The Chemistry of Solidagenone and Peucenin Congeners.

THESIS

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Summary

Part 1

In an attempt to clarify a botanical classification problem, the chemical constituents of Ptaeroxylon obliquum were investigated. Structures are proposed for three chromones and four coumarins isolated from the timber.

Two novel chromones, karenin and desoxykarenin (ptaeroxylin), have been shown to possess a seven-membered oxide ring fused to a 5-hydroxy-2-methylchromone nucleus. The linear direction of fusion was uniquely defined by the isolation of dihydropeucenin as a hydrogenolysis product of both karenin and desoxykarenin. Peucenin was also isolated and its structure confirmed.

The functionality and position of substituents for the coumarins was derived from spectral data. The structure of 7-O-(3,3-dimethylallyl)scopoletin was verified by acid hydrolysis to scopoletin and synthesis from aesculin. Two further coumarins, nieshoutin and nieshoutol, from spectral behaviour, are very similar; both possess a fused trimethyldihydrofuran system but have not been related chemically. On biogenetic and other evidence, structures have been proposed and a recent synthesis in this laboratory confirms the constitution of the former.

Part 2

A systematic study of the carbonyl and hydroxyl regions of the infrared solution spectra of twenty-seven chromones has been undertaken. The results of a qualitative survey of the effects of progressive structural changes on the spectral profile of the carbonyl region are presented.

Although the hydroxyl stretching frequencies were easily identified, the complexity of the carbonyl spectrum did not allow a unique assignment of the carbonyl stretching frequency. Variation of solvent polarity and temperature proved to be of little value in this assignment. The spectra of seven chromones containing a deuterium-enriched 5-hydroxyl group indicated that two maxima, ca. 1660 and ca. 1630 $cm.^{-1}$, had high carbonyl character. The possible dependence of spectral splitting on an intramolecular vibrational effect (Fermi resonance) is discussed with reference to two nuclearly deuterated derivatives of 5-hydroxy-2-methylchromone and to oxygen-18 enriched 5-hydroxy-2-methylchromone.

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PART 3.

The Chemistry of Solidagenone

PART 1.

Naturally occurring Chromones.

1. The Biogenesis of Chromones.

The chromone nucleus (1), the benzo homologue of 4-pyrone, is widely represented in natural products. However, the majority of chromones possess a phenyl substituent in the 2- or 3-positions and are separately classified as flavones (2) and isoflavones (3). A small remaining group of about forty compounds contain methyl (or hydroxymethyl) at position 2, are generally unsubstituted at position 3 and are based on a resorcinol or phloroglucinol skeleton; these are regarded as the naturally occurring chromones.[1] The basic oxygenated chromone nucleus is often substituted at positions 5-8 by methyl or isopentenyl groups and sometimes occurs in a 2,3-dihydro form (chromanones). Linear and angular annellation are common: fusion of a six-membered carbocyclic ring to ring A (1) produces the naphthopyrones while cyclised five-carbon chains give rise to 5-, 6-, and 7-membered oxide rings. Dinuclear chromones are also known. It may be noted that fusion of a benzene ring between positions 2 and 3 of the chromone nucleus gives xanthone (4). While there are many naturally occurring xanthones, discussion of these is outwith the scope of this review.

The occurrence of chromones is by no means widespread. No less than twenty-two are found in only three plant species and a further eight are metabolites of six fungal species. In all cases, chromones co-occur with other phenolic compounds, notably, acetophenones, flavonoids, and coumarins, the analogous benzo homologues of 2-pyrone.

Phenols have been shown to originate biogenetically from acetate[2,3,4] or shikimate[4,5] by one of two pathways. It seems logical, therefore to expect that the hydroxylated chromones could have similar derivation. However, owing to the lack of extensive feeding studies, the current postulates for the biogenesis of chromones are largely speculative. At present, three distinct pathways can be visualised.

1. Acetate-malonate pathway

The chromone nucleus may arise by cyclisation and aromatisation of a suitably arranged polyketide chain (Scheme 1). Bu'Lock[6] found that carboxyl labelled acetate was incorporated as predicted into 5-hydroxy-2-methylchromanone (5) by strains of Daldinia concentrica,

a mould parasitic on ash trees. It is significant that the co-metabolites of this compound include the corresponding chromone (6), the naphthol derivatives (7; R^1=H, R^2=CH_3; R^1=R^2=CH_3), and the acyl resorcinols (8 to 10). This series might represent successive stages of reduction, the effective side chains being acetoacetyl, β-hydroxybutyryl, crotonyl, and butyryl respectively, a sequence similar to that in fatty acid biogenesis.

In the biogenesis of polyketide chains it is quite common for an acid other than acetic acid to act as the initial condensing unit. Cinnamic acid has been shown[7] to have this role in the genesis of some flavonoid compounds (Scheme 2). In an analogous way, crotonic acid could act as chain initiator to produce oxygenated 2-methylchromanones and 2-methylchromones (Scheme 3). This scheme offers no apparent advantages over the previous acetate-initiated pathway and differs only in time sequence. Both routes would be expected to give the same incorporation of labelled acetate and could only be distinguished by feeding labelled crotonate as precursor.

2. Shikimate pathway

Geissman[8a] has suggested that the naturally occurring chromones, eugenin (11), eugenitin (12), isoeugenitin (13), and peucenin (14), together with the furochromones, visnagin (15) and khellin (16) have a shikimate-derived nucleus, but no research has been conducted *in vitro* to support this postulate. (More recently,[8b] Geissman favours an acetate derivation for these compounds.) The origin of the three carbon atoms necessary to convert shikimic acid (C_6-C_1) into the C_6-C_4 unit, necessary for the 2-methylchromonone nucleus, is obscure. A plausible pathway (Scheme 4) involves the condensation of shikimic acid (17) with two acetate units followed by decarboxylation, cyclisation, and dehydration-aromatisation. An alternative possible scheme is the condensation of a C_6-C_3 unit (18), derived from shikimate via prephenic acid (19), with a C_1 moiety followed by cyclisation, aromatisation, and oxygenation. The relative sequence of events in these schemes is not predictable. Certain naturally occurring acetophenones such as euparin (20), phloracetophenone (21), and its 2,4-dimethylether have been assigned a shikimate derivation. Compounds of this type may, therefore,

represent intermediates in 2-methylchromone synthesis (or degradation); significantly, the β-diketone (22) co-occurs with the simple chromones in Eugenia species. Since chromones, 2,4,6-trioxygenated acetophenones, and similarly oxygenated coumarins are found together in many Rutaceous and Umbelliferous species, it is possible that chromones are derived from shikimate by way of a coumarin nucleus[9] as in Scheme 5.

3. Biogenesis in Higher plants

Recently, Dean and Taylor[10] have suggested that the C_6-C_4 skeleton of chromones is derived in higher plants by insertion of a C_5 substituent (related to isoprene) into a phloroglucinol nucleus followed by loss of one carbon atom and cyclisation to the 2-methyl- or 2-hydroxymethylchromone (Scheme 6). The terminal carbon atom is presumed to be lost by a process similar to the osmium tetroxide-periodic acid cleavage of glycols. The biogenesis of coumarins is accommodated in this scheme by invoking a further loss of a C_1 unit (by the same process) to produce the necessary C_6-C_3 skeleton. It is claimed that this scheme explains why chromones and coumarins are

frequently substituted by further C_5 residues and that it avoids the difficulties inherent in routes to derivatives of 5,7-dihydroxycoumarin based on oxidative cyclisations.[11] The hypothesis excludes the micro-organisms which normally do not utilise C_5 insertions; their coumarins are unlike those of the higher plants and the structures of their chromones are more easily rationalised by the cyclisation of polyketide chains.

Naphthopyrones

The oxygenation pattern of the naphthopyrones suggests an acetate-malonate derivation as shown for eleutherinol (23) and rubrofusarin (24). The dinuclear chromones aurofusarin (25) and ustilaginoidins A, B, and C (26, 27, 28) are probably formed from the simpler naphthopyrone units by phenol oxidative coupling.

Origin of substituents

The substituents most commonly encountered in naturally occurring chromones are (a) the methyl group and (b) alkyl groups and rings of isoprenoid origin. Recently, the occurrence of a chlorine substituent in the

chromone sordidone (29) has been reported.[12] C-methyl groups are currently thought to originate from methionine but there is little positive evidence for the biological source of the C-methyl units.[8b] Isoprenoid residues are considered to be derived from mevalonic acid.

Numerous C-methylated chromones are known.[13] For the 5,7-dioxygenated chromones, positions 6 and 8 are available for methylation as shown by eugenin (11), eugenitin (12), isoeugenitin (13), isoeugenitol (30), angustifolionol (31), lepraric acid (32), and sordidone (29). It is not certain at which stage in the construction of the chromone nucleus the introduction of the 'extra' carbon atoms takes place. The co-occurrence of eugenitin (12) and isoeugenitin (13) is explained by the two modes of cyclisation available to the precursor (33; Scheme 7) or by the interconversion of the 6-alkylated chromone (12) to the 8-alkylated (13) by a process similar to the Wessely-Moser rearrangement.[14] The structure of lepraric acid may be in error and is at present under review.[15]

Isoprene units appear intact in ring A of the chromone nucleus of peucenin (14) and heteropeucenin (34) but various modifications of the C_5 residue are evident throughout the chromone series. Cyclisation between position 2' of the 3,3-dimethylallyl chain and a hydroxyl group on the ortho position (as in Scheme 8)[16] gives rise to the 2-isopropyldihydrofuran systems of visamminol (35) and umtatin (36). A related cyclisation (Scheme 8) with loss of the three terminal carbon atoms[17] furnishes the furano-chromone nucleus of visnagin (15), khellin (16), khellinol (37), khellol glucoside (38), and ammiol (39). Isomerisation of the double bond to the 1',2'-position of the C_5 chain followed by cyclisation (Scheme 8) between position 3' and the neighbouring phenolic hydroxyl group[16] produces a fused dimethylchromene ring as found in ptaerochromenol (40). A unique mode of cyclisation of an isoprene unit is encountered in the seven-membered oxide rings (oxepins) of ptaeroxylin (41), karenin (42), ptaeroxylinol (43), ptaeroglycol (44), dehydroptaeroxylin (45), and ptaeroxylone (46). Formation of the seven-membered ring would be expected to require isomerisation of the double bond to the 3',4'-position of

the isoprene residue (Scheme 9) followed by cyclisation between position 4' and the ortho hydroxyl group.

Up till 1963, only seventeen naturally occurring chromones were known and, as a class of compounds, had received little attention. Recent work has indicated that the occurrence of chromones may be more widespread than implied by their present number. It would seem at this juncture that clarification (by the feeding of labelled precursors) of the biogenetic routes to these compounds and especially to the derivation of the seven-membered oxide ring, would be of chemotaxonomic value. A list of the naturally occurring chromones and their origin is presented on pages 10 and 11.

Chromone	Structure	Origin	Ref.
Ammiol	(39)	Ammi visnaga L.	18
Angustifolionol	(31)	Backhousia angustifolia Benth.	13
Aurofusarin	(25)	Fusarium culmorum Sacc.	19
Dehydroptaeroxylin	(45)	Ptaeroxylon obliquum (Thumb.) Radlk.	10
Eleutherinol	(23)	Eleutherine bulbosa (Mill.) Urb.	20
Eugenitin	(12)	Eugenia caryophyllata Thunbg.	13
Eugenin	(11)	-do-	13
Heteropeucenin	(34)	P. obliquum	21
Heteropeucenin 7-methyl ether	(47)	-do-	22
Heteropeucenin 5,7-dimethyl ether	(48)	-do-	21
Isoeugenitin	(13)	E. caryophyllata	13
Isoeugenitol	(30)	-do-	13
5-Hydroxy-2-methyl-chromanone	(5)	Daldinia concentrica	6
5-Hydroxy-2-methyl chromone	(6)	-do-	6
Karenin	(42)	P. obliquum	23
Khellin	(16)	A. visnaga	24

Chromone	Structure	Origin	Ref.
Khellinol	(37)	A. visnaga	24
Khellol glucoside	(38)	-do-	25
Lepraric acid	(32)	Lepraria latebrarum Ach.	26
Peucenin	(14)	P. obliquum Peucedanum ostruthium Koch.	23, 27
Ptaerochromenol	(40)	P. obliquum	21
Ptaerocyclin	(49)	-do-	21
Ptaeroglycol	(44)	-do-	10
Ptaeroxylin	(41)	-do-	23,10
Ptaeroxylinol	(43)	-do-	10
Ptaeroxylone	(46)	-do-	10
Rubrofusarin (nor)	(24)	F. culmorum	19
Sorbifolin	(76)	Spathelia sorbifolia L.	28
Sordidone	(29)	Lecanora sordida (Pers.) Th. Fr.	12
Umtatin	(36)	P. obliquum	21
Ustilaginoidin A	(26)	Ustilaginoidea virens (Cooke) Takahashi	29
-do- B	(27)		
-do- C	(28)		
Visamminol	(35)	A. visnaga	24
Visnagin	(15)	-do-	30

2. The Constituents of Sneezewood,

Ptaeroxylon obliquum

(Thunb.) Radlk.

Considerable study has been devoted, in this department, to the elucidation of the structures of the heartwood constituents of Meliaceous plants.[31] In continuance, an investigation of this family from a botanical viewpoint disclosed that three genera, Chloroxylon, Flindersia, and Ptaeroxylon, at times included in the Meliaceae, have proved difficult for botanists to classify.[32] Bentham and Hooker[33] have placed Chloroxylon and Flindersia with the Meliaceae, whereas placement in the Rutaceae is favoured by others.[34] Chemical evidence strongly supports the latter classification.[35] Ptaeroxylon has been placed in the Sapindaceae by Bentham and Hooker,[33] whereas Harvey and Sonder[36] favour a separate small sub-family, the Ptaeroxylaceae, in the Meliaceae. Radlkofer[32] places Ptaeroxylon in the Meliaceae because of the presence of secretory cells in the leaf tissue and in the pith and cortex of the axes. Kribs,[32] on the other hand, having examined the wood of this monotypic genus, suggests that it more closely resembles the Rutaceae, plants of this family being characterised by the presence of schizogenous secretory cavities in the ground tissue of the branches and leaf.

It was felt that identification of the constituents present in the wood might help to clarify the botanical relationship, since, if it were indeed Meliaceous, degraded triterpenes might be expected to be present.[31] During the course of this work, it was found that other authors[22,37] had approached this problem for similar reasons.

Ptaeroxylon obliquum (Thunb.) Radlk. or Ptaeroxylon utile Eckl. and Zeyh., more commonly known as sneezewood, nieshout, or umTati, is a small tree endemic to South Africa. The distribution of this species extends from Port Elizabeth to the forests of the Anatolas, Pondoland, Natal, and the Northern Transvaal. Trees may grow to a height of over fifty feet with a girth of around three feet but in some districts there is a tendency to bushiness. The bark of sneezewood is smooth and almost white. When freshly cut, the trunk-wood is crimson and shows a curled grain. The timber, valuable for its extreme durability and resistance to attack by wood pests, has a strong peppery smell and, when sawn dry, the dust causes violent sneezing, thus restricting its commercial use.[38]

The ethyl acetate extract of ground heartwood, on cooling, deposited crystals, m.p. 212-214°, which were subsequently shown to be the known[27] chromone, peucenin (14),

found previously in the roots of Peucedanum ostruthium Koch (Umbelliferae). After washing with acid and base, the mother liquors were chromatographed over silica gel. Preparative thin layer chromatography of the fractions led to the isolation of six new compounds. The early fractions yielded a new chromone, desoxykarenin (41), m.p. 133-135°, two new isomeric coumarins, 7-O-(3,3-dimethylallyl)-scopoletin (50), m.p. 81-82° and nieshoutin (51), m.p. 125-127°, and the ubiquitous β-sitosterol. Later fractions contained peucenin (14), nieshoutol (52), m.p. 143-144° (a third new coumarin), and a novel chromone, karenin (42), m.p. 203-204°. With the exception of karenin (42), all these compounds were present in a light petroleum extract of the heartwood. In addition, the petrol extract afforded a further coumarin, 8-(1,1-dimethylallyl)-scopoletin (53), m.p. 141-143°. Although analytical t.l.c. indicated the presence of many further natrual products, their isolation was not attempted since Dr. Dean had informed us of his preliminary results on these chromones and coumarins.[10,21]

It was imperative to ascertain that the compound m.p. 212-214°, a major constituent of P. obliquum, was, in fact, peucenin (14) and not the as yet unknown isomer, heteropeucenin (34) since a recent investigation[22] had demonstrated the presence of heteropeucenin derivatives in this species. Several chemical transformations were thus carried out; these were, in essence, the reactions employed by Späth and Eiter[27] in their proof of structure of peucenin. The products gave identical physical data in all cases, with those recorded in the literature and the structures were substantiated spectroscopically. These derivatives were to prove invaluable as model compounds in elucidating the structures of karenin (42) and desoxykarenin (41).

The existence of a 5,7-dihydroxy-2-methylchromone system in the compound, m.p. 212-214°, $C_{15}H_{16}O_4$, was evident from the ultraviolet spectrum.[39] The infrared spectrum (see Table 1) confirmed the nature of the heterocyclic ring[40] showing the strongly hydrogen-bonded 5-hydroxyl as a broad band around 3000 cm.$^{-1}$. In the nuclear magnetic resonance (n.m.r.) spectrum in deuterochloroform (see Table 2), the methyl group at position 2

appears as a singlet (τ 7·68, 3H) [with pyridine as solvent, this shifts to τ 7·91], while a singlet at τ 4·02 (1H) shows that the olefinic proton is α to the carbonyl group. The presence of the 3,3-dimethylallyl grouping attached to an aromatic ring carrying but one proton, singlet at τ 3·69 (1H), was readily deduced from the n.m.r. spectrum.[41] There are two non-equivalent olefinic methyls at τ 8·17 (3H) and τ 8·23 (3H), one vinyl hydrogen, triplet centred at τ 4·73 (J = 8 c./sec.), and a methylene group both allylic and benzylic, two-proton doublet at τ 6·55 (J = 8 c./sec.).

The mass spectrum confirmed the molecular weight, the dimethylallyl grouping, and the 2-methyl-γ-pyrone ring. Normal fission[42] of a 3,3-dimethylallyl substituent was evident, fragment ions of m/e 205 (base peak), 217, and 192 arising from benzylic cleavage with loss of C_4H_7, rearrangement and elimination of C_3H_7, and complete fission of C_5H_8 respectively. The fragment ion m/e 205 undergoes two modes of fission characteristic of 2-methylchromones.[43] It shows retro-Diels-Alder elimination of C_3H_4, a metastable peak at 132·9, corresponding to the transition $205^+ \rightarrow 165^+$. The alternative loss of carbon monoxide, followed by a hydrogen atom, is evident from the peaks at m/e 177 and 176.

The points of attachment of the dimethylallyl side chain and one further hydroxyl group were still in doubt. This more acidic hydroxyl, which led to the facile preparation with diazomethane of a monomethyl ether (54), was probably in position 7 since the diol gave a characteristic blue colour with alkaline hydrogen peroxide.[27,44] In position 7, the hydroxyl is influenced vinylogously by the highly polar carbonyl group, giving rise to a very strong OH-π intramolecular interaction with the dimethylallyl side chain. This hydrogen bridge explains the two hydroxyl stretching frequencies in the infrared spectrum (in chloroform) at 3588 [$\nu(OH)^{free}$ and 3387 cm.$^{-1}$ $\nu(OH)^{bonded}$], showing no change on dilution. The $\Delta\nu(OH)$ value of 201 cm.$^{-1}$ in chloroform corresponds to a hydrogen bond energy, on Badger's hypothesis,[45,46] of approximately 3 kcal./mole, an outstandingly stable OH-π interaction. The 3387 cm.$^{-1}$ band is absent in the derived methyl ether (54) and in dihydropeucenin (55) (see Table 1). Corroboration of position 7 for the second hydroxyl and position 6 for the dimethylallyl substituent was effected unambiguously by the acid-catalysed cyclisation of peucenin into two known isomers, isopeucenin (56) and

allopeucenin (57). The spectral data of these derivatives accord with the structural assignments of Späth and Eiter.[27] Acid-catalysed cyclisation of heretopeucenin, with the dimethylallyl group in position 8, can furnish only one product. This conclusively verifies that the isolated compound, m.p. 212-214° is peucenin; an independent investigation by Pachler and Roux[37] confirms this assignment.

The two new related chromones, karenin (42) and desoxykarenin (41) showed markedly different solubilities and chromatographic behaviour. The extreme insolubility of the more abundant, but structurally more complex, karenin (42) posed technical difficulties in the obtention and interpretation of spectral data. Conversely, spectral information on the less accessible, but more soluble, desoxykarenin (41) was readily acquired. Nevertheless, the spectral data which were obtained indicated that the two compounds were very closely related structurally.

Desoxykarenin (41), $C_{15}H_{14}O_4$, present only in small amounts in the heartwood, could be isolated by careful use of thin layer chromatography from a column chromatographic fraction of a complex mixture which included nieshoutin and dimethylallylscopoletin. The separation technique made use of slight differences in mobility on chromatoplates combined with differing intensities of fluorescence of the individual components under ultraviolet light of 350 mμ.

The similarity between desoxykarenin and peucenin is evident from their infrared spectra (see Table 1) and characteristic reactions with ferric chloride. These require the existence of a 5-hydroxychromone nucleus. A methyl singlet at τ 7·66 in the n.m.r. spectrum (see Table 2) in deuterochloroform (this shifts to τ 7·86 in pyridine), taken with a one proton singlet at τ 3·99, additionally defines this partial structure. Like peucenin, the aromatic ring has one unsubstituted position (one proton singlet at τ 3·49) but, since desoxykarenin is neutral and gave no colour reaction with alkaline hydrogen peroxide, the hydroxyl at C-7 has been functionalised. Taking into account the presence of one

more double bond equivalent, the remainder of the n.m.r. data can be readily accommodated on the basis of structure (41) for desoxykarenin. Thus, an ill-defined two-proton doublet at τ 6·52 (J = 6 c./sec.) and a diffuse one-proton triplet at τ 4·33 (J = 6 c./sec.) is satisfactorily explained by a benzylic methylene adjacent to one vinylic hydrogen. A three-proton singlet at τ 8·40 is in accord with one vinylic methyl, while a broad two-proton singlet at τ 5·47 is assigned to the allylic methylene protons which in addition are adjacent to oxygen. Confirmation of the unique seven-membered ring was imparted by n.m.r. (and n.m.d.r.) at 100 Mc./sec. of dihydrodesoxykarenin (58). That the 2-methylchromone was still present in this hydrogenation product was established by the occurrence of a singlet at τ 3·56 (one aromatic proton), a methyl singlet at τ 7·72 (2-methyl), and a broad one-proton singlet at τ 3·98 (H-3), which sharpened on irradiation at τ 7·72. This indicated that the γ-pyrone system had survived hydrogenation, whereas the double bond in the seven-membered ring had been saturated. A two-proton multiplet around τ 8·0 contains the new 3'-methine proton and one of the methylene protons at the 2' position, the

other appearing as a diffuse multiplet about τ 8·8. A double resonance experiment confirmed the assignment of the methine proton, since irradiation at τ 7·9 resulted in collapse to a singlet of the three-proton doublet at τ 9·07 (J = .6 c./sec.), assigned to the secondary methyl. This experiment also located the two protons at the 4' position. On irradiation, the smaller (vincinal) couplings were removed from this geminal pair of protons, one-proton quartets at τ 5·82 (J_{gem} = 12, J_{vic} = 4 c./sec.) and τ 6·54 (J_{gem} = 12, J_{vic} = 8 c./sec.), each collapsing to a doublet. A separate irradiation experiment confirmed the existence of coupling between the two geminal protons. The multiplicity of the benzylic 1' protons, two ill-defined octets centred at τ 6·90 and 7·51, is also in agreement with the proposed structure. Although the functionality of desoxykarenin had now been defined, the direction of fusion of the seven-membered oxide ring remained equivocal. As before, two positions, namely C-6 and C-8, were possible sites for the location of the five-carbon residue. Conclusive evidence for the former, together with chemical support for the allylic ether was obtained by the isolation of

a minor hydrogenolysis product (17% yield) from catalytic hydrogenation of desoxykarenin. This compound was identical in all respects with dihydropeucenin (55) and differed significantly, in physical and spectral properties, from dihydroheteropeucenin (59), prepared by rearrangement of dihydropeucenin with hydrogen iodide.[47] Desoxykarenin has the same physical constants as those quoted for ptaeroxylin (60) the structure of which, a derivative of heteropeucenin (34), was based initially[22] on the untrustworthy similarity of its ultraviolet spectrum to that of 8-substituted rather than 6-substituted chromones. With such chromophores, it is unwise to draw definite conclusions from ultraviolet spectra when only minor structural differences are involved. Thus, it can be seen (Fig. 1) that, in the region 200-270 mμ, the ultraviolet spectrum of dihydrodesoxykarenin (58) is like that of dihydropeucenin methyl ether (61), whereas, between 270 and 350 mμ, the spectrum more closely resembles that of the 8-substituted isomer, dihydroheteropeucenin methyl ether (62). By personal communication, it was shown that ptaeroxylin and desoxykarenin were, in fact, identical (mixed m.p. and t.l.c.).

Re-investigation of the hydrogenation of ptaeroxylin under the conditions described above has led Dean and Taylor[10] to retract their proposed angular structure (60) in favour of the linear structure (41). By mutual agreement, the name 'ptaeroxylin' has been adopted to replace 'desoxykarenin'.

The close similarity of the spectral properties of karenin (42) to those of desoxykarenin (ptaeroxylin) (41) suggested the presence of the same ring system in both compounds. Taken with the mass spectrum, which shows analogous fragment ions sixteen mass units heavier, element analysis of karenin ($C_{15}H_{14}O_5$) indicated the existence of one additional oxygen atom. Absorption at 3615 $cm.^{-1}$ ($\varepsilon \sim 90$) in the infrared spectrum (see Table 1) of a rigorously dried sample showed this oxygen to be present as a primary and possibly allylic[48] alcohol. Karenin possesses one vinylic methyl group (three-proton singlet at τ 8·46) (see Table 2) and one vinylic methylene adjacent to oxygen (two-proton singlet at τ 5·21) in the n.m.r. spectrum in pyridine. Therefore, of the two vinylic methyls in desoxykarenin, one must be oxygenated in karenin, limiting possible structures to (42) or (43).

The former seemed more probable on the basis of the n.m.r. assignments (in pyridine) made for desoxykarenin, τ 7·86 for the 2-methyl, absent in karenin, and τ 8·42 for the 3'-methyl.

The structure of karenin was uniquely defined by the three main products obtained on catalytic reduction. Hydrogenolysis of the allylic alcohol with concomitant hydrogenation of one double bond gave dihydrodesoxykarenin (58) in 43% yield, thus confirming the carbon skeleton in karenin. This was substantiated by the co-formation, in 7% yield, of dihydropeucenin (55), resulting from two allylic cleavages. The major compound (46%), dihydrokarenin (63), which shows characteristic chromone absorption in the ultraviolet spectrum, must have the oxide ring, rather than the pyrone ring, saturated. Moreover, since the chemical shift (τ 5·30 in pyridine) of the methylene group bearing the hydroxyl has approximately the same value as found in karenin (τ 5·21 in pyridine), the hydroxymethyl group is, therefore, located at position 2. Structure (43) for karenin can be discounted, for, on formation of dihydrokarenin these protons would then be expected to shift substantially to

higher field. In conclusion, dihydrokarenin contains a secondary methyl group, three-proton doublet at τ 9·07 (J = 6 c./sec.) in deuterochloroform, and the observed chemical shifts and multiplicities for the protons of the seven-membered ring closely parallel those found for dihydrodesoxykarenin, which additionally supports structure (42) for karenin. A natural product, ptaeroxylinol, $C_{15}H_{14}O_5$, m.p. 135°, recently isolated from P. obliquum, has been shown[10] to have the structure (43) isomeric with karenin.

The existence of a coumarin nucleus in the compound (50), m.p. 81-82°, $C_{15}H_{16}O_4$, was evident from the multipeaked[49] u.v. spectrum, the major bands of which were 210, 231, and 346 mμ, and from the broad intense carbonyl stretching absorption at 1720 $cm.^{-1}$ ($CHCl_3$) in the i.r. spectrum.[50] That the lactone ring contained no substituents was shown by the appearance in the n.m.r. spectrum of two doublets (J = 10 c./sec.) centred at τ 2·42 (1H) and 3·79 (1H) for the protons on positions 3 and 4. The benzene ring of the coumarin system was 6,7-disubstituted, the two remaining aromatic proton signals at τ 3·17 (s, 1H) and 3·22 (s, 1H) indicating a para relationship. A methoxyl

substituent was evident from a three-proton singlet at τ 6·10 and the remaining n.m.r. peaks were accountable in terms of a 3,3-dimethylallyloxy unit attached to an aromatic ring. Thus, an ill-defined triplet (J = 7 c./sec.) at τ 4·55 (1H) was attributed to an olefinic proton adjacent to an allylic methylene group, two-proton doublet centred at τ 5·4 (J = 7 c./sec.), which is also attached to oxygen. The geminal methyl groups appear as a singlet at τ 8·21 (6H).

On addition of alkali, the u.v. spectrum did not undergo a bathochromic shift, indicating the absence of free phenolic substituents, an observation which was in accord with the i.r. spectrum.

Fragmentation of the dimethylallyl residue gave[42] mass spectral peaks at m/e 217, 216, 205, and 192 (base peak) through loss of C_3H_7, C_3H_8, C_4H_7, and C_5H_8 respectively from the parent ion (m/e 260). Further fission of the fragment ion m/e 192 by consecutive loss of CH_3 and carbon monoxide (or by loss in the reverse order) furnished peaks at m/e 177, 164, and 149.

The arrangement of substituents on the coumarin benzene ring was determined by hydrolysis of the dimethylallyl ether with hydrochloric acid in refluxing methanol. Isolation of the known coumarin, scopoletin (64),[51] m.p. 203°, as the only hydrolysis product allowed assignment of the methyl ether substituent to position 6 and the 3,3-dimethylallyloxy group to position 7 of the coumarin nucleus as in (50).

An unambiguous synthesis of this coumarin corroborated the structure. Aesculetin (65), obtained from aesculin (66) by acid-catalysed hydrolysis, was converted into scopoletin (64) by methylation of the derived aesculetin 7-tosylate (67) and subsequent removal of the protecting group (method of Desai and Desai[52]). Reaction of scopoletin with 3,3-dimethylallyl bromide in presence of potassium carbonate afforded 7-O-(3,3-dimethylallyl)-scopoletin (50) which was identical to the sample extracted from P. obliquum. In an alternative synthesis[53] in this department, aesculetin (65) has been reacted with 3,3-dimethylallyl bromide to form the 7-(3,3-dimethylallyl) ether (68), reaction time being adjusted to minimise the amount of 6,7-di-(3,3-dimethylallyl) ether (69)

formed, and the 6-hydroxyl group was then methylated (methyl iodide - potassium carbonate). By a reverse order of reaction, aesculetin 7-methyl ether (70), formed from aesculin by diazomethylation and hydrolysis, was treated with 3,3-dimethylallyl bromide to yield 6-(3,3-dimethylallyloxy)-7-methoxycoumarin (71), m.p. 116-117°. This compound is similar to its naturally occurring isomer (50) in the i.r., u.v., and n.m.r. but possesses a somewhat different fragmentation pattern in the mass spectrum.

The related coumarin m.p. 141-143° (53), once more possessed a u.v. spectrum characteristic of an aesculetin derivative.[54] In this case, a profound change in the spectrum accompanied the addition of base, indicating the presence of a free phenolic hydroxyl probably at C-7; thus, the major peaks at 211 and 346 mμ were replaced by bands at 221 and 410 mμ in presence of alkali. Acidification of the solution returned the spectrum to its original contour.

The coumarin nucleus, unsubstituted at positions 3 and 4 and possessing one aromatic proton, was evident from two doublets (J = 10 c./sec.) centred at τ 2·47 (1H) and 3·82 (1H) and from a sharp one-proton singlet (τ 3·27) in the n.m.r. spectrum at 100 Mc./sec. Singlets at τ 3·32 (1H), 6·14 (3H), and 8·32 (6H) were assigned to hydroxyl, methoxyl, and two identical tertiary methyl groups respectively. The ABX system formed by the three olefinic protons of the 1,1-dimethylallyl side chain gave[55] a seven-line spectrum; four lines (1H) of unequal intensity at τ 3·49, 3·60, 3·67, and 3·78 were coupled to a group of three lines (2H), situated at τ 4·98, 5·04, and 5·15. Double irradiation at τ ~5 and ~5·1 resulted, in both cases, in collapse to a triplet of the four-line spectrum in the τ 3·5-4 region. Similarly, double irradiation at τ ~3·7 caused considerable sharpening of the group of three lines around τ 5.

The foregoing spectral data do not indicate the arrangement of substituents on the aromatic ring of the coumarin nucleus. However, a biogenetic relationship between (50) and (53) can be envisaged on the basis of an <u>in vitro</u> Claisen rearrangement of the 7-(3,3-dimethyl-

allyl) ether of (50) to give a hydroxyl group at position 7 and a 1,1-dimethylallyl residue at position 8. Thus, a plausible structure for the coumarin, m.p. 141-143°, would be (53). This structure has recently been confirmed[53] in this department by Claisen rearrangement of 7-O-(3,3-dimethylallyl)scopoletin at 195° in vacuo. Separated from the mixture of products, this synthetic sample showed identical spectral properties to the naturally occurring material. Dean and Taylor have also isolated this compound (obliquetin)[56] and their assignments are in complete agreement with structure (53) proposed above.

Nieshoutin, m.p. 125-127°, a coumarin isomeric with (50) and (53), possessed the AB system of the unsubstituted lactone ring. One aromatic proton and a methoxyl group were evident from the singlets at τ 3·27 (1H) and 6·12 (3H). The remainder of the n.m.r. evidence was interpreted in terms of a trimethyl-substituted dihydrofuran system fused to the coumarin benzene ring (51). Thus, two tertiary methyl groups appeared at τ 8·44 (3H) and 8·71 (3H) and a secondary methyl group [τ 8·56 (d, 3H); J = 6 c./sec.] was coupled

to the proton [τ 5·41 (q, 1H); J = 6 c./sec.] of a methine unit, which was also adjacent to oxygen.

Fragmentation with loss of CH_3 (one or two), C_2H_5, and C_3H_7 was demonstrated by the peaks at m/e 245, 231, 230, and 217 in the mass spectrum, the latter fragment ion, decaying by loss of carbon monoxide to m/e 189. The abundant ion m/e 245 (70%) showed elimination of water and methanol, resulting in the ionic fragments m/e 227 and 213. Metastable peaks at m/e 231, 192, and 186·5 governed the transitions $260^+ \rightarrow 245^+$ (loss of CH_3), $245^+ \rightarrow 217^+$ (loss of carbon monoxide), and $245^+ \rightarrow 214^+$ (loss of CH_3O).

The close resemblance of nieshoutin to the above coumarins (50) and (53) was exhibited by the carbonyl stretching frequency [$\nu_{max.}(CCl_4)$ 1739 $cm.^{-1}$ (ε 1060)] and almost identical u.v. absorption. In presence of base the u.v. spectrum showed no bathochromic effect, corroborating the absence of phenolic substituents.

As before, although the functionality of nieshoutin was known, the placement of the aromatic substituents remained equivocal. Assuming a 6,7 arrangement of oxygen atoms on the coumarin nucleus, the biogenetically based

structure (51) can be proposed for nieshoutin, derived from (53) by cyclisation of the 7-hydroxyl group and the terminal double bond of the side chain. The validity of this proposal was demonstrated by Dean and Taylor[56] by partial synthesis of nieshoutin (cyclo-obliquetin) in this way; acid-catalysed cyclisation of obliquetin [8-(1,1-dimethylallyl)scopoletin] furnished cyclo-obliquetin, m.p. 124°, which was subsequently shown by mixed m.p., t.l.c., and spectral comparison, to be identical to nieshoutin.

Nieshoutol, m.p. 143-144°, $C_{15}H_{16}O_5$, containing one oxygen atom more than nieshoutin, exhibited phenolic properties. Thus, a hydroxyl stretching band is present in the i.r. spectrum at 3556 $cm.^{-1}$ (ε 120); a signal situated at τ 3·62 in the n.m.r. spectrum disappeared when the sample was shaken with deuterium oxide; and reaction of nieshoutol with diazomethane produced an oily monomethyl ether. The auxochromic effect of the additional oxygen substituent resulted in a u.v. spectrum different from those of the previous coumarins; the peaks were situated at 217, 231, 260, and 340 mμ which may be loosely attributed to a 5,6,7- or an 8,6,7-trioxygenated

coumarin.[54] On addition of alkali, the spectrum changed markedly, the appearance of new peaks at 256 and 404 mμ accompanying the disappearance of the 231 mμ band.

The benzene ring of the coumarin system was completely substituted, as shown by the absence of aromatic proton absorption in the n.m.r. spectrum, but the remaining spectral properties of nieshoutol closely resemble those of nieshoutin (51). Unsubstituted at positions 3 and 4 [τ 2·16 (d, 1H) and 3·86 (d, 1H); J = 10 c./sec.], the α-pyrone ring showed a broad carbonyl stretching absorption at 1720 $cm.^{-1}$ (ε 950) in chloroform. An aromatic methoxyl group was apparent at τ 6·01 (s, 3H) and a fused dihydrofuran ring possessed three methyl substituents, two of which were tertiary, τ 8·48 (s, 3H) and τ 8·75 (s, 3H), the third [τ 8·61 (d, 3H)] being coupled (J = 7 c./sec.) to a methine proton [τ 5·42 (q, 1H)] adjacent to oxygen.

The mass spectrum of nieshoutol portrayed a fragmentation pattern analogous to that of nieshoutin but fragment ions were sixteen mass units heavier. Thus, peaks at m/e 261, 247, 246, and 233 arose from loss of CH_3 (one or two), C_2H_5 and C_3H_7 from the parent ion

(m/e 276), further elimination of carbon monoxide from the m/e 233 fragment resulting in an ion at m/e 205. The m/e 261 ion (base peak) decayed by loss of water, methanol, and C_3H_5 to give peaks at m/e 243, 229, and 220 respectively. Three metastable peaks at m/e 247, 232, and 217 were in accord with three consecutive losses of a CH_3 unit from the parent ion ($276^+ \rightarrow 261^+$, $261^+ \rightarrow 246^+$, and $246^+ \rightarrow 231^+$). Two further metastable ions at m/e 208 and 202·5 represented the processes $261^+ \rightarrow 233^+$ (loss of carbon monoxide) and $261^+ \rightarrow 230^+$ (loss of CH_3O).

Once more, the spectral data give little information on the relative arrangement of substituents. However by an extension of the biogenetic assumptions already proposed for the derivation of the structure of nieshoutin, it is reasonable to expect that an <u>in vitro</u> hydroxylation could take place at the free aromatic 5-position of nieshoutin thus giving structure (52) for nieshoutol. (Conversely, the process might occur in the opposite direction, the deoxygenation of nieshoutol yielding nieshoutin.) It was hoped to perform this deoxygenation by a degradative sequence in which the S-aryldimethyl-thiocarbamate (72) of nieshoutol, derivable from the

O-aryl isomer (73) by thermal rearrangement,[57] could be hydrolysed and the resulting thiol (74) desulphurised with Raney nickel; the product of this reaction series, if successful, should be nieshoutin (or an isomer of nieshoutin). However, the O-aryldimethylthiocarbamate (73), formed from nieshoutol by treatment with dimethylthiocarbamoyl chloride in dimethylformamide, failed to give any recognisable product when heated in vacuo at 250°.

As an alternative to chemical degradation, an X-ray crystal structure analysis was attempted. To this end, the synthesis of three heavy atom derivatives was undertaken; the bromoacetyl derivative proved to be rather unstable and partially degraded during crystallisation to the starting phenol; the p-bromobenzene sulphonate was crystallographically unsuitable because crystals were imperfectly formed and belonged to the triclinic system. The p-bromobenzoate crystallised in the orthorhombic system but layer lines (rotation photograph) were alternately intense and diffuse. This arose from a pseudosymmetric property of the crystal; the weakness of the reflections from layers higher than the sixth rendered

impossible the compilation of sufficient data (Weissenberg photograph) to circumvent the pseudosymmetry. The synthesis of further derivatives has not been attempted.

Nieshoutol is apparently the active constituent, or one of the active constituents, of sneezewood. Its irritancy towards the nasal passages was particularly noticeable when inhalation of finely divided chromatoplate silica, containing adsorbed nieshoutol, caused violent sneezing. None of the other constituents, either in a pure state or in mixtures, showed similar behaviour. The inactivity of nieshoutin would appear to indicate that the irritant nature of nieshoutol is connected with the presence of the phenolic hydroxyl.

The optical inactivity of nieshoutol, its derivatives, and nieshoutin implies that the natural coumarins are racemic. Because of this, Dean and Taylor have suggested[56] that nieshoutin (cyclo-obliquetin) is an artefact, formed from obliquetin (53) during isolation. However, this explanation cannot hold for nieshoutol since it was claimed above to be the active constituent of the tree. It seems reasonable to expect that some nieshoutin and nieshoutol are present as natural products but that

enhanced amounts are isolated through induced cyclisation of the 1,1-dimethylallyl analogues (53) and (75). (It is noteworthy that the open chain analogue (75) of nieshoutol has not yet been reported as a constituent of sneezewood.) If the formation of nieshoutin and nieshoutol is enzymatically controlled, the enzyme surface cannot be capable of performing a stereospecific cyclisation.

The occurrence of chromones and coumarins[34] as relatively abundant heartwood constituents may exclude P. obliquum from the Meliaceae. This conclusion is substantiated by the apparent absence of degraded triterpenes in the timber. Coumarins are so widespread that classification on these alone is unreliable. Chromones, albeit relatively rare, co-occur with coumarins in the Umbelliferae, a family taxonomically close to those under consideration, but there has been no report of the appearance of chromones in the Rutaceae.[58] Recently, Chan, Taylor, and Willis,[28] in a private communication disclosed that they had isolated the chromone, sorbifolin (76), from Spathelia sorbifolia L., a Rutaceous plant. Thus, it seems that P. obliquum may have closer affinities

with the Rutaceae and Umbelliferae than with the Meliaceae. However, both chromones and coumarins may be formed from a single biosynthetic pathway[59] so classification merely on the presence of chromones is also tenuous. Moreover, Pachler and Roux[37] have pointed out that the isolated occurrence of peucenin in P. obliquum and Peucedanum ostruthium (Umbelliferae) cannot be of taxonomic significance. Dean and Taylor,[10] having isolated ptaeroxylin, as a major constituent, from Cedrelopsis grevei Baill., favour separate classification of these similar genera in a new family, the Ptaeroxylaceae, distinct from the Meliaceae. In the solution of this chemotaxonomic paradox, the presence of a unique mode of cyclisation of the C-5 side-chain, as in karenin and ptaeroxylin, may prove to be important. Although numerous heterocyclic oxygen compounds occur in P. obliquum, only the heartwood has been investigated; before definite taxonomic conclusions can be drawn, it may be necessary to examine other parts of the tree.

