Pergamon

0031-9422(95)00352-5

Phytochemistry, Vol. 40, No. 3, pp. 793-796, 1995

0031-9422/95 $9.50 + 0.00

BIOTRANSFORMATION OF SESQUITERPENOIDS, (+)-AROMADENDRENE AND (−)-ALLOAROMADENDRENE BY *GLOMERELLA CINGULATA*

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(*Received in revised form* 22 *February* 1995)

Key Word Index—Biotransformation; *Glomerella cingulata*; (+)-aromadendrene; (−)-alloaromadendrene; (−)-10α,13,14-trihydroxyaromadendrane; (−)-10β,13,14-trihydroxyalloaromadendrane; plant pathogenic microorganism

Abstract—The biotransformation of (+)-aromadendrene and (−)-alloaromadendrene by a plant pathogenic microorganism, *Glomerella cingulata*, was investigated. *Glomerella cingulata* oxidized (+)-aromadendrene and (−)-alloaromadendrene at the double bond and at one of the geminal methyl groups on the cyclopropane ring regioselectivity to form triols which were hydroxylated at C-10, C-13 and C-14. The structures of the new compounds have been elucidated on the basis of their spectral data coupled with some chemical evidence.

INTRODUCTION

We have been studying the biotransformation of terpenoids by the plant pathogenic microorganisms *Glomerella cingulata*, *Rhizoctonia solani* and *Botrytis allii*. In our previous papers, (+)-cedrol [1], (−)-α-bisabolol [2], 1,8-cineole [3] and (−)-globulol [4] were transformed to novel terpenes via stereoselective oxidation by *G. cingulata*. (+)-(1*R*)-aromadendrene (**1**) and (−)-(1*S*)-alloaromadendrene (**3**) are sesquiterpene hydrocarbons with an aromadendrane skeleton. They differ from each other only in the configuration at the C-1 position. We were interested in comparing the biotransformation of the (1*R*)-form and the (1*S*)-form of the hydrocarbons. We were interested in stereospecific oxidation and the oxidation of the geminal methyl groups of the cyclopropane ring of tricyclic sesquiterpenoids with an aromadendrene skeleton by *G. cingulata*. The biotransformation of **1**. by *Diplodia gossypina* gives 10α, 13,14-trihydroxyaromadendrane as a major metabolite and transformation of **3** by *Mycobacterium smegmatics* gives 1-hydroxyalloaromadendrane [5], but there are no reports on the metabolism of **1** and **3** by *G. cingulata*.

This paper describes the transformation of **1** and **3** by *G. cingulata*.

RESULTS AND DISCUSSION

Compounds **1** and **3** were used as starting substrates. *Glomerella cingulata* oxidized **1** at the double bond and at one of the geminal methyl groups on the cyclopropane ring regioselectivity and hydroxylated at the 10α,13 and 14-positions to form the triol (**2**). The ^{1}H NMR spectra of **2** displayed two signals at δ3.36 and 3.28 due to the primary alcohol and disappearance of the methyl signal at H-13. The methylene signal at H-14 had moved to δ3.62 with an upfield shift of 1.02 ppm. The ^{13}C NMR spectrum of **2** had a signal at δ73.3 for C-13, which had shifted 44.6 ppm. The signal for C-12 had moved to δ11.5 with an upfield shift of 4.3 ppm. The structure of **2** was determined by comparing ^{1}H NMR and ^{13}C NMR spectral data with those in ref. [5]. The metabolite **2** was identified as (−)-10α,13,14-trihydroxyaromadendrane.

Glomerella cingulata oxidized **3** at the double bond and at one of the geminal methyl groups on the cyclopropane ring and hydroxylated at 10β,13 and 14-positions to form a triol. The ^{1}H NMR spectra of **4** displayed two signals at δ3.47 (1H, *d*, J = 11.0 Hz, H-13) and 3.26 (1H, *d*, J = 11.0 Hz, H-13′) due to the primary alcohol and disappearance of the methyl signal at H-13. The signals for H-14 had moved to δ3.29 (1H, *d*, J = 10.5 Hz, H-14) and 3.42 (1H, *d*, J = 10.5 Hz, H-14′) with an upfield shift from δ4.73 and 4.70, respectively. The ^{13}C NMR spectrum of **4** had a signal at δ73.7 for C-13. The ^{13}C NMR signal for C-13 had moved to δ45.0 (from δ28.6 to 73.6). The ^{13}C NMR signal for C-12 in **4** was at δ11.9 with an upfield shift of 4.2 ppm (from δ15.8 to 11.9). The signal for C-14 was at δ70.7 with an upfield of 36.3 ppm (from δ109.7 to 70.7). The structure of **4** was determined by comparing its spectral data with that of the similar compound in ref. [6]. The metabolite **4** was identified as 10β,13,14-trihydroxy alloaromadendrane.

