SECTION C
Organic Chemistry

The Structure of Leucogenenol

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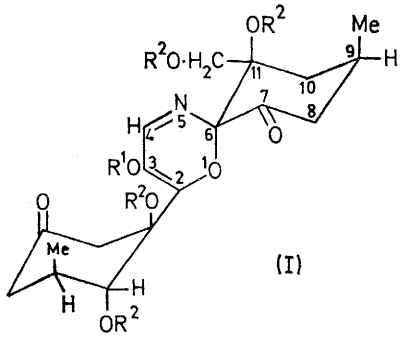
The structure of leucogenenol, a blood cell stimulatory substance has now been determined. The compound forms a number of salts, a monomethyl enol ether, a derived tetra-acetate, and a hexabenzoate. Leucogenenol is hydrolysed to form 1,2-dihydroxy-3-methyl-5-oxocyclohexanecarboxylic acid, 3-hydroxy-3-hydroxymethyl-5-methylcyclohexane-1,2-dione, glycolaldehyde, aminoacetaldehyde, and ammonia. This evidence and spectroscopic data indicate that leucogenenol is 2-(1,2-dihydroxy-3-methyl-5-oxocyclohexyl)-3,11-dihydroxy-11-(hydroxymethyl)-9-methyl-1-oxa-5-azaspiro[5,5]undeca-2,4-dien-7-one.

A PREVIOUS communication [1] reported the isolation from the metabolic products of *Penicillium gilmanii* of a compound that, when injected into animals, induced a neutrophilia without a concurrent febrile response. This compound, for which the name leucogenenol was suggested, also stimulated the production of those cells that are the precursors of the peripheral blood cells,[2] and in general stimulated the regeneration of myeloid and lymphoid tissues.[3] Leucogenenol was shown to occur in normal bovine and human liver [4] and hence could be a compound that normally plays a role in the regulation of the number and type of blood cells in the body.

Leucogenenol (I; $R^1 = R^2 = H$), $C_{18}H_{25}NO_8$, is an enol which consumes 1 equiv. of hydroxide (pK 3·8) and which reacts with diazomethane to give an enol ether



(I)

(I; $R^1 = Me$, $R^2 = H$). That this is an enol ether and not an ester is shown by the n.m.r. singlet at δ 3·8 p.p.m. (C=C–OMe) (rather than δ 3·6—3·75) [5,6] and by the negative reaction with hydroxylamine followed by iron(III) chloride. Confirmation of the absence of a carboxy-group comes from the i.r. spectra of leucogenenol and its methylation product, both of which show a maximum at 1709 cm^{-1}. A methyl ester would be expected to absorb at a higher frequency than its acid.[6-8] In fact, this maximum is due to two keto-groups. The presence of these and of four hydroxy-groups is demonstrated by the formation of a methyl ether tetra-acetate (I; $R^1 = Me$, $R^2 = OAc$), which forms a bis-2,4-dinitrophenylhydrazone. The n.m.r. spectrum of the tetra-acetate shows a singlet at δ 3·78 p.p.m. (C=C–OMe) and singlets at δ 2·05, 2·10, 2·15, and 2·20 arising from four non-equivalent acetoxy-groups. Benzoylation of the methyl ether gives a hexabenzoate whose formation must involve reaction with the four acetylatable hydroxy-groups and enolization of the two keto-groups Doublets at δ 1·22 and 1·35 p.p.m. in the n.m.r. spectrum of leucogeneneol (I; $R^1 = R^2 = H$) and at δ 1·20 and 1·30 p.p.m. in the spectrum of the methyl ether tetra-acetate (I; $R^1 = Me$, $R^2 = OAc$) indicate that leucogenenol possesses two >CH*Me* groups.[9,10] The lack of strong i.r. absorption in the ranges 1640—1659, 1550—1565, 800—666, 728—750, and 700—736 cm^{-1} indicates the absence of an amide or amino-group in leucogenenol.[6-8] This is confirmed by the absence of *N*-acetyl resonance [11] in the n.m.r. spectrum of the tetra-acetate.

Hydrolysis of leucogenenol with boiling water affords ammonia and aminoacetaldehyde, a monobasic carboxylic acid $C_8H_{12}O_5$ (II), and a dione $C_8H_{12}O_4$ (III), these products accounting for all the carbon atoms in the molecule.

The carboxylic acid (II), pK 3·7, reacts with diazomethane giving an ester (positive hydroxamic reaction) which shows signals at δ 3·75 p.p.m., in its n.m.r. spectrum and at 1745 cm^{-1} in the i.r., both characteristic of an ester group. The formation of an ester is confirmed by the mass spectrum, which in addition to the weak

[1] F. A. H. Rice, *Proc. Soc. Expt. Biol. Med.*, 1966, **123**, 189.
[2] F. A. H. Rice and J. H. Darden, *J. Infectious Diseases*, 1968, **118**, 289.
[3] F. A. H. Rice, J. Lepick, and J. H. Darden, *Radiation Res.*, 1968, **36**, 144.
[4] F. A. H. Rice and B. Shaikh, *Biochem. J.*, 1970, **116**, 709.
[5] 'Varian Associates N.M.R. Spectra Catalog,' 1963.
[6] R. M. Silverstein and G. C. Bassler, 'Spectrometric Identification of Organic Compounds,' Wiley, New York, 1967.
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[8] M. St. C. Flett, 'Characteristic Frequencies of Chemical Groups in the Infrared,' Elsevier, New York, 1962.
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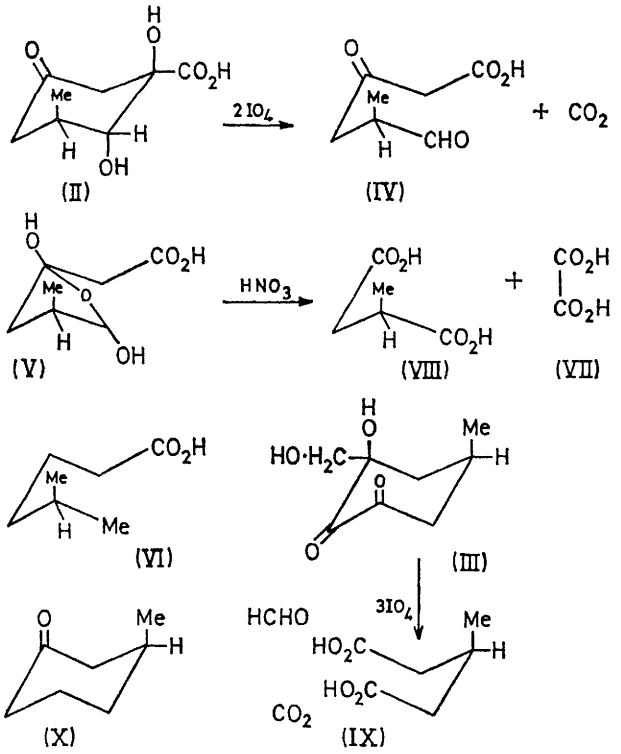
[10] F. A. Bovey, 'Nuclear Magnetic Resonance Spectroscopy,' Academic Press, New York, 1968.
[11] F. A. L. Anet, R. A. B. Bonnard, and D. Hall, *Canad. J. Chem.*, 1963, **41**, 2331.

