# THE STRUCTURAL ELUCIDATION AND PARTIAL SYNTHESIS OF PHACIDIN, A FUNGAL GROWTH INHIBITOR FROM POTEBNIAMYCES BALSAMICOLA SMERLIS VAR. BOYCEI FUNK

BY

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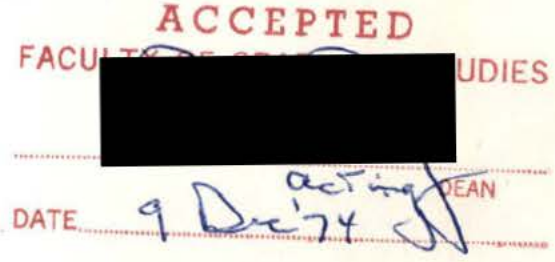


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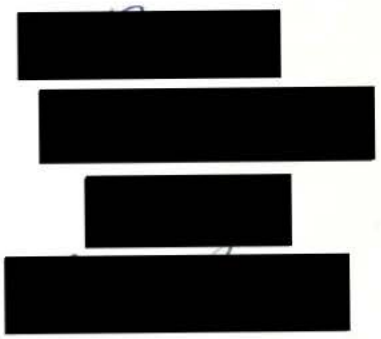
ABSTRACT

An antibiotic substance called phacidin has been isolated from the fungus Potebniamyces balsamicola which infects fir trees in British Columbia. Biochemical tests have shown that phacidin inhibits the growth of other fungi and for this reason may be useful to medicine in the future. By the use of spectra and degradation reactions we have been able to identify the components of the molecule and propose a structure for phacidin: 6-methoxy-5-n-nonanoyl-4-oxo-4H-pyran-2-carboxaldehyde. The synthesis of phacidin and its isomers has been partially completed. A number of interesting properties of γ-pyrones have been encountered during the progress of this work and have been investigated.



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# PART I

## THE STRUCTURAL ELUCIDATION OF PHACIDIN

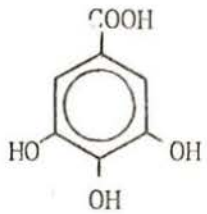
# INTRODUCTION

## 1. Discovery of an Antibiotic

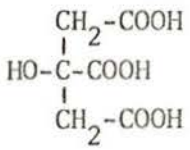
That fungi play an integral part in life on earth has been known for many hundreds of years, but only in recent times has their economic importance really been felt. Fungi belong to the subkingdom Thallophyta, a very large group of plants characterized by lack of true roots, stems, leaves or flowers. Instead, they have a vegetative body which can vary from a single cell to a large colony of cells. Further classification confines true fungi to the phylum Eumycophyta which distinguishes them from other thallophytes such as algae, bacteria, and slime moulds. Unlike algae, fungi lack the necessary mechanisms by which to synthesize photosynthetic pigments such as chlorophyll. They are thus unable to synthesize food, and are compelled to live either as saprophytes, feeding on dead plant or animal tissues, or as parasites, obtaining essential nutrients from living organisms.

Both parasitic and saprophytic fungi have wide spread economic significance. Saprophytic fungi, along with bacteria, are the principal agents of decay of complex plant and animal remains in the soil. Through this essential process, fungi play an important part in the nutrition of green plants by providing a source of simple nutrients. An increasing number of fungi are being used industrially in the

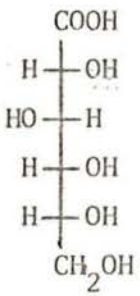
familiar processes of fermentation, cheese making, baking, antibiotic production, and the synthesis of a variety of organic compounds such as gallic, citric, gluconic, itaconic and kojic acids and glycerol.



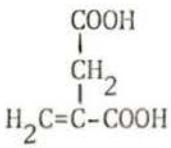

gallic acid



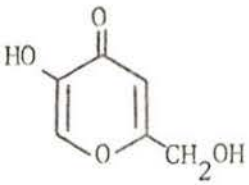

citric acid



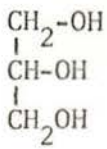

gluconic acid




itaconic acid




kojic acid




glycerol

As well as the beneficial uses to which fungi may be put, their harmful effects are forever evident. Such materials as timber and wood products, leather, paper goods, textiles, and a host of food products are destroyed by a variety of fungi, and millions of dollars are spent yearly on methods of product treatment which will prevent fungal growth.

The parasitic fungi are also well known to man as the cause of diseases in plants and animals. While most diseases in man and animals are caused by bacteria and viruses, the majority of plant diseases are caused by fungi. These organisms have been known to cause extensive damage to food and timber crops leaving a number of countries

in economic ruin. Such notable examples as the potato blight in Europe and the chestnut blight in the United States were the result of fungal infections not checked in their early stages.

Not all fungal infections in plants are as disastrous as the examples given, but there are, nevertheless, many which have economic significance. The forest industry in British Columbia provides a major source of income for the province and because of this, any disease affecting commercial timber stands is looked at with particular care.

A recent study has revealed that a fungal growth is responsible for a bark disease common to commercial forests in northern British Columbia and the southern interior at higher elevations. This disease is characterized by stem cankers, dieback syndrome or "red top", and red flagging. The most common and conspicuous form of the infection is the red flagging which occurs when the end of a branch is killed and turns a red-brown colour in contrast with the natural green foliage. From studies of several closely related species of fir found in British Columbia, notably alpine fir or spruce (Abies lasiocarpa), grand fir (Abies grandis), and amabilis fir (Abies amabilis), it was found that the chief cause of the sumptoms is Potebniamyces balsamicola Smerlis var. boycei Funk,[1] an ascomycetous fungus. This fungus was isolated from both cankered stems and flagged branches of young alpine fir trees by removing a small amount of the inner bark and placing it on agar containing 3% malt extract. Incubation at 20-23° in the dark afforded rapid growth of Potebniamyces balsamicola. In a more recent study,[2] ascospores and conidia of the fungus were obtained from diseased specimens of grand fir (Abies grandis (Dougl.) Lindl) gathered near Salmo

and Yahk, B.C. Under optimum growth conditions of 15-20°, dark, and 2% malt agar, fungal colonies of Potebniamyces balsamicola grew rapidly producing conidia within six weeks. The petri plate cultures were then ground in distilled water and the macerated mycelium used to inocculate solutions of 5% malt extract in distilled water. The cultures were allowed to incubate at 15° in the dark and after several weeks it was noted that a pale yellow substance was being produced by the fungus and being deposited on the surface of the mycelial mats. Maximum production of the crystalline material occurred in five to six weeks. The name "phacidin" has been proposed for this substance from Phacidiales, the order of ascomycetes to which the fungus belongs.

In a recent publication by Funk and McMullan,[3] the isolation and purification of phacidin are described in detail. Crude extracts of the metabolite were found to have significant inhibitory effects on fungi of all major groups, especially yeasts and moulds. Subsequent tests of the effects of the purified substance, carried out at the Royal Jubilee Hospital, Victoria, B.C., showed that none of the medical bacteria were significantly inhibited by phacidin.

From the way in which phacidin is produced by the fungus and deposited on the surface of the mycelial mats in culture, we are led to assume that phacidin is a product of secondary, rather than primary metabolism. Primary metabolism, on the one hand, refers to the complicated series of reactions which provide an organism with energy, synthetic intermediates, and key macromolecules. Nearly all living systems have basically the same primary metabolism. On the other hand,

secondary metabolism is normally restricted to lower forms of life and is usually species, and often strain, specific. It involves the utilization of a relatively small number of intermediates, arising from primary metabolism, for synthesis of products which play no obvious role in the economy of the organism.[4]

Since phacidin indeed has an inhibitory effect on fungi, it can be conveniently described as an antibiotic. Zähner and Maas[5] have defined antibiotics as "...substances produced by living organisms, which, in low concentrations are able to inhibit growth of (other) organisms". They have also stated that most antibiotics are secondary metabolites produced during the stationary phase of microbial growth; that is, after the cultured fungus has reached the maximum number of cells possible in a given environment.

While not all antibiotics produced by fungi are chemotherapeutically important at this time, most of those which are useful to man are synthesized by fungi of the class Ascomycetae, to which Potebniamyces balsamicola belongs. For example, fungi of the genus Penicillium produce a variety of penicillins, the first class of antibiotics used extensively in medicine. Since phacidin is both a secondary fungal metabolite and an antibiotic, its potential importance to medicine gives study of its chemical structure particular significance.

2. Isolation and Purification

The technique used in isolation and purification of phacidin proved to be relatively simple, giving good yields of antibiotic (one gram of pure antibiotic from 1500 $cm^3$ of culture medium). Phacidin was scraped from mycelial mats of the fungus and the crude product suction filtered and stirred in a large volume of benzene for 25 hr. Filtration to remove residual fungus was followed by concentration in vacuo at 40° and addition of hexane to precipitate the crude antibiotic. Repeated extraction of the culture broth with benzene yielded a small amount of phacidin which was combined with the other crude product. Centrifugation proved to be the most facile method of collecting the precipitate. Crude phacidin was then recrystallized from benzene/hexane and finally acetone/water to give a pale yellow crystalline product (mp 112-113°).

Phacidin is soluble in most organic solvents as well as 5% NaOH, conc. $H_2SO_4$, and syrupy $H_3PO_4$. It is, however, only slightly soluble in 5% $NaHCO_3$ and 5% HCl, and insoluble in water and petroleum ether. A preliminary investigation of the spectral properties of the antibiotic indicated that phacidin was indeed an unknown compound. This stimulated a study of the chemical structure of phacidin and led to partial synthesis of the proposed system.

## RESULTS AND DISCUSSION

In attempting to identify a newly found compound, one must first consider all the possible classes of organic compounds and, by a process of elimination, narrow the list down to a few possible structures which best fit the data gathered on the new compound. Zähner and Maas[6] have produced a list of classes of compounds in which secondary metabolites have been found (Table I).

| | | |
|---|---|---|
| Amino sugars | Lactones | Pyrones |
| Anthocyanins | Macrolides | Pyrroles |
| Anthraquinones | Naphthalenes | Pyrrolines |
| Aziridines | Naphthoquinones | Pyrrolizines |
| Benzoquinones | Nucleosides | Quinolines |
| Coumarins | Oligopeptides | Quinolinols |
| Diazines | Phenazines | Quinones |
| Epoxides | Phenoxazinones | Salicylates |
| Ergoline alkaloids | Phthaldehydes | Terpenoids |
| Flavonoids | Piperazines | Tetracyclines |
| Glutaramides | Polyacetylenes | Tetronic acids |
| Glycosides | Polyenes | Triazines |
| Hydroxylamines | Pyrazines | Tropolones |
| Indole derivatives | Pyridines | |

Table I. Classes of organic compounds in which secondary metabolites have been found.

It is important, nevertheless, to remember that while such a list is helpful in considering possible structures, it is not absolute, since

new compounds and new systems are rapidly being discovered.

As described in the Introduction, the new antibiotic was purified by a series of recrystallizations before any attempt was made to characterize it.

1. Spectral Analysis

(a) Elemental Composition

From the accurate mass measurement (294.1514) and the fact that the mass spectrum is not characteristic of a chlorine, bromine, or sulfur containing compound, the molecular formula was proposed to be $C_{16}H_{22}O_5$. The theoretical accurate mass must then be 294.1467, a value which agrees, within experimental error, with the value found for phacidin. Supporting evidence for this formula was obtained from the elemental analysis (found: C 65.10%, H 7.61%; $C_{16}H_{22}O_5$ requires C 65.29%, H 7.53%).

(b) Nuclear Magnetic Resonance Spectrum (NMR) (Fig. 1)

A preliminary nuclear magnetic resonance spectrum of phacidin did not include a search to lower field than δ 10.0. Reinvestigation of the spectrum revealed a one proton singlet at δ 10.17. Initial interpretation of the NMR spectrum allows the following assignments: the one proton singlet at δ 10.17 due to -C<u>H</u>O (aldehydic); the one proton singlet at δ 7.11 due to a proton greatly deshielded either by being on an aromatic or pseudoaromatic ring, or on a double bond with additional deshielding; the three proton singlet at δ 4.17 due to -O-C<u>H</u>$_3$; the two proton triplet (J = 6.7 Hz) at δ 2.96 due to X-C<u>H</u>$_2$-CH$_2$-,

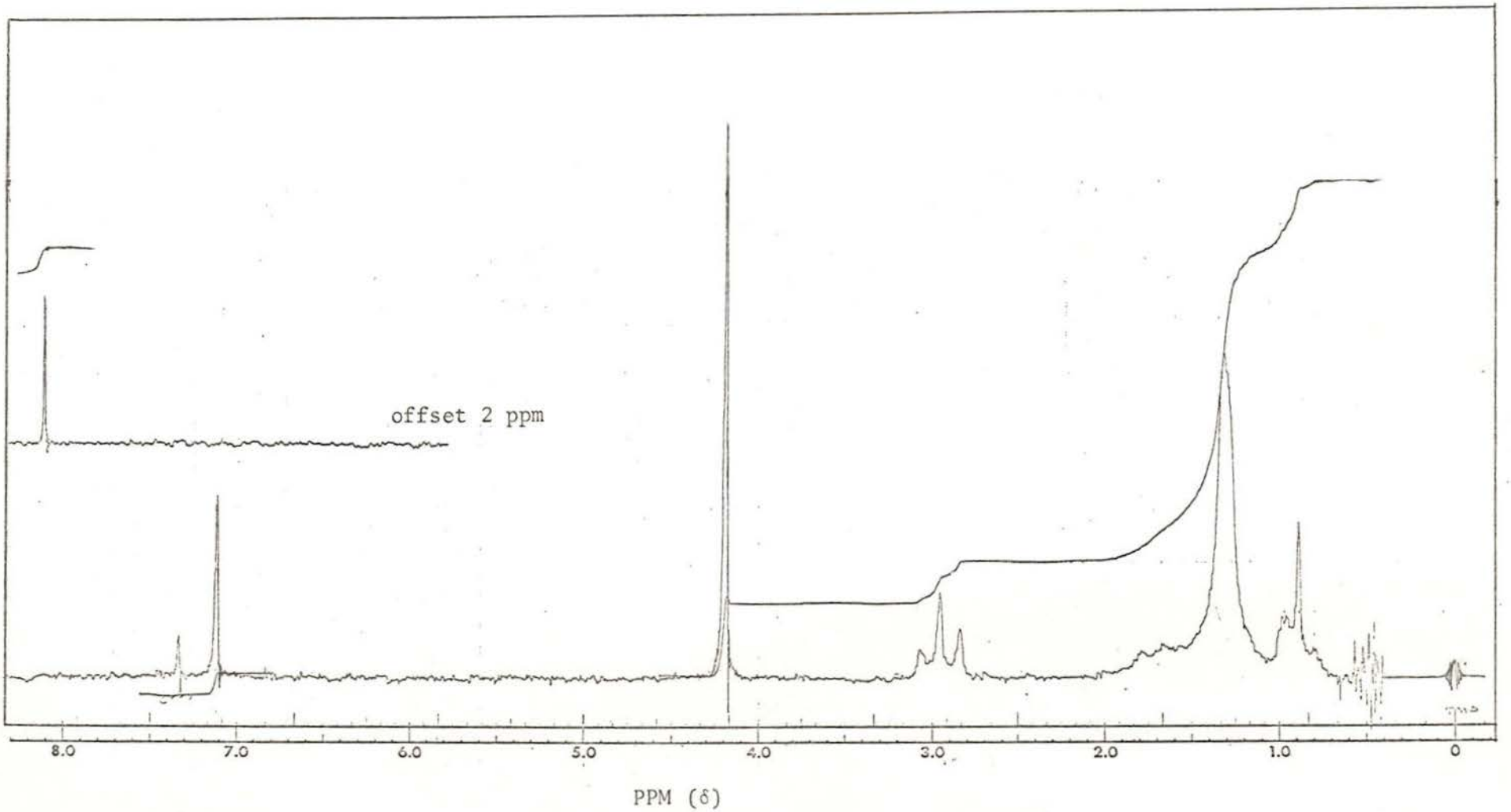



Fig. 1. $^{1}H$ Nuclear magnetic resonance spectrum of phacidin ($CDCl_3$).

X being a deshielding group; the twelve proton multiplet from δ 2.0 to 1.0 due to methylene protons (-$\underline{CH}_2$'s); and the three proton triplet at δ 0.88 (J = 6.1 Hz) due to a methyl group -$CH_2$-$\underline{CH}_3$ not deshielded from its normal position at the end of a hydrocarbon chain (normally J = 6.7 to 7.2 Hz; δ 0.9).[7] Thus, analysis of the NMR spectrum suggests the presence of a methoxyl group, an aldehyde function, and a hydrocarbon chain.

(c) Mass Spectrum (Fig. 2)

The base peak in the mass spectrum appears at m/e 153 and has the formula $C_7H_5O_4$ (found 153.0208, calculated 153.0188), which we shall call Part A. A complementary peak appears at m/e 141, corresponding to $C_9H_{17}O$ (found 141.1283, calculated 141.1279) which we shall call Part B, indicating that the molecule splits into two halves, the more stable part A giving rise to the most intense peak.

$$[C_7H_5O_4\text{-}H\text{-}C_9H_{17}O]$$

Part A Part B

None of the other peaks in the spectrum were of an intensity greater than 10% of the base peak. Metastable ion peaks (m*) were found corresponding predominantly to mass losses of 28 and 32.

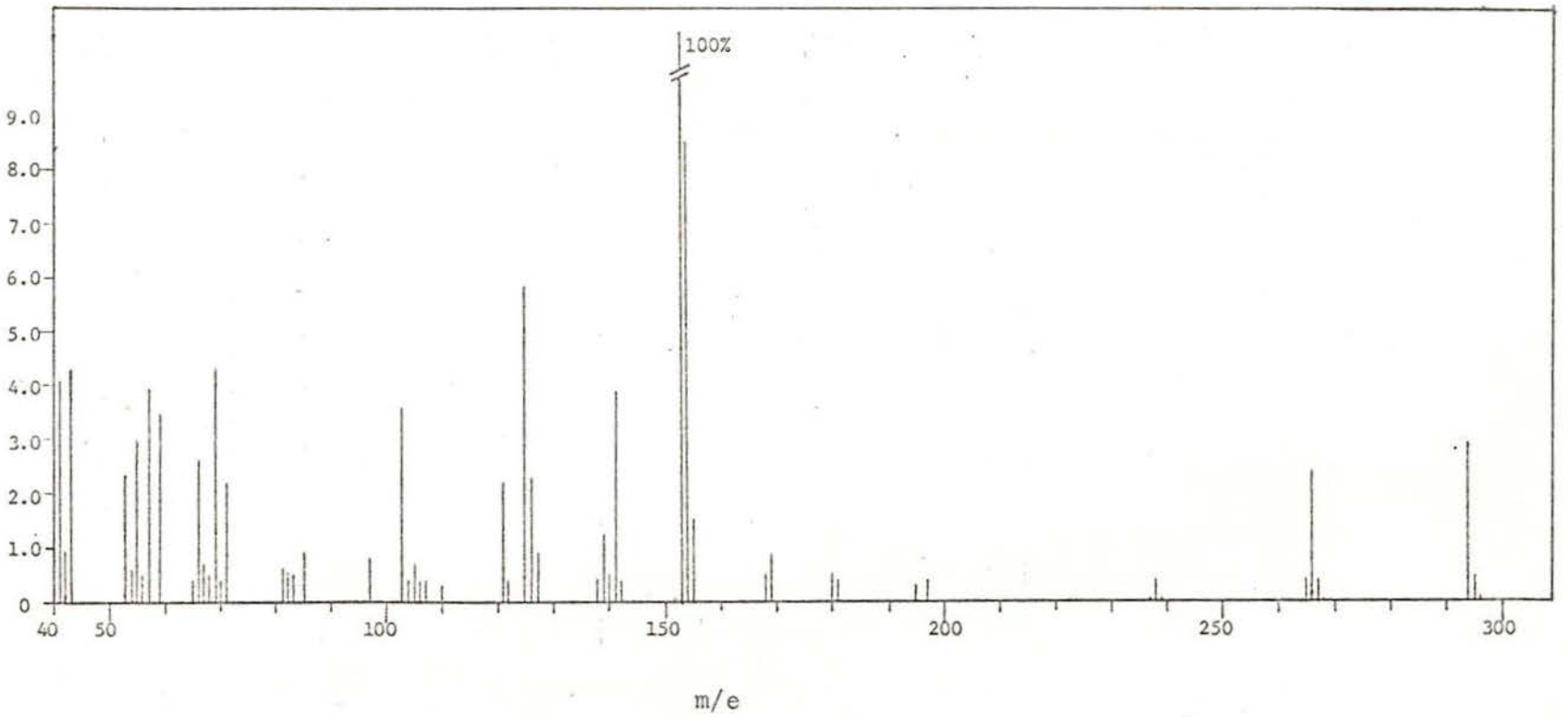
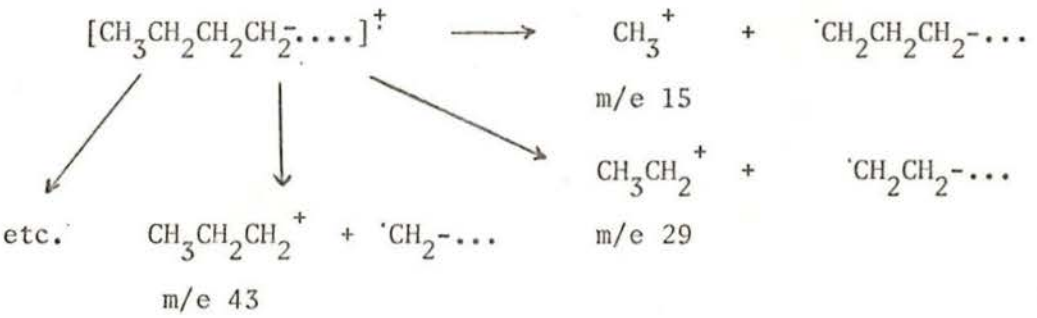



Fig. 2. Mass spectrum of phacidin.

| m* | parent ion | daughter ion | Δ mass |
|---|---|---|---|
| 240.7 | 294 | 266 | 28 |
| 212.9 | 266 | 238 | 28 |
| 102.1 | 153 | 125 | 28 |
| 95.7 | 153 | 121 | 32 |
| 69.2 | 125 | 93 | 32 |

Table II. Fragmentation of phacidin from metastable ion peaks in the mass spectrum.

A mass loss of 28 could correspond to loss of either $C_2H_4$ or CO, while loss of 32 could only reasonably be due to loss of $CH_4O$ ($CH_3OH$). The other major characteristic of the mass spectrum is the presence of a series of peaks in the low molecular weight region (m/e < 100) appearing at intervals of 14 mass units (85, 71, 57, 43, 29; 83, 69, 55, 41, 27). This fragmentation pattern is characteristic of saturated hydrocarbon chains which exhibit simple carbon-carbon bond cleavages at successive positions along the chain to give a series of peaks representing $[C_nH_{2n+1}]^+$ fragments:




A second series of peaks of lower intensity is produced by ions of the formula $[C_nH_{2n-1}]^+$. These ions arise by loss of two hydrogen atoms (probably as a hydrogen molecule) from the $[C_nH_{2n+1}]^+$ series ions.[8] Peaks corresponding to $[C_nH_{2n}]^+$ are also evident at low molecular

weights but are of very low intensity. Studies of a great number of saturated hydrocarbons have shown that the intensities of fragments of m/e > 100 are very small. Thus the length of the hydrocarbon chain in phacidin cannot be deduced from the mass spectrum.

(d) Infrared Spectrum (Fig. 3)

The infrared spectrum of phacidin (1% in KBr) shows the absence of absorptions due to such groups as hydroxyls, acetylenes, and anhydrides. The absorptions are usually assigned as follows[9]: 3110 $cm^{-1}$ (w) $\nu_{CH}$ for an aromatic ring or proton on a double bond; 2975 (sh), 2940 (s) $\nu_{as}$ CH for methyl and methylene groups; 2870 (s) $\nu_{s}$ CH for methyl and methylene groups and/or $\nu_{CH}$ for an aldehyde; 1730 (m), 1700 (s), 1690 (m) all $\nu_{C=O}$ absorptions; 1620 (m), 1530 (m), 1520 (s) all $\nu_{C=C}$ absorptions; 1480 (m), 1340 (m) $\nu_{CH}$ typical of an aromatic methoxy group; 1387 (m) $\delta_{CH}$ for methyl groups; and 1239 (m) $\nu_{as}$ C-O-C and 1041 $\nu_{s}$ C-O-C for vinyl or aromatic ethers. In summary, the infrared spectrum indicates the presence of a number of methyl and methylene groups, three carbonyl groups, at least one alkene, and at least one ether linkage.

(e) Ultraviolet Spectrum (Fig. 4)

The ultraviolet spectrum exhibits absorptions at 224 (log $\varepsilon$ = 4.15), 274 (3.36) and 342 nm (3.88) in 95% EtOH. The longer wavelength absorptions would tend to indicate conjugation between a carbonyl group and one or more carbon-carbon double bonds.

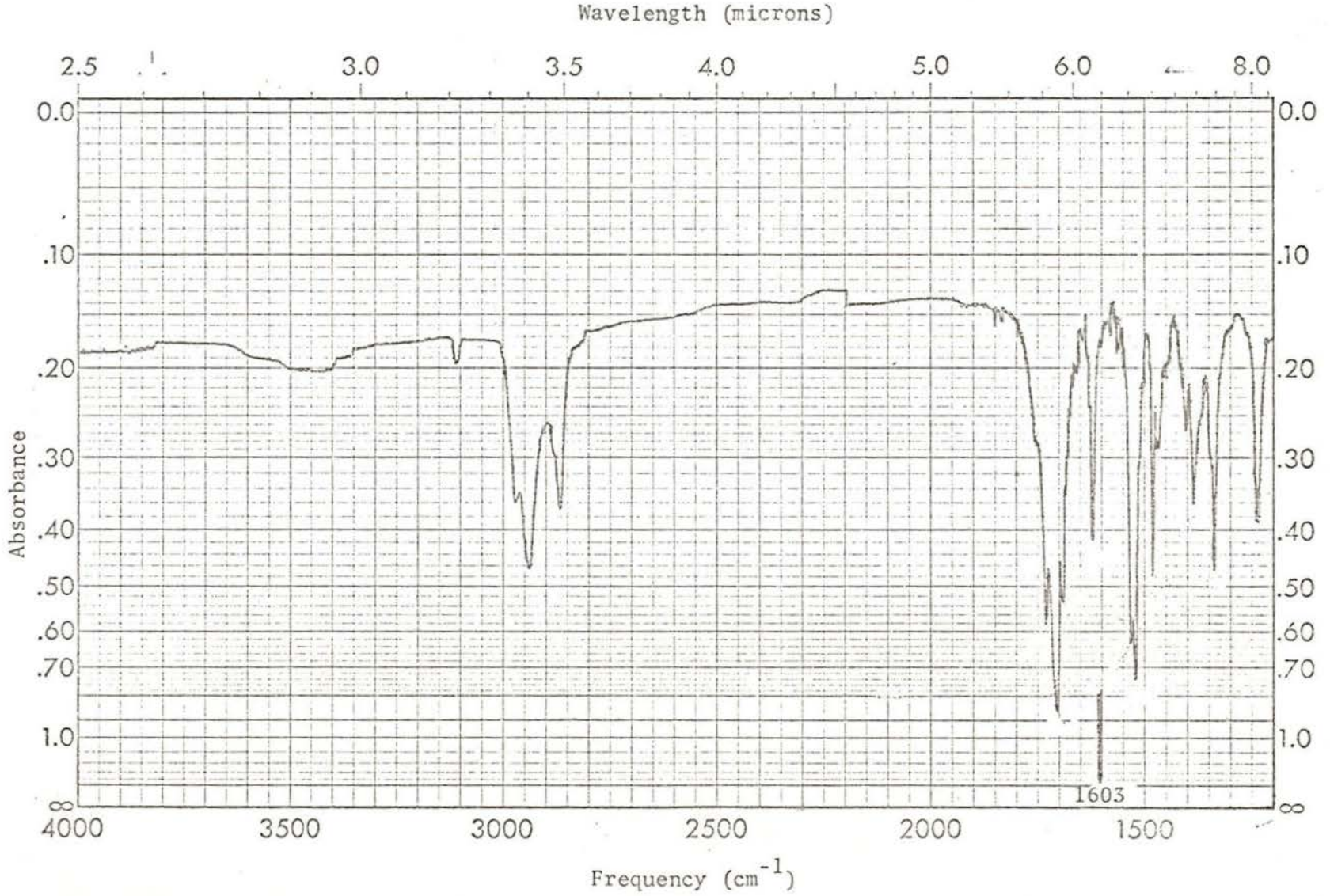



Fig. 3a. Infrared spectrum of phacidin (1% in KBr) 4000-1200 $cm^{-1}$.

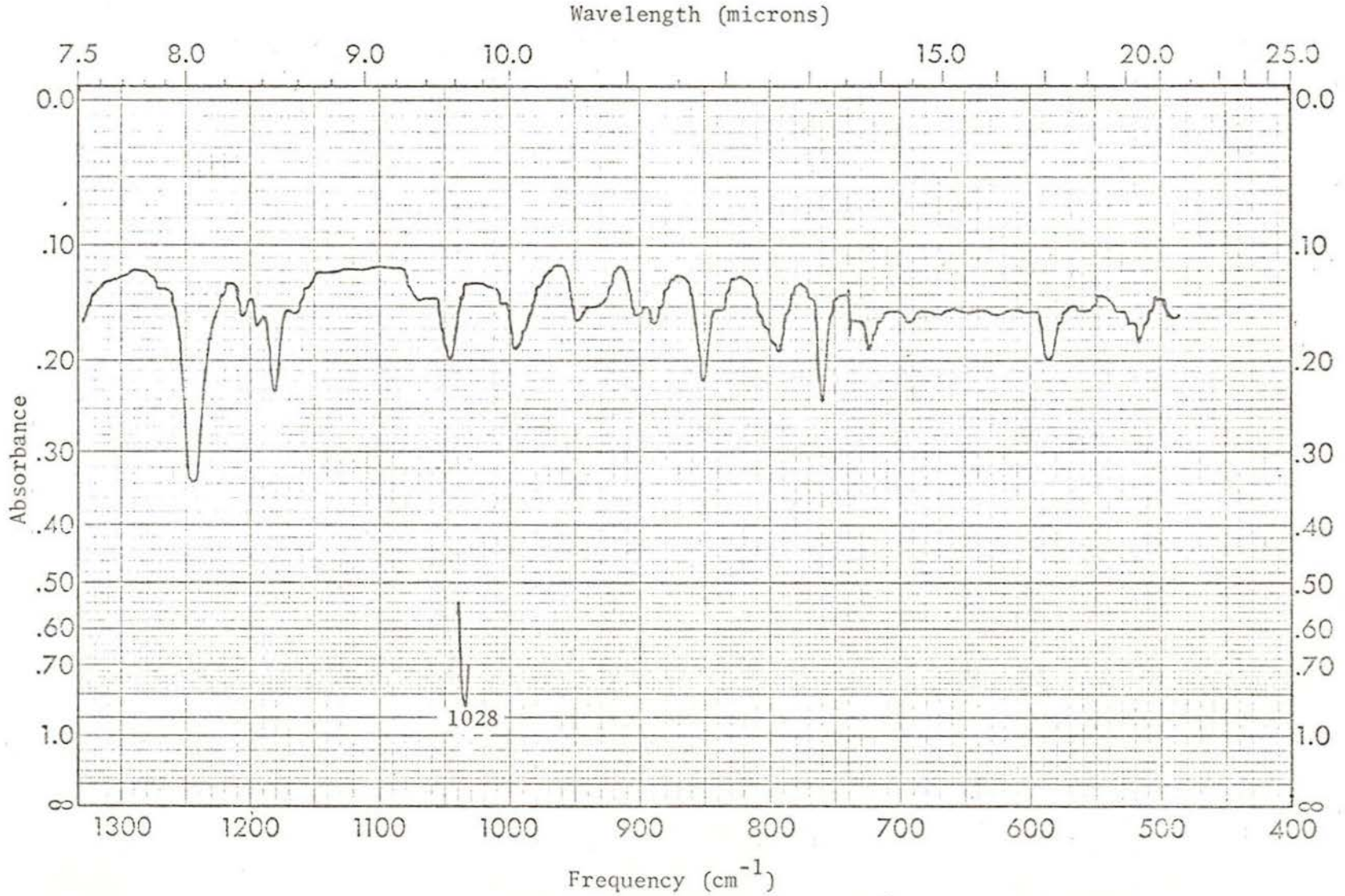



Fig. 3b. Infrared spectrum of phacidin (1% in KBr) 1350-400 cm$^{-1}$.

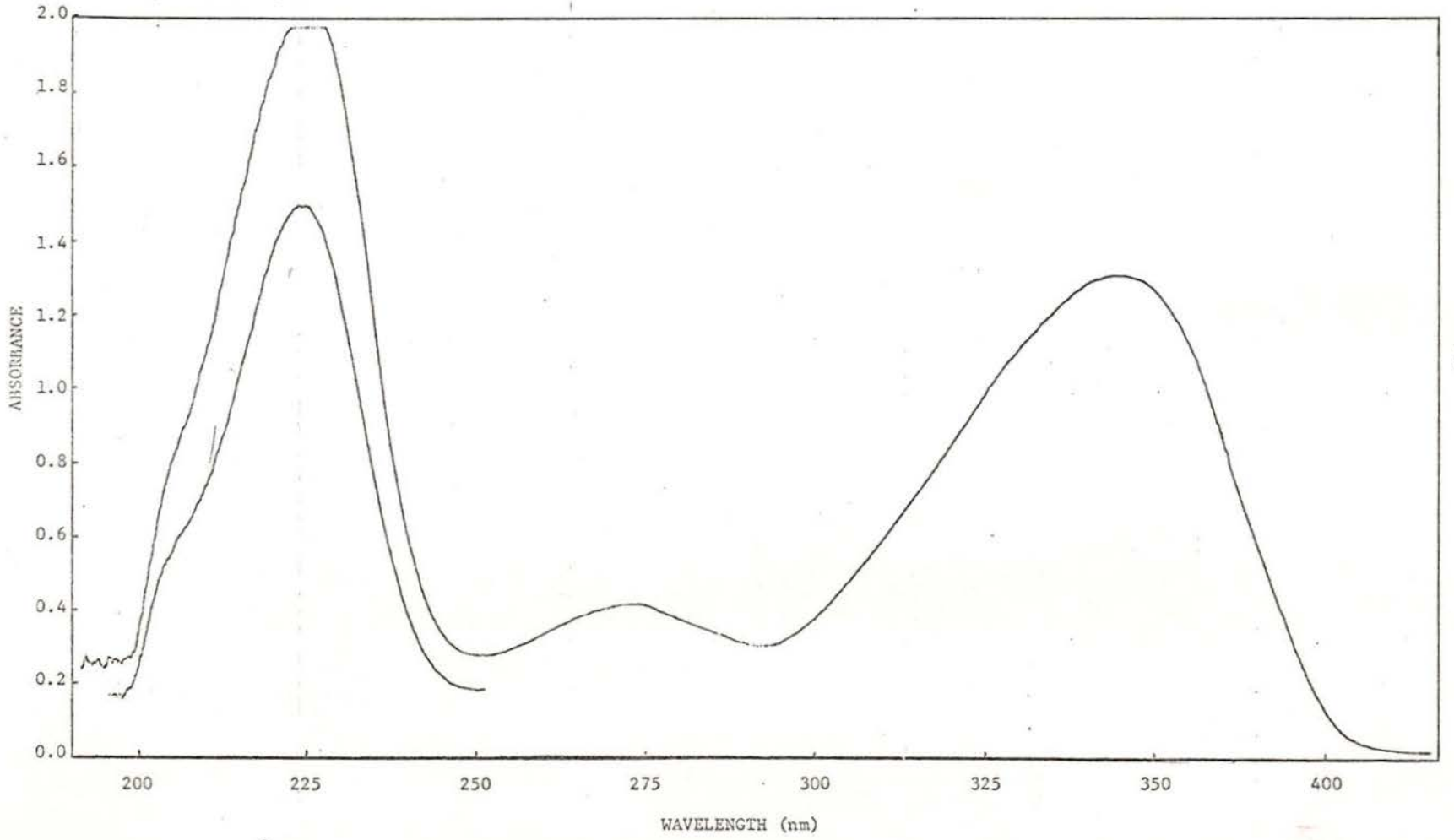



Fig. 4. Ultraviolet spectrum of phacidin (95% EtOH).

(f) Optical Rotation

No optical rotation was displayed by a solution of phacidin in chloroform at a variety of wavelengths (589 to 365 nm). From this, it must be assumed either that phacidin is optically inactive, or that the material with which we are dealing is a racemic mixture, a very unlikely proposition.

2. Chemical Analysis

While phacidin is relatively stable in acids at room temperature, it readily decomposes in bases to give a red oily mixture of products. A similar red oil is formed when the fungus is grown in a shaken culture. Decomposition also occurs on heating (50°) under vacuum for prolonged periods.

Phacidin affords a red phenylhydrazone on treatment with phenylhydrazine hydrochloride and a yellow oxime on treatment with hydroxylamine hydrochloride. It gives a positive Tollens' test, indicative of an aldehyde.

