Tetrahedron Vol 24, pp 3949 to 3953. Pergamon Press 1968 Printed in Great Britain

23ξ-HYDROXY-LANOSTEROL

A NEW TRITERPENE FUNGAL METABOLITE OF THE BASIDIOMYCETE *SCLERODERMA AURANTIUM* PERS

N. ENTWISTLE and A. D. PRATT

School of Chemistry, University of Bradford

(*Received in the UK* 13 *November* 1967; *accepted for publication* 11 *December* 1967)

Abstract—Extraction of the peridium of *Scleroderma Aurantium* yields a new dihydroxy tetracyclic triterpene of the lanostane series-23ξ-hydroxylanosterol (I).

THE Basidiomycete *Scleroderma aurantium* is a common fungus and is known as the Common Earth-ball. The metabolites of this fungus were first investigated by Bamberger and Landsiedl[1] who obtained from the peridium crystalline substances $C_{22}H_{36}O_2$ and $C_{21}H_{34}O_2$, which gave positive Liebermann–Burchardt reactions, and mannitol. Apparently no further tests were carried out. Zellner[2] reported the isolation from the fungus of fumaric acid, glycerine and mannitol as well as a crystalline substance which gave positive Salkowski and Liebermann reactions. Our own work does not support the formulae quoted above.

Specimens were collected from oak woods near Shipley and Wakefield. Petroleum spirit (b.p. 60–80°) extraction of the dried, ground peridium yielded a crude, crystalline extract which on TLC examination was seen to consist of four components. The major component (estimated as >98% of the mixture) was isolated by thick layer chromatography (PLC) and crystallization as colourless needles $C_{30}H_{50}O_2$, m.p. 158–160·5°, ν_{max} ($CHCl_3$) 3616 cm^{-1} (ν-OH) and 1024 cm^{-1} (ν-C—O).

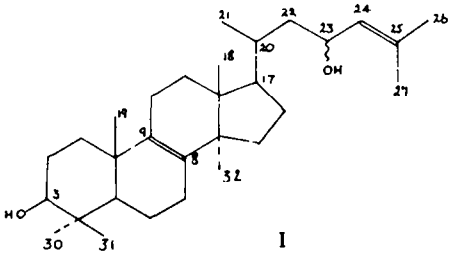
The Liebermann–Burchardt test indicated that this compound is steroidal or triterpenoid. Confirmation was obtained from the NMR spectrum which shows 7 intense, sharp peaks (in the high field region) which are characteristic signals of the Me groups of triterpenoid compounds. The compound shows no significant UV absorption above 210 mμ.

The compound formed a diacetate $C_{34}H_{54}O_4$, m.p. 165–166·5°, confirming the presence of two OH groups. The tetranitro-methane unsaturation test was positive and the number of double bonds was estimated by titrations with perbenzoic acid in chloroform. These titrations indicated the presence of two double bonds. From the above data it was concluded that the compound is a dihydroxy tetracyclic triterpene.

The diol (I) absorbed one mole of hydrogen over platinum to form a dihydro derivative $C_{30}H_{52}O_2$, m.p. 179–180°. This compound still showed a positive tetranitromethane reaction. The remaining double bond is resistant to normal hydrogenation techniques. Such a double bond in a tetracyclic triterpene is most likely to be in the C-8, C-9 position.

The carbon skeleton of the diol (I) was determined as follows: the dihydro diol was oxidized to the corresponding crystalline diketone $C_{30}H_{48}O_2$, m.p. 101·5–103°, ν_{max} 1707 cm^{-1} (ν-C=O), by means of aqueous chromic acid solution; Wolff–

Kishner reduction of this diketone then yielded a hydrocarbon $C_{30}H_{52}$ which crystallized as plates m.p. 73–74°. This hydrocarbon was found to be identical to lanost-8-ene (obtained by Wolff–Kishner reduction of lanost-8-ene-3-one) by comparison of m.p., $[\alpha]_D$, IR and mass spectra. The diol (I) thus belongs to the (13β, 14α, 17α H)-lanostane series. This evidence along with the observation that there are no olefinic proton signals in the NMR spectrum of the dihydro diol establishes the presence of a C-8, C-9 double bond and confirms the existence of only two double bonds in the diol (I).



