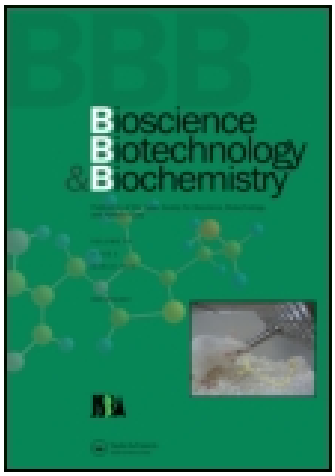

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Absolute Stereochemistry of Yeast Reduction Products from 2-(3′,4′-Dimethoxycinnamyl)-2-methylcyclopentane-1,3-dione

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Absolute Stereochemistry of Yeast Reduction Products from 2-(3′,4′-Dimethoxycinnamyl)-2-methylcyclopentane-1,3-dione

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The absolute stereochemistry of yeast reduction products 2s and 2a from 2-(3′,4′-dimethoxycinnamyl)-2-methylcyclopentane-1,3-dione (1) was investigated. The results from several spectral studies and chemical correlations enabled their absolute configurations to be concluded as (2*S*,3*S*) and (2*R*,3*S*), respectively.

The trends in medicinal chemistry over the past two decades or so have been the optical resolution of pharmacoactive racemic molecules, pharmacological evaluation of both enantiomers, and establishment of preparative methods for only the useful enantiomer. In the course of a search for new cardiovascular drugs, optically active hydroxyketone **2** with unambiguous absolute stereochemistry became necessary as a versatile intermediate for several terminal target molecules. Among several possible methods leading to **2**, yeast reduction of symmetrical diketone **1**[1] seemed most attractive. While investigating the results of the preceding studies on similar substrates, we found that most micro-organisms produced (2*S*,3*S*)- and/or (2*R*,3*S*)-hydroxyketones from 2,2-disubstituted 1,3-dicycloalkanones through reduction on the *re*-face.[2,4,8] However, we were confused with one case,[3] in which the major and minor hydroxyketones possesses (2*S*,3*S*)- and (2*S*,3*R*)-configurations. Chemical correlation through inversion of the hydroxyl group between one set of major and minor hydroxyketones was the most important point to determine the absolute configuration of the minor product, although the conclusion about the absolute stereochemistry of the minor product was corrected later by alternative chemical correlation studies and a brief comment that mirror images were illustrated for the minor products.[4] We wondered where the results of the chemical correlation study initially reported would lead. Since a kind of ambiguity seemed to remain and it thus seemed somewhat risky to determine the absolute configuration of our products based on a deduction according to precedents, we decided to clarify the absolute stereochemistry of the products for our own purpose. We report here our results and conclusion.

The diastereomeric ratio of the yeast reduction product **2** was first determined from ^{1}H-NMR studies on the acetates **3**. Among the tested strains, only KI0117, a strain related to *Pichia terricola* in our collection, gave an almost single diastereomer assigned to **2s** as colorless crystals, while bakers' yeast, brewers' yeast, or KI0116 related to *Saccharomyces bailii*, gave a mixture of **2s** and **2a**. The results are summarized in Table I.

NOE experiments suggested that the KI0117 product possessed *syn*-stereochemistry as illustrated in **2s**; a marked NOE difference between an oxymethine proton and quaternary methyl protons was observed. This was clarified by a ^{13}C-NMR study[5]; the KI0117 product showed a signal at 19.9 ppm for a methyl carbon atom attached to the quaternary carbon, while the major product obtained from bakers' yeast reduction showed a signal at 15.3 ppm as shown in Table II. Thus, **2a** must have been an *anti*-isomer.

A relative ^{1}H-NMR study of the (+)-MTPA ester[6] derived from the yeast reduction products and a $NaBH_4$ reduction product of **1** indicated the optical purity of **2s** and **2a** to both be >98% ee, the results being summarized in Table I. In order to determine the absolute configurations of **2s** and **2a**, a modification of Mosher's method[7] was used. The results illustrated in Fig. 2 show that the absolute configurations of **2s** and **2a** were (2*S*,3*S*) and (2*R*,3*S*). This conclusion is supported by the chemical conversion of **2s** into known optically active lactone **5**[3,4,8] and by the

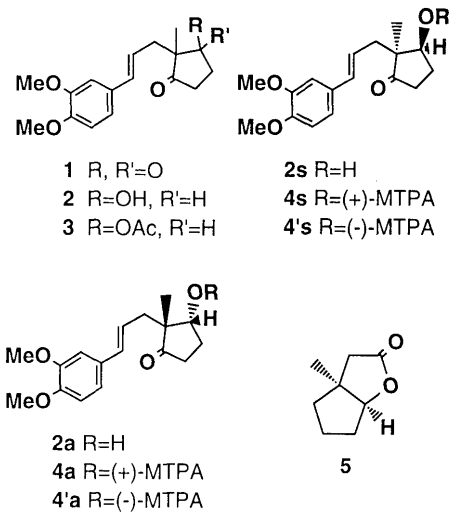

Fig. 1. Compounds Studied.

