Punctatins B and C (Antibiotics M95154 and M95155): Further Sesquiterpene Alcohols from the Fungus *Poronia punctata*

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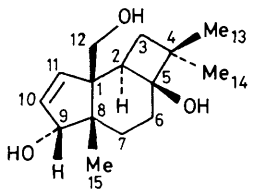
Further studies on metabolites of the dung fungus *Poronia punctata* (Linnaeus *ex* Fries) have furnished the structures of punctatins B (**2**) and C (**3**) as a trihydroxycaryophyllene isomer of punctatin A (**1**) and a novel tetracyclic hemi-succinate ester of close biogenetic origin, respectively.

Still culture of the dung fungus *Poronia punctata* (Linnaeus *ex* Fries) (Whalley collection) has proved a rich source of new sesquiterpenes, of which punctatin A (**1**) has been the subject of a recent communication.[1] A number of biogenetically-related compounds have now been isolated, the proportions of which vary from grow to grow. Several isomers of punctatin A are produced and the structure of one of these, punctatin B, has been established as (**2**) from chemical transformation and spectral† and *X*-ray crystallographic analyses. Similar procedures have allowed the structure of a further metabolite, punctatin C, to be determined as (**3**), a novel tetracyclic skeleton. This compound was not always produced in significant amounts.

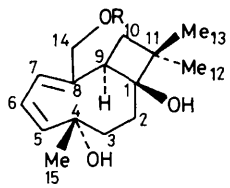
Punctatin B (**2**) was obtained by direct crystallisation of the crude extract or by silica gel chromatography (as described previously[1]) and recrystallisation from ethyl acetate to give colourless needles (520 mg; 21.7 mg/l), m.p. 187.5—188.5 °C; $[\alpha]_D^{20}$ −221° (*c* 1.0, MeOH). The presence of several hydroxy groups and a conjugated double bond system was indicated by the i.r. (KBr) bands at 3340(s), 3245(s), and 1620(w) cm^{-1}, and a u.v. (EtOH) absorption at 212 nm (ε 6346). The ^{13}C n.m.r. spectrum in CD_3OD revealed three signals due to carbon atoms bearing oxygen at δ 80.4 (s), 73.8 (s), and 65.5 (t,C-14), and four olefinic type signals at δ 141.7 (d), 139.6 (s, C-8), 124.9 (d), and 123.3 (d). This was compatible with a primary alcohol (as in punctatin A), two double bonds, and two tertiary hydroxy groups. The alternative possibility of ether linkages was excluded by conversion (acetic anhydride–pyridine) into an oily hydroxy-monoacetyl derivative (**4**), $[\alpha]_D^{20}$ −55°(*c* 1.0, MeOH); $\nu_{max.}$ ($CHCl_3$) 3500, 1740, 1260 (sh), and 1240 cm^{-1}; δ ($CDCl_3$) *inter alia* 2.1 (3H, s), 4.64 (1H, d, *J* 12 Hz), and 4.92 (1H, d, *J* 12 Hz); *m/z* 294 ($M^{+\cdot}$). Loss of 60 mass units (AcOH) to *m/z* 234 [($M^{+\cdot}$ − ROH) as also seen in the mass spectrum of (**2**) (R = H)] is followed by loss of a further 18 mass units to the base peak, *m/z* 216. Both this fragment and that at *m/z* 201 undergo elimination of isobutene (56 mass units), which has been ascribed to the dimethylcyclobutanol system of punctatin A (**1**).

Oxidation of punctatin B with pyridinium dichromate

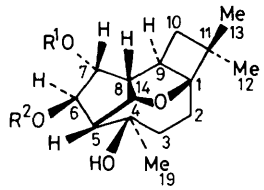
† All new compounds gave spectral data in good agreement with the assigned structures and satisfactory microanalytical data were obtained.