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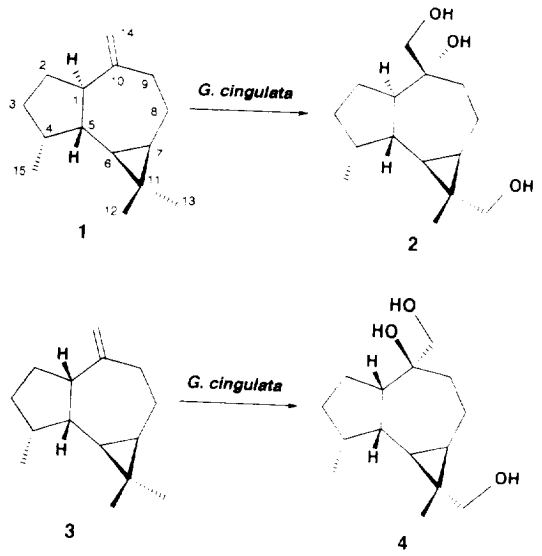

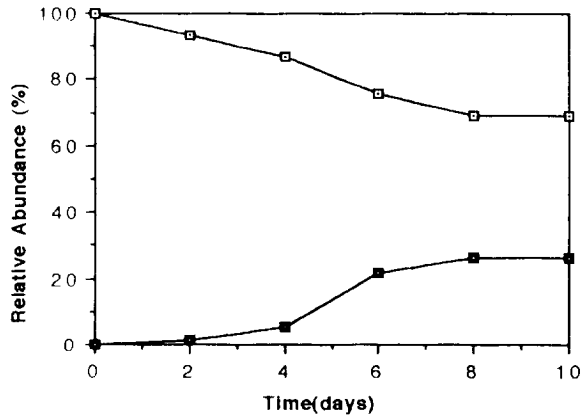

Fig. 1. Time course of (+)-aromadendrene (**1**) metabolism and (−)-10α,13,14-trihydroxy aromadendrane (**2**) formation after incubation with *Glomerella cingulata*. □, (+)-aromadendrene (**1**); ■, (−)-10α,13,14-trihydroxyaromadendrane (**2**).

The time courses for the biotransformation of (+)-aromadendrene and (−)-alloaromadendrene by *G. cingulata* are shown in Figs. 1 and 2. These were determined by GC (OV-1, 140–260 . 4 min $^{-1}$) analysis of the extract from the fermentation. The production of these compounds seems to be closely related to the decline of **1** and **3**. The production of **2** and **4** reached a maximum (both about 25%) after eight days. Compounds **1** and **3** were rapidly oxidized to the 10,13,14-trihydroxy forms (**2** and **4**) by *G. cingulata*, **2** was the (10*R*)-form and **4** was the (10*S*)-form. No epoxide, monool and diol products were detected. The difference in the configuration of the C-10 position for **2** [(10*R*)-form] and **4** [(10*S*)-form] occurs due to the configuration of the C-1 position for **1** [(1*R*)-form] and **3** [(1*S*)-form]. The difference of oxidation of these compounds is due to the configuration of the C-1 position. In the cases of **1** and **3**, there are no products with a carboxyl group. In the biotransformation of these two aromadendrane type sesquiterpenoids by *G. cingulata*, the configuration of the C-1 position affects the oxidation system.

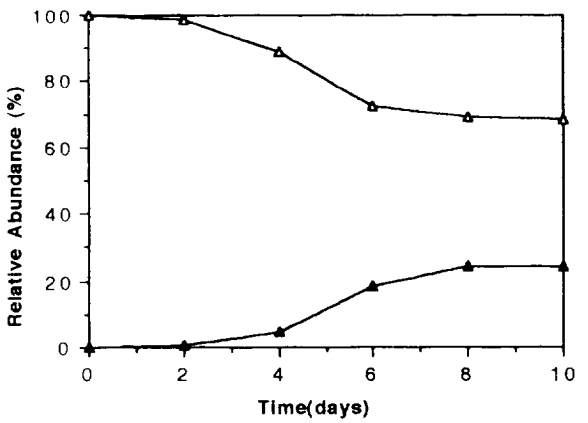

Fig. 2. Time course of (−)-alloaromadendrene (**3**) metabolism and (−)-10β,13,14-trihydroxyalloaromadendrane (**4**) formation after incubation with *Glomerella cingulata*. △, (−)-alloaromadendrene (**3**); ▲, (−)-10β,13,14-trihydroxyalloaromadendrane (**4**).

