SYNTHESIS AND ANTIMICROBIAL SCREENING OF SOME C-4-HYDROXYBENZO[C]PYRANQUINONES

by

TEBOGO MASENYA

B.Tech: Chemistry (Cape Technikon)

Thesis submitted in fulfilment of the requirements for the degree of

MASTER IN TECHNOLOGY: CHEMISTRY

in the

DEPARTMENT OF CHEMICAL AND FOOD TECHNOLOGY

at the

CAPE TECHNIKON

CAPE TOWN
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ABSTRACT

Many naturally occurring antibiotics have been identified and their synthesis successfully carried out in laboratories. It was envisaged by our group that by preparing a variety of, for example, quinoid compounds and then comparing their biological properties and activities, a better insight would be gained into a molecular structure – activity relationship.

Chapter 1 deals with the attempted synthesis of 2-(1′-hydroxyethyl)-3-(prop-2′-enyl)-1,4-benzoquinone **(17)** which may be converted by known methods into benzopyran derivatives.

The second chapter describes a synthetic route to (±)(3R, 4R)-3,4-dihydro-3-methyl-4-hydroxy-1H-benzo[c]pyran-5,8-dione **(42)** and its (±)(4S)-diastereomer **(43)**, both of which were found to be active against Gram negative and Gram positive organisms. The *trans*-1,3-dimethyl-4-hydroxy-pyranquinones **(45)** and **(46)** were also successfully synthesised.
A different route of synthesis for the compounds **(53)**, **(54)**, **(55)**,and **(56)** was also investigated.

Several compounds were evaluated for biological activity. It was found that the quinones synthesised during this study were active against Gram negative and

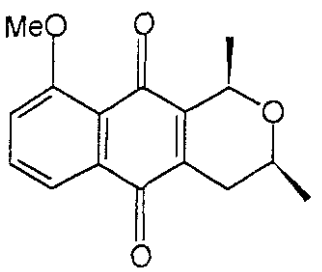
Gram positive bacteria, with the exception of compound (**25**) which had an acetate group in place of a hydroxy group. It was found that this trend was carried through all of the quinone derivatives tested.

INTRODUCTION

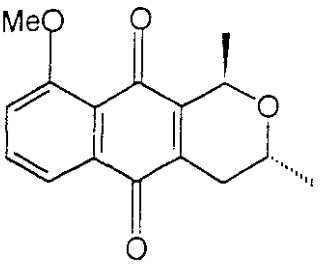
The biological potential of naturally occurring naphtho[2, 3 -*c*]pyranquinones as antineoplastic agents has been recognised for many years and the synthesis of some of these compounds has been successfully undertaken by several groups [1-9].

Many of these compounds are found as naturally occurring compounds in the plant and animal kingdom[10].

Eleutherin[11] **(1)** and isoeleutherin[12] **(2)** were isolated from the tubers of *Eleutherin bulbosa* (Iradaceae). Extracts of *Eleutherin americana* of which **(1)** and **(2)** are the major constituents, have been used to treat heart diseases such as angina pectoris[13, 14].



(1)



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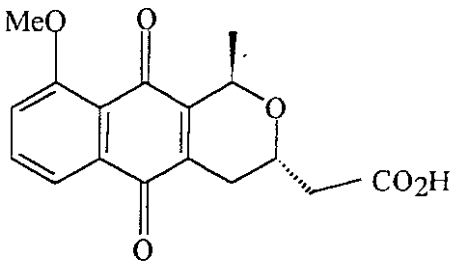
Other naturally occurring pyranquinones include nanomycin A [6, 7] **(3)**, D **(4)**, hongconin **(5)** and naphthocyclinone [15] **(6)**.



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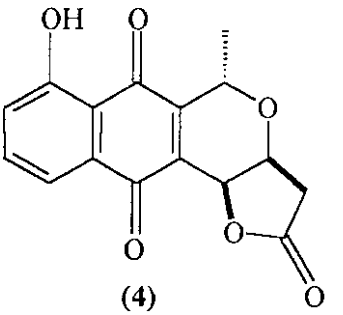

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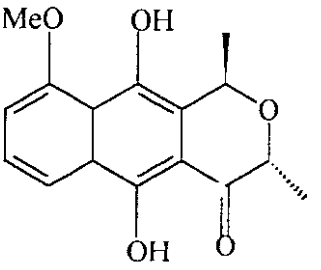

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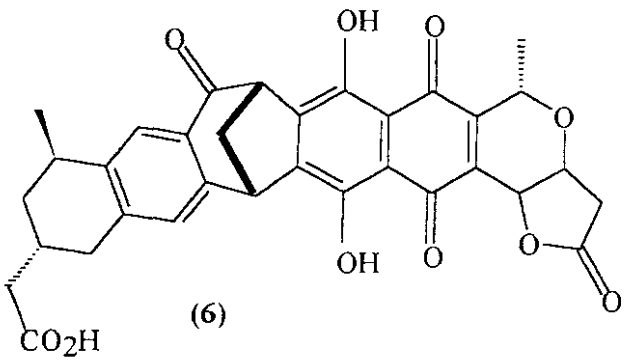


(6)

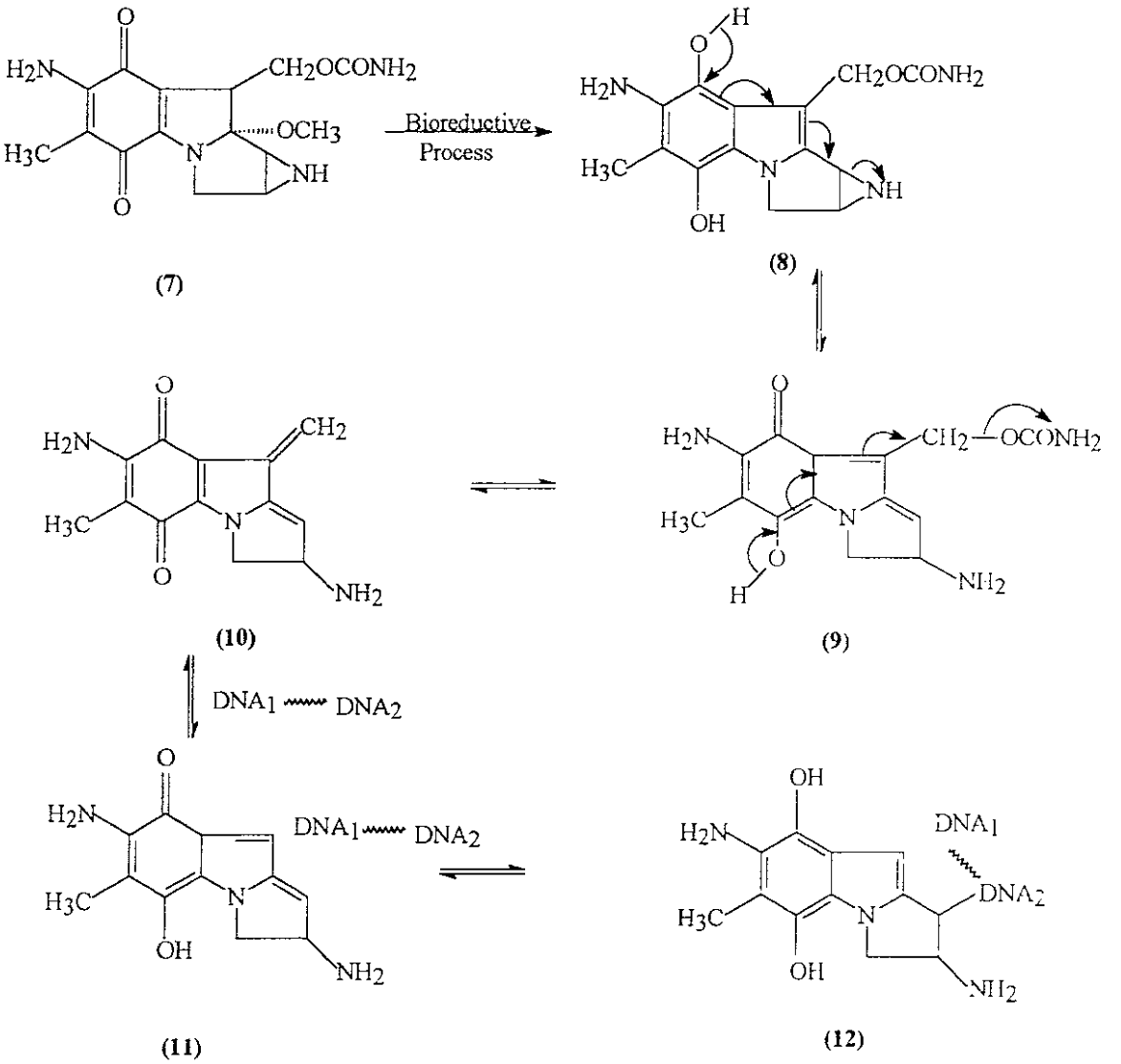
These pyranonaphthoquinones have been shown to exhibit activity against a variety of Gram-positive and some Gram-negative bacteria, pathogenic fungi and yeast, as well as exhibiting antiviral activity [16].

Moore[17], based on the work by Sartorelli [18, 19] proposed that these pyranonaphthoquinones can act as alkylating agents upon bioreduction in a mode of action resembling that of the antitumor drug mitomycin C [18,20] **(7)**. Mitomycin C is believed to cross-link the complementary strands of cellular DNA, thereby inhibiting tumour growth or bacterial multiplication [21] as shown in Scheme I.

Mytomycin C is reduced *in vivo* to the corresponding hydroquinone. The elimination of CH_3OH gives the indole **(8)**. The steric strain of the aziridine ring is released during the formation of compound **(9)**. This is followed by the elimination of HO-$CONH_2$ to give **(10),** which is the proposed biologically active form of mitomycin C.

Previous research work [22] 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 *in vivo* bioreductive alkylation process.

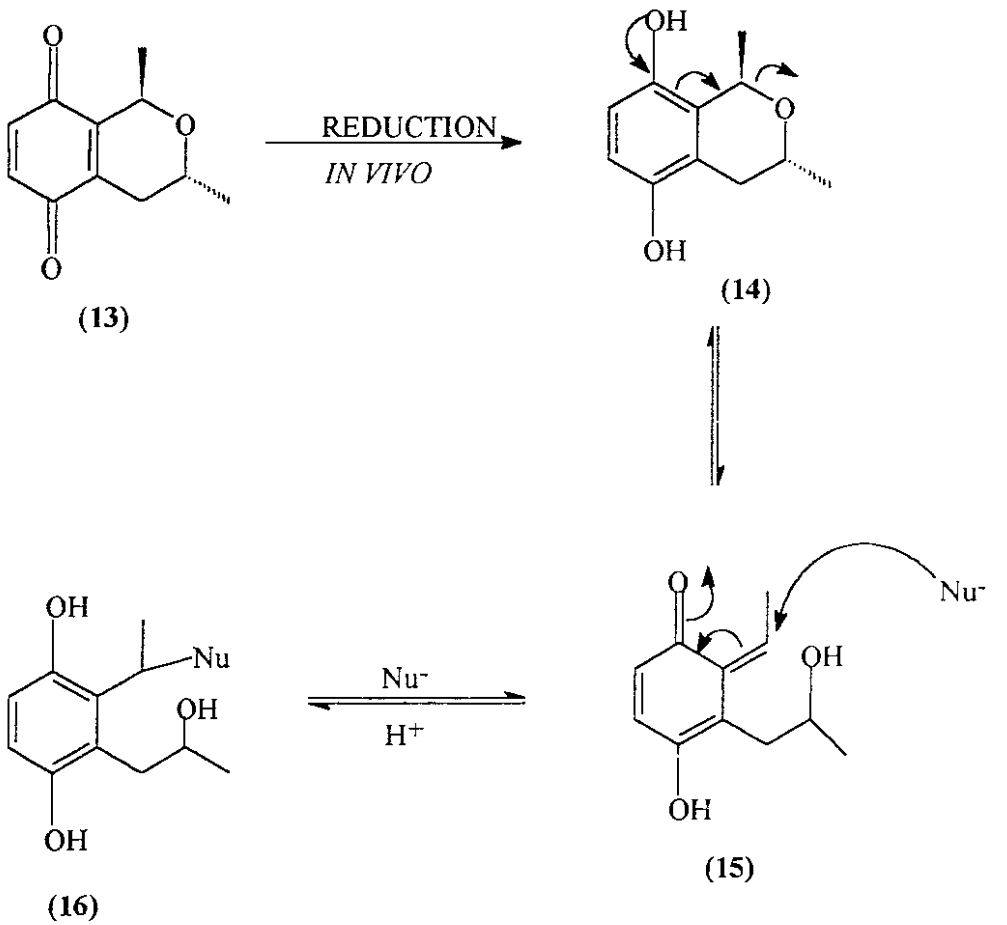
SCHEME 1



The [2,3-*c*]pyranquinone, *viz.* **(13)** may also first undergo an *in vivo* reduction to the quinol **(14)** which could ring open as in **Scheme 2** to give a highly active

monoquinone-methide system **(15)**. Attack on this system by nucleophilic centres in DNA and RNA would inhibit tumour growth or bacterial multiplication.

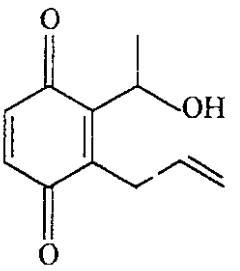
SCHEME 2



It was envisaged by our group that the most important structural feature for biological activity in these systems is the aryl[2,3-*c*]pyranquinone nucleus and that a leaving group L at C-4 of the pyran ring would increase the activity.

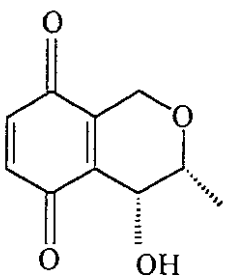
The objectives of this study were:

(i) to establish a viable synthetic route to the hydroxyethylquinone **(17)** via the regioselective allylation of the precursor quinonod nucleus **(25)** or **(23)**.



(17)

(ii) To establish a viable synthetic route to the C 4–hydroxypyran-quinones **(42)** and **(43)** and the *trans*-1,3-dimethyl-4-hydroxy-pyranquinones **(45)** and **(46)**



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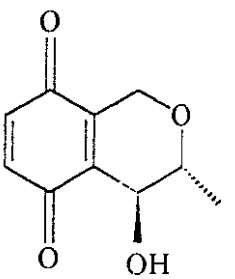

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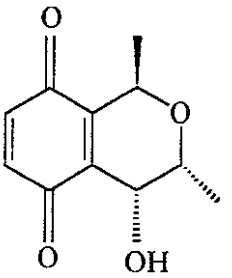

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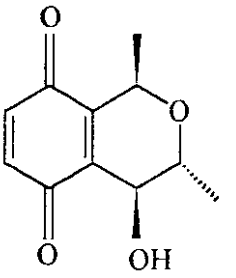


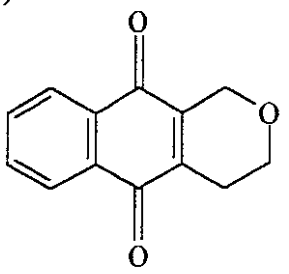
(46)

(iii) To evaluate the synthesised target quinones and some precursors obtained during synthesis (i) and (ii) above for antimicrobial activity. The main objective in this regard was the synthesis of compounds for antibiotic activity and specificity (whether the compound is active against Gram positive and / or Gram negative micro-organisms) *in vitro*, rather than to quantify their bioactivity or establish their mechanism of action. As mentioned earlier it was envisaged that by preparing a variety of these quinonoid compounds and then comparing their antimicrobial specificity, a better insight would hopefully be gained into a molecular structure activity-specificity relationship.

