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BIOTRANSFORMATION OF (+)- AND (−)-CAMPHORQUINONES TO CAMPHANEDIOLS BY *GLOMERELLA CINGULATA*

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Key Word Index—*Glomerella cingulata*; fungus; biotransformation; (+)-camphorquinone; (−)-camphorquinone; (+)-2-*exo*-3-*exo*-camphane-2,3-diol; (+)-2-*endo*-3-*exo*-camphane-2,3-diol; reduction; stereoselectivity; enantioselectivity.

Abstract—The reduction of (+)-camphorquinone by the plant pathogen *Glomerella cingulata* produced (+)-2-*exo*-3-*exo*-2,3-diol with high stereoselectivity, whereas (−)-camphorquinone yielded (+)-2-*endo*-3-*exo*-2,3-diol with high stereo- and enantioselectivity. Copyright © 1996 Elsvier Science Ltd

INTRODUCTION

In our previous paper, (+)- and (−)-camphorquinones (**1a, 1b**) were shown to be readily reduced to keto alcohols by short time (24 hr) fermentation with various fungi [1]. In particular, *Glomerella cingulata* gave high yields of hydroxycamphors in a short time. However, further reduction to diols in the biotransformation of compounds **1a** and **1b** have not been reported.

This report deals with the biotransformation of **1a** and **1b** to camphanediols by *G. cingulata*.

RESULTS AND DISCUSSION

In time-course experiments, small amounts of either (+)-camphorquinone (**1a**) or (−)-camphorquinone (**1b**) were incubated with *G. cingulata* for 10 days, respectively (Figs 1 and 2). The biotransformation of compounds **1a** and **1b** proceeded readily to give the α-ketoalcohols (+)-2*R*-*exo*-hydroxyepicamphor(**1a-1**), (−)-2*S*-*endo*-hydroxyepicamphor (**1a-2**), (−)-3*S*-*exo*-hydroxycamphor (**1a-3**) and (+)-3*R*-*endo*-hydroxycamphor (**1a-4**) from **1a**, and (−)-2*S*-*exo*-hydroxyepicamphor (**1b-1**), (+)-2*R*-*endo*-hydroxyepicamphor (**1b-2**), (+)-3*R*-*exo*-hydroxycamphor (**1b-3**) and (−)-3*S*-*endo*-hydroxycamphor (**1b-4**) from **1b** with reduction of the carbonyl group [1]. These compounds of absolute configuration **1a-1–4** and **1b-1–4** were described in a previous paper [1]. It was confirmed that the α-keto-alcohols were further transformed by *G. cingulata* to give **1a-5** and **1b-6** over the ten-day period of the experiment. Two secondary metabolites (**1a-5** and **1b-6**) were detected by TLC and GC analysis. Camphorquinone (**1a** and **1b**) disappeared after 9 hr, as shown in a previous paper [1], and α-ketoalcohols were produced in about 100% yield at 9 hr, and then decreased from 12 hr to 10 days. Compounds **1a-5** and **1b-6** were obtained 10–20% yield at 10 days (Figs 1 and 2).

In order to isolate these metabolites, a large scale incubation of **1a** and **1b** with *G. cingulata* was carried out. After the biotransformation, the metabolites were extracted and purified as described in the Experimental. The structures of these compounds were determined by spectral methods. Metabolites **1a-5** and **1b-6** were identified as (+)-(1*R*, 2*R*, 3*R*)-3-*exo*-1,7,7-trimethylbicyclo [2.2.1] heptane-2,3-diol((+)-2-*exo*-3-*exo*-camphane-2,3-diol, **1a-5**) and (+)-(1*S*, 2*R*, 3*R*)-3-*exo*-hydroxy-1,7,7-trimethyl-bicyclo [2.2.1] heptane-2,3-diol ((+)-2-*endo*-3-*exo*-camphane-2,3-diol, **1b-6**) by comparison with literature data [2]. It was considered that compound **1a** was transformed to (+)-2-*exo*-3-*exo*-2,3-diol (**1a-5**), yield 10%) via α-ketoalcohols (+)-2*R*-*exo*-hydroxyepicamphor (**1a-1**) and/or (−)-3*S*-*exo*-hydroxycamphor (**1a-3**), and compound **1b** to (+)-2-*endo*-3-*exo*-2,3-diol (**1b-6**), yield 20%) via (+)-2*R*-*endo*-hydroxyepicamphor (**1b-2**) and/or (+)-3R-*exo*-hydroxyepicamphor (**1b-3**) (Scheme 1). These bioconversion processes exhibited high regio- and stereoselective reduction.