parent ion at *m/e* 202 shows strong peaks at *M* — Ac (*m/e* 159), CO_2Me (*m/e* 59), and OMe (*m/e* 31).[6,12] A *C*-methyl group gives rise to a doublet in the n.m.r. spectrum. That the carboxylic acid possesses two hydroxy-groups is indicated by the formation of a diacetate, δ 2·08 and 2·18 (2 × OAc) and 1·30 p.p.m. (d, CMe). Treatment with boiling acetic anhydride and fused sodium acetate yields a mixture whose n.m.r. spectrum shows a singlet at δ 5·9 p.p.m. (C=CH–CO), suggesting that a β-hydroxy-ketone has been dehydrated. Assignment of structure (II) to the acid is specifically indicated by the following reactions. Treatment with periodate (uptake 2 equiv.) liberates carbon dioxide (1 equiv.) and



an acidic reducing compound $C_7H_{10}O_4$ (IV), 5-methyl-3,6-dioxohexanoic acid as a cyclic hydrate $C_7H_{12}O_5$ (V), whose further reaction with periodate is presumably prevented by the cyclization.[13,14] The mass spectrum of the methyl ester of compound (V), in addition to a weak parent ion at *m/e* 196, shows peaks characteristic of a methyl ester, and a strong peak at *m/e* 167 (*M* — CHO), suggesting the presence of an aldehyde or potential aldehyde group. A *C*-methyl signal appears in the n.m.r. spectrum of compound (V) at δ 1·2 p.p.m. (d), and in that of its methyl ester at δ 1·35 (d). The presence of two hydroxy-groups in the methyl ester is indicated by formation of a diacetate. The methyl group must be on the same carbon atom as the formyl group since treatment of compound (V) with ethanethiol followed by reduction with Raney nickel yields 5-methylhexanoic acid (VI). Oxidation of compound (V) with nitric acid yields oxalic acid (VII) and (—)-methylsuccinic acid (VIII), known to be of the L-configuration.[15] This not only confirms the position of the *C*-methyl group but establishes the configuration as L.

Because of the energy considerations, the conformer of compound (II) in which the carboxy-group is equatorial will predominate.[16] The change in the resonance of the *C*-methyl protons from δ 1·35 to 1·30 p.p.m. caused by acetylation indicates a 1,3-diaxial relationship between the *C*-methyl and the tertiary hydroxy-group.[17] The acetoxy-signal at δ 2·08 p.p.m. corresponds to that found for an axial tertiary acetoxy-group on the same carbon atom of a cyclohexane ring that carries a carboxy-group.[17] The acetoxy-signal at δ 2·18 therefore represents the group at C-2 and the chemical shift is as expected for axial acetoxy-groups.[18] The coupling of 4 Hz observed for the proton (δ 4·3 p.p.m.) on the carbon atom bearing the secondary hydroxy-group confirms the *eq–eq* relationship between it and the proton on the carbon atom bearing the methyl group.[6,9] A model shows that the dihedral angle between the hydroxy-groups is 180°.

The dione $C_8H_{12}O_4$ (III) can be titrated with hydroxide, and forms a diacetate, a dibenzoate, and a bisphenylhydrazone of the dibenzoate, indicating the presence of two hydroxy-groups and two keto-groups, one of which is enolizable. The n.m.r. spectrum of the dione shows signals for a *C*-methyl group at δ 1·20 (d), a CH_2·O group at δ 4·16, and two hydroxy-groups at δ 5·6 p.p.m.; i.r. absorption at 1707 cm^{-1} is in the normal range for a 1,2-dione.[6-8] The u.v. spectrum shows a bathochromic shift on neutralization with alkali. In agreement with the assignment of structure (III), treatment with periodate yields carbon dioxide, formaldehyde, and 3-methylglutaric acid (IX). Furthermore, treatment with ethanethiol and reduction of the product with Raney nickel yields 1-hydroxy-1-hydroxymethyl-3-methylcyclohexane, which reacts with periodate to yield the known 3-methylcyclohexanone (X).

The n.m.r. spectrum of the diacetate of the dione (III) shows resonances for an equatorial primary acetoxy-group[18] (δ 2·02) and an axial tertiary acetoxy-group[18] (δ 2·10). The change in the chemical shift of the *C*-methyl protons from δ 1·25 to 1·20, caused by acetylation indicates a 1,3-diaxial relationship between the methyl and the tertiary hydroxy-group.[17]

The singlet at δ 8·1 p.p.m. in the n.m.r. spectrum of leucogenenol suggests that an azomethine group (CH=N) is present.[6,8] This would also account for the i.r. absorption at 1634 cm^{-1} (C=N str.).[6-8] Azomethines show no absorption in the near u.v. unless conjugated.[6] The molar absorptivity (ε 9000) at *ca.* 220 nm could therefore indicate that the azomethine group is conjugated.[6,8]

[12] H. Budzikiewicz, C. Djerassi, and D. H. Williams, 'Interpretation of Mass Spectra of Organic Compounds,' Holden-Day, San Francisco, 1965.
[13] J. M. Bobbitt, *Adv. Carbohydrate Chem.*, 1956, **11**, 1.
[14] R. D. Guthrie, *Adv. Carbohydrate Chem.*, 1961, **16**, 105.
[15] S. Stallberg-Stenhagen and E. Stenhagen, *Arkiv. Kemi*, 1947, **24***B*, 6.
[16] E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, 'Conformational Analysis,' Wiley, New York, 1965, p. 436.
[17] M. R. Harnden, *J. Chem. Soc.*, 1969, 960.
[18] L. D. Hall, *Adv. Carbohydrate Chem.*, 1964, **19**, 51.

The reaction of leucogenenol with periodate in neutral solution yields formaldehyde, indicating that the 11-hydroxy- and 11-hydroxymethyl groups are available for oxidation. The chemical shifts of the protons in leucogenenol (Tables 1 and 2) are essentially the same as those of the carboxylic acid (II) and the dione (III). Therefore in the leucogenenol molecule the 1-keto-group of 3-hydroxy-3-hydroxymethyl-5-methylcyclohexane-1,2-dione (III) is not likely to be involved in a union with any other hydrolytic fragments or the chemical shifts of the protons in compound (III), particularly those of the C-6 protons, would not be the same as those of leucogenenol. However, a union involving the C-2 keto-group would not result in a marked change in chemical shifts. If this group is involved in an azomethine linkage such as CH=N–*C*, where *C* is the 2-carbon atom, the azomethine proton would resonate as observed at 8·1 p.p.m., and the C=N linkage would furnish the i.r. absorption at 1634 cm^{-1}. The formulation of a conjugated system to account for the u.v. absorption and the inclusion of an acidic enol group leads to two possible structures, (A) and (B). The formulation of an oxazine or a dihydro-oxazole ring is necessary since the two fragments $C_8H_{12}O_5$ (II) and $C_8H_{12}O_4$ (III) together account for all the hydroxy-groups in leucogenenol except that of the enol. Either of structures (A) and (B) should hydrolyse with loss of u.v. absorption (see Figure) to yield the system RC(OH):C(OH)·CHO together with ammonia and the dione (III). The group R would necessarily be of such a nature that further hydrolysis would yield glycolaldehyde and the acid (II). The n.m.r. signals of R would also need to correspond to those of the acid (II) (Tables 1 and 2). Leucogenenol could thus be formulated as (I; $R^1 = R^2 = H$). The oxazine rather than the dihydro-oxazole structure is preferred since the latter would require the presence of two adjacent hydroxy-groups that would react rapidly with periodate.