Table I. Results of Yeast Reduction

Yeast strain	Reaction time (h)	Yield (%)	Recovery (%)	*d.e.* (%)[a]	*e.e.* (%)[b]
Bakers' yeast	19	60.3	22.9	−18	>98
Brewers' yeast	48	64.9	17.9	78	>98
KI 0116	70	64.8	16.3	44	>98
KI 0117	192	25.8	69.3	96	>98

[a] Diastereomeric excess for *syn*-hydroxyketone **2s** is shown.
[b] Enantiomeric excess for both of *syn*- and *anti*-hydroxyketones, **2s** and **2a** is shown.

chemical correlation between **2s** and **2a** as shown in Fig. 3.

First of all, **2s** was esterified with 3,5-dinitrobenzoyl chloride to give (2*S*,3*S*)-3,5-dinitrobenzoate **6s**, which showed a specific rotation of $+132.2°$ and a singlet absorption for methyl protons at δ 1.23 ppm. A mixture enriched with **2a** by chromatographic purification of the bakers' yeast reduction products was then esterified in the same manner. The esterified product thus obtained showed two singlet signals at δ 1.23 and 1.25. Thus, *syn*- and *anti*-3,5-dinitrobenzoates **6s** and **6a** could obviously be distinguished. Major product **6a** showed a specific rotation of $+27.0°$ and a singlet absorption at δ 1.25 ppm, after chromatographic purification. Finally, the same mixture was treated under modified Mitsunobu conditions.[9] The reaction proceeded very slowly, because the reaction center was a neopentyl carbon which was resistant to nucleophilic attack by 3,5-dinitrobenzoate anion, and anyway gave the other *syn*-3,5-dinitobenzoate **6's** after TLC separation. The obtained **6's** showed a singlet absorption at δ 1.23 ppm and a specific rotation of $-135.4°$. These values indicate **6's** to be the antipode of **6s** and thus the absolute stereochemistry of **6's** is (2*R*,3*R*). Consequently, original hydroxyketone **2a** before the inversion reaction should have possessed (2*R*,3*S*)-stereochemistry. In summary, all the strains used in the present investigation reduced both two carbonyl groups from the *re*-face against each carbonyl group. Our conclusion on the absolute stereochemistry of the yeast reduction products is summarized in Fig. 4.

Table II. ^{13}C-NMR Data for Hydroxyketones **2s** and **2a**

2s	2a
19.9	15.3
27.9	27.6
34.0	35.0
34.6	38.8
53.6	53.5
55.8	55.9
55.9	55.9
Unidentified[a]	75.2
108.8	108.7
111.1	111.1
119.1	119.2
123.7	122.8
130.3	130.1
132.8	133.3
148.6	148.7
149.0	149.0
220.6	219.9

[a] An unidentified signal overlapping with those of $CDCl_3$ must have been present.

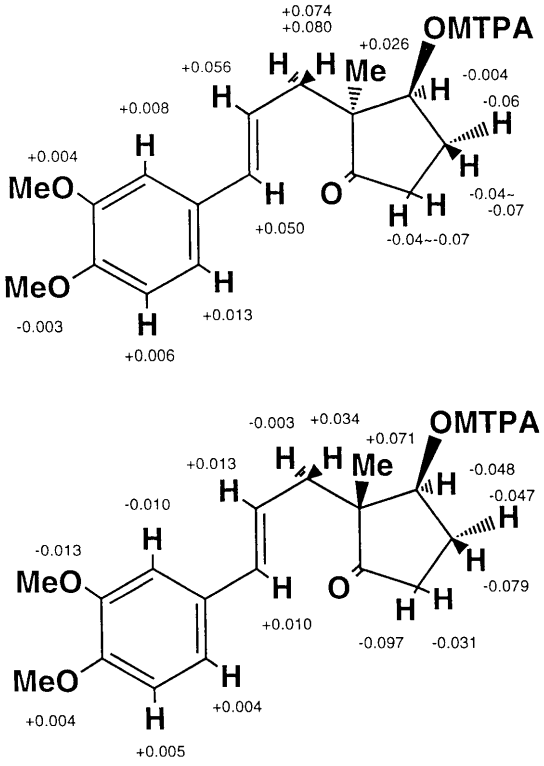

Fig. 2. ^{1}H-NMR Chemical Shift Differences for Two Sets of MTPA Esters of *syn*- and *anti*-Hydroxyketones **2s** and **2a**: $\Delta\delta$ (ppm) = δ {(−)-MTPA ester} − {(+)-MTPA ester}.

