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NEW SESQUITERPENES FROM *PLEOCARPHUS REVOLUTUS**

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Key Word Index—*Pleocarphus revolutus*; Compositae flavone; pleocarpanone; new guaiane and isopatchoulenone sesquiterpenes; cytotoxicity; TLX5 mouse mammary lymphoma.

Abstract—Six new sesquiterpenes of the guaiane and isopatchoulenone types have been isolated from the Chilean plant *Pleocarphus revolutus*. Of these, the compound hydroxy-isopatchoulenone and related esters showed cytotoxicity towards TLX5 mouse mammary lymphoma cells.

INTRODUCTION

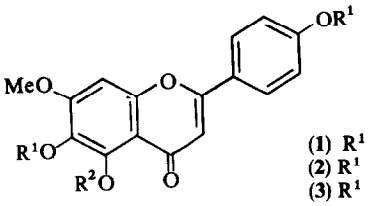
The plant *Pleocarphus revolutus*, a member of the Compositae, occurs as a small shrub growing near La Serena, Chile. Extracts of the powdered leaves and stems have been shown to possess antitumour properties in the primary screen operated by the Cancer Chemotherapy National Service Centre, Maryland, U.S.A. [2]. We now wish to report the results of a more detailed examination of the constituents of this plant. Isolation of active compounds was aided by screening fractions against a rapid, convenient cytotoxicity test developed from a method used at the Chester Beatty Research Institute, London [3, 4].

RESULTS AND DISCUSSION

Although activity against the *in vitro* KB human epidermoid carcinoma of the nasopharynx had been established for the petrol, benzene and ethyl acetate extracts of *P. revolutus*, this study concentrated on the highly active benzene extract. The assayed fractionation was preceeded by an examination of this extract, in order to establish the major components (Fig. 1.A). Thus, column chromatography over silica gel afforded six crystalline compounds. These were a polyalkane, a flavone, α-amyrin, α-amyrin acetate, and two sesquiterpenes.

The crystalline polyalkane, mp 76–78° appeared to be a mixture by MS examination. Although it showed a weak carbonyl absorption in its IR spectrum (ν_{co} 1705 cm^{-1}) as well as saturated alkane bands, the rest of the spectrum was featureless. Since its NMR spectrum only showed a broad singlet at τ 8.7, characteristic of a saturated hydrocarbon, no further work on this fraction was carried out. α-Amyrin and its acetate were characterised by both spectral means and direct comparison with authentic samples.

The flavone (1), mp 272–274° analysed as $C_{16}H_{12}O_6$. Its NMR spectrum indicated one methoxy group, three hydroxyl protons and six aromatic protons Extensive ultraviolet measurements, using the methods of Mabry *et al.* [5], confirmed that this compound was a flavone and indicated the presence of a 4′ and 5-hydroxyl group as well as a 7-methoxy function. Methylation of the flavone, with methyl iodide in acetone containing potassium carbonate, initially yielded the known trimethoxy-derivative (2) [6], whilst extended alkylation times eventually produced the tetramethyl ether (3) [6], thus confirming the general oxidation pattern of the starting flavone (1). Further evidence for the placing of the methoxy substituent at C-7 was obtained by measuring the chemical shift differences for the bis-trimethylsilyl ether in D_6-benzene compared to $CDCl_3$ [7].



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

Two earlier reports on flavones possessing structure (1) have been published. In the first [8], only a mp (190–192°) is quoted, which is considerably lower than that of our compound. The second mention, by Arisawa *et al.* [9], records a mp of 290–292°, somewhat higher than that of our material, and slight differences in its UV properties; the derived tetramethyl ether of Arisawa's flavone was also identical to the authentic compound. Since a sample of Arisawa's material was not available for direct comparison, the reasons for these differences remain unexplained; it should be noted, however, that crystallisation of the flavone (1) proved extremely difficult, the material tending to precipitate from solution as a microcrystalline powder.

Two sesquiterpenes were also isolated from the initial

* Part 2 in the series. For Part 1 see ref [1].

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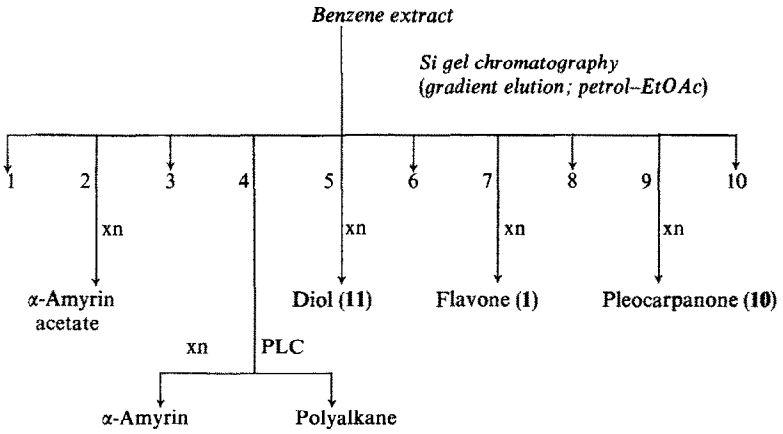
¶ Taken from Ph.D. thesis of T.W.T., Imperial College, University of London, 1976.

examination of the benzene extract. The first, mp 133–135°, analysed as $C_{15}H_{26}O_2$ and was shown to be the diol (**4**). The MS of this compound did not show a parent ion; a weak peak at *m/e* 223 (M^+-15), as well as the one at *m/e* 220 (M^+-18) belied the molecular mass of 238. Loss of a second molecule of water was also observed. three singlet methyl signals with chemical shifts between τ 8.8–9.0, indicative of attachment to quaternary carbon atoms bearing hydroxyl groups. Attempted acetylation with acetic anhydride in pyridine also failed, thus confirming this assignment. Use of the lanthanide induced shift reagent, $Eu(fod)_3$, failed to simplify the spectrum,