CHAPTER 1

THE SYNTHESIS OF 2-(1′-HYDROXYETHYL)-3-(PROP-2′-ENYL)-1,4-BENZOQUINONE

The class of compounds known as pyranonaphthoquinone antibiotics, which are isolated from various strains of bacteria and fungi, have a basic skeleton of the naphtho[2, 3-*c*]pyran-5-10-dione ring system [16] **(28)**. Some members of the group contain a carboxylic acid side chain bonded to the dihydropyran moiety as in **(28a)**.



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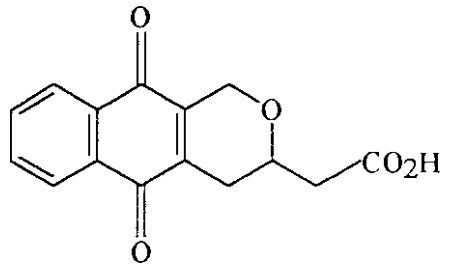
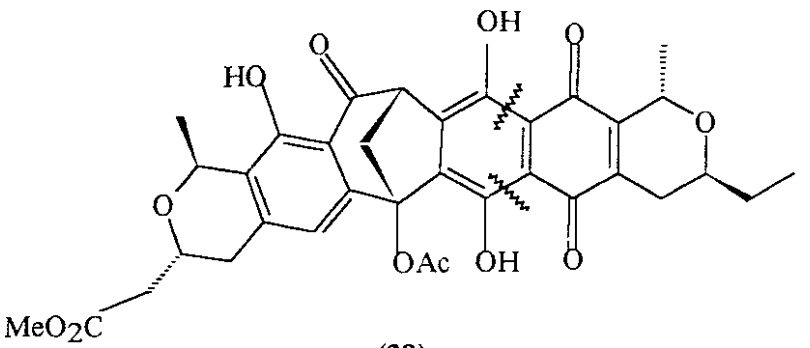


(28a)

The biological activities of some of the naphthocyclinones have been investigated. β- **(29)** and γ-naphthocyclinone **(30)**



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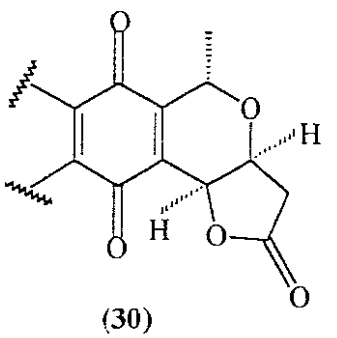
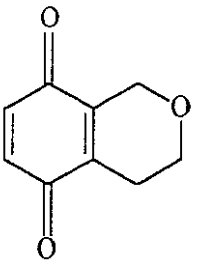


(30)

were found to be active against Gram-positive bacteria [23] suggesting a minimal structure **(31)** was required as α-naphthocyclinone **(32)** displayed no activity.



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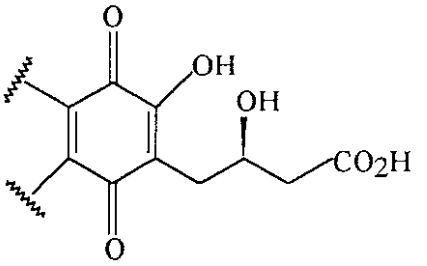
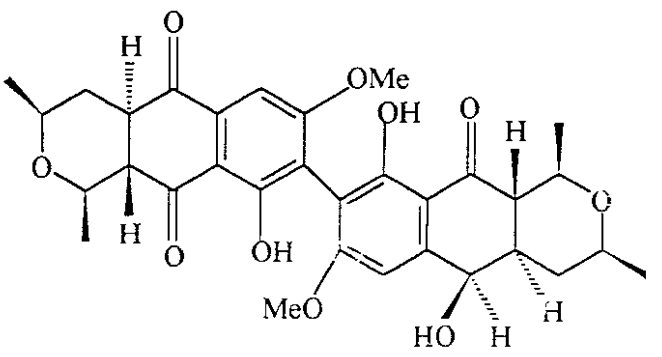


(32)

A new series of dimeric quinones, the cardinalins, represent the first class of pyranonaphthoquinones to be discovered in higher order fungi. Examples of these are Cardinalin 1**(33)** and cardinalin 3 **(34)** which has the bis-naphthoquinone skeleton. These were isolated [24] from the ethanol extract of the New Zealand toadstool *Dermocybe cardinalis*. Cardinalin 1 **(33)** was obtained as a colourless solid and the structure was assigned based on extensive NMR analysis [25]. Some of the signals closely resembled that of eleutherin (1).



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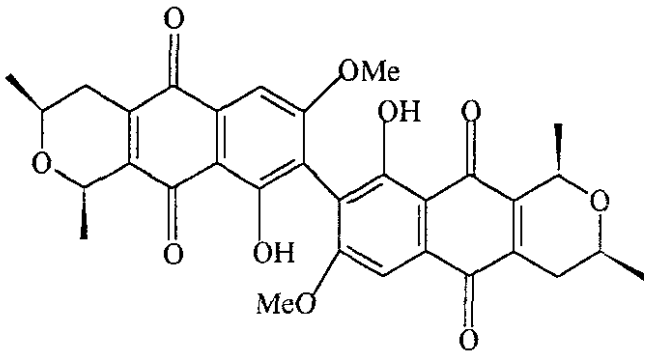
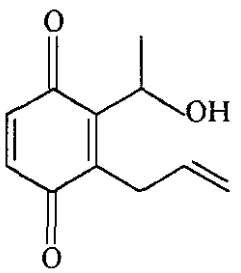


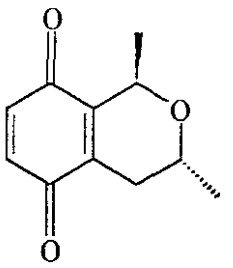
(34)

The aim of this work was to synthesise the alcohol (**17**) *which has the*



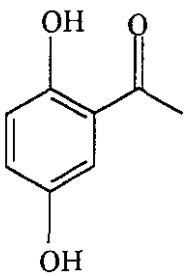
(17)

potential to be converted by known methods [5] to afford, for example, the pyranquinone (**27**) which has been shown to be active against Gram negative and Gram-positive bacteria [26]. This moiety is also found in various natural products such as isoeleutherin (**2**) and the cardinalins (**33**) and (**34**).



(27)

Hydroquinone **(18)** was converted in good yield to the diacetate **(19)** by reacting with acetic anhydride [27]. Compound **(20)** was obtained in a yield of 53 % from ester **(20)** after a Fries rearrangement employing aluminium chloride as the Lewis acid.



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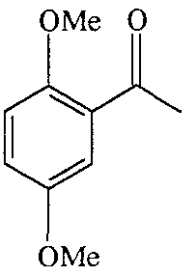
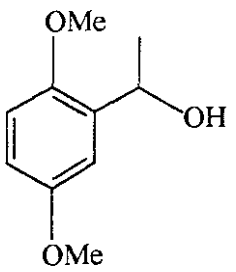


(21)

Refluxing with excess potassium carbonate and iodomethane for 24 hours effected methylation of **(20)**. The reaction gave **(21)** as a red oil (98 %), the structure of which was confirmed by ^{1}H nmr which gave singlets at δ 3.79 and δ 3.87 for the 5-OMe and 2-OMe respectively.

The dimethoxy-ketone (**21**), was dissolved in dry ether. The solution was added dropwise to lithium aluminium hydride (1:6 molar ratio) suspended in ether. Addition of a saturated solution of ammonium chloride quenched the reaction and after work-up and chromatography, the alcohol (**22**) was obtained in a yield of 50 %. The excess lithium aluminium hydride reacted violently with the water in the ammonium chloride solution at times resulting in combustion.

The molar ratio was reduced to 1:1. This resulted in a more efficient reaction giving the alcohol (**22**) in a yield of 78 %.



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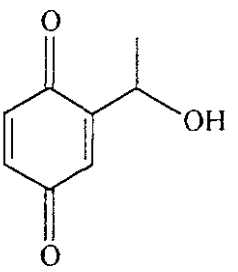


(23)

Oxidative demethylation of compound (**22**) was carried out using cerium (IV) ammonium nitrate (2 equivalents) in acetonitrile to afford quinone (**23**) in a yield of 27.5 %.

The structure was supported by spectroscopic data. High resolution mass spectrometry found M^- at 152.04711 and its IR spectrum showed a broad peak at 3460 cm^{-1} for the OH. Its ^1H nmr spectrum showed a D_2O exchangeable singlet at δ 2.48 corresponding to the OH group.

A doublet at δ 1.4 corresponded to the methyl protons with *J* 6.4 Hz and another quartet at δ 4.85 corresponding to the methine proton (*J* 6.6 Hz).

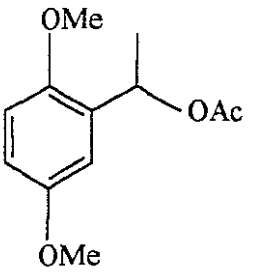
The yield obtained for the last step was considered too low, thus an alternative oxidising agent was sought.

The alcohol **(22)** was dissolved in dioxan and reacted with argentic(II)oxide in 6N nitric acid for 10 minutes to yield the quinone **(23)** in a yield of 30 %. There was no significant increase in the yield of quinone.

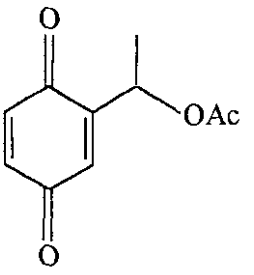
It was then decided to protect the OH group from being oxidised. The alcohol **(22)** was dissolved in an excess amount of acetic anhydride, and the mixture was refluxed under nitrogen for 20 minutes. Work-up and chromatography gave compound **(24)** as a clear oil in a yield of 94.7 %. Oxidation of compound **(24)** to afford **(25)** was effected using argentic(II)oxide as above. The yield of **(25)** ranged from 18-25-99 % and none were readily reproducible.

The infra-red spectrum of **(25)** showed a peak at 1725 cm^{-1} indicating the presence of the carbonyl groups. ^1H nmr spectrum indicated a doublet at δ 1.44 corresponding to the methyl protons and a singlet at δ 2.10 corresponding to the acetate methyl protons. A doublet of quartets was obtained at δ 5.80 with *J* 6.8

and 1.4 Hz corresponding to the methine proton. The smaller *J* value is due to the long range coupling between this proton and the methyl protons of the ester group.

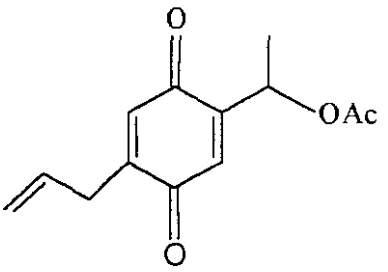


(24)

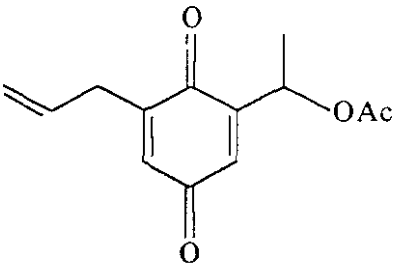


(25)

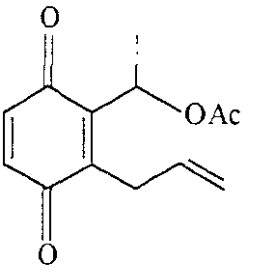
The quinone **(25)** was allylated using vinyl acetic acid[5]. Tlc showed four products including the starting material **(25)**. The following isomeric possibilities may arise for allylation of **(25)**.



(26a)



(26b)

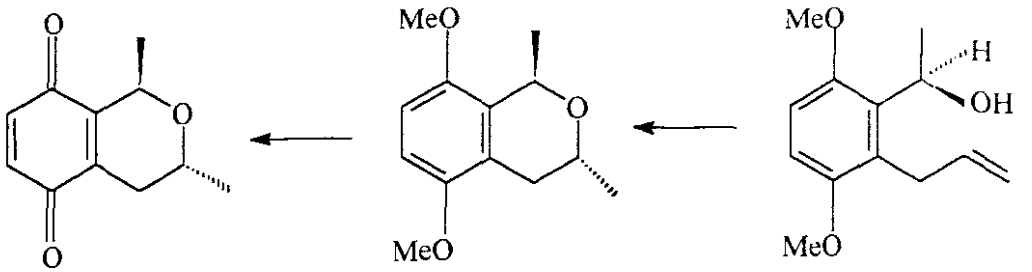


(26)

These could not be separated. However, a mass spectrum of the mixture confirmed the molecular mass of our target quinone. As the quinonoid nucleus was not regiospecific, it was decided to abandon this route towards our target pyranquinones.

It was envisaged that if the regiospecific allylation of **(25)** *proceeded* successfully, optical resolution of alcohol **(22)**, would ultimately provide a chiral route to pyranquinone **(27)** via the following sequence.

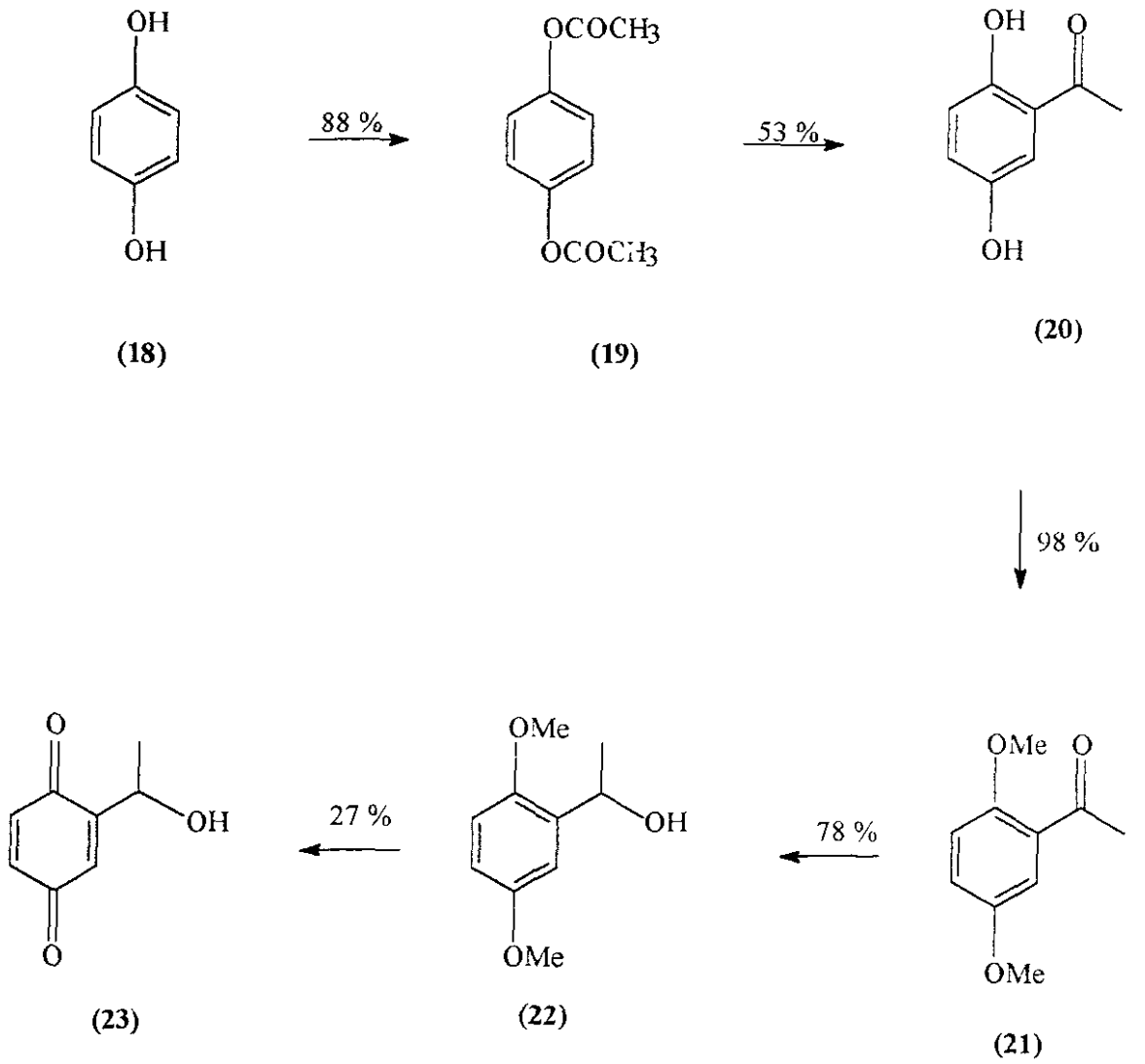
(R-22) → (R-24) → (R-25) → (R-26)



