New Metabolic Products of *Verticillium lecanii*. Part 1. 3β-Hydroxy-4,4,14α-trimethyl-5α-pregna-7,9(11)-diene-20*S*-carboxylic Acid and the Isolation and Characterisation of some Minor Metabolites

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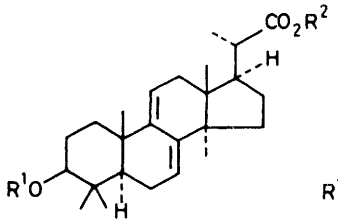
The isolation is described of two insecticidal hydroxycarboxylic acids, $C_{25}H_{38}O_3$ and $C_{25}H_{38}O_4$, from entomopathogenic strains of the fungus *Verticillium lecanii* grown on Czapek Dox medium. Minor products from these fermentations include a number of related C_{25} compounds and 3-methyl-lumichrome. The $C_{25}H_{38}O_3$ acid is shown by *X*-ray crystallography to be 3β-hydroxy-4,4,14α-trimethyl-5α-pregna-7,9(11)-diene-20*S*-carboxylic acid.

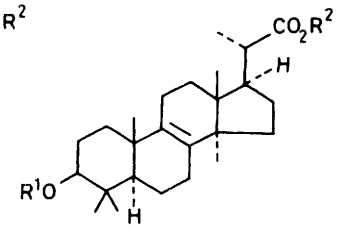
Although the principal insecticidal metabolic product of the entomopathogenic fungus *Verticillium lecanii* (Zimm.) was shown [1] to be pyridine-2,6-dicarboxylic acid, the 'neutral' fractions from two strains (UICP 64 and 65) of this organism, grown on Czapek Dox medium, contained,[1] in yields of 5—10 mg/l, a mixture of insecticidal compounds, the major components of which had the composition $C_{25}H_{38}O_3$ and $C_{25}H_{38}O_4$. This paper describes the isolation and characterisation of these products and some related minor metabolites. The major products were insoluble in most organic solvents and n.m.r. spectra and other physical data were obtained for suitable derivatives. Although the 'neutral' fractions were obtained, initially, by solvent extraction of the culture filtrate at pH 7—8,[1] both major components of the insecticidal mixture were shown to be carboxylic acids. Thereafter these compounds were obtained by extraction of the culture filtrate at pH 5: the overall yield was not increased by re-extraction at pH 3. After some months the yield steadily declined, eventually reaching the unacceptable level of <1 mg/l, and lack of material thus restricted, and ultimately prevented, further chemical work. The structure of the $C_{25}H_{38}O_3$ compound was then determined by *X*-ray crystallography of the monoacetate. This revealed, instead of the hoped-for novel sesterterpene skeleton, the pentanortriterpene structure (1; R^1 = Ac, R^2 = H).

Initially,[1] the mixture of C_{25} compounds was obtained by trituration of the crude 'neutral' extract with methanol; subsequently, individual components were separated by column chromatography on silica and purified further by t.l.c. The major components, and a minor product (*ca.* 0.5 mg/l), a keto acid ($\nu_{max.}$ 3 200br, 1 720, and 1 700 cm^{-1}) of composition $C_{25}H_{36}O_3$, showed C=O absorption in the i.r. spectrum, and characteristic u.v. absorptions at 235, 243, and 252 nm (log ε 4.2), attributed to a conjugated heteroannular diene chromophore. The $C_{25}H_{38}O_4$ compound was separated with difficulty from a $C_{25}H_{40}O_4$ impurity which had only end absorption in the u.v.

During the period when the yields were acceptable, only the $C_{25}H_{38}O_3$ compound was obtained when the fermentation was harvested after 22—24 days. It was sometimes associated with a yellow impurity, composition $C_{13}H_{12}N_4O_2$, from which it was readily separated by preparative t.l.c. Fermentations carried out for a longer period of time, up to 42 days, yielded the mixture of diene carboxylic acids, together with additional more polar neutral hydroxy-containing minor products (*ca.* 0.1 mg/l) (i.r., see Experimental section). These products were of similar composition to the components of the insecticidal mixture, but had broad maxima in the region 255—260 nm (log ε 3.8), assigned to αβ-unsaturated ketone chromophores. These keto alcohols had the compositions $C_{25}H_{38}O_4$, $C_{25}H_{40}O_4$, and $C_{25}H_{40}O_5$.



(1)



(2)

The $C_{25}H_{38}O_3$ diene (1; R^2 = R^2 = H) contained hydroxy and carboxylic acid functions ($\nu_{max.}$ 3 390 and 1 700 cm^{-1}). It formed a methyl ester (1; R^1 = H, R^2 = Me), a monoacetate (1; R^1 = Ac, R^2 = H), and a diacetyl derivative, shown to be the acyclic mixed anhydride (1; R^1 = R^2 = Ac) by the i.r. spectrum ($\nu_{max.}$ 1 820 and 1 730 cm^{-1}). This anhydride was unstable and after preparative t.l.c. only the monoacetate (1; R^1 = Ac, R^2 = H), present as a *CH*OAc group [δ_H 4.5; δ_C 80.8 (d)] was recovered. The conjugated diene chromophore, which carried two hydrogen atoms [δ_H 5.50 and 5.35; δ_C 145.8 (s), 142.2 (s), 120.5 (d), and 116.1 (d)], remained unchanged in all the derivatives; it accounted for both the ethylenic linkages indicated by the ^{13}C n.m.r. spectrum of the acetate (1; R^1 = Ac, R^2 = H).

