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# 23,24,25,26,27-PENTANORLANOST-8-EN-3β,22-DIOL FROM *VERTICILLIUM LECANII**

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**Key Word Index**—*Verticillium lecanii*; fungus; biosynthesis; pentanorlanost-8-en-3,22-diol.

**Abstract**—23,24,25,26,27-Pentanorlanost-8-en-3β,22-diol has been isolated from the mycelium of the fungus *Verticillium lecanii*. Addition of lanosterol to the culture medium did not significantly increase the yields either of the pentanorlanostane metabolites or of ergosterol.

## INTRODUCTION

The pentanorlanost-7,9(11)-diene acids (**1**; R = H and OH) have been identified, albeit in low yield, among the insecticidal metabolic products from the culture media of certain strains of the entomopathogenic fungus *Verticillium lecanii* (Zimm.) [1–3]. In the course of an unsuccessful attempt to increase the yields of these acids by incorporating lanosterol in the fermentation medium, a novel pentanorlanost-8-ene, shown by spectroscopic methods to be the diol (**2**; R = H), has been isolated from the mycelium from both lanosterol-enriched and control fermentations.

## RESULTS AND DISCUSSION

Although the position of lanosterol in the biosynthetic pathway from mevalonate to the fungal steroids is secure [4, 5], there are no reports of the successful use of lanosterol as a fermentation substrate for increasing the yield of steroidal fungal metabolic products. Thus in the present work the yield of ergosterol isolated from the mycelium was essentially the same for both lanosterol-enriched and control fermentations.

Although the total yield of the acids (**1**; R = H and OH), obtained by solvent extraction of the culture medium at pH 5.0, was increased from ~0.5 to ~1 mg/l, this increase is not considered significant in relation to the amount of lanosterol added (50 mg/l), 75% of which was recovered unchanged from the mycelium. However, it would be interesting to repeat this work using agnosterol as substrate since 7,9(11)-dienes were present to the extent of 2.5% in the lanosterol used and there was analytical evidence that this value had been diminished in the recovered material.

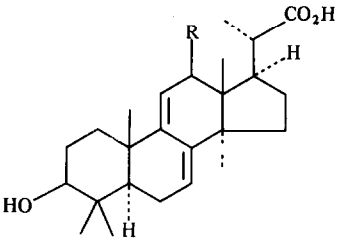
The petrol extract of the dried powdered mycelium was subjected to column chromatography and, after glycerides, lanosterol and ergosterol had been eluted, benzene–diethyl ether (1:1) furnished a novel $C_{25}H_{42}O_2$ diol ($\nu_{max}$ 3380 $cm^{-1}$) which formed a diacetate ($\nu_{max}$ 1740, 1728 $cm^{-1}$). The $^1H$ NMR spectrum of the diol showed the presence of one CHMe and five C–Me groups. Except for the resonances assigned to C-17, C-20 and C-21, the $^{13}C$ NMR spectrum corresponded closely with that of lanosterol (Table 1) and showed the presence of one tetrasubstituted ethylenic linkage [$\delta_C$ 134.3 (0), 134.6 (0)] assigned to position C-8 (9) of a lanostane ring system. One hydroxyl group was secondary [$\delta_C$ 79.0 (1); $\delta_H$ 3.21 ($J$ = 11,5 Hz)] and the chemical shifts and coupling constants were consistent with 3β-substitution. The

Table 1. $^{13}C$ NMR resonances (δ, number of bonded H in parentheses) for lanosterol and the diol (**2**; R = H)

| | Compound | |
|---|---|---|
| Position | Lanosterol | (**2**; R = H) |
| 1 | 35.7 (2) | 35.6 (2) |
| 2 | 27.9 (2) | 27.6 (2)* |
| 3 | 79.0 (1) | 79.0 (1) |
| 4 | 39.0 (0) | 38.9 (0) |
| 5 | 50.6 (1) | 50.4 (1) |
| 6 | 18.3 (2) | 18.3 (2) |
| 7 | 28.2 (2) | 27.9 (2)* |
| 8 | 134.0 (0) | 134.3 (0)† |
| 9 | 134.4 (0) | 134.6 (0)† |
| 10 | 37.2 (0) | 37.0 (0) |
| 11 | 21.1 (2) | 21.0 (2) |
| 12 | 26.6 (2) | 26.5 (2) |
| 13 | 44.6 (0) | 44.7 (0) |
| 14 | 49.9 (0) | 49.6 (0) |
| 15 | 30.9 (2) | 30.9 (2)‡ |
| 16 | 31.1 (2) | 31.0 (2)‡ |
| 17 | 50.6 (1) | 46.8 (1) |
| 18 | 15.8 (3) | 15.9 (3)§ |
| 19 | 19.1 (3) | 19.1 (3) |
| 20 | 36.3 (1) | 39.5 (1) |
| 21 | 18.7 (3) | 16.7 (3) |
| 22 | 36.4 (2) | 68.2 (2) |
| 28 | 24.3 (3) | 24.3 (3) |
| 29 | 28.0 (3) | 28.0 (3) |
| 30 | 15.4 (3) | 15.4 (3)§ |

