SHORT PAPER

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The biotransformation of the diterpenoid, rosenonolactone by *Mucor plumbeus*

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The biotransformation of rosenonolactone by the fungus, *Mucor plumbeus* involves hydroxylation at C-2α C-6β C-12α and epoxidation of the Δ^{15}-alkene.

Keywords: biotransformation, *Mucor plumbeus*, rosenonolactone

Rosenonolactone **1** is a major metabolite of *Trichothecium roseum*.[1] It is the best known and the most readily available of the rosane family of diterpenoids.[2] This structure possesses quaternary centres at C-4, C-9, C-10 and C-13 and consequently it is difficult using conventional chemical means, to functionalise rings A and C. A number of rosanes are biologically active and recently as a result of screening studies, it was concluded that rosenonolactone analogues may be good models for the design of new inhibitors of the cholesteryl ester transfer protein.[3] It was therefore of interest to examine the use of microbiological transformation in order to introduce hydroxyl groups into rings A and C.

The fungus, *Mucor plumbeus*, has been used for the biotransformation of terpenoids. Incubation of rosenonolactone **1** with *M.plumbeus* for seven days on shake culture gave five metabolites which were separated by chromatography. The first metabolite to be isolated from the column was identified as 6β-hydroxyrosenonolactone 2.[4] There was an additional CH(OH) resonance in the ^{1}H NMR spectrum [δH 3.96 (double-doublet *J* 4.9 and 1.9 Hz)] which sharpened to a doublet (*J* 4.9 Hz) on addition of $^2H_20$. The structure and

Table 1 ^{13}C NMR data (determined in $CDC1_3$ at 75 MHz)

Carbon number	Compound 1	2	3	4	5	6
1	30.2	31.3	27.1	30.4	44.1	27.4
2	19.8	19.7	19.7	19.7	66.1	19.6
3	35.4	36.3	35.4	35.3	39.5	35.2
4	47.3	45.4	47.2	47.2	45.6	47.0
5	50.9	54.8	50.8	50.9	50.6	50.5
6	30.8	68.5	29.5	31.9	31.1	28.5
7	210.2	212.8	210.2	209.5	209.7	211.2
8	47.3	47.1	46.9	46.8	47.3	47.2
9	38.8	40.5	38.9	40.7	38.9	38.7
10	87.0	86.5	86.8	86.5	87.1	87.1
11	35.8	31.3	35.7	38.0	35.4	35.7
12	31.4	31.4	28.6	70.5	31.4	28.6
13	35.1	35.0	32.5	41.2	35.0	35.4
14	31.7	31.6	30.2	35.7	31.6	29.0
15	149.6	149.5	60.5	146.2	149.4	70.8
16	109.9	109.9	43.3	114.1	110.0	62.1
17	21.8	21.8	18.5	30.8	21.8	18.0
18	16.9	16.4	16.7	16.8	16.6	16.6
19	179.3	179.7	179.2	179.1	178.4	179.6
20	16.9	17.0	16.9	18.3	16.7	16.7

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† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M).*

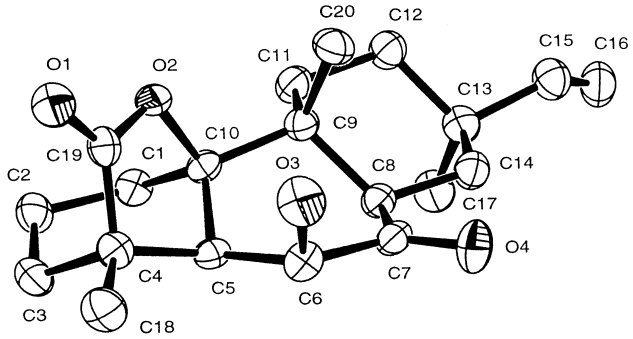
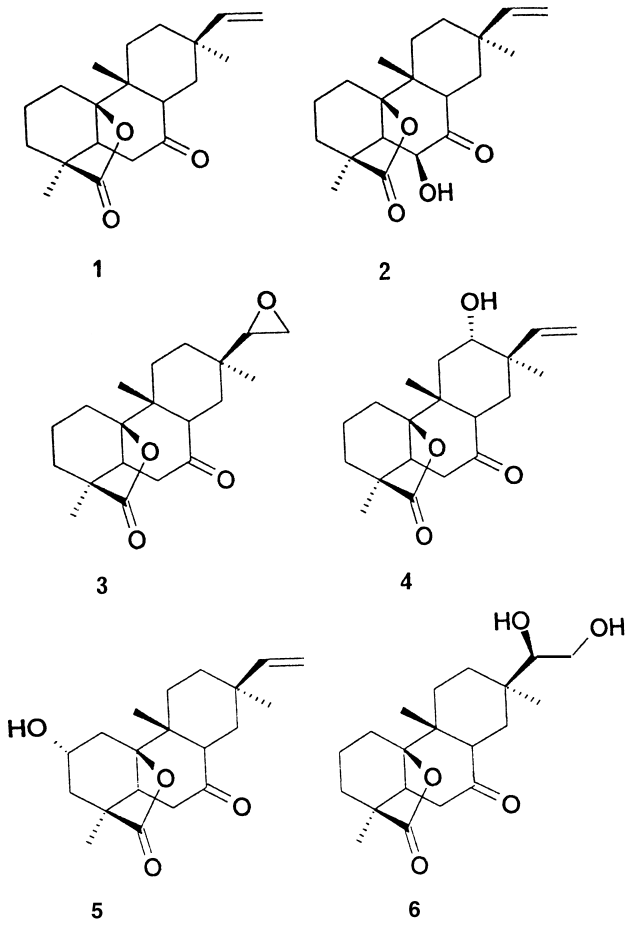