On this basis, this derivative of the $C_{25}H_{38}O_3$ diene contained four rings. It had one CH*Me* [δ_H 1.20 (d)] and five CMe groups (δ_H 0.59, 0.89, 0.92, 0.95, and 1.00). All of this spectroscopic evidence was consistent with the trimethylpregnadiene carboxylic acid structure (1; R^1 = Ac, R^2 = H) obtained by the *X*-ray crystallographic analysis of the hydrate.

The *X*-ray structure was determined by direct methods using diffractometer data. Least-squares refinement converged to *R* 5.94% over the 990 independent observed reflections. The structure and relative stereochemistry were confirmed as shown in structure (1) while Figure 1 shows the conformation of the molecule and includes the atomic numbering scheme adopted for crystallographic purposes. Bond lengths and angles are listed in Tables 1 and 2, respectively, together

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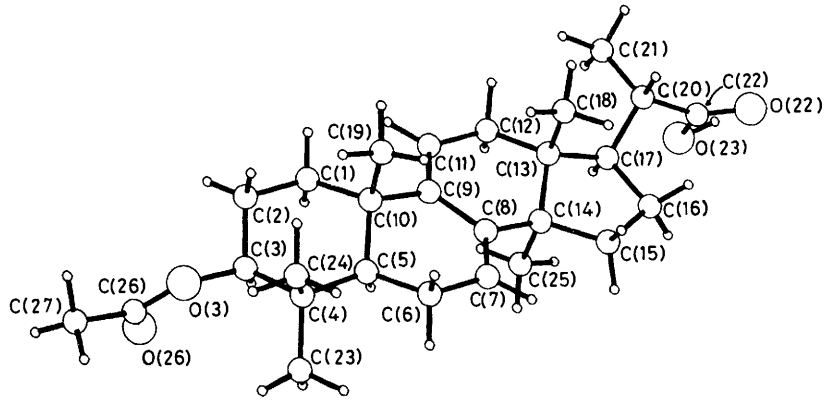


Figure 1. Conformation of the acetate (1; R^1 = Ac, R^2 = H) and the numbering scheme used for crystallographic purposes

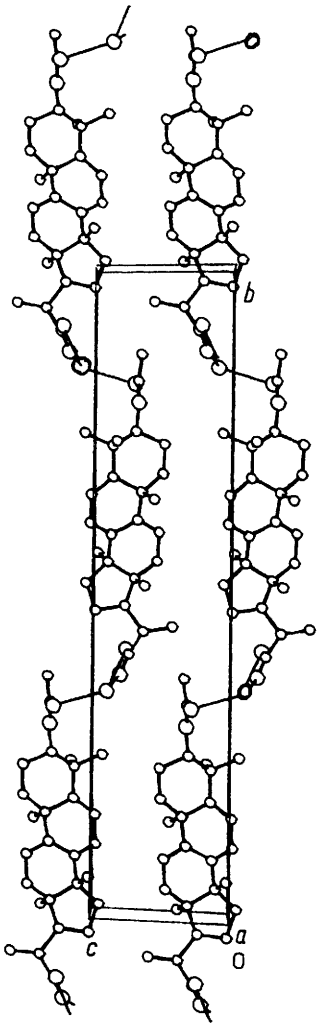
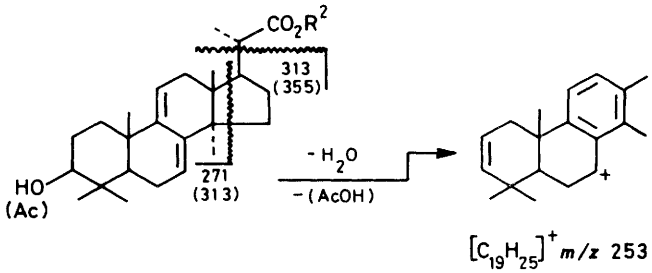


Figure 2. Molecular packing in the crystal of the acetate (1; R^1 = Ac, R^2 = H)



Scheme. Mass spectral fragmentation of the acid (1; R^1 = R^2 = H) and its derivatives (1; R^1 = Ac, R^2 = H, Me)

with their standard deviations. A study of the torsion angles within the ring systems confirmed that the molecular conformation was unexceptional. Figure 2 shows the molecular packing viewed down the short *a* axis of the unit cell. This clearly shows that the structure is linked in layers perpendicular to the *c* axis by the hydrogen bonding associated with the solvent water molecule. Hydrogen bonds of 2.61 Å were found from the acid [O(23)H] to the water O(28) at $(2 - x, -\frac{1}{2} + y, 1 - z)$, and of 2.85 and 2.84 Å respectively from the water [O(28)H] to the acid carbonyl O(22) at $(1 - x, \frac{1}{2} + y, 1 - z)$ and to the acetate carbonyl O(26) at $(x, y, 1 + z)$. All of the hydrogen atoms in these bonds were located in a difference map.