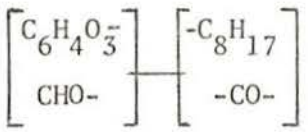
(a) Sodium Borohydride Reduction[10]

Treatment of phacidin with excess sodium borohydride in anhydrous methanol gave a crystalline tetrahydro derivative purified by preparative thin layer chromatography. Reduction of two of the three carbonyl functions to alcohols was evident from the infrared spectrum which showed a broad hydroxyl absorption at 3400 $cm^{-1}$ and a single carbonyl absorption at 1688 $cm^{-1}$. The mass spectrum showed a molecular weight of 298 with predominant water loss (m/e 280), and a base peak at m/e 155.

Since the base peak appears at two mass units higher than it does in phacidin, it can be assumed that one reducible carbonyl group resides in each half of the molecule. Metastable ion peaks support direct loss of 18 (263.1), 29 (242.8) and 143 mass units (80.6) from the parent compound.

(b) Phenylhydrazone Formation

Treatment of phacidin with excess phenylhydrazine hydrochloride reagent gave an immediate red precipitate characterized by mass spectrometry as a monophenylhydrazone ($M^+ = 384$). The infrared spectrum showed an N-H absorption at 3280 $cm^{-1}$, and a broadened carbonyl absorption at 1677 $cm^{-1}$. The NMR spectrum is very similar to that of phacidin with the additional aromatic protons and showing a large shift in the aldehydic proton from $\delta$ 10.17 to $\delta$ 7.87 (normal for a phenylhydrazone). Appearance of the base peak at m/e 243, due to loss of 141 from the molecular ion, indicates formation of the phenylhydrazone in part A of the molecule leaving the second reducible carbonyl group unreacted. It can thus be deduced that there is an aldehyde function in part A, and a ketone function in part B. This latter part must then be $C_8H_{17}CO-$ and therefore contain a saturated hydrocarbon chain.



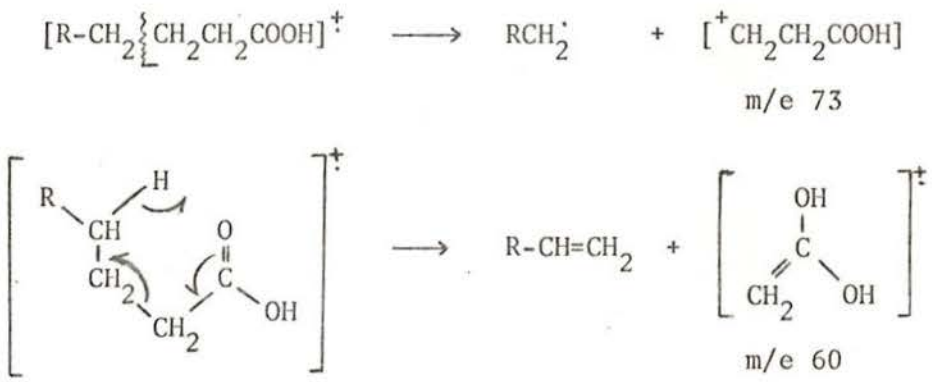

A B

(c) Oxime Formation

Treatment of phacidin with excess hydroxylamine hydrochloride reagent in methanol/water gave yellow needles of a mono-oxime ($M^+ = 309$). The mass spectrum and NMR were consistent with formation of the aldoxime in a reaction analogous to the phenylhydrazone formation ($B^+$ 168 = $M^+$ - 141; 1 H at δ 8.34, shifted upfield from δ 10.17.)

(d) Permanganate Oxidation

A hot solution of phacidin in aqueous acetone reacted immediately with neutral permanganate to give, after thin layer chromatographic separation, a colourless oil. This oil was identified spectrally as n-nonanoic acid and then compared with an authentic sample. The mass spectrum identified the molecular weight as 158 ($C_9H_{18}O_2$) with base peaks at m/e 73 and 60; both characteristic of aliphatic carboxylic acids.




The IR spectrum showed a broad hydroxyl absorption at 3400-2400 $cm^{-1}$ and a carbonyl absorption at 1712 $cm^{-1}$. The NMR spectrum showed a two proton triplet at δ 2.32 for the methylene group adjacent to the acid function. For comparison, an authentic sample of n-nonanoic acid

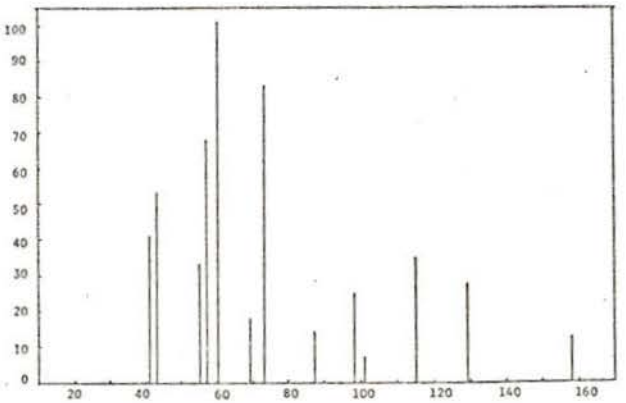
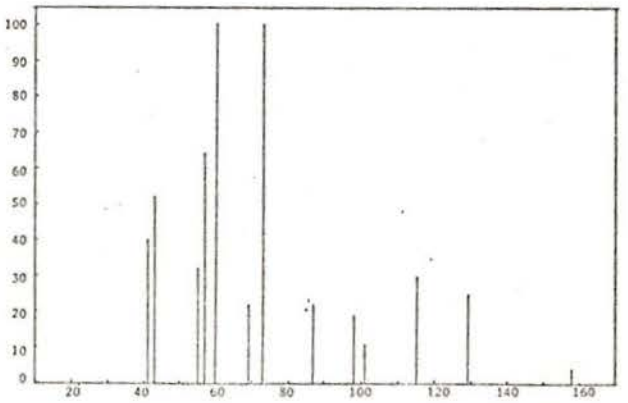
| (a) n-Nonanoic Acid | | (b) Oxidation Product |
|---|---|---|
| | Mass Spectra | |
|  | |  |
| | Accurate Masses | |
| 158.130 | | 158.125 |
| | NMR (δ) | |
| 2.33 (2H t)<br>1.8 to 1.2 (12H m)<br>0.88 (3H t) | | 2.33 (2H t)<br>1.8 to 1.2 (12H m)<br>0.88 (3H t) |
| | Infrared ($cm^{-1}$) | |
| 3400-2400 (broad)<br>2950 (sh)<br>2915 (s) 1285 (m)<br>2845 (m) 1110 (w)<br>1713 (s) 930 (m)<br>1445 (m)<br>1411 (m) | | 3400-2400 (broad)<br>2950 (sh)<br>2920 (s) 1280 (m)<br>2850 (m) 1108 (w)<br>1712 (s) 925 (m)<br>1448 (m)<br>1410 (m) |

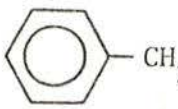
Table IV. Comparison of n-nonanoic acid and the oxidation product of phacidin.

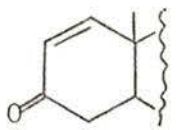
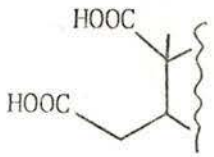
was prepared from n-octanol (see experimental) and was found to be identical to the above sample as shown by the spectra in Table IV.

(e) Ruthenium Tetroxide Oxidation

Since permanganate oxidation gave only one isolable product which could be used to identify half of the phacidin molecule, a method of oxidation was required which would preserve a larger portion of the molecule, or give isolable products of part A of the phacidin molecule. Ruthenium tetroxide is a reactive oxidizing agent which has been used to oxidize aliphatic ethers to esters (a)[11], and to oxidatively cleave aromatic (b)[12] and olefinic (c)[13] double bonds to give diacids:

(a) $R\text{-}CH_2\text{-}O\text{-}R' \xrightarrow[CCl_4]{RuO_4} \underset{\underset{O}{\|}}{RC\text{-}OR'}$ R = n-Pr , R' = n-Bu

(b)  $\xrightarrow[H_2O]{RuO_4}$ $HOOC\text{-}CH_2\text{-}CH_2\text{-}COOH$

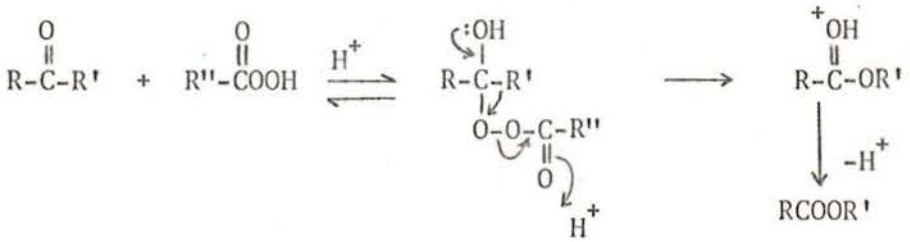
(c)  $\xrightarrow[CH_3COCH_3]{RuO_4}$ 


In a method developed by Wolfe, Hasan and Campbell,[12] ruthenium tetroxide can conveniently be generated by addition of sodium hypochlorite (5%) to a solution of ruthenium trichloride in water.

Since the oxidant can be regenerated either from ruthenium trichloride or ruthenium dioxide (generated in the reaction) by the action of NaOCl, only a catalytic amount of $RuCl_3$ is required. If the reaction is carried out by titrating a solution of reactant and $RuCl_3$ with NaOCl, the end point can readily be detected by persistence of the yellow/orange $RuO_4$ colour. Following the method of Wolfe et al., phacidin reacted with $RuO_4$ to again give n-nonanoic acid as the only isolable product.

(f) Baeyer-Villiger Rearrangement

The Baeyer-Villiger rearrangement[14] effects conversion of ketones into esters by treatment with hydrogen peroxide or organic peracids. The mechanism of this reaction involves migration of one of the carbonyl substituents onto oxygen as shown below:



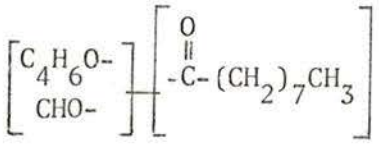
R'' = H, $CH_3$, $CF_3$, m-Cl-$C_6H_4$-

When an unsymmetrical ketone is oxidized, two possible isomeric esters could be formed. Although the more electronegative group normally migrates to the oxygen, steric effects can markedly affect the ease of migration. This reaction appeared to provide a possible route to identification of part A of the molecule, since cleavage of the resulting

ester(s) would give both an alcohol and an acid. The reaction, however, is complicated by the presence of an aldehyde function in phacidin which could also be oxidized in the Baeyer-Villiger reaction to give either an acid or a formate ester. On oxidation of phacidin with m-chloroperbenzoic acid* a complex mixture of products resulted, one of which could be identified as n-nonanoic acid by its NMR spectrum. The other products could not successfully be separated by thin layer chromatography.

Treatment of phacidin with hydrogen peroxide once again gave the same product : n-nonanoic acid.

From the oxidation reactions described, it is clear that part B of the molecule contains a saturated straight chain adjacent to an electron withdrawing group. Combining all available spectral data, it becomes apparent that the portion of the molecule oxidized to n-nonanoic acid must be an n-nonanoyl group [$CH_3$-$(CH_2)_7$-CO-]. This is supported by the facile loss of 141 ($C_9H_{17}O$) in the mass spectrometer, the NMR spectrum ($\delta$ 2.96, 2H t, -$CH_2\underline{CH_2}$CO-; $\delta$ 1.3, 12 H m, -$(\underline{CH_2})_6$-; $\delta$ 0.88, 3 H t, -$CH_2$-$\underline{CH_3}$), and the infrared spectrum (strong $\nu_{CH_2,CH_3}$ in the 3000-2800 $cm^{-1}$ region, $\nu_{C=O}$ 1700 $cm^{-1}$).

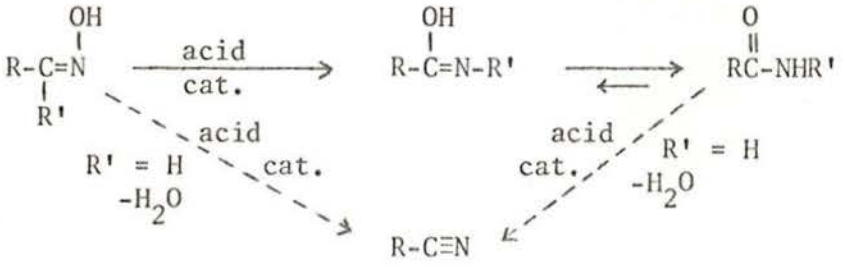


A B

* Prepared and standardized by the method of R.N. McDonald, R.N. Steppel, and J.E. Dorsey, Org. Synthesis, 50, 15 (1970).

(g) Beckmann Rearrangement of Phacidin Oxime

In the Beckmann rearrangement, Lewis acids catalyse the conversion of oximes to amides. In the case of aldoximes (R' = H), Lewis acids can also catalyse the dehydration of the oxime to a nitrile, although whether the dehydration proceeds directly or via the intermediate primary amide is not well known.




Upon treatment with $PCl_5$, phacidin oxime gave a product with a strong absorption in the infrared spectrum at 2245 $cm^{-1}$, indicative of a nitrile group ($\nu_{C\equiv N}$) . This was also accompanied by loss of the hydroxyl absorption as well as loss of the aldoxime proton in the NMR spectrum. The mass spectrum was also consistent with formation of the dehydration product ($M^+$=291; $B^+$= 150). This evidence confirms the presence of an aldehyde function in phacidin.

(h) Catalytic Hydrogenation

Catalytic hydrogenation was carried out using either 10% palladium on charcoal or platinum oxide as catalyst. Both reactions gave complex mixtures of hydrogenation products contaminated by highly coloured decomposition products. Separation by thin layer chromatography afforded a few milligrams of each of the following products:

(i) demethylphacidin ($M^+$ = 280), which has a base peak in the mass spectrum at m/e 139 indicating loss of 141 mass units, the nonanoyl group. This can be interpreted as hydrogenolysis of a methoxyl substituent leaving a hydroxyl group ($\nu_{OH}$ 3400 $cm^{-1}$).

(ii) demethyldihydrophacidin ($M^+$ = 282) which, upon electron impact, splits in half to give a base peak at m/e 141. This corresponds to hydrogenation of an alkene in product (i); the product also shows a broad OH stretch in the infrared spectrum. Neither (i) nor (ii) shows loss of 32 mass units from the base peak in the mass spectrum as does the parent compound which contains a methoxyl substituent.

(iii) dihydrophacidin ($M^+$ = 296), which was isolated in very small quantity, has a base peak in the mass spectrum at m/e 155, also corresponding to loss of the n-nonanoyl group ($M^+$ = 141). Clearly then, one unsaturation has been reduced in part A of the molecule. Appearance of a hydroxyl stretch in the infrared spectrum ($\nu_{OH}$ 3400 $cm^{-1}$) and loss of the carbonyl stretch at 1725 $cm^{-1}$ indicates reduction of one of the carbonyl groups to an alcohol. The mass spectrum shows facile loss of $H_2O$ from the molecular ion ($M^+$ - 18), also indicative of an alcohol.

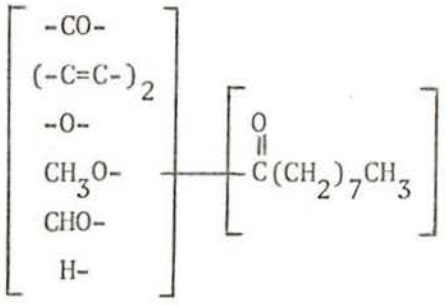
(iv) A tetrahydrophacidin ($M^+$ = 298), which loses 143 in the mass spectrum to give a base peak at m/e 155. Reduction of one unsaturation has occurred in each part of the molecule. Comparison of infrared and mass spectra with those of the product of sodium

borohydride reduction indicates that the same product, resulting from reduction of the two reactive carbonyl functions, has been formed (see experimental).

(v) a second tetrahydrophacidin ($M^+$ = 298), which was different from (iv). The infrared spectrum indicates that the reactive carbonyl functions are retained ($\nu_{C=O}$ 1724, 1700 $cm^{-1}$); thus, reduction of two carbon-carbon double bonds must have occurred. A base peak in the mass spectrum at m/e 113, and smaller peaks at m/e 141 and 157 imply that the relative stability of part A has been greatly decreased on reduction of both carbon-carbon double bonds.

While it is evident that there are two carbon-carbon double bonds present in the molecule, they are indeed difficult to reduce. Hydrogenolysis and reduction of the carbonyl groups occur in competitive reactions. Throughout the reduction reactions (both catalytic and non-catalytic) there remains one carbonyl group in part A which is not reduced.

A more complete partial structure can now be written which accounts for all of the atoms as components of functional groups or as substituents:

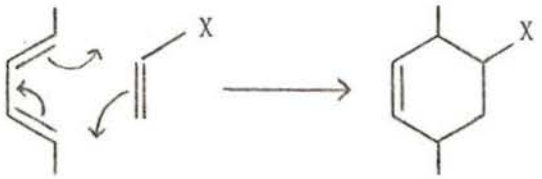



A B

We then needed to distinguish between conjugated and isolated carbon-carbon double bonds, since conjugation of some type is indicated by the strong absorptions in the ultraviolet spectrum.

(i) The Diels-Alder Reaction

Dienes in which the double bonds are conjugated and can attain a cisoid configuration normally react with dienophiles (activated double bonds) to form Diels-Alder adducts



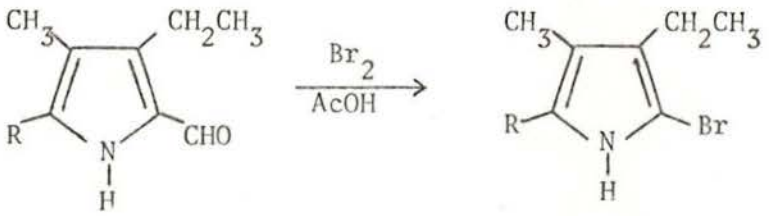

X = activating group

To determine whether the two double bonds in phacidin were in conjugation and cis, the antibiotic was treated with maleic anhydride and allowed to react over a week. No reaction occurred, indicating that phacidin does not contain a cisoid diene system.

(j) Bromination

An initial test for unsaturation in phacidin resulted in slow decolourization of a solution of bromine in carbon tetrachloride. However, attempted thin-layer chromatographic separation of the products resulted in decomposition. When a saturated solution of bromine in water was used, a crystalline solid could be isolated from a methanolic solution of phacidin.[15] The bromination product would not form an oxime under the conditions used to form the mono-oxime

of phacidin. From the mass spectrum ($M^+$=344, $C_{15}H_{21}O_4{}^{79}Br_1$) and NMR spectrum (no aldehyde proton), the product was identified as a bromination-deformylation product in which one bromine atom had replaced the formyl group. Bromination-deformylation is not unknown; for example, Markovac and MacDonald[16] have reported the following transformation:




(k) Hydrolysis[15]

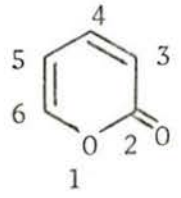
Treatment of a methanolic solution of phacidin with 20% HCl yielded a product with a molecular weight of 280, corresponding to loss of a methyl group. Like the hydrogenation product [h(i)], it shows a base peak at m/e 139 in the mass spectrum which indicates loss of the methyl group from part A of the molecule. An orange mono-phenylhydrazone derivative of this product was readily formed with spectral data confirming derivatization of the aldehyde function.

3. Structure Elucidation

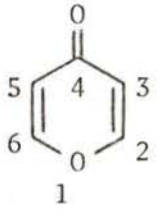
To summarize the evidence gathered from spectral data and chemical reactions, it is apparent that phacidin must contain (i) a nonanoyl group (part B), (ii) an aldehyde function which is readily replaced by bromine, (iii) a methoxyl group which is particularly labile, (iv) an unreactive carbonyl function and (v) two carbon-carbon double bonds not in conjugation with each other and difficult to reduce. Together with these functional groups, phacidin has a single proton which is not coupled and appears in the olefinic-aromatic region of the NMR spectrum. This leaves a single oxygen atom which must, therefore, exist as an ether linkage to complete the molecular formula of $C_{16}H_{22}O_5$.

(a) γ-Pyrone Nucleus

Phacidin thus appears to be composed of a highly unsaturated nucleus around which several substituents are arranged. There is one class of optically inactive, naturally occurring compounds which is consistent with the data, namely the pyrones. There are two isomeric pyrones:

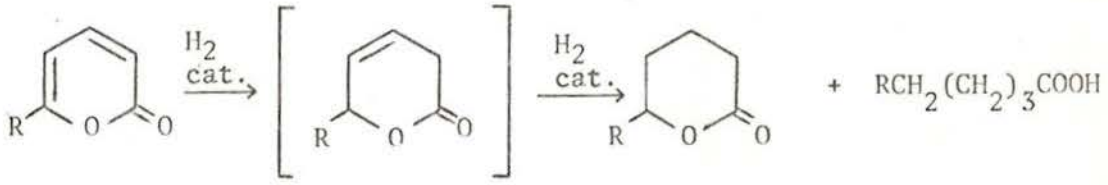



α-pyrone
2H-pyran-2-one




γ-pyrone
4H-pyran-4-one

In general, both of these types of compounds are relatively stable in acid but are cleaved or decomposed in base (see Part II, Introduction, for a more complete discussion of reactions). α-Pyrones are noted for the ease with which they undergo Diels-Alder addition reactions with maleic anhydride,[17] while γ-pyrones do not react at all. On catalytic hydrogenation,[18] α-pyrones act as dienes which first add hydrogen 1,4 then are either completely reduced to saturated lactones or, as allylic esters, suffer hydrogenolysis to give saturated acids.




γ-Pyrones, on the other hand, give complex mixtures of products which may contain the di- or tetrahydropyrones but which are difficult to separate. Similarly, bromine addition to the double bonds of γ-pyrones does not occur. Instead, substitution similar to that in aromatic systems takes place. Both α- and γ-pyrones suffer ring cleavage in base, as do normal lactones (γ -pyrone can be considered a vinylogue of a lactone), however the results of base attack on γ-pyrones are normally much more severe than the corresponding attack on α-pyrones. Neither α- nor γ-pyrones give normal carbonyl reactions and both are generally resistent to chemical oxidation and reduction.

Considering the chemical reactions of both α- and γ-pyrones, phacidin best fits the description of a γ-pyrone nucleus, around which are

arranged the three substituents and the single proton. Examination of the spectral properties of both $\alpha$- and $\gamma$-pyrones gives further support for the $\gamma$-pyrone nature of phacidin.

(i) Infrared Spectra[19,20]

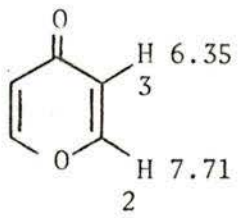
Support for the $\gamma$-pyrone structure is found in the non-reactive carbonyl absorption at 1690 $cm^{-1}$. In $\gamma$-pyrones, this absorption occurs at 1690-1650 $cm^{-1}$ whereas, in $\alpha$-pyrones, it occurs at 1750-1720 $cm^{-1}$ (reglardless of the substituents). The two carbon-carbon double bond absorptions occur in the region 1650-1540 $cm^{-1}$ for both types of pyrone.

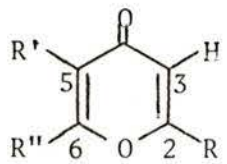
(ii) Mass Spectra[21]

Although the mass spectra of $\alpha$- and $\gamma$-pyrones have been investigated by use of various labelling techniques, distinction is not possible since, in both types, a major fragmentation involves expulsion of carbon monoxide ($M^+$-28) (although this fragmentation is less important in the $\gamma$-pyrones). Phacidin shows loss of 28 mass units from both the parent molecular ion (294 $\longrightarrow$ 266, m* 240.7), and the base peak (153 $\longrightarrow$ 125, m* 102.1), and in the absence of both isomeric pyrones, no definite assignment can be made on this basis. Fragmentation of the pyrones depends greatly upon the nature and position of substituents on the rings, and can thus not be readily used in structure determination.

(iii) Nuclear Magnetic Resonance Spectra (NMR)[22]

It is difficult to distinguish between the isomeric α- and γ-pyrones by means of NMR spectroscopy. Protons of both isomers appear in the aromatic region, well below the position expected for protons on "normal" double bonds (δ 4.6 to 5.9).[23] In γ-pyrones, protons at $C_2$ and $C_6$ appear at lower field (δ 7.5 to 8.2) than do those at $C_3$ and $C_5$ (δ6.1 to 7.0).* A similar situation arises for the α-pyrones where $C_4$ and $C_6$ protons are found downfield (δ 7.3 to 8.8) of the protons on $C_3$ and $C_5$ (δ 6.0 to 6.5). The chemical shifts of the γ-pyrone protons are affected greatly by substituents on the same side of the ring, and only slightly by substituents on the opposite side of the ring as these examples illustrate.**




| R | $\Delta\delta_{2,3}$ |
|---|---|
| -H | 0 |
| -CHO | +0.65 |
| -CN | +0.57 |
| $-CH_3$ | -0.21 |
| **R'** | **$\Delta\delta_{5,3}$** |
| -CONHAr | +0.16 to +0.22 |
| **R"** | **$\Delta\delta_{6,3}$** |
| -COOH | +0.17 |
| $-CH_3$ | -0.08 |

Table III. Effects of substituents on the chemical shifts of γ-pyrone protons.

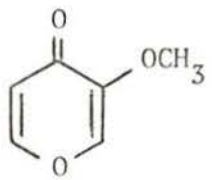
* A table of observed shifts is appended.

** Calculations by G.A. Poulton.
$\Delta\delta_{2,3}$ = change in chemical shift of the proton at $C_3$ due to the effect of the substituent at $C_2$ (relative to γ-pyrone $-C_3-H$ at δ 6.35). The effects of other substituents on the ring have been accounted for. A negative value denotes an upfield shift; positive, a downfield shift.

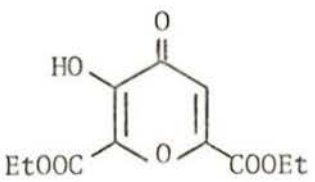
It is evident that electron withdrawing groups deshield their adjacent protons. For this reason, the proton which is found at δ 7.11 in the NMR of phacidin must be located at $C_{3(5)}$ since all of the substituents are expected to be deshielding. In order for the deshielding to be large enough, there must be a carbonyl substituent at $C_2$. A second carbonyl substituent at $C_5$ would account for additional deshielding, placing the proton in the region in which it is observed in the NMR of phacidin.

(iv) Ultraviolet Spectra[20]

γ-Pyrones absorb in the region of 250 nm, with substitution shifting this band to longer wavelength ($\lambda_{max}$ = 250 to 280 nm). Some substituted γ-pyrones exhibit an additional absorption at longer wavelength[24]:




$\lambda_{max}$ 258, 310-350 (heptane)




$\lambda_{max}$ 236, 302, 370 (heptane)

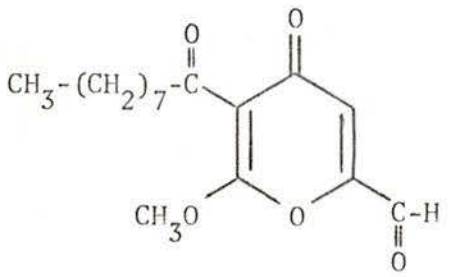
α-Pyrones normally absorb between 280 and 330 nm, the longer wavelength band reflecting the extended conjugation of the α-pyrone ring. Substitution effects are similar to those of γ-pyrones and extinction coefficients for both systems are essentially the same (log ε = 3.7 to 4.1). Phacidin exhibits absorptions at 224, 274 and 342 nm. Distinction between substituted α- and γ-pyrones is not generally possible unless both isomers are at hand.

(v) Optical Activity

It is obvious from the structures of the pyrones that they will be optically inactive if their substituents have no centers of asymmetry. This is true of phacidin.

(b) Proposed Structure of Phacidin

After careful consideration of spectral and chemical properties of phacidin and a number of α- and δ-pyrones, we propose the following structure for phacidin:



Although there are twelve possible isomers for a γ-pyrone nucleus having four different substituents, the proposed structure was chosen for the following reasons:

(i) as explained previously, the ring proton is placed at $C_3$ since protons at $C_2$ occur at lower field (δ 7.5 to 8.2) and any of the substituents would deshield a proton causing the resonance to appear at even lower field.

(ii) the group adjacent to the proton must be the formyl group. Analogous halide exchange reactions have been carried out on γ-pyrone derivatives having $C_2$ formyl groups (see Part II, Results and Discussion).

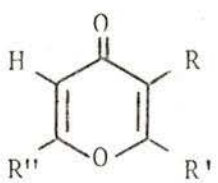
The methoxy group could not be placed at $C_2$ adjacent to the proton since it is not normally deshielding enough to account for the observed shift (from δ 6.5 to 7.11).

(iii) the methoxy group must be placed at $C_6$ since it is particularly labile to acid. From work described in Part II, we have found that methoxy substituents at $C_3$ or $C_5$ are particularly stable and that these compounds exhibit a characteristic loss of 18 mass units ($M^+$ -18) in the mass spectrometer. Phacidin does not.

(iv) thus, the n-nonanoyl group must occupy the $C_5$ position to give the final structure 6-methoxy-5-n-nonanoyl-4-oxo-4H-pyran-2-carboxaldehyde.

(c) Occurence of γ-Pyrones in Nature

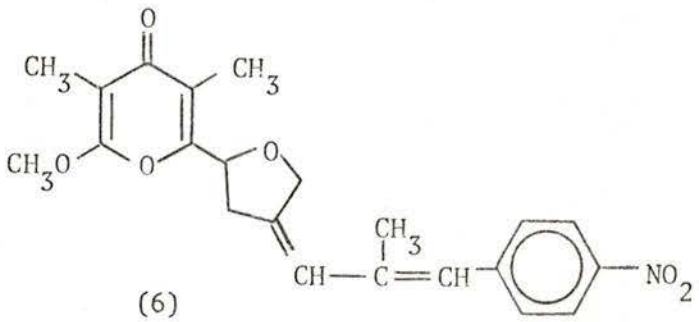
While some γ-pyrones have long been recognized as plant products, the major source now appears to be the fungi.[25] Some of the well known naturally occurring γ-pyrones include chelidonic acid (1) from plants of more than one hundred genera, meconic acid (2) from the waste liquors of opium manufacture, comenic acid (3), from Acetobacter action on glucose, maltol (4), from larch and silver fir needles, and kojic acid (5) from fermentation of a number of substrates by fungi of the genus Aspergillus.




(1) R = H, R' = R'' = COOH
(2) R = OH, R' = R'' = COOH
(3) R = OH, R' = H, R'' = COOH
(4) R = OH, R' = $CH_3$, R'' = H
(5) R = OH, R' = H, R'' = $CH_2OH$

Kojic acid is by far the most extensively studied of the monocyclic γ-pyrones although no commercial use has yet been made of it. First discovered by Saito[26] during investigation of the fermentation of steamed rice (koji) by A. oryzae, it was later identified by Yabuta[27] as having the γ-pyrone nucleus. Extensive investigation into the biosynthesis of kojic acid[28] indicates that a number of fungi can produce the compound from substrates varying in C-skeleton size from $C_2$ to $C_{18}$ and larger polysaccharides. Labelling studies by Arnstein and Bentley[29] indicate that Aspergillus fungi convert D-glucose directly to the γ-pyrone without ring opening.

Several other γ-pyrones have been isolated and identified in recent years, most of which are biologically active. Aureothin,[30] produced by Streptococcus thioluteus has the structure (6) and is very toxic to animals.



(6)

(d) Conclusions

In conclusion, the structure which we have proposed for phacidin fits the spectral data, and is supported by chemical evidence accumulated from a number of degradative reactions. The structure seems reasonable in the light of the occurrence of γ-pyrones in nature and their production by fungi. The small quantities of antibiotic available,

however, made the task of separating and identifying the products of many reactions very difficult, and severely limited us in the execution of further reactions. With increased production of phacidin expected in the near future, these problems may be resolved and the structure proved beyond question.

## EXPERIMENTAL

Mass spectra were obtained using a Hitachi Perkin-Elmer RMU-7 mass spectrometer at an ionizing voltage of 70 eV (except where indicated otherwise) and metastable ions were studied in the defocused mode by variation of sector potential. The number in parenthesis following each m/e value indicates its percentage relative to the base peak (100%). Infrared spectra were recorded on a Beckman IR-20 and calibrated with polystyrene. Proton magnetic resonance spectra were determined in deuterochloroform containing 1% (v/v ) tetramethylsilane (TMS) (unless otherwise specified) using a Varian HA-60-IL, a Perkin-Elmer R 12 A (60 MHz) or a Perkin-Elmer R 32 (90 MHz) NMR spectrometer. Chemical shifts are given as $\delta$ values relative to TMS (0.00 ppm), the internal standard. Ultraviolet spectra were recorded in 95% ethanol (except where noted) on a Unicam SP 800B spectrophotometer.

Melting points were determined using a Monoscop VS apparatus and are uncorrected. Preparative thin layer chromatography was carried out on Camag Silica Gel (DS-5) activated at 100°. All samples were eluted using a 1:1 mixture of chloroform and ethyl acetate (v/v) and components detected by ultraviolet light. Compounds are listed in order of elution, those travelling the farthest being listed first. Column chromatography was carried out on Baker Analysed Silica Gel (40-140

mesh).

In some cases, the nomenclature does not conform to I.U.P.A.C. rules. The numbering scheme used places at position 2 of the γ-pyrone ring the substituent whose ultimate fate is to become the aldehyde function. This approach was taken to allow reaction sequences to be more easily followed.

1. Phacidin Oxime

A solution of phacidin (0.050 g, $1.7 \times 10^{-4}$ mole) in methanol (5 $cm^3$) was treated with hydroxylamine hydrochloride reagent* and a yellow precipitate formed immediately. The mixture was allowed to stand overnight, and then the precipitate was filtered and dried (0.034 g, 69%) yellow plates mp 173-175°. $\nu_{max}$ 3265 (broad), 1727 (s), 1695 (s), 1628 (m), 1392 $cm^{-1}$ (s); δ (accumulated in $CDCl_3$) 8.34 (1H s, -C<u>H</u>=NOH), 7.12 (1H s, -$C_3$-<u>H</u>), 4.06 (3H s, -O<u>$CH_3$</u>), 2.95 (2H t, -$CH_2$-<u>$CH_2$</u>-CO-), 2.0 to 1.2 (12H m, $\{CH_2\}_6$), 0.88 (3H t, -$CH_2$-<u>$CH_3$</u>); m/e 309 ($M^+$, 6), 291 ($M^+$-$H_2O$, 3), 263(2), 207(3), 194(7), 193(10), 168 ($M^+$-141, 100), 152(60), 150(74), 141 ($M^+$-168, 6), 125(10).

2. Permanganate Oxidation of Phacidin

A solution of potassium permanganate (0.071 g, $4.5 \times 10^{-4}$ mole) in water (10 $cm^3$) was added dropwise to a warm (70°), stirred solution of phacidin (0.081 g, $2.8 \times 10^{-4}$ mole) in aqueous acetone (5 $cm^3$ acetone,

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* This reagent was prepared by dissolving 0.100 g hydroxylamine hydrochloride and 0.150 g sodium acetate in 4 $cm^3$ 50% aqueous methanol.

10 $cm^3$ water). Reaction occurred immediately on addition of the permanganate, with precipitation of black $MnO_2$. After refluxing for 15 min, the reaction mixture was made alkaline with a few drops of 10% KOH then filtered to remove the $MnO_2$. No precipitation of product occurred on acidification with aq. HCl. The solution was neutralized, and extracted with diethyl ether which was dried over $MgSO_4$ and evaporated *in vacuo*. The crude product (0.031 g), a yellow oil, was separated by thin layer chromatography into four fractions, the second of which was identified as *n*-nonanoic acid (0.005 g), a colourless oil (see spectra, Table IV).

3. Preparation of *n*-Nonanoic Acid

1-Octanol (10.0 g, 0.077 mole) was cooled to -5° and $PBr_3$ (3 $cm^3$, 0.03 mole) added dropwise with stirring, keeping the temperature below 5°. After allowing the mixture to warm to room temperature, water was added and the reaction mixture extracted with ether. The ether extracts were washed with conc. $H_2SO_4$, and water, then dried over $K_2CO_3/MgSO_4$. Evaporation of the solvent *in vacuo* left a colourless liquid identified spectrally as 1-bromooctane (6.13 g, 42%).

A solution of the 1-bromooctane (6.00 g, 0.031 mole) in anhydrous ether (75 $cm^3$) was added slowly to magnesium powder (1.0 g, 0.041 mole) in ether (25 $cm^3$). Addition of a small crystal of iodine and refluxing were required to initiate the reaction. When most of the magnesium had been consumed, the mixture was cooled to room temperature and poured over dry ice. Water was then added, followed by 25% $H_2SO_4$ (40 $cm^3$), and the brown-orange ether phase separated. The aqueous phase was extracted with ether (3 x 75 $cm^3$) and the combined extracts

washed with water, dried over $MgSO_4$ and evaporated *in vacuo*. The pale yellow oil (2.56 g, 52%) was identified as *n*-nonanoic acid (see spectra Table IV).