Hydrolysis of leucogenenol could be considered to occur in steps, yielding first the dione (III) and the fragment (XI). A retroaldol-type reaction *via* structure (XII) would then yield the acid (II) and aminoacetaldehyde. Loss of ammonia from (XI) or (XII) would yield glycolaldehyde.

The continued slow uptake of periodate by leucogenenol after the consumption of 1 equiv. by reaction with the 11-hydroxy- and 11-hydroxymethyl groups could result from the oxidation of the 1-hydroxy- and 2-hydroxy-groups of the methyloxocyclohexyl ring. The dihedral angle between them is 180° and they would thus react slowly with periodate.[13,14]

It is considered that the nitrogen atom rather than the oxygen atom in the disubstituted 2*H*-1,3-oxazine ring is located contiguous to the primary alcohol group because acetylation of leucogenenol causes a shift in the signal

TABLE 1

Comparison of the n.m.r. spectrum of leucogenenol with that of its hydrolysis products after removal of volatile and basic constituents (solutions in deuterium oxide with tetramethylsilane as external standard)

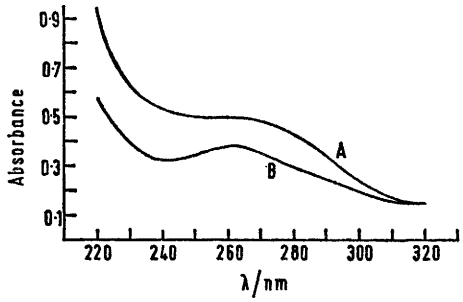
Leucogenenol	After hydrolysis
1·13 (3H, d, *J* 7)	1·13 (3H, d, *J* 7)
1·24 (3H, d, *J* 6)	1·24 (3H, d, *J* 6)
1·95 (1H, d, *J* 10)	1·95 (1H, d, *J* 10)
2·6 (3H, m)	2·6 (3H, m)
3·5 (1H, d, *J* 10)	3·5 (1H, d, *J* 10)
3·7 (2H, s)	3·7 (2H, s)
3·9 (6H, m)	3·9 (6H, m)
4·1 (1H, d, *J* 14)	4·1 (1H, d, *J* 14)
4·6 (DHO)	4·6 (DHO)
7·5 (1H, s)	

J in Hz; δ in p.p.m.

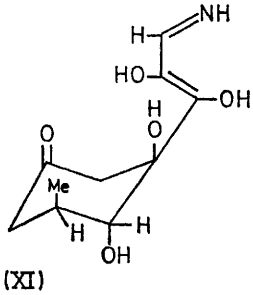
TABLE 2

Comparison of the n.m.r. spectra of the two hydrolysis products (II) and (III) with that of leucogenenol after hydrolysis and removal of volatile and basic constituents (solvent [$^2H_6$]acetone; δ values in p.p.m. from internal tetramethylsilane; *J* in Hz)

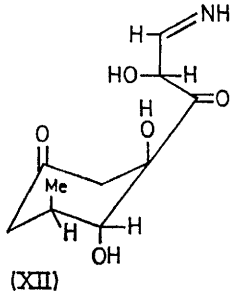
(II)	(III)	Leucogenenol after hydrolysis
	1·20 (3H, d, *J* 7)	1·20 (3H, d, *J* 7)
1·35 (3H, d, *J* 6)		1·30 (3H, d, *J* 6)
2·10 (1H, d, *J* 10)		2·10 (1H, d, *J* 10)
2·6 (2H, m)	2·6 (1H, m)	2·6 (3H, m)
	3·4—3·7 (2H, m)	3·4—3·7 (2H, m)
4·0 (1H, d, *J* 10)		4·0 (1H, d, *J* 10)
	4·10 (2H, complex s)	4·10 (2H, complex s)
4·15 (1H, d, *J* 4)		4·15 (1H, d, *J* 4)
	4·25 (2H, s)	4·25 (2H, s)
4·6 (1H, m)		4·6 (1H, m)
5·9br (3H)	5·6br (2H)	5·7br (5H)



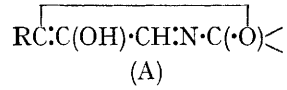
U.v. spectrum of leucogenenol in acid; A, spectrum taken immediately; B, spectrum taken after 9 days at room temperature (2·8 mg in 10 ml of 0·001N-HCl)



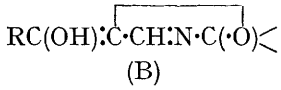
(XI)



(XII)



(A)



(B)

at δ 8·1 to 7·9 p.p.m. A model shows that the primary alcohol group should be near enough to affect the chemical shift of the azomethine proton.

Table 3 gives the suggested n.m.r. assignments for leucogenenol.

EXPERIMENTAL

M.p.s were determined with a Kofler micro hot-stage apparatus. Silicic acid (Merck) was used for preparative chromatography. Silica gel (Research Specialities Co., Richmont, California) was used for t.l.c. Zinc silicate (250 mg) was added to the silica gel (30 g) to produce a fluorescent adsorbent. Zones were made visible by u.v. illumination, by spraying where appropriate with a saturated solution of 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid and by exposing slides to iodine vapour. The P and N model 700 Laboratory Chromatograph (Hewlett–Packard) was used for g.l.c. It was equipped with a model 5771A electrometer and dual flame detectors. A coiled glass column (5 ft. × 2 mm. i.d.) was packed with 3% Ov-17 Gas-Chrom. Q(80—100 mesh). N.m.r., i.r., u.v., and mass spectra were taken with Varian A-60, Beckman IR-8, Coleman–Hitachi, and Hitachi–Perkin-Elmer RMU-66 spectrometers, respectively. Optical rotations were measured with a Perkin-Elmer 141 spectrometer (1 dm tube holding *ca.* 1 ml).

Isolation and Properties of Leucogenenol.—Leucogenenol was obtained from *Penicillium gilmanii* grown as a surface culture on Czapek-Dox medium with added trace quantities of zinc and copper ions.[19] Cultures were grown for 4—6 weeks [20] in flasks (2 l) containing 1 l of medium, sterilized in an autoclave for 30 min at 20 lb in^{-2} and inoculated with *ca.* 10^6 spores obtained from a slant culture grown on the same medium hardened with *ca.* 1% agar.

Details of the isolation of leucogenenol have been reported.[1] It was obtained as a syrup, and formed crystalline salts with sodium, potassium, and calcium. Solutions of the salts in water or isotonic saline retained their biological activity for over a year. An aqueous solution of leucogenenol itself, however, lost its biological activity in 1 week at room temperature.

