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THE STRUCTURE OF LOLIOLIDE

A TERPENE FROM *LOLIUM PERENNE*

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Abstract—Loliolide, a compound $C_{11}H_{16}O_3$ obtained from *Lolium perenne*, has been shown to be 1,3-dihydroxy-3,5,5-trimethylcyclohexylidene-4-acetic acid lactone (II).

THE compound formulated $C_{20}H_{30}O_6$ (m.p. 149°), was first isolated[1] from ether extracts of New Zealand pasture. This same compound, now named loliolide, has now been obtained from *Lolium perenne* (perennial ryegrass) in a yield of 8×10^{-4} % using a modified extraction procedure, and it has also been isolated in 4×10^{-6} % yield from the effluent of silage made from a predominantly ryegrass pasture.†

The mass spectrum of loliolide shows that its m.wt. is 196 and that it contains one hydroxyl group, the hydrogen atom in this group being replaceable by deuterium in the inlet system of a mass spectrometer. Hence the formula quoted above should be revised to $C_{11}H_{16}O_3$. The presence of the secondary hydroxyl group is confirmed by the formation of a monoacetate derivative and by oxidation of loliolide to an optically active ketone which has absorption maxima, in methanol, at 212 mμ, $\varepsilon = 14{,}200$ and, in CCl_4, at 1750, 1720, 1630, 856 cm^{-1}. Loliolide itself shows absorption maxima at 215 mμ, $\varepsilon = 14{,}800$ and, in $CHCl_3$, at 1745, 1630 and 856 cm^{-1}. Catalytic hydrogenation of loliolide yields a dihydro derivative, which shows no maxima in the UV, and has no peaks in the IR which can be assigned to a trisubstituted double bond: it does however exhibit a carbonyl peak, now at 1775 cm^{-1} in CCl_4 solution. These results suggest that loliolide is an $\alpha\beta$-unsaturated γ-lactone possessing a trisubstituted double bond and a secondary non-allylic hydroxyl group. This hydroxyl group is either acyclic or else is attached to a ring containing more than five atoms.

The lactone ring in loliolide opens only after prolonged heating with strong alkali and is reformed after this treatment on acidifying the resultant product with acetic acid. The ketone obtained from loliolide is, however, very unstable to aklali; on treating it with sodium hydroxide it gives the sodium salt of an acid which has a maximum absorption, in methanol, at 292 mμ, $\varepsilon = 11{,}400$. Acidification of this sodium salt produces a different, isomeric, ketone whose IR spectrum in solution is the same as that of the original ketone. However, the IR spectra examined as nujol mulls are different, the two ketones have different melting points and the latter, isomeric,

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† Dr. G. Snatzke of the University of Bonn has informed us that R. Tschesche and G. Buschauer, isolated a compound from *Digitalis Lanata* leaves which was shown (R. Tschesche and G. Buschauer, unpublished; cf. G. Buschauer, Dissertation Hamburg, 1957) to be identical with the neutral substance isolated by R. H. F. Manske from *Fumaria officinalis* [*Canad. J. Res.* **B16**, 438 (1938)]. Dr. Snatzke has shown that this compound is identical with loliolide.

[1] E. P. White, *N.Z. J. Agric. Res.* **1**, 859 (1958).

ketone exhibits no optical rotation. Each of these observations can readily be accounted for if the ketone is partially formulated as I, and racemizes on treatment with alkali, as shown; seven atoms of deuterium can be introduced into the racemic ketone by refluxing it with sodium deuteroxide. This partial formula is supported by the instanteous opening of the lactone ring in the dihydroketone (ν_{max} in CCl_4: 1780, 1725 cm^{-1}) obtained from loliolide. When the dihydroketone is treated with cyclohexylamine, an optically active product is obtained whose spectrum (λ_{max}: 242 mμ, ε = 12,900 ν_{max}: 2700, 2620, 2570, 2230, 1660; 1620, 840 cm.$^{-1}$) shows that it is a salt rather than an amide. This salt can be converted into a liquid acid (λ_{max}: 238 mμ, ε = 11,250). The presence of a strong absorption peak at 840 cm^{-1} in the IR spectrum, in both this acid and in its salt, suggests that the double bond conjugated with the ketonic carbonyl group is trisubstituted, e.g. in I, either R_1 or R_2 = H. Furthermore, the optical activity of these last two compounds is consistent with the suggested trisubstitution (856 cm^{-1}) of the original double bond in loliolide, i.e., in I either R_3 or R_4 = H.