4. Ruthenium Tetroxide Oxidation of Phacidin

An aqueous solution of $RuCl_3 \cdot 3H_2O$ (3.6 $cm^3$ [2%], 2.8 x $10^{-4}$ mole) was added to water (15 $cm^3$) and enough NaOCl (29 $cm^3$ [0.5%]) added to produce a clear yellow solution of $RuO_4$. A solution of phacidin (0.075 g, 2.55 x $10^{-4}$ mole) in $CH_2Cl_2$ was added dropwise with rapid stirring, and sufficient NaOCl solution was added to keep the reaction mixture yellow. Each drop of phacidin solution produced a dark precipitate of $RuO_2$ which was reoxidized with NaOCl. The reaction mixture was acidified with HCl, the two phases separated, and the aqueous phase extracted with $CH_2Cl_2$. Extracts were combined, dried over $MgSO_4$, filtered through celite and evaporated *in vacuo*. The product was *n*-nonanoic acid, a colourless oil (0.013 g, 33%). Spectra were identical to those of the *n*-nonanoic acid prepared in the laboratory.

5. Baeyer-Villiger Reaction of Phacidin

To a solution of phacidin (0.058 g, 1.96 x $10^{-4}$ mole) in $CH_2Cl_2$ (15 $cm^3$) was added dropwise a solution of *m*-chloroperbenzoic acid in $CH_2Cl_2$ (10 $cm^3$) (containing 2.03 x $10^{-4}$ mole "active" oxygen)*.

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* The *m*-chloroperbenzoic acid was prepared and "active oxygen" content iodometrically determined by the method of R.N. McDonald, R.N. Steppel, J.E. Dorsey, Org. Syn. 50, 15 (1970).

Stirring was continued for 48 hrs followed by refluxing for 12 hr. After cooling to room temperature the reaction mixture was extracted with NaOH (25 $cm^3$, 0.5 N), brine (2 x 25 $cm^3$) and water (3 x 25 $cm^3$). After drying over $MgSO_4$ and evaporation in vacuo, the ether phase yielded a pale yellow liquid (0.039 g). Purification by thin layer chromatography yielded a buff-coloured oil (0.009 g) identified spectrally as n-nonanoic acid.

6. Hydrogen Peroxide Oxidation of Phacidin

To a solution of phacidin (0.023 g, $7.8 \times 10^{-5}$ mole) in methanol/acetone (1:1), was added hydrogen peroxide (0.20 g [30%], $1.8 \times 10^{-3}$ mole). The mixture was allowed to stir overnight then refluxed for 1 hr. Excess peroxide was destroyed by addition of $MnO_2$, the solution filtered, and the dried solvent evaporated in vacuo. A complex mixture of products resulted, from which n-nonanoic acid could be separated by thin layer chromatography.

7. Beckmann Rearrangement of Phacidin Oxime

Phacidin oxime (0.045 g, $1.45 \times 10^{-4}$ mole) was suspended in ether (50 $cm^3$) and the stirred suspension cooled in an ice bath. Phosphorous pentachloride was added in small portions with rapid stirring until excess remained in the flask. After stirring for an additional hour, the clear solution was decanted and neutralized by rapid stirring with a solution of $Na_2CO_3$ (3 hr). The ether phase was then washed with water, dried over $Na_2SO_4$ and evaporated in vacuo. The product was an off-white semi-solid which could be purified by thin layer chromato-

graphy. The main fraction was identified as a dehydration product of the oxime, phacidin nitrile (0.032 g, 76%), off-white crystals mp 105°. $\nu_{max}$ 2245 (m), 1745 (s), 1715 (s), 1470 (s), 1395 $cm^{-1}$ (s); $\delta$ 7.03 (1H s, $-C_3-\underline{H}$), 4.17 (3H s, $-OC\underline{H}_3$), 2.92 (2H t, $-CH_2-C\underline{H}_2-CO-$), 2.0 to 1.2 (12H m, $\{C\underline{H}_2\}_6$), 0.88 (3H t, $-CH_2C\underline{H}_3$); m/e 291 ($M^+$, 9), 273 ($M^+-H_2O$, 3), 193 (17), 150 ($M^+$-141, 100), 141 (16), 57(42), 43(48).

8. Catalytic Reduction of Phacidin

(a) A solution of phacidin (0.050 g, 1.7 x $10^{-4}$ mole) in benzene (10 $cm^3$) containing 10% palladium on charcoal (0.030 g) was stirred at room temperature in an atmosphere of hydrogen for 24 hr. during which time 11 $cm^3$ hydrogen was taken up. After filtration, and removal of the solvent in vacuo, the brown oily product was separated by thin layer chromatography to yield a few milligrams of each of three products; (i), (ii), and (v).

(b) When the reaction was repeated using a $PtO_2$ catalyst, five products were isolated by thin layer chromatographic separation of the resulting brown oil; (i) to (v).

(i) demethylphacidin [h(i)] $\nu_{max}$ ($CHCl_3$) 3400 (broad), 1718 (m), 1704 (m), 1640 (m), 1465 (m), 1256 $cm^{-1}$ (s); m/e 280 ($M^+$, 7), 252 ($M^+$-28, 4), 139 ($M^+$-141, 100), 111(8), 97(11), 83(29).

(ii) demethyldihydrophacidin [h(ii)] $\nu_{max}$ ($CHCl_3$) 3400 (broad), 1700 (broad), 1660 (w), 1583 (m), 1528 (m), 1475 (m), 1268 $cm^{-1}$ (w);

m/e 282 ($M^+$, 22), 155(24), 141 ($M^+$-141, 100), 139(85), 113(38).

(iii) dihydrophacidin [h(iii)] $\nu_{max}$ ($CHCl_3$) 3400 (broad), 1695 (sl. broadened), 1635 (s), 1558 (w), 1463 (m), 1387 (m), 1255 $cm^{-1}$ (m); m/e 296 ($M^+$, 1), 278 ($M^+$-$H_2O$, 5), 155 ($M^+$-141, 100), 141(17), 127(19), 71(24).

(iv) tetrahydrophacidin [h(iv)] $\nu_{max}$ ($CHCl_3$) 3400 (broad), 1688 (s), 1632 (m), 1658 (m), 1568 (m), 1465 $cm^{-1}$ (m); m/e 298 ($M^+$, 3), 280 ($M^+$-$H_2O$, 19), 255(7), 167(24), 155(80), 139(20), 126(37), 83(43), 44(100).

(v) second tetrahydrophacidin [h(v)] $\nu_{max}$ ($CHCl_3$) 1724 (m), 1709 (m), 1636 (m), 1465 (m), 1385 (m), 1259 $cm^{-1}$ (m); m/e 298 ($M^+$, < 1), 266(1), 145(44), 141(86), 139(49), 113(100), 111(61), 99(37), 83(49).

9. Diels-Alder Reaction

Phacidin (0.054 g, $1.8 \times 10^{-4}$ mole) and maleic anhydride (0.050 g, $5.1 \times 10^{-4}$ mole) were dissolved in p-xylene (10 $cm^3$) and enough benzene added to allow the solution to reflux between 110° and 112°. After refluxing for five hours, the red solution was allowed to stand at room temperature for a week. Additional benzene was added (75 $cm^3$) to dissolve the precipitate which had formed, then the reaction mixture was extracted with aqueous $K_2CO_3$. The aqueous phase was reextracted with benzene and chloroform, and all of the organic extracts were combined, dried over $MgSO_4$ and the solvent removed in vacuo. Unreacted

phacidin was recovered (0.052 g), as identified by mass spectrometry and N.M.R.

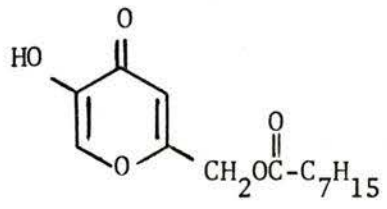
# PART II

# THE PARTIAL SYNTHESES OF PHACIDIN AND ITS ISOMERS

## INTRODUCTION

We have assigned to the nucleus of phacidin a γ-pyrone structure on the basis of spectral and chemical properties (see Part I). However, as in the identification of all previously unknown substances, there remains an element of doubt as to the authenticity of the proposed structure until it can be synthesized in the laboratory from known starting materials. It was thus necessary to carry out a literature study of the methods of preparation and characteristic reactions of γ-pyrones and their derivatives.

Since Wilde[31] first prepared γ-pyrone itself in 1863, a wide variety of substituted γ-pyrones has been synthesized. A number of these have commercial importance, and new and cheaper methods of synthesis are constantly in demand. Extensive work, for example, has been carried out on new syntheses of maltol (4)[32] a flavour enhancer used in high carbohydrate foods. Some γ-pyrones, both natural and synthetic, exhibit antibiotic activity, and their commercial preparation may soon be in demand. For example H. Kato[33] found 7-octanoylkojic acid (6) to be an effective antimicrobial agent for food preservation. More recently, 2-(1,4-dioxocin-6-yl)-4H-pyran-4-one (7) prepared from a fermentation product,[34] has been found to have significant antifungal activity.



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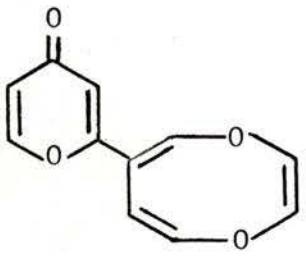


(7)

1. Methods of Preparation of Substituted γ-Pyrones

There are three main routes by which the syntheses of substituted γ-pyrones (such as phacidin) have been approached:

(a) by modification of existing γ- and α-pyrones,

(b) from carbohydrates, and

(c) from acyclic compounds.

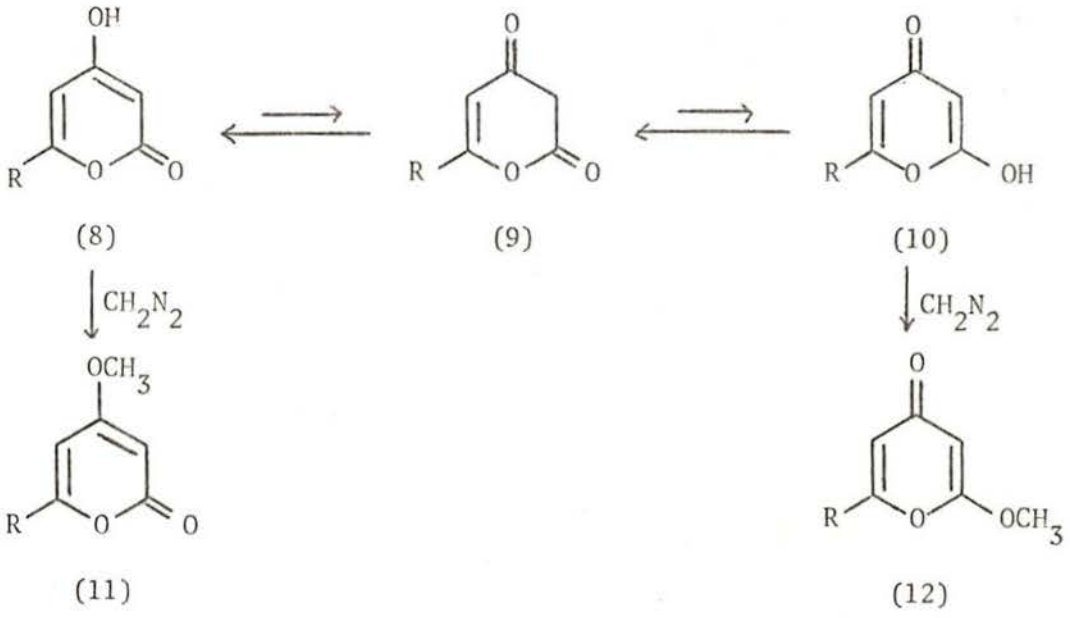
(a) Modification of Existing γ- or α-Pyrones

The first and probably most straightforward method utilizes γ-pyrone itself or a readily available substituted γ-pyrone as the starting material. Thus, by substituting groups onto the ring and modifying existing substituents, the desired product might be obtained. (Specific reactions of γ-pyrones applicable to synthesis will be discussed later in the text.)

There are several naturally occurring γ-pyrones which are commercially available, and from which a great number of the γ-pyrone compounds found in the literature have been derived. Kojic acid (5), maltol (4), chelidonic acid (1), and meconic acid (2) are all obtained

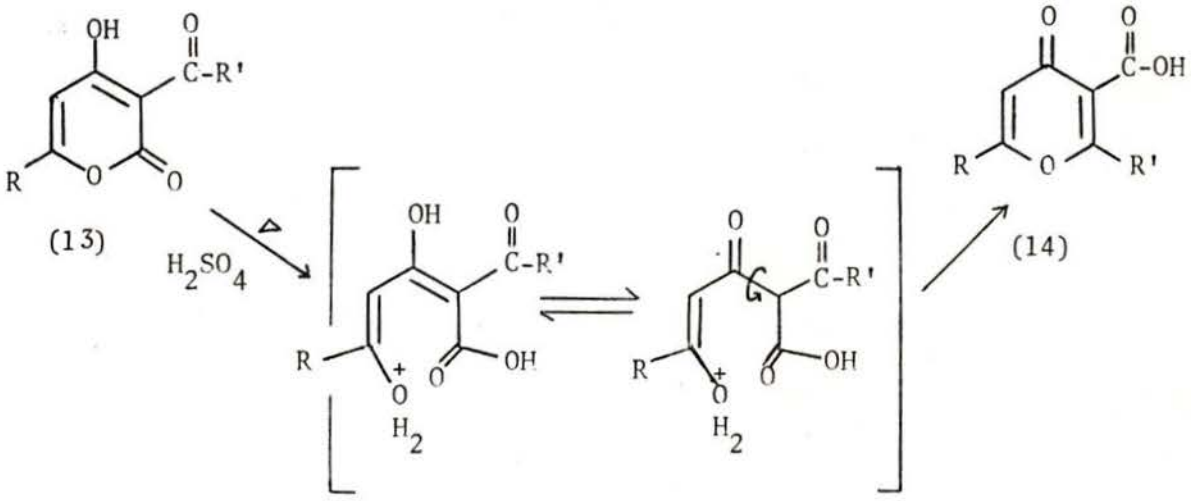
from natural sources and can be purchased quite inexpensively. A number of other naturally occurring γ-pyrones mentioned previously are not available commercially due either to restricted sources or to lack of demand.

It is interesting to note that some α-pyrones can be used to obtain the desired γ-pyrone skeleton. 4-Hydroxy-α-pyrones (8) must be regarded as existing in tautomeric equilibrium with the corresponding ketonic form (9) (a pyronone) and with the 2-hydroxy-γ-pyrone (10).



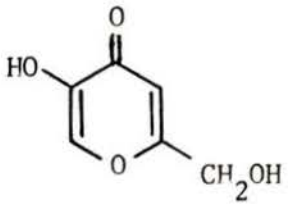

Evidence for this equilibrium comes from diazomethane methylation of triacetic acid lactone[35] (8, R = $CH_3$), which gives not only the 4-methoxy-α-pyrone (11), but also some of the 2-methoxy-γ-pyrone (12). The actual ratio of these two products depends upon the nature of the substituent (R), the solvent used, and the rate of addition of the reagent ($CH_2N_2$), but normally the α-pyrone product predominates.[35]

The desired γ-pyrone nucleus can also be obtained from another 4-hydroxy-α-pyrone which undergoes an interesting rearrangement in concentrated acids. Dehydroacetic acid (13, R = R' = $CH_3$) and its analogues undergo a ring opening reaction in hot concentrated sulfuric acid[36] to give an intermediate which may ring close in the alternate fashion to yield the γ-pyrone product (14).

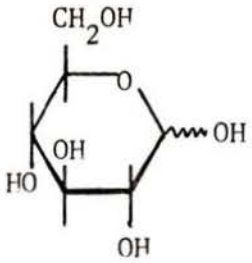



(b) From Carbohydrates

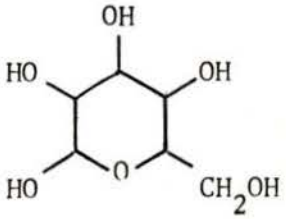
A second method of synthesizing substituted γ-pyrones utilizes monosaccharides as the major starting compounds. This method, in effect, parallels the biosynthesis of γ-pyrones from various carbohydrates. The structural similarity between kojic acid (5) and glucopyranose (15) was recognized as early as 1927 by Kinoshita.[37] Since the γ-pyrone nucleus has no centre of asymmetry, the configuration about each asymmetric centre in the starting pyranose is unimportant. Glucose, and all of its configurational isomers, can then be drawn as (16) and the structural similarity to kojic acid (5) and other γ-pyrones becomes obvious.



(5)

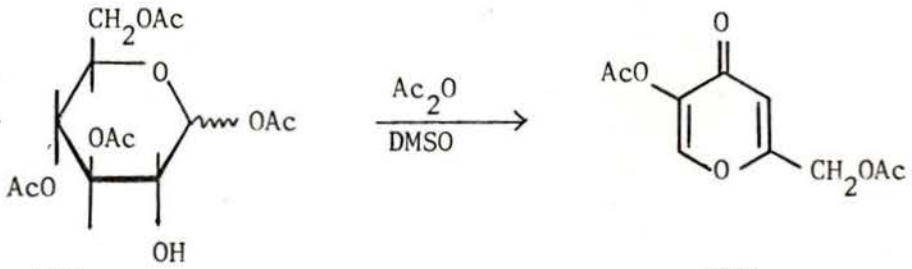


(15)



(16)

My review of the literature concerning the preparation of γ-pyrones from carbohydrates revealed that little work has been reported in this field. Generally low yields have been obtained and complex isolation procedures were required.[38] One notable exception is the facile preparation of the diacetate of kojic acid (17) from 1,3,4,6-tetra-acetylglucopyranose (18, α or β) and acetic anhydride.[39] It is



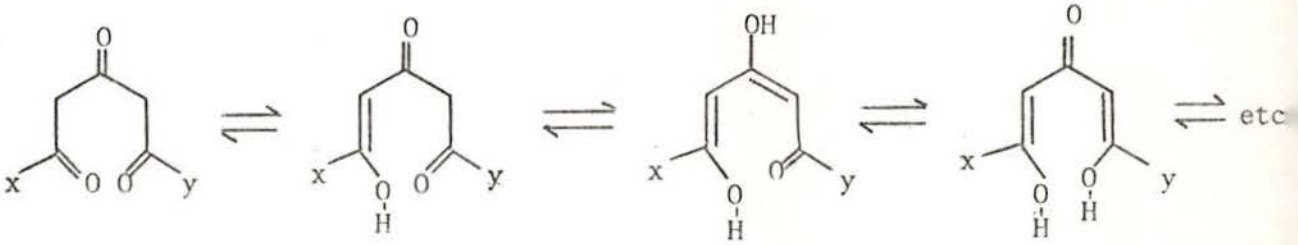
(18) (17)

therefore not surprising that most reports concern only the preparation of simple γ-pyrones from carbohydrates in which little elaboration of the monosaccharide structure, prior to conversion to the pyrone, is required.

(c) From Acyclic Compounds

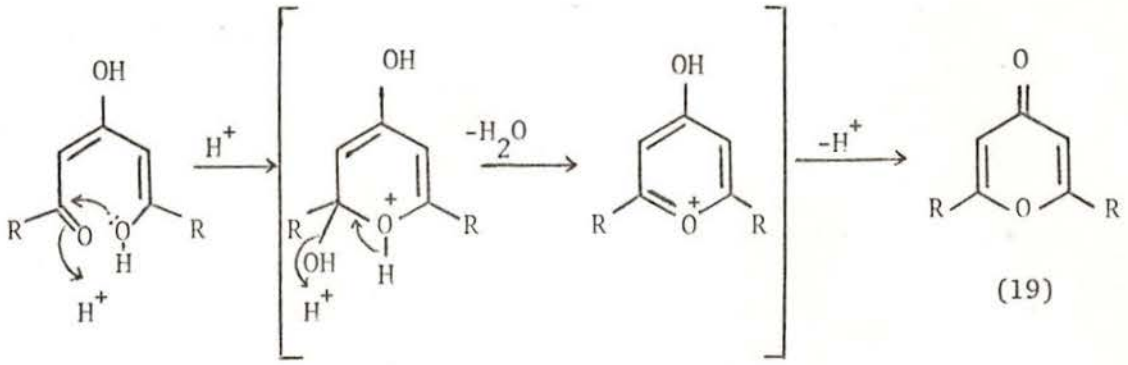
The third general method of preparing substituted γ-pyrones involves the use of acyclic starting materials. Although a wide variety

of ketonic compounds has been utilized over the years to synthesize γ-pyrones, this general method can be greatly simplified by recognizing that most of the reactions involve a 1,3,5-tricarbonyl compound, either as the main reactant or as an intermediate. The tricarbonyl compound may exist as such, or as one of its enol tautomers. Such



x,y = R, Ar, -COR

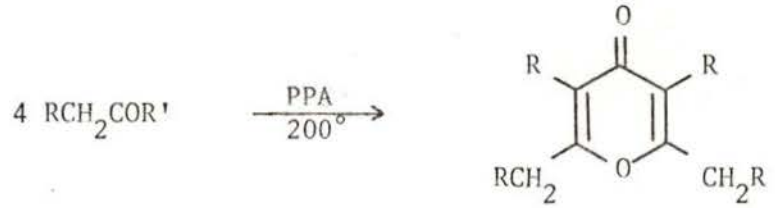
tricarbonyl compounds are well known to be very reactive and often intermediates such as acetylenes, vinyl ethers, and vinyl halides are used and converted to the triketone intermediate <u>in situ</u>. It is now well known that 1,3,5-tricarbonyl compounds readily dehydrate and cyclize upon treatment with acids, a reaction which can be reversed by treatment with base. A variety of acids has been successfully employed including cold concentrated sulfuric acid,[40] liquid hydrofluoric acid,[41] hydrochloric acid,[42] phosphorous pentachloride,[43] and polyphosphoric acid.[44,45]



(19)

for (19), R = COOH chelidonic acid
= COOEt diethyl chelidonate
= OH 2,6-dihydroxy-γ-pyrone
= H γ-pyrone
= $CH_3$ 2,6-dimethyl-γ-pyrone
= $C_6H_5$ 2,6-diphenyl-γ-pyrone

In independent studies of the reactions of aliphatic carboxylic acids and their anhydrides in polyphosphoric acid (PPA), both Sagredos[44] and Mullock and Suschitzky[45] found a convenient method of synthesizing symmetrically tetrasubstituted γ-pyrones (20).

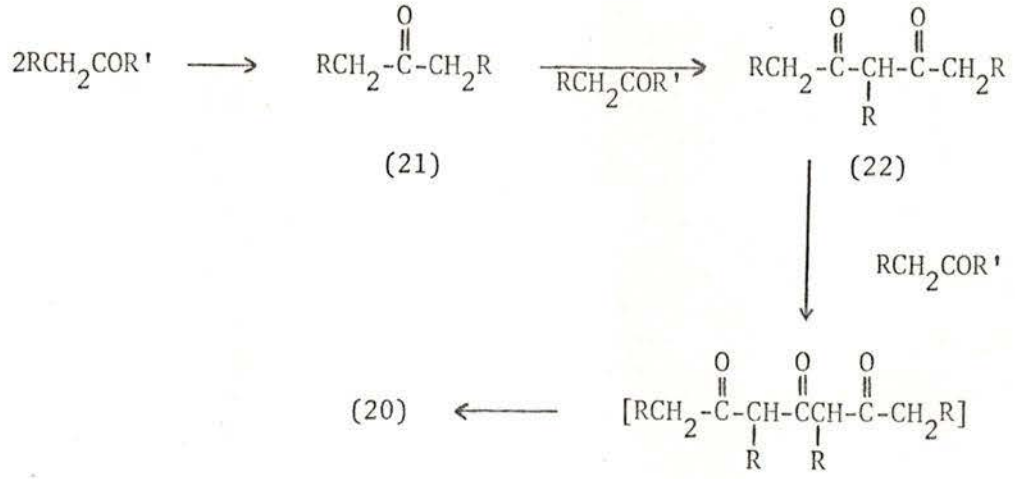


(20)

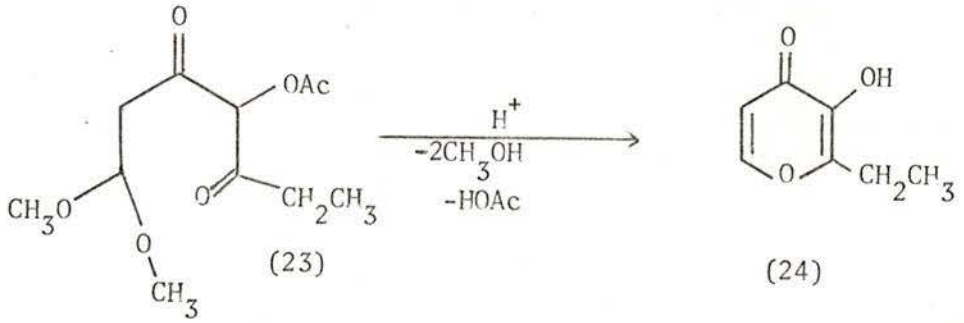
R = $-(CH_2)_nCH_3$ n = 0 to 5,7

R'= -OH, -Cl, $-NH_2$, -OEt, $-OCH_3$, -OCOR

The isolation of ketonic intermediates (21) and (22) by Sagredos supports his mechanistic proposal of a 1,3,5-tricarbonyl intermediate.

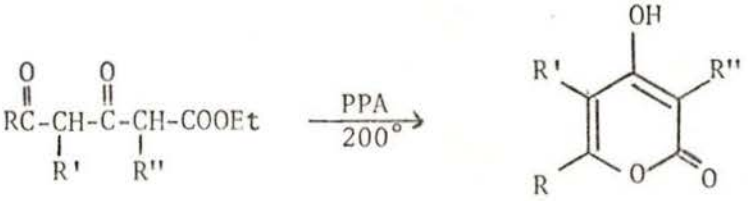


These two methods of preparation lead to symmetrically substituted γ-pyrones. By using an unsymmetrical tricarbonyl compound, however, γ-pyrone products having the desired substitution pattern might be produced. An acetal derivative of γ-acetoxy-β,δ-dioxoheptanal (23) was used by Higuchi and Suzuki[46] to prepare a flavouring agent similar to maltol: 2-ethyl-3-hydroxy-4H-pyran-4-one (24).



However, 1,3,5-tricarbonyls in which one of the terminal carbonyls is an acid or an ester tend to lead to α- rather than γ-pyrones. For example, Suzuki[47] has synthesized a number of alkylated 4-hydroxy-α-

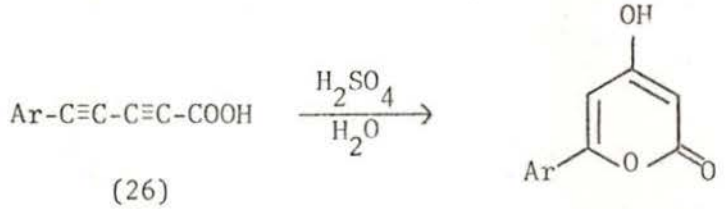
pyrones from intermediates (25):



(25)

R, R', R" = alkyl

Similarly, the diacetylenic acids (26), on hydration and cyclization, form 4-hydroxy-α-pyrones.[48]



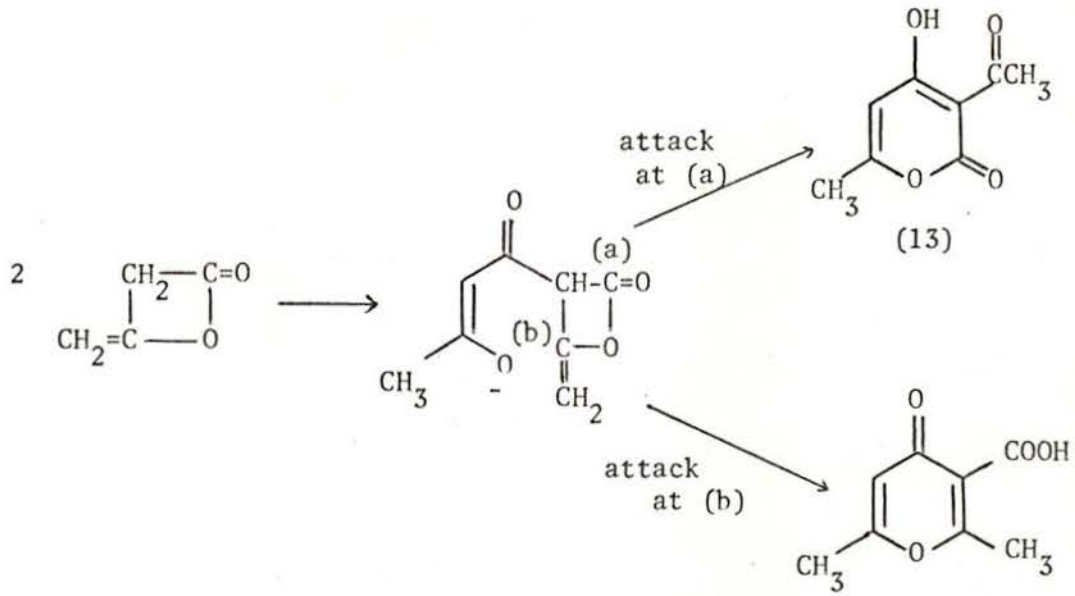
(26)

There are two other routes to synthesis of γ-pyrones of which infrequent use has been made.

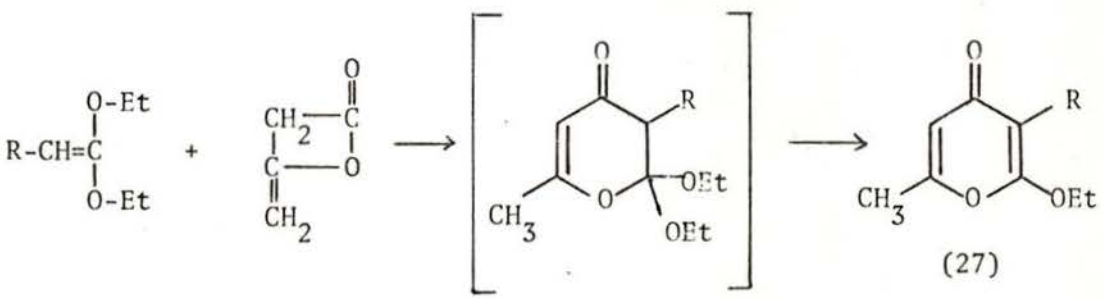
(d) From Diketene

For a number of years, diketene has been known to dimerize to dehydroacetic acid (13) in the presence of a basic catalyst such as sodium ethoxide.[49] More recently, however, Oda[50] isolated 2,6-dimethyl-γ-pyrone-3-carboxylic acid (14) as well as dehydroacetic acid from an aluminum tribromide catalyzed dimerization. This can be explained by the formation of a common intermediate anion (or its protonated form) with a possibility for attack at two positions

[(a) and (b)]:




Another group of workers[51] utilized diketene and a variety of ketene acetals to prepare a series of substituted γ-pyrones (27):

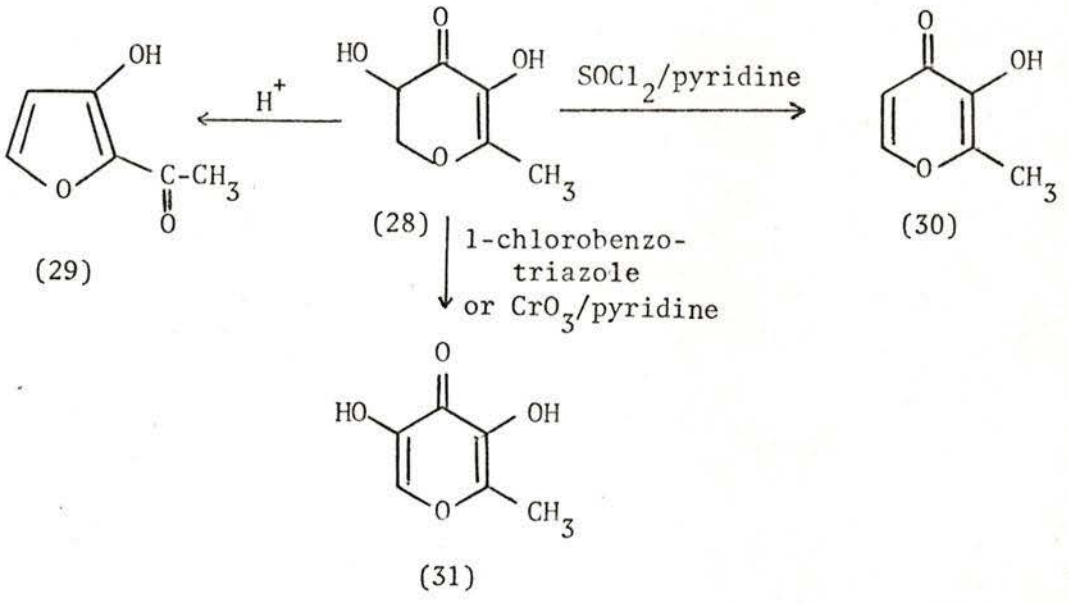



R = H, $-CH_3$, -Ph, -Cl, -Br, -CN

(e) From Dihydro- and Tetrahydro-γ-pyrones

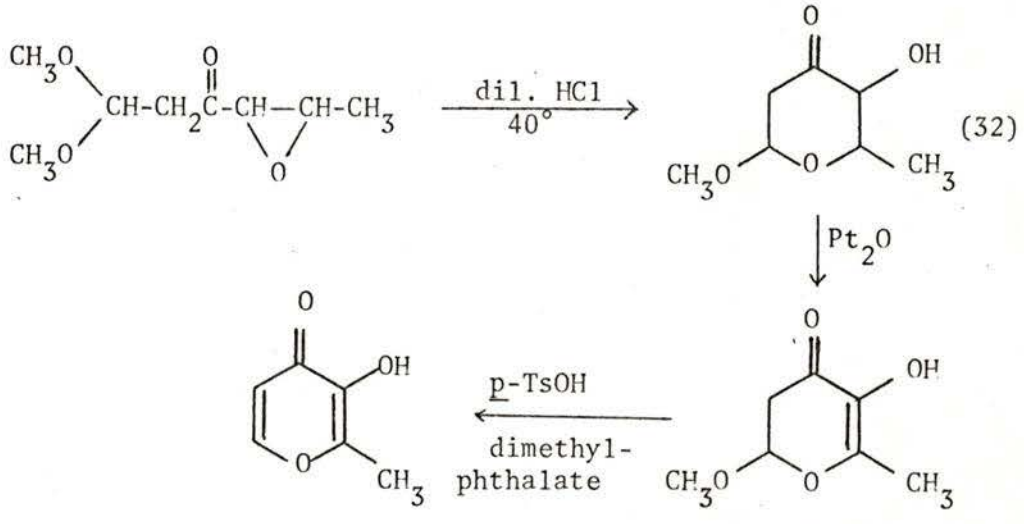
Although a small number of dihydropyrones have been isolated from natural sources, little has been reported on the oxidation of di- and tetrahydropyrones to pyrones. Conversion of several of the hexoses to γ-pyrones involves the isolation of intermediate dihydropyrones[52,53] (e.g. 28) which can either be dehydrated to give the hydroxypyrone [53]

(30), or dehydrogenated to give the dihydroxypyrone[54] (31):




(31)

In an elaborate synthesis of maltol, Schleppnik[55] succeeded in preparing a tetrahydropyrone (32) which he subsequently catalytically oxidized to the dihydropyrone (33) and further reacted with p-toluene-sulfonic acid (p-TsOH) to eliminate methanol:




(33)

## 2. Properties and Reactions of γ-Pyrones

### (a) Aromaticity

The aromatic character of γ-pyrones has been the subject of debate for many years. According to the classical definition of aromaticity, described in terms of chemical properties, γ-pyrones must possess some aromatic stabilization. In general, γ-pyrones contain unsaturated ring systems which are not easily oxidized, undergo substitution rather than addition reactions, and resist catalytic hydrogenation. All of these reactions are typical of "aromatic" compounds in which delocalization of the π-electrons gives the ring increased stability. γ-Pyrones do not undergo reactions characteristic of their functional groups and have a carbonyl function which is unusually basic.

| | | $pK_a$* |
|---|---|---|
| γ-pyrone[56] | | 0.1 |
| kojic acid[57] | (5) | 7.8 |
| acetone[56] | | -7.2 |

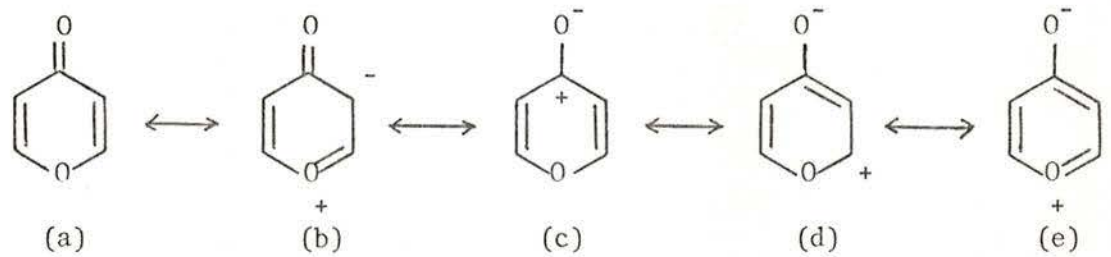
Furthermore, substituents react as groups attached to a benzene ring (see Part II 2(i)).

Contribution to the resonance hybrid of forms having charge separation was first proposed by Arndt[58] in 1924. Support for this

---

* $pK_a$ refers to the loss of a proton from the protonated carbonyl oxygen of each compound.

proposal came when Hunter and Partington[59] reported an unusually high dipole moment for γ-pyrone (4.05 D). The calculated dipole moment for either structure (a) or the fully aromatic zwitterion (e) (1.75 D and 22 D respectively) cannot account for the observed dipole moment. In order to do so, one must assume a resonance hybrid of these two structures, with the participation of other forms having less charge separation. Participation of structures such as (e) in the resonance



(a) (b) (c) (d) (e)

hybrid, which possess the full aromatic sextet, will result in an increased stability for the molecule.