I

The presence of one of the OH groups at C-3 was confirmed from the 100 MHz NMR spectrum of the diol (I). This spectrum exhibits a one proton quartet at 6·82 τ, with observed splittings of 10 and 4·5 Hz, the X component of an ABX system. This signal is also present in the spectrum of lanost-8-ene-3β-ol and is due to the C-3 proton. The large splitting of 10 Hz is indicative of an axial–axial interaction between the C-3 proton and the C-2 axial proton. The smaller splitting of 4·5 Hz is clearly indicative of an axial–equatorial interaction between the C-3 proton and the C-2 equatorial proton. The C-3 OH group is therefore equatorial.[3] On acetylation the quartet due to the C-3 axial proton appears at 5·56 τ.

Further evidence for a 3β-OH group was shown by the occurrence of a 3-proton singlet at 9·22 τ due to the C-31 Me group and a 6-proton singlet at 9·03 τ produced by the overlapping signals of the C-30 and C-19 Me groups. On acetylation the C-30 and C-31 Me signals move to 9·14 τ which is the position of .he C-32 resonance peak. This convergence, on acetylation of the C-3-OH group, of the C-30 and C-31 Me signals is characteristic, with certain exceptions, of triterpenes of the lanostane series containing a 3β-OH group.[4,5]

Ozonolysis of the diol (I) yielded acetone which was isolated as its 2,4-dinitrophenylhydrazone. This result is indicative of the presence of an isopropylidene group in the side chain. Confirmation of this was obtained in the 100 MHz spectrum of the diol (I) by the occurrence of a 6-proton singlet at 8·35 τ, due to the C-26 and C-27 Me resonances of a side chain isopropylidene group. This singlet is not present in the spectrum of the dihydro diol. It is replaced by two new 3-proton peaks at 9·08 τ and 9·14 τ, typical of a side chain isopropyl grouping;[4] as the signal at 9·14 τ is coincident with the C-32 Me signal, the peak appears as a 6-proton singlet. This evidence confirms the presence of a Δ^{24-25} double bond.

The IR CO absorption of the 24,25-dihydro diketone at 1707 cm^{-1} precludes the positions C-15 and C-16 as possible sites for the second OH group since 5-membered ring ketones absorbed at 1745 cm^{-1}. In the mass spectra of C-17 sub-

stituted steroids and tetracyclic triterpenes a characteristic peak is that corresponding to the elimination of the side chain plus 42 mass units due to cleavage of the C-13, C-17 and C-14, C-15 bonds. In the spectrum of the diol (I) the fragment left after this cleavage is at *m/e* 273 [M—($C_8H_{15}O$) + 42]. The second OH group must therefore be situated in the side chain; as it is secondary it is at C-22 or C-23.

Oxidation of the diol (I) with chromium trioxide in pyridine gave a crystalline diketone $C_{30}H_{46}O_2$, 151–153·5°, λ_{max} 2386 Å (ε 13,700) (αβ-unsaturated ketone), ν_{max} (KBr disc) 1707 cm^{-1} (6-membered ring ketone), 1675 cm^{-1} (αβ-unsaturated ketone), 1608 cm^{-1} (ν-C=C). The second keto group is therefore conjugated with the side chain double bond. As the oxidation was carried out under conditions where one would not expect double bond migration the second OH group in the diol (I) must be situated at C-23. In accord with this conclusion the C-26 and C-27 Me signals in the 100 MHz spectrum of the diketone appear at 7·89 and 8·15 τ. The Me groups are no longer equivalent and are more strongly deshielded than in the case of the diol (I), a consequence of the magnetic anisotropy of the C-23 keto group.