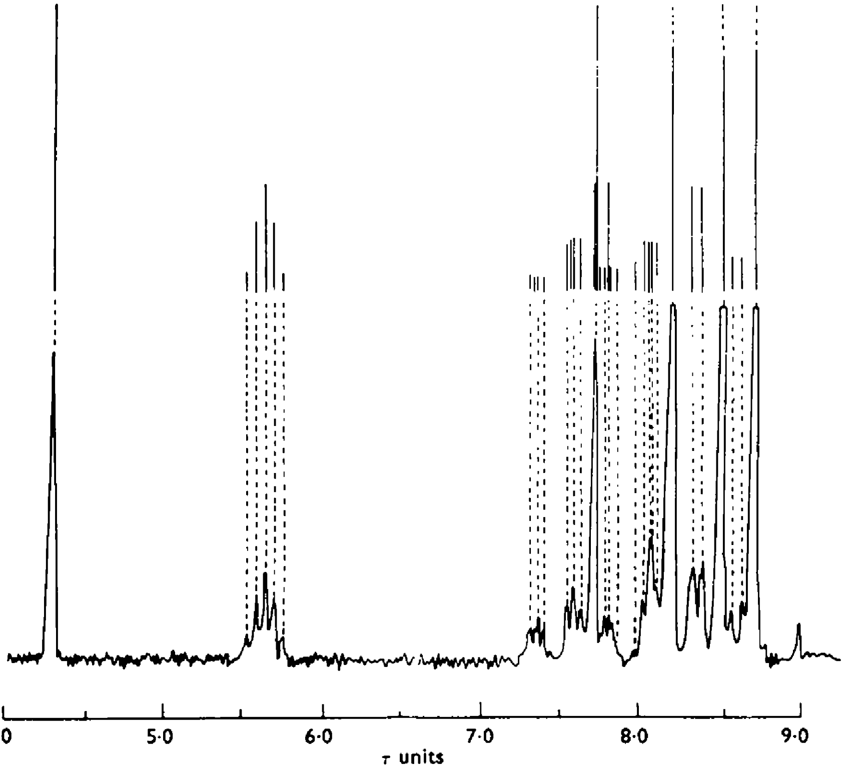


Fig. 1. Proton magnetic resonance spectrum of loliolide.

When loliolide is subjected to vigorous oxidation 2,2-dimethylsuccinic anhydride is obtained, thus accounting for a further three carbon atoms in the molecule.

PMR spectra of loliolide and its dihydro derivative are shown in Figs. 1 and 2 respectively. Figure 1 shows that R_4 = H and since the olefinic region of this spectrum consists of a sharp singlet it immediately follows that R_3 is a fully substituted carbon atom and that $R_2 \neq$ H. Furthermore, the C*H*OH resonance consists of a

1:4:6:4:1 quintet originating at $\tau = 5{\cdot}65$. Hence $R_1 = H$, the magnetic resonance results thus confirming the deductions made from the IR spectra, and R is a methylene group. The combination of the chemical and PMR studies show that loliolide is 1,3-dihydroxy-3,5,5-trimethylcyclohexylidene-4-acetic acid lactone (II).

Structure II is confirmed by more complete analyses of the PMR spectra of loliolide and its dihydro derivative, III. The assignments of functional groups to the observed absorption peaks and the appropriate chemical shifts and coupling constants extracted from these spectra are listed in Tables 1 and 2. First-order line spectra calculated from these chemical shifts and coupling constants are shown in Figs. 1 and 2, but because of the extensive overlap of peaks which arise from the C(2)H_2 and C(6)H_2 protons in dihydrololiolide, we can not assign accurate chemical shifts to these protons nor can we extract the magnitude of the geminal coupling constant within these CH_2 residues. Consequently, that part of the line spectrum due to these protons has been left out of Fig. 2.

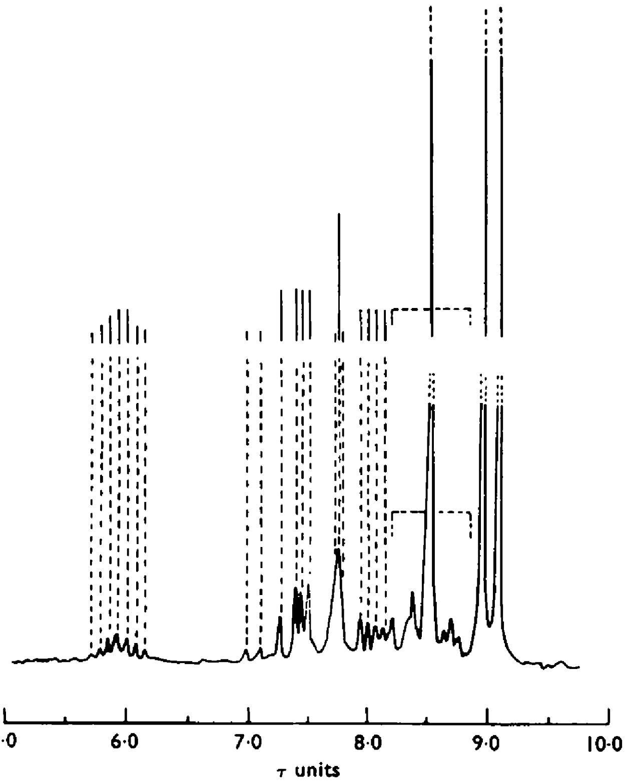
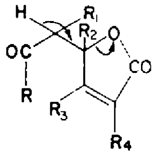


