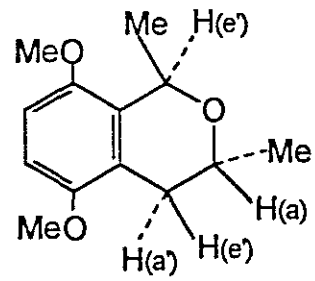
Harwood[25] has shown that acid catalysed Claisen rearrangements are dependant on internal hydrogen bonding, while the above described thermal Claisen rearrangements are independent of internal hydrogen bonding.

The ketone (28) was dimethylated by refluxing with a large excess of distilled methyl iodide (15 mol. equiv.) in acetone with potassium carbonate (5 mol. equiv.) for 24 hours. Yields in excess of 88 % were obtained throughout. The red-brown oil was readily converted to the corresponding alcohol (30)(95%) by reduction with lithium aluminium hydride (5 mol. equiv.) in dry ether at room temperature for 15 minutes.

Alcohol (30) was treated with potassium tertiary butoxide in dimethyl formamide under nitrogen at room temperature. Analysis of the spectra (^{1}Hnmr, ir, ms) of the obtained product confirmed that alcohol (30) had undergone base induced cyclisation[16] to afford the *trans* pyran (31)[17,29] in a yield of 94 %. Its ^{1}Hnmr spectrum agreed entirely with that published earlier[17,29].

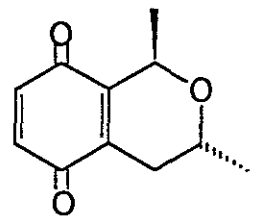


(30)



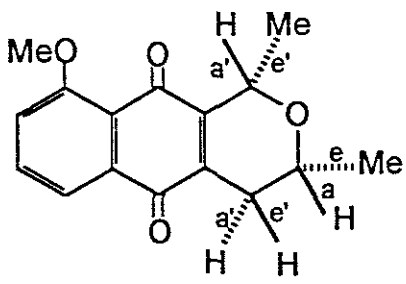
(31)

Dimethoxybenzopyran (31) was dissolved in a mixture of acetonitrile and water. This solution was treated with cerium(IV) ammonium nitrate (2 mol. equiv.). Spectroscopic data proved that the expected oxidative demethylation had occurred. Purification by column chromatography afforded the pure pyranquinone (13) as yellow crystals (99 %). Its spectroscopic data [17,29] were in accord with the assigned structure.

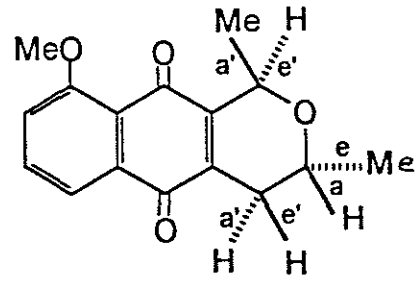


(13)

To assist in the stereochemical assignment of compound (13) the stereochemistry of eleutherin (1) and isoeleutherin (2) was considered carefully with respect to their respective ^{1}H nmr spectra as shown by Schmid et al.[3,4]



Eleutherin (1)



Isoeleutherin (2)

The *symbols a' and e' denote the pseudo* - axial and *pseudo* - equatorial configurations of the bonds in question in the chair - like conformation of the partially unsaturated ring. Karplus equation[32,33] is a useful tool to determine the stereochemistries of protons on adjacent carbon atoms. The factor that is ostensibly most easy to predict is its influence on J_{vic} is the dihedral angle Φ between the two vicinal C-H bonds; the equations due to Karplus give frequent agreement with the observed values.

Karplus equations :

Φ between 0° and 90° : $J_{vic} = 8.5 \cos^2 \Phi - 0.28$

Φ between 90° and 100° : $J_{vic} = 9.5 \cos^2 \Phi - 0.28$

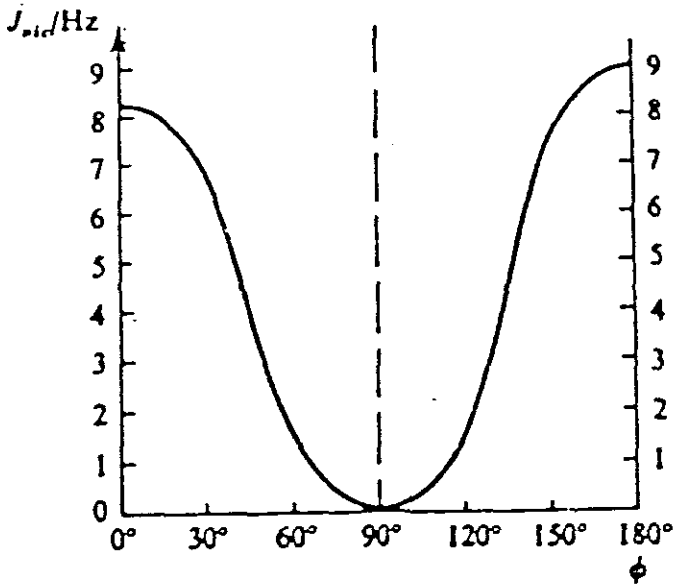
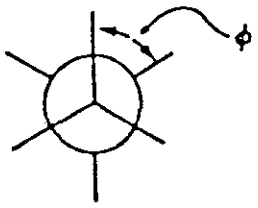
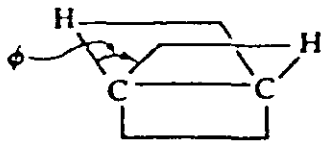


FIGURE 1. : VARIATION OF VICINAL COUPLING CONSTANT J_{vic}, WITH DIHEDRAL ANGLE Φ





GRAPHICAL EXPLANATION OF KARPLUS'S EQUATIONS

From the reasonable assumption that the C-3 methyl adopts the less crowded equatorial position it follows that the adjacent axial - *pseudo* axial protons at C-3 and C-4 respectively, having a dihedral angle of ≈180° are strongly coupled, whereas axial - *pseudo* equatorial protons having a dihedral angle Φ of about 60°, should only be moderately coupled.