That these structures probably contribute to the resonance hybrid can be seen from the nature of the chemical reactions which γ-pyrones undergo. For example, contribution of structure (d) gives the ring π-deficient properties and allows the activation of methyl groups at the α-positions, as shown by their ready condensation with aromatic aldehydes.[60] Contribution of structure (b) to the hybrid gives π-excessive character to the ring, and may facilitate electrophilic substitution Molecular orbital calculations indicate a large delocalization energy for γ-pyrone[61] (-2.9 β; benzene[62] -2 β) and increased charge density at the β-positions, consistent with the observation of electrophilic substitution at the β-carbons and nucleophilic substitution

at the carbon atoms α and γ to the ring oxygen.

In terms of the ring current definition of aromaticity, γ-pyrones meet most of the requirements for an aromatic system.[63] Delocalization of the π-electrons is seen in the NMR spectrum of γ-pyrones: chemical shifts for β-protons of γ-pyrones with respect to their dihydro analogues are greater than can be simply attributed to the electronegativity effect of a second double bond. The observed chemical shift differences (Δδ) are similar to those observed in the benzene series (Fig. 5).

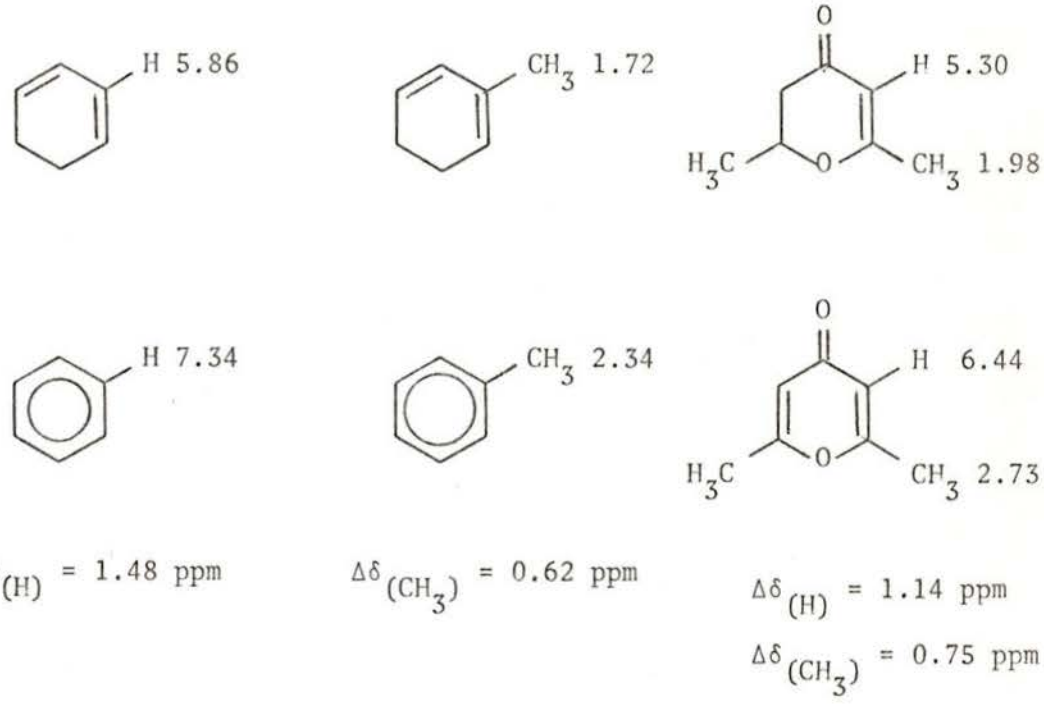



Fig. 5. Chemical Shifts of Benzene and γ-Pyrone Derivatives

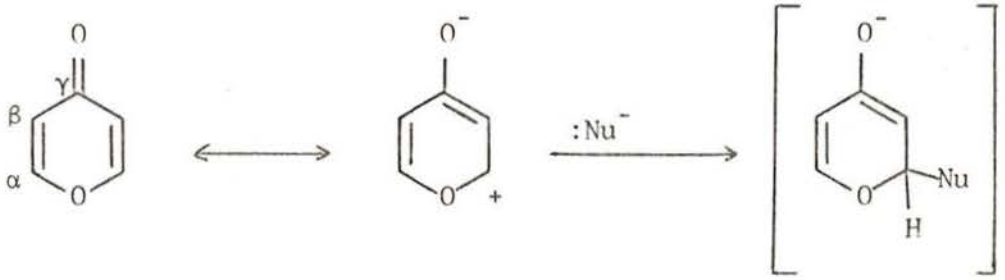
In an elaborate calculation on benzene and γ-pyrone, Smitherman[63] has found the magnitudes of the ring currents to be approximately the same.

Although these NMR data support the presence of a ring current in γ-pyrones, magnetic susceptibility measurements are not as yet clear enough to confirm this.[63] The aromatic model does, however, help to rationalize the chemical and spectral properties of γ-pyrones.

In general, then, γ-pyrones do not undergo reactions typical of their functional groups (carbonyl, alkene, ether). They can variously be regarded as either α,β-enones, divinyl ethers, or vinylogues of acid lactones or esters. However, unlike α,β-enones, γ-pyrones do not undergo Michael condensation reactions. Unlike vinyl ethers, they are relatively stable in acids. They do not form Schiff bases in any reasonable yield and give "abnormal" ring-opened Grignard reaction products. γ-Pyrones resist both oxidation and reduction, giving complex mixtures and poor yields of isolable products.

(b) Reactions with Bases and Nucleophiles

Nucleophilic attack at the α-positions can readily be explained by consideration of the structures contributing to the resonance hybrid of γ-pyrone. Molecular orbital and electron density calculations[61] (which locate increased charge density at the α-carbons) favour nucleophilic attack at the carbon atoms α or γ to the heteroatom.




Like esters or lactones, of which γ-pyrones are analogues, these

compounds suffer ring fission in base. The resulting 1,3,5-triketones can be recyclized by treatment with acid, a reaction found useful in γ-pyrone preparation. However, most strong bases cause complete degradation of the triketones to a mixture of all possible acids and ketones resulting from fission of the β-ketonic linkages.[64] 2,6-Dialkyl-γ-pyrones, for example, have been shown to decompose to at least six different products [Fig. 6 1(a)].

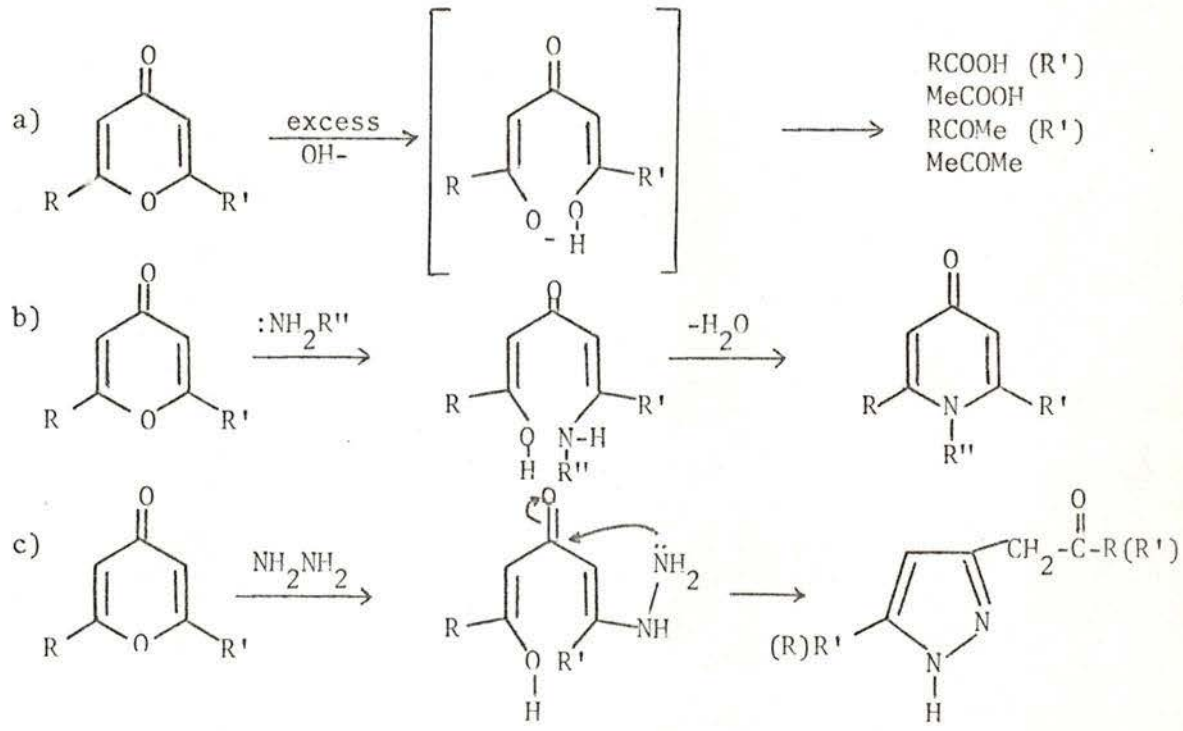
Ammonia and primary amines also attack γ-pyrones at the α-position but the intermediate enolate readily recyclizes with loss of water to give pyridones [Fig. 6 1(b)]. This reaction provides a particularly useful method of preparing substituted 4-pyridones and their N-substituted derivatives, as well as being a useful reaction for the characterization of γ-pyrones.

Although γ-pyrones do not form the normal ketone derivatives, they will react with hydrazine to form pyrazoles[65] [Fig. 6 1(c)]. Attack again occurs at the α-carbon to give a ring-opened compound in which the carbonyl group can then be attacked by the free nitrogen to give a mixture of isomeric pyrazoles. The remaining carbonyl substituent would then react with excess hydrazine to give a normal hydrazone.

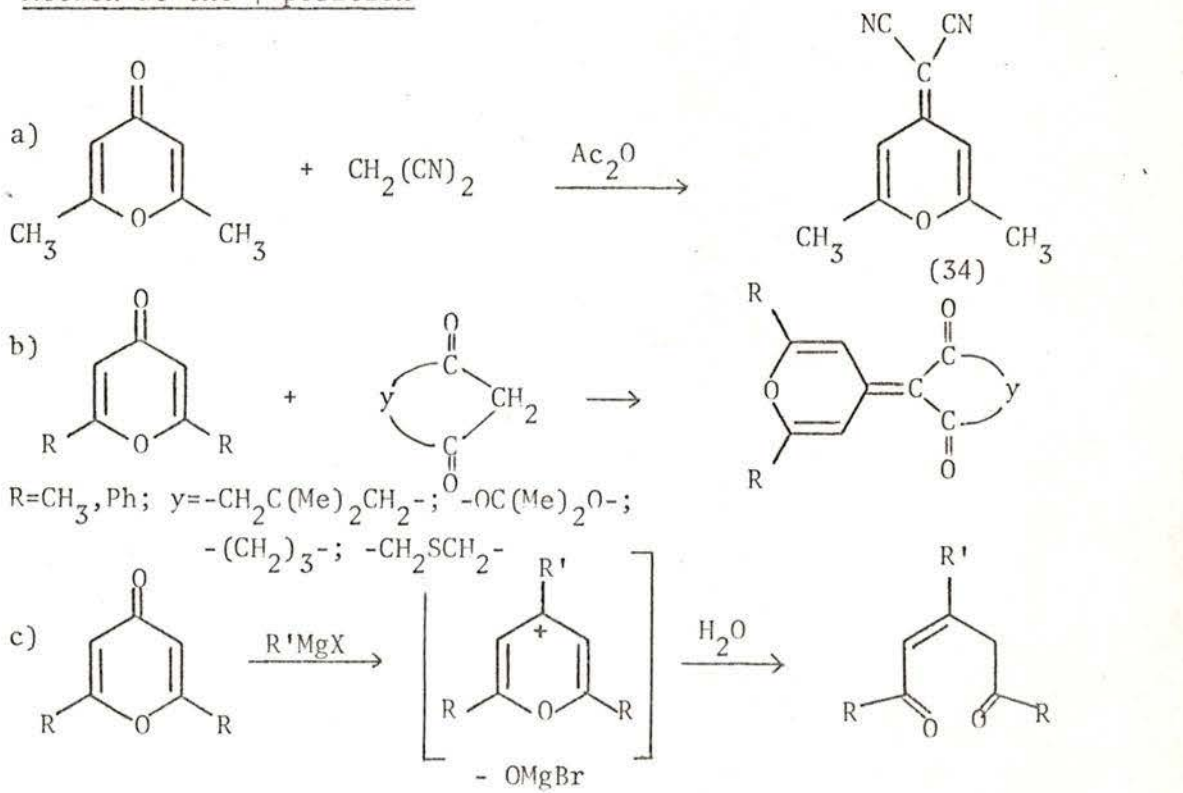
While γ-pyrones do not undergo "normal" carbonyl reactions, attack at the carbonyl carbon can be accomplished by using strong nucleophiles such as those which attack amides. Malononitrile has been shown[60] to react with 2,6-dimethyl-γ-pyrone to give the pyran derivative 4-(dicyanomethylene)-2,6-dimethyl-4H-pyran (34) [Fig. 6 2(a)]. More recently, Seitz and Moennighoff[66] successfully reacted γ-pyrones

Fig. 6. Nucleophilic Reactions of γ-Pyrones.

1. Attack at the α-position

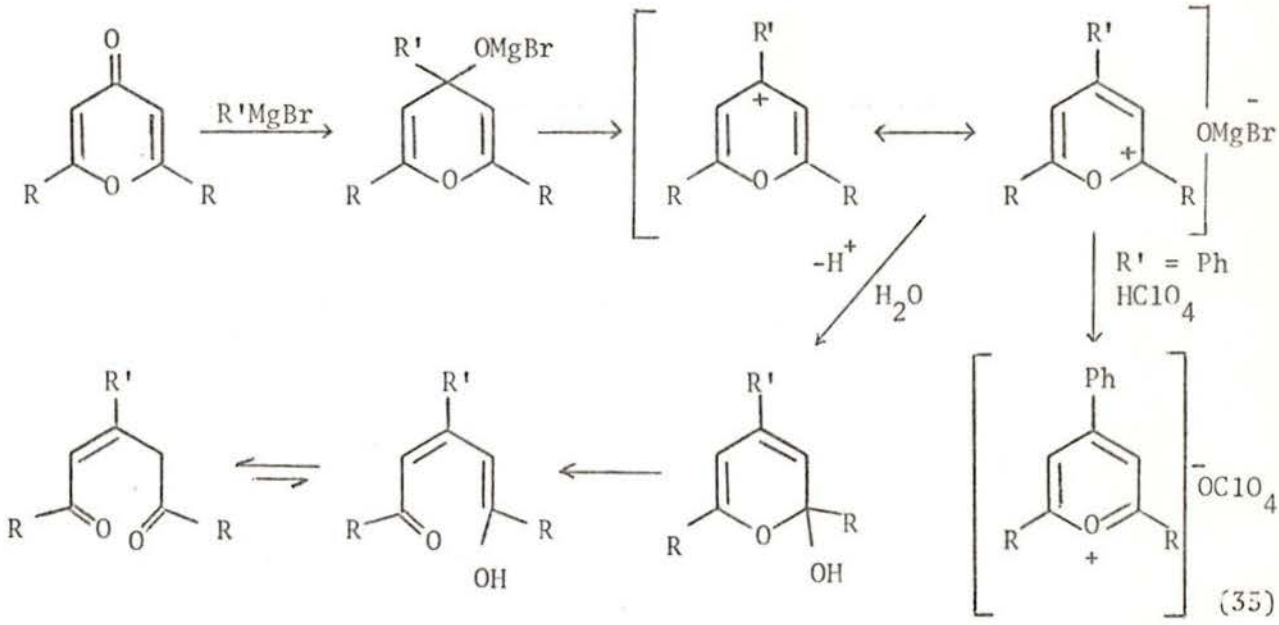



2. Attack at the γ-position



with 1,3-dicarbonyls to form the analogous vinyl compounds [Fig. 6 2(b)].

Grignard reagents do not give normal ketone addition products with γ-pyrones, but instead, by a process of attack at the carbonyl carbon and subsequent hydrolysis, they give ring-opened substituted enones[67] [Fig. 6 2(c)]. A mechanism for this reaction has been proposed which was substantiated by isolation of the intermediate pyrylium ion as the perchlorate salt (35):

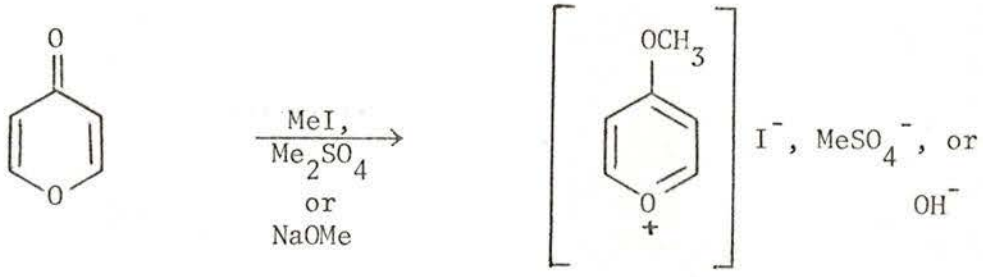



(35)

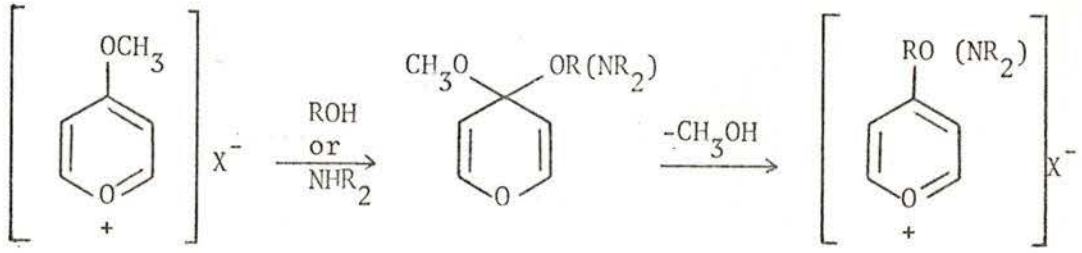
(c) Reactions with Acids

The basicity of γ-pyrones was first investigated by Collie and Tickle[68] in 1899 and has since been the subject of a number of studies. Unlike vinyl ethers such as 4H-pyran, γ-pyrones are not cleaved by acids but form stable salts with Lewis acids. For many years no definite structure could be assigned to the protonated form of γ-pyrone and its derivatives, but recently Cook[69]

showed that protonation occurs at the carbonyl oxygen. From a study of the vibrational spectra of a variety of Lewis acid complexes of 2,6-dimethyl-γ-pyrone, he identified hydroxyl bending and stretching modes for protium and deuterium salts having a variety of anions ($Cl^-$, $Br^-$, $I^-$, $HgCl_3^-$, $ClO_4^-$, $TiCl_6^=$, $ZrCl_6^=$, $SnBr_6^=$, $SnCl_6^=$, $PtCl_6^=$). Salts have also been formed with such methylating agents as methyl iodide and dimethyl sulfate and also with sodium methoxide.

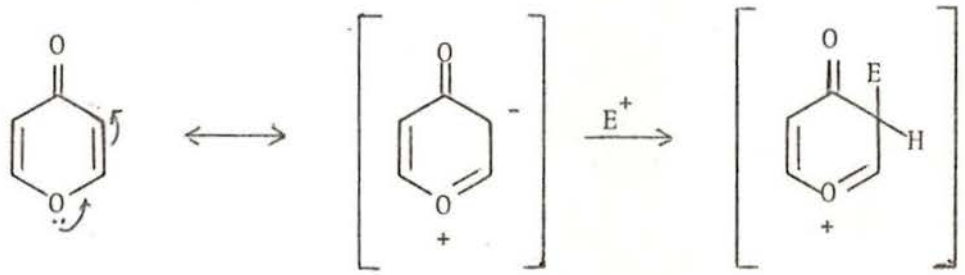



In their protonated forms, γ-pyrones are much more susceptible to nucleophilic attack, as can be seen by the facile displacement of the methoxy group by alcohols or amines.[70]



(d) Electrophilic Reactions; 3-Hydroxy-γ-pyrones

By again examining the structures contributing to the resonance hybrid of γ-pyrone, it can be seen that increased electron density should be found at the β-carbons.

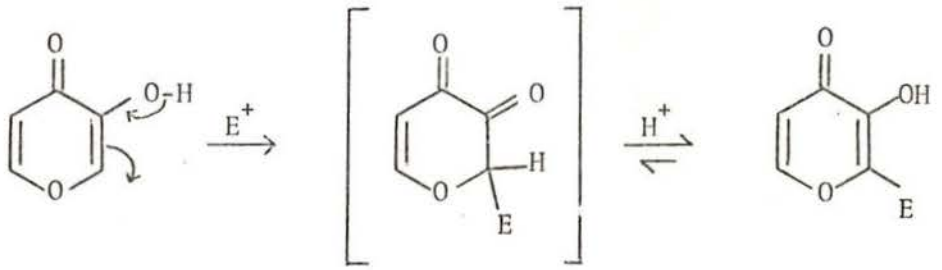


As might be predicted from their aromatic properties, γ-pyrones do not add bromine across their carbon-carbon double bonds, but are known to undergo nitration, bromination, diazonium coupling, and hydroxyalkylation reactions. γ-Pyrone itself, chelidonic acid (1, R = R' = COOH, R'' = H), 2,6-dimethyl-4H-pyran-4-one (36, R = R' = $CH_3$, R'' = H), and maltol ( 4, R = H, R' = $CH_3$, R'' = OH) all can be brominated at the β-position in the presence of a trace of iodine or ferric chloride:

With the exception of this bromination reaction which occurs more readily than in benzene, electrophilic substitution is difficult, often because the γ-pyrones exist as unreactive cations in strongly acidic media.

3-Hydroxy-γ-pyrones, however, are an exception and deserve special consideration. Like phenols, 3-hydroxy-γ-pyrones give positive ferric chloride tests, indicating significant chelation, and readily form ethers and esters indicating almost complete enolization.

These compounds, when unsubstituted at the 2-position, undergo a variety of electrophilic substitution reactions at $C_2$, adjacent to the hydroxyl substituent, due to activation of this position by the neighbouring hydroxyl group:



Other reactions, such as coupling with diazonium salts and Mannich reactions, are possible with the adjacent activating hydroxyl group. The scheme outlined in Fig. 7 illustrates some of the reactions which these compounds undergo. The increased reactivity ortho to the hydroxyl group is more easily explained in basic media since the phenolic proton is relatively easily removed in base. Contribution of the $C_2$ carbanion to the resonance hybrid of the resulting salt increases the likelihood of electrophilic attack at that position.

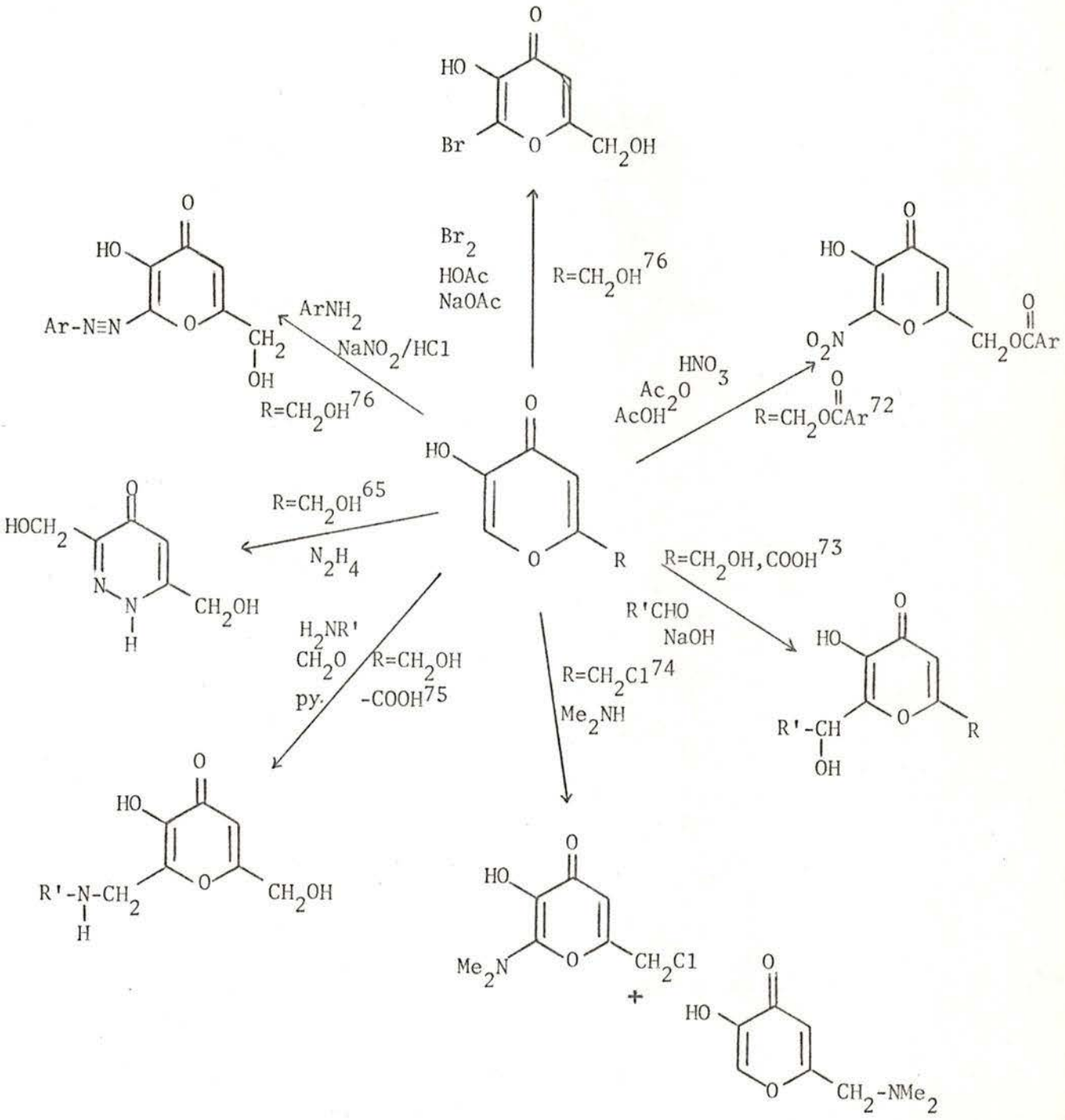
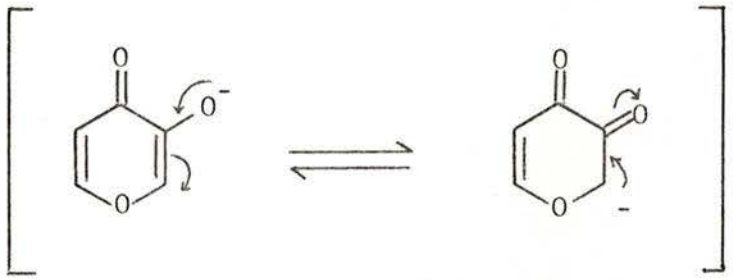
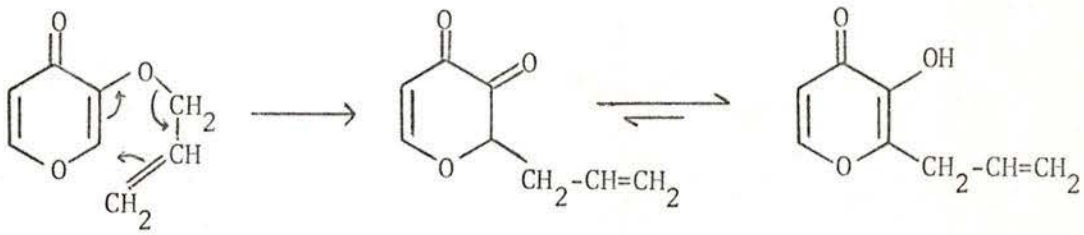


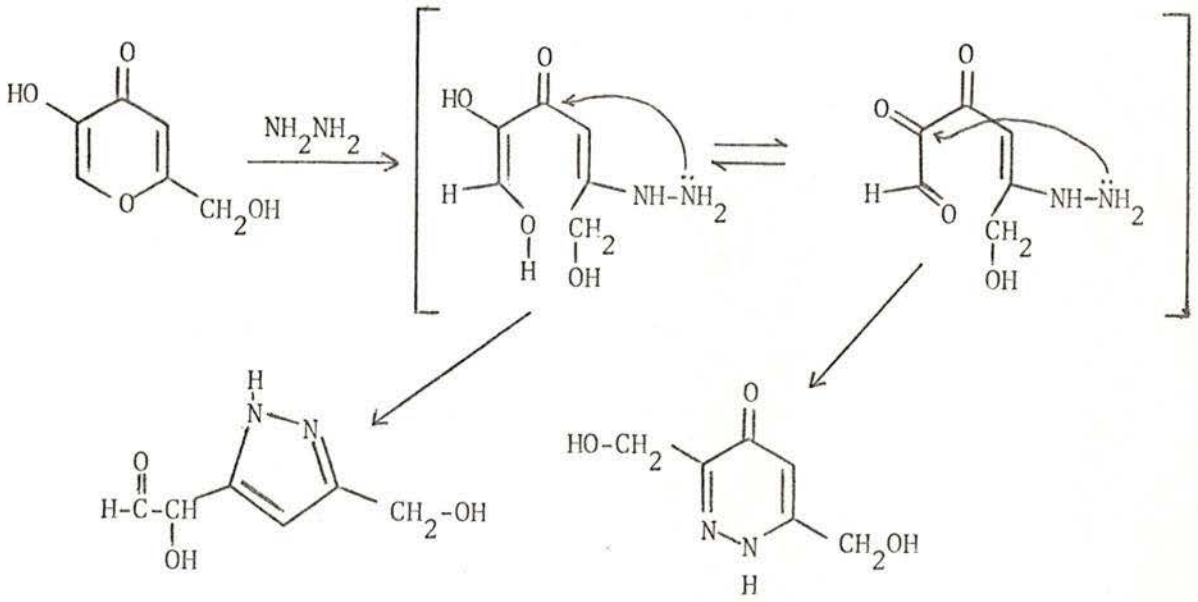

Fig. 7. Reactions of 3-Hydroxy-4H-pyran-4-ones.



Several other reactions of 3-hydroxy-γ-pyrones have proved interesting and useful. Their allyl ethers have been found[71] to undergo Claisen rearrangements to give the $C_2$ alkylation and allylation products, without concurrent rearrangement to the $C_6$ position (para):

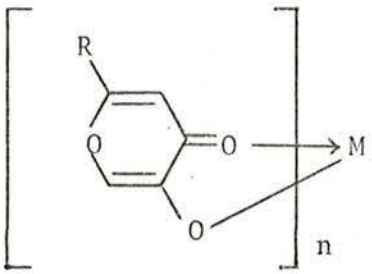


Kojic acid gives an additional product (37) (a pyridazinone) not given by γ-pyrone, on treatment with hydrazine,[77] since the hydroxyl group represents an incipient carbonyl group and hence another point of nucleophilic attack.



(37)

3-Hydroxy-γ-pyrones react with a variety of metal salts and metal oxides to form a number of stable, isolable chelates:



R = $-CH_2OH$[78,79], -COOR'[80]

M(n) = Al(3), Cu(2), Cd(2) Co(2), Ni(2), Mn(2), etc.

(e) Deuterium and Oxygen-18 Exchange Reactions

Although γ-pyrone has been known for some time to exchange two protons on treatment with $D_2O$ in acidic media, it was not until 1964 that Beak and Carls[81] established that substitution occurs at the β-positions. In an effort to determine intermediates in this reaction, the same authors treated γ-pyrone with $^{18}O$-enriched water and found

incorporation at both the carbonyl and ring oxygens. This confirmed their proposal that a ring-opened intermediate must be involved. Later studies by Ichimoto[82] on $^{18}O$ incorporation into several γ-pyrones using acidic, neutral, and basic media gave similar results which they explained by a slightly different mechanism to accommodate both acid and base catalysis. The intermediates they proposed could also account for the deuterium exchange reaction (see Fig. 8):

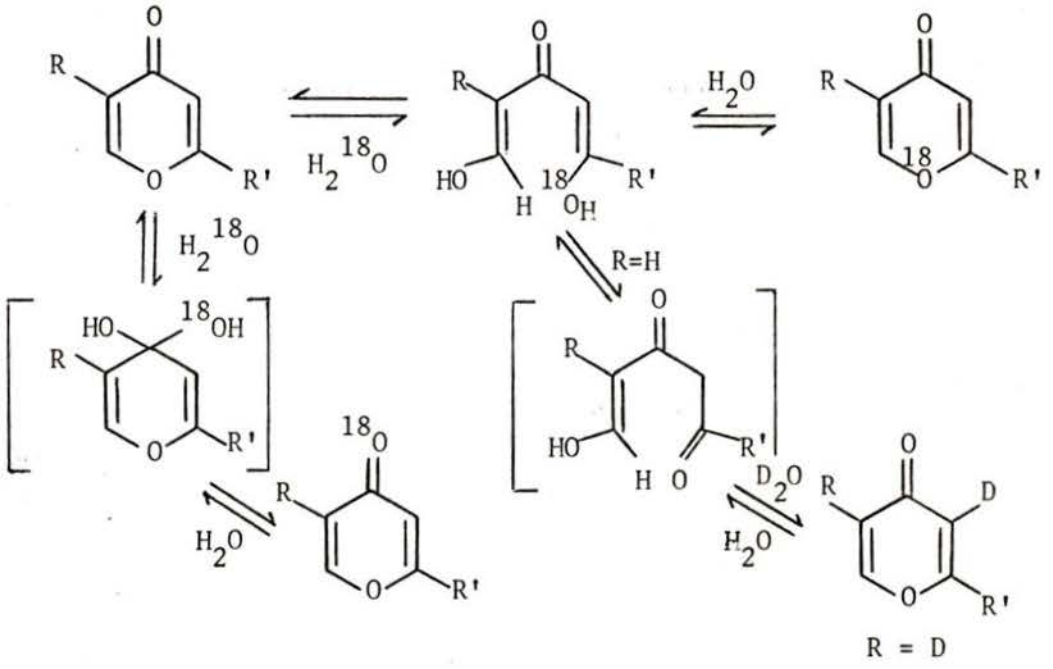



Fig. 8 Isotopic Exchange in γ-Pyrones

(f) Reduction Reactions

The γ-pyrone skeleton is not readily altered by non-catalytic reducing agents (such as Zn/HCl, $NaBH_4$ etc.) resulting in low yields and the formation of decomposition products. Substituents can easily be reduced without altering the γ-pyrone ring.

Catalytic hydrogenation similarly gives decomposition products, mixtures of di- and tetrahydropyrones, and, in some cases, tetrahydropyranols, in poor yields. A variety of products has been isolated[83] from the catalytic reduction of kojic acid (see Fig. 9), although some doubt still exists as to their absolute structures.

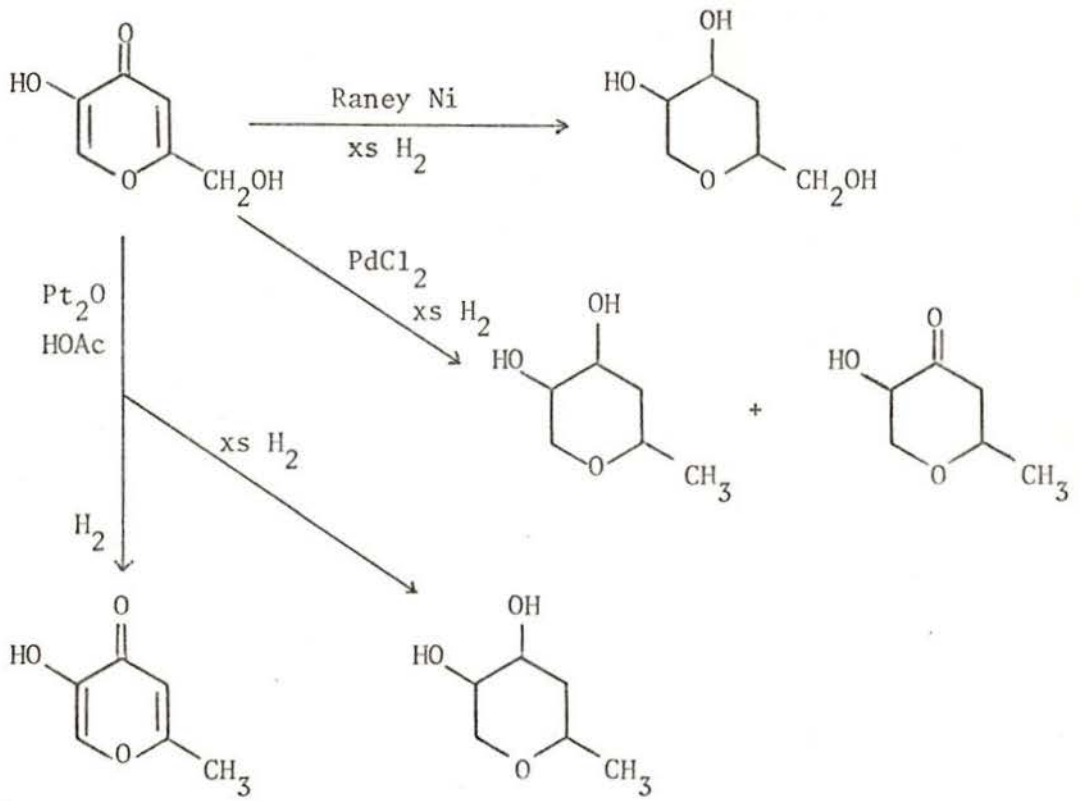



Fig. 9. Catalytic Hydrogenation Products of Kojic Acid.