Freshly isolated leucogenenol showed a single spot on t.l.c. (5% v/v methanol–benzene), R_F 0·18, and g.l.c.[21] of the trimethylsilyl derivative [22] showed a single component; $\nu_{max.}$ (film) 3279m, 3145sh, 2967s, 2907s, 2841sh, 1709s, 1634s, 1524w, 1427m, 1346m, 1252sh, 1196s, 1129m, 1075m, 1047s, and 1002w cm^{-1}, δ [$(CD_3)_2CO$] 1·22 (3H, d, *J* 7 Hz), 1·35 (3H, d, *J* 6 Hz), 2·10 (1H, d, *J* 10 Hz), 2·6 (1H, m, *J* 6 Hz), 2·62 (1H, m, *J* 7 Hz), 2·55 (1H, d, *J* 14 Hz), 3·3—3·7 (2H, m), 3·9 (1H, d, *J* 10 Hz), 4·10 (2H, complex s), 4·15 (1H, d, *J* 4 Hz), 4·25 (2H, s), 4·6 (1H, dd, *J* 2 and 14 Hz), 5·7br (5H), and 8·15 p.p.m. (1H, s), and a band at 5·5 p.p.m. that disappeared on adding D_2O. The u.v. spectrum has been reported [20]; ε_{220} 9000, ε_{266} 420; in 0·001N-HCl (pH 2·9) or water ε_{262} 980 (Found: C, 45·75; H, 7·7; N, 3·0. $C_{18}H_{25}NO_8,5H_2O$ requires C, 45·65; H, 7·45; N, 2·95%); *M* (thermal diffusion in EtOAc) 420 (Calc. 473).

Leucogenenol (9·6 mg) was titrated with aqueous 0·01N-potassium hydroxide, added in 0·1 ml portions (pH measured with a Radiometer pH meter). Neutralization (to pH 7) required 2·07 ml. (Calc. 2·03 ml.); p*K* 3·8.

Calcium Salt of Leucogenenol.—Calcium hydroxide (*ca.* 5 mg) was added to leucogenenol (*ca.* 40 mg) dissolved in methanol (5 ml). Water (1 ml) was added and the mixture was stirred until neutral to Alk-Acid test paper. Excess of base was filtered off, the filtrate was evaporated to dryness under reduced pressure, and the dry residue was crystallized from methanol–propanol or water–dioxan [Found: C, 43·1; H, 7·3; N, 2·75. $(C_{18}H_{25}NO_8,5H_2O)_2Ca$ requires C, 43·8; H, 7·2; N, 2·85%], $\nu_{max.}$ (KBr) 3390s, 2970m, 2907m, 2874m, 1709s, 1600s, 1418sh, 1379s, 1282m,

TABLE 3

Suggested ^{1}H n.m.r. assignments for leucogenenol, the methyl enol ether of leucogenenol, and the methyl enol ether of leucogenenol tetra-acetate

	Leucogenenol [a]	Methyl enol ether of leucogenenol [b]	Methyl enol ether of leucogenenol tetra-acetate [b]
Protons of 3,11-hydroxy-11-(hydroxymethyl)-9-methyl-1-oxa-5-azaspiro[5,5]undeca-2,4-dien-7-one system			
C(3)OCH_3		3·80 (3H, s)	3·78 (3H, s)
C(4)H	8·15 (1H, s)	8·15 (1H, s)	7·9br (1H)
C(8)H_2	4·10 (2H, complex s)	4·10 (2H, complex s)	3·8 (2H, complex s)
C(9)CH_3	1·22 (3H, d)	1·22 (3H, d)	1·20 (3H, d)
C(9)H	2·62 (1H, m)	2·6 (1H, m)	2·6 (1H, m)
C(10)H_2	3·4—3·6 (2H, m)	3·4—3·6 (2H, m)	3·4—3·6 (2H, m)
C(11)OAc			2·15 (3H, s)
C(11)CH_2·O	4·25 (2H, s)	4·25 (2H, s)	4·30 (2H, s)
C(11)CH_2·O*Ac*			2·05 (3H, s)
Protons of 1,2-dihydroxy-3-methyl-5-oxocyclohexane system			
C(1)O*Ac*			2·10 (3H, s)
C(2)CHO	4·15 (1H, d)	4·15 (1H, d)	4·90 (1H, d)
C(2)CH·O*Ac*			2·20 (3H, s)
C(3)CH_3	1·35 (3H, d)	1·35 (3H, d)	1·30 (3H, d)
C(3)CH	2·6 (1H, m)	2·6 (1H, m)	2·6 (1H, m)
C(4)CH*eq*	4·6 (1H, m)	4·6 (1H, m)	4·6 (1H, m)
C(4)CH*ax*	2·55 (1H, m)	2·55 (1H, m)	2·6 (1H, m)
C(6)CH*eq*	3·9 (1H, d)	4·0 (1H, d)	3·9 (1H, d)
C(6)CH*ax*	2·10 (1H, d)	2·10 (1H, d)	2·10 (1H, d)

[a] In $[^2H_6]$acetone. [b] In $[^2H]$chloroform. *J* in Hz.

[19] G. Smith, *Trans. Brit. Mycol. Soc.*, 1949, **32**, 280.
[20] F. A. H. Rice and M. Barrow, *Appl. Microbiol.*, 1967, **15**, 790.
[21] S. Dal Nogare and R. S. Juvet, jun., 'Gas–liquid chromatography,' 3rd edn., Interscience, New York, 1965.
[22] C. C. Sweeley, R. Bently, M. Makita, and V. V. Wells, *J. Amer. Chem. Soc.*, 1963, **85**, 2497.

1258m, 1121m, 1081m, and 1010m cm^{-1}, δ (D_2O) 1·13 (3H, d, *J* 7 Hz), 1·24 (3H, d, *J* 6 Hz), 1·95 (1H, d, *J* 10 Hz), 2·6 (3H, m), 3·5 (1H, d, *J* 10 Hz), 3·7 (2H, s), 3·9 (6H, m), 4·1 (1H, d, *J* 14 Hz), and 7·5 p.p.m. (1H, s); the u.v. spectra of solutions in ethanol and 0·001N-HCl were indistinguishable from those of free leucogenenol.

Hydrogenation of Leucogenenol.—Leucogenenol (6—12 mg) in absolute ethanol (10 ml) was hydrogenated in a micro-hydrogenation apparatus [23] at room temperature and atmospheric pressure over Adams catalyst (3—6 mg). Six determinations gave an average uptake of 0·10 ∓ 0·01 ml at S.T.P. per mg of leucogenenol (Calc. for two double bonds: 0·092 ml).

Monomethyl Enol Ether of Leucogenenol.—Leucogenenol (15 mg) in anhydrous diethyl ether (5 ml) was treated with a freshly distilled solution of diazomethane prepared from nitrosomethylurea [24] until a permanent colour was obtained and the evolution of gas had ceased. The solution was filtered and concentrated under reduced pressure to yield a syrup that was dissolved in methylene chloride (1 ml) and chromatographed on a silicic acid column (1 × 10 cm). Elution with methanol–benzene (5% v/v) gave the *ether* (14 mg), which was homogeneous on t.l.c. (five systems) (Found: C, 57·4; H, 6·9; N, 3·45. $C_{19}H_{27}NO_8$ requires C, 57·4; H, 6·85; N, 3·5%); u.v. spectrum similar to that of leucogenenol, with increasing absorption below 240 nm, ε_{270} 700, $[\alpha]_{589}^{20}$ −1° (*c* 0·7 in MeOH), $\nu_{max.}$ 3367s, 2976m, 2941m, 2957m, 2841sh, 1709s, 1639w, 1420m, 1366m, 1252s, 1193s, 1131s, 1070s, and 1000m cm^{-1}, δ ($CDCl_3$) 1·22 (3H, d, *J* 7 Hz), 1·35 (3H, d, *J* 6 Hz), 2·10 (1H, d, *J* 10 Hz), 2·6 (3H, m), 3·4—3·6 (2H, m), 3·80 (3H, s), 4·0 (1H, d, *J* 10 Hz), 4·10 (2H, s), 4·15 (1H, d, *J* 4 Hz), 4·25 (2H, s), 4·6 (1H, dd, *J* 2 and 14 Hz), 4·8br (5H, exchangeable), and 8·15 p.p.m. (1H, s).