In the case of the diastereomers eleutherin (1) and isoeleutherin (2) we should observe a relatively large $J^{e'a}$ and a smaller $J^{e'a}$ vicinal coupling constant. In addition long range proton - proton (H-1 to H-4) couplings in the CH-C=C-CH system should be greatest where the CH bonds are perpendicular to the plane of the double bond[34]. The di*pseudo* - axial configurations (a') will give rise to a greater angle between the direction of the CH bond and the plane of the double bond than would the di*pseudo* - equatorial (e') configuration.

The magnitude of the coupling constants ($J_{3,4}$) should therefore be as follows : $J_{3,4}$: $J^{a'a} > J^{a'e'} \approx J^{e'a'} > J^{e'e'}$. The following table lists the assignments of coupling constants for eleutherin (1) and isoeleutherin (2)

COUPLING CONSTANT (Hz)	
ELEUTHERIN	ISOELEUTHERIN
$J_{4,4}$ (gem) 17.8	$J_{4,4}$ (gem) 18.8
$J_{4,3}$ (a'a) 9.2	$J_{4,3}$ (a'a) 8.8
$J_{4,3}$ (e'a) 2.9	$J_{4,3}$ (e'a) 4.5
$J_{1,4}$ (a'a') 3.5	$J_{1,4}$ (e'a) 2.0
$J_{1,4}$ (a'e') 2.9	$J_{1,4}$ (e'e') 1

For compound (13) the following spectral characteristics were observed : a doublet (J 6 Hz) at δ 1.33 due to the C-3 methyl coupled to the axial 3-H.

The *pseudo* - axial H-4 resonated as a multidoublet (J 19, 10 and 2 Hz). J 19 Hz is due to *geminal* coupling between the *pseudo* - axial H-4 and the *pseudo* - equtorial H-4. J 10 Hz is thus due to *vicinal* coupling between the *pseudo* - axial H-4 and the axial H-3. The smaller value (2 Hz) is no doubt then due to

long - range coupling between the *pseudo* - axial H-4 and the *pseudo* equatorial H-1. Had this H-1 proton been *pseudo* - axial, one would have observed long - range coupling to the *pseudo* - equatorial H-4 as well. The latter resonated as a doublet of doublets (*J* 19 and 4 Hz) due to *geminal* coupling to the *pseudo* axial H-4 and *vicinal* coupling to the axial H-3. This indicates that H-1 adopts the *pseudo* equatorial position and thus the methyl group at C-1 occupies the *pseudo* - axial position as indicated in the structure.

Compound (13) was biologically evaluated in these laboratories by the Bauer - Kirby method and showed significant inhibitory activity against both Gram positive and Gram negative organisms.

The results are as shown in Table I.

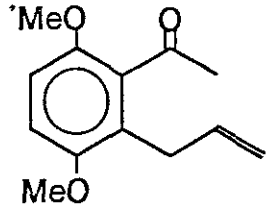
TABLE I

RELATIVE INHIBITION USING COMPOUND (13)

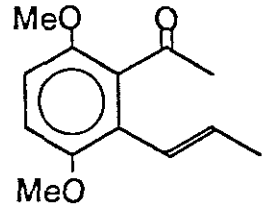
ORGANISM	NUMBER OF ORGANISMS/ML	DOSE OF COMPOUND (µmol)	MASS OF COMPOUND IN mg/0,1 ml	INHIBITION ZONE IN mm
Gram pos.				
Staphylococcus aureus	$7,21 * 10^{12}$	2,60	0,50	25
		1,30	0,25	24
		0,65	0,125	16
Bacillus subtillus	$1,4 * 10^{9}$	2,60	0,50	35
		1,30	0,25	30
		0,65	0,125	24
Candida albicans Fungus	$1,2 * 10^{7}$	2,60	0,50	28
		1,30	0,25	20
		0,65	0,125	18
Gram neg.		2,60	0,50	0
Pseudomonas aeruginosa	$1,2 * 10^{10}$	1,30	0,25	0
		0,65	0,125	0
Proteus mirabilis	$5,5 * 10^{9}$	2,60	0,50	20
		1,30	0,25	16
		0,65	0,125	11
Eschericia coli	$3,6 * 10^{13}$	2,60	0,50	20
		1,30	0,25	14
		0,65	0,125	11

CHAPTER 3

SYNTHESIS OF (±) (1R, 3R, 4R)-3,4-DIHYDRO-4-HYDROXY-1,3-DIMETHYL-1H-BENZO[*c*]PYRAN-5,8-QUINONE (17) AND ITS 4S-DIASTERIOMER (18).



(29)



(32)

6 - Allyl - 2,5 - dimethoxyacetophenone (29) was used as starting material (Scheme C) and converted to its conjugated analogue (32).

This conversion was attempted by dissolving compound (29) in freshly distilled tetrahydrofuran (THF) and treating the solution with 4 mol. equiv. of potassium *tertiary* butoxide. The mixture was left stirring at room temperature under nitrogen for 30 minutes. ^{1}H nmr analysis of the product after work up showed that no conjugation had taken place. Instead only starting material was recovered.

The above procedure was repeated at 60°C for 2 hours. Analysis of the reaction mixture by thin layer chromatography (tlc) showed that the product had a slighty lower R_f than the starting material. The reaction mixture was worked up and chromatographed and spectroscopic analysis (^{1}H nmr, ir, ms) of the oil recovered showed that the expected product, 2,5 - dimethoxy - 6 - (*trans* - 1 - propenyl) acetophenone (32) had formed in a yield of 99 %. Its ^{1}H nmr spectrum showed *inter alia* a doublet at δ 1.85 corresponding to the methyl of the propenyl group, a singlet at δ 2.42 corresponding to the methyl of the keto group and two more singlets at δ 3.77 and 3.80 corresponding to the two methoxy groups.

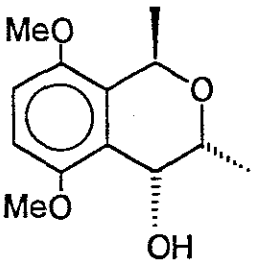
The conjugated ketone (32) was reduced to the alcohol (33), in good yield (95 %), by treating compound (32) with 5 mol. equiv. lithium aluminium hydride in dry ether.

Compound (33) underwent oxidative cyclisation when treated with 4 mol. equiv. of cerium (IV) ammonium nitrate (CAN) to afford a diastereomeric mixture of benzopyranquinones (17) and (18). Compound (17) in which the hydroxy group occupies the *pseudo* axial position was the major product (60 %) while compound (18) was recovered in a yield of 11 %.