In addition to ions resulting from the loss of H_2O and CH_3, the mass spectra of the acid (1; R^1 = R^2 = H) and its derivatives showed the normal fragmentation of a lanostane [2] with (see Scheme) loss of the C_{17} substituent followed by 42 mass units (ring D + H) giving [from the acid (1; R^1 = R^2 = H)] fragments at *m/z* 313 $(C_{22}H_{33}O)^+$, 271 $(C_{19}H_{27}O)^+$, and 253 $(C_{19}H_{25})^+$. The same fragments are present in the mass spectrum of dihydroagnosterol.[3]

The $C_{25}H_{38}O_4$ diene was also a hydroxy acid, forming a methyl ester, a diacetate, and a triacetyl derivative which was shown to be a mixed anhydride by its i.r. spectrum ($\nu_{max.}$ 1 820 and 1 723 cm^{-1}). The elucidation of the structure of this compound is described elsewhere.[4]

Triterpenoids of the lanostane group containing the 7,9(11)-diene system are frequently accompanied by the corresponding (8-ene) dihydro compounds from which they have not readily been separated.[5] This generalisation applies also to the *V. lecanii* metabolites. The $C_{25}H_{38}O_4$ acid was accompanied by the $C_{25}H_{40}O_4$ acid, and the acid (1; R^1 = R^2 = H) by a compound $C_{25}H_{40}O_3$ (*m/z* 388) seen only in the mass spectra of crude material and not isolated, but presumed to be the acid (2; R^1 = R^2 = H). This acid, characterised as its derivatives (2; R^1 = H, R^2 = Me) and (2; R^1 = Ac, R^2 = H and Me), is a known side-chain degradation product of lanosterol.[6-8] At one time lanosta-7,9(11)-dienes were considered to be artifacts resulting from the autoxidation of the 8-enes, but, in addition to the compounds described here, there are

Table 1. Bond lengths (Å) for the acetate (1; R^1 = Ac, R^2 = H) with e.s.d.s in parentheses

C(1)–C(2)	1.54(2)
C(1)–C(10)	1.56(1)
C(2)–C(3)	1.49(2)
C(3)–C(4)	1.51(2)
C(3)–O(3)	1.48(1)
C(4)–C(5)	1.58(2)
C(4)–C(23)	1.53(2)
C(4)–C(24)	1.55(1)
C(5)–C(6)	1.53(2)
C(5)–C(10)	1.53(1)
C(6)–C(7)	1.48(2)
C(7)–C(8)	1.34(1)
C(8)–C(9)	1.45(1)
C(8)–C(14)	1.53(1)
C(9)–C(10)	1.55(2)
C(9)–C(11)	1.38(1)
C(10)–C(19)	1.56(1)
C(11)–C(12)	1.51(1)
C(12)–C(13)	1.52(1)
C(13)–C(14)	1.51(1)
C(13)–C(17)	1.57(1)
C(13)–C(18)	1.55(1)
C(14)–C(15)	1.53(1)
C(14)–C(25)	1.59(2)
C(15)–C(16)	1.56(2)
C(16)–C(17)	1.53(2)
C(17)–C(20)	1.55(1)
C(20)–C(21)	1.51(2)
C(20)–C(22)	1.50(2)
C(22)–O(22)	1.19(1)
C(22)–O(23)	1.33(1)
C(26)–C(27)	1.50(2)
C(26)–O(3)	1.34(2)
C(26)–O(26)	1.20(1)

now several examples, *e.g.* polycarpol,[9] where the 7,9(11)-diene is the major product, accompanied by little or none of the 8-ene.

With the notable exceptions of agnosterol, dihydroagnosterol, polycarpol, and the sapogenins related to holothurinogenin,[10] the naturally occurring lanosta-7,9(11)-dienes are metabolic products of fungi, and have hitherto, with one possible exception, been obtained from Basidiomycetes, usually from the fruiting bodies. The presence of lanosta-7,9(11),24-triene-3β,21-diol in the mycelium of the imperfect fungus *Trichoderma pseudokoningii* has been claimed,[11] but the u.v. absorption of the material isolated [$\lambda_{max.}$ 274 and 325 nm (ε 20 157 and 31 152)] is inconsistent with the structure assigned. Deuteromycetes, notably *Cephalosporium* and *Fusidium* sp., are well-recognised producers of triterpenoids with the fusidane skeleton, but the formation of lanostanes by this class of fungi is unusual.

The majority of fungal lanostanes are 24-methylene compounds (C_{31}), or their simple derivatives, but some are based on the C_{30} skeleton. Many have the 21-methyl group oxidised to CH_2OH or, more often, CO_2H,[12] but these compounds have the 20*R* configuration. No lanostane side-chain degradation products strictly analogous to the acid (1; R^1 = R^2 = H) have been isolated from natural sources, but two 4,4,14-trimethylpregn-8-en-20-ones have been obtained as minor products from *Fomes officinalis*.[13,14]

Antibacterial activity is commonly found in the fungal triterpenoid carboxylic acids. The *V. lecanii* acids showed toxicity to insects[1] but lack of material prevented a more extended evaluation of the biological activity. In general, the lanostane analogues of physiologically active steroids are without activity, but a possible hormonal role for the *V. lecanii* compounds cannot be excluded.

Table 2. Bond angles (°) for the acetate (1; R^1 = Ac, R^2 = H) with e.s.d.s in parentheses

