Metabolites of the Higher Fungi. Part 25.[1] Punctaporonin G from the Fungus *Poronia Punctata* (Linnaeus: Fries) Fries

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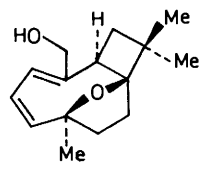
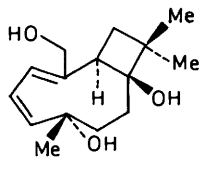
Punctaporonin G is a new naturally occurring tricyclic sesquiterpene; it can also be produced from punctaporonin B by acid catalysed dehydration involving the 1- and 4-hydroxy substituents. The properties of punctaporonin G are compared with those of B and an additional major acid degradation product of B is identified.

In part 23[2] of this series we described the isolation and properties of the punctaporonins, a series of bi-, tri-, and tetracyclic sesquiterpenoids from the fungus *Poronia punctata*. These compounds were accompanied by a quantity of less polar gummy material which failed to crystallise. From this material we have now isolated a new metabolite, which we name punctaporonin G (**1**). The compound occurs as a low-melting solid and appears as a brown spot on SiO_2 with the detecting reagent.[2] In the electron-impact (e.i) mass spectrum of the punctaporonins A, B, D, E, and F, the molecular ion at *m/z* 252 is not seen; instead the highest ion occurs at *m/z* 234, corresponding to the spontaneous loss of water. Punctaporonin G also shows the highest ion at *m/z* 234, but unlike the former compounds elemental analysis shows this to be the molecular ion.

Punctaporonin G (**1**), $C_{15}H_{22}O_2$, m.p. 32—38 °C; $[\alpha]_D^{23}$ − 231° (*c* 1.0 in MeOH), in the ^{13}C n.m.r. spectrum (Table 1) shows three methyl, four methylene, four methine, and four quaternary resonances; three of the methines and one of the quaternary carbons are unsaturated and one of the methylene and two of the quaternary carbons are attached to oxygen.



(**1**)



(**2**)

These resonances are similar to those of punctaporonin B (**2**) and the close structural analogy is confirmed (Table 2) by the H^1 n.m.r. resonances in the range δ 1.03—3.19; which appear as three singlet methyls, four single-proton multiplets, and a three proton multiplet; these absorptions identify the three methyl, three methylene, and the methine group characteristic of the punctaporonin nucleus. The primary alcohol protons occur as two separate doublets at δ 4.34 and 4.42. Three olefin protons at δ 5.19 (d, *J* 12.6 Hz, 5-H), 5.72 (dd, *J* 12.6 and 7.0 Hz, 6-H) and 6.30 (dd, *J* 7.0 and 1.2 Hz, 7-H) constitute a conjugated diene; except for 7-H these signals are significantly upfield compared with those of B. Unlike punctaporonin B which forms a monoacetate as a gum, the new compound yields a crystalline derivative under the same experimental conditions. The downfield shifts of the individual primary alcohol proton signals of punctaporonin G in going from alcohol to acetate are quite different (Δδ 0.25 and 0.38), whereas in B both have similar downfield shifts (Δδ 0.25 and 0.24). Punctaporonin G and B form dienals as the only identifiable products of pyridinium dichromate oxidation. Unlike B, G (**1**) is quite stable to treatment with mineral acid; it can be recovered unchanged after refluxing with acid over 1.5 h, whereas B under the same conditions is decomposed completely within 10—15 min. The characteristic red colouration observed when B is heated with all but a trace of mineral acid is not observed with G.

Table 1. ^{13}C N.m.r. chemical shifts (δ relative to Me_4Si in C_5D_5N) of punctaporonins G (**1**) and B (**2**)