TABLE 1

Hydroxyl, carbonyl, and C=C absorptions of related chromones in chloroform

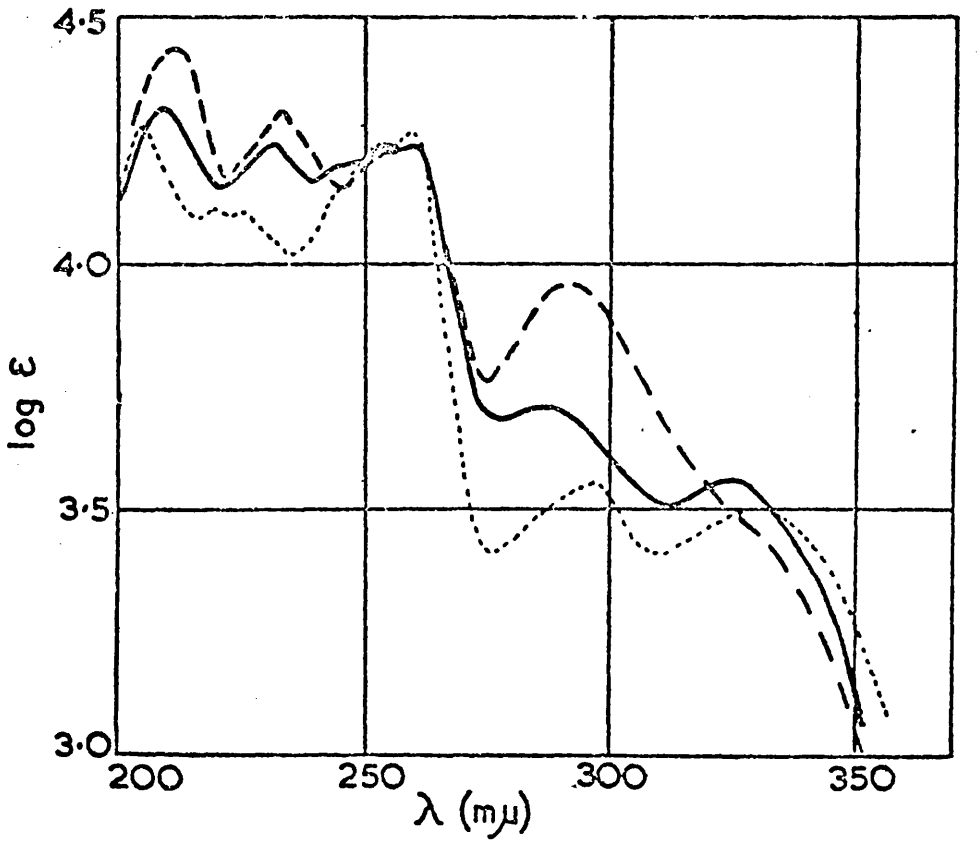
	ν(OH)	$\Delta\nu_{\frac{1}{2}}$[a]	ε	ν(CO), ν(C:C)	$\Delta\nu_{\frac{1}{2}}$[a]	ε
Peucenin	3588	45	70	1659	15	870
	3387	129	75	1633	25	560
	~2970	~450	~60	1589	19	480
Peucenin methyl ether	—	—	—	1661	12	1120
	—	—	—	1627	28	490
	~2970	~450	~60	1593	25	410
Dihydropeucenin	3626	32	95	1659	15	810
	3598	47	90	1630	29	440
	—	—	—	1590	24	360
	~2970	~450	~60			
Karenin	3615	48	98	1658	14	1010
	—	—	—	1629	28	545
	—	—	—	1602	26	370
	~2970	~450	~60			
Desoxykarenin	—	—	—	1656	14	1010
	—	—	—	1627	29	560
	~2970	~450	~60	1595	23	390

Table 2

Chemical shifts (τ) at 60 Mc./sec. for chromone derivatives

Numbers in the column headings refer to positions and numbers of chromone protons

	Solvent	8 or 6(1H)	3(1H)	2'(1H)	3'(1H)	1'(2H)	2-Me(3H)	4'(3H)	5'(3H)
Peucenin	$CDCl_3$	3·69(s)	4·02(s)	4·73(t) J = 8 c./sec.	—	6·55(d) J = 8 c./sec.	7·68(s)	8·17(s)	8·23(s)
	Pyridine	3·86(s)	?	?	—	6·18(d)	7·91(s)	8·05(s)	8·28(s)
Peucenin methyl ether	$CDCl_3$	3·69(s)	4·02(s)	4·80(t) J = 8 c./sec.	—	6·68(d) J = 8 c./sec.	7·69(s)	8·22(s)	8·33(s)
	Pyridine	3·86(s)	4·18(s)	4·44(m)	—	6·42(d)	8·22(s)	8·17(s)	8·33(s)
Dihydropeucenin	$CDCl_3$	3·66(s)	3·95(s)	*ca.* 8·50	(m, 3H)	7·40(m)	7·66(s)	8·08(s)	9·07(s)
Dihydropeucenin methyl ether	$CDCl_3$	3·60(s)	3·92(s)	*ca.* 8·70	(m, 3H)	7·29(m)	7·67(s)	9·00(s)	9·09(s)
Dihydroheteropeucenin	$CDCl_3$	3·66(s)	3·92(s)	*ca.* 8·60	(m, 3H)	7·25(m)	7·68(s)	9·04(s)	9·12(s)
Dihydroheteropeucenin methyl ether	$CDCl_3$	3·68(s)	4·01(s)	*ca.* 8·65	(m, 3H)	7·35(m)	7·63(s)	9·00(s)	9·09(s)
Isopeucenin	$CDCl_3$	3·83(s)	4·11(s)	8·21(t) J = 7 c/sec.	—	7·33(t) J = 7 c./sec.	7·70(s)	8·67(s, 6H)	
Karenin	Pyridine	?	?	4·30(t) J = 6 c./sec.	—	6·37(d) J = 6 c./sec.	5·21(s, 2H)	5·40(s, 2H)	8·47(s)
Dihydrokarenin (100 Mc./sec.)	$CDCl_3$	3·59(s)	3·67(s)	*ca.* 8·0 *ca.* 8·7	(m, 1H) (m, 2H)	6·89(m, 1H) 7·52(m, 1H)	5·55s, 2H)	5·83(q, 1H) 6·53(q, 1H)	9·07(d) J = 6 c./sec.
Desoxykarenin	$CDCl_3$	3·49(s)	3·99(s)	4·33(t) J = 6 c./sec.	—	6·52(d) J = 6 c./sec.	7·66(s)	5·47(s, 2H)	8·40(s)
	Pyridine	?	3·81(s)	4·23(t) J = 6 c./sec.	—	6·37(d) J = 6 (c./sec.	7·86(s)	5·43(s, 2H)	8·42(s)
Dihydrodesoxykarenin (100 Mc./sec.)	$CDCl_3$	3·56(s)	3·98(s)	*ca.* 8·0 *ca.* 8·8	(m, 1H) (m, 2H)	6·90(m, 1H) 7·51(m, 1H)	7·72(s)	5·82(q, 1H) 6·54(q, 1H)	9·07(d) J = 6 c./sec.



Ultraviolet spectra of dihydrodesoxykarenin (——), dihydropeucenin methyl ether (— — —), and dihydroheteropeucenin methyl ether (– – – –)

Fig. 1.

Experimental.

Alumina for column chromatography was Woelm Grade I (neutral). Chromatoplates were spread with Merck silica gel G. Light petroleum used was of b.p. 40-60° unless otherwise stated. Melting points were determined on a Kofler hot-stage apparatus. Quantitative infrared solution spectra (10^{-2} - 10^{-3} molar) were recorded with a Unicam SP 100 double beam spectrophotometer (see Part 2); qualitative spectra were recorded on Unicam SP 200, Perkin-Elmer 237, or Perkin-Elmer 257 instruments. Ultraviolet spectra were run in ethanol (10^{-3} - 10^{-4} molar) with a Unicam SP 800 spectrophotometer. N.m.r. spectra were determined by Mr. J. Gall on a Perkin-Elmer R 10 and a Varian HA 100 spectrometer on solutions in deuterochloroform or pyridine, with tetramethylsilane as internal standard. Mass spectra were measured by Dr. T.A. Bryce, using an A.E.I. MS 9 spectrometer and microanalyses were carried out by Mr. J.M.L. Cameron, and his staff. Ferric chloride colour tests[44,47] on chromones were carried out by adding aqueous ferric chloride to cold ethanolic solutions of suspected 5-hydroxychromones. Complexes of 5,7-dihydroxy-2-methylchromones with hydrogen peroxide[27] were formed by treatment of cold dilute

sodium hydroxide solutions of test samples with hydrogen peroxide (30%). Specific rotations refer to chloroform solutions at room temperature.

Extraction of P. obliquum

(i) With ethyl acetate. The ethyl acetate extract of ground heartwood (2 kg.) (provided by Mr. J.H. van Wyk, Forest Research Institute, Pretoria), on cooling gave a brown solid (15 g.), m.p. 170-210°. Repeated fractional crystallisation of this solid from methanol afforded peucenin (14) (12 g.) as pale yellow needles m.p. 210-212°, which sublimed under vacuum as colourless needles m.p. 212-214°. The ethyl acetate-soluble material, on evaporation of solvent, furnished a red-brown gum (242 g.), a portion (188 g.) of which was separated into acid-soluble (3 g.), carbonate-soluble (22 g.), alkali-soluble (39 g.), and neutral (71 g.) fractions, by washing successively with 1N hydrochloric acid (2 x 500 ml.), 2% aqueous potassium carbonate (3 x 500 ml.), and 5% aqueous potassium hydroxide (5 x 500 ml.). The neutral fraction deposited an amorphous precipitate (12 g.), mainly peucenin, which was removed,

the residue being chromatographed over silica gel (1·5 kg.). Early fractions, ethyl acetate-light petroleum (1:3) to (3:7), gave a mixture (1·5 g.) of essentially four compounds. Separation of this mixture was effected by preparative t.l.c., yielding ptaeroxylin (desoxy-karenin) (41) (80 mg.), nieshoutin (51) (300 mg.), 7-O-(3,3-dimethylallyl)scopoletin (50) (500 mg.), and β-sitosterol (70 mg.), m.p. 135-138°. Later fractions, ethyl acetate-light petroleum (1:1) to (9:1), were combined (20 g.) and rechromatographed over silica gel (1·5 kg.). Preparative t.l.c. of the fractions (7 g.) eluted with ethyl acetate-light petroleum (3:2) to (2:1) from this second column gave karenin (42) (300 mg.), and nieshoutol (52) (1·5 g.).

(ii) With petroleum ether. The green-brown gum (12 g.), extracted from ground heartwood (2 kg.) with light petroleum (b.p. 60-80°), was chromatographed over silica gel (700 g.). The presence of compounds (41), (50), and (51) in early fractions (800 mg.) eluted with ethyl acetate-light petroleum (1:9) to (1:1) was detected by mobility, ultra-violet fluorescence, and

characteristic staining properties on t.l.c. Continued elution with ethyl acetate-light petroleum (1:1) gave peucenin (2 g.), m.p. 210-212°. Fractions eluted with ethyl acetate-light petroleum (1:1) to (9:1) contained a mixture (6 g.) of at least seven compounds, from which nieshoutol (52) (2·3 g.) was partly removed by fractional crystallisation from carbon tetrachloride. Preparative t.l.c. of the mother liquors (3·7 g.) yielded 8-(1,1-dimethylallyl)scopoletin (obliquetin) (53) (575 mg.).

Peucenin (14)

Peucenin, m.p. 212-214° (lit.[27] m.p. 212°), occurred as 2% of dried heartwood of P. obliquum (Found: C, 68·95; H, 6·3. Calcd. for $C_{15}H_{16}O_4$ C, 69·2; H, 6·2%), $\nu_{max.}^{CHCl_3}$ (see Table 1); $\nu_{max.}^{KCl}$ & nujol 1658, 1628, and 1570 $cm.^{-1}$; $\lambda_{max.}$ 215 (log ε 4·45), 233 (4·30), 255 (4·23), 260 (4·24), and 299 mμ (3·97) mass spectral peaks at m/e 260 (molecular ion), 245, 217, and 205 (relative abundance 42, 25, 85, and 100%). Peucenin showed violet ferric chloride and blue hydrogen peroxide colour tests.

Peucenin 7-methyl ether (54)

Obtained by diazomethylation (30 hr.) of peucenin (100 mg.), peucenin 7-methyl ether was eluted from alumina (10 g.) with ethyl acetate-light petroleum (3:7) and crystallised from methanol as colourless needles (54 mg.), m.p. 106-107° (lit.[27] m.p. 108-109° or 101-102°); $\nu_{max.}^{CHCl_3}$ (see Table 1); $\nu_{max.}^{KCl}$ 1666, 1641, 1613, 1595, and 1589 $cm.^{-1}$; $\lambda_{max.}$ 213 (log ε 4·30), 233 (4·22), 255 (4·13), 260 (4·14), and 293 mμ (3·86), mass spectral peaks at m/e 274 (molecular ion), 259, 231, 219, and 189 (relative abundance 41, 24, 100, 87, and 16%). Peucenin methyl ether gave a red-violet colour with ferric chloride; the hydrogen peroxide colour test was negative. The derived 5-acetate obtained by refluxing peucenin methyl ether in acetic anhydride for four hours, crystallised from ether-light petroleum as colourless needles, m.p. 124-126° (lit.[27] m.p. 125-126°). Both colour tests were negative.

Dihydropeucenin (55)

Peucenin (1·16 g.) in acetic acid (100 ml.) was hydrogenated over 10% palladium-charcoal; after 15 min., the hydrogen uptake was 1·3 mol. The product, freed from catalyst and solvent, crystallised from methanol as colourless needles (0·86 g.) m.p. 205-207° (lit.[27] m.p. 207°); $\nu_{max.}^{CHCl_3}$ (see Table 1); $\nu_{max.}^{KCl}$ 1658, 1630, and 1570 cm.$^{-1}$, $\lambda_{max.}$ 213 (log ε 4·46), 232 (4·27), 255 (4·24), 260 (4·25), 298 (4·02), and 330 mμ (3·54), mass spectral peaks at m/e 262 (molecular ion), 219, 206, and 205 (relative abundance 16, 15, 100, and 75%). Dihydropeucenin gave ferric chloride (blue-violet) and hydrogen peroxide (blue) colour tests.

Dihydropeucenin 7-methyl ether (61)

The reaction product of diazomethylation (25 hr.) of dihydropeucenin (62 mg.) was adsorbed on alumina. Dihydropeucenin 7-methyl ether, eluted with ethyl acetate-light petroleum (1:16), crystallised from ether-light petroleum as colourless plates (45 mg.), m.p. 105-107° (lit.[27] m.p. 105-106°); $\nu_{max.}^{CHCl_3}$ 1661, 1627, and 1591 cm.$^{-1}$; $\lambda_{max.}$ (see Fig. 1). Dihydropeucenin methyl ether gave a violet colour with ferric chloride; the hydrogen peroxide colour test was negative.

Acid-catalysed Rearrangement of Dihydropeucenin

Dihydropeucenin (55), (395 mg.) was heated under gentle reflux for 2 hr., with constant-boiling aqueous hydrogen iodide (20 ml.). The reaction mixture was poured into ice-water, containing methanol (5 ml.) and sodium dithionite (10 mg.) and the product extracted with ethyl acetate. Dihydroheteropeucenin (59), isolated by preparative t.l.c. (development in chloroform), crystallised from ether-light petroleum as pale yellow needles (298 mg.), m.p. 191-193° (lit.[47] m.p. 191-192°); $\nu_{max.}^{CHCl_3}$ 3625, 3600, 1663, 1624, and 1591 cm.$^{-1}$; $\nu_{max.}^{KCl}$ 1660, 1650, 1618, 1595, and 1560 cm.$^{-1}$; $\lambda_{max.}$ 205 (log ε 4·32), 219 (4·21), 227 (4·16), 255 (4·30), 261 (4·32), 301 (3·72), and 328 mμ (3·58), mass spectral peaks at m/e 262 (molecular ion), 205, and 165 (relative abundance 17, 100, and 15%). Dihydroheteropeucenin showed violet ferric chloride and blue hydrogen peroxide colour reactions.

Dihydroheteropeucenin 7-methyl ether (62)

The product of diazomethylation (20 hr.) of dihydroheteropeucenin (42 mg), the 7-methyl ether (62), was isolated by preparative t.l.c. (development with chloroform) and crystallised from ether-light petroleum or methanol as colourless needles (18 mg.), m.p. 91-93° (Found: C, 69·85; H, 7·35. $C_{16}H_{20}O_4$ requires C, 69·55; H, 7·30%); $\nu_{max.}^{CHCl_3}$ 1661, 1622, and 1594 cm.$^{-1}$; $\lambda_{max.}$ (see Fig. 1).

Acid-catalysed Cyclisation of Peucenin

Peucenin[27] (200 mg.) was heated under reflux for 1½ hr., in a mixture of acetic acid (5 ml.) and concentrated sulphuric acid (5 drops). The residue, after removal of solvent and neutralisation with 2% aqueous potassium carbonate, was extracted with ethyl acetate, washed with 1% aqueous potassium hydroxide (2 x 10 ml.) then with water and dried. Evaporation of the ethyl acetate yielded isopeucenin (56), which crystallised from ether-light petroleum as colourless needles (100 mg.), m.p. 132-134° (lit.[27] m.p. 132°); $\nu_{max.}^{CHCl_3}$ 1659, 1628, and 1584 cm.$^{-1}$; $\lambda_{max.}$ 211 (log ε 4·45), 230 (4·27), 252 (4·31), 259 (4·31),

and 298 mμ (4·02); mass spectral peaks at m/e 260 (molecular ion), 217, and 205 (relative abundance 53, 37, and 100%). The potassium hydroxide washings, on acidification and extraction with ethyl acetate, gave allopeucenin (57), which crystallised from methanol as colourless needles (40 mg.), m.p. 303-304° (lit.[27] m.p. 303-304°), mass spectral peaks at m/e 260 (molecular ion), 217, and 205 (relative abundance 32, 19, and 100%). The extreme insolubility of allopeucenin rendered difficult the compilation of spectral data in solution. Isopeucenin gave a violet colour with ferric chloride; the hydrogen peroxide colour reaction was negative. Allopeucenin showed negative colour tests.

Ptaeroxylin (Desoxykarenin) (41)

Ptaeroxylin, colourless needles m.p. 133-135°, from ether-light petroleum, occurred as 0·004% of dried heartwood of P. obliquum (Found: C, 69·95; H, 5·65. $C_{15}H_{14}O_4$ requires C, 69·75; H, 5·45%); $\nu_{max.}^{CHCl_3}$ (see Table I); $\lambda_{max.}$ 209 (log ε 4·23), 232 (4·27), 241 (4·22), 255 (4·24), 284 (3·69), and 321 mμ (3·59); mass spectral peaks at m/e 258 (molecular ion), 243, and 217 (relative abundance 73, 100, and 10%). Desoxykarenin gave a green-brown ferric chloride colour reaction; the hydrogen peroxide colour test was negative.

Hydrogenation of Ptaeroxylin (Desoxykarenin)

Ptaeroxylin (41) (23 mg.) in acetic acid (20 ml.) was hydrogenated over 10% palladium charcoal; after 1½ hr., hydrogen (1·5 mol.) had been absorbed. The mixture of products, after removal of catalyst and solvent, was adsorbed on a preparative-scale chromatoplate, development of which with chloroform gave two concentrated, fluorescent bands. The upper band, after extraction (ethyl acetate) and crystallisation from chloroform-light petroleum (or from methanol), yielded dihydroptaeroxylin (58) (15 mg.) as pale yellow plates, m.p. 81-83° (Found: C, 69·5; H, 6·35. $C_{15}H_{16}O_4$ requires C, 69·2; H, 6·2%); $\nu_{max.}^{CHCl_3}$ 1654, 1623, and 1592 cm.$^{-1}$; $\nu_{max.}^{KCl}$ 1660, 1620, 1592, and 1574 cm.$^{-1}$; $\lambda_{max.}$ (see Fig. 1); mass spectral peaks at m/e 260 (molecular ion), 217, 205, 192, and 190 (relative abundance 90, 100, 31, 43, and 23%). Dihydroptaeroxylin gave a violet colour with ferric chloride; the hydrogen peroxide colour test was negative. The lower band contained dihydropeucenin (55) (4 mg.), identical [mixed m.p., infrared (KCl) and ultraviolet absorption, mass spectrum and chromatoplate mobility] with the sample prepared from peucenin (14). This

hydrogenolysis product of ptaeroxylin showed depression of m.p., when mixed with dihydroheteropeucenin (59), from which it also differed in infrared, ultraviolet, mass spectral, and t.l.c. behaviour (Rf, ultraviolet fluorescence, iodine stain).

Karenin (42)

Occurring as 0·02% of dried heartwood karenin, pale yellow needles, m.p. 202-204°, from methanol or ethyl acetate, showed a tendency to solvate when crystallised from hydroxylic solvents. Vacuum sublimation ($154^{\circ}/10^{-4}$ mm.) gave a non-solvated sample, m.p. 204-205° (Found: C, 65·6; H, 5·4. $C_{15}H_{14}O_5$ requires C, 65·7; H, 5·15%); $\nu_{max.}^{CHCl_3}$ (see Table 1); $\lambda_{max.}$ 211 (log ε 4·24), 232 (4·28), 243 (4·23), 255 (4·24), 286 (3·55), and 324 mμ (3·59); mass spectral peaks at m/e 274 (molecular ion), 259, and 233 (relative abundance 46, 100, and 19%). Karenin gave a green-brown ferric chloride colour; the hydrogen peroxide test was negative.

Hydrogenation of Karenin

Karenin (42) (30 mg.) in acetic acid (15 ml.) was hydrogenated in the cold over 10% palladium-charcoal; after 1½ hr. the uptake of hydrogen was 2·8 mol. The mixture of products, freed from catalyst and solvent, was adsorbed on a preparative-scale chromatoplate, which, after development with chloroform, showed three main, fluorescent bands. The band of lowest Rf, on extraction (ethyl acetate) and crystallisation from chloroform-light petroleum, yielded dihydrokarenin (63) as pale yellow needles (14 mg.), m.p. 130-131° (Found: C, 64·95; H, 5·8. $C_{15}H_{16}O_5$ requires C, 65·2; H, 5·85%); $\lambda_{max.}$ 208 (log ε 4·25), 231 (4·14), 245 (4·09), 254 (4·12), 259 (4·12), 289 (3·62), and 325 mμ (3·43). Dihydrokarenin gave a green colour with ferric chloride and showed no action with alkaline hydrogen peroxide. The middle t.l.c. band contained dihydropeucenin (55) (2·3 mg.), identical (mixed m.p., infrared, ultraviolet, and mass spectra, chromatoplate mobility) with the sample prepared from peucenin (14). The m.p. of this minor hydrogenolysis product was depressed by dihydroheteropeucenin (59) and t.l.c. behaviour (Rf., ultraviolet fluorescence, iodine

stain) was different; the infrared, ultraviolet, and mass spectra also showed significant differences. The band of highest Rf afforded dihydroptaeroxylin (58), which crystallised from chloroform-light petroleum as pale yellow plates (12 mg.), m.p. 81-83°, identical (mixed m.p., infrared, ultraviolet, and mass spectra, chromatoplate behaviour) with the specimen obtained by hydrogenation of ptaeroxylin (41).

7-O-(3,3-dimethylallyl)scopoletin (50)

7-O-(3,3-dimethylallyl)scopoletin, pale yellow needles, m.p. 80-81°, from ether-light petroleum, occurred as 0·03% of dried heartwood of P. obliquum (Found: C, 69·1; H, 6·1. $C_{15}H_{16}O_4$ requires C, 69·2; H, 6·2%); $\nu_{max.}^{CHCl_3}$ 1720 cm.$^{-1}$ (ε 860); $\lambda_{max.}$ 210 (log ε 4·42), 231 (4·25), 252 (3·76), 260 (3·69), 296 (3·75), and 346 mμ (4·09); mass spectral peaks at m/e 260 (molecular ion), 192, 177, 164, and 149 (relative abundance 4, 100, 29, 16, and 10%).

Hydrolysis of 7-O-(3,3-dimethylallyl)scopoletin

7-O-(3,3-dimethylallyl)scopoletin (18 mg.) in methanol (5 ml.) and concentrated hydrochloric acid (5 drops) was refluxed for 1½ hr. After evaporation to dryness, the reaction mixture was washed with aqueous sodium bicarbonate giving a fluorescent alkaline extract which was acidified and re-extracted with ethyl acetate. Removal of solvent furnished scopoletin (64) as a colourless solid (12 mg.) which crystallised from chloroform-light petroleum as needles, m.p. 202-203° (lit.[51] m.p. 204°); $\nu_{max.}^{KCl}$ 1705, 3318 cm.$^{-1}$; $\lambda_{max.}$ 211 (log ε 4·14), 230 (4·11), 254 (3·68), 260 (3·63), 298 (3·68), and 346 mμ (4·07). No depression of m.p. was observed when this hydrolysis product was mixed with an authentic sample of scopoletin. Both samples showed identical t.l.c. characteristics and all peaks in the i.r. (KCl) and u.v. spectra were superposable.

Hydrolysis of aesculin (66)[60]

Hydrogen chloride was passed for 5 min. into a solution of aesculin (1 g.) in methanol (25 ml.) and water (3 ml.) and the reaction mixture was left at room temperature for 60 hr. After removal of solvent under reduced pressure, the residual yellow solid was filtered and washed with water. Crystallisation from aqueous ethanol afforded aesculetin (65) (454 mg.) as yellow needles, m.p. 270-272° (lit.[61] m.p. 276°).

Aesculetin 7-p-toluenesulphonate (67)[52]

Anhydrous potassium carbonate (AnalaR) (4·8 g.) was added to a solution of aesculetin (1·2 g.) and p-toluenesulphonyl chloride (1·2 g.) in anhydrous acetone (AnalaR) (140 ml.) and the mixture was shaken at room temperature for 4 hr. The reaction mixture, freed from solvent, was extracted with dilute aqueous sodium hydroxide and the solution was filtered to remove the base-insoluble 6,7-di-p-toluenesulphonate. The required 7-p-toluene-sulphonate (67) (990 mg.), precipitated on acidification of the filtrate, crystallised from ethanol as needles, m.p. 220-223° (lit.[52] m.p. 203°).

Methylation of aesculetin 7-p-toluenesulphonate (67)[52]

Dimethyl sulphate (2 ml.) was added to a solution of aesculetin 7-p-toluenesulphonate (1 g.) in anhydrous acetone (Analar) (50 ml.) and the solution was refluxed for 24 hr. with anhydrous potassium carbonate (6 g.). The residue, after removal of the acetone, was washed with water and crystallised from aqueous ethanol, affording 6-methoxy-7-tosyloxycoumarin (77) as colourless needles (540 mg.), m.p. 193-195° (Lit.[52] m.p. 195°) (Found: C, 58·9; H, 4·35. Calcd. for $C_{17}H_{14}O_6S$ C, 58·95; H, 4·05%).

Scopoletin (64)[52]

6-Methoxy-7-tosyloxycoumarin (77) (200 mg.) was hydrolysed for 24 hr. at room temperature with concentrated sulphuric acid (9 ml.). The reaction mixture was poured on to ice and extracted with ethyl acetate, the organic layer washed with water and dried over magnesium sulphate. Removal of solvent furnished scopoletin (95 mg.) which crystallised from methanol as colourless needles, m.p. 204-206° (lit.[51] m.p. 204°).

Dimethylallylation of scopoletin[62]

Scopoletin (300 mg.) and potassium carbonate (AnalaR) (272 mg.) were stirred for 15 hr. at 50° in AnalaR acetone (70 ml.) and freshly distilled 3,3-dimethylallyl bromide (330 mg.). After removal of the acetone under suction, the reaction product was extracted with ethyl acetate, the organic layer washed with dilute sodium bicarbonate solution, with water to neutrality and dried over magnesium sulphate. Freed from solvent, the resulting oil (390 mg.) solidified on trituration with ether and crystallised from ether-light petroleum as pale yellow needles, m.p. 80-81°, $\nu_{max.}^{CHCl_3}$ 1719 cm.$^{-1}$; $\lambda_{max.}$ 209 (log ε 4·41), 231 (4·16), 252 (3·67), 259 (3·59), 295 (3·65), and 345 mμ (3·99). This compound was identical, by t.l.c. and spectral (i.r., u.v., n.m.r.) comparison, to the naturally-occurring 7-O-(3,3-dimethylallyl)scopoletin (50).

Aesculin 7-methyl ether (78)

Prepared by reaction of aesculin (66) with diazomethane, aesculin 7-methyl ether crystallised from methanol as colourless needles, m.p. 230-233° (lit.[51] m.p. 230°).

Aesculetin 7-methyl ether (70)

Hydrogen chloride was passed into a solution of aesculin 7-methyl ether (78) (600 mg.) in methanol (25 ml.) and water (3 ml.) for 15 mins. and the mixture set aside for 24 hr. Removal of solvent and crystallisation of the resulting solid from methanol furnished 6-hydroxy-7-methoxycoumarin (70) as pale yellow needles (390 mg.), m.p. 185-187° (lit.[51] m.p. 185°).

Dimethylallylation of aesculetin 7-methyl ether (70)[62]

A suspension of 6-hydroxy-7-methoxycoumarin (300 mg.) and AnalaR potassium carbonate (272 mg.) in AnalaR acetone (70 ml.) and 3,3-dimethylallyl bromide (300 mg.) was stirred at 50° for 20 hr. The crude product, freed from solvent, was extracted with ethyl acetate, washed with aqueous sodium bicarbonate, with water to neutrality and dried. Removal of the ethyl acetate furnished 6-O-(3,3,-dimethylallyl)-7-methoxycoumarin (71) as colourless needles (350 mg.), m.p. 116-117° (Found: C, 69·2 ; H, 6·25. $C_{15}H_{16}O_4$ requires C, 69·2; H, 6·2%); $\nu_{max.}^{CHCl_3}$ 1719 cm.$^{-1}$ (ε 870); $\lambda_{max.}$ 208 (log ε 4·50), 232 (4·33), 251 (3·87), 260 (3·79), 287 (3·70), 295 (3·77),

and 344 mμ (4·07); n.m.r. signals at τ 2·46 (1H, d; J = 10 c./sec.), 3·84 (1H, d; J = 10 c./sec.), 3·19 (1H, s), 3·28 (1H, s), 4·56 (1H, t; J = 7 c./sec.), 5·48 (2H, d; J = 7 c./sec.), 6·14 (3H, s), 8·24 (3H, s), and 8·28 (3H, s); mass spectral peaks at m/e 260 (molecular ion), 208, 194, 178, 164, and 149 (relative abundance 3, 2, 100, 5, 22, and 14%).

8-(1,1-dimethylallyl)scopoletin (53)

8-(1,1-dimethylallyl)scopoletin, pale yellow needles, m.p. 141-143°, from ethyl acetate-light petroleum, occurred as 0·03% of dried heartwood of P. obliquum; $\lambda_{max.}$ 211 (log ε 4·44), 230 (4·08), 254 (3·60), 262 (3·56), 308 (3·74), and 346 mμ (3·99). This compound was identical (t.l.c., u.v., and n.m.r.) to one of the products of Claisen rearrangement of 7-O-(3,3,-dimethylallyl)scopoletin (50).[53]

Nieshoutin (51)

Nieshoutin, isolated from P. obliquum as 0·02% of dried heartwood, crystallised from ether-light petroleum as pale yellow needles, m.p. 125-127° (Found: C, 69·5; H, 6·1. $C_{15}H_{16}O_4$ requires C, 69·2; H, 6·2%); $\nu_{max.}^{CCl_4}$ 1739 cm.$^{-1}$ (ε 1060); $\lambda_{max.}$ 211 (log ε 4·45), 232 (4·19), 254 (3·50), 262 (3·46), 309 (3·74), and 346 mμ (4·11); mass spectral peaks at m/e 260 (molecular ion), 245, 231, 230, 227, 217, 213, 204, and 189 (relative abundance 100, 70, 11, 11, 15, 37, 16, 17, and 22%). This compound was identical to one of the products of Claisen rearrangement of 7-O-(3,3,-dimethylallyl)scopoletin (50)[53] and, by direct comparison, was also shown to be the same as cyclo-obliquetin.[56]

Nieshoutol (52)

Nieshoutol, pale yellow plates, m.p. 143-144°, from carbon tetrachloride, occurred as 0·1% of dried heartwood of P. obliquum (Found: C, 64·95; H, 5·85. $C_{15}H_{16}O_5$ requires C, 65·2; H, 5·85%); $\nu_{max.}^{CHCl_3}$ 1720 (ε 950), 3556 cm.$^{-1}$ (120); $\lambda_{max.}$ 217 (log ε 4·37), 231 (4·19), 260 (3·53), and 340 mμ (4·12); mass spectral

peaks at m/e 276 (molecular ion), 261, 247, 246, 243, 233, 229, 220, and 205 (relative abundance 98, 100, 9, 15, 7, 32, 56, 12, and 10%); $[\alpha]_D$ 0°.

Nieshoutol methyl ether (79)

Obtained by diazomethylation (65 hr.) of nieshoutol (100 mg.), nieshoutol methyl ether (85 mg.) was eluted from alumina (7 g.) with ethyl acetate-light petroleum (1:49) as a colourless viscous oil (Found: C, 65·95; H, 6·2. $C_{16}H_{18}O_5$ requires C, 66·2; H, 6·25%); $\nu_{max.}^{CCl_4}$ 1740 cm.$^{-1}$ (ε 1140); $\lambda_{max.}$ 212 (log ε 4·43), 230 (4·16), 251 (3·75), 259 (3·70), and 334 mμ (4·01); n.m.r. signals at τ 2·13 (1H, d; J = 10 c./sec.), 3·86 (1H, d; J = 10 c./sec.), 5·44 (1H, q; J = 7 c./sec.), 6·02 (3H, s), 6·09 (3H, s), 8·46 (3H, s), 8·59 (3H, d; J = 7 c./sec.) and 8·73 (1H, s); mass spectral peaks at m/e 290 (molecular ion), 275, 261, 260, 257, 247, 244, 234, and 219 (relative abundance 82, 100, 4, 7, 2, 15, 23, 2, and 4%); $[\alpha]_D$ 0°.

Nieshoutol bromoacetate (80)

Nieshoutol (63 mg.) and bromoacetyl bromide (1 ml.) were refluxed gently for 20 hr. in anhydrous benzene (AnalaR) (15 ml.). On cooling, ice was added and the mixture was extracted with ethyl acetate, the organic layer being washed with aqueous sodium bicarbonate, with water till neutral, and dried. The crude product, on removal of solvent, was adsorbed on a preparative chromatoplate, development of which in chloroform gave a broad, blue-fluorescent band higher in Rf than nieshoutol. Extraction (ethyl acetate) of this band and attempted crystallisation from carbon tetrachloride furnished an impure yellow semi-solid, which was (analytical t.l.c.) a mixture of the blue-fluorescent compound and nieshoutol. Repeated chromatography and crystallisation always gave the same proportion of nieshoutol as impurity; thus, a crystalline sample of nieshoutol bromoacetate was not obtained.

Nieshoutol p-bromobenzoate (81)

Nieshoutol (110 mg.) was heated at 65° for 18 hr. with p-bromobenzoyl chloride in anhydrous pyridine (12 ml.). On cooling, ice was added and the mixture was extracted with ethyl acetate as in the preparation of the bromo-acetate. The crude reaction product was separated by t.l.c. in chloroform and the broad fluorescent upper band, on extraction, afforded nieshoutol p-bromobenzoate (83 mg.) which crystallised from chloroform-light petroleum as colourless needles, m.p. 213-215° (Found: C, 57·3; H, 3·95. $C_{22}H_{19}O_6Br$ requires C, 57·5; H, 4·2%); n.m.r. signals at τ 1·90 (1H) and 2·34 (1H) (doublets, J = 9 c./sec.), 2·10 (1H) and 3·82 (1H) (doublets, J = 10 c./sec.), 5·44 (1H, q; J = 7 c./sec.), 6·07 (3H, s), 8·41 (3H, s), 8·62 (3H, d; J = 7 c./sec.), and 8·66 (3H, s); $[\alpha]_D$ 0°.

Nieshoutol p-bromobenzenesulphonate (82)

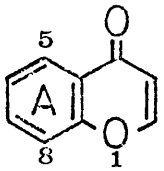
Nieshoutol (140 mg.) was heated at 80° for 16 hr. with p-bromobenzenesulphonyl chloride (145 mg.), m.p. 75°, in anhydrous pyridine (15 ml.). Isolated from the reaction mixture as before, nieshoutol p-bromobenzene-

sulphonate was purified by preparative t.l.c. in chloroform and crystallised from chloroform-light petroleum as colourless needles (115 mg.), m.p. 175-176° (Found: C, 51·0; H, 4·3. $C_{21}H_{19}O_7SBr$ requires C, 50·9; H, 3·9%); $\nu_{max.}^{nujol}$ 1721 (lactone carbonyl), 1624 and 1573 (coumarin skeletal modes), 1604 (benzene skeletal mode), 1515 and 1138 [ν(S=O) region], 770 and 730 $cm.^{-1}$ [ν(C-Br) region]; n.m.r. signals at τ 2·14 (1H) and 3·86 (1H) (doublets, J = 10 c./sec.), multiplet centred on 2·24 (4H), 5·73 (1H, q; J = 7 c./sec.), 6·05 (3H, s), 8·50 (3H, s), 8·79 (3H, d; J = 7 c./sec.), and 8·80 (3H, s); $[\alpha]_D$ 0°.

Nieshoutol O-(dimethylthiocarbamate) (73)

A suspension of nieshoutol (52) (276 mg.) and sodium hydride [obtained from a 50% dispersion (50 mg.) washed with anhydrous petroleum ether (b.p. 80-100°)] in freshly distilled dimethylformamide (10 ml.) showed complete hydrogen evolution within 1 hr. Dimethylthiocarbamoyl chloride (Me_2NCSCl) (160 mg.) was added and the solution heated at 100° for 50 min. The reaction mixture, on cooling, was treated with aqueous potassium hydroxide

(1%; 10 ml.) and, after a short delay, extracted with ether, the organic layer being washed with aqueous potassium hydroxide (1%), water to neutrality and dried over magnesium sulphate. Removal of solvent furnished the O-aryldimethylthiocarbamate (73), which crystallised from methanol as plates (140 mg.), m.p. 178-180°, (Found: C, 59·75; H, 5·75; N, 4·2. $C_{18}H_{21}O_5NS$ requires C, 59·5; H, 5·8; N, 3·9%). Ether extraction of the acidified alkaline aqueous layer yielded mainly unreacted nieshoutol (150 mg.). Pyrolysis of the O-aryldimethylthiocarbamate (73) *in vacuo* at 250° for 30 min. gave a brown resin; only a trace of yellow distillate was observed.



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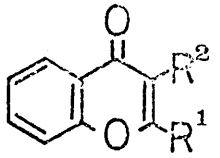

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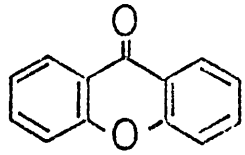

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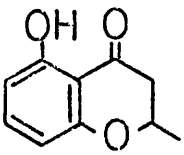

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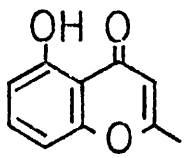

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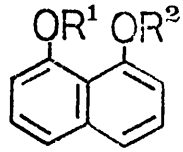

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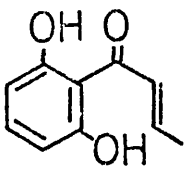

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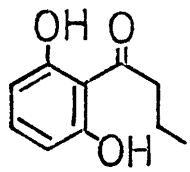

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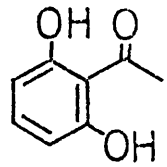

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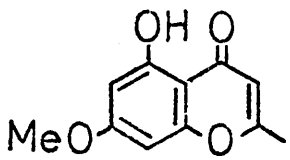

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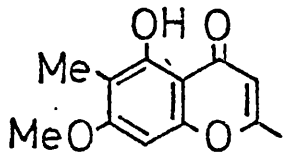

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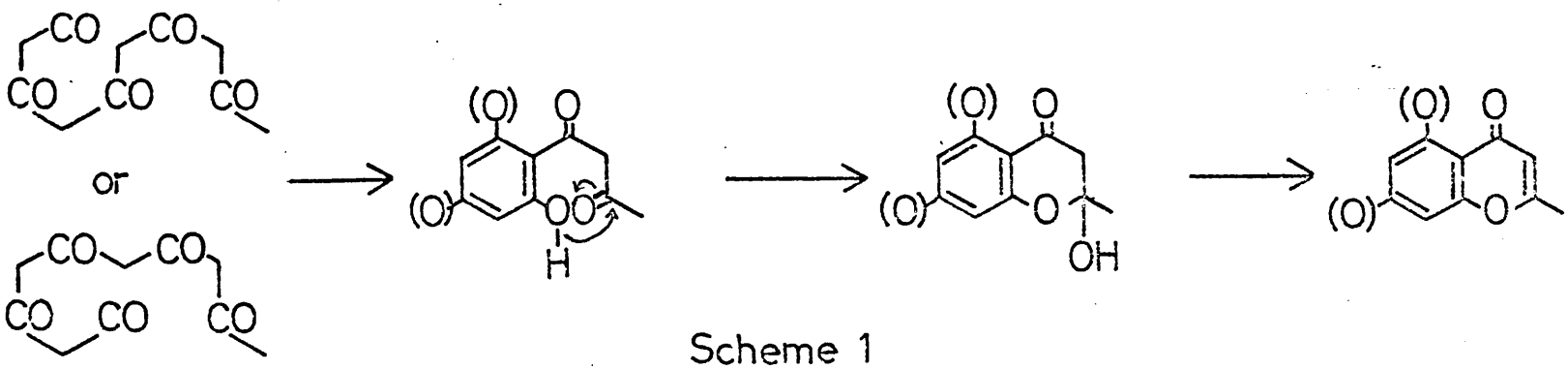
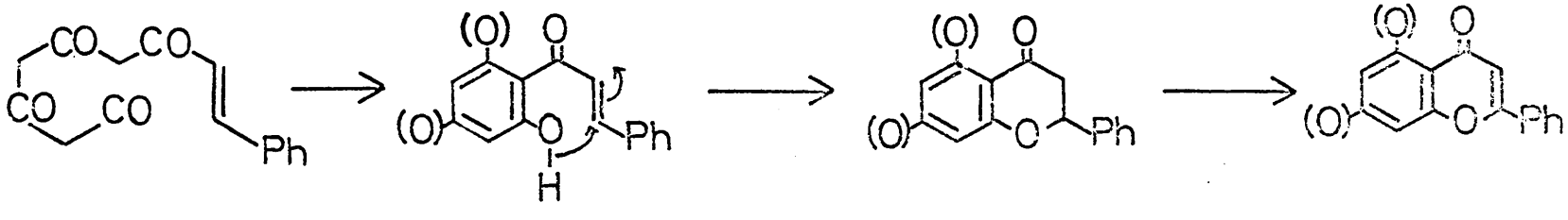
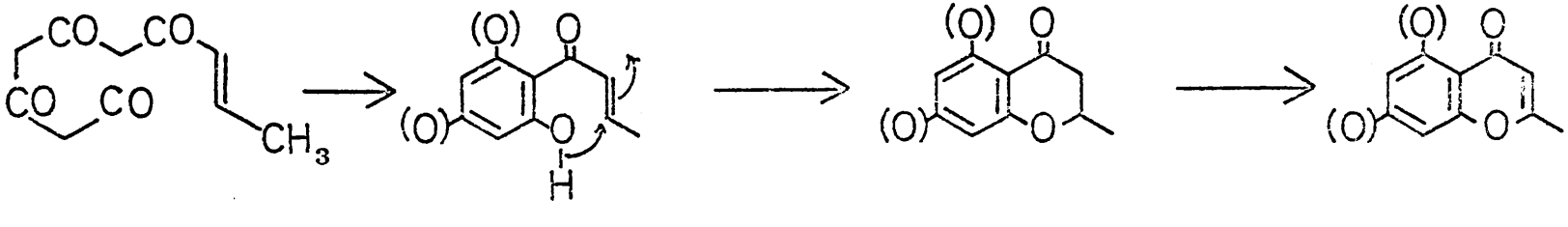


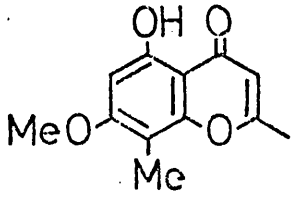
Scheme 1



Scheme 2



Scheme 3



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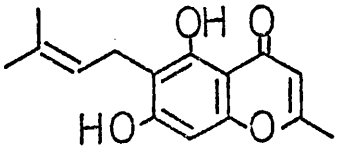

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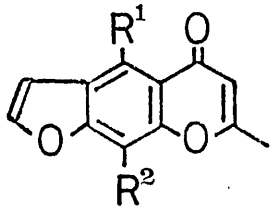
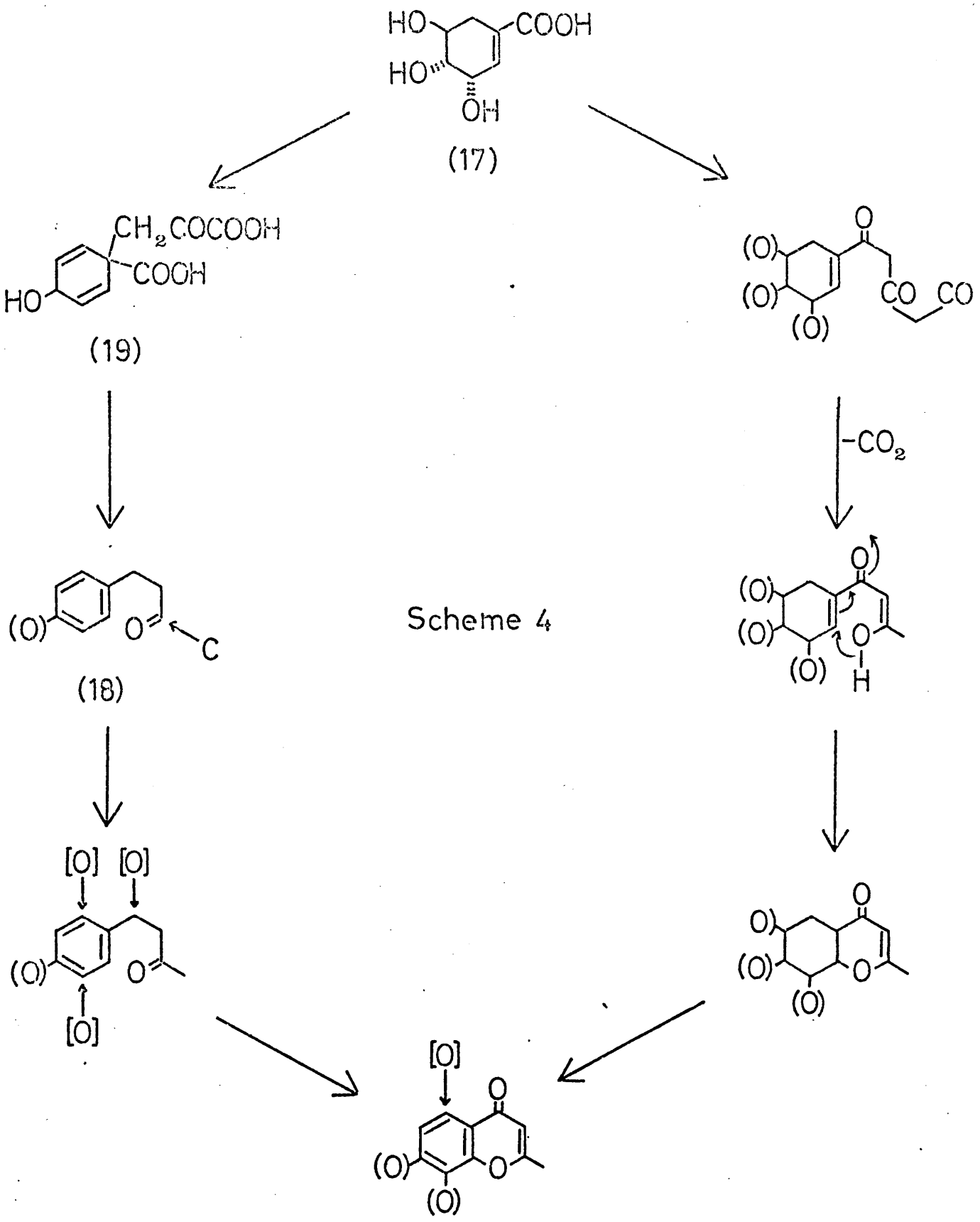
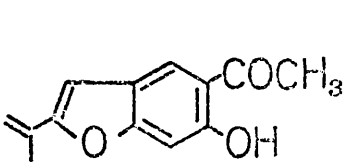


(15) R^1=OMe, R^2=H

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


HO
COOH
HO
OH
(17)
CH2COCOOH
COOH
HO
(19)
(O)
(O)
(O)
CO
CO
-CO2
(O)
O
C
(18)
Scheme 4
(O)
(O)
(O)
O
O
H
[O]
[O]
(O)
O
[O]
(O)
(O)
(O)
O
(O)
(O)
(O)
O
[O]
(O)
O
(O)