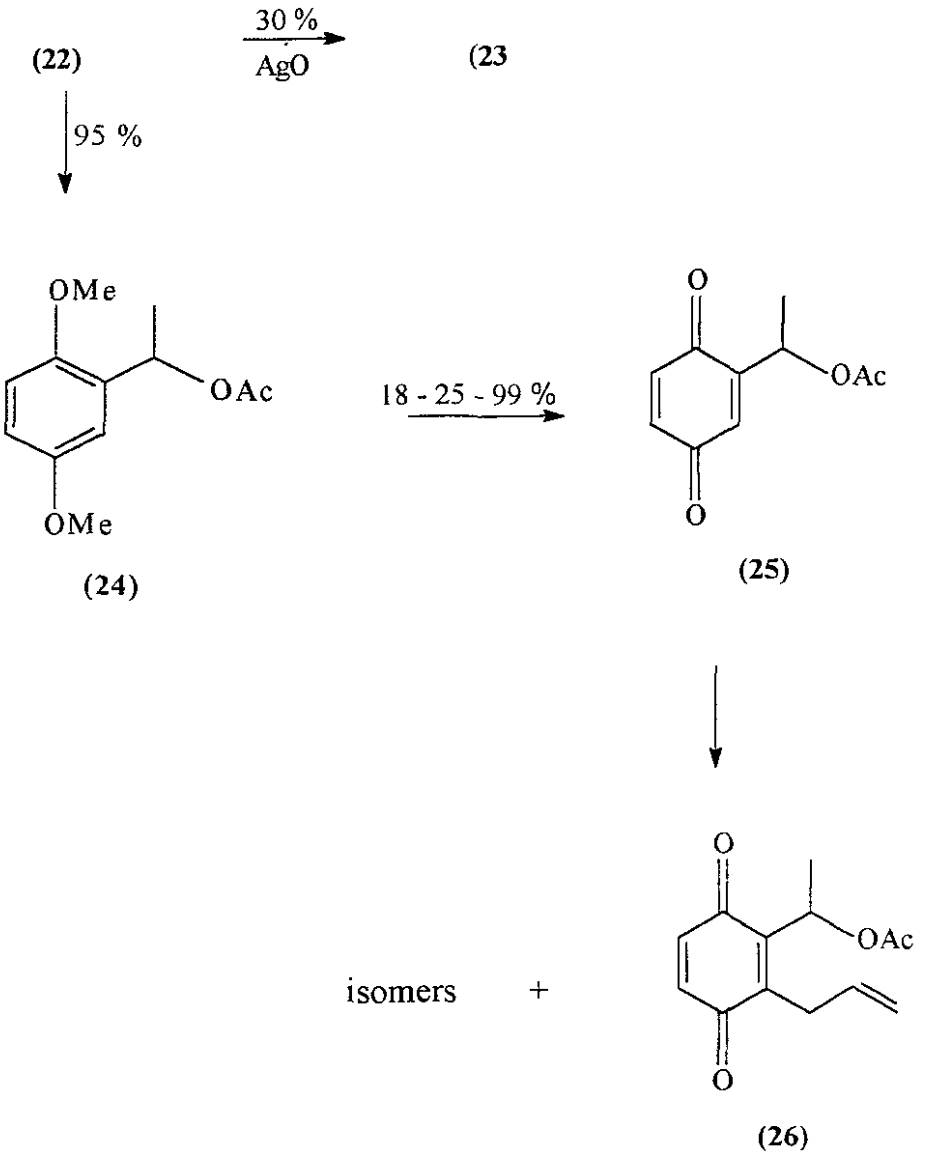
(1R, 3R-27) **(1R, 3R-27b)** **(R-27a)**

A summary is depicted in **Scheme 3.**

SCHEME 3



(18) **(19)** **(20)** **(21)** **(22)** **(23)**



(24)

(25)

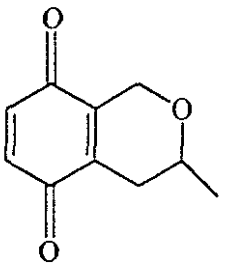
(26)

Antimicrobial activity tests were performed on compounds **(19)**, **(23)** and **(25)** using the Bauer-Kirby method. Compounds **(23)** and **(25)** showed a significant inhibitory activity against both Gram negative and Gram p ›sitive organisms, while ester **(19)** was inactive against all of our standard organisms. (See Chapter 4).

CHAPTER 2

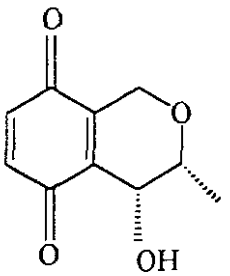
2.1 SYNTHESIS OF (±)-(3R,4R)-3,4-Dihydro-3-methyl-4-hydroxy-1H-benzo[c]pyran-5,8-dione(42) and (±)-(4S)-diastereomer (43)

Pyran (**44**) has been successfully synthesised [21,28] in good yield as its racemate and it was found to be active against Gram positive and Gram negative bacteria [29].

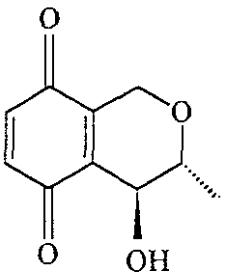


(44)

It was envisaged that the introduction of a leaving group at C-4 in the form of a hydroxy group, might increase the activity of the compound (see scheme 2). A study was thus undertaken to synthesise the C-4-hydroxypyranquinones (**42**) and (**43**)

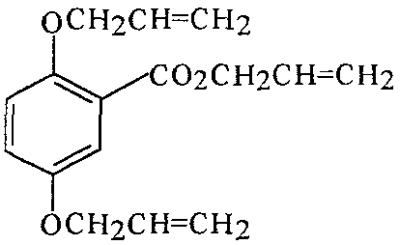


(42)

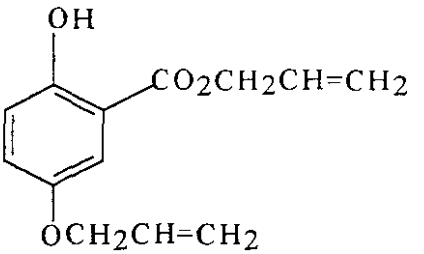


(43)

The readily available gentisic acid **(35)** was boiled in acetone together with potassium carbonate and allyl bromide for 24 hours. After work-up and chromatography, product **(37)** [30] was obtained in a yield of 72 % together with a second component, the tri-allylated compound **(36)**.

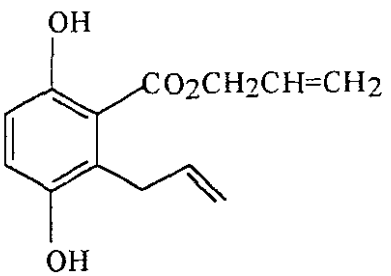


(36)

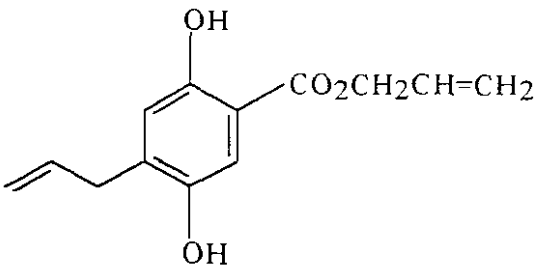


(37)

Claissen rearrangement of **(37)** at 210 °C for five hours afforded quinol **(38)** (68 %) as a result of an *ortho* migration of the allyl group to the more sterically hindered position.



(38)



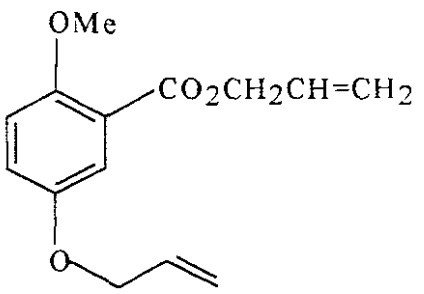
(38a)

^{1}H nmr showed two *ortho* coupled doublets (*J* 9 Hz) at δ 6.8 and δ 7.0 corresponding to 5- and 6-ArH's. The hydroxy protons each gave D_2O exchangeable singlets at δ 4.78 and δ 10.37 corresponding to 4-OH and 1-OH respectively.

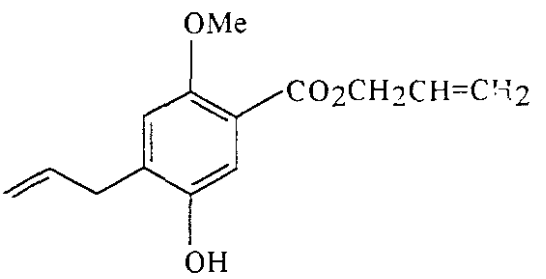
It is theoretically possible for the for the allyl group to migrate to position 4 as in **(38)** or 6, as in **(38a)** on the benzene ring.

Bruce and Ali [31] had previously studied this type of thermal Claissen rearrangement and found that when the group at C-2 is electron accepting [as in **(37)**], migration to the *ortho* position nearest to this electron accepting group is favoured.

It was found in previous work [29,32] that during the Claissen rearrangement, when hydrogen bonding between the ester carbonyl group and the hydroxyl on C-2 [as in **(38b)**] was absent, migration of the allyl group to the less crowded alternative *ortho* position occurred exclusive [as in **(38c)**].



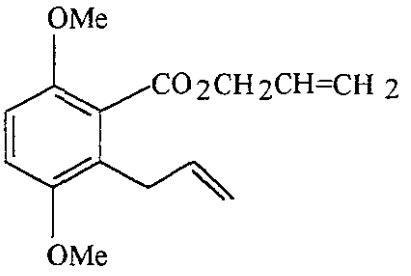
(38b)



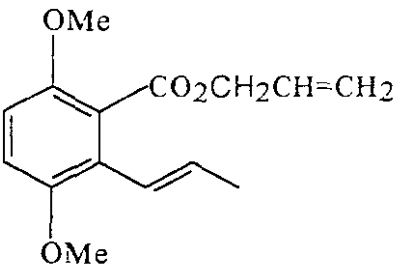
(38c)

The formation of **(38c)** 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 **(37)**], the ester group is not freely rotating and thus migration to the sterically less favoured position is possible.

The ester **(38)** was methylated using excess amounts of iodomethane and potassium carbonate to afford compound **(39)** (87.5 %) as a yellow oil. The methylated ester was treated with palladium chloride bisacetonitrile [33] as the catalyst (80 % loading) over 17 hours (in an inert atmosphere) to give compound **(40)** (90 %) as a light yellow oil.



(39)



(40)

Different amounts of catalyst loading and reaction times were investigated in an attempt to optimise the reaction yields. (See Table 2.1)

With a 1 : 1 molar ratio, it was found that the yields decreased with an increase in reaction time (entries 1-3), whereas a 1 : 0.9 molar ratio, there was no significant change in yields with the increasing reaction time.

The optimum yields were obtained with a 1 : 0.8 molar ratio. As can be seen for entries 7-9, reaction time has a major effect on the product yields.

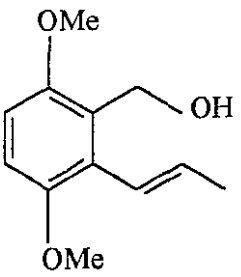
Table 2.1: Optimisation reactions for palladium chloride bisacetonitrile complex.

	s.m. (mg)	Catalyst loading (%)	Time (hours)	Yield (%)
1	171.2	100	15	50.2
2	202.2	100	17.5	46.7
3	168.9	100	18	38.0
4	189.3	90	13	63.0
5	160.0	90	15	62.6
6	199.7	90	18	62.0
7	123.6	80	13.5	64.8
8	196.9	80	18	84.3
9	209.0	80	17	90.0
10	305.6	50	15.5	43.4
11	307.0	40	38.5	44.7

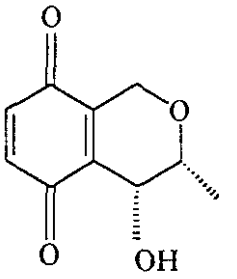
s.m. = starting material

Mass spectrometry gave a molecular ion at *m/z* 262.12068. ^{1}H nmr gave a doublet of doublets at δ 1.85 with *J* 6.6 and 1.8 Hz corresponding to the methyl protons of the olefinic chain. The methoxy groups each gave a singlet at δ 3.77 and δ 3.79. The proton at C-1′ gave a doublet of quartets at δ 6.42 with *J* 16 and 1.8 Hz.

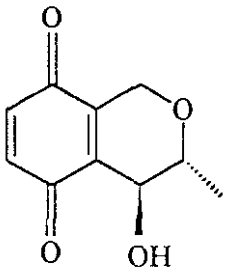
Compound **(40)** was readily reduced to the alcohol **(41)** by employing lithium aluminium hydride in dry ether.



(41)



(42)



(43)

The alcohol **(41)** was then dissolved in acetonitrile and water and a solution of 4 mol equivalents of CAN was added slowly to this. The reaction mixture was

stoppered and the reaction carried out at ambient temperature for 20 minutes. After work up and chromatography, two products were isolated.

The pyranaphthoquinones **(42)** (67.5 %) and **(43)** (21.8 %) were obtained as oils. The minor isomer **(43)** had a higher R_f value than the major isomer **(42)**. This could be as a result of the intramolecular hydrogen bonding between the *pseudo*-equatorial hydroxyl group of **(43)** and the quinonoid oxygen.

Complete characterisation of the minor product failed due to contamination. Mass spectrometry did confirm the presence of the product with M^+ at 194.01335. ^1H nmr showed a doublet at δ 1.40 (*J* 6.2 Hz) corresponding to the C-3 CH_3 protons.

The structure of compound **(42)** was confirmed by spectroscopic data. The C-3 -CH_3 protons gave a doublet at δ 1.4 with *J* 6.6 Hz. The hydroxy proton resonated at δ 2.2. The 3-H proton which is vicinally coupled to 4-H, gave a doublet of a quartet at δ 3.61, *J* 6.6 and 1.8 Hz. The small coupling constants can be ascribed to the narrower dihedral angle between the axial 3-H and the *pseudo*-equatorial 4-H proton. 4-H appeared as a doublet at δ 4.28 with *J* 1.8 Hz.

This is a confirmation that 4-OH is in a *pseudo*-axial position. The two protons at C-1 appeared as a doublet at δ 4.71 with J 19.0 Hz (geminal coupling). The shape of the pyran ring is distorted from that of the normal chair confirmation (see Fig. 2.1). It is assumed that the methyl group at C-3 adopts the less crowded equatorial position. The 1-H and 4-H protons are in the *pseudo*-axial (a') and *pseudo*-equatorial (e') positions, respectively.

