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NOVEL TRITERPENOIDS FROM THE FUNGUS PISOLITHUS TINCTORIUS[†]

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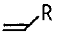
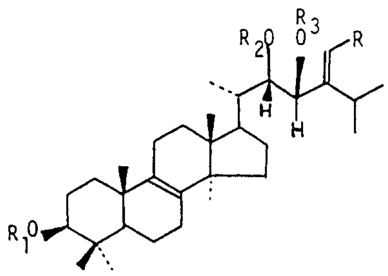
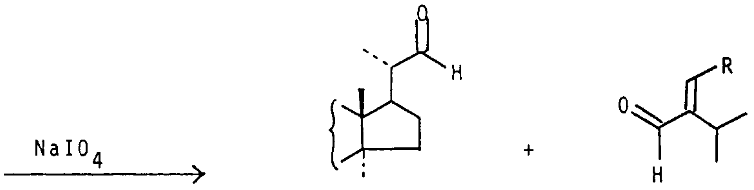
Summary The structures of two triterpenes, possessing novel side chains, were elucidated by chemical correlations with known compounds and physical methods involving in particular nuclear Overhauser effect difference spectroscopy.

Amongst various compounds present in the fruits and bodies of *Pisolithus tinctorius*[1] (var. *crassipes* (VITTADINI *et* PINTO LOPES) two new closely related lanostene derivatives (I) and (II) were isolated by extraction with dichloromethane and chromatographic purification. The crude mixture (I) and (II) (present in 1:4 ratio by ^1H n.m.r.[±], i.r. absorption band at 1735 cm^{-1}) upon mild hydrolysis yielded (III) and (IV) (no carbonyl absorption, broad OH band) that were separated by preparative t.l.c. The alcohol (III), $C_{32}H_{54}O_3$ (by mass spectrum), m.p. 192-196 ºC, $[\alpha]_D^{20}$ +39 º (*c* 1.1 , $CHCl_3$) showed in its ^1H n.m.r. spectrum five singlets at δ 0.745, 0.819, 0.921, 0.990 and 1.005 that were attributed respectively to C(18), C(30), C(31), C(32) and C(19) tertiary methyl groups. Signals at δ 0.936 (3H, d, J 6 Hz) and 1.078 (3H, d, J 6 Hz), 1.152 (3H, d, J 6 Hz), 2.833 (1H, h, J 6 Hz) were assigned to the secondary methyl group at C(21) and the isopropyl group attached to C(24). Resonances at δ 1.470 (3H, d, J 6 Hz) and 5.636 (1H, q, J 6 Hz) suggested the presence of a CH_3-CH=CR_2group. A multiplet at δ 3.225 (1H, 3α-H) as well as two doublets at 3.984 (1H, J 9 Hz) and 3.627 (1H, J 9 Hz) were attributed to protons attached to carbons bearing the three secondary OH groups. (III) formed a crystalline triacetate (V), m.p. 160-165 ºC, $[\alpha]_D^{20}$ +30 º (*c* 0.9 , $CHCl_3$). That the closely related triol (IV), $C_{31}H_{52}O_3$ (by mass spectrum), m.p. 193-195 ºC, $[\alpha]_D^{20}$ +38 º(*c* 1.1 , $CHCl_3$) contained a CH_2=CR_2 function was suggested by the presence of characteristic singlets at δ 5.146 and 5.096. The occurrence of a common ion at 314 in the mass spectrum of both (III) and (IV) (M^+ - side chain - H) indicated that these compounds differ only in the degree of substitution in the steroid side chain.[2] The provisional structures that (III) and (IV) were 3 β - hydroxy-lanost- 8 (9) - ene derivatives, with a vicinal diol and  group (R = CH_3 , H) in the side chain, were confirmed by the following chemical reactions (Scheme 1). Thus (IV) on oxidation with $NaIO_4$ yielded the aldehyde (VII), m.p. 177-180 ºC, $[\alpha]_D^{20}$ +38 (*c* 1.4 , $CHCl_3$) and 3-methyl-2-methylene-butanal (IX), isolated



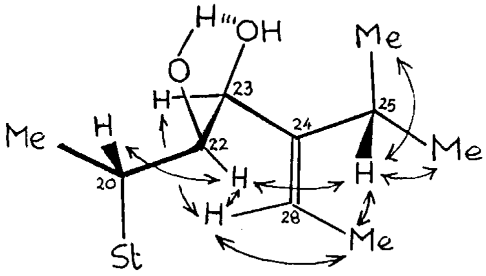
	R	R_1	R_2	R_3
I	CH_3	H	Ac	H
II	H	H	Ac	H
III	CH_3	H	H	H
IV	H	H	H	H
V	CH_3	Ac	Ac	Ac
VI	H	Ac	Ac	Ac

