Discovery of Aromatic Components with Excellent Fragrance Properties and Biological Activities: β-Ionols with Antimelanogenetic Effects and Their Asymmetric Syntheses

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Both enantiomers of dihydro-β-ionol and β-ionol, contained in the aromatic components of Osmanthus flower and of Hakuto peach, were obtained with high optical purity by lipase-catalyzed kinetic resolution of the racemates. It was found that all these enantiomers had different characteristic favorable scents and high antimelanogenetic effects. The absolute configuration and the enantiomer ratios of dihydro-β-ionol in the aromatic components of Osmanthus flower and of Hakuto peach were determined. The asymmetric synthesis of (R)-dihydro-β-ionol, one of the most valuable raw materials for fragrance and flavor, was performed from inexpensive β-ionone via lipase-catalyzed dynamic kinetic resolution followed by reduction.

Key words dihydro-β-ionol; antimelanogenetic effect; aromatic component; Osmanthus flower; asymmetric synthesis; lipase-catalyzed kinetic resolution

It has long been known that spices such as cloves have antibacterial and antioxidative effects, and they have been used as food preservatives.1–5) It has been reported that one of the main components of cloves, eugenol, is also present in the essential oils of flowers such as jasmine and rose, and serves as a major aromatic component.6–11) However, research on fragrances has focused only on their aroma, and no attention has been paid to their biological activities. Some flower and leaf extracts have antioxidant and antimelanogenic effects and have been used as cosmetic raw materials; however, they are mainly non-volatile compounds and have been studied without regard to their aromatic properties.12–16)

The aromatic components in Osmanthus flower are known to contain various β-ionone and its derivatives, which have outstanding scents.17–23) Dihydro-β-ionol11–21) and β-ionol22,23) are representative of these compounds. However, their absolute configurations remained unknown until quite recently,24) and synthetic racemates [(±)-1 and (±)-2] have been used as raw materials of fragrances.

Based on the above facts, we thought that some of the volatile aromatic components obtained from flowers should have excellent properties in terms of scent and biological activities. In addition, it is highly probable that the two enantiomers of an optically active compound will have different aromatic characters and different biological activities. However, the aromatic components obtained from natural sources are present in minute amounts, therefore little has been discovered about the aromatic properties and biological activities of both enantiomers of naturally occurring aromatic components. We have therefore been undertaking a project on the discovery of such compounds from natural essential oils and on the asymmetric synthesis of sufficient quantities of the natural components and their enantiomers to identify their structures and absolute configurations, and to evaluate their biological activities. This paper reports some results of a study of the aromatic components in Osmanthus flower and in Hakuto peach.25)

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Results and Discussion

The bulb-to-bulb distillation of the essential oil of Osmanthus absolute (OSM1) obtained from the flowers of Osmanthus fragrans var. thunbergii was conducted under reduced pressure (0.2 kPa). The bath temperature was raised from 50 to 200°C at 50°C intervals. Four distillate fractions were obtained under individual conditions. GC and GC-MS analyses of these fractions showed that the distillate (OSM2) obtained at 200°C contained ionone derivatives, namely dihydro-β-ionol 1, β-ionol 2, dihydro-β-ionone 3, and β-ionone 4. γ-Decalactone 5 was also present as another main ingredient in the same fraction (Fig. 1). Five fractions, i.e., the above-mentioned four and the distillation residue, were submitted to antimelanogenic tests using B16 melanoma cells; OSM2 had the strongest antimelanogenic effect.

In order to clarify the antimelanogenic effects of these components, we investigated the degrees of activity of the commercial products 3–5 and those of (±)-1 and (±)-2, obtained by NaBH₄ reduction of 3 and 4, respectively. The IC₅₀ values of these compounds are as follows: (±)-1: 0.005 mM<IC₅₀<0.050 mM, (±)-2: 0.050 mM<IC₅₀<0.500 mM, 3: 0.050 mM<IC₅₀<0.500 mM, 4: 0.050 mM<IC₅₀<0.500 mM, and 5: IC₅₀≥0.500 mM.