The compound gave a negative test for an ester when treated with hydroxylamine followed by iron(III) chloride.[25]

Methyl Enol Ether Hexabenzoate of Leucogenenol.—The methyl enol ether (*ca.* 10 mg) dissolved in pyridine (0·5 ml) was cooled in ice–water. Freshly distilled benzoyl chloride (50 mg) was added and after 1 h at 0° the solution was set aside at room temperature overnight. It was then again cooled to 0° and water (1 drop) was added. After 30 min at 0° the solution was left at room temperature for 1 h and was then added to benzene (10 ml). The solution was extracted with water (1 ml × 3) and the organic layer was shaken with saturated aqueous sodium hydrogen carbonate (3 ml × 3), dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was dried overnight at room temperature and 0·05 mmHg. The resulting syrup was extracted with n-hexane (5 ml × 3); the solution was filtered and evaporated to *ca.* 2 ml. The crystals formed yielded the *hexabenzoate* (27 mg.), m.p. 109° (from n-hexane) (Found: C, 71·6; H, 5·05; N, 1·25. $C_{61}H_{51}NO_{14}$ requires C, 71·7; H, 5·05; N, 1·35%), δ ($CDCl_3$) 1·40 (3H, d, *J* 7 Hz), 1·60 (3H, d, *J* 6 Hz), 2·10 (1H, d, *J* 10 Hz), 2·55 (3H, m), 3·70 (2H, m), 3·8 (3H, s), 3·9 (1H, d, *J* 10 Hz), 4·8 (2H, s), 5·6 (1H, d, *J* 4 Hz), 5·9 (1H, d, *J* 4 Hz), 7·6 (21H, m), and 8·2 p.p.m. (10H, m), $\nu_{max.}$ 3100m, 2967m, 2907m, 2841m, 1681s, 1439m, 1361m, 1307m, 1276m, 1198m, 1163w, 1105w, 1063w, 1015w, 926w, 704s, and 654m cm^{-1}.

Tetra-acetate of the Methyl Enol Ether of Leucogenenol.—The freshly chromatographed methyl enol ether (*ca.* 10 mg) dissolved in pyridine (0·1 ml) was cooled in ice–water, and acetic anhydride (0·1 ml) was added. After 1 h in the ice–water bath the solution was set aside at room temperature for 2 days. It was then cooled again in ice–water and a few drops of water were added. After 1 h the solution was diluted with methylene chloride (20 ml) and shaken with water (2 ml × 3), then with saturated aqueous sodium hydrogen carbonate (1 ml × 3). The methylene chloride solution was then dried (Na_2SO_4), filtered, and evaporated at 50° (bath temperature). The resulting syrup in benzene (2 ml) was chromatographed on a column (1 × 10 cm) of silicic acid. Elution with 2% (v/v) ethanol–benzene (*ca.* 45 ml) gave the acetate, which was homogeneous on t.l.c. (five systems; detection with iodine vapour) (Found: C, 57·25; H, 6·20; N, 2·5. $C_{27}H_{35}NO_{12}$ requires C, 57·35; H, 6·25; N, 2·45%), $\nu_{max.}$ 2976m, 2941s, 2857m, 1727s, 1695sh, 1667sh, 1626sh, 1429m, 1316s, 1266sh, 1220s, and 1058m cm^{-1}, δ ($CDCl_3$) 1·20 (3H, d, *J* 7 Hz), 1·30 (3H, d, *J* 6 Hz), 2·10 (1H, d, *J* 10 Hz), 2·05 (3H, s), 2·10 (3H, s), 2·14 (3H, s), 2·20 (3H, s), 2·6 (2H, m; 1H with *J* 7, and 1H with *J* 6 Hz), 2·62 (1H, d, *J* 14 Hz), 3·4—3·6 (2H, m), 3·78 (3H, s), 3·80 (2H, complex d), 3·9 (1H, d, *J* 10 Hz), 4·30 (2H, s), 4·6 (1H, dd, *J* 2 and 14 Hz), 4·9 (1H, d, *J* 4 Hz), and 7·9br p.p.m. (1H), ε_{265} 2500 (EtOH) with increasing absorption below 240 nm, $[\alpha]_{589}^{20}$ 0·4° (*c* 2 in MeOH).

Bis-2,4-dinitrophenylhydrazone of the Methyl Enol Ether of Leucogenenol Tetra-acetate.—To the methyl enol ether tetra-acetate (*ca.* 23 mg) in acetic acid (0·1 ml) was added 2,4-dinitrophenylhydrazine (30 mg) in acetic acid (1 ml) and water (1 ml). The mixture was heated on a boiling-water bath for *ca.* 20 min then cooled in ice–water. The crystalline precipitate had m.p. 198° (decomp.) (from chloroform–hexane) (Found: C, 50·4; H, 4·5; N, 13·5. $C_{39}H_{43}N_9O_{18}$ requires C, 50·6; H, 4·7; N, 13·6%).

Hydrolysis of Leucogenenol.—(*a*) Leucogenenol (2·8 mg) was dissolved in 0·001N-hydrochloric acid (10 ml). The u.v. spectrum (Figure) was taken immediately and then at daily intervals for 9 days. After 5 days no further changes were observed and the solution was biologically inactive. The same results were obtained when leucogenenol (3·8 mg) in water (2 ml) was heated overnight on a boiling-water bath. Neutral solutions of leucogenenol showed no change in their absorption spectrum or biological activity when set aside at room temperature for a year.

(*b*) Leucogenenol (20 mg) was dissolved in water (1 ml) and heated on a boiling-water bath for 24 h. The solution was then lyophilized and the residue in methanol (10 ml) was passed through a column (1 × 5 cm) of Amberlyst-15 (H^+) (Rohn and Haas Co., Philadelphia). The eluate was evaporated to dryness under reduced pressure. T.l.c. showed two components (EtOAc). The n.m.r. spectrum was compared with that of leucogenenol (Table 1).

(*c*) Leucogenenol (65·9 mg) dissolved in water (5 ml) was heated at boiling water temperature for 24 h; the solution was then lyophilized. The distillate was collected in a flask surrounded by solid carbon dioxide. The residue, a light yellow syrup, was acidic and gave a positive test for ammonia with Nessler's reagent.[25] It was dissolved in methanol (10 ml) and passed through a column (2 × 35 cm) of Amberlyst-15 (H^+). The column was washed with methanol (50 ml) and the methanolic solutions were combined and evaporated to dryness under reduced pressure. The resulting syrup (*ca.* 60 mg) dissolved in ethyl acetate (10 ml) was filtered through silicic acid (10 g), which was then washed

[23] C. L. Ogg and F. J. Cooper, *Analyt. Chem.*, 1949, **21**, 1400.
[24] E. A. Warner, *J. Chem. Soc.*, 1919, **115**, 1098.
[25] F. Feigl, 'Qualitative Analysis by Spot Tests,' Elsevier, New York, 3rd English edn., 1947.

with ethyl acetate (80 ml). The ethyl acetate solution was evaporated to yield fraction (1) (*ca.* 30 mg). The silicic acid was eluted with 1 : 1 (v/v) methanol–benzene (*ca.* 100 ml.) to yield fraction (2) (*ca.* 30 mg). The Amberlyst-15 column was then eluted with 2N-hydrochloric acid (20 ml) followed by distilled water (100 ml) to yield a semi-crystalline mass, fraction (3) (*ca.* 6 mg).

