Pergamon

Tetrahedron: *Asymmetry* 12 (2001) 3185–3187

TETRAHEDRON: *ASYMMETRY*

Microbial resolution of racemic 2-*endo*-acetoxy-1,8-cineole by *Glomerella cingulata*

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Received 19 November 2001; accepted 6 December 2001

Abstract—Resolution of (±)-2-*endo*-acetoxy-1,8-cineole by *Glomerella cingulata* is described. Both (+)-2-*endo*-acetoxy-1,8-cineole and (−)-2-*endo*-hydroxy-1,8-cineole could be quantitatively obtained in enantiomerically pure form (yield 50%; e.e. 100%). In addition, the odor differences between the enantiomers are also described. In both compounds (acetoxy and hydroxy), the (+)-enantiomers tended to have more bright, light and sweet odors than their (−)-antipodes.

1. Introduction

It is well known that many terpenoids have odor differences between enantiomers, therefore, it is very important to obtain enantiomerically pure compounds when establishing the odor and thus, economical methods for the resolution of terpenoid enantiomers are increasingly required.

It has been reported that (±)-2-*endo*-hydroxy-1,8-cineole **1**, one of the terpene alcohols, had the characteristic terpenoid odor.[1] However, the compound **1** evaluated in the study was a racemic mixture and thus, the odors of the enantiomerically pure compounds **1** are not yet known. The odor differences between both enantiomers of 2-*endo*-acetoxy-1,8-cineole **2** have been evaluated.[2,3] In the reports, however, each enantiomer of **2** was prepared via enantiomerically pure **1** (which was prepared from enantiomerically pure α-terpineol). Thus, methods for the resolution of (±)-**1** and (±)-**2** are desired in order to obtain enantiomerically pure compounds from the inexpensive racemates.

In order to obtain the enantiomerically pure alcohols, we have previously utilized enantioselective esterification with the plant-pathogenic fungus, *Glomerella cingulata*[4] as a biocatalyst. As a result, the enantiomerically pure alcohols and their malonates were obtained.[5,6] Through these studies, we have found that *G. cingulata* has the ability to esterify secondary terpene alcohols enantioselectively.

In contrast to our previous work, in the study reported herein we have attempted to obtain enantiomerically pure secondary terpene alcohol **1** by utilizing the ability of *G. cingulata* to hydrolyze enantioselectively the corresponding acetate **2**. The racemate (±)-**2** was hydrolyzed with *G. cingulata* to give compounds **1** and **2** in enantiomerically pure form. Moreover, the odor differences between enantiomers **1** and **2** were evaluated and it was found that in both compounds the (+)-enantiomers tended to have more light and sweet odors than the (−)-enantiomers.

2. Results and discussion

The racemic mixture, (1*RS*,2*RS*,4*SR*)-**2**, dissolved in DMSO, was added to the culture medium of *G. cingulata* and hydrolyzed to give, after 18 h, the enantiomerically pure alcohol (−)-(1*R*,2*R*,4*S*)-**1** in 50% yield (e.e. 100%) (Scheme 1, Table 1). The recovered acetate **2** was also obtained in enantiomerically pure form in 50% yield (e.e. 100%), this was the (+)-(1*S*,2*S*,4*R*)-enantiomer. Thus, the microbial resolution of compounds **1** and **2** was successfully achieved via enantioselective hydrolysis by the use of *G. cingulata* in this study.

Nishimura and Noma reported that (±)-**1** had a slightly medicinal odor.[1] Kubota et al. reported that (+)-

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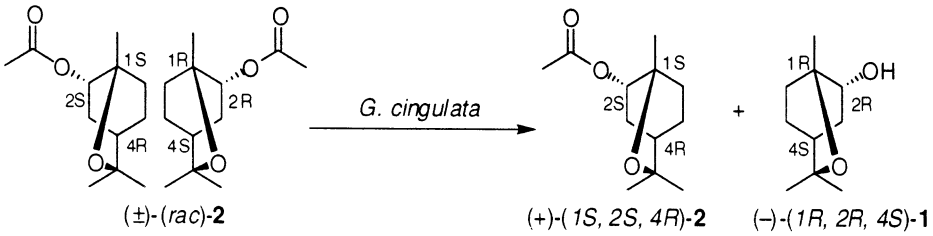
0957-4166/01/$ - see front matter
PII: S0957-4166(01)00555-9



Scheme 1.