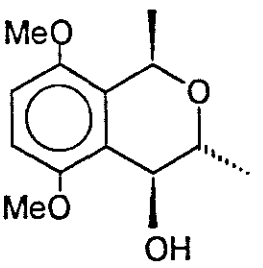
For compounds (17)and (18) it was possible to assign the relative stereochemistry at the three chiral centres as drawn by virtue of their respective ^1H nmr spectra, using the same arguments as for compound (13) in Chapter Two.

Compounds (17) was biologically evaluated in these laboratories and found to be active against both Gram negative and Gram positive organisms. The results are shown in Table II. Since compound (18) could not be obtained in a pure form, biological evaluation was not performed.

It was found that when only 2 mol. equiv. CAN was used cyclisation also occurred to afford pyrans (54) and (55). The stereochemical assignments were established similarly to that of compunds (17 and (18)



(54)



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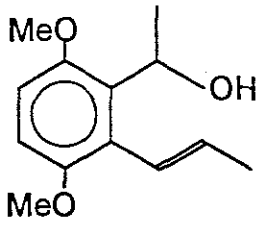

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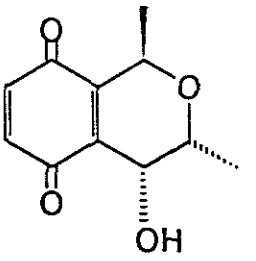
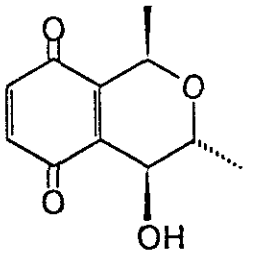


(17)



(18)

Here again compound (54) was the major product (65,4 %) while compound (35) was formed in a yield of 20 %.

In the above described reactions it is evident that in both cases, compounds {(18) and (55)} in which the hydroxyl groups occupy the *pseudo* equatorial position were obtained in moderate yields. It was also difficult to obtain a pure product.

In view of the above it was decided to attempt a different approach to obtain a good yield of pure benzopyranquinone (18).

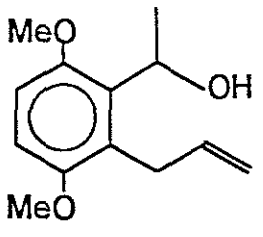
Benzopyranquinone (13) was dissolved in dry distilled dimethyl sulphoxide (DMSO) and dry air was passed through the solution for 15 minutes. Potassium *tertiary* butoxide was added to the mixture which was stirred for a further 20 minutes under a constant stream of dry air at ambient temperature. The reaction was quenched after 40 minutes by the addition of water. Work-up of the reaction mixture showed that no reaction had occurred.

It was then attempted to hydroxylate the dimethoxybenzopyran (31) as per the above procedure with dry air and *tertiary* butoxide in DMSO. Compound (31) was insoluble in DMSO and no reaction occurred.

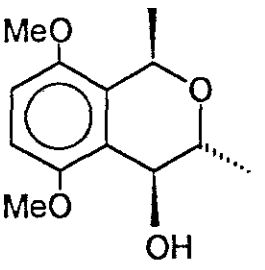
The above reaction was repeated using dimethylformamide (DMF) as solvent. Compound (31) was soluble in the DMF but it was found on work up of the reaction mixture that only starting material was present and that no reaction had occurred.

The above was now repeated with the reaction vessel in an oil bath at 60°C, but no change in the starting material was observed on work up of the reaction mixture.

It was then attempted to cyclise the alcohol (30) under aerobic conditions.



(30)



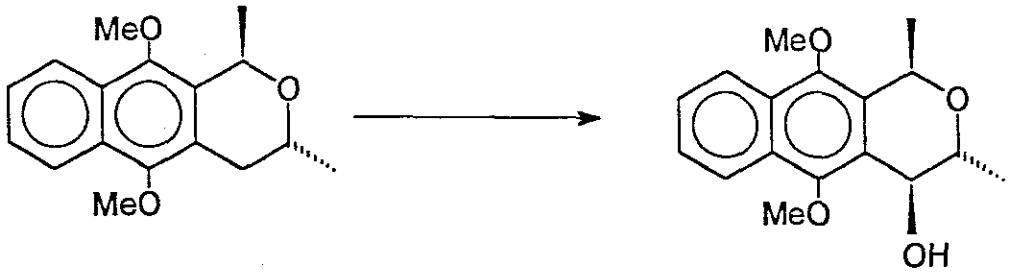
(55)

Compound (30) was dissolved in DMF and dry air was blown through for 15 minutes. Potassium *tertiary* butoxide (5 mol. equiv.) was added and the mixture was stirred at room temperature for an hour with the constant stream of dry air passing through the reaction mixture. On work up of the reaction mixture and purification of the product, the ^{1}H nmr spectrum showed that the product obtained was the conjugated alcohol (33) (62,5 %) and not the desired pyran (55).

This product (33) was dissolved in DMF and treated as above with *tertiary* butoxide and dry air at elevated temperature (60°C) for 1 hour.

The product was obtained in the usual manner and study of the ^{1}H nmr spectrum proved that the compound obtained was in fact the pyran (31), and that no hydroxylation had taken place.

As a control the naphthopyran (56) was subjected to the above experimental conditions. The reaction was worked up after 2 hours. The major fraction obtained by preparative layer chromatography (plc) was found to be the hyroxylated analogue (40)[16](20 %).



(56) (40)

It is not clear at the time of writing why pyran (56) underwent hydroxylation whereas pyran (13) did not.

Its ^1H nmr spectrum showed the following signals : Two doublets at δ 1.43 and δ 1.70 corresponding to the methyls at C-1 and C-3 respectively (*J* 6.5 and 7.0 Hz) and two singlets corresponding to the two methoxy groups at δ 3.90 and δ 4.02; a multiplet between δ 3.90 - 4.3 corresponding to the proton at C-3 and the hydroxyl proton.