*†‡§Assignments may be reversed.

*"New Metabolic Products of *Verticillium lecanii*", Part 3. For Part 2 see ref. [1].

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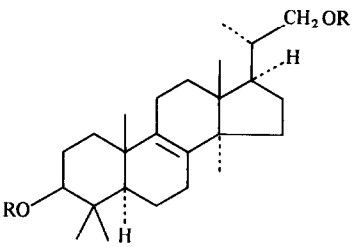


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second hydroxyl group was primary [$\delta_C$ 68.2(2); $\delta_H$ 3.34, 3.63 ($J_{AB}$ = 10.5 Hz)] and, since the $^1$H NMR signals formed the AB part of an ABX system, was located at C-21 or C-22. Of these two possibilities C-22 is favoured on biogenetic grounds.

In addition to ions resulting from the loss of $CH_3$ and $H_2O$, the mass spectrum showed the normal lanost-8-ene fragmentation [3] with loss of the C-17 substituent followed by 42 mass units giving fragments at *m/z* 315, 273 and (loss of $H_2O$ from ring A) 255.

All this spectroscopic evidence is consistent with the pentanorlanost-8-ene structure (**2**; R = H) for the diol. The compound is mentioned in the literature [6] but only as a mixture with the 25,26,27-trisnorlanost-8-ene analogue.

The $^{13}$C NMR resonance assignments for lanosterol (Table 1) are those deduced by Knight [7] except that the INEPT subspectra indicated that his C-18 and C-6 assignments needed to be reversed.

## EXPERIMENTAL

Experimental details have been recorded previously [1, 3]. The lanosterol used in the fermentation contained 7,9(11)-diene impurities equivalent (UV absorbance) to 2.5% agnosterol.

*Isolation of metabolic products. V. lecanii* strain 64 was grown on Czapek-Dox medium (200 ml layer) in Roux bottles (100) as described elsewhere [2]. After 14 days lanosterol (10 mg) in EtOH (0.5 ml) was added to each of 50 bottles and dispersed by gentle rocking. The remaining 50 bottles constituted the control fermentation. The pH and optical rotation of the culture fluid from the lanosterol-enriched and control fermentations were measured twice weekly [2] but no significant differences were seen.

After 36 days the fermentations were harvested in the usual way [2] and the culture filtrates (7.5 l. each) were extracted at pH 5.0 with EtOAc. The extracts (253 mg, control 327 mg) were absorbed on silica gel (3 g) from EtOAc (5 ml) and added to columns of silica gel (8 g, 1.2 × 20 cm) made up in petrol. The columns were eluted with (i) $Et_2O$–petrol (1:4, 50 ml) followed by $Et_2O$ (25 ml portions) giving the following fractions (i) 10 mg, control 18 mg, (ii) 12, 18 mg, (iii) 52, 53 mg, (iv) 28, 30 mg, (v) 18, 19 mg. Fractions (i), (iii) and (v) were discarded. Trituration of fraction (ii) with MeOH afforded the acid (**1**; R = H) (3.5, 2.0 mg). Fraction (iv) crystallised from MeOH in felted needles (3.0, 1.5 mg) of the acid (**1**; R = OH). Both acids were identified by their IR and mass spectra.

The mycelium was dried *in vacuo*, and, after cooling in liquid $N_2$, was powdered in a steel mortar. The powder (83.9 g, control 77.9 g) was extracted in a Soxhlet apparatus with petrol for 8 hr (2.97 g, control 2.50 g), followed by EtOAc (8 hr, 1.52 g, control 1.14 g). The extracts (1.50 g portions) in $C_6H_6$ (3 ml) were chromatographed on silica gel (30 g, 30 × 1.6 cm) made up in $C_6H_6$ and the columns were eluted with (i) $C_6H_6$, 100 ml (ii) and (iii) $C_6H_6$–$Et_2O$ (4:1), 100 ml (iv) and (v) $C_6H_6$–$Et_2O$ (1:1), 50 ml and (vi) $Et_2O$, 100 ml.

