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BIOTRANSFORMATION OF (−)- AND (+)-ISOPINOCAMPHEOL BY THREE FUNGI

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Key Word Index—*Glomerella cingulata*; *Rhizoctonia solani*; *Aspergillus niger*; plant pathogenic fungi; (−)-isopinocampheol; (+)-isopinocampheol; (1*S*, 2*S*, 3*R*, 5*S*)-2,3-pinanediol; (1*R*, 2*R*, 3*S*, 4*S*, 5*R*)-3,4-pinanediol; (1*R*, 2*R*, 3*R*, 5*R*)-3,5-pinanediol; (1*S*, 2*S*, 3*S*, 5*S*)-3,5-pinanediol; (1*S*, 2*S*, 3*S*, 5*R*, 7*R*)-3,7-pinanediol; (1*S*, 2*S*, 3*S*, 5*R*)-3,9-pinanediol; hydroxylation; biotransformation.

Abstract—The biotransformation of (−)-isopinocampheol and (+)-isopinocampheol by *Glomerella cingulata* has been studied. Both substrates were converted to three pinanediols, respectively. The major metabolic products obtained were diols. The main product of (−)-isopinocampheol was (1*R*, 2*R*, 3*S*, 4*S*, 5*R*)-3,4-pinanediol, and that of the (+)-enantiomer was (1*S*, 2*S*, 3*S*, 5*R*, 7*R*)-3,7-pinanediol. These results confirmed that the oxidation by *G. cingulata* took place with enantioselectivity. In addition, the same substrates were also converted by other fungi such as *Rhizoctonia solani* and *Aspergillus niger*. Some similarities exist between the main products of the metabolism of *R. solani*, while those of *A. niger* were somewhat different.

INTRODUCTION

We are studying the microbial transformation of monoterpene alcohols possessing a pinane skeleton by *Glomerella cingulata*. We have already reported on the transformation of (−)-nopol [1], (−)-*cis*-myrtanol [2] and (+)-*trans*-myrtanol [2]. In this paper, we report on the biotransformation of (−)-isopinocampheol (**1**) and (+)-isopinocampheol (**2**) into novel terpenes by this fungus. In addition, we describe the biotransformation of these compounds by the fungi *Rhizoctonia solani* and *Aspergillus niger*.

The biotransformation of isopinocampheols by cultured cells of *Nicotiana tabacum* has been reported and the product was determined to be isopinocamphone [3]. However, the microbial transformation of **1**, and **2** has not been dealt with before. Compounds possessing structures resembling isopinocampheol are of interest because some are known to possess useful biological activities such as insect antifeedant activity [4].

RESULTS AND DISCUSSION

The biotransformation of compounds **1** and **2** by *G. cingulata* was followed over a 12 day time period.

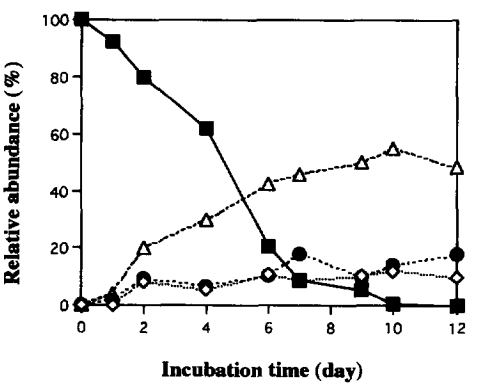

Fig. 1. Biotransformation of (−)-isopinocampheol (**1**) by *G. cingulata*. ■, (−)-isopinocampheol (**1**); ◇, (1*S*, 2*S*, 3*R*, 5*S*)-2,3-pinanediol (**1-1**); △, (1*R*, 2*R*, 3*S*, 4*S*, 5*R*)-3,4-pinanediol (**1-2**); ●, (1*R*, 2*R*, 3*R*, 5*R*)-3,5-pinanediol (**1-3**).

The extracts were examined by TLC and GC (Figs 1 and 2). Each compound was gradually converted into three metabolic products.

In order to obtain sufficient amounts of the metabolites of **1** for characterization, **1** (4.10 g) was incubated with *G. cingulata* for 12 days. The culture was extracted and the extract purified by silica gel chromatography to give **1-1**–**1-3**.