C(2)–C(1)–C(10)	112(1)
C(1)–C(2)–C(3)	109(1)
C(2)–C(3)–C(4)	116(1)
C(2)–C(3)–O(3)	106(1)
C(4)–C(3)–O(3)	107(1)
C(3)–C(4)–C(5)	107(1)
C(3)–C(4)–C(23)	108(1)
C(3)–C(4)–C(24)	112(1)
C(5)–C(4)–C(23)	108(1)
C(5)–C(4)–C(24)	115(1)
C(23)–C(4)–C(24)	107(1)
C(4)–C(5)–C(6)	112(1)
C(4)–C(5)–C(10)	117(1)
C(6)–C(5)–C(10)	110(1)
C(5)–C(6)–C(7)	110(1)
C(6)–C(7)–C(8)	124(1)
C(7)–C(8)–C(9)	121(1)
C(7)–C(8)–C(14)	123(1)
C(9)–C(8)–C(14)	115(1)
C(8)–C(9)–C(10)	117(1)
C(8)–C(9)–C(11)	122(1)
C(10)–C(9)–C(11)	121(1)
C(1)–C(10)–C(5)	110(1)
C(1)–C(10)–C(9)	111(1)
C(1)–C(10)–C(19)	108(1)
C(5)–C(10)–C(9)	108(1)
C(5)–C(10)–C(19)	115(1)
C(9)–C(10)–C(19)	106(1)
C(9)–C(11)–C(12)	122(1)
C(11)–C(12)–C(13)	112(1)
C(12)–C(13)–C(14)	109(1)
C(12)–C(13)–C(17)	118(1)
C(12)–C(13)–C(18)	108(1)
C(14)–C(13)–C(17)	102(1)
C(14)–C(13)–C(18)	111(1)
C(17)–C(13)–C(18)	109(1)
C(8)–C(14)–C(13)	113(1)
C(8)–C(14)–C(15)	118(1)
C(8)–C(14)–C(25)	103(1)
C(13)–C(14)–C(15)	104(1)
C(13)–C(14)–C(25)	113(1)
C(15)–C(14)–C(25)	106(1)
C(14)–C(15)–C(16)	103(1)
C(15)–C(16)–C(17)	107(1)
C(13)–C(17)–C(16)	103(1)
C(13)–C(17)–C(20)	118(1)
C(16)–C(17)–C(20)	113(1)
C(17)–C(20)–C(21)	113(1)
C(17)–C(20)–C(22)	108(1)
C(21)–C(20)–C(22)	111(1)
C(20)–C(22)–O(22)	124(1)
C(20)–C(22)–O(23)	112(1)
O(22)–C(22)–O(23)	123(1)
C(27)–C(26)–O(3)	110(1)
C(27)–C(26)–O(26)	127(1)
O(3)–C(26)–O(26)	123(1)
C(3)–O(3)–C(26)	119(1)

The $C_{13}H_{12}N_4O_2$ compound was identified as 3-methyllumichrome by its spectroscopic properties.[15,16] This flavin pigment was unknown as a natural product until recently[16] when it was isolated from the bird's nest fungus *Cyanthus bulleri*.

Experimental

M.p.s were taken on a Kofler hot-stage apparatus and are corrected. I.r. spectra were determined for Nujol mulls and

u.v. spectra for methanolic solutions. ^{1}H N.m.r. spectra were obtained at 90 MHz and ^{13}C n.m.r. spectra at 25.2 MHz in $CDCl_3$, with $SiMe_4$ as internal standard. ^{13}C Chemical shifts are recorded in p.p.m. downfield from $SiMe_4$. Molecular weights and compositions were taken from the high resolution mass spectra recorded with a Varian CH5D (double-focusing) mass spectrometer interfaced with a Varian 620L computer. Optical rotations were measured in MeOH in a 1-dm cell. Merck silica gel HF_{254} was used in analytical t.l.c. with chloroform–methanol (95 : 5). In preparative t.l.c. silica layers 20 × 20 × 0.075 cm were developed in this solvent system and examined in u.v. light. Merck silica gel 7734 was used in column chromatography. Light petroleum had b.p. 60—80 °C. Fermentation details have been recorded elsewhere.[1]

Isolation of the Major Metabolites.—A. The culture filtrate (7.2 l) from a fermentation harvested after 33 days was adjusted to pH 5.0 and extracted with ethyl acetate giving a gum (343 mg). This in ethyl acetate (5 ml) was adsorbed on silica gel (3 g) and placed on top of a column of silica gel (8 g; 20 × 1.2 cm) made up in light petroleum. After elution with diethyl ether–light petroleum (1 : 4; 50 ml) of (i) a yellow oil (33 mg), elution of the column with diethyl ether (25 ml portions) furnished semi-solid fractions (ii) (41 mg) and (iii) (70 mg), followed by (iv) gums (50 mg) which were retained for further purification (see section D below).

Trituration of fraction (ii) with methanol furnished 3β-*hydroxy*-4,4,14α-*trimethyl*-5α-*pregna*-7,9(11)-*diene*-20S-*carboxylic acid* (1; $R^1 = R^2 = H$) (8 mg), obtained, on concentration of a methanol solution, as an amorphous powder, m.p. 300—303 °C (decomp.), R_F 0.25 (Found: *M*, 386.2806. $C_{25}H_{38}O_3$ requires *M*, 386.2820); $\nu_{max.}$ 3 390 and 1 700 cm^{-1}; $\lambda_{max.}$ 236, 243, and 252 nm (log ε 4.22, 4.29, and 4.12); *m/z* (% base peak) 386 (58), 371 (19), 368 (10), 353 (32), 313.2491 (12), 295 (15), 271.2030 (21), 253.1957 (25), and 55 (100) ($C_{22}H_{33}O^+$, $C_{19}H_{27}O^+$, and $C_{19}H_{25}^+$ require 313.2531, 271.2061, and 253.1956 respectively). It was insoluble in chloroform and acetone and only sparingly soluble in methanol and ethyl acetate.