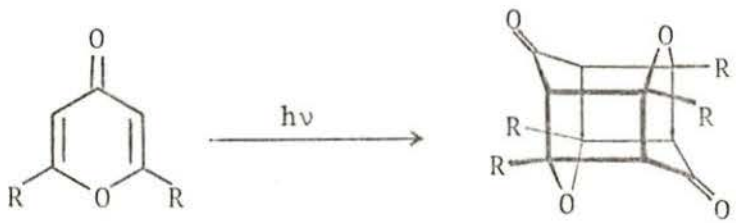
(g) Oxidation Reactions

As well as resisting reduction reactions, γ-pyrones also resist oxidation reactions.[63] Various oxidative degradation reactions have

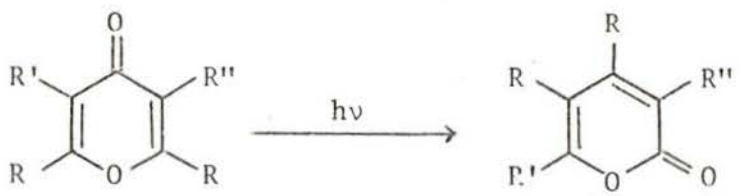
been tried, most of which have led to low molecular weight cleavage products similar to those obtained on alkali degradation.

(h) Photochemical Reactions

γ-Pyrones are relatively heat stable but are sensitive to irradiation, both in the solid state and in solution. Since not all substituted γ-pyrones react in the same way under irradiation, Pavlik and Kwong[84] suggested a method for determining the substitution patterns in these systems: 2,6-disubstituted-γ-pyrones give cage dimers on irradiation,



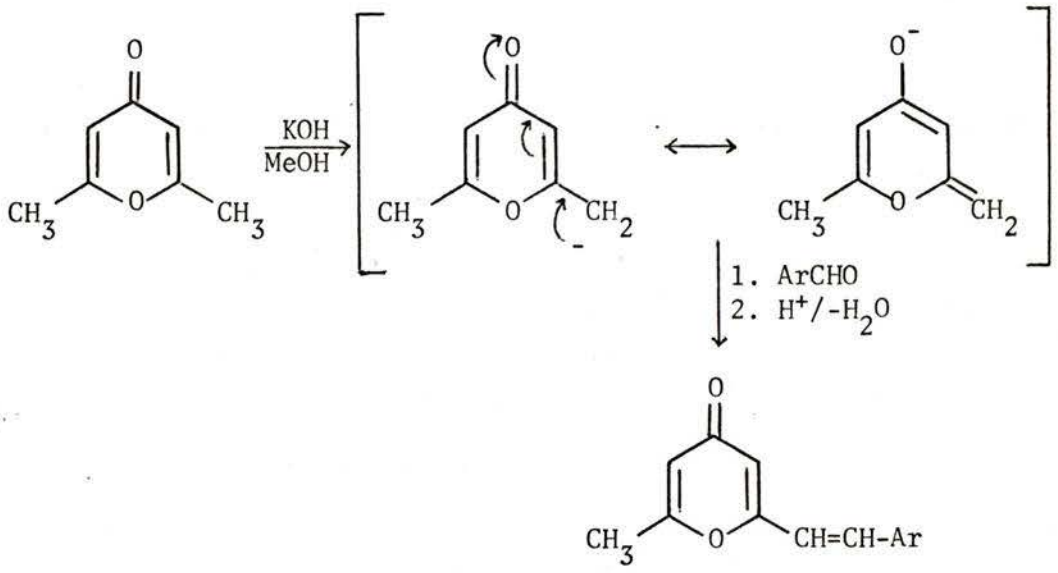

trisubstituted-γ-pyrones are not affected, and tetrasubstituted-γ-pyrones rearrange to form tetrasubstituted-α-pyrones, the rearrangement being specific with respect to the positions of the functional groups in the reactants and products.



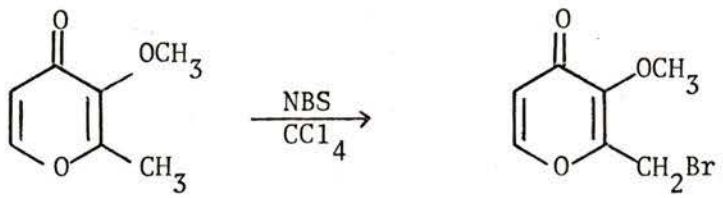

R, R', R'' = alkyl, aryl

(i) Reactions of the α-Methyl Substituent

The presence of a methyl substituent α- to the heteroatom of the γ-pyrone ring can be of particular synthetic importance since, in this position, it is sufficiently activated to condense with aromatic aldehydes.[60] The intermediate carbanion, formed in the presence of a strong base, is stabilized by delocalization of negative charge onto the carbonyl oxygen; this carbanion then attacks the aldehyde carbon:




Bromination at the α-methyl group has been accomplished[85] by treatment of the methyl ether of maltol with N-bromosuccinimide:



## RESULTS AND DISCUSSION

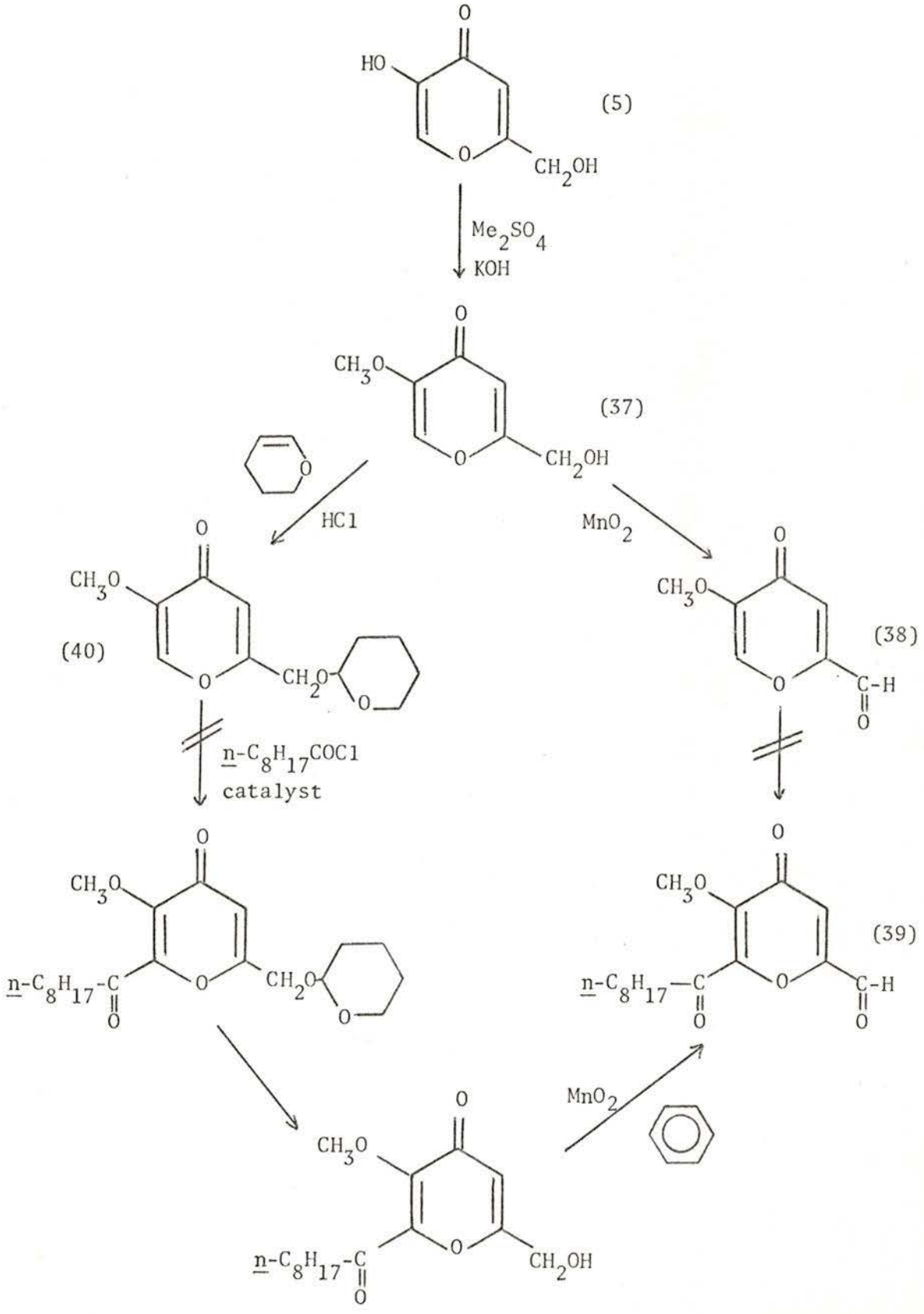
### 1. Synthetic Routes to Phacidin

As described in the introduction, there are several appproaches which can be taken to the synthesis of a substituted γ-pyrone. With the possibility of twelve different isomers of phacidin, it seemed reasonable that the synthesis of any one of the isomers would provide either proof of the structure or valuable information as to the actual substitution pattern. Since there are several substituted γ-pyrones readily available, the first approach we took was to start with a naturally occurring γ-pyrone.

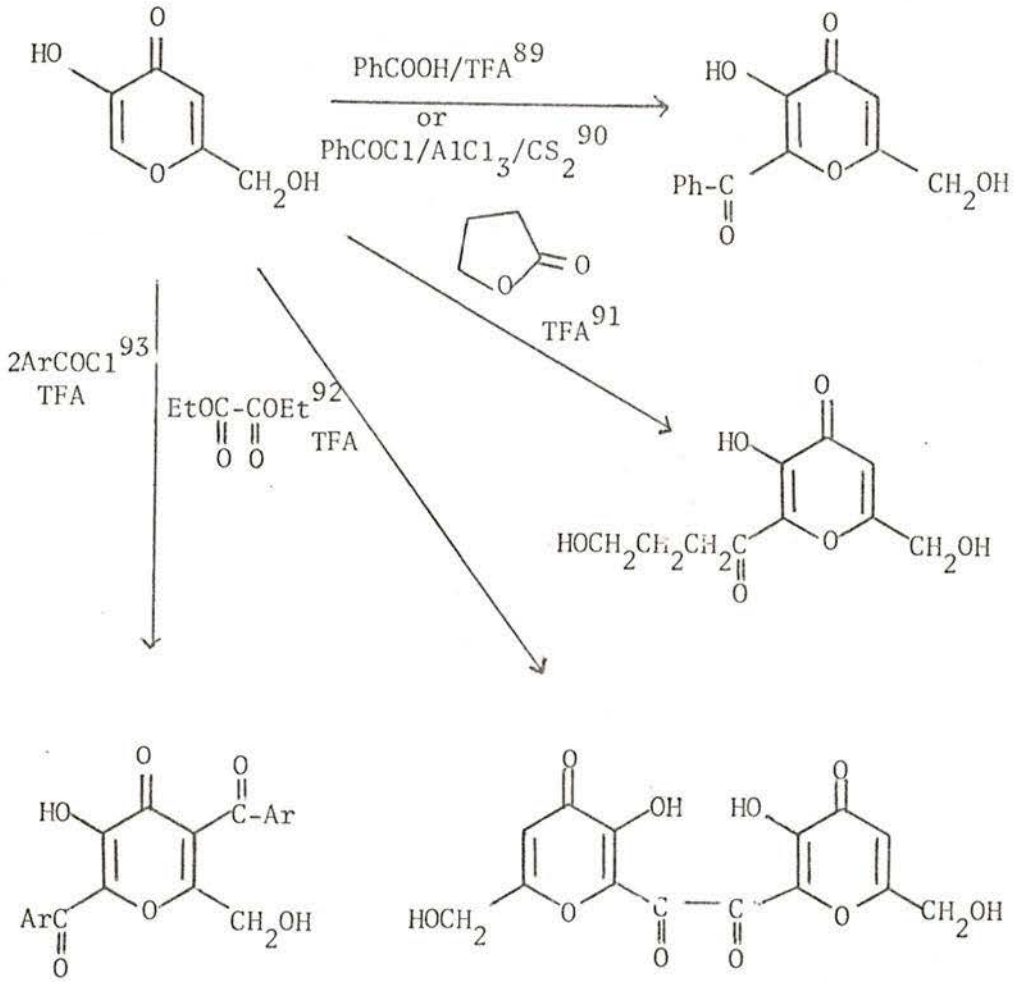
#### (a) C-Acylation of Kojic Acid Derivatives (Scheme I)

Kojic acid (5) was chosen as the starting material since it has been studied more extensively than any other γ-pyrone, and possesses two functional groups which can be modified to give two of the three desired substituents (see Scheme I). The method of Campbell[86] was used to methylate the phenolic hydroxyl group of kojic acid to give 2-hydroxymethyl-5-methoxy-4H-pyran-4-one (37). The primary alcohol was then readily oxidized[87] to an aldehyde by active manganese dioxide to give 5-methoxy-4-oxo-4H-pyran-2-carboxaldehyde (38).

SCHEME I

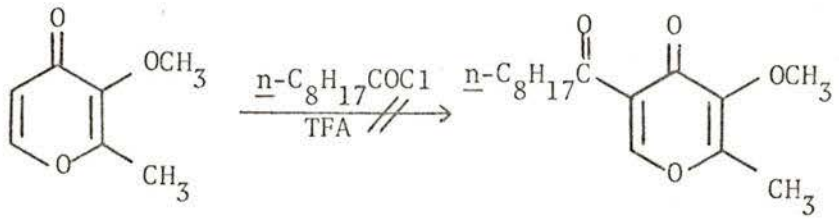

HO
(5)
$CH_2OH$
$Me_2SO_4$
KOH
$CH_3O$
(37)
$CH_2OH$
HCl
$MnO_2$
(40)
$CH_3O$
$CH_2O$
(38)
C-H
$\underline{n}$-$C_8H_{17}COCl$
catalyst
$\underline{n}$-$C_8H_{17}$-C
(39)
$MnO_2$

Until relatively recently, γ-pyrones were thought to undergo Friedel-Crafts reactions to give C-acylation and C-aroylation products. Woods and co-workers,[88] in a large number of papers, described the reactions of kojic acid and other γ-pyrones with acyl halides, anhydrides, lactones, acids and esters. In the presence of trifluoroacetic acid (or $AlCl_3$ or $ZnCl_2$), kojic acid reacted readily with the acylating agents to give products which Woods identified as the $C_6$-acyl derivatives. A few examples are listed below:



Following this work, the aldehyde (38) was reacted, in trifluoroacetic acid, with n-nonanoyl chloride in an attempt to form the C-acylation product 5-methoxy-6-n-nonanoyl-4-oxo-4H-pyran-2-carboxaldehyde (39) which would have all the component functional groups of phacidin. However, no reaction occurred and the starting aldehyde was re-isolated after several hours of refluxing.

With an aldehyde function at the $C_2$-position, it is reasonable to suspect that the ring might be sufficiently deactivated to prevent electrophilic substitution by the n-nonanoyl group. The tetrahydropyran derivative of monomethyl kojic acid, 5-methoxy-2-tetrahydropyranyloxymethyl-4H-pyran-4-one (40), was then prepared and reacted with n-nonanoyl chloride in pyridine. No reaction occurred in pyridine, in trifluoroacetic acid, or in diethyl ether with boron trifluoride as catalyst. Neither did the reaction of methyl maltol (41) under the same conditions give a C-acylation product.



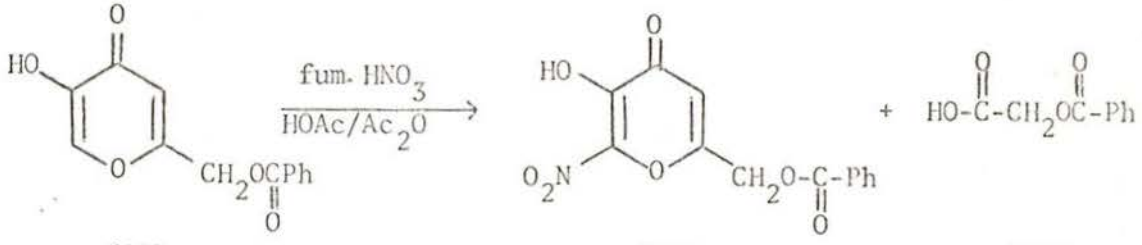

(41)

A further search of the recent literature confirmed our results that C-acylation of γ-pyrones is not the facile reaction Woods demonstrated it to be. Repeating much of the work of Woods, Tolentino and Kagan[94] found that the acylation products Woods reported were indeed O-acylation products of unprotected alcohol substituents.

Since direct acylation of the γ-pyrone nucleus could not be accomplished, a different method of synthesis of phacidin was needed.

(b) Electrophilic Substitution of Kojic Acid (Scheme II)

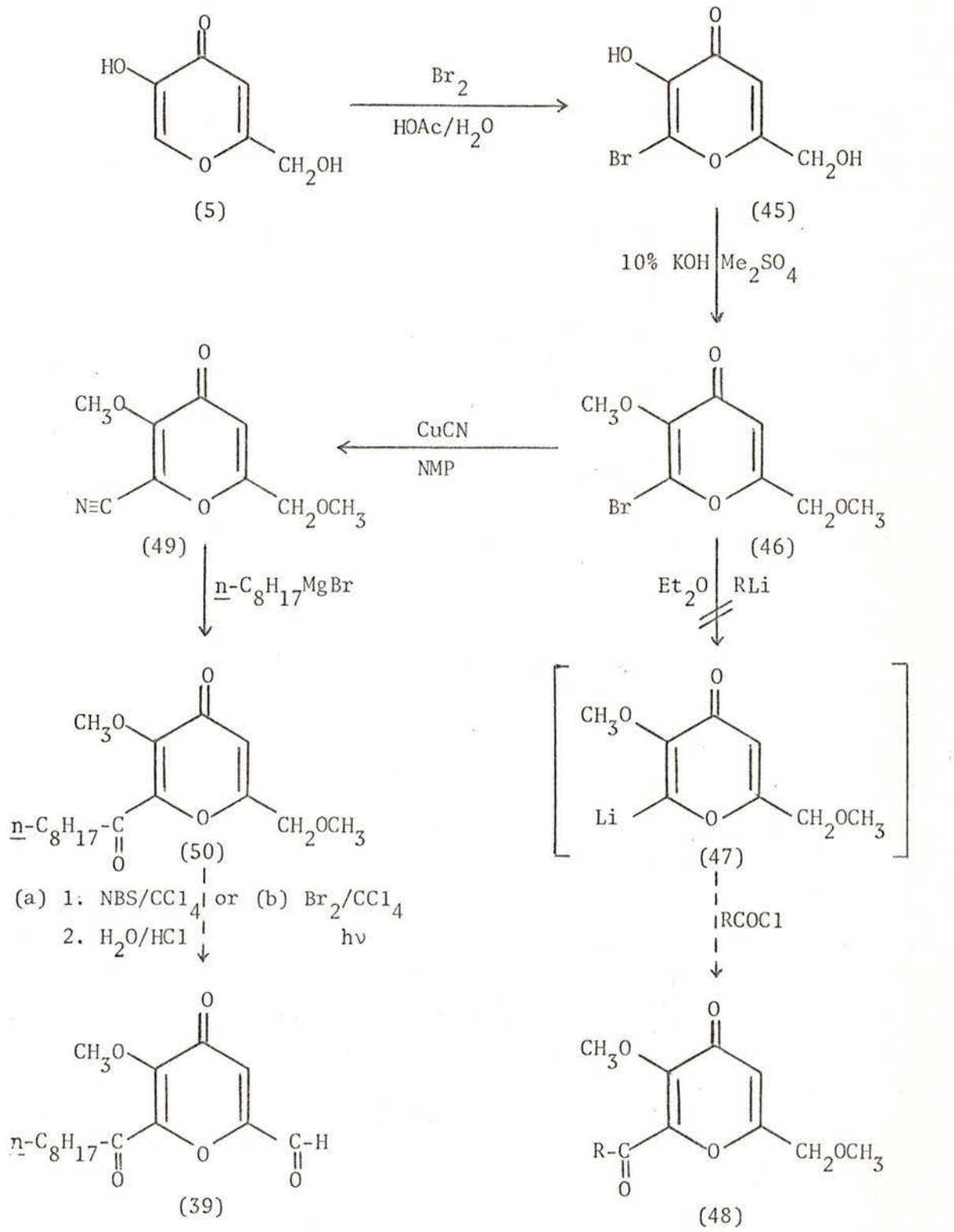
The second approach involved introduction, onto the kojic acid nucleus, of a third substituent which could be modified or displaced to give the desired acyl substituent (see Scheme II). As described in the introduction (Part II), there are a number of electrophilic reactions of kojic acid which can be used to introduce a substituent at the $C_6$-position of the γ-pyrone ring. Nitration of the benzoate ester of kojic acid (42, 2-benzoyloxymethyl-5-hydroxy-4H-pyran-4-one) by the method of Eiden[72] (see Fig. 7) gave the desired $C_6$-nitro compound (43, 2-benzoyloxymethyl-5-hydroxy-6-nitro-4H-pyran-4-one). An additional product, resulting from oxidative cleavage of the γ-pyrone ring was isolated and identified as the benzoate ester of glycolic acid (43).




(42) (43) (44)

Kojic acid is readily brominated at the $C_6$-position to give (45) 6-bromo-5-hydroxy-2-hydroxymethyl-4H-pyran-4-one. This was accomplished using bromine in acetic acid by the method of Ichimoto.[76] The bromokojic acid (45) was then dimethylated with excess dimethyl sulfate

SCHEME II


HO
O
CH2OH
(5)
Br2
HOAc/H2O
Br
(45)
10% KOH
Me2SO4
CH3O
(46)
CuCN
NMP
N≡C
CH2OCH3
(49)
n-C8H17MgBr
Et2O
RLi
Li
(47)
RCOCl
(50)
(a) 1. NBS/CCl4
2. H2O/HCl
or (b) Br2/CCl4
hν
C-H
(39)
R-C
(48)

by a method similar to that described by Thomas[95] for the dimethylation of kojic acid. To displace the bromine substituent and introduce an acyl group into its place, the bromo compound (46) was first treated with an equimolar amount of n-butyllithium in diethyl ether in an attempt to form the lithium derivative (47). Appearance of a bright red colour on addition of the n-butyllithium indicated the probable formation of an anion (lithium salt), but quenching of the reaction mixture with n-nonanoyl chloride gave only starting compound (46), n-nonanoic acid, and a small amount of unidentifiable decomposition product. The reaction was then repeated, quenching instead with water or alternately with a saturated solution of ammonium chloride in water. Instead of forming an anion at the $C_6$-position and generating dimethyl kojic acid on hydrolysis, the n-butyllithium preferentially attacked the carbonyl group to give ring-opened butylated products. Attempts at anion formation at $C_6$ using ethyllithium again gave ring-opened ethylated products rather than the desired acylated compound (48).

A second attempt to displace the bromine substituent proved successful. In a reaction common to most aromatic compounds,[96] the bromo compound (46) was heated with cuprous cyanide in N-methyl-2-pyrrolidone to give the cyano compound (49, 5-methoxy-2-methoxymethyl-4-oxo-4H-pyran-6-carbonitrile) resulting from bromine-cyanide exchange.

Although time did not permit the reaction sequence to be continued beyond this point, the following reactions have been proposed to complete the synthesis of 5-methoxy-6-n-nonanoyl-4-oxo-4H-pyran-2-carboxaldehyde (39).

(i) Addition of the appropriate Grignard reagent (n-octanoyl-

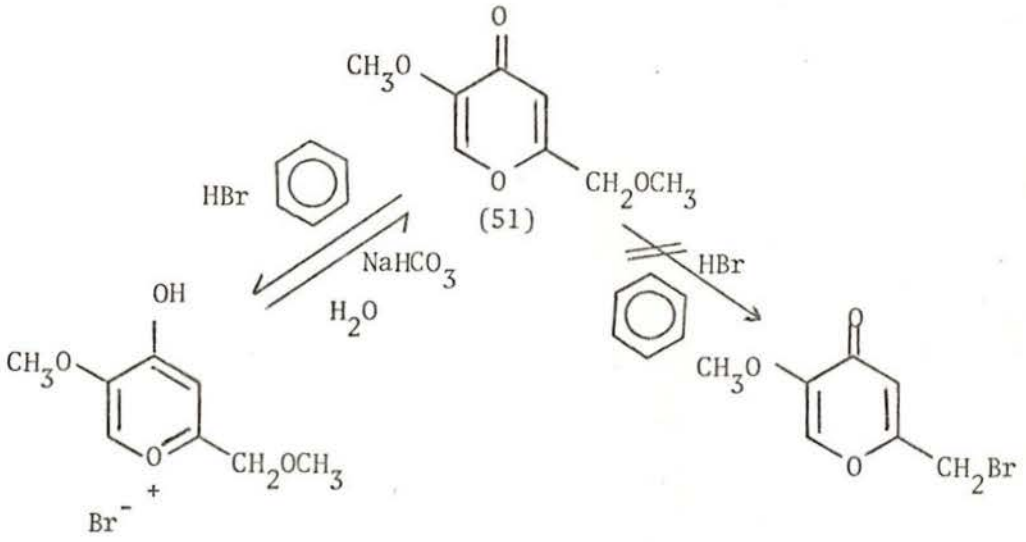
magnesium halide) to the cyano compound (49), and subsequent hydrolysis of the ketimine salt should readily give the acylation product (50, 5-methoxy-2-methoxymethyl-6-n-nonanoyl-4H-pyran-4-one). This is a well known reaction for preparation of ketones from nitriles[97]:

$$R\text{-}C{\equiv}N \xrightarrow{R'\text{-}MgX} R\text{-}\underset{\displaystyle R'}{\underset{|}{C}}{=}N\text{-}MgX \xrightarrow{\text{hydrolysis}} R\text{-}\overset{\displaystyle O}{\overset{\|}{C}}\text{-}R'$$

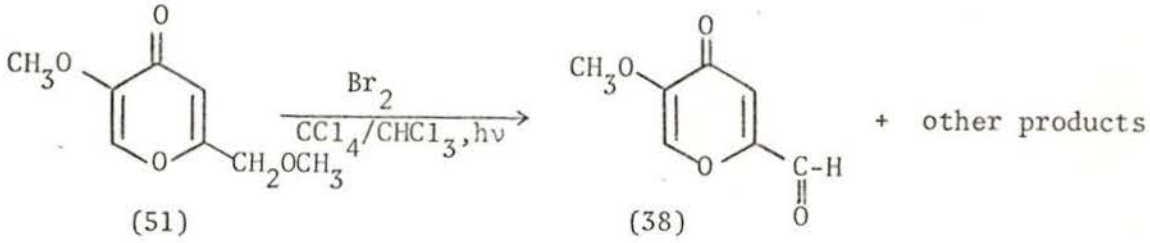
ketimine

(ii) Finally, conversion of the methoxymethyl substituent to an aldehyde function must be accomplished to give the desired product (39). There are several possibilities for this conversion which have been explored using model reactions carried out on the dimethyl ether of kojic acid (51).

It is well known that ethers may be cleaved by heating with concentrated hydriodic acid, hydrogen bromide, or other Lewis acids.[98] Since γ-pyrones form stable salts with the hydrogen halides and with a large number of Lewis acids, their ethers are not readily cleaved by acids. Anhydrous HBr was bubbled through a solution of dimethyl kojic acid (51) in dry benzene and immediate precipitation of the colourless HBr salt (52) occurred. Refluxing the solution for several hours gave only a trace of benzene soluble product which was not the desired bromomethyl compound (53).

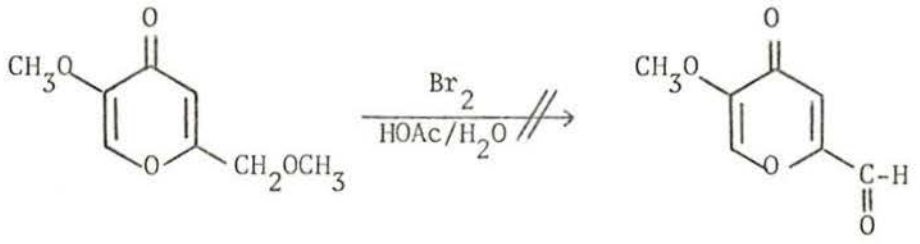



Markees[99] has described a method for preparing aldehydes directly from benzyl methyl ethers by treatment with bromine in carbon tetrachloride in the presence of light. Reaction of dimethyl kojic acid (51) under the same conditions yielded a mixture of products, one of which gave a mass spectrum identical to that of the aldehyde prepared from monomethyl kojic acid (38), although the yield was poor.

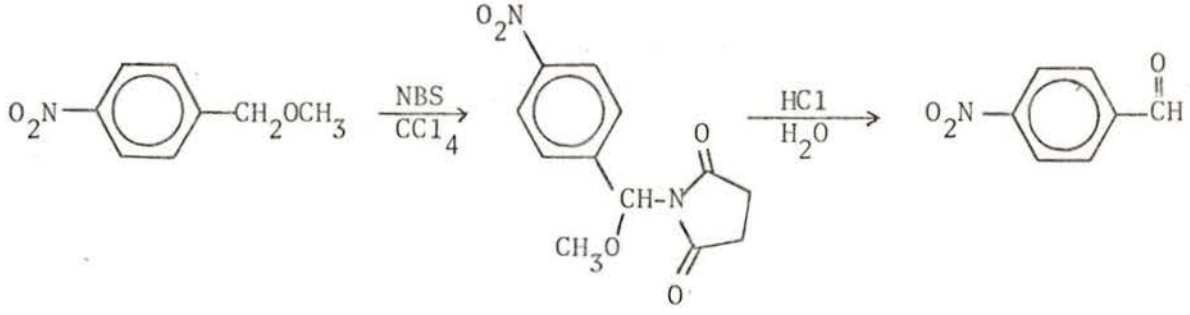



A similar method of oxidative cleavage was described by Deno and Potter[100] who used aqueous bromine to cleave a variety of ethers. While most aliphatic ethers give acids as their oxidation products, benzyl ethers were found to produce benzaldehydes with equimolar amounts

of bromine in aqueous acetic acid. Dimethyl kojic acid (51) in 60% acetic acid was treated with bromine at room temperature for twenty-four hours but failed to give any of the desired aldehyde. Instead, a mixture of oxidation and decomposition products, not identified, was produced.

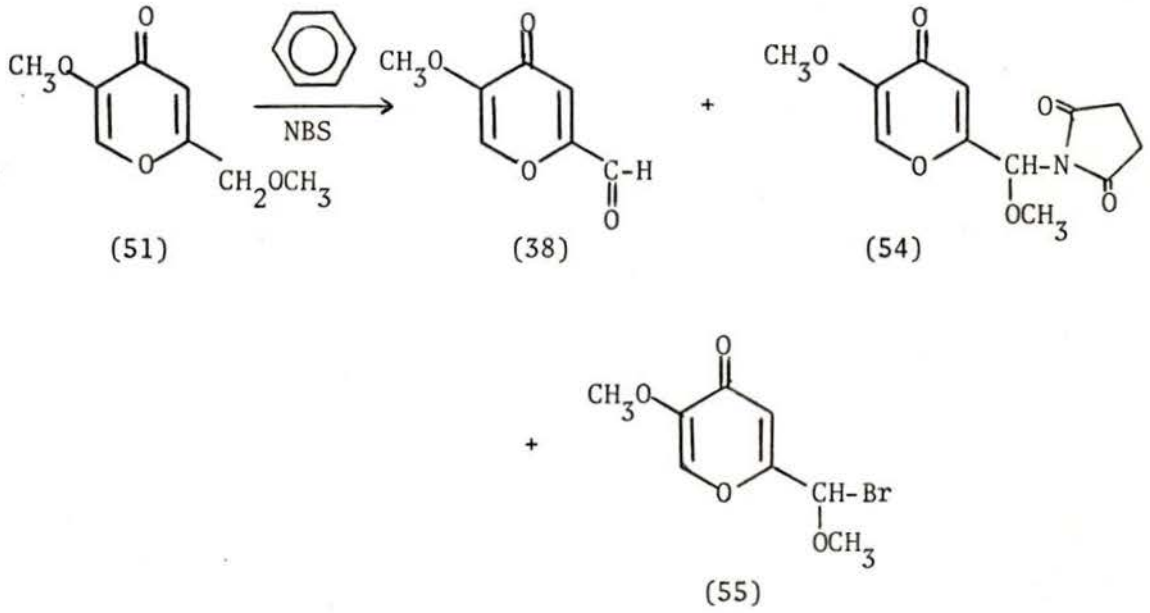



A third method by which aldehydes can be prepared directly from ethers has also been described by Markees.[99] He found that reaction of benzyl methyl ethers with N-bromosuccinimide (NBS) in diffuse light gave intermediate succinimides which could be hydrolyzed by hydrochloric acid to give benzaldehydes:




The dimethyl ether of kojic acid (51) was dissolved in benzene and treated with an equimolar amount of NBS at reflux. After fifteen minutes, a yellow colour was produced which was discharged on further refluxing (15 min). Evaporation of the solvent gave a yellow product which, upon analysis by NMR, was found to be a mixture of the desired

aldehyde (38), and two other 2,5-disubstituted γ-pyrones which appear to be the intermediate succinimide (54) and an intermediate bromide (55).




(51) (38) (54) (55)

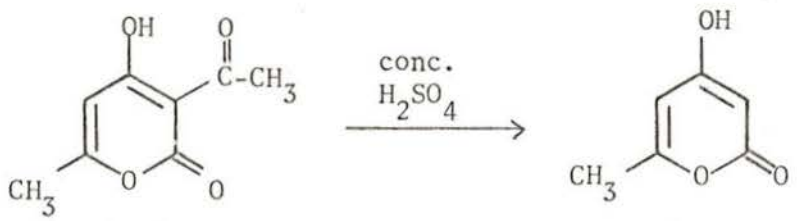
Although the sequence illustrated in Scheme II has not been completed, the NBS and bromine induced oxidations, done as models for the final step, indicate that oxidation of the methoxymethyl ether to an aldehyde function can possibly be accomplished by this method. Using conditions suitable for free radical reaction, bromination should not occur on the nonanoyl side chain α to the carbonyl function, although the possibility of this reaction occurring cannot be excluded completely. The sequence of the reactions is of particular importance since the Grignard reagent would attack an aldehyde function preferentially to the nitrile. As we have seen, attempts to brominate the dimethyl ether of kojic acid led to oxidation products rather than

ring substitution products, thus making bromination of the ring essentially the first step of the reaction sequence.

(c) From an α-Pyrone (Scheme III)

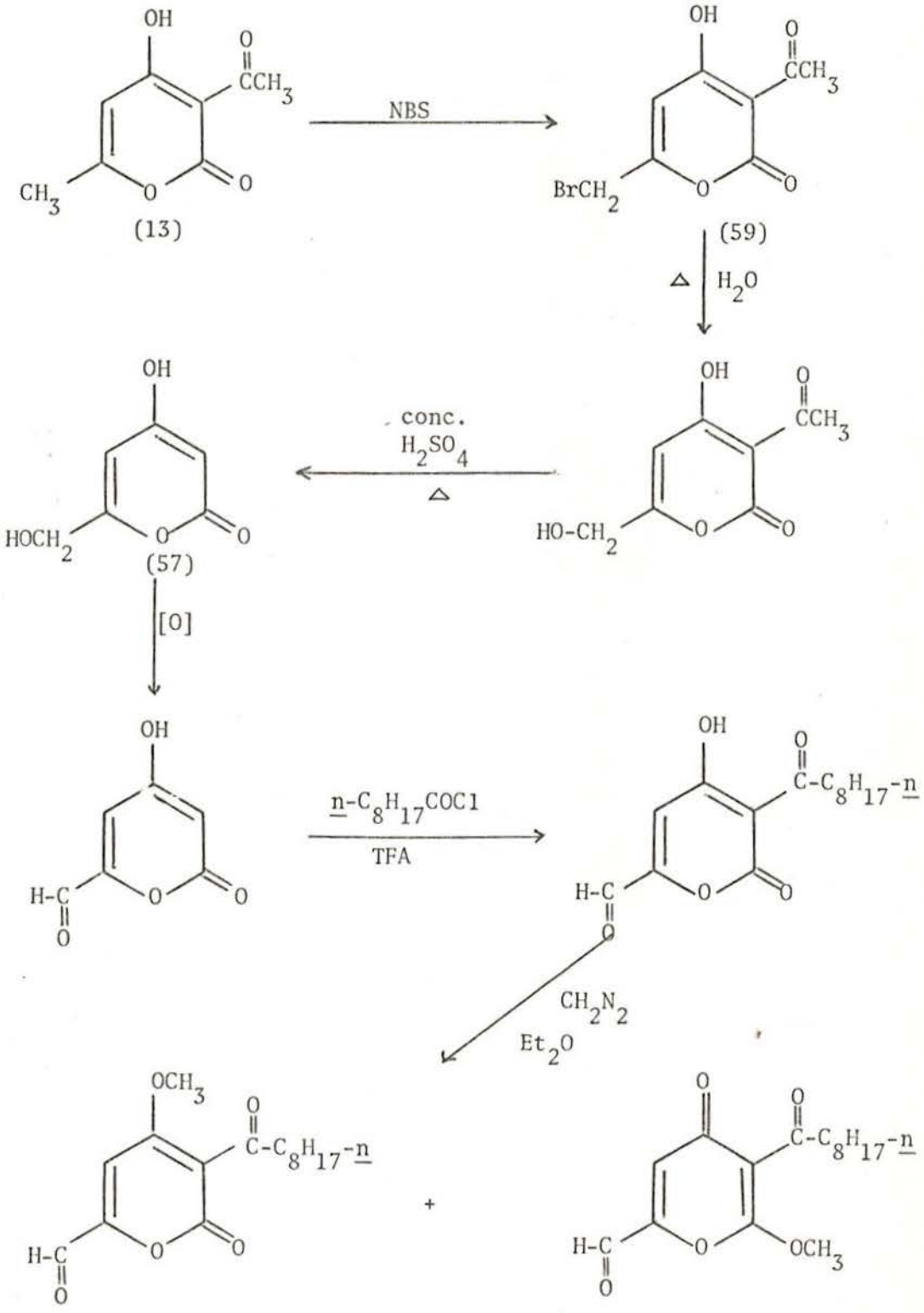
Neither of the first two reaction sequences outlined would give rise to a product with the substitution pattern we have deduced for phacidin. A third scheme, which has resulted from work on a number of α-pyrones, is proposed (see Scheme III). This sequence depends ultimately on the existence of tautomers of the 4-hydroxy-α-pyrone system. Thus, the final step in the reaction, methylation with diazomethane, would give a mixture of α- and γ-isomers. However, even if no γ-pyrone were formed, the resulting α-pyrone would provide negative evidence for the existence of an α-pyrone nucleus possessing the requisite substituents. Again, several reactions have been carried out as models for the sequence outlined in Scheme III, although it has not been completed.

