Punctatin A (Antibiotic M95464): *X*-Ray Crystal Structure of a Sesquiterpene Alcohol with a New Carbon Skeleton from the Fungus, *Poronia punctata*

John R. Anderson,[a] Clive E. Briant,[b] Raymond L. Edwards,*[a] Richard P. Mabelis,[c] J. Philip Poyser,*[c] Harry Spencer,[c] and Anthony J. S. Whalley[d]

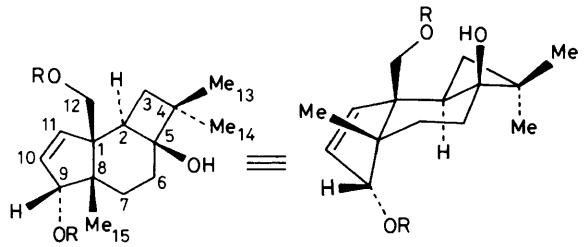
[a] *School of Chemistry, University of Bradford, Bradford BD7 1DP, U.K.*
[b] *University of Oxford, Chemical Crystallography Laboratory, 9 Parks Road, Oxford OX1 3PD, U.K.*
[c] *Imperial Chemical Industries p.l.c., Pharmaceuticals Division, Microbial Metabolites Group, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, U.K.*
[d] *Department of Biology, Liverpool Polytechnic, Liverpool L3 3AF, U.K.*

Physical methods and *X*-ray diffraction analysis have been used to establish the structure of a novel trihydroxysesquiterpene, punctatin A, produced, with a series of related compounds, by the dung fungus *Poronia punctata* (Linnaeus *ex* Fries).

Two strains of the dung fungus, *Poronia punctata* (Linnaeus *ex* Fries), one CBS 459.48, the other recently isolated from samples from the New Forest, give rise to a family of sesquiterpene alcohols when grown on malt solution in still culture for 56 days at 23 °C. The structure of the major sesquiterpene reported here has a caryophyllene-related tricyclic skeleton, which, to our knowledge, has not previously been found in nature.

Extraction of culture filtrate (24 l) at natural pH with ethyl acetate (3 × 4 l), removal of solvent at reduced pressure, and silica gel chromatography (benzene–ethyl acetate–acetic acid 50 : 49 : 1) allowed the isolation of several compounds. The least polar component was a pale yellow oil (660 mg; 27.5 mg/l), a new natural product, identified as (*E*)-methyl 3-(4′-methoxyphenyl)propenoate, ν_{max} ($CHCl_3$) 1710 cm^{-1}; λ_{max} (EtOH) 213 (ε 11000) and 246 nm (9900); δ ($CDCl_3$) 3.62 (3H, s), 3.68 (3H, s), 5.32 (1H, d, *J* 12 Hz), 6.76 (4H, d, *J* 6 Hz), and 7.58 (1H, d, *J* 12 Hz); *m/z* 208 (100%). Further elution effected the separation of a crystalline solid (**1**), assigned the trivial name, punctatin A, and several congeners, the structures of which are the subject of separate publications. Punctatin A was recrystallised from ethyl acetate to give large rhomboid crystals (57 mg; 2.4 mg/l),† m.p. 187—192 °C; $[\alpha]_D^{20}$ −26° (*c* 1.0, MeOH). By electron-impact mass spectroscopy, the molecular formula was at first thought to be $C_{15}H_{22}O_2$ (*m/z* 234.1630; calc. 234.1619). However, the formation of a hydroxydiacetate (**2**) {m.p. 125 °C (1 °C/min) [light petroleum (b.p. 80—100 °C)]; $[\alpha]_D^{23}$ −138° (*c* 1.0, MeOH); ν_{max} (KBr) 3420, 1740, and 1720 cm^{-1}} on treatment with acetic anhydride–pyridine indicated the presence of a third oxygen atom in the molecule.

† Satisfactory elemental analyses were obtained for punctatin A and its diacetate, and for (*E*)-methyl 3-(4′-methoxyphenyl)propenoate.



(1) R = H
(2) R = COMe

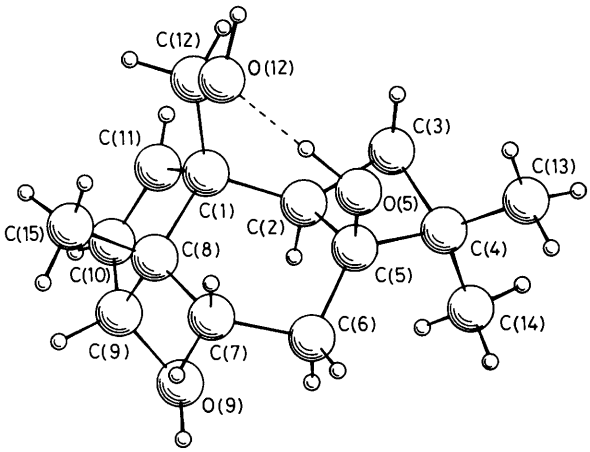
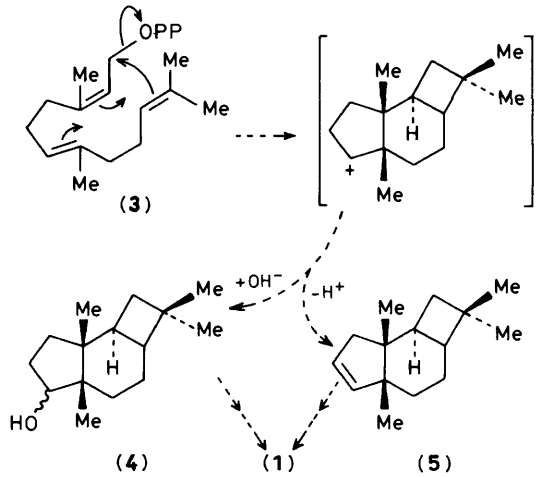