EXPERIMENTAL

Compounds **1** and **3** were purchased from Fluka. The ^{1}H and ^{13}C NMR spectra were determined for solns in $CDCl_3$ with TMS as the int. standard at 500 and 125 MHz, respectively. MS: 70 eV. GC: OV-1 (25 m × 0.25 mm) capillary column.

Preculture of Glomerella cingulata. Spores of *G. cingulata* were inoculated onto a plate containing 15 ml of the following medium: 1.5% sucrose, 1.5% glucose, 0.5% polypeptone, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, 0.1% K_2HPO_4, 0.001% $FeSO_4 \cdot 7H_2O$. Culture was incubated at 28° under a static condition for 3 days.

Addition of (+)-aromadendrene (**1**). After the growth of *G. cingulata*, 2 g of **1** (20 mg/15 ml of medium) was added to the medium, and the plate was left for 8 days.

Isolation of metabolite **2**. The culture broth was filtered to remove the mycelial mat. The filtrate was acidified to pH 2.0 and extracted continuously with CH_2Cl_2 for 72 hr. The organic layer was dried over Na_2SO_4. After evapn of the solvent, an oily residue (1.41 g) was obtained. This was dissolved in CH_2Cl_2, washed with 5% $NaHCO_3$ soln and sepd into neutral (980 mg) and acidic (271 mg) frs in the usual manner. For isolation of metabolite **2**, the neutral fr. was purified by silica gel CC with hexane containing increasing concns of EtOAc (0, 20, 40, 60, 80 and 100%), and **2** (155 mg) was isolated in the 80% EtOAc eluate. No acidic metabolite was identified from GC (140–260°, 4° min^{-1}) or TLC: (*n*-hexane–EtOAc, 1:1). Unutilized **1** (439 mg) was recovered.

Addition of (−)-alloaromadendrene (**3**). After the growth of *G. cingulata*, 4 g of **3** (20 mg/15 ml of medium)

was added to the medium, and the plate was left for 8 days.

Isolation of metabolite **4**. The culture broth was filtered to remove the mycelial mat. The filtrate was acidified to pH 2.0 and extracted continuously with CH_2Cl_2 for 72 hr. The organic layer was dried over Na_2SO_4. After evapn of the solvent an oily residue (1.42 g) was obtained, which was dissolved in CH_2Cl_2, washed with 5% $NaHCO_3$ soln. and sepd into neutral (986 mg) and acidic (272 mg) frs in the usual manner. For isolation of metabolite **4**, the neutral fr. was purified by silica gel CC with hexane containing increasing concns of EtOAc (0, 20, 40, 60, 80 and 100%), and **4** (159 mg) was isolated in the 80% EtOAc eluate. No metabolite in the acidic fr. was identified from GC (140 to 260°, 4° min^{-1}) or TLC: *n*-hexane–EtOAc. Unutilized **3** (492 mg) was recovered.

Biotransformation of (+)*-aromadendrene* (**1**). Fermentation of **1** (2.0 g) with *G. cingulata* gave, after 7 days, unutilized **1** (439 mg) and **2** (155 mg).

(−)-10α,13,14-*Trihydroxyaromadendrane* (**2**). Crystals. Mp: 135°. $[\alpha]_D^{20}$: − 38.2° ($CHCl_3$; *c*1), EIMS *m/z* (rel. int.): $[M]^+$ 254 (1), 236 (3), 223 (29), 205 (35), 187 (36), 147 (26), 121 (36), 107 (47), 95 (86), 81 (100); IR ν_{max} cm^{-1}: 3338, 2953, 2870, 1459, 1017; ^{1}H NMR: δ0.92 (3H, *d*, *J* = 7.0 Hz, H − 15), 1.09 (3H, *s*, H-12), 3.29 (1H, *d*, *J* = 11.0 Hz, H-13), 3.36 (1H, *d*, *J* = 11.0 Hz, H-13'), 3.62 (2H, *s*, H-14). ^{13}C NMR data are listed in Table 1.