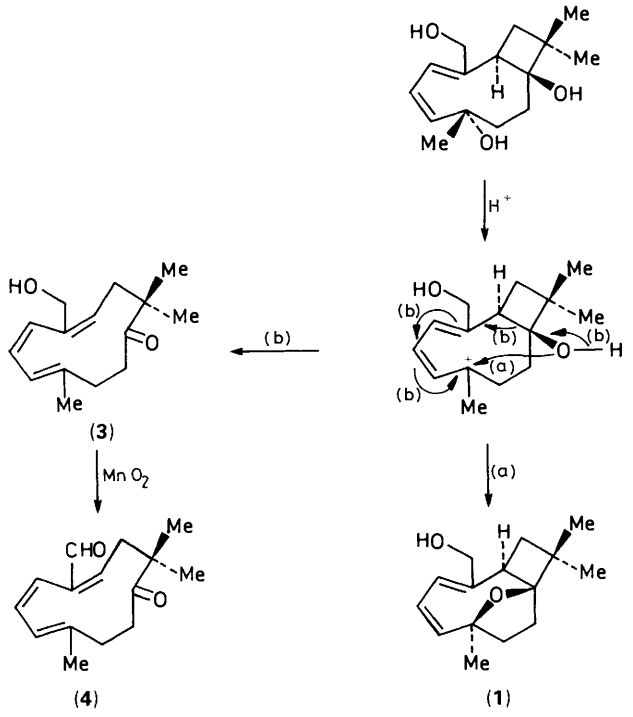
	G	B
C-1	88.45	81.4
C-2, C-3, C-10	38.26, 35.97, 32.38	35.7, 34.2, 30.8
C-4	84.35	74.2
C-5	134.78	144.5
C-6, C-7	119.28, 123.44	124.40, 124.44
C-8	142.14	142.0
C-9	44.10	43.0
C-11	40.08	40.9
C-12, C-13	26.46, 26.00	24.6, 23.3
C-14	66.04	65.1
C-15	23.9	32.65

We have shown that, when punctaporonin B is treated with hot mineral acid, punctaporonins A, E, D, and F are produced in low yield.[2] However, the major product of this reaction is a gum of relatively high R_F compared with the parent; this consists of a mixture of three components; that showing the lowest R_F corresponded with the new metabolite but separation was unsatisfactory. Separation *via* the acetates overcame this problem. The oily acetylation product comprised three components; that of intermediate R_F yielded crystalline punctaporonin G acetate. Punctaporonin G is thus produced in small quantity, like A, D, E, and F, as the result of the formation of a car-

Table 2. ^{1}H N.m.r. chemical shifts (δ, relative to Me_4Si in C_5D_5N)[a,b] of punctaporonins G (**1**) and B (**2**)

	G	B
1-H		1.99 (m, 2 H)
*2-H_2	1.34 (m, 1 H)	
	1.69 (m, 1 H)	
*3-H_2	1.83 (m, 1 H)	2.39 (m, 1 H)
	2.09 (m, 1 H)	3.00 (m, 1 H)
4-H		5.92 (dd, 13.0, 2.0, 1 H)
5-H	5.19 (d, 12.6, 1 H)	6.32 (d, 13.0, 1 H)
6-H	5.72 (dd, 12.6, 7.0, 1 H)	6.14 (s, 1 H)
7-H	6.30 (dd, 7.0, 1.2, 1 H)	
8-H		3.76 (dd, 11.6, 8.8, 1 H)
9α-H	3.19 (br, dd, 8.4, 9.5, 1 H)	1.60 (dd, 8.8, 10.0, 1 H)
10-H_2	1.89 (dd, 8.4, 4.2, 2 H)	2.51 (dd, 11.6, 10.0, 1 H)
11-H		
**12-H_3	1.07 (s, 3 H)	1.21 (s, 3 H)
**13-H_3	1.03 (s, 3 H)	1.14 (s, 3 H)
14-H_2	4.34 (d, 13.4, 1 H)	4.39 (d, 12.2, 1 H)
	4.42 (dd, 13.7, 1.2, 1 H)	4.68 (d, 12.2, 1 H)
15-H_3	1.42 (s, 3 H)	1.47 (s, 3 H)

*, ** Assignments may be interchanged within a column. [a] Bruker WM-400 spectrometer. [b] Connectivities determined at 270 MHz by spin-decoupling.



Scheme.

bocation [Scheme, path (a)]. Since in the formation of A, D, E, and F the carbocation site can be located at position 4- of B with certainty, in the formation of G the stereochemistry of the new ring is dependent on carbocation formation at either position 1 or 4. Whilst it is difficult to be precise, we suggest on the basis of previously observed patterns of reactivity, that the carbocation is formed at position 4 and leads to the formation of (**1**).