TABLE II

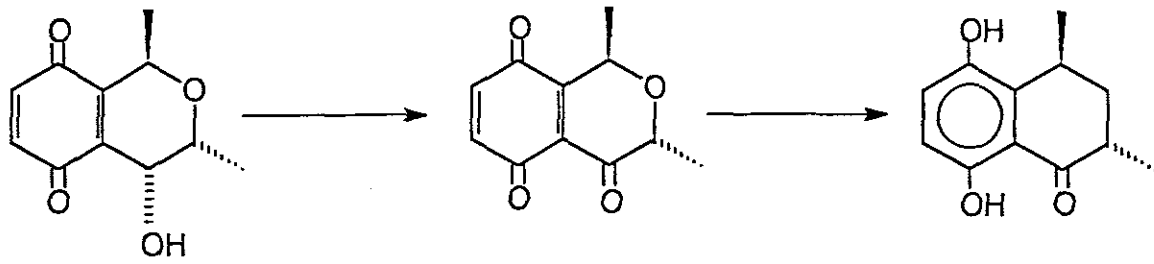
RELATIVE INHIBITION USING COMPOUND (17)

ORGANISM	NUMBER OF ORGANISMS/ML	DOSE OF COMPOUND (µmol)	MASS OF COMPOUND IN mg/0,1 ml	INHIBITION ZONE IN mm
Gram pos.				
Staphylococus aureus	$7,21 * 10^{12}$	2,40	0,50	20
		1,20	0,25	20
		0,60	0,125	20
Bacillus subtillus	$1,4 * 10^{9}$	2,40	0,50	29
		1,20	0,25	26
		0,60	0,125	19
Candida albicans Fungus	$1,2 * 10^{7}$	2,40	0,50	17
		1,20	0,25	17
		0,60	0,125	0
Gram neg.		2,40	0,50	15
Pseudomonas aeruginosa	$1,2 * 10^{10}$	1,20	0,25	0
		0,60	0,125	0
Proteus mirabilis	$5,5 * 10^{9}$	2,40	0,50	15
		1,20	0,25	13
		0,60	0,125	11
Eschericia coli	$3,6 * 10^{13}$	2,40	0,50	15
		1,20	0,25	11
		0,60	0,125	0

CHAPTER 4

SYNTHESIS OF (1R, 3R)-5,8-DIHYDROXY-1,3-DIMETHYL-4-OXO-(1H,3H)-BENZO[c]PYRAN.

It was decided to attempt the following route :



(17) (19) (20)

The first step would entail the oxidation of the hydroxyl group and the second step selective reduction of the quinone system (19) to afford the diol (20).

Pyridinium dichromate (PDC) has been proved to be a useful oxidant of alcohols[26] to ketones or aldehydes. PDC can be easily prepared by dissolving chromium trioxide in a minimum of water, adding pyridine and collecting the precipitated product.

Compound (17) was treated with PDC (3 mol. equiv.) at 0°C under a nitrogen blanket and was left stirring in dimethylformamide for four hours. After work up of the reaction mixture and analysis of the reaction mixture by tlc it was evident that no reaction had taken place.

Next the dimethoxyhydroxpyran (54) was subjected to the same conditions as above. On work-up and inspection of the tlc analysis of the product mixture it was evident that some reaction had occurred as a compound with a higher R_f than the starting material (54) was clearly visible. This compound was purified by chromatography and was assigned structure (57) based on its spectoscopic properties (ir, ms, ^1H nmr, see experimental) (11,4 %). Its ^1H nmr spectrum showed the following signals : Two doublets at δ 1.41 and δ 1.49 *J* 7Hz each corresponding to the protons of the methyls at C-3 and C-1 respectively, two singlets at δ 3.75 and δ 3.85 corresponding to the methoxy groups protons and two quartets at δ 4.47 and δ 5.24 corresponding to the two coupled protons at C-3 and C-1 respectively (*J* 7 Hz). The two doublets corresponding to the aromatic protons were also visible at δ 6.78 and δ 6.96.

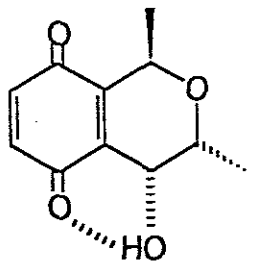
The above spectrum was compared with that of its precursor and thereby established that the stereochemistry at C-1 and C-3 had remained in tact.

The ir spectrum showed Vmax 1690 cm^{-1} corresponding to the carbonyl group at C-4.

By experimentation with various solvents, temperatures and reaction times the yield was improved to 37,4 % by treating compound (54) with PDC (15 mol. equiv.) in dry dichloromethane at room temperature, under nitrogen for 12 hours.

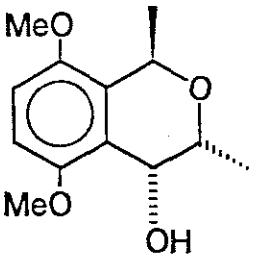
The finding above indicates that the strong hydrogen bonding between the hydroxy group and the carbonyl group of compound (17) causes this compound to resist oxidation of the hydroxy group.

The dimethoxy analogue (57) readily undergoes oxidation at the hydroxy group as less strong hydrogen bonding presumably exists here.

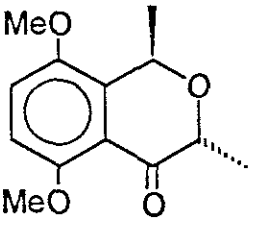


(17)

Hydrogen bonding in compound (17)

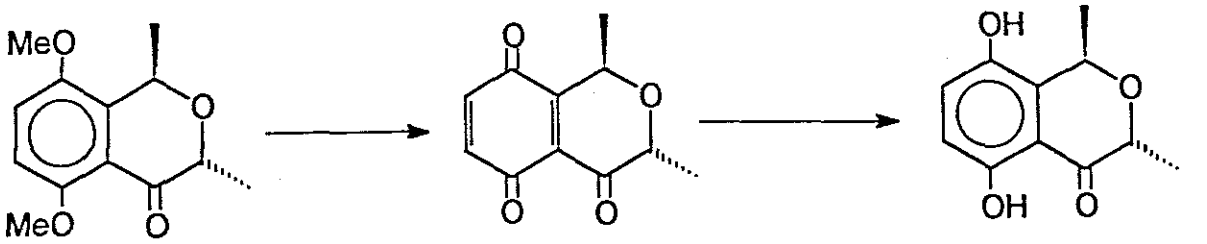


(54)



(57)

As a result of the above findings it was decided to oxidise the dimethoxy keto pyran (57) to the quinone (19) [using silver (II) oxide (AgO)[27]] wich could then be reduced to the target compound (20).