Identification of glycolaldehyde. The distillate from the hydrolysis was treated with a saturated aqueous solution of 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid (5 ml) and set aside at room temperature for a week. The precipitate, crystallized from nitrobenzene–xylene, had m.p. 236° and was identical with the bis-2,4-dinitrophenylhydrazone of glyoxal (mixed m.p., i.r. and u.v. spectra) (Found: C, 40·2; H, 2·4; N, 26·7. Calc. for $C_{14}H_{10}N_8O_8$: C, 40·2; H, 2·4; N, 26·8%).

In a second experiment a portion of the distillate (2 ml) from the hydrolysis of leucogenenol (65 mg) in water (25 ml) was tested for glycolaldehyde.[26] The test was positive. A portion of the distillate (23 ml) was heated at reflux with an excess (22 mg) of mercury(II) oxide for 4 h and then treated with hydrogen sulphide. The mixture was filtered and evaporated to dryness to yield glycolic acid, m.p. 77° (lit.,[27] 78°), identified by i.r. spectrum and mixed m.p.

Identification of ammonia and aminoacetaldehyde. The eluate of the Amberlyst-15 column [fraction (3)] was evaporated to dryness and the residue was dissolved in 0·1N-sodium hydroxide (1 ml) and heated under reflux for 3 h. Volatile products were swept with nitrogen into 0·01N-hydrochloric acid (10 ml). Titration of the acid solution caused liberation of ammonia (*ca.* 10% of theory). Treatment of the non-volatile portion with a saturated solution of sodium periodate (5 ml) liberated more ammonia (*ca.* 85% of theory) and treatment of the volatile portion with a saturated solution of 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid yielded the 2,4-dinitrophenylhydrazone of formaldehyde, m.p. 167° (from methanol) (Found: C, 40·2; H, 2·35; N, 26·8. Calc. for $C_7H_5N_4O_4$: C, 40·2; H, 2·4; N, 26·8%), identified by mixed m.p. and i.r. spectrum.

1,2-Dihydroxy-3-methyl-5-oxocyclohexanecarboxylic acid (II) [*fraction* (1)]. Fraction (1) was a light golden syrup. T.l.c. showed only one spot (five systems; detection by u.v. and iodine vapour) and g.l.c. of the trimethylsilyl derivative [22] showed a single component with a retention time of 3·94 min (diethyl ether standard; conditions as for leucogenenol) (Found: C, 51·2; H, 6·4. $C_8H_{12}O_5$ requires C, 51·05; H, 6·45%), $[\alpha]_{589}^{20}$ −2·1° (*c* 0·9 in MeOH), $\lambda_{max.}$ 265 nm (ε 95) and terminal absorption below 220 nm, $\nu_{max.}$ 3390—3333m, 2941m, 2934m, 2937m, 1712s, 1613w, 1429m, 1479w, 1370w, 1282m, 1205m, 1176sh, 1136s, 1064m, 1000m, and 975m cm^{-1}, δ [$(CD_3)_2CO$ or $CDCl_3$] 1·35 (3H, d, *J* 6 Hz), 2·10 (1H, d, *J* 10 Hz), 2·55—2·6 (2H, 1H, m, *J* 6 and 14 Hz), 4·0 (1H, d, *J* 10 Hz), 4·15 (1H, d, *J* 4 Hz), 4·6 (1H, dd, *J* 2 and 14 Hz), and 5·9br (3H, exchangeable).

The *acid* (II) (18·0 mg) required 10·08 ml of 0·01N-potassium hydroxide to neutralize it (calculated for $C_8H_{12}O_5$: 9·57 ml); p*K* 3·7.

3-Hydroxy-3-hydroxymethyl-5-methylcyclohexane-1,2-dione (III) [*fraction* (2)]. Fraction (2) was a light golden syrup. T.l.c. showed a single component (five systems; detection by u.v. and iodine vapour) and g.l.c. of the trimethylsilyl derivative [22] showed a single component with a retention time of 2·8 min (ether standard; conditions as for leucogenenol) (Found: C, 55·4; H, 7·35. $C_8H_{12}O_4$ requires C, 55·8; H, 7·05%), $\nu_{max.}$ 3390—3333m, 2941m, 2934s, 2927m, 1707s, 1418m, 1361m, 1258m, 1205m, 1130m, 1068m, and 1000w cm^{-1}, δ [$(CD_3)_2CO$] 1·20 (3H, d, *J* 7 Hz), 2·60 (1H, m, *J* 7 Hz), 3·4—3·7 (2H, m), 4·10 (2H, complex s), 4·25 (2H, s), and 5·60br p.p.m. (2H, exchangeable). $\lambda_{max.}$ (EtOH) 265 nm (ε 655) and increasing absorption below 220 nm, $\lambda_{max.}$ (0·001N-HCl) 262 nm (ε 815), $\lambda_{max.}$ (0·001N-NaOH) 270 nm (ε 3000).

An aqueous solution of the *dione* (III) was acidic. Titration of 8·9 mg with 0·01N-sodium hydroxide required 5·03 ml (Calc. 5·17 ml).

Monomethyl Ester of the Acid (II).—The acid (15 mg) dissolved in ether (2 ml) was treated with a freshly distilled ethereal solution of diazomethane.[24] The solution was then filtered and evaporated to dryness to yield a *syrup* that showed a single spot on t.l.c. The mass spectrum showed a weak parent ion at *m/e* 202 and strong peaks at *M* − Ac (*m/e* 159), CO_2Me (*m/e* 59), and OMe (*m/e* 31), indicative of a methyl ester (Found: C, 53·35; H, 6·85. $C_9H_{14}O_5$ requires C, 53·45; H, 6·95%), $\lambda_{max.}$ 276 nm (ε 859) with increasing absorption below 220 nm, $\nu_{max.}$ 3333m, 2941m, 1745s, 1709s, 1650m, 1428m, 1258sh, 1205s, 1134s, 1062m, and 1000m cm^{-1}, δ ($CDCl_3$) 1·35 (3H, d, *J* 6 Hz), 2·03 (1H, d, *J* 10 Hz), 2·6 (1H, m, *J* 6 Hz, and 1H uncertain), 3·73 (1H, d, *J* 10 Hz), 3·75 (3H, s), 4·2 (1H, m), 4·15 (1H, d, *J* 4 Hz), and 5·6br p.p.m. (2H, exchangeable).