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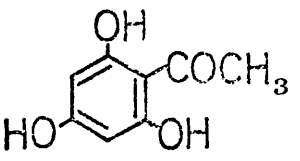

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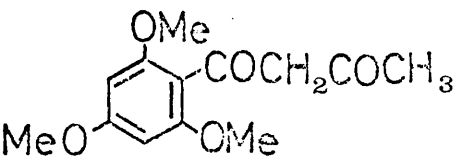

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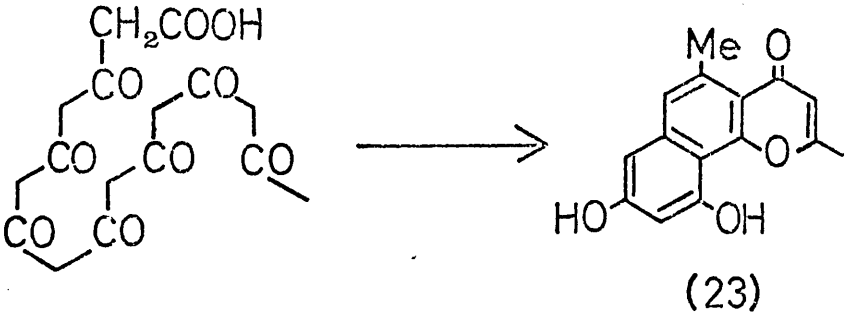

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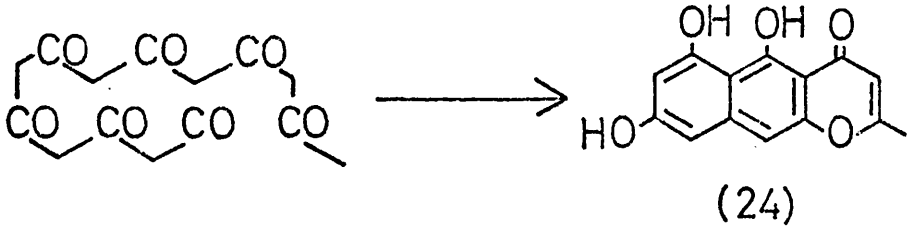
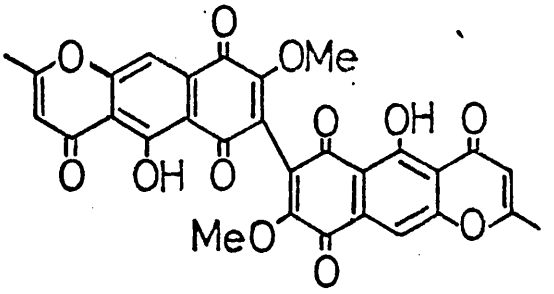


(24)



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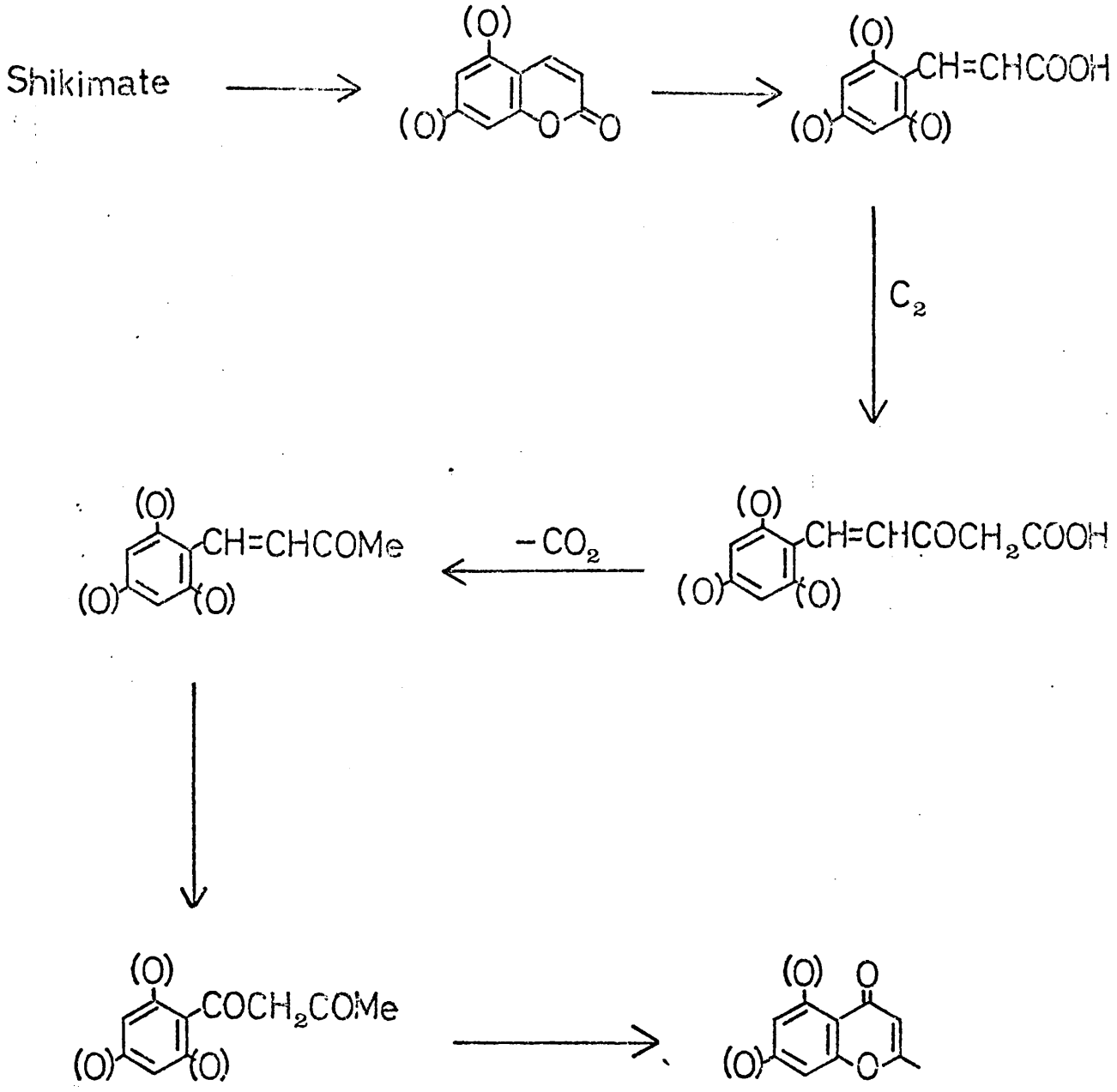
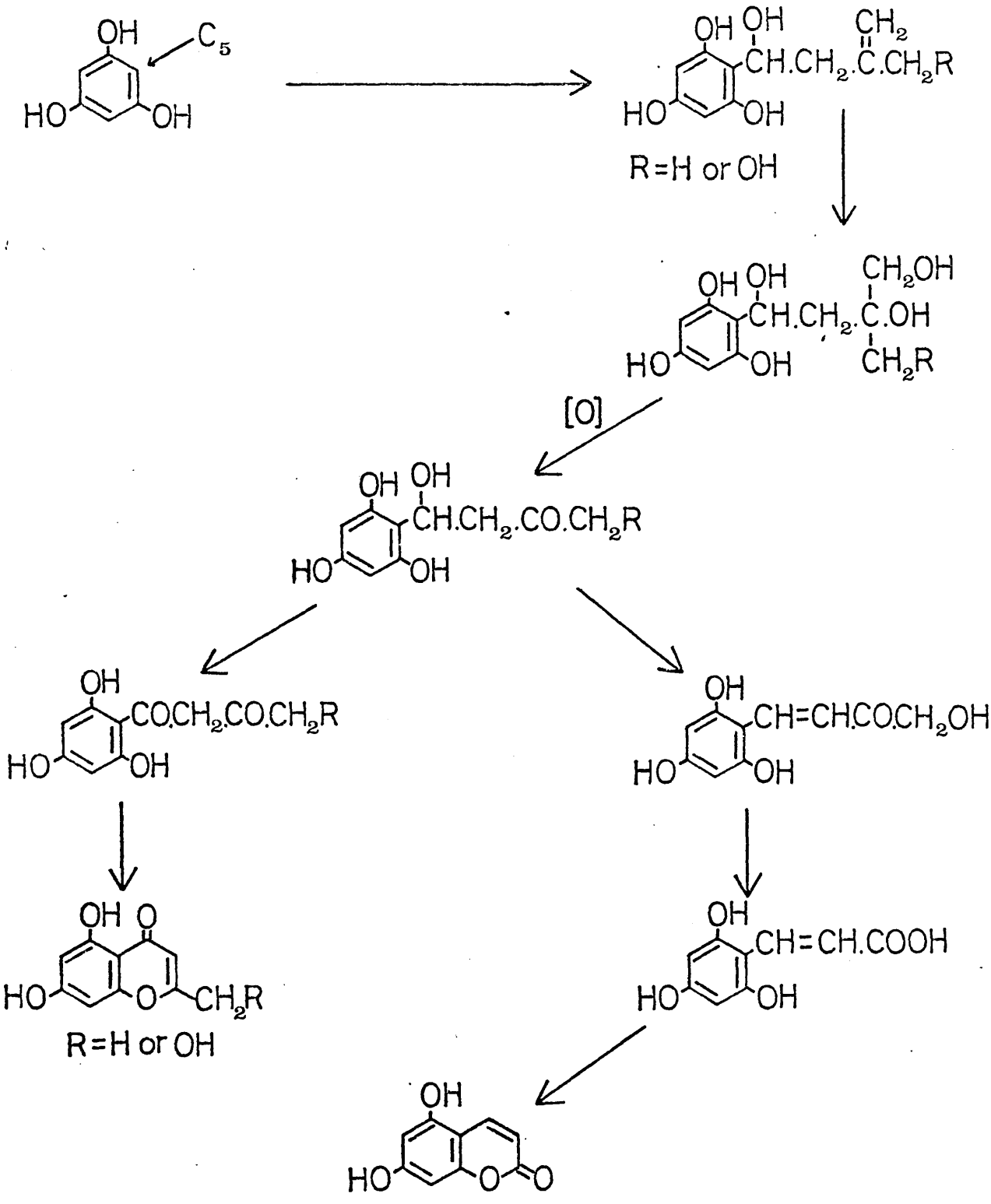
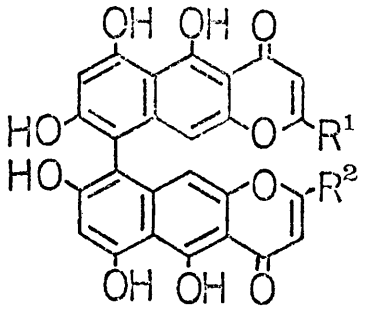

Shikimate
(O)
(O)
O
O
(O)
CH=CHCOOH
(O)
(O)
C2
(O)
CH=CHCOCH2COOH
(O)
(O)
−CO2
(O)
CH=CHCOMe
(O)
(O)
(O)
COCH2COMe
(O)
(O)
(O)
O
(O)
O

Scheme 5


OH
C5
HO
OH
OH OH
CH2
CH.CH2.C.CH2R
HO
OH
R=H or OH
CH2OH
CH.CH2.C.OH
CH2R
[O]
CH.CH2.CO.CH2R
CO.CH2.CO.CH2R
CH=CH.CO.CH2OH
O
CH2R
R=H or OH
CH=CH.COOH

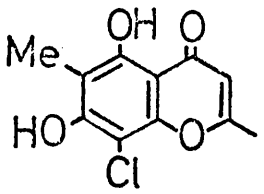
Scheme 6



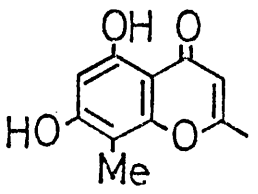
(26) A: $R^1=R^2=Me$

(27) B: $R^1=CH_2OH$, $R^2=Me$

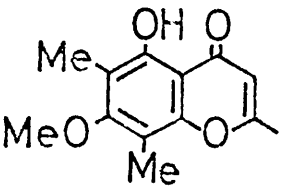
(28) C: $R^1=R^2=CH_2OH$



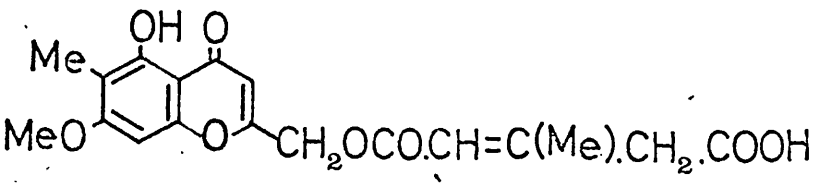
(29)



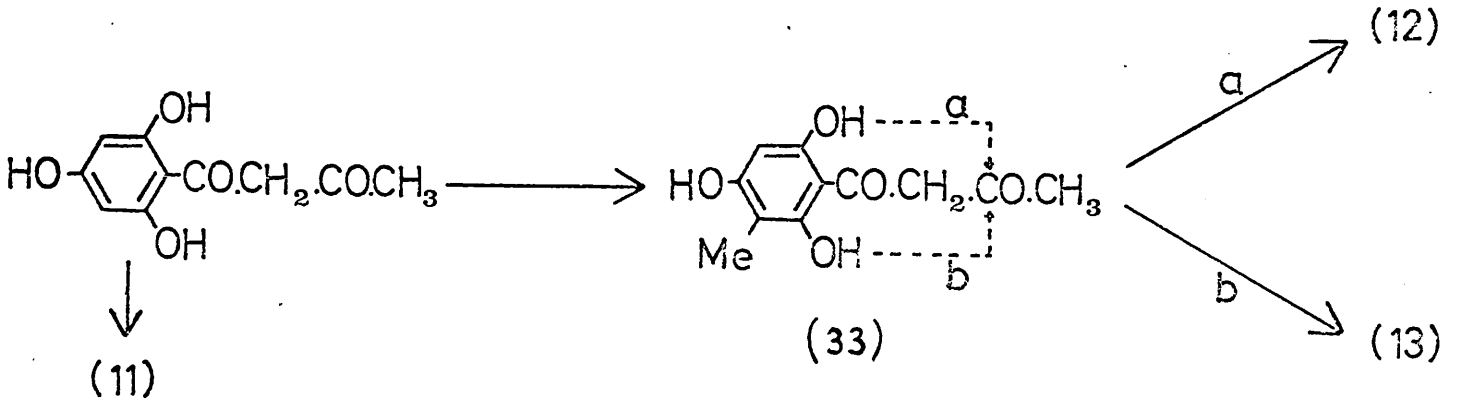
(30)



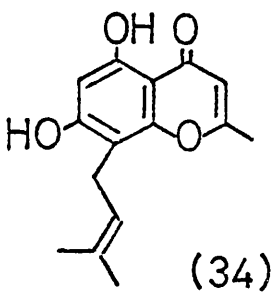
(31)



(32)



Scheme 7



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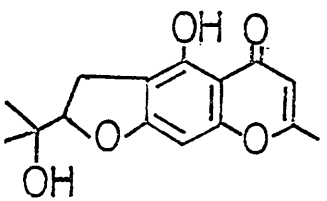

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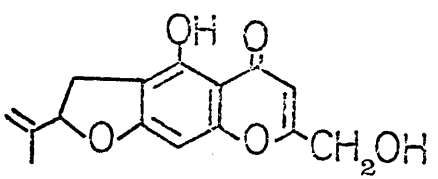
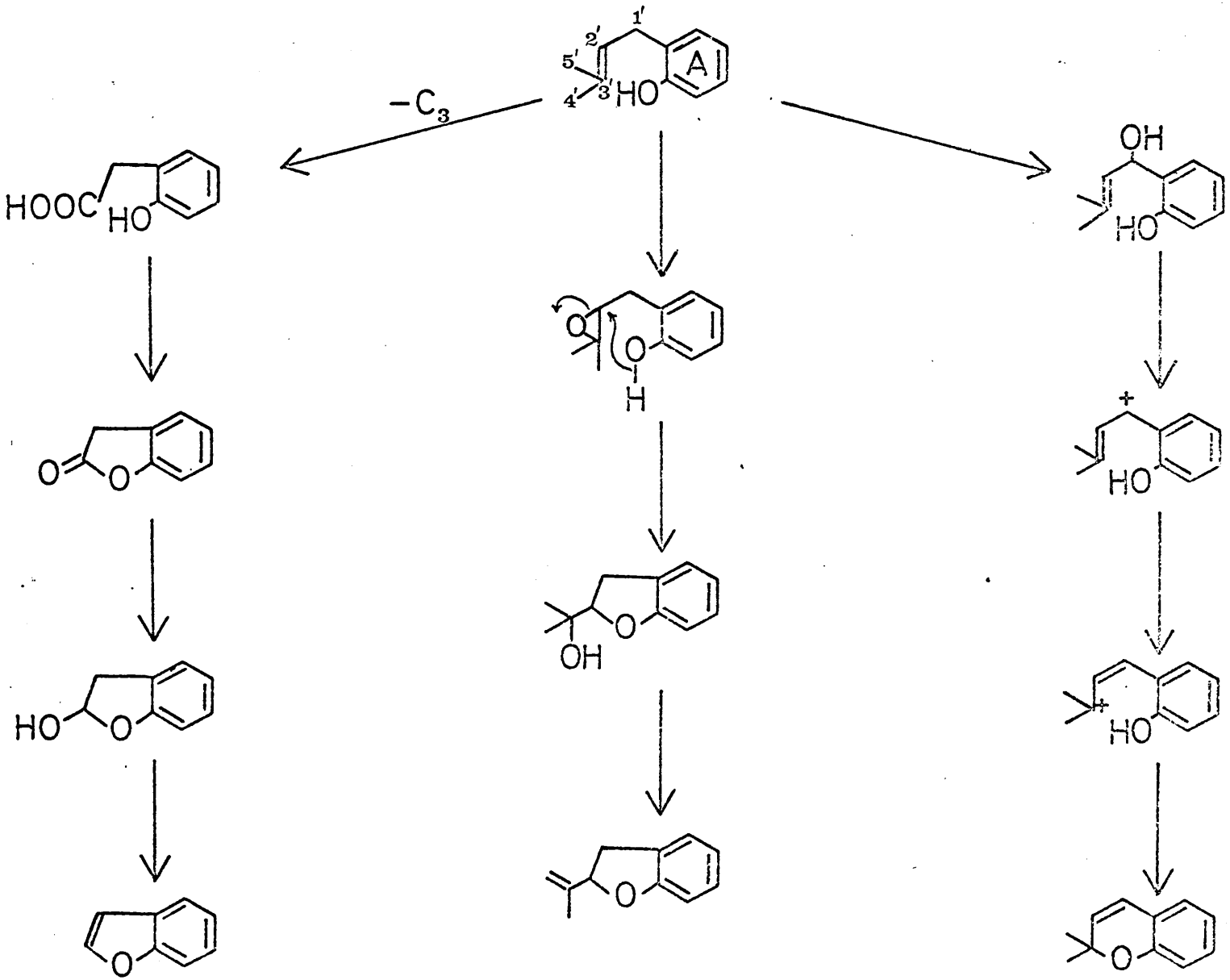
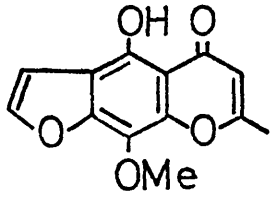


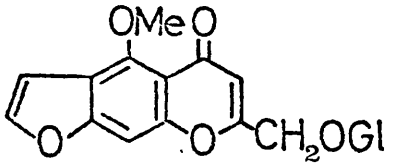
(36)



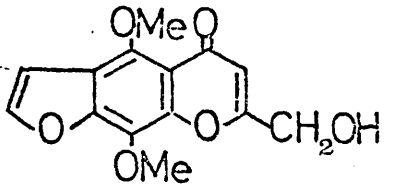
Scheme 8



(37)



(38)



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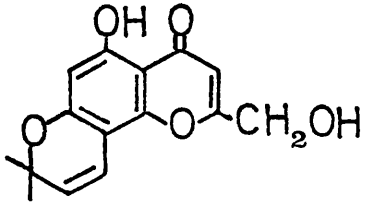

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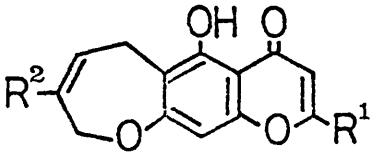
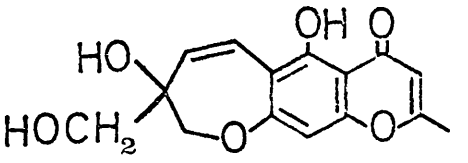


(41) $R^1 = R^2 = Me$

(42) $R^1 = CH_2OH$, $R^2 = Me$

(43) $R^1 = Me$, $R^2 = CH_2OH$



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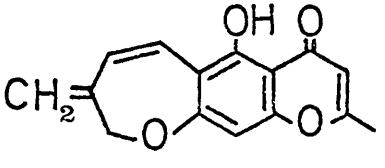

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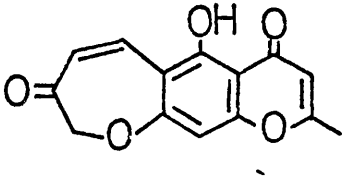
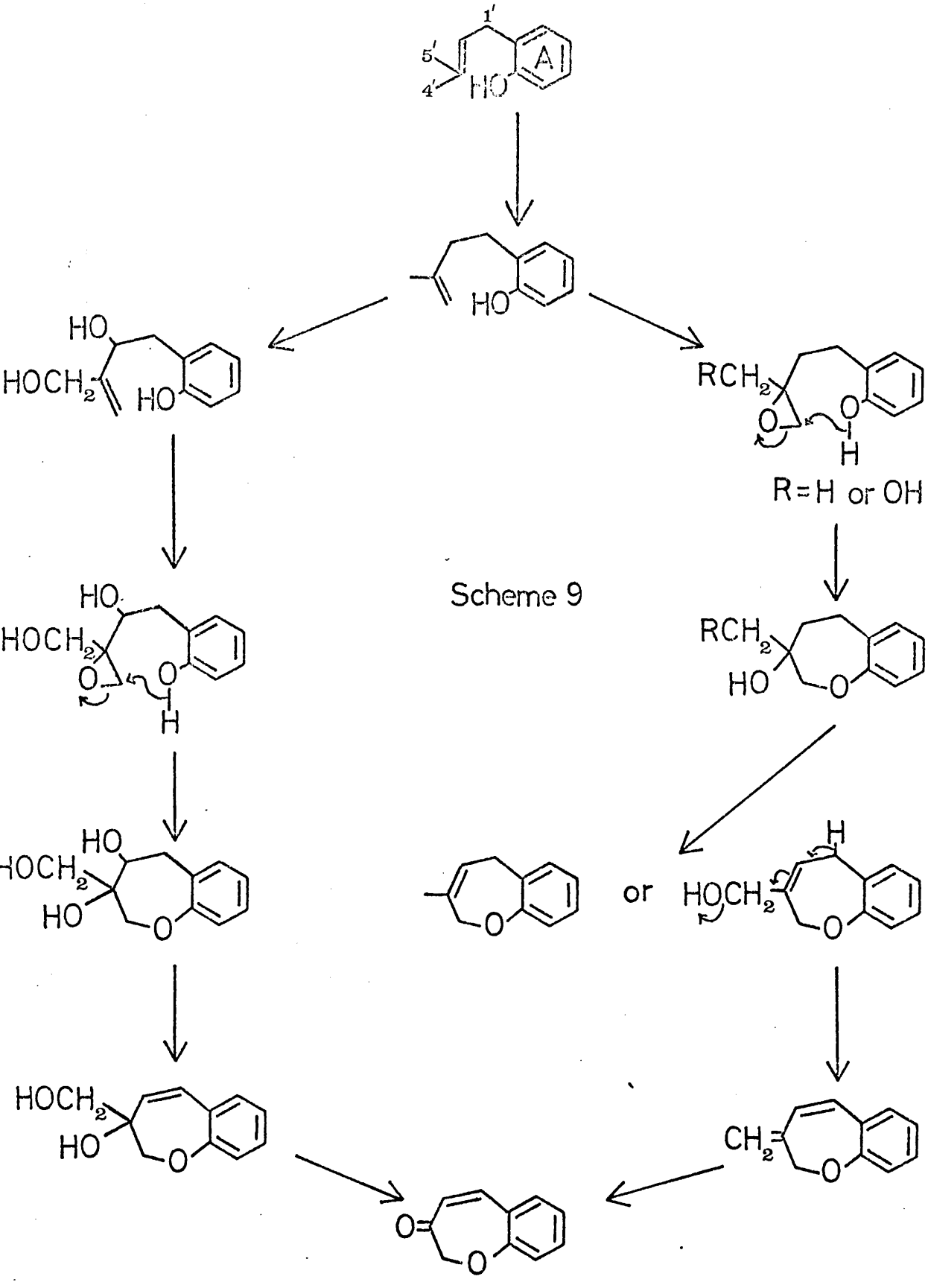
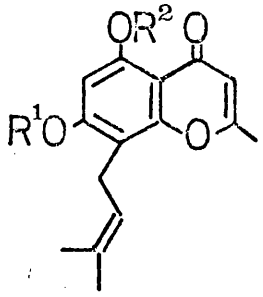


(46)

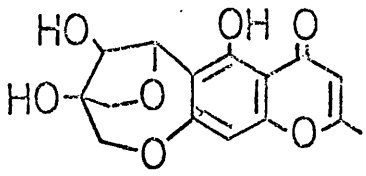

1'
5'
4'
A
HO
HO
HOCH2
HO
RCH2
O
H
R=H or OH
HO
HOCH2
O
O
H
RCH2
HO
O
HO
HOCH2
HO
O
O
or
H
HOCH2
HOCH2
HO
O
CH2
O
O

Scheme 9

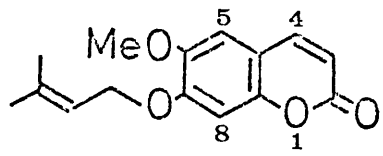


(47) R^1=Me, R^2=H

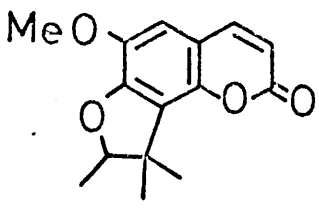
(48) R^1=R^2=Me



(49)



(50)



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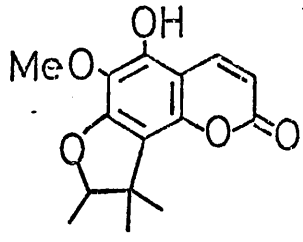
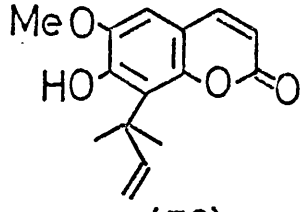
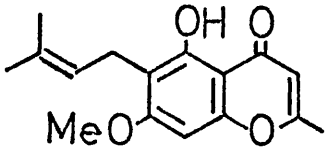


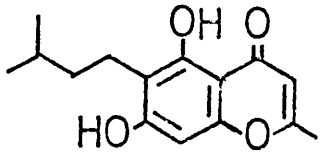
(52)



(53)



(54)



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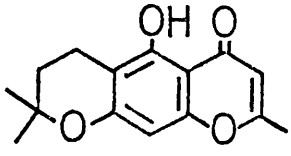

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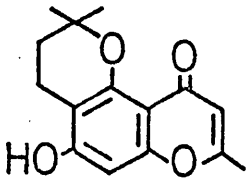
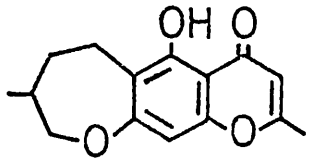


(57)



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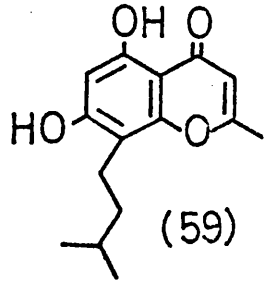

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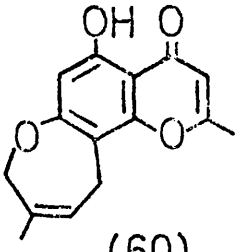

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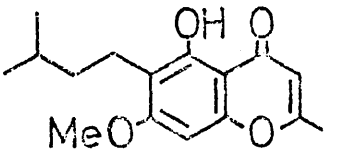
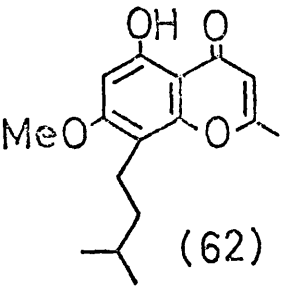


(61)



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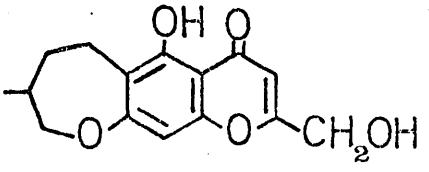

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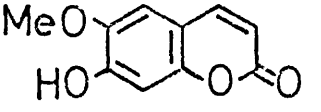
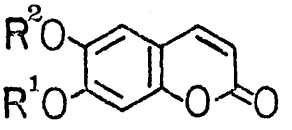


(64)



(65) $R^1 = R^2 = H$

(66) $R^1 = H$, $R^2 = \beta$-glucose

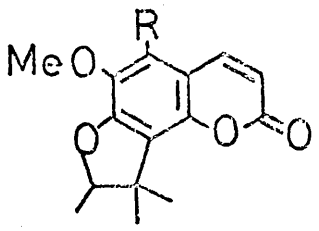
(67) $R^1 = SO_2.C_6H_4.CH_3\text{-}\underline{p}$, $R^2 = H$

(68) $R^1 = CH_2.CH{:}CMe_2$, $R^2 = H$

(69) $R^1 = R^2 = CH_2.CH{:}CMe_2$

(70) $R^1 = Me$, $R^2 = H$

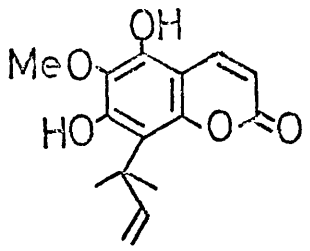
(71) $R^1 = Me$, $R^2 = CH_2.CH{:}CMe_2$



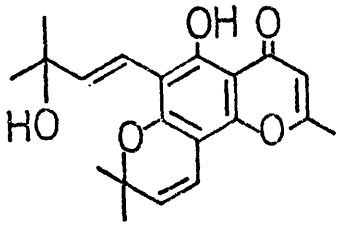
(72) R = S.CO.NMe_2

(73) R = O.CS.NMe_2

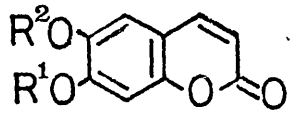
(74) R = SH



(75)

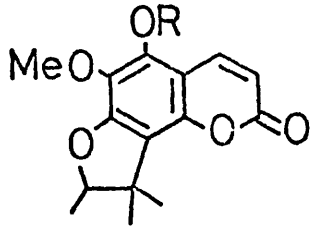


(76)



(77) R^1 = $SO_2.C_6H_4.CH_3$-p, R^2 = Me

(78) R^1 = Me, R^2 = β-glucose



(79) R = Me

(80) R = $CO.CH_2Br$

(81) R = $CO.C_6H_4Br$-p

(82) R = $SO_2.C_6H_4Br$-p

PART 2.

An Infrared Study of Chromones.

During the structural elucidation[23] of the chromones previously described, quantitative solution infrared spectra were systematically recorded. It was not, at first glance, apparent how the small changes in these complex spectra could be related to molecular architecture. In general, more than one band with extinction coefficient greater than 500 was present in the 1600-1700 $cm.^{-1}$ region and thus the assignment of the carbonyl frequency was unclear. Conversely, from the simpler nature of the hydroxyl stretching region, the differing hydroxyl absorptions could be easily recognised. The i.r. spectra of several structurally simpler chromones were recorded for reference but, with the exception of chromone and 2-methylchromone, these spectra showed the same anomalies as those of the more complex compounds. The effects of solvent polarity, temperature, and progressive structural changes on the spectral pattern of the carbonyl region were investigated.

Infrared of systems related to chromones

Literature Survey

There is very little information in the literature regarding the i.r. spectra of chromones. However, a number of closely related systems have been investigated: acetophenones, flavanones, chalcones, flavones, isoflavanones, isoflavones, and simple 4-pyrones. Moreover due to solubility problems, solution frequencies and quantitative data have not often been recorded; most frequently, only one peak, the most intense, is quoted and assumed to be the carbonyl stretching frequency.

Hergert and Kurth[63] found that for acetophenones (Table 3), a hydroxyl group ortho to the ketone lowered the carbonyl stretching frequency by ~ 50 cm.$^{-1}$ relative to the unsubstituted parent compound and an ortho methoxyl had a lowering effect of 40 cm.$^{-1}$ while a similarly placed acetoxy group depressed the carbonyl frequency by only 10 cm.$^{-1}$. This sequence was explained by the participation of resonance forms like (83) and (84) in the ground state of the oxygenated acetophenones. Their carbonyl, by partial loss of double bond character in this fashion should thus absorb at a lower frequency than that of the

parent. Moreover, the greater electron releasing power of the hydroxyl group is responsible for the lower carbonyl frequency in the 2-hydroxy derivative, while the 2-acetoxy group, because of its meagre ability to release electrons, precludes the possibility of such resonance structures and causes o-acetoxyacetophenone to absorb at approximately the same frequency as acetophenone itself. Chelation through hydrogen bonding between the 2-hydroxyl and the carbonyl group does not alone explain the observations, since hydrogen-bonding cannot occur for the 2-methoxy compound yet a lowering of the carbonyl frequency is observed. For 2-hydroxyacetophenone, no obvious hydroxyl stretching band appears in the region 2500-3500 $cm.^{-1}$; this was taken to indicate a large downward shift and broadening of the hydroxyl frequency due to chelation, an effect already observed for the peri-hydroxynaphtha- and anthraquinones.[64]

Resonance structure (85) was taken to account for the carbonyl shift to 1635 $cm.^{-1}$ of 4-hydroxyacetophenone, the methyl ether and acetate of which absorb at 1657 and 1685 $cm.^{-1}$ respectively. In 2,4-dihydroxyacetophenone, the separate effects of the oxygen substituents reinforce

to give a carbonyl stretching frequency of 1620 cm.$^{-1}$; the 2,4-dimethoxy compound absorbs at 1643 cm.$^{-1}$ and the diacetate at 1683 cm.$^{-1}$, close to the parent absorption. In contrast, a meta electron releasing substituent, which cannot conjugate with the carbonyl group, has little effect on the carbonyl frequency; thus, 3-methoxyacetophenone absorbs at 1681 cm.$^{-1}$.

Several independent studies on flavanones have been reported;[63,65-67] all authors are agreed that flavanones (Table 4) behave in a parallel fashion to acetophenones. Hergert and Kurth noted that, since the carbonyl stretching frequency (1680 cm.$^{-1}$) of the parent, flavanone, is only slightly lower than that of acetophenone [1687 (liq.)[63] or 1695 (CCl_4)[65]], the hetero-oxygen atom cannot have the same influence on the carbonyl group as a 2-methoxyl and resonance structures such as (86) are therefore of minor importance. 5-Hydroxyflavanones absorb (KBr) in the range 1645-1629 cm.$^{-1}$ (35-51 cm.$^{-1}$ lower than unsubstituted flavanone) and 7-hydroxyflavanones absorb around 1647 cm.$^{-1}$. It was deduced[63,65,66] that these downward shifts indicate an important contribution to the resonance hybrid of canonical forms like (87) and

(88). A 7-methoxyl group causes a smaller carbonyl shift [from 1695 to 1685 cm.$^{-1}$ (CCl_4)[65]] and 5,7-dihydroxyflavanone [1629 cm.$^{-1}$ (CCl_4);[65] 1620 cm.$^{-1}$ (KBr)[63]] shows almost additive effects of the 5- and 7-hydroxyls separately. 5,7-Dihydroxyflavanone has the same carbonyl frequency (1620 cm.$^{-1}$) as 2,4-dihydroxyacetophenone. Chalcones, derivatives of phenyl styryl ketone (89), show similar behaviour.[63]

The i.r. spectra of flavones (Table 5) differ markedly[63,65-72] from the simple spectral patterns of the flavanones, anomalous frequency shifts on substitution being more difficult to interpret. Although a hydroxyl on position 5 of the flavone nucleus (90) chelates strongly with the carbonyl group, this has apparently very little effect on the carbonyl frequency; the wavenumber lowering of 5-hydroxyflavones is not greater than 5 cm.$^{-1}$ and in many cases an increase rather than a decrease is observed. Thus, flavone and 5-hydroxyflavone absorb (CCl_4)[65] at 1649 and 1652 cm.$^{-1}$ respectively and other 5-hydroxyflavones[65] absorb (CCl_4) in the region 1645-1659 cm.$^{-1}$ with a median of 1654 cm.$^{-1}$. It would appear that the solid state carbonyl frequencies have similar values;

the carbonyl bands of eighteen 5-hydroxyflavones in potassium bromide[66] fall in the range 1641-1664 cm.$^{-1}$ with a median of 1654 cm.$^{-1}$. In contrast to the increase of ~ 15 cm.$^{-1}$ in the carbonyl frequency in passing from a 2-hydroxy- to a 2-methoxyacetophenone, 5-methoxyflavones absorb ~ 25 cm.$^{-1}$ lower (i.e. around 1630 cm.$^{-1}$) than the 5-hydroxyflavones.[63,66] Moreover, acetylation of 5-hydroxyflavones decreases (by ~ 15 cm.$^{-1}$) rather than increases the stretching frequency; thus 5-acetoxyflavones absorb around 1640 cm.$^{-1}$. Introduction of a hydroxyl group at position 7 of the flavone nucleus[65,66,68] causes a decrease in carbonyl frequency of ~ 20 cm.$^{-1}$. Methylation and acetylation of a 7-hydroxyl raises the carbonyl frequency by ~ 15-25 cm.$^{-1}$ and by ~ 10 cm.$^{-1}$ respectively. Isoflavones show similar characteristics.[74-77]

Briggs and Colebrook[66] explain the discordant flavone behaviour in terms of the statistical weights which canonical forms contribute to the ground state of the molecule under the stabilising or destabilising influence of the oxygen substituents. It is suggested that flavone (90) has a polar character because of contribution of the canonical form (91). Dipole measurements on 4-pyrone

(92)[73] and the formation of flavylium salts support this postulate. Flavanone, by the absence of the 2,3-double bond, has no canonical structure parallel to (91). Of the two canonical forms (94) and (95) of 5-hydroxyflavone (96), the pyrilium form (94) makes a greater contribution to the ground state than the conjugate-chelate form (95); the strong hydrogen bond between the 5-hydroxyl and the negatively charged carbonyl oxygen atom stabilises the pyrilium form (94) at the expense of (95), in which disturbance of the benzenoid resonance of ring A lowers the resonance energy. Similarly, a greater contribution to 5-acetoxyflavone (97, R = Ac) is made by (98, R = Ac) than by (99, R = Ac); the presence of an electron withdrawing group (CH_3CO-) must reduce the ability of the 5-oxygen atom to donate electrons to the flavone nucleus, the polar form (99, R = Ac) thus being disfavoured. Conversely, for 5-methoxyflavone (97, R = Me), the major polar contributor is considered to be (99, R = Me) since, in absence of the strong hydrogen bond between the 5-oxygen atom and the carbonyl group, there is no marked preference for the pyrilium form (98, R = Me). Thus, since (94) and (96), the main canonical forms of 5-

hydroxyflavone are substantially the same as the canonical structures (91) and (90) of unsubstituted flavone, the close similarity of the carbonyl stretching frequencies is explained. The same reasoning applies for the 5-acetoxy compound. In contrast, since the major contributing forms (97 and 99, R = Me) of 5-methoxyflavone represent a hybrid essentially different from the flavone hybrid, a different carbonyl frequency would be expected. It does not seem obvious, however, that this difference should be a <u>decrease</u> in frequency for the 5-methoxy derivative. The contribution of resonance structures like (100) to the ground state of 7-oxygenated flavones is responsible[65,66] for the observed lowering of frequency; the consistently lower carbonyl bands of 7-hydroxyflavones suggests that the contribution of (100, R = H) is greater than that of the corresponding form (100, R = Me) of the methoxy compound.

Lebreton and Chopin[71] treat the apparent anomalies in the flavone series from a rather different standpoint. They consider that the carbonyl group is acted on by diverse influences which are competitive but not totally independent; the combination of these influences is

complex but can be rationalised on an additive basis in conjunction with certain selection rules. The parent absorption is taken as the carbonyl frequency of flavanone, 1685 $cm.^{-1}$ (KBr); this value differs from the stretching frequency of saturated ketones (~ 1715 $cm.^{-1}$) through the conjugation of the flavanone carbonyl with a phenyl substituent (ring A). The various influences and their characteristic lowering effects on this frequency can be tabulated as follows:

	lowering of frequency
hydroxylation at 5:	35 (± 5) $cm.^{-1}$;
" " 7:	30 (± 5) $cm.^{-1}$;
" " 3:	30 (± 5) $cm.^{-1}$;
methoxylation at 7:	15 (± 5) $cm.^{-1}$;
double bond 2,3 :	30 (± 5) $cm.^{-1}$.

These frequency lowerings are governed by the following empirical rules of selection or exclusion:

(a) Hydroxylation at position 5, which confers great stability through formation of a hydrogen-bonded, six-membered chelate ring, excludes all other influences; thus flavone absorbs at 1649 $cm.^{-1}$ [1685 - 30 (± 5)] and 5-hydroxyflavone has a carbonyl frequency of 1652 $cm.^{-1}$

[1685 - 35 (± 5)] which shows that, in the presence of a 5-hydroxyl, the 2,3-double bond has no influence.

(b) Hydroxylation at position 7 excludes the influence of hydroxylation at position 3, which in turn excludes the effect of methoxylation at position 7.

(c) The 2,3-double bond adds its effect to those of substituents (other than 5-hydroxyl) in ring A or in the pyrone ring.

(d) The 2,3-double bond annuls the influence of ring B substituents.

Lebreton and Chopin appear to have omitted the lowering effect, on the parent absorption, of methylation at position 5 of the flavone nucleus. As quoted previously, 5-methoxyflavones absorb around 1630 $cm.^{-1}$; this would suggest that the 5-methoxyl group exerts a lowering influence of 25 $cm.^{-1}$ in addition to the 30 $cm.^{-1}$ lowering of the 2,3-double bond. Thus the following entry can be added to the table:

methoxylation at 5:	25 (± 5) $cm.^{-1}$.

4-Pyrones possess several peaks of high intensity in the 1600-1700 cm.$^{-1}$ region; this complicating feature has led some authors[78-80] to make uncertain assignments. Katritzky and Jones,[81] using the solvent shift technique, have attributed bands at 1634 and 1660 cm.$^{-1}$, in the spectrum ($CHCl_3$) of 4-pyrone,(92), to ring stretching modes. Of the two remaining bands cited, 1674 and 1613 cm.$^{-1}$, the latter appears to be favoured for the carbonyl stretching frequency. More recently, it has been demonstrated[82,83] by isotopic substitution that the carbonyl stretching absorption of 4-pyrone is the doublet at 1674 (ε 980) and 1661 cm.$^{-1}$ (1210). The inference of these latter results is that conclusions based only on solvent shift data in such systems must be treated with great reserve.

To summarise, many publications on the infrared characteristics of flavonoid compounds only record the observed data and, as Briggs and Colebrook[66] have pointed out, do not attempt to explain anomalous behaviour. The tabulation of Lebreton and Chopin,[71] although useful, makes the dangerous assumption that flavone and flavanone are closely related. However, these must represent,

chemically and spectroscopically, two entirely different systems because of the pseudo-aromatic character conferred on the former by the presence of the 2,3-double bond, apparently a simple difference on paper. It is notable that, even in the most recent investigations[40b,68,84] only one value is quoted for the carbonyl stretching frequency of flavonoids. Low resolution and solid-state spectra of chromones possess one or two broad absorptions in the carbonyl region but careful solution measurements suffice to show that this region is extremely complex. This may also apply to flavonoids.

Table 3.

Carbonyl absorption of acetophenone derivatives

	Frequency ($cm.^{-1}$)	
	nujol	liquid
Acetophenone	1687	-
2-hydroxy-	-	1635
2-methoxy-	-	1649
2-acetoxy-	1762, 1678	-
4-hydroxy-	1638	-
4-methoxy-	-	1657
4-acetoxy-	1763, 1685	-
2,4-dihydroxy-	1620	-
2,4-dimethoxy-	1643	-
2,4-diacetoxy-	1764, 1683	-

Table 4.

Carbonyl absorption of flavanone derivatives

	Frequency ($cm.^{-1}$)	
	KBr	CCl_4
Flavanone	1680	1695
5-hydroxy-	-	1648
7-hydroxy-	1647	-
7-methoxy-	-	1685
5,7-dihydroxy-	1620	1629
7,4'-dihydroxy-	1656	-
7,4'-dimethoxy-	-	1686
5,7,4'-trihydroxy-	1631	-

Table 5

Carbonyl absorption of flavone derivatives

	Frequency ($cm.^{-1}$)	
	nujol	CCl_4
Flavone	1646	1652[a] or 1649[b]
5-hydroxy-	1651	1652
5-methoxy-	1649	1653
5-acetoxy-	1756, 1648	1774, 1655
7-hydroxy-	1626	-
7-methoxy-	1653	1650[a] or 1640[b]
7-acetoxy-	1759, 1638	1774, 1658
5,7-dihydroxy-	1648	1650
5-hydroxy-7-methoxy-	1672(KBr)	1656
5,7-dimethoxy-	1646	-
5,7-diacetoxy-	1640	1656

a Value quoted by Looker and Hanneman.[68]

b Value quoted by Shaw and Simpson.[65]

Infrared of Chromones

Results

(i) Carbonyl region.