The structures of the metabolic products were determined by NMR, IR and MS spectroscopy. Metabolic

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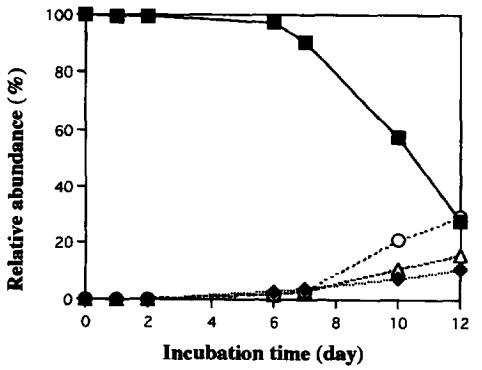


Fig. 2. Biotransformation of (+)-isopinocampheol (**2**) by *G. cingulata*. ■, (+)-isopinocampheol (**2**); ◆, (1*S*, 2*S*, 3*S*, 5*S*)-3,5-pinanediol (**2-1**); ○, (1*S*, 2*S*, 3*S*, 5*R*, 7*R*)-3,7-pinanediol (**2-2**); △, (1*S*, 2*S*, 3*S*, 5*R*)-3,9-pinanediol (**2-3**).

product **1-1** was assigned the molecular formula $C_{10}H_{18}O_2$, based on the EI-mass (*m/z* 155 $[M-Me]^+$ and 152 $[M-H_2O]^+$) and NMR data. The ^{13}C NMR spectrum showed the presence of a second hydroxyl group (δ_C 73.79). While in the ^1H NMR spectrum, there was no signal for a proton adjoining a hydroxyl group, just that for Me-10. In addition, the coupling constant (J = 7.3 Hz) between Me-10 and H-2 was absent. Therefore, the hydroxylated position was presumed to be at C-2. The absolute configuration at C-2 was revealed by a previously described method [5, 6]. Compound **1-1** was converted to its acetonide and analyzed by GC-mass spectrometry. The peak at *m/z* 210 $[M]^+$ supported the formation of the acetonide of **1-1** (Fig. 3). Thus, the product **1-1** was determined to be (1*S*, 2*S*, 3*R*, 5*S*)-2,3-pinanediol.

The main product, **1-2**, was assigned the molecular formula $C_{10}H_{18}O_2$, by means of the EI-mass (*m/z* 152 $[M-H_2O]^+$) and NMR data. The ^{13}C NMR spectrum showed the presence of a second hydroxyl group (δ_C 81.01 or 82.71). In the ^1H NMR spectrum, the coupling constants between H-4 and H-3 (J = 9.3 Hz) were absent, therefore the hydroxylation occurred at C-4. The absolute configuration at C-4 was established as follows. Firstly, no acetonide derivative of **1-2** was produced by the method used to make the acetonide of **1-1** (Fig. 3) [5, 6]. This result indicated that the

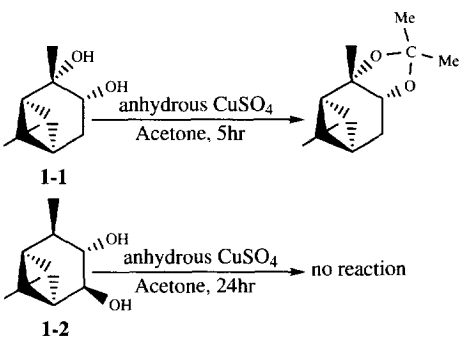


Fig. 3. The formation of the acetonide of **1-1**.

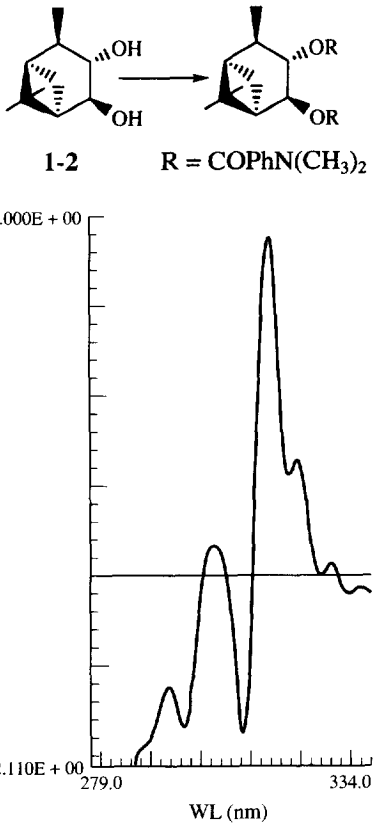


Fig. 4. The formation of the dimethylaminobenzoate of **1-2** and its CD curve.

