New Metabolic Products of *Verticillium lecanii*. Part 2.[1] 3β,12β,-Dihydroxy-4,4,14α-trimethyl-5α-pregna-7,9(11)-diene-20*S*-carboxylic Acid and 4,4,14α-Trimethyl-3-oxo-5α-pregna-7,9(11)-diene-20*S*-carboxylic Acid

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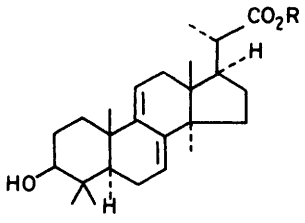
An insecticidal hydroxy acid ($C_{25}H_{38}O_4$) isolated from certain entomopathogenic strains of *Verticillium lecanii* is shown to be 3β,12β-dihydroxy-4,4,14α-trimethyl-5α-pregna-7,9(11)-diene-20*S*-carboxylic acid. 4,4,14α-Trimethyl-3-oxo-5α-pregna-7,9(11)-diene-20*S*-carboxylic acid and, probably, 3β,20,21-trihydroxy-4,4,14α,20-tetramethyl-5α-pregn-8-en-7-one were identified as minor metabolic products of this fungus.

Two insecticidal C_{25} hydroxy carboxylic acids were isolated[1,2] from certain entomopathogenic strains of the fungus *Verticillium lecanii* and the structure of the less polar, $C_{25}H_{38}O_3$, compound was shown to be 3β-hydroxy-4,4,14α-trimethyl-5α-pregna-7,9(11)-diene-20*S*-carboxylic acid (**1**; R = H) by *X*-ray crystallography.[1] The more polar, $C_{25}H_{38}O_4$, compound is shown in the present paper to be the 12β-hydroxy derivative (**2**; $R^1 = R^2 = H$).



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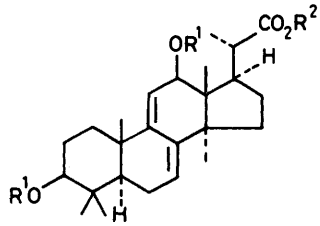

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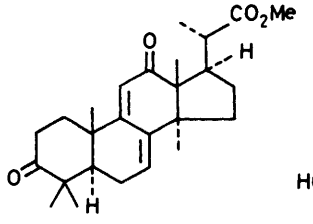

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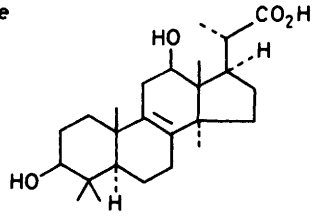

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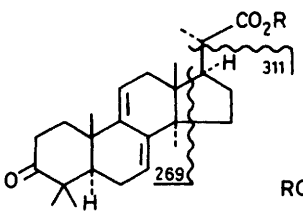

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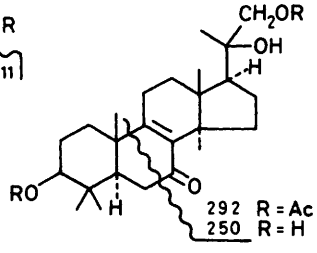

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The close similarity between the n.m.r. spectra of the two sets of derivatives of the two hydroxy acids[1] suggested that they were closely related, the additional oxygen atom in the $C_{25}H_{38}O_4$ compound being present in a second >C*H*OH group [acetate: δ_H 5.03, δ_C 77.4(d)]. The chemical shift (δ_H 4.25) of this hydrogen in the methyl ester (**2**; $R^1 = H$, $R^2 = Me$) suggested an allylic alcohol, consistent both with a small bathochromic shift in the diene u.v. absorption of the dihydroxy acid and its derivatives compared with the acid (**1**; R = H), and with the formation of a conjugated dienone (**3**), $\lambda_{max.}$ 291 nm (log ε 4.08) on oxidation of the methyl ester (**2**; $R^1 = H$, $R^2 = Me$) with the chromic oxide–sulphuric acid reagent.[3] The second hydroxy substituent must, therefore, be present at position 6 or 12. Initially it was hoped that the allylic hydrogens at these positions in the ester (**1**; R = Me) would be well resolved in the 360 MHz n.m.r. spectrum, and that they would be readily assigned from the spin-coupling patterns.[4,5] A comparison with this region of the spectrum of the hydroxylated derivative (**2**; $R^1 = H$, $R^2 = Me$) would then reveal which position had been substituted in the latter ester.