A. *Initial separation*



B. *Separation via cytotoxicity assay*

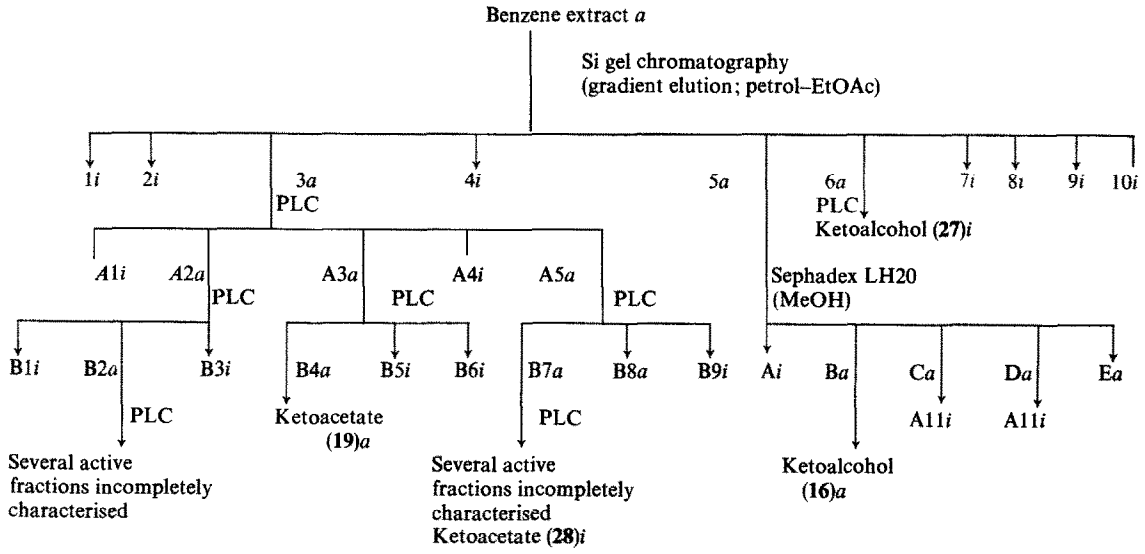


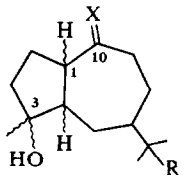
Fig. 1. Fractionation of the benzene extract from *P. revolutus*. xn: crystallisation; *a*: active; *i*: inactive.

This evidence suggested the compound was a diol, since ethers do not readily lose water [10]. The base peak at *m/e* 59 (C_3H_7O) suggested that one of these alcohol functions was part of a 2-hydroxy-propyl group, typical of many sesquiterpenes. The presence of an exocyclic methylene group was indicated by bands at 1640 and 895 cm^{-1} in the IR spectrum; no carbonyl groups were present, confirming the deductions from the MS. The NMR spectrum confirmed the presence of the exocyclic methylene group but, since no other resonance peaks were present below τ 8.0, the hydroxyl groups must both be tertiary. The high field region indicated probably because of complications arising from two competing, complexing sites.

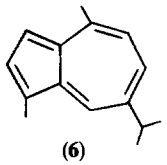
Hydrogenation of the diol gave a dihydro-derivative (**5**) indicating a bicyclic structure. Dehydrogenation of the natural product over palladium readily gave a blue oil, with a UV spectrum identical to that of *S*-guaiazulene (**6**), suggesting a guaiane skeleton for the diol. Only two substitution patterns are consistent with these results, structures **4** and **7**. Since ozonolysis afforded a ketone having ν_{co} 1695 cm^{-1}, characteristic of a six or higher-membered ring ketone, the former structure is favoured. Ozonolysis thus produces the cycloheptanone (**10**).

^{13}C-NMR spectroscopy (see Table 1 and Experimental) was entirely consistent with the diol structure **4**.

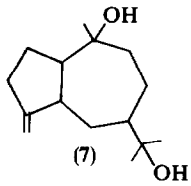
The diol (**4**) was extremely sensitive to acid; reaction with traces of toluene-*p*-sulphonic acid in benzene at room temperature readily gave a black tar. Dehydration was effected more cleanly by use of phosphorus oxychloride in pyridine. The major product was a triene, assigned structure **9**, although this was also unstable to storage at room temperature.



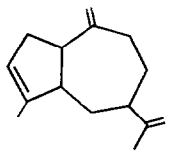
(**4**) R = OH, X = CH_2
(**5**) R = OH, X = H, Me
(**8**) R = H, X = CH_2



(**6**)



(**7**)



(**9**)

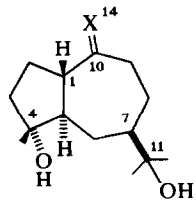
To our knowledge this diol (**4**) appears to be a new sesquiterpene, although the monoalcohol nardol (**8**) has been described [11].

The other, major sesquiterpene obtained from the benzene extract was a crystalline solid, mp 106–108° which analysed as $C_{14}H_{24}O_3$. This nor-sesquiterpene proved to be identical to the ketone (**10**) obtained by ozonolysis of the diol (**4**). Spectral identification of this compound was accompanied by an X-ray analysis [12] which showed the stereochemical structure depicted by **10**. Since ozonolysis of the diol (**4**) gave the ketone (**10**) directly, with no sign of any isomerisation, the stereochemical relationships must be the same, thus giving **4** the stereochemistry depicted by **11**. Treatment of the ketone (**10**) with base did not induce epimerisation about C-1. It has been given the trivial name pleocarpanone [12]. The seven-membered ring adopts a twist-chair conformation with C-9 as the axis carbon.