The side chain protons of the diol (I) give rise to a complex spin system. We find that we can apply to this system a simplified treatment which is justified since our predictions of the effects of double resonance are verified by experiments on a 100 MHz instrument.

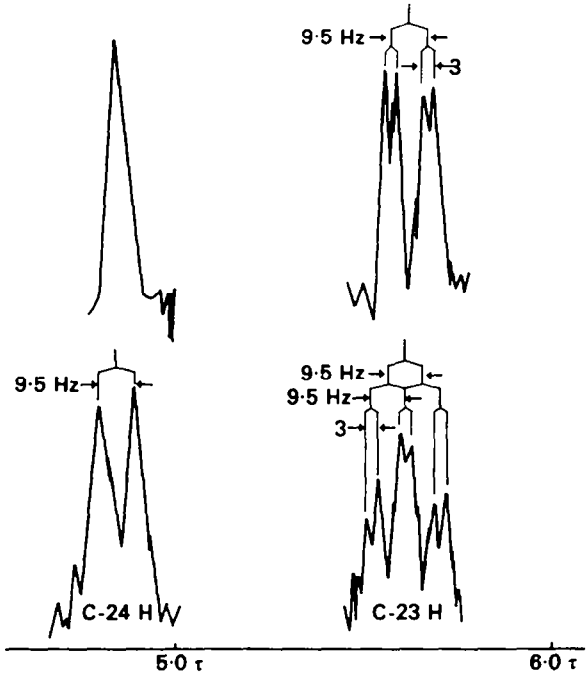


FIG. 1 Double resonance experiment on 23ξ-hydroxylanosterol showing the collapse of the C-24 H doublet to a singlet on irradiation at 5·64 τ, the C-23 H sextet collapsing to a quartet on irradiation at 4·88 τ.

The side chain protons of the diol (I) can be considered to give rise to an ABMX system where A and B are the non-equivalent C-22 protons whose signals are obscured by the methylene envelope.

The C-23(M) proton gives rise to a sextet centered at 5·64 τ (Fig. 1). On acetylation of the diol this signal moves downfield to 4·46 τ confirming that the M proton is situated on the C atom carrying a secondary OH group.[6] The X (C-24) proton gives rise to a doublet at 4·88 τ, J_{MX} 9·5 Hz; on irradiation at 5·64 τ with a frequency of 2942·7 Hz this doublet collapses to a singlet. On irradiation at 4·88 τ with a frequency of 3018·3 Hz the sextet at 5·64 τ collapses to a quartet with observed splittings of 9·5 and 3 Hz. This quartet can now be regarded as the four line X portion of an ABX system ($J_{AX} \neq J_{BX}$) where the C-23 proton becomes the X component.

During the course of the structural elucidation it was observed that the diol (I) is extremely acid labile. Work is proceeding on the artefacts from acid treatment, and their possible relationship to the minor components of the crude extract, as well as on the absolute configuration at C-23.

EXPERIMENTAL

M.ps were determined on a Kofler hot-stage apparatus and are uncorrected. Rotations were determined for $CHCl_3$ solns at room temp on a photoelectric polarimeter. UV spectra were determined on EtOH solns. TLC was carried out on unactivated silica gel on glass, with a stationary phase thickness of 0·2 mm, the developing solvent being benzene–EtOAc (4:1). The positions of the triterpenes were determined by spraying with an ethanolic soln of Ac_2O and conc H_2SO_4, followed by heating at 110° for 10 min. PLC was carried out on unactivated silica gel (Merck Kieselgel $PF_{254+366}$) supported on glass with a stationary phase thickness of 2 mm, the developing solvent being benzene–EtOAc (4:1).

