Aust. J. Chem., 1979, **32**, 2455–71

Novel Sesquiterpene Lactones from *Callitris columellaris* Heartwood

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Abstract

Five new sesquiterpene lactones, callitrin (1) (an elemanolide), callitrisin (2) and dihydrocallitrisin (6) (eudesmanolides), and columellarin (4) and dihydrocolumellarin (5) (guaianolides) have been isolated from *Callitris columellaris* heartwood along with the germacranolide (3) which is a new natural product previously only known synthetically. These compounds are the first sesquiterpene lactones isolated from the Cupressaceae. Lactones (1), (2) and (6) all share a novel *cis*-fused lactone ring in which the C 7–C 11 bond is axial to a cyclohexane ring.

Introduction

Callitris columellaris F. Muell. (var. *glauca*) (white cypress pine) is native to a wide area of eastern Australia. Its decorative and highly aromatic timber is resistent to termites and fungi. Chemical investigation of the oleoresin yielded mainly diterpene acids,[1] while the heartwood contains several sesquiterpene alcohols,[2] monoterpene acids,[3] and the cytotoxic compound deoxypodophyllotoxin.[4] We now report the isolation of six sesquiterpene lactones (1)–(6) from *C. columellaris* heartwood.[5] These compounds represent the first sesquiterpene lactones isolated from the Cupressaceae. Three of them, callitrin (1), callitrisin (2) and dihydrocallitrisin (6), share a novel stereochemical arrangement of the lactone ring, in which the C 7–C 11 bond has an axial orientation relative to a cyclohexane ring.

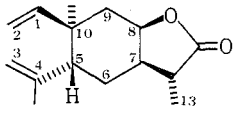
Note on Nomenclature

The structure determinations reported in this paper depend in part on correlations with structures of compounds widely reported in the literature under trivial names. We have thus obtained the Editor's agreement to continue within the traditional system

[1] Carman, R. M., and Deeth, H. C., *Aust. J. Chem.*, 1967, **20**, 2789; Carman, R. M., and Deeth, H. C., *Aust. J. Chem.*, 1971, **24**, 353; Atkinson, P. W., and Crow, W. D., *Tetrahedron*, 1970, **26**, 1935; Gough, L. J., *Chem. Ind. (London)*, 1964, 2059; Gough, L. J., *Tetrahedron Lett.*, 1968, 295.
[2] Baker, R. T., and Smith, H. G., 'A Research on the Pines of Australia' (Government Printer: Sydney 1910); Rudman, P., *Chem. Ind. (London)*, 1964, 808.
[3] Trikojus, N. M., and White, D. E., *Proc. R. Soc. N.S.W.*, 1932, **66**, 284; Neuhaus, J. W. G., and Reuter, F. H., *Proc. R. Aust. Chem. Inst.*, 1948, **15**, 185; Deeth, H. C., Ph.D. Thesis, University of Qld., 1965, p. 115.
[4] Aynehchi, V., *J. Pharm. Sci.*, 1971, **60**, 121.
[5] Brecknell, D. J., and Carman, R. M., *Tetrahedron Lett.*, 1978, 73.

of sesquiterpene nomenclature (i.e. we have given our compounds (1)–(6) trivial names and have used the traditional numbering) even though we are aware that this system is in conflict with Section F of the IUPAC Rules on the nomenclature of organic chemistry.

The systematic names of the new compounds are as follows: (1) is [3*R*-(3α,3aα,5α,-6β,7aα)]-6-ethenyl-3,6-dimethyl-5-(1-methylethenyl)hexahydrobenzofuran-2(3*H*)-one; (2) is [3*R*-(3α,3aα,4aβ,8aα,9aα)]-3,8a-dimethyl-5-methylene-3a,4,4a,5,6,8a,9,9a-octahydronaphtho[2,3-*b*]furan-2(3*H*)-one; (3) is [3*R*,5*E*,9*E*-(3α,3aα,11aβ)]-3,6,10-trimethyl-3a,4,7,8,11,11a-hexahydrocyclodeca[*b*]furan-2(3*H*)-one; (4) is [3a*R*-(3aα,4aα,-5α,9aβ]-5,8-dimethyl-3-methylene-3a,4,4a,5,6,7,9,9a-octahydroazuleno[6,5-*b*]furan-2(3*H*)-one; (5) is [3*R*-(3α,3aα,4aα,5α,9aβ)]-3,5,8-trimethyl-3a,4,4a,5,6,7,9,9a-octahydroazuleno[6,5-*b*]furan-2(3*H*)-one; (6) is [3*R*-(3α,3aα,4aβ,8aα,9aα)]-3,8a-dimethyl-5-methylenedecahydronaphtho[2,3-*b*]furan-2(3*H*)-one.



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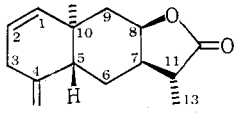

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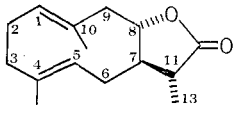

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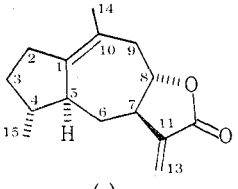

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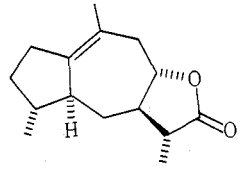

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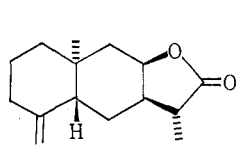

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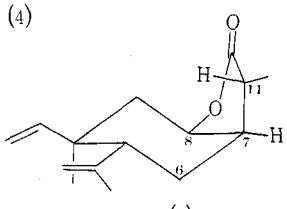

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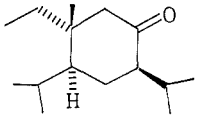


(8)

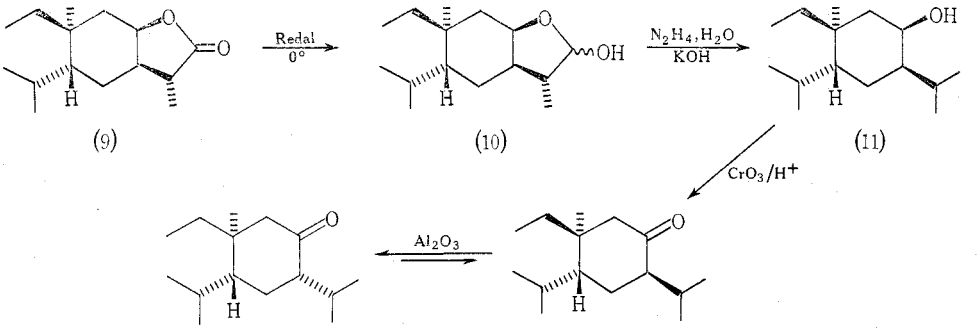
Discussion and Results

Extraction of the hexane solution of heartwood extract from *C. columellaris* with aqueous silver nitrate solution[6] yielded a mixture consisting mainly of callitrin (1), callitrisin (2) and the germacradienolide (3),[7] which were separated by chromatography over alumina impregnated with silver nitrate. The silver-nitrate-insoluble portion of the hexane extract yielded, after preliminary fractionation over silica gel and then careful chromatography over Merck 'Lobar' prepacked silica gel columns, another three sesquiterpene lactones—columellarin (4), dihydrocolumellarin (5) and dihydrocallitrisin (6).

Callitrin ($C_{15}H_{22}O_2$, M 234) was a γ-lactone (1780 cm^{-1}), with an ^1H n.m.r. spectrum showing a quaternary methyl group (δ 1·02) and vinyl and isopropenyl groups

[6] Jones, R. V. H., and Sutherland, M. D., *Aust. J. Chem.*, 1968, **21**, 2255.
[7] Takeda, K., and Horibe, I., *J. Chem. Soc., Perkin Trans. 1*, 1975, 870.

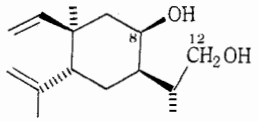
(five vinyl protons, 4·5–5·7, allylic methyl group at 1·69). This suggested an elemene carbon skeleton. A one-proton multiplet at 4·47 due to a proton geminal to a lactone oxygen atom exhibited couplings of 6·5, 6·5 and 11 Hz; this indicated lactone ring closure to C8 rather than the alternative C6, which would show only two vicinal couplings. The magnitudes of the H8 coupling constants were consistent only with those of an axial proton coupled to one axial and two equatorial neighbours, which in turn required the new type of lactone orientation shown in structure (7). The relative stereochemistry at C11 was determined with the aid of the n.m.r. shift reagent $Eu(fod)_3$,[8] which gave H11 as a six-line multiplet having three couplings of 6·5 Hz ($J_{11,13}$) and one of 13 Hz ($J_{7,11}$), showing H7 and H11 to be approximately anti-periplanar.



Scheme 1

The elemene carbon skeleton for callitrin (1) was confirmed, and its absolute configuration was determined, by relating callitrin to the known elemanone (8)[9] by the reaction sequence shown in Scheme 1.[10] Partial reduction of tetrahydrocallitrin (9) with sodium bis(methoxyethoxy)aluminium dihydride ('Redal') at ice temperatures gave the hemiacetal (10), which was not characterized but was further reduced by the Huang Minlon method to the alcohol (11). The couplings of H8 in (11), as well as in the derived diols (14) and (15a) and the diacetate (15b), were about 4, 4 and 11 Hz (cf. 6·5, 6·5 and 11 Hz in (1) and (9)); this suggested that some strain was released on cleavage of the lactone ring. Jones oxidation of (11) afforded the ketone (12), which showed a strong positive Cotton effect, indicative of an axial isopropyl group in a positive quadrant.[11] Epimerization of (12) at room temperature over neutral alumina[12] yielded a mixture of the ketones (13) and (12) in the ratio 3 : 1. The infrared spectrum of the mixture was almost identical with that published for ketone (8),[9] and it gave a semicarbazone, m.p. 173° (lit.[9] 174° for the semicarbazone of (8)). The equilibrium ratio of (12) and (13) corresponds to a free energy difference of *c.* 2·5 kJ mol^{-1}, in good agreement with that between the *cis*- and *trans*-2-isopropyl-4-t-butyl-cyclohexanones.[13] Attempts to isolate pure ketone (13) were unsuccessful.