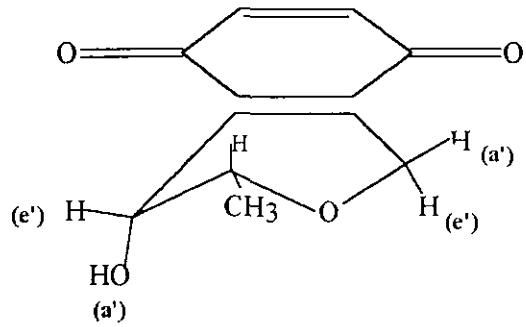


Figure 2.1: Chair confirmation for compound (**42**)

The Karplus equation [34,35] is a useful tool to determine the stereochemistry of protons on adjacent carbon atoms J_{vic} is a function of the dihedral angle, ϕ, between 3-H and 4-H of the pyran ring and its magnitude is given by the Karplus equations.

Karplus equations:

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

ϕ between 0° and 100° : $J_{vic} = 9.5 \cos^2 \phi - 0.28$

The largest vicinal couplings arise with protons in the *trans* co-planar position (ϕ = 180°), while vicinal coupling for *cis* co-planar protons are almost as large (ϕ = 0°). In contrast very small couplings arise between protons at 90° to each other.

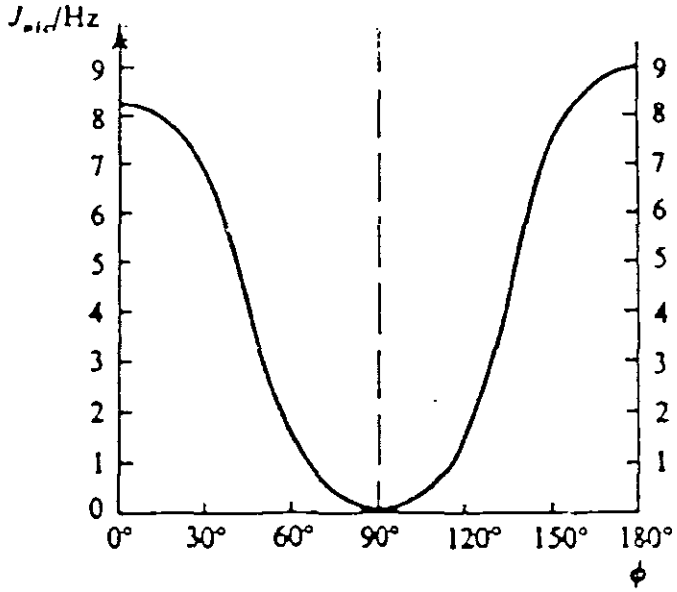
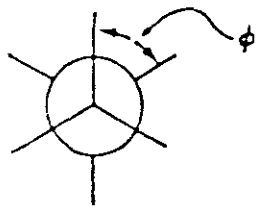
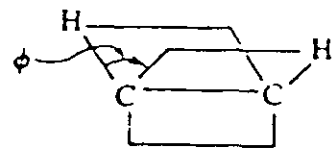


Figure 2.2: Variation of vicinal coupling constant J_{vic}, with the dihedral angle ϕ

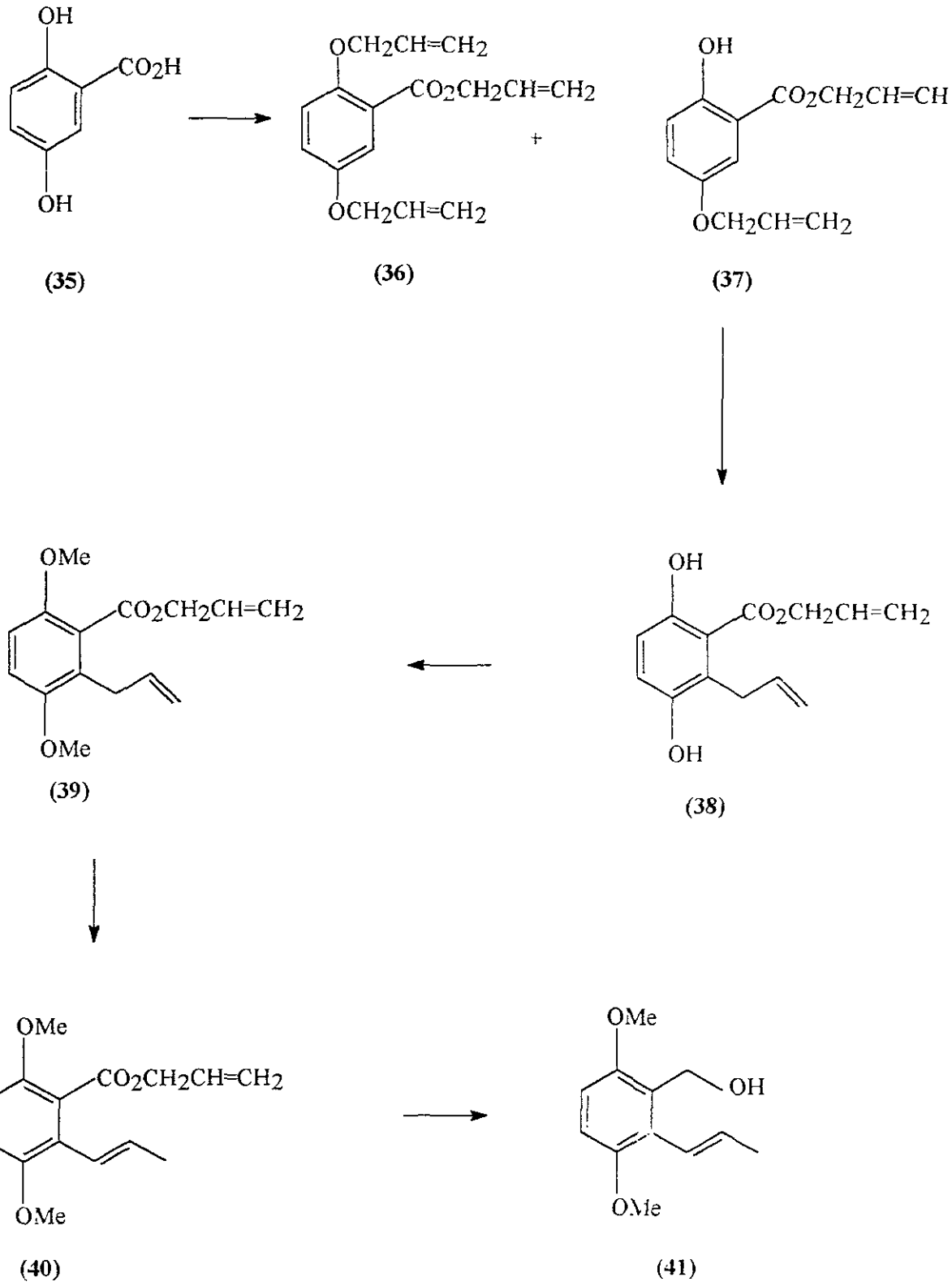


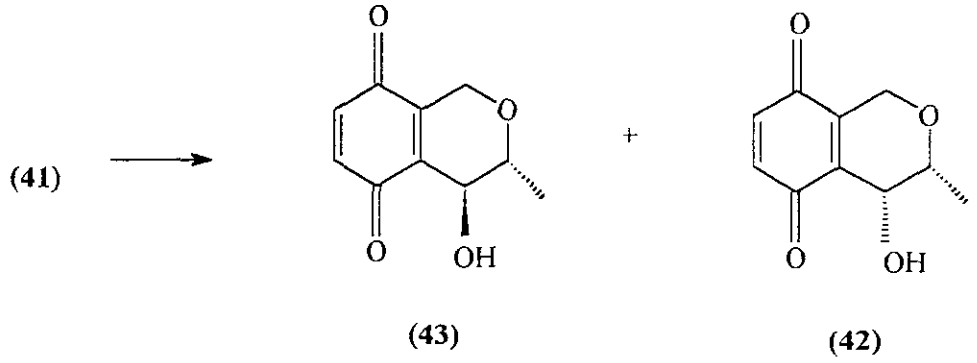


Graphical explanation of Karplus's equations

The synthetic route to the target quinones is summarised in Scheme 4.

SCHEME 4





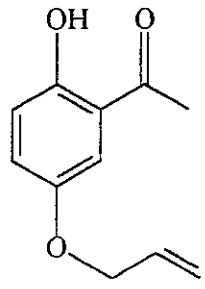
(43) **(42)**

Compounds **(42)** and **(43)** were evaluated for biological activity using the Bauer-Kirby method. Both compounds were found to be active against Gram positive and Gram negative organisms. Results can be seen in Table 4.6 and Table 4.7.

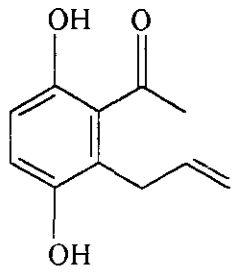
2.2 SYNTHESIS OF (±)-(1R,3R,4R)-3,4-Dihydro-1,3-dimethyl-4-hydroxy-1H-benzo[c]pyran-5,8-dione(45) and (±)-(4s)-diastereomer (46)

2,5-Dihydroxyacetophenone **(20)** was boiled with an excess amount of allyl bromide and potassium carbonate in acetone. Upon work-up, product **(47)** was obtained in a yield of 88 %. The Claissen rearrangement afforded the diol **(48)** in a yield of 59 % with a melting point of 108.9 - 109.6°C.

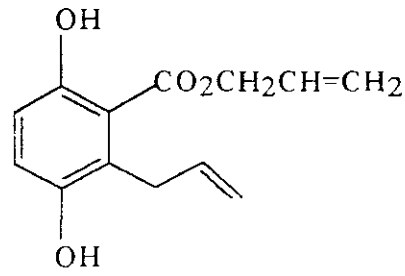
Migration of the allyl group was again to the more sterically hindered position as discussed previously for compound **(38)**.



(47)

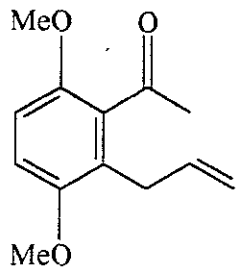


(48)



(38)

Reaction with iodomethane and potassium carbonate gave compound **(49)** in a good yield of 96 %. Its ^{1}H nmr showed two singlets at δ 3.77 and δ 3.78 corresponding to the two methoxy groups. The two aromatic protons each resonated as a doublet with *J* 9.2 Hz at δ 6.74 and δ 6.82.



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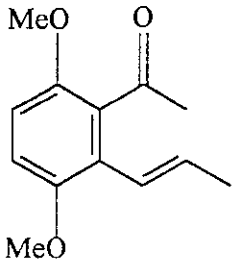


(50)

Conjugation of the allyl double bond was achieved by reacting compound **(49)** with palladium chloride bis-acetonitrile (50 % loading) complex over 18 hours in an inert atmosphere. Compound **(50)** was obtained as a yellow oil in a yield of 87 %. Its mass spectrum gave a molecular ion at *m/z* 220.70970 ($C_{13}H_{16}O_3$ requires 220.10994). The protons of the methyl group of the olefinic chain showed a doublet at δ 1.85 with *J* 6.6 Hz in the ^{1}H nmr spectrum.

A number of experiments were run using the palladium catalyst in order to optimise the reaction yields (see Table 2.2).

From the results obtained, it was concluded that the 50 % catalyst loading was the most efficient.

Table 2.2: Optimisation reactions for palladium chloride bisacetonitrile complex.

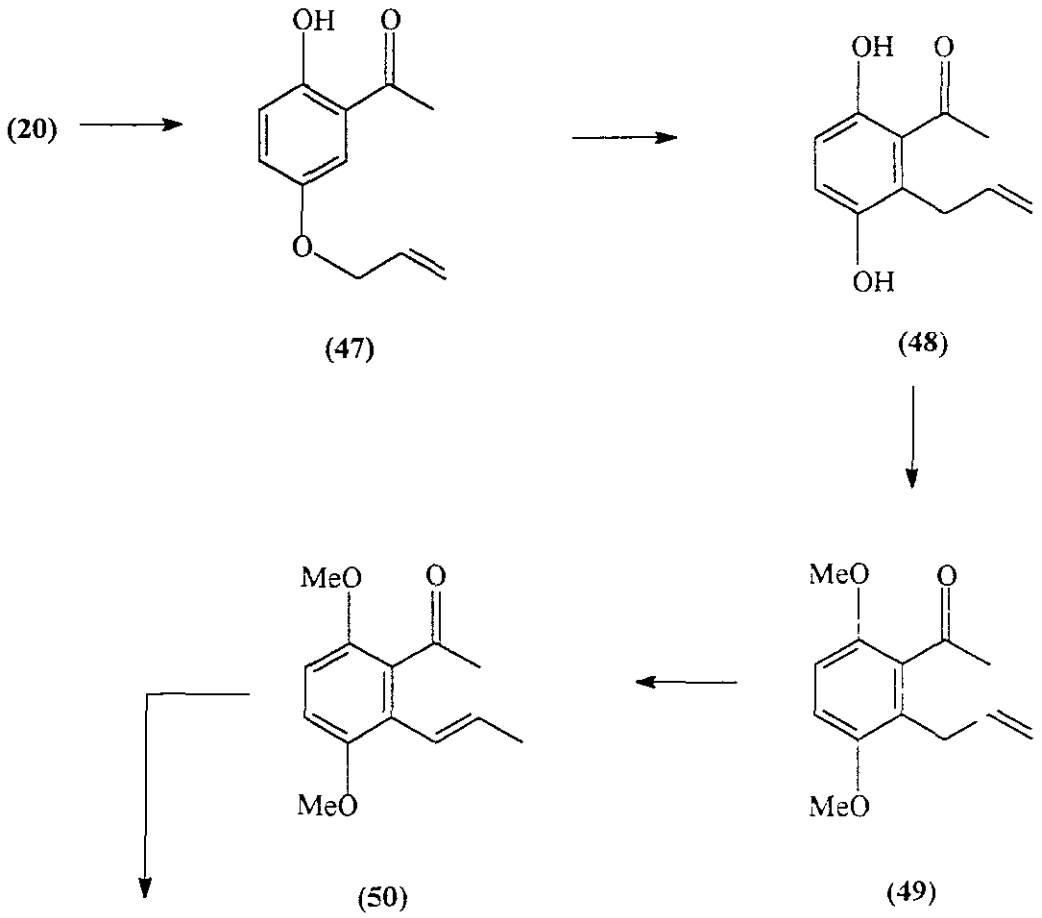
	s.m. (mg)	Catalyst loading (%)	Time (hours)	Yield (%)
1	141.1	100	40	-
2	121.7	70	13	72.6
3	155.8	70	18	67.1
4	160.5	50	15	79.9
5	195.4	50	17	89.4
6	167.7	50	18.5	92.7
7	151.1	30	23	81.7
8	313.3	30	61	23.5

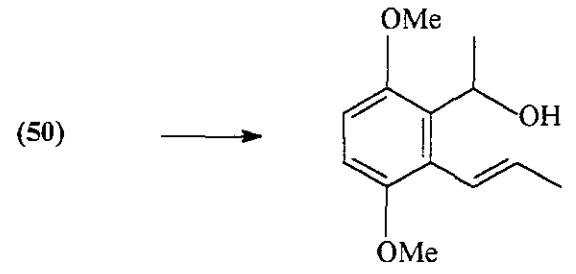
s.m. = starting material

The conjugated ketone **(50)** was reduced to the alcohol **(51)** with lithium aluminium hydride. Finally, reaction of the alcohol with 4 molar equivalents of CAN produced the target compounds **(45)** and **(46)** in a yield of 51.5 % and 37.3 % respectively. See **Scheme 5** for a summary of the reaction sequence.