Table 1. Microbial resolution of compound **2** by *G. cingulata*

Time (h)	**2**			**1**		
	Yield (%)	E.e. (%)	Abs. configuration	Yield (%)	E.e. (%)	Abs. configuration
12	51.2	95.3	(+)-(1*S*,2*S*,4*R*)	48.8	100	(−)-(1*R*,2*R*,4*S*)
18	50.0	100	(+)-(1*S*,2*S*,4*R*)	50.0	100	(−)-(1*R*,2*R*,4*S*)

(1*S*,2*S*,4*R*)-**2** and (−)-(1*R*,2*R*,4*S*)-**2** showed weakly woody odor and *Alpinia galanga*-like woody odor, respectively, on GC-sniffing of the essential oil of *A. galanga*.[2,3] In the reports by Kubota et al., (+)-(1*S*,2*S*,4*R*)-**2** showed weaker odor than (−)-(1*R*,2*R*,4*S*)-**2**, because (+)-(1*S*,2*S*,4*R*)-**2** was less abundant than (−)-(1*R*,2*R*,4*S*)-**2** ((+)/(−)=18/82). To compare the odor of these enantiomers, (−)-(1*R*,2*R*,4*S*)-**2**, (+)-(1*S*,2*S*,4*R*)-**2**, (−)-(1*R*,2*R*,4*S*)-**1** and (+)-(1*S*,2*S*,4*R*)-**1** were prepared by hydrolysis and acetylation. The odor evaluation in this study was performed by comparing the same amount of enantiomers, and the odor description is shown in Table 2. (−)-(1*R*,2*R*,4*S*)-**2** showed camphorous, dry odor. (+)-(1*S*,2*S*,4*R*)-**2** showed sharp, fruity, sweet odor. (−)-(1*R*,2*R*,4*S*)-**1** showed camphorous odor. (+)-(1*S*,2*S*,4*R*)-**1** had cineole-like, sweet odor. This study is the first report of the odor differences between the enantiomers of **1**. In both compounds **1** and **2**, (+)-(1*S*,2*S*,4*R*)-enantiomers tended to have more bright, light and sweet odors than the (−)-(1*R*,2*R*,4*S*)-enantiomers. Of these enantiomerically pure compounds, (−)-**1** and (−)-**2**, which had the characteristic camphorous odor, are now available for flavor and fragrance applications.

3. Conclusions

The racemic acetate (1*RS*,2*RS*,4*SR*)-**2** was hydrolyzed enantioselectively using *G. cingulata* to give the enantiopure compounds **1** and **2** in high yield. The odors of each of the enantiomeric acetates **2** and alcohols **1** were also evaluated.

4. Experimental

4.1. Gas chromatography conditions

Gas chromatography was carried out using a Hewlett–Packard 5890 Series II plus equipped with a flame ionization detector on a capillary chiral column: CHROMPACK WCOAT Fused silica Cp-Cyclodextrin-β-236-M-19 (50 m×0.25 mm i.d.). The column temperature was programmed from 80 to 210°C at the rate of 2°C/min. The injector and the detector temperatures were 210°C. Helium at a flow rate of 1.52 mL/min was used as a carrier gas.

4.2. Microbial resolution

A nutrient broth was prepared by dissolving saccharose (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%) in distilled water (100 mL). The sterilized broth was inoculated with *G. cingulata*. The culture medium was incubated at 27°C on a reciprocatory shaker. After 72 h, the pH value of the culture medium was adjusted at 7.0, and a solution of acetate **2** (50.0 mg) in DMSO, was added to the medium. After 18 h, the reaction mixture was extracted with diethyl ether and evaporated. The residue was chromatographed on silica gel (hexane/diethyl ether gradient system). Finally, (+)-**2** (25.0 mg, 50%; colorless oil) and (−)-**1** (20.0 mg, 50%; colorless needle-like crystal) were obtained. Compounds **1** and **2** were identified with spectral data obtained in this study (shown below) and in the reports by other workers.[1,2] The absolute configurations of compounds **1** and **2**, which were obtained as a result of the microbial resolution by *G. cingulata*, were determined by comparing the retention times on gas chromatography with authentic samples of (−)-(1*R*,2*R*,4*S*)-**1** and (+)-(1*S*,2*S*,4*R*)-**2**.