[8] Rondeau, R. E., and Sievers, R. E., *J. Am. Chem. Soc.*, 1971, **93**, 1522.
[9] Joshi, G. D., Paknikar, S. K., Kulkarni, S. N., and Bhattacharyya, S. C., *Tetrahedron*, 1966, **22**, 1651.
[10] Wagh, A. D., Paknikar, S. K., and Bhattacharyya, S. C., *Tetrahedron*, 1964, **20**, 2647.
[11] Eliel, E., 'Stereochemistry of Carbon Compounds' p. 427 (McGraw–Hill: New York 1962).
[12] Acklin, W., Prelog, V., Schenker, F., Serdarevic, B., and Walter, P., *Helv. Chim. Acta*, 1965, **48**, 1725.
[13] Allinger, N. L., and Blatter, H. M., *J. Am. Chem. Soc.*, 1961, **83**, 994.



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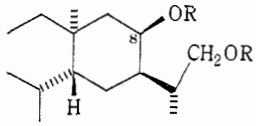
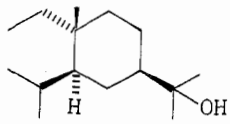


(15a) R = H
(15b) R = Ac



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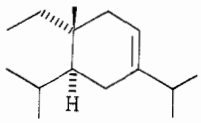

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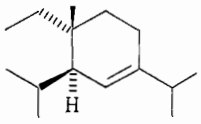

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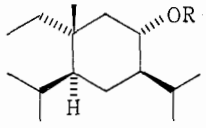
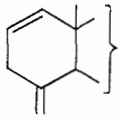


(19a) R = H
(19b) R = $C_6H_3(NO_2)_2$-3,5



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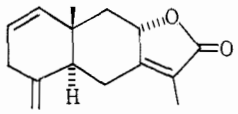

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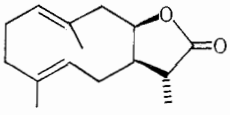

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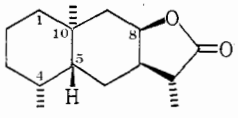

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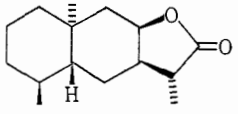

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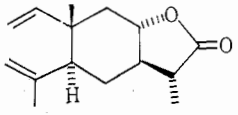

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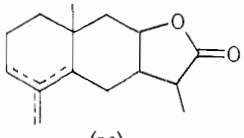

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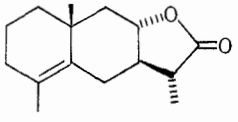

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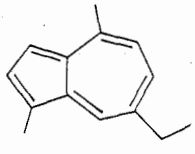

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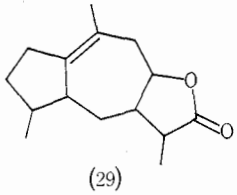

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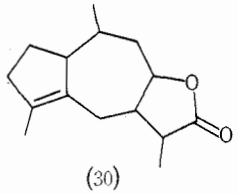

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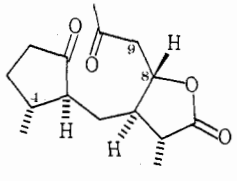

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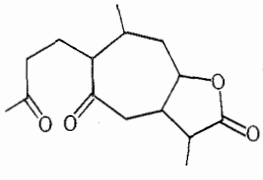

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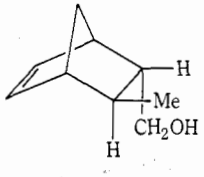


(33)

Authentic ketone (8) was prepared for comparison by a method based on a published procedure.[9] Tetrahydroelemol (16) was dehydrated with perchloric acid to yield a mixture of olefins consisting mainly of (17) and (18). Chromatography over silver nitrate–alumina showed that the difficult separation was possible, but this tedious operation was unnecessary since hydroboration of the mixture of olefins by means

of the highly selective 9-borabicyclo[2,2,1]nonane[14] proceeded only with the more accessible olefin (17) to yield, after oxidation, the alcohol (19a). Jones oxidation produced the ketone (8). Epimerization of (8) over alumina gave a mixture of ketones which was identical with that described above by gas chromatography and infrared spectroscopy, but which exhibited a mirror-image o.r.d. curve, thus establishing the structure and absolute configuration of callitrin (1) as in conformer (7).

There are suggestions in the literature[6,15] that elemene-type sesquiterpenoids isolated from natural sources are often artefacts derived from germacrene derivatives by thermal Cope rearrangements incurred during the isolation procedures. However, callitrin itself is present in the heartwood, since the yield of callitrin from a particular tree was not dependent on the temperatures (up to 100°) to which the extract was subjected.

Callitrisin ($C_{15}H_{20}O_2$, M 232) was a γ-lactone (1760 cm^{-1}) containing a 1,1-disubstituted double bond (3070, 1650, 895 cm^{-1}, two broad one-proton singlets at δ 4·74, 4·97). The ^1H n.m.r. spectrum revealed two additional vinyl protons on a 1,2-disubstituted double bond as a very narrow AB system at δ 5·5, in which the lower-field doublet was further coupled. A second broad AB system was centred at 2·89, which suggested a doubly allylic methylene group. The large value of the coupling constant (J_{AB} 19 Hz) for this system confirmed that the methylene group was adjacent to two sp^2-hybridized carbon atoms.[16] Irradiation at the frequency of this methylene group removed the splitting from the lower-field doublet of the AB system at 5·5, giving a clean AB quartet. The above n.m.r. data, together with the observation of a quaternary methyl group at 0·86, suggested a eudesmanolide structure containing the arrangement (20) for ring A. The olefinic chemical shifts of callitrisin agree well with those of lindestrenolide (21).[17]

The proton geminal to the lactone ether oxygen of callitrisin gave an n.m.r. multiplet at δ 4·70, with couplings of 6·5, 6·5 and 11 Hz. It follows that the lactone ring is closed to C 8, with the same arrangement as that in callitrin (1). Two four-line one-proton systems at δ 1·37 and 2·07, significantly coupled only to each other and to H 8, were due to a C 9 methylene group adjacent to a quaternary C 10 atom, while a three-proton doublet at 1·19 was assigned to a C 11 methyl group. Decoupling experiments on $Eu(fod)_3$-doped samples confirmed all of these assignments, and showed that H 7 and H 11 were approximately antiperiplanar ($J_{7,11}$ 13 Hz).

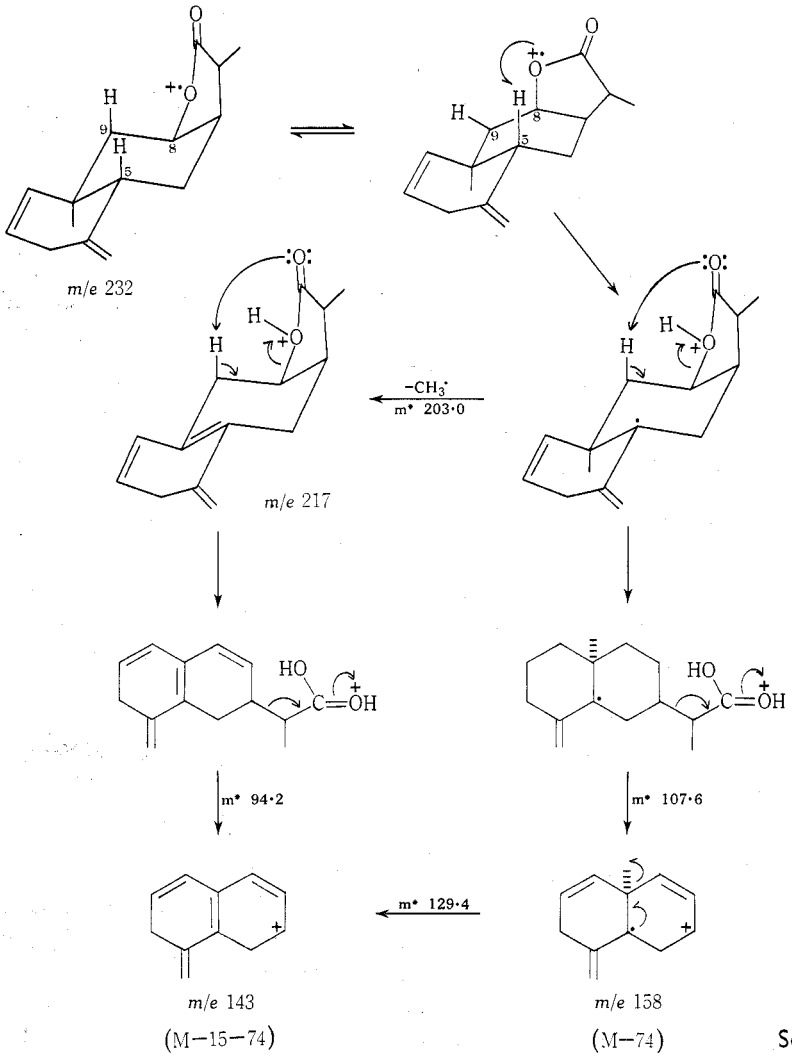
These data suggested structure (2) for callitrisin, with both compounds (1) and (2) bioderivable from the same germacradienolide precursor (22).[18]

Hydrogenation of callitrisin produced two tetrahydro derivatives (23) and (24) in the ratio 3 : 1, from which the major isomer was isolated by fractional crystallization. The chemical shift of H 8 in the tetrahydro compounds was virtually unchanged from that in callitrisin, showing that the C 10–C 1 bond cannot be axial to ring B, since the C 1–C 2 double bond in callitrisin should then significantly shield H 8. Thus callitrisin has the same relative configuration at C 10 as callitrin (1).