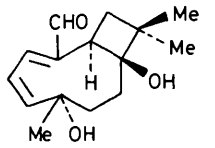
(1)



(2) R = H
(4) R = Ac



(3) R^1 = H, R^2 = $HO_2C[CH_2]_2CO$
(6) R^1 = H, R^2 = $MeO_2C[CH_2]_2CO$
(7) R^1 = Ac, R^2 = $MeO_2C[CH_2]_2CO$
(8) R^1 = H, R^2 = H,



(5)

(CH_2Cl_2, 24 h, room temperature) gave a 35% yield of an unsaturated aldehyde (**5**), m.p. 74—75 °C; $[\alpha]_D^{20}$ −364° (*c* 1.0, MeOH); $\nu_{max.}$ ($CHCl_3$) 3590, 3460, and 1670 cm^{-1}; $\lambda_{max.}$ (EtOH) 263 nm (ε 5470); δ (C_5D_5N) *inter alia* 1.06 (3H,s), 1.14 (3H,s), 1.36 (3H,s), 5.80 (1H, dd, *J* 3 and 11 Hz, 6-H), 6.24 (1H, d, *J* 11 Hz, 5-H), 6.74 (1H, d, *J* 3 Hz, 7-H), and 9.60 (1H, s, 14-H). If punctatin B contains two double bonds and a cyclobutane ring, it must possess one other ring only. Furthermore, in order to account for the coupling of the vinyl protons, the carbon α to the disubstituted double bond must be quaternary (C-4). On the basis of its relationship with punctatin A and the above facts, structure (**2**) was proposed for punctatin B. The complete structure and relative stereochemistry were confirmed by a single-crystal *X*-ray analysis.

Crystal data: $C_{15}H_{24}O_3$, *M* = 252.4, orthorhombic, space group $P2_12_12_1$, *a* = 7.432(5), *b* = 11.017(4), *c* = 17.699(5) Å, *U* = 1449.2 $Å^3$, *F*(000) = 552, D_c = 1.15 g cm^{-3}, *Z* = 4, μ(Mo-K_α) = 0.85 cm^{-1}.

1196 Independent reflections [$I > 3.0\sigma(I)$] were measured on an Enraf-Nonius CAD-4 automatic diffractometer. The structure was elucidated by direct phasing techniques (MULTAN) and refined by least squares calculations to a final *R* of 0.044.‡ The molecular structure of (**2**), illustrated in Figure 1, exhibits only slight delocalisation of the conjugated double bonds along C(5)–C(6)–C(7)–C(8) with subsequent opening of the angles around atoms C(5), C(6), and C(7). The shortest intermolecular contacts are O(1)–O(4) 2.75 and O(4)–O(8) 2.82 Å.

Other caryophyllene derivatives of fungal origin are known.[2,3] 13-Hydroxycaryophyllene 4,5-oxide[2] from *Lactarius camphoratus* gives an indication as to how the two allylic alcohol systems (5-en-4-ol and 7-en-14-ol) of punctatin B might be introduced.

Punctatin C (**3**) crystallised from ethyl acetate as large rods, (22.5 mg; 0.94 mg/l), m.p. 158—161 °C; $[\alpha]_D^{20}$ −14° (*c* 1.0, MeOH), suitable for *X*-ray crystallographic analysis (see later). The chemical ionisation mass spectrum (m.s.) showed an ion at *m/z* 369 corresponding to a molecular formula of $C_{19}H_{28}O_7$, with ready loss of 18 (*m/z* 351) and 100 (251) mass units. The electron-impact m.s. had no molecular ion, but rather the punctatin-characteristic elimination of isobutene to give *m/z* 312 (30%) and again losses of 18 (*m/z* 294), 100 (194, 40%), and a further 18 (176, 34). The hemi-succinate group of punctatin C, probably lost as succinic anhydride, gives rise to the 100 mass unit losses. Its presence can be seen in the i.r. spectrum (KBr) as carbonyl absorptions at $\nu_{max.}$ 1733 and 1709 cm^{-1}, in the ^{13}C n.m.r. spectrum at δ173.9 and 172.1, and as a four hydrogen singlet at δ2.63 in the ^1H n.m.r. spectrum. Furthermore treatment with ethereal diazomethane§ gave a monomethyl ester (**6**), m.p. 84—87 °C (ethyl acetate–light petroleum, b.p. 60—80 °C), $[\alpha]_D^{20}$ + 39.9° (*c* 1.0, $CHCl_3$); $\nu_{max.}$ (Nujol) 3430, 3400, 1730, 1705, 1176, and 1160 cm^{-1}; δ($CDCl_3$) *inter alia* 3.70 (3H, s); *m/z* 382 ($M^{+\cdot}$), 326 ($M^{+\cdot}-C_4H_8$) (22%), 194 (15), 176 (27), and 115 ($MeO_2CCH_2CH_2CO$) (100). Conversion (acetic anhydride–pyridine) of the methyl ester into a monoacetate diol (**7**) gave needles, m.p. 102—104 °C (ethyl acetate–light petroleum, b.p. 60—80 °C); $[\alpha]_D^{20}$ −3.8°(*c* 1.06, $CHCl_3$); $\nu_{max.}$ (Nujol) 3550, 1735, and 1700(sh) cm^{-1}; δ ($CDCl_3$) *inter alia* 2.05 (3H, s), 3.66 (3H,s), and 5.35 (1H, t, *J* 7 Hz); *m/z* 424 ($M^{+\cdot}$), 368