Next, in order to clarify the differences between the aromatic properties and antimelanogenetic effects of the enantiomers
of (±)-1 and (±)-2, we conducted lipase-catalyzed kinetic resolutions of (±)-1 and (±)-2. First, the screening of suitable lipases was carried out based on the reaction of (±)-1 with various commercial lipases in the presence of vinyl acetate in i-Pr₂O at room temperature. We found that *Alcaligenes* sp. lipase (Meito QLM) showed very high enantioselectivity. Thus, (S)-1 (49%, 96% ee) and (R)-6 (45%, 94% ee) were obtained by reaction for 4.5 h (E value = 136). *Burkholderia cepacia* lipase (Roche Diagnostics, CHIRAZYME L-1), *Burkholderia* sp. lipase (Toyobo LIP), *Burkholderia cepacia* lipase (Amano PS-C), and *Candida antarctica* lipase B (Roche Diagnostics, CHIRAZYME L-2) also gave high enantioselectivities (E value = 38 to 72). (R)-6 was then hydrolyzed with K₂CO₃ in MeOH to obtain (R)-1 (83%, 94% ee) (Chart 1).

For the kinetic resolution of (±)-2, *Candida antarctica* lipase B was found to be suitable for providing (S)-2 (45%, 99% ee) and (R)-7 (46%, 99% ee). The alkaline hydrolysis of (R)-7 then produced (R)-2 (94%, 99% ee) (Chart 2).

GC analysis using a chiral column (Beta DEX™ 325, 30 m × 0.25 mm, Spelco) showed that (S)-1 and (R)-1 were present in the volatile aromatic components of Osmanthus flower in a ratio of 9 : 1. It was found that (S)-1 and (R)-1 were also present in the aromatic components of Hakuto peach in a ratio of 1 : 10. However, it is not possible at present to determine the absolute configuration and content ratio of 2 in these natural aromatic components because it is present in very small amounts.

Sensory evaluation of the aromatic properties of these compounds showed that (S)-1 had a woody ambery scent and a powdery floral aroma of violet and cassis. We also found that (R)-1 had an outstanding high-class musky leathery scent and also had a powdery floral aroma of violet and cassis. In contrast, (S)-2 had a floral, ambery, and woody scent, whereas (R)-2 had a floral, fruity, and woody scent.

These compounds showed very high antimelanogenetic effects in tests using B16 melanoma cells (Table 1). In particular, (S)-1 and (R)-1 had powerful antimelanogenetic effects equivalent to or better than that of a typical antimelanogenetic...
compound, N-phenylthiourea (PTU).

(R)-1 has excellent value as a fragrance raw material because its scent is high-class and superior to that of (S)-1, and also because it has a very high antimelanogenetic effect. We examined another method for the asymmetric synthesis of (R)-1. We already reported the lipase-catalyzed dynamic kinetic resolution of (±)-2, which is readily and quantitatively available from the less expensive β-ionone 4 rather than dihydro-β-ionone 3, to give (R)-7 (79%, >99% ee).27) The chemoselective reduction of the external double bond of (R)-2, which was obtained from (R)-7 in almost quantitative yield, was therefore studied to produce (R)-1. After intensive trials, the catalytic hydrogenation of (R)-2 using 5% Pd/C (20 wt%) in MeOH produced a mixture (77% total yield) of (R)-1 and some other reduced products. (R)-1 (99% ee) was the largest component, constituting 46% of the product mixture, and therefore, its yield was calculated as 35% from (R)-2. Tetrahydro-β-ionol29) and dihydro-α-ionol29) were also found in the mixture as minor components (Chart 2). Although this method still needs improvement in terms of its chemoselectivity, we believe that the asymmetric synthesis of (R)-1 from relatively inexpensive 4 will open up a new pathway to the practical synthesis of optically pure (R)-1.

Conclusion

Both enantiomers of dihydro-β-ionol 1 and of β-ionol 2, contained in the aromatic components of Osmanthus flower and of Hakuto peach, were synthesized, and the absolute configuration and enantiomer ratios of 1 in these natural aromatic components were determined. It was found that (S)-1, (R)-1, (S)-2, and (R)-2 had different characteristic favorable scents, which would be applicable in the fragrance, flavor, cosmetics, and food industries.