However, in the spectrum of the ester (**1**; R = Me), only one A hydrogen (δ 2.38, J_{AB} = 17 Hz, J_{AX} = 0) of the AB part of an ABX system was completely resolved although the corresponding B hydrogen (J_{AB} = 17 Hz, J_{BX} = 6 Hz) could be distinguished as part of a broad multiplet centred at δ 2.05. These signals were assigned to the 12α- and 12β-hydrogens respectively, and were absent from the spectrum of the hydroxylated ester (**2**; $R^1 = H$, $R^2 = Me$). The signals from the hydrogens at the 6-position, which constitute the AB part of an ABMX system, were masked at δ 1.9—2.1 by signals from five other hydrogens including the 12β-hydrogen. The appearance of this 7 H multiplet was altered by irradiation at δ 1.10 of a well resolved double doublet (*J* 10, 5 Hz) assigned to 5-H. This signal, with identical spin coupling, was present in both esters. In the spectrum of the hydroxylated ester (**2**; $R^1 = H$; $R^2 = Me$) removal from the δ 2.05 multiplet of the 12β-H resonance did not clarify the interpretation, other than to suggest that the masking signals emanated from the ring D A_2B_2C system since a multiplet at δ 2.21 assigned to 17-H could be readily distinguished.

The vinylic hydrogens at δ 5.48 and 5.32 in the spectrum of the ester (**1**; R = Me) were assigned to the 7 and 11 positions, respectively, by the appropriate decoupling experiments. The introduction of the second hydroxy substituent into this ester caused a downfield shift of the signals assigned 20- and 21-H (Table 1). All this evidence suggests hydroxy substitution at the 12 position in the $C_{25}H_{38}O_4$ compound. In the methyl ester (**2**; $R^1 = H$, $R^2 = Me$) the signals from 11- and 12-H both appeared as singlets, consistent only with a 12β-substituent ($\varphi_{11,12\alpha}$ *ca.* 90°).

It follows that the $C_{25}H_{38}O_4$ compound has structure (**2**; $R^1 = R^2 = H$) and the di- and tri-acetyl derivatives[1] are represented by (**2**; $R^1 = Ac$, $R^2 = H$) and (**2**; $R^1 = R^2 = Ac$) respectively. Formation of a 22 ⟶ 12 δ-lactone on treatment of the acid (**2**; $R^1 = R^2 = H$) with acetic anhydride presumably does not occur because of the unfavourable 1,3-diaxial

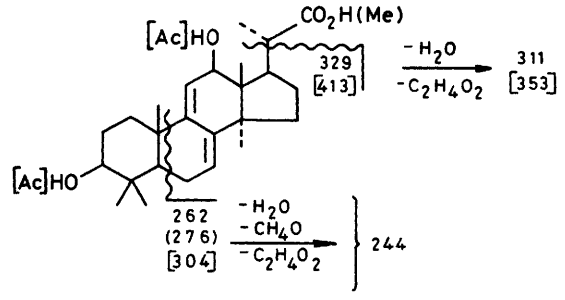
Table 1. ^{1}H N.m.r. resonances (δ, *J* in parentheses) for the esters (**1**; R = Me) and (**2**; R^1 = H, R^2 = Me)

Compound	Position 3	5	7	11	12	20	21	18	Other Me(s)				OMe(s)
(**1**; R = Me)	3.44 dd (11.2,4.6)	1.10 dd (10.6,5.0)	5.48 m	5.32 d (6.1)	2.15 AB (17,6)	2.43 dq (6.8,10.7)	1.17 d (6.8)	0.58 s	0.88	0.92	0.98	1.01	3.66
(**2**; R^1 = H, R^2 = Me)	3.25 dd (11.2,4.6)	1.12 dd (10.9,4.7)	5.54 m	5.15 s	4.25 s	2.55 dq (6.9,8.5)	1.34 d (6.9)	0.51 s	0.89	0.95	1.01	1.01	3.65

Table 2. ^{13}C N.m.r. resonances (δ, number of bonded H in parentheses) for the esters (**1**; R = Me) and (**2**; R^1 = H, R^2 = Me) and for dihydroagnosterol