(57) (19) (20)

Compound (57) was dissolved in dioxane and treated with argentic oxide. The reaction was worked up after 10 minutes and the residue chromatographed in the usual manner.

On inspection of the spectroscopic data, it was evident that the product formed was in fact the target compound (20) and not the expected intermediate (19).

The ^1H nmr spectrum showed *inter alia* two D_2O exchangeable singlets at δ 4.92 and 11.30 and two doublets corresponding to the protons of the two methyl groups at δ 1.70 for C-3 and δ 1.75 for C-1 each 7 Hz.

The ir spectrum showed the hydroxy absorption at 3400 cm^{-1} and the carbonyl absorption at 1650 cm^{-1}.

The reason that the oxidation of the dimethoxy pyran (57) did not go to completion as proposed by Snyder and Rapport[27] is not clear at this stage. The significance of this finding is however that it was possible to synthesize compound (20) directly from compound (57) in one easy step and that the reaction was reproducible with a moderate yield of 50 %.

Compounds (57) and (20) were biologically evaluated. Compound (57) showed no inhibition zones while compound (20) showed inhibitory activity against most of the selected Gram positive and Gram negative organisms (See Table III).

TABLE III

RELATIVE INHIBITION USING COMPOUND (20)

ORGANISM	NUMBER OF ORGANISMS/ML	DOSE OF COMPOUND (µmol)	MASS OF COMPOUND IN mg/0,1 ml	INHIBITION ZONE IN mm
Gram pos.				
Staphylococus aureus	$7,21 * 10^{12}$	2,40	0,50	19
		1,20	0,25	17
		0,60	0,125	11
Bacillus subtillus	$1,4 * 10^{9}$	2,40	0,50	20
		1,20	0,25	17
		0,60	0,125	14
Candida albicans Fungus	$1,2 * 10^{7}$	2,40	0,50	20
		1,20	0,25	0
		0,60	0,125	0
Gram neg.		2,40	0,50	0
Pseudomonas aeruginosa	$1,2 * 10^{10}$	1,20	0,25	0
		0,60	0,125	0
Proteus mirabilis	$5,5 * 10^{9}$	2,40	0,50	14
		1,20	0,25	0
		0,60	0,125	0
Eschericia coli	$3,6 * 10^{13}$	2,40	0,50	12
		1,20	0,25	0
		0,60	0,125	0

SUMMARY

The target compounds of this study, namely compounds (13), (17), (18) and (20) were successfully synthesised via new synthetic routes. Yields were fair to good. The sytheses of the above mentioned compounds were accepted for publication in the *South African Journal of Chemistry*.

The biological evaluations done thus far show that these compounds are inhibiting the growth of both Gram positive and Gram negative organisms.

Compound (20), the benzo analogue of the naturally occurring naphthopyran, Hongconin (21), was readily synthesised in one step from compound (57) by treatment with argentic oxide.

Hongconin (21) may now be prepared in a few steps by using the known naphthopyran (34) as starting material and following the route proposed in this work.

EXPERIMENTAL

GENERAL :

^1H nmr Spectra were recorded on a 60 MHz Varian EM 360 Spectrometer and a Varian 200 MHz Spectrometer. All nmr spectra were recorded at ambient temperature in deuterochloroform using tetramethylsilane as internal standard. Mass spectra were recorded on a modified AEI analyser (902). IR - spectra were measured for nujol mulls on a Beckman Aculab IR spectrophotometer. Melting points quoted are uncorrected and were recorded on an electrothermal digital melting point apparatus.

Column chromatography was carried out on dry columns with Merck Kieselgel 60 (70 - 230 mesh) as adsorbent. Preparative layer chromatography (plc) was performed on glass plates coated with Merk Kieselgel F254, while thin layer chromatography (tlc) was carried out on aluminium plates coated with the same material.

Petroleum spirit refers to light petroleum, the fraction of boiling point 60 - 80°C, and ether to diethyl ether. Anhydrous magnesium sulphate ($MgSO_4$) was used to dry the organic solvents after extraction procedures, and most organic solvents and liquid reagents were distilled prior to use.

As in the text some solvents and reagents have been abbreviated. THF, DMF, CAN, PDC refers to tetrahydrofuran, N,N-dimethylformamide, cerium(IV)ammonium nitrate and pyridinium dichromate respectively. The phrase, "residue obtained upon work up", refers to the residue when the organic layer was separated, dried and the solvent evaporated.

Hydroquinone diacetate. (24)

Hydroquinone (23) 27,5 g (0,25 mol) was stirred in acetic anhydride 51 g (47,5 ml; 0,5 mol). One drop of concentrated sulphuric acid was added to the mixture. After five minutes the solution was poured onto 200 ml of crushed ice. The resulting crystals were filtered at the pump and washed with 250 ml cold water. The product was recrystallised from 200 ml 50 % aqueous ethanol to give clear needle -like crystals (18 g, 70 %) with a melting point of 121,6°C (Lit[20] m.p. 122°C).

2,5-Dihyroxyacetophenone. (25)

A mixture of hydroquinone diacetate (24) (10 g, 0,05 mol) and anhydrous aluminium chloride (21,75 g, 0,16 mol) was finely powdered, homogenated and introduced into a 250 ml flask fitted with an air condenser protected by a calcium chloride drying tube and connected to a gas absorption trap. The flask was lowered into an oil bath and the temperature was slowly increased from ambient temperature to 110°C at which point hydrogen chloride gas began to evolve. This temperature was maintained until all visible HCl evolution had ceased. The temperature was then slowly increased to 164°C where it was

maintained for a further three hours. The flask was removed from the oil and cooled. 70 g of crushed ice was inserted carefully into the flask followed by 5 ml of concentrated hydrochloric acid to decompose the excess aluminium chloride. The resulting solid was filtered under reduced pressure and washed with a small amount of water. Recrystallisation of this solid from 50 % aqueous ethanol yielded 4,6 g (58,7 %) product (25) m.p. 202 -203°C (Lit.[20] m.p. 202 -203°C).