Dreiding models indicate that severe crowding makes it unlikely that the C 5 configuration of callitrisin should be the reverse of that in callitrin (1). Striking differences

[14] Knights, E. F., and Brown, H. C., *J. Am. Chem., Soc.*, 1968, **90**, 5280.
[15] Jones, R. V. H., and Sutherland, M. D., *Chem. Commun.*, 1968, 1229.
[16] Jackman, L. M., and Sternhell, S., 'Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry' 2nd Edn, p. 273 (Pergamon Press: London 1969).
[17] Takeda, K., Horibe, I., and Minato, H., *J. Chem. Soc. C*, 1968, 569.
[18] Brecknell, D. J., and Carman, R. M., *Aust. J. Chem.*, 1979, **32**, 2097.

in the mass spectra of the *cis*-fused lactones (1), (2), (6), (9) and (23) on the one hand, and the *trans*-fused lactones (3), (4), (5), (25) and (27) on the other, support the assignment of a common stereochemistry within the former group. A prominent M–74 peak is common to the *cis*-lactones (base peak in callitrisin (1)), but is virtually absent from the mass spectra of the *trans*-lactones (in particular (25)), showing that this cleavage



Scheme 2

is related to stereochemistry. The mass spectral difference is unlikely to be due to the mode of lactone fusion (i.e. *cis* or *trans*) alone, since no such difference was reported for the *cis* and *trans* santonins.[19] A fragmentation pattern which accounts for the M−74 peak together with the large M−15−74 peak in the *cis* lactones above, is shown in Scheme 2. This scheme requires H 5 and the C 8 oxygen to be in a *cis* relationship, while a *cis*-fused lactone ring is necessary to permit the boat conformation

[19] Wasada, N., Tsuchiya, T., Yoshii, E., and Watanabe, E., *Tetrahedron*, 1967, **23**, 4623.

required for abstraction of H 5. These arguments support structure (2) for callitrin. The major tetrahydro isomer is assigned structure (23) on the basis of the chemical shifts of the C 10 and C 4 methyl groups, since in the more abundant isomer these groups appear at lower field than in the minor isomer, as a result of the 1,3-diaxial interaction between them.[20]

Callitrisin (2) exhibited a negative c.d. maximum at 222 nm ($\Delta\varepsilon$ −1·27), showing that it has the same absolute configuration as callitrin (1) (λ_{max} 217 nm, $\Delta\varepsilon$ −1·72).

Dihydrocallitrisin (6) ($C_{15}H_{22}O_2$, M 234) was a γ-lactone (1769 cm^{-1}) showing the same n.m.r. splitting pattern for H 8 as did callitrin and callitrisin. The quaternary methyl (δ 0·76) and exocyclic double bond (two broad singlets at 4·52 and 4·78) suggested a eudesmane carbon skeleton, and hence a dihydro derivative of callitrisin (2). Hydrogenation of lactone (6) produced the same mixture of tetrahydrocallitrisin derivatives as was obtained by hydrogenation of callitrisin. The major isomer was identical (i.r., n.m.r., g.l.c., m.m.p., o.r.d.) with that described above, so that dihydrocallitrisin has structure (6). The structure of dihydrocallitrisin, and hence of callitrisin, has very recently been confirmed[21] by the total synthesis of (±)-dihydrocallitrisin, identical with the natural product by g.l.c., i.r. and n.m.r. spectrometry.

Callitrin, callitrisin and dihydrocallitrisin are the first natural sesquiterpene lactones to be reported with an axial C 7–C 11 bond in the lactone ring, although this stereochemistry is not unknown in other sesquiterpenoids.[22]

The germacranolide (3) ($C_{15}H_{22}O_2$, M 234) was a γ-lactone (1765 cm^{-1}) which gave a broad, poorly resolved ^1H n.m.r. spectrum characteristic of the flexible ten-membered ring of a germacranolide, but showing a three-proton doublet (δ 1·12, C 11 methyl), a broad six-proton singlet (1·57, two allylic methyls), a broad triplet (4·21, proton geminal to the lactone rather oxygen) and two very broad vinyl protons (4·68, 5·05). The compound partially rearranged at 160° to produce an elemadienolide whose ^1H n.m.r. spectrum showed a multiplet at 3·88 (*J* 4, 9, 11 Hz) assigned to a proton geminal to the lactone ether oxygen, and indicative of a *trans*-fused lactone ring closed to C 8. This suggested structure (25) for the elemanolide and (3) for its germacranolide precursor. These compounds have both been synthesized by Takeda *et al.*[7] and spectral comparisons confirmed their identities.

Acid treatment of germacra-1(10),4-dienolides normally produces a mixture of eudesmanolide isomers, e.g. (26).[23] Lactone (3) with perchloric acid gave a good yield of the single eudesmanolide (27), whose ^1H n.m.r. spectrum showed an allylic methyl (δ 1·62) but no vinyl protons. The couplings of H 8 (δ 4·01, *J* 4, 10, 12 Hz) confirmed the presence of a *trans*-7,8 lactone ring fusion.

Columellarin (4) ($C_{15}H_{20}O_2$, M 232) was an α-methylene γ-lactone (1768 cm^{-1}, two low-field ^1H n.m.r. doublets both with *J c.* 3 Hz[24]) with an allylic methyl (δ 1·75), a secondary methyl (1·07) and a one-proton multiplet at 3·78. Dihydrocolumellarin (5) showed a similar spectrum, with a doublet methyl at 1·23 replacing the two low-

[20] Abraham, R. J., and Holker, J. S. E., *J. Chem. Soc.*, 1963, 806.
[21] Schultz, A. G., personal communication; *Tetrahedron Lett.*, in press.
[22] Schildknecht, H., Holtkotte, H., Krauss, D., and Tacheci, H., *Justus Liebigs Ann. Chem.*, 1975, 1850; Chetty, G. L., Zalkow, V. B., and Zalkow, L. H., *Tetrahedron Lett.*, 1968, 3223; Klein, E., and Rojahn, W., *Tetrahedron Lett.*, 1970, 279.
[23] Barton, D. H. R., Böckman, O. C., and de Mayo, P., *J. Chem. Soc.*, 1960, 2263; Rao, A. S., Kelkar, G. R., and Bhattacharyya, S. C., *Tetrahedron*, 1960, **9**, 275.
[24] Herz, W., Romo de Viver, A., Romo, J., and Viswanathan, N., *J. Am. Chem. Soc.*, 1963, **85**, 19.

field doublets. Hydrogenation of columellarin at low pressure produced crystalline dihydrocolumellarin which resisted further low pressure hydrogenation over palladized charcoal.

Both columellarin and dihydrocolumellarin lacked a quaternary methyl group, suggesting a guaiane skeleton, and this was confirmed by catalytic dehydrogenation to give dark blue azulenic material from which chamazulene (28) was separated and identified by its trinitrobenzene adduct. Dihydrocolumellarin possessed a tetrasubstituted double bond with an allylic methyl group but no vinyl protons. Lactone closure to C 8 was required by the six- or eight-line system (dependent on solvent) observed for H 8, giving two possible basic structures (29) and (30). Ozonolysis of dihydrocolumellarin produced the diketo lactone (31), distinguishable from the alternative (32) by its infrared and ^{1}H n.m.r. spectra. The product showed carbonyl bands at 1775 (γ-lactone) and 1736 cm^{-1} (cyclopentanone), with a shoulder at 1720 cm^{-1} (acyclic ketone), while the n.m.r. spectrum showed a sharp two-proton doublet at δ 2·83 (J 5·5 Hz) for the C 9 protons in isomer (31). Irradiation of the H 8 signal collapsed this doublet to a singlet, while irradiation of the H 9 doublet collapsed H 8 to a doublet with J 9 Hz. H 8 in columellarin (4) and dihydrocolumellarin (5) falls at an unusually high field position (3·80, as against 4·2–4·8 normally), but reverts to a normal chemical shift (4·45) in the diketo lactone (31). The difference is ascribed to the anisotropy of the C 1–C 10 double bond in (4) and (5), with H 8 located 'above' the double bond.

Table 1. ^{1}H n.m.r. parameters (Hz) for ABX systems in columellarin (4) and dihydrocolumellarin (5)

Compound	Conditions[A]	δ_{AB}	$J_{8,9\alpha}$	$J_{8,9\beta}$	$J_{9\alpha,9\beta}$
(4)	$\rho = 0{\cdot}4$	17	10·8	2·2	−14·5
(4)	$\rho = 0{\cdot}35$	13	10·4	2·4	−14·5
(4)	$\rho = 0{\cdot}31$	11	10·3	2·7	−14·6
(4)	$\rho = 0{\cdot}26$	8	10·7	2·3	−14·2
(5)	C_6D_6	30	10·2	3·0	−14·0
(5)	$\rho = 0{\cdot}4$	35	10·4	2·6	−14·0
Av. (4)			10·4	2·4	−14·4
Av. (5)			10·3	2·8	−14·0

[A] ρ = moles Eu(fod)$_3$/moles lactone in CCl_4.

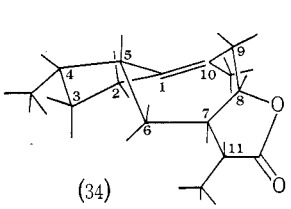
In both columellarin and dihydrocolumellarin, H 8 showed variations in n.m.r. pattern on change of solvent and with increasing concentration of Eu(fod)$_3$. The other lactones studied did not show this effect and conformational changes induced by solvents or lanthanide shift reagents were not expected.[25] The overall width of the H 8 multiplet did not change as the pattern varied, and H 8 was behaving[26] as the X-region (further split by H 7) of an ABX system also involving H 9α and H 9β, where solvent- or l.s.r.-induced changes to δ_{AB} (the chemical shift between H 9α and H 9β) were causing the observed spectral changes. Coupling constants from analysis of a range of these splitting patterns are given in Table 1. They show good consistency,

[25] Hofer, O., *Top. Stereochem.*, 1976, **9**, 111.