Tribenzoate of Methyl Ester of the Acid (II).—The methyl ester (20 mg) dissolved in freshly distilled pyridine (0·5 ml) was cooled in ice–water and freshly distilled benzoyl chloride (0·2 ml) was added. After 1 h it was set aside at room temperature overnight, then neutralized with sodium hydrogen carbonate and extracted with methylene chloride. The extract was dried (Na_2SO_4), evaporated, and distilled at 0·05 mmHg (bath temperature 160°). The *distillate* crystallized (30 mg); m.p. 205° (from n-hexane) (Found: C, 69·95; H, 5·2. $C_{30}H_{26}O_8$ requires C, 70·0; H, 5·1%), δ ($CDCl_3$) 1·55 (3H, d, *J* 6 Hz), 2·03 (1H, d, *J* 11 Hz) 2·6, 1H, m, *J* 6 Hz), 3·75 (3H, s), 4·0 (1H, d), 4·5 (1H, d, *J* 4 Hz), 5·5 (1H, d, *J* 3 Hz), 7·5 (10H, m), and 8·1 p.p.m. (5H, m).

Diacetate of Methyl Ester of the Acid (II).—The methyl ester (10 mg) in pyridine (0·1 ml) was cooled in ice–water and acetic anhydride (0·05 ml) was added. The solution was set aside overnight, then poured into ice–water (5 ml), neutralized with sodium hydrogen carbonate, and extracted with methylene chloride. The extract was dried (Na_2SO_4), evaporated to dryness, and distilled at 65—70° (bath temperature; 0·05 mmHg). T.l.c. showed a single spot (five systems; detection by u.v. and iodine vapour) (Found: C, 54·45; H, 6·25. $C_{13}H_{18}O_7$ requires C, 54·55; H, 6·35%), δ ($CDCl_3$) 1·30 (3H, d, *J* 6 Hz), 2·08 (3H, s), 2·10 (1H, d, *J* 10 Hz), 2·18 (3H, s), 2·60 (1H, m, *J* 6 Hz, and 1H uncertain), 3·75 (3H, s), and 3·90 (1H, d, *J* 10 Hz), 4·6 (1H, dd, *J* 2 and 14 Hz), and 5·15 p.p.m. (1H, d, *J* 4 Hz).

Treatment of the methyl ester (10 mg) with boiling acetic anhydride saturated with anhydrous sodium acetate (1 ml) yielded a mixture (t.l.c.) that showed resonance at δ 5·9 p.p.m. The i.r. spectrum of the distillate collected at 65—70° (*ca.* 1 mg) was the same as the spectrum of the diacetate. The mixture was not further investigated.

Periodate Oxidation of the Acid (II).—To the acid (19·1 mg) in water (10 ml) was added 0·3N-sodium periodate (5 ml). The solution was set aside at room temperature with nitrogen sweeping any carbon dioxide formed into aqueous barium hydroxide (0·2N; 15 ml). At intervals of 6, 12, and

[26] Z. Dische and E. Borenfreund, *J. Biol. Chem.*, 1949, **180**, 1297.

24 h a sample (1 ml) of the solution was estimated by adding excess of aqueous sodium arsenite (0·071N; 10 ml) and saturated aqueous sodium hydrogen carbonate (2 ml) and titrating the excess of sodium arsenite with iodine solution (0·0135N) to a starch end-point. Carbon dioxide evolution was determined by titrating the barium hydroxide solution with 0·1N-hydrochloric acid (phenolphthalein). The sample consumed 2 equiv. (Found: 30·02 ml of 0·0135N-I_2. Calc.: 30·0 ml). One equiv. of carbon dioxide was liberated (Found: 2·10 ml of 0·1N-HCl. Calc.: 2·03 ml). A portion of the periodate solution (2 ml.) was titrated with 0·1N-sodium hydroxide to pH 7 (pH meter) and the result was subtracted from that obtained with a blank containing no sample (Found: 1·00 ml of 0·1N-NaOH. 1 Equiv. of acid requires 1·02 ml).

A portion of the neutralized periodate solution (2 ml) was evaporated to dryness at room temperature under reduced pressure, and the distillate was collected in a trap surrounded by crushed solid carbon dioxide. The distillate gave no reaction with a saturated solution of 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid. The neutral residue from the distillation was dissolved in water (10 ml), acidified with toluene-*p*-sulphonic acid (pH 2), and evaporated to dryness. The distillate was neutral.

2,3,4,5-*Tetrahydro*-2,5-*dihydroxy*-4-*methylfuran*-2-*acetic Acid* (V) *from the Periodate Oxidation of the Acid* (II).—The acid (39·5 mg) was dissolved in 0·3N-sodium periodate (5 ml). After 10 h at room temperature the solution was acidified to pH 2 with 2N-hydrochloric acid and extracted with methylene chloride (5 ml × 4). The extract was dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure to yield a syrup (*ca.* 23 mg). T.l.c. showed a single component. G.l.c. showed a single peak with a retention time of 4 min. (standard ether).

Titration of a sample (12·2 mg) with 0·01N-sodium hydroxide showed the presence of one acidic group. The sample was recovered by adding of 0·1N-hydrochloric acid (1 ml) and extracting with methylene chloride; ε_{260} 1400.

The mass spectrum did not show a parent ion but did show strong peaks at *m/e* 147 (*M* — CHO) and 131 (*M* — CO_2H). Treatment with diazomethane formed the methyl ester, that showed a parent ion at *m/e* 190 and strong peaks at *m/e* 161 (*M* — CHO), 159 (*M* — OMe), 131 (*M* — CO_2Me), 59 (OMe), 58 (MeCH=CH·OH), and 29 (HCO); δ ($CDCl_3$) 1·2 (3H, d, *J* 6 Hz), 2·4 (1H, m, *J* 6 Hz), 3·8 (2H, s), 4·2br (2H), 5·3 (1H, s), and 5·9br p.p.m. (2H, exchangeable).

Diacetate of the Methyl Ester of the Acid (V).—The methyl ester (10 mg), prepared by treating the acid with diazomethane in ether, was dissolved in pyridine (0·5 ml) and acetylated with acetyl chloride (0·3 ml). The product showed δ ($CDCl_3$) 1·15 (3H, d, *J* 6 Hz, CMe) and 2·15 p.p.m. (6H, s, 2 × OAc).

Reaction of the Acid (V) *with Nitric Acid*.—The acid (10 mg), dissolved in concentrated nitric acid (0·2 ml), was heated on a steam-bath for 3 h. The solution was then evaporated to dryness on a water-bath under reduced pressure. Ethanol (0·1 ml) was added and the solution was again evaporated to dryness. Water (0·2 ml) was added and the aqueous solution was extracted with ether (1 ml × 5). The aqueous phase was evaporated to dryness under reduced pressure on a boiling-water bath and then sublimed (0·02 mmHg). The product had m.p. 190° (hydrate 99°), as reported for oxalic acid and its dihydrate (lit.,[27] 189·5 and 100°). The compound gave a positive diphenylamine test,[25] and the i.r. spectrum of the dihydrate was identical with the spectrum of an authentic sample.

The ethereal extract of the aqueous solution was evaporated to dryness at room temperature and the residue was recrystallized from benzene; yield *ca.* 6 mg, m.p. 112°. (+)-Methylsuccinic acid [28] melts at 114—115°. The optical rotation, $[\alpha]_{589}^{25}$ −9° (*c* 0·5 in H_2O), was equal but opposite to that of (+)-methylsuccinic acid.[28] The mass spectrum, identical to that of methylsuccinic acid, showed peaks at *m/e* 114 (*M* — H_2O), 87 (*M* — CO_2H), 86, 73, 69, 60, 58, 55, 44, 43, 41, 39, 28, and 18.