5-Allyloxy-2-hydroxyacetophenone. (26)

3,074 g (0,02 mol) 2,5 Dihydroxyacetophenone (25) was mixed with allyl bromide 2.34 g (0,02 mol) and potassium carbonate 2,2 g (0,016 mol) in acetone (50 ml). The mixture was stirred under reflux for six hours in a round bottomed flask and a condenser with a calcium chloride drying tube. The reaction mixture was filtered and the residue obtained was chromatographed (eluent 20 % ethyl acetate in light petroleum) to afford the product (26) (3,6 g; 0.019 mol; 93%), m.p. 57-58°C (Lit.[23], 59-60°C).

Vmax 3350(OH), 1640(C=O), 1610cm^{-1}(C=C) ; $\delta(CDCl_3)$ 2.60 (3H, s,Ar-CO$\underline{CH_3}$), 4.50 (2H, dt, *J* 6 and 2 Hz, $\underline{CH_2}CH=CH_2$), 5.10 - 5.70 (2H, m, $CH_2CH=\underline{CH_2}$), 5.80 - 6.50 (1H, m, $CH_2\underline{CH}=CH_2$), 6.70 - 7.30 (3H, m, Ar-H), 11.80 (1H, s, OH, D_2O-exchangeable).

2,5-Dihyroxy-6-(2-propenyl)acetophenone. (28)

Compound (26) 1 g (0,08 mol) was added to a 50 ml round bottomed flask, the flask flushed with nitrogen and immersed into an oil bath preheated to 220°C. After two hours the flask was removed, cooled and the residue dissolved in acetone, preabsorbed onto kieselgel and chromatographed (eluent 20 % ethyl acetate in light petroleum) to afford the product (28) (0,616 g, 61,6 %), m.p. 108,9 - 109,6°C (Lit.[28] 104 - 106°C). Vmax. 3270(OH), 1670(C=O), 1610cm^{-1}(C=C); $\delta(CDCl_3)$ 2.44 (3H,s,$CO\underline{CH_3}$), 3.35 (2H, dt, *J* 6 and 2 Hz, $\underline{CH_2}CH=CH_2$), 4.70 - 5.20 (2H, m, $CH_2CH=\underline{CH_2}$), 5.60 - 6.27 (1H, m, $CH_2\underline{CH}=CH_2$), 6.60 (1H, d, *J* 9.5 Hz, Ar-H), 6,80 (1H, d, *J* 9.5 Hz, Ar-H) 8.05 (1H, s, OH, D_2O-exchangeable), 11.80 (1H, s, OH, D_2O-exchangeable).

2,5-Dimethoxy-6-(2-propenyl)-acetophenone. (29)

Compound (28) (0,616 g, 3,21 mmol) was dissolved in acetone and gently boiled under reflux in the presence of methyl iodide (6,82 g, 48,05 mmol) and potassium carbonate (2,21 g, 16 mmol) for 24 hours. The reaction mixture was then filtered, preabsorbed onto kieselgel and chromatographed using 15 % ethyl acetate in petroleum spirit on a short column. The product was obtained as a red/brown oil (0,784 g, 90 %). (Found : M^+ 220,1088. $C_{13}H_{16}O_3$ requires M, 220,1099); (Found : C 71,1 %; H 7,35 %. $C_{13}H_{16}O_3$ requires C 70,9 %; H 7,27 %)

Vmax. 1690(C=O), 1630 cm^{-1}(C=C); $\delta(CDCl_3)$ 2.48 (3H, s, $CO\underline{CH_3}$), 3.30 (2H, dt, *J* 6 and 2 Hz, $\underline{CH_2}CH=CH_2$), 3.80 (6H, s, 2 x OCH_3), 4.70 - 5.20 (2H, m, $CH_2CH=\underline{CH_2}$), 5.52 - 6.30 (1H, m, $CH_2\underline{CH}=CH_2$), 6.65 (1H, d, *J* 10.50 Hz, Ar-H), 6.85 (1H, d, J 10.5 2 Hz, Ar-H).

1-(1-Hydroxyethyl)-2,5-dimethoxy-6-(2-propenyl)benzene. (30)

Compound (29) (300 mg, 1,36 mmol)was dissolved dry ether (10 ml). This solution was added dropwise to a stirring suspension of lithium aluminium hydride (259 mg, 8,63 mol) in dry ether (3 ml). The reaction vessel was stoppered and stirring continued for a further 15 minutes. The reaction was then quenched by the addition of saturated ammonium chloride solution (1 ml). The reaction mixture was dried, ($MgSO_4$), filtered and the solvent

evaporated. The mixture was purified by chromatography on a short column using an eluent of 15 % ethyl acetate in petroleum spirit to give the product (30) as a light brown oil (287 mg, 95 %). (Found : C 70,63 %; H 8,99 %. $C_{13}H_{18}O_3$ requires C 70,27 %; H 8,11 %).

Vmax. 3520 cm^{-1} (OH), $\delta(CDCl_3)$ 1.55 (3H, d, *J* 6.5 Hz, Ar-CH<u>CH_3</u>OH), 3.50 (2H, d, *J* 6 Hz, Ar-<u>CH_2</u>$CH=CH_2$), 3,80 (3H, s, OCH_3), 4.05 (1H, s, OH, D_2O exchangeable), 4.75 - 5.25 (Ar-<u>CH</u>$CH_2=CH_2$), 6.77 (2H, s, Ar-H).

5,8-Dimethoxy-1,3-dimethyl-1H-benzo[*c*]pyran. (31)

100 mg (0,45 mmol) of compound (30) was dissolved in freshly distilled DMF and stirred in a nitrogen atmosphere. 202 mg (1,80 mmol) potassium *tertiary* butoxide was added and the mixture was stirred at room temperature under nitrogen for 15 minutes. 1 ml ammonium chloride solution was added and the reaction mixture was worked up, extracted with dichloromethane and chromatographed on a short column (eluent 20 % ethyl acetate in petroleum ether) to afford the product (31) (94 mg, 0,42 mmol, 94 %) as a light brown oil.