Table 2. Odor description of enantiomers **1** and **2**

Compound	Odor description
(−)-(1*R*,2*R*,4*S*)-**2**	Camphorous, dry odor
(+)-(1*S*,2*S*,4*R*)-**2**	Sharp, fruity, sweet odor
(−)-(1*R*,2*R*,4*S*)-**1**	Camphorous odor
(+)-(1*S*,2*S*,4*R*)-**1**	Cineole-like, sweet odor

4.2.1. (−)-2-*endo*-Hydroxy-1,8-cineole 1. (−)-(1*R*,2*R*,4*S*)-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-6-ol; a colorless needle-like crystal; $[\alpha]_D^{25}$ −19.6 (*c* 1.08 in $CHCl_3$); HRMS *m*/*z* 170.1305 ([M$^+$], calcd for $C_{10}H_{18}O_2$, 170.1307); EI-MS *m*/*z* (rel. intensity) 170 [M$^+$] (10), 155 (tr), 137 (1), 126 (44), 111 (34), 109 (12), 108 (65), 93 (34), 83 (40), 71 (63), 69 (37), 58 (19), 55 (21), 43 (100); IR ν_{max} (KBr) cm^{-1} 3445, 2966, 1457, 1364, 1063, 1034, 978; ^{1}H NMR ($CDCl_3$) δ_H 3.73 (1H, ddd, *J* = 10.0, 4.0, 2.0, H-2$_{exo}$), 2.52 (1H, dddd, *J* = 15.0, 10.0, 4.0, 3.0, H-3$_{exo}$), 2.01–1.85 (2H, m, H-5, H-6), 1.58–1.48 (3H, m, H-4, H-5, H-6), 1.32 (1H, ddd, *J* = 15.0, 4.0, 3.0, H-3$_{endo}$), 1.28 (3H, s, H-9), 1.20 (3H, s, H-10), 1.10 (3H, s, H-7); ^{13}C NMR ($CDCl_3$) δ_C 73.4 (s, C-8), 72.5 (s, C-1), 71.1 (d, C-2), 34.6 (t, C-3), 34.2 (d, C-4), 29.0 (q, C-10), 28.6 (q, C-9), 24.9 (t, C-6), 24.0 (q, C-7), 22.1 (t, C-5).

4.2.2. (+)-2-*endo*-Acetoxy-1,8-cineole 2. Acetate, (+)-(1*S*,2*S*,4*R*)-1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-6-ol; a colorless oil; $[\alpha]_D^{25}$ +74.2 (*c* 0.96 in $CHCl_3$); HRMS *m*/*z* 212.1414 ([M$^+$], calcd for $C_{12}H_{20}O_3$, 212.1413); EI-MS *m*/*z* (rel. intensity) 212 [M$^+$] (9), 197 (tr), 170 (tr), 155 (2), 137 (2), 126 (17), 111 (15), 109 (17), 108 (36), 93 (19), 82 (17), 71 (24), 55 (10), 43 (100); IR ν_{max} (KBr) cm^{-1} 2973, 1742, 1457, 1376, 1241, 1027; ^{1}H NMR ($CDCl_3$) δ_H 4.69 (1H, ddd, *J* = 10.0, 4.0, 2.0, H-2$_{exo}$), 2.63 (1H, dddd, *J* = 15.0, 10.0, 4.0, 3.0, H-3$_{exo}$), 2.06 (3H, s, H-12), 2.05–1.96 (1H, m, H-5), 1.87 (1H, ddd, *J* = 14.0, 11.0, 4.0, H-6$_{endo}$), 1.64–1.57 (1H, m, H-6$_{exo}$), 1.57–1.49 (2H, m, H-4, H-5), 1.32–1.27 (1H, m, H-3), 1.28 (3H, s, H-9), 1.22 (3H, s, H-10), 1.04 (3H, s, H-7); ^{13}C NMR ($CDCl_3$) δ_C 170.5 (s, C-11), 73.7 (s, C-8), 72.8 (d, C-2), 70.8 (s, C-1), 33.8 (d, C-4), 32.7 (t, C-3), 28.8 (q, C-10), 28.5 (q, C-9), 25.9 (t, C-6), 24.1 (q, C-7), 21.9 (t, C-5), 21.2 (q, C-12).

Acknowledgements

We thank Dr. M. Ishihara of Shiono Koryo Co., Ltd. for evaluating the odor characteristics.

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