It is also worth mentioning that (S)-1, (R)-1, (S)-2, and (R)-2 were all found to show high antimelanogenetic effects. In particular, (R)-1 is expected to find use as a fragrance raw material with high added value because it has superior floral aromatic properties and also because it has a remarkable antimelanogenetic effect. Although the supply of high-purity (R)-1 from natural sources is very limited, the asymmetric synthesis of (R)-1 from inexpensive 4, which we developed in this study, will provide the possibility of mass production of (R)-1 once further improvements have been made to the synthesis. Because the synthetic product (R)-1 is identical to the natural product, it has high value as a safe raw material for fragrance and flavor.

Experimental

General All reactions were carried out under an argon or nitrogen (or hydrogen for hydrogenation) atmosphere in an oven-dried flask, containing a stirring-bar, with a rubber septum or with an inlet adaptor with a three-way stopcock. Candida antarctica lipase B was gifted by Roche Diagnostics K.K., Japan, and Lipase QLM was obtained from Meito Sangyo,
Co., Ltd., Japan. Pd/C (5%) was purchased from Wako Pure Chemical Industries, Ltd. MeOH was used as a solvent without further purification, and all other reagents were purchased from commercial sources (Aldrich, TCI, Wako, Kanto, Kishida and Nacalai) and used without further purification. All reactions were monitored using thin-layer chromatography on glass-backed silica gel 60 F254 0.2-mm plates (Merck) or GC-MS performed using an Agilent Technologies 6890N (GC) combined with a JEOL J-AMSUN (MS) or JMS-Q1000GC K9 (MS). GC analysis was performed using Agilent Technologies 7890N (GC) equipped with a column (DB-WAX, 60m×0.25mm) in an oven (65 to 120°C, 3°C/min and then 120 to 245°C, 5°C/min) or with a chiral column (Beta DEX™ 325, 30m×0.25 mm, Spelco) in an oven (40 to 85°C, 2°C/min and then 85°C for 8h) with a flame ionization detector. Flash chromatography was performed with silica gel 60N, spherical neutral (40–50 μm), purchased from Kanto Chemical Co. 1H- and 13C-NMR spectra were recorded on a JEOL JMN-A500 or ECA-500 (1H: 500 MHz, 13C: 125 MHz) instrument with chemically shifted tetramethylsilane as the internal standard.

An essential oil from *Osmanthus fragrans* var. *thunbergii* flowers, produced in the Zhuang Zu Autonomous Region of Guang Xi Provence, People’s Republic of China, was purified by column chromatography on silica gel (hexane–EtOAc 10 : 1) to afford (S)-β-ionol (0.90 g, 45%, 99% ee) and (R)-O-acetyl-β-ionol (R)-7 (1.1 g, 46%, 99% ee). The optical purity of (S)-2 was determined by HPLC analysis using a chiral column (Daicel CHIRALCEL OD-H; hexane–i-PrOH = 99.9:0.1).

A colorless oil. The 1H-NMR data were in good agreement with those reported in the literature.27

(R)-β-ionol [(S)-2]30 A colorless oil, [α]D26 = −7.8 (c=0.60, CHCl3) [ref. 30]. [α]D24 = −8.0 (c=0.158, CHCl3). 1H-NMR (500 MHz, CDCl3) δ: 0.98 (3H, s), 0.99 (3H, s), 1.31 (3H, d, J=6.5Hz), 1.43–1.45 (2H, m), 1.57–1.62 (2H, m), 1.66 (3H, s), 1.97 (2H, t, J=6.5Hz), 4.34–4.39 (1H, m), 5.49 (1H, dd, J=6.5, 16.0Hz), 6.05 (1H, d, J=16.0Hz).

(R)-O-Acetyl-β-ionol [(R)-7] A colorless oil. The 1H-NMR data were in good agreement with those reported in the literature.