Experimental

All melting point (mp) values are uncorrected. IR spectra were determined with a JEOL Diamond-20 FT-IR spectrophotometer, and ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a JEOL JNM-A500 FT NMR spectrometer. Chemical shifts are expressed in ppm downfield from TMS as an internal standard, unless otherwise noted. Mass spectra were measured with a JEOL JMS-SX/SX 102A tandem mass spectrometer, and specific rotation values were determined with a JASCO DIP-140 digital polarimeter. Elemental analyses were carried out with a Perkin-Elmer 240C elemental analyzer. Oriental Dry Yeast® from Oriental Yeast Co., Ltd. was used as bakers' yeast. Raw brewers' yeast was obtained as a wet cake from Yeast Business Development Group of Kirin Brewery Co., Ltd.

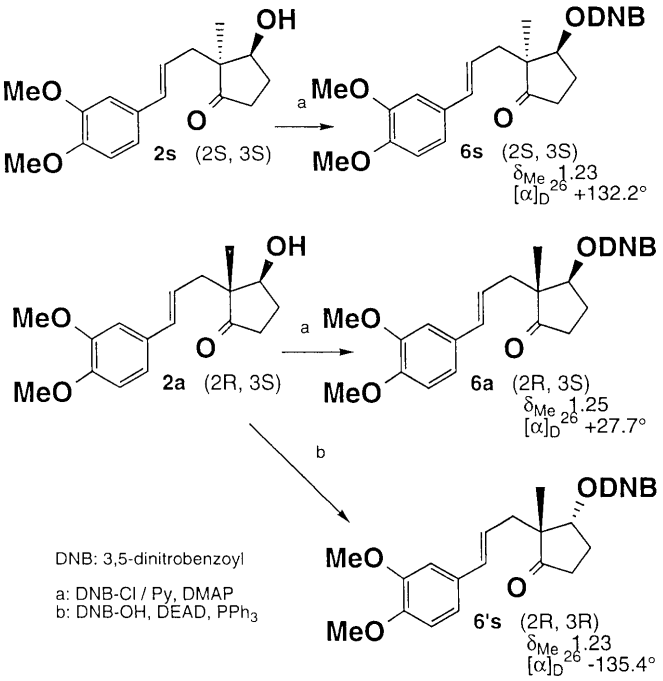

Fig. 3. Chemical Correlation between **2s** and **2a**.

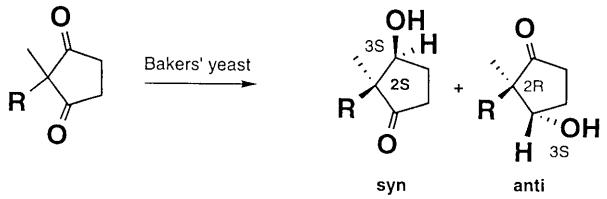

Fig. 4. Conclusion on the Stereochemical Aspects of Yeast Reduction Products.

KI0116 and 0117 strains were precultivated to grow cells.

Reduction of **1**. Bakers' yeast (14 g), wet brewers' yeast (70 g), or the pre-cultivated culture of KI0116 or 0117 strain was added to a solution comprising sucrose (30 g) and tap water (100 ml) and warmed to 30°C. The mixture was swirled at 27°C for 10 min. A suspension of **1** (1.0 g) in EtOH (10 ml) and Triton X100 (2 ml) was homogenized by sonication and added to the foregoing mixture. The mixture thus obtained was swirled at 27°C and then filtered together with Celite 545 and ethyl acetate. The solid residue was washed with EtOAc. The organic layer was separated, and the aqueous layer saturated with NaCl was extracted with EtOAc. The organic layers were combined, dried over Na_2SO_4, and evaporated *in vacuo* to give a colorless oil. This was purified through a column (35 mmϕ × 250 mm) of silica gel (120 g), using hexane–EtOAc (7 : 2 and 7 : 3) as the eluent, to give **2** as a colorless gum, together with the recovery of **1**. Yields and recoveries are shown in Table I.