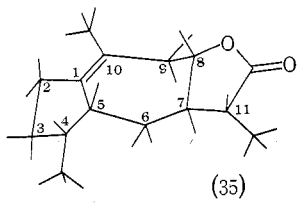
[26] Pople, J. A., Schneider, N. G., and Bernstein, H. J., 'High Resolution Nuclear Magnetic Resonance' p. 132 (McGraw–Hill: New York 1959).

in view of difficulties in measuring line positions (due to peak overlap and to line broadening of H 9α by homoallylic coupling with H 5 and H 2β). Calculations employing the average coupling constants from Table 1 with varying H 9α and H 9β chemical shifts accurately reproduced the patterns observed for H 8 throughout the whole range of Eu(fod)$_3$ concentrations. A similar change occurred in the observed line pattern for an AB_3 system in alcohol (33) and again the coupling constants were shown to be invariant with changing concentrations of shift reagent.[27]

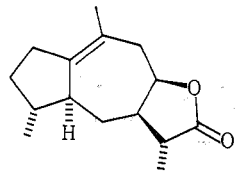
The above analysis allowed a value of 10–12 Hz to be assigned to $J_{7,8}$ in the columellarins. This suggests, on the basis of coupling constants reported for the pseudoguaianolides, a *trans*-7,8 lactone ring fusion,[28] although the ambiguity of such assignments by using a single coupling constant in seven-membered rings is recognized.[29] Support for a *trans*-fused lactone ring also comes from the two $J_{7,13}$ values for columellarin (4) (3·1 and 3·2 Hz), since values in excess of 3 Hz have been associated with *trans* lactone rings.[28]



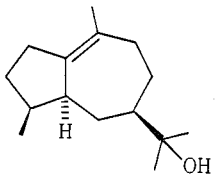
(34)



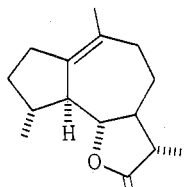
(35)



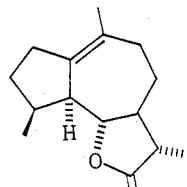
(36)



(37)



(38)



(39)

The whole set of coupling constants of the protons H 6, H 7, H 8 and H 9 in dihydrocolumellarin support the above assignments and also indicated that H 5 has an α orientation. Models show only three arrangements of the seven-membered ring with a *trans*-fused lactone that could in any way be consistent with the complete set of observed couplings: two conformers (34) and (35) having the 5β-H arrangement and relatively rigid 5α-H isomer (5). Although a *trans* lactone ring was favoured (see above), a *cis*-fused structure (36)—the only one likely to fit the observed coupling constants—was also included in the following analysis. Coupling constants for each structure were calculated from angles read from molecular models, according to the empirical relationship of Vorontsova and Bochkov:[30]

$$J = (4\cdot37+4\cdot39\cos\psi+3\cdot32\cos 2\psi)[1\cdot28-0\cdot041(\delta_1+\delta_2)]$$

[27] Abraham, R. J., Coppell, S. M., and Ramage, R., *Org. Magn. Reson.*, 1974, **6**, 658.
[28] Herz, W., Aota, K., Holub, M., and Samek, Z., *J. Org. Chem.*, 1970, **35**, 2611.
[29] Holub, M., Samek, Z., Vasickova, S., and Masojidkova, M., *Collect. Czech. Chem. Commun.*, 1978 **43**, 2444.
[30] Vorontsova, L. G., and Bochkov, A. F., *Org. Magn. Reson.*, 1974, **6**, 654; 1975, **7**, 313.

where ψ is the smaller of the angles between one C–H bond and a line drawn through the same carbon atom parallel to the other C–H bond, and δ_1 and δ_2 are the chemical shifts of the two protons. The calculated and observed values are listed in Table 2. The best agreement is clearly for structure (5) having a *trans*-lactone ring and the 5α-H configuration.

Table 2. Calculated and observed coupling constants for possible dihydrocolumellarin structures

Parameter	Protons	J_{obs} (Hz)	J_{calc} (Hz) for (5)	(34)	(35)	(36)
	5α,6α	2·5, 11	1·8	—	—	1·4
	5β,6α		—	8·1	14·5	—
	5α,6β		12·4	—	—	12·9
	5β,6β		—	11·3	3·7	—
	6α,7	2·5, 11	1·5	3·8	11·5	3·1
	6β,7		11·9	14·2	8·8	14·2
	7,8	10–12	12·4	10·1	10·8	8·7
	8,9α	10·8, 2·8	12·4	6·5	12·4	1·4
	8,9β		2·3	8·8	4·7	11·9
Corr. coeff. r^2			0·994	0·654	0·749	0·891
Standard error			0·46	2·17	2·20	2·03

Attempts to measure the H 5 coupling constants with the aid of lanthanide shift reagents were unsuccessful, since H 5 could not be clearly observed throughout the whole usable range of concentrations of both $Eu(fod)_3$ and $Pr(fod)_3$. $Eu(fod)_3$, however, clarified H 11 of dihydrocolumellarin (5), and double irradiation experiments showed $J_{7,11}$ *c.* 12 Hz, so that H 7 and H 11 must be antiperiplanar.

Table 3. ^{1}H n.m.r. data for the 4-methyl group

	(4)	(5)	(38)	(37)	(39)
δ ($CDCl_3$) (ppm)	1·07	1·06	1·17	0·91	0·91
$J_{4,15}$ (Hz)	5·9	5·5	5·8	6·7	6·5

The orientation of the C 4 methyl group of columellarin (4) and dihydrocolumellarin (5) was deduced by comparison of its chemical shift and coupling constant with those of related compounds. Methyl groups equatorial to a cyclohexane ring exhibit smaller coupling constants with the geminal ring proton ($J < 6$ Hz) than do axial methyl groups ($J > 6$ Hz).[31] The coupling constants of methyl groups attached to cyclopentane rings show a similar dependence on stereochemistry, since a methyl in a *cis*-1,2 relationship with another substituent shows a larger coupling constant (6·0–6·6 Hz) than does a methyl in a *trans*-1,2 system (4·8–5·4 Hz).[32] In the *trans* situation, both substituents will occupy pseudoequatorial positions, while in the *cis* arrangement, the smaller substituent (usually the methyl) will be forced into a pseudo-axial position, so that the results from methyl-substituted cyclopentane rings parallel those of cyclohexane systems. Table 3 compares the ^{1}H n.m.r. data for the C 4 methyl

[31] Johnston, F., Starkovsky, N. A., and Gurowitz, W. D., *J. Am. Chem. Soc.*, 1965, **87**, 3492.
[32] Wolinsky, J., Gibson, T., Chan, D., and Wolf, H., *Tetrahedron*, 1965, **21**, 1247.

groups of columellarin (4) and dihydrocolumellarin (5) with those of closely related compounds bulnesol (37),[33] and the lactones (38) and (39).[34] There is a clear distinction between the results for the β methyl groups of (37) and (39) and those for the methyls of (4), (5) and (38). The larger coupling constants of (37) and (39) indicate the presence of pseudoaxial C4 methyl groups, while (4), (5) and (38) have pseudoequatorial, and hence α C4 methyls. The difference between the C4 methyl chemical shifts of (38) and those of (4) and (5) is expected, since in lactone (38) the C6 oxygen atom is close to and deshielding the pseudoequatorial methyl group.

The absolute configurations shown for columellarin (4) and dihydrocolumellarin (5) are assigned on biogenetic grounds. The negative Cotton effect of columellarin is then contrary to that predicted by the empirical rule of Stöcklin *et al.*[35] but other exceptions are known.[36]

Experimental

Melting points were determined on a Yanagimoto hot stage and are uncorrected. Preparative liquid chromatography was performed in open columns containing Merck (silica gel) and Woelm (alumina) adsorbents, or on Merck 'Lobar' prepacked silica gel columns, which were operated at solvent pressures of 10–40 p.s.i., with peak detection by a variable wavelength ultraviolet monitor. Infrared spectra were obtained as Nujol mulls or liquid films. ^{1}H n.m.r. spectra were recorded at 100 MHz on Jeol MH-100 and PS-100 spectrometers. O.r.d. data were obtained in Perkin–Elmer 141 and 141MC automatic polarimeters operating at the wavelengths of sodium and mercury lamp lines. C.d. curves were measured by using a Jobin–Yvon Dichrographic III and are reported in the form λ ($\Delta\varepsilon$). Mass spectra were recorded on an AEI MS902S spectrometer at 70 eV; high-resolution mass measurements were carried out at a resolution of 10000. Microanalyses were performed by the Chemistry Department's Microanalytical Laboratory.

Extraction of Callitris columellaris *Heartwood*

Logs of *C. columellaris* were collected by the Queensland Department of Forestry, from Compartment 1, Cutthroat L.A., S.F.R. 302, Chinchilla, in Central Queensland; trees were about 50 years old. The logs were stripped of bark and sapwood, and reduced to sawdust. The sawdust was extracted by soaking overnight in hexane at room temperature. The solvent was run off and evaporated under reduced pressure, to give a brown oil (yield variable, 1–6% by weight of wood). The oil was redissolved in hexane, and washed with sodium carbonate solution and with water.

Isolation of the Silver-Nitrate-Soluble Lactones

The neutral hexane extract above (40 g in 100 ml of hexane) was extracted with aqueous silver nitrate solution (40%, 3 × 40 ml). The aqueous extract was washed with hexane (30 ml), and then treated with excess concentrated ammonia solution (d 0·88). The oil which separated was extracted into hexane, washed with water, dried and evaporated (3·5 g). G.l.c. analysis (5% DC710, 220°) showed three major components. The mixture was chromatographed over silver nitrate–alumina prepared by shaking neutral alumina (activity I, 200 g) with a solution of silver nitrate (25 g) in water (30 ml) until homogenous. The resulting free-running white powder was packed directly into a chromatography column without drying. Elution with hexane containing increasing proportions of ether gave, in order:

(i) *Callitrisin** (2), m.p. 163·5–164·5° (from methanol) (Found: C, 77·6; H, 8·8. $C_{15}H_{20}O_2$ requires C, 77·5; H, 8·7%). ν_{max} (Nujol): 1760, 1650, 1380, 1328, 1295, 1238, 1225, 1196, 1170, 1129, 1106, 1050, 995, 979, 960, 895, 731, 683 cm^{-1}. ^{1}H n.m.r. δ ($CDCl_3$): 0·84, s, 10-Me; 1·21,

* See Note on Nomenclature, p. 2455.