Position	Dihydroagnosterol[6]	(**1**; R = Me)	(**2**; R^1 = H, R^2 = Me)
1	35.8	35.9(2)	35.7(2)
2	28.2	27.9(2)	27.8(2)
3	79.0	79.0	78.8
4	38.5	38.8(0)	38.7(0)
5	49.2	49.3(1)†	49.1(1)†
6	23.0	23.1(2)	23.1(2)
7	120.1	120.8	121.0*
8	142.7	141.9	141.5
9	145.9	145.8	147.9
10	37.4	37.5(0)	37.3(0)
11	116.3	115.9	122.4*
12	37.9	37.8(2)	76.5
13	43.8	43.9(0)	48.8(0)
14	50.4	50.3(0)	51.2(0)
15	28.1	26.9(2)	25.9(2)
16	31.6	31.7(2)	31.7(2)
17	51.1	48.2(1)†	49.3(1)†
18	15.7	15.8(3)*	9.7(3)
19	22.8	22.7(3)	22.7(3)
20	36.5	43.1(1)	41.6(1)
21	18.6	17.0(3)	18.4(3)
22	36.3	177.5	178.2
28	25.6	25.6(3)	25.3(3)
29	27.9	28.2(3)	28.1(3)
30	15.8	16.0(3)*	15.8(3)
OMe		51.3(3)	51.3(3)

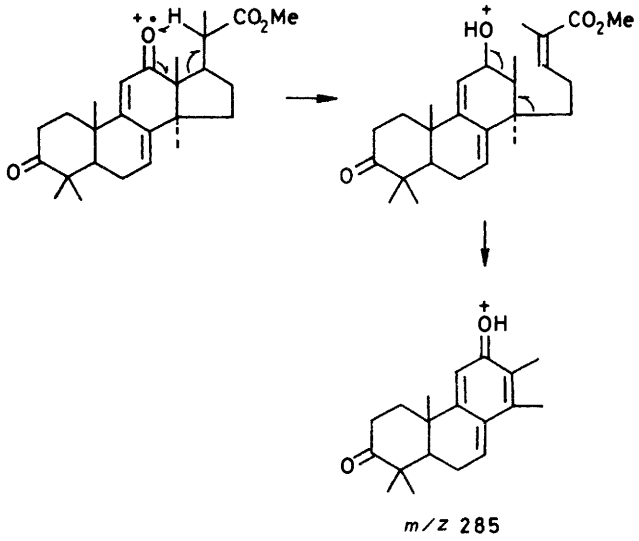
*† Assignments may be reversed.



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

interaction between the 18- and 21-Me groups. The diketone which results from chromic oxide oxidation of the methyl ester (**2**; R^1 = H, R^2 = Me) has structure (**3**).

Equally convincing evidence for 12β-substitution was obtained from a study (Table 2) of the ^{13}C n.m.r. spectra of the esters (**1**; R = Me) and (**2**; R^1 = H, R^2 = Me). Except for positions 17, 20, and 21, where the differences resulting from the replacement of a CH_2 at position 22 by CO_2Me were as expected, the ^{13}C resonances for the ester (**1**; R = Me) agreed



Scheme 2. Mass spectral fragmentation of the ester (**3**)

well with those reported[6] for dihydroagnosterol and were assigned without serious ambiguity with the aid of the spin-echo subspectra. The resonance at δ 37.8 assigned to position 12 in the ester (**1**; R = Me) was eliminated in the ester (**2**; R^1 = H, R^2 = Me) by the introduction of the second OH group. Other resonances affected by this substitution were those assigned to positions 11, 13, and 18 and the upfield shift of C-18 was consistent only with the 12-OH in a β-configuration by analogy with the spectra of the epimeric androstan-12-ols.[7] The resonance at δ 23.1 assigned to position 6 in the ester (**1**; R = Me) was seen unchanged in the ester (**2**; R^1 = H, R^2 = Me), as were those resonances assigned to positions 4, 5, 29, and 30, all positions likely to be affected by the introduction of a 6-hydroxy substituent.[7]

The mass spectral fragmentation pattern of the acid (**1**; R = H), and its derivatives,[1] underwent a marked change on the introduction of a 12-hydroxy substituent. With the acid (**2**; R^1 = R^2 = H) and its derivatives, although loss of the C-17 substituent was still an important pathway (see Scheme 1), the elision of ring A was now of equal prominence. These pathways gave, from the acid (**2**; R^1 = R^2 = H), after the additional loss of the elements of water, fragments of composition $C_{22}H_{31}O^+$ (*m/z* 311) and $C_{16}H_{20}O_2{}^+$ (*m/z* 244) respectively.

The mass spectrum of the ester (**3**) also provided strong support for the presence of a 12-ketone. Although loss of the C-17 substituent, to give an ion of *m/z* 325, still occurred, the main fragmentation process was a McLafferty rearrangement leading to the ion *m/z* 285 (Scheme 2).

The $C_{25}H_{38}O_4$ hydroxy acid is therefore considered to have structure (**2**; R^1 = R^2 = H). The accompanying $C_{25}H_{40}O_4$ hydroxy acid[1] is probably the corresponding 8-ene (**4**), but with insufficient material available for n.m.r. spectroscopy there is no

solid evidence for an identical hydroxylation pattern in this compound.