syn-Hydroxyketone **2s**. The reduction product with KI0117, a colorless gum, formed crystals of **2s** in a refrigerator: mp 90.8–91.0°C, $[\alpha]_D^{25}$ 44.70° (*c* 0.860, EtOH). ν_{max} (Nujol) cm^{-1}: 3537, 1741, 1603, 1585, 1516. ^{1}H-NMR δ: 1.05 (s, 3H), 1.98 (ddt, *J* = 3.5, 10.0, and 14.0 Hz, 1H), 2.23 (ddt, *J* = 5.0, 9.0, and 14.0 Hz, 1H), 2.34 (ddd, *J* = 3.5, 9.0, and 19.0 Hz, 1H), 2.40 (ddd, *J* = 1.5, 7.0, and 14.5 Hz, 1H), 2.50 (ddd, *J* = 1.0, 7.5, and 14.5 Hz, 1H), the other 2.50 (ddd, *J* = 9.0, 10.0, and 19.0 Hz, 1H), 3.88 (s, 3H), 3.90 (s, 3H), 4.18 (dd, *J* = 3.5 and 5.0 Hz, 1H), 6.11 (dt, *J* = 7.5 and 15.5 Hz, 1H), 6.42 (d, *J* = 15.5 Hz, 1H), 6.80 (d, *J* = 9.0 Hz, 1H), 6.89 (dd, *J* = 2.0 and 9.0 Hz, 1H), the other 6.89 (d, *J* = 2.0 Hz, 1H). HRMS *m/z*: 290.1480 (observed); 290.1518 (theoretical ion distribution for $C_{17}H_{22}O_4$). *Anal.* Observed: C, 70.13; H, 7.68%. Calcd.: C, 70.32; H, 7.64%. Crystalline **2s** showed a circular spot on a TLC plate, corresponding to the top half of an oval spot observed in other cases.

anti-Hydroxyketone **2a**. From bakers' yeast reduction product **2** (3.7 g) just described, **2a** was obtained as colorless crystals (11 mg) after repetitive column and thin-layer chromatography; mp 85.5–86.0°C, $[\alpha]_D^{24}$ −22.6° (*c* 1.0, EtOH). ν_{max} (Nujol) cm^{-1}: 3539, 1730, 1601, 1585, 1516. ^{1}H-NMR δ: 1.06 (s, 3H), 1.88 (m, 1H), 2.19 (ddd, *J* = 8.0, 9.0, and 19.0 Hz, 1H), 2.27 (dddd, *J* = 4.0, 5.0, 9.0, and 12.5 Hz, 1H), 2.32 (d, *J* = 8.0 Hz, 1H), 2.33 (d, *J* = 8.0 Hz, 1H), 2.49 (ddd, *J* = 4.0, 9.0, and 19.0 Hz, 1H), 3.87 (s, 3H), 3.89 (s, 3H), 4.28 (br. t, *J* = 6.5 Hz, 1H), 5.99 (dt, *J* = 8.0 and 16.0 Hz, 1H), 6.37 (d, *J* = 16.0 Hz, 1H), 6.80 (d, *J* = 9.0 Hz, 1H), 6.87 (dd, *J* = 2.0 and 9.0 Hz, 1H), the other 6.87 (d, *J* = 2.0 Hz, 1H). FDMS *m/z*: 290 (M^+). HRMS *m/z*: 290.1556 (observed); 290.1518 (theoretical ion distribution for $C_{17}H_{22}O_4$). *Anal.* Observed: C, 70.85; H, 7.77%. Calcd. for $C_{17}H_{22}O_4$: C, 70.32; H, 7.64%.

Evaluation of the diastereomeric excess for **2**. The yeast reduction product **2** was acetylated in the usual manner to give acetate **3** as a colorless gum, which showed a single spot by TLC analysis. The diastereomeric excess was calculated on the basis of the integration ratio of quaternary methyl protons from the ^{1}H-NMR spectrum and the results are shown in Table I. The *syn*-isomer **2s** was also acetylated in the same manner. Two examples of the spectral data are described next.