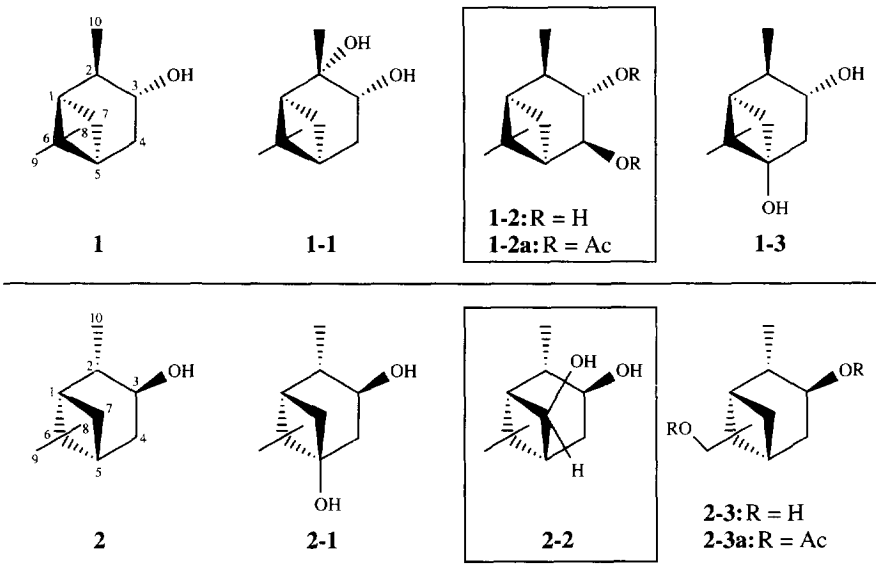
position of the hydroxyl group at C-3 was far away from that at C-4. Secondly, the dimethylaminobenzoate derivative of **1-2** was prepared and its CD spectrum measured. The compound was considered to have positive chirality, because the CD spectra showed positive first and negative second Cotton effects (Fig. 4) [7]. These findings indicated that C-4 was in the *S*-configuration. Thus, metabolite **1-2** was identified as (1*R*, 2*R*, 3*S*, 4*S*, 5*R*)-3,4-pinanediol.

The molecular formula of compound **1-3** was determined as $C_{10}H_{18}O_2$ based on the EI-mass (*m/z* 152 $[M-H_2O]^+$) and NMR data. The ^{13}C NMR spectrum indicated the presence of a new hydroxyl group (δ_C 76.21). In the ^1H NMR spectrum, the coupling constants (J = 2.5 and 3.5 Hz) between H-4 and H-5 were absent. Therefore, this metabolite was determined to be (1*R*, 2*R*, 3*R*, 5*R*)-3,5-pinanediol hydroxylated at C-5.

The metabolic products of **2** were obtained by incubating **2** (4.10 g) with *G. cingulata* for 12 days. The culture was then extracted and compounds **2-1**–**2-3** isolated from the extract.

The compound **2-1** was very similar to **1-3** in many of the spectral data and physical properties. Besides the specific rotation shown, **2-1** had a dextrorotatary value. Therefore, this metabolite was determined to be the enantiomer of **1-3**, (1*S*, 2*S*, 3*S*, 5*S*)-3,5-pinanediol.

The major metabolic product, **2-2**, was assigned the



Scheme. 1. The metabolic products formed from **1** and **2** by *G. cingulata*.

molecular formula $C_{10}H_{18}O_2$ (EIMS). The ^{13}C and ^1H NMR spectra showed a new hydroxyl group at C-7. According to the literature [8], the coupling constants of 7a-H (1.04 ppm, axial) and 7s-H (2.37 ppm, equatorial) of **2** are 9.8 Hz (7a-7s) and 9.8 Hz (7s-7a), 6.0 Hz (7s-1), 6.0 Hz (7s-5), respectively. In the spectral data of **2-2**, the proton adjoining the hydroxyl group at C-7 had two coupling constants, 5.7 and 5.7 Hz. This showed that the hydroxyl group was 7a(axial position). Accordingly, compound **2-2** was determined to be (1*S*, 2*S*, 3*S*, 5*R*, 7*R*)-3,7-pinanediol.

Compound **2-3** was determined to have the molecular formula $C_{10}H_{18}O_2$ based on EI-mass spectrometry of its diacetate **2-3a**. The ^1H and ^{13}C NMR data indicated that hydroxylation had occurred either at C-8 or at C-9. To determine the location of the hydroxyl group, ^{13}C NMR spectroscopy was used. A comparison was made between the calculated shift values that would be obtained if the acetate was substituted at the C-8 position vs the C-9 position. The results suggested that the most likely substitution position was C-9. Thus, **2-3** was determined to be (1*S*, 2*S*, 3*S*, 5*R*)-3,9-pinanediol.