[33] Marshall, J. A., and Partridge, J. J., *Tetrahedron*, 1969, **25**, 2159.
[34] Vokac, K., and Samek, Z., *Collect. Czech. Chem. Commun.*, 1974, **39**, 480.
[35] Stöcklin, W., Waddell, T. G., and Geissman, T. A., *Tetrahedron*, 1970, **26**, 2397.
[36] Cox, P. J., Sim, G. A., and Herz, W., *J. Chem. Soc., Perkin Trans. 2*, 1975, 459.

d, *J* 6·5 Hz, 11-Me; 1·37, dd, *J* 13 and 11 Hz, H 9*ax*; 2·07, dd, *J* 13, and 6·5 Hz, H 9*eq*; 2·5, partially obscured m, H 11; 2·81 and 2·97, broadened AB system, $|J_{AB}|$ 19 Hz, two H 3; 4·7, partly obscured m, H 8; 4·47 and 4·97, two br s, exocyclic methylene; 5·45, d, *J* 10 Hz, H 1; 5·56, dd, *J* 10 and 3 Hz, H 2. Addition of Eu(fod)$_3$ gave observable H 11 and H 8 signals yielding $J_{7,11}$ 13 Hz, $J_{11,13}$ 6·5 Hz, $J_{8,9eq}$ 6·5 Hz, $J_{8,9ax}$ 11 Hz and $J_{7,8}$ 6·5 Hz. ^{1}H n.m.r. δ (C_6D_6): 0·56, s, 10-Me; 0·94, d, *J* 6·5 Hz, 11-Me; 1·04, dd, *J* 13 and 11 Hz, H 9*ax*; 1·70, dd, *J* 13 and 6·5 Hz, H 9*eq*; 2·0, obscured m, H 11; 2·70, 2·54, broadened AB system, $|J_{AB}|$ 19 Hz, two H 3; 4·16, dt, *J* 11, 6·5 and 6·5 Hz, H 8; 4·79 and 4·50, two br s, exocyclic methylene; 5·2, 2H m, H 1 and H 2. Irradiation of the H 3 AB system at δ 2·62 collapsed the multiplet at 5·2 to a simple AB system, J_{AB} 10 Hz. O.r.d. (*c*, 0·52 in methanol): $[\phi]_{589}$ −41°, $[\phi]_{578}$ −47°, $[\phi]_{436}$ −121°, $[\phi]_{365}$ −241°, $[\phi]_{265}$ −1690°, $[\phi]_{254}$ −2370°. C.d. λ_{max} ($\Delta\varepsilon$) (hexane): 222 (−1·27). M.s. (*m/e*): 232 (M, 25%), 217 (3·2), 204 (3·2), 203 (3·2), 171 (9·4), 159 (80), 158 (63), 145 (20), *143* (100), 131 (40), 129 (28), 119 (92), 117 (35), 105 (53), 91 (74), 79 (40), 77 (46), 67 (21), 65 (25), 55 (41), 53 (40), 51 (19).

(ii) *Callitrin* (1), m.p. 82–83° (from hexane) (Found: C, 76·9; H, 9·6. $C_{15}H_{22}O_2$ requires C, 76·9; H, 9·5%). ν_{max} (Nujol): 3050, 1780, 1645, 1638, 1338, 1307, 1195, 1173, 1136, 1055, 1005, 907, 890 cm^{-1}. ^{1}H n.m.r. δ (CCl_4): 1·02, s, 10-Me; 1·15, d, *J* 6·5 Hz, 11-Me; 1·69, br s, 4-Me; 4·47, dt, *J* 6·5, 6·5 and 11 Hz, H 8; 4·5–5·0, 4H, complex vinyl system; 5·67, X region of an ABX system, H 1. Addition of Eu(fod)$_3$ showed H 11 as a six-line multiplet which collapsed to a doublet, *J* 13 Hz, on irradiation of the 11-methyl. ^{1}H n.m.r. δ (C_6D_6): 0·65, s, 10-Me; 0·93, d, *J* 6·5 Hz, 11-Me; 1·56, br s, 4-Me; 2·02, obscured m, H 11; 4·12, dt, *J* 6·5, 6·5 and 11 Hz, H 8; 4·5–5·0, 4H, complex vinyl system; 5·55, X region of an ABX system, H 1. O.r.d. (*c*, 0·58 in ethanol): $[\phi]_{589}$ +225°, $[\phi]_{578}$ +240°, $[\phi]_{436}$ +470°, $[\phi]_{365}$ +760°, $[\phi]_{265}$ +1840°. C.d. λ_{max} ($\Delta\varepsilon$) (methanol): 215 (−1·72). M.s. (*m/e*): 234 (M, 19%), 219 (27), 206 (12), 205 (8), 193 (39), 192 (38), 173 (16), 161 (95), *160* (100), 151 (18), 145 (64), 134 (32), 133 (24), 121 (44), 119 (78), 108 (36), 107 (23), 105 (29), 95 (32), 93 (59), 91 (24), 81 (25), 79 (21), 68 (89), 55 (40), 41 (48).

(iii) (1*E*,4*E*,7*R*,8*S*,11*R*)-Germacra-1(10),4-dien-12,8-olide (deacetoxydihydrolaurenobiolide) (3), as needles, m.p. 132–133° from hexane (lit.[7] 132·5–133°) (Found: C, 77·0; H, 9·5. Calc. for $C_{15}H_{22}O_2$: C, 76·9; H, 9·5%). $[\alpha]_D$ +139° (lit.[7] +135°). λ_{max} (Nujol): 1765, 1334, 1300, 1248, 1207, 1180, 1136, 1120, 1058, 1048, 978, 937, 892, 863, 851, 767, 721, 670 cm^{-1}. ^{1}H n.m.r. δ (CCl_4): 1·26, d, *J* 7 Hz, 11-Me; 1·57, br s, 4-Me and 10-Me; 2·86, br d, *J c.* 13 Hz, H 6α; 4·21, br t, *J c.* 9 Hz, H 8; 4·68 and 5·05, two br d, H 1 and H 5. O.r.d. (*c*, 1·2 in ethanol): $[\phi]_{589}$ +319°, $[\phi]_{578}$ +331°, $[\phi]_{436}$ +720°, $[\phi]_{365}$ +1300°, $[\phi]_{254}$ +9230°. C.d. λ ($\Delta\varepsilon$) (methanol): 214 (31·3), 200 (0), 192 (−18·8). M.s. (*m/e*): 234 (M, 58%), 219 (11), 206 (4), 205 (5), 177 (6), 161 (19), 160 (6), 138 (23), 122 (40), 107 (32), 95 (18), 93 (26), 81 (21), *68* (100), 55 (26), 53 (24), 41 (47).

Tetrahydrocallitrin (*9*)

Callitrin (1) (1·5 g) in ethanol (20 ml) with 5% palladized charcoal (50 mg) was hydrogenated at atmospheric pressure to give *tetrahydrocallitrin* (9) (1·2 g) as colourless prisms, m.p. 69–61° (from hexane and after sublimation at 56°/0·1 mm) (Found: C, 75·5; H, 11·1. $C_{15}H_{26}O_2$ requires C, 75·6; H, 11·0%). ν_{max} (Nujol): 1761, 1347, 1327, 1297, 1227, 1185, 1150, 1139, 1114, 1071, 1047, 985, 828, 807, 778, 724, 705, 680 cm^{-1}. ^{1}H n.m.r. δ (CCl_4): 0·89, s, 10-Me; 1·14, d, *J* 6·5 Hz, 11-Me; 2·3, obscured m, H 11; 4·45, dt, *J* 6·5, 6·5 and 11 Hz, H 8. Addition of Eu(fod)$_3$ gave H 11 as a six-line multiplet with $J_{7,11}$ 13 Hz. O.r.d. (*c*, 0·72 in ethanol): $[\phi]_{589}$ +56°, $[\phi]_{578}$ +58°, $[\phi]_{436}$ +107°, $[\phi]_{365}$ +156°, $[\phi]_{265}$ +207°. M.s. (*m/e*): 238 (M, 45%), 223 (5), 220 (5), 209 (87), 195 (37), 191 (12), 165 (14), 153 (32), 151 (30), *135* (100), 121 (62), 110 (30), 107 (33), 95 (60), 93 (93), 81 (33), 69 (64), 55 (82), 41 (83).

(*1*R,*2*R,*4*R,*5*S)-*5-Ethyl-2,4-diisopropyl-5-methylcyclohexanol* (*11*)

Tetrahydrocallitrin (9) (0·51 g) in dry tetrahydrofuran (15 ml) was cooled to 3°, and a solution of 'Redal' (70% sodium bis(methoxyethoxy)aluminium dihydride in benzene) in dry tetrahydrofuran (0·5 ml in 5 ml) was added slowly at 0–5°. The reaction was monitored by quenching small aliquots in water, ether-extracting the product and subjecting it to g.l.c. analysis (DC 710, 200°). When the tetrahydrocallitrin peak had been almost completely replaced by a shorter retention-time peak, the mixture was quenched with water, filtered and ether-extracted to give a colourless oil (0·45 g). This oil in diethylene glycol (5 ml) with potassium hydroxide (2 g) and hydrazine hydrate (3 ml) was heated at 130–140° for 3 h, and then cooled and poured into water. Ether-extraction followed by preparative

thin-layer chromatography over silica gel (hexane/ethyl acetate 7 : 3) gave (*1*R,2R,*4*R,*5*S)-*5-ethyl-2,4-diisopropyl-5-methylcylcohexanol* (11) which was sublimed (45°/0·05 mm) to give colourless needles, m.p. 61–62° (Found: C, 79·8; H, 13·4. $C_{15}H_{30}O$ requires C, 79·6; H, 13·4%). ν_{max} (Nujol): 3360, 1380, 1367, 1343, 1100, 1045, 916 cm^{-1}. ^1H n.m.r. δ ($CDCl_3$): 0·92, s, 10-Me; 4·01, dt, J 4·1, 4·1 and 10·6 Hz, H 8. O.r.d. (*c*, 0·26 in pentane): $[\phi]_{589}$ +97°, $[\phi]_{578}$ +99°, $[\phi]_{365}$ +295°, $[\phi]_{265}$ +690°, $[\phi]_{254}$ +780°. M.s. (*m/e*): 226 (M, 0·7%), 208 (7), 179 (39), 165 (83), 138 (14), *123* (100), 99 (28), 95 (39), 83 (35), 81 (30), 69 (60), 57 (28), 55 (54), 43 (58), 41 (63).