The *methyl ester* (1; $R^1 = H$, $R^2 = Me$), prepared with diazomethane in diethyl ether, crystallised from methanol in plates or prisms, m.p. 220—223 °C, R_F 0.57 (Found: *M*, 400.2999. $C_{26}H_{40}O_3$ requires *M*, 400.2977); $\nu_{max.}$ 3 540, 3 460, 1 737, and 1 710 cm^{-1}; *m/z* 400 (50), 385 (10), 367 (8), 313(10), 271 (10), 253 (10), and 55 (100).

Fraction (iii) crystallised from methanol in felted needles (13 mg) of a hydroxy acid, m.p. 229—233 °C (subl.), R_F 0.12 [Found (dried at 80 °C): C, 72.5; H, 10.0%; *M*, 402.2788. $C_{25}H_{38}O_4$ requires *M*, 402.2769. $C_{25}H_{38}O_4 \cdot CH_3OH$ requires C, 71.9; H, 9.7%]; $\nu_{max.}$ 3 320, 3 190br, 1 665, and 1 620w cm^{-1}; $\lambda_{max.}$ 239, 246, and 255 nm (log ε 4.20, 4.27, and 4.10).

The methyl ester, prepared with diazomethane, crystallised from methanol in needles, m.p. 151—152 °C and 180 °C, R_F 0.34 (Found: *M*, 416.2926. $C_{26}H_{40}O_4$ requires *M*, 416.2926).

The gum (292 mg) obtained by re-extraction of the culture filtrate at pH 3 was subjected to column chromatography as described above. None of the gummy fractions (total 112 mg) so obtained showed the characteristic u.v. absorption of the C_{25} hydroxy acids.

B. The extract (1.04 g) from a fermentation (30 l) harvested after 22—24 days was adsorbed on silica (5 g) and chromatographed on a column of silica (31 g; 21 × 1.7 cm) made up in light petroleum. After elution with (i) diethyl ether–light petroleum (1 : 4; 300 ml, 96 mg) and (ii) diethyl ether (100 ml, 4 mg), diethyl ether–ethyl acetate (99 : 1; 200 ml) gave (iii) a gum (264 mg) which furnished the acid (1; $R^1 = R^2 = H$) (32 mg) on trituration with methanol. Further elution of the column with increasing proportions of ethyl acetate in diethyl ether gave the following gummy fractions (eluant composition in square brackets): (iv) (116 mg) [99 : 1; 200 ml]; (v) (61 mg) [97:3; 200 ml]; (vi) (19 mg) [20 : 1; 100 ml]; and (vii) (34 mg) [9 : 1; 200 ml]. None of these fractions yielded the $C_{25}H_{38}O_4$ hydroxy acid or showed the u.v. chromophore associated with this compound.

Isolation of the Minor Metabolites.—C. The residue (100 mg) from the accumulated mother liquors of fractions corresponding to fractions A(ii) and B(iii) (see above) from the 'neutral' extracts from several fermentations (601) were subjected to preparative t.l.c. giving material corresponding to three bands, R_F 0.45 (9 mg), 0.38 (10 mg), and 0.33 (44 mg). The material with R_F 0.45 crystallised (from benzene) in yellow needles, m.p. 300 °C (subl.) of 3,7,8-trimethylbenzo[*g*]pteridine-2,4(1*H*,3*H*)-dione (3-methyl-lumichrome), R_F 0.42 (Found: C, 60.3; H, 4.9; N, 21.3%; *M*, 256.0952. Calc. for $C_{13}H_{12}N_4O_2$: C, 60.9; H, 4.7; N, 21.9%; *M*, 256.0947); $\nu_{max.}$ 3 170, 3 060w, 1 730, 1 690, 1 582, and 1 560 cm^{-1}; $\lambda_{max.}$ 220, 250, *ca.* 259, 343, and 387 nm (log ε 4.60, 4.59, 4.55, 3.93, and 3.91); δ 2.51 (s, 3 H), 2.55 (s, 3 H), 3.57 (s, 3 H NMe), 7.80 (s, 1 H), 8.07 (s, 1 H), and 8.70 (1 H NH); *m/z* 256 (100), 227 (20), 199 (75), 171 (75), 156 (63), 123 (24), 105 (28), 95 (63), and 81 (75), identified by comparison with an authentic specimen provided by Professor W. A. Ayer. The material of R_F 0.38 crystallised from methanol in prisms, m.p. 266 °C, of an oxocarboxylic acid (Found: *M*, 384.2666. $C_{25}H_{36}O_3$ requires *M*, 384.2664) $\nu_{max.}$ 3 200br, 1 720, 1 700, and 1 650w; $\lambda_{max.}$ 237, 244, and 253 nm; *m/z* 384 (100), 369 (39), 325 (20), 311 (23), and 269 (42). The methyl ester, prepared with diazomethane, crystallised from methanol in plates, m.p. 184—186 °C. The band with R_F 0.33 yielded the hydroxy acid (1; $R^1 = R^2 = H$).

D. The residue (166 mg) from the accumulated mother liquors of fractions corresponding to fractions A(iii) (see above) from the same fermentations which generated the residue used in C, were subjected to repetitive preparative t.l.c. In addition to the hydroxy acid, $C_{25}H_{38}O_4$, R_F 0.12 (14 mg), a hydroxy acid (2 mg), R_F 0.14 was obtained which crystallised from methanol in felted needles, m.p. 235—240 °C (Found: *M*, 404.2917. $C_{25}H_{40}O_4$ requires *M*, 404.2925); $\nu_{max.}$ 3 465, 3 370, 3 270, and 1 695 cm^{-1}. It reacted vigorously in diethyl ether with diazomethane.