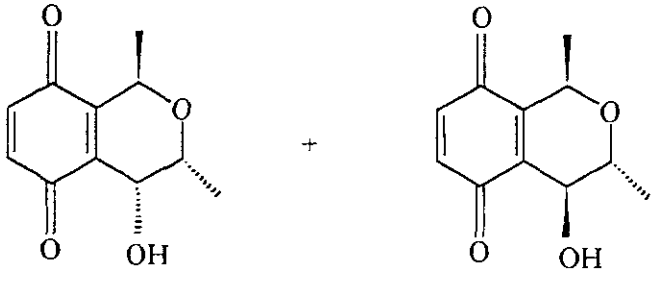
Infrared spectra of both compounds showed sharp bands at 3490 and 1650 cm^{-1} which confirmed the presence of an alcohol group and quinone moiety respectively. ^1H nmr for compound **(45)** had a doublet of quartets at δ 3.95 (*J* 6.6 and 1.6 Hz), a doublet of doublets at δ 4.35 (*J* 8.0 and 1.6 Hz) and a quartet at δ 4.81 (*J* 7.0 Hz) assigned to 3-H, 4-H and 1-H respectively. The relative stereochemistry between the 3-H and 4-H pyran ring protons was established as axial and *pseudo*-equatorial, respectively, because of the magnitude of the common coupling constant of 1.6 Hz.

SCHEME 5



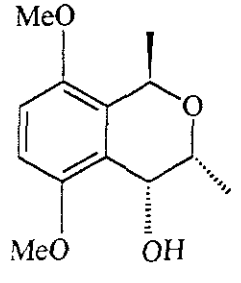


(51)

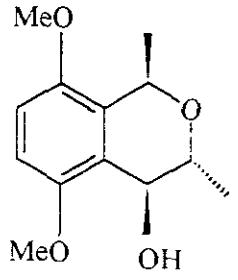


(45) (46)

Compound **(51)** was previously treated with 2 molar equivalents of CAN [5, 29, 36] in aqueous acetonitrile to produce compounds **(53)** and **(54)** with the former as the major product.



(53)



(54)

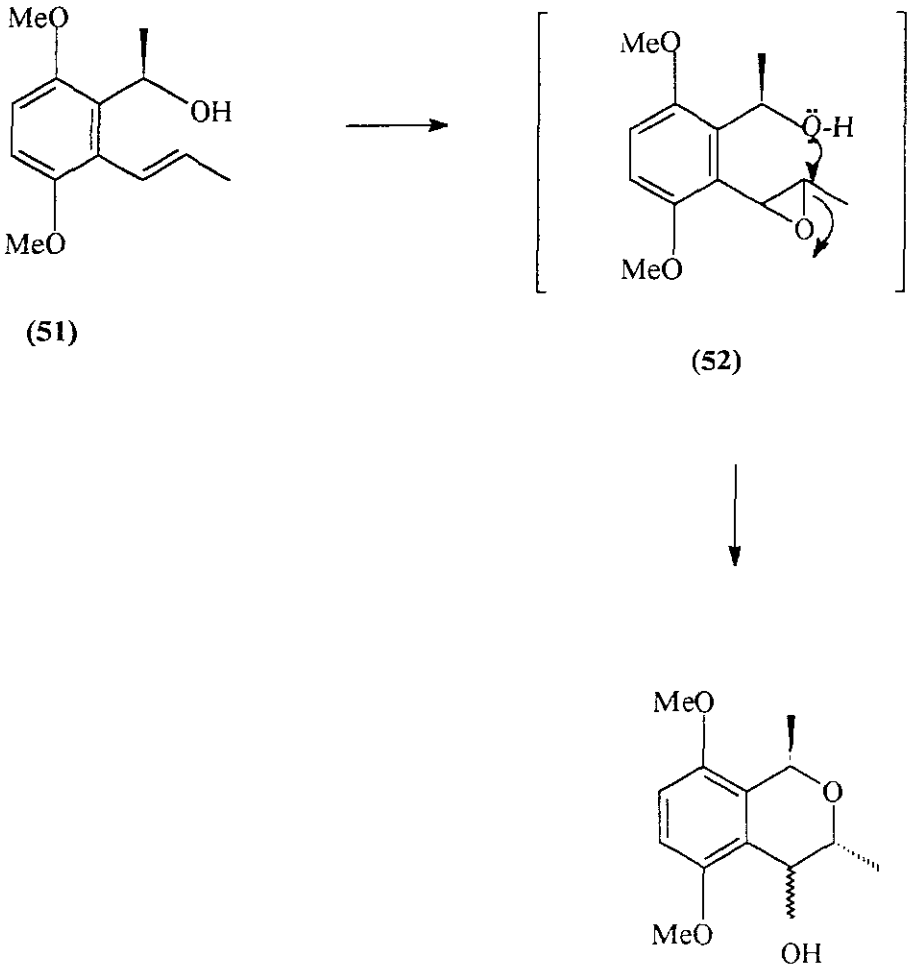
Krupadanam *et al* [37] reported the successful use of *meta* chloroperbenzoic acid (mCPBA) in the oxidative cyclisation of hydroxy chromenes.

It was envisaged that treatment of the conjugated alcohol **(51)** with mCPBA might yield compounds **(53)** and **(54)**.

The alcohol was dissolved in dichloromethane and immersed in ice. A solution of mCPBA was added dropwise to the stirred alcohol solution. Reaction time was 2 hours in an inert atmosphere. After work-up and chromatography, compound **(53)** was obtained as an oil in a yield of 46.2 %, together with product **(54)** which was isolated in a yield of 35.5 %.

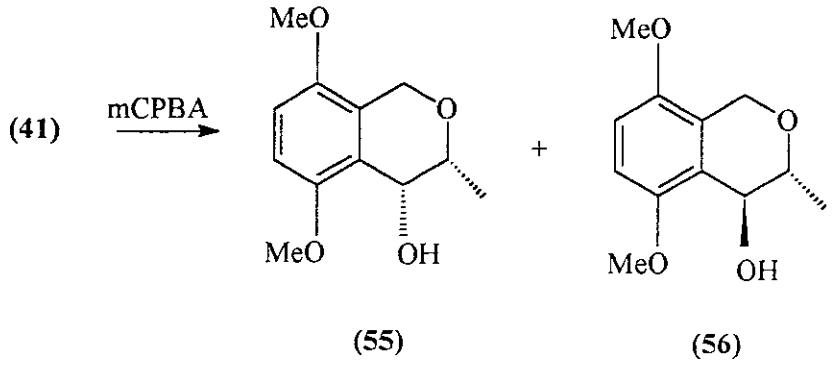
The reaction is thought to proceed by initial epoxidation of the olefinic bond giving the intermediate **(52),** which was not isolated. Intramolecular nucleophilic attack leads to opening of the epoxide ring giving rise to the six membered hydroxypyran ring system. Infrared spectra gave a peak at 3400 cm^{-1} confirming the presence of a hydroxy group. Mass spectroscopy gave a molecular ion *m/z* 238.1290 for both the *pseudo*-equatorial **(54)** and *pseudo*-axial **(53)** hydroxy pyrans. It was possible to assign the relative stereochemistry at the three chiral centres for compound **(54)** as drawn by virtue of their respective ^1H nmr spectra, using the same arguments as for compound **(46)**. The structure of compound **(53)** was not completely characterised due to contamination.

SCHEME 6



Similarly, compound **(41)** was also reacted with mCPBA to afford compounds **(55)** and **(56)** at a yield of 55.7 % and 23.1 % respectively (see **Scheme** 7).

SCHEME 7



(55) (56)

Quinones (**42**) and (**43**) were also evaluated for biological activity. The results are tabulated in Tables 4.6 and 4.7.

CHAPTER 3

EXPERIMENTAL

GENERAL:

^{1}H nmr Spectra were recorded on a 60 MHz Varian EM 360 Spectrometer and a Varian 200 MHz Spectrometer. All nmr 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 were 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. Thin layer chromatography was carried out on aluminium plates coated with Merck Kieselgel F254.

Petroleum spirit refers to light petroleum, the fraction of boiling point 60 – 80 ^{O}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; CAN and mCPBA refers to cerium (IV) ammonium nitrate and meta-chloro-perbenzoic acid respectively. The phrase "residue obtained upon work up", refers to the residue when the organic layer was separated, dried and the solvents evaporated.

The palladium chloride bisacetonitrile complex was prepared by weighing out ~1 g of palladium chloride and dissolving it in 20 ml of dry distilled acetonitrile. The solution was stirred at ambient temperature in an inert atmosphere for 5 hours, after which it was filtered. The resultant yellow powder was rotavapped at reduced pressure (temperature 30 – 40 °C) to rid the complex of excess acetonitrile.

Hydroquinonediacetate (19)

55.01 g (0.5001 mol) of hydroquinone was weighed out into a 250 ml round bottom flask. To this, acetic anhydride (95 ml, 1.0003 mol), was added. A drop of concentrated sulphuric acid was added to the mixture, which was lightly shaken by hand until it became clear. The solution was allowed to stand for 5 min. The solution was then poured onto 400 ml of crushed ice. The crystals were filtered under vacuum and washed thoroughly with water. The collected crystals were recrystallised in 400 ml of 50 % ethanol giving clear needle like crystals (86.64 g, 88.40 %) with a melting point of 122.7 °C (Lit.[27] m.p. 122 °C)

2,5-Dihydroxyacetophenone (20)

Hydroquinone diacetate (40 g, 0.2 mol), was weighed out and transferred into a mortar. Anhydrous aluminium chloride (87 g, 0.67 mol) was mixed with the diacetate by grounding the two together to a fine powder. The resultant mixture was transferred to a 500 ml round bottom flask. The flask was connected to a gas trap flask and a calcium chloride tube. The mixture was heated (using an oil bath) to 110 – 120 °C during which time hydrogen chloride gas was evolved.

After approximately 30 minutes (when gas evolution was complete) the temperature was slowly increased to 165 °C and maintained for 3 hours. The flask was removed from the bath and allowed to cool. To the reaction product, 140g of crushed ice was added, followed by approximately 10 ml of concentrated HCl. The mixture was allowed to stand overnight. It was then filtered and recrystallised from 95 % Ethanol giving compound **(20)** in a yield of 16.56 g (53.4 %) with a melting point of 201.9-203.1 °C (lit. 202-203 °C).

2,5-Dimethoxyacetophenone (21)

2,5-Dihydroxyacetophenone (7.05 g, 46.3816 mmol) was weighed out together with potassium carbonate (25.64 g, 185.5 mmol). The reactants were dissolved in approximately 50 ml acetone. Iodomethane (26.35 g, 185.5 mmol) was added to the mixture and the whole system was flushed with nitrogen (N_2). The reaction mixture was refluxed under N_2 for 24 hours. At the end of the reaction time, the mixture was worked up and chromatographed using 20 % Ethyl acetate in petroleum ether. The methylated product **(21)** was obtained as a light yellow oil in a yield of 8.19g (98 %). (Found: M^+ 180. $C_{10}H_{12}O_3$ requires 180) ν_{max} 1754 cm^{-1} (C=O); δ ($CDCl_3$) 2.61 (3H, s, Ar-CO$\underline{CH_3}$), 3.79 (3H, s, 5-OMe), 3.87 (3H, s, 2-OMe), 6.87-7.23 (3H, m, 3 x Ar-H).

2,5-Dimethoxy–1–(1′-hydroxyethyl)-benzene (22)

Lithium aluminium hydride ($LiAlH_4$) (42.17 mg, 1.1111 mmol) was weighed out under N_2 and then suspended in dry, distilled ether. 200 mg (1.111 mmol) of the ketone **(21)** was dissolved in ether and added dropwise to the continuously stirred suspension of $LiAlH_4$. The reaction was allowed to carry on for a further 15 minutes at the end of which the reaction was quenched using a saturated solution of ammonium chloride. The mixture was filtered and the filtrate was dried using $MgSO_4$. The product was chromatographed on a short column using 30 % Ethyl acetate and giving 77.74 % yield of the product **(22)**.

2-(1′-hydroxyethyl)–1,4 -benzoquinone (23)

Method A

140 mg (0.7692 mmol) of the methylated alcohol **(22)** was dissolved in 10 ml of acetonitrile. 1 ml of water was added to the soluti ,n. A solution of CAN (0.8434 g, 1.5385 mmol) was slowly added to the continuously stirred solution of the methylated alcohol. Addition was carried out over 10 minutes. The reaction was allowed to carry on for a further 5 minutes at the end of which the solution was thrown into water. The organic components were extracted

with dichloromethane. The residue was worked up and chromatographed on a short column using 40 % ethyl acetate in petroleum ether giving a yield of 32.19 mg (27.53 %) of compound **(23)**.

Method B

100 mg (0.5494 mmol) of the alcohol **(22)** was dissolved in 7 ml of distilled dioxan. To this, argentic oxide (AgO) (456 mg. 3.6804 mmol) was added. 6M HNO_3 was added dropwise to the continuously stirred mixture until all the AgO had dissolved. The reaction was allowed to carry on for a further 5-10 minutes. At the end of the reaction time, the reaction solution was thrown into a chloroform-water mixture (4:1). The organic components were extracted with chloroform and washed with water. The solution was dried ($MgSO_4$) and the residue obtained was chromatographed using 30 % ethyl acetate in Petroleum ether giving the product **(23)** as a red oil in a yield of 25.05 mg (30 %).

(Found M^+: 152.04711. $C_8H_8O_3$ requires 152.04734).

ν_{max} 3460 (OH) and 1670 cm^{-1} (C=O); δ ($CDCl_3$) 1.4 (3H, d, *J* 6.4 Hz, $CHCH_3OH$), 2.45 (1H, s, OH), 4.85 (1H, q, *J* 6.6 Hz, $CHCH_3OH$) and 6.75 (3H, d, *J* 7.6 Hz, 3-, 5- and 6-H).

2-(1′-Acetoxyethyl)-1,4-dimethoxybenzene (24)

156.3 mg (0.8588 mmol) of product **(22)** was dissolved in an excess amount of distilled acetic anhydride (in a test tube). The test tube was connected to a condenser and the mixture was refluxed under nitrogen for 20 minutes (using a bunsen burner). At the end of reaction time, the mixture was thrown into water and dichloromethane was used to extract the organic components. The organic layer was washed with water and then dried with $MgSO_4$. The solvents were evaporated under reduced pressure and the residue was chromatographed on a short column using 30 % ethyl acetate in petroleum ether. The product **(24)** was obtained in a yield of 182.2 mg (94.7 %). (Found $M^{+\cdot}$: 224. $C_{12}H_{16}O_4$ requires 224).

ν_{max} 1765 cm^{-1} (C=O); δ ($CDCl_3$) 1.25 (3H, d, *J* 6.4 Hz, Ar-CH$\underline{CH_3}$OAc), 2.08 (3H, s, Ar-CHCH$_3$OCO$\underline{CH_3}$), 3.79 and 3.77 (3H each, s, 2 x OCH_3), 6.19 (1H, q, *J* 6.4 Hz, Ar-$\underline{CH}$CH$_3$OAc), 6.76-6.95 (3H, m, 3-, 5- and 6-H).

2-(1′-Acetoxyethyl)-1,4-benzoquinone (25)

Compound **(24)** (182.2 mg, 0.8134 mmol) was oxidised using argentic oxide (675.2 mg, 5.450 mmol) as for compound **(23)**. After chromatography on a short column, 29.2 mg (18 %) of compound **(25)** was obtained.