In the case of biotransformation of racemic camphorquinones by *G. cingulata* for 10 days, optically active camphanediol (**1b-6** yield 10%, optical purity 99.9%) was isolated from the extract. This revealed that the reduction of racemic camphorquinones by *G. cingulata* proceeded with high stereo- and enantioselectivity. The yield of compound **1a-5** was very poor (about 5%, optical purity 99.9%). Thus, the racemic camphorquinones (**1a, 1b**) were reduced by *G. cingulata* to

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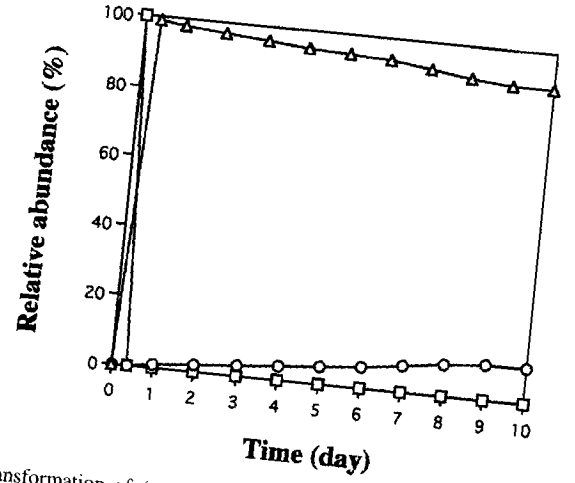


Fig. 1. Time course of the biotransformation of (+)-camphorquinone (**1a**) by *Glomerella cingulata*. □, **1a**; △, ketoalcohols (**1a-1–4**); ○, 2-*exo*-3-*exo*-camphane-2, 3-diol (**1a-5**).