(Found : M^+ 222,2694. $C_{13}H_{18}O_3$ requires M, 222,2700

$\delta(CDCl_3)$ 1.31 (3 H, d, *J* 6 Hz, 3 - Me), 1.49 (3 H, d, *J* 6 Hz, 1 - Me), 2,28 (1 H, dd, *J* 17 and 11 Hz, 4a' - H), 2.78 (1 H, dd, *J* 17 and 3 Hz, 4e' - H), 3.76 (6 H, s, 5- and 8- OMe), 3.90 - 4.20 (1 H, m, 3 - H), 5.10 (1 H, q, *J* 6 Hz, 1-H), and 6.64 (2 H, s, Ar-H)

3,4-Dihydro-1,3-dimethyl-1H-benzo[*c*]pyran-5,8-quinone. (13)

Dimethoxypyran (31) (200 mg,0,901 mmol) was dissolved in acetonitrile (10 ml). Water (1 ml) was added to the solution. A solution of CAN (987 mg, 1,8 mmol in 1 ml water) was added dropwise to the stirred solution over 10 minutes. The reaction mixture was thrown into water and extracted with dichloromethane. The residue obtained upon work-up was chromatographed (eluent : 15 % ethyl acetate in petroleum ether). The product (13) was obtained as yellow crystals (187 mg, 99 %) mp 101,9-104,7°C (petroleum spirit) (Lit.[29] 102,5 - 105,5°C).

(Found : M^+ 192,0767. $C_{11}H_{12}O_3$ requires M, 192,0786)
Vmax. 1606 cm^{-1} (C=O) 1406 cm-1 (C-O), $\delta(CDCl_3)$ 1.33 (3 H, d, *J* 6 Hz, C3 - Me), 1.45 (3 H, d, *J* 6 Hz, 1 - Me) 2.08 (1 H dddd, *J* 19, 10 and 2 Hz, 4a' - H), 2.56 (1 H, dd, *J* 19 and 4 Hz, 4 - H), 3.70 - 4.10 (1 H, m, 3 - H), 4,80 br (1 H, q, *J* 6 Hz, 1 - H), and 6.66 (2 H, s, Ar-H)

2,5-dimethoxy-6-(*trans*-1-propenyl)acetophenone. (32)

Compound (29) (100 mg, 0,455 mmol) was dissolved in freshly distilled THF (20 ml) and the solution was stirred at 60°C under nitrogen. Potassium *tertiary* butoxide (204 mg, 1,82 mmol) was added and the reaction mixture was stirred under nitrogen at 60°C for 2 hours. By spotting (tlc) the reaction mixture against the starting material (29) a slightly reduced R_f was observed. The reaction mixture was worked up (by quenching the reaction with 20 % ammonium chloride solution and exhaustive extraction with dichloromethane) and chromatographed on a short column with 15 % ethyl acetate in petroleum spirit as an eluent to afford (32) as a clear oil (100 mg, 100 %).

(Found : C 71,15 %; H 7,2 %. $C_{13}H_{16}O_3$ requires C 70,9 %, H 7,27 %).

(Found : M^+ 220,1078. $C_{13}H_{16}O_3$ requires M, 220,1099).

Vmax. 1700 cm^{-1}(C=O); δ($CDCl_3$) 1.85 (3H, d, *J* 6 Hz, CH=CH$\underline{CH_3}$), 2.42 (3H, s, $COCH_3$), 3.77 (3H, s, OCH_3), 3.80 (3H, s, OCH_3) 5.60 - 6.70 [2H, m, olefinic H collapsed to 2 x d (*J* 16 Hz) on double irradiation at δ 1.85], 6.80 (2H, s, Ar-H).

1-(Hydroxyethyl)-2,5-dimethoxy-6-(*trans*-1-propenyl)benzene. (33)

The dimethoxyketone (32) (389 mg, 1,77 mmol) was dissolved in dry distilled ether (10 ml). This solution was added dropwise to a suspension of lithium aluminium hydride (336 mg, 8,84 mmol) in dry ether (2 ml). After 15 minutes the reaction was quenched by the addition of 1 ml saturated ammonium chloride solution. Anhydrous magnesium sulphate was added to dry the mixture. The slurry was then filtered and washed with dichloromethane. The solvent was evaporated and the residue was chromatographed on a short column (eluent : 15 % ethyl acetate in petroleum spirit) to give the product (33) as white crystals (370 mg, 95%) with m.p. 83,5 - 84,5°C.

(Found : C 70,4 %; H 8,35 %. $C_{13}H_{18}O_3$ requires C 70,27 %; H 8,1 %)

(Found : M^+ 222,1263. $C_{13}H_{18}O_3$ requires 222,1256.)

Vmax. 3520 cm^{-1} (OH); δ($CDCl_3$) 1.55 (3H, d, *J* 6.5 Hz, Ar-$CH\underline{CH_3}OH$), 1.80 (3H, dd, *J* 6.5 and 2 Hz, $CH=CH-\underline{CH_3}$), 3.80 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.05 (1H, s, OH, D_2O-exchangeable), 4.95 - 5.65 (1H, m, Ar-$\underline{CH}OHCH_3$), 5.75 - 6.65 [2H, m, olefinic H, collapsed to 2 x d (*J* 16 Hz) on double irradiation at δ 1.80], 6.77 (2H, s, Ar-H).

(+-) (1R,3R,4R,)-3,4-Dihydro-4-hydroxy-1,3-dimethyl- 1H-benzo [2,3-c]pyran-5,8-quinone (17) and its 4S-diastereomer (18).

Dimethoxy alcohol (33) (500 mg, 2,25 mmol) was dissolved in 20 ml acetonitrile containing 10 ml water. CAN (4,94 g, 9,01 mmol) in 10 ml water was added dropwise to the stirred solution at room temperature. The reaction flask was stoppered and left stirring for 40 minutes. Water (100 ml) was added and the organics were extracted with dichloromethane. The solvent was evaporated and the residue upon work up was chromatographed to afford firstly compound (18) (51,5 mg, 11 %)as an oil, followed by compound (17) (281 mg, 60 %) with a melting point of 98,5 - 99,2°C .

(17) (Found : M^+ 208,0742. $C_{11}H_{12}O_4$ requires 208,0763).

Vmax. 3490 (OH), 1650 cm^{-1}(C=O); δ (CDCl3) 1.38 (3H, d, *J* 6.0 Hz, 3-CH_3), 1.45 (3H, d, *J* 7.0 Hz, 1-CH_3), 2.24 (1H, d, *J* 7.0 Hz, 4-OH), 3.95 (1H, d x quartet, *J* 6.0 and 2.0 Hz, 3-H), 4.37 (1H, d, *J* 2.0 Hz, 4-H), 4.85 (1H, quartet, *J* 7.0 Hz, 1-H), 6,72 (1H, d, *J* 10.0 Hz, Ar-H), 6.80 (1H, d, *J* 10.0 Hz, Ar-H).