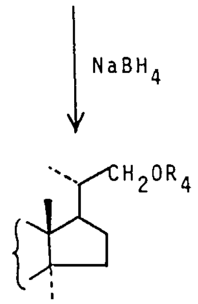
III R = CH_3
IV R = H



VII R_1= H
X R_1= Ac

VIII R = CH_3
IX R = H





XI R_1 = H, R_4 = H
XII R_1 = Ac,R_4 = Ac

Scheme 1

as its DNP derivative, m.p. 166-168 ºC [^1H n.m.r. δ 9.136 (1H, s , -CH=N-), 5.607 and 5.450 (2H, $R_2C{=}C\underline{H}_2$), 3.044 (1H, h, J 6 Hz , $-C\underline{H}Me_2$), 1.213 (6H, d, J 6 Hz, $-CH(C\underline{H}_3)_2$]. (X), m.p. 150-154 ºC (lit.[3] m.p. 154-155 ºC), $[\alpha]_D^{20}$ +40 º (*c* 2.1 , $CHCl_3$) [lit.[4] $[\alpha]_D^{20}$+32 (*c* 2.9)], obtained by acetylation of (VII) formed a DNP derivative the physical constants of which were in close agreement with those reported in the literature.[3] Reduction of (X) afforded the diol (XI), m.p. 200-207 ºC (lit.[5] m.p. 206-207 ºC), $[\alpha]_D^{20}$ +62 (*c* 0.78 , $CHCl_3$) which yielded the diacetate (XII), m.p. 171-176 ºC, $[\alpha]_D^{20}$ +45 º (*c* 0.61 , $CHCl_3$), confirming that the sterol isolated by Grove[5] is 23,24,25,26,27-pentanorlanost-8-ene-3 β,22-diol.[¶] Oxidation of the triol (III) with $NaIO_4$ generated the dialdehyde (VII) and 2-isopropyl-but-2-enal (VIII) characterised as its DNP derivative.

The stereochemistry of the substituents in the steroid side chain of (III) was elucidated by nOe [Σ] experiments (Fig. 1). While experiment (a) confirms the 24(28)-Z geometry of the olefin because of nOe of H_{28} to H_{22} and H_{23} , and <u>not</u> to H_{25} , experiments (b) and (c) suggest the conformation as depicted (intramolecular hydrogen bonding apparent in dilution i.r. studies). Inspection of the Dreiding model for this conformation [&] reveals a dihedral angle between H_{20} and H_{22} of *ca*. 90º, which would account for the apparent lack of coupling between these two protons. Other salient features are the <u>lack</u> of nOe between H_{22} and H_{23}, and nOe between H_{22} and H_{20}, which together with the observed value of $J_{H_{22},H_{23}}$ 9 Hz, points towards a close antiperiplanar arrangement of H_{22} and H_{23}. The presence of diastereotopic methyls groups at C(25) is attributed to chirality at C(23). An analogous conformation is attributed to the side chain of (IV). The doublets at 5.2 and 4.2 in both (I) and (II) were assigned to H_{22} and H_{23} respectively, by comparison with those of compounds (III) and (IV) and the corresponding triacetates (V) and (VI), enabling the identification of the natural products

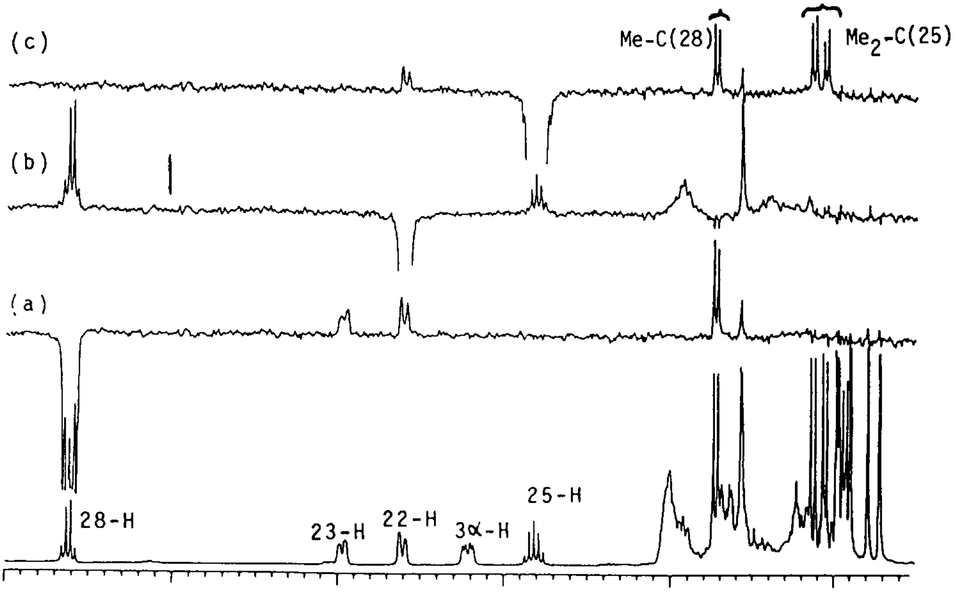


Fig. 1 (a) Irradiation at 28-H : nOe to 23-H, 22-H and 28-Me
(b) Irradiation at 22-H : nOe to 28-H, 25-H and 20-H
(c) Irradiation at 25-H : nOe to 22-H, 25-Me and 28-Me

(I) and (II) as the 22-acetates derivatives.[§]

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[†] This paper constitutes Part 2 in the series "Terpenoids from Basidiomycetes"; ref. 1a is Part 1.

[‡] All n.m.r. spectra refer to $CDCl_3$ solutions.

[¶] Both i.r. spectra of (XI) and (XII) were identical with those of authentic samples kindly provided by Dr. J.F.Grove (Sussex).

[Σ] Nuclear Overhauser effect difference spectra were obtained on a Bruker WM 250 spectrometer, as previously described (cf. A.M.Lobo, S.Prabhakar, M.A.Santos, H.S.Rzepa and D.J.Williams, *J.C.S. Perkin 2*, **1984**, 1511).

[&] The 22 *S* chirality, postulated on biogenetic grounds may well be reversed [*cf.* J.P. Poyser, F.Reinach-Hirtzbach and G.Ourisson, *Tetrahedron*, **30**, 977 (1974)].

[§] The natural products are thus: (I), 22ξ-acetoxy-3β, 23ξ-dihydroxy- 24(28)Z-ethylidenelanost-8-ene, and (II), 22ξ-acetoxy- 3β, 23ξ-dihydroxy- 24 -methylenelanost-8-ene.

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