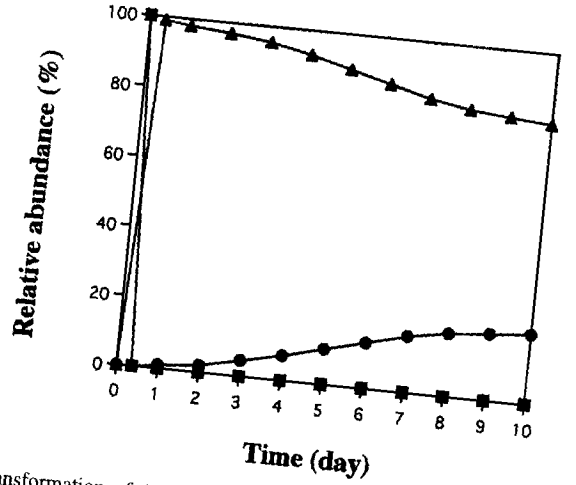
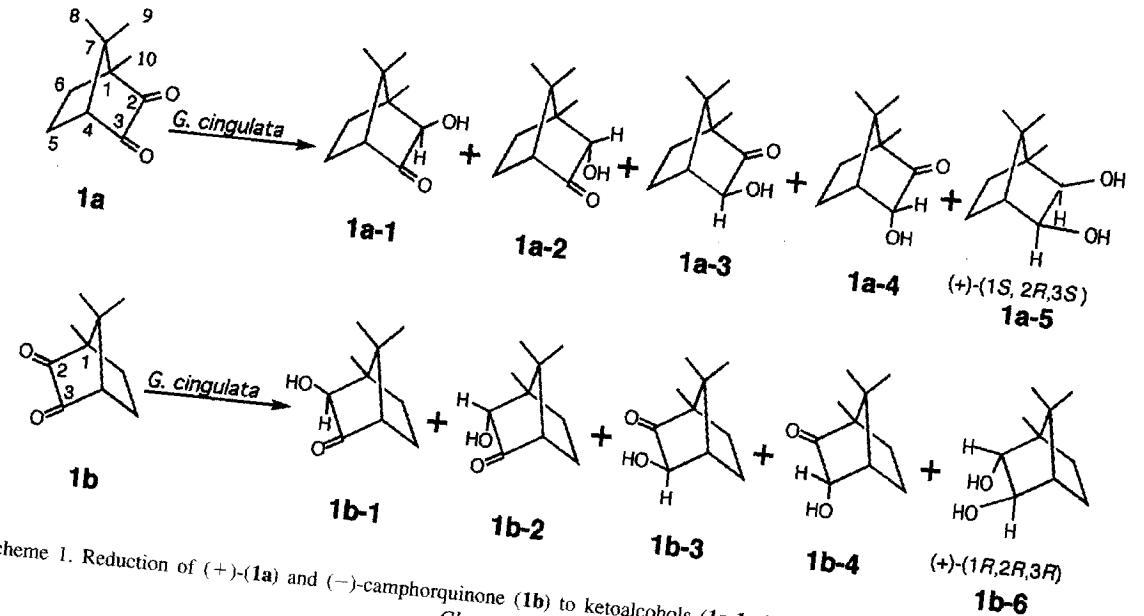


Fig. 2. Time course of the biotransformation of (−)-camphorquinone (**1b**) by *Glomerella cingulata*. ■, **1b**; ▲, ketoalcohols (**1b-1–4**); ●, 2 *endo*-3-*exo*-camphane-2,3,-diol (**1b-6**).



Scheme 1. Reduction of (+)-(**1a**) and (−)-camphorquinone (**1b**) to ketoalcohols (**1a-1–4**, **1b-1–4**) and diols (**1a-5**, **1b-6**) by *Glomerella cingulata*.

yield 2,3-diol with high-stereo- and enantioselectivity, respectively.

In 1971, Robertson *et al.* [3] reported the reduction of camphorquinone in rabbits. This reduction provided 2-*endo*-3-*endo*-camphanediol and 2-*endo*-3-*exo*-camohanediol. However, there have been no detailed reports of the stereoselectivity. To our knowledge, this is the first report of the reduction of compound **1a** to (+)-2-*exo*-3-*exo*-camphane-2, 3-diol (**1a-5**) with microorganisms. In this work, it is clear that the reduction of camphorquinones to diols via α-ketoalcohols occurs with higher stereo- and enantioselectivity than the reduction to α-ketoalcohols.

EXPERIMENTAL

General. (+)- and (−)-camphorquinones were purchased from Kanto Chemical Co., Inc. ^{1}H and ^{13}C NMR: 270.05 and 67.80 MHz, respectively, GC-MS: 20 eV (ion voltage) and 250° (ion source) using OV-1 (0.25 mm × 30 m) capillary column GC; column temp. 4° min^{-1} from 140 to 240°, injection temp. 240°. TLC: silica gel 60 F_{254} pre-coated (layer thickness 0.25 mm, Merck) with *n*-hexane–EtOAc (1:1). CC: silica gel with *n*-hexane–EtOAc gradient).

Microorganisms and culture condition. Glomerella cingulata was purchased from Gifu University. The fungus was maintained on nutrient agar slants at 10° and was used to inoculate autoclaved culture medium (g l^{-1}); sucrose 15, glucose 15, polypeptone 5, KCl 0.5 $MgSO_4 \cdot 7H_2O$ 0.5, K_2HPO_4 1, $FeSO_4 \cdot 7H_2O$ 0.01.

