PUNCTATINS A, D, E, AND F (ANTIBIOTICS M95464, M167906, M171950, AND M189122), ISOMERIC ALLYLIC ALCOHOLS FROM THE FUNGUS *PORONIA PUNCTATA*: X-RAY CRYSTAL STRUCTURES OF D AND OF E ACETONIDE

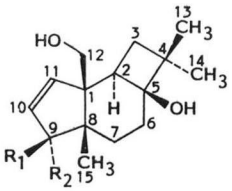
Sir:

Punctatins A (**1a**) and B (**2**) are isomeric sesquiterpene alcohols produced by still cultures of the dung fungus *Poronia punctata* (Linnaeus *ex* Fries) (Whalley collection). Their structures and that of the related metabolite punctatin C (**3**) have been reported previously[1,2]. We now wish to describe three further isomers of A: punctatins D (**1b**); E (**4a**); and F (**4b**), which together with A, constitute the four allylic alcohols possible in the five-membered ring.

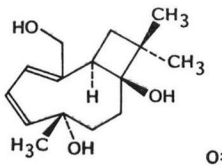
A strain of *Poronia punctata* isolated from horse dung from the New Forest, U.K., was grown for 56 days at 23°C in 4% aqueous Boot's Liquid Malt Extract using surface culture in 21 Thompson bottles. Growth was sparse. The felt was discarded, and the culture filtrate (24 liters) was extracted with EtOAc (3×4 liters) without adjusting the pH. The combined extracts were dried and the solvent was removed at reduced pressure. The residue was separated into its components by column chromatography on Merck silica gel, eluting with benzene - EtOAc - AcOH (50: 49: 1).

The most polar compound, punctatin D (**1b**), of similar polarity to B in several TLC systems, crystallised from ethyl acetate as plates (9 mg/liter), suitable for X-ray crystallographic studies (see below): mp 199~201°C; $[\alpha]_D^{20}$ +125° (*c* 1, MeOH); IR ν_{max} (KBr) 3240, 1088 and 1012 cm^{-1}. The EI mass spectrum was virtually identical with that of punctatin A (Calcd for $C_{15}H_{22}O_2$, $M^{+\cdot}-H_2O$ (30%): 234.1619. Found: 234.1618), showing the characteristic loss of isobutene from 221 *m/z* (19%) to the base peak 165 *m/z* (100%). ^{13}C and ^1H NMR spectra, whilst generally similar to those of punctatin A, had some differences, of which those in the ^1H spectrum centred around the C-9 position (1H, dd, at δ 5.36 compared to δ 4.60) and the adjacent methyl group (3H, s at δ 1.64 compared to δ 1.38) (C_5D_5N).

Punctatin D formed a hydroxydiacetate, crystallising from aqueous EtOH: mp 112~113°C; $[\alpha]_D^{24}$ +33° (*c* 1, MeOH), closely resembling diacetylpunctatin A. Simple transformations established the structure of D as 9-*epi*-punctatin A. Oxidation with pyridinium dichromate in CH_2Cl_2 for 2 hours at room temperature gave an α,β-unsaturated ketone (**1c**) and a ketolactone (**5**), identical in all respects to the oxidation products from A. Meerwein-Ponndorf-



1a R_1 = H, R_2 = OH
1b R_1 = OH, R_2 = H
1c $R_1 + R_2$ = O



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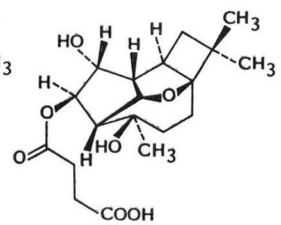

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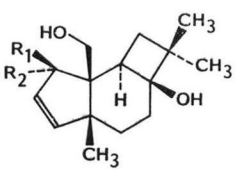
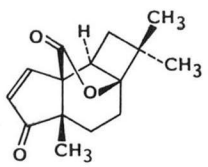


4a R_1 = OH, R_2 = H
4b R_1 = H, R_2 = OH
4c $R_1 + R_2$ = O



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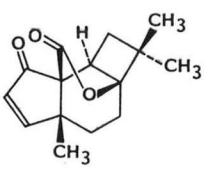

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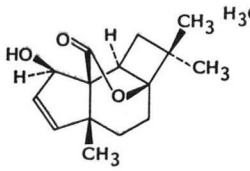

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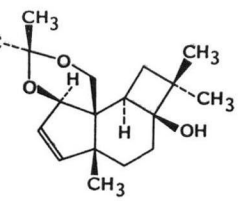

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Verley reduction of **1c** with aluminium isopropoxide for 8 hours in refluxing isopropanol gave a mixture of D and A, thus confirming their relationship. Other methods of reduction were unsuccessful.

An X-ray crystal structure of D revealed three crystallographically independent molecules per unit cell, though these were conformationally similar (Fig. 1): Crystal data: $C_{15}H_{24}O_3$, M=252.0, triclinic, space group P1, a=8.701(1), b=10.924(2), c=12.574(2) Å, α=70.30(1)°, β=80.56(1)°, γ=82.43(1)°, U=1106.2 Å^3, Z=3, D_c=1.135 g/cm^3, F (000)=414.0, λ=1.5418 Å, μ(Cu-K$_\alpha$)=5.85 cm^{-1}. 3375 observed data out of 3764 unique measured reflections [$I \geq 1.5\sigma(I)$] to sin θ/λ=0.588 Å^{-1}.