The two petrol extracts gave the following materials: lanosterol-enriched (L) (i) gum, 64 mg (ii) gel, 1221 mg (iii) solid, 121 mg (iv) gum, 7 mg (v) solid, 18 mg (vi) gum, 13 mg; control (C) (i) gum, 64 mg (ii) oil, 1054 mg (iii) solid, 162 mg (iv) gum, 7 mg (v) solid, 22 mg (vi) gum, 17 mg. Crystallisation of fractions L (iii) and C (iii) from MeOH furnished ergosterol, 73 mg (19 mg/l.) and 75 mg (17 mg/l.) respectively, identified by the IR spectrum. Crystallisation of fractions L(v) and C(v) from MeOH furnished the diol (**2**; R = H) 4 mg and 5 mg respectively (see below).

The IR spectrum of fraction L(ii) was similar to C(ii) (glycerides $\nu_{max}$ 1740 cm$^{-1}$) but had a band at 1022 cm$^{-1}$, absent from C(ii) but present in lanosterol. The gel was repeatedly extracted with boiling MeOH and the extract crystallised twice from $C_6H_6$ giving needles (230 mg, 92% recovery) mp 110–130°, the IR spectrum of which corresponded closely to that of lanosterol. A portion (10.0 mg) of this material was subjected to preparative TLC in $CHCl_3$–MeOH (95:5). Elution of a band $R_f$ 0.57 and crystallisation of the recovered material from MeOH furnished needles (8.1 mg, 75% recovery overall) mp 142–143° of lanosterol identified by the IR spectrum. The UV spectrum showed 7,9(11)-diene impurities equivalent to 1.8% agnosterol. No lanosterol was obtained when the same procedure was applied to fraction C(ii).

The EtOAc extract of the powdered mycelium from the lanosterol-enriched fermentation gave the following fractions: (i) gum, 15 mg (ii) oil, 730 mg, the IR spectrum of which was similar to fraction Cii (above) (iii) semi-solid, 102 mg (iv) gum, 28 mg (v) oil, 82 mg (vi) oil, 71 mg. No crystalline products were obtained from these fractions and the corresponding extract from the control fermentation was not examined.

23,24,25,26,27-*Pentanorlanost*-8-*en*-3$\beta$,22-*diol* (**2**; R = H). The diol (**2**; R = H) crystallised from MeOH in prisms or needles mp 206—207° after a transition at 90° (loss of solvent) $R_f$ 0.37. [Found (dried at 100°): C, 79.6; H, 11.4%; $[M]^+$ at *m/z* 374.3186. $C_{25}H_{42}O_2$ requires C, 80.1; H, 11.3%; $[M]^+$ 374.3185.] $\nu_{max}$ 3380, 1622 (w) cm$^{-1}$. UV: end absorption only. $^1$H NMR (360 MHz): $\delta$ 0.70, 0.79, 0.86, 0.96, 0.98 (all *s*, 3H), 1.01 (*d*, *J* = 6 Hz, 3H), 1.1–2.0 (19H), 2.0 (*br*, 2OH), 3.21 (*dd*, *J* = 11,5 Hz, H-3), 3.34, 3.63 (AB, *J* = 10.5, 6.5, 2.0 Hz, H-22); MS *m/z* (rel. int.): 374 (30), 359 (100), 341 (65), 324 (25), 315 (3), 273 (5), 255 (5).

The diacetate (**2**; R = Ac), prepared in pyridine with $Ac_2O$ during 2 hr at room temp, crystallized from MeOH in plates mp 175–177°. Found: $M^+$ at *m/z* 458.3381 $C_{29}H_{46}O_4$ requires $M^+$ 458.3396. IR $\nu_{max}$ 1740, 1729 cm$^{-1}$; $^1$H NMR (90 MHz): $\delta$ 0.70 (*s*, 3H) 0.86 (*s*, 9H) 0.99 (*m*, 6H) 2.03 (*s*, 6H, 2Ac) 3.75, 4.05 (AB, *J*

= 10.5, 7, 2.5 Hz H-22) 4.5 (*dd*, *J* = 10.5 Hz); MS *m/z* (rel. int.): 458 (22), 443 (35), 383 (80), 323 (80), 43 (100).

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