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Resolution of racemic 2,6,6-trimethyl-7-oxa-bicyclo[3.1.1]octan-2-ol and 1,6,6-trimethyl-7-oxa-bicyclo[3.1.1]octan-2-ol by microbial esterification

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Abstract: Resolution of racemic 2,6,6-trimethyl-7-oxa-bicyclo[3.1.1]octan-2-ol and racemic 1,6,6-trimethyl-7-oxa-bicyclo[3.1.1]octan-2-ol via esterification with malonic acid by *Glomerella cingulata*, is described. Both of alcohols and malonic esters could be obtained in enantiomerically pure states. © 1997 Elsevier Science Ltd

In our laboratory, as a continuing efforts of research on the optical resolution of various terpen-alcohols using a plant pathogenic fungus, *Glomerella cingulata* as biocatalyst, the resolution of (±)-*cis* and *trans*-linalool oxide-pyranoid was achieved.[1] In this context, we became interested in resolution of racemic form of 2,6,6-trimethyl-7-oxa-bicyclo[3.1.1]octan-2-ol **1** and 1,6,6-trimethyl-7-oxa-bicyclo[3.1.1]octan-2-ol **3** whose structures are similar to that of (±)-*cis* and *trans*-linalool oxide-pyranoid respectively, by *Glomerella cingulata* mediated. These compounds in enantiomerically enriched forms would be unique as flavour and fruity and/or wood fragrance. The results obtained are summarized in Table 1.

Racemic 2,6,6-trimethyl-7-oxa-bicyclo[3.1.1]octan-2-ol **1**,[2] dissolved in DMSO, was added to a culture of *G. cingulata*,[2] After 24h of incubation, the products, (2*R*)-2,6,6-trimethyl-7-oxa-bicyclo-[3.1.1]octan-2-malonyl ester **2** was obtained in 50% yield (100% ee) (Scheme 1, Table 1).

The recovered alcohol (1*S*,2*S*,5*S*)-enantiomer also showed an of 100% ee. In the same manner, the microbial esterification of racemic 1,6,6-trimethyl-7-oxa-bicyclo[3.1.1]octan-2-ol **3** (Scheme 2) was carried out and the result was shown in Table 1. In this study, although malonic acid was not externally added to the culture of *G. cingulata*, the fact that compounds **1** and **3** were converted to malonyl ester **2** and **4** respectively clearly show malonic acid was produced in a metabolic pathway of *G. cingulata*.

As the resolution produced in a highly enantioselective manner, then the odor of both of pure enantiomer of **1** and **3** was evaluated. Although both of enantiomer of the alcohols emitted a similar camphoraceous-cool odor that did not last for a long time. The odor of the (+)-form of **1** and **3** had a notably fresher than that of the (−)-form.

Experimental

Esterification on analytical scale

A nutrient broth was prepare dissolving sucrose (15 g), glucose (15 g), polypeptone (5 g), KCl (0.5 g), $MgSO_4$ (0.5 g), K_2PO_4 (1 g), $FeSO_4 \cdot 7H_2O$ (0.01 g) in 1 L of distilled water. A sterilized

Table 1. Resolution of racemic tertiary alcohols **1** and **3** by *Glomerella cingulata*

alcohol	time (hr)	ester (%)	ee (abs conf)	alcohol (%)	ee (abs conf)
1	24	**2** (50)	100 (1*R*,2*R*,5*R*)	**1** (50)	100 (1*S*,2*S*,5*S*)
3	24	**4** (50)	100 (1*R*,2*R*,5*R*)	**3** (50)	100 (1*S*,2*S*,5*S*)