(Found M^+: 194.05598. $C_{10}H_{10}O_4$ requires 194.05791). (m.p.120 °C). ν_{max} 1725 cm^{-1} (C=O); δ ($CDCl_3$) 1.44 (3H, d, *J* 6.8 Hz, ArCHC<u>H</u>$_3$OAc), 2.10 (3H, s, Ar-CH-CH$_3$OCOC<u>H</u>$_3$), 5.80 (1H, dq, *J* 6.8 and 1.4 Hz, ArC<u>H</u>CH$_3$OAc), 6.65-6.77 (3H, m, 3-, 5- and 6-H).

2-(1′-Acetoxyethyl)-3-(prop-2′-enyl)-1,4-benzoquinone (26)

50.4 mg (0.2598 mmol) of quinone **(25)** was dissolved in acetonitrile (3 ml) and water (1.2 ml). Vinylacetic acid (0.022 ml, 0.2598 mmol) was added to the solution, followed by a solution of silver nitrate (26.48 mg, 0.1559 mmol) in 0.5 ml of water. The reaction flask was flushed with N_2 for approximately 3 minutes. A solution of $K_2S_2O_8$ (132.38 mg, 0.4897 mmol) in 3 ml of water was added to the reaction flask (with continuous stirring) in a nitrogen atmosphere, over a period of 20 minutes. The reaction flask was then immersed in an oil bath at 80 - 85 °C and the solution was stirred for a further 30 minutes. At the end of the reaction time, the cooled solution was poured into water and extracted with dichloromethane (3 x 50 ml). The organic layer was washed with aqueous sodium hydrogen carbonate.

Four components were obtained but their separation was not effective. Mass spectroscopy showed that compound **(26)** or its isomers were present in the mixture.

Allyl-4-alloxy-1-hydroxybenzoate (37) and allyl-1,4-bis(allyloxy)benzoate (36)

Gentisic acid **(35)** (15.00g, 0.0974 mol) was dissolved in a 100 ml acetone. To this solution, potassium carbonate (20.19 g, 0.1461 mol) and allyl bromide (17.68 ml, 0.2045 mol) were added. The reaction flask was immersed in an oil bath and the continuously stirred solution was refluxed under nitrogen for 24 hours, after which the reaction mixture was filtered and the filter cake washed with ether. The solvents were removed under reduced pressure. The resulting solid residue was triturated with ether (3 x 100 ml) and the combined ethereal extracts were washed with a saturated solution of sodium hydrogen carbonate and dried with $MgSO_4$. Filtration and removal of solvents gave a yellow oil which was chromatographed using 10 % ethyl acetate in petroleum ether. This afforded a 72 % (16.63 g) yield of **(37)** as white crystals with a melting point of 42.5 – 44.5 °C (pentane), (Lit.[33] 43.5 – 44.5 °C). (Found M^+: 234.09018. $C_{13}H_{14}O_4$ requires 234.8921).

ν_{max} 3230 (OH), 1685 (C=O), 1640 cm^{-1} (C=C); δ ($CDCl_3$) 4.5 (2H, dt, *J* 5.2 and 1.4 Hz, Ar-$CO_2\underline{CH_2}CH{=}CH_2$), 4.85 (2H, dt, *J* 6 and 1.6 Hz, Ar-$O\underline{CH_2}CH{=}CH_2$), 5.26-5.49 (2H, m, 2 vinyl CH_2), 5.95-6.18 (1H, m, 2 vinyl CH), 6.90 (1H, d, *J* 9.2 Hz, 6-H), 7.10 (1H, dd, *J* 9.2 & 3.2 Hz, 5-H), 7.35 (1H, d, *J* 2.8, 3-H) and 10.36 (1H, s, OH, D_2O exchangeable).

Allyl–1,4–dihydroxy–2–prop–2′-enylbenzoate (38)

1.1051 g of **(37)** was weighed out into a flask, which was immersed in an oil bath at 210 °C. The temperature was maintained, in a nitrogen atmosphere, for 5 hours. The resulting residue was chromatographed using 15 % ethyl acetate in petroleum ether. Product **(38)** was obtained as a light brown oil in a yield of 751.5 mg (68 %). (Found M^+ 234.08906. $C_{13}H_{14}O_4$ requires 234.08921) ν_{max} 3440 (OH), 1670 (C=O) and 1470 (C=C); δ ($CDCl_3$) 3.73 (2H, dt, *J* 6 and 1.8 Hz, Ar-$CH_2CH{=}\underline{CH_2}$), 4.78 (1H, s, 4-OH, D_2O exchangeable), 4.86 (2H, dt, *J* 6 and 1.8 Hz, Ar-$CO_2\underline{CH_2}CH{=}CH_2$), 4.99-5.25 (4H, m, Ar-$CH_2CH{=}\underline{CH_2}$ and Ar-$CO_2CH_2CH{=}\underline{CH_2}$), 5.90-6.12 (2H, m, 2 vinyl CH), 6.8 (1H, d, *J* 9.2 Hz, 5-H), 7.0 (1H, d, *J* 9 Hz, 6-H) and 10.37 (1H, s, 1-OH, D_2O exchangeable).

Allyl-3,6-dimethoxy-2-prop-2′enylbenzoate (39)

Compound **(38)** (1.3737 g, 5.87 mmol) was reacted with iodomethane (8.25 g, 0.05812 mmol) and potassium carbonate (8.03 g, 0.05812 mmol) as for product **(21)** to afford product **(39)** (1.35 g, 87.5 %) as a yellow oil. Chromatography was carried out using 30 % ethyl acetate in petroleum ether. (Found M^+: 262.12097. $C_{15}H_{18}O_4$ requires 262.12051).

ν_{max} (film) 1735 cm^{-1}; δ ($CDCl_3$) 2.84 (2H, dt, *J* 6.6 and 1.6 Hz, Ar-$\underline{CH_2}CH=CH_2$), 3.77 and 3.78 (3H each, s, 2 x OCH_3), 4.85 (2H, dt, *J* 5.8 and 1.4 Hz, Ar-$CO_2\underline{CH_2}CH=CH_2$), 4.99-5.25 (4H, m, Ar-$CH_2CH=\underline{CH_2}$ and Ar-$CO_2CH_2CH=\underline{CH_2}$), 5.8-6.1 (2H, m, 2 vinyl CH), 6.75 (1H, d, *J* 8.8 Hz, 5-H) and 6.85 (1H, d, *J* 8.8 Hz, 6-H).

Allyl-3,6-dimethoxy-2-*trans*-prop-1′-enylbenzoate (40)

209.0 mg (0.7977 mmol) of the ester **(39)** was dissolved in dry, distilled dichloromethane. Palladium chloride bisacetonitrile (165.5 mg, 0.6382 mmol) was added to the stirred solution. The reaction was carried out over 18 hrs, at ambient temperature in a nitrogen atmosphere. At the end of the reaction time, the reaction mixture was thrown into water and the organic components were extracted with dichloromethane. The organic layer was backwashed with water

and then dried with $MgSO_4$. The residue was chromatographed using 30 % ethyl acetate in petroleum ether. The conjugated ester **(40)** was obtained at a yield of 188.1 mg (90 %) as a light yellow oil. (Found M^+: 262.12068. $C_{15}H_{18}O_4$ requires 262.12051).

ν_{max} 1845 and 1700 cm^{-1} (C=O); δ ($CDCl_3$) 1.85 (3H, dd, *J* 6.6 and 1.8 Hz, Ar-CH=CH$\underline{CH_3}$), 3.32 (2H, dt, *J* 5.8 and 1.4 Hz, Ar-$CO_2\underline{CH_2}CH=CH_2$), 3.77 and 3.79 (3H each, s, 2 x OCH_3), 4.75-4.82 (2H, m, Ar-$CO_2CH_2CH=\underline{CH_2}$), 5.32-5.48 (1H, m, Ar-CH=$\underline{CH}CH_3$), 5.80-6.25 (1H, m, Ar-$CO_2CH_2\underline{CH}=CH_2$), 6.42 (1H, dq, *J* 16 and 1.8 Hz, Ar-$\underline{CH}$=$CHCH_3$), 6.76 (1H d, *J* 9 Hz, 5-H), 6.82 (1H, d, *J* 9 Hz, 6-H).

3,6-Dimethoxy-1-hydroxymethyl-2-prop-1'-enylbenzene (41)

103.9 mg (0.3966 mmol) of the ester **(40)** was reduced with lithium aluminium hydride (75.25 mg, 1.9828 mmol) as for compound **(22)** with the reaction being carried out over 30 minutes. A similar chromatographic procedure afforded the product **(41)** (73 mg, 88.5 %) as an oil. (Found M^+: 208.11095. $C_{12}H_{16}O_3$ requires 208.10994).

δ ($CDCl_3$) 1.94 (3H, dd, *J* 6.6 and 1.8 Hz, Ar-CH=CH$\underline{CH_3}$), 2.28 (1H, br s, OH, D_2O exchangeable), 3.78 (3H, s, 6-OCH_3), 3.83 (3H, s, 3-OCH_3), 4.77 (2H,s, Ar-$\underline{CH_2}$OH), 5.89-6.07 (1H, m, Ar-CH=$\underline{CH}CH_3$), 6.48 (1H, dq, *J* 16 and 1.8 Hz, Ar-$\underline{CH}$=$CHCH_3$) and 6.74 (2H, s, 5- and 6-H)

(±)(3R,4R)-3,4-Dihydro-3-methyl-4-hydroxy-1H-benzo[c]pyran-5,8-dione (42) and (±)(4S)-diastereomer (43)

114.4 mg (0.5500 mmol) of the alcohol **(41)** was dissolved in 6 ml of acetonitrile and 6 ml of water. CAN (1.2061 g, 2.2000 mmol) was dissolved in 6 ml of water. The CAN solution was added dropwise to the alcohol solution over a period of 7 minutes. The reaction mixture was stoppered and the reaction allowed to carry on for a further 20 minutes. Pouring the reaction mixture into 100 ml of water quenched the reaction and the organic components were extracted using dichloromethane. The crude mixture was worked up and chromatographed using 30 % ethyl acetate in petroleum spirits giving a yield of 34.7 mg (21.8 %) for product **(43)** and 107.7 mg (67.5 %) for product **(42)**.

For (**42**):

(Found M^+: 194.05874. $C_{10}H_{10}O_4$ requires 194.05791)

ν_{max} 3510 (OH), 1680 cm^{-1} (C=O); δ ($CDCl_3$)1.4 (3H, d, *J* 6.6 Hz, 3-CH_3), 2.12-2.30 (1H, br s, OH, D_2O exchangeable), 3.61 (1H, dq, *J* 6.2 and 1.8 Hz, 3-H), 4.28 (1H, d, *J* 1.8 Hz, 4-H), 4.71 (2H, d, *J* 19.0 Hz,2 x 1-H) and 6.79 (2H, d, *J* 10.2 Hz, 6- and 7-H)

For (**43**):

(Found M^+: 194.01335. $C_{10}H_{10}O_4$ requires 194.05791).

Samples were too contaminated to afford an appropriate ^1H nmr spectrum.

5-Allyloxy-2-hydroxyacetophenone (47)

5.03 g (0.0331 mol) of 2,5-dihydroxyacetophenone (**20**) was mixed with allyl bromide (4.80 g, 0.0397 mol) and potassium carbonate (5.48 g, 0.0397 mol) in acetone (80 ml). The mixture was continuously stirred in a r ɔund bottom flask and refluxed under nitrogen for 24 hours. after which the mixture was filtered and the residue obtained was chromatographed using 15 % ethyl acetate in light petroleum. Product (**47**) was obtained in a yield of 5.59 g (87.96 %). (Found M^+: 192.07976. $C_{11}H_{12}O_3$ requires 192.07862).

ν_{max} 3350 (OH), 1640 (C=O), 1610 cm^{-1} (C=C); δ ($CDCl_3$) 2.60 (3H, s, Ar-CO$\underline{CH_3}$), 4.48 (2H, dt, *J* 6 and 2 Hz, Ar-$\underline{CH_2}$CH=CH_2), 5.12-5.69 (2H, m, Ar-CH_2CH=$\underline{CH_2}$), 5.80-6.48 (1H, m, $CH_2\underline{CH}$=CH_2), 6.65-7.28 (3H, m, Ar-H), 11.77 (1H, s, OH, D_2O-exchangeable).

2,5-Dihydroxy-6-(2'-propenyl)-acetophenone (48)

2.03 g (10.57 mmol) of compound **(47)** was weighed out into a round bottom flask. The flask was flushed with nitrogen and immersed into an oil bath preheated at 220 °C. The compound was pyrolysed at this temperature for 2 hours, after which the flask was cooled, the residue dissolved in dichloromethane, preabsorbed onto kieselgel and chromatographed (20 % ethyl acetate in light petroleum) to afford the product **(21)** (1.218 g , 59.13 %), m.p. 108.9 - 109.6 °C (Lit.[39] 104 - 106 °C).

ν_{max} 3270 (OH), 1670 (C=O) and 1610 cm^{-1} (C=C); δ ($CDCl_3$) 2.44 (3H, s, ArCO$\underline{CH_3}$), 3.35 (2H, dt, *J* 6 and 2 Hz, Ar$\underline{CH_2}$CH=CH_2), 4.70-5.20 (2H, m, ArCH_2CH=$\underline{CH_2}$), 5.60-6.27 (1H, m, Ar$CH_2\underline{CH}$=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, 5-OH, D_2O-exchangeable), 11.80 (1H, s, 2-OH, D_2O-exchangeable).

2,5-Dimethoxy-6-(2′-propenyl)-acetophenone (49)

Compound **(48)** (2.03 g, 0.0106 mol) was mixed with potassium carbonate (5.84 g, 0.0423 mol) in a round bottom flask. The dry reagents were dissolved in acetone (50 ml) and iodomethane (6.00 g, 0.0423 mol) was added to the stirred solution. The flask was flushed with nitrogen. The reaction mixture was refluxed in an inert atmosphere for 24 hours. The reaction mixture was then filtered, preabsorbed onto kieselgel and chromatographed on a short column using 15 % ethyl acetate in petroleum ether. The reaction gave a yield of 2.2327g (95.99 %.) (Found M^+: 220.10897. $C_{13}H_{16}O_3$ requires 220.10994). ν_{max} 1690 cm^{-1} (C=O), 1630 cm^{-1} (C=C); δ ($CDCl_3$) 2.46 (3H, s, Ar-CO$\underline{CH_3}$), 3.32 (2H, dt, *J* 6 and 2 Hz, Ar-$\underline{CH_2}$CH=CH_2), 3.77 and 3.78 (3H each, s, 2 x OCH_3), 4.89-4.99 (2H, m, Ar-CH_2CH=$\underline{CH_2}$), 5.79-5.99 (1H, m, Ar-$CH_2\underline{CH}$=CH_2), 6.74 (1H, d, *J* 9.2 Hz, Ar-H) and 6.82 (1H, , d, *J* 9.2 Hz, Ar-H).

2,5-Dimethoxy-6-(*trans*-1′-propenyl)-acetophenone (50)

Compound **(49)** (202.0 mg, 0.9182 mmol) was reacted using 119.1 mg (0.4591 mmol) of palladium chloride-acetonitrile complex as for compound **(40)** to afford the product **(50)** (179.1 mg, 88.66 %) as a yellow oil. (Found M^+: 220.10970. $C_{13}H_{16}O_3$ requires 220.10994).