Cultivation of G. cingulata. The spores were shaken with culture medium at 27° for 72 hr in an incubator. Mycelia were then transferred to the culture medium (150 ml in a 300 ml Erlenmeyer flask) and stirred for 72 hr at 27°. After the growth of the fungus, the substrates (**1a** and **1b**) were added directly to each medium (400 mg per 200 ml) and the incubation continued under the same conditions for 10 days.

Purification of the metabolic products. At the end of the incubation period, the culture medium was collected, saturated with NaCl, and extracted with EtOAc. The mycelia were also collected and extracted with EtOAc. The EtOAc extracts were combined and the solvent removed under red. pres. The extract was chromatographed on silica gel using *n*-hexane–EtOAc and the substrate and metabolites isolated. In the case of compound **1a**: **1a-1–4**: 161mg; **1a-5**: 82 mg. In the case of **1b**: **1b-1–4**: 188 mg **1b-6**: 40mg).

(+)-2-Exo-3-exo-*camphane*-2,3-*diol* (**1a-5**). Mp 256.5–258.5°; $[\alpha]_D^{20}$ + 17.4° (EtOH; c = 6.0); EI-MS *m/z*: 170 $[M]^+$ ($C_{10}H_{18}O_2$); IR ν_{max} cm^{-1}: 3641(OH); ^{1}H NMR (270.05 MHz, $CDCl_3$, TMS as int. standard): δ 0.94 (3H, *s*, H-10) 0.80 (3H, *s*, H-9), 1.11(3H, *s*, H-8), 0.80 (1H, *m*, H-6-endo, 1.40 (1H, *m*, H-6-exo), 0.85 (1H, *m*, H-5-endo), 1.60 (1H, *m*, H-5-exo), 1.70 (1H, *d*, J = 4.7 Hz, H-4) 3.84 (1H, *d*, J = 7.0 Hz, H-3), 3.51 (1H, *d*, J = 7.0 Hz, H-2); ^{13}C NMR (67.80 MHz, $CDCl_3$: δ 79.9 (*d*, C-2), 76.2 (*d*, C-3), 51.5 (*d*, C-4), 48.8 (*s*, C-7), 46.43 (*s*, C-1), 33.1 (*t*, C-6), 24.1 (*t*, C-5), 21.8 (*q*, C-9), 21.0 (*q*, C-8), 11.1 (*q*, C-10).

(+)-2-Endo-3-exo-*camphane*-2,3-*diol* (**1b-6**). Mp 254.0–256.0°; $[\alpha]_D^{20}$ + 15.0° (EtOH; c = 1.5); EI-MS *m/z*: 170 $[M]^+$ ($C_{10}H_{18}O_2$); IR ν_{max} cm^{-1}: 3628 (OH); ^{1}H NMR (270.05 MHz, $CDCl_3$, TMS as int. standard): δ 0.88(3H, *s*, H-10), 1.07 (3H, *s*, H-9), 0.85 (3H, *s*, H-8), 1.72 (1H, *m*, H-6-*endo*), 1.19 (1H, *m*, H-6-*exo*), 1.17 (1H, *m*, H-5-*endo*), 1.72 (1H, *m*, H-5-*exo*), 1.66 (1H, *m*, J = 4.7 Hz, H-4), 3.54 (1H, *d*, J = 2.5 Hz, H-3), 3.96 (1H, *d*, J = 2.5 Hz, H-2), ^{13}C NMR (67.80 MHz, $CDCl_3$): δ 86.1 (*d*, C-2), 84.6 (*d*, C-3), 52.5 (*d*, C-4), 50.3 (*s*, C-7), 47.2 (*s*, C-1), 25.4 (*t*, C-6), 25.1 (*t*, C-5), 21.0 (*q*, C-8), 19.3 (*q*, C-9), 12.9 (*q*, C-10).

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