(*2*R,*4*R,*5*S)-*5-Ethyl-2,4-diisopropyl-5-methylcyclohexanone* (*12*)

Jones reagent (8 N chromic acid) was added to alcohol (11) (0·25 g) in acetone (10 ml) at 10–20°, until an orange coloration persisted. Workup gave the *ketone* (12) as a colourless oil, b.p. 50°/0·1 mm (Found: C, 80·3; H, 12·6. $C_{15}H_{28}O$ requires C, 80·3; H, 12·6%). ν_{max} (film): no OH, 1714, 1465, 1428, 1390, 1370, 1332, 1302, 1287, 1254, 1235, 1205, 1185, 1168, 1081, 1027, 949, 851, 820, 779 cm^{-1}. ^1H n.m.r. δ ($CDCl_3$): 1·78, 2·39, AB system, J_{AB} 12·3 Hz, two H 9. O.r.d. (*c*, 0·2 in ethanol: $[\phi]_{589}$ +200°, $[\phi]_{578}$ +211°, $[\phi]_{436}$ +526°, $[\phi]_{365}$ +1200°.

Epimerization of Ketone (*12*)

Ketone (12) (0·1 g) was shaken in ether (10 ml) with alumina (activity I, 2 g) while being monitored by g.l.c. (DEGS, 160°). After three days, the product composition was steady as a mixture of C 7 epimer (13) and starting material (12) (ratio 74 : 26). The ether was filtered and evaporated to give an oil (0·07 g) after distillation (50°/0·1 mm) (Found: C, 80·3; H, 12·6. Calc. for $C_{15}H_{28}O$: C, 80·3; H, 12·6%). ν_{max} (film): 1711, 1467, 1380, 1367, 1304, 1281, 1254, 1236, 1210, 1182, 1169, 1083, 1070, 1034, 1027, 1001, 946, 918, 777 cm^{-1}. O.r.d. (*c*, 0·96 in ethanol): $[\phi]_{589}$ +52°, $[\phi]_{578}$ +56°, $[\phi]_{436}$ +150°, $[\phi]_{365}$ +330°. The mixture gave a crude semicarbazone, m.p. 168–170°, which after several recrystallizations (aqueous ethanol) increased to 173° (lit.[9] for semicarbazone of enantiomer (8), 174°).

Dehydration of Tetrahydroelemol (*16*)

Elemol (4·5 g) obtained by silver nitrate extraction of *Hedycarya augustifolia* oil[15] was hydrogenated over palladized charcoal. The resulting tetrahydroelemol (16) (4 g) in ethyl acetate was treated dropwise with perchloric acid (70%, 5 ml) and stirred for 1 h. The solution was washed with sodium bicarbonate solution, then with water, dried, and evaporated to give a colourless oil (3·5 g), which was passed through a column of neutral alumina (100 g, activity I), eluting with hexane. The resulting oil showed two peaks (ratio *c.* 1 : 1) on g.l.c. analysis (DEGS, 120°). A portion (1 g) was chromatographed on alumina impregnated with silver nitrate (40 g $AgNO_3$ on 100 g alumina), eluting with hexane. The early fractions were repeatedly rechromatographed to give pure (3*R*,4*R*)-4-ethyl-1,3-diisopropyl-4-methylcyclohexene (18) as a colourless oil which distilled (60°/0·1 mm) (Found: C, 86·3; H, 13·4. Calc. for $C_{15}H_{28}$: C, 86·5; H, 13·5%). $[\alpha]_D$ (*c*, 2·13 in $CHCl_3$) −90° (lit.[37] −20·9°). ν_{max} (film): 2955, 2925, 2865, 1665, 1465, 1388, 1328, 1307, 1293, 1170, 1159, 1141, 1114, 1098, 1086, 1052, 1011, 981, 970, 918, 894, 884, 855, 800, 776 cm^{-1}; similar to that reported.[38] ^1H n.m.r. δ (CCl_4): 5·26, br s, $W_{h/2}$ 6 Hz, H 6; 1·6–2·4, four allylic protons. O.r.d. (*c*, 2·13 in $CHCl_3$): $[\phi]_{589}$ −187°, $[\phi]_{578}$ −189°, $[\phi]_{436}$ −388°, $[\phi]_{365}$ −621°. Purification of the second component (17) of the above olefin mixture was not pursued to completion, but successive passes down a silver nitrate–alumina column did improve its purity.

Hydroboration of the Mixture of Olefins (*17*) *and* (*18*)

(i) *With diborane.*—Diborane gas was generated by adding sodium borohydride (4 g) in dry diglyme (50 ml) dropwise to boron trifluoride etherate (25 ml) in diglyme (30 ml). The generated diborane was swept by nitrogen into the reaction flask containing a mixture (*c.* 1 : 1) of (17) and (18) (3 g) in dry tetrahydrofuran (45 ml) at 0°. After 1 h, the reaction mixture was allowed to warm to room temperature, and diborane was bubbled into it for another hour. Excess diborane was destroyed

[37] Joshi, B. N., Seshadri, R., Chakravarti, K. K., and Bhattacharyya, S. C., *Tetrahedron*, 1964, **20**, 2911.
[38] Gough, J., and Sutherland, M. D., *Aust. J. Chem.*, 1964, **17**, 1270.

with ice, and then aqueous potassium hydroxide solution (3 M, 30 ml) was added, followed by slow addition of hydrogen peroxide (30 ml, 30%). The mixture was stirred overnight and then extracted with ether to give a colourless oil (3 g) which showed two major peaks on g.l.c. analysis. Chromatography of the mixture over silica gel failed to give complete separation.

The mixture (2 g) in dry pyridine (15 ml) with 3,5-dinitrobenzoyl chloride (3 g) gave a white solid which was fractionally crystallized (ethanol) producing as colourless needles (*1S,2R,4S,5R*)-*5-ethyl-2,4-diisopropyl-5-methylcyclohexyl 3,5-dinitrobenzoate* (19b), m.p. 142·5–143·5° (Found: C, 62·9; H, 7·5; N, 6·6. $C_{22}H_{32}N_2O_6$ requires C, 62·8; H, 7·7; N, 6·7%). ν_{max} (Nujol): 3115, 1720, 1632, 1552, 1345, 1292, 1180, 1073, 997, 962, 928, 918, 771, 731, 718 cm^{-1}. ^1H n.m.r. δ ($CDCl_3$): 5·16, dt, *J* 5, 11 and 11 Hz, H 8; 9·06, complex m, aryl H. O.r.d. (*c*, 0·32 in $CHCl_3$): $[\phi]_{589}$ +286°, $[\phi]_{578}$ +307°, $[\phi]_{436}$ +608°, $[\phi]_{365}$ +750°.

Addition of potassium hydroxide solution (10 M, 5 ml) to a solution of 3,5-dinitrobenzoate (19b) (0·1 g) in warm ethanol (20 ml) produced an immediate deep red coloration. After 3 h, ether was added. Normal workup yielded a pale yellow oil which was distilled (100°/0·1 mm) to give (1*S*,2*R*,4*S*,5*R*)-5-ethyl-2,4-diisopropyl-5-methylcyclohexanol (19a) (40 mg) as a colourless oil (Found: C, 79·5; H, 13·5. Calc. for $C_{15}H_{30}$: C, 79·6; H, 13·4%). $[\alpha]_D$ +18° (*c*, 0·35 in pentane) (lit.[9] +14·7°). ν_{max} (film): 3300, 1465, 1381, 1368, 1260, 1181, 1147, 1097, 1057, 1043, 1026, 977, 918, 779 cm^{-1}. ^1H n.m.r. δ ($CDCl_3$): 1·57, dd, *J* 4·2 and 12·1 Hz, H 9*eq*; 1·89 and 2·15, two isopropyl methine m; 3·56, dt, *J* 4·2, 10 and 10 Hz, H 8. O.r.d. (*c*, 0·35 in pentane): $[\phi]_{589}$ +42°, $[\phi]_{578}$ +43°, $[\phi]_{436}$ +77°, $[\phi]_{365}$ +120°, $[\phi]_{265}$ +265°, $[\phi]_{254}$ +315°.

(ii) *With borabicyclononane.*—Olefins (17) and (18) (*c*, 1 : 1) (1 g) were refluxed for 24 h with a solution of 9-borabicyclo[3,3,1]nonane (0·5 M, 30 ml) in dry tetrahydrofuran. G.l.c. analysis showed that only olefin (17) was consumed. Potassium hydroxide solution (6 M, 15 ml) and hydrogen peroxide (30%, 15 ml) were added, and the mixture refluxed for 48 h. Workup gave an oil which was chromatographed over alumina, eluting with hexane and hexane/ether mixtures. Early fractions contained non-polar material, while later fractions gave the alcohol (19a) as a colourless oil, identical with that described above and giving a 3,5-dinitrobenzoate m.p. 143° also identical with (19b) described above.

(2R,4S,5R)-5-Ethyl-2,4-diisopropyl-5-methylcyclohexanone (8)

Jones oxidation of alcohol (19a) (0·2 g) in acetone (15 ml) at 10–20° gave, on distillation, (2*R*,4*S*,5*R*)-5-ethyl-2,4-diisopropyl-5-methylcyclohexanone (8) as a colourless oil (0·17 g) (Found: C, 80·3; H, 12·6. Calc. for $C_{15}H_{28}O$: C, 80·3; H, 12·6%). $[\alpha]_D$ −2° (*c*, 0·3 in ethanol) (lit.[9] −4·2°). ν_{max} (film): 1713, 1468, 1382, 1370, 1304, 1281, 1236, 1209, 1180, 1170, 1071, 1032, 946, 917 cm^{-1}. O.r.d. (*c*, 0·3 in ethanol): $[\phi]_{589}$ −4·5°, $[\phi]_{578}$ −8·5°, $[\phi]_{436}$ −22·5°, $[\phi]_{365}$ −31·4°. Epimerization of the product over alumina as described for compound (12) resulted in an equilibrium mixture which was identical with that from (12) (by g.l.c. and i.r.) but which showed a mirror-image o.r.d. curve.