Fig. 1 X-ray crystal structure of 6β-hydroxyrosenonolactone **2**.



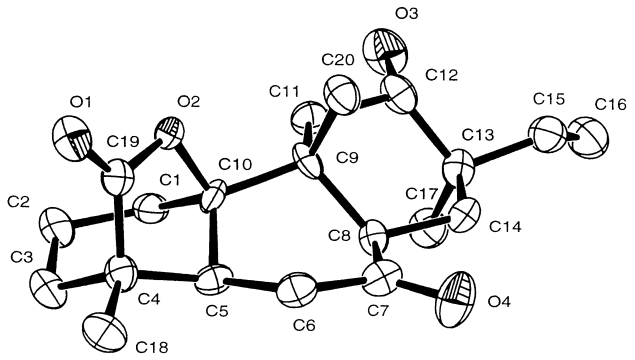


Fig. 2 X-ray crystal structure of 12α-hydroxyrosenonolactone **4**.

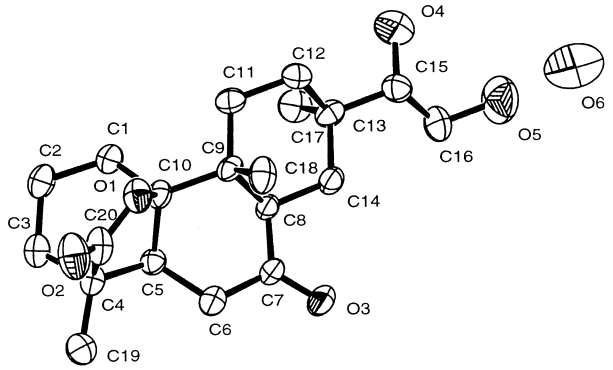


Fig. 3 X-ray crystal structure of 15S, 16-dihydroxyrosenonolactone **6**.

stereochemistry of the metabolite were confirmed by X-ray crystallography (see Fig. 1).

In the second metabolite the characteristic alkene ^{1}H NMR signals had been replaced by those of an epoxide [δ_H 2.66 (2H) and 2.75 (1H)] and hence the metabolite was assigned the structure **3**. The third metabolite possessed a CH(OH) signal at δ_H 3.62 (double-doublet, *J* 4.2 and 11.5 Hz). This multiplicity, arising from an axial:equatorial and a diaxial coupling together with the changes in the position of the ^{13}C NMR signals[5] (*e.g.* for C-13) (see Table 1) suggested that the hydroxyl group was at the 12α position. The structure **4** was confirmed by X-ray crystallography (see Fig. 2). The fourth metabolite was a gum. The change in position of the C-l and C-3 ^{13}C NMR signals (see Table 1) and the multiplicity (triplet of triplets) of the CH(OH) resonance (δ_H 4.12, *J* 4.4 and 10.9 Hz) suggested that the compound was 2α-hydroxyrosenonolactone. A 2α-hydroxyrosane, isorosenolic acid, has been isolated[6] from *Trichothecium roseum.* In the final metabolite, the alkene resonances were replaced by those of a primary and a secondary alcohol [δ_H 3.27 and 3.43 (each 1H doublets, *J* 8.9 and 9 Hz) and 3.74 (double-doublet, *J* 8.9 and 9 Hz)]. The structure and stereochemistry of **6** particularly at C-15, were established by X-ray crystallography (see Fig. 3). This metabolite,15S,16-dihydroxyrosenonolactone, which crystallised with a molecule of water of crystallisation, may arise by hydrolysis of the epoxide **3**.

The isolation of the metabolites **4** and **5**, albeit in low yield, suggests that microbiological hydroxylation is a potential method for introducing functionality into rings A and C of rosenonolactone.