E. The bulked 'neutral' fractions (1.18 g) from several fermentations (total 37 l) harvested after 38 days were adsorbed on silica gel (4 g) and chromatographed on a column of silica gel (35 g; 25 × 1.7 cm) as described above. After elution of ergosterol, m.p. and mixed m.p. 150—154 °C, and the hydroxy acid (1; $R^1 = R^2 = H$), further elution with diethyl ether–ethyl acetate (99 : 1; 200 ml and 95 : 5; 300 ml) yielded gums (i) (48 mg) and (ii) (155 mg), respectively.

Crystallisation of fraction (i) from methanol gave plates (1 mg), m.p. 226—230 °C of a keto alcohol, R_F 0.08 (Found: *M*, 420.2860. $C_{25}H_{40}O_5$ requires *M*, 420.2876); $\nu_{max.}$ 3 505, 3 370br, 1 650, and 1 625w; $\lambda_{max.}$ 257 nm (log ε 4.10).

The residue from the mother liquors was subjected to repeated preparative t.l.c. A band with R_F 0.20 (9 mg) crystallised from methanol in prisms of a keto alcohol (1 mg), double m.p. 230—242 °C and 266 °C (Found: *M*, 402.2803. $C_{25}H_{38}O_4$ requires *M*, 402.2769); $\nu_{max.}$ 3 500, 3 330br, 1 700w, and 1 680w; $\lambda_{max.}$ 255 nm (log ε 3.81).

Fraction (ii) was also subjected to repeated preparative t.l.c. A band with R_F 0.10 (12 mg) gave, from methanol, needles of a keto alcohol (1 mg), double m.p. 140 °C and 232 °C (Found: *M*, 404.2926. $C_{25}H_{40}O_4$ requires *M*, 404.2925); $\nu_{max.}$ 3 320br and 1 650; $\lambda_{max.}$ 255 nm (log ε 3.70). Further

Table 3. Atomic co-ordinates (e.s.d.s in parentheses) for the acetate (1; R^1 = Ac, R^2 = H)

Atom	*x/a*	*y/b*	*z/c*
C(1)	0.668(2)	0.1837	0.500(2)
C(2)	0.580(2)	0.2297(6)	0.507(2)
C(3)	0.506(2)	0.2489(5)	0.299(2)
C(4)	0.310(2)	0.2261(5)	0.135(2)
C(5)	0.388(2)	0.1778(5)	0.136(2)
C(6)	0.202(2)	0.1503(6)	−0.019(2)
C(7)	0.293(2)	0.1064(5)	−0.027(2)
C(8)	0.475(2)	0.0891(5)	0.122(2)
C(9)	0.587(2)	0.1114(5)	0.314(2)
C(10)	0.481(2)	0.1551(5)	0.343(2)
C(11)	0.769(2)	0.0931(5)	0.471(2)
C(12)	0.852(2)	0.0479(5)	0.460(2)
C(13)	0.673(2)	0.0212(5)	0.296(2)
C(14)	0.587(2)	0.0465(5)	0.100(2)
C(15)	0.437(2)	0.0140(5)	−0.056(2)
C(16)	0.576(2)	−0.0298(5)	0.012(2)
C(17)	0.757(2)	−0.0215(5)	0.226(2)
C(18)	0.471(2)	0.0108(5)	0.371(2)
C(19)	0.293(2)	0.1440(5)	0.436(2)
C(20)	0.795(2)	−0.0617(5)	0.364(2)
C(21)	0.975(2)	−0.0551(5)	0.575(2)
C(22)	0.862(2)	−0.0982(5)	0.258(2)
C(23)	0.284(2)	0.2454(5)	−0.072(2)
C(24)	0.073(2)	0.2326(5)	0.163(2)
C(25)	0.789(2)	0.0607(5)	0.024(2)
C(26)	0.558(3)	0.3264(6)	0.312(2)
C(27)	0.442(3)	0.3674(5)	0.336(2)
O(3)	0.425(1)	0.2928(4)	0.317(1)
O(22)	0.746(1)	−0.1294(4)	0.196(2)
O(23)	1.065(1)	−0.0916(4)	0.235(2)
O(26)	0.739(2)	0.3229(5)	0.286(2)
O(28)	0.728(2)	0.3479(5)	0.894(2)
H(1A)	0.8153	0.1861	0.4612
H(1B)	0.7248	0.1702	0.6412
H(2A)	0.6932	0.2465	0.6104
H(2B)	0.4336	0.2255	0.5446
H(3)	0.6574	0.2441	0.2711
H(5)	0.5380	0.1733	0.1070
H(6A)	0.1570	0.1639	−0.1640
H(6B)	0.0539	0.1490	0.0096
H(7)	0.2176	0.0869	−0.1499
H(11)	0.8545	0.1107	0.5989
H(12A)	1.0052	0.0470	0.4329
H(12B)	0.8922	0.0315	0.5990
H(15A)	0.4279	0.0215	−0.1992
H(15B)	0.2758	0.0109	−0.0568
H(16A)	0.6539	−0.0382	−0.0852
H(16B)	0.4668	−0.0536	0.0166
H(17)	0.9145	−0.0166	0.2223
H(18A)	0.5297	−0.0079	0.5013
H(18B)	0.3961	0.0362	0.3953
H(18C)	0.3486	−0.0086	0.2649
H(19A)	0.3743	0.1303	0.5765
H(19B)	0.2180	0.1715	0.4603
H(19C)	0.1734	0.1243	0.3507
H(20)	0.6415	−0.0667	0.3874
H(21A)	0.9963	−0.0812	0.6707
H(21B)	1.1370	−0.0481	0.5763
H(21C)	0.9378	−0.0299	0.6579
H(23A)	0.2261	0.2750	−0.0907
H(23B)	0.4317	0.2429	−0.1000
H(23C)	0.1607	0.2271	−0.1900
H(24A)	0.0395	0.2633	0.1659
H(24B)	0.0920	0.2199	0.3058
H(24C)	−0.0513	0.2167	0.0603
H(25A)	0.7264	0.0761	−0.1063
H(25B)	0.9089	0.0779	0.1294
H(25C)	0.8754	0.0334	−0.0011
H(27A)	0.3741	0.3625	0.4575
H(27B)	0.5472	0.3922	0.3646
H(27C)	0.3011	0.3737	0.2041
H(O23)	1.0858	−0.1217	0.2441
H(O28A)	0.8474	0.3404	1.0862
H(O28B)	0.6186	0.3479	0.8309