The component of lowest R_F from the above acetylation mixture was an oil; and ^{1}H and ^{13}C n.m.r. spectra of this showed it to be still a mixture. A significant feature of the ^{13}C n.m.r. spectrum was the appearance of quaternary carbon atoms at δ 210.7 and 217.0 indicative of the presence of either two different monoketones or less likely a diketone admixed with other non-carbonyl containing material. The problem was resolved by pyridinium dichromate oxidation of the crude acid-treated punctaporonin B mixture. The reaction was virtually complete in 15 min and the oxidation with MnO_2 in benzene solution was complete in 2 h; both gave the same product. This latter reaction is faster than the similar oxidation of punctaporonin B and proves the retained but different allylic nature of the primary OH group. Chromatographic separation of the oxidised mixture gave punctaporonin G aldehyde and a gum, which yielded a mixture of 2,4-dinitrophenylhydrazones; this was separated to yield the major product as a monoderivative. The ^{1}H n.m.r. of this product showed three singlet methyls; two of them superimposed at δ 1.28 and the third at δ 1.77. The latter is markedly downfield compared with that of either punctaporonin B (δ 1.47) or G(δ 1.38) and the superimposition of the 12-and 13-methyl groups indicates a change in their environment compared with those in the naturally occurring punctaporonins. The derivative shows four unsaturated protons, three of which constitute a diene appearing as two doublets and a double doublet; the additional fourth resonance at δ 5.54 (dd, *J* 8.1 and 8.4 Hz) is coupled to a broad unresolved two proton signal at δ 3.4. Since the unoxidised compound (**3**) is a ketone, the carbonyl of which must arise from the 1-hydroxy group, its formation can be explained by cation formation at 4-C, rearrangement of the diene, which induces cleavage of the four membered ring to produce a triene and loss of a proton to produce the ketotriene (**3**) [Scheme, path (b)]. In the 2,4-D.n.p derivative of (**4**) the 2- and 3-methylene signals both occur as unresolved broad absorptions at δ 2.0 and 2.3. The lowfield position of the 15-methyl group at δ 1.77 supports structure (**4**).

Experimental

M.p.s were determined on a Kofler hot-stage apparatus, i.r. spectra on a Perkin-Elmer 681 spectrophotometer, u.v. spectra on a Unicam S.P. 800 spectrophotometer, ^{1}H and ^{13}C n.m.r. spectra on a JEOL JNM GX-270 spectrometer unless otherwise specified (C_5D_5N with $SiMe_4$ as internal standard, connectivities in the ^{1}H n.m.r. spectra were established by spin-spin decoupling experiments, ^{13}C n.m.r. operating frequency 67.8 MHz, ^{1}H broad-band decoupled and DEPT 135 spectra), mass spectra on an AEIMS 902 spectrometer, and optical rotations on a Perkin-Elmer 141 polarimeter. All t.l.c., preparative t.l.c. (p.l.c.) and column chromatography was done on Merck Kieselgel PF 256 + 366. Components on t.l.c. plates were identified by the colours produced when sprayed with EtOH–H_2SO_4 (98:2) and heated at 100—110 °C for 1—2 min. Extracts were dried over Na_2SO_4.

Isolation of Punctaporonin G from Poronia punctata.—The brown gum (1.2 g) obtained as described previously[2] from tubes 11—39 of the chromatographic separation, was dissolved in the mixed solvent light petroleum (b.p. 60—80 °C)–diethyl ether–acetic acid (70:30:3) and applied to a column of SiO_2 (15 × 1.5 cm). Elution with the same solvent gave two main fractions. The eluate containing the upper fraction (brown spot) was evaporated to yield *puntaporonin G* as a pale yellow oil which slowly solidified to a waxy, pale yellow solid (0.28 g), m.p. 32—38 °C (Found: C, 77.3; H, 9.5. $C_{15}H_{22}O_2$ requires C, 76.9; H, 9.4%); *m/z* 234 (2.9%), 216 (5.7), 201 (5.3), 178 (193), 177 (6.2), 176 (5.3), 173 (11.5), and *inter alia* 41 (100%).

Punctaporonin G Acetate.—A mixture of punctaporonin G (100 mg), acetic anhydride (1 ml), and pyridine (1 drop), was set aside overnight and then poured into water (10 ml) and the oily mixture set aside at 5 °C. After 2 h the colourless solid was filtered off and recrystallised from aqueous ethanol to yield