ν_{max} 1700 cm^{-1} (C=O); δ ($CDCl_3$) 1.85 (3H, d, *J* 6.6 Hz, Ar-CH=CHC<u>H</u>$_3$), 2.41 (3H, s, Ar-CO<u>CH</u>$_3$), 3.76 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 6.04 (1H, dq, *J* 16.2 and 6.6 Hz, Ar-CH=<u>CH</u>CH_3) and 6.38 (1H, dq, *J* 16.2 and 1.8 Hz, Ar-<u>CH</u>=$CHCH_3$), 6.72 (1H, d, *J* 9 Hz, Ar-H), 6.80 (1H, d, *J* 9 Hz, Ar-H).

1-(Hydroxyethyl)-2,5-dimethoxy-6-(*trans*-1-propenyl) benzene (51)

168.4 mg (0.7655 mmol) of the ketone **(50)** was dissolved in dry distilled ether. Lithium aluminium hydride (145.2 mg, 3.8273 mmol) was weighed out under nitrogen and suspended in dry ether. The ketone **(50)** was reduced as for compound **(41)** with a similar chromatographic procedure to afford the product **(51)** as white crystals in a yield of 151.1 mg (88.9 %) with melting point 83.5-84.4 °C. *(Found* M^+*: 222.1260.* $C_{13}H_{18}O_3$ *requires 222.1256).*

ν_{max} 3520 cm^{-1} (OH); δ ($CDCl_3$) 1.55 (3H, d, *J* 6.6 Hz, Ar-<u>CH</u>$_3$CHOH), 1.90 (3H, dd, *J* 6.6 and 2 Hz, CH=CH<u>CH</u>$_3$), 3.75 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 5.22-5.36 (1H, br s, OH, D_2O exchangeable), 5.64-5.82 (1H, dq, *J* 16.0 and 6.6 Hz, CH=<u>CH</u>CH_3), 6.35 (1H, dq, *J* 16.0 and 2.0 Hz, <u>CH</u>=$CHCH_3$), 6.69 (1H, d, *J* 8.8 Hz, Ar-H) and 6.77 (1H, d, *J* 8.8Hz, Ar-H).

(±)-(1R,3R,4R)-3,4-Dihydro-1,3-dimethyl-4-hydroxy-1H-benzo[c]pyran-5,8-dione (45) and its (±)-(4S)-diastereomer (46)

115.1 mg (0.5185 mmol) of the alcohol **(51)** was dissolved in 6 ml of acetonitrile and 6 ml of water. CAN (1.1370 g, 2.0739 mmol) was dissolved in 5 ml of water. The CAN solution was added dropwise to the alcohol solution over a period of 7 minutes. The reaction mixture was stoppered and the reaction allowed to carry on for a further 20 minutes, after which it was quenched by pouring into 100 ml of water and extracted with dichloromethane. The organic layer was collected and dried with $MgSO_4$ and chromatographed using 30 % ethyl acetate in petroleum ether. Product **(45)** was obtained in a yield of 55.1 mg (51.5 %) and product **(46)** in a yield of 40.2 mg (37.3 %).

For **(45)**:

(Found M^+: 208.07506. $C_{11}H_{12}O_4$ requires 208.07356).

ν_{max} 3490 (OH), 1650 cm^{-1} (C=O); δ ($CDCl_3$) 1.37 (3H, d, *J* 6.6 Hz, 3-CH_3), 1.44 (3H, d, *J* 7.0 Hz, 1-CH_3), 2.20-2.35 (1H, br s, *pseudo*-axial 4-OH, D_2O exchangeable), 3.95 (1H, dq, *J* 6.6 and 1.6 Hz, 3-H), 4.35 (1H, dd, *J* 8 and 1.6 Hz, 4-H), 4.81 (1H, q, *J* 7 Hz, 1-H), 6.73 (2H, s, 6-and 7-H).

For **(46)**:

(Found M^+: 208.07506. $C_{11}H_{12}O_4$ requires 208.07356).

ν_{max} 3490 (OH), 1650 cm^{-1} (C=O); δ ($CDCl_3$) 1.37 (3H, d, *J* 6.6 Hz, 3-CH_3), 1.52 (3H, d, *J* 7.0 Hz, 1-CH_3), 3.52 (1H, d, *J* 2.5 Hz, *pseudo*-equatorial 4-OH, D_2O exchangeable), 3.85 (1H, dq, *J* 8.0 and 6.0 Hz, 3-H), 4.35 (1H, dq, *J* 8.0 and 2.5 Hz, *pseudo*-axial 4-H), 4.75 (1H, dq, *J* 7 and 1.2 Hz, 1-H), 6.73 (2H, s, 6-and 7-H).

(1R, 3R, 4R)-3,4-Dihydro-4-hydroxy-5,8-dimethoxy-1,3-dimethyl-1H-benzo[2,3-*c*]pyran (53) and its 4S-diastereomer (54)

The conjugated alcohol **(51)** (205.9 mg, 0.9275 mmol) was dissolved in dichloromethane and immersed in ice. mCPBA (240.1 mg, 1.3912 mmol) was also dissolved in dichloromethane and added dropwise to the stirred alcohol solution. The reaction was carried out over 2 hours under nitrogen. The reaction mixture was then thrown into 0.1 M sodium hydroxide to precipitate the acid. The organic components were extracted with dichloromethane and dried with $MgSO_4$. The residue was chromatographed on a short column using 30 % ethyl acetate in petroleum ether to afford 94.2 mg (46.2 %) of **(53)** and 72.4 mg (35.5 %) of **(54)**. (Found M^+: 238.1290. $C_{13}H_{18}O_4$ requires 238.12051).

For **(54)**:

ν_{max} 3400 cm^{-1} (OH); δ ($CDCl_3$) 1.08 (3H, d, *J* 6.2 Hz, 3-CH_3), 1.50 (3H, d, *J* 6.2 Hz, 1-CH_3), 3.36 (1H, br s, 4-OH, D_2O-exchangeable), 3.77 and 3.79 (3H each, s, 2 x OCH_3), 4.02-4.16 (1H, m, 3-H), 5.14 (1H, dq, *J* 6.0 and 1.6 Hz, 4-H), 5.25-5.48 (1H, m, 1-H), 6.71 (2H, s, 6- and 7-H).

^{1}H nmr spectra for **(53)** was not conclusive due to impurities.

(3R, 4R)-3,4-Dihydro-4-hydroxy-5,8-dimethoxy-3-methyl-1H-benzo[2,3-*c*]pyran (55) and its 4S-diastereomer (56)

Alcohol **(41)** (151.6 mg, 0.7288 mmol), was cyclised using a solution of mCPBA (188.7 mg, 1.0933 mmol) as for compounds **(53)** and **(54)** above. After work up and chromatography, two products were collected **(56)** (23.1 %) and **(55)** (55.7 %). For each product (Found M^+: 224.10535. $C_{12}H_{16}O_4$ requires 224.10486).

^{1}H nmr data was inconclusive due to impurities.

CHAPTER 4

IN VITRO ANTIMICROBIAL ACTIVITY SCREENING OF SYNTHETIC COMPOUNDS

4.1 GENERAL

Bioactive quinonoid systems occurring naturally have proven to be of vital importance over the years. Due to the limited amount of these compounds obtained in nature, chemists all over the world have embarked on synthesising these compounds in the laboratory to have them readily available for bioactivity analyses.

There is an ongoing search for cheaper and more efficient medicines, especially antibiotics. It was envisaged that by synthesising potential antibiotic compounds, a better understanding of activity and structure relationships could be reached.

4.2 OBJECTIVES

The objectives were to:

(i) screen the synthetic compounds for antimicrobial activity and specificity against a few select Gram positive and Gram negative organisms employing the Bauer-Kirby[38] method, and

(ii) to establish a possible molecular structure-specificity relationship .

4.3 GENERAL METHODOLOGY

Filter paper discs with diameter of 10 mm were impregnated with the compounds dissolved in triple distilled dichloromethane. The discs were dried under reduced pressure and placed onto the surface of nutrient agar plates inoculated with the test organisms. The plates were incubated at 37 °C for 24 hours and the diameter of the zones of inhibition (including that of the impregnated discs) was measured. Inhibition of microbial growth was indicated by a clear zone around the disc. The discs were impregnated to contain 2.81, 1.40 and 0.70 μmol of compound.

It must be noted that zone sizes are not comparable and cannot be used to determine the potency of relative activity of the compound under investigation because different antibiotics diffuse through agar gells at different rates.

The following test organisms were obtained from the South African Bureau of Standards (SABS) in Pretoria for experimental work.

Staphylococcus aureus	SATCC Sta 53
Bacillus subtilus	SATCC Bac 96
Candida albicans	Fungus
Eschericia coli	SATCC Esc 25
Pseudomonas aeruginosa	SATCC Pse 2
Proteus mirabilis	SATCC Pre 1

4.4 DISCUSSION OF RESULTS

The following may be concluded with respect to a structure –activity-specificity relationship.

(i) Reduction of the ketone group of the inactive aryl system (**21**) and the consequent conversion of the resulting alcohol (**23**) into the acetate (**24**) resulted in a broadening of activity against all of the test organisms.

(ii) On the other hand, the hydroxy quinone (**23**) was active against Gram negative and Gram positive organisms. After replacing the hydroxy group with an acetate group, compound (**25**) was rendered inactive. This was also observed for compounds (**59**) and (**60**), and compounds (**61**) and (**62**).

(iii) The *pseudo*-axial and *pseudo*-equatorial C-4 hydroxy naphthopyrans (**42**) and (**43**) showed activity against both Gram negative and Gram positive organisms. The most significant of these being against *Bacillus Subtilus*. Similar results were obtained for the trans-1,3-dimethyl derivarive[26] (**45**) and (**46**).

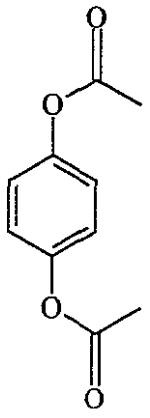
(iv) Introduction of a double bond to C-3of compound **(65)** to afford compound **(66)** did not have a significant impact to the activity of the compound, neither did the position of the alkyl chain [as in **(67)**].

(v) Compound **(71)**, with extra conjugation on the alkyl chain is less active than the non-conjugated compound **(72)** which showed activity towards *Candida albicans*

(vi) The chiral pyran compounds **(73)** and **(74)** are active only against Gram positive organisms. The chiral *cis* shows a bit more activity as compared to its *trans* derivative. The activities of the two compounds are not significantly different to the activity of the racemic *trans* compound **(75).**

(vii) Compound **(27)**[29] showed activity against both Gram negative and Gram positive organisms – with the exception of *Pseudomonas aeruginosa*. It can be deduced that addition of a methoxy group at C-6 as in compounds **(73)** and **(74)** rendered the compo nds inactive against all Gram negative organisms tested. There was a significant reduction in activity against *Bacillus subtilus* and *Candida albicans* with the addition of the methoxy group.

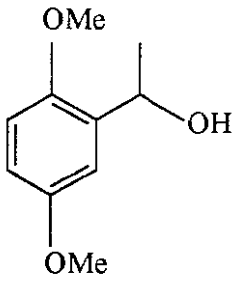
Table 4.1: Inhibitory Activity of Compound against SABS Organisms



(19)

Gram Positive Organism and SABS culture number	**Dose in μmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81	0
Bacillus Subtilus SATCC Bac 96	2.81	0
Candida albicans fungus	2.81	0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

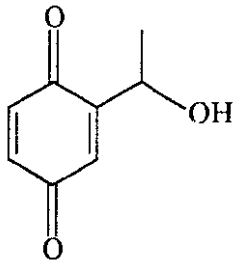
Table 4.2: Inhibitory Activity of Compound against SABS Organisms



(21)

Gram Positive Organism and SABS culture number	**Dose in μmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81	0
Bacillus Subtilus SATCC Bac 96	2.81	0
Candida albicans fungus	2.81	0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

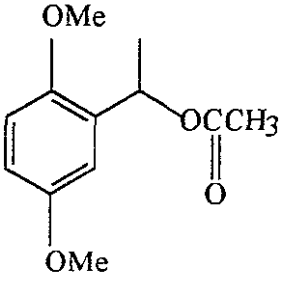
Table 4.3: Inhibition Activity of Compound against SABS Organisms



(23)

Gram Positive Organism and SABS culture number	**Dose in μmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81 1.40 0.70	22 20 18
Bacillus Subtilus SATCC Bac 96	2.81 1.40 0.70	20 16 15
Candida albicans fungus	2.81	0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81 1.40 0.70	12 11 0
Proteus mirabilis SATCC Pre 1	2.81 1.40 0.70	20 18 15
Eschericia coli SATCC Esc 25	2.81 1.40 0.70	24 20 15

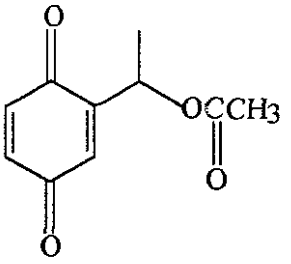
Table 4.4: Inhibition Activity of Compound against SABS Organisms



(24)

Gram Positive Organism and SABS culture number	**Dose in μmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81 1.40	18 17
Bacillus Subtilus SATCC Bac 96	2.81 1.40	17 15
Candida albicans fungus	2.81 1.40	11 0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81 1.40	14 15
Proteus mirabilis SATCC Pre 1	2.81 1.40	15 12
Eschericia coli SATCC Esc 25	2.81 1.40	20 18

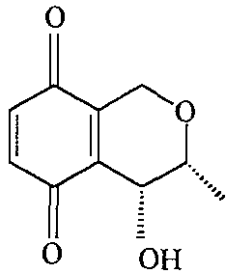
Table 4.5: Inhibition Activity of Compound against SABS Organisms



(25)

Gram Positive Organism and SABS culture number	**Dose in μmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81	0
Bacillus Subtilus SATCC Bac 96	2.81	0
Candida albicans fungus	2.81	0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

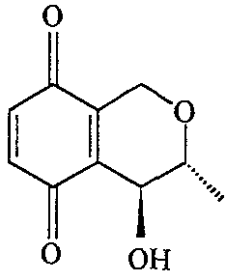
Table 4.6: Inhibition Activity of Compound against SABS Organisms



(42)

Gram Positive Organism and SABS culture number	**Dose in µmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81 1.40 0.70	20 17 14
Bacillus Subtilus SATCC Bac 96	2.81 1.40 0.70	24 19 17
Candida albicans fungus	2.81 1.40 0.70	11 0 0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81 1.40 0.70	12 11.5 0
Proteus mirabilis SATCC Pre 1	2.81 1.40 0.70	19 16 12
Eschericia coli SATCC Esc 25	2.81 1.40 0.70	17 15 11

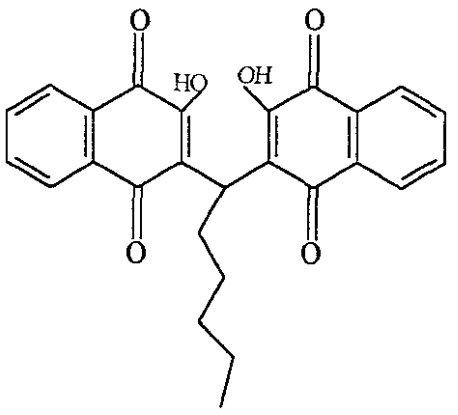
Table 4.7: Inhibition Activity of Compound against SABS Organisms



(43)

Gram Positive Organism and SABS culture number	**Dose in μmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	1.40	16
Bacillus Subtilus SATCC Bac 96	1.40	25
Candida albicans fungus	1.40	0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	1.40	10.5
Proteus mirabilis SATCC Pre 1	1.40	13
Eschericia coli SATCC Esc 25	1.40	14