small quantities of these keto alcohols were obtained by preparative t.l.c. of fractions A(iv) and B(iv)—(vii).

Acetyl Derivatives.—(*a*) The acid (1; $R^1 = R^2 = H$) (4 mg) in pyridine (0.1 ml) and acetic anhydride (0.1 ml) was left at room temperature for 2 days. The solvent was removed under reduced pressure at 40 °C and the residue was crystallised from benzene–light petroleum giving prisms (3 mg), double m.p. 180 °C and 255 °C (decomp.) of *acetic* 3β-*hydroxy*-4,4,14α-*trimethyl*-5α-*pregna*-7,9(11)-*diene*-20S-*carboxylic anhydride* (1; $R^1 = R^2$ = Ac), R_F 0.72 (Found: *M*, 470.3037. $C_{29}H_{42}O_5$ requires *M*, 470.3032); $\nu_{max.}$ 1 820, 1 730, and 1 625w cm^{-1}; $\lambda_{max.}$ 236, 243, and 252 nm (log ε 4.14, 4.20, and 4.03); δ_H 0.58 (s, 3 H, 18-Me), 0.89 (s, 3 H), 0.91 (s, 3 H,) 0.95 (s, 3 H), 1.00 (s, 3 H), 1.24 (d, 3 H, 21-Me), 1.0—2.5 (15 H), 2.05 (s, 3 H, Ac), 2.24 (s, 3 H, Ac), 4.53 (m, 1 H, C*H*OAc), 5.35 (m, 1 H, HC=), and 5.50 (m, 1 H, =CH); δ_C(>70 p.p.m.) 80.7 (C–OAc), 115.8, 120.7, 142.0, 145.8 (C=C), 166.8 (CO_2R), 170.9 (AcO), and 172.2 p.p.m. (AcO); *m/z* 470 (4%), 427 (10), 410 (3), 367 (10), 355 (27), 353 (11), 314 (15), 313 (30), 295 (8), 253 (69), and 55 (100).

(*b*) The acid (1; $R^1 = R^2 = H$) (12 mg) was acetylated as described above but the product was submitted to preparative t.l.c. giving two bands, R_F 0.3 and 0.7, which were extracted with chloroform. The band with R_F 0.3 afforded rhombs (4 mg), m.p. 252—253 °C (decomp.) from benzene–light petroleum of the 3β-*acetoxy*-4,4,14α-*trimethyl*-5α-*pregna*-7,9(11)-*diene*-20S-*carboxylic acid* (1; R^1 = Ac, R^2 = H), R_F 0.33, $[\alpha]_D^{22}$ + 69° (*c*, 0.0145) (Found: C, 75.6; H, 9.0%; *M*, 428.2928. $C_{27}H_{40}O_4$ requires C, 75.7; H, 9.4%; *M*, 428.2926); $\nu_{max.}$ 1 730, 1 710, 1 640w, and 1 625w cm^{-1}; $\lambda_{max.}$ 235, 243, and 252 nm (log ε 4.09, 4.16, and 4.01); δ_H 0.59 (s, 3 H, 18-Me), 0.89 (s, 3 H), 0.92 (s, 3 H), 0.95 (s, 3 H), 1.00 (s, 3 H), 1.20 (d, 3 H, 21-Me), 1.0—2.5 (16 H), 2.05 (s, 3 H, Ac), 4.50 (m, 1 H, CHOAc), 5.35 (m, 1 H, HC=), and 5.50 (m, 1 H, =CH); δ_C (> 70 p.p.m.) 80.8 (d, C–OAc), 116.1 (d), 120.5 (d), 142.2 (s), 145.8 (s), (C=C), 171.0 (AcO), and 182.1 p.p.m. (CO_2H); *m/z* 428 (29%), 413 (14), 368 (34), 355 (36), 353 (66), 313 (14), 295 (23), 253 (62), and 43 (100). It crystallised from methanol in plates, m.p. 255—258 °C (decomp.) after a transition at 130 °C, of a solvate shown by *X*-ray crystallographic analysis to be the *hydrate* (Found: C, 73.0; H, 9.3. $C_{27}H_{40}O_4 \cdot H_2O$ requires C, 72.6; H, 9.5%); $\nu_{max.}$ 3 530, 3 420, 1 718, 1 700, and 1 650w cm^{-1}.

Methyl 3β-*acetoxy*-4,4,14α-*trimethyl*-5α-*pregna*-7,9(11)-*diene*-20S-*carboxylate* (1; R^1 = Ac, R^2 = Me), prepared with diazomethane, crystallised from methanol in prisms, m.p. 200—204 °C, R_F 0.70 (Found: *M*, 442.3092. $C_{28}H_{42}O_4$ requires *M*, 442.3083).