Chromones exhibit spectral characteristics similar to the flavones but frequency shifts accompanying structural changes are not so large. However, as for the monocyclic 4-pyrones, the chromone spectra also possess more than one intense band in the carbonyl region and it is not obvious which or how many of these originate from carbonyl stretching vibrations. This problem is considered in detail later; for the present, the highest frequency band (usually the most intense) is taken to represent, at least in part, the carbonyl stretching frequency. The absorption frequencies, apparent extinction coefficients (l.mole^{-1} cm.$^{-1}$), and half-band widths for the chromone spectra are presented in Table 6.

Chromone (1) and 2-methylchromone (101), possessing no oxygen substituents, show a broad, intense carbonyl band around 1650 cm.$^{-1}$ in chloroform (cf. 1650 cm.$^{-1}$ for flavone), which undergoes a hypsochromic shift of 15 cm.$^{-1}$ when the spectrum is recorded in carbon tetrachloride (Figs. 2 and 3). No other major absorptions appear in the 1600-1700 cm.$^{-1}$ region.

A change in the contour of the spectrum accompanies hydroxylation of the 2-methylchromone nucleus at position 5 (6); the major band shifts to 1661 cm.$^{-1}$ (CCl_4) and sharpens considerably but two further intense peaks appear at 1625 and 1606 cm.$^{-1}$ (Fig. 4). The spectrum in chloroform is virtually identical. Methylation of the 5-hydroxyl (102) causes a further marked change (Fig. 5). The major peak at 1665 cm.$^{-1}$ (CCl_4) shows a large solvent effect [1658 cm.$^{-1}$ ($CHCl_3$)] and there is only one other significant maximum at 1607 cm.$^{-1}$.

Introduction of a 7-methoxyl group (103) into the 5-hydroxy-2-methylchromone nucleus makes only a little difference to the spectrum (Fig. 6); however, the peaks at 1663 and 1626 cm.$^{-1}$ (again barely affected by solvent) are broader and show partial resolution into doublets. It is notable that, for both 5-hydroxy-2-methylchromone (6) and 5-hydroxy-7-methoxy-2-methylchromone (103), the smallest peak [1606 and 1602 cm.$^{-1}$ (CCl_4)]respectively shows the largest solvent shift [1601 and 1590 cm.$^{-1}$ ($CHCl_3$)]; this is contrary to expectation for association of the polar solvent molecules around a polar (e.g. carbonyl) group and probably reflects the solvent effect shown by the 1613

cm.$^{-1}$ band of 4-pyrone.[81] A marked change in the spectrum of 5-hydroxy-7-methoxy-2-methylchromone is observed on methylation of the 5-hydroxyl group. The chloroform spectrum (Fig. 7) of 5,7-dimethoxy-2-methylchromone (104) has an appearance similar to that of 5-methoxy-2-methylchromone, two intense maxima existing at 1659 and 1610 cm.$^{-1}$ ($CHCl_3$). In carbon tetrachloride, the former moves to 1666 cm.$^{-1}$ and becomes more intense while the latter now appears at 1620 cm.$^{-1}$ diminished in intensity. There is unresolved absorption around 1627 cm.$^{-1}$ ($CHCl_3$) which may correspond to the 1644 cm.$^{-1}$ peak in the carbon tetrachloride spectrum (vide infra).

Various substituted and modified 5,7-dihydroxy-2-methylchromones occur naturally. In general, they exhibit spectral characteristics similar to the parent compound but minor variations with structure were noted. 5,7-Dihydroxychromones related to peucenin (14) and heteropeucenin (34) (Figs. 8-15) possess the major absorption band at 1660 ($\pm$ 2) cm.$^{-1}$ in chloroform showing an increase in frequency of 2-3 cm.$^{-1}$ on methylation of the 7-hydroxyl group. (This increase, although consistent, is close to experimental error and cannot be regarded as highly significant.)

The heteropeucenin series is slightly anomalous in that dihydroheteropeucenin (59) ($\nu_{max.}$ 1663 cm.$^{-1}$) shows a lowering of 2 cm.$^{-1}$ on methylation of the 7-hydroxyl (Figs. 14 and 15).

The spectral pattern of peucenin 5,7-dimethyl ether (105) is analogous to that of 5,7-dimethoxy-2-methyl-chromone, two almost equally intense absorptions appearing at 1656 and 1602 cm.$^{-1}$ ($CHCl_3$) and, in this case, a small maximum is resolved at 1627 cm.$^{-1}$ (Fig. 12). In carbon tetrachloride, the 1656 cm.$^{-1}$ peak shifts to 1663 cm.$^{-1}$ with an increase in intensity while the 1602 cm.$^{-1}$ absorption appears at ~1616 cm.$^{-1}$, considerably reduced in intensity. (The last value (1616 cm.$^{-1}$) must be regarded as tentative as a loss of energy occurred close to the absorption maximum.)

8-Alkylated chromones of the heteropeucenin (34) series differ from the 6-alkylated peucenin (14) series in that the absorption at ~1592 cm.$^{-1}$ is noticeably more intense than the neighbouring 1622 cm.$^{-1}$ peak for hetero-peucenins whereas it is less intense for the peucenins. From this variation in intensity it may be interpreted that the vibrational mode associated with the 1592 cm.$^{-1}$

absorption has aromatic ring stretching character, the change in dipole moment during vibrational transition being affected by the position of the alkyl substituent. This effect in the 8-position is also shown by a methoxy substituent; thus, 6-ethyl-5,7-dihydroxy-8-methoxy-2-methylchromone (106) (Fig. 16) possesses a substituent in both 6- and 8-positions but the profile of the spectrum more closely resembles that of a heteropeucenin rather than a peucenin derivative, the peak at 1597 $cm.^{-1}$ ($CHCl_3$) being of greater intensity than the 1629 $cm.^{-1}$ absorption. Only a minor contribution appears to be made to the chromophore by the extra 8-oxygen atom since the position of the peaks differ little from those of heteropeucenin 7-methyl ether (47) (Fig. 13). The derived 5-methyl ether, 6-ethyl-7-hydroxy-5,8-dimethoxy-2-methylchromone (107) shows a spectral contour (Fig. 17) close to that of 5,7-dimethoxy-2-methylchromone; the second major absorption, at 1596 $cm.^{-1}$, is however 15 $cm.^{-1}$ lower.

A number of naturally occurring chromones or their derivatives contain five-, six-, or seven-membered oxide rings fused to the chromone nucleus. Some regularity in their spectral features has been found.

Linear annellation of the 5-hydroxy-2-methylchromone system with a dihydrofuran ring, as in visamminol (35) and umtatin (36) (Figs. 18 and 19) gives a broad spectrum consisting of three well separated and approximately symmetrical peaks similar to the parent system. The most intense band at 1672 $cm.^{-1}$ ($CHCl_3$) is however 13 $cm.^{-1}$ higher than the major peak in the spectrum of 5-hydroxy-2-methylchromone and 9 $cm.^{-1}$ higher than that of 5-hydroxy-7-methoxy-2-methylchromone. Dihydrokhellin (108), similarly annellated but containing methoxyl groups in the 5- and 8-positions does not show a spectrum of this type but rather possesses the spectral characteristics of a 5-methoxychromone (Fig. 21). Since it was previously demonstrated that an 8-methoxyl group makes only a minor contribution to the spectrum, it seems likely that the presence of a 5-methoxyl destroys the annellation influence of the dihydrofuran ring.

The spectrum of the 5-hydroxyfuranochromone, khellinol (37) (Fig. 22) is similar to that of 5-hydroxy-7-methoxy-2-methylchromone (103) (Fig. 6) in that the main absorption is 1661 $cm.^{-1}$ but a strong peak at 1592 $cm.^{-1}$, possibly due to extension of the chromophore with

respect to this reference compound, is present in addition to that at 1604 cm.$^{-1}$, which is now visible only as an inflection. Only a small solvent shift is observed in all peaks. The 5-methyl ether, khellin (16) (Fig. 23), shows a spectrum markedly different in contour, the dominating influence being the 5-methoxyl group as expected. Isovisnagin (109) (Fig. 20), containing an angularly fused furan ring, shows a considerably lower absorption frequency (1648 cm.$^{-1}$) that the linearly annellated khellin, the spectral contour resembling that of a 5-methoxychromone.

Six-membered rings appear to have no significant annellation effect. Thus, isopeucenin (56) (Fig. 24) absorbs at 1659 cm.$^{-1}$ ($CHCl_3$), the spectral pattern being similar to peucenin 7-methyl ether (54) (Fig. 9), and ptaerochromenol (40) (Fig. 25) absorbs at 1664 cm.$^{-1}$ ($CHCl_3$). The unusual contour of the latter spectrum is assumed to arise from extension of conjugation by the double bond in the six-membered oxide ring.

By comparison with 5-hydroxy-7-methoxy-2-methylchromone (103), which absorbs at 1663 cm.$^{-1}$ ($CHCl_3$), a small lowering effect is produced by annellation of a seven-membered unsaturated oxide ring. Thus, ptaeroxylin (41) (Fig. 26) and karenin (42) (Fig. 28) show their major absorption at 1656 and 1657 cm.$^{-1}$ ($CHCl_3$) respectively. The effect of the saturated oxide ring of dihydroptaeroxylin (58) (Fig. 27) is to cause a further small lowering.

Although it is known that allyl alcohols normally show weak intramolecular OH-π hydrogen bonding,[48,85] the presence of a 2-hydroxymethyl group has little effect on the carbonyl region of the chromone spectrum. Thus karenin (42) (Fig. 28) and ptaeroxylin (41) (Fig. 26) show very similar absorption and umtatin (36) (Fig. 19) and visamminol (35) (Fig. 18) have almost superposable spectra.

The apparent extinction coefficients (l. mole^{-1} cm.$^{-1}$) and half-band widths for the chromone spectra are listed in Table 6. The large extinction coefficients of the main bands once more demonstrate the polar nature of these compounds. Chromone and 2-methylchromone differ from the more complex members of the series in that they each possess a single broad and intense absorption maximum while the spectra of the substituted chromones, although equally intense, are multipeaked and considerably sharper. All chromones bearing oxygen substituents exhibit two maxima with extinction coefficients greater than 500 implying that both these peaks have considerable carbonyl character. (It is possible, however, that one of these peaks may be due to a stretching mode of a highly polar

carbon-carbon double bond.) Thus all 5-hydroxychromones possess two major absorptions, ~1660 and ~1630 $cm.^{-1}$, which show a constant wavenumber separation of 34 (± 5) $cm.^{-1}$ in both chloroform and carbon tetrachloride. The larger separations of the main maxima of various 5-methoxychromones do not show the same constancy, varying between 42 and 64 $cm.^{-1}$ with a median of 50 $cm.^{-1}$.

By recording the spectra in a non-polar solvent (carbon tetrachloride) and in a polar solvent (chloroform) it was hoped to demonstrate that the two major peaks, if predominantly carbonyl in character, would show a considerable alteration in absorption frequency through the solvation effect of the polar chloroform molecules around the carbonyl group. This was not, in fact, realised. For the 5-hydroxychromones, the smallest band showed the largest solvent shift (~ 10 $cm.^{-1}$), the main peaks varying by not more than 3 $cm.^{-1}$. Moreover, an attempt to solvate the carbonyl group of dihydropeucenin 7-methyl ether (61), by successive additions of methanol to a carbon tetrachloride solution, caused no significant change in the spectrum. The 5-methoxychromones, while showing a solvent effect of ~ 7 $cm.^{-1}$ for the upper band and between 4 and 11 $cm.^{-1}$ for the lower, undergo in addition considerable change of contour.

Since the chromone nucleus is almost planar, there are no apparent stereochemical features which could cause a splitting of the carbonyl frequency. The 5-hydroxyl group, forming a stable six-membered chelate ring through strong hydrogen bonding (OH resonance at $\tau \sim -3$ in the n.m.r. spectrum) with the pyrone carbonyl group, does not possess a free stretching frequency; thus, the existence of a free and a bonded carbonyl absorption seems most unlikely.

It is possible that an intramolecular vibrational effect such as Fermi resonance is responsible for the observed multiplicity of spectral peaks. This is supported by the appearance of the carbonyl frequency of 4-pyrone as a doublet, an observation which has been attributed[82,83] to Fermi resonance. Jones et al. have shown[82] that, for thirteen lactones exhibiting doublet carbonyl absorption due to Fermi resonance, the wave-number separation of the components varies between 11 and 43 with a median of 26. As previously stated, the two major peaks of the 5-hydroxychromone spectra are separated by ~ 34 cm.$^{-1}$, which is within the above range, but the separation of the two main maxima of the 5-methoxychromones is considerably greater (~ 50 cm.$^{-1}$).

For the split lactone spectra, the relative intensities of the components are independent of concentration but are sensitive to solvent polarity and temperature. It has already been noted that, although a certain solvent effect is found in the spectra of the 5-methoxychromones, the marked inversion of relative intensities which occurs for 4-pyrone (and other lactones) is not observed in the chromone spectra. The spectra of 5-hydroxy-2-methylchromone (6), 5-methoxy-2-methylchromone (102), 5-hydroxy-7-methoxy-2-methylchromone (103), and 5,7-dimethoxy-2-methylchromone (104) showed no change of contour when recorded at temperatures between 20° and 100°. It appears that, in general, the chromone spectra do not respond to solvent polarity and temperature in the manner required for Fermi resonance; therefore, the operation of a Fermi resonance effect is not proved.

In a qualitative interpretation of the 5-hydroxychromone spectra, it is tempting to assign the band at $\sim$1660 cm.$^{-1}$ to the carbonyl stretching vibration, the weakest absorption (1590-1600 cm.$^{-1}$) to a ring stretching mode, and the centre intense peak ($\sim$ 1630 cm.$^{-1}$) to the stretching vibration of a polar carbon-carbon double bond

(e.g. the 2,3-double bond). This interpretation is oversimplified since the 5-methoxychromone spectra do not possess an intense band around 1630 $cm.^{-1}$ and the lowest absorption ($\sim$ 1600-1615 $cm.^{-1}$) in these spectra seems too intense to be attributed to an aromatic-type ring stretching mode. From the spectra of some isotopically substituted chromones (vide infra) it becomes less plausible to assign the 1660 $cm.^{-1}$ band alone to the carbonyl stretching vibration; the possibility also arises that some of the bands do not represent pure vibrational modes but arise from combinations of several simpler molecular vibrations.

(ii) Hydroxyl region.

The hydroxyl groups most commonly encountered in the naturally occurring chromones are the phenolic 5- and 7-hydroxyl groups, the allylic 2-hydroxymethyl groups, and the aliphatic tertiary hydroxyl groups situated at the 3-position of an isopentyl side chain.

The 5-hydroxyl group: 5-Hydroxychromones possess no absorption maxima in the 3300-3600 $cm.^{-1}$ region; however, a weak absorption envelope, underlying the sharp C-H stretching frequencies, is evident around 2970-2980 $cm.^{-1}$

showing no definite maxima and possessing a half-band width of ~450 cm.$^{-1}$ (Figs. 29 and 30). By comparison with the 5-hydroxyflavones, which show similar absorption,[40b,68,84] this envelope is assigned to the stretching mode (or modes) of the chelated 5-hydroxyl group. This assignment is supported by the absence in 5-methoxychromone spectra (Figs. 29 and 30) of absorption of this kind. Moreover, the spectrum of 5-hydroxy-2-methylchromone (6), after treatment at room temperature with deuterium oxide in chloroform, shows a diminution in intensity of the broad absorption around 2970 cm.$^{-1}$ and possesses a new, considerably sharper ($\Delta\nu_{\frac{1}{2}}$ ~85 cm.$^{-1}$) maximum at ~2220 cm.$^{-1}$ (Fig. 29). If the latter band is assumed to be the stretching frequency of the 5-deuteroxyl group, the position of the 5-hydroxyl stretching maximum can be estimated by multiplication of the deuteroxyl frequency by 1·35.[86,87] The computed maximum, 2995 cm.$^{-1}$, is in reasonable agreement with the estimated midpoint (~ 2970-2980 cm.$^{-1}$) of the broad hydroxyl envelope. It has also been noted in the 5-hydroxyflavone spectra that the deuteroxyl stretching bands are sharper than the corresponding hydroxyl absorptions.[87]

The 7-hydroxyl group: 6-ethyl-5,7-dihydroxy-8-methoxy-2-methylchromone (106) and 6-ethyl-7-hydroxy-5,8-dimethoxy-2-methylchromone (107) each exhibit a sharp, intense 7-hydroxyl stretching frequency around 3515 $cm.^{-1}$. This value, 95 $cm.^{-1}$ lower than the free hydroxyl absorptions of simple phenols,[88] is not wholly accountable by intramolecular hydrogen-bonding to the 8-methoxyl substituent since 2-methoxyphenol [ν(OH) 3558 $cm.^{-1}$] absorbs only 52 $cm.^{-1}$ lower than phenol [ν(OH) 3610 $cm.^{-1}$].[90a] It is suggested that for chromones the polar pyrone carbonyl group exerts a vinylogous electron-withdrawing influence on the 7-hydroxyl group causing enhanced acidity of this function. This has the effect of strengthening the OH...OMe hydrogen-bond in (106) and (107) thus producing a lower bonded 7-hydroxyl frequency. The polar character of 7-hydroxychromones has also been noted in their chromatographic properties and in their insolubility in non-polar solvents [e.g. 5,7-dihydroxy-2-methylchromone (110) is insoluble in carbon tetrachloride and sparingly soluble in chloroform and methanol]. These characteristics closely parallel the properties of 7-hydroxyflavones.[40b,68]

When the 7-hydroxyl group is flanked by a bulky substituent in the ortho-position, a steric buttressing effect is observed. Thus, for the 7-hydroxychromones, dihydropeucenin (55) and dihydroheteropeucenin (59), possessing a large isopentyl group in the 6- and 8-

positions respectively, the free hydroxyl band appears as a doublet centred at ~3615 cm.$^{-1}$, the separation of the components being ~ 26 cm.$^{-1}$ (Fig. 31). This phenomenon has been reported[88] for o-t-butylphenols, the two hydroxyl peaks being attributed to two conformations of the hydroxyl group relative to the t-butyl group. Other investigations[89] have demonstrated that the lower band is probably due to a 'trans' conformation in which the hydroxyl group lies in the plane of the benzene ring and is oriented away from the ortho substituent. This conformation, allowing maximal overlap of the lone-pair orbitals of the oxygen atom with the π-electron system of the benzene ring is of lowest energy and gives rise to the 'normal' hydroxyl stretching frequency. In the 'cis' conformation, where the hydroxyl group once more lies in the plane of the aromatic ring but is oriented towards the ortho substituent, the severe non-bonded steric interaction between this substituent and the hydroxyl group either twists the latter slightly out of planarity with the aromatic nucleus or causes a small opening out of the C-O-H angle whereby the coplanarity (and thus the lone-pair delocalisation) is maintained. In either case, this

conformation is of higher energy than the 'trans' conformation and produces the higher hydroxyl stretching frequency.

When a dimethylallyl residue is adjacent to the 7-hydroxyl group as in peucenin (14), an intramolecular OH-π interaction takes place, resulting in two hydroxyl stretching frequencies at 3588 and 3387 $cm.^{-1}$ (Fig. 32), which show no alteration in relative intensity on dilution. Both these peaks are absent in peucenin 7-methyl ether (54) (Fig. 32). Although a separation of 120 $cm.^{-1}$ between the free and the bonded hydroxyl frequencies of o-(3,3-dimethylallyl)phenol has been recorded,[45,48,90] the $\Delta\nu$(OH) value for peucenin is much greater ($\sim$ 205 $cm.^{-1}$). From the hypothesis[45,46] that a frequency difference of $\sim$ 70 $cm.^{-1}$ represents a hydrogen-bond energy of 1 kcal./mole, the $\Delta\nu$(OH) for peucenin implies a hydrogen-bond energy of $\sim$ 3 kcal./mole for the intramolecular association of the 7-hydroxyl and the dimethylallyl substituent. It is reasonable to assume that this outstandingly strong OH-π interaction arises from the polar character of the 7-hydroxyl group aided by the high basicity of the allyl double bond through the positive inductive effect of the two methyl substituents.[90a]

The 2-hydroxymethyl group: The 2-hydroxymethyl group of karenin (42), ptaerochromenol (40), and umtatin (36) exhibits a free hydroxyl stretching frequency at $\sim$3615 cm.$^{-1}$ ($\varepsilon \sim 90$). At concentrations greater than $\sim 1{\cdot}5 \times 10^{-2}$ molar, this hydroxyl participates in intermolecular hydrogen-bonding, a broad bonded hydroxyl frequency appearing at $\sim$3400 cm.$^{-1}$ accompanied by a diminution in intensity of the free hydroxyl band. The bonded maximum disappears on dilution. It is notable that the free hydroxyl peak at 3615 cm.$^{-1}$ is rather broad ($\Delta\nu_{\frac{1}{2}} \sim 45$ cm.$^{-1}$); this may suggest that, being an allylic alcohol, the 2-hydroxymethyl group is undergoing intramolecular OH-π hydrogen-bonding with the 2,3-double bond[48,85] but that the bonded conformations are not sufficiently rigid to produce a splitting of the hydroxyl spectrum. The corresponding deuteroxyl absorption at 2663 cm.$^{-1}$, the wavenumber ratio, 3615/2663 = 1·36, being close to the theoretical value (1·38),[86] is considerably sharper ($\Delta\nu_{\frac{1}{2}} \sim 30$ cm.$^{-1}$).

Side chain hydroxyl group: The aliphatic tertiary hydroxyl group of visamminol (35) gives a stretching frequency at 3603 cm.$^{-1}$ ($\varepsilon \sim 40$). The reported value (3300 cm.$^{-1}$)[28] of a similar type of hydroxyl group in tetrahydrosorbifolin (111) is not in agreement with this value; the 3300 cm.$^{-1}$ absorption may indicate that, at the concentration employed, the tertiary hydroxyl of tetrahydrosorbifolin is totally intermolecularly hydrogen bonded.

(iii) Isotopic substitution.

From a qualitative examination of intensity data, the possible existence of two carbonyl peaks in the i.r. spectra of 5-hydroxychromones has been inferred. It was thought that, if the chelated hydrogen-bond could be slightly altered in some way, a small change in the position of part of the spectrum would indicate which absorptions are associated with the carbonyl stretching vibration.

To this end, the 5-hydroxyl group of seven chromones was enriched with deuterium by shaking at room temperature a chloroform or benzene solution of the chromone with deuterium oxide. In all cases, a definite change in the spectrum was observed (Table 7). For 5-hydroxy-2-methylchromone (6) (Fig. 33), 5-hydroxy-7-methoxy-2-methylchromone (103) (Fig. 34), peucenin 7-methyl ether (54) (Fig. 35), khellinol (37) (Fig. 36), ptaeroxylin (41) (Fig. 37), and karenin (42) (Fig. 38), the two upper peaks of the spectrum are broadened and resolved into doublets, the components of which show a separation of 6-9 $cm.^{-1}$. The upper components have the same frequency as before and represent the spectrum of the undeuterated compound present. The lower components must be due to the deuterated compound and increase in intensity with increasing incorporation of deuterium; the spectrum of a highly enriched sample of ptaeroxylin (Fig. 37) shows that as incorporation tends to 100% the original peaks diminish in intensity being gradually replaced by those 6-9 $cm.^{-1}$ lower. The spectrum of enriched ptaerochromenol (40) (Fig. 39) exhibits only a broadening and slight lowering of the main maximum (1664 $cm.^{-1}$) which was taken

to indicate that the peak due to the 5-deuteroxy compound was not capable of resolution in this solvent ($CHCl_3$). Little change was observed in the remainder of the spectrum.

It is significant that for 5-hydroxyflavone (87),[84] the carbonyl frequency (1652 $cm.^{-1}$) suffers a decrease of 7 $cm.^{-1}$ on deuteration of the 5-hydroxyl. Therefore, by analogy, deuterium exchange of the 5-hydroxychromones indicates that the two main peaks ($\sim$ 1660 $cm.^{-1}$ and $\sim$1630 $cm.^{-1}$) of the spectra are largely carbonyl in character. This deuteration experiment, however, allows no assignment to be made for the remaining absorption (1590-1605 $cm.^{-1}$) since this remained unchanged in the deuterated samples.

In an attempt to determine the cause of the doublet carbonyl absorption of the 5-hydroxychromone spectra, deuteration of the 3-position of the chromone nucleus was undertaken. It has been shown[83] for 4-pyrone that Fermi resonance occurs between the carbonyl stretching vibration and the overtone of an absorption at $\sim$ 850 $cm.^{-1}$, assigned to the out-of-plane deformation of the protons α to the carbonyl group. By replacement of these protons by

deuterium atoms,[83,91] the deformation overtone was moved sufficiently in energy to cause an interruption of the Fermi resonance and consequently a collapse of the doublet carbonyl absorption to a singlet. Since, in the chromone series, the investigation of the influences of solvent polarity and increasing temperature failed to prove or disprove the dependence of spectral multiplicity on Fermi resonance, it was expected that, if an effect of this type were operative, the spectrum of 5-hydroxy-2-methylchromone-3D (112) should show a single carbonyl stretching frequency, by analogy with the simple 4-pyrones. (Fermi resonance is feasible in the 5-hydroxychromone spectra since a sharp maximum at 837 cm.$^{-1}$ (KBr), possibly the out-of-plane deformation of the proton in the 3-position, would possess an overtone in the required region.) Reaction of deuterium oxide with 5-hydroxy-2-methylchromone in an acidic medium[83] gave mainly two deuterated species. The product of short-time reaction was undeuterated at position 3 of the chromone nucleus (n.m.r.) but was shown by mass spectral analysis to be 53 ± 2% $C_{10}H_5D_3O_3$, 20 ± 2% $C_{10}H_4D_4O_3$, and 16 ± 2% $C_{10}H_6D_2O_3$; the fragmentation pattern indicated that these three species possessed two

deuterium atoms in ring A of the chromone nucleus and differed only in incorporation into the 2-methyl group. This was in accord with the n.m.r. spectrum which indicated a replacement by deuterium of the protons at positions 6 and 8 of the nucleus, the remaining 7-proton appearing as a singlet at τ 2·54 (1H). The singlet at τ 7·64, assigned to the 2-methyl group in 5-hydroxy-2-methylchromone, now integrated for ~ 2 protons but the olefinic 3-H signal at τ 3·94 (s, 1H) showed no diminution in intensity. The major component of this mixture is therefore 5-hydroxy-2-monodeuteromethylchromone-6,8-D_2 (113). [Deuterium enrichment in the 5-hydroxyl group would be lost by exposure to atmospheric moisture during isolation.] The product of prolonged reaction was 68 ± 2% $C_{10}H_2D_6O_3$, 22 ± 2% $C_{10}H_3D_5O_3$, and 5 ± 2% $C_{10}H_4D_4O_3$. The n.m.r. spectrum of this mixture possessed a singlet at τ 2·53 (1H) (the C-7 resonance), the area of which was ~ 5 times greater than that of a small residual absorption (the 3-H resonance) at τ 3·93; no further signals were discernible. Thus the major product of this reaction is 5-hydroxy-2-trideuteromethylchromone-3,6,8,-D_3 (114).

In contrast to the i.r. spectrum of 5-hydroxy-2-methylchromone, the carbonyl region (Fig. 40) of 5-hydroxy-2-trideuteromethylchromone-3,6,8-D_3 (114) possesses only two absorption bands, 1649 and 1610 cm.$^{-1}$ (Table 8), and no maximum appears at 837 cm.$^{-1}$ in the solid state spectrum. The sharp, intense maximum at 1649 cm.$^{-1}$ is in approximately the same region as the carbonyl band of 5-hydroxyflavone (1652 cm.$^{-1}$),[68] the spectrum of which does not appear to possess a split carbonyl frequency. It is also close to the position of absorption of chromone and 2-methylchromone, both of which are unsubstituted at position 5. The inference of these comparisons is that the doublet ($\sim$ 1660 and 1630 cm.$^{-1}$) of the 5-hydroxychromone spectra arises by a Fermi resonance interaction between the carbonyl stretching vibration and the overtone of the out-of-plane deformation mode of the proton at position 3. Replacement of this proton by deuterium renders impossible the Fermi coupling by lowering the energy of the deformation overtone and results in collapse of the carbonyl doublet to a singlet at 1649 cm.$^{-1}$ That the Fermi resonance occurs only when a 5-hydroxyl group is present is shown by the spectra of chromone (1)

(Fig. 2) and 2-methylchromone (101) (Fig 3), which possess only one peak in the carbonyl region. However, interpretation of the spectrum of 5-hydroxy-2-trideuteromethylchromone-3,6,8-D_3 is not altogether straightforward; comparison with the spectrum of 5-hydroxy-2-monodeuteromethylchromone-6,8-D_2 (113) (Fig. 41; Table 8) tends to suggest that the lower two peaks (1625 and 1606 $cm.^{-1}$) of the 5-hydroxy-2-methylchromone spectrum (Fig. 4) rather than the upper two are undergoing collapse on deuteration. This could be interpreted as implying that the 1606 $cm.^{-1}$ peak of 5-hydroxy-2-methylchromone is largely associated with the combination of the stretching vibration of a polar double bond, e.g. the 2,3-double bond, and an aromatic ring mode, the energy of these modes being affected by the increase in the mass of substituents on deuteration. It is possible, of course, that the two above effects, viz., the destruction of Fermi resonance and the change in energy of the double bond stretching-ring stretching combination, could be operating simultaneously on deuteration.

The collapse observed in the spectrum of 5-hydroxy-2-methylchromone on deuteration at the 3-position bears a certain resemblance to the results of similar experiments on p-benzoquinone.[92(a)] The complex absorption which this compound displays in the 1600-1700 cm.$^{-1}$ region would not be expected from molecular symmetry considerations. Two bands, 1668 and 1656 cm.$^{-1}$, arise from a Fermi resonance effect involving the C=O fundamental and show a dependence on solvent polarity analogous to that of 4-pyrone. A further peak below 1600 cm.$^{-1}$, moderately weak and solvent-insensitive, is assigned to the carbon-carbon double bond stretching fundamental. Incorporation of deuterium into position 2 of the quinone nucleus (quinone-2D) results in a single carbonyl absorption at 1664 cm.$^{-1}$. (The authors stress that the frequency of the unsplit carbonyl vibration should occur between the Fermi resonance components.) The temperature effects observed in the spectra of quinone and its deuterated derivatives are not ascribed directly to the presence of Fermi resonance. Since the actual perturbation responsible for the resonance interaction should not be temperature dependent, the components of a Fermi resonance

multiplet change in relative intensities only if one of the vibrations involved (e.g. the carbonyl fundamental) varies significantly with temperature. The data presented shows that the quinone carbonyl stretching frequency is not, in fact, temperature dependent. Thus, it is reasonable to expect that the stretching mode of the strongly hydrogen-bonded carbonyl group of the 5-hydroxychromones could be temperature independent; this would explain the constancy of the 5-hydroxychromone spectra when recorded under variable temperature. However, if a Fermi resonance interaction is responsible for the multiplicity of these spectra, the reason for their apparent independence of solvent polarity remains unclear.

One further convincing parallel can be drawn between the spectra of the 5-hydroxychromones and p-benzoquinone. Incorporation (incomplete) of oxygen-18 into both carbonyl groups of p-benzoquinone results in the appearance of a single intense carbonyl band at 1634 $cm.^{-1}$, 30 $cm.^{-1}$ lower than the unsplit carbonyl frequency of quinone-2D. A similar situation obtains for oxygen-18 enriched 5-hydroxy-2-methylchromone. Reaction of 5-hydroxy-2-methylchromone (6) with oxygen-18 enriched

water[92] in an acidic medium gave, by mass spectral assay, ~50% incorporation of oxygen-18 into the molecule. 23 ± 2% of the recovered material contained oxygen-18 in the carbonyl group and 30 ± 2% in one or other of the 5-hydroxyl oxygen and the heterocyclic ether oxygen. Only ~1% possessed two oxygen-18 atoms and 40 ± 2% was unlabelled. The hydroxyl region (Fig. 29) of the oxygen-18 enriched sample is closely similar to that of 5-hydroxy-2-methylchromone but possesses a small ill-defined maximum at 2350 cm.$^{-1}$, probably associated with the O^{18}-H stretching vibration. The carbonyl region (Fig. 42) is identical to that of the unlabelled compound with respect to the position of absorption of the original bands of the oxygen-16 compound present. Significantly, however, a sharp new peak appears at 1593 cm.$^{-1}$, the high intensity of which suggests that this is a carbonyl stretching frequency, presumably that of the oxygen-18 enriched carbonyl group. No further absorptions were observed between 1590 and 1500 cm.$^{-1}$. From this single oxygen-18 carbonyl stretching frequency it is possible to calculate the position of absorption of the equivalent oxygen-16 carbonyl group, by employing equation (1), which relates the stretching

frequency (ν, cm.$^{-1}$) with the stretching-force constant (k, millidynes/Å) of the bond and the reduced mass $\mu = \frac{m_1 m_2}{m_1 + m_2}$ of the atoms (atomic mass units m_1 and m_2) containing the bond under consideration.[86,92]

$$\nu = \frac{1}{2\pi} \cdot \sqrt{\frac{k}{\mu}} \qquad \ldots\ldots(1)$$

$$\nu_{CO^{16}} = \frac{1}{2\pi} \cdot \sqrt{\frac{k(12{\cdot}0 + 16{\cdot}0)}{12{\cdot}0 \times 16{\cdot}0}}$$

$$\nu_{CO^{18}} = \frac{1}{2\pi} \cdot \sqrt{\frac{k(12{\cdot}0 + 18{\cdot}0)}{12{\cdot}0 \times 18{\cdot}0}}$$

$$\frac{\nu_{CO^{16}}}{\nu_{CO^{18}}} = \sqrt{\frac{28{\cdot}0 \times 12{\cdot}0 \times 18{\cdot}0}{12{\cdot}0 \times 16{\cdot}0 \times 30{\cdot}0}}$$

$$= \sqrt{\frac{21}{20}}$$

$$= \sqrt{1{\cdot}05}$$

$$= 1{\cdot}03$$

$$\nu_{CO^{16}} = \nu_{CO^{18}} \times 1{\cdot}03$$

$$= 1593 \times 1{\cdot}03$$

$$= 1641 \text{ cm.}^{-1}$$

This calculated oxygen-16 carbonyl frequency is remarkably close to the wavenumber value (1649 $cm.^{-1}$) of the single carbonyl absorption of 5-hydroxy-2-trideuteromethyl-chromone-3,6,8-D_3 (114). This relationship suggests that, by insertion of the heavy oxygen atom, the fundamental carbonyl stretching mode has been changed sufficiently to render Fermi resonance coupling impossible; thus, an unsplit O^{18}-carbonyl band is obtained.

The apparent diminution of intensity of the 1606 $cm.^{-1}$ peak could be associated with incorporation of O^{18} into the ether oxygen of the heterocyclic ring; increase in mass of this atom would be expected to have a small effect on the stretching frequency of the 2,3-double bond, to which the band at 1606 $cm.^{-1}$ has already been partly assigned. However, the possibility that this band may possess carbonyl character cannot be excluded, despite its weak intensity. In addition to the observed reduction in intensity in the spectrum of the oxygen-18 enriched compound, this band gives, as demonstrated previously, the largest solvent shift when the spectrum of 5-hydroxy-2-methylchromone is recorded in carbon tetrachloride and in chloroform and also undergoes marked change on deuteration

of the chromone nucleus. In support of this assignment, recent work[93] on dihydro-1,2-benzocyclohepten-3-ones has shown that chelated carbonyl absorptions can be lowered to the region of 1610 cm.$^{-1}$

Attempts to incorporate deuterium and oxygen-18 into 5-methoxy-2-methylchromone were unsuccessful as, under the acidic conditions of the exchange reaction, partial demethylation took place and a mixture of products was obtained.

The dominant characteristics of the chromone spectra can be summarised in terms of the various influences which perturb the enone orbitals and hence affect the carbonyl stretching frequency. Chromone and 2-methylchromone display absorption characteristic of an enone system perturbed only slightly by the effects of the hetero-oxygen atom and the benzene ring (ring A); thus, the carbonyl stretching frequency ($\sim$ 1650 cm.$^{-1}$) appears 20-30 cm.$^{-1}$ lower than the normal position of absorption of unsaturated ketones. When a hydroxyl group is present in the 5-position of the nucleus, the enone system can suffer three perturbing effects, viz. extended mesomerism involving the 5-hydroxyl, strong hydrogen bonding between the hydroxyl and the

carbonyl group with the formation of a 6-membered chelate ring, and a Fermi resonance interaction between the carbonyl stretching fundamental and an energetically suitable overtone. The combination of these effects is a doublet carbonyl absorption, ~1660 and ~1630 $cm.^{-1}$, the contour of which is independent of both solvent polarity and temperature. For the 5-methoxychromones, a large perturbation causes the appearance of a carbonyl frequency near 1600 $cm.^{-1}$ in addition to that at ~1655 $cm.^{-1}$. It seems unlikely that this large wavenumber separation could be the outcome of a Fermi resonance interaction. It is possible, however, that the non-bonded steric repulsion between the methoxyl and the carbonyl group, enhanced by rotation of the methoxyl around the carbon (aromatic)-oxygen bond, could have a perturbing influence on the enone orbitals. In a '<u>cis</u>' rotamer, in which the methyl group is oriented towards the carbonyl group, there would be severe steric interaction between the methyl group and the carbonyl oxygen atom; a perturbation of this kind could conceivably be solvent dependent.

Table 6.

I.r. absorption of chromones in chloroform and carbon tetrachloride (10^{-2} - 10^{-3} molar). Absorption frequency (ν) in cm.$^{-1}$; apparent half-band width ($\Delta\nu_{\frac{1}{2}}^{a}$) in cm.$^{-1}$; apparent extinction coefficient (ε^{a}) (l. mole^{-1} cm.$^{-1}$).

	Chloroform						Carbon tetrachloride					
	Carbonyl			Hydroxyl			Carbonyl			Hydroxyl		
	ν	$\Delta\nu_{\frac{1}{2}}^{a}$	ε^{a}	ν	$\Delta\nu_{\frac{1}{2}}^{a}$	ε^{a}	ν	$\Delta\nu_{\frac{1}{2}}^{a}$	ε^{a}	ν	$\Delta\nu_{\frac{1}{2}}^{a}$	ε^{a}
Chromone	1654	16	1050	-			1668	12	1060	-		
	1621	13	235				1620	10	210			
	1606	13	170	-			-			-		
2-Methyl-chromone	1650	14	1060	-			1665	12	1050	-		
	1618	13	230				1618	12	200			
	1607	13	170	-						-		
5-Hydroxy-2-methylchromone	1659	9	1360	-			1661	8	1500	-		
							1639	-	165			
	1624	10	680				1625	9	585			
	1601	14	390	~2975	~450	~60	1606	10	395	~2970	~400	~60
5-Methoxy-2-methylchromone	1658	12	1330	-			1665	9	1620	-		
							1650[i]					
							1642[i]					
	1628	21	360									
	1608	10	660	-			1607	9	580	-		

Table 6 contd.

	Chloroform						Carbon tetrachloride					
	Carbonyl			Hydroxyl			Carbonyl			Hydroxyl		
	ν	$\Delta\nu_{\frac{1}{2}}$[a]	ε[a]	ν	$\Delta\nu_{\frac{1}{2}}$[a]	ε[a]	ν	$\Delta\nu_{\frac{1}{2}}$[a]	ε[a]	ν	$\Delta\nu_{\frac{1}{2}}$[a]	ε[a]
5,7-Dihydroxy-2-methyl-chromone	~1664	Sparingly soluble					Insoluble					
5-Hydroxy-7-methoxy-2-methylchromone	1663[d]	14	1180	-			1666[d]	15	1240	-		
	1626[d]	18	770				1630	17	790			
	1590	16	490	~2980	~450	~60	1602	13	495	~2970	~400	~60
5,7-Dimethoxy-2-methyl-chromone	1659	9	1290	-			1666	9	1640	-		
	1610	13	930				1644	17	270			
	1574	12	150	-			1620	11	395	-		
Peucenin	1659	15	870	3588	45	70	1661			3598		
	1633	25	560	3387	129	75	1635			3405		
	1589	19	480	~2975	~450	~60	Sparingly soluble					
Peucenin 7-methyl ether	1661	12	1120	-			1663	10	1170	-		
	1627	28	490				1630	23	440			
	1593	25	410	~2970	~450	~60				~2965	~400	~60
Dihydro-peucenin	1659	15	810	3626	32	95						
	1630	29	440	3598	47	90						
	1590	24	360	~2970	~450	~60						

Table 6 contd.

	Chloroform						Carbon tetrachloride					
	Carbonyl			Hydroxyl			Carbonyl			Hydroxyl		
	ν	$\Delta\nu_{\frac{1}{2}}$ [a]	ε [a]	ν	$\Delta\nu_{\frac{1}{2}}$ [a]	ε [a]	ν	$\Delta\nu_{\frac{1}{2}}$ [a]	ε [a]	ν	$\Delta\nu_{\frac{1}{2}}$ [a]	ε [a]
Dihydropeucenin 7-methyl ether	1661	13	1090				1663	10	1140	-		
	1627	27	410				1630	19	415			
	1591	24	350	~2975	~450	~60				~2965	~450	~60
Peucenin 5,7-dimethyl ether	1656	11	1230	-			1663	9	1670	-		
	1627						1641	16	170			
	1602	12	1160	-						-		
Heteropeucenin 7-methyl ether	1661	10	1180	-			1663	10	1175	-		
	1622	14	510				1624	16	470			
	1593	15	550	~2970	~450	~60				~2960	~400	~60
Dihydroheteropeucenin	1663	12	1010	3625	33	107						
	1624	25	340	3600	50	85						
	1591	23	360	~2970	~450	~60						
Dihydroheteropeucenin 7-methyl ether	1661	12	825	-								
	1622	20	355									
	1594	15	390	~2975	~450	~60						
6-Ethyl-5,7-dihydroxy-8-methoxy-2-methylchromone	1661	9	970	3513	35	160	1663	10	840	3517	23	130
	1629	22	425				1631	23	375			
	1597	19	465	~2980	~450	~60	1604	17	420	~2970	~400	~60

Table 6 contd.

	Chloroform						Carbon tetrachloride					
	Carbonyl			Hydroxyl			Carbonyl			Hydroxyl		
	ν	$\Delta\nu_{\frac{1}{2}}$ [a]	ε [a]	ν	$\Delta\nu_{\frac{1}{2}}$ [a]	ε [a]	ν	$\Delta\nu_{\frac{1}{2}}$ [a]	ε [a]	ν	$\Delta\nu_{\frac{1}{2}}$ [a]	ε [a]
6-Ethyl-7-Hydroxy-5,8-dimethoxy-2-methylchromone	1657	10	1210	3517	33	165	1664	9	1290	3523	23	140
	1631	12	410				1643	13	310			
	1596	15	850	-			-			-		
Visamminol	1671	14	925	3603	45	35						
	1636	17	770									
	1593	13	420	~2975	~450	~60						
Umtatin	1672	14	973	3617[a]	45	60	1674	11	1180	3628	38	91
				3392[a]	180	30	1643	20	600			
	1639	21	555	3617[b]	43	93						
	1595	13	525	~2970	~450	~60	-			~2975	~400	~60
Isovisnagin	1648	14	690	-								
	1631	26	170									
	1607	14	410	-								
Dihydrokhellin	1657	12	1120	-			1662	9	1160	-		
							1637	16	320			
	1615	23	918	-			1620	17	570	-		

Table 6 contd.

	Chloroform						Carbon tetrachloride					
	Carbonyl			Hydroxyl			Carbonyl			Hydroxyl		
	ν	$\Delta\nu_{\frac{1}{2}}$ [a]	ε [a]	ν	$\Delta\nu_{\frac{1}{2}}$ [a]	ε [a]	ν	$\Delta\nu_{\frac{1}{2}}$ [a]	ε [a]	ν	$\Delta\nu_{\frac{1}{2}}$ [a]	ε [a]
Khellinol	1661	16	695	-			1663	15	700	-		
	1636	27	325				1639	25	335			
	1604[i]						1606	23	160			
	1592	23	580	~2970	~450	~60	1596	15	550	~2970	~450	~60
Khellin	1655	14	1090	-			1661	11	1260	-		
	1636[i]						1645	23	150			
	1620	18	630				1624	15	335	-		
	1598	17	170	-			-					
Isopeucenin	1659	12	1015	-								
	1628	26	540									
	1584	22	400	~2970	~450	~60						
Ptaerochromenol	1664	11	1390	3615[c]	50	30	1666	10	~1400	3625	34	~90
	1644	21	290	3407[c]	200	65						
	1615	13	310	3615[a]	42	83			Sparingly soluble			
	1585	15	720	~2970	~450	~60						
Ptaeroxylin	1656	14	1010	-			1658	11	1260	-		
	1627	29	560				1629	27	560			
	1595	23	390	~2940	~450	~60	-			~2930	~480	~50

Table 6 contd.

	Chloroform						Carbon tetrachloride					
	Carbonyl			Hydroxy			Carbonyl			Hydroxy		
	ν	$\Delta\nu_{\frac{1}{2}}$ [a]	ε [a]	ν	$\Delta\nu_{\frac{1}{2}}$ [a]	ε [a]	ν	$\Delta\nu_{\frac{1}{2}}$ [a]	ε [a]	ν	$\Delta\nu_{\frac{1}{2}}$ [a]	ε [a]
Dihydro-	1654	12	1105	-								
ptaeroxylin	1623	25	550									
	1592	23	385	~2950	~450	~60						
Karenin	1658	14	1010	3615	48	98						
	1629	28	545									
	1602	26	370	~2950	~450	~60						

a, $1{\cdot}53 = 10^{-2}$ M. b, $4{\cdot}74 \times 10^{-3}$M. c, $2{\cdot}00 \times 10^{-2}$ M.

d, doublet. i, inflection.

Table 7.

Carbonyl absorption of 5-deuteroxy enriched chromones.

	Frequency ($cm.^{-1}$)	
	CCl_4	$CHCl_3$
5-Hydroxy-2-methylchromone (6)	1660 1654	
	1624	-
	1618	
	1605	
5-Hydroxy-7-methoxy-2-methylchromone (103)	1669^s 1663 1656^s	
	1630	-
	1621^s	
	1601	
Peucenin 7-methyl ether (54)	1663 1655	
	1629	-
	1620	
	1598	
Khellinol (37)	1663 1655^s	
	1639	-
	1631^s	
	1607^i	
	1596	

Table 7 contd.