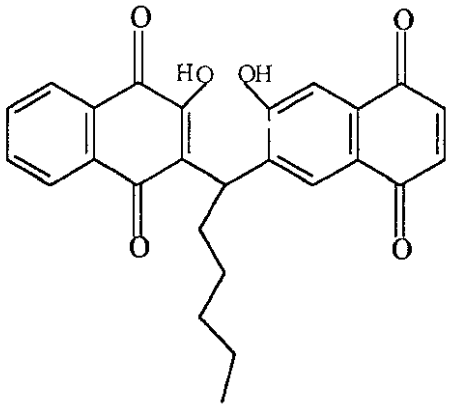
Table 4.8: Inhibition Activity of Compound against SABS Organisms



(57)

Gram Positive Organism and SABS culture number	**Dose in μmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81	0
Bacillus Subtilus SATCC Bac 96	2.81	0
Candida albicans fungus	2.81	0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

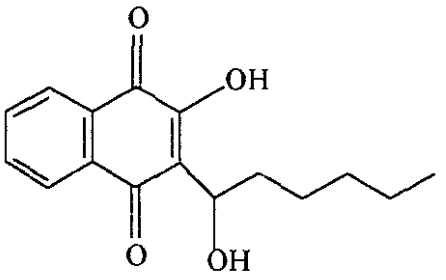
Table 4.9: Inhibition Activity of Compound against SABS Organisms



(58)

Gram Positive Organism and SABS culture number	**Dose in µmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81	0
Bacillus Subtilus SATCC Bac 96	2.81	0
Candida albicans fungus	2.81	0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

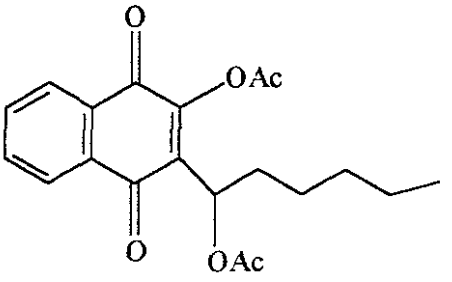
Table 4.10: Inhibition Activity of Compound against SABS Organisms



(59)

Gram Positive Organism and SABS culture number	**Dose in μmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	1.40	0
Bacillus Subtilus SATCC Bac 96	1.40 0.70	10.5 10.5
Candida albicans fungus	2.81	0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

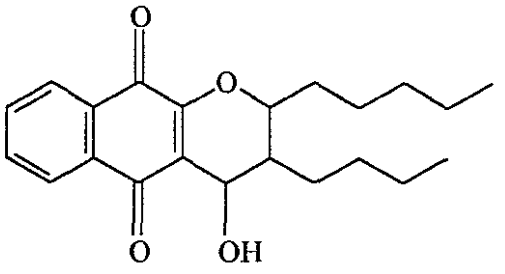
Table 4.11: Inhibition Activity of Compound against SABS Organisms



(60)

Gram Positive Organism and SABS culture number	**Dose in µmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81	0
Bacillus Subtilus SATCC Bac 96	2.81	0
Candida albicans fungus	2.81	0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

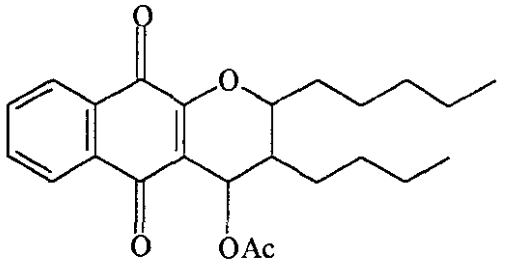
Table 4.12: Inhibition Activity of Compound against SABS Organisms



(61)

Gram Positive Organism and SABS culture number	**Dose in μmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81	0
Bacillus Subtilus SATCC Bac 96	2.81 1.40 0.70	11 11 22
Candida albicans fungus	2.81	0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

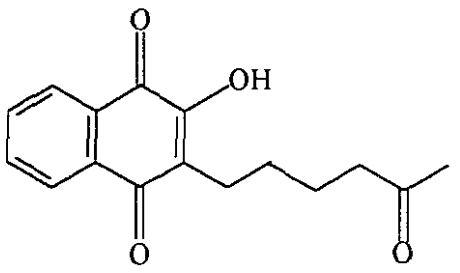
Table 4.13: Inhibition Activity of Compound against SABS Organisms



(62)

Gram Positive Organism and SABS culture number	**Dose in μmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81	0
Bacillus Subtilus SATCC Bac 96	2.81	0
Candida albicans fungus	2.81	0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

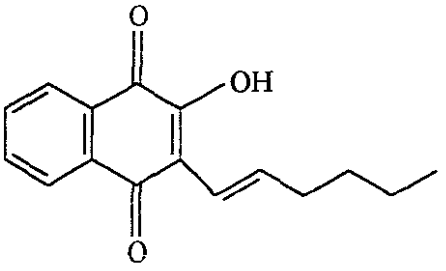
Table 4.14: Inhibition Activity of Compound against SABS Organisms



(63)

Gram Positive Organism and SABS culture number	**Dose in μmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81 1.40 0.70	13 12 0
Bacillus Subtilus SATCC Bac 96	2.81 1.40 0.70	16 14 12
Candida albicans Fungus	2.81	0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

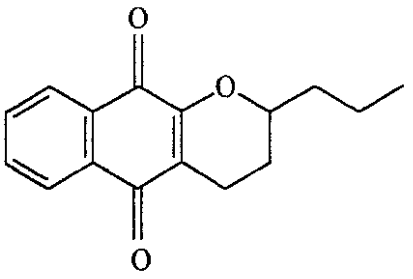
Table 4.15: Inhibition Activity of Compound against SABS Organisms



(64)

Gram Positive Organism and SABS culture number	**Dose in μmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81 1.40 0.70	12 13 0
Bacillus Subtilus SATCC Bac 96	2.81 1.40 0.70	18 17 17
Candida albicans fungus	2.81	0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

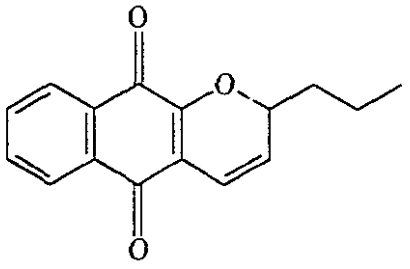
Table 4.16: Inhibition Activity of Compound against SABS Organisms



(65)

Gram Positive Organism and SABS culture number	**Dose in µmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81 1.40 0.70	17 14 13
Bacillus Subtilus SATCC Bac 96	2.81 1.40 0.70	20 18 16
Candida albicans fungus	2.81	0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

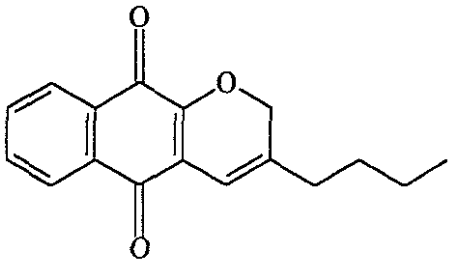
Table 4.17: Inhibition Activity of Compound against SABS Organisms



(66)

Gram Positive Organism and SABS culture number	**Dose in μmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81 1.40 0.70	16 13 13
Bacillus Subtilus SATCC Bac 96	2.81 1.40 0.70	16 16 16
Candida albicans fungus	2.81	0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

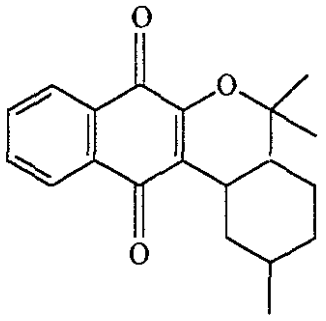
Table 4.18: Inhibition Activity of Compound against SABS Organisms



(67)

Gram Positive Organism and SABS culture number	**Dose in μmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81 1.40 0.70	12 14 0
Bacillus Subtilus SATCC Bac 96	2.81 1.40 0.70	16 15 13
Candida albicans fungus	2.81	0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

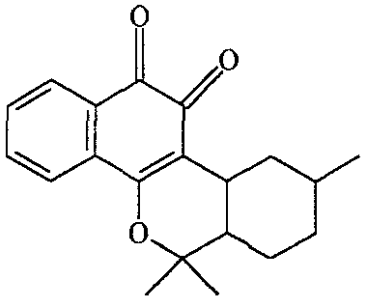
Table 4.19: Inhibition Activity of Compound against SABS Organisms



(68)

Gram Positive Organism and SABS culture number	**Dose in μmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81	0
Bacillus Subtilus SATCC Bac 96	2.81	0
Candida albicans fungus	2.81	0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

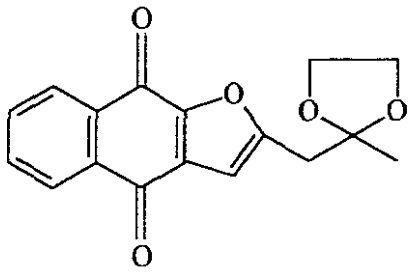
Table 4.20: Inhibition Activity of Compound against SABS Organisms



(69)

Gram Positive Organism and SABS culture number	**Dose in μmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81 1.40 0.70	11 10.5 10.5
Bacillus Subtilus SATCC Bac 96	2.81 1.40 0.70	12 12 11
Candida albicans fungus	2.81	0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

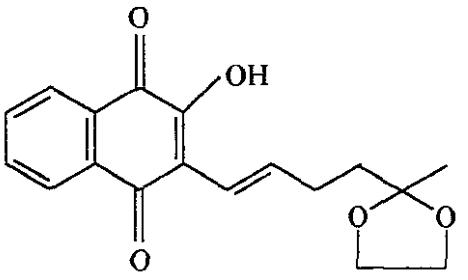
Table 4.21: Inhibition Activity of Compound against SABS Organisms



(70)

Gram Positive Organism and SABS culture number	Dose in μmol	Zone of inhibition in mm
Staphylococcus aureus SATCC Sta 53	1.40	16
Bacillus Subtilus SATCC Bac 96	1.40	20
Candida albicans fungus	1.40	14
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	1.40	0
Proteus mirabilis SATCC Pre 1	1.40	0
Eschericia coli SATCC Esc 25	1.40	0

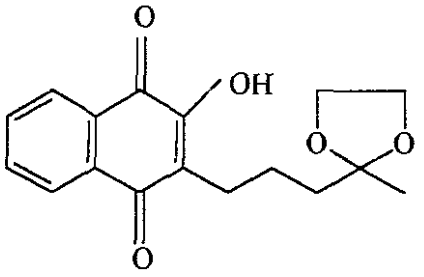
Table 4.22: Inhibition Activity of Compound against SABS Organisms



(71)

Gram Positive Organism and SABS culture number	**Dose in μmol**	***Zone of inhibition in* mm**
Staphylococcus aureus SATCC Sta 53	2.81 1.40 0.70	11 12 0
Bacillus Subtilus SATCC Bac 96	2.81 1.40 0.70	19 16 15
Candida albicans fungus	2.81	0
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

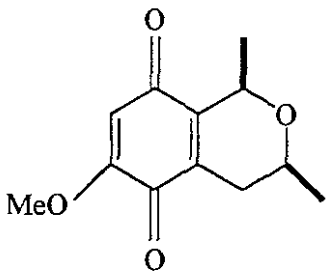
Table 4.23: Inhibition Activity of Compound against SABS Organisms



(72)

Gram Positive Organism and SABS culture number	**Dose in μmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81 1.40 0.70	24 20 19
Bacillus Subtilus SATCC Bac 96	2.81 1.40 0.70	23 22 20
Candida albicans fungus	2.81 1.40 0.70	15 13 12
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

Table 4.24: Inhibition Activity of Compound against SABS Organisms

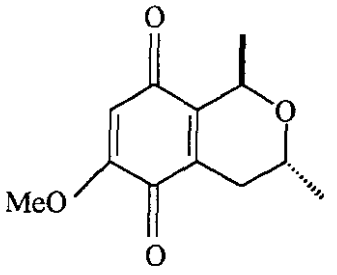


Chiral *cis* [40]

(73)

Gram Positive Organism and SABS culture number	**Dose in µmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81 1.40 0.70	26 25 20
Bacillus Subtilus SATCC Bac 96	2.81 1.40 0.70	26 28 21
Candida albicans fungus	2.81 1.40 0.70	20 15 15
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

Table 4.25: Inhibition Activity of Compound against SABS Organisms

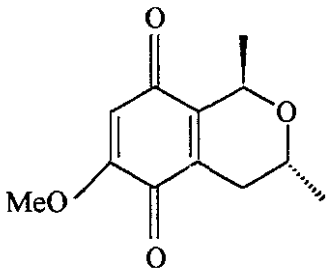


Chiral *trans* [40]

(74)

Gram Positive Organism and SABS culture number	**Dose in μmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81 1.40 0.70	21 21 21
Bacillus Subtilus SATCC Bac 96	2.81 1.40 0.70	22 23 20
Candida albicans fungus	2.81 1.40 0.70	18 14 13
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

Table 4.26: Inhibition Activity of Compound against SABS Organisms

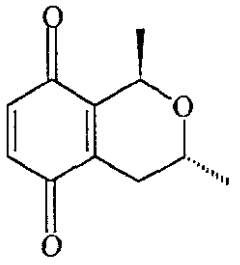


Racemic *trans* [40]

(75)

Gram Positive Organism and SABS culture number	**Dose in µmol**	**Zone of inhibition in mm**
Staphylococcus aureus SATCC Sta 53	2.81 1.40 0.70	26 25 23
Bacillus Subtilus SATCC Bac 96	2.81 1.40 0.70	20 20 19
Candida albicans fungus	2.81 1.40 0.70	17 16 13
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81	0
Eschericia coli SATCC Esc 25	2.81	0

Table 4.27: Inhibition Activity of Compound against SABS Organisms



(27) [29]

Gram Positive Organism and SABS culture number	Dose in µmol	Zone of inhibition in mm
Staphylococcus aureus SATCC Sta 53	2.81 1.40 0.70	25 24 16
Bacillus Subtilus SATCC Bac 96	2.81 1.40 0.70	35 30 24
Candida albicans fungus	2.81 1.40 0.70	28 20 18
Gram Negative Organism and SABS culture number		
Pseudomonas aeruginosa SATCC Pse 2	2.81	0
Proteus mirabilis SATCC Pre 1	2.81 1.40 0.70	20 16 14
Eschericia coli SATCC Esc 25	2.81 1.40 0.70	20 14 14

CONCLUSION

Allylation of compound **(25)** was not regiospecific and thus this route of synthesis towards our target quinone **(27)** was abandoned. The intermediated compounds **(23)** and **(25)** showed activity against both Gram negative and Gram positive organisms.

The C-4-hydroxypyranquinone **(42)** was successfully synthesised and isolated. This compound was active against all our standard organisms. The (±)-(4S)-diastereomer **(43)** was confirmed by high resolution mass spectroscopy. An appropriate ^{1}H nmr spectrum was not obtained due to contamination.

Compounds **(45)** and **(46)** were synthesised successfully. These compounds were also found to be active against all the organisms tested.

Compounds **(53)** and **(54)**, which were previously synthesised via reactions of the conjugated alcohol **(51)** with two molar equivalents of CAN, were successfully synthesised using mCPBA. The *pseudo*-equatorial hydroxy pyran was obtained in good yield.
Reaction of the demethylated conjugated alcohol **(41)** with mCPBA yielded two C-4-hydroxy pyrans **(55)** and **(56)**. Unfortunately, the structures were not fully characterized due to impurities.

The compounds tested for antimicrobial activity showed a wide range of activity against the tested organisms. Replacement of a hydroxy group with an acetate group in most compounds rendered them inactive against all tested organisms.

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