Table 1. ^{13}C-NMR chemical shifts for the diol **11** (≡**4**)

Peak no.	Chemical shift*	Off resonance pattern	Assignment
1	152.28	*s*	C-10
2	109.90	*t*	C-14
3	81.63	*s*	C-4 or C-11
4	73.83	*s*	C11 or C-4
5	54.04	*d*	C-1 or C-5
6	52.02	*t*	CH_2
7	47.34	*d*	C-5 or C-1
8	40.24	*t*	CH_2
9	37.02	*t*	CH_2
10	32.73	*t*	CH_2
11	28.33	*q*	Me
12	27.59	*d*	C-7
13	26.73	*t*	CH_2
14	25.34	*q*	Me
15	24.39	*q*	Me

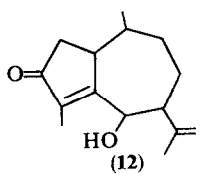
* From ref. tetramethylsilane.



(**10**) X = O
(**11**) X = CH_2

Subsequent fractionation of the *Pleocarphus revolutus* extract was based on an assay method using a simple cytotoxicity test against TLX5 mouse mammary lymphoma cells. The advantages of this assay procedure lay in the rapidity with which assay results could be obtained; fractions could be tested and activity established within two days. This prevented delays in the fractionation procedure and enabled us to concentrate on the active material. The test was shown to be a reliable oi. fo: cytotoxicity. In testing the validity of the assay method a variety of known anti-tumour compounds proved active whilst a selection of known, inactive compounds gave the expected, negative tests [4].

The active fractions (Fig. 1B) 3, 5 and 6 were selected for further purification. Separation of fraction 5 over Sephadex LH20 gave three active subfractions, two of which proved unstable and, on subsequent fractionation only gave inactive material. The remaining fraction afforded a new crystalline substance which showed moderate activity in the assay. This material analysed as $C_{15}H_{22}O_2$ and its IR spectrum showed hydroxyl (3480 cm^{-1}) and carbonyl (1700 cm^{-1}) groups, accounting for both oxygen atoms, and an exocyclic double bond (1640 and 895 cm^{-1}). The UV spectrum proved that the carbonyl group was conjugated (λ_{max} 243.5 nm, ε 13400) having two double bond equivalents shown to be due to the bicyclic nature of the compound. The hydroxyl group was secondary. It could be either acetylated or formylated under mild conditions. The ^{13}C-NMR spectrum of this compound, together with Eu(fod)$_3$ experiments, suggested structure **12** for the ketone. The Eu(fod)$_3$, experiments were particularly informative. The induced shift of the proton at C-6 was very small,indicating a very weak interaction of this proton with the europium complex. The primary site of complexing is probably the car-

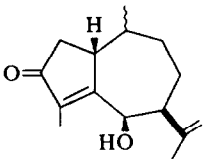


(**12**)

bonyl group. This rather surprising result suggests that the hydroxyl group at C-6 is in a relatively hindered site.

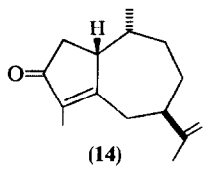
ORD data for a number of guaiane-type compounds containing cyclopentenone systems similar to the keto-alcohol (**12**) have been obtained by Piers and Cheng [13]. Since the cyclopentenone ring is essentially planar these authors considered that, in the absence of other conjugated chromophores, the sign of the Cotton effect was principally determined by the configuration about C-1. A negative Cotton effect, and thus a negative CD curve, was considered to be diagnostic for systems with the hydrogen at C-1 on the β-face. Since the keto-alcohol (**12**) also possessed a negative Cotton effect, it has been assigned a β-orientation at C-1. The configuration of the isopropenyl group at C-7 is assumed to be β, in agreement with the configuration found at this centre for the vast majority of known guaianes, as well as on the isolation of the related compounds **10** and **11**.

From previous conformational analyses of perhydroazulenes [14], the isopropenyl group would favour a pseudo-equatorial position. This assumption was made in the following configurational arguments. Molecular models indicate that the hydroxyl group at C-6 must be *cis* to the isopropenyl group in order to explain the small coupling constant of 1 Hz observed between the protons at C-6 and C-7; a dihedral angle of approximately 90° is required. These deductions lead to the partial stereochemical assignment of the keto-alcohol as **13**.

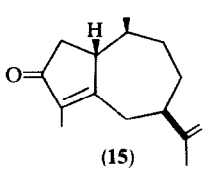


(13)

Reduction of the keto-alcohol with zinc dust in acetic acid afforded an $\alpha\beta$-unsaturated ketone (**15**), in which the 6-hydroxy group was eliminated. This was similar in its properties, but not identical, to the known ketone (**14**),



(14)



(15)

prepared by Büchi *et al.* [15] in a synthetic approach to cyclocolorenone. Since their optical rotations differ, the stereochemistry about C-10 must vary. Hence the keto-alcohol is completely represented by structure **16**.

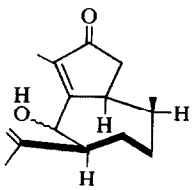
Attempted dehydration of the keto-alcohol, using toluene-*p*-sulphonic acid in benzene, gave one major product which proved to be an isomer. The NMR spectrum of this material was almost identical to that of the starting keto-alcohol except for the shift to higher field (τ 9.40) of the 10-methyl doublet. The upfield shift of the 10-methyl group indicates epimerisation about C-1. The isomeric keto alcohol (**17**) thus has the 10-methyl group held over the shielding zone of the cyclopentenone ring. A similar effect was observed for the enone (**18**) prepared by Büchi *et al.* [15]. The orientation about C-6 was undefined for our isomer. The epimerisation of **16** to **17** probably proceeds by acid-catalysed enolisation of the cyclopentenone function.

