Pergamon

0031-9422(93)E0090-2

Phytochemistry, Vol. 35, No 6, pp. 1465–1467, 1994

0031-9422/94 $6.00+0.00

BIOTRANSFORMATION OF (−)- AND (+)-NEOMENTHOLS AND ISOMENTHOL BY *ASPERGILLUS NIGER*

HIRONOBU TAKAHASHI, YOSHIAKI NOMA,* MASAO TOYOTA and YOSHINORI ASAKAWA

Faculty of Pharmaceutical Science; *Faculty of Domestic Science, Tokushima Bunri University, Yamashiro-cho, Tokushima 770, Japan

(*Received in revised form* 23 *September* 1993)

Key Word Index—*Aspergillus niger*; fungus; biotransformation; (−)- and (+)-neomenthol; (+)-isomenthol; menthol; hydroxylation.

Abstract—Three menthols, (+)- and (−)-neomenthols and (+)-isomenthol were biotransformed by *Aspergillus niger* which converted nonspecifically (−)- and (+)-neomenthols to more hydroxylated compounds, whereas, (+)-isomenthol was smoothly converted into 1-hydroxyisomenthol and 6-hydroxyisomenthol by the same fungus.

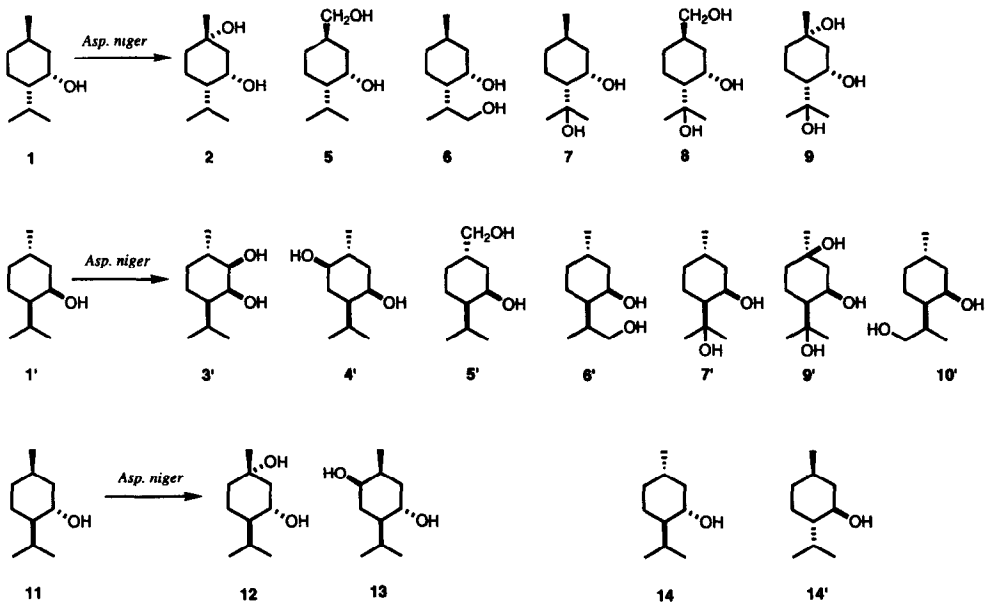
INTRODUCTION

In the previous paper [1], we reported the biotransformation of (+)- **14** and (−)-menthols (**14'**) by *Aspergillus niger*. We have further investigated the biotransformation of three related compounds [(+)- **1** and (−)-neomenthol (**1'**), (+)-isomenthol (**11**)] by *A. niger*, and isolated 11 new compounds. We report the structure determination of the new metabolites and compare their metabolites with those of (−)- and (+)-menthols [1].