The main metabolic products formed as a result of the biotransformation of **1** and **2** by *G. cingulata* are shown in Scheme. 1. They are all monohydroxy derivatives of the parent compound. The formation of (−)- and (+)-isopinocamphone from **1** or **2** was reported in the literature [3], but this was not confirmed. With regard to the speed of the metabolism, the relative rate of reaction with *G. cingulata* was different from that with *Nicotiana tabacum*. The metabolism of **1** was faster than that of **2**. In the present experiment, the position of the hydroxyl group incorporation was different between **1** and **2**. The main product of **1** was (1*R*, 2*R*, 3*S*, 4*S*, 5*R*)-3,4-pinanediol, and that of **2** was (1*S*, 2*S*, 3*S*, 5*R*, 7*R*)-3,7-pinanediol. It is, therefore, concluded that *G. cingulata* has the ability to recognize an enantiomer and oxidize it regioselectively.

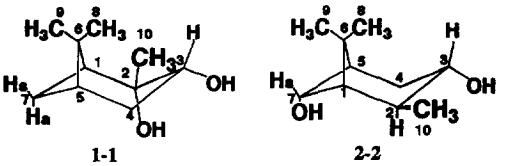


Fig. 5. The configuration of **1-1** and **2-2**.

The direction of hydroxyl group incorporation in **1-1** and **2-2** was *trans* to the geminal methyl group (Fig. 5). This is also the case with isopinocampheol, (−)-nopol [1], (−)-*cis*-myrtanol [2] and (+)-*trans*-myrtanol [2]. Therefore, the geminal methyl group has an effect on the selectivity of the direction of hydroxyl group incorporation. However, in **1-2**, it was introduced *cis* to the geminal methyl group. The reason for this may be that the hydroxyl group adjoining C-4 influences the direction of hydroxylation at C-4.

To further examine the biotransformation of **1** and **2**, by fungi, their biotransformation by *R. solani* and *A. niger* was examined. Each biotransformation was carried out under the same conditions. The extracts were analyzed by GC and GC-mass spectrometry (Table 1). In the case of *R. solani*, both **1** and **2** were predominantly oxidized at C-5. The ratio of the regioselectivity was above 60%, respectively. Whereas, for *A. niger*, which is not a plant pathogenic fungus, the extract was deficient in metabolic products **1-1**–**2-3**.

EXPERIMENTAL

General. (−)-Isopinocampheol (**1**) and (+)-isopinocampheol (**2**) were purchased from Fluka Chem. NMR: 270 MHz (^1H) or 67.8 MHz (^{13}C), $CDCl_3$ or

Table 1. The biotransformation of **1** and **2** by *G. cingulata*, *R. solani* and *A. niger* (after incubation for 12 days)

	1	**1-1**	**1-2**	**1-3**	**Others**
G. cingulata	0%	9.9%	48.2%	17.8%	24.1%
R. solani	0%	0%	0%	67.4%	32.6%
A. niger	0%	16.1%	2.0%	24.5%	57.4%
	2	**2-1**	**2-2**	**2-3**	**Others**
G. cingulata	27.9%	10.8%	29.2%	15.9%	16.2%
R. solani	0%	61.9%	0%	1.5%	36.6%
A. niger	0%	12.4%	2.2%	6.4%	79.0%

CD_3OD with TMS as int. standard; MS: H.P. 5972 series (Hewlett packard), 70 eV (ion voltage), 250° (ion source); GC: H.P. 5890 series II plus (Hewlett packard), FID, 1.18 ml min^{-1} (flow rate), HP-5MS (0.25 mm × 25 m) capillary column at 80–240° (2° min^{-1}); TLC: silica gel 60 F_{254} pre-coated (layer thickness 0.25 mm, Merck); CC: silica gel developed with *n*-hexane–EtOAc gradient; CD spectra: J-700 series (Japan spectroscopic Co. Ltd), 10 mm cell, MeOH as solvent.