Examination of fraction 3 yielded three active subfractions and, after further purification, a pure, active oil was isolated. This proved to be identical to the acetate ester (**19**), formerly prepared from the keto-alcohol (**16**). Hydrolysis of this acetate ester with base produced the crystalline alcohol (**16**). The CD curves of the two acetate compounds were also superimposable, indicating configurational identity.

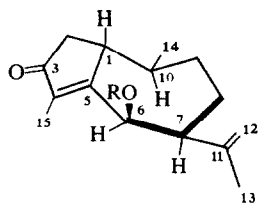
The acetate ester proved to be unstable. On leaving the oil, in Pyrex vessels exposed to subdued sunlight and air, a new compound formed. In the absence of light no reaction occurred. Isolation by PLC, using the multiple elution technique, gave a pure oil, analysing as $C_{17}H_{24}O_5$. Its IR spectrum indicated a sharp hydroxyl band (3420 cm^{-1}), a broad, strong carbonyl peak (1760 cm^{-1}) and carbon–carbon double bond absorptions (1665, 1645 and 960 cm^{-1}). The material gave a positive starch–iodide test, indicating a hydroperoxide. Reduction of this material with potassium iodide gave an alcohol. The latter possessed two carbonyl absorptions (1720 cm^{-1}) as well as a band for the hydroxyl group. Neither compound showed new vinylic protons in their NMR spectra but resonances similar to that of the starting material for the protons at C-6 and C-7 and for the methyl groups. The hydroperoxide thus has structure **20** and the corresponding alcohol formula **21**. Although it is tempting to consider a singlet oxygen process for the oxidation, attempts to repeat the reaction under more controlled conditions failed. Use of methylene blue in either dichloromethane or benzene failed, although traces of the peroxide formed when the keto-acetate was irradiated, in an oxygenated-benzene solution, with light from a medium pressure mercury lamp.

Instances of specific autoxidation of sesquiterpenes in the absence of sensitizers are rare. McMurray and Mollan [16], however, have noted that santonene formed a hydroperoxide in ethanol solution.

By contrast to the acetate, the crystalline formate (**22**) did not form a hydroperoxide on exposure to sunlight and air. Samples of the hydroperoxide (**20**) were eventually obtained from the crude benzene extract, but these could well be an artefact formed after the extraction process.



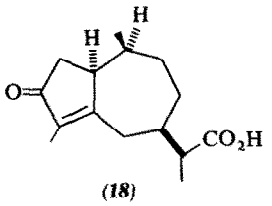
(17)



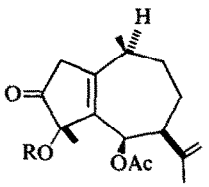
(16) R = H
(19) R = MeCO
(22) R = HCO.
(23) R = Ph.CH=CH.CO

In order to gain insight into structure-activity relationships between the pure alcohol (**16**) and its esters these, as well as the cinnamate ester (**23**), were assayed by the TLX5 method. Lee *et al.* [17] have shown that

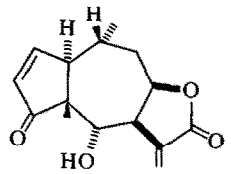
variation of ester functions can make dramatic changes to the level of activity in derivatives of helenalin (**24**), the most active being the cinnamate ester. The observed order of activity [4] was alcohol (**16**) ≪ formate (**22**) ~ acetate (**19**) < cinnamate (**23**), which agrees with the type of order found by Lee [17]. One further derivative in this series was also examined. The chloride (**25**), obtained in an attempted dehydration of the keto-alcohol with phosphorus oxychloride in pyridine, was similar to the acetate in the assay.



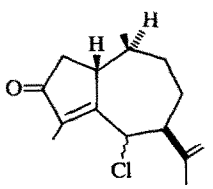
(*18*)



(**20**) R = OH
(**21**) R = H

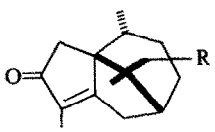


(**24**)



(**25**)

Although other active fractions were isolated from fraction 3 (Fig. 1B) these were not full characterised. One other, inactive compound was isolated from fraction 6. Obtained as a colourless oil this analysed as $C_{15}H_{22}O_2$. The appearance of IR bands at 3440, 1700, and 1670 cm^{-1} indicated both an $\alpha\beta$-unsaturated ketone and an alcohol group, the former being confirmed by its UV absorption (λ_{max} 261; ε 13 500). This information suggested a structure similar to the keto-alcohol (**16**). Attempted reduction with zinc dust in acetic acid failed, ruling out the presence of an oxygen function at C-6. The NMR spectrum included an AB pattern centred at τ 6.2, indicative of a hydroxymethylene group. The absence of the exocyclic double bond and no other vinylic resonances suggested a tricyclic structure of the isopatchoulane type. A comparison of the NMR pattern of isopatchoul-4(5)-en-3-one (**26**) with this ketol indicated a close similarity. The major difference was the absence of the C-12 methyl group and the replacement by the hydroxymethylene function. On this basis the compound was assigned structure **27**. The configuration of the 10-methyl group follows from its high field position (τ 9.38), indicative of the shielding by the cyclopentenone and in agreement with that of the known isopatchoulone (**26**). The alcohol (**27**) readily formed an acetate (**28**). Neither the alcohol nor its ester showed activity in the TLX5 assay. A small sample of the acetate ester (**28**) was eventually obtained from the fraction B7 (Fig. 1B), thus it, too, is a natural product.



(**26**) R = H
(**27**) R = OH
(**28**) R = OAc

EXPERIMENTAL

^{13}C-NMR spectra were recorded on a Varian XL 100 instrument. TLC was carried out on kieselgel GF 254 (Merck). Yields of natural products are expressed as %wt of dried plant material.