Experimental

General experimental details: IR spectra were determined as nujol mulls. ^{1}H and ^{13}C NMR spectra were determined for solutions in deuteriochloroform at 300 and 75 MHz respectively. Silica for chromatography was Merck 9385. Light petroleum refers to the fraction, b.p. 60–80°C.

Rosenonolactone 1, isolated from *Trichothecium roseum*, had m.p. 208–210°C (lit.,[1] 213–214°C).

Fermentation details: The fungus, *Mucor plumbeus*, *was* grown on shake culture (100 cm^3 medium per 250 cm^3 conical flask) on a medium comprising (per litre): glucose (30 g), potassium dihydrogen phosphate (2 g), magnesium sulfate (2 g ammonium tartrate (2 g), yeast extract (1 g), calcium chloride (0.1 g), sodium chloride (1 g), ferrous ammonium sulfate (0.2 g) and a trace elements solution (2 cm^3). The latter comprised (per litre): zinc sulfate (1.6 g), ferrous sulfate (1 g), cobalt nitrate (1 g), ammonium molybdate (1 g), copper sulfate (0.1 g) and manganese sulfate (0.1 g). Rosenonolactone (500 mg) in ethanol (18 cm^3) and dimethylsulfoxide (18 cm^3) was evenly distributed between 18 flasks of a 2-day old culture and the fermentation was continued for a further 7 days. The mycelium was filtered and the broth acidified to pH 2 with dilute hydrochloric acid and extracted with ethyl acetate. The extract was dried and the solvent evaporated to give a residue which was chromatographed on silica. Elution with a gradient of increasing (from 0 to 100%) concentrations of ethyl acetate in light petroleum gave rosenonolactone **1** (260 mg), 6β-hydroxyrosenonolactone **2** (45 mg), 15,16-epoxy-rosenonolactone **3** (15 mg); 12α-hydroxyrosenonolactone **4** (35 mg) 2α-hydroxy-rosenonolactone **5** (35 mg) and 15S,16-dihydroxy-rosenonolactone **6** (30 mg).

6β-Hydroxyrosenonolactone **2**: m.p.185–187°C (lit.,[4] 180–181°C), (Found:M$^+$ 332.197 Calc. for $C_{20}H_{28}0_4$ M$^+$ 332.199), υ_{max}/cm^{-1} 3431, 1763, 1713, 1637; δH 0.97 (3H,s,H-17), 1.11(3H,s, H-20), 1.40(3H,s,H-18), 2.60(1H,dd, *J* 4.1 and 11.8 Hz, H-8), 2.88(1H,br.s.OH) (disappears on treatment with $^2H_20$), 3.96(1H,dd, *J* 1.9 and 4.9 Hz, H-6) (collapses to doublet *J* 4.9 Hz, on treatment with $^2H_20$), 4.92 (lH,dd, *J* 10.7 and 0.9 Hz), 5.00 (lH,dd, *J* 17.5 and 0.9 Hz) (each H-16), 5.82 (lH,dd, *J* 10.7 and 17.5 Hz, H-15).

15,16-Epoxyrosenonolactone **3**: m.p, 168–172°C, (Found: M$^+$ 332.197 $C_{20}H_{28}0_4$ requires 332.199), υ_{max}/cm^{-1} 1756, 1714; δ_H 0.86(3H,s, H-17), 0.92(3H,s, H-20), 1,11(3H,s, H-18), 2.62 (2H,d, *J* 3.2 Hz, H-16), 2.75(1H,t, *J* 3.2 Hz, H-15).

12α-Hydroxyrosenonolactone **4**: m.p. 200–204°C, (Found: M$^+$ 332.201 $C_{20}H_{28}0_4$ requires M$^+$ 332.199) υ_{max}/cm^{-1} 3447, 1763, 1716; δ_H 0.98 (3H,s, H-20), 1.03 (3H,s, H-17), 1,14(3H,s, H-18), 3.65(1H,dd, *J* 4.2 and 11.5 Hz, H-12), 5.14(2H,m,H-16), 5.82(1H,dd, *J* 11.1 and 17.3 Hz, H-15).

2α-Hydroxyrosenonolactone **5**: a gum (Found, M$^+$ 332.200 $C_{20}H_{28}0_4$ requires M$^+$ 332.199), υ_{max}/cm^{-1} 3410, 1755, 1713, 1659; δ_H 0,94(3H,s, H-17), 0.97(3H,s, H-20), 1.17(3H,s, H-18), 4.12 (lH,tt, *J* 6.5 and 9.9 Hz, H-2), 4.93(1H,dd, *J* 10.8 and 0.9 Hz), 5.00(1H,dd, *J* 17.5 and 0.9 Hz) (each H-16), 5.82 (1H,dd, *J* 10.8 and 17.5 Hz, H-15).