Preculture of fungi. Spores of *Glomerella cingulata* (provided by Prof. M. Hyakumachi, Gifu Univ.), *Aspergillus niger* IFO 4414 (purchased from the Institute of Fermentation Osaka) and *Rhizoctonia solani* AG1-1C189 (gift from Prof. M. Hyakumachi, Gifu Univ.) which had been stored at low temp. were inoculated into sterilized culture medium in a shaking flask and shaken at 28° for 2 days, at 28° for 2 days and at 26° for 3 days, respectively. The components of the culture medium of *G. cingulata* and *A. niger* were: sucrose 3.75 g, glucose 3.75 g, polypeptone 1.25 g, $MgSO_4 \cdot 7H_2O$ 0.125 g, KCl 0.125 g, K_2HPO_4 0.25 g, $FeSO_4 \cdot 7H_2O$ 0.0025 g and H_2O 250 ml. Those of the culture medium of *R. solani* were: KNO_3 2.5 g, KH_2PO_4 1.25 g, $MgSO_4 \cdot 7H_2O$ 0.625 g, $FeCl_3 \cdot 6H_2O$ 0.005 g, sucrose 12.5 g and H_2O 250 ml. The active growing mycelia were transplanted into petri dishes which contained 16 ml culture media (pH 7.2) and were incubated at 28° without shaking for 3 days (*G. cingulata* and *A. niger*) or at 26° without shaking for 5 days (*R. solani*).

Addition of (−)-isopinocampheol (**1**). After growth of *G. cingulata*, **1** (4.10 g) was dissolved in 31.5 ml EtOH and 100 μl of this soln. (per petri dish) added to the culture medium (after confirming that EtOH did not adversely affect **1** or the mycelia). The cultures were then incubated under the same conditions for 12 days.

Purification of the metabolites **1-1**–**1-3**. After incubation, the culture media was collected, saturated with NaCl and extracted with CH_2Cl_2 and EtOAc. The mycelia were also collected and extracted with CH_2Cl_2 for 3 days. Both extracts were mixed, and the solvent was evapd under red. press. The extract (3.58 g) was dissolved in CH_2Cl_2 and washed with 5% aq. $NaHCO_3$, and sepd into a neutral (2.62 g) and an acid part (0.34 g).

Addition of (+)-isopinocampheol (**2**). This was carried out in the same manner as that described for **1**.

Purification of the metabolites **2-1**–**2-3**. Standard work up (see above) gave 3.13 g of extract which was sepd into a neutral (2.14 g) and an acid part (0.50 g).

Biotransformation of **1** *and* **2** *by the three fungi.* To the pre-culture of each fungus, **1** or **2** in EtOH was added to the medium [EtOH soln (8 mg), medium (16 mg)] as described above, and the cultures incubated for 12 more days. Every second day, the culture media was collected, saturated with NaCl and extracted with EtOAc. The extracts were analyzed by GC and GC-MS, respectively.

Metabolites from (−)-isopinocampheol (**1**). The neutral fraction (2.62 g) was chromatographed over silica gel repeatedly with a hexane–EtOAc gradient to give the metabolic products **1-1** (184 mg), **1-2** (897 mg) and **1-3** (331 mg).

(1S, 2S, 3R, 5S)-2,3-*Pinanediol* (**1-1**). Oil, $[\alpha]_D^{20}$ −4.55° ($CHCl_3$; *c* 1.0). EIMS *m/z* (rel. int.): 155 [M-Me]$^+$(0.5), 152(0.5), 137(3), 126(24), 111(38), 99(68), 43(base); IR ν_{max} cm^{-1}: 3355(O-H), 2929, 1734, 1450, 1365, 1120, 1042, 1014; ^1H ^{13}C NMR: Tables 2 and 3.

(1R, 2R, 3S, 4S, 5R)-3,4-*Pinanediol* (**1-2**). Colourless crystal, mp 121.5–121.8°, $[\alpha]_D^{20}$ +2.75° (MeOH; *c* 1.0). EIMS *m/z* (rel. int.): 152 [M-H_2O]$^+$(0.5), 137(2), 109(8), 95(22), 85(base), 71(38), 55(48), 43(71), 41(75); IR ν_{max} cm^{-1}: 3319(O-H), 2921, 1450, 1365, 1057, 1028, 1000; ^1H ^{13}C NMR: Tables 2 and 3.

3,4-*Diacetate of* **1-2** (**1-2a**). Compound **1-2** (45 mg) was acetylated in the usual manner to yield **1-2a** (58 mg). Oil, $[\alpha]_D^{20}$ −17.93° ($CHCl_3$; *c* 0.75). EIMS *m/z* (rel. int.): 239 [M − Me]$^+$(0.1), 152(15), 136(8), 134(18), 127(32), 85(48), 43(base); IR ν_{max} cm^{-1}: 2943, 1741(C=O), 1365, 1244, 1025, 978, 603; ^1H ^{13}C NMR: Tables 2 and 3.