The band R_F 0.7 also gave rhombs, m.p. 248—256 °C (decomp.) (5 mg), R_F 0.34, identified as the acetate (1; R^1 = Ac, R^2 = H) by comparison of the i.r. and mass spectra.

(*c*) The $C_{25}H_{38}O_4$ hydroxy acid (12 mg) was acetylated as described in (*a*). The product crystallised from benzene–hexane in rosettes of needles (7 mg), m.p. 156—160 °C, R_F 0.71, of an anhydride diacetate [Found: C, 72.3; H, 9.5%; *M* − 60, 468.2875. $C_{31}H_{44}O_7 \cdot C_6H_{14}$ requires C, 72.3; H, 9.5%; $C_{29}H_{40}O_5$ ($C_{31}H_{44}O_7 - C_2H_4O_2$) requires *M*, 468.2876]; $\nu_{max.}$ 1 820, 1 723, and 1 635w cm^{-1}; $\lambda_{max.}$ 237, 245, and 254 nm

(log ε 4.08, 4.17, and 4.00); δ_H 0.67 (s, 3 H), 0.89 (s, 3 H), 0.96 (s, 3 H), 1.03 (s, 6 H), 1.25 (d, 3 H), 1.3—2.5 (13 H), 2.06 (s, 3 H, Ac), 2.10 (s, 3 H, Ac), 2.23 (s, 3 H, Ac), 4.56 (dd, 1 H, C*H*OAc), 5.03 (s, 1 H, C*H*OAc), 5.44 (s, 1 H, HC=), and 5.60 (m, 1 H, =CH); δ_C (>70 p.p.m.) 77.4d (C–OAc), 80.3d (C–OAc), 117.2 (d), 122.9 (d), 140.6 (s), 147.9 (s) (C=C), 166.6 (s, CO_2Ac), 170.73 (s, AcO), 170.85 (s, AcO), and 172.1 p.p.m. (s, AcO). It was unstable at room temperature and on storage underwent decomposition to the diacetate $C_{29}H_{42}O_6$ (below).

The residue from the mother liquors was subjected to preparative t.l.c. giving two bands, R_F 0.7 and 0.35. The material from both bands crystallised from benzene–light petroleum in prisms (2 mg), m.p. 230—232 °C, of a diacetate (Found: *M*, 486.2979. $C_{29}H_{42}O_6$ requires *M*, 486.2981); $\nu_{max.}$ 3 200br, 1 730, and 1 695 cm^{-1}; $\lambda_{max.}$ 237, 246, and 255 nm.

Crystallographic Analysis of the Acetate (1; R^1 = Ac, R^2 = H).—The space group and preliminary cell parameters were determined photographically. For the intensity measurement the crystal was then mounted on an Enraf-Nonius CAD4 diffractometer. Accurate lattice parameters were obtained by least squares refinement of the positions of 25 reflections measured on the diffractometer with θ *ca.* 25°. Intensity data were collected with Cu-K_α radiation using an $\omega - 2/3\theta$ scan for $1° \leqslant \theta \leqslant 60°$. A total of 1 895 independent reflections were measured of which only 990 had $I \geqslant 2\sigma(I)$ and were considered observed and used in the subsequent refinement. The data were corrected for Lorentz and polarisation factors, but no absorption corrections were applied. Data reduction and subsequent crystallographic calculations were performed using the CRYSTALS [17] system of programs.

Crystal Data.—$C_{27}H_{40}O_4 \cdot H_2O$, $M = 446.6$. Monoclinic, $a = 6.162(2)$, $b = 31.110(12)$, $c = 7.028(2)$ Å, $\beta = 111.16(3)°$, $U = 1256.4$ Å^3, $z = 2$, $D_c = 1.18$ g cm^{-3}, $F(000) = 488$. Space group $P2_1$ from systematic absences. Cu-K_α radiation, $\lambda = 1.54178$ Å, μ(Cu-K_α) = 6.42 cm^{-1}.

Structure Solution and Refinement.—The structure was solved by direct methods using the MULTAN [18] program. 281 Reflections with $E > 1.3$ were used and the *E* map based on the best set of phases revealed the positions of all 31 non-hydrogen atoms in the molecule among the largest peaks in the map. The solvent atom was readily located in a subsequent difference map. Full-matrix isotropic least squares refinement of these positions gave an *R* value of 12.2%.

Refinement was continued with anisotropic thermal parameters for all non-hydrogen atoms. A difference map next revealed the approximate positions of many of the hydrogen atoms. Geometric considerations were then used to calculate the accurate positions of all of the hydrogen atoms whose location could be fixed in this way. The remaining hydrogen atom positions were taken directly from the peaks in the difference map. The hydrogen atoms were then included in the calculations bit without refinement. Analysis of the agreement between F_o and F_c suggested the adoption of a weighting scheme based on a Chebyshev polynomial. Refinement finally converged with the largest parameter shifts 0.1σ after 31 cycles of least squares refinement. The final *R* value at convergence was 5.94% with R_w 0.0682. A final difference map was calculated which showed no peaks or depressions >0.2 e Å^{-3}. Final atomic co-ordinates are listed in Table 3. Temperature factors and observed and calculated structure factors are available as a Supplementary Publication (SUP No. 23806, 15 pp.).*

* For details of the Supplementary Publications scheme, see Instructions for Authors (1984) in *J. Chem. Soc., Perkin Trans. 1*, 1984, Issue 1.

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