Biotransformation of (−)*-alloaromadendrene* (**3**). Fermentation of **3** (2.0 g) with *G. cingulata* gave, after 7 days, unutilized **3** (492 mg) and **4** (159 mg).

(−)-10β,13,14-*Trihydroxyalloaromadendrane* (**4**). Crystals. Mp: 135°. $[\alpha]_D^{20}$ − 31.3° ($CHCl_3$; *c*1). EIMS *m/z* (rel. int.): $[M]^+$ 254(1), 236(3), 223(6), 205(14), 187(13), 147(40), 133(24), 107(45), 95(77), 81(100); IR ν_{max} cm^{-1}: 3336, 2957, 2871, 1460, 1017; ^{1}H NMR: δ0.95 (3H, *d*, *J* = 7.0 Hz, H-15), 1.15 (3H, *s*, H-12), 3.26 (1H, *d*, *J* = 10.5 Hz, H-13), 3.29 (1H, *d*, *J* = 10.5 Hz, H-14), 3.42 (1H, *d*, *J* = 10.5 Hz, H-14'), 3.47 (1H, *d*, *J* = 10.5 Hz, H-13'). ^{13}C NMR data are listed in Table 1.

Table 1. ^{13}C NMR data for **1**–**4** (125 MHz, $CDCl_3$)

C	1	2	3	4
1	53.8	55.2	50.8	53.5
2	29.4	25.7	28.2	24.5*
3	35.1	34.6	31.2	29.1*
4	35.3	36.2	37.8	38.5
5	43.8	37.3	42.2	38.9
6	29.2	25.1	23.6	25.7†
7	27.5	23.4	24.8	19.4†
8	24.8	19.2	22.2	17.9*
9	39.1	37.3	35.7	31.7*
10	154.7	76.1	152.6	76.1
11	20.0	26.3	17.2	26.1
12	15.8	11.5	15.9	11.7
13	28.7	73.3	28.6	73.6
14	105.3	61.4	109.7	70.7
15	17.1	15.7	16.4	16.3

Chemical shifts in ppm downfield from TMS.
*,†The assignments for these signals within the same column may be interchanged.

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Table 2. ^{1}H NMR data for **1**–**4** (500 MHz, $CDCl_3$)

H	1	2	3	4
1	2.24 *m*	2.09 *m*	2.67 *m*	1.89 *m*
2	1.59 *m*	1.53 *m**	1.88 *m*	1.52 *m*†
	1.70 *m*	1.80 *m**	1.73 *m*	1.68 *m*†
3	1.21 *m*	1.28 *m**	1.32 *m*	1.30 *m*†
	1.88 *m*	1.64 *m**	1.73 *m*	1.72 *m*†
4	2.12 *m*	2.01 *m*	2.07 *m*	2.02 *m*
5	1.39 *m*	1.32 *m**	1.86 *m*	1.83 *m*†
6	0.60 *dd* (9, 11)	0.68 *dd* (9.5, 10.5)	0.24 *d* (9, 10.5)	0.32 *dd* (9.5, 10.5)
7	0.67 *ddd* (6, 9.5, 11)	0.79 *ddd* (6, 9.5, 10.5)	0.55 *ddd* (6, 9.5, 11)	0.83 *ddd* (6, 9.5, 10.5)
8	1.02 *m*	0.99 *m**	1.84 *m*	1.68 *m*†
	1.98 *m*	1.83 *m**	1.24 *m*	1.49 *m*†
9	2.08 *m*	1.34 *m**	2.34 *m*	1.54 *m*†
	2.43 *m*	2.14 *m**	2.28 *m*	1.71 *m*†
12	1.00 *s*	1.09 *s*	0.96 *s*	1.15 *s*
13	1.06 *s*	3.28 *d* (11)	1.00 *s*	3.26 *d* (11)
		3.36 *d* (11)	--	3.47 *d* (11)
14	4.64 *s*	3.62 *s*	4.70 *s*	3.29 *d* (10.5)
			4.73 *s*	3.42 *d* (10.5)
15	0.94 *d* (7)	0.92 *d* (7)	0.94 *d* (7)	0.95 *d* (7)

Chemical shifts in ppm downfield from TMS, coupling constants in Hz.
*,†The assignments for these signals within the same column may be interchanged.

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