The methyl ester, obtained by treatment with diazomethane, had the reported rotation,[29] $[\alpha]_{589}^{25}$ −2° (*c* 0·5 in MeOH), and the mass spectrum showed a parent ion at *m/e* 160 and strong peaks at 129 (*M* — Me) and 70 (*M* — CO_2Me).

Reduction of the Acid (V) *to* 5-*Methylhexanoic Acid*.—Anhydrous hydrogen chloride was bubbled for 1 min through the acid (V) (*ca.* 15 mg) dissolved in ethanethiol (1 ml) at −10°, and the solution was set aside at room temperature overnight. Evaporation of the ethanethiol and hydrogen chloride under reduced pressure, and reduction with Raney nickel yielded 5-methylhexanoic acid, whose methyl ester, formed by treatment with diazomethane, showed a parent ion at *m/e* 144 and prominent peaks at 115, 101, 87, 85 (*M* — CO_2Me), 73, 71, 59 (CO_2Me), 57, 43, and 31 (OMe); δ ($CDCl_3$) 0·9 (6H, complex d), 1·26 (7H, s), 3·75 (1H, s), and 5·1 p.p.m. (1H, s). The amide, made by treatment with liquid ammonia, had m.p. 102° (from ethanol) (lit.,[30] 102°).

Diacetate of the Dione (III).—Acetyl chloride (0·2 ml) was added to the dione (*ca.* 10 mg) dissolved in pyridine (0·2 ml) and cooled in ice–water. The solution was set aside at room temperature for 6 h, then poured into water (1 ml) and extracted with methylene chloride. The extract was dried (Na_2SO_4), evaporated to dryness, and chromatographed on silicic acid with 1 : 1 (v/v) ethanol–benzene to yield a syrup (*ca.* 15 mg) that showed a single spot on t.l.c. The mass spectrum showed a weak parent ion at *m/e* 256 with peaks characteristic of acetates [12] at 61 [$MeC(OH)_2$], 43 (MeCO), and 15 (Me); δ ($CDCl_3$) 1·20 (3H, d, *J* 7 Hz), 2·02 (3H, s), 2·10 (3H, s), 2·6 (1H, m, *J* 7 Hz), 3·9 (2H, m), 4·0 (2H, s), and 4·2 p.p.m. (2H, s).

Dibenzoate of the Dione (III).—Benzoyl chloride (0·1 ml) was added to the dione (10 mg) in pyridine (0·2 ml) at 0° and the solution was set aside at room temperature for 6 h. After neutralization with saturated sodium hydrogen carbonate the mixture was extracted with methylene chloride (5 ml × 5); the extract was washed with water, dried (Na_2SO_4), and evaporated to dryness. The *residue* was sublimed at 100° (0·01 mmHg); m.p. 67° (Found: C, 69·35; H, 5·2. $C_{22}H_{20}O_6$ requires C, 69·45; H, 5·3%). The *bisphenylhydrazone* [from reaction with phenylhydrazine in 50% aqueous acetic acid (v/v)] had m.p. 164° (from hexane–benzene) (Found: C, 72·5; H, 6·1; N, 10·2. $C_{34}H_{32}N_4O_4$ requires C, 72·85; H, 5·75; N, 10·0%), $\lambda_{max.}$ (EtOH) 239 (ε 6·5 × 10^4) and 274 nm (ε 4·4 × 10^4).

Reaction of the Dione (III) *with Periodate*.—The dione (13·4 mg) in water (1 ml) was treated with 0·3N-sodium periodate (5 ml); carbon dioxide evolution and periodate consumption were determined as before. The sample

[27] E. H. Huntress and S. P. Mulliken, 'Identification of Pure Organic Compounds,' Wiley, New York, 1946.
[28] V. H. T. James, *J. Chem. Soc.*, 1955, 637.
[29] E. Berner and R. Leonardsen, *Annalen*, 1939, **538**, 1.
[30] F. Sorm and J. Arient, *Coll. Czech. Chem. Comm.*, 1950, **15**, 160 (*Chem. Abs.*, 1951, **45**, 8481).

consumed 3 equiv. of periodate and liberated 1 equiv. of carbon dioxide.

Formaldehyde and 3-Methylglutaric Acid from the Action of Periodate on the Dione (III).—Sodium periodate (5 ml; 0·3N) was added to the dione (40·2 mg) in water (1 ml). After 24 h the solution was neutralized (pH 7) with 0·1N-sodium hydroxide and lyophilized. The distillate, collected at solid carbon dioxide temperature, on treatment with a saturated solution of 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid, yielded (identified by i.r. and mixed m.p.) the 2,4-dinitrophenylhydrazone of formaldehyde (*ca.* 5 mg), m.p. 167° (Found: C, 40·1; H, 2·35; N, 26·7. Calc. for $C_7H_5N_4O_4$: C, 40·2; H, 2·4; N, 26·8%).

The residue from the evaporation of the periodate solution, in water (2 ml), was adjusted to pH 2 with 2N-hydrochloric acid, and extracted with diethyl ether (5 ml × 5). The extract was dried (Na_2SO_4) and evaporated to dryness, and the residue crystallized from 10% hydrochloric acid to yield 3-methylglutaric acid, m.p. 84° (lit.,[31] 85°) (Found: C, 49·2; H, 6·8. Calc. for $C_6H_{10}O_4$: C, 49·3; H, 6·9%), identical (mass, n.m.r., and i.r. spectra) with an authentic sample.

Reduction of the Dione (III).—The dione (*ca.* 10 mg) in ethanethiol (1 ml) was saturated with dry hydrogen chloride at −10° and set aside at room temperature for 3 days. The solution was then evaporated to dryness under reduced pressure. The residue was dissolved in absolute ethanol (5 ml) and reduced with Raney nickel (100 mg) at reflux temperature. Filtration and evaporation yielded a syrup, which was treated with saturated aqueous sodium periodate (0·5 ml). After 8 h at room temperature the solution was extracted with ether (1 ml × 5). The aqueous phase was neutralized (0·1N-NaOH) to pH 7 and evaporated to dryness under reduced pressure, and the distillate, which was collected at solid carbon dioxide temperature, was treated with 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid to yield the 2,4-dinitrophenylhydrazone of formaldehyde (i.r. and mixed m.p.).

The ether phase was evaporated to dryness at room temperature and distilled at 168°. The mass spectrum of the product was the same as that of authentic 3-methylcyclohexanone and the 2,4-dinitrophenylhydrazone had m.p. 154° (lit.,[27] 154°) (identified by i.r. spectrum and mixed m.p.).

Reaction of Leucogenenol with Neutral Sodium Periodate.—The calcium salt of leucogenenol (18·1 mg) was dissolved in saturated aqueous sodium periodate at pH 7 (5 ml) and set aside overnight. The solution remained neutral and no carbon dioxide was evolved. After 12 h the periodate consumption amounted to 1 equiv.

The periodate solution was evaporated to dryness under reduced pressure. The distillate, collected at solid carbon dioxide temperature, gave the 2,4-dinitrophenylhydrazone of formaldehyde (i.r. spectrum, mixed m.p.) (Found: C, 40·2; H, 2·4; N, 26·1. Calc. for $C_7H_5N_4O_4$: C, 40·2; H, 2·4; N, 26·7%).

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