(1R, 2R, 3R, 5R)-3,5-*Pinanediol* (**1-3**). Crystal, mp 98.4–102.4°, $[\alpha]_D^{20}$ −2.63° (MeOH; *c* 1.0). EIMS *m/z* (rel. int.): 155 [M − Me]$^+$(0.5), 152(5), 137(8), 109(17), 84(38), 69(47), 55 (52), 43(base); IR ν_{max} cm^{-1}: 3333(O-H), 2922, 1706, 1457, 1372, 1294, 1010, 989; ^1H ^{13}C NMR: Tables 2 and 3.

Preparation of acetonide of **1-1**. Compound **1-1** (10 mg) was treated with distilled Me_2CO (3.0 ml) in the presence of anhydrous $CuSO_4$ at 60° for 5 hr [5, 6]. The residue was evapd, saturated with NaCl, extracted with Et_2O, evapd and dried. GC-EIMS *m/z* (rel. int.): 210 [M]$^+$(1.0), 195([M − Me]$^+$,32), 153(7), 135(50).

Preparation of dimethylaminobenzoate of **1-2**. A soln of **1-2** (17 mg), *p*-dimethylaminobenzoic acid (49.6 mg) and *n*-Bu_3N (0.171 ml) in distilled CH_2Cl_2 was treated with 2-chloro-1-methylpyridinium-*p*-toluenesulphonate (108 mg), and refluxed for 12 hr. The residue was saturated with NaCl, extracted with Et_2O, evapd, dried and purified by silica gel CC to give the dimethylaminobenzoate of **1-2** (15 mg). EIMS *m/z* (rel. int.): 317 [M − $(CH_3)_2$NPhCO + H]$^+$(23); IR ν_{max}

Table 2. ^{1}H NMR spectral data for (−)-isopinocampheol (**1**), its metabolites and their derivatives (δ, TMS, in $CDCl_3$ and CD_3OD (**1**, **1-2**, **1-3**) at 270 MHz)

H	1	1-1	1-2	1-2a	1-3
1	1.78 *dt* (6.2, 6.2, 2.0)	2.01 *t* (6.0, 6.0)	1.80 *ddd* (6.3, 5.5, 1.8)	1.88 *ddd* (6.0, 5.5, 1.8)	1.63 *dd* (7.5, 1.5)
2	1.91 *m*	—	1.80 *ddd* (7.3, 5.5, 1.8)	2.00 *ddd* (7.3, 6.0, 1.8)	1.76 *m*
3	3.98 *ddd* (9.3, 5.4, 4.8)	3.99 *dd* (9.3, 5.2)	3.88 *dd* (5.5, 4.6)	5.34 *dd* (6.4, 4.0)	4.02 *dt* (9.6, 5.0, 5.0)
4	2.47 *dddd* (14.0, 9.3, 3.5, 2.3)	2.46 *dddd* (14.2, 9.3, 3.6, 2.3)	3.90 *dd* (4.6, 2.5)	5.17 *dd* (4.3, 3.0)	2.38 *ddd* (13.2, 9.6, 3.3)
	1.70 *ddd* (14.0, 4.8, 2.5)	1.64 *ddd* (14.2, 5.2, 2.6)	—	—	1.75 *dd* (13.2, 5.0)
5	1.91 *m*	1.93 *ddt* (6.0, 6.0, 3.6, 2.6)	2.03 *ddd* (6.3, 5.5, 2.3)	2.13 *ddd* (6.5, 5.5, 3.0)	—
7a	1.05 *d* (9.8)	1.37 *d* (10.5)	0.85 *d* (10.4)	1.01 *d* (10.5)	1.45 *d* (9.3)
7b	2.37 *ddt* (9.8, 6.2, 6.2, 2.3)	2.21 *ddt* (10.5, 6.0, 6.0, 2.3)	2.37 *dt* (10.4, 6.3, 6.3)	2.44 *ddd* (10.5, 6.5, 6.0)	2.31 *ddd* (9.3, 7.5, 3.3)
8-Me	0.93[a] *s*	0.94[b] *s*	1.05[c] *s*	1.12[d] *s*	0.93[f] *s*
9-Me	1.22[a] *s*	1.28[b] *s*	1.25[c] *s*	1.27[d] *s*	1.11[f] *s*
10-Me	1.11 *d* (7.3)	1.31 *s*	1.15 *d* (7.3)	1.15 *d* (7.3)	1.10 *d* (7.4)
2-OH	—	Not found	—	—	—
3-OH	Not found	Not found	Not found	—	Not found
4-OH	—	—	Not found	—	—
5-OH	—	—	—	—	Not found
3-OCOMe	—	—	—	2.03[e] *s*	—
4-OCOMe	—	—	—	2.08[e] *s*	—

Coupling constants in Hz.
[a-f] Values are interchangeable within each column.