FIG. 2. Proton magnetic resonance spectrum of dihydrololiolide.

If it is assumed that the cyclohexane ring is in the chair conformation in both loliolide and in dihydrololiolide, then the relative configurations at the asymmetric carbon atoms can be deduced for both compounds from their PMR spectra. This follows by making use of Karplus's relationships concerning the changes which occur in proton spin-spin coupling constants within an ethane-like system as the dihedral angles within the system are varied.[2] These arguments show that the configurations in loliolide and in dihydroloplliolide are those depicted in IV and V respectively.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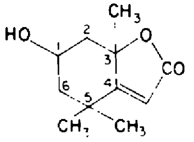
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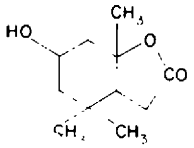
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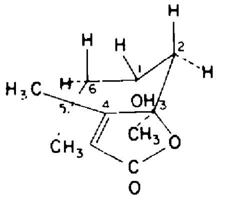
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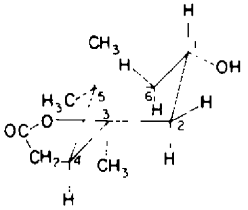
V

Table 1. Assignments for loliolide

Functional group	τ value	Functional group	τ value
C(1)H	5·65	C(5)CH_3 (α; β)	8·52; 8·72
C(2)H_2 (α; β)	7·48; 7·94	C(6)H_2 (α; β)	8·0; 8·5
C(3)CH_3	8·21	C(1)OH	7·73
C(4)=CH	4·32		

Coupling constants (c/sec, all ±0·3 c/s)

J[C(1)H—C(2)H(α)] = 3·6
J[C(1)H—C(2)H(β)] = 3·6
J[C(1)H—C(6)H(α)] = 3·6
J[C(1)H—C(6)H(β)] = 3·6
J[C(2)H(α)—C(2)H(β)] = 14·4
J[C(6)H(α)—C(6)H(β)] = 13·8
J[C(2)H(α)—C(6)H(α)] = 2·7

Table 2. Assignments for dihydrololiolide

Functional group	τ value	Functional group	τ value
C(1)H	5·92	C(4)CH_2 (α; β)	7·24; 7·57
C(2)H_2 (α; β)	~8·2–8·6	C(5)CH_3 (α; β)	8·95; 9·07
C(3)CH_3	8·52	C(6)H_2 (α; β)	~8·2–8·6
C(4)H	8·03	C(1)OH	7·75

Coupling constants (c/sec, all ±0·3 c/s)

J[C(1)H—C(2)H(α)] = 10
J[C(1)H—C(2)H(β)] = 5
J[C(1)H—C(6)H(α)] = 10
J[C(4)H—C(4)CH$_2$(α)] = 8
J[C(4)H—C(4)CH$_2$(β)] = 4
J[C(4)CH$_2$(α)—C(4)CH$_2$(β)] = 17·7

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[2] M. Karplus, *J. Chem. Phys.* **30**, 11 (1959).

EXPERIMENTAL

Isolation of loliolide from Lolium perenne. Dry leaves (1 kg) of *Lolium perenne*, obtained from ryegrass grown as individual plants to avoid contamination by other species, were extracted in a Soxhlet with ether. After evaporation, the extract was heated under reflux with aqueous (50%) methanolic NaOH (25%) for 4 hr. The acidic products, in chloroform, were washed with NaOH aq and the residual neutral material was adsorbed from benzene on alumina deactivated with water (10%). Elution with benzene–ether (9:1) gave loliolide as plates (8 mg) from carbon tetrachloride, m.p. 148–149°, $[\alpha]_D$ −92° (*c*, 1·1 in $CHCl_3$), IR spectrum identical with the original sample.[1] Similar treatment of the chloroform extract of silage effluent (260 kg) gave loliolide (10 mg).