	Frequency ($cm.^{-1}$)	
	CCl_4	$CHCl_3$
Ptaeroxylin (41)	1658	
	1648	
	1628^s	-
	1619	
	1598	
Karenin (42)		1656
		1650
	-	1627
		1621
		1595
Ptaerochromenol (40)		1660^b
		1644^s
	-	1610
		1587
		1566^w

b, broad. i, inflection. s, shoulder,

w, weak absorption.

Table 8.

Carbonyl absorption of nuclearly deuterated 5-hydroxy-2-methylchromone and oxygen-18 enriched 5-hydroxy-2-methylchromone.

	Frequency ($cm.^{-1}$)	
	CCl_4	$CHCl_3$
5-Hydroxy-2-trideutero-methylchromone-3,6,8-D_3 (114)	1649 1610	-
5-Hydroxy-2-monodeutero-methylchromone-6,8-D_2 (113)	1656 1616 1603	-
Oxygen-18 enriched 5-hydroxy-2-methylchromone	1659 1637^s 1624 1606 1593	1657 1639^s 1623 1600 1587 1581^w

s, shoulder. w, weak absorption.

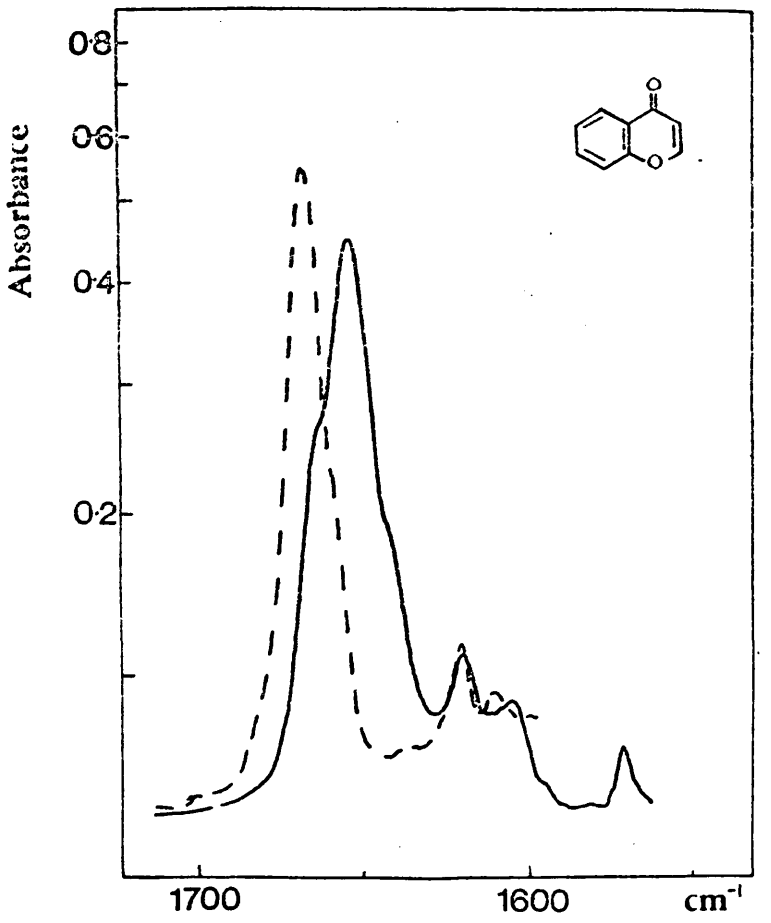


Fig.2.

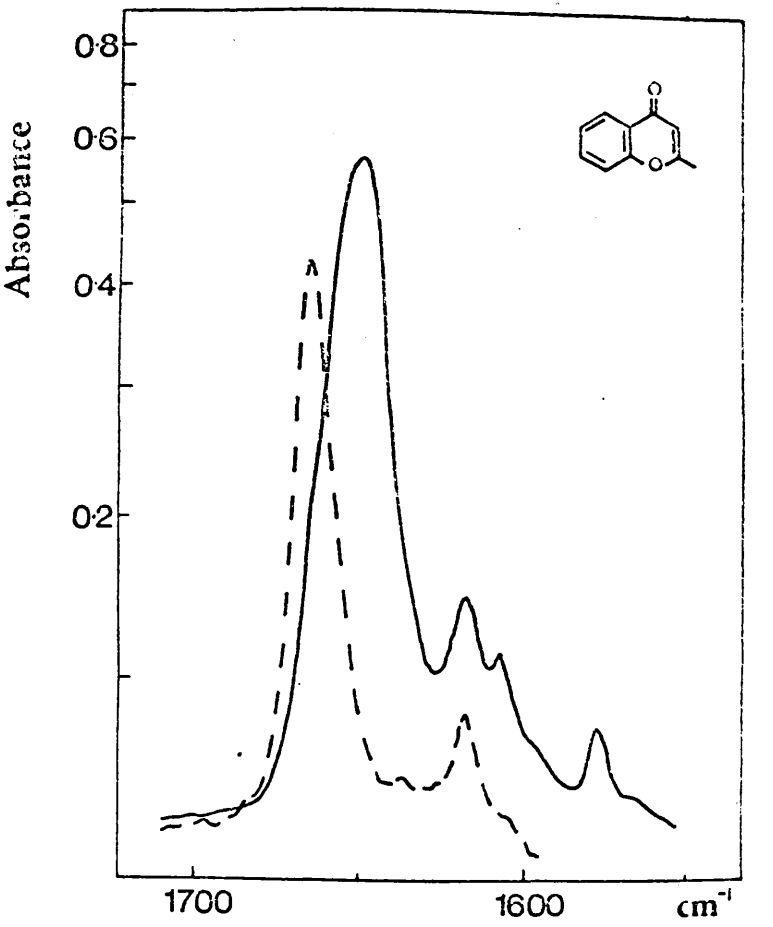


Fig. 3.

Chloroform solution (——). Carbon tetrachloride solution (----).

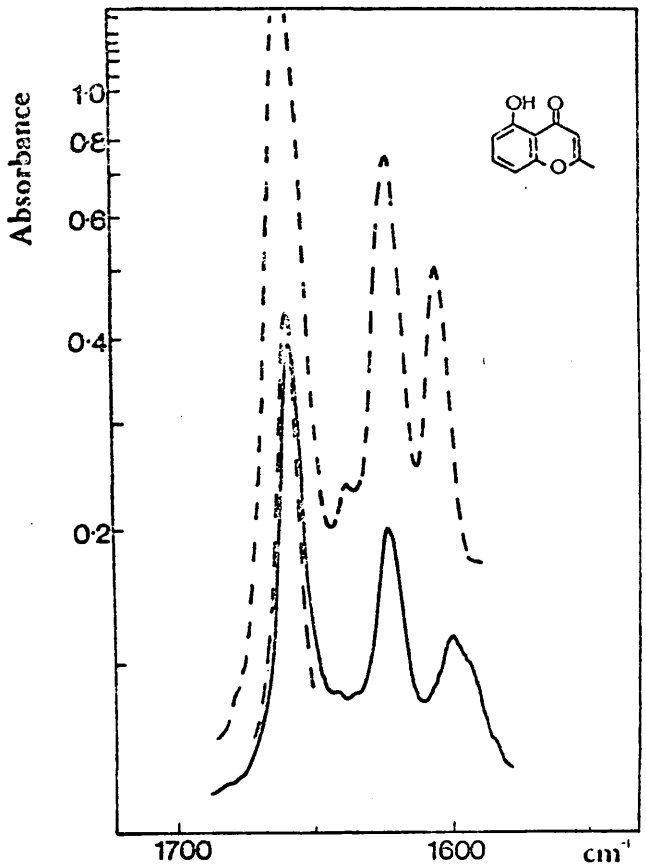


Fig. 4.

Chloroform solution (——).
Carbon tetrachloride solution (----),
[0·5 mm. and 0·125 mm. cell paths].

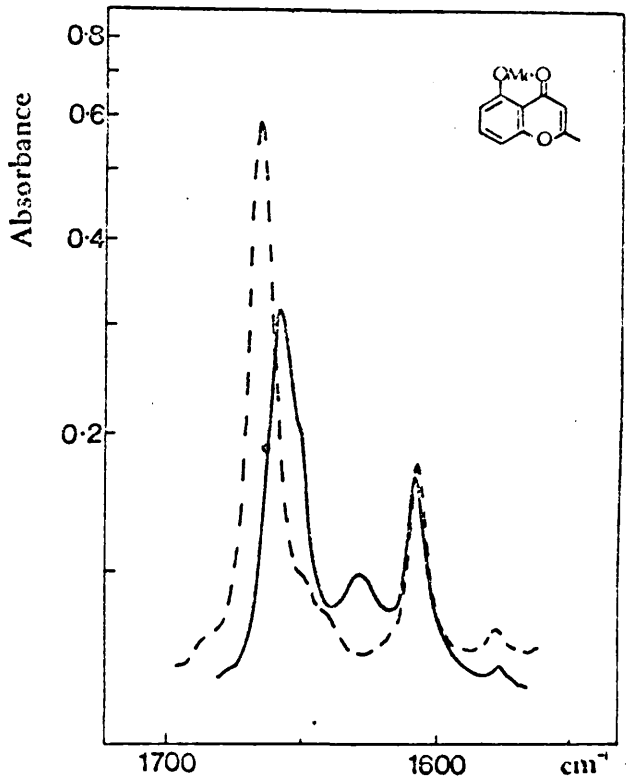


Fig. 5.

Chloroform solution (——).
Carbon tetrachloride solution (----).

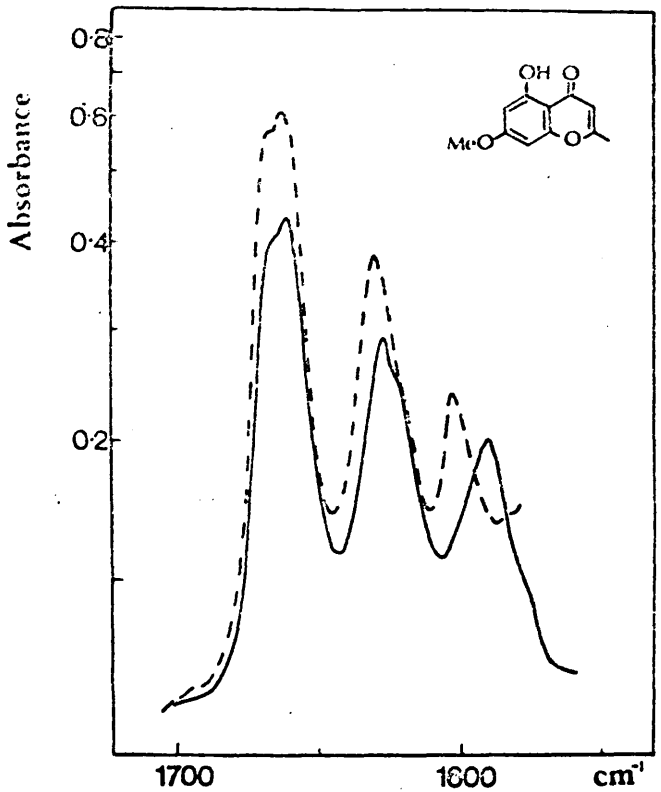


Fig. 6.

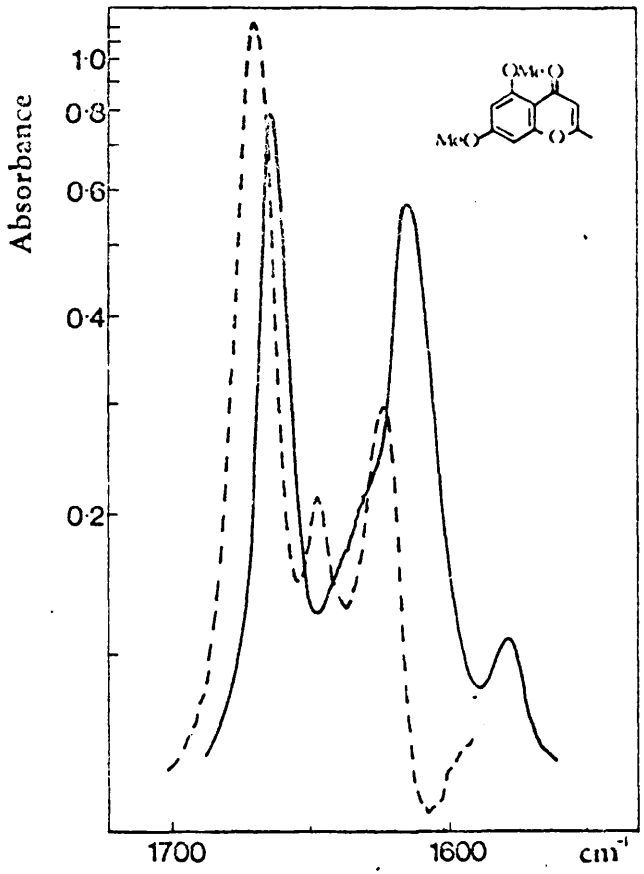


Fig. 7.

Chloroform solution (——). Carbon tetrachloride solution (----).

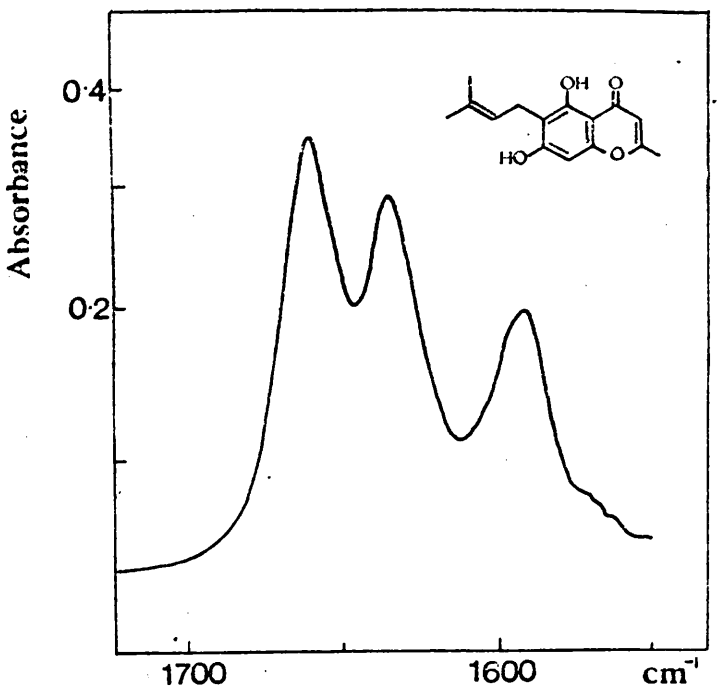


Fig. 8.

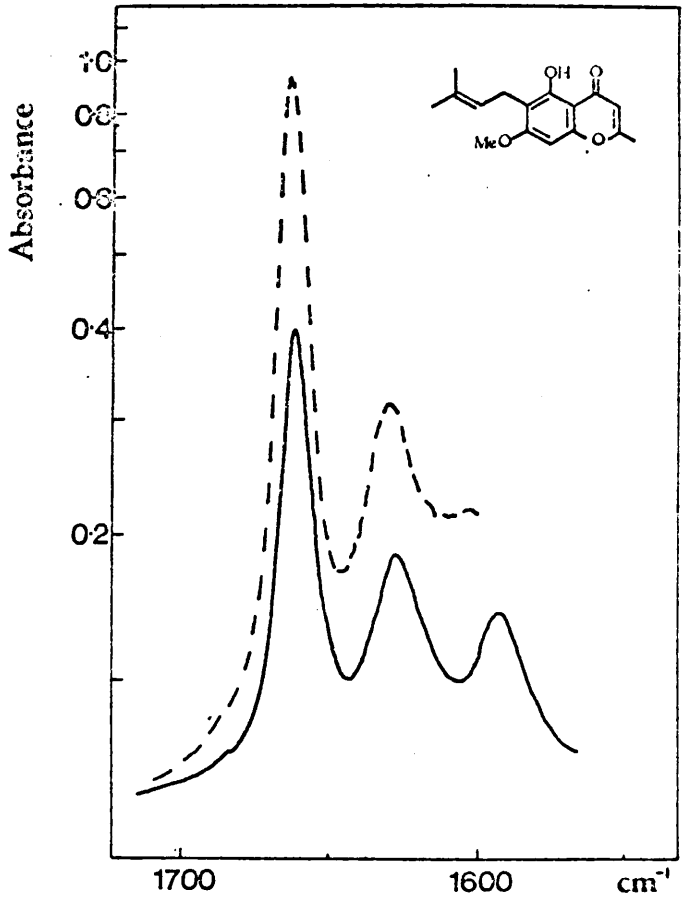


Fig. 9.

Chloroform solution (——). Carbon tetrachloride solution (----).

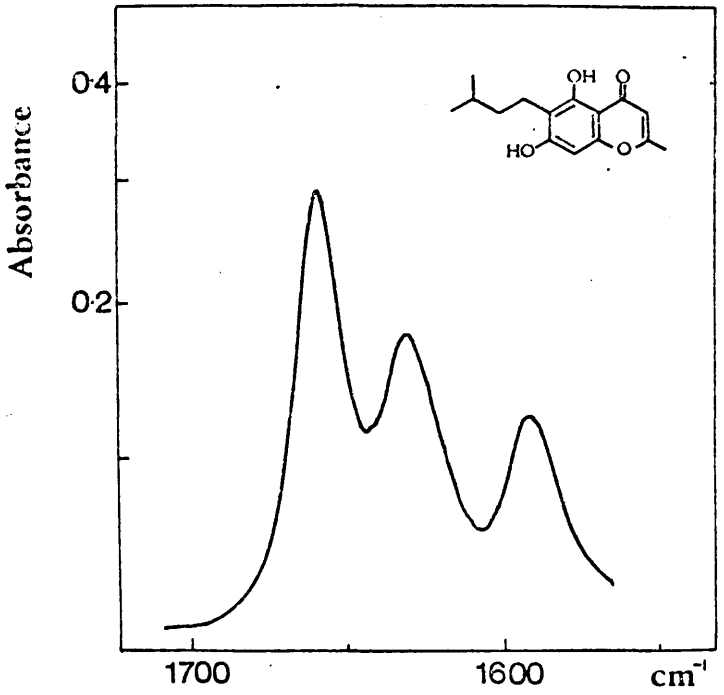


Fig. 10.

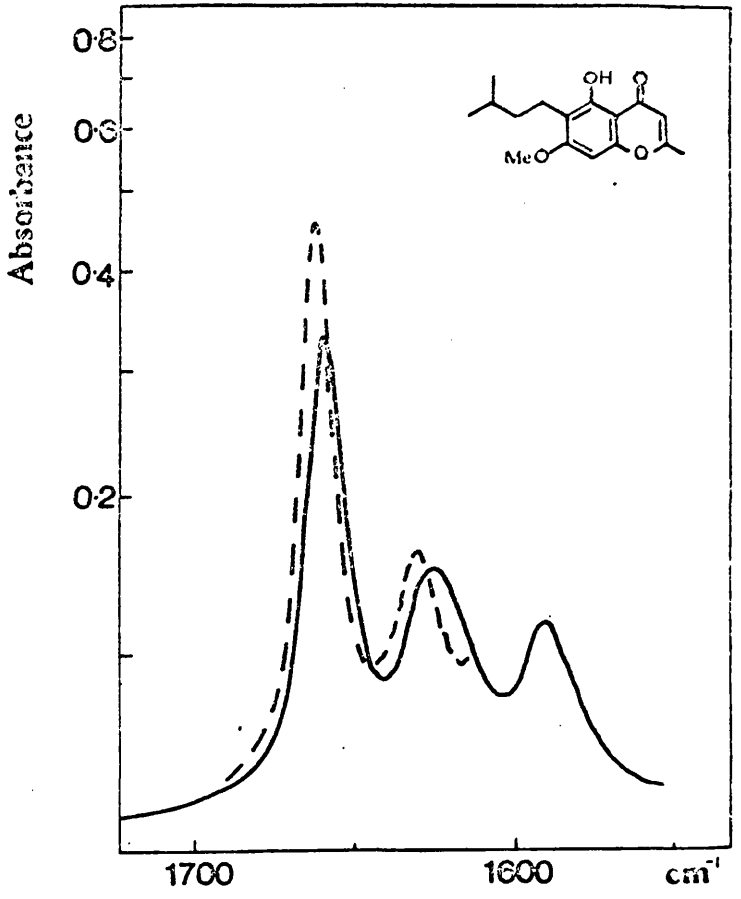


Fig. 11.

Chloroform solution (——). Carbon tetrachloride solution (----).

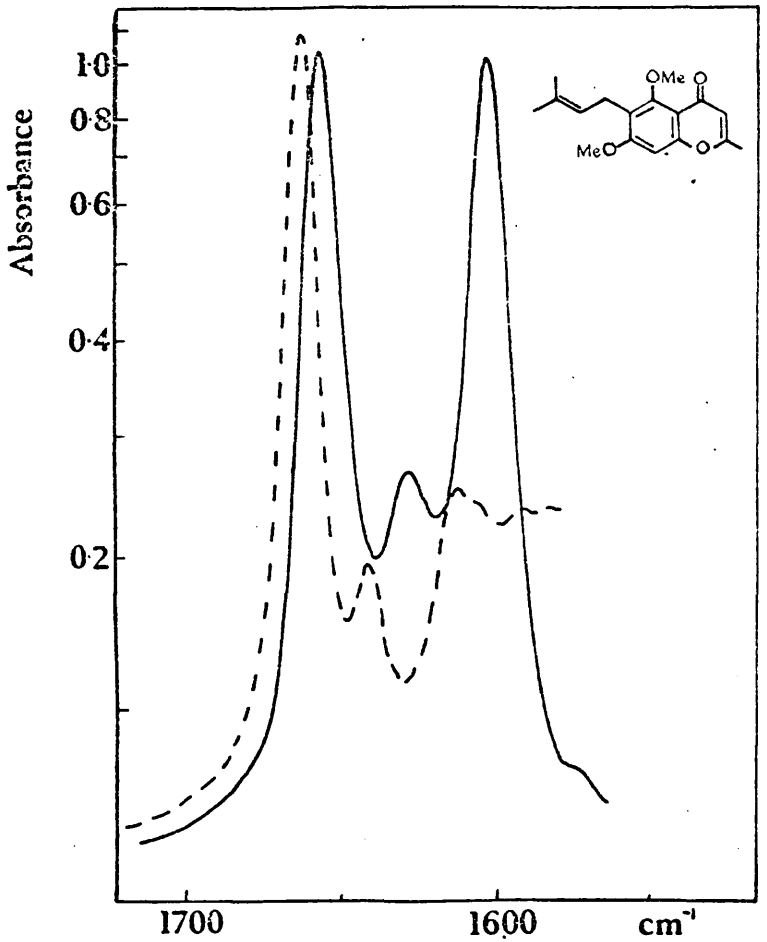


Fig. 12.

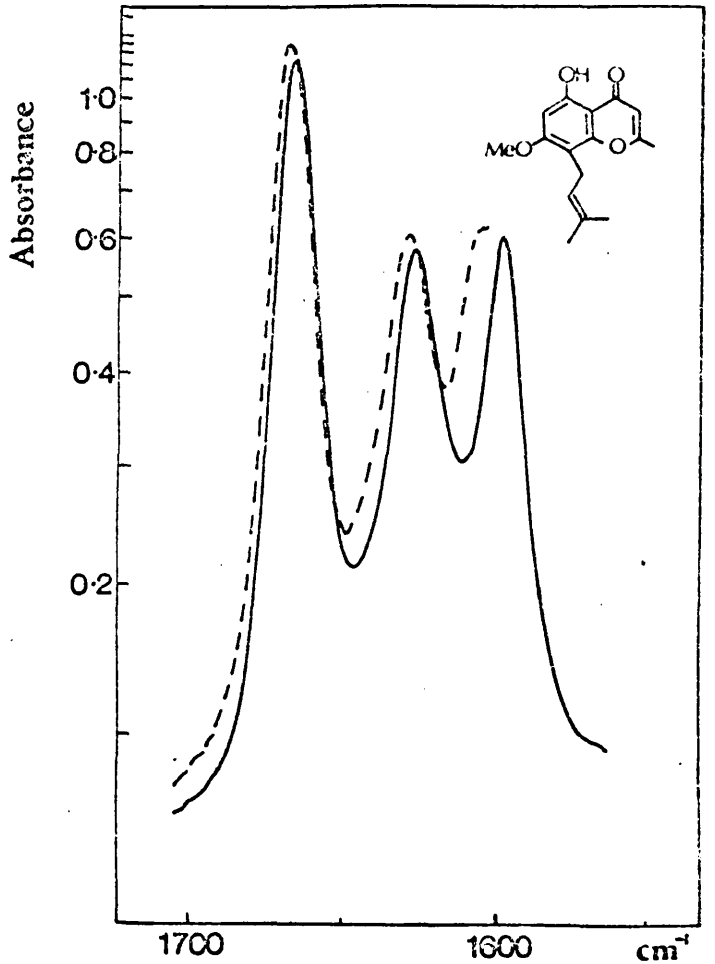


Fig. 13.

Chloroform solution (——). Carbon tetrachloride solution (----).

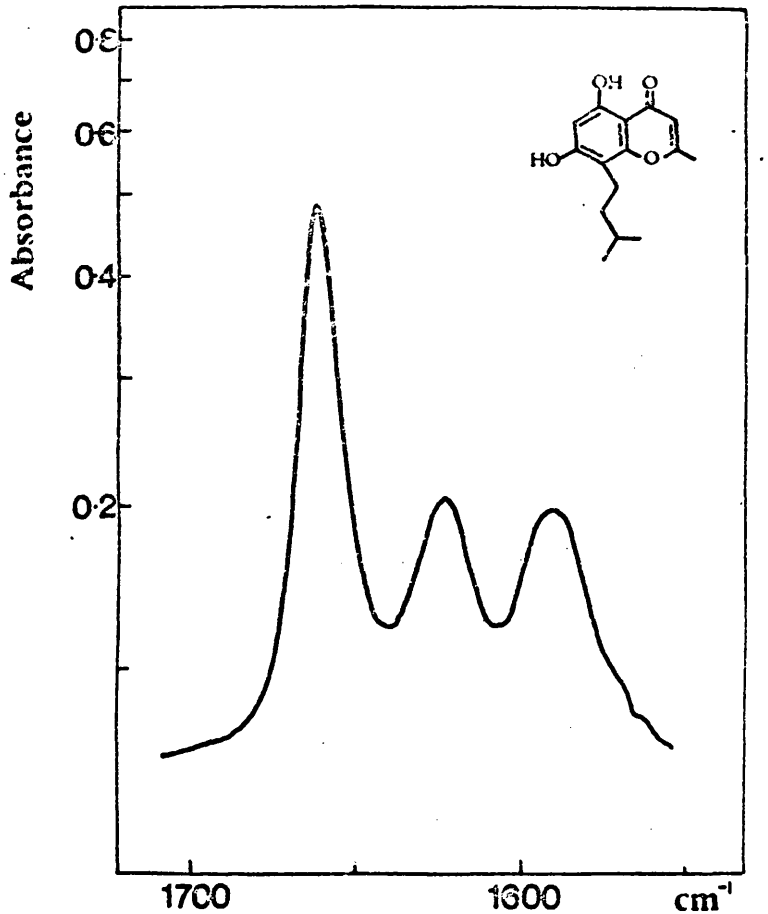


Fig. 14.

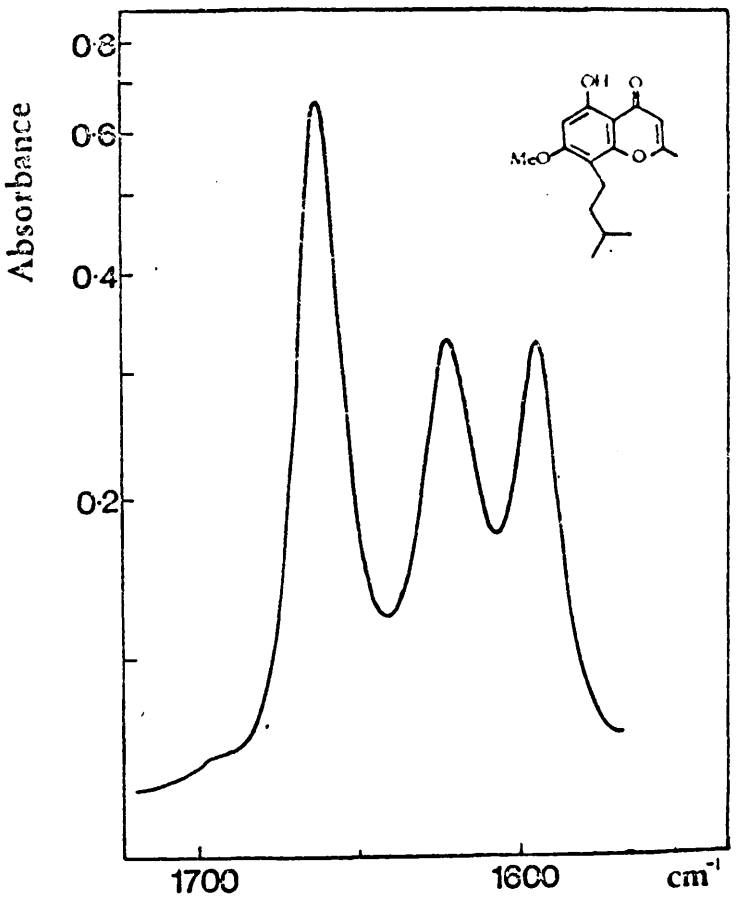


Fig. 15.

Chloroform solution (——).

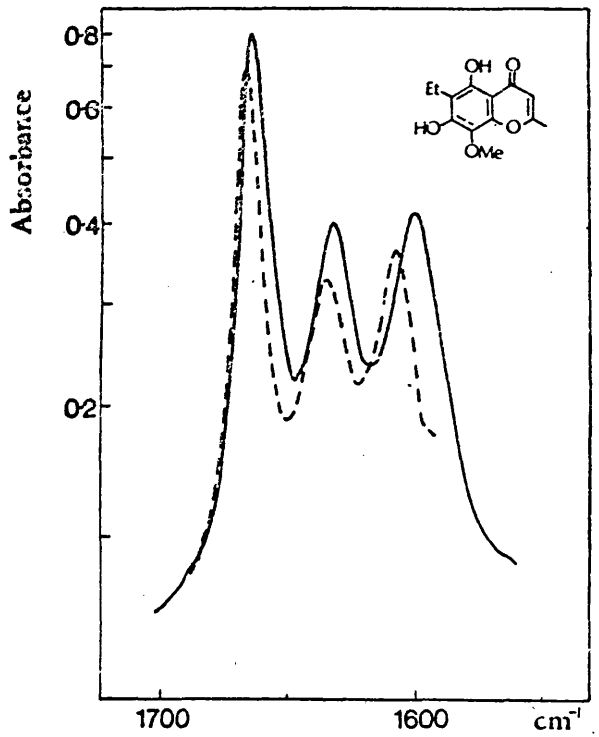


Fig. 16.

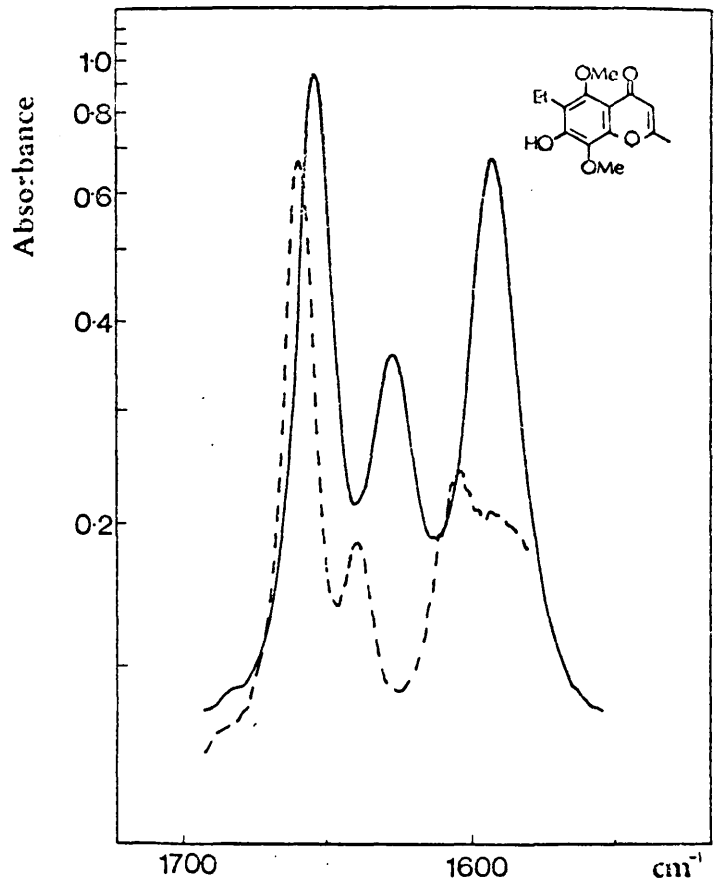


Fig. 17.

Chloroform solution (——). Carbon tetrachloride solution (----).

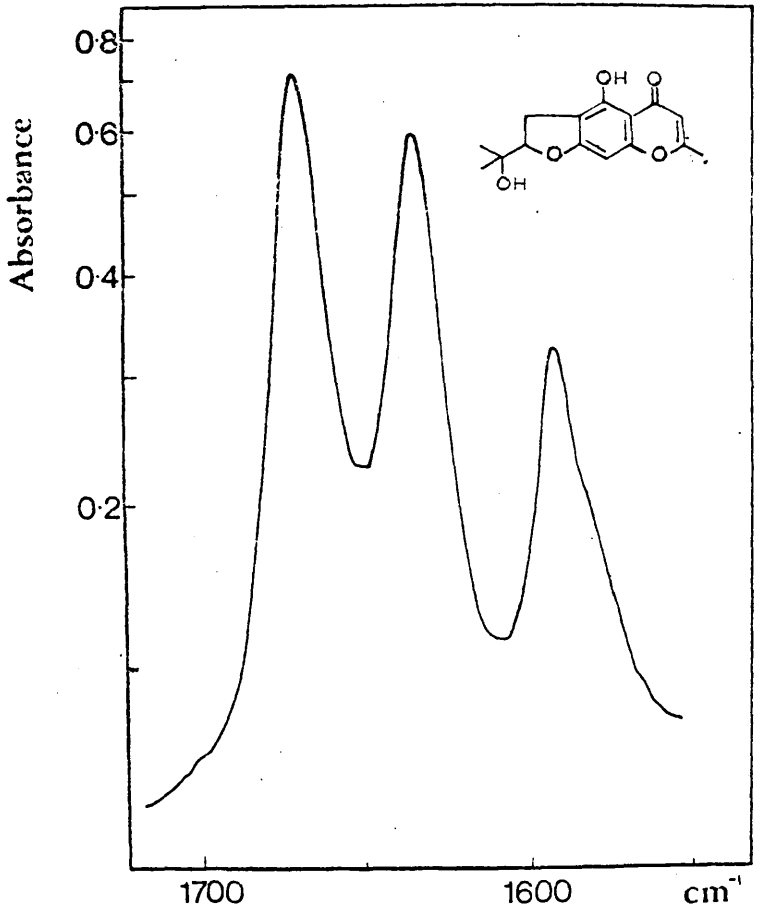


Fig. 18.

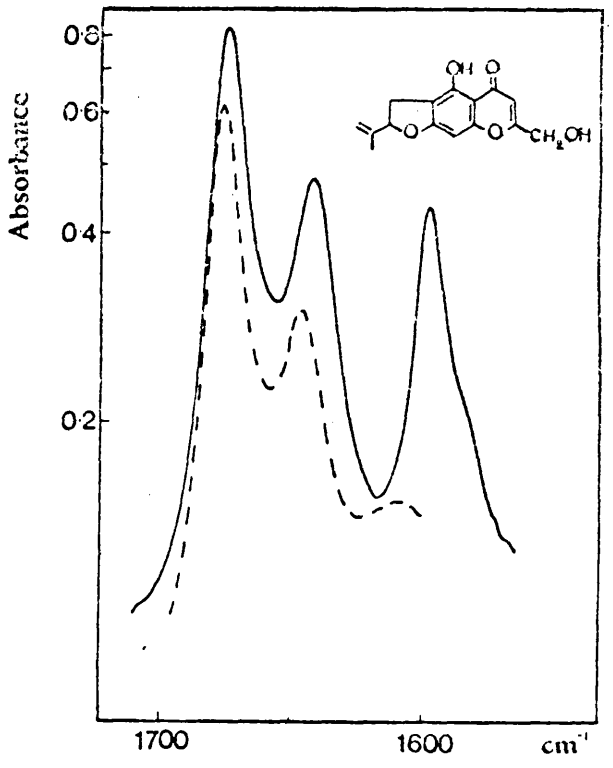


Fig. 19.

Chloroform solution (——). Carbon tetrachloride solution (----).

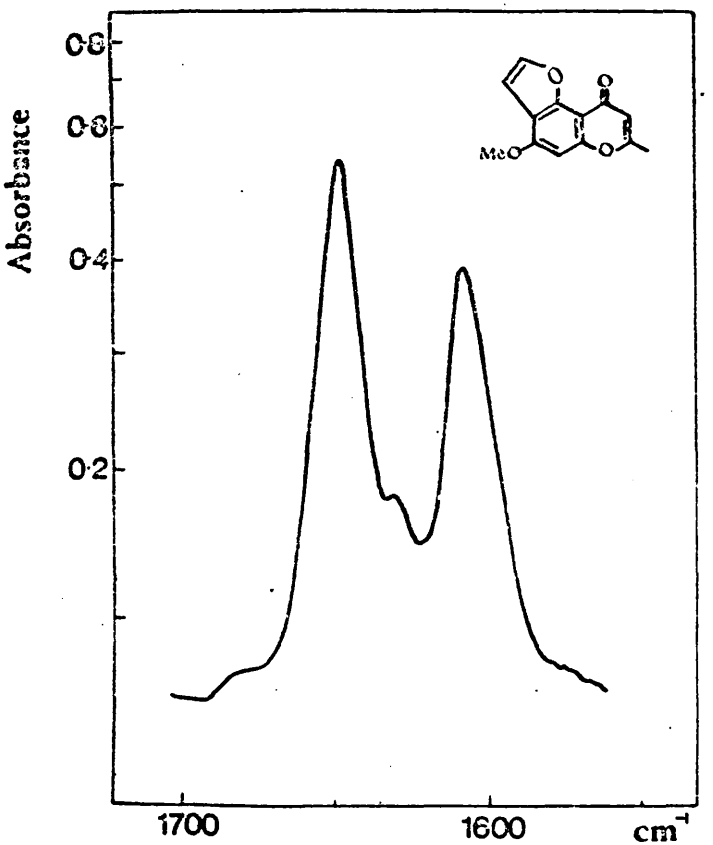


Fig. 20.

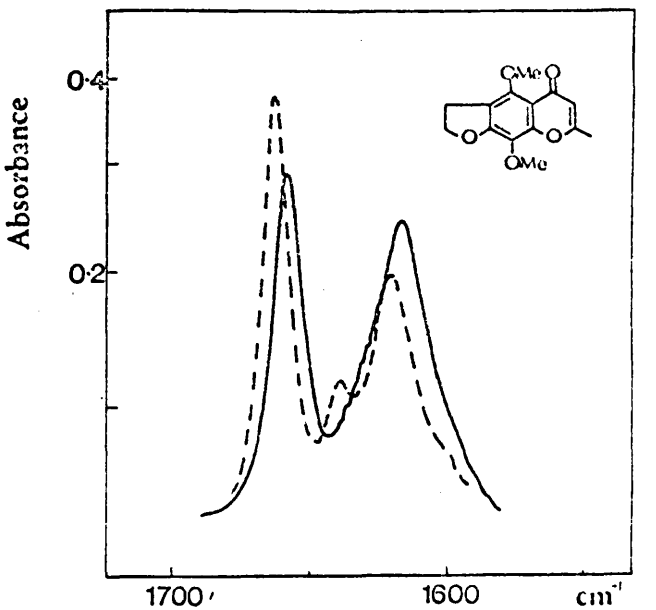


Fig. 21.

Chloroform solution (——). Carbon tetrachloride solution (----).

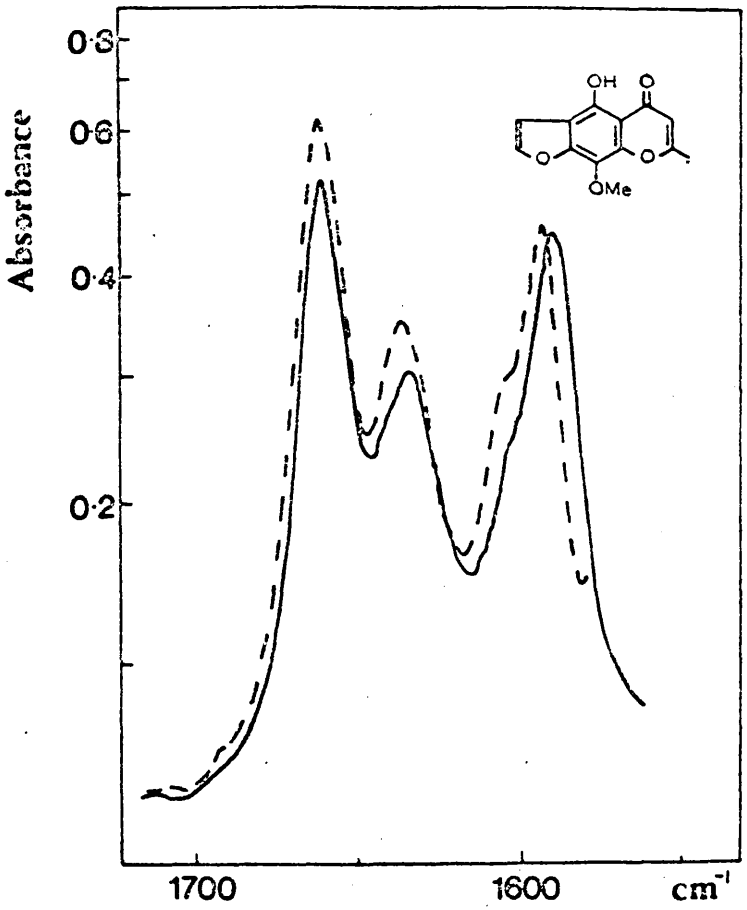


Fig. 22.

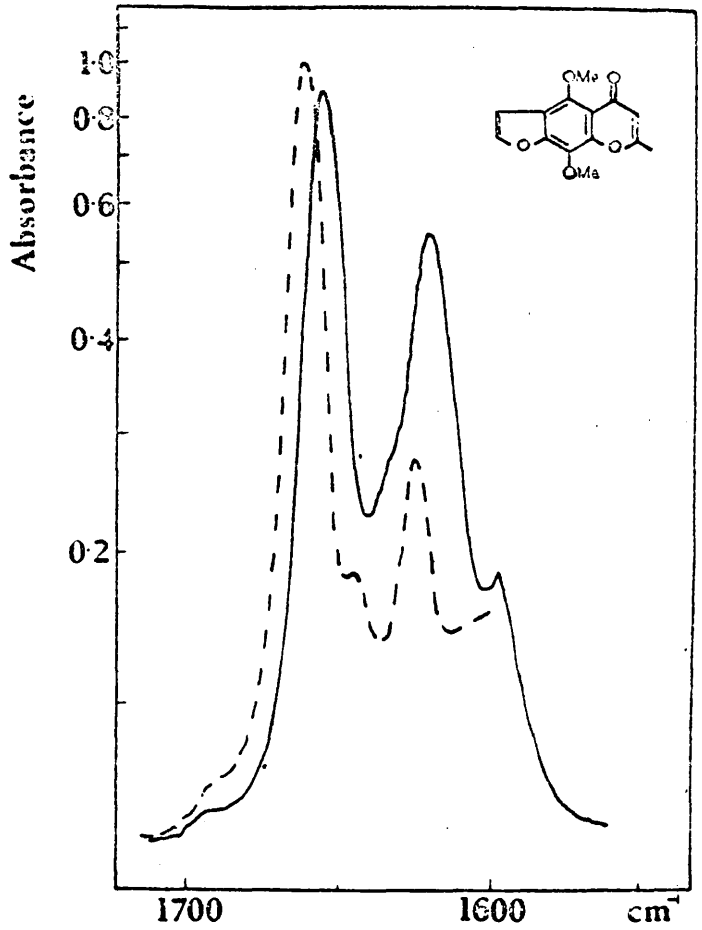


Fig. 23.

Chloroform solution (——). Carbon tetrachloride solution (----).

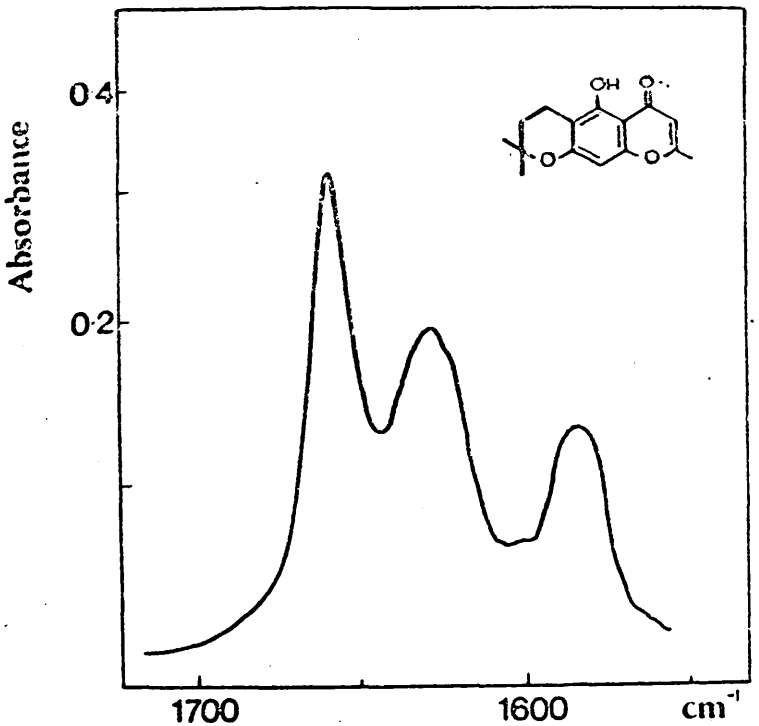


Fig. 24.