RESULTS AND DISCUSSION

Three substrates, (+)- **1** and (−)-neomenthol (**1'**) and (+)-isomenthol (**11**) were biotransformed by *Aspergillus niger*. Metabolites were obtained after liquid–liquid extraction of cells and medium using diethyl ether. The crude extract was chromatographed on silica gel and prep. LPLC to give 1-hydroxy-**2**, 7-hydroxy-**5**, 9-hydroxy-**6**, 8-hydroxy-**7** [2], 7,8-dihydroxy-**8** and 1,8-dihydroxy-neomenthol (**9**) from **1**, and 2-hydroxy-**3'**, 6-hydroxy **4'**, **5'**–**7'**, **9'** and 10-hydroxyneomenthol (**10'**) from **1'**. While compound **11** was regiospecifically hydroxylated to 1-hydroxyisomenthol (**12**) as major product, **1** and **1'** were nonspecifically utilized.

In the ^{1}H NMR spectrum of product **3'**, a double doublet signal (δ3.06) assignable to H-2 newly appeared. The position of the newly introduced hydroxyl group was supported by an irradiation experiment. The ^{1}H NMR



spectrum of the second product **4'** showed a broad double doublet signal (δ3.86) assignable to a methine-bearing hydroxyl group, the position of which was confirmed to be at C-6 from the H/H COSY spectrum.

The ^{1}H NMR spectrum of **5**, M_r 172, showed the presence of two secondary methyl groups and a hydroxymethyl group. Irradiation at δ1.56, led to a singlet of the hydroxymethyl group signal. Further irradiation at δ1.88 caused two secondary methyl group proton signals to collapse to a singlet, indicating the presence of a newly introduced hydroxymethyl group at C-7.

The ^{1}H and ^{13}C NMR spectra of **6** also showed two secondary methyl groups and one hydroxymethyl group. No valuable information was obtained from irradiation experiments. However, compound **5** has already been obtained, thus the location of the hydroxyl methyl group was assigned at C-9. The structures of compounds **8** and **9** were also determined by comparison of the spectral data with those of **2**, **5** and **7**.

The mixture of **6'** and **10'** obtained from **1'**, could not be isolated by low pressure liquid chromatography. The ^{13}C NMR and DEPT spectra showed 20 carbon signals, 10 of which were identical to those of **6**, the enantiomer of **6'**. The structure of **10'**, the diastereomer of **6'**, was confirmed by the remaining 10 carbon signals including one hydroxymethyl group (δ64.4) and one carbon- (δ66.1) bearing secondary hydroxyl group. The stereochemistry of **6'** and **10'** at C-8 remains to be clarified.

1-Hydroxy-**12** [2] and 6-hydroxyisomenthols (**13**) were obtained from isomenthol (**11**). ^{1}H and ^{13}C NMR spectra of **13** (M_r 172) showed a methine proton (δ3.95). As in the case of **4'**, the position of the methine proton was assigned at C-6 from the H/H COSY spectrum.

The evidence from the present and previous work [1] shows that *Aspergillus niger* biotransformed menthols to give more hydroxylated compounds. Neomenthols (**1** and **1'**) and (−)-menthol (**14'**) were converted into 9-hydroxymenthols preferentially, but isomenthol (**11**) was not converted to 9-hydroxyisomenthol. Isomenthol (**11**) and (+)-menthol (**14**) were regiospecifically converted into 1-hydroxy- and 7-hydroxymenthols, respectively, but other menthols were nonspecifically hydroxylated.

EXPERIMENTAL

General. Mps: uncorr. *n*-Hexane–EtOAc gradients were used for CC on silica gel. Low pressure liquid chromatography (LPLC) was performed on a Lichroprep Si60 (type B, 40–63 μm, Merck) column using *n*-hexane–EtOAc (1:1). Solvents for spectral measurements were TMS–$CDCl_3$, pyridine-d_5 [^{1}H (400, 200); ^{13}C (100, 50 MHz)] and $CHCl_3$ or MeOH ($[\alpha]_D$). GC-MS was carried out at 70 eV using DB-17 (0.25 mm × 30 m), oven temp. prog. from 70 to 250° at 10° min^{-1}, inj. temp. 250°, He 0.9 ml min^{-1}.

