Tetrahedron Letters,Vol.24,No.21,pp 2205-2208,1983 0040-4039/83/212205-04$03.00/0
Printed in Great Britain

PISOLACTONE, A NOVEL TRITERPENOID ISOLATED FROM THE FUNGUS PISOLITHUS TINCTORIUS

Ana M. Lobo, P. Macedo de Abreu, Sundaresan Prabhakar and Lîcio S. Godinho

Department of Chemistry, F.C.T., New University of Lisbon, Quinta da Torre, 2825 Monte da Caparica, Portugal

David J. Williams

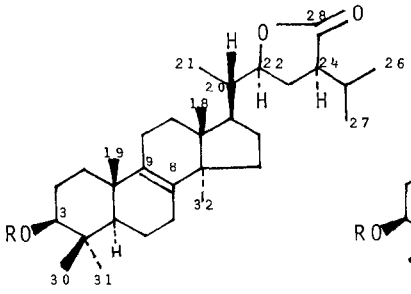
Chemical Crystallography Laboratory, Imperial College of Science and Technology, London SW7, U.K.

Summary Pisolactone, shown to possess structure (I) on the basis of spectral evidence and X-ray analysis, represents the first member of a lanosterol derivative with a carboxylic acid function at C(24).

The fungi of the Basidiomycetes are a source of tetracyclic triterpene carboxylic acids incorporating the lanostane skeleton and are characterized by the presence of a carboxylic acid group, usually at C(21), as in eburicoic acid, or C(26) as in quercinic acid.[1] Amongst various compounds present in the ether extract of the spores of the fungus Pisolithus tinctorius[2] (collected in the Portuguese Province of Alentejo) a new triterpene lactone was isolated which is the first member of a C(28) carboxylic acid derivative.

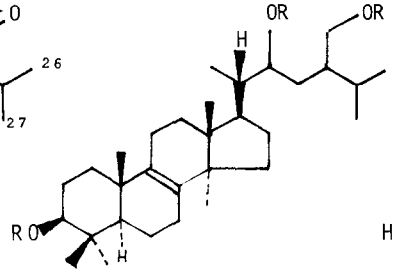
Pisolactone (I), $C_{31}H_{50}O_3$ (by mass spectrum and elemental analysis) obtained as colourless prisms, after chromatographic purification, had m.p. 279-280 ºC (from MeOH), $[\alpha]_D^{28}$ +60 º (c 1, $CHCl_3$) and ν_{max} (KBr) 3410 and 1745 cm^{-1}. Acetylation of (I) yielded the monoacetate (Ia), m.p. 280 ºC (from MeOH), $[\alpha]_D^{28}$ +53 º (c 1, $CHCl_3$) and ν_{max} (KBr) 1746 and 1725 cm^{-1}. Reduction of (I) (LAH-THF) afforded the triol (II) (no carbonyl absorption, strong OH band at 3400 cm^{-1}) which was characterised as the triacetate (IIa), m.p. 112-115 ºC (from MeOH), $[\alpha]_D^{28}$ +30 º, (c 1, $CHCl_3$) suggesting that (I) is a lactone. A careful study of the ^1H n.m.r. (100 MHz) and ^{13}C n.m.r. spectra[+] of (I) led to the conclusion that it possessed the basic lanosterol skeleton.[3] Thus four singlets [δ 0.72 (3H), 0.97 (6H), 0.81 (3H) and 0.88 (3H)] were attributed to methyl groups