Extraction. Powdered, dried leaves and stems of *Pleocarphus revolutus* (5 kg) were extracted to completion by percolation with EtOH–H_2O (1:1). Solvent was removed *in vacuo*, residue (550 g) macerated with H_2O and the ppt. obtained was discarded. The aq. soln was then extracted with petrol (60–80°), C_6H_6, EtOAc, *n*-BuOH, and methyl ethyl ketone. On evaporation these extracts respectively weighed 36, 60, 35, 28 and 23 g, all as dark tars. Column chromatography of the dried C_6H_6 extracts (5 g) on Si gel (200 g) yielded 10 fractions by gradient elution with petrol–EtOAc mixtures. Fraction 2 (Fig. 1A) crystallised to an oily white solid. Recrystallisation (MeOH) yielded white needles of α-amyrin acetate (73 mg, 0.018%); mp 274–276° (lit [18] mp 224°), ν_{max}^{Nujol} cm^{-1}: 3450, 1730, 1470, 1255. Crystallisation of fraction 4 yielded α-amyrin (242 mg, 0.058%) mp 181–183° (lit [18] mp 186°). From the mother liquors was obtained, by PLC ($CHCl_3$), a solid mp 76–78° (hexane–EtOAc), τ 8.70 (broad *s*, strong), ν_{max}^{Nujol} cm^{-1}: 1705 (weak), 1305, 735, 725. MS of this portion showed no discrete parent ion and a fragmentation pattern typical for a long-chain hydrocarbon.

7-Methoxy-4′,5,6-trihydroxyflavone, (**1**). Obtained as a yellow solid, precipitated from fraction 7 (Fig. 1A). Purification by PLC (MeOH–$CHCl_3$, 1:9) followed by crystallisation from C_6H_6–MeOH gave fine yellow crystals, mp 272–274°; IR ν_{max}^{Nujol} cm^{-1}: 3400 (broad), 2950, 2880, 1710, 1655, 1610, 1580, 1470, 1385, 1285, 1255, 1170, 1100; NMR (d_6-DMSO): τ 3.2 (1H, *s*, 5-OH), −0.28 (2H, *br*, OH), 2.24 (2H) and 3.12 (2H) as AA′ XX′ system (J_{AX} = 9 Hz), 3.33 (1H, *s*, 8-H), 3.48 (1H, *s*, 3-H), 6.28 (3H, *s*); MS *m/e* 300.0625 ($C_{16}H_{12}O_6$ requires 300.0634), 285 (M^+-Me), 282 (M^+-H_2O); UV λ_{max}^{MeOH} nm: 273.5, 335.5 + $AlCl_3$: 302, 258; +NaOAc; 275, 388; +NaOAc + H_3BO_3: 272, 341; +NaOMe: 276, 326.5, 394.5.

Methylation and silylation of the flavone (**1**) The flavone (30 mg), K_2CO_3 (4 mg) and methyl iodide (1 ml) in Me_2CO (20 ml) was heated to reflux for 6 hr. Filtration, evap. and PLC ($CHCl_3$) gave yellow crystals of 6,7,4′-trimethoxy-5-hydroxyflavone (**2**) (21 mg, 65%) mp 192–4° (MeOH), (lit [6] mp 187–188°); UV λ_{max}^{EtOH} nm: 277, 330 NMR: 2.20 (1H, *s*), 218 (2H, *m*), 3.00 (2H, *m*), 3.45 (1H, *s*), 3.50 (1H, *s*), 6.05 (3H, *s*), 6.10 (3H, *s*), 6.15 (3H, *s*). Further alkylation under similar conditions, but for 24 hr, gave, after work up, 5,6,7,4′-tetra-methoxyflavone (**3**) (59%) mp 158–159° (MeOH), (lit [6] mp 161–162°); λ_{max}^{EtOH} nm: 267, 320 NMR: τ 2.11 (2H, *m*), 2.97 (2H, *m*), 3.17 (1H, *s*), 3.38 (1H, *s*), 6.00 (6H, *s*), 6.07 (3H, *s*), 6.10(3H, *s*). Treatment of the flavone (**1**) (30 mg) with hexamethyldisilazane (0.5 ml) and trimethylchlorosilane (0.5 ml) in C_5H_5N (3 ml) for 16 hr gave, after evap and extraction with CCl_4, the bistrimethylsilyl ether, NMR (CCl_4): τ 2.16, (2H, *m*), 3.15 (2H, *m*), 3.50 (1H, *s*), 3.70 (1H, *s*), 6.34, (3H, *s*), NMR (C_6D_6): τ 3.12 (2H, *m*), 3.82 (2H, *m*), 4.03 (1H, *s*), 4.10 (1H, s), 6.77 (3H, *s*). Further samples of the flavone were subsequently obtained from the EtOAc extract of the plant (126 mg, 0.0087%). The *diol* (**11**, 542 mg, 0.13%), after sublimation at 45–50° under red. pres. had mp 133–135°; $[\alpha]_D^{24}$ −4.77° (0.41, $CHCl_3$); IR ν_{max}^{Nujol} cm^{-1}: 3350, 2950, 1640, 1470, 1380, 1190, 1135, 1080, 950, 895; UV λ_{max}^{EtOH}: end absorption; ^{13}C-NMR: see Table 1; NMR ($CDCl_3$): τ 5.28 (1H, broad *s*), 5.36 (1H, broad *s*), 8.88 (3H, *s*), 8.92 (3H, *s*), 8.96 (3H, *s*); MS *m/e* 223 (M^+-methyl), 220 (M^+-H_2O), 202 (M^+-$2H_2O$), 59 (base peak). (Found: C, 75.50; H, 11.04; $C_{15}H_{26}O_2$ requires C, 75.63; H, 10.92%.) Attempted acetylation of the diol, with Ac_2O in C_5H_5N for 24 hr failed.