Oxidation of the methyl ester (**1**; R = Me) with chromic oxide–sulphuric acid furnished the 3-ketone (**5**; R = Me) identical with the methyl ester of the $C_{25}H_{36}O_3$ keto-acid isolated[1] as a minor metabolic product of *V. lecanii*. This acid, therefore, has structure (**5**; R = H) and the mass spectral fragmentation of this compound,[1] giving ions at *m/z* 311 and 269, is consistent with the structure proposed.

In addition to the acids (**1**; R = H), (**2**; $R^1 = R^2 = H$), and (**5**; R = H), three neutral keto alcohols of composition $C_{25}H_{38}O_4$, $C_{25}H_{40}O_4$, and $C_{25}H_{40}O_5$ were isolated[1] from the *V. lecanii* culture filtrate in very low yield. The $C_{25}H_{40}O_4$ keto alcohol (**6**; R = H) ($\nu_{max.}$ 3 320br and 1 650 cm^{-1}) formed a diacetate (**6**; R = Ac) which contained one, presumably tertiary, hydroxy function ($\nu_{max.}$ 3 540 cm^{-1}). This is assigned to position 20 since, in the ^1H n.m.r. spectrum, the 20-Me signal (δ 1.40) was a singlet. This spectrum also showed the absence of vinylic hydrogens supporting the presence of the tetrasubstituted enone chromophore indicated by the u.v. absorption ($\lambda_{max.}$ 255 nm, log ε 3.7). A major fragment (*m/z* 250) in the mass spectrum of the keto alcohol had the composition $C_{15}H_{22}O_3^+$, indicating that the keto group was most probably at position 7,[8] and that the compound contained the 8-en-7-one group. The ^1H n.m.r. spectrum of the diacetate was consistent with the presence of CH_2OAc and CHOAc groupings. The latter was shown by the mass spectrum (fragment at *m/z* 292) to be present in ring A: the ^1H n.m.r. coupling constants indicated that the hydrogen was axial and at position 1 or 3 and the chemical shift was consistent with that expected for position 3. 3β,20,21-Trihydroxy-4,4,14α,20-tetramethyl-5α-pregn-8-en-7-one (**6**; R = H), without specifying the configuration at position 20, is therefore the most probable structure for the parent keto alcohol, but there is no formal proof of the position of the ring A hydroxy group.

The mass spectrum of the $C_{25}H_{40}O_5$ keto alcohol also contained the $C_{15}H_{22}O_3^+$ fragment suggesting that this compound was also an 8-en-7-one, but with two hydroxy substituents in ring A. Lanost-8-en-7-ones are well recognised auto-oxidation products of the lanosta-7,9(11)-diene system, but 7,9(11)-dienes corresponding to these keto alcohols were not isolated.

Experimental

Unless stated otherwise, n.m.r. spectra were obtained in $CDCl_3$ on a Bruker WH360 FT instrument at 360 MHz (^1H) and 90.55 MHz (^{13}C). Chemical shifts were recorded as δ(p.p.m.) from $SiMe_4$ (internal standard). CH_3/CH and CH_2/Cq ^{13}C subspectra for the aliphatic region were generated by spin-echo pulse sequences. Within these subspectra quaternary carbons (Cq) were identified by their relative relaxation times in the proton noise decoupled spectra and CH_3 resonances on the basis of chemical shift. Other experimental conditions have been described previously.[1]

3β,12β-Dihydroxy-4,4,14α-trimethyl-5α-pregna-7,9(11)-diene-20S-carboxylic Acid (**2**, $R^1 = R^2 = H$).—The acid was obtained as before,[1] *m/z* (% base peak) 402(7), 384(69), 369(22), 329.2450(8), 311.2357(100), 246(57) and 244.1472(82). $C_{22}H_{33}O_2^+$, $C_{21}H_{31}O^+$, and $C_{16}H_{20}O_2^+$ require 329.2480, 311.2375, and 244.1463 respectively. Mass spectra of the methyl ester (**2**; $R^1 = H$, $R^2 = Me$),[1] *m/z* 416(11), 398(12), 384(16), 329(7), 311(11), 276(5), 244(8), and 43(100); the diacetate (**2**; $R^1 = Ac$, $R^2 = H$),[1] *m/z* 486(1), 443(11), 426(22), 366(9), 353(58), 244(35), and 43(100) and the triacetyl derivative (**2**; $R^1 = R^2 = Ac$);[1] *m/z* 468(5), 426(18), 371(35), 353(37), 329(30), 311(10), 288(16), 253(29) and 43(100) were also obtained.