As a starting compound, dehydroacetic acid (13) was chosen since it is readily available and has been studied more extensively than any of the other α-pyrones. Dehydroacetic acid can readily be deacylated to give triacetic acid lactone (8, 6-methyl-4-hydroxy-2H-pyran-2-one) by the action of concentrated sulfuric acid at elevated temperatures[101] (120-140°).

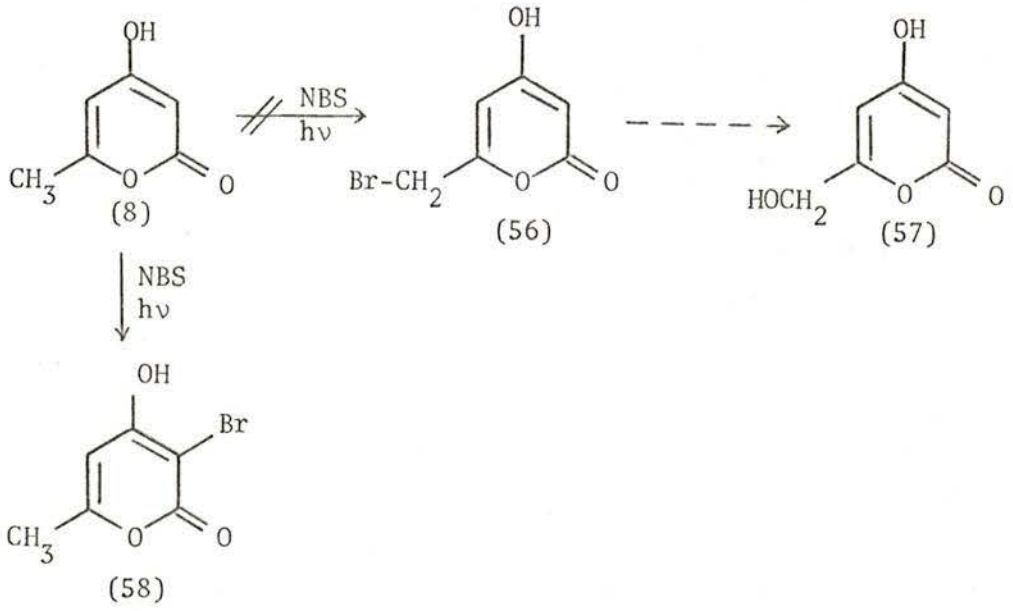



(13) (8)

SCHEME III

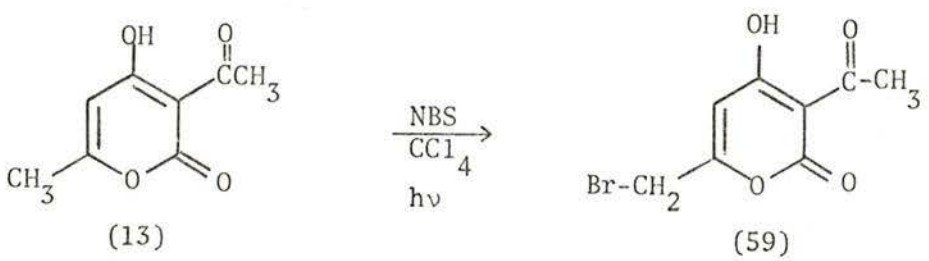

OH
O
CCH3
CH3
O
O
(13)
NBS
BrCH2
(59)
Δ
H2O
HO-CH2
conc.
H2SO4
HOCH2
(57)
[O]
H-C
n-C8H17COCl
TFA
C-C8H17-n
CH2N2
Et2O
OCH3
+

It was first thought that bromination of triacetic acid lactone (8) under free radical conditions would give the 6-bromomethyl compound (56) which could then be hydrolysed to 4-hydroxy-6-hydroxymethyl-2H-pyran-2-one (57).

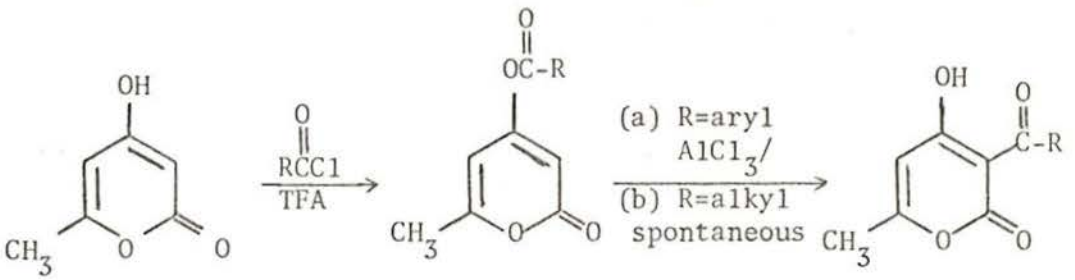



However, Harris et al.[102] have demonstrated that bromination of triacetic acid lactone and its methyl ether both give bromination products in which the bromine is substituted at $C_3$ (58).

Bromination of the $C_6$-methyl can nevertheless be accomplished by treatment of dehydroacetic acid with an equimolar amount of N-bromosuccinimide (NBS) in the presence of light to give (59) 3-acetyl-6-bromomethyl-4-hydroxy-2H-pyran-2-one.[17]



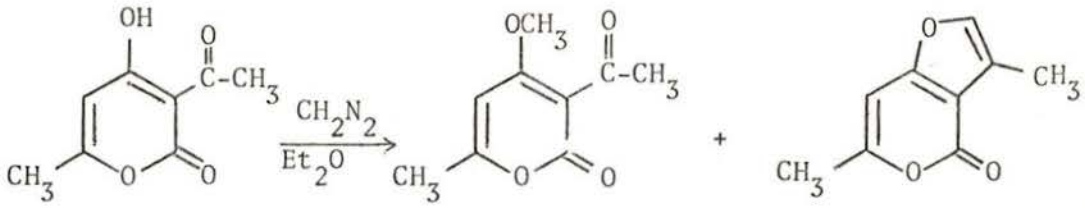
Introduction of an acyl substituent onto the α-pyrone nucleus of triacetic acid lactone proved easier than expected. Marcus[103] has described the reaction of triacetic acid lactone (8) with acyl chlorides and anhydrides in trifluoroacetic acid. He found that aromatic acyl chlorides or anhydrides gave the aromatic esters of triacetic acid lactone (60) while aliphatic acyl chlorides gave 3-acyl-α-pyrones (61) by a Fries rearrangement reaction. The aromatic esters could be rearranged by heating with aluminum chloride:




When triacetic acid lactone was refluxed with n-nonanoyl chloride in trifluoroacetic acid, the product was found to be exclusively the $C_3$ acylation compound (61, R = n-$C_8H_{17}$). The NMR spectrum of this compound showed a two proton triplet at δ 3.06, due to the -$CH_2$- adjacent to the substituent carbonyl function, and similar in position to the analogous triplet in phacidin (δ 2.96). The n-nonanoyl group in phacidin must indeed be present as a ketone substituent.

As a model reaction for the methylation of the ring hydroxyl group, dehydroacetic acid (13) was treated with diazomethane. The product was found to be mainly the expected 3-acetyl-4-methoxy-6-methyl-2H-pyran-2-one (62), with none of the desired γ-pyrone isomer being detected. The main product (62) was accompanied by a dehydration

product later identified as 4-oxo-3,6-dimethyl-4H-furo[3,2-c]pyran (63).[104]




(13) (62) (63)

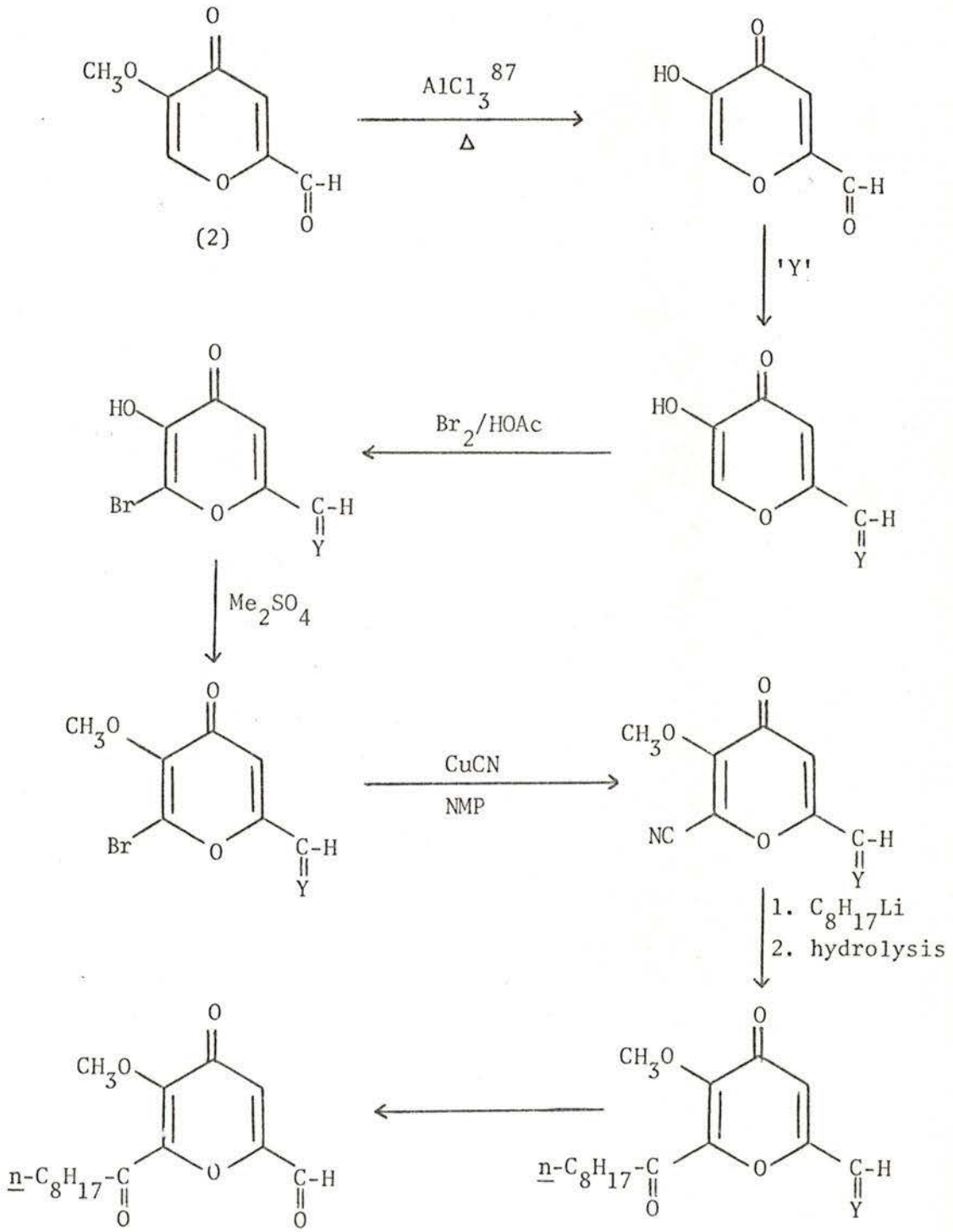
Although Scheme III has not been completed, it is proposed as a feasible means of preparing a trisubstituted pyrone having all of the desired functional groups.

During the process of this work, a number of other schemes for the preparation of phacidin were proposed, all of which ran into difficulties at one stage or another. There are, as well, a large number of synthetic schemes, such as Scheme IV, which can be proposed but have not yet been investigated to any extent. Little work has been done utilizing either of the other main methods of synthesis of γ-pyrones; that is, ring closure of a tricarbonyl system or preparation from a carbohydrate.

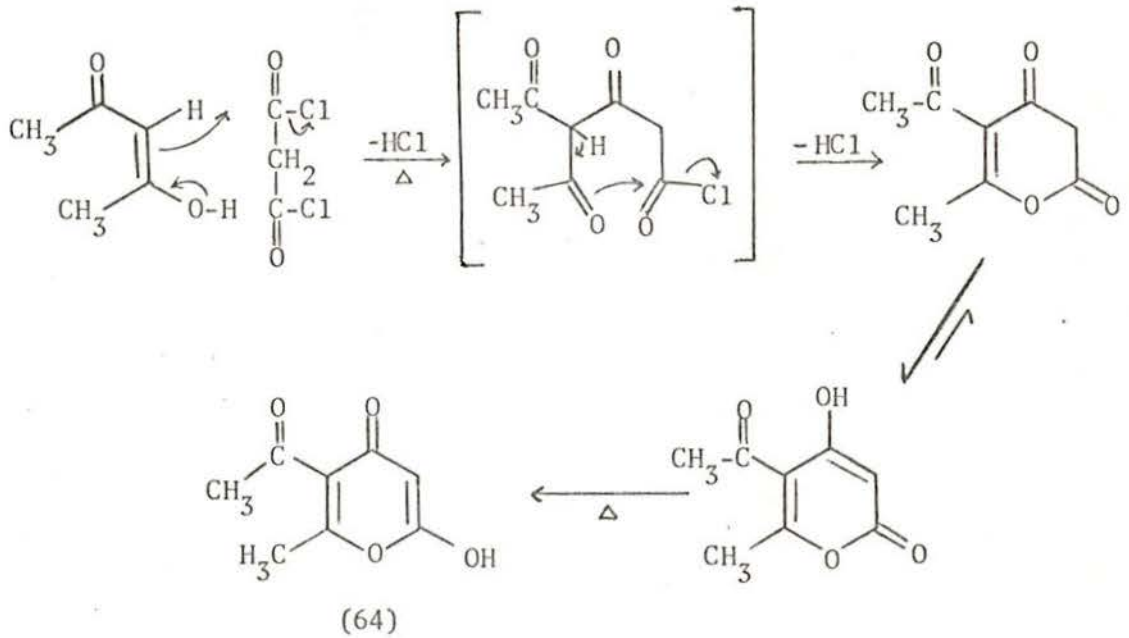
(d) From an Acyclic Polycarbonyl Compound (Scheme V)

Butt and Elvidge[105] have prepared an isomer of dehydroacetic acid (64) by condensing acetylacetone with malonyl dichloride.

SCHEME IV

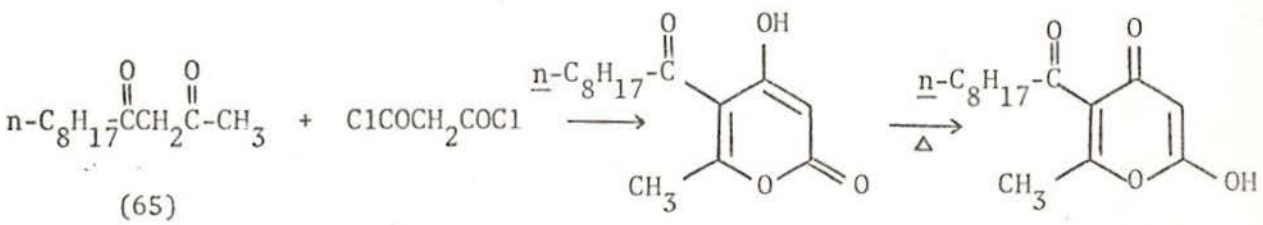



'Y' = protecting group e.g. -S-$(CH_2)_3$-S-, -O-$CH_2CH_2$-O-



(64)

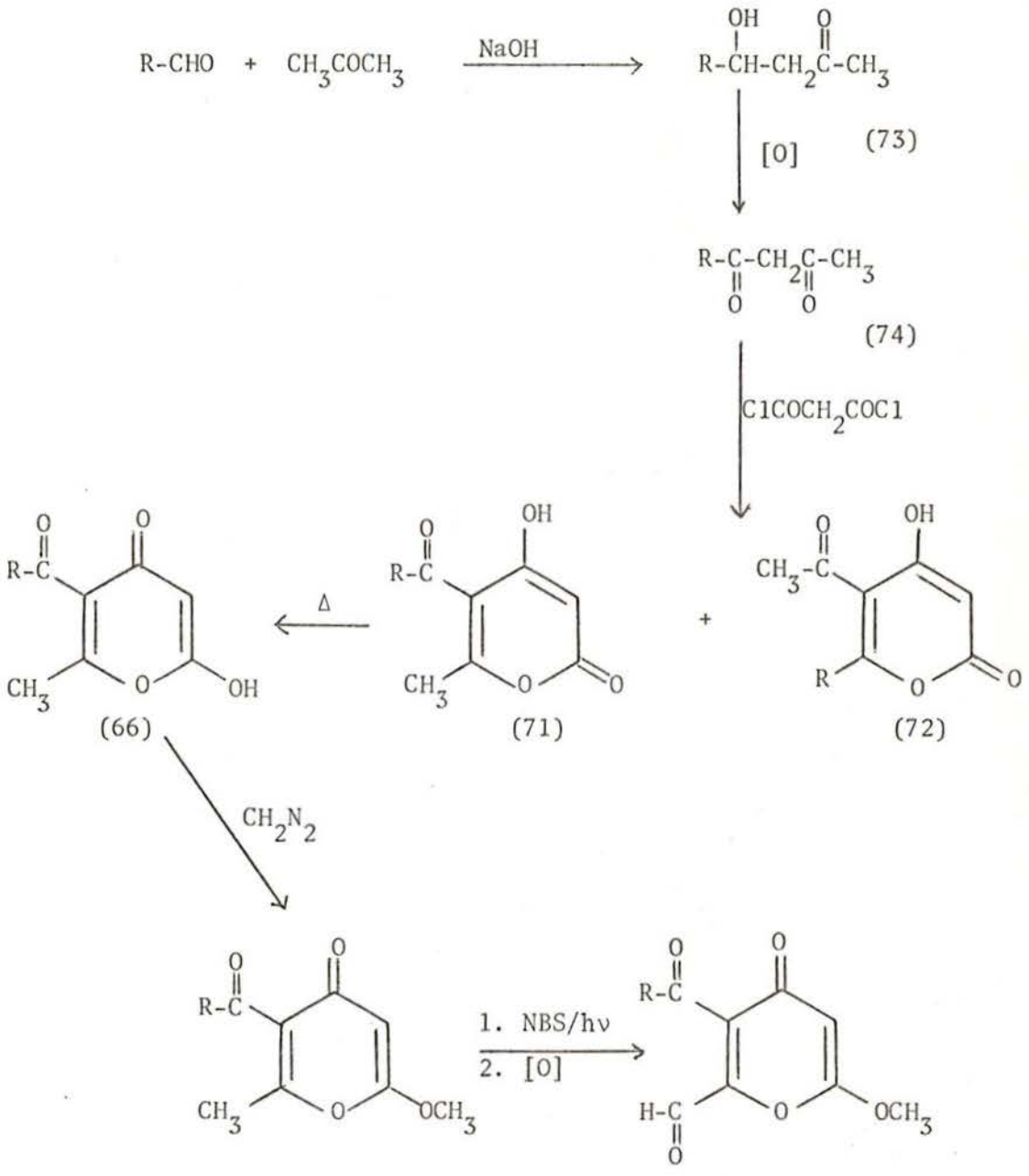
Assuming a similar reaction would occur using malonyl dichloride and n-nonanoyl acetone (65, R = n-$C_8H_{17}$-) (Scheme V), a γ-pyrone product (66) having the desired n-nonanoyl substituent should readily be prepared:



(65) (66)

After careful consideration of the mechanism of the reaction, it is evident that the direction of enolization of the acylacetone (65) will determine the direction of cyclization. Butt and Elvidge[105]

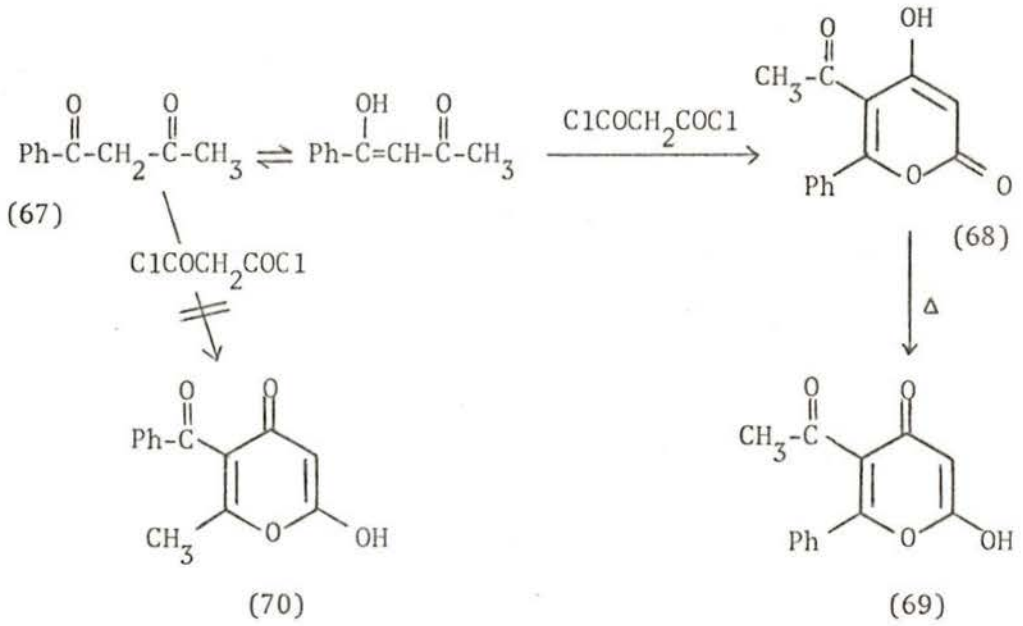
SCHEME V




R = n-$C_6H_{13}$ model

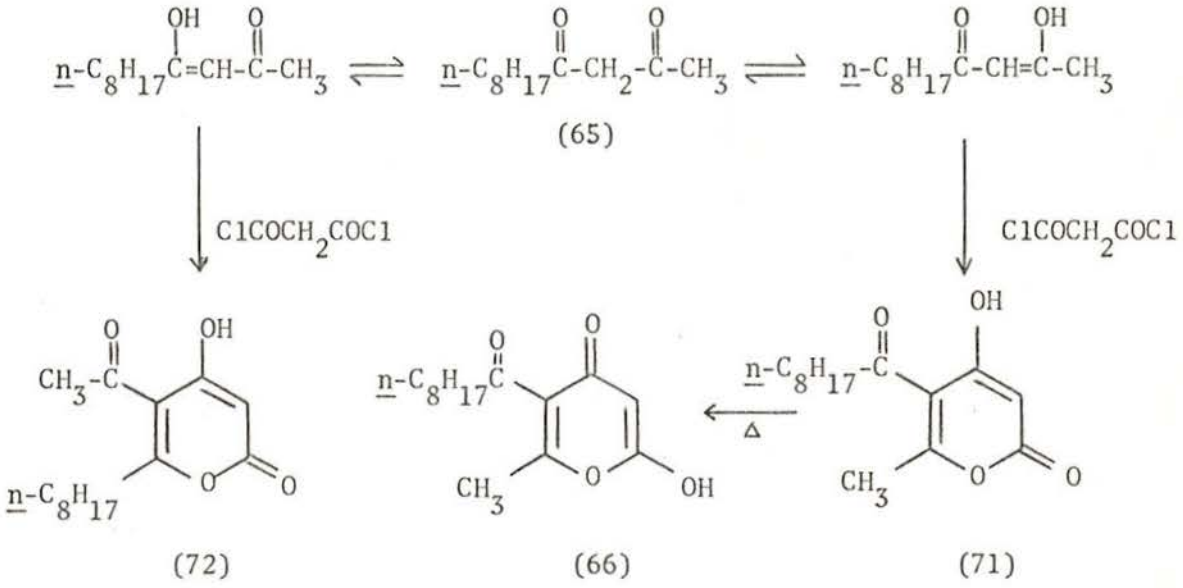
n-$C_8H_{17}$ proposed

found that benzoylacetone (67) enolized towards the phenyl ring to give exclusively the α-isomer, 5-acetyl-4-hydroxy-6-phenyl-2H-pyran-2-one (68), which tautomerized to the γ-form (69) above its melting point.




No evidence was obtained for the presence of a 5-benzoyl-6-methyl isomer such as (70) in the reaction product.

Since nonanoylacetone (65) could enolize in either direction (towards the methyl end of the molecule or towards the nonanoyl end), a mixture of products (71) and (72) would be expected to be formed. This reaction, however, was not completed since difficulties arose in the preparation of the acyl acetone to be used in the condensation reaction. Since n-heptanal was immediately available, it was used as a model for the reactions of n-nonanal. Reaction of n-heptanal and acetone in dilute sodium hydroxide[106] gave the expected aldol

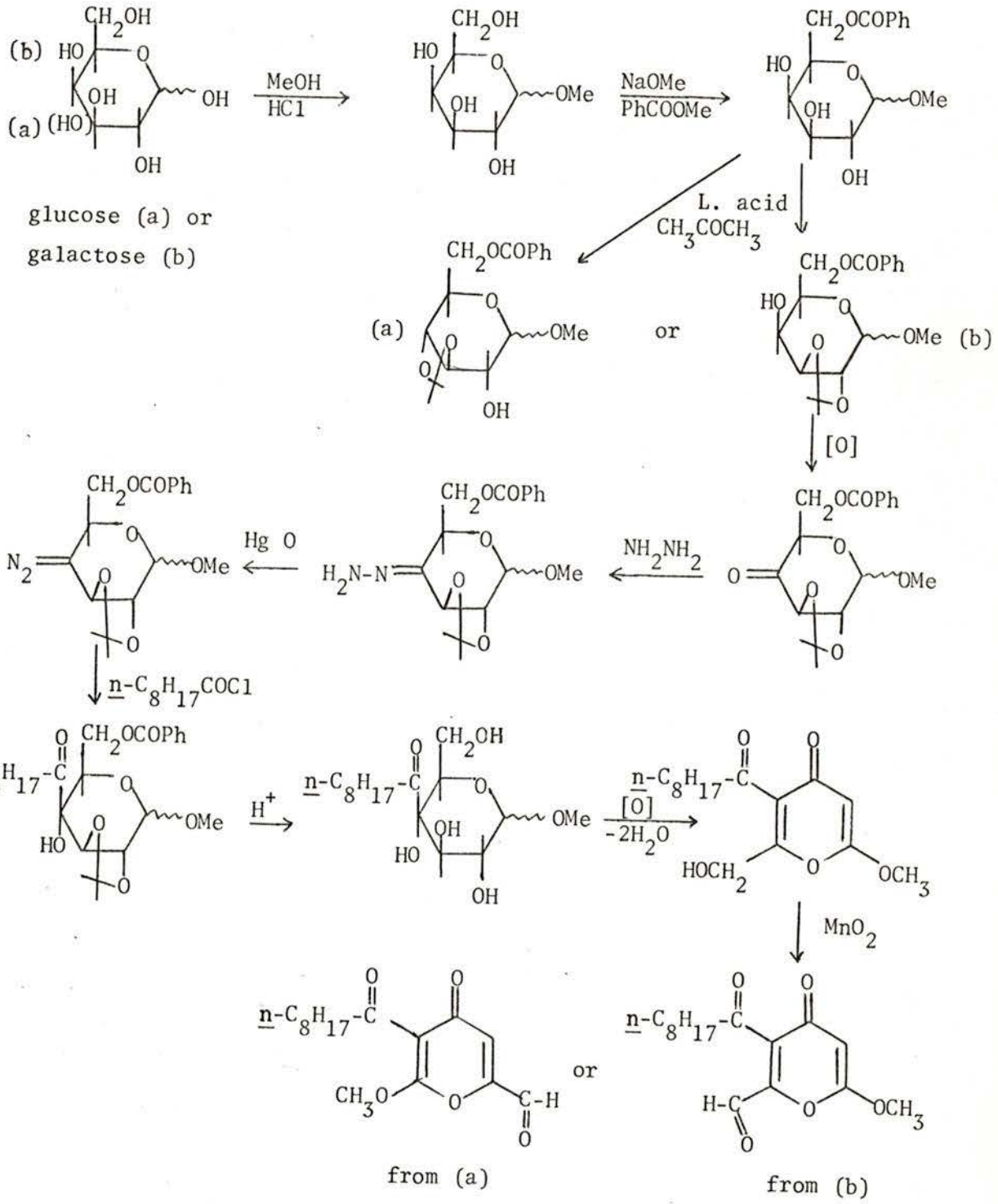



condensation product (73, R = n-$C_6H_{13}$-) but oxidation of the alcohol to the ketone (74) proved more difficult than expected. Treatment of (73) with a variety of oxidizing agents including silver carbonate, chromium trioxide/pyridine, and sodium dichromate (at room temperature and at reflux) allowed only reisolation of the starting alcohol with no evidence of oxidation or dehydration having occurred. This reaction sequence was not pursued further.

(e) From a Pyranose Sugar (Scheme VI)

The possibilities for synthesis of γ-pyrones from carbohydrates have been outlined in the introduction. One advantage of this method is that the introduction or modification of substituent functional groups can possibly be accomplished prior to oxidation/dehydrogenation of the

SCHEME VI


glucose (a) or
galactose (b)
MeOH
HCl
NaOMe
PhCOOMe
L. acid
CH3COCH3
or
[O]
Hg O
NH2NH2
n-C8H17COCl
H+
[O]
-2H2O
MnO2
or
from (a)
from (b)

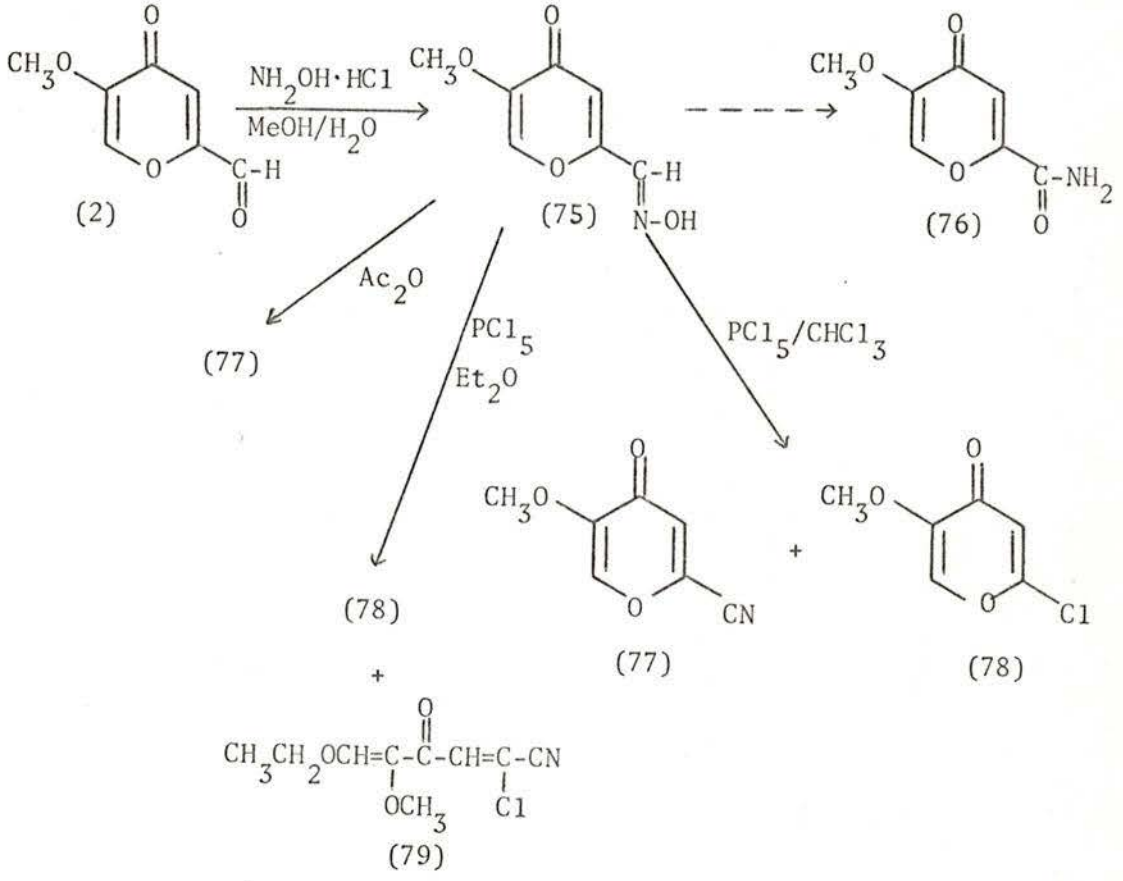
pyranoside to the γ-pyrone ring system. However, syntheses such as the one outlined in Scheme VI are long and elaborate making other schemes appear much more favourable.

## 2. Novel Reactions and Spectral Properties of γ-Pyrones

While attempting to synthesize phacidin and its isomers, a number of interesting reactions and spectral properties of γ-pyrones were encountered.

### (a) Reactions of 5-Methoxy-4-oxo-4H-pyran-2-carboxaldehyde-oxime (75)

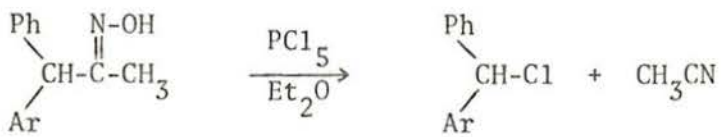
In order to gain further information about the chemical reactivity of phacidin, we decided to investigate the properties of a simple γ-pyrone carboxaldehyde. The oxime (75) of 5-methoxy-4-oxo-4H-pyran-2-carboxaldehyde was thus prepared in the same way as the oxime of phacidin. The oxime was similarly treated with phosphorous pentachloride in chloroform to produce the expected Beckmann rearrangement products, the primary amide (76) and/or its dehydration product (77), which could be examined spectrally. Two products were formed, the first of which was readily identified as the dehydration product, the nitrile (77), by its infrared ($\nu_{C\equiv N}$ 2250 $cm^{-1}$), NMR (δ 7.61, 1Hs, $-C_6-\underline{H}$; 6.88, 1Hs, $-C_3-\underline{H}$; 3.79, 3Hs, $-OC\underline{H}_3$), and mass spectra ($M^+$ 151.027, calculated for $C_7H_5NO_3$ 151.027). The second product had an NMR very similar to that of the first and typical of a 2,5-disubstituted γ-pyrone (δ 7.14, 1Hs, $-C_6-\underline{H}$; 6.48, 1Hs, $-C_3-\underline{H}$). The mass spectrum was characteristic of a chlorine containing substance (m/e 162, 13.7%; $M^+$ 159.986, 37.2%; calculated for $C_6H_5{}^{35}ClO_3$ 159.993) and exhibited a fragmentation pattern which supported the identification of the product as 2-chloro-5-methoxy-4H-pyran-4-one (78).