Reduction of the diol (**11**). Hydrogenation of the diol (50 mg) over PtO_2 (10 mg) in EtOH (10 ml) at atm. pres. and room temp. afforded colourless crystals of the *dihydrodiol* (**5**) (48.2 *mg*, 97%); *mp* 156–7° (3:1 hexane–EtOAc); $[\alpha]_D^{25}$ −56.5° (1.1, $CHCl_3$); IR ν_{max}^{Nujol} cm^{-1}: 3350, 2950, 2880, 1470, 1385, 1325, 1255, 1170, 1145, 1085, 1075, 940, NMR ($CDCl_3$): τ 8.83 (6H, *s*), 8.87 (3H, *s*); MS *m/e*: 225 (M^+-methyl), 222 (M^+-H_2O), 206 (M^+-$2H_2O$). (Found:

C, 75.25; H, 11.39; $C_{15}H_{28}O_2$ requires C, 75.00; H, 11.67%.)

Dehydrogenation of diol (**11**). Sesquiterpene (25 mg) was sealed under vacuum in a glass tube containing 5% Pd(C (25 mg) and heated at 198° for 4 hr. PLC (EtOAc) of the product afforded *S*-guaizaulene (**6**) (8.3 mg, 40%), UV: λ_{max}^{EtOH} nm; 244, 284, 289, 304, 349, 367, identical to that recorded in the literature [19, 20].

Ozonolysis of the diol (**11**). The diol (500 mg) in CH_2Cl_2 (50 ml) at −10 to −15° was treated with a stream of ozonised O_2 for 2 hr. The soln was allowed to warm to room temp. over 4 hr before separating the nor-guaiane *pleocarpanone* (**10**) by PLC. This ketone (246 mg, 49%) crystallised from hexane–EtOAc as a hemi-hydrate, mp 96–100°. After prolonged drying under vacuum at 70° the dry keto-diol formed, mp 106–108°. This had $[\alpha]_D$ −4.77° (1.0, $CHCl_3$); IR ν_{max}^{Nujol} cm^{-1}: 3460, 1695, 1465, 1405, 1390, 1370, 1315, 1255, 1245, 1225, 1200, 1180, 1150, 1125, 1110, 1075, 1045, 960, 925, 880, 850; MS *m/e*: 240 (M^+), 222, (M^+-H_2O), 204 (M^+-2H_2O), 59 (base peak). (Found for the hemihydrate C, 67.49; H, 10.05. $C_{14}H_{24}O_3 \cdot \frac{1}{2}H_2O$ requires C, 67.47; H, 10.04%.) The nor-ketone was also obtained by PLC (EtOAc) of fraction 10 from the Si gel column chromatography. The yield of white crystals was 72 mg (0.017%), identical in all respects to the ozonolysis product (**10**).

Dehydration of the diol (**11**). The diol (50 mg) in C_5H_5N (5 ml) was stirred with $POCl_3$ (0.2 ml) for 1 hr before working up by neutralising with dil. acid and extraction into CH_2Cl_2. The major product, isolated by PLC ($CHCl_3$) was the *triene* (**9**) (45 mg, 81%), as an oil; IR ν_{max}^{film} cm^{-1}: 3080, 2950, 2880, 1640, 1455, 1385, 1330, 1275, 1180, 960 and 905; NMR: τ 4.76 (1H, *br s*), 5.34 (4H, broad *m*), 8.30 (6H, *br s*); MS *m/e*: 202 (M^+). This material gradually absorbed oxygen with exposure to air.

Keto alcohol (**16**). The keto-alcohol (**16**) (630 mg, 0.076%) had mp 108–109° (hexane–EtOAc); $[\alpha]_D^{22}$ −145° (1.65, $CHCl_3$); CD (MeOH): $\Delta\varepsilon_{304}$ −2.35, $\Delta\varepsilon_{204}$ −10.3, $\Delta\varepsilon_{203}$ +5.4; IR ν_{max}^{Nujol} cm^{-1}: 3480, 2950, 1700, 1640, 1450, 1415, 1385, 1340, 1320, 1285, 1190, 1155, 1145, 1115, 1090, 1070, 1065, 1030, 1000, 960, 950, 910, UV λ_{max}^{EtOH} nm: 243.5 (ε 13 400); NMR: τ 5.18 (3H, *br m*), 6.88 (1H, *m*), 8.16 (3H, *s*), 8.37, *d*, *J* = 2 Hz), 9.04 (3H, *d*, *J* = 7 Hz) ^{13}C-NMR: see Table 2; MS *m/e*: 234 (M^+), 216 (M^+-H_2O), 109 (Base peak). (Found: C, 77.10; H, 9.32. $C_{15}H_{22}O_2$ requires C, 76.92; H, 9.40%.) Treatment of the alcohol (25 mg) with Ac_2O in C_5H_5N afforded the *acetate* (**19**), isolated by PLC ($CHCl_3$) as a colourless oil (18.8 mg, 63%); IR ν_{max}^{film} cm^{-1}: 1740, 1705, 1645, 1235; NMR: τ 4.6 (1H. *s*), 5.27 (2H, *br m*), 7.12 (1H, *m*), 8.05 (3H, *s*), 8.22 (3H, *s*), 8.29 (3H, *d*, *J* = 2Hz), 9.04 (3H, *d*, *J* = 7Hz), *m/e* 276 (M^+), 216 (M^+-AcOH). This acetate proved to be identical to the naturally occurring keto-acetate subsequently isolated from the plant (148 mg, 0.018%); $[\alpha]_D$ −110.0° (1.0, $CHCl_3$); CD (MeOH): $\Delta\varepsilon_{307}$ −0.55, $\Delta\varepsilon_{238}$ −3.1, $\Delta\varepsilon_{196}$ +3.1. Cinnamoylation of the alcohol, using cinnamoyl chloride, was likewise carried out to give the *cinnamate ester* (**23**) as a colourless oil (33.1 mg, 90%). After separation by PLC ($CHCl_3$) this had UV λ_{max}^{EtOH} nm: 223, 240, 280 (ε 21 500, 17 500, 26 000); NMR: τ 2.20 (1H, *d*, *J* = 16Hz), 2.50 (5H, *m*), 3.48 (1H, *d*, *J* = 16 Hz), 3.90 (3H, *d*, *J* = 7 Hz); MS *m/e* 364 (M^+), 216 (M^+-cinnamic acid); $[\alpha]_D^{20}$ −175° (1.05, $CHCl_3$). (Found: C, 78.85; H, 7.67. $C_{24}H_{28}O_3$ requires C, 79.12; H, 7.69%.) Finally, formylation, with formic–acetic anhydride in C_5H_5N, gave the *formate ester* (**22**) as a crystalline solid, mp 103–105° (hexane–EtOAc); $[\alpha]_D^{23}$ −127.7° (0.62, $CHCl_3$), UV λ_{max}^{EtOH} nm: 238 (ε 17 600); IR ν_{max}^{Nujol} cm^{-1}: 1735, 1710, 1645, 1445, 1410, 1380, 1335, 1280, 1200, 1160, 1110, 1085, 1055, 955, 925, 905; NMR: τ 1.83 (1H, *s*), 3.90 (1H, *br. s*), 5.13 (2H, *m*), 8.16 (6H, *m*), 9.05 (3H, *d*, *J* = 7Hz); MS *m/e*: 262 (M^+), 216 (M^+-HCO_2H). (Found: C, 73.16; H, 8.62. $C_{16}H_{22}O_3$ requires C, 73.28; H, 8.40%).