Extraction of the fungus. The central spore mass of each freshly collected, mature sporophore of the fungus was removed. The dried, ground peridium (1300 g) was then extracted in a Soxhlet apparatus with petrol (b.p. 60–80°) to yield the crude, solid extract (80 g). Crystallization from EtOAc (2×) yielded colourless needles m.p. 150–180°.

Preliminary investigation of the crude extract. In the Liebermann–Burchardt colour test the extract gave a very intense fluorescent green $CHCl_3$ layer and a brown acid layer. A TLC investigation showed the crystalline extract to comprise 4 components: in order of decreasing predominance A (R_f 27·6), B (R_f 19·2), D (R_f 42·9) and C (R_f 36·5).

Purification of the extract. In a typical PLC run, 172 mg of pure I was recovered from 200 mg crude fungal extract. PLC and crystallization from EtOAc yielded the major component (the diol I) in a chromatographically pure state as needles m.p. 158–160·5°, $[\alpha]_D$ + 66° (c, 0·30), (Found: C, 81·8; H, 11·3. $C_{30}H_{50}O_2$ requires: C, 81·5; H, 11·3%), M^+. *m/e* 442, ν_{max} ($CHCl_3$) 3616, 2950, 2878, 2841, 1455, 1373, 1024 and 832 cm^{-1}.

Acetylation of the diol (I). Acetylation with Ac_2O–pyridine afforded the diacetate which crystallized from EtOAc as plates m.p. 165–166·5°, $[\alpha]_D$ + 55° (c, 0·31). (Found: C, 78·0; H, 10·25. $C_{34}H_{54}O_4$ requires: C, 77·6; H, 10·3%), ν_{max} (KBr disc) 1735, 1640, 1240, 938 and 836 cm^{-1}.

Hydrogenation of the diol (I). The diol (104 mg) in EtOH (95%, 20 ml) was hydrogenated at 20° and atmo press over Adams' catalyst (30 mg) until the uptake of H_2 was complete, ca. 1·1 mol. After PLC the product crystallized from EtOAc to give the dihydro diol (83 mg) as fine needles m.p. 179–180°, $[\alpha]_D$ + 60° (c, 0·32), (Found: C, 81·2; H, 11·8. $C_{30}H_{52}O_2$ requires: C, 81·1; H, 11·7%). The product still showed a positive tetranitromethane reaction.

Oxidation of the dihydro diol. The diol (503 mg) in acetone (30 ml) was oxidized at room temp by the dropwise addition of aqueous chromic acid soln (8N w.r.t.O_2). Excess oxidant was destroyed with MeOH, the whole diluted with water and extracted (5×) with ether. The diketone (403 mg) crystallized from MeOH as rods m.p. 101·5–103°, $[\alpha]_D$ + 64° (c, 0·31), (Found: C, 81·4; H, 10·9. $C_{30}H_{48}O_2$ requires: C, 81·8; H, 10·9%); ν_{max} ($CHCl_3$) 1707 cm^{-1}; RD (c, 0·12; MeOH) $[\Phi]_{400}$ 0°; $[\Phi]_{314}$ −570°; $[\ ]_{270}$ +3780° (shoulder); $[\Phi]_{222}$ +11700°.

Wolff–Kishner reduction of the dihydro diketone. The diketone (120 mg), hydrazine hydrate 99–100%

(1 ml) and abs EtOH (2·5 ml) containing Na (200 mg) were heated in a sealed tube at 180° for 18 h. The product was filtered through alumina in petrol (b.p. 40–60°) and crystallized as irregular plates from MeOH–$CHCl_3$ m.p. 73–74°, $[\alpha]_D$ +63° (c, 0·3). (Found: C, 87·5; H, 12·5. $C_{30}H_{52}$ requires: C, 87·4; H, 12·6%). This hydrocarbon was found to be identical with lanost-8-ene (mixed m.p., $[\alpha]_D$, IR, mass spectra and TLC) obtained by Wolff–Kishner reduction of an authentic sample of lanost-8-ene-3-one.

