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Biotransformations of 1′,2′-Dihydrorotenone by *Streptomyces griseus*

F. S. SARIASLANI AND J. P. ROSAZZA*

Division of Medicinal Chemistry and Natural Products, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52242

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Dihydrorotenone yields three major products when incubated with growing cultures of *Streptomyces griseus*. These were isolated by solvent extraction and characterized by spectral methods as 1′,2′-dihydro-6aβ-hydroxyrotenone, 1′,2′-dihydro-2′,6aβ-dihydroxyrotenone, and 1′,2′-dihydro-1′,6aβ-dihydroxyrotenone.

Rotenoids are widely used as natural pesticides, and they are subject to microbial (6), enzymatic (5a), and photochemical oxidations (2). In a previous report (6), metabolites of rotenone and 1′,2′-dihydrorotenone formed by cultures of the fungus *Cunninghamella blakesleeana* were isolated and identified. This organism introduced hydroxyl groups into the isopropylene and isopropyl moieties of the two substrates. Screening studies revealed that *Streptomyces griseus* also formed metabolites of dihydrorotenone. *S. griseus* has been widely used in biotransformation studies with numerous other natural products including alkaloids, terpenes, and coumarins (7). Thus, we sought to identify the types of metabolites formed by this organism with dihydrorotenone.

S. griseus ATCC 10137 was grown in 11 liters of a soybean meal-glycerol medium by a standard microbial transformation protocol (1). 1′,2′-Dihydrorotenone (compound 1) (5 g) was added to 24-h-old, second-stage cultures as before (1). Samples of the culture were taken at various time intervals and extracted with one-fourth volumes of ethyl acetate. The progress of microbial transformation was followed by thin-layer chromatography of the extracts (30 μl) on Silica Gel GF-254 layers with ethyl acetate-cyclohexane-methanol (40:10:0.1). At 72 h, thin layer chromatography indicated that approximately one-half of the substrate was used and that three products were present in nearly equal amounts. The incubation beer was exhaustively extracted with ethyl acetate to concentrate the fermentation products. After evaporation to a thick oil, the extract was subjected to extensive silica gel column chromatography with benzene-methanol (40:1) and cyclohexane-ethyl acetate (1:8) as the eluting solvents. Final purification of the transformation products was achieved by preparative thin-layer chromatography with the same solvent used for analytical thin-layer chromatography.

Analytically pure samples of 1′,2′-dihydro-6aβ-hy-droxyrotenone (compound 2) (117 mg; R_f, 0.63), 1′,2′-dihydro-2′,6aβ-dihydroxyrotenone (compound 3) (53 mg; *Rf*, 0.51), and 1′,2′-dihydro-1,6aβ-dihydroxyrotenone (compound 4) (23 mg; R_f, 0.42) were subjected to high-resolution mass spectrometry and proton and ^{13}C nuclear magnetic resonance (NMR) spectroscopy as before (4–6). High-resolution mass spectrometry of compound 2 gave a molecular ion at *m/e* 412.1501 for $C_{23}H_{24}O_7$ (calculated value, 412.1522), or one more oxygen atom than the starting material, compound 1. The base peak in the mass spectrum occurred at *m/e* 208 for $C_{11}H_{12}O_4$, indicating that the additional oxygen atom was located in the A or B ring of the fermentation product (5). The ^{13}C NMR spectrum of compound 2 was similar to that of compound 1 (Table 1), except for the signal at C-6a, which resonated as a singlet at 67.5 ppm from the original doublet at 44.89 ppm. Accompanying downfield shifts of carbon signals for positions 6, 6b, 12, and 12a indicate the location of the hydroxyl group at C-6a. These spectral shifts due to a hydroxyl group at this position are nearly identical to those observed in the structure of 6aβ,12aβ-rotenolone (5a). Proton NMR spectral findings were also consistent for the structure of the fermentation product as compound 2. The ^1H NMR spectrum was similar to that of compound 1, except for the position 7 aromatic proton signal, which serves as an indicator for the presence of a β-hydroxyl group at position 6a (6).

The high-resolution mass spectrum of compound 3 gave a molecular ion at *m/e* 428.1461 for $C_{23}H_{24}O_8$ (calculated

TABLE 1. ^{13}C NMR spectral properties of 1′,2′-dihydrorotenone and its fermentation products[a]