Table 3. ^{13}C NMR spectral data for (−)- and (+)-isopinocampheol (**1**, **2**) and their metabolites and derivatives (67.8 MHz, $CDCl_3$, CD_3OD (**1-2**, **1-3**, **2-1**))

C	1	1-1	1-2	1-2a	1-3	2	2-1	2-2	2-3a
1	47.84(*d*)	53.96(*d*)	49.71(*d*)	47.57(*d*)[e]	43.41(*d*)	49.27(*d*)	43.50(*d*)	52.29(*d*)[l]	43.21(*d*)[n]
2	47.73(*d*)	73.79(*s*)	46.33(*d*)	42.53(*d*)	46.74(*d*)	48.44(*d*)	46.88(*d*)	40.73(*d*)[l]	43.52(*d*)[n]
3	71.63(*d*)	69.15(*d*)	81.01(*d*)[c]	77.85(*d*)	73.32(*d*)	72.05(*d*)	73.41(*d*)	67.32(*d*)[m]	73.73(*d*)
4	39.02(*t*)	38.08(*t*)	82.71(*d*)[c]	80.26(*d*)	45.98(*t*)	39.60(*t*)	46.11(*t*)	33.42(*t*)	35.39(*t*)
5	41.77(*d*)	40.50(*d*)	50.24(*d*)	46.33(*d*)[e]	76.21(*s*)	43.17(*d*)	76.29(*s*)	46.72(*d*)[l]	37.19(*d*)
6	38.14(*s*)	38.92(*s*)	39.19(*s*)	37.75(*s*)	44.02(*s*)	39.38(*s*)	44.09(*s*)	32.11(*s*)	42.07(*s*)
7	34.37(*t*)	28.00(*t*)	32.63(*t*)	30.93(*t*)	42.45(*t*)	35.08(*t*)	42.51(*t*)	69.56(*d*)[m]	33.23(*t*)
8	23.65(*q*)[a]	24.09(*q*)[b]	25.07(*q*)[d]	24.63(*q*)[f]	21.49(*q*)[i]	24.16(*q*)[j]	21.49(*q*)[k]	23.19(*q*)	19.08(*q*)
9	27.66(*q*)[a]	27.80(*q*)[b]	29.08(*q*)[d]	27.75(*q*)[f]	23.56(*q*)[i]	28.22(*q*)[j]	23.57(*q*)[k]	27.21(*q*)	70.98(*t*)
10	20.70(*q*)	29.54(*q*)	20.25(*q*)	19.45 (*q*)	20.84(*q*)[i]	21.15(*q*)	20.82(*q*)[k]	19.93(*q*)	21.44(*q*)
3-COMe				21.32(*q*)[g]					20.31(*q*)[o]
3-COMe				170.7(*s*)[h]					170.98(*s*)[p]
4-COMe				21.22(*q*)[g]					
4-COMe				170.50(*s*)[h]					
9-COMe									20.95(*q*)[o]
9-COMe									171.57(*s*)[p]

[a-p] Values are interchangeable within each column.

Table 4. ^{1}H NMR spectral data for (+)-isopinocampheol (**2**), its metabolites and derivatives (δ, TMS, in $CDCl_3$ and CD_3OD (**2-1**) at 270 MHz)

H	2	2-1	2-2	2-3a
1	1.80 *dt* (6.2, 6.2, 2.0)	1.63 *dd* (7.5, 1.5)	2.00 *dt* (5.7, 5.7, 2.5)	1.96 *dt* (6.0, 6.0, 2.2)
2	1.93 *m*	1.76 *m*	2.10 *ddd* (7.6, 3.0, 2.5)	2.17 *ddd* (7.6, 5.0, 2.2)
3	4.06 *ddd* (9.3, 5.4, 4.8)	4.02 *dt* (9.8, 5.0, 5.0)	3.76 *dt* (9.3, 3.0, 3.0)	5.06 *ddd* (9.3, 5.0, 4.2)
4	2.51 *dddd* (14.2, 9.3, 3.5, 2.4)	2.38 *ddd* (13.2, 9.6, 3.3)	2.47 *ddd* (14.8, 9.3, 3.0)	2.59 *dddd* (14.5, 9.3, 2.7, 2.2)
	1.71 *ddd* (14.2, 4.8, 2.6)	1.75 *dd* (13.2, 5.0)	1.79 *dt* (14.8, 3.0, 3.0)	1.73 *ddd* (14.5, 4.2, 3.0)
5	1.93 *m*	—	2.15 *tt* (5.7, 5.7, 3.0, 3.0)	2.08 *m*
7a	1.04 *d* (9.8)	1.45 *d* (9.3)	4.40 *t* (5.7, 5.7)	1.15 *d* (9.8)
7b	2.37 *ddt* (9.8, 6.2, 6.2, 2.3)	2.31 *ddd* (9.3, 7.8, 3.0)	—	2.37 *ddt* (9.8, 6.2, 6.2, 2.2)
8-Me	0.92[a] *s*	0.93[b] *s*	0.94[c] *s*	1.03 *s*
9-Me	1.22[a] *s*	1.11[b] *s*	1.14[c] *s*	
9-CH_2-				4.20 *s*
10-Me	1.13 *d* (7.3)	1.10 *d* (7.4)	1.17 *d* (7.6)	1.12 *d* (7.6)
3-OH	1.78 *s*	Not found	2.74–3.30 *br s*	—
5-OH	—	Not found	—	—
7-OH	—	—	2.74–3.30 *br s*	—
9-OH	—	—	—	—
3-OCOMe	—	—	—	2.06[d] *s*
9-OCOMe	—	—	—	2.08[d] *s*