Lipase-Catalyzed Kinetic Resolution of (±)-1 (Chart 1) A mixture of (±)-1I20 (49 g, 0.25 mol), vinyl acetate (16 g, 0.19 mmol), and lipase QLM (6.0 g, 12 wt%) in i-PrO (0.63 L) was stirred under nitrogen atmosphere at room temperature for 4.5h and filtered using a Celite pad. The filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (hexane–EtOAc = 30:1) to afford (S)-4 (2.6 mmol), (S)-5 (2.4 g, 94%, 96% ee) and (R)-4 (2.6 mmol), (R)-5 (2.4 g, 94%, 96% ee). The optical purity of (S)-5 was determined by HPLC analysis using a chiral column (Daicel CHIRALCEL OD-H; hexane–i-PrOH = 99:1). [α]D26 = +8.7 (c=0.66, CHCl3) [ref. 30]. [α]D24 = +7.6 (c=0.176, CHCl3). Its 1H-NMR data were in good agreement with those of (S)-2.

(S)-4 (2.6, 6-Trimethylcyclohex-1-enyl)butan-2-ol [(S)-1] A colorless oil, [α]D26 = +4.1 (c=0.19, CHCl3 for 96% ee).

A mixture of (R)-6 (10.3 g, 20 mmol), vinyl acetate (2.0 mL, 20 mmol) and MeOH (8 mL) was stirred at room temperature for 4.5h. The reaction mixture was concentrated under reduced pressure and filtered through a Celite pad. The filtrate was evaporated and purified by column chromatography on silica gel (hexane–EtOAc = 5:1) to provide (R)-2 (0.34 g, 83%) as a colorless oil [14% recovery of (R)-6]. The optical purity of (R)-1 was determined to be 94% ee by HPLC analysis using the procedure described for (S)-1. [α]D26 = −3.7 (c=0.17 CHCl3). Its 1H, 13C-NMR and IR data were in good agreement with those of (S)-1. HR-MS m/z: 197.1933 [Calcd for C13H25O (M+H)+: 197.1905].
total yield), in which (R)-1 was found as the largest component. Tetrahydro-β-ionol\(^{29}\) and dihydro-α-ionol\(^{29}\) were also found in the mixture as minor products. The GC, GC-MS, \(^1\)H-, \(^1\)C-NMR, and HPLC analysis of the product mixture showed that (R)-1 constituted 46% of the mixture (35% yield from (R)-2) and that its optical purity was 99% ee. The above mentioned flash column chromatography also provided tetrahydro-β-ionone (30 mg, 15%).

Preparation of B16 Melanoma Cells Cryopreserved B16 cells were revived, and subcultures were prepared using 10% fetal bovine serum and Dulbecco’s modified Eagle’s medium (DMEM). Culturing was performed in an incubator (5% CO\(_2\), 37°C). B16 melanoma cells were seeded on 24-well plates at 3×10\(^4\) (mL/well), and cultured for 48h on the next day in a medium (1 mL) containing various concentrations of the experimental samples dissolved in dimethyl sulfoxide. The amounts of intracellular melanin in all the cells were then measured.

Measurement of Intracellular Melanin After removing the medium supernatant, the cells pasted on the wells were washed in 1 mL of phosphate-buffered saline 1 (PBS). A 0.25% trypsin-ethylenediaminetetraacetic acid solution (100 µL) was added to the cells, and the cells were allowed to stand at 37°C in 5% CO\(_2\) for 1 min. The cells exfoliated by trypsinization in 1 mL of PBS were collected and centrifuged at 1000 rpm at 4°C. The cell pellets were resuspended in 110 µL of PBS, the intracellular melanin content was computed.

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References and Notes
24) The absolute configuration and the enantiomer ratio of 1 contained in the aromatic components of \textit{Osmanthus fragrans} flowers were reported in 2011; however, no comments were given on the aromatic property and biological activity of each enantiomer of 1. See: Nakayama Y., Abe S., Yamaguchi Y., Sakurai K., 55th Symposium on the Chemistry of Terpenes, Essential Oils, and Aromatics, Tsukuba, Japan (2011). Abstract Book, pp. 22–24.