Reduction of the keto alcohol (**16**). Zn dust (25 mg) was added to the keto alcohol (50 mg) in HOAc (10 ml). The mixture was heated to reflux for 18 hr, fresh portions of Zn dust being added after 13 and 15 hr. Work up. by neutralising with aq. NaOH, extraction into CH_2Cl_2 and evap, gave an oil (45 mg). After PLC the reduction product (**15**) (25 mg, 63%) was obtained as a colourless oil, $[\alpha]_D^{21}$ +29°, uv λ_{max}^{EtOH} nm: 239 (ε 15000); IR ν_{max}^{film} cm^{-1}: 3070, 2960, 2930, 2880, 1705, 1648, 1460, 1445, 1412, 1385, 1345, 1285, 1180, 1155, 1075, 1045, 945, 910, 895; NMR: τ 5.2 (2H, *m*), 8.0 (3H, *s*), 8.05 (3H, *d*, *J* = 2 Hz) 9.00 (3H, *d*, *J* = 7 Hz); MS *m/e*: 218 (M^+), (Found: C, 82.51; H, 10.15. $C_{15}H_{22}O$ requires C, 82.57, H, 10.09%).

Attempted dehydration of the keto-alcohol (**16**). The keto-alcohol (100 mg) and toluene-*p*-sulphonic acid (100 mg) were heated in dry CH_2Cl_2 (20 ml) to reflux for 6 hr. After washing the sol. with aq. $NaHCO_3$ and H_2O, evap of the organic phase gave an oil. PLC ($CHCl_3$) afforded recovered starting material (18 mg, 18%) and a more polar alcohol, shown to be the isomer (**17**) (11 mg, 11%), IR ν_{max} cm^{-1}: 3540, 2980, 2910, 1710, 1645, 1410, 1385, 1312, 908, NMR: τ 5.1 (3H, *m*), 8.1 (3H, *br s*), 8.2 (3H, *br s*), 9.40 (3H, *d*, *J* = 7 Hz); MS *m/e*: 252 (M^+), 234 (M^+-H_2O), 109 (base peak).

Autoxidation of the acetate (**19**). A freshly prepared sample of the acetate (50 mg) was sealed in a Pyrex flask and exposed to direct daylight (diffuse sunlight) at room temp. for 48 hr. A control sample, stored in the dark showed no change after this time. The exposed sample was separated by PLC (petrol–$CHCl_3$, 1:2; 3 elutions) to give, as a slightly more polar material than the starting ester, the *hydroperoxide* (**20**) as a colourless oil (20 mg), UV λ_{max}^{EtOH} nm: 233, 284 (ε 6400, 250); IR ν_{max}^{film} cm^{-1}: 3420, 2960, 2930, 2850, 1740, 1665, 1645, 1460, 1440, 1380, 1330, 1295, 1235, 1155, 1115, 1080, 1055, 1020, 975 and 960; NMR: τ 2.12 (1H, *m*, exchanged by D_2O), 4.49 (1H, *br s*), 5.18 (2H, *br s*), 7.89 (3H, *s*), 8.18 (3H, *s*), 8.20 (3H, *d*, *J* = 3 Hz), 8.98 (3H, *d*, *J* = 7 Hz): MS *m/e*: 308 (M^+); $[\alpha]_D^{24}$ −26.3° (0.63, $CHCl_3$). (Found: C, 66.3; H, 7.89. $C_{17}H_{24}O_5$ requires C, 66.23; H, 7.79%.) This material gives a positive KI–starch test. Reduction of hydroperoxide (10 mg) in Et_2O (3 ml) with aq. KI for 2 hr followed by washing with sodium thiosulphate soln, and evap of the organic layer and PLC ($CHCl_3$) gave the *alcohol* (**21**) (3.7 mg, 43%) as a colourless oil, IR ν_{max} cm^{-1}: 3460, 2930, 2860, 1745, 1720, 1645, 1460, 1375, 1330, 1230, 1120, 960, 900; MS *m/e*: 292.1673 (M^+), ($C_{12}H_{24}O_4$ requires 292.1674), 232 (M^+-AcOH), 214 (M^+-AcOH–H_2O).