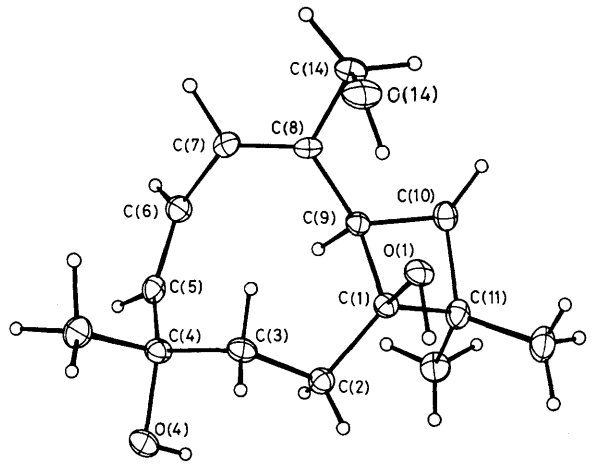


Figure 1. The molecular structure of (**2**), $C_{15}H_{24}O_3$.

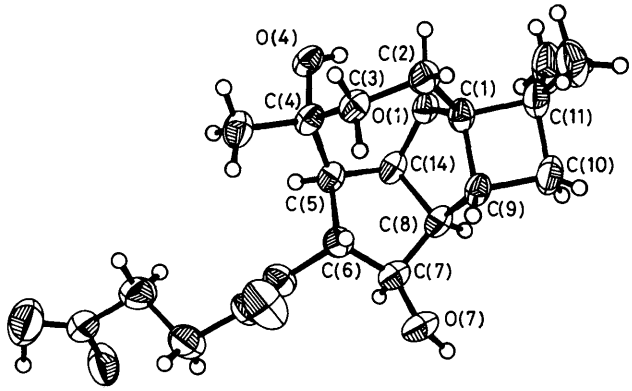


Figure 2. The molecular structure of (**3**), $C_{19}H_{28}O_7$.

‡ The atomic co-ordinates for this work are available on request from the Director of 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.

§ A methylation product of a minor component, in which the tertiary alcohol had been eliminated, was also isolated and evidence for the structure will be discussed elsewhere.

($M^{+\cdot}-C_4H_8$) (98%), 194 (18), 176 (41), 115 (96), and 43 (100).

Alternatively, exposure to mild basic conditions (0.3% K_2CO_3, 90% aqueous methanol, room temperature, 60 h), cleaved the hemisuccinate ester of punctatin C to furnish after flash chromatography the sesquiterpene triol parent (**8**), as a colourless solid, m.p. 145—160 °C; $\nu_{max.}$ (Nujol) 3400, 3200, 1110, 1070, and 1037 cm^{-1}; *m/z* 268 ($M^{+\cdot}$; $C_{15}H_{24}O_4$) (5%), 251 (19), 212 ($M^{+\cdot}-C_4H_8$) (8), 194 (20), 99 (52), and 95 (100).

The novel tetracylic structure of (**8**), and its hemi-succinate, punctatin C (**3**), was established by *X*-ray diffraction studies on the latter.

Crystal data: $C_{19}H_{28}O_7$, M = 368.4, monoclinic, space group $P2_1$, a = 5.932(3), b = 17.509(3), c = 9.166(2) Å, β = 99.23(2)°, U = 939.7 Å^3, $F(000)$ = 396, D_c = 1.30 g cm^{-3}, Z = 2, μ(Mo-K_α) = 1.06 cm^{-1}.

The crystal structure was solved by direct phasing procedures and least squares adjustment of the atomic parameters converged to R = 0.038 over 1959 independent reflections [$I > 3.0\sigma(I)$] measured with Mo-K_α radiation.‡ The molecular structure of (**3**) is depicted in Figure 2. The shortest intermolecular contact is O(1)–O(7) 2.61 Å.

Punctatins A (**1**), B (**2**), and C (**3**) would appear to be closely related biosynthetically, but their exact relationship remains to be elucidated. The structures of other *Poronia punctata* metabolites are being investigated. Those of D, E, and F, containing the punctatin A skeleton, will be reported shortly.

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