15S,16-Dihydroxyrosenonolactone **6**: m.p. 140–144°C, (Found: M$^+$ 350.209 $C_{20}H_{30}O_5$ requires M$^+$ 350.209), υ_{max}/cm^{-1} 3409, 1756, 1709; δ_H 0.80(3H,s, (H-17), 0,85(3H,s, H-20), 1.07 (3H,s, H-18), 3.31(1H,d, *J* 8.9 Hz), 3.47 (lH,d, *J* 9 Hz)(each H-16), 3.74(1H,dd, *J* 8.9 and 9.0 Hz, H-15).

X-Ray crystallographic data and structure determinations

(a) 6β-Hydroxyrosenonolactone **2**: $C_{20}H_{28}0_4$, M_r 332.42, orthorhombic, space group $P2_12_12_1$ (No.19), *a* = 11.1079(10), *b* = 11.5247(10), *c* = 13.3132(14)Å α = β = γ = 90°, *V* = 1704.3(3)Å^3, *Z* = 4, D_{calc} 1.30g cm^{-3}, μ = 0.09 mm^{-1} F(000) = 720. Data were collected using a crystal of size 0.30 x 0.20 x 0.05 mm^3 on a Nonius Kappa CCD diffractometer. A total of 4262 reflections were collected for 3.85 < θ < 22.98° and –12 < = *h* < = 10, –11 <= *k* <= 12, –14 <= *1* <= 9. There were 2272 independent reflections and 2048 reflections with $I > 2\sigma(I)$ were used in the refinement. No absorption correction was applied. The structure was solved by direct methods and refined by SHELXL-97. The drawings used ORTEP-3 for Windows. The final *R* indices were [$I > 2\sigma(I)$] R_1 = 0.044, $^{wR}2$ = 0.102 and all data R_1 = 0.051, $^{wR}_2$ = 0.106. The goodness-of-fit on F^2 was 1.071 and the largest difference peak and hole was 0.26 and –0.25eÅ^{-3}.

(b) 12α-hydroxyrosenonolactone **4**: $C_{20}H_{28}0_4$, M_r 332.42, orthorhombic, space group $P2_12_12_1$ (No.19), *a* = 10.9607(5), *b* = 11.9800(16), *c* = 13.153(2)Å α = β = γ = 90°, *V* = 1727.1(4)Å^3, *Z* = 4, D_{calc} = 1.28g cm^{-3}, μ = 0.09 mm^{-1} *F*(000) = 720. Data were collected using a crystal of size 0.05 x 0.05 x 0.05 mm^3 on a Nonius Kappa CCD diffractometer. A total of 5917 reflections were collected for 3.72 < θ < 21.95° and –9 <= *h* <= 11, –12 <= *k* <= 12, –13 <= *1* <= 13. There were 2085 independent reflections and 1402 reflections with $I > 2\sigma(I)$ were used in the refinement. No absorption correction was applied. The structure was solved by direct methods and refined by SHELXL-97. The drawings used ORTEP-3 for Windows. The

final *R* indices were [$I > 2\sigma(I)$] $R_1 = 0.066$, $wR_2 = 0.132$ and all data $R_1 = 0.113$, $wR_2 = 0.157$. The goodness-of-fit on F^2 was 1.011 and the largest difference peak and hole was 0.24 and -0.21eÅ^{-3}.

(c) 15S,16-dihydroxyrosenonolactone **6**: $C_{20}H_{30}0_5.H_20$, M_r 368.46, orthorhombic, space group $P2_12_12_1$ (No.19), a = 6.2949(4), b = 11.0304(8), c = 26.9217(17)Å. $\alpha = \beta = \gamma = 90°$, V = 1869.3(2)Å^3, Z = 4, D_{calc} = 1.31g cm^{-3}, μ = 0.10 mm^{-l}, $F(000)$ = 800. Data were collected using a crystal of size 0.3 x 0.3 x 0.1 mm^3 on a Nonius Kappa CCD diffractometer. A total of 6685 reflections were collected for $3.77 < \theta < 21{,}97°$ and –6 <= *h* <= 6, –11 <= *k* <= 10, –28 = *1* <= 28. There were 2270 independent reflections and 1774 reflections with $I > 2\sigma(I)$ were used in the refinement. No absorption correction was applied. The structure was solved by direct methods and refined by SHELXL-97. The drawings used ORTEP-3 for Windows. The final R indices were [$I > 2\sigma(I)$] $R_1 = 0.069$, wR_2 = 0,168 and all data R_1 = 0.093, $wR_2 = 0.185$. The goodness-of-fit on F^2 was 1.08 and the largest difference peak and hole was 0.42 and –0.29eÅ^{-3}. The compound crystallised with a molecule of water of crystallisation. The crystallographic data have been deposited at the Cambridge Crystallographic Data Centre.

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