1-*Acetoxy*-3-*hydroxy*-3,5,5-*trimethylcyclohexylidene*-4-*acetic acid lactone*. Loliolide (18 mg), acetic anhydride (0·1 ml) and pyridine (0·3 ml) were allowed to react at 20° for 18 hr. The product was adsorbed from benzene on alumina and eluted with benzene–ether (1:1) as prisms (16 mg) from light petroleum–chloroform, m.p. 86–87°. (Found: C, 65·35; H, 7·15; O, 26·55; Ac, 18·4. $C_{13}H_{18}O_4$ requires: C, 65·55; H, 7·6; O, 26·85; Ac, 18·05%).

1,3-*Dihydroxy*-3,5,5-*trimethylcyclohexyl*-4-*acetic acid γ-lactone*. Loliolide (46 mg) in acetic acid (4 ml) was hydrogenated (1 mole) using Adams' catalyst at 20° and atm. press. The product was adsorbed from carbon tetrachloride on deactivated alumina and eluted with benzene–ether (9:1) as plates of dihydroloILiolide (32 mg) from carbon tetrachloride, m.p. 86–87°, $[\alpha]_D$ +8° (*c*, 1·6 in $CHCl_3$). (Found: C, 66·55; H, 9·1; O, 24·55. $C_{11}H_{18}O_3$ requires: C, 66·65; H, 9·15; O, 24·2%).

3-*Hydroxy*-3,5,5-*trimethyl*-1-*oxocyclohexylidene*-4-*acetic acid lactone*. Loliolide (21 mg) in acetone, was oxidized with Jones' chromium trioxide reagent. The product crystallized from light petroleum as prisms (17 mg) of the ketone, m.p. 101–102° $[\alpha]_D$ −151° (*c*, 0·4 in $CHCl_3$). (Found: C, 68·4; H, 7·65; O, 24·45. $C_{11}H_{14}O_3$ requires: C, 68·0; H, 7·25; 0, 24·75%). This ketone in alkaline methanol (0·001 N NaOH) had λ_{max} at 292 mμ and on evaporation of the alkaline solution gave a sodium salt with IR absorption at 1660 and 1610 cm^{-1}. Acidification of an alkaline solution of the ketone with acetic acid yielded the racemic form as plates from light pet. ether, m.p. 73–75°. (Found: C, 68·1; H, 7·4; O, 24·55. $C_{11}H_{14}O_3$ requires: C, 68·0; H, 7·25; O, 24·75%).

Oxidation of 1,3-*dihydroxy*-3,5,5-*trimethylcyclohexyl*-4-*acetic acid γ-lactone*. The lactone (27 mg) was oxidized as before, giving the corresponding ketone as crystals (14 mg) from light petroleum, m.p. 92–96°, $[\alpha]_D$ −68° (*c*, 0·5 in $CHCl_3$). These were unstable, could not be recovered from chloroform solution and in methanol had λ_{max} 241 mμ, $\varepsilon = 1{,}200$ increasing in 1 hr to 7,600 and reaching a value of 13,200 on treatment with NaOH.

When this ketolactone (12 mg) in benzene (1 ml) was treated with cyclohexylamine (0·05 ml) a salt was immediately precipitated. Crystallization from benzene–methanol gave needles (12 mg), m.p. 140–146°, $[\alpha]_D$ −36° (*c*, 0·7 in MeOH). (Found: C, 68·75; H, 9·95; O, 16·5. $C_{17}H_{29}NO_3$ requires: C, 69·1; H, 9·9; O, 16·25%). This cyclohexylamine salt was converted on acidification with acetic acid to the corresponding acid which was isolated as an oil, $[\alpha]_D$ −54° (*c*, 1·8 in $CHCl_3$), ν_{max} 1725, 1665, 1635, 840 cm^{-1}.

Oxidation of loliolide to 2,2-*dimethylsuccinic anhydride*. Loliolide (130 mg) was dissolved in nitric acid (95%, sp. gr. 1·5) and kept at 90° for 48 hr. The ether soluble acidic products were sublimed, giving 2,2-dimethylsuccinic anhydride (11 mg) identical with an authentic specimen.

Deuteration of 3-*hydroxy*-3,5,5-*trimethyl*-1-*oxocyclohexylidene*-4-*acetic acid lactone*. (−)3-Hydroxy-3,5,5-trimethyl-l-oxocyclohexylidene-4-acetic acid lactone (55 mg) was heated under reflux for 5 min with sodium deuteroxide (20 mg) in dioxan–deuterium oxide (1 ml, 2:1). The solvents were then evaporated under reduced pressure and the residue heated as before with a further 1 ml of deuterium oxide. This solution was then acidified with deuteroacetic acid, extracted with light petroleum, the extract washed with deuterium oxide and crystallized, giving the (±) deuterated ketone (23 mg), m.p. 73–74°.

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