Figure 1. The molecular structure of (**1**), $C_{15}H_{24}O_3 \cdot 0.5\ H_2O$, showing the intramolecular hydrogen bond between O(5)–H(5) · · · · · · · O(12). The water molecule positions have been omitted for clarity but are involved in hydrogen bonds with O(9) and O(12). In addition, there is a hydrogen bond between O(9) and O(5) of a neighbouring molecule.

Downfield shifts in the ^1H n.m.r. spectra for the signals due to hydrogen atoms on carbons bearing acylatable hydroxy groups suggested the presence of one primary, one secondary, and one tertiary alcohol group. The probable molecular formula of $C_{15}H_{24}O_3$ for punctatin A was confirmed by pyridinium dichromate oxidation in methylene dichloride to give an α,β-unsaturated ketone {m.p. 154—155 °C [light petroleum (b.p. 60—80 °C)–ethyl acetate]; ν_{max} (KBr) 3440, 3160, and 1705 cm^{-1}; λ_{max} (EtOH) 228 nm (ε 6125); *m/z* 250} and a keto-lactone {m.p. 122—123 °C [light petroleum (80—100 °C)]; $[\alpha]_D^{20}$ + 91° (*c* 1.0, MeOH); ν_{max} (KBr) 1775 and 1712 cm^{-1}; *m/z* 246}. The primary alcohol function appeared to show some resistance to oxidation, compared to the secondary alcohol group, which is allylic as borne out by the generation of the chromophore. Despite chemical equivalence of the olefinic protons of punctatin A in CD_3OD, ^1H (in C_5D_5N) and ^{13}C n.m.r. spectroscopy allowed the –O–CHR–CH=CH–C≤ and $-OCH_2-C\leq$ system to be clearly distinguished. Other features, *e.g.* the three methyl singlets, were also readily identifiable. In the absence of methoxy groups, the losses of 31 mass units in the mass spectrum of punctatin A can be attributed to fragmentation of the CH_2OH unit. The base peak at *m/z* 165.0928 (calculated for $C_{10}H_{13}O_2$, 165.0915) arises in this way after the ion at *m/z* 221 has undergone the loss of a further 56 units (C_4H_8). This subsequently proved to be extrusion of isobutene from the dimethylcyclobutane, typical of punctatin A and its congeners. The complete structure and relative stereochemistry of punctatin A were solved by *X*-ray crystallography.

Crystal data: $C_{15}H_{24}O_3 \cdot 0.5H_2O$, M = 261.4, tetragonal, space group $P42$, $a = b$ = 10.117(3), c = 28.786(7) Å, U = 2946.4 Å^3, Z = 8, D_c = 1.18 g cm^{-3}, μ = 0.47 cm^{-1} (Mo-K_α, λ = 0.71069 Å).‡



Scheme 1. Cyclisation of farnesyl pyrophosphate (**3**) to possible intermediates in the biosynthesis of punctatin A (**1**). OPP = pyrophosphate.

Intensity data were collected to θ_{max} = 20° on an Enraf–Nonius CAD-4 diffractometer using graphite monochromated Mo-K_α radiation. The structure was solved (direct and Fourier methods) and refined anisotropically by full matrix least squares. All hydrogen atom positions were located from difference Fourier maps. The final R value was 3.38% (R_w = 4.14%) over 864 reflections with $I > 3\sigma(I)$. The molecular structure of (**1**), illustrated in Figure 1, contains a relatively short intramolecular hydrogen bond. In the crystal structure the molecules are held together by a three dimensional network of intermolecular O–H · · · O hydrogen bonds. The H_2O molecule lies on a crystallographic two-fold axis, and is involved in four hydrogen bonds, while each hydroxy group is involved in two hydrogen bonds.

Acyclic and mean ring bond lengths and angles are close to values normally found, though in the 6-membered ring, which adopts the stable 'chair' conformation, there is a wide variation. The 4- and 5-membered ring system exists in the 'folded' and 'envelope' forms.

The tricyclic skeleton of punctatin A can be envisaged as arising from a cyclisation of farnesyl pyrophosphate (**3**) to hypothetical precursors such as (**4**) and (**5**) (Scheme 1). Cyclisations of an analogous type have been suggested as the origin of other related fungal sesquiterpene structures.[1]

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1 W. B. Turner and D. C. Aldridge, 'Fungal Metabolites II,' Academic Press, London and New York, 1983, ch. 6.

‡ The atomic co-ordinates for this work are available on request from the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.