Methyl 4,4,14α-Trimethyl-3,12-dioxo-5α-pregna-7,9(11)-diene-20S-carboxylate (**3**).—The ester (**2**; $R^1 = H$, $R^2 = Me$) (3 mg) in acetone (0.2 ml) at 0 °C was treated with the chromic oxide–sulphuric acid reagent[3] (3 μl) during 20 min. After most of the solvent had been removed in a stream of N_2, water was added, and the emulsion was extracted with ethyl acetate. The organic layer was washed with aqueous sodium hydrogen carbonate and dried. The recovered product crystallised from methanol as prisms (2 mg), m.p. 214—217 °C, R_F 0.67, of the *diketone* (**3**) (Found: C, 75.2; H, 8.8%; *M*, 412.2591. $C_{26}H_{36}O_4$ requires C, 75.7; H, 8.8%; *M*, 412.2613); $\nu_{max.}$ 1 738, 1 712, 1 678 and 1 640 cm^{-1}; $\lambda_{max.}$ 291 nm (log ε 4.08); *m/z* 412(20), 397(5), 381(3), 325(10), 285(35), and 55(100).

Methyl 4,4,14α-Trimethyl-3-oxo-5α-pregna-7,9(11)-diene-20S-carboxylate (**5**; R = Me).—The ester (**1**; R = Me)[1] (3 mg) was oxidised with chromic oxide as described above. The solid product crystallised from methanol as plates (2 mg) of the *keto ester* (**5**; R = Me), m.p. 186—188 °C, R_F 0.67 (Found: C, 78.3; H, 9.7%; *M*, 398.2829. $C_{26}H_{38}O_3$ requires C, 78.3; H, 9.6%; *M*, 398.2821); $\nu_{max.}$ 1 740 and 1 712 cm^{-1}. It was identical (mixed m.p.) with the methyl ester of the $C_{25}H_{36}O_3$ keto acid isolated[1] from *V. lecanii*.

C_{25} *Keto Alcohols from* V. lecanii.—The $C_{25}H_{40}O_4$ keto alcohol (**6**; R = H)[1] showed *m/z* 404(87) 386(34), 371(42), 353(19), 250.1561(71), and 95(100) ($C_{15}H_{22}O_3^+$ requires *m/z* 250.1569). The *diacetate* (**6**; R = Ac), prepared in pyridine with acetic anhydride,[1] crystallised from benzene–light petroleum as prisms, m.p. 230—240 °C (decomp.), R_F 0.70 (Found: *m/z* 488.3136. $C_{29}H_{44}O_6$ requires *M*, 488.3138); $\nu_{max.}$ 3 540, 1 740, 1 735, and 1 645 cm^{-1}; $\lambda_{max.}$ 255 nm; *m/z* 488(31), 473(11), 470(9), 428(18), 413(20), 395(18), 292(82), and 95(100); δ_H (90 MHz) 0.8—1.2 (15 H, 5 C-Me) 1.40 (s, 3 H), 1.4—2.3 (13 H), 2.06 (s, 6 H, 2 Ac), 2.42 (m, 3 H), 3.95 (AB, 2 H, *J* 11 Hz), 4.5 (dd, 1 H, *J* 11, 5 Hz), and 4.6 (br s, 1 H, OH).

The $C_{25}H_{40}O_5$ keto alcohol[1] showed *m/z* 420(12), 402(29), 387(19), 369(9), 250.1573(23), 95(89), and 55(100) ($C_{15}H_{22}O_3^+$ requires *m/z* 250.1569).

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References

1 Part 1, N. Claydon, J. F. Grove, M. Pople, and M. J. Begley, *J. Chem. Soc., Perkin Trans. 1*, 1984, 497.
2 N. Claydon and J. F. Grove, *J. Invertebr. Pathol.*, 1982, **40**, 413.
3 K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 1946, 39.
4 J. J. H. Simes, M. Woolton, B. J. Ralph, and J. T. Pinhey, *Chem. Commun.*, 1969, 1150.
5 J. T. Pinhey, B. J. Ralph, J. J. H. Simes, and M. Woolton, *Aust. J. Chem.*, 1971, **24**, 609.
6 S. A. Knight, *Org. Magn. Reson.*, 1974, **6**, 603.
7 H. Eggert, C. L. Van Antwerp, N. S. Bhacca, and C. Djerassi, *J. Org. Chem.*, 1976, **41**, 71.
8 J. R. Dias, *Org. Mass Spec.*, 1973, **11**, 333.

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