Acetate **3** *derived from the bakers' yeast reduction product.* δ: 1.05 (s, 3 × 3/5H), 1.07 (s, 3 × 2/5H), 1.91–2.09 (m, 2H), 2.05 (s, 3 × 3/5H), 2.08 (s, 3 × 2/5H), 2.22–2.50 (m, 4H), 3.87 (s, 3H), 3.89 (s, 3 × 3/5H), the other 3.89 (s, 3 × 2/5H), 5.22 (dd, *J* = 3.0 and 5.0 Hz, 2/5H), 5.34 (t, *J* = 5.5 Hz, 3/5H), 5.93 (ddd, *J* = 2.5, 8.0, and 16.0 Hz, 2/5H), 5.95 (ddd, *J* = 2.5, 8.0, and 16.0 Hz, 3/5H), 6.33 (d, *J* = 16.0 Hz, 2/5H), 6.66 (d, *J* = 16.0 Hz, 3/5H), 6.79 (d, *J* = 8.0 Hz, 3/5H), the other 6.79 (d, *J* = 8.0 Hz, 2/5H), 6.85 (dd, *J* = 2.0 and 8.0 Hz, 2/5H), 6.86 (d, *J* = 2.0 Hz, 2/5H), 6.87 (d, *J* = 2.0 Hz, 3/5H), the other 6.87 (dd, *J* = 2.0 and 8.0 Hz, 3/5H).

Acetate **3s** *derived from* **2s**. δ: 1.08 (s, 3H), 2.04 (dddd, *J* = 3.0, 4.5, 9.0, and 14.0 Hz, 1H), 2.09 (s, 3H), 2.30 (ddt, *J* = 5.0, 9.0, and 14.0 Hz, 1H), 2.38 (ddd, *J* = 8.0 and 14.0 Hz, 1H), 2.40 (ddd, *J* = 4.5, 9.0, and 19.0 Hz, 1H), 2.41 (dd, *J* = 7.5 and 14.0 Hz, 1H), 2.45 (dt, *J* = 9.0 and 19.0 Hz, 1H), 3.87 (s, 3H), 3.90 (s, 3H), 5.22 (dd, *J* = 3.0 and 5.0 Hz, 1H), 5.95 (dt, *J* = 7.5 and 15.5 Hz, 1H), 6.34 (d, *J* = 15.5 Hz, 1H), 6.80 (d, *J* = 8.5 Hz, 1H), 6.86 (dd, *J* = 2.0 and 8.0 Hz, 1H), 6.87 (d, *J* = 2.0 Hz, 1H). HRMS *m/z*: 332.1599 (observed); 332.1624 (theoretical ion distribution for $C_{19}H_{24}O_5$).

Determination of the relative configuration of **2s** *and* **2a**.

(1) *Difference NOE experiment for* **2s**: a marked difference NOE was observed between an oxymethine proton and quaternary methyl protons.

(2) ^{13}*C-NMR data for* **2s** *and* **2a**: these are shown in Table II.

Evaluation of the enantiomeric excesses for **2s** *and* **2a**. Each of yeast reduction products **2** was esterified with (*S*)-(+)-MTPA chloride in pyridine in the presence of DMAP in the usual manner. A typical procedure involved (*S*)-(+)-MTPA chloride (50 mg, 0.2 mmol) and then DMAP (a piece of crystal) being added to a solution of hydroxyketone **2** (29 mg, 0.1 mmol) in dry pyridine while stirring in an ice bath. The mixure was stirred at ambient temperature for 17–24 h, poured into ice-cooled water, and extracted twice with ether. The combined extracts were successively washed twice with aqueous $CuSO_4$, twice with water, aqueous $NaHCO_3$, and twice with brine, dried over anhydrous $MgSO_4$ and evaporated to give a colorless gum (66 mg), which was purified by TLC, developed with hexane–EtOAc (1 : 1), to afford a colorless gum (51 mg, quantitative yield). The enantiomeric excesses of both **2s** and **2a** were calculated on the basis of the integration ratio of quaternary methyl protons from the ^{1}H-NMR spectrum of the (+)-TMPA ester thus obtained, in comparison with the spectrum for the (+)-MTPA ester derived from racemic **2**. The results are shown in Table I, two examples of the spectral data being described next.

(+)-*MTPA ester* **4s** *derived from* **2s**. δ: 1.06 (s, 3H), 2.12 (m, 1H), 2.20 (ddd, *J* = 1.0, 7.0 and 14.0 Hz, 1H), 2.26 (ddd, *J* = 0.5, 8.0, and 14.0 Hz, 1H), 2.31–2.44 (m, 3H), 3.53 (d, *J* = 1.0 Hz, 3H), 3.88 (s, 3H), 3.89 (s, 3H), 5.39 (t, *J* = 4.0 Hz, 1H), 5.79 (ddd, *J* = 7.0, 8.0, and 16.0 Hz, 1H), 6.24 (d, *J* = 16.0 Hz, 1H), 6.81 (m, 3H), 7.39 (m, 3H), 7.53 (m, 2H).

Mixture of (+)-*MTPA esters* **4s** *and* **4a