Reduction of Callitrin (1)

Callitrin (1·05 g) in dry ether (25 ml) with lithium aluminium hydride (0·5 g) over 1 h yielded *diol** (14) as fine needles, m.p. 102·5–103·5° (from hexane) (Found: C, 75·4; H, 10·9. $C_{15}H_{26}O_2$ requires C, 75·6; H, 11·0%). ν_{max} (Nujol): 3205 (br), 3085, 1639, 1328, 1200, 1139, 1110, 1075, 1063, 1048, 1030, 1021, 998, 918, 907, 888, 885 cm^{-1}. ^1H n.m.r. δ ($CDCl_3$): 0·91, d, *J* 6·5 Hz, 11-Me; 1·03, s, 10-Me; 1·41, dd, *J* 4 and 13 Hz, H 9*eq*; 1·70, s, 4-Me; 3·48, dd, *J* 6 and 11 Hz, H 12a; 3·67, dd, *J* 3 and 11 Hz, H 12b; 4·07, dt, *J* 4, 4 and 11 Hz, H 8; 4·26, br s, two OH; 4·45–5·10, four vinyls; 5·88, X region of ABX system, H 1. O.r.d. (*c*, 0·32 in ethanol): $[\phi]_{589}$ +125°, $[\phi]_{578}$ +129°, $[\phi]_{436}$ +250°, $[\phi]_{365}$ +411°, $[\phi]_{265}$ +1260°, $[\phi]_{254}$ +1460°.

Reduction of Tetrahydrocallitrin (9)

Tetrahydrocallitrin (9) (1·17 g) in dry ether was reduced with lithium aluminium hydride (0·5 g) over 30 min to give *diol*† (15a) as colourless needles (0·8 g), m.p. 95–95·5° (from hexane) (Found: C, 74·3; H, 12·5. $C_{15}H_{30}O_2$ requires C, 74·3, H, 12·5%). ν_{max} (Nujol): 3150 (br), 1501, 1331,

* Systematic name (1*R*,2*R*,4*R*,5*R*)-5-ethenyl-2-[(*R*)-2-hydroxy-1-methylethyl]-5-methyl-4-(1-methylethenyl)cyclohexanol.

† Systematic name (1*R*,2*R*,4*R*,5*S*)-5-ethyl-2-[(*R*)-2-hydroxy-1-methylethyl]-5-methyl-4-(1-methylethyl)cyclohexanol.

1301, 1289, 1161, 1150, 1138, 1099, 1081, 1057, 1023, 971, 944, 938, 888 cm^{-1}. O.r.d. (*c*, 0·4 in ethanol): $[\phi]_{589}$ +84°, $[\phi]_{578}$ +96°, $[\phi]_{436}$ +186°, $[\phi]_{365}$ +287°, $[\phi]_{265}$ +720°, $[\phi]_{254}$ +810°.

Acetylation of Diol (15a)

Diol (15a) (71 mg) was acetylated with acetic anhydride (1 ml) in dry pyridine (5 ml) overnight to give the *diacetate* (15b) which distilled as a colourless oil (Found: C, 69·7; H, 10·6. $C_{19}H_{34}O_4$ requires C, 69·9; H, 10·6%). ν_{max} (film): 1738, 1516, 1364, 1240, 1029 cm^{-1}. ^1H n.m.r. δ (CCl_4): 0·78, d, *J* 7 Hz, 11-Me; 0·97 s, 10-Me; 1·95, 1·98, two s, acetyl; 3·80, dd, *J* 7 and 13 Hz, H 12a; 4·33, dd, *J* 3·5 and 13 Hz, H 12b; 5·00, dt, *J* 4, 4 and 12 Hz, H 8.

Cope Rearrangement of the Germacranolide (3)

Lactone (3) (110 mg) was refluxed in diglyme (15 ml) for 10 h. G.l.c. monitoring showed that the composition remained constant after about 4 h, and contained starting material (3) (72%) and a new compound (28%). Chromatography over alumina containing 10% by weight of silver nitrate and elution with hexane/ether (1 : 1) gave the elemanolide (25) (25 mg), which sublimed (65°, 0·1 mm) as prisms, m.p. 92–93° (lit.[7] 89–90°), $[\alpha]_D$ −15° (lit.[7] −8·5°) (Found: C, 76·9; H, 9·5. Calc. for $C_{15}H_{22}O_2$: C, 76·9; H, 9·4%). ν_{max} (Nujol): 3075, 1775, 1637, 1334, 1245, 1224, 1193, 1144, 1117, 1090, 1018, 1000, 927, 906, 726, 706 cm^{-1}. ^1H n.m.r. δ (CCl_4): 1·09, s, 10-Me; 1·17, d, *J* 7·5 Hz, 11-Me; 1·75, d, *J* 1 Hz, 4-Me; 3·88, m, *J* 4, 9·1 and 11·3 Hz, H 8; 4·5–6·0, 5H, vinyl. O.r.d. (*c*, 0·77 in ethanol): $[\phi]_{589}$ −35°, $[\phi]_{578}$ −39°, $[\phi]_{436}$ −104°, $[\phi]_{265}$ −1395°. M.s. (*m/e*): 234 (M, 20%), 219 (7), 206 (4), 165 (6), 161 (12), 160 (5), 138 (9), 122 (18), 109 (10), 108 (11), 107 (12), 93 (21), *68* (100), 55 (15), 41 (26).

Cope Rearrangement of Elemanolide (25)

Lactone (25) (2 mg) was heated at 220° in a sealed tube for 1 h. G.l.c. analysis (SE30, 160°; DEGS 160°) showed lactones (3) and (25) in the ratio 3 : 1.

Acid Cyclization of the Germacranolide (3)

Perchloric acid (70%, 0·2 ml) was added to a solution of lactone (3) (0·25 g) in ethyl acetate. The solution slowly became deep red in colour. G.l.c. analysis showed that the starting material had largely been converted into a new compound after 45 min. Workup gave a pale yellow oil which after chromatography over silver nitrate–alumina gave the *eudesmanolide** (27), m.p. 69–70° (prisms from aqueous methanol and after sublimation at 50°/0·1 mm) (Found: C, 76·7; H, 9·4. $C_{15}H_{22}O_2$ requires C, 76·9; H, 9·5%). ν_{max} (Nujol): 1778, 1380, 1331, 1246, 1228, 1195, 1175, 1162, 1139, 1120, 1088, 1056, 1004, 994, 837, 807, 723, 699 cm^{-1}. ^1H n.m.r. δ (CCl_4): 1·12, s, 10-Me; 1·17, d, *J* 7·5 Hz, 11-Me; 1·35, dd, *J* 10 and 12 Hz, H 9*ax*; 1·62, s, 4-Me; 2·20, m, H 11; 2·76, dd, *J* 3 and 13·5 Hz, H 6*eq*; 4·01, m, *J* 4, 10 and 12 Hz, H 8. O.r.d. (*c*, 0·54 in ethanol): $[\phi]_{589}$ +164°, $[\phi]_{578}$ +170°, $[\phi]_{436}$ +320°, $[\phi]_{365}$ +517°, $[\phi]_{265}$ +1070°. M.s. (*m/e*): 234 (M, 28%), *219* (100), 191 (7), 173 (5), 163 (6), 161 (10), 145 (11), 133 (6), 123 (11), 107 (17), 93 (10), 91 (9), 81 (9), 67 (5), 55 (10), 41 (11).

Tetrahydrocallitrisin (23)

Callitrisin (2) (0·5 g) was hydrogenated in ethanol (10 ml) over 5% palladized charcoal to give an oil (0·5 g) which crystallized on standing. G.l.c. analysis (SE30, 190°) showed a mixture of two compounds (3 : 1). Fractional crystallization (hexane) gave the *major isomer*† (23) as needles, m.p. 109–110° (Found: C, 76·3; H, 10·4. $C_{15}H_{26}O_2$ requires C, 76·2; H, 10·2%). ν_{max} (Nujol): 1776, 1445, 1434, 1407, 1400, 1190, 1174, 1145, 1128, 1114, 1074, 1036, 1005 cm^{-1}. ^1H n.m.r. δ (CCl_4): 0·91, d, *J* 7 Hz, 4-Me; 1·16, d, *J* 6·5 Hz, 11-Me; 2·43, partly obscured m, H 11; 4·46, dt, *J* 6·5, 6·5 and 11·5 Hz, H 8. O.r.d. (*c*, 0·15 in pentane): $[\phi]_{589}$ +79°, $[\phi]_{578}$ +79°, $[\phi]_{436}$

* Systematic name [3*R*-(3α,3aα,8aβ,9aβ)]-3,5,8a-trimethyl-3a,4,6,7,8,8a,9,9a-octahydronaphtho-[2,3-*b*]furan-2(3*H*)-one.

† Systematic name [3*R*-(3α,3aα,4aβ,5α,8aα,9aα)]-3,5,8a-trimethyldecahydronaphtho[2,3-*b*]furan-2(3*H*)-one.

+157°, $[\phi]_{365}$ +220°, $[\phi]_{302}$ +300°, $[\phi]_{265}$ +45°, $[\phi]_{254}$ −315°. M.s. (*m/e*): 236 (M, 38%), 221 (46), 218 (3), 177 (29), 163 (38), 162 (52), 149 (42), *123* (100), 109 (50), 95 (38), 81 (38), 68 (30), 67 (31), 55 (51), 41 (54).