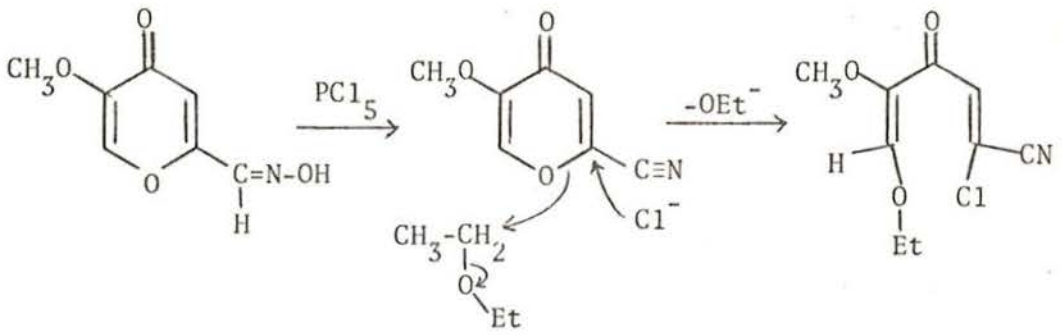

In an attempt to clarify the course of the reaction, the oxime was then reacted with $PCl_5$ in diethyl ether at low temperature. Again two products were isolated which could be separated by column chromatography. Using these conditions, neither the nitrile nor the corresponding amide rearrangement product was isolated. One of the products was identified as the chloride (78), while the other product was found to be different from the disubstituted γ-pyrones so far encountered. The mass spectrum indicated a molecular formula of $C_9H_{10}ClNO_3$ (m/e 217, 8.2%; $M^+$ 215.031, 24.4%; calculated 215.035)

with a parent ion cluster characteristic of a compound containing one chlorine atom. An absorption in the infrared spectrum at 2225 $cm^{-1}$ indicated the presence of a nitrile group, while the NMR showed signals due to an ethoxyl group (δ 3.86, 2H q; 1.27, 3H t), a methoxyl group (δ 3.86, 3H s), and two vinyl protons (δ 6.19, 1H s; 5.69, 1H s) shifted upfield too far to be γ-pyrone ring protons. On the basis of this spectral evidence, the compound was assigned the structure (79), 2-chloro-6-ethoxy-5-methoxy-4-oxo-2,5-hexadienoic acid nitrile.

Formation of a chloro-substituted compound by displacement of an oxime is not a new reaction. Under Beckmann rearrangement conditions ($PCl_5/Et_2O$), oximes have been found to cleave to give a nitrile plus another fragment which either loses a proton to give an alkene, or picks up a chloride ion to form an alkyl chloride. Hassner and Nash[107] prepared a number of benzhydryl chlorides by treating 1,1-diaryl-2-propanone oximes with $PCl_5$ in diethyl ether:

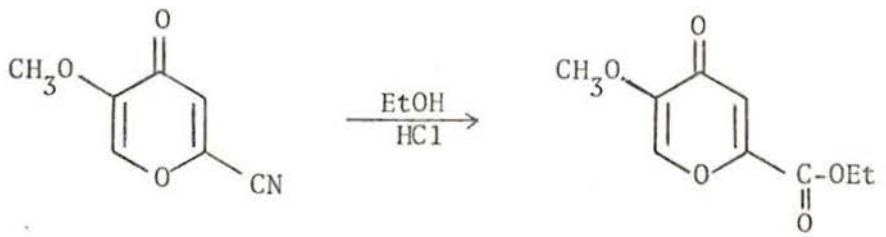


While both the chloride and the nitrile arise through reactions which have previously been explored, ring-opened products such as (79) arising under Beckmann rearrangement conditions have not been reported. The cleavage product could represent a trapped "intermediate" in the substitution of chloride for cyanide, although the mechanism is at present purely speculative.



The reaction was repeated in glyme (ethylene glycol monomethyl ether) in an attempt to prepare the methoxy analogue of (79). However, this reaction gave a quantitative yield of the chloride (78). The normal Beckmann rearrangement of the oxime to the nitrile was found to occur in high yield using acetic anhydride/acetic acid.

A further attempt to clarify the mechanism of formation of (79) involved heating the nitrile (77) in 100% ethanol containing dry HCl. The product, however, was not a ring opened compound, but the ethyl ester of comenic acid (80) formed on solvolysis of the nitrile function.



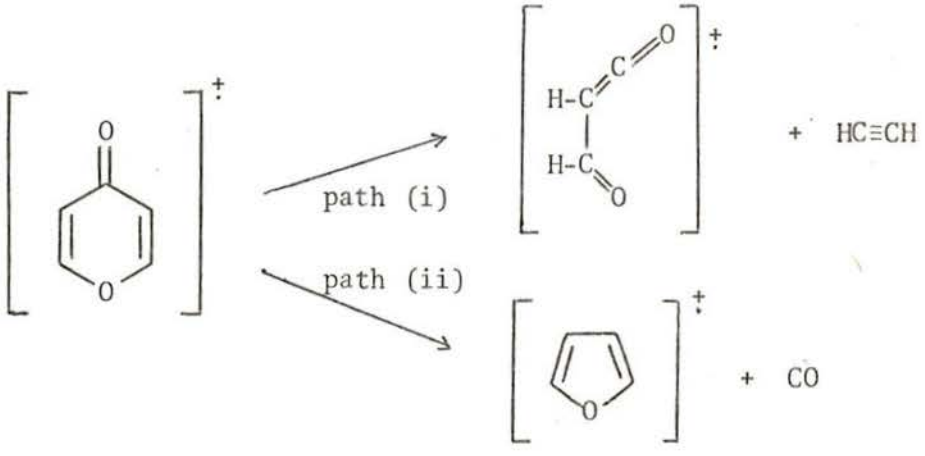
(77) (80)

(b) Mass Spectral Fragmentation of 3-Methoxy-γ-Pyrones

Throughout the investigation of the reactions of γ-pyrones, mass spectra were used to help elucidate structures of new compounds and to confirm formation of known reaction products. The mass spectra of simple γ-pyrones have been studied extensively and two primary pathways of fragmentation found to occur in both γ-pyrone itself and a number of substituted γ-pyrones[21]:

(i) The main fragmentation occurs by a retro-Diels-Alder mechanism[108] which leads to the expulsion of acetylene or a substituted acetylene;

(ii) A second primary fragmentation occurs involving the expulsion of carbon monoxide, leaving a furan-like species, the structure of which has recently been challenged.[109]




The mass spectra of kojic acid (5), 6-bromo-kojic acid (45), kojic acid-2-methyl ether (81), and maltol (4) all show major fragmentations by retro-Diels-Alder type cleavage, and by expulsion of carbon monoxide and a hydrogen radical. On comparison of the mass spectra of these β-hydroxy-γ-pyrones with the mass spectra of

their methyl ethers and other β-methoxy-γ-pyrones, one striking and unexpected difference was noticed: almost all of the β-methoxy-γ-pyrones studied showed a significant peak at $M^+$ - 18, indicative of water loss (see Table V). This feature was not observed to any great extent in the β-hydroxy compounds, while both the β-hydroxy and β-methoxy compounds had the normal peaks at $M^+$ - 28 (loss of carbon monoxide), and at $M^+$ - 29 (loss of carbon monoxide and a hydrogen radical). A second feature of the β-methoxy-γ-pyrones which distinguished them from their β-hydroxy analogues was the distinct decrease in, or absence of, peaks corresponding to retro-Diels-Alder cleavages. This problem, however, was not investigated further.

Since no simple fragmentation could be deduced to explain the water loss, a study of deuterated species was undertaken to help elucidate what must be a complex rearrangement process. Methyl maltol was chosen as a model compound for these studies since it is readily prepared and shows an intense $M^+$ - 18 peak in the mass spectrum (m/e 122, 39%) [Fig. 10(a)]. The position in methyl maltol most amenable to introduction of a deuterium label is the methoxyl methyl group, during methylation.

Several different methods of preparing methyl-$d_3$ maltol were used giving varying amounts of label incorporation. Methyl iodide-$d_3$ was prepared from trimethylsulfoxonium iodide (82) by the method of Cotton *et al*[110] illustrated below:

| Substituents | | | | | Peak Heights (as % of base peak) | | | |
|---|---|---|---|---|---|---|---|---|
| $R_2$ | $R_3$ | $R_5$ | $R_6$ | MWt | $M^+$ | $M^+$-18 | $M^+$-28 | $M^+$-29 |
| $CH_2OH$ | | OH | | 142 | 100 | | 3 | 50 |
| $CH_3$ | OH | | | 126 | 100 | | 5 | 28 |
| $CH_2OH$ | | OH | Br | 220 | 79 | | <1 | 2 |
| $CH_2OCH_3$ | | OH | | 156 | 100 | | 3 | 26 |
| $CH_2OH$ | | $OCH_3$ | | 156 | 100 | 29 | 2 | 5 |
| $CH_2OCH_3$ | | $OCH_3$ | | 170 | 33 | 7 | | 3 |
| $CH_3$ | $OCH_3$ | | | 140 | 50 | 39 | | 10 |
| CHO | | $OCH_3$ | | 154 | 87 | 33 | 5 | 59 |
| $CH_2OTHP$ | | $OCH_3$ | | 240 | 51 | * | | |
| Cl | | $OCH_3$ | | 160.5 | 57 | 1 | 43 | 11 |
| CN | | $OCH_3$ | | 151 | 40 | 4 | 1 | 10 |
| CH=NOH | | $OCH_3$ | | 169 | 43 | 33 | | 3 |
| $CH_2OCH_3$ | | $OCH_3$ | Br | 249 | 100 | 9 | | 5 |
| $CH_2Br$ | $OCH_3$ | | | 219 | 20 | 3 | | |
| COOEt | | $OCH_3$ | | 198 | 38 | 7 | 4 | 11 |

* $M^+$ -  = 156; 156-18 = 138 100%; 156-28 = 128 3%; 156-29 = 127 6%

Table V. Primary Fragmentation of β-Hydroxy and β-Methoxy-γ-pyrones.

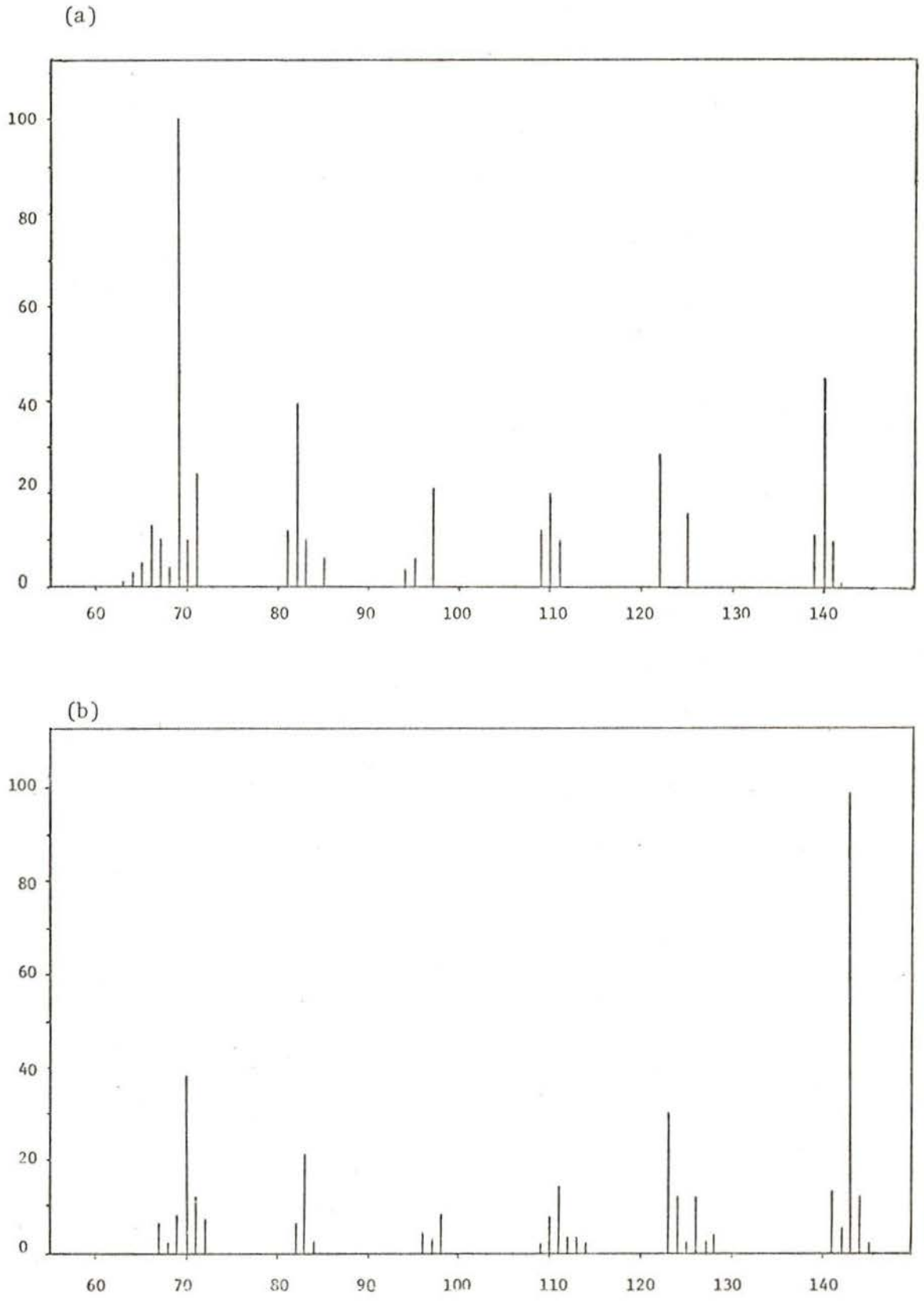



Fig. 10. Mass Spectra of (a) Methyl-$d_o$ Maltol and (b) Methyl-$d_3$ Maltol (mass range 900).

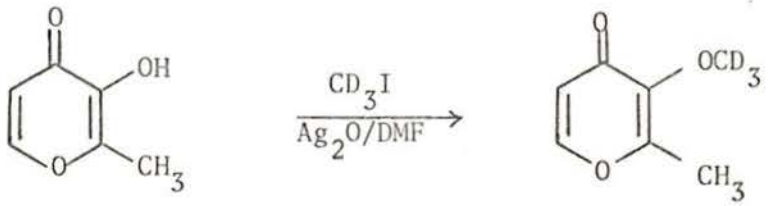
$$(CH_3)_2SO + CH_3I \longrightarrow (CH_3)_3\overset{+}{S}O\ \overset{-}{I}$$

(82)

$$\downarrow \; K_2CO_3 \;|\; D_2O \;(x3)$$

$$(CD_3)_2SO + CD_3I \longleftarrow (CD_3)_3\overset{+}{S}O\ \overset{-}{I}$$

Methyl-$d_3$ maltol (83) was then prepared from maltol and $CD_3I$ by a silver oxide catalysed process, and purified by column chromatography. Mass spectral examination indicated essentially 100% $d_3$ labelling and NMR was used to confirm location of the labels in the methoxyl group (no randomization).




(4) (83)

Direct measurement of the daughter ion peaks [Fig. 10(b)] corresponding to all possible water losses from the molecular ion (with correction for $^{13}C$), indicated that only $D_2O$ and HDO were lost; no $H_2O$ loss occurred. Using the metastable defocusing technique, we were able to observe water losses occurring in the first field free region (1st F.F.R.) of the mass spectrometer (see Fig. 11). Again it was evident that only $D_2O$ and HDO were lost, and metastable ion peaks gave the same values for water loss as those obtained directly from the spectrum (Table VI). Metastable ions formed in the second field free region (2nd F.F.R.) of the mass spectrometer are observed as broad

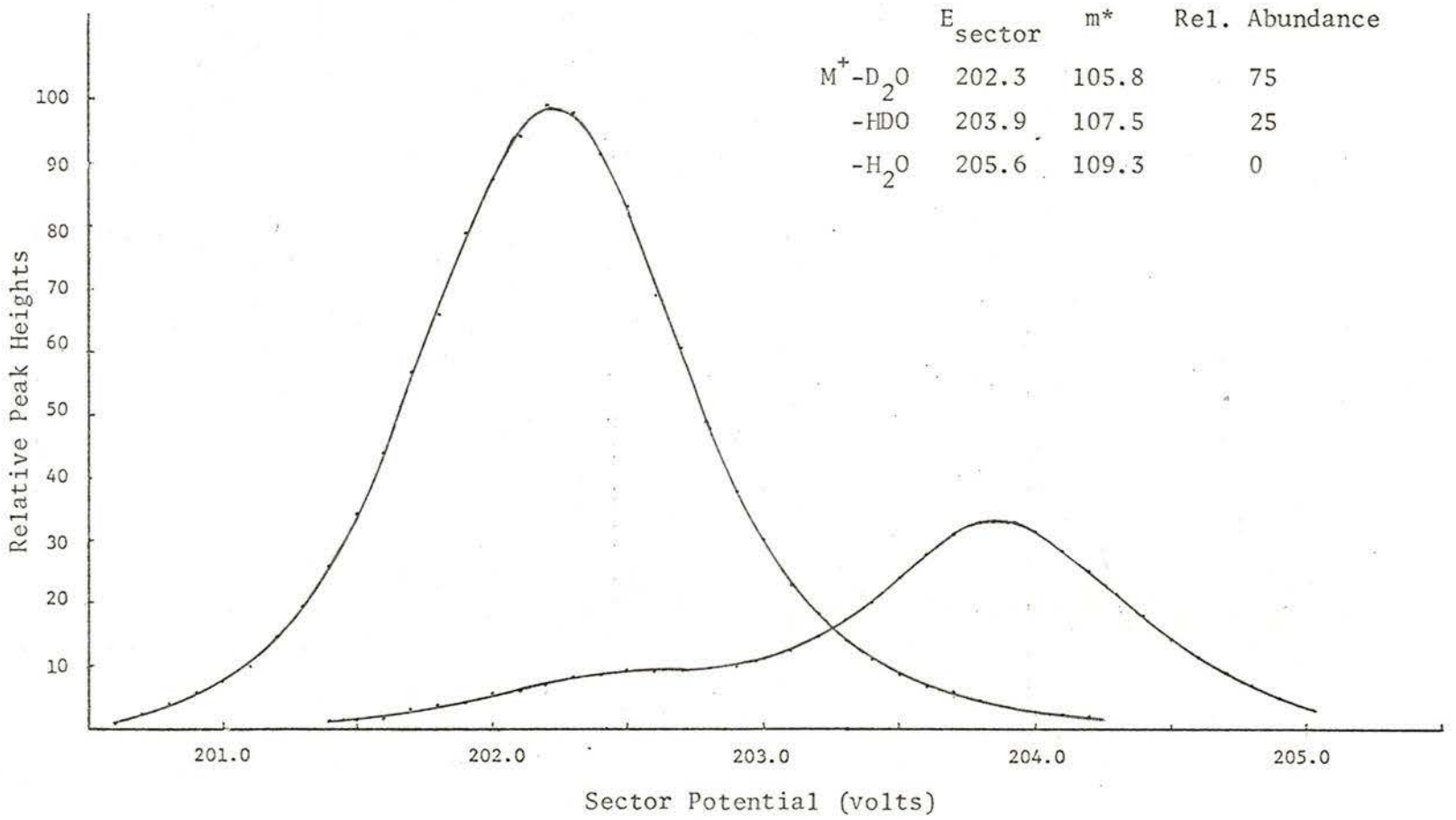



Fig. 11. Defocused metastables for water losses from methyl-$d_3$ maltol.

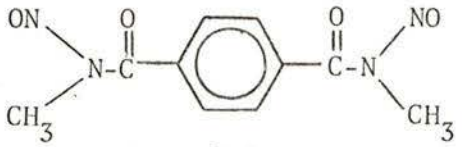
peaks in the normal mass spectrum. The relative intensities of these metastable ion peaks for water loss were also measured and found to give the same results as the other two methods. These results were

| | daughter ion | 1st F.F.R. m* (d focused mode) | 2nd F.F.R. m* |
|---|---|---|---|
| $D_2O$ | 74 | 75 | 72 |
| HDO | 24 | 25 | 28 |
| $H_2O$ | 2 | 0 | 0 |

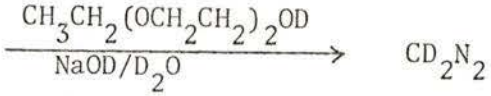
Table VI. Relative abundances of water loss from the molecular ion of methyl-$d_3$ maltol (%).

surprising since we had first proposed a scheme where only one of the hydrogens lost as water originated from the methoxyl group.

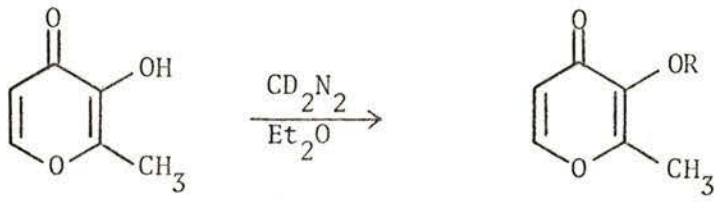
In an attempt to prepare methyl-$d_2$ maltol, maltol was reacted with deuteriodiazomethane ($CD_2N_2$), prepared from N,N'-dimethyl-N,N'-dinitrosoterephthalamide (84) by a method similar to that of Campbell.[111]



(84)




The reaction of $CD_2N_2$ with maltol gave a product which, by NMR, contained approximately 50% deuterium at the methoxyl position. Analysis by mass spectrometry showed a mixture of partially deuterated products in the relative amounts shown in Table VII.




(4)

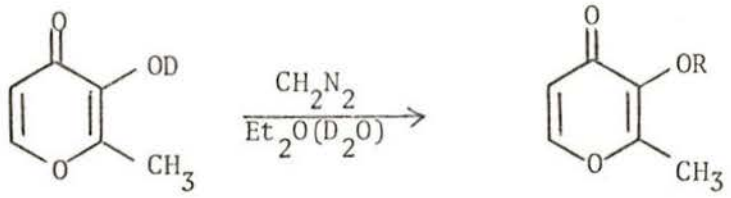
| R | % Formed | Relative Water Losses from Each Species (%) | | |
|---|---|---|---|---|
| | | $H_2O$ | HDO | $D_2O$ |
| $CD_3$ | 17 | | 30 | 70 |
| $CHD_2$ | 38 | 10 | 64 | 26 |
| $CH_2D$ | 34 | 35 | 65 | |
| $CH_3$ | 11 | 100 | | |

Table VII.

The relative abundances of metastable ion peaks (defocused mode) for water loss from the molecular ions of the partially labelled compounds indicate the relative losses of $H_2O$, HDO and $D_2O$ from each species (Table VII). It was not possible to determine the relative water losses directly from the daughter ion peaks because of the number of different fragmentations leading to each daughter ion (e.g. methyl-$d_3$ maltol-$D_2O$ ≡ methyl-$d_2$ maltol-HDO).

A third preparation of labelled methyl maltol involved methylation of maltol-CD (85) with diazomethane ($CH_2N_2$). Although this method was expected to produce methyl-$d_1$ maltol, essentially the same

results as those produced from deuteriodiazomethane methylation were obtained (Table VIII).

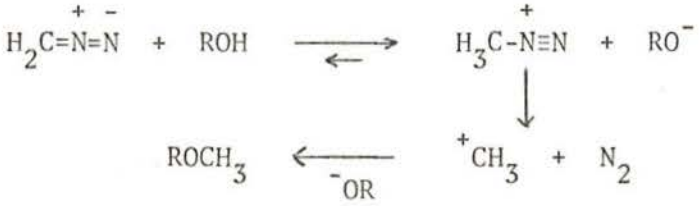



(85)

| R | % Formed | Relative Water Losses from Each Species (%) | | |
|---|---|---|---|---|
| | | $H_2O$ | HDO | $D_2O$ |
| $CD_3$ | 16 | | 31 | 69 |
| $CHD_2$ | 37 | 10 | 64 | 26 |
| $CH_2D$ | 35 | 39 | 61 | |
| $CH_3$ | 12 | 100 | | |

Table VIII.

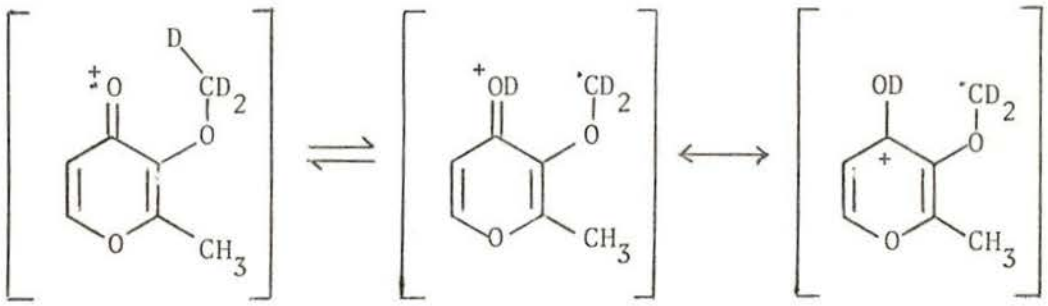
From the mechanism of diazomethane methylation, one would expect the incorporation by the first method ($CD_2N_2$ + ROH) to be approximately 67% instead of 55%, and, by the second method ($CH_2N_2$ + ROD), 33% instead of 53%.



The observed discrepancies can be explained fairly readily. In the preparation of deuteriodiazomethane, the carbitol-OD contained a small amount of carbitol-OH and possibly some HDO, both of which would exchange the $CD_2N_2$ (to $CHDN_2$ and $CH_2N_2$) as it was being formed. Any traces of water in the ether would also reduce the proportion of $CD_2N_2$ present and lower the isotope content of the product.

In the second preparation, the hydroxy proton of maltol was first exchanged using $D_2O$, then the maltol-OD was methylated with $CH_2N_2$. Traces of $D_2O$ in the reaction mixture would rapidly exchange the $CH_2N_2$ protons (to $CHDN_2$ and $CD_2N_2$) and increase the total isotope incorporation into the product.

The most plausible mechanism by which $D_2O$ (HDO) loss from methyl-$d_3$ maltol could be explained involves transfer of a hydrogen from the methoxyl group to the carbonyl oxygen:




The mechanism would then require migration of a second hydrogen to the same oxygen to allow water loss. In order to explain $D_2O$ loss from the $d_2$ and $d_3$ compounds, both of these hydrogen atoms must arise from the methoxy methyl group. Since transfer of a hydrogen from the $-\dot{C}D_2$ group is unlikely, it seems reasonable to assume that another hydrogen atom must first migrate to the methoxy methyl carbon ($-O\dot{C}D_2 \xrightarrow{H\cdot} -OCD_2H$).

Subsequently, a second hydrogen transfer from $-CD_2H$ to the carbonyl oxygen would account for loss of HDO and $D_2O$ from both the $d_2$ and $d_3$ labelled methyl maltols.

Support for the proposed first hydrogen migration can be gained from inspection of the mass spectra of the $d_3$ labelled compound [Fig. 10(b)] and unlabelled methyl maltol [Fig. 10(a)]. Clearly, an isotope effect must be involved in all of the major fragmentation processes since the fragment ions of the labelled compound are all reduced in intensity, with respect to the parent molecular ion. (The relative intensities of the fragment ions with respect to each other remain essentially the same.) The isotope effect is large in magnitude ($\sim$ 6) and must arise from the breaking of a C-D bond prior to or during fragmentation (primary isotope effect). Since all of the major fragmentation processes are affected to approximately the same extent, the formation of an intermediate involving C-D bond cleavage prior to fragmentation would best explain this effect. As can be seen in Fig. 12, the proposed intermediate could readily fragment to lose $OCD_2$ (143 $\rightarrow$ 111). By a second hydrogen migration, an intermediate Ion A would be formed containing a $-OCD_2H$ group which could readily lose $CD_2H$ (143 $\rightarrow$ 126) or transfer either a protium or deuterium atom to allow loss of $D_2O$ and HDO (143 $\rightarrow$ 123,124).

The other fragmentation processes of methyl maltol shown in Fig. 12 were substantiated by identification of metastable ion peaks for the unlabelled compound in conjunction with isotopic labelling. It is interesting to note that the base peak at m/e 69 in the unlabelled compound does not arise, as expected, from a retro-Diels-Alder

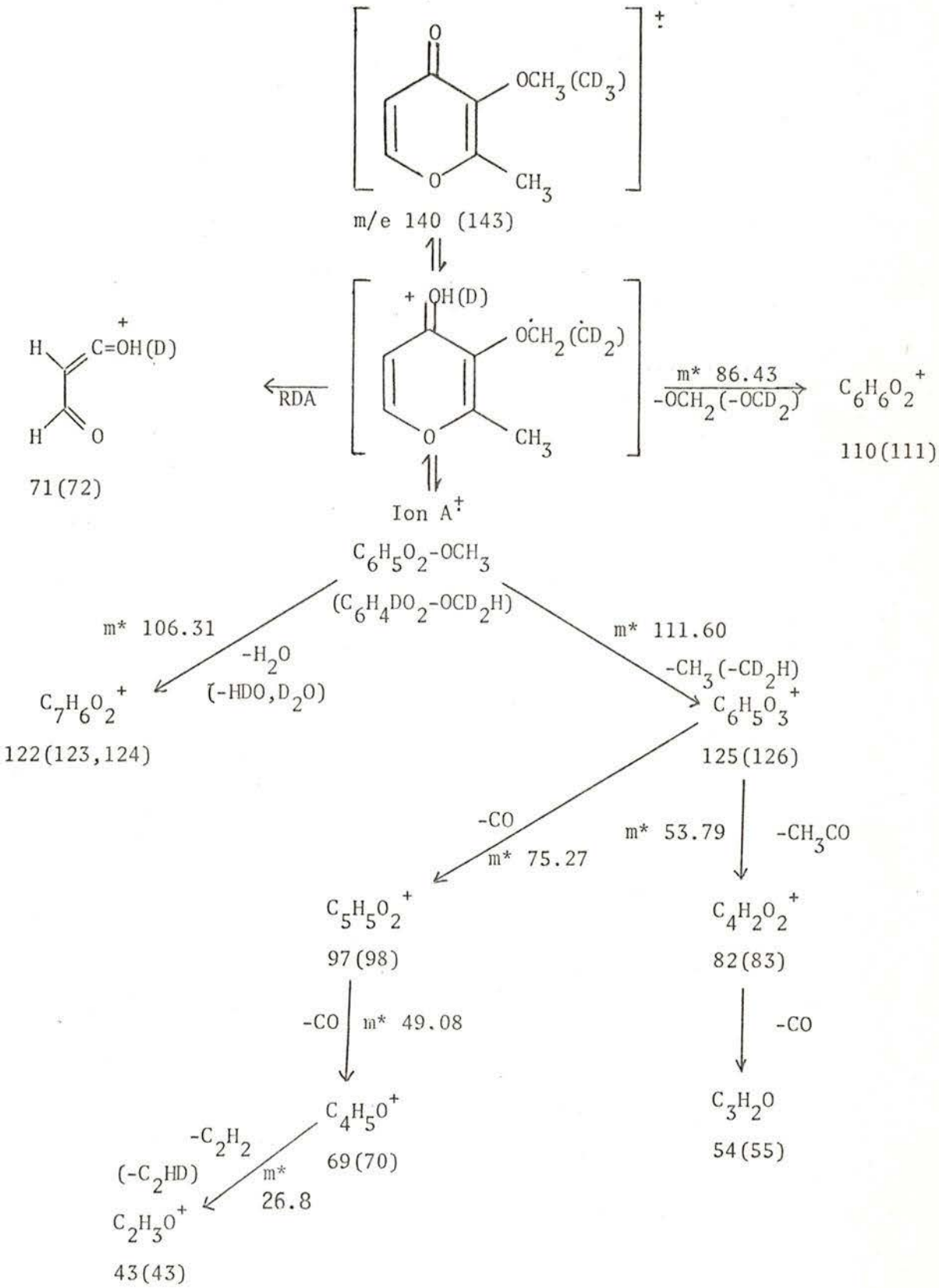



Fig. 12. Mass spectral fragmentation of methyl-$d_o$ ($-d_3$) maltol.

cleavage of the ring. Instead, it is produced by a series of fragmentations involving methyl and carbon monoxide losses (similar to the fragmentation of α-pyrones).

While this mechanism appears to be a reasonable one, the ratio of $D_2O$:HDO loss from methyl-$d_3$ maltol is larger than would be predicted. One would expect a ratio of 2:1 favouring loss of $D_2O$ by the proposed mechanism (67:33). Although the data obtained for the partially labelled products agrees fairly closely with this value (70:30), the fully labelled compound differs significantly (75:25 or 3:1). With a second hydrogen migration from $-CD_2H$, one would expect an isotope effect to favour protium transfer over dueterium transfer. This would thus decrease the 2:1 ratio rather than produce the observed increase. Thus, we must conclude that either there is more than one mechanism operating for water loss, or that the proposed mechanism needs some refinement.

There is still considerable work which must be done to prove a mechanism for this interesting fragmentation. Introduction of deuterium and possibly $^{18}O$ labels at different positions in the methyl maltol molecule and study of a number of the other β-methoxy-γ-pyrones would no doubt aid in elucidating the mechanism of fragmentation.

## EXPERIMENTAL

### 1. 2-Hydroxymethyl-5-methoxy-4H-pyran-4-one (37)

The methyl ether of kojic acid (37) was prepared by the method of Campbell.[86] To a well stirred solution of kojic acid (11.4 g, 0.080 mole) in 10% KOH (50 $cm^3$)* was added freshly distilled dimethyl sulfate (10.1 g, 0.080 mole) in small portions over 30 min. The temperature was kept below 25° with occasional cooling and stirring was continued for an additional 30 min. Cooling to below 20° gave a pale yellow precipitate of (37) which was filtered and recrystallized from ethanol/diethyl ether (7.55 g, 60%) mp 160-161° (lit.[86] mp 161°). $\nu_{max}$ 3200 (broad), 3085 (m), 1650 (s), 1612 (s), 1590 (sh), 1460 (m), 1270 (s), 1232 (s), 1160 $cm^{-1}$ (s); $\delta$ ($CDCl_3$/acetone-$d_6$) 7.64 (1H s, $-C_6-\underline{H}$), 6.40 (1H broad s, $-C_3-\underline{H}$), 4.41 (2H m, $-C\underline{H}_2-O$), 3.71 (3H s, $-OC\underline{H}_3$), 2.58 (1H s, $-O\underline{H}$); m/e 156 ($M^+$, 100), 155 ($M^+$-H, 37), 138 ($M^+$-$H_2O$, 27), 125 ($M^+$-$CH_2OH$, 80), 95 (100), 83(13), 69(24).

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* It was found that in an excess of 10% KOH (80 $cm^3$) methylation occurred preferentially at the primary hydroxyl group to give 5-hydroxy -2-methoxymethyl-4H-pyran-4-one.

2. 5-Methoxy-4-oxo-4H-pyran-2-carboxaldehyde (38)

The aldehyde was prepared from the monomethyl ether (37) by a method similar to that of Becker.[87] To a suspension of the methyl ether (1.00 g, 6.4 x $10^{-3}$ mole) in boiling benzene (1000 $cm^3$) was added finely powdered active* manganese dioxide (5 g). The mixture was refluxed for three hours and filtered while warm. An additional 200 $cm^3$ warm benzene was used to wash the $MnO_2$ and the filtrates were combined. Benzene was removed in vacuo leaving colourless needles of the aldehyde (38) (0.642 g, 65%) decomp. > 240°. $\nu_{max}$ 3095 (m), 1715 (s), 1667 (s), 1650 (sh), 1625 (m), 1612 (m), 1438 (m), 1235 $cm^{-1}$ (m); $\delta$ ($CDCl_3$) 9.70 (1H s, -C<u>H</u>O), 7.69 (1H s, $-C_6-\underline{H}$), 6.97 (1H s, $-C_3-\underline{H}$), 3.82 (3H s, $-OC\underline{H}_3$); m/e 154 ($M^+$, 86), 153 ($M^+$-H, 21), 136 ($M^+-H_2O$, 33), 125(59), 95(100), 83(22), 69(33).

3. 5-Methoxy-2-tetrahydropyranyloxymethyl-4H-pyran-4-one (40).

To a stirred solution of conc. hydrochloric acid (5 drops) in dihydropyran (12 $cm^3$) was added 5-methoxy-2-hydroxymethyl-4H-pyran-4-one (37) (0.400 g, 2.56 x $10^{-3}$ mole). Acetone (5 $cm^3$) was added to aid in solubilizing the alcohol (37), and the mixture was heated to 65°. After stirring at room temperature for 24 hr the reaction mixture was extracted with a saturated solution of sodium carbonate, and dried over $Na_2SO_4$. Most of the dihydropyran was removed in vacuo and addition of petroleum ether (30/60, 5 $cm^3$) completed precipitation of the white needles of tetrahydropyranyl ether; (0.275 g, 45%) mp 105-106°

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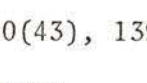
* Active $MnO_2$ was prepared from $MnSO_4 \cdot H_2O$ and $KMnO_4$ by the method of Rosenkranz, J. Chem. Soc., 2198 (1953).

from acetone/pet. ether. $\nu_{max}$ 3080 (m), 2950 (m), 2865 (m), 1658 (s), 1630 (s), 1606 (m), 1463 (s), 1443 (s), 1276 (s), 1235 $cm^{-1}$ (s); δ ($CDCl_3$) 7.59 (1H s, $-C_6-\underline{H}$), 6.52 (1H s, $-C_3-\underline{H}$), 4.71 (1H m, -O-C<u>H</u>-O), 4.40, 4.47 (both 1H s, $-C\underline{H}_2-O-$), 3.79 (3H s, $-OC\underline{H}_3$), ∿3.7 (2H m, $-CH_2-C\underline{H}_2-O-$), 1.90 to 1.40 (6H m, $\{C\underline{H}_2\}_3$); m/e 240 ($M^+$, 51), 210(4), 183(5), 167(13), 156(18), 140(43), 139(38), 138 ($M^+$ - HO-  , 100), 125(20), 111(31), 95(22), 85(76).