The crystal structure was solved using direct methods and refined by least squares using anisotropic thermal parameters for all non-hydrogen atoms. The positions of the hydrogen atoms, except H (**8**) and all hydroxyl hydrogens, were held in idealised geometry while refining with isotropic thermal parameters. The final R value, $\Sigma|\Delta F|/\Sigma|F_0|$, was 0.034.

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.

An analogous relationship was established for the two other punctatin A isomers. Punctatin E (**4a**) crystallised from EtOAc (27 mg/liter): mp 176°C (subliming 157~165°C); $[\alpha]_D^{20}$ −39° (*c* 1, MeOH); IR ν_{max} (KBr) 3320, 3200, 1088 and 1056 cm^{-1}; MS M$^{+\cdot}$ at 252 *m/z* (trace), M$^{+\cdot}$ −H_2O at 234 *m/z*. Punctatin F (**4b**) also crystallised from EtOAc (1 mg/liter): mp 205~208°C (subliming at 150°C); $[\alpha]_D^{20}$ +83° (*c* 1, MeOH); IR ν_{max} (KBr) 3280~3160 and 1088 cm^{-1}. Pyridinium dichromate oxidation of **4a** gave the ketone (**4c**), purified by sublimation at 100°C/ 0.01 mmHg: mp 207°C; $[\alpha]_D^{24}$ −35.5° (*c* 0.4, MeOH); IR ν_{max} (KBr) 3170 and 1693 cm^{-1}, NMR δ ($CDCl_3$) *inter alia* 3.91 (2H, q, J=12.5 and 5.5 Hz), 5.90 (1H, d, J=3 Hz) and 7.42 (1H, d, J=3 Hz). A keto-lactone (**6**) was also formed, crystallising from EtOAc - petroleum ether 60~80 fraction: mp 137°C; $[\alpha]_D^{24}$ +127° (*c* 0.4, MeOH); IR ν_{max} (KBr) 1786 and 1691 cm^{-1}. Reaction with barium manganate for 5 days at room temperature furnished the ketone (**4c**), together with the hydroxy-lactone (**7**). Aluminium isopropoxide reduction of (**4c**) this time led almost exclusively to punctatin F (**4b**). Both E and F could be converted into hydroxy-diacetates. Diacetylpunctatin E crystallised from aqueous EtOH: mp 132~133°C; $[\alpha]_D^{24}$ −102° (*c* 1, MeOH), as did diacetylpunctatin F: mp 108°C; $[\alpha]_D^{24}$ +50° (*c* 0.3, MeOH). The ^{1}H NMR spectra of punctatins A, D, E, and F were helpful in assigning the relative stereochemistry around the five-membered ring (Table 1). In particular the signals due to the C15-methyl groups in D and E ("β"-OH) are downfield, *ca.* δ 1.6, of those in A and F ("α"-OH), *ca.* δ 1.35.

The structural assignments for E and F were confirmed indirectly, but unambiguously. In a single instance, column chromatography of the crude broth extract involving acetone had led to the isolation of a non-polar compound, crystallising as needles from MeOH: mp 177~178°C; $[\alpha]_D^{24}$ −11° (*c* 1, MeOH), IR ν_{max} ($CHCl_3$) 3600 cm^{-1}; MS M$^{+\cdot}$ at *m/z* 292. The additional 40 mass units was attributed to acetonide formation, a deduction supported by the ^{1}H NMR (5 Me singlets compared to 3 in A, D, E or F) and ^{13}C NMR (2 additional Me signals and an extra quaternary carbon) spectra, which otherwise resembled those of the parent punctatins. Brief exposure of the acetonide (**8**) to 10% aqueous AcOH at room temperature allowed punctatin E (**4a**) to be identified by TLC as the major product. Furthermore, treatment of E in acetone with a little dilute sulphuric acid, gave an

Table 1. Selected peaks from 90 MHz ^{1}H NMR spectra of punctatins F, E, A, and D in C_5D_5N.

Compound	9 or 11-H (α-OH) (δ)	One of 3-CH_2 protons (δ)	12-CH_2OH (δ)	ΔCH_2	15-CH_3 (δ)
4b	5.00	*ca.* 3.0	4.2, 4.5	0.3	1.36
4a	4.50	Both upfield	4.2, 4.8	0.6	1.6
1a	4.60	*ca.* 3.0	3.8, 4.1	0.3	1.38
1b	5.36	Both upfield	3.95, 4.1	0.15	1.58

acetonide identical in all respects to that described above, presumably obtained as an artefact of manipulating column fractions in this one instance. An X-ray crystal structure confirmed that the compound was punctatin E acetonide (**8**): Crystal data: $C_{18}H_{28}O_3$, M=292.0, orthorhombic, a=7.284(1), b=11.488(2), c=19.823(3) Å, U=1658.6 Å^3, space group $P2_12_12_1$, Z=4, D_c=1.169 g/cm^3, F (000)=640.0, λ=1.5418 Å, μ(Cu-K$_\alpha$)=5.40 cm^{-1}. 2749 observed data out of 3134 unique measured reflections [$I \geq 1.5\sigma(I)$] to sin θ/λ=0.609 Å^{-1}. The final R value, $\Sigma|\Delta F|/\Sigma|F_0|$, was 0.041. Other details are as described for punctatin D.

The production of four such isomers as punctatins A, D, E and F by an organism is unusual, and, depending on the biosynthetic pathway(s) involved, could imply poorly specific (enzymic) steps at some stage.

Biological activities of punctatins A, D, E, and F revealed to date have been of little interest. Punctatin D was marginally active (1 ppm) *in vitro* against a mycelial form of *Candida albicans*, and punctatin E was inhibitory to *Trichomonas vaginalis* at 100 ppm *in vitro*.

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