punctaporonin G acetate as needles (44 mg), m.p. 65—66 °C (Found: C, 73.7; H, 8.8. $C_{17}H_{24}O_3$ requires C, 73.9; H, 8.7%); $\nu_{max.}$(KBr) 1 740 and 1 638 cm^{-1}; $\nu_{max.}$($CHCl_3$) 1 727 and 1 647—1 632 cm^{-1}; *m/z* 276 (M^+, 7.4%), 220 (12), 216 (21), 201 (5.2), 177 (10.2), 173 (14.5), 166 (24), 146 (48), 145 (51), 132 (39), 131 (82), 118 (31), 117 (22), 105 (47), 91 (24), and 43 (100); δ_H 1.01 (3 H, s, 12- or 13-H_3), 1.05 (3 H, s, 12- or 13-H_3, 1.32 (1 H, m, one of 2-H_2 or 3-H_2), 1.38 (3 H, s, 15-H_3) 1.68 (1 H, m, one of 2-H_2 or 3-H_2), 1.77 (2 H, dd, *J* 8.3 and 3.1 Hz, 10-H_2), 1.80 (1 H, m, one of 2-H_2 or 3-H_2), 2.03 (3 H, s, OAc), 2.08 (1 H, m, one of 2-H_2 or 3-H_2), 3.06 (1 H, br, dd, *J* 8.8 and 8.3 Hz, 9-αH), 4.58 (1 H, d, *J* 12.3 Hz, 1 H of 14-H_2), 4.80 (1 H, dd, *J* 12.3 and 0.9 Hz, 1 H of 14-H_2), 5.21 (1 H, d, *J* 12.8 Hz, 5-H), 5.62 (1 H, dd, *J* 12.6 and 6.6 Hz, 6-H), 6.03 (1 H, dd, *J* 6.0 and 1.65 Hz, 7-H); δ_C 20.89, 23.85, 25.77, and 26.40 (CH_3), 32.35, 35.79, and 38.18 (CH_2), 39.88 (C), 43.83, (CH), 69.11 (O-CH_2), 84.47, and 88.07 (C-O), 122.57, 124.34, and 136.13 (CH=), 135.92 (C=), and 170.56 (CO_2).

Punctaporonin G Aldehyde.—A mixture of punctaporonin G (300 mg), pyridinium dichromate (500 mg), molecular sieve 3A (1 g), and dichloromethane (10 ml) was stirred (2 h). The mixture turned dark within 1 min. The solution was diluted with diethyl ether (30 ml), filtered, and evaporated. The residue was separated by p.l.c. on SiO_2 in the solvent system benzene–ethyl acetate–acetic acid (75:25:1) to yield *punctaporonin G aldehyde* as a pale yellow solid (123 mg), which recrystallised from aqueous ethanol (× 2) as colourless needles, m.p. 71—73 °C (Found: C, 77.25; H, 8.7. $C_{15}H_{20}O_2$ requires C, 77.6; H, 8.6%); $\nu_{max.}$(KBr) 1 684 cm^{-1}; $\lambda_{max.}$(EtOH) 274 nm (log ε 4.30); *m/z* 232 (M^+, 6.6%), 176 (100), 161 (12.8), 148 (14.3), 147 (31.3); δ_H 0.98 (3 H, s, 12- or 13-H_3), 1.04 (3 H, s, 13-or 12-H_3), 1.29 (1 H, m, one of 2-H_2 or 3-H_2), 1.40 (3 H, s, 15-H_3), 1.62 (2 H, m, two of 2-H_2 and/or 3-H_2), 1.85 (1 H, dd, *J* 10.3 and 10.4 Hz, β10-H_2), 2.02 (1 H, dd, *J* 7.7 and 11.9 Hz, one of 2-H_2 or 3-H_2), 2.37 (1 H, dd, *J* 9.2 and 10.8 Hz, α10-H_2), 3.15 (1 H, dd, *J* 9.2 and 9.9 Hz, 9α-H), 5.52 (1 H, d, *J* 12.6 Hz, 5-H), 5.82 (1 H, ddd, *J* 12.6, 7.1 and 1.4 Hz, 6-H), 6.59 (1 H, dd, *J* 7.1 and 2.4 Hz, 7-H), 9.5 (1 H, s, CHO); δ_C 23.55, 25.45, and 26.30 (CH_3), 31.38, 36.94, and 38.98 (CH_2), 40.41 (CH), 40.54 (C), 84.48, and 89.04 (C-O), 121.68, 141.54, and 144.51 (=CH), 143.64 (C=), and 196.13 (CHO).

Acid Decomposition of Punctaporonin B.—A solution of punctaporonin B (1 g) in tetrahydrofuran (THF) (10 ml) and sulphuric acid (0.3 ml; 1 M) was refluxed (15 min.). The solution was diluted with water (5 ml) and the THF removed under reduced pressure. The oily suspension was extracted with ethyl acetate (2 ×) and the extracts were washed with saturated aqueous sodium hydrogen carbonate and water and dried. Evaporation gave a gum, which t.l.c. in the solvent system chloroform–methanol (99:1) showed to be a mixture of three components (red, orange, and brown spots). Chromatographic separation of 100 mg by p.l.c. resulted in a major loss of material, but yielded punctaporonin G (brown spot, slowest component) as a waxy solid (8 mg); identical by ^1H, ^{13}C n.m.r. and m.s. with the natural product.