at C(18), C(19) + C(30), C(31) and C(32) respectively. The remaining three methyl groups C(21), C(26) and C(27) were identified as an isopropyl group and a secondary methyl group respectively, by irradiating in the vicinity of a 2 proton multiplet at δ 1.66 which caused the appearance of additional singlets at δ 1.02 (3H) and 0.94 (6H). The multiplet at δ 3.21 ($w_{1/2} \simeq 5$ Hz) was shifted downfield to δ 4.37 (J_{aa} 9 Hz, J_{ae} 4.5 Hz) in the spectrum of (Ia) and is consistent with the presence of a 3β - hydroxyl group.[4] This was further supported by the presence of an absorption at 78.9 ppm in the ^{13}C n.m.r. spectrum of (I) and at 80.6 ppm in that of (Ia). The characteristic resonance at 178.5 ppm in the ^{13}C n.m.r. spectrum of (Ia) strongly indicated the existence of a lactone ring (the 3-acetate carbonyl appears at 170.7 ppm) and signals at 134.7 and 134.2 ppm were suggestive of the presence of a $\Delta^{8,9}$ tetrasubstituted double bond.[5] The presence of one proton multiplets at δ 4.45 and 2.50 in the ^{1}H n.m.r. spectra of both (I) and (Ia) were ascribed to hydrogens attached to C(22) and C(24) respectively. The mass spectra of both (I) and (Ia) showed loss of a species m^+/e 155 ($C_9H_{15}O_2$), revealing a diagnostic fragmentation[1] between the steroidal framework and the side-chain, indicating that the lactone ring is a part of the latter. Of two possible structures for pisolactone, (I) and (III), the former was shown to be correct by X-ray analysis of (Ia). Crystal data: $C_{33}H_{52}O_4$, crystallises as clear long fibrous needles, monoclinic, a = 9.391(3), b = 6.217(1), c = 25.960(6) Å, β = 95.43(2), U = 1509Å^3, space group $P\,2_1$, Z = 2, D_c = 1.42 g cm^{-3}. A crystal of dimensions ca. 0.04 x 0.75 x 0.04 mm was mounted approximately about its b direction. Data were measured using monochromatised Cu-K_α radiation (graphite monochromator) on a Nicolet R 3m diffractometer. A total of 2259 independent reflections were measured ($\theta \leq 58°$) using the omega-scan measuring routine, and of these 1852 had $|F_o| > 3\,(|F_o|)$ and were considered to be observed. The structure was solved by direct methods and the non-hydrogen atoms refined anisotropically. The hydrogen atoms, with the exception of the methyl groups which were refined as rigid bodies, were placed at calculated positions and allowed to ride on their parent carbons. Refinement was by full-matrix block cascade least-
-sequence to R = 0.052. Computations were carried-out on an Eclipse S 140 computer using the SHELXTL program system. A view of the structure of (Ia) is shown in Figure 1. Salient features of the structure are : a) the C(8)-C(9) double bond length is 1.332(6) Å, while other C-C bonds have normal values; b) the packing is consistent with Van der Waals with only three contacts less than 3.4 Å between [O(28) ... C(22) 3.28 Å]§ , [O(28) ... C(23)^{I} 3.30 Å] and [O(28) ... C(24)^{II}



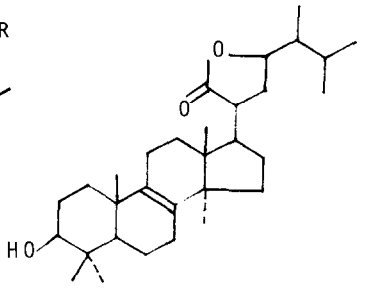
(I) R = H

(Ia) R = Ac

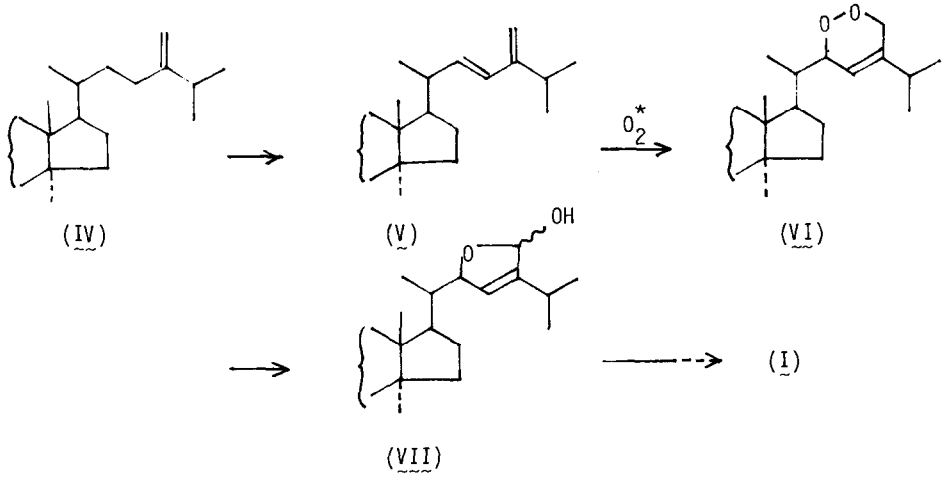


(II) R = H

(IIa) R = Ac



(III)



(IV) (V) (VI) (VII) (I)