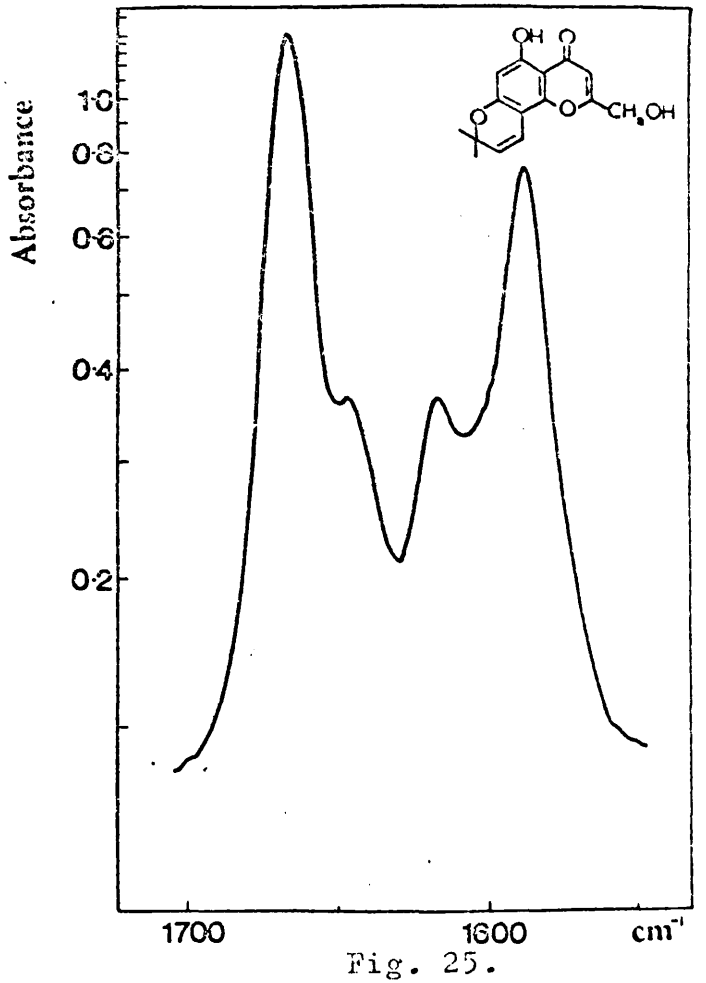


Fig. 25.

Chloroform solution (——).

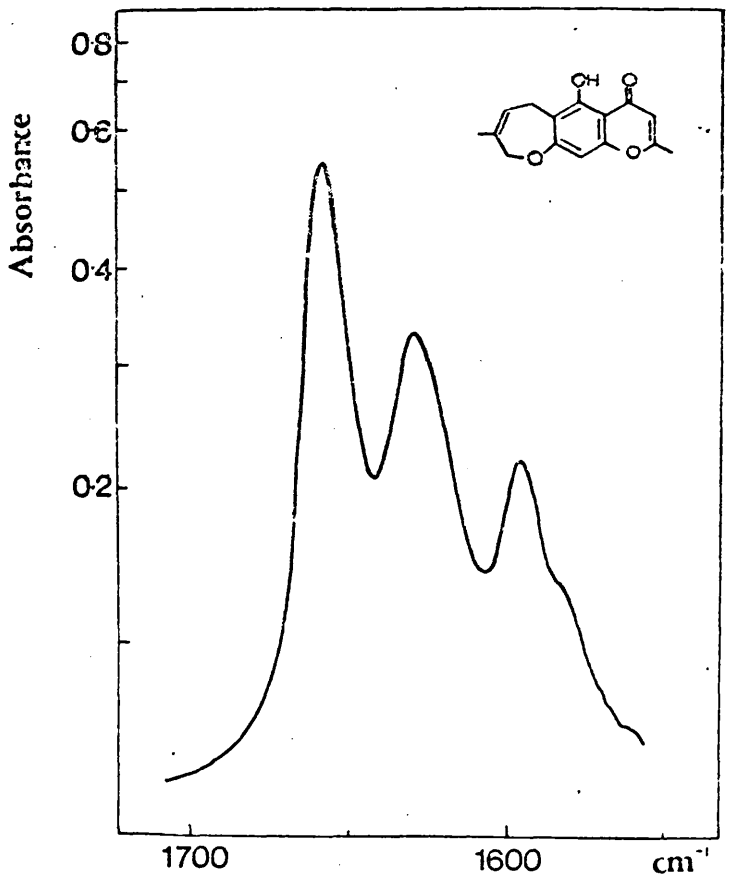


Fig. 26.

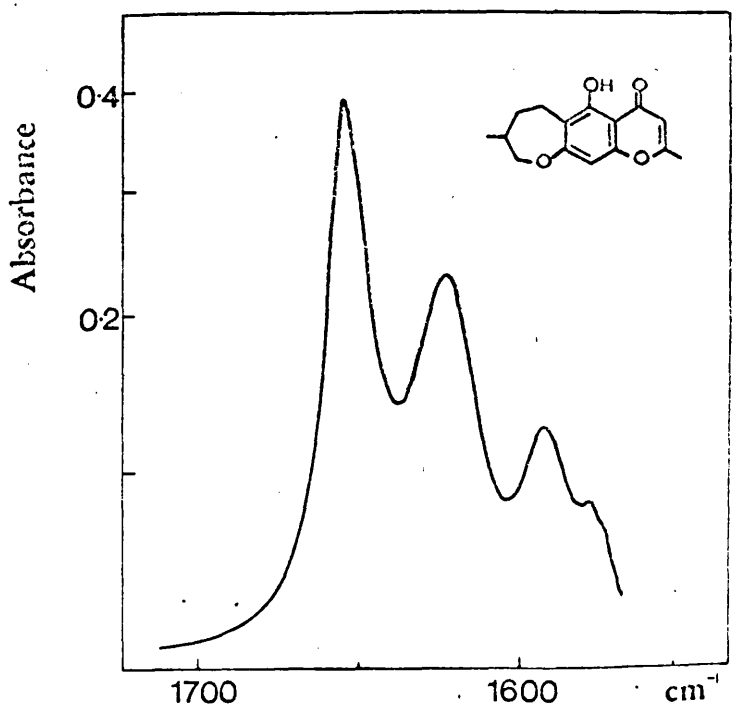


Fig. 27.

Chloroform solution (——).

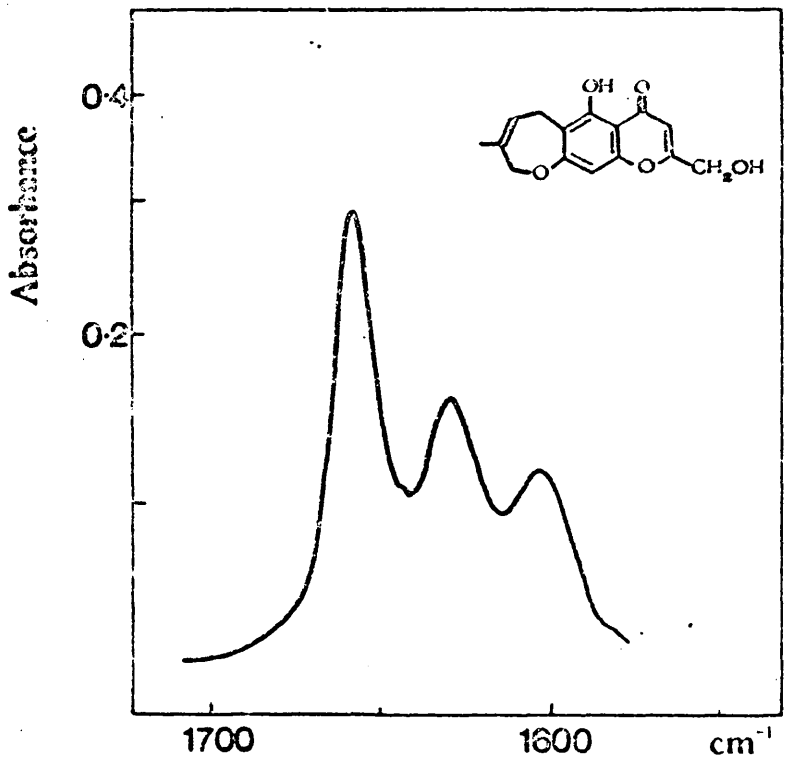


Fig. 28.

Chloroform solution (——).

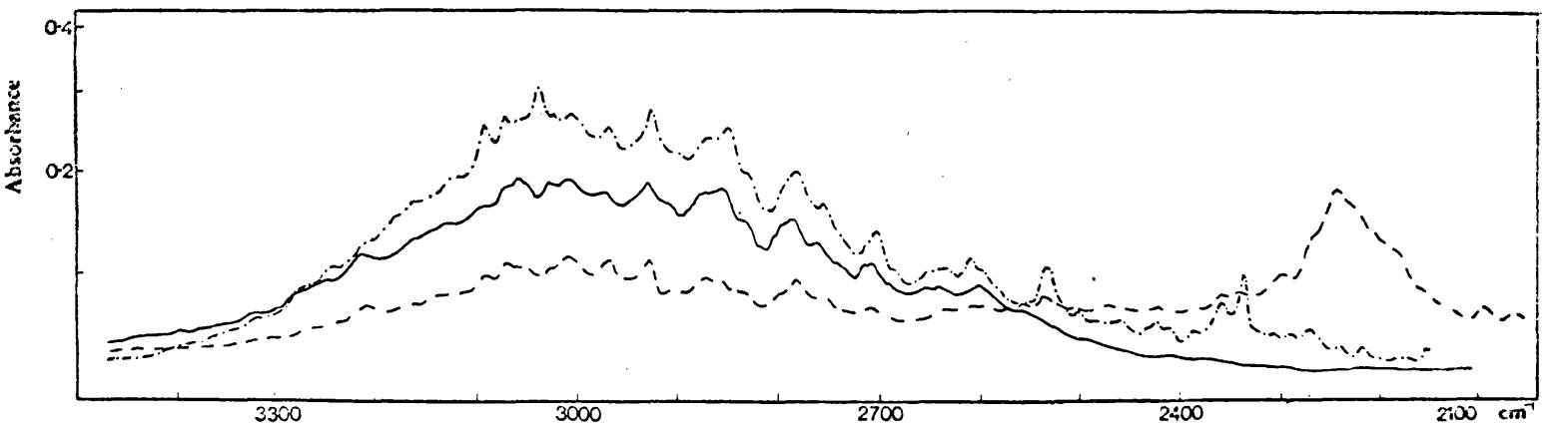


Fig. 29.

5-Hydroxy-2-methylchromone (——). 5-Hydroxy-2-methylchromone, deuterium-enriched 5-hydroxyl (----). Oxygen-18-enriched 5-hydroxy-2-methyl-chromone (-·-·-·-·). Solutions in carbon tetrachloride.

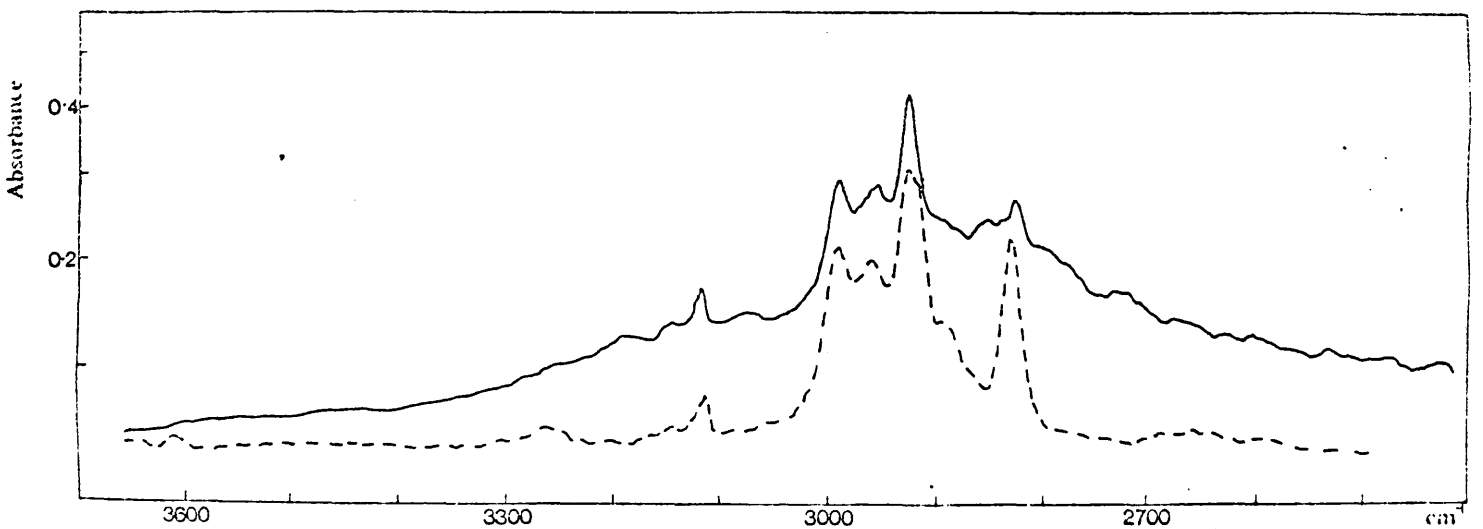


Fig. 30.

Khellinol (——). Khellin (----). Solutions in carbon tetrachloride.

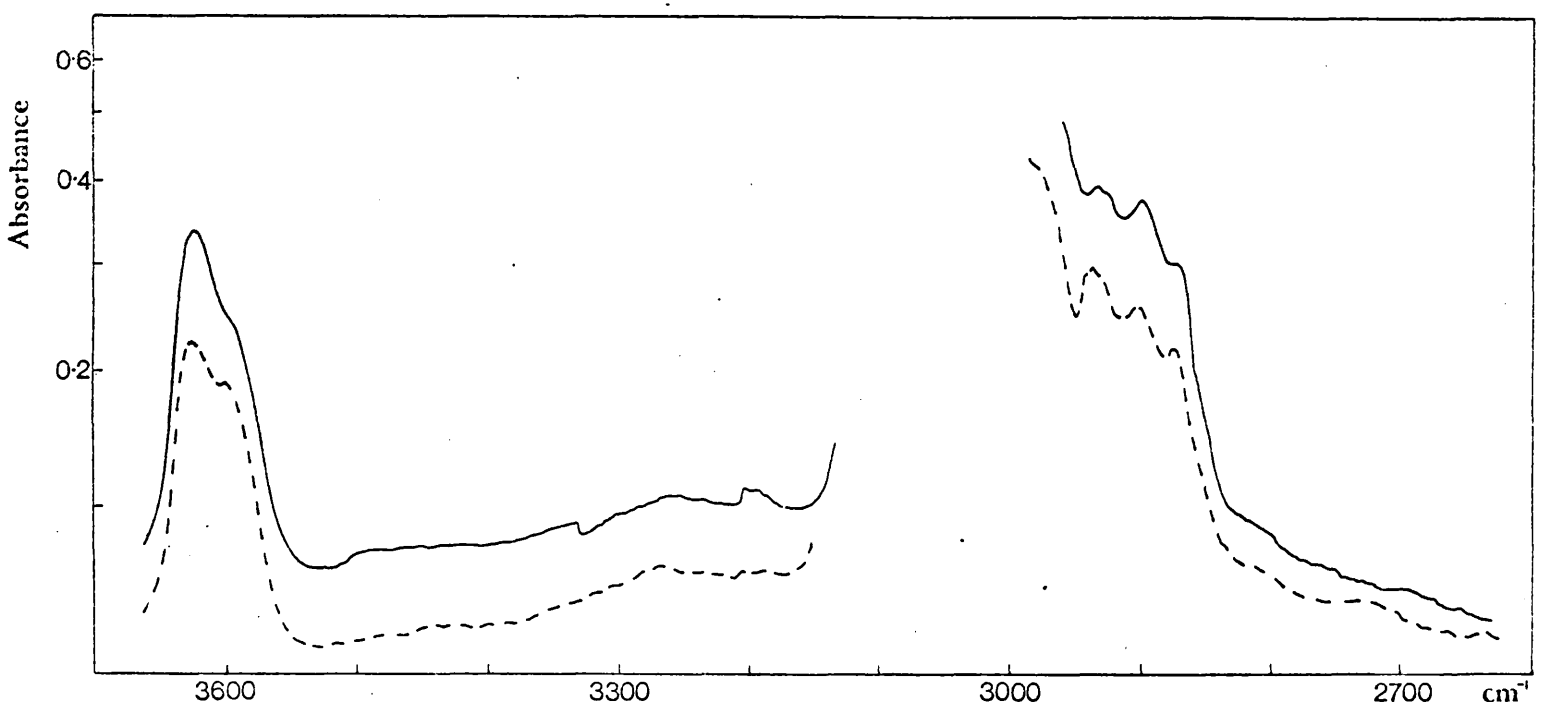


Fig. 31.

Dihydroheteropeucenin (——). Dihydropeucenin (----). Solutions in chloroform. (The discontinuity in the curves is the region in which chloroform transmits less than 25% of the incident radiation.)

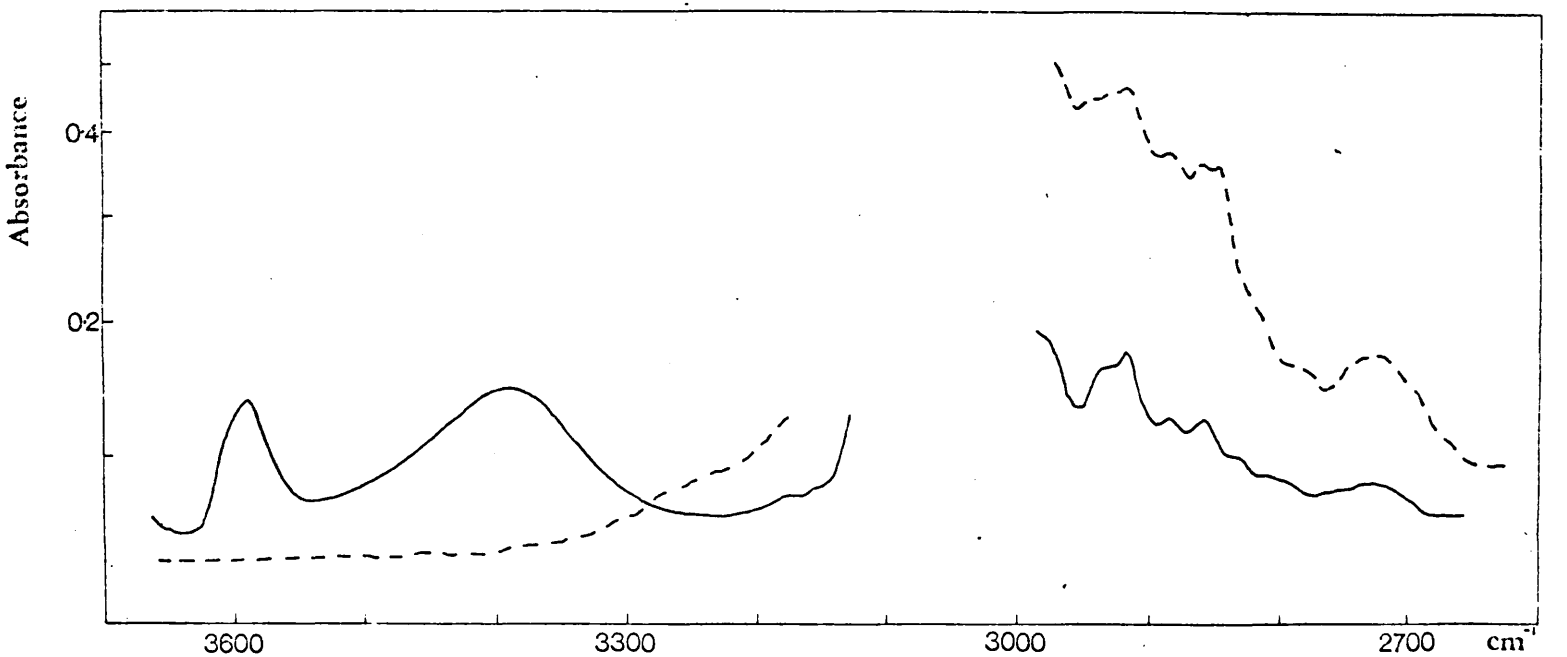


Fig. 32.

Peucenin (——). Peucenin 7-methyl ether (----). Solutions in chloroform.

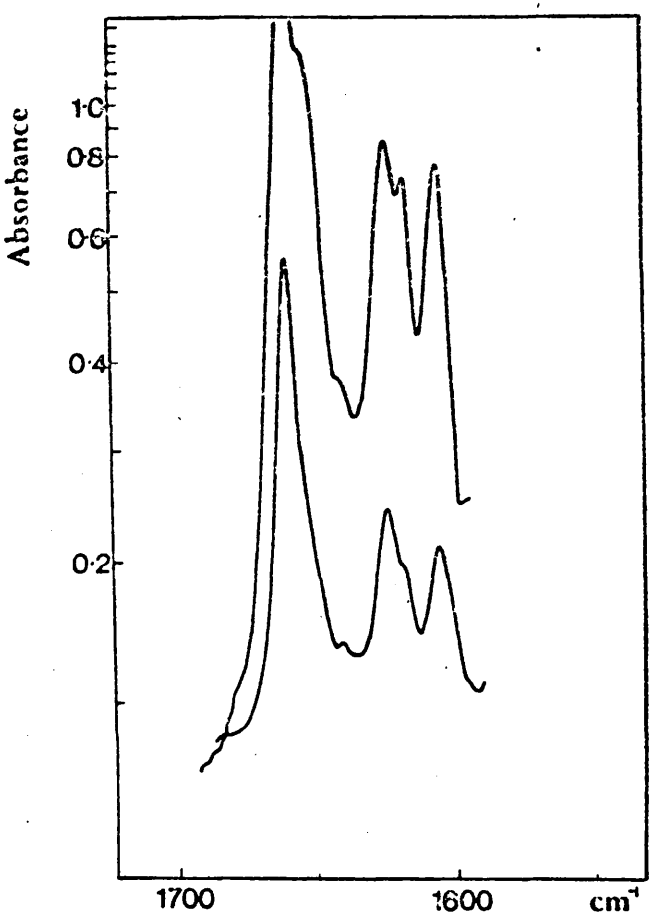


Fig. 33.

5-Hydroxy-2-methylchromone, deuterium-enriched 5-hydroxyl, in carbon tetrachloride [0·5 mm. and 0·125 mm. cell paths].

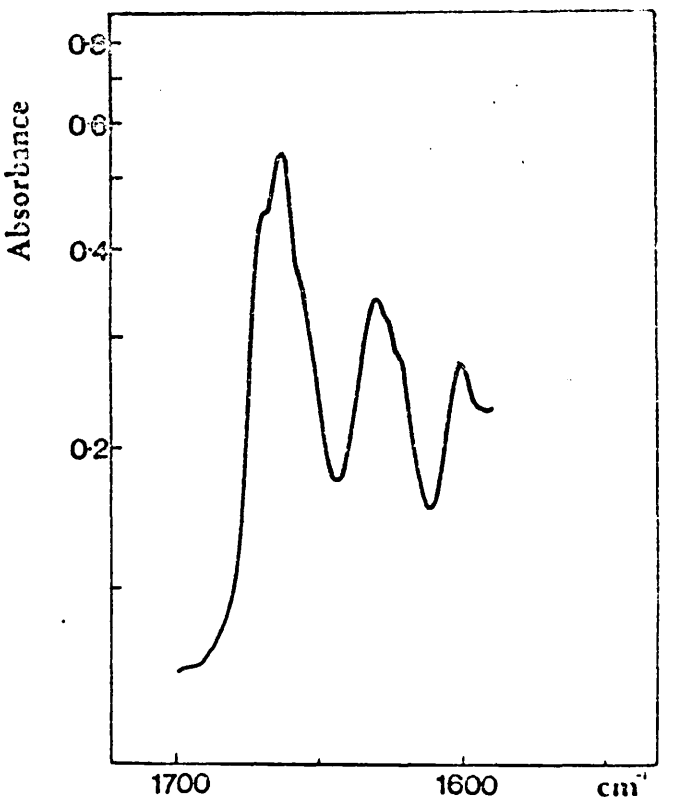


Fig. 34.

5-Hydroxy-7-methoxy-2-methylchromone, deuterium-enriched 5-hydroxyl, in carbon tetrachloride.

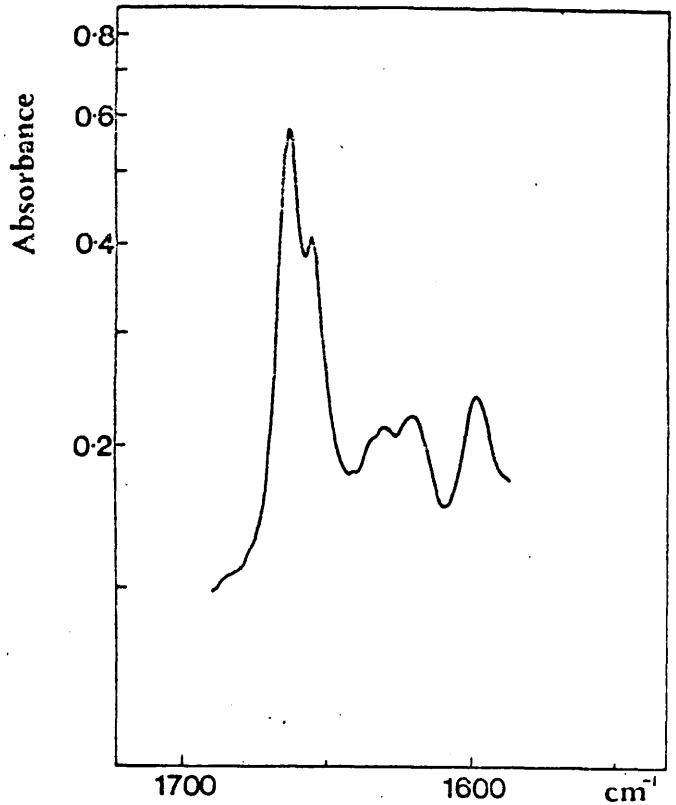


Fig. 35.

Peucenin 7-methyl ether, deuterium-enriched 5-hydroxyl, in carbon tetrachloride.

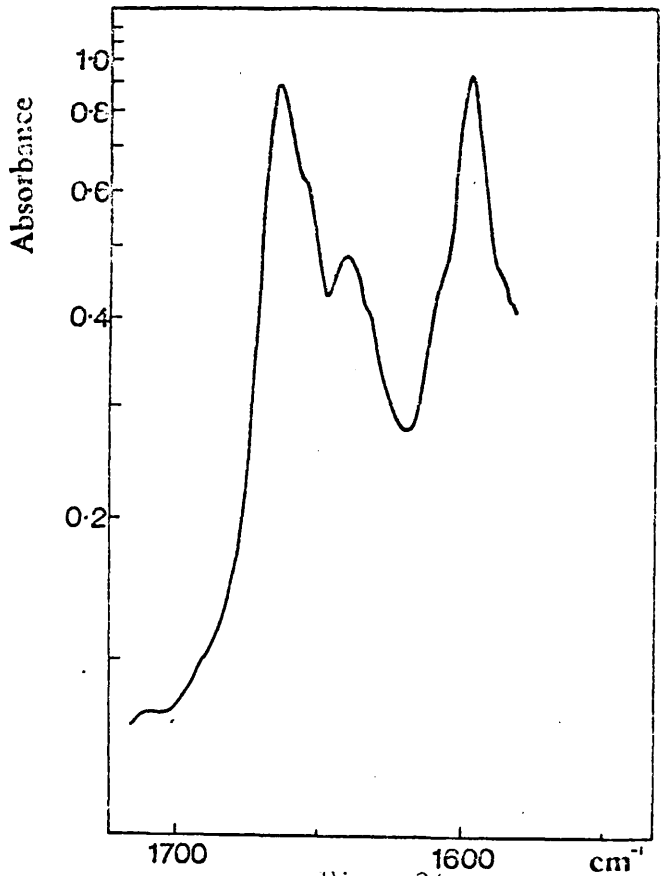


Fig. 36.

Khellinol, deuterium-enriched 5-hydroxyl, in carbon tetrachloride.

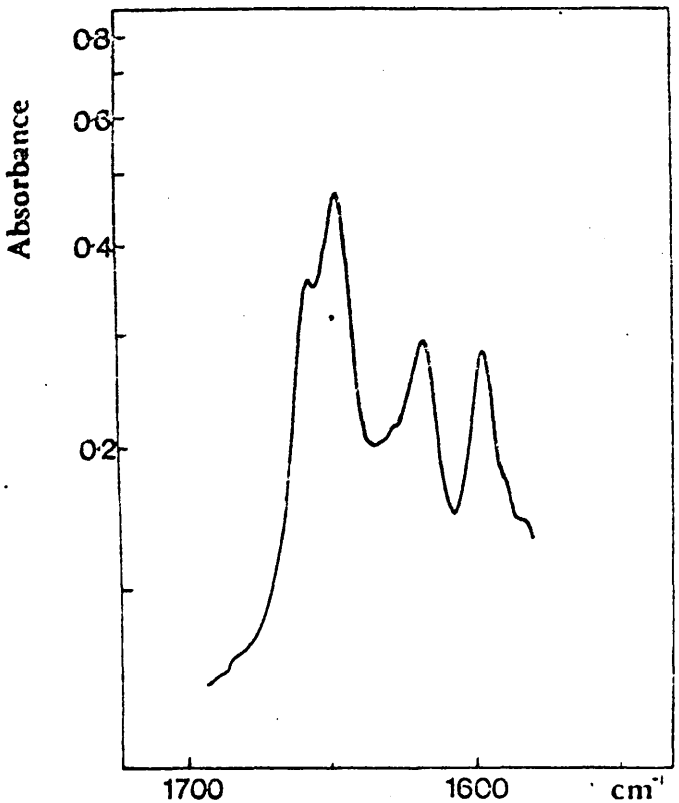


Fig.37.

Ptaeroxylin, deuterium-enriched 5-hydroxyl, in carbon tetrachloride.

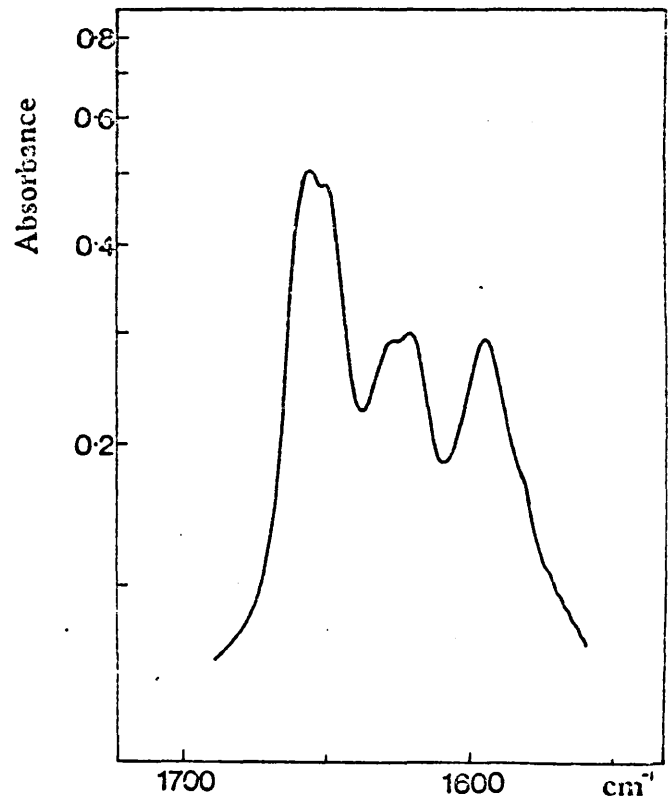


Fig. 38.

Karenin, deuterium-enriched 5-hydroxyl, in chloroform.

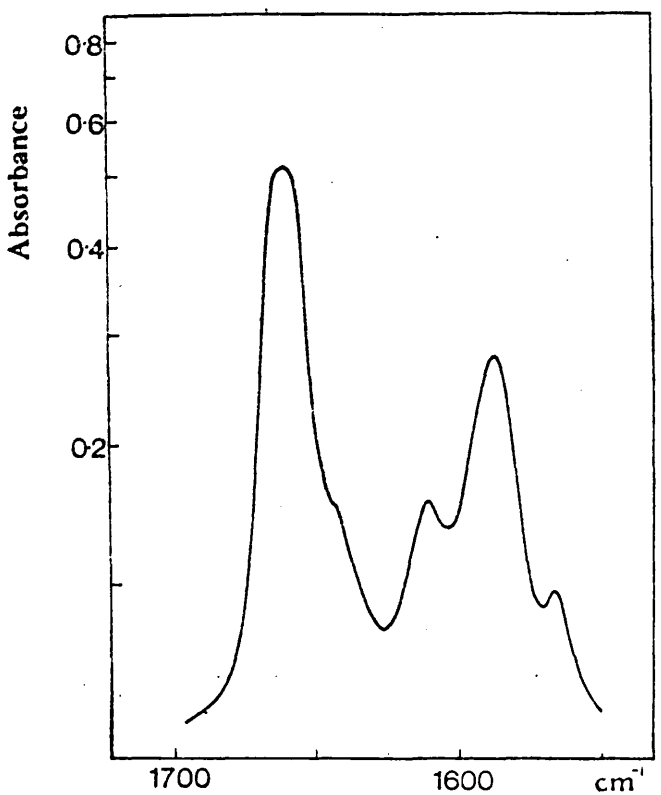


Fig. 39.

Ptaerochromenol, deuterium-enriched 5-hydroxyl, in chloroform.

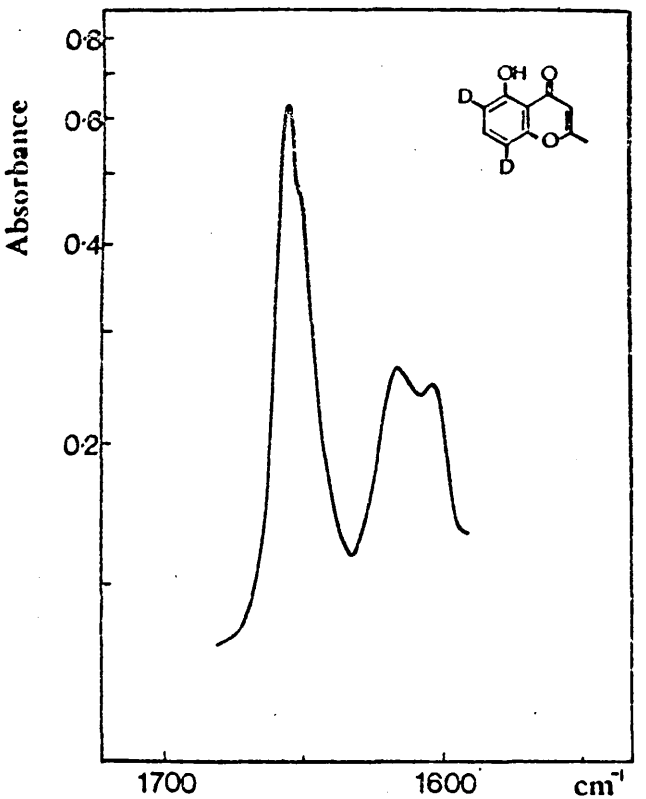


Fig. 41.

5-Hydroxy-2-monodeutero-methylchromone-6,8,-D_2 in carbon tetrachloride.

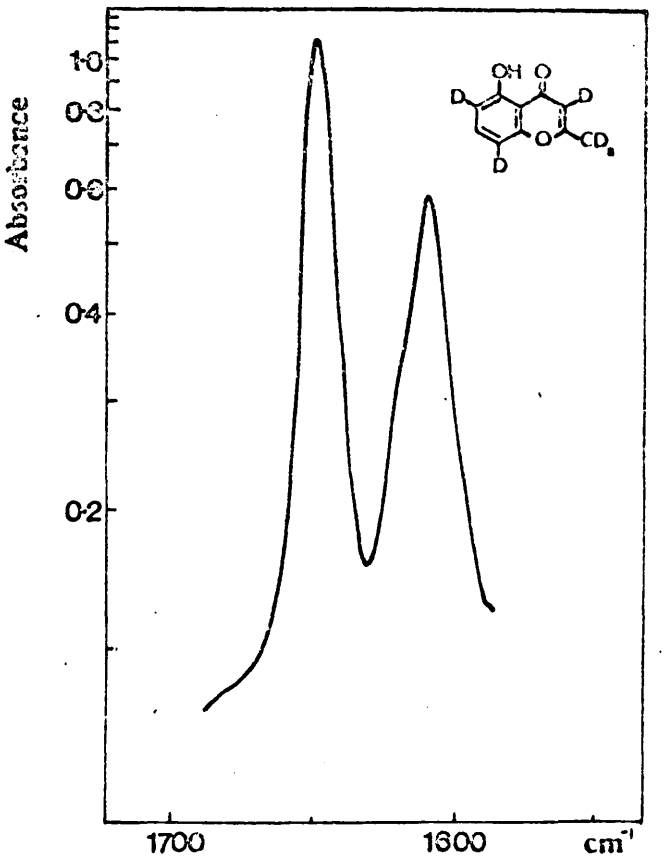


Fig. 40.

5-Hydroxy-2-trideutero-methylchromone-3,6,8-D_3 in carbon tetrachloride.

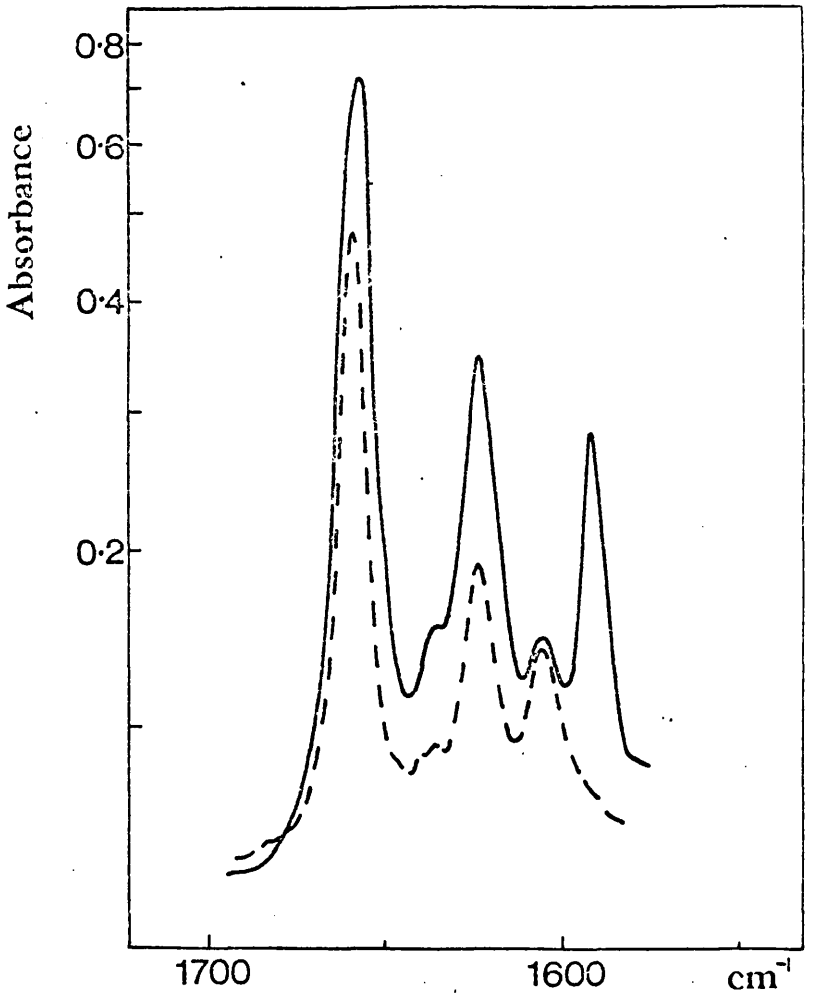


Fig. 42.

Oxygen-18-enriched 5-hydroxy-2-methylchromone (——). 5-Hydroxy-2-methylchromone (----). Solutions in carbon tetrachloride.

Experimental.

Infrared solution measurements were carried out by Mrs. F. Lawrie and Miss A.M. Robertson, Glasgow, on a Unicam S.P. 100 Mark II spectrophotometer (prism/grating monochromator) operated with evacuated optics. The spectra were recorded linearly in wavenumbers as optical density and calibration was checked against the spectrum of water vapour after each set of measurements. Frequency determinations for resolved carbonyl and hydroxyl bands are believed to be accurate to ± 1 cm.$^{-1}$. Resolved peaks were approximately symmetrical. The apparent half-band widths, $\Delta\nu_{\frac{1}{2}}^{a}$, are quoted to the nearest integer; where necessary, they were determined by reflection of the undisturbed wings of unsymmetrical bands. Intensities are specified as apparent extinction coefficients, ε^{a} (l. mole^{-1} cm.$^{-1}$), rounded to the nearest five units, measured from a solvent-solvent base line and corrected, where necessary, for enhancement of intensity by contiguous bands. Infrared spectra (625-4000 cm.$^{-1}$) in the solid state (pressed disc) were recorded on a Perkin-Elmer 225 double beam spectrophotometer. A blank disc was inserted in the reference beam and calibration was made against the spectrum of polystyrene. Solution spectra at elevated temperature were

recorded in tetrachloroethylene on the Perkin-Elmer 225 instrument. Melting points were determined on a Kofler hot-stage apparatus. N.m.r. spectra were recorded by Mr. J. Gall and Mrs. S.J. Hamilton on a Perkin-Elmer R 10 and a Varian HA 100 on solutions in deuterochloroform with tetramethylsilane as internal standard. Mass spectra were measured by Drs. T.A. Bryce and J.D. Roberts on A.E.I. MS 9 and MS 12 spectrometers and micro-analysis were carried out by Mr. J.M.L. Cameron and his staff.

(i) Synthesis and Characterisation of Compounds

The simple 2-methylchromones were prepared by the standard technique[94] of condensation (Scheme 10) of an o-hydroxyacetophenone derivative (115) with a C_2 unit (from acetic anhydride or ethyl acetate) and mild base hydrolysis of the resulting 3-acetylchromone (116). Chromatographic techniques were employed where appropriate and, in general, replaced the methods of isolation recommended in the literature. It was found that the efficiency of the condensation step improved with increasing oxygenation of the starting acetophenone.

Chromone (1).[95]

Sample, m.p. 55°, supplied by Dr. R.I. Reed (Glasgow).

2-Methylchromone (101).

2-Hydroxyacetophenone (83) (2 g.) and sodium acetate (1·5 g.) were refluxed for 25 hr. in acetic anhydride (15 ml.). On cooling, water (20 ml.) was added and the mixture extracted with ethyl acetate. The crude product, a mixture of 2-hydroxyacetophenone, 3-acetyl-2-methylchromone, and 2-methylchromone, was gently refluxed for 2 hr. with 10% aqueous sodium carbonate (30 ml.) and a portion of the resulting mixture (2-hydroxyacetophenone and 2-methylchromone) was separated by preparative t.l.c. in chloroform. 2-Methylchromone (225 mg.), the more polar component, crystallised from light petroleum as pale yellow needles, m.p. 70-71° (lit.[96] m.p. 71°); n.m.r. signals at τ 2·04 (m, ~ 1H) and 2·75 (m, ~3H) [4 aromatic protons], 4·06 (s, 1H) [olefinic proton] and 7·63 (s, 3H) [2-methyl group].

5-Hydroxy-2-methylchromone (6)

2,6-Dihydroxyacetophenone (117) (1·5 g.) and sodium acetate (1·6 g.) were refluxed for 15 hr. in acetic anhydride (20 ml.). The reaction mixture was poured on to ice and extracted with ethyl acetate. After removal of solvent, the discoloured crystalline solid was refluxed for 2 hr. with 10% aqueous sodium carbonate (20 ml.) and, on cooling, deposited 5-hydroxy-2-methylchromone as brown, greasy needles. These were purified by treatment with activated charcoal and crystallised from aqueous ethanol as pale yellow needles (1 g.), m.p. 90-91° (lit.[97] m.p. 92°); n.m.r. signals at τ 7·64 (s, 3H) [2-methyl group], 4·04 (s, 1H) [olefinic proton], and seven peaks (τ 2·49, 2·62, 2·76, 3·22, 3·30, 3·35, 3·45)[3 aromatic protons]; mass spectral peaks at m/e 176 (molecular ion), 148, 136, and 108 (relative abundance 100, 39, 27, and 46%).

The 2,6-dihydroxyacetophenone (117) employed in this synthesis was prepared from 4-methylumbelliferone (7-hydroxy-4-methylcoumarin) by the method of Limaye and Baker.[98] 7-Acetoxy-4-methylcoumarin (118) was rearranged in an aluminium chloride melt at 160° (Fries conditions) to a mixture of the 6- and 8-acetyl-7-hydroxy-4-methylcoumarins

(119 and 120), which were separated by fractional crystallisation from ethanol. The efficiency of the separation was checked by analytical t.l.c. in methanol-chloroform (1:19) in which the components were marginally separated. The purified 8-acetyl isomer (120), m.p. 160-162° (lit. m.p. 163°) was hydrolysed in refluxing aqueous sodium hydroxide (12%) in a nitrogen atmosphere to 2,6-dihydroxyacetophenone m.p. 153-154° (lit. m.p. 155°).