4. <u>2-Benzoyloxymethyl-5-hydroxy-4H-pyran-4-one (42)</u>

The benzoate ester of kojic acid was prepared by the method of Ghosal[112] and obtained as white crystals (42%) from 100% ethanol; mp 179-181 (lit.[112] mp 181°). $\nu_{max}$ (KBr) 3245 (broad), 3090 (w), 1733 (s), 1664 (m), 1622 (s), 1603 (s), 1458 (m), 1262 (s), 1215 $cm^{-1}$ (s); δ ($CDCl_3$) 7.99 and 7.60 (6H m, phenyl protons and $-C_6-\underline{H}$), 6.52 (1H s, $-C_3-\underline{H}$), 5.21 (2H s, $-C\underline{H}_2-O-$), 2.70 (1H m, $-O\underline{H}$, $D_2O$ exchanges); m/e 246 ($M^+$, 25), 105 ($PhC\overset{+}{O}$-, 100), 77 ($Ph^+$-, 25), 67(4), 51(6).

5. <u>2-Benzoyloxymethyl-5-hydroxy-6-nitro-4H-pyran-4-one (43)</u>

Nitration of (42) was carried out by a method similar to that of Eiden.[72] To a solution of kojic ester (42) (0.200 g, $8.13 \times 10^{-4}$ mole) in glacial acetic acid (5 $cm^3$) containing acetic anhydride (0.25 $cm^3$) at 40° was added fuming nitric acid (0.5 $cm^3$) dropwise with rapid stirring. After 10 min at 40°, the solution was cooled to 15° and stirring continued for one hour. The mixture was poured into 10 $cm^3$ ice water and a white solid precipitated. After standing at 4° for 24 hr, the precipitate was filtered, washed with a few $cm^3$ of water, and

dried in vacuo (0.114 g, 48%) yellow needles from EtOH/$H_2O$, mp 170° with decomp. $\nu_{max}$ (KBr) 3230 (broad), 1738 (m), 1642 (s), 1556 (m), 1454 (m), 1280 (s), 720 $cm^{-1}$ (m); δ (acetone-$d_6$) 8.2 to 7.4 (5H m, Ph-), 6.77 (1H s, $-C_3-\underline{H}$), 5.38 (2H s, $-\underline{CH_2}-O-$); m/e 291 ($M^+$, 1), 169(5), 167(5), 123(11), 122(7), 105 ($PhC\overset{+}{O}-$, 100), 77(48), 51(16).

The filtrate was concentrated in vacuo to 3 $cm^3$ then poured over 3 $cm^3$ ice water. Precipitation of a white solid occurred immediately. After standing at 4° overnight, the precipitate was filtered, washed with 1 $cm^3$ water and dried in vacuo (0.049 g, 33%) colourless crystals mp 110-112° (lit.[113] mp 112°). This product was identified spectrally as the phenyl ester of glycolic acid (44). $\nu_{max}$ (KBr) 3200-2800 (broad), 1730 (s), 1430 (m), 1245 (s), 1120 (s), 728 $cm^{-1}$ (s); δ (acetone-$d_6$), 8.20 to 7.49 (5H m, Ph-), 6.8 (broad 1H s, -O$\underline{H}$), 4.86 (2H s, $-\underline{CH_2}-O$); m/e 180 ($M^+$, 17; 180.0508, calculated for $C_9H_8O_4$ 180.0423), 122(9), 105 ($PhC\overset{+}{O}-$, 100), 92(25), 77($Ph^+$, 80), 51(11).

6. 6-Bromo-5-hydroxy-2-hydroxymethyl-4H-pyran-4-one (45)

The method of Ichimoto[76] was followed to prepare the 6-bromo derivative of kojic acid. Recrystallization from water gave buff-coloured crystals in 23% yield; mp 165-167° (lit.[76] mp 166-167°). $\nu_{max}$ 3350 (broad), 1660 (s), 1656 (s), 1642 (s), 1600 (s), 1457 (s), 1310 $cm^{-1}$ (s); δ (acetone-$d_6$) 6.42 (1H s, $-C_3-\underline{H}$), 4.45 (2H s, $-\underline{CH_2}-O-$); m/e 222(94), 220 ($M^+$, 100), 193(3), 191(3), 113 ($M^+$-Br, 45), 85(55), 84(33), 80(20), 69(43), 67(53), 57(98).

7. 6-Bromo-5-methoxy-2-methoxymethyl-4H-pyran-4-one (46)

6-Bromokojic acid (45) (3.00 g, $1.36 \times 10^{-2}$ mole) was dissolved in 10% potassium hydroxide (10 $cm^3$) and dimethyl sulfate (1.29 $cm^3$, $1.36 \times 10^{-2}$ mole) added dropwise with stirring and cooling. Stirring was continued for 30 min after which time an additional 1.29 $cm^3$ dimethyl sulfate was added dropwise. The mixture was heated to 50° then allowed to stir for 30 min. An additional 0.65 $cm^3$ dimethyl sulfate was added and the mixture again heated to 50° and allowed to stir overnight. The solution was made strongly alkaline with solid KOH then extracted with chloroform (3 x 50 $cm^3$). The crude methylation product was obtained as a pale yellow solid (1.08 g, 32%) which was purified by column chromatography to give colourless crystals; mp 65°. $\nu_{max}$ (KBr) 3075 (w), 1673 (s), 1640 (m), 1592 (m), 1453 (m), 1403 (m), 1385 $cm^{-1}$ (m); $\delta$ ($CDCl_3$) 6.37 (1H s, $-C_3-\underline{H}$), 4.21 (2H s, $-\underline{CH_2}-O-$), 3.87 (3H s, $-O-\underline{CH_3}$), 3.41 (3H s, $-O-\underline{CH_3}$); m/e 250(37), 248 ($M^+$, 37; 247.9754, calculated for $C_8H_9O_4{}^{79}Br$ 247.9684), 232(3), 230 ($M^+-H_2O$, 3), 220(6), 218(6), 190(4), 188(4), 175(96), 173(100), 169 ($M^+$-Br, 19), 153(13), 139(14), 137(29), 109(16).

8. 5-Methoxy-2-methoxymethyl-4-oxo-4H-pyran-6-carbonitrile (49)

A mixture of CuCN (0.057 g, $6.4 \times 10^{-4}$ mole) in N-methylpyrrolidinone (10 $cm^3$) was heated to 50° with stirring and a solution of 6-bromo-5-methoxy-2-methoxymethyl-4H-pyran-4-one (46) (0.080 g, $3.4 \times 10^{-4}$ mole) in N-methylpyrrolidinone (3 $cm^3$) was added slowly as the mixture was heated to 75°. The mixture was refluxed for 2 hr then maintained at 150° for 14 hr and cooled to 50°. A solution of ferric chloride (0.2 g) in aqueous HCl (0.4 $cm^3$, 10%) was added and the

reaction kept at 50° with stirring for 30 min. The hot solution was diluted with 10 $cm^3$ water and extracted with 20% diethyl ether in benzene (6 x 20 $cm^3$). The organic phase was washed with dil. HCl (25 $cm^3$), water (2 x 10 $cm^3$), a dilute solution of $NaHCO_3$, and finally with water until neutral. The organic phase was then dried over $Na_2SO_4$, and evaporated in vacuo to leave a pale brown oil (0.047 g, ∿ 70%) containing predominantly the desired nitrile (49), $\nu_{max}$ 2200 (w), 1660 $cm^{-1}$ (s); δ ($CDCl_3$) 6.50 (1H s, $-C_3-\underline{H}$), 4.22 (2H s, $-\underline{CH_2}-O-$), 4.14 (3H s, $-OC\underline{H_3}$), 3.43 (3H s, $-OC\underline{H_3}$); m/e 195 ($M^+$, 34; 195.0536, calculated for $C_9H_9O_4N$ 195.0531), 194(6), 120(36), 113(12), 108(28), 71(20), 69(19), 45(100).

9. 5-Methoxy-2-methoxymethyl-4H-pyran-4-one (51)

The dimethyl ether of kojic acid was prepared by the method of Thomas[95] in 56% yield. Colourless needles were obtained from toluene mp 89-90° (lit.[95] mp 90-91°). $\nu_{max}$ (KBr) 3090 (s), 1660 (s), 1640 (s), 1612 (s), 1475 (m), 1465 (m), 1283 (s), 1240 (s), 1128 (s), 895 $cm^{-1}$ (m); δ ($CDCl_3$) 7.59 (1H s, $-C_6-\underline{H}$), 6.45 (1H s, $-C_3-\underline{H}$), 4.22 (2H s, $-\underline{CH_2}-O-$), 3.75 (3H s, $-OC\underline{H_3}$), 3.41 (3H s, $-OC\underline{H_3}$); m/e 170 ($M^+$, 56), 169 ($M^+-H$, 12), 152 ($M^+-H_2O$, 10), 140(70), 139 ($M^+-CH_3O$, 8), 125(28), 111(10), 95(100), 83(14), 69(24).

10. Treatment of Dimethyl Kojic Acid (51) with HBr

Anhydrous HBr was bubbled through a cooled solution of kojic acid dimethyl ether (51) (0.50 g, 2.9 x $10^{-3}$ mole) in dry benzene (100 $cm^3$) for 3 hr. A white precipitate formed after approximately 15 min. The

bubbling was discontinued and the mixture refluxed for 3 hr. After the reaction mixture was cooled to room temperature, the white hygroscopic precipitate of 5-methoxy-2-methoxymethyl-4H-pyran-4-one hydrobromide (52) was filtered off and dried in vacuo (0.72 g, 97%). The product was insoluble in organic solvents but dissolved readily in dilute aqueous sodium carbonate. Extraction with diethyl ether gave back the starting compound (51). The benzene filtrate was evaporated in vacuo leaving a brown oil (0.018 g) which, by thin layer chromatography, was found to be mostly the starting compound with at least four contaminants.

11. Reaction of Dimethyl Kojic Acid (51) with Bromine in $CCl_4$

To a solution of dimethyl kojic acid (51) (0.130 g, $7.6 \times 10^{-4}$ mole) in hot carbon tetrachloride (150 $cm^3$) was added dropwise bromine (0.04 $cm^3$, $7.5 \times 10^{-4}$ mole) in $CCl_4$ (10 $cm^3$). The reaction mixture was illuminated with a 150 watt bulb while refluxing and when colourless (30 min) was allowed to cool to room temperature. Evaporation in vacuo gave a yellow solid which, on recrystallization from chloroform yielded a few milligrams of a product identified as 5-methoxy-4-oxo-4H-pyran-2-carboxaldehyde (38), m/e 154 ($M^+$, 62), 153 ($M^+$-1, 13), 136 ($M^+-H_2O$, 20), 125 ($M^+$-CHO, 33), 95(100), 83(10), 53(43).

12. Reaction of Dimethyl Kojic Acid (51) with N-Bromosuccinimide

To a hot solution of dimethyl kojic acid (51) (1.00 g, $5.88 \times 10^{-3}$ mole) in $CCl_4$ (200 $cm^3$) was added freshly recrystallized N-bromosuccinimide (NBS) (1.04 g, $5.84 \times 10^{-3}$ mole) and the mixture refluxed for 45 min. The orange-yellow colour which appeared after 10 min was discharged in

15 min and a white precipitate of succinimide formed. After the reaction mixture was cooled to 4°, the precipitate was filtered off and the filtrate evaporated in vacuo leaving an orange semisolid which decomposed overnight in air (1.06 g). NMR analysis ($CDCl_3$) indicated that the product contained a mixture of the desired aldehyde (38) [δ 9.65 (1H s, -CHO), 7.75 (1H s, $-C_6-H$), 7.01 (1H s, $-C_3-H$), 3.90 (3H s, $-OCH_3$)], and two other products tentatively identified as the intermediate succinimide compound (54) [δ 7.84 (1H s, $-C_6-H$), 7.01 (1H s, $-C_3-H$), 4.46 (1H s, -N-CH-O-)] and an intermediate bromo compound (55) [δ 8.05 (1H s, $-C_6-H$), 7.01 (1H s, $-C_3-H$), 5.20 (1H s, -CH-Br ]].

13. Preparation of Triacetic Acid Lactone (8) from Dehydroacetic Acid (13)

A method similar to that of N.C. Brown[101] was used to prepare triacetic acid lactone (8) from dehydroacetic acid. A solution of dehydroacetic acid (5.00 g, $2.98 \times 10^{-2}$ mole) in conc. $H_2SO_4$ (10 $cm^3$) was heated rapidly to 140°, allowed to cool to 90° and poured into ice water (10 $cm^3$). The white precipitate which formed immediately was filtered, dried, and identified spectrally as triacetic acid lactone (3.35 g, 89%); recrystallized to colourless needles (water) mp 187-9°, (with decomposition; lit.[101] mp 186-8°); $\nu_{max}$ 3450 (broad), 1725 (s), 1660 (s), 1630 (s), 1594 (m), 1540 (m), 1305 (m), 1260 (s), 990 (m), 840 $cm^{-1}$ (m); δ ($CDCl_3$/acetone-$d_6$) 5.85 (1H m, $-C_3-H$), 5.37 (1H d, J = 2.4 Hz, $-C_5-H$), 2.86 (1H broad s, -OH), 2.20 (3H broad s, $-CH_3$); m/e 126 ($M^+$, 43), 111 ($M^+-CH_3$, 18), 98 ($M^+$-CO, 66), 85(37), 69(90), 43(100).

14. 3-Acetyl-6-hydroxy-2-methyl-4H-pyran-4-one (64)

The γ-pyrone (64) was prepared by a method similar to that of Butt and Elvidge.[105] Malonyl dichloride (2 $cm^3$, 2.1 x $10^{-2}$ mole) and freshly distilled acetylacetone (2 $cm^3$, 1.9 x $10^{-2}$ mole) were heated at 70° for 15 min. The resulting dark brown semisolid was triturated with ether. The ether extract was evaporated to dryness and the resulting yellow semisolid recrystallized from ether/THF to give colourless needles of an unidentified product (mp 153-155°); $\nu_{max}$ (KBr) 3400 (broad), 2980 (w), 2940 (w), 1827 (s), 1806 (m), 1779 (sh), 1756 (s), 1720 (s), 1445 (m), 1363 (m), 1270 (w), 1165 (s), 1088 $cm^{-1}$ (m); δ (acetone-$d_6$) 4.10 (1H s), 3.35 (3H s), 2.75 (2H d), 1.79 (3H s), 1.76 (3H s).

Sublimation of the brown residue left after ether wash yielded a yellow solid which was recrystallized from THF/ether and found to be the desired γ-pyrone (64), mp 159-160° (lit.[105] mp 162°); $\nu_{max}$ (KBr), 1695 (m), 1668 (s), 1617 (s), 1604 (m), 1554 (m), 1498 (m), 1277 $cm^{-1}$ (s); δ ($CDCl_3$), 12.1 (1H broad s, -O<u>H</u>), 5.47 (1H s, $-C_5-\underline{H}$), 2.62 (3H s, $-\underline{CH}_3$), 2.59 (3H s, $-\underline{CH}_3$); m/e 168 ($M^+$, 11), 140(45), 125(31), 111(8), 98(9), 85(17), 69(27), 43 (100).

15. Preparation of Triacetic Acid Lactone (8) from 3-Acetyl-6-hydroxy-2-methyl-4H-pyran-4-one (86)

The deacylation of (86) was carried out by a method similar to that described by Butt and Elvidge.[105] A solution of 3-acetyl-6-hydroxy-2-methyl-4H-pyran-4-one (86) (0.350 g, 2.08 x $10^{-3}$ mole) in 90% sulfuric acid (1.2 $cm^3$) was heated rapidly to 140° by immersion in an

oil bath. Heating at that temperature was continued for 10 min, then the mixture cooled slightly and poured over ice water (5 $cm^3$). After filtration to remove a small amount of black precipitate, the filtrate was neutralized with a solution of sodium hydroxide and extracted with diethyl ether (4 x 25 $cm^3$). The ether was dried and evaporated in vacuo leaving a yellow solid, triacetic acid lactone (8) (0.086 g, 33%), mp 189° (lit.[105] mp 192° with decomp.) (water).

16. 4-Hydroxy-6-methyl-3-nonanoyl-2H-pyran-2-one (61)

A mixture of triacetic acid lactone (8) (0.77 g, 6.1 x $10^{-3}$ mole) and n-nonanoyl chloride (1.13 g, 6.00 x $10^{-3}$ mole) in trifluoroacetic acid (3 $cm^3$) was heated slowly to reflux where it was maintained for 3 hr. The mixture was cooled slightly and poured into ice water (10 $cm^3$). A buff-coloured precipitate formed immediately which was filtered and dried in vacuo. The product (61) was recrystallized from methanol as colourless needles (1.27 g, 79%) mp 81-2°. $\nu_{max}$ (KBr) 3090 (w), 1730 (s), 1660 (m), 1626 (m), 1570 (s), 1463 (m), 1001 $cm^{-1}$ (s); $\delta$ ($CDCl_3$) 8.2 (1H broad s, -OH), 5.94 (1H s, $-C_5-H$), 3.06 (2H t, $-CO-CH_2-$), 2.26 (3H s, $-C_6-CH_3$), 1.3 (12H m, $(CH_2)_6$), 0.88 (3H t, $-CH_2-CH_3$); m/e 266 ($M^+$, 8; 266.2111 calculated for $C_{15}H_{22}O_4$ 266.2144), 248 ($M^+-H_2O$, 3), 181(55), 168(100), 153(79), 71(3), 69(10), 43(23).

17. Methylation of Dehydroacetic Acid (13)

To a solution of dehydroacetic acid (13) (1.00 g, 5.95 x $10^{-3}$ mole) in chloroform (15 $cm^3$) was added diazomethane in ether (0.05 mole) at 0°. The mixture bubbled vigorously for 10 min then was stirred for 16 hr as the solution warmed to room temperature. Evaporation of the

solvent in vacuo left a brown solid (∿ 1 g) which was purified and separated into its two main components by column chromatography. The first product was eluted with benzene and identified as the dehydration product 4-oxo-3,6-dimethyl-4H-furo[3,2c]pyran (63) (0.120 g, 12.5%) mp 129-130° (MeOH/$H_2O$); $\nu_{max}$ 3105 (m), 1735 (s), 1633 (s), 1608 (s), 1565 (m), 1203 (m), 960 $cm^{-1}$ (s); δ 7.24 (1H m, J ∿ 2 Hz), 6.37 (1H m, J ∿ 2 Hz), 2.32 (6 H d, J ∿ 2 Hz); m/e 164 ($M^+$, 39; 164.0478, calculated for $C_9H_8O_2$ 164.0473) 150(19), 149(60), 135(4), 93(6), 65(21), 43(100).

The second product, which was eluted with chloroform, was identified as 3-acetyl-4-methoxy-6-methyl-2H-pyran-2-one (62); $\nu_{max}$ 3108(m), 1720 (s), 1683 (s), 1655 (sh), 1510 (s), 1352 (s), 1245 (s), 1023 $cm^{-1}$ (s); δ 6.11 (1H s, $-C_5-\underline{H}$), 3.89 (3H s, $-O\underline{CH_3}$), 2.43 (3H s, $-\underline{CH_3}$), 2.27 (3H s, $-\underline{CH_3}$); m/e 182 ($M^+$, 30; 182.0579, calculated for $C_9H_{10}O_3$ 182.0579), 168(9), 167 ($M^+-CH_3$, 100), 154(18), 153(29), 139 ($M^+-COCH_3$, 33), 125(38), 111(16), 43(56).

18. Aldol Condensation of n-Heptaldehyde and Acetone

A mixture of freshly distilled n-heptaldehyde (5.0 g, 0.044 mole) and acetone (5.0 g, 0.086 mole) in 1.5% NaOH (125 $cm^3$) was kept overnight at 4° then stirred for 4 hr at 10°. The mixture was extracted with diethyl ether, dried, and evaporated in vacuo leaving a yellow oil (4.93 g). Column chromatographic purification yielded the desired 4-hydroxydecan-2-one (73) (1.62 g, 22%), a colourless liquid; $\nu_{max}$ 3450 (broad), 2940 (s), 2865 (m), 1730 (s), 1375 (m), 1177 $cm^{-1}$ (m); δ ($CDCl_3$) 3.95 (1H quintet, J = 6 Hz, $-C(OH)-\underline{H}$), 2.95 (1H broad s, $-\underline{OH}$),

2.50 (2H d, J = 6 Hz, -C$\underline{H}_2$CO-), 2.12 (3H s, -COC$\underline{H}_3$), 1.3 (10H m, {$CH_2$}$_5$), 0.85 (3H t, -$CH_2$-C$\underline{H}_3$); m/e 172(0), 154 ($M^+$-$H_2O$, 2), 139 ($M^+$-$CH_3CO$; 3), 125(3), 111(5), 96(14), 87(79), 58(25), 43(100).

19. 5-Methoxy-4-oxo-4H-pyran-2-carboxaldehydeoxime (75)

The oxime of aldehyde (38) was prepared by dissolving the aldehyde (0.150 g, 9.74 x $10^{-4}$ mole) in a solution of hydroxylamine hydrochloride (0.20 g) and sodium acetate (0.30 g) in 60% aqueous methanol (5 ml). Heating (15 min) induced precipitation of white crystals of the oxime which were collected by filtration after the mixture was cooled (0.141 g, 86%) mp 130°; $\nu_{max}$ (KBr) 3080 (m), 3400-2400 (broad), 1660 (sh), 1630 (s), 1605 (s), 1575 (m), 1525 (m), 1446 (m), 1277 (s), 1240 (s), 1158 (m), 1020 (s), 970 $cm^{-1}$ (s); δ (acetone-$d_6$, computer averaged due to insolubility) 7.93 (1H s, -$C_6$-$\underline{H}$), 7.89 (1H s, -C$\underline{H}$=N-), 6.50 (1H s, -$C_3$-$\underline{H}$), 3.70 (3H s, -OC$\underline{H}_3$); m/e 169 ($M^+$, 44), 153(26), 151(34), 95(100), 78(24), 69(37), 53(26).

20. Treatment of (75) with $PCl_5$ in Chloroform

The oxime (75) (0.10 g, 5.9 x $10^{-4}$ mole) was suspended with rapid stirring in chloroform (50 $cm^3$) and cooled in an ice bath. Phosphorus pentachloride was added slowly until excess remained in the flask. After stirring for 5 hr at room temperature, the solution was decanted and stirred with an aqueous solution of sodium carbonate until neutral. The organic phase was dried and evaporated in vacuo leaving an off-white solid (0.065 g). Column chromatography was used to separate the products. Elution with benzene gave white prisms of

2-chloro-5-methoxy-4H-pyran-4-one (78) (0.022 g, 23%) mp 122-4°. $\nu_{max}$ (KBr) 1728 (s), 1647 (s), 1560 (s), 1477 (m), 1425 (m), 1334 (m), 1258 (s), 1147 (s), 1015 $cm^{-1}$ (s); δ 7.14 (1H s, $-C_6-\underline{H}$), 6.48 (1H s, $-C_3-\underline{H}$), 3.73 (3H s, $-O\underline{CH_3}$); m/e 162(14), 160 ($M^+$, 37; 159.986, calculated for $C_6H_5{}^{35}ClO_3$ 159.993), 132(43), 119(33), 117(100), 89(76), 61(86), 60(35), 53(97). Elution with benzene:chloroform (3:1) gave white crystals of 5-methoxy-4-oxo-4H-pyran-2-carbonitrile (77) (0.021 g, 24%) mp 154-154.5°. $\nu_{max}$ (KBr) 1650 (s), 1625 (s), 1597 (m), 1297 (s), 1245 (s), 997 (s), 900 $cm^{-1}$ (s); δ ($CDCl_3$) 7.61 (1H s, $-C_6-\underline{H}$), 6.88 (1H s, $-C_3-\underline{H}$), 3.79 (3H s, $-O\underline{CH_3}$); m/e 151 ($M^+$, 100; 151.0269, calculated for $C_7H_5O_3N$ 151.0269), 133 ($M^+-H_2O$, 16), 122(18), 121(53), 95(20), 94(27), 51(17).

21. Treatment of (75) with $PCl_5$ in Diethyl Ether

The reaction outlined above was repeated using diethyl ether as solvent (40 $cm^3$). Elution with petroleum ether (30/60°):benzene (2:1) gave 2-chloro-6-ethoxy-5-methoxy-4-oxo-2,5-hexadienoic acid nitrile (79) (0.018 g, 14%) mp 60-61°. $\nu_{max}$ (KBr) 3090 (m), 2225 (m), 1655 (s), 1600 (s), 1280 $cm^{-1}$ (s); δ ($CDCl_3$) 6.19 (1H s, vinyl $\underline{H}$), 5.69 (1H s, vinyl $\underline{H}$), 3.86 (3H s, $-O\underline{CH_3}$), 3.85 (2H q, $-O-\underline{CH_2}-CH_3$), 1.27 (3H t, $-O-CH_2-\underline{CH_3}$); m/e 217(8), 215($M^+$, 24; 215.031, calculated for $C_9H_{10}{}^{35}ClNO_3$ 215.035), 186(8), 184(16), 172(45), 170(100). Elution with benzene gave 0.013 g (14%) of 2-chloro-5-methoxy-4H-pyran-4-one (78).

22. Treatment of (75) with $PCl_5$ in Glyme

The oxime (75) was treated with $PCl_5$ in glyme (glycol dimethyl ether, 50 $cm^3$) using the reaction outlined above. The product was identified as exclusively 2-chloro-5-methoxy-4H-pyran-4-one (78) (0.090 g, 95%).

23. Treatment of (75) with Acetic Anhydride

The oxime (75) (0.027 g, $1.6 \times 10^{-4}$ mole) in acetic anhydride (5 $cm^3$) containing acetic acid (5 drops) was heated gradually with stirring to 140° and refluxed for 3 hr. The reaction mixture was cooled slightly then poured into water and neutralized with sodium carbonate. The aqueous solution was extracted with ethyl acetate and the extracts dried and evaporated in vacuo to yield exclusively 5-methoxy-4-oxo-4H-pyran-2-carbonitrile (77) (0.023 g, 95%).

24. Reaction of (77) with HCl in Ethanol

5-Methoxy-4-oxo-4H-pyran-2-carbonitrile (77) (0.084 g, $5.6 \times 10^{-4}$ mole) was dissolved in 100% ethanol (50 $cm^3$) through which HCl had been bubbled vigorously for 1 min. After 24 hr stirring at room temperature, additional HCl was bubbled through the solution and the mixture stirred for 24 hr. Evaporation of the solvent in vacuo left a chloroform insoluble white salt which dissolved readily in aqueous sodium carbonate. The aqueous phase was then extracted with chloroform which was dried and evaporated in vacuo. The product, an off-white solid (0.077 g, 70%), was purified by column chromatography and identified as the solvolysis product ethyl 5-methoxychelidonate (80),

mp 155.5-156.5°. $\nu_{max}$ (KBr) 3065 (m), 1753 (s), 1645 (s), 1620 (s), 1436 (m), 1270 (s), 1227 (m), 993 $cm^{-1}$ (s); δ ($CDCl_3$) 7.68 (1H s, $-C_6-\underline{H}$), 7.16 (1H s, $-C_3-\underline{H}$), 4.41 (2H q, $-\underline{CH_2}-CH_3$), 3.80 (3H s, $-O\underline{CH_3}$), 1.40 (3H t, $-CH_2\underline{CH_3}$); m/e 198 ($M^+$, 38), 180 ($M^+-H_2O$, 7), 169 (11), 153(8), 125(19), 96(8), 95(100), 69(30), 53(21).

25. Methyl Maltol (41) (3-Methoxy-2-methyl-4H-pyran-4-one)

To a stirred solution of maltol (4) (0.520 g, 4.13 x $10^{-3}$ mole) in chloroform (20 $cm^3$) was added slowly a solution of diazomethane in diethyl ether (0.045 mole). The solution was stirred for 48 hr then the solvent was removed in vacuo leaving crude methyl maltol (41) as an orange oil (0.542 g, 95%). The crude product was purified by column chromatography. Elution with benzene gave methyl maltol as a colourless oil (lit.[112] bp 114°/15 mm). $\nu_{max}$ (liquid film) 3080 (m), 1660 (s), 1590 (m), 1457 (s), 1260 (s), 1220 (s), 832 $cm^{-1}$ (s); δ ($CDCl_3$) 7.60 (1H d, J = 7.0 Hz , $-C_6-\underline{H}$), 6.32 (1H d, J = 7.0 Hz, $-C_5-\underline{H}$), 3.82 (3H s, $-O\underline{CH_3}$), 2.31 (3H s, $-\underline{CH_3}$); m/e see Fig. 10(a).

26. Preparation of Methyl-$d_3$ Iodide

Methyl-$d_3$ iodide was prepared by the method of Cotton et al.[110] The colourless liquid obtained was distilled (41-42°) to remove traces of dimethyl-$d_6$ sulfoxide (12.5 g, 50% from $(CH_3)_3S^+O\ I^-$).

27. Preparation of Methyl-$d_3$ Maltol (83)

Maltol (2.00 g, 1.59 x $10^{-2}$ mole) was dissolved with stirring in dimethylformamide (DMF, 40 $cm^3$) and silver oxide (6.0 g) added.

Methyl-$d_3$ iodide (1.6 $cm^3$, 1.5 x $10^{-2}$ mole) was added dropwise and stirring continued for 12 hr. Additional methyl-$d_3$ iodide (1.6 $cm^3$) was added and the reaction mixture stirred for an additional 12 hr. Silver oxide was removed by centrifugation and washed with DMF (15 $cm^3$). The reaction liquid and DMF washing were combined, filtered, and the solvent removed in vacuo leaving a brown semisolid. This mixture was washed with diethyl ether (3 x 50 $cm^3$) to extract the methylated product. Evaporation of the ether in vacuo yielded a brown liquid (0.948 g, 43%). Column chromatography was used to obtain methyl-$d_3$ maltol (83) as a colourless oil. $\nu_{max}$ 3450 (broad), 3085 (w), 2070 (m), 1660 (s), 1640 (sh), 1585 (m), 1438 (m), 1264 (s), 1209 (s), 1100 $cm^{-1}$ (m); $\delta$ ($CDCl_3$, 90 MHz), 7.66 (1H d, J = 7.0 Hz, $-C_6-\underline{H}$), 6.36 (1H d, J = 7.0 Hz, $-C_5-\underline{H}$), 2.31 (3H s, $-\underline{CH_3}$); m/e see Fig. 10(b).

28. Preparation of Deuteriodiazomethane ($CD_2N_2$)

A method similar to that of Campbell[111] was used to prepare deuteriodiazomethane. A mixture of carbitol-OD* (40 $cm^3$), 30% sodium deuteroxide (55 g) in $D_2O$, and sodium dried diethyl ether (200 ml) was cooled to 0° in a continuous reaction/distillation vessel. N,N'-Dimethyl-N,N'-dinitrosoterephthalamide (16 g) was added in one portion and heating commenced. The yellow distillate ($CD_2N_2$ in $Et_2O$) was passed into dry ether (50 $cm^3$) to produce 150 $cm^3$ of a 0.21 M solution.

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* Carbitol-OD was prepared by refluxing carbitol with $D_2O$ and distilling off the $HOD/D_2O$. This procedure was repeated four times.

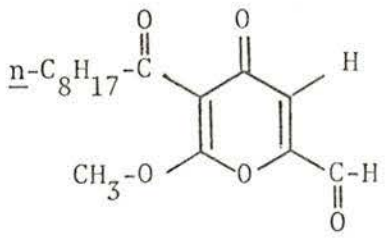
29. Methylation of Maltol with $CD_2N_2$

To a solution of maltol (0.500 g, $3.97 \times 10^{-3}$ mole) in diethyl ether (25 $cm^3$) and chloroform (20 $cm^3$) was added freshly prepared deuteriodiazomethane in diethyl ether (50 $cm^3$, 0.025 mole). After the reaction mixture was stirred at room temperature for 36 hr, the solvent was removed in vacuo and the methylation repeated. The slightly cloudy reaction mixture was filtered and evaporated in vacuo yielding a pale yellow oil (0.482 g, 86%), identified spectrally as partially labelled methyl maltol.

Conclusions

From a locally occurring fungus Potebniamyces balsamicola was isolated a fungal growth inhibitor, phacidin. Since phacidin exhibits antibiotic activity and can readily be isolated and purified, it may in the future be of significant importance to medicine. For this reason, a study of its chemical structure and proof by synthesis was undertaken.

We have assigned a γ-pyrone structure to this new antibiotic on the basis of spectral and chemical evidence. The substitution pattern proposed best fits the data we have accumulated with one



possible alteration: the two carbonyl substituents could conceivably be interchanged ($C_2$-nonanoyl, $C_5$-aldehyde). Supporting evidence for their original assignment comes from the $^{13}C$ NMR spectrum of phacidin in which the carbonyl and α-methylene carbons of the n-nonanoyl group exhibit chemical shifts which suggest steric interference with an adjacent substituent (i.e. methoxyl)[121].

Several attempts to synthesize phacidin have been unsuccessful, while other methods look particularly promising in the light of model reactions and partially completed synthetic schemes. Although several of the reaction sequences outlined in Part II lead to isomers of phacidin rather than to the antibiotic itself, they can still

provide useful information as to the exact substitution pattern of phacidin and can be used as valuable models for chemical investigation. While most of the synthetic work completed has involved the use of γ- or α-pyrones as starting compounds, there still exists opportunity for synthesis of phacidin from acyclic systems or carbohydrates.

During the investigation and synthesis of γ-pyrones, two particularly interesting features were encountered. A ring-opened compound, thought to be an "intermediate" of chloride/nitrile substitution, was isolated and identified. Such ring cleavage reactions have not previously been reported and work is continuing in an effort to clarify the reaction mechanism.

Study of the electron impact mass spectra of a number of β-hydroxy and β-methoxy-γ-pyrones revealed that the β-methoxy compounds exhibit an unexpected water loss from their molecular ions, a fragmentation process which does not occur in their β-hydroxy analogues. Labelling studies have been used to help propose a mechanism for this fragmentation, and further experiments are being carried out to help clarify the mechanism.

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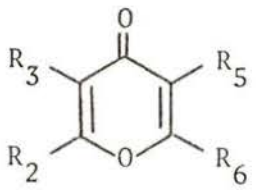
δ Chemical shifts of γ-pyrone protons ($CDCl_3$; * indicates acetone-$d_6$)




| $R_2$ | $R_3$ | $R_5$ | $R_6$ | 2,6 | 3,5 | Reference |
|---|---|---|---|---|---|---|
| H | H | H | H | 7.71 | 6.35 | 114 |
| $CH_3$ | H | H | Ph | | 6.19,6.69 | 114 |
| $CH_3$ | H | H | $CH_3$ | | 6.04 | 114 |
| $CH_3$ | OH | H | H | 7.73 | 6.43 | 114 |
| $CH_3$ | $OCH_3$ | H | H | 7.89 | 6.23 | 115 |
| H | $CH_3$ | H | H | 7.52 | 6.15 | 116 |
| $CH_3$ | OH | H | $CH_3$ | | 6.25 | 26 |
| $OCH_2CH_3$ | H | H | $CH_3$ | | 5.95(5) | 51 |
| $OCH_2CH_3$ | $CH_3$ | H | $CH_3$ | | 5.96 | 51 |
| $OCH_2CH_3$ | Ph | H | $CH_3$ | | 6.10 | 51 |
| $OCH_2CH_3$ | Cl | H | $CH_3$ | | 6.10 | 51 |
| $OCH_2CH_3$ | Br | H | $CH_3$ | | 6.10 | 51 |
| $OCH_2CH_3$ | CN | H | $CH_3$ | | 6.10 | 51 |
| $CH_2OCH_3$ | H | $OCH_3$ | H | 7.59 | 6.45 | This work |
| $CH_2OCH_3$ | H | OH | H | 7.85 | 6.51 | This work |
| $CH_2OH$ | H | $OCH_3$ | H | 7.64 | 6.40* | This work |
| CHO | H | $OCH_3$ | H | 7.69 | 6.97 | This work |
| $CH_3$ | COOH | H | $CH_3$ | | 6.39 | This work |
| $CH_2OTHP$ | H | OMe | H | 7.59 | 6.52 | This work |
| $CH_2OCOPh$ | H | OH | H | 7.99 | 6.54* | This work |
| $CH_2OCOPh$ | H | OH | $NO_2$ | | 6.77* | This work |
| $CH_2OH$ | H | OH | Br | | 6.42* | This work |
| Cl | H | $OCH_3$ | H | 7.14 | 6.48 | This work |
| CN | H | $OCH_3$ | H | 7.61 | 6.88 | This work |
| CHNOH | H | $OCH_3$ | H | 7.88 | 6.50* | This work |
| $CH_2Br$ | $OCH_3$ | H | H | 7.85 | 6.66 | This work |

Continued....

| $R_2$ | $R_3$ | $R_5$ | $R_6$ | 2,6 | 3,5 | Reference |
|---|---|---|---|---|---|---|
| $CH_2Cl$ | H | OH | H | 7.86 | 6.56 | This work |
| $CH_2OCH_3$ | H | $OCH_3$ | Br | | 6.44 | This work |
| $CH_3$ | H | CONHAr | $CH_3$ | | 6.29 | 117 |
| $CH_3$ | H | CONHAr' | $CH_3$ | | 6.23 | 117 |
| $CH_3$ | H | COSePh | $CH_3$ | | 6.23 | 118 |
| $CH_3$ | H | $COOCH_2CH_3$ | $CH_3$ | | 6.10 | 119 |
| $COOCH_2CH_3$ | H | $OCH_3$ | H | 7.65 | 7.15 | This work |

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Title of Thesis/Dissertation

The Structural Elucidation and Partial Synthesis of Phacidin, a Fungal Growth Inhibitor from Potebniamyces balsamicola

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November, 1974