Scheme 1

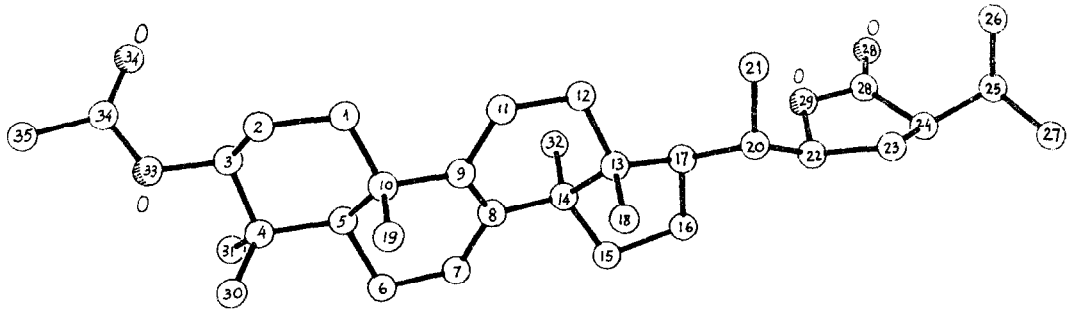


Figure 1

3.40 Å]. The lactone ring is inclined 87 ° to the mean plane[¶] of the steroid framework C(1) to C(17). A reasonable biosynthesis of (I) (Scheme 1) would involve an initial dehydrogenation of eburicol (IV) to give (V)[6] which by pigment sensitised photo-oxidation [7] leads to (VI). Cleavage of the peroxide [8], ring closure to the hemiacetal (VII) and subsequent prototropic shift would generate (I).[Σ]

Acknowledgements We are grateful to Dr. S. Natori, Meiji College of Pharmacy (Japan), Dr. P. A. Diassi, Squibb (U.S.A) and Dr. J. S. E. Holker , University of Liverpool (U.K.) for authentic samples of triterpene acids for chemical comparisons.

REFERENCES and NOTES

1. A.Yokoyama, S.Natori and K.Aoshima, Phytochemistry, 1975, 14, 487 ; P.Pant and R.P.Rastogi, ibidem, 1979, 18, 1095.
2. Previous chemical study of this fungus relates to its fatty acid composition (cf. J.H. Melhuish and E.Hacskaylo, Mycologia, 1980, 72, 1041, and references therein).
3. A.I.Cohen, D.Rosenthal, G.W.Krakower and J.Fried, Tetrahedron, 1965, 21, 3171.
4. J.Bridgeman, P.C.Cherry, A.S.Clegg, J.Evans, E.R.H.Jones, A.Kasal, V.Kumar, G.D.Meakins, Y.Morisawa, E.E.Richards, P.D.Woodgate, J.Chem.Soc. (C), 1970, 250.
5. G.Lukacs, K.Khuong-Huu, C.R.Bennett, B.Buckwalter and E.Wenkert, Tetrahedron Lett., 1972, 3515 ; S.A.Knight, ibidem, 1973, 83 ; S.A.Knight, Org.Magn.Reson., 1974, 603.
6. A similar introduction of Δ^{22} was shown to occur in the conversion of episterol to ergosterol: M.Freyberg, A.O.Oehlschlager and A.M.Unrau, Biochem.Biophys.Res.Comm., 1972, 48, 593.
7. J.Arditti, R.E.M.Fish and B.H.Flick, J.C.S., Chem.Comm., 1972, 1217.
8. Pisolithus tinctorius is known to possess an enzyme active in the decomposition of hydrogen peroxide (cf. J.L.Lamaison, A.Pourrat and H.Pourrat, Ann.pharm.franç., 1975, 33, 441).

† All n.m.r. spectra refer to $CDCl_3$ solutions.

§ Symmetry operators: I, x, $1 + y$, z ; II, $2 - x$, $1/2 + y$, $-z$.

¶ Equation of the plane comprising atoms C(1) to C(17) in direct space:
$-4.1155\,x + 5.5783\,y - 0.3050\,z = 1.8791$.
Equation of lactone ring phase C(22), C(23), C(24), C(28), O(29) in direct space:
$5.1342\,x + 2.2840\,y + 18.1021\,z = 9.7155$.

Σ With this assumption the absolute configuration of pisolactone would be (20 S, 22 S, 24 R) - 3 β -hydroxy - 5 α -lanost- 8 - en - 22 , 28 - olide.

(Received in UK 18 February 1983)