5-Methoxy-2-methylchromone (102)

Methyl iodide (2 ml.) was added to a stirred suspension of 5-hydroxy-2-methylchromone (6) (110 mg.) and anhydrous potassium carbonate (AnalaR) (850 mg.) in dry acetone (AnalaR) (12 ml.) and the mixture was refluxed under stirring for 11 hr. After filtration and removal of solvent, the crude 5-methyl ether (102) was purified by preparative t.l.c. [methanol-chloroform (1:19)] and crystallised from chloroform-light petroleum as pale yellow flakes (100 mg.), m.p. 98-100° (lit.[97c] m.p. 105°). A portion of this material sublimed at 95°/0·01 mm. as colourless prisms, m.p. 100-102°; n.m.r. signals at τ 7·71 (s, 3H), 6·07 (s, 3H) [methoxyl group], 4·01 (s, 1H), and seven peaks (τ 2·40, 2·55, 2·68, 3·04, 3·19, 3·22, 3·38) [3 aromatic protons].

5,7-Dihydroxy-2-methylchromone (110)

Reaction of phloroacetophenone (21) (858 mg.) with acetic anhydride (10 ml.) and sodium acetate (940 mg.) was conducted as in the preparation of 2-methylchromone. In this case, however, it was necessary to acidify (hydrochloric acid) the sodium carbonate hydrolysis solution to precipitate the acidic 7-hydroxychromone product. The collected 5,7-dihydroxy-2-methylchromone crystallised from methanol as discoloured flakes (750 mg.), m.p. $\sim 275^{\circ}$ (lit.[99] m.p.290°), which were purified by sublimation at $160^{\circ}/0{\cdot}004$ mm. The colourless microcrystalline sublimate, m.p. $282\text{-}285^{\circ}$ (decomp.), on account of its insolubility, was characterised after methylation (vide infra).

5-Hydroxy-7-methoxy-2-methylchromone (103)

5,7-Dihydroxy-2-methylchromone (110) (108 mg.) was reacted with excess ethereal diazomethane for 20 hr. After evaporation to dryness and filtration in chloroform through alumina (Grade I; neutral) to remove polymethylene, the crude product was purified by t.l.c. (two developments in chloroform). The less polar band furnished 5-hydroxy-7-methoxy-2-methylchromone (103) (80 mg.) which crystallised

from ether as pale yellow needles, m.p. 119-120° (lit.[100] m.p. 120°) (Found: C, 64·25; H, 4·9. Calcd. for $C_{11}H_{10}O_4$ C, 64·1; H, 4·9%); n.m.r. peaks at τ 7·68 (s, 3H), 6·19 (s, 3H) [methoxyl group], 4·00 (s, 1H), and 3·69 (s) [2 aromatic protons]. The band of lower Rf contained unreacted substrate (15 mg.).

5,7-Dimethoxy-2-methylchromone (104)

5,7-Dihydroxy-2-methylchromone (110) (110 mg.) was reacted for 15 hr. with methyl iodide (3 ml.) in a stirred suspension of AnalaR potassium carbonate (800 mg.) in refluxing anhydrous acetone (AnalaR) (12 ml.). The product, 5,7-dimethoxy-2-methylchromone (104) (85 mg.), was isolated by preparative t.l.c. in chloroform and crystallised from ether as yellow needles, m.p. 123-124° (lit.[101] m.p. 124°) (Found: C, 65·5; H, 5·3. Calcd. for $C_{12}H_{12}O_4$ C, 65·4; H, 5·5%); n.m.r. peaks at τ 7·76 (s, 3H), 6·15 (s, 3H) [methoxyl group], 6·09 (s, 3H) [methoxyl group], 4·03 (s, 1H), 3·71 (d, 1H; J = 2 c./sec.) [aromatic proton], and 3·62 (d, 1H; J = 2 c./sec.) [aromatic proton].

Peucenin (14), ptaeroxylin (41), and karenin (42), were isolated from the heartwood of Ptaeroxylon obliquum. The preparation of peucenin derivatives was described in Part 1. Heteropeucenin-7-methyl ether (47), m.p. 110°, ptaerochromenol (40), m.p. 175°, and umtatin (36), m.p. 178°, also natural products of P. obliquum,[10,22,56] were supplied by Dr. F.M. Dean.

Khellin (16) and visamminol (35) were isolated from the seeds of Ammi visnaga L. The extraction procedure and the preparation of khellin derivatives are described below. A sample of isovisnagin, m.p. 240°, was donated by Dr. R.I. Reed.

Extraction of Ammi visnaga L.

The ether extract of finely ground seeds (1·2 kg.), on removal of solvent, gave a brown gum (120 g.). Trituration of this gum with light petroleum (b.p. 60-80°) allowed khellin (16) to precipitate as a greenish amorphous solid (20 g.), which crystallised from methanol as pale yellow needles, m.p. 155-156° (lit.[24,25,102] m.p. 153°); n.m.r. signals at τ 7·70 (s, 3H), 6·06 (s, 3H) [methoxyl group], 5·94 (s, 3H) [methoxyl group], 4·10 (s, 1H), 3·12 (d, 1H; J = 2 c./sec.) [furan proton], and 2·50 (d, 1H;

J = 2 c./sec.) [furan proton]; mass spectral peaks at m/e 260 (molecular ion), 245, 231, 217, 216, and 189 (relative abundance 100, 97, 40, 45, 78, and 27%). The seeds, after extraction with ether, were re-extracted with methanol. A portion of the dark brown viscous oil obtained was separated by t.l.c. [methanol-chloroform (1:19)] and a compound, slightly lower in Rf than khellin, was eluted from the most concentrated band. This compound, visamminol (35), was further purified by preparative t.l.c. in chloroform and crystallised from ethyl acetate-ether as pale yellow needles (50 mg.), m.p. 159-160° (lit.[24,103] m.p. 160°); n.m.r. signals at τ 8·79 (s, 3H), 8·68 (s, 3H), 8·15 [hydroxyl proton], 7·69 (s, 3H), 6·89 (d, 2H; J = 9 c./sec.) [benzyl protons], 5·25 (t, 1H; J = 9 c./sec.), 4·01 (s, 1H), and 3·73 (s) [aromatic proton].

A portion (8 g.) of the ether extract after removal of the crystalline khellin was chromatographed over silica gel (400 mg.) by elution with ethyl acetate-light petroleum mixtures of increasing polarity. However, efficient separation of the components (at least fifteen) was not achieved and further isolation was not attempted.

Khellinol (37)

Khellin (16) (1 g.) in methanol (15 ml.) was refluxed for 40 min. with 50% hydrobromic acid (10 ml.). The product, khellinol (37), was filtered, washed with water, and crystallised from ethyl acetate as orange needles, m.p. 201-203° (lit.[24,104] m.p. 201°) n.m.r. signals at τ 7·58 (s, 3H), 5·91 (s, 3H) [methoxyl group], 3·97 (s, 1H), 3·02 (d, 1H; J = 2 c./sec.) [furan proton], and 2·40 (d, 1H; J = 2 c./sec.) [furan proton]; mass spectral peaks at m/e 246 (molecular ion), 231, 191, and 163 (relative abundance 43, 100, 10, and 26%).

Dihydrokhellin (108)

Khellin (16) (320 mg.) in ethyl acetate (15 ml.) was hydrogenated over 5% rhodium-charcoal[105] for 22 min. After filtration, the product was adsorbed on a preparative chromatoplate, development of which in methanol-chloroform (1:49) gave four concentrated bands. The most polar furnished dihydrokhellin (108) (140 mg.), needles, m.p. 146-147° (lit.[106] m.p. 150°) from methanol (Found: C, 64·1; H, 5·4. Calcd. for $C_{14}H_{14}O_5$ C, 64·1; H, 5·4%); n.m.r. peaks at τ 7·67 (s, 3H), 6·69 (t, 2H; J = 8 c./sec.),

6·13 (s, 3H) [methoxyl group], 6·07 (s, 3H) [methoxyl group], 5·27 (t, 2H; J = 8 c./sec.), and 4·06 (s, 1H).

Unreacted khellin (70 mg.) was eluted from the band immediately above dihydrokhellin. The band of second highest Rf contained a tetrahydro-reduction product (121) (33 mg.), n.m.r. signals at τ 8·50 (d, 3H; J = 6 c./sec.) [2-Me], 7·49 (s, 1H) [3-H], 7·37 (s, 1H) [3-H], 6·78 (t, 2H; J = 8 c./sec.) [benzyl protons], 6·17 (s, 3H) [methoxyl group], 6·15 (s, 3H) [methoxyl group], 5·32 (t, 2H; J = 8 c./sec.), and ~5·42 (m, 1H) [2-H]. The uppermost band contained the corresponding chromanol (122) (16 mg.), n.m.r. absorption at τ 8·74 [hydroxyl proton], 8·59 (d, 3H; J = 6 c./sec.) [2-Me], 6·74 (t, 2H; J = 8 c./sec.) [benzyl protons], 6·21 (s, 3H) [methoxyl group], 6·16 (s, 3H) [methoxyl group], and 5·42 (t, 2H; J = 8 c./sec.).

Dehydrogenation of the dihydrofuranochromanone (121) (33 mg.) by gently refluxing for 2 hr. in benzene (6 ml.) with 2,3-dichloro-5,6-dicyanoquinone[107] (25 mg.) furnished dihydrokhellin (108). The product was isolated by filtration of the reaction mixture in chloroform through a short column of alumina (Grade I; neutral) and identified by mixed m.p., t.l.c., and n.m.r.

6-Ethyl-5,7-dihydroxy-8-methoxy-2-methylchromone (106)

Khellinol (37) (215 mg.) in ethyl acetate (10 ml.) was hydrogenated for 25 min. over Adams catalyst.[108] Isolated by preparative t.l.c. in chloroform, the hydrogenolysis product (106) crystallised from methanol as pale yellow needles (160 mg.), m.p. 177-179° (Found: C, 62·5; H, 5·6. $C_{13}H_{14}O_5$ requires C, 62·4; H, 5·6%); n.m.r. peaks at τ 8·86 (t, 3H; J = 8 c./sec.) [side chain terminal methyl], 7·63 (s, 3H) [2-Me], 7·29 (q, 2H; J = 8 c./sec.) [benzyl protons], 6·08 (s, 3H) [methoxyl group], 4·01 (s, 1H), 3·49 (s) [hydroxyl proton].

6-Ethyl-7-hydroxy-5,8-dimethoxy-2-methylchromone (107)

Khellin (16) (208 mg.) in ethyl acetate (15 ml.) was hydrogenated for 20 min. over Adams catalyst.[108] The hydrogenolysis product (107), freed from catalyst and solvent, was purified by preparative t.l.c. [methanol-chloroform (1:19)] and crystallised from methanol as pale yellow needles (106 mg.), m.p. 169-171° (lit.[108] m.p. 173°); n.m.r. signals at τ 8·82 (t, 3H; J = 8 c./sec.) [side chain terminal methyl], 7·66 (s, 3H) [2-Me], 7·26 (q, 2H; J = 8 c./sec.) [benzyl protons], 6·17 (s, 3H) [methoxyl group], 6·03 (s, 3H) [methoxyl group], 4·03 (s, 1H), and 3·13 [hydroxyl proton].

(ii) Isotopic substitution of chromones

Deuterated chromones were obtained by exchange reactions with deuterium oxide (99·8 atom %) (Koch-Light). Isotopic exchange of oxygen atoms was carried out using oxygen-18 enriched water (normalised, H_2O^{18}; 60 atom %) (Miles Laboratories, inc.).

5-Deuteroxy enriched chromones

Samples (8-10 mg.) of the following chromones in anhydrous chloroform (1-2 ml.) (AnalaR; dried over silica gel) were shaken with deuterium oxide (five drops) at room temperature for 3 min. With the minimum exposure to moist air, the deuteration mixtures were evaporated to dryness under suction and the entire procedure was repeated four times. Residual deuterium oxide was removed in anhydrous benzene vapour under vacuum. Infrared spectra were recorded for 5-deuteroxy enriched samples of:

5-hydroxy-2-methylchromone (6)

5-hydroxy-7-methoxy-2-methylchromone (103)

peucenin-7-methyl ether (54)

ptaeroxylin (41)

khellinol (37)

karenin (42)

ptaerochromenol (40).

Chromones (40) and (42) contain a 2-hydroxymethyl group; in the above procedure, this hydroxyl group also became enriched.

From the mild conditions under which deuteration was carried out, it was not considered necessary to verify the structures of the deuterated samples by spectroscopic means other than infrared; in all cases, the i.r. spectra possessed the absorption bands of the original undeuterated compounds because of incomplete exchange. For the same reason, deuterium analysis was not attempted.

5-Hydroxy-2-monodeuteromethylchromone-6,8-D_2 (113).

5-Hydroxy-2-methylchromone (6) (14 mg.) in dioxan (0·1 ml.), deuterium oxide (0·6 ml.), and acetyl chloride (0·2 ml.) was heated at 96 ± 3° (oil bath temperature) for 70 hr. in a sealed tube. On cooling, the reaction solution was extracted with anhydrous chloroform (AnalaR). Filtration and evaporation to dryness furnished 5-hydroxy-2-monodeuteromethylchromone-6,8-D_2 as a pale yellow solid, m.p. 86-89°, n.m.r. signals at τ 7·64 (s, ~2H) [2-CH_2D], 3·94 (s, 1H) [3-H], and 2·54 (s, 1H) [7-H]. This sample was used directly for i.r. measurements without purification.

The acetyl chloride employed in the above preparation had been refluxed for 3 hr. over phosphorus pentachloride and distilled at 52° and the dioxan (AnalaR) was dried over sodium. The partial incorporation of deuterium, under the above conditions, into the 5-hydroxyl group of the chromone sample would be nullified by exposure to moist air during isolation and compilation of spectral data.

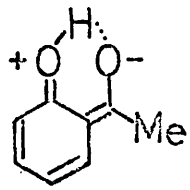
Alternative conditions in which 5-hydroxy-2-methylchromone (12 mg.) was heated in a sealed tube in presence of deuterium oxide (0·4 ml.) and 12N hydrochloric acid (0·06ml.) (pD $\sim$ -0·2), with and without added dioxan, were ineffective in promoting deuteration.

5-Hydroxy-2-trideuteromethylchromone-3,6,8-D_3 (114)

5-Hydroxy-2-methylchromone (6) (14 mg.) in dioxan (0·1 ml.), deuterium oxide (1·2 ml.), and acetyl chloride (0·8 ml.) was heated at 90 $\pm$ 3° in a sealed tube for 116 hr. Isolated by extraction with chloroform as before, 5-hydroxy-2-trideuteromethylchromone-3,6,8-D_2 sublimed at 76°/0·04 mm. as a colourless semi-solid, m.p. 87-89°, which showed one peak (τ 2·53) in the n.m.r. spectrum. A diffuse signal at τ 3·92 had 15-20% the area of the τ 2·53 peak.

Oxygen-18 enriched 5-hydroxy-2-methylchromone

A suspension of 5-hydroxy-2-methylchromone (12 mg.) in oxygen-18 enriched water (0·2 ml.) was treated with dry hydrogen chloride in absence of moist air until an increase in weight of approx. 100 mg. was obtained (~ 15 sec.) (pH of solution ~ - 1·1). The pyrilium chloride solution was heated in a sealed apparatus at 96 ± 3° (oil bath temperature) for 95 hr. and, after cooling, extracted with anhydrous chloroform (AnalaR). Removal of solvent under suction and sublimation of the residue at 75°/0·01 mm. furnished a crystalline product (10 mg.), m.p. 88-90° which was shown by mass spectral analysis to be 53 ± 2% $C_{10}H_8O_2^{16}O^{18}$, 2 ± 2% $C_{10}H_8O^{16}O_2^{18}$, and 40 ± 2% $C_{10}H_8O_3^{16}$. Examination of the M-28 (carbon monoxide) region of the mass spectrum showed that 23 ± 2% of the material contained oxygen-18 in the carbonyl group. From the mechanism of incorporation,[83,109] it is expected that, of the remaining 30 ± 2%, 15 ± 2% is located in the 5-hydroxyl group and 15 ± 2% in the heterocyclic ether oxygen. The infrared carbonyl region contained all the peaks of 5-hydroxy-2-methylchromone (due to the unlabelled compound present) in addition to a new peak at 1593 $cm.^{-1}$.



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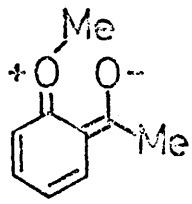

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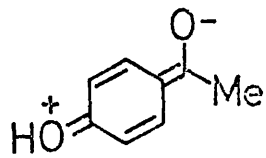

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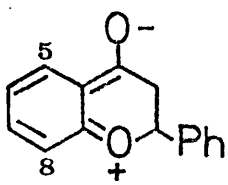

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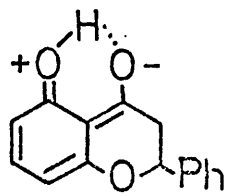

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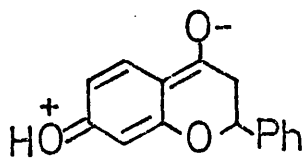

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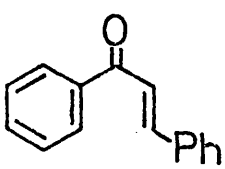

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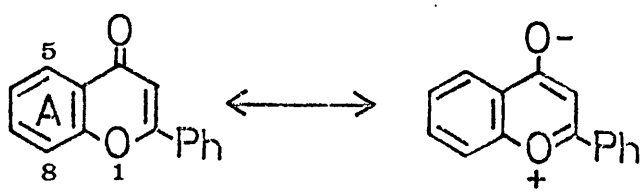

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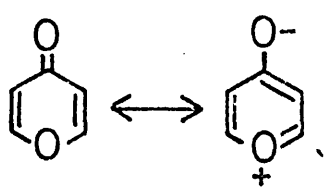

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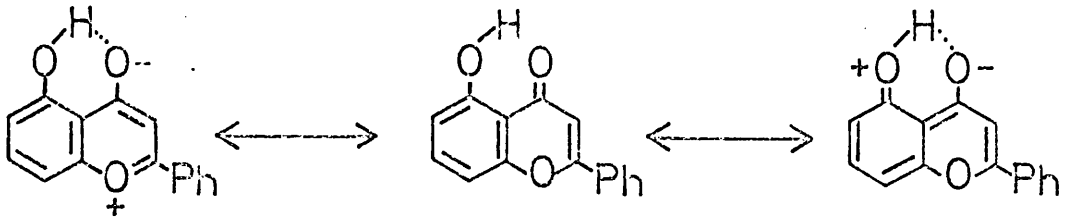

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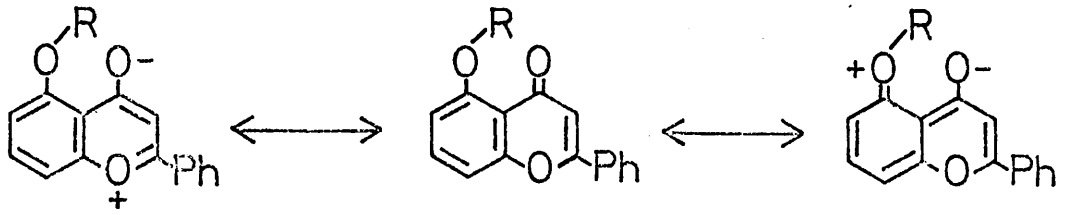

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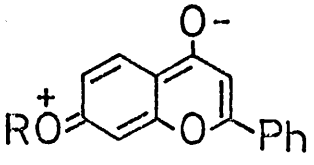

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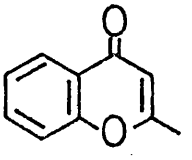

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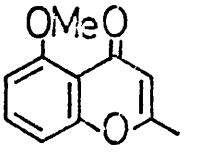

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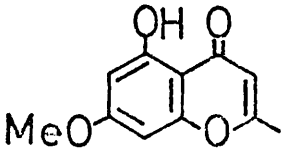

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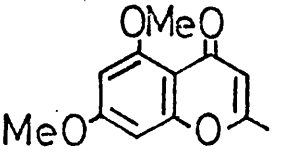

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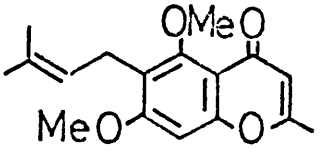

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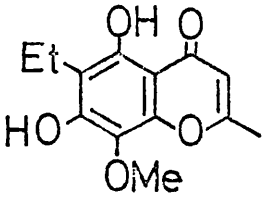

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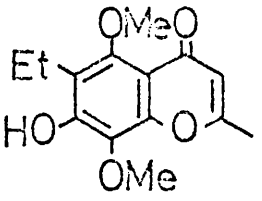

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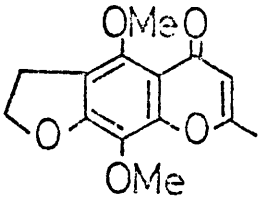

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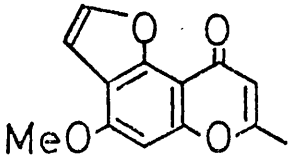

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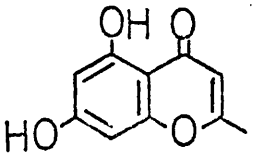

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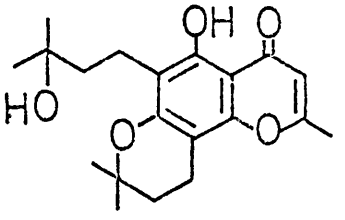

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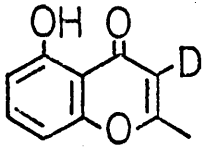

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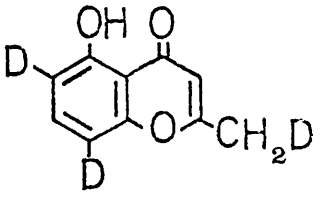

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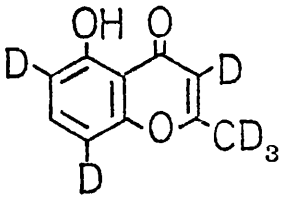

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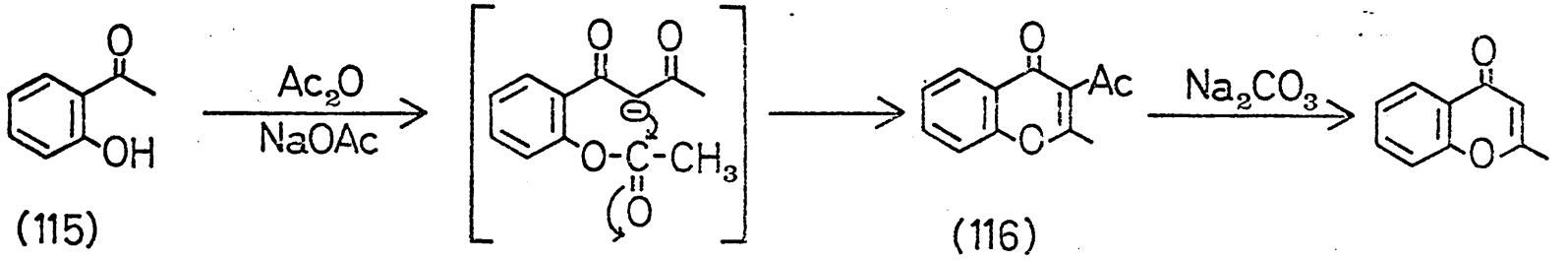
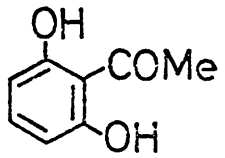


Scheme 10



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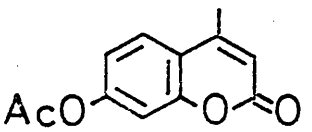

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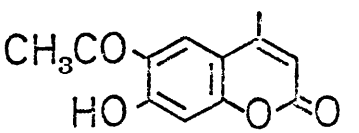

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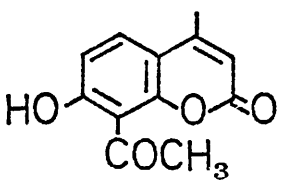

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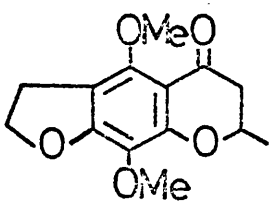

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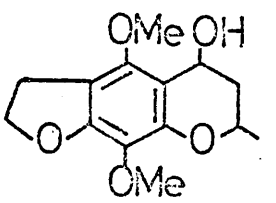


(122)

PART 3.

The Chemistry of Solidagenone.

Previous studies

The chemical constitution of Solidagenone, the major diterpenoid constituent of Golden Rod, Solidago canadensis L. (Compositae), has remained for some time inadequately solved.[110-112] In 1948 Houston and Burrell[110] found that, on extraction of S. canadensis root, the m.p. of the crystals first obtained was 90°, changing to 132° on recrystallisation. Ketonic and hydroxylic functional groups were assumed to be absent because of the inability of this compound to form carbonyl derivatives and because of its unreactivity toward metallic sodium in benzene. In 1965, Gerlach,[111] from chemical and mass spectral analysis, quoted the molecular formula of the diterpenoid as $C_{20}H_{28}O_3$, in agreement with the assignment of Houston and Burrell, but proposed structure (123), corresponding to a molecular formula of $C_{20}H_{26}O_3$. It is not obvious why a compound with this structure should not show the characteristics of a hydroxy-diketone. Moreover, the u.v. spectrum ($\lambda_{max.}$ 222 mμ) is incompatible with structure (123), which would be expected to show strong absorption in the 295 ($\varepsilon \sim 2000$) and 250 mμ ($\sim 14,000$) regions.[112,113]

More recently, Anthonsen[112] has verified the molecular formula $C_{20}H_{28}O_3$; his interpretation of spectral data and preliminary chemical reactions were recorded (vide infra) but no structure was proposed for the diterpenoid.

The appearance of signals at τ 2·62, 2·73, and 3·67 in the n.m.r. spectrum, taken with the u.v. data, were assigned to a β-substituted furan ring.[114] This was confirmed by the sharp C-H deformation frequencies in the i.r. spectrum at 763 and 873 $cm.^{-1}$ and by the less pronounced double bond stretching vibrations at 1502 and 1567 $cm.^{-1}$. The mass spectrum has peaks at m/e 81 (C_5H_5O) and 95 (C_6H_7O), typical of diterpenoids containing a 3-substituted furan residue.[115] Reaction of the diterpene with maleic anhydride smoothly yielded an adduct, the i.r. spectrum of which shows no furan absorptions; as expected, the adduct was reversibly reconverted into the starting materials by heating in vacuo. The diterpene further gives a positive Erlich test for furans and complexes with acetic acid-sulphuric acid producing an orange-red colour.[116]

By analogy with the fragmentation pattern of known diterpenoids, the mass spectrum shows that, contrary to the assignment of Gerlach, the diterpene $C_{20}H_{28}O_3$ possesses a normal ring A bearing no oxygen substituents. Thus a peak at m/e 192 arises from loss of C_9H_{16} from the parent ion, a metastable peak at m/e 117 corresponding to the transition $316^+ \rightarrow 192^+$. The m/e 192 ion undergoes further fission to fragments m/e 82 (C_5H_6O; base peak) and 110 ($C_6H_6O_2$), a metastable absorption at m/e 35 governing the transition $192^+ \rightarrow 82^+$.

The presence of one hydroxyl group is indicated by a fragment ion m/e 298 (M-18) in the mass spectrum, by a stretching vibration at $\sim$3550 cm.$^{-1}$ in the i.r., and by a sharp solvent-dependent peak in the n.m.r. at $\tau \sim 8$. The inability of the diterpenoid to form a trimethylsilyl ether demonstrated that the hydroxyl group is sterically hindered. Ready dehydration occurs in presence of acid catalysts but a complex mixture of products was obtained.

In agreement with the findings of Houston and Burrell, Anthonsen encountered no reactions characteristic of carbonyl groups and attributed the intense absorption at 1667 cm.$^{-1}$ in the i.r. spectrum to a vinyl ether

(HC=C-C-).[117] One of the major products of reduction of the diterpenoid with lithium aluminium hydride was a saturated ketone ($\nu_{max.}$ 1704 cm.$^{-1}$) which shows retention of all furan absorptions. Anthonsen ascribed the formation of this compound to the hydrogenolysis of the vinyl ether function.[118]

In the following discussion, chemical and spectroscopic evidence is presented leading to the assignment of the structure and stereochemistry to the diterpenoid $C_{20}H_{28}O_3$, now termed Solidagenone. This work, a continuance of the study in this department of similar diterpenoid compounds, was carried out in collaboration with T. Anthonsen.

The Structure of Solidagenone

The foregoing investigation has shown that solidagenone possess a normal terpenoid ring A and a 3-substituted furan system. These functions incorporate thirteen carbon atoms and one oxygen atom. The remaining C_7 moiety contains two oxygen atoms, one of which is a hindered hydroxyl group, the other associated with the intense i.r. absorption at 1678 cm.$^{-1}$ in the i.r. spectrum.

The nature of this chromophore was imparted by the u.v. spectrum. Contrary to Anthonsen's claims that the spectrum contains a single sharp maximum, the absorption at 223 mμ was found to be a broad composite band, the extinction coefficient of which was too large to be accountable merely in terms of a furan ring.[119] Subtraction of the furan absorption of the diterpenoid, marrubiin (124),[120] gave a single sharp maximum at 234 mμ (ε 9,800). This derived maximum is consistent with a β,β-disubstituted enone system (Woodward's rules) possessing a γ-hydroxyl group which has a hypsochromic effect (of ~ 6 mμ) on the chromophore.[121,122] That one of the β-substituents is a methyl group as shown by the doublet at

τ 7·98 in the n.m.r. spectrum at 100 Mc./sec., its coupling ($J \sim 1$ c./sec.) with the α-vinyl proton (τ 4·26) confirmed by double irradiation. The intense i.r. band at 1678 $cm.^{-1}$ is in agreement with the presence of an enone system.

The close proximity of the hydroxyl group and the furan ring was suggested by the i.r. absorptions (high resolution; CCl_4) at 3611 and 3567 $cm.^{-1}$, the relative intensities of which do not alter on dilution. These are assigned to free and intramolecularly hydrogen-bonded (OH-π) hydroxyl stretching frequencies respectively. Moreover, the absence of proton resonance of the type H-C-OH in the n.m.r. shows the hydroxyl to be tertiary.

Assuming a labdane skeleton,[123] structure (125) can now be postulated for solidagenone, $C_{20}H_{28}O_3$. The proposed arrangement of functional groups is as required by the i.r. and u.v. spectra and the remainder of the n.m.r. data can be readily accommodated by this structure. Thus, the methylene groups at positions 11 and 12 appear as two triplets ($J = 8$ c./sec.) centred at τ 7·98 and 7·30 respectively, the former protons being deshielded by the 9-hydroxyl group, the latter having an allylic relation to

the furan double bond. The singlet at τ 7·25 (1H) is attributed to the proton at C-5 and the quaternary methyl singlets appear at τ 9·01, 8·85, and 8·82.

Structure (125) is in accord with the chemical properties of solidagenone. The ketone on position 6 of the labdane terpenoids is known to be sterically hindered [cf. 6-oxogrindelic acid (126)[121] and 6-oxocativic acid (127)[124]] and would not thus be expected to react easily with bulky reagents (e.g. 2,4-dinitrophenylhydrazine). However, the enone system should be capable of reduction with lithium aluminium hydride to at least one saturated ketone, as is observed. The intramolecular association of the furan ring and the 9-hydroxyl group, which is also tertiary, explains the inability of solidagenone to form hydroxyl derivatives. Finally, structure (125) is biogenetically plausible since 6-oxogrindelic acid is present[121] in a member of the Grindelia genus (robusta) which is closely related to Solidago.

Solidagenone may well be an artefact. It was found that the concentrated light-petroleum extract of a Solidago hybrid deposited crystalline material, which melted unsharply around 103°. Recrystallisation of this

from light petroleum furnished colourless prisms, m.p. 108-110°, which contained no solidagenone but consisted of a mixture (approximately 1:1) of two compounds (128; epimers at C-13) [cf. the spiro-hemiacetal from Marrubium vulgare[125]]. Further concentration of the mother liquors gave small amounts of solidagenone (needles, m.p. 132°), which significantly could also be obtained in high yield by refluxing an ethanolic solution of the mixed spiro-ethers (128). These findings may explain the apparent discrepancy[110,111] in the melting points previously quoted for recrystallised samples of solidagenone.

The Stereochemistry of Solidagenone

An initial approach to the assignment of the stereochemistry of solidagenone envisaged a correlation with the diterpene lactone, marrubiin (124)[126] by converting both compounds into the dihydrosolidagenone (129). The keto-aldehyde (130),[126] derived from marrubiin by lithium aluminium hydride reduction and chromium trioxide-pyridine (Sarett) oxidation, reacted with ethane dithiol (Scheme 11) to form an oily thio-acetal (131), which underwent reduction with Raney nickel in acetone to the saturated

ketone (129), m.p. 89-90°. Solidagenone, on reduction with lithium aluminium hydride was expected to form two saturated ketones, epimeric at C-8, one of which (8α-methyl) would be identical to the ketone (129) derived from marrubiin (provided that the stereochemical configurations at positions 5, 9, and 10 were the same in both compounds). However, the only ketonic product obtained from hydride and catalytic (H_2; Pd/C; ethanol; triethylamine) reduction of solidagenone was solidaganone (132), m.p. 110-111°, possessing an 8β-methyl group (axial) and differing from (129) in chromatoplate mobility. Up till now conditions under which solidagenone can be reduced to compound (129), containing an 8α-methyl, have not been found. Thus, the proposed stereochemical interrelation of solidagenone and marrubiin was not achieved. The absolute stereochemistry of the two saturated ketones (129) and (132) is considered in detail later.

An alternative derivation of stereochemistry involved the interconversion of solidagenone into isoambrenolide (133) both C-8 epimers of which are known.[127] To this end solidaganone (132) was oxidised by chromic acid in acetic acid[128] to the keto-lactone (134) but attempts to remove the

highly hindered 6-ketone of this compound by modified Wolff-Kishner techniques were unsuccessful. Thus, once more, stereochemical interrelation was not secured.

The absolute configurations at C-5 and C-10 of solidagenone were shown to be the same as in marrubiin by the following sequence of reactions. Reduction of solidagenone with lithium in liquid ammonia or zinc in acetic acid furnished as the major product the βγ-unsaturated ketone (135), m.p. 78-79°, $[\alpha]_D$ +139°. Phosphoryl chloride-pyridine dehydration of the ketol (129) from marrubiin furnished two enones [Δ^8 and $\Delta^{9(11)}$] the more abundant (Δ^8) isomer, m.p. 77-79°, $[\alpha]_D$ +132°, being identical (mixed m.p., i.r., u.v., m.s., and t.l.c.) to the βγ-unsaturated ketone (135) from solidagenone. This verifies the A-B trans ring fusion in solidagenone.

The configuration at the remaining asymmetric centre (C-9) of solidagenone was derived as follows. The enone (135) on reaction with m-chloroperbenzoic acid in chloroform gave one furan-containing epoxide (136), which was isomerised in high yield to solidagenone (125) with β-naphthalene sulphonic acid in refluxing benzene. Formation of the α-epoxide and thus ring opening to the

9α-hydroxyl group can be predicted on the basis of earlier work[127] with closely analogous compounds. Thus, the stereochemistry of solidagenone is as shown in (137).

To support the stereochemical assignments made for solidagenone, a comparison of the methyl signals in the n.m.r. spectra of solidaganone (132), ketone (129) derived from marrubiin, and the solidaganol (138) was carried out. The methyl resonances of the two saturated ketones (132) and (129) were identified (Tables 9 and 10) by recording the n.m.r. spectra after progressive additions of benzene to deuterochloroform solutions and utilisation of the 'plane rule'.[126,129] Assignment of the resonances of the C-8 methyl groups (secondary) in both ketones is straightforward since they appear as doublets, τ 8·92 for (132) and 8·99 for (129) in deuterochloroform. The most probable assignments of the resonances of the quaternary methyl groups at position 4 were derived by comparison of their solvent shifts with those observed for hopan-6-one (139),[130] in which the equatorial 4-methyl group undergoes a downfield shift of 15 c./sec. (from solution in deuterochloroform to solution in benzene) and the axial 4-methyl group a downfield shift of 10 c./sec.

Thus, from the shifts observed for solidaganone (132) and the marrubiin ketone (129), the equatorial and axial 4-methyl resonances of solidaganone (132) can be assigned to the signals at τ 8·78 and 9·05 respectively and for ketone (129) at τ 8·77 and 9·02. The remaining unassigned resonances are those of the C-10 methyl groups which must therefore occur at τ 9·00 for solidaganone and τ 9·09 for the ketone (129). It can be seen that the solvent shifts exhibited by the C-4 and C-10 methyl groups are very similar in both ketones indicating that they are situated in similar stereochemical environments. Conversely, there is a considerable difference in the magnitude of the solvent shifts experienced by the C-8 methyl groups implying that, since the C-8 methyl of marrubiin ketone (129) is equatorial, that of solidaganone (132) is axial.

Conclusive evidence for the axial orientation of the C-8 methyl group of solidaganone was derived by comparison (Table 11) of the methyl resonances (in deuterochloroform) of solidaganone (132) and the solidaganol (138), derived from lithium aluminium hydride reduction of solidagenone. It has been demonstrated[131] that, if the C-8 and C-10 methyl groups are axial, they should undergo

a similar upfield shift of ~15-20 c./sec. on conversion of a 6-hydroxyl group into a 6-ketone. The C-8 methyl resonances of the solidaganol (138) and solidaganone (132) occur at τ 8·65 and 8·92 respectively, which represents an upfield shift of 16 c./sec. Thus, the C-8 methyl groups of solidaganone and the solidaganol (138) are axial (assuming a ring B chair conformation). Although there is ambiguity in the assignment of the C-10 methyl resonance (τ 8·77 or 8·62) of the solidaganol (138), the two possible values give rise to shifts of 13·8 or 22·8 c./sec. for replacement of a 6-hydroxyl by a 6-ketone. Both shifts are of the magnitude characteristic of an axial methyl group. Thus, the relationship of the C-8 and C-10 methyl groups of solidaganone [and of the solidaganol (138)] is 1,3-diaxial. For comparison, the hydroxy-acetate (140) derived from marrubiin [126] shows an upfield shift of 22 c./sec. of the C-10 (axial) methyl resonance on replacement of the 6-hydroxyl group by a 6-ketone (141), whereas the C-8 methyl resonance suffers a downfield shift of 3·5 c./sec., indicating that the latter is equatorial.

Reduction of Solidagenone

A certain specificity of reduction of the enone system of solidagenone has already been noted. While only one saturated ketone might be expected from catalytic reduction by preferential adsorption of the less hindered face (usually the α face) of the molecule on to the catalyst surface, hydride reduction would be expected to be non-stereospecific and to yield two saturated ketones epimeric at C-8 as a result of attack of the small hydride ion from both the α- and β-faces. That only one ketone, solidaganone (132) containing an 8β-methyl group, is obtained by hydride reduction might imply that there is great steric hindrance towards hydride attack at position 8 from the β-face of the molecule or that there is some structural feature exerting an influence on the direction of attack. An investigation of a molecular model of solidagenone indicates that there is, in fact, severe steric crowding in the 8β-region produced by the C-8 and C-10 methyl groups and the methylene group at position 11; the crowding is probably enhanced by the 1,3-diaxial interaction between the C-4 and C-10 axial methyl groups.

Conversely, although the hydrogen-bonded system formed by the 9-hydroxyl group and the furan ring is oriented towards the α-face, the 8-carbon atom is much more easily accessible from this direction. However, it is also highly probable that the 9-hydroxyl group could form an aluminium hydride complex with the reducing agent and, since this hydroxyl is α-oriented, addition of a hydride ion at position 8 would take place from the α-face. It is significant that catalytic reduction of the enone-lactone (142), obtained from chromic acid in acetic acid oxidation of solidagenone is non-stereospecific and produces two keto-lactones (134 and 143) epimeric at C-8. A model of the enone-lactone shows that formation of the rigid spiro-lactone system has considerably removed the crowding influence of the 11-methylene group and the 7,8-double bond is now much more open from the β-face. Moreover, since the bulky lactone carbonyl group projects towards the α-face of the molecule, adsorption on the catalyst surface from this face will be less efficient.

Steric crowding is probably also responsible for the anomalous dissolving-metal reductions of the enone system of solidagenone. Reduction of solidagenone with lithium

in liquid ammonia, zinc in acetic acid, or sodium in ethanol does not produce a saturated ketone but yields as the major product the βγ-unsaturated ketone (135). In this type of reduction, formation of a saturated ketone depends on axial protonation of the β-carbon atom of the enone system.[132] In order to unite axially with the 8-carbon atom of solidagenone, the attacking proton must approach the 8-position from the β-face of the molecule. Since there is a considerable steric barrier to this, it is conceivable that the primary addition of an electron from the metal surface[132] could take place at position 7 rather than 8. Concomitant isomerisation of the double bond and elimination of a hydroxide ion from the 9-position, as in Scheme 12, produces 9-deoxysolidag-8-ene-6-one (135).

The preceding investigations into the structure, stereochemistry, and properties of solidagenone have been outlined in two communications.[133]

Table 9.

Solidaganone (132); methyl resonances.

	Chemical Shift		Chemical shift difference	
	$CDCl_3$	Benzene	p.p.m.	c./sec.
C-8	8·92	9·17	+0·25	+15·0
C-4 axial	9·05	8·88	-0·17	-10·2
C-4 equatorial	8·78	8·53	-0·25	-15·0
C-10	9·00	9·13	+0·13	+ 7·8

Table 10.

Ketone (129) derived from marrubiin ; methyl resonances.

	Chemical shift		Chemical shift difference	
	$CDCl_3$	Benzene	p.p.m.	c./sec.
C-8	8·99	9·34	+0·35	+21·0
C-4 axial	9·02	8·83	-0·19	-11·4
C-4 equatorial	8·77	8·55	-0·22	-13·2
C-10	9·09	9·27	+0·18	+10·8

Table 11.

Comparison of methyl resonances of solidagan-6β-ol (138) and solidaganone (132)

	Chemical shift, $CDCl_3$		Chemical shift difference	
	Solidagan-6β-ol	solidaganone	p.p.m.	c./sec.
C-8	8·65	8·92	+0·27	+16·2
C-4 axial	9·01	9·05	+0·04	+ 2·4
C-4 equatorial	8·62 or 8·77	8·78	+0·16 or +0·01	+ 9·6 or + 0·6
C-10	8·77 or 8·62	9·00	+0·23 or +0·38	+13·8 or +22·8

Experimental.

Melting points and spectral data were determined as previously described. Specific rotations refer to chloroform solutions at room temperature. Woelm Grade I alumina, deactivated to the appropriate grade, was used for chromatography. For analytical and preparative thin-layer chromatography (t.l.c.), chromatoplates were spread with Kieselgel G (Merck). Light petroleum was of b.p. 40--60° unless otherwise stated.

Extraction of S. canadensis

(i) With petroleum ether.

Dried, finely powdered root and root hair (108 g.) was Soxhlet extracted for four hours with light petroleum. The concentrated extract, on standing, deposited large yellow prisms (2 g.), m.p. 101-106°. Recrystallisation from light petroleum yielded the spiro-ether mixture (128) as colourless prisms, m.p. 108-110° (Found: C, 75·8; H, 8·6. $C_{20}H_{28}O_3$ requires C, 75·9; H, 8·9%); $\nu_{max.}^{CCl_4}$ 1677 cm.$^{-1}$; $\lambda_{max.}$ 210 (log ε 3·97) and 232 mμ (4·00); n.m.r. signals at 9·09 (s, 3H), 9·07 (s, 3H), and 8·90 (s, 12H) [6 quaternary methyls], 8·17 (d, 3H; J = 1 c./sec.) and 8·10 (d, 3H; J = 1 c./sec.) [two C(8)-methyls], 7·35

(s, 1H) and 7·28 (s, 1H) [two C-5 protons], 5·99 (d, 2H; J = 10 c./sec.), 5·71 (d, 1H; J = 10 c./sec.), and 5·55 (d, 1H; J = 10 c./sec.) [four C-16 protons], 4·99 (d, 1H; J = 2 c./sec.) and 4·92 (d, 1H; J = 2 c./sec.) [two C-14 protons], 4·42 (m, 2H) [C-7], 3·58 (d, 1H; J = 2 c./sec.) and 3·55 (d, 1H; J = 2 c./sec.) [two C-15 protons].

The mother liquor furnished solidagenone (137) as an amorphous deposit (0·5 g.) which crystallised from ether-light petroleum as colourless needles, m.p. 131-133° (Found: C, 75·9; H, 8·8. Calcd. for $C_{20}H_{28}O_3$ C, 75·9; H, 8·9%); $[\alpha]_D$ -15·2 (c 1·0); $\nu_{max.}^{CCl_4}$ 3611, 3567, and 1678 $cm.^{-1}$; $\lambda_{max.}$ 223 mμ (log ε 4·03); n.m.r. signals at τ 9·01 (s, 3H), 8·85 (s, 3H), 8·82 (s, 3H) [quaternary methyls], 7·98 (d, 3H; J = 1 c./sec.) [C(8)-Me], 7·98 (t, 2H; J = 8 c./sec.) [C-11], 7·30 (t, 2H; J = 8 c./sec.) [C-12], 7·25 (s, 1H) [C-5], 4·26 (d, 1H; J = 1 c./sec.) [C-7], 3·67 (m, 1H) [C-14], 2·73 (m, 1H), and 2·63 (m, 1H) [3 furan protons]. The remainder of the light petroleum extract, after removal of the solid material, was evaporated to dryness and a portion (2 g.) of the residual light brown gum (4·5 g.) was chromatographed over silica gel (110 g.). Fractions eluted with ether-light petroleum

(2:3) to (1:1) contained mainly solidagenone (700 mg.) and solidified spontaneously.

In an alternative procedure, the light petroleum extract of ground root (280 g.) was evaporated to dryness without first allowing any material to deposit. , The entire extract, a brown gum, was adsorbed on silica gel (1 kg.) from ether-light petroleum (1:4). Fractions eluted with ether-light petroleum (11:9) to (4:1) were predominantly solidagenone (6·5 g.).

(ii) With benzene

The benzene extract (3 g.) of root and root hair (55 g.), on trituration and crystallisation from ether-light petroleum yielded impure prisms of the spiro-ether mixture (1·2 g.), m.p. 98-106°. T.l.c. indicated that the benzene and light petroleum extracts were identical in composition.