Hydroxy-Isopatchoulenone (**16**). The *alchol* was obtained as a pale yellow oil (181 mg, 0.022%) $[\alpha]_D^{19}$ +22.9° (1.52, $CHCl_3$), UV λ_{max}^{EtOH} nm: 241 (ε 13500); IR ν_{max}^{film} cm^{-1}: 3440, 2930, 2880, 1710, 1670. 1465. 1430, 1415, 1380, 1330, 1305, 1250, 1165, 1135, 1025, 910; NMR: τ 6.2 (3H, *br m*), 7.8 (3H, *s*), 8.28 (3H, *s*), 9.15 (3H, *s*), 9.38 (3H, *d*, *J* = 6.5 Hz); MS *m/e*: 234 (M^+), 216 (M^+-H_2O). (Found: C, 71.48; H, 9.47. $C_{15}H_{22}O_2 + H_2O$ requires C, 71.43; H, 9.52%.) Acetylation of the alcohol (30 mg) in C_5H_5N (3 ml) with Ac_2O (0.1 ml) for 12 hr at room temp. gave, after evaporation and PLC ($CHCl_3$), the *acetate* (**27**) (10 mg, 31%), $[\alpha]_D^{23}$ −44° (0.5, $CHCl_3$); UV λ_{max} nm: 239.5 (ε 9700),

Table 2. ^{13}C-NMR chemical shifts for the ketoalcohol **16**

Peak no.	Chemical shift*	Off resonance pattern	Assignment
1	200.48	*s*	C-3
2	176.76	*s*	C-5
3	147.00	*s*	C-4
4	134.78	*s*	C-11
5	111.61	*t*	C-12
6	70.80	*d*	C-6
7	51.95	*d*	C-1 or C-7
8	43.76	*d*	C-7 or C-1
9	36.95	*t*	CH_2
10	33.52	*d*	C-10
11	30.18	*t*	CH_2
12	29.05	*t*	CH_2
13	23.19	*q*	Me
14	20.87	*q*	Me
15	7.79	*q*	C-14

* From ref. tetramethylsilane.

IR ν_{max} cm^{-1}: 2960, 1748, 1710, 1670, 1465, 1430, 1420, 1320, 1380, 1365, 1330, 1302, 1275, 1235, 1180, 1035; NMR: τ 5.7 (2H, AB*q*, J = 11 Hz), 7.85 (3H, *s*), 7.98 (3H, *s*), 8.29) (3H, *s*), 9.18 (3H, *s*), 9.38 (3H, *d*, J = 6.0 Hz), MS *m/e*: 276 (M^+-AcOH). (Found: C, 73.75; H, 8.62 $C_{17}H_{24}O_3$ requires C, 73.91; H, 8.70%). Attempted reduction of the acetate with zinc dust in refluxing glacial HOAc failed. Attempted dehydrogenation of either the alcohol (**26**) or its acetate ester (Pd/C), as described above, gave no azulene derivatives.

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REFERENCES

1. Bhakuni, D. S., Bittner, M., Marticorena, C., Silva, M., Hoeneisen, M. and Hartwell, J. L. (1976) *Lloydia* **39**, in press.
2. *Cancer Chemother. Rep.* **25**, 1 (1962).
3. Connors, T. A., Mandel, H. G. and McGack, D. H. (1972) *Int. J. Cancer* **9**, 126.
4. Tyler, T. W. (1976) Ph.D. thesis, London University.
5. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavanoid Compounds*. Springer-Verlag, Berlin.
6. Fukui, K., Matsumoto, T. and Kineshita, T. (1964) *Bull. Chem. Soc. Japan*, **37**, 662.
7. Mabry, T. J., Rodriguez, E. and Carman, N. J. (1972) *Phytochemistry* **11**, 409.
8. Gritsenko, E. N., Litivenko, V. I. and Kovalev, Z. P. (1970) *Chem. Abstr.* **73**, 6316p.
9. Arisawa, M., Takakuwa, T. and Nakaoki, T. (1970) *Chem. Pharm. Bull.* **18**, 916.
10. McLafferty, F. W. (1966) *Interpretation of Mass Spectra*, p. 132. W. R. Benjamin, New York.
11. Hiremath, S. V., Kulkarni, G. H., Kelkar, G. R. and Bhattacharyya, S. C. (1968) *Indian J. Chem.* **6**, 243.
12. Taira, Z., Watson, W. H., Sammes, P. G. and Silva, M. (1976) *Crypt. Structure Comm.* **5**, 601.
13. Piers, E. and Cheng, K. F. (1967) *Can. J. Chem.* **54**, 1591.
14. Hendrickson, J. B. (1963) *Tetrahedron* **19**, 1387.
15. Büchi, G., Kauffman, J. M. and Lowenthal, H. J. E. (1966) *J. Am. Chem. Soc.* **88**, 3403.
16. McMurry, T. B. H. and Mollan, R. C. (1969) *J. Chem. Soc.* (*C*) 1619.
17. Lee, K. H., Meck, R., Piantadosi, C. and Huang, E. S. (1973) *J. Med. Chem.* **16**, 299.
18. Ruzicka, L. and Wirz, W. (1939) *Helv. Chim. Acta* **22**, 948.
19. Neville, G. A., Nigan, I. C. and Holmes, J. L. (1968) *Tetrahedron* **24**, 3891.
20. Hikino, H., Aota, K. and Takemoto, T. (1966) *Chem. Pharm. Bull.* **14**, 890.