Acetylation.—A mixture of the crude acid degradation product (1 g), acetic anhydride (10 ml), and pyridine (4 drops) was set aside overnight at room temperature and then poured into water. The oily mixture was extracted into ether, and the extract washed with sulphuric acid (1M), saturated aqueous sodium hydrogen carbonate and water, and then dried and evaporated to yield an oil. Column chromatography on SiO_2 in the solvent system light petroleum (b.p. 60—80 °C)–ether–acetic acid (70:30:3) gave three fractions (pink, brown and intense orange-brown spots). The first fraction (pink spots) gave negligible material upon evaporation. The second fraction (brown spot) yielded an oil, which rapidly solidified. Crystallisation from aqueous ethanol afforded punctaporonin G acetate as needles (56 mg), m.p. 65—66 °C. The third fraction (intense orange brown spot) gave an oil (260 mg), which failed to crystallise; δ_C 17.79, 20.82, 22.67, and 25.66 (CH_3), 27.93, 30.02, 30.26, 34.44, 35.05, 39.35, and 40.41 (CH_2), 46.46 and 48.32 (C), 62.70 and 67.68 (OCH_2), 121.21 (CH), 122.75, 136.73, 138.44, and 138.80 (C), 123.28, 124.72, 127.95, 130.10, 130.18, 133.57, and 134.70 (CH), 170.56 (OAc), and 210.71 and 217.05 (CO).

Oxidation with Manganese Dioxide.—A solution of the punctaporonin B acid derived gum (1 g) in benzene (30 ml) was stirred with manganese dioxide (6 g). After 2 h the mixture was filtered and evaporated and the resulting gum (0.86 g) separated by column chromatography with a mixture of light petroleum (b.p. 60—80 °C)–diethyl ether–acetic acid (70:30:3) to yield: fraction (i) of highest R_F which after evaporation gave a gum (92 mg); this yielded *punctaporonin G aldehyde* as needles, m.p. and mixed m.p. 71—73 °C, identical with a sample prepared from naturally occurring punctaporonin G, and fraction (ii) of lowest R_F; this yielded a gummy solid which after trituration with ethanol and filtration gave an amorphous polymeric residue (22 mg). The gum resulting from evaporation of the filtrate was treated with an acidified (H_2SO_4) ethanolic solution of 2,4-dinitrophenylhydrazine (DNP). The red precipitate was filtered off and chromatographed in chloroform on SiO_2; evaporation of the eluate gave the least mobile component as an orange–red powder. Crystallisation from toluene yielded stout needles of (6*Z*)-8-*formyl*-4,11,11-*trimethylcycloundeca*-4,6,8-*trien*-1-*one* (**4**) 8-(2,4-*dinitrophethylhydrazone*), m.p. 183 °C (Found: C, 61.1; H, 5.8; N, 13.2. $C_{21}H_{24}N_4O_5$ requires C, 61.15; H, 5.9; N, 13.6%); δ_H 1.28 (6 H, s, 12- and 13-H_3), 1.77 (3 H, s, 15-H_3) 1.83—2.16 (2 H, br, s), 2.16—2.52 (2 H, br, s), 3.22—3.6 (2 H, br, s), 5.54 (1 H, dd, *J* 8.1 and 8.4 Hz, 9-H), 5.86 (1 H, dd, *J* 6.6 and 12.5 Hz, 6-H), 6.12 (1 H, d, *J* 12.5 Hz, 5-H), 6.25 (1 H, d, *J* 6.6 Hz, 7-H), 7.95 (1 H, d, *J* 9.5 Hz, 6-ArH), 8.16 (1 H, s, NH), 8.51 (1 H, dd, *J* 9.5 and 2.6 Hz, 5-ArH), 9.11 (1 H, d, *J* 2.6 Hz, 3′-ArH), and 11.71 (1 H, s, CHN); δ_C 18.00 × 2, and 25.8 (CH_3), 26.45, 35.51, and 39.43 (CH_2), 46.33 (C), 116.50, 121.43, 123.74, 124.31, 130.25, 134.80, and 136.51 (CH=), 153.59 (NCH=), 129.79, 138.03, 138.26, 138.82, and 145.15 (C=), and 210.60 (CO).

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References

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