Substrates. Commercially available (+)-neomenthol (**1**), $[\alpha]_D$ +16.6° (*c* 11.8), (−)-neomenthol (**1'**), $[\alpha]_D$ −17.8° (*c* 1.3) and (+)-isomenthol (**11**), $[\alpha]_D$ +23.4° (*c* 1.8) were used. The purity of the substrates was checked by GC-MS and TLC. The substrates were sterilized under a UV lamp for 10 min before being added to a medium.

Cultivation of A. niger. The same medium (200 ml) was prepd for *A. niger* as described earlier [1] and oily substrates (100 mg) were added directly to the medium and the culture further cultivated for 1 week. Crystalline substrate **11** (100 mg) was dissolved in EtOH (1 ml) and then added to the medium. The crude extracts were obtained by liquid–liquid extraction of cells and medium using Et_2O and each extract was purified by a combination of CC on silica gel and prep. LPLC.

Metabolites from (+)-neomenthol (**1**). The crude extract (1.5 g) obtained from **1** (4 g) was chromatographed on silica gel and each fr. further purified by LPLC to afford **2** (11 mg) [2], **5** (17 mg), **6** (97 mg), **7** (106 mg), **8** (57 mg) [2] and **9** (13 mg).

Metabolites from (−)-neomenthol (**1'**). The crude extract (1.2 g) obtained from **1'** (4 g) was chromatographed on silica gel and each fr. further purified by LPLC to afford **3'** (11 mg), **4'** (5 mg), **5'** (23 mg), **7'** (68 mg), **9'** (17 mg) and a mixt. of **6'** and **10'** (175 mg).

Compound **3'**. $[\alpha]_D$ +24.2° (*c* 1.1); IR ν_{max} cm^{-1}: 3385, 1458, 1366, 1159, 1124, 1060, 949; EIMS *m/z* (rel. int.): 172 $[M]^+$ (2), 157 (6), 154 (25), 139 (100), 121 (25), 111 (69), 84 (62), 71 (50), 69 (49), 55 (53), 43 (56), 43 (56), 41 (38); ^{1}H and ^{13}C NMR (Tables 1 and 2).

Table 1. ^{1}H NMR spectral data for compounds **3–6**, **8**, **9** and **13** ($CDCl_3$–TMS)

H	3'	4'	5	6	8	9	13*
1	—	—	—	—	—	—	—
2	3.06 *dd* (10.3, 2.8)	—	—	—	—	—	—
3	4.01 *br s*	4.12 *d* (1.5)	4.18 *d* (2.2)	3.94 *br s*	4.45 *br s*	4.49 *br s*	3.95 *td* (11.0, 4.4)
4	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—
6	—	3.86 *dd* (5.4, 2.4)	—	—	—	—	4.14 *ddd* (11.0, 4.4, 5.1)
7	0.93 *d* (7.0)	0.93 *d* (6.8)	3.46 *d* (5.9)	0.91 *d* (6.7)	3.38 *dd* (10.7, 6.4) 3.43 *dd* (10.7, 5.9)	1.20 *s*	0.96 *d* (7.3)
8	—	—	—	—	—	—	—
9	0.97 *d* (7.3)	0.95 *d* (7.3)	0.93 *d* (5.1)	3.43 *m*	1.21 *s*	1.25 *s*	0.97 *d* (6.6)
10	1.00 *d* (6.2)	0.96 *d* (6.8)	0.97 *d* (6.6)	0.87 *d* (6.6)	1.35 *s*	1.36 *s*	1.22 *d* (7.3)

*Measured in pyridine-d_5.

Table 2. ^{13}C NMR spectral data for compounds **3**, **5**, **6**, **8**–**10** and **13** ($CDCl_3$–TMS)

C	3′	5	6	8	9	10′	13*
1	32.7	33.8	26.1	33.4	70.4	26.0	26.1
2	78.6	36.9	42.5	36.8	42.7	42.5	41.0
3	71.0	67.1	70.3	67.3	69.3	66.1	65.7
4	47.9	48.3	46.7	48.4	47.7	46.0	49.3
5	23.3	23.6	20.0	19.8	16.2	25.4	27.4
6	32.8	29.2	35.3	29.1	38.7	35.4	71.7
7	21.2	68.4	22.3	67.9	30.6	22.3	12.3
8	28.9	29.2	39.0	73.3	73.3	38.1	35.2
9	18.4	20.7	64.0	28.4	28.5	15.8	16.4
10	20.7	21.1	17.6	28.9	29.0	64.4	21.0

*Measured in pyridine-d_5.