Carbon	Spectral properties of compound:			
	1	2	3	4
1	30.64t	29.25t	29.68	33.79t
2	90.77d	90.87d	91.65d	88.61d
3a	167.72s	168.3s	167.77s	167.60s
4	104.7d	105.2d	105.15d	105.34d
5	129.81d	129.81d	129.90d	129.98d
5a	113.3s	111.6s	109.62s	111.80s
6	188.6s	190.97s	191.10s	191.08s
6a	44.89d	67.5s	67.62s	67.62s
6b	105.4s	108.87s	108.78s	108.83s
7	111.7d	109.41d	109.62d	109.68d
8	144.3s	143.9s	144.02s	144.03s
9	150.1s	151.1s	151.21s	151.21s
10	101.4d	101.07d	101.51d	101.16d
10a	147.74s	148.3s	148.3s	148.42s
12	66.38t	63.86t	63.85t	65.42t
12a	72.47d	76.01d	76.65d	77.2d
13a	157.9s	157.62s	157.0s	157.65s
13b	113.2s	113.54s	113.8s	113.27s
1′	17.84q	17.88q	26.10q	63.84t
2′	29.44d	29.25d	71.67s	29.63d
3′	17.56q	17.52q	24.28q	22.64q
OMe	55.99q	55.85q	55.87q	55.86q
OMe	56.67q	56.4q	56.45q	56.43q

[a] All spectra were recorded at 90.562 MHz on a Bruker WM-360 FTQNMR spectrometer with $CDCl_3$ as the solvent.

* Corresponding author.

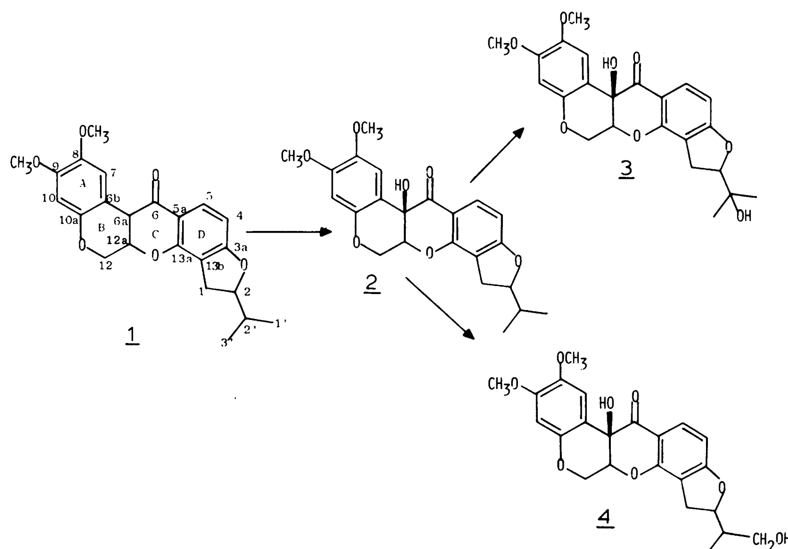

FIG. 1. Pathways for biotransformations of compound 1 by *S. griseus* ATCC 10137.

value, 428.14712), or a new structure incorporating two more oxygen atoms that the substrate compound 1. The base peak *m/e* 208 indicated that one oxygen atom was present on either the A or B ring (5) of the metabolite. The ^{13}C NMR spectrum displayed two major changes relative to compound 1. These were two singlet signals at 67.2 and 71.6 ppm replacing doublet signals for carbons 6a and 2′, respectively. Accompanying deshielding of carbons 2, 1′, 3′, 6, 6b, 12a, and 12 and the expected changes in the proton NMR spectrum confirm the structure of the metabolite as compound 3. This compound is identical to one of the biotransformation products of compound 1 formed by *C. blakesleeana* and previously identified in our laboratory (6).

The third compound gave high-resolution mass spectral fragmentation patterns nearly identical to those for compound 3. The molecular ion occurred at *m/e* 428.1465 for $C_{23}H_{24}O_8$. The base peak at *m/e* 208 for $C_{11}H_{12}O_4$ confirmed the presence of one oxygen atom in the dimethoxychroman moiety. One oxygen atom was fixed at position 6aβ by ^{13}C NMR, and the second oxygen atom was introduced into position 1′. The latter hydroxyl-group location was confirmed by the observation of a triplet signal at 63.04 ppm in the metabolite replacing a quartet signal at 17.84 ppm in the spectrum of compound 1. Proton NMR spectral data also supported the structure as compound 4. Signals for H-7, H-1′, and H-3′ appeared at 6.48, 0.968 (t, $2H$, J = 6.7 Hz), and 0.818 ppm (d, $3H$, J = 6.7 Hz). Therefore, the structure of the metabolite was compound 4. Hydroxylation of the methyl group by *S. griseus* introduces a new chiral position at 2′ of the metabolite with uncertain stereochemistry.

Rotenoids undergo air oxidation with the incorporation of a hydroxyl group at position 6a (5–6). Control incubations with dihydrorotenone in buffer alone or in sterile medium without *S. griseus* revealed that compound 2 was formed in good yield. Thus, in incubations with *S. griseus*, compound 2 is most probably a simple artifact of incubation whose formation precedes the formation of biotransformation products compounds 3 and 4 (Fig. 1). The major metabolic focus of *S. griseus* is on the isopropyl functional group of dihydrorotenone. This finding parallels previous results in our laboratory with the same microorganism in biotransformations of the terpene compound bruceantin (3).

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