(18) (Found : M^+ 208,0742. $C_{11}H_{12}O_4$ requires 208,0763).

Vmax. 3500 (OH), 1650 cm^{-1} (C=O); δ (CDCl3) 1.38 (3H, d, *J* 6.0 Hz, 3-CH_3), 1.52 (3H, d, *J* 7.0 Hz, 1-CH_3), 3.52 (1H, d, *J* 2.5 Hz, 4-OH), 3.85 (1H, d x quartet, *J* 8.0 and 6.0 Hz, 3-H), 4.35

(1H, d x quartet, J 8.0 and 2.5 Hz, 4-H), 4,73 (1H, d x quartet, J 6 and 1 Hz, 1H), 6.72 (2H, s, Ar-H).

(1R,3R,4R)-3,4-Dihydro-4-hydroxy-5,8-dimethoxy-1,3-dimethyl-1H-benzo[2,3-c]pyran (54) and its 4S-diasteriomer (55).

Dimethoxy alcohol (33) (200 mg, 0,90 mmol) was dissolved in acetonitrile (10 ml) containing water (10 ml). CAN (984 mg, 1,8 mmol) was dissolved in 5 ml water and added to the stirred solution dropwise over seven minutes. The solution was stoppered and stirred for a further 20 minutes. The reaction was quenched by the addition of water (100 ml). On extraction of the organic portion with dichloromethane and further work up, the residue was chromatographed (eluent : 20 % ethyl acetate at first then 30 % ethyl acetate in petroleum spirit) to afford, firstly a mixture of (55) and (33), followed by product (54) (150 mg, 70%) with a melting point of 113,3 - 114,5 °C.

(54) Found : M^+ 238,1184. $C_{13}H_{18}O_4$ requires 238,1205)

Vmax. 3400 cm^{-1} (OH), δ ($CDCl_3$) 1.40 (3H, d, *J* 6.5 Hz, 3-CH_3), 1.49 (3H, d, *J* 7,0 Hz, 1 CH_3), 2.05 (1H, d, *J* 7.5 Hz, 4a'-OH, D_2O-exchangeable), 3.75 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.90 - 4.14 (1H, m, 3-H), 4.54 (1H, dd, *J* 7.5 and 2 Hz, 4e'-H), 5.10 (1H , quartet, *J* 7.0 Hz, 1-H), 6.75 (2H, s, Ar-H).

Compound (55) had an almost similar R_f to the starting material (33) therefor these two compounds were separated by plc (15 % ethyl acetate in petroleum spirit).

(55) (Found : M^+ 238,1180. $C_{13}H_{18}O_4$ requires 238,1205)

δ ($CDCl_3$) 1.38 (3H, d, *J* 6.5 Hz, 3-CH_3), 1.55 (3H, d, *J* 7.0 Hz, 1- CH_3), 3.74 and 3.82 (3H each, s, 2 x OCH_3), *ca.* 3.80 - 4.0 (2H, broad m, 3-H and OH), 4.55 (1H, d, *J* 8.0 Hz, 4a'-H), 5.05 (1H, q, *J* 7.0 Hz, 1-H), 6.68 and 6.76 (each 1H, d, *J* 9.0 Hz, 6- and 7-H).

(1R, 3R)-5,8-Dimethoxy-1,3-dimethyl-4-oxo-(1H,3H)-benzo [c]pyran. (57)

The dimethoxy hydroxy pyran (34) (211,8 mg; 0,890 mmol) was dissolved in dichloromethane (20 ml). The solution was stirred at room temperature. PDC (5,020 g, 13,351 mmol) was added to the solution and stirring continued under nitrogen for a further 12 hours. The reaction mixture was then filtered and the residue upon evaporation of the solvent was chromatographed to afford product (36) (78,9 mg 37,4 %) as cream coloured crystals with a melting point of 82 - 84°C.

(57) (Found : M^+ 236,10151. $C_{13}H_{16}O_4$ requires 236,1048)

Vmax. 1690 cm^{-1} (C=0); δ ($CDCl_3$) 1.41 (3H, d, *J* 7.0 Hz, 3-CH_3), 1.49 (3H, d, *J* 7.0 Hz, 1-CH_3) 3.75 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 4.47 (1H, quartet, *J* 7.0 Hz, 3-H), 5.24 (1H, q, *J* 7 Hz, 1-H) 6.78 (1H, d, *J* 9.0 Hz, Ar-H) 6.96 (1H, d, *J*, 9.0 Hz, Ar-H).

(1R,3R)-5,8-Dihydroxy-1,3-dimethyl-4-oxo-(1H,3H)-benzo[c]pyran. (20)

The pyran (36) (78 mg, 0,331 mmol) was dissolved in dioxane (5 ml). Argentic oxide (AgO) (206 mg, 1,66 mmol) was added to the stirring solution. 6 M Nitric acid solution was added dropwise until the AgO had dissolved. The solution was stirred for a further 10 minutes. A "mixture" of 5 ml dichloromethane and 50 ml water was added and the mixture transferred to a separating funnel where the organic portion was removed by extraction with dichloromethane. The residue obtained upon work-up was chromatographed (eluent 25 % ethyl acetate in petroleum spirit) to afford product (20) 31,6 mg (47,3 %) with a melting point of 93,4-95°C (petroleum spirit).

(Found : M^+ 208,0742. $C_{11}H_{12}O_4$ requires 208,0736)

Vmax. 3400 (OH), 1650 cm^{-1}(C=O), δ ($CDCl_3$), 1.70 (3H, d, *J* 7.0 Hz, 3-CH_3), 1.75 (3H, d, *J* 7.0 Hz, 1-CH_3), 4.57 (1H, quartet, *J* 6.5 Hz, 3-H), 4.92 (1H, s, 8-OH, D_2O-exchangeable), 5.23 (1H, quartet, *J* 7.0 Hz, 1-H), 6.67 (1H, d, *J* 9.0 Hz, Ar-H), 6,87 (1H, d, *J* 9.0 Hz, Ar-H), 11.30 (1H, s, 5-OH, D_2O-exchangeable).

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