Perbenzoic acid titrations of the diols. A large excess of perbenzoic acid (47 mg/ml) in $CHCl_3$ (6 ml) was added to I (45 mg) and stored at 0°. Aliquot portions were withdrawn at intervals and treated with excess KI, the solns were acidified with AcOH and the liberated I_2 titrated against 0·1N $Na_2S_2O_3$. Blank values were also determined. After 21 hr 2·07 atoms of O_2 per molecule of the diol had been absorbed, the value rising to 2·19 after 26 hr. On repetition with a more concentrated soln of perbenzoic acid (97·3 mg/ml) 2·68 atoms of O_2 per molecule of the diol were absorbed after only 5 hr.

The diol dihydro on perbenzoic acid (97·3 mg/ml) titration absorbed 1·7 atoms of O_2 per molecule after 5 hr the value rising to 1·9 after 21 hr.

It should be noted that an epoxidized Δ^{8-9} double bond can undergo, in the presence of mineral acid, an elimination reaction to form a 7,9(11)-diene which will react with more peracid. Compounds with a Δ^{8-9} double bond can therefore give spuriously high results.

Ozonolysis of the diol. The diol (100 mg) in glacial AcOH (10 ml) was treated with ozonized O_2 at −5° until ozonolysis was complete as indicated by the extensive liberation of I_2 from an aqueous soln of KI–boric acid. The soln was diluted with water and distilled. The distillate, when treated with 2,4-DPH hydrochloric gave acetone-2,4-dinitrophenylhydrazone, in 36% yield, purified by chromatography on alumina in benzene and crystallized from EtOH, m.p. 125° (undepressed on admixture with an authentic sample). (Found: C, 45·9; H, 4·25. Calc. for $C_9H_{10}N_4O_4$: C, 45·4; H, 4·20%).

Chromium trioxide–pyridine oxidation of the diol (I). The diol (200 mg) was added to pyridine (15 ml) containing CrO_3 (150 mg) and stored at room temp for 24 hr. The mixture was diluted with ether and the insoluble Cr residue filtered off. Dilution of the filtrate with water and extraction with ether (3 ×) yielded the diketone which crystallized from MeOH as rods (126 mg) m.p. 151–153·5°, $[\alpha]_D$ +59° (c, 0·29) (Found: C, 82·1; H, 10·5. $C_{30}H_{46}O_2$ requires: C, 82·2; H, 10·5%); λ_{max} 2386 Å (ε 13,700), ν_{max} (KBr disc) 1707, 1675, 1608 and 833 cm^{-1}, RD (c, 0·12, MeOH), $[\Phi]_{400}$ + 300°; $[\Phi]_{358}$ + 80°; $[\Phi]_{296}$ + 2660°; $[\Phi]_{270}$ +1390°.

Acknowledgements—The authors wish to thank Professor W. Klyne for ORD determinations, Dr. T. G. Halsall for some mass spectral measurements and Dr. R. L. Edwards for his interest in this work. One of us (A.D.P.) acknowledges the receipt of an S.R.C. Research Studentship.

REFERENCES

[1] M. Bamberger and A. Landsiedl, *Monatsch* **27**, 963 (1906).
[2] J. Zellner, *Ibid.* **39**, 603–615 (1918).
[3] N. S. Bhacca and D. H. Williams, *Application of NMR Spectroscopy in Organic Chemistry* p. 81. Holden-Day, San Francisco (1964).
[4] A. I. Cohen, D. Rosenthal, G. W. Karkower and J. Fried, *Tetrahedron* **21**, 3179 (1965).
[5] D. Lavie, Y. Shvo and E. Glotter, *Ibid.* **19**, 2257 (1963).
[6] N. S. Bhacca and D. Williams, *Applications of NMR Spectroscopy in Organic Chemistry* p. 77. Holden-Day, San Francisco (1964).