Isolation of Columellarin (4), Dihydrocolumellarin (5) and Dihydrocallitrisin (6)

The hexane extract of *C. columellaris* heartwood, after extraction with silver nitrate solution, was chromatographed over silica gel (20 g extract on 300 g silica gel). The eluents were hexane (500 ml), and hexane/ether mixture (9 : 1, 4 : 1 and 1 : 1, 500 ml each). The second fraction (3 g) was chromatographed (in 1·5 g lots) on a Merck 'Lobar' silica gel column (size C). Elution with hexane/ether (4 : 1) produced, in order:

(i) *Columellarin* (4) as an oil which crystallized from pentane at −20°. Sublimation (40°/0·1 mm) produced prisms, m.p. 43·5–44° (Found: C, 77·3; H, 8·7. $C_{15}H_{20}O_2$ requires C, 77·5; H, 8·7%). ν_{max} (Nujol): 3100, 1768, 1667, 1632, 1443, 1406, 1380, 1307, 1256, 1161, 1149, 1140, 1127, 1066, 1048, 993, 983, 963, 935, 924, 902, 816, 712 cm^{-1}. ^1H n.m.r. δ ($CDCl_3$): 1·07, d, *J* 5·9 Hz, 4-Me; 1·75, s, 10-Me; 2·19, dt, *J* 2·5, 2·5 and 12·5 Hz, H 6α; 3·78, m, *J* 5·5, 8 and 10 Hz, H 8; 5·42, d, *J* 3·2 Hz, H 13a; 6·11, d, *J* 3·1 Hz, H 13b. ^1H n.m.r. δ (C_6D_6): 0·91, d, 5·6 Hz, 4-Me; 1·53, s, 10-Me; 3·35, dt, *J* 3·3, 9·6 and 9·6 Hz, H 8; 4·91, d, *J* 3·2 Hz, H 13a; 6·00, d, *J* 3·0 Hz, H 13b. Values of $J_{8,9\alpha}$, $J_{8,9\beta}$ and $J_{9\alpha,9\beta}$ derived from analysis of ABX systems in spectra measured in the presence of Eu(fod)$_3$, are listed in Table 1. O.r.d. (*c*, 0·4 in hexane): $[\phi]_{589}$ −104°, $[\phi]_{578}$ −112°, $[\phi]_{436}$ −222°, $[\phi]_{365}$ −368°, $[\phi]_{280}$ −1460°, $[\phi]_{265}$ −531°, $[\phi]_{254}$ +856°. M.s. (*m/e*): 232 (M, 100%), 217 (11), 203 (5), 199 (7), 189 (8), 175 (6), 147 (32), 136 (28), 122 (58), 107 (73), 105 (40), 91 (44), 81 (52), 69 (20), 53 (37), 41 (44).

Continued elution of the column produced (ii) *dihydrocolumellarin* (5) which crystallized from hexane at −20°, and on sublimation gave prisms, m.p. 77–77·5° (Found: C, 77·0; H, 9·4. $C_{15}H_{22}O_2$ requires C, 76·9; H, 9·5%). ν_{max} (Nujol): 1775, 1461, 1435, 1380, 1342, 1262, 1222, 1189, 1181, 1132, 1055, 1047, 1005, 990, 940, 730 cm^{-1}. ^1H n.m.r. δ ($CDCl_3$): 1·06, d, *J* 5·5 Hz, 4-Me; 1·23, d, *J* 6·6 Hz, 11-Me; 1·72, br s, 10-Me; 3·80, dt, *J* 4·6, 9 and 9 Hz, H 8. ^1H n.m.r. δ (C_6D_6): 0·88, d, *J* 5·7 Hz, 4-Me; 1·02, d, *J* 7 Hz, 11-Me; 1·56, br s, 10-Me; 3·39, dt, *J* 3·2, 9·8 and 9·8 Hz, H 8. Values of $J_{8,9\alpha}$, $J_{8,9\beta}$ and $J_{9\alpha,9\beta}$ derived from analysis of ABX systems in spectra measured in the presence of Eu(fod)$_3$ are included in Table 1. O.r.d. (*c*, 0·8 in ethanol): $[\phi]_{589}$ +44°, $[\phi]_{578}$ +49°, $[\phi]_{365}$ +152°, $[\phi]_{297}$ +245°, $[\phi]_{265}$ +73°, $[\phi]_{254}$ −24°. M.s. (*m/e*): 234 (M, 64%), 219 (14), 205 (9), 191 (5), 173 (5), 161 (16), 160 (9), 147 (12), 145 (12), 138 (27), *122* (100), 107 (68), 105 (25), 93 (31), 91 (30), 81 (28), 79 (28), 55 (26), 41 (24).

Further elution of the column produced (iii) *dihydrocallitrisin* (6) as needles, m.p. 127–128° (from hexane) (Found: C, 76·8; H, 9·5. $C_{15}H_{22}O_2$ requires C, 76·9; H, 9·5%). ν_{max} (Nujol): 3080, 1769, 1648, 1456, 1385, 1332, 1300, 1188, 1151, 1115, 1001, 990, 959, 896 cm^{-1}. ^1H n.m.r. δ ($CDCl_3$): 0·76, s, 10-Me; 1·22, d, *J* 6 Hz, 11-Me; 4·52, s, H 15a; 4·64, partly obscured m, H 8; 4·78, s, H 15b. ^1H n.m.r. δ (C_6D_6): 0·43, s, 10-Me; 0·95, d, *J* 6 Hz, 11-Me; 4·15, dt, *J* 6·7, 6·7 and 11·3 Hz, H 8; 4·39, s, H 15a; 4·72, s, H 15b. O.r.d. (*c*, 0·4 in ethanol): $[\phi]_{589}$ −91°, $[\phi]_{578}$ −94°, $[\phi]_{436}$ −199°, $[\phi]_{365}$ −346°, $[\phi]_{265}$ −1500°, $[\phi]_{254}$ −2020°. M.s. (*m/e*): 234 (M, 19%), 219 (8), 206 (47), 161 (21), 160 (12), 145 (26), *133* (100), 121 (17), 107 (14), 105 (24), 91 (25), 79 (22), 67 (13), 55 (21), 41 (29).

Continued elution of the column produced a small quantity of callitrisin (2).

Hydrogenation of Columellarin

Columellarin (4) (0·1 g) in ethanol took up 1 molar equivalent of hydrogen over 5% Pd/C to give dihydrocolumellarin (5) (0·07 g), m.p. 77° (from hexane at −20°), identical with the natural compound by mixed m.p., g.l.c. (SE30 and DC710), i.r. and n.m.r.

Ozonolysis of Dihydrocolumellarin

Dihydrocolumellarin (5) (0·11 g) in methanol (15 ml) was ozonized at −70° for 1 hour. Work-up with powdered zinc (1 g) and acetic acid (2·5 ml) gave the *diketo lactone** (31) (0·06 g) as a colourless

* Systematic name for indexing: [3*R*-(3α,4β(1′*S*,2′*R*),5α)]-3-methyl-4-[(2-methyl-5-oxocyclopentyl)-methyl]-5-(2-oxopropyl)dihydrofuran-2(3*H*)-one. 'Best' name under IUPAC rules: (2*R*,3*R*,4*S*)-2-methyl-3-[{(1′*S*,2′*R*)-2-methyl-5-oxocyclopentyl}methyl]-6-oxoheptan-4-olide.

oil (single g.l.c. peak; SE30, 200°) which was distilled (160°/0·1 mm) (Found: C, 67·2; H, 8·3. $C_{15}H_{22}O_4$ requires C, 67·7; H, 8·3%). ν_{max} (film): 1775, 1736, 1720sh, 1463, 1411, 1383, 1361, 1331, 1298, 1260, 1228, 1170, 1053, 1037, 977 cm^{-1}. ^1H n.m.r. δ ($CDCl_3$): 1·16, d, *J* 5·2 Hz, 4-Me; 1·28, d, *J* 7 Hz, 11-Me; 2·21, s, 10-Me; 2·83, sharp d, *J* 5·5 Hz, two H 9; 4·45, m, *J* 5·5, 5·5 and 9 Hz, H 8. O.r.d. (*c*, 0·1 in pentane): $[\phi]_{589}$ +8·2°, $[\phi]_{578}$ +13·7°, $[\phi]_{436}$ +41°, $[\phi]_{365}$ +245°, $[\phi]_{334}$ +850°, $[\phi]_{313}$ +1165°, $[\phi]_{302}$ −77°, $[\phi]_{297}$ −820°, $[\phi]_{280}$ −1960°, $[\phi]_{254}$ −1760°. M.s. (*m/e*): 266 (M, 62%), 251 (7), 248 (6), 238 (8), 209 (56), *208* (100), 193 (20), 169 (22), 163 (22), 154 (49), 152 (31), 149 (12), 141 (39), 137 (24), 123 (19), 111 (64), 110 (55), 98 (99), 97 (95), 95 (68), 93 (30), 85 (19).

Dehydrogenation of Dihydrocolumellarin

Dihydrocolumellarin (5) (0·2 g) when heated (300°, 2 h) with 10% palladized charcoal (0·15 g) showed a blue liquid condensing on the cooler parts of the tube. Workup gave an intense blue oil which was chromatographed over neutral alumina. T.l.c. (silica gel, hexane) of the fast-moving blue band showed two overlapping blue spots of unequal intensity. A solution of trinitrobenzene in warm methanol produced black crystals of the trinitrobenzene adduct of chamazulene (28), m.p. 131–132° (after several recrystallizations from methanol) (lit.[39] 132°).

Hydrogenation of Dihydrocallitrisin

Dihydrocallitrisin (6) (75 mg) was hydrogenated in ethanol (10 ml) over 5% palladized charcoal until uptake of hydrogen ceased (uptake 7 ml, 1 molar equivalent). The product showed two peaks (2 : 1) on g.l.c. analysis (SE30 and DC710) identical with those obtained from the hydrogenation of callitrisin (2). Fractional crystallization (hexane) yielded tetrahydrocallitrisin (23), m.p. 109–110°, identical by mixed m.p., g.l.c., i.r., o.r.d., with that described above (Found: C, 76·3; H, 10·2. Calc. for $C_{15}H_{24}O_2$: C, 76·2; H, 10·2%).

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