Coupling constants in Hz.
[a–d] Values are interchangeable within each column.

cm^{-1}: 3496, 2801, 1681, 1610, 1369, 1283, 1184, 1113, 769.

Metabolites from isopinocampheol (**2**). The neutral fr. (2.14 g) was chromatographed over silica gel repeatedly with a hexane–EtOAc gradient to give the metabolic products **2-1** (164 mg), **2-2** (444 mg). Compound **2-3** was isolated as its acetylated product **2-3a** (242 mg).

(1S, 2S, 3S, 5S)-3,5-*Pinanediol* (**2-1**). Crystal, mp 98.8–99.8°, $[\alpha]_D^{20}$ +5.13° (MeOH; *c* 1.0). EIMS *m/z* (rel. int.): 152 $[M-H_2O]^+$(6.7), 137(10), 84(65), 69(63), 55(52), 43(base); IR ν_{max} cm^{-1}: 3326(O-H), 2929, 1450, 1301, 1223, 1096, 1046, 989; ^{1}H, ^{13}C NMR: Tables 4 and 3.

(1S, 2S, 3S, 5R, 7R)-3,7-*Pinanediol* (**2-2**). Oil, $[\alpha]_D^{20}$ +11.89° ($CHCl_3$; *c* 1.0). EIMS *m/z* (rel. int.): 155 $[M-Me]^+$(4.2), 152(1.7), 137(5), 84(93), 81(83), 69(97), 55(92), 43(base), 41(90); IR ν_{max} cm^{-1}: 3369(O-H), 2915, 1457, 1372, 1223, 1120, 1074, 1042; ^{1}H, ^{13}C NMR: Tables 4 and 3.

Diacetate of (1S, 2S, 3S, 5R)-3,9-*pinanediol* (**2-3a**). Oil, $[\alpha]_D^{20}$ +31.77° ($CHCl_3$; *c* 0.35). EIMS *m/z* (rel. int.): 195 $[M-OAc]^+$(5), 152(10), 134(25), 119(33), 82(18), 43(base); IR ν_{max} cm^{-1}: 2936, 1741, 1454, 1375, 1224, 1032, 978, 950, 599; ^{1}H, ^{13}C NMR: Tables 4 and 3.

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REFERENCES

1. Miyazawa, M., Suzuki, Y. and Kameoka, H., *Phytochemistry*, 1995, **39**, 337.
2. Miyazawa, M., Suzuki, Y. and Kameoka, H., *Phytochemistry* (in press).
3. Suga, T., Hirata, T., Hamada, H. and Futatsugi, M., *Plant Cell Reports*, 1983, **2**, 186.
4. Bardyshev, I. I., Pereguda, T. A., Kozlov, N. G., Kalechits, G. V. and Degtyarenko, A. S., *Vestsi Akademic Navuk BSSR, Series Khimicheskii Navuk*, 1984, **1**, 69.
5. McCloskey, J. A. and McClelland, M. J., *Journal of the American Chemical Society*, 1965, **87**, 5090.
6. Baragliu, A., Grandolini, G. and Rossi, C., *Tetrahedron*, 1980, **36**, 645.
7. Harada, N., Sow-mei Lai Chen and Nakanishi, K., *Journal of the American Chemical Society*, 1975, **97**, 5345.
8. Badjah-Hadj-Ahmwd, A. Y., Meklati, B. Y., Waton, H. and Pham, Q. T., *Magnetic Resonance Chemistry*, 1992, **30**(7), 807.