Benzene and petroleum ether extracts of ground leaves and stem of S. canadensis contained neither solidagenone nor the spiro-ether mixture.

Conversion of the spiro-ether mixture (128) into solidagenone (137).

The epimeric spiro-ethers (128) (40 mg.) were refluxed for 30 min. in ethanol (15 ml.) and acetic acid (4 drops). The product (36 mg.), on removal of solvent, crystallised from ether-light petroleum as colourless needles, m.p. 131-132° and was identical (i.r. (KCl), n.m.r., mixed m.p., and t.l.c.) with solidagenone (137) extracted from S. canadensis root. The interconversion could also be achieved quantitatively by filtration of the spiro-ether mixture, in chloroform, through a short column of alumina (Grade III; neutral).

Reduction of solidagenone.

(i) With lithium aluminium hydride.[122]

Solidagenone (137) (1 g.) in anhydrous ether (40 ml.) stood at room temperature for 25 hr. in presence of lithium aluminium hydride (203 mg.). Reduction was terminated by the dropwise addition of saturated aqueous sodium sulphate and the suspension, dried over magnesium sulphate, was filtered. The crude product (920 mg.) in chloroform-benzene (1:19) was chromatographed over alumina (90 g.)

(Grade I; neutral). Fractions eluted with chloroform-benzene (1:4) to (9:11) yielded solidaganone (132) (450 mg.), which crystallised from ether-light petroleum as colourless needles, m.p. 110-111° (Found: C, 75·2; H, 9·3. $C_{20}H_{30}O_3$ requires C, 75·4; H, 9·5%); $\nu_{max.}^{CCl_4}$ 1713, 3621, 3578 cm.$^{-1}$; $\nu_{max.}^{KBr}$ 1690 cm.$^{-1}$; $\lambda_{max.}$ 207 mμ (log ε 3·80); n.m.r. signals at τ 9·05 (s, 3H), 9·00 (s, 3H), 8·78 (s, 3H) [quaternary methyls], 8·92 (d, 3H; J = 8 c./sec.) [C(8)-Me], 7·00 (s, 1H) [C-5], 3·71 (m, 1H), 2·76 (m, 1H), and 2·64 (m, 1H) [3 furan protons]. The methyl resonances progressively shifted on addition of benzene to final values of τ 8·88, 9·13, 8·53, and 9·17 respectively.

In an alternative procedure, solidagenone (555 mg.) was refluxed for 1 hr. with excess lithium aluminium hydride in tetrahydrofuran (30 ml.). The oily product, isolated as above, was chromatographed over alumina (40 g.) (Grade I; neutral). Elution with chloroform-benzene (1:1) first gave a mixture (100 mg.) of solidaganone (132) and the solidagan-6β-ol (138) while later fractions (300 mg.) eluted by the same polarity of solvent contained solidaganone and both solidaganols (138 and 144). A portion of both mixtures was oxidised by the Sarett pro-

cedure (described later) and, in each case, only one ketone, solidaganone (132), was produced. On preparative t.l.c. of the later fractions [ether-light petroleum (3:7)], the 6β(axial) alcohol (138) separated partially as the higher Rf front of a broad band. The tail of this band contained the equatorial alcohol (144) and a substantial amount of solidaganone but no axial alcohol. Solidagan-6β-ol (138) (102 mg.), eluted from the band front, crystallised with difficulty from ether-light petroleum as colourless prisms, m.p. 100-102° (Found: C, 75·0; H, 10·0. $C_{20}H_{32}O_3$ requires C, 75·0; H, 10·1%); n.m.r. signals at τ 9·01 (s, 3H), 8·77 (s, 3H), 8·62 (s, 3H) [quaternary methyls], 8·65 (d, 3H; J = 8 c./sec.) [C(8)-Me], 5·62 (m, 1H) [6-H], 3·81 (m, 1H), 2·86 (m, 1H), and 2·75 (m, 1H) [3 furan protons]. A t.l.c. phenomenon, from a practical point of view, is that solidaganone (132) has approximately the same mobility as solidagan-6α-ol (144) in ether-light petroleum (3:7) as eluant whereas in chloroform or methanol-chloroform (1:49) it possesses an Rf close to solidagan-6β-ol (138). Thus, it was found possible to separate the 6β(axial) alcohol (138) from a mixture of solidaganone and the equatorial alcohol (144) by t.l.c. in

ether-light petroleum (3:7) as above. The remaining mixture of solidaganone and the 6α(equatorial) alcohol (144) was then readsorbed on silica (t.l.c.). Two developments in chloroform afforded only a marginal separation but by division of the broad band into three portions it was possible to elute solidaganone (132) (80 mg.) from the uppermost portion and, from the lowest, the oily solidagan-6α-ol (144) (42 mg.); n.m.r. signals at τ 9·00 (s, 3H), 8·69 (s, 3H), 8·38 (s, 3H) [quaternary methyls], 8·74 (d, 3H; J = 8 c./sec.) [C(8)-Me], 5·78 (m, 1H) [6-H], 3·71 (m, 1H), 2·75 (m, 1H), and 2·65 (m, 1H) [3 furan protons].

(ii) With palladium-charcoal

Solidagenone (82 mg.) in ethanol (8 ml.) and triethylamine (2·5 ml.) was hydrogenated over 10% palladium-charcoal for 15 min. Filtration through celite and removal of solvent gave an oily mixture (87 mg.), which was adsorbed on a preparative chromatoplate. Development in chloroform-methanol (49:1) gave two concentrated bands, the less polar of which contained solidaganone (132) (55 mg.), identified by mixed m.p. and t.l.c., i.r., and n.m.r.

The lower component (25 mg.) failed to crystallise and showed no furan-type resonance in the n.m.r. spectrum. On n.m.r. evidence, this was deduced to be a mixture of the C-13 epimers of the hexahydro-reduction product (145) and was not further investigated.

(iii) With lithium in liquid ammonia.[134]

Solidagenone (100 mg.) and anhydrous ether (20 ml.) were added separately to a solution of lithium in liquid ammonia (80 ml.). After a stirring period of 30 min., ammonium chloride (1 g.) was added and the solvents were allowed to volatilise. The residue was treated with water (40 ml.) and extracted with ether (100 ml.), the organic layer being washed with 1N hydrochloric acid (30 ml.), with water to neutrality and dried over magnesium sulphate. On removal of solvent, the crude product (97 mg.) was adsorbed on a preparative chromatoplate, two developments of which in ether-petroleum ether (3:7) gave three bands. The uppermost band yielded 9-deoxysolidag-8-en-6-one (135) (25 mg.), which crystallised from ether-light petroleum as colourless needles, m.p. 78-79° (Found: C, 79·8; H, 9·5. $C_{20}H_{28}O_2$ requires C, 79·95; H, 9·4%); $\nu_{max.}^{CCl_4}$ 1718 cm.$^{-1}$;

$\nu_{max.}^{KBr}$ 1706 cm.$^{-1}$; $\lambda_{max.}$ 204 mμ (log ε 4·13); $[\alpha]_D$ +139·4° (c 1·0); n.m.r. signals at τ 9·05 (s, 3H), 9·00 (s, 3H), 8·74 (s, 3H) [quaternary methyls], 8·34 (s, 3H) [C(8)-Me], 3·68 (m, 1H), 2·71 (m, 1H), and 2·60 (m, 1H) [3 furan protons]; mass spectral peaks at m/e 300 (molecular ion), 285, 219, 177, 149, 135, 121, and 109 (relative abundance 42, 12, 28, 28, 100, 49, 32, and 57%). The band of intermediate polarity contained 9-deoxysolidag-8-en-6α-ol (146) (35 mg.); n.m.r. peaks at τ 8·99 (s, 3H), 8·92 (s, 3H), 8·83 (s, 3H) [quaternary methyls], 8·39 (s, 3H) [C(8)-Me], 5·85 (q, 1H; J = 9 c./sec.) [6-H(axial)], 3·70 (m, 1H), 2·74 (m, 1H), and 2·63 (m, 1H) [3 furan protons]. The lowest band gave a mixture of more highly reduced material (30 mg.), the n.m.r. of which showed no vinylic methyl resonance but still possessed the ABX pattern of the β-substituted furan ring. Tentative structures for the components of this mixture are (147) or (148) but, as these compounds were not of immediate importance, they were not further examined.

In a larger scale reduction, solidagenone (1 g.) was reduced as above, but in this case, the crude product (950 mg.) was chromatographed over alumina (Grade I; neutral) (100 g.). Elution with chloroform-benzene (1:9) to (3:17)

yielded pure 9-deoxysolidag-8-en-6-one (135) (280 mg.) while a mixture (30 mg.) of 9-deoxysolidag-8-en-6-one and a lower Rf impurity were eluted in the fractions chloroform-benzene (3:17) to (3:2). At least four more polar reduction products (520 mg.) were contained in the fractions chloroform-benzene (4:1) to ethyl acetate-chloroform (3:1) but were not well separated and have not been investigated.

Under alternative conditions, solidagenone (106 mg.) in anhydrous tetrahydrofuran (10 ml.) was added to a solution of lithium (20 mg.) in liquid ammonia (50 ml.). After being stirred for 30 min., the reaction was terminated by the addition of ammonium chloride (0·5 g.) and the product (102 mg.) was isolated as before. This was separated into three components by preparative t.l.c., using chloroform as solvent. The uppermost band yielded 9-deoxysolidag-8-en-6-one (135) (7 mg.) and the lowest Rf band consisted of a mixture (10 mg.) of highly reduced material (147 and 148) as before. The intermediate band (70 mg.), in this case, was a mixture of solidaganone (132) and the two solidaganols (138 and 144). Analytical t.l.c. of this mixture showed an elongate spot, the foremost tip of which had the same Rf and stain as solidaganone (brown with

Ce^{IV}) while the remainder of the spot showed the characteristic mobility and purple stain of the solidaganols (138 and 144). The composition of this mixture was verified by oxidation (Sarett procedure) to one compound as follows. The mixture (70 mg.) in anhydrous pyridine (15 ml.) stood at room temperature for 16 hr. in presence of excess chromium trioxide. Oxidation was terminated by the addition of methanol (1 ml.) and, after a short delay, an ethyl acetate-water mixture (10 ml.). The suspension was filtered through celite and further diluted with ethyl acetate and water. After removal of the aqueous layer, the organic layer was washed with 1N hydrochloric acid (4 x 30 ml.), with water to pH7 and dried over magnesium sulphate. The product (30 mg.) was solidaganone, verified by n.m.r., mixed m.p., and t.l.c.

(iv) With zinc in acetic acid.[134b]

A suspension of solidagenone (1·2 g.) and zinc (1 g.) in glacial acetic acid (10 ml.) was gently refluxed for 12 hr. The acetic acid was removed under vacuum and the residue extracted with ether. The crude product (1·1 g.) was chromatographed over alumina (80 g.) (Grade I; neutral).

9-Deoxysolidag-8-en-6-one (135) (408 mg.) was eluted in benzene and benzene-chloroform (19:1) while fractions eluted with benzene-chloroform (9:1) to (7:3) yielded the isomeric 9-deoxysolidag-7-en-6-one (149) (285 mg.), colourless needles, m.p. 58-59°, from pentane (Found: C, 80·1; H, 9·4. $C_{20}H_{28}O_2$ requires C, 80·0; H, 9·4%); $\nu_{max.}^{CCl_4}$ 1675 $cm.^{-1}$; $\lambda_{max.}$ 215 (log ε 3·80) and 240 mμ (4·03); n.m.r. absorption at τ 9·18 (s, 3H), 8·89 (s, 3H), 8·86 (s, 3H) [quaternary methyls], 8·06 (d, 3H; J = 1 c./sec.)[C(8)-Me], 4·22 (m, 1H) [C-7], 3·69 (m, 1H), 2·71 (m, 1H), and 2·61 (m, 1H) [3 furan protons]. Although later fractions (120 mg.), chloroform-benzene (1:1) to ethyl acetate-chloroform (1:4), contained two further compounds, isolation was not attempted.

Equilibration of 9-deoxysolidag-8-en-6-one (135) and 9-deoxysolidag-7-en-6-one (149) was carried out as follows. 9-Deoxysolidag-8-en-6-one (135) (50 mg.) in AnalaR methanol (30 ml.) was refluxed for 35 min. in presence of potassium hydroxide (10 mg.). After removal of solvent, the reaction mixture was extracted with ether, the ether solution being washed with water and dried over magnesium sulphate. The oily product, mainly two compounds, was adsorbed on a

preparative chromatoplate, developed twice in ether-light petroleum (3:17). Unchanged 9-deoxysolidag-8-en-6-one (135) (10 mg.) was eluted from the upper band, while the lower band gave 9-deoxysolidag-7-en-6-one (149) (30 mg.), which crystallised from pentane as colourless needles, m.p. 58-59°. The latter was shown (mixed m.p., t.l.c., and i.r.) to be identical to the 9-deoxysolidag-7-en-6-one obtained from the reduction of solidagenone with zinc in acetic acid.

(v) With sodium in ethanol.[134b]

To a solution of solidagenone (20 mg.) in anhydrous ethanol (5 ml.) sodium metal (84 mg.) was added over a period of 20 min. and the suspension stood at room temperature for 1 hr. The reaction mixture, freed from ethanol, was extracted with ether, the organic layer being washed with water till neutral and dried over magnesium sulphate. Since analytical t.l.c. of the crude product showed the characteristic deep purple stain of alcoholic rather than ketonic products, over-reduction was assumed to have occurred. Accordingly, the crude product was oxidised by the Sarett method as described above. The

pattern of spots on analytical t.l.c. of the oxidised mixture showed a close similarity to the products of zinc-acetic acid reduction of solidagenone, the uppermost spot, which possessed the same Rf and mauve ceric stain as 9-deoxysolidag-8-en-6-one (135), being the most abundant product. A substantial quantity of unreacted solidagenone remained.

In a modified procedure, a suspension of solidagenone (105 mg.) and sodium amalgam (5%; 500 mg.) in anhydrous ethanol (15 ml.) was stirred at room temperature for 15 hr. The ethanol was removed under vacuum without heating and the reaction mixture was extracted with ether, washed with water to neutrality, and dried over magnesium sulphate. On removal of solvent, the crystalline product (95 mg.) showed, on analytical t.l.c., an approximately (1:1) mixture of two compounds. The lower component was unreacted solidagenone (brown ceric stain), while the upper showed the characteristic mauve stain of 9-deoxysolidag-8-en-6-one (135). Although solidagenone had not completely reacted, the product, under the above conditions, was free from the by-products of the other dissolving-metal reductions described.

(vi) With sodium borohydride.

Solidagenone (100 mg.) in methanol (10 ml.) stood at room temperature for 15 min. in presence of sodium borohydride (40 mg.). After removal of methanol, the reaction mixture was extracted with ether, the organic layer being washed with water and dried over magnesium sulphate. The recovered material was unreacted solidagenone, verified by m.p. and t.l.c. and showed no evidence of reduction products. The reaction was re-attempted using three times the quantity (120 mg.) of sodium borohydride. After a reaction time of 20 hr., the isolated material was again unreacted solidagenone.

Marrubiin (124).

The sample of marrubiin, m.p. 159-160°, employed in the following section, was extracted from Marrubium vulgare L. (White Horehound) in this laboratory. The reduction of marrubiin to marrubenol (150) by lithium aluminium hydride and the chromic oxidation of marrubenol to the keto-aldehyde (130)(Scheme 11) have been previously described.[120, 125, 126]

Marrubenol (150).

Marrubiin (515 mg.) in anhydrous tetrahydrofuran (40 ml.) was refluxed for 2 hr. in presence of excess lithium aluminium hydride. After cooling, the reaction mixture was decomposed with a small volume of saturated aqueous sodium sulphate added dropwise and dried with anhydrous sodium sulphate. Filtration and removal of solvent yielded a clear gum (550 mg.), which was chromatographed over alumina (Grade I; neutral) (25 g.). Pure marrubenol (380 mg.) was eluted in the fractions chloroform-benzene (3:2) to (4:1) and later fractions, chloroform-benzene (9:1) to methanol-chloroform (1:9), yielded marrubenol (60 mg.) containing a trace of a more polar compound. Marrubenol (150) crystallised from ether-light petroleum as colourless needles, m.p. 143-145° (lit. m.p. 138°).

Keto-aldehyde (130).

Marrubenol (150) (440 mg.) in anhydrous pyridine (40 ml.) stood at room temperature for 18 hr. in presence of chromium trioxide (700 mg.) (Sarett procedure). The reaction was terminated by the addition of methanol (1 ml.)

and, after a short delay a water-ethyl acetate mixture (10 ml.). The suspension was filtered through celite under suction and, after the removal of the aqueous layer, the organic layer was washed with 1N hydrochloric acid (5 x 30 ml.), aqueous sodium carbonate (2 x 30 ml.) and water (4 x 40 ml.) and dried over anhydrous magnesium sulphate. The oily product was chromatographed over alumina (25 g.) and the keto-aldehyde (130)(380 mg.), eluted with chloroform-benzene (7:3), crystallised from chloroform-light petroleum as colourless needles, m.p. 109-111° (lit. m.p. 110-111°); $\nu_{max.}^{CCl_4}$ 1713, 1704, and 872 cm.$^{-1}$; aldehydic proton at τ -0·42 (d) (separation 1 c./sec.).

Ketone (129).

Keto-aldehyde (130) (380 mg.) in anhydrous ether (20 ml.) reacted for 30 hr. with ethane dithiol (0·5 ml.) in presence of freshly distilled boron trifluoride etherate (4 ml.). The reaction mixture, after dilution with ether (40 ml.), was washed with 1N sodium hydroxide (5 x 50 ml.), with water to neutrality and dried over magnesium sulphate. The crude product (240 mg.) was chromatographed over alumina (Grade I; neutral) (20 g.). Fractions eluted with chloro-

form-benzene (3:7) to (2:3) afforded the keto-thioacetal (131) (140 mg.) as an oil; n.m.r. signals at τ 8·99 (d, 3H; J = 8 c./sec.) [C(8)-Me], 8·94 (s, 3H), 8·72 (s, 3H) [quaternary methyls], 6·92 (m, 4H) [$-S-CH_2-CH_2-S-$], 4·78 (s, 1H) [$-S-\overset{|}{C}H-S$], 3·79 (m, 1H), 2·82 (m, 1H), and 2·70 (m, 1H) [3 furan protons]. The thioacetal was reduced by refluxing with acetone-deactivated Raney nickel (2 g.) in AnalaR acetone (20 ml.) for 15 min. The product (130 mg.) recovered from the Raney nickel by Soxhlet extraction, was chromatographed over alumina (Grade III; neutral) (12 g.). Fractions eluted with benzene and benzene-chloroform (19:1) contained mainly the ketone (129) (110 mg.) but failed to crystallise because of lower Rf impurities. These were removed by preparative t.l.c. using ether-light petroleum (3:7) as solvent. The <u>ketone</u> (129) (98 mg.) crystallised from ether-light petroleum as colourless flakes, m.p. 89-90° (Found: C, 75·6; H, 9·3. $C_{20}H_{30}O_3$ requires C, 75·4; H, 9·5%); $\nu_{max.}^{CCl_4}$ 1711, 3628, and 3588 cm.$^{-1}$; $\nu_{max.}^{KBr}$ 1685 cm.$^{-1}$; $\lambda_{max.}$ 207 mμ (log ε 3·79); n.m.r. signals at τ 9·09 (s, 3H), 9·02 (s, 3H), 8·77 (s, 3H) [quaternary methyls], 8·99 (d, 3H; J = 8 c./sec.) [C(8)-Me], 7·20 (s, 1H) [C-5], 3·73 (m, 1H), 2·77 (m, 1H), and 2·67 (m, 1H) [3 furan protons]. The methyl resonances shifted progressively on addition of benzene to final values of τ 9·27, 8·83, 8·55, and 9·34 respectively.

Dehydration of ketone (129).[125]

Ketone (129) (32 mg.) in anhydrous pyridine (12 ml.) was gently refluxed for 6 hr. with phosphoryl chloride (0·5 ml.). The cooled reaction mixture, poured on to ice, was extracted with ethyl acetate, the organic layer being washed with 1N hydrochloric acid (4 x 40 ml.), with water to pH7, and dried over magnesium sulphate. Removal of solvent gave an oily mixture (30 mg.) which was freed from polar by-products by preparative t.l.c. using ether-light petroleum as solvent. The recovered mixture (15 mg.) of Δ^{8}- and $\Delta^{9(11)}$-dehydro-compounds was adsorbed on a preparative chromatoplate and the components were narrowly separated by two developments in ether-light petroleum (1:9). Eluted from the upper band, the Δ^{8}-enone (7 mg.) crystallised from ether-light petroleum as colourless needles, m.p. 77-79°, mixed m.p. with 9-deoxysolidag-8-en-6-one (135) 78-79°; $\nu_{max.}^{KBr}$ 1707 cm.$^{-1}$; $[\alpha]_D$ +131·6° (c 0·4); n.m.r. signals at τ 9·05 (s, 3H), 9·00 (s, 3H), 8·73 (s, 3H), 8·35 (s, 3H), 3·68 (m, 1H), 2·72 (m, 1H), and 2·61 (m, 1H); mass spectral peaks at m/e 300 (molecular ion), 285, 219, 177, 149, 135, 121, and 109 (relative abundance 28, 10, 23, 30, 100, 58, 44, and 74%).

Epoxidation of the Δ^{8}-enone (135).

The Δ^{8}-enone (135) (100 mg.) and m-chloroperbenzoic acid (88 mg.) in AnalaR chloroform (35 ml.) stood at room temperature for 35 min. The reaction mixture was filtered in chloroform through a short column of alumina (Grade I; basic) and the eluate was adsorbed on a preparative chromatoplate, two developments of which in ether-petroleum ether (1:3) gave two close, concentrated bands. The higher Rf band contained unreacted enone (135) (30 mg.) and the lower band yielded its 8α, 9α-epoxide (136) (40 mg.). A mixture (20 mg.) of at least three further compounds was eluted from a diffuse band, lower in Rf than the epoxide (136). The n.m.r. spectrum of this crude mixture showed no furan-type resonance but a broad multiplet, possibly attributable to protons on epoxide rings, existed at τ 7-7·5. Significantly, the proportion of these furan-degraded compounds increased with increasing reaction time and/or increase in concentration of reactants. Epoxide (136), isolated as above, contained a trace of solidagenone as impurity and failed to crystallise. During rechromatography of this mixture over silica (t.l.c.), the same minor proportion of solidagenone, as a rearrangement product of the epoxide (136), was produced.

This impure sample of epoxide distilled at 85° under vacuum (8×10^{-3} mm.) to give a clear oil, which was free from impurity (t.l.c.) and solidified slowly on trituration with pentane. Redistillation followed by seeding of the distillate, yielded the epoxide (136) as colourless, semi-solid prisms, m.p. 52-55° (Found: C, 75·6; H, 8·6. $C_{20}H_{28}O_3$ requires C, 75·9; H, 8·9%); $\nu_{max.}^{CCl_4}$ 1714 cm.$^{-1}$; n.m.r. signals at τ 8·98 (s, 6H), 8·80 (s, 3H), 8·72 (s, 3H) [4 quaternary methyls], 3·73 (m, 1H), 2·78 (m, 1H), and 2·66 (m, 1H) [3 furan protons].

Rearrangement of epoxide (136) to solidagenone (137).

A saturated solution (5 ml.) of naphthalene 2-sulphonic acid in benzene was added to epoxide (136) (32 mg.) in benzene (45 ml.) and the mixture was refluxed for 10 min. After evaporation to small volume, the reaction mixture was filtered in chloroform through a short column of alumina (Grade I; basic). The eluate, mainly two compounds but containing some naphthalene sulphonic acid reagent, was adsorbed on a preparative chromatoplate, developed twice in ether-light petroleum (3:7). Of the two major bands, the upper gave unrearranged epoxide (136) (12 mg.)

and the lower contained solidagenone (137) (16 mg.), identified by n.m.r., i.r., mixed m.p., and t.l.c. A small amount of material remained in three other bands, barely discernible on staining with iodine. The proportion of these by-products increased with prolonged reaction time.

Alternative conditions, in which ether solutions of epoxide (136) were shaken in the cold or refluxed with concentrated solutions of iodine in ether, were found to be less efficient in the rearrangement to solidagenone.

Osmylation of the Δ^{8}-enone (135).[128]

The Δ^{8}-enone (135) (90 mg.) and osmium tetroxide (100 mg.) in ether (6 ml.) stood at room temperature for 65 hr. After the addition of AnalaR benzene (25 ml.), the osmate was decomposed by treatment (1 hr.) with gaseous hydrogen sulphide, the excess hydrogen sulphide being removed with nitrogen, bubbled through the solution for 30 min. On filtration through celite and removal of solvent, the product, mainly one compound but containing a trace of a less polar by-product with the same Rf as solidagenone, was adsorbed on a preparative chromatoplate, developed twice in

ether-petroleum ether (1:1). From the major band, the 8α, 9α-diol (151) (68 mg.) was obtained as a clear oil, $\nu_{max.}^{CHCl_3}$ 1709, 3401, and 3544 $cm.^{-1}$, the latter two peaks, broadened due to intramolecular hydrogen-bonding, showing on dilution an approximately constant intensity ratio of (1:1·83); n.m.r. signals at τ 9·08 (s, 6H), 8·80 (s, 3H), 8·68 (s, 3H) [4 quaternary methyls], 3·72 (m, 1H), 2·75 (m, 1H), and 2·64 (m, 1H) [3 furan protons], with broad hydroxyl proton resonance around τ 8·7, disappearing on addition of deuterium oxide.

The diol (151) failed to form an acetonide when stirred for 65 hr. with anhydrous copper sulphate in dry acetone (AnalaR).

Under mild dehydrating conditions, conversion of diol (151) to solidagenone (137) was unsuccessful: no apparent reaction occurred when the diol in AnalaR benzene was stirred for 18 hr. in the presence of acidic or basic alumina (Grade I); no significant dehydration took place when a test sample of the diol (151) stood at room temperature for 2 hr. in anhydrous pyridine (1 ml.) and phosphoryl chloride (4 drops). More stringent conditions, under which the pyridine-phosphoryl chloride solution stood at room

temperature for 15 hr. or was gently refluxed for 1 hr. gave multiple products, of which the compound showing the same Rf as solidagenone was not the major component.

Oxidation of solidagenone (137) with chromic acid in acetic acid.[128]

Solidagenone (137) (990 mg.) in glacial acetic acid (22 ml.) reacted at room temperature for 50 hr. with a solution of chromium trioxide (2·6 g.) in water (5 ml.) and acetic acid (13 ml.). After removal of acetic acid under vacuum, water was added and the suspension extracted with ethyl acetate. The organic layer, washed with water till neutral, was extracted with aqueous sodium carbonate, washed with water to pH 7, and dried over magnesium sulphate. The neutral organic fraction, on evaporation of solvent, gave the enone-lactone (142) as a clear oil (300 mg.), which was purified by preparative t.l.c. [ether-light petroleum (3:2)]. The enone-lactone crystallised from ether-light petroleum as needles, m.p. 143-144° (Found: C, 74·0; H, 8·6. $C_{17}H_{24}O_3$ requires C, 73·9; H, 8·75%); $\nu_{max.}^{CCl_4}$ 1783 [lactone] and 1681 cm.$^{-1}$ [enone]; $\lambda_{max.}$ 231 mμ (log ε 4·10); n.m.r. signals at τ 8·98 (s, 3H), 8·84 (s, 6H) [3 quaternary methyls], 8·09 (d, 3H; J = 1 c./sec.) [C(8)-Me], 7·26 (s, 1H) [C-5], 4·21 (d, 1H J = 1 c./sec.) [C-7].

Hydrogenation of the enone-lactone (142).

(i) Over Adams catalyst

Enone-lactone (142) (110 mg.) in ethanol (30 ml.) was hydrogenated for 20 min. over Adams catalyst. Filtration and removal of solvent gave an oily mixture (100 mg.) of mainly two compounds, which were separated by preparative chromatoplate [two developments in ether-light petroleum (4:1)]. The less polar compound (25 mg.) was the keto-lactone (143) (8α-methyl), which crystallised from ether-light petroleum as colourless needles, m.p. 109-110° (Found: C, 73·6; H, 9·6. $C_{17}H_{26}O_3$ requires C, 73·35; H, 9·4%); $\nu_{max.}^{CCl_4}$ 1781 [lactone] and 1717 cm.$^{-1}$ [ketone]; n.m.r. peaks at τ 9·09 (s, 3H), 9·02 (s, 3H), 8·79 (s, 3H) [quaternary methyls], 9·04 (d, 3H; J = 8 c./sec.) [C(8)-Me], 7·71 (m, 1H) [C-7], 7·50 (s, 1H) [C-5], and 7·35 (m, 1H) [C-7]. The more polar product (45 mg.) was the isomeric keto-lactone (134) (8β-methyl), needles, m.p. 146-148°, from ether-light petroleum (Found: C, 73·4; H, 9·2%); $\nu_{max.}^{CCl_4}$ 1782 [lactone] and 1716 cm.$^{-1}$ [ketone]; n.m.r. absorption at τ 9·05 (s, 3H), 9·02 (s, 3H), 8·78 (s, 3H) [quaternary methyls], 8·89 (d, 3H; J = 8 c./sec.)[C(8)-Me], 7·70 (m, 1H) [C-7], 7·48 (s, 1H) [C-5], and 7·20 (m, 1H)

[C-7]. The keto-lactone (134) was identical (mixed m.p., t.l.c., i.r. (CCl_4), and n.m.r.) to the major product of chromic acid-acetic acid oxidation of solidaganone (132) (*vide infra*).

(ii) Over palladium-charcoal

Enone-lactone (142) (50 mg.) in ethanol (15 ml.) was hydrogenated over 10% palladium-charcoal for 10 min. Freed from catalyst and solvent, the reaction mixture contained some unreacted enone-lactone (142) but showed one hydrogenation product with the same t.l.c. characteristics as the 8α-methyl lactone (143). In addition, an elongate spot, lower in Rf than the enone-lactone (142), indicated that hydrogenolysis possibly to carboxylic acid by-products had taken place. Only a trace of a compound with the same Rf as the 8β-methyl lactone (134) was visible.

Reduction of the enone-lactone (142) with lithium in liquid ammonia.

Enone-lactone (142) (40 mg.) and anhydrous ether (5 ml.) were added separately to a solution of lithium (9 mg.) in liquid ammonia (20 ml.). The solution was stirred for

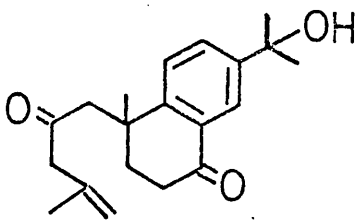
30 min. and ammonium chloride (1 g.) was added. On evaporation of the ammonia, water (20 ml.) was added and the suspension was extracted with ether, the organic layer being washed with 1N hydrochloric acid (20 ml.), with water to neutrality, and dried over magnesium sulphate. Analytical t.l.c. showed an elongate, blue-staining spot lower in Rf than the enone-lactone substrate (142), possibly indicating that degradation of the lactone ring to carboxylic acid products rather than reduction of the enone had occurred. Some unreacted enone-lactone was present but there were no significant less polar reduction products.

Oxidation of solidaganone (132) with chromic acid in acetic acid.[128]

Solidaganone (132) (270 mg.) in acetic acid (6 ml.) reacted for 50 hr. with a solution of chromium trioxide (708 mg.) in water (1·2 ml.) and acetic acid (3·6 ml.). The acetic acid was removed under vacuum without heating and the residue, after the addition of water (100 ml.), was extracted with ether, washed with aqueous sodium carbonate (2 x 50 ml.), with water to neutrality, and dried over

magnesium sulphate. Removal of solvent furnished the keto-lactone (134) (120 mg.) as a clear oil, which was freed from traces of impurities by preparative t.l.c. (chloroform). The keto-lactone (134) crystallised from ether-light petroleum as colourless needles, m.p. 148-149° $\nu_{max.}^{CCl_4}$ 1782, 1716 cm.$^{-1}$; n.m.r. spectrum identical to that of the more polar hydrogenation (Adams catalyst) product of the enone-lactone (142).

Preliminary attempts to convert the keto-lactone (134) into isoambrenolide (133) were unsuccessful: the highly hindered 6-ketone failed to form a thioketal when dry hydrogen chloride was bubbled through an ethanedithiol solution of the keto-lactone (method of Hauptmann[135]); no recognisable product was obtained when the keto-lactone, in triethylene glycol, was heated at 130° in presence of hydrazine hydrate (80%) and hydrazine dihydrochloride (method of Nagata and Itazaki[136]).



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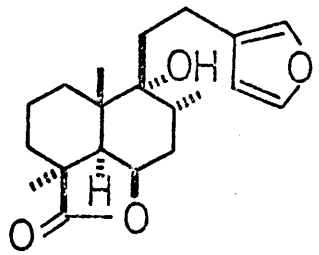

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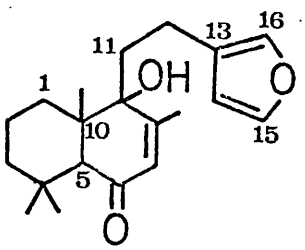

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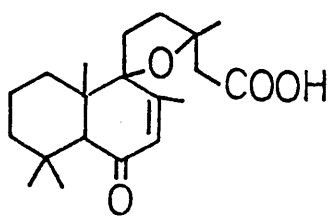

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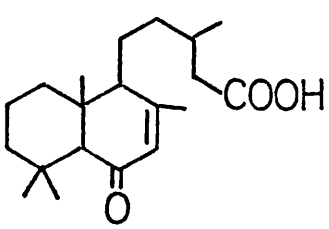

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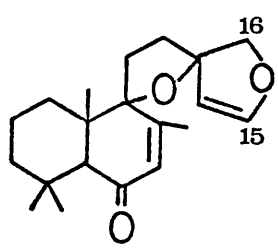

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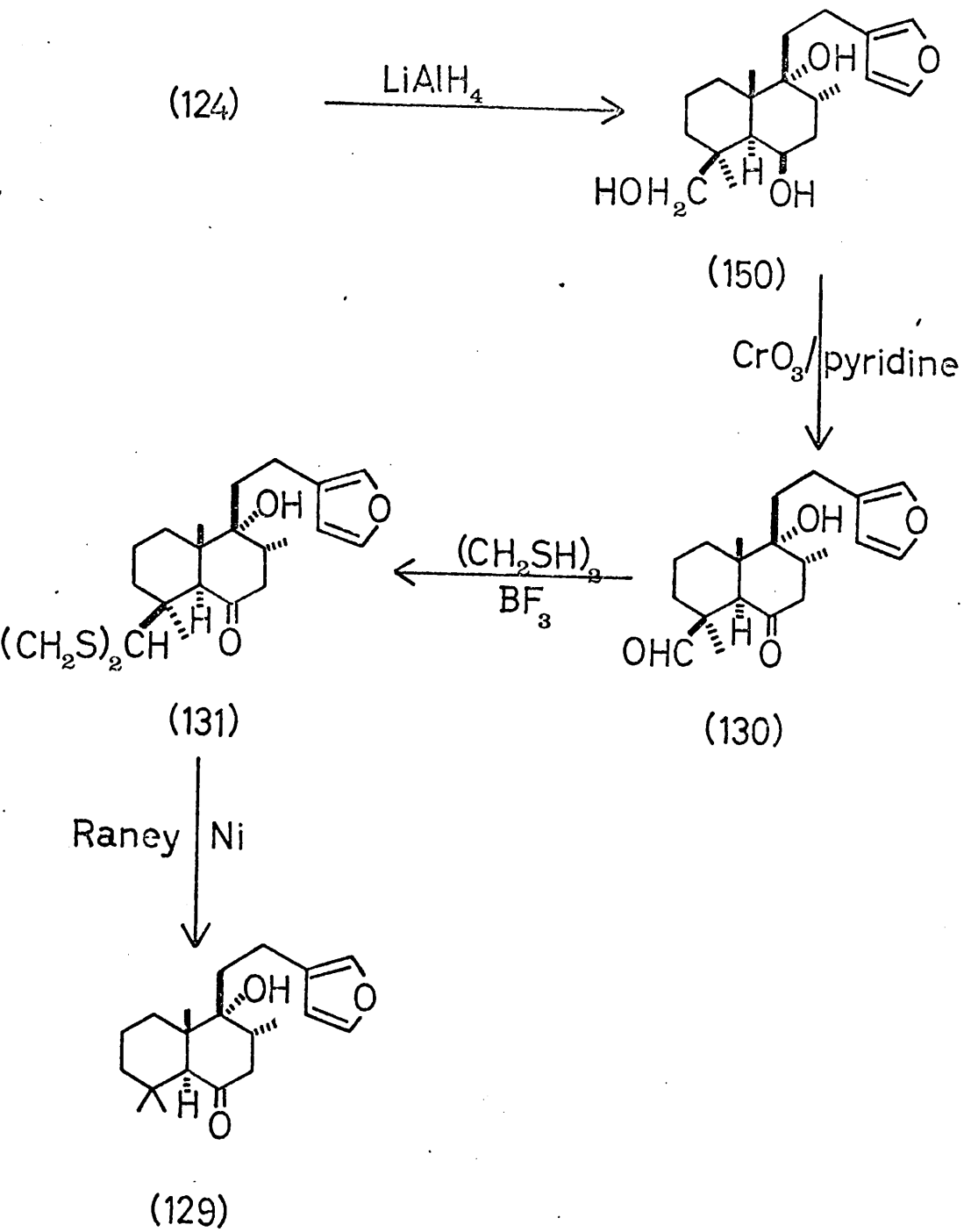
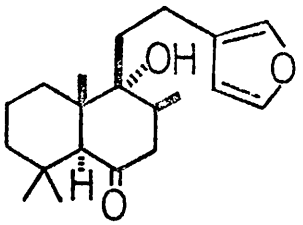

(124)
LiAlH4
OH
O
HOH2C
H
OH
(150)
CrO3/pyridine
OH
O
OHC
H
O
(130)
(CH2SH)2
BF3
OH
O
(CH2S)2CH
H
O
(131)
Raney Ni
OH
O
H
O
(129)

Scheme 11



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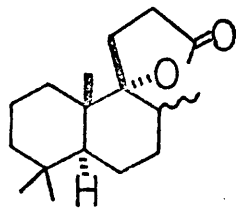

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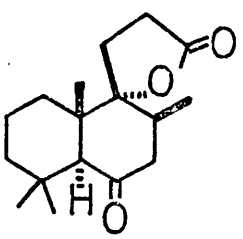

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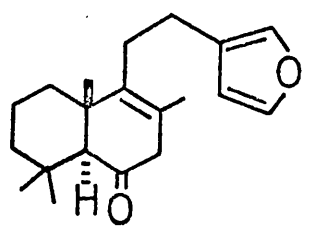

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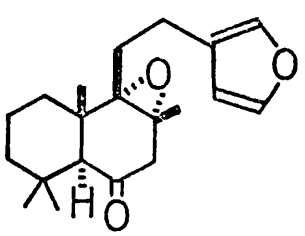

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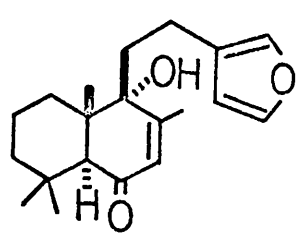

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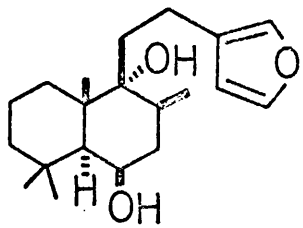

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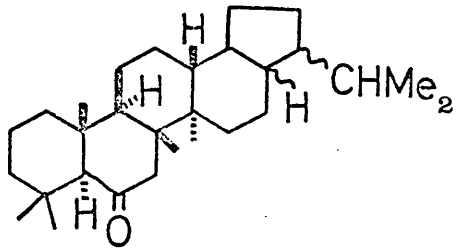

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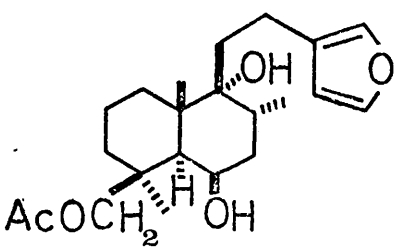

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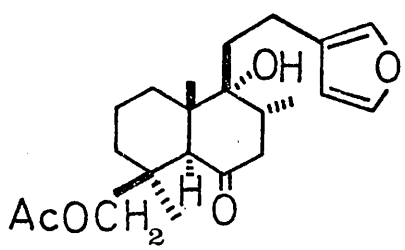

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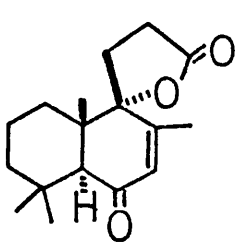

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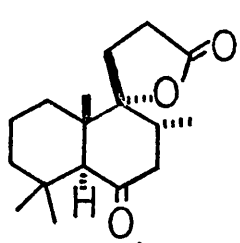

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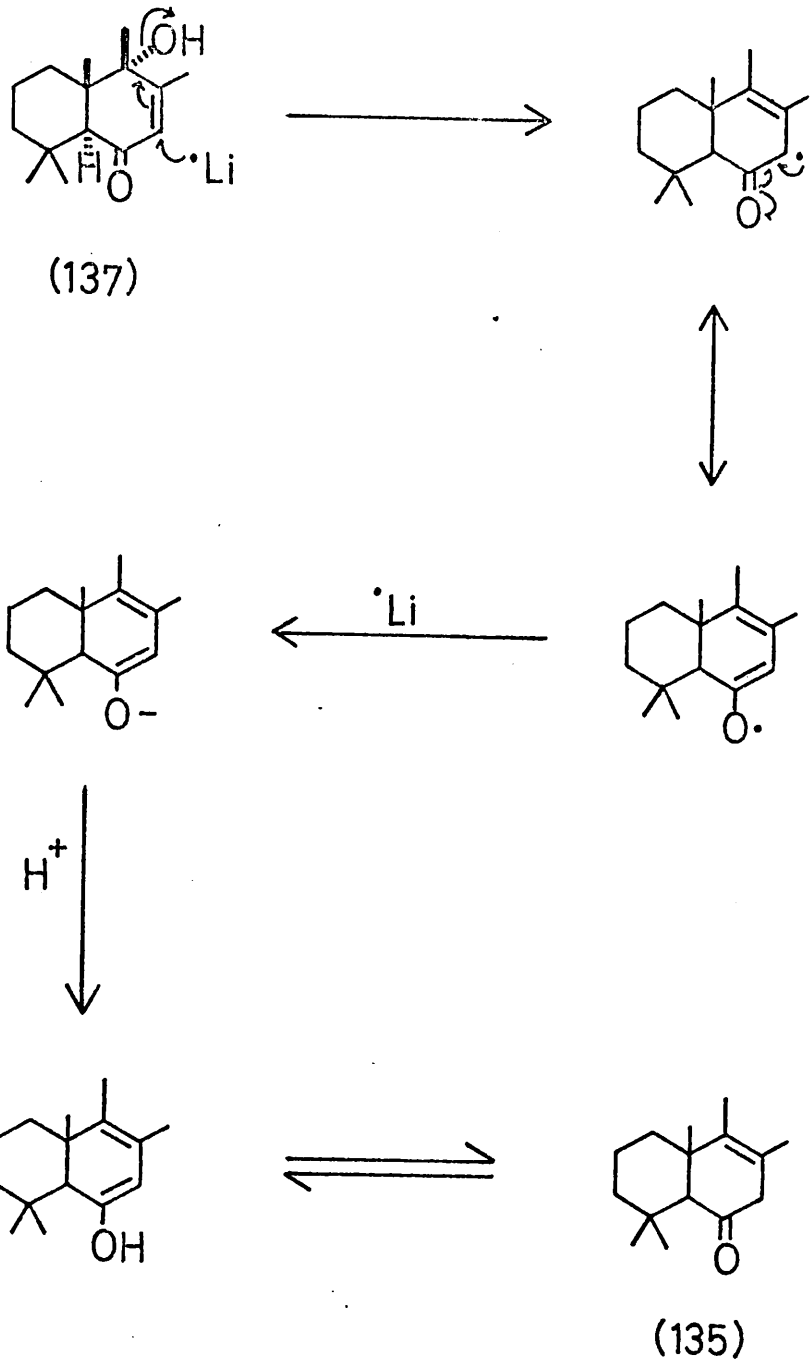
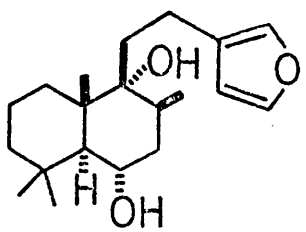


(137)

(135)

Scheme 12



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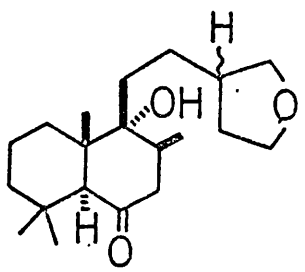

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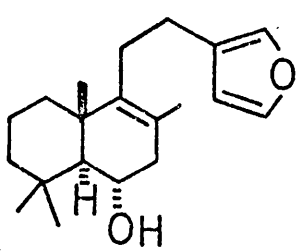

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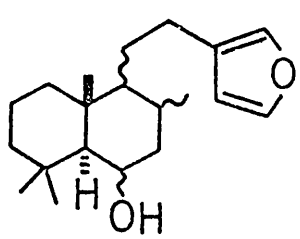

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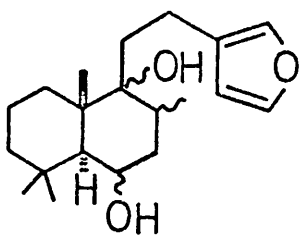

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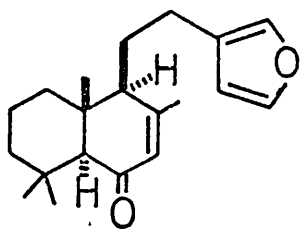
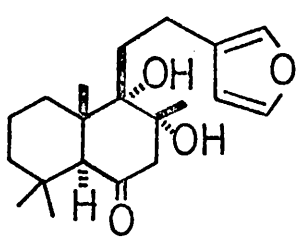


(149)



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