Compound **4′**. $[\alpha]_D$ +7.2° (*c* 0.5); EIMS *m/z* (rel. int.): 154 $[M-H_2O]^+$ (45), 139 (100), 111 (55), 97 (42), 95 (45), 83 (48), 72 (44), 55 (62), 43 (57); ^{1}H NMR (Table 1); ^{13}C NMR: δ17.9, 20.7, 21.1, 28.9, 29.2, 31.7, 35.6, 40.7, 67.3, 70.6.

Compound **5**. $[\alpha]_D$ +7.3° (*c* 1.68); IR ν_{max} cm^{-1}: 3358, 1446, 1383, 1032; EIMS *m/z* (rel. int.): 172 $[M]^+$ (17), 154 (16), 139 (53), 123 (100), 111 (33), 93 (46), 83 (89), 67 (69), 55 (74), 41 (56); ^{1}H and ^{13}C NMR (Tables 1 and 2).

Compound **6**. Mp 55–58°; $[\alpha]_D$ +13.7° (*c* 3.76); IR ν_{max} cm^{-1}: 3258, 1454, 1375, 1065, 1034; EIMS *m/z* (rel. int.): 172 $[M]^+$ (10), 154 (16), 139 (50), 136 (16), 123 (86), 112 (28), 95 (45), 93 (41), 83 (66), 81 (100), 71 (55), 69 (70), 55 (86); ^{1}H and ^{13}C NMR (Tables 1 and 2).

Compound **8**. $[\alpha]_D$ +10.8° (*c* 2.87); IR ν_{max} cm^{-1}: 3333, 1446, 1379, 1159, 1038, 951, 899; EIMS *m/z* (rel. int.): 173 $[M-Me]^+$ (2), 152 (10), 94 (94), 79 (100), 59 (44), 43 (30); ^{1}H and ^{13}C NMR (Tables 1 and 2).

Compound **9**. $[\alpha]_D$ ±0° (*c* 1.26); IR ν_{max} cm^{-1}: 3352, 1448, 1377, 1165, 941, 906, 849. EIMS *m/z* (rel. int.): 173 $[M-Me]^+$ (3), 170 (3), 155 (4), 137 (14), 112 (46), 94 (92), 87 (55), 59 (60), 58 (79), 43 (100); ^{1}H and ^{13}C NMR (Tables 1 and 2). The spectral data of the enantiomers (**5′**, **6′** and **9′**) were identical to those of **5**, **6** and **9**.

Metabolites from (+)-*isomenthol* (**11**). The crude extract (2.14 g) obtained from **11** (1.5 g) was chromatographed on silica gel to afford **12** (1181 mg) [2] and **13** (12 mg).

Compound **13**. $[\alpha]_D$ +50.2° (MeOH; *c* 1.21); IR ν_{max} cm^{-1}: 3231, 1454, 1383, 1304; EIMS *m/z* (rel. int.): 154 $[M-H_2O]^+$ (21), 139 (100), 121 (18), 111 (27), 97 (42), 83 (35), 69 (40), 57 (44), 55 (54), 43 (53), 41 (31); ^{1}H and ^{13}C NMR (Tables 1 and 2).

Acknowledgement—A part of this work was supported by a Grant-in-Aid for cancer research from the Ministry of Health and Welfare.

REFERENCES

1. Asakawa, Y., Takahashi, H., Toyota, M. and Noma, Y. (1991) *Phytochemistry* **30**, 3981.
2. Asakawa, Y., Matsuda, R., Tori, M. and Hashimoto, T. (1988) *Phytochemistry* **27**, 3557.