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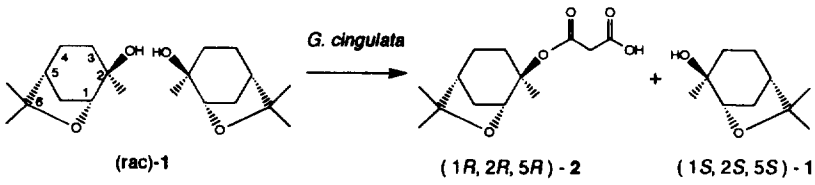
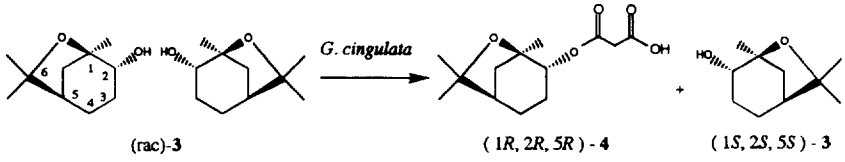


Scheme 1.



Scheme 2.

nutrient broth (50 ml) was inoculated with *Glomerella cingulata*.[3] The mixture was incubated for 2 days at 28°C on a reciprocatory shaker. Mycelia were transplanted on to the culture medium (15 ml in a Petri dish) and incubated for 36–48 h, until mycelia occupied 100% of 5 the surface area of the culture medium. To the resulting mycelia the racemic alcohol **1** (10 mg) and **3** (10 mg) in DMSO (0.1 ml) were added, corresponding to 5 mg of substrate per Petri dish. Aliquots were withdrawing periodically and monitored by GLC on a chiral column: CHROMPACK WCOAT Fused silica Cp-Cyclodextrin-B-236-M-19 (50 m×0.25 mm, N_2 flow rate 1 ml/min). In the case of malonic esters **2** and **4** separation of the alcohols were achieved after alkaline hydrolysis.

Esterification on preparative scale

To the resulting mycelia of grown cell **1** (500 mg) and **3** (500 mg) in DMSO (1.0 ml) were added same as analytical scale. After 2 days, the reaction mixtures were extracted with dichrolomethane with continuous liquid–liquid extractor, dried over anhydrous sodium sulphate and concentrated under reduced pressure. The products were purified by column chromatography (silica gel, hexane/dimethyl ether ratio) to give the malonic ester and the unreacted alcohols. The absolute configurations of compounds **1** and **3** were assigned on the basis of specific rotations in the literature.[2] Rotations were measured on a Japan Spectroscopic CO. LTD DIP-1000 in C_2H_5OH: (1*R*,2*R*,5*R*)-1, $[\alpha]_D=-93$ (*c*=0.2), (1*S*,2*S*,5*S*)-**3**, $[\alpha]_D=-79$ (*c*=0.2). The enantiomeric excesses are determined by GLC on chiral column [retention time (min) of alchohols: (1*R*,2*R*,5*R*)-**1**, 34.2, (1*S*,2*S*,5*S*)-**1**, 34.9, (1*R*,2*R*,5*R*)-**3**, 32.2, (1*S*,2*S*,5*S*)-**3**, 32.8]. Compound **2**; ^{1}H NMR (270 MHz, in $CDCl_3$, TMS as int. standard, δ 0.00): malonyl group (δ_H 3.40, 2H *s*, δ_H 7.2, 1H *br s*). ^{13}C NMR 67.80 MHz, in $CDCl_3$, residual $CHCl_3$ as int. standard, δ 77.00): malonyl group (δ_c 40.1, δ_c 165.3 and 170.2). $[\alpha]_D=-79.0$ (*c* 0.2 in C_2H_5OH). Compound **4**; ^{1}H NMR (270 MHz, in $CDCl_3$, TMS as int. standard, δ 0.00): malonyl group (δ_H 3.37, 2H *s*, δ_H 8.1, 1H *br s*). ^{13}C NMR 67.80 MHz, in $CDCl_3$, residual $CHCl_3$ as int. standard, δ 77.00): malonyl group (δ_c 41.5, δ_c 166.7 and 171.2). $[\alpha]_D=-65.1$ (*c* 0.2 in C_2H_5OH).

References

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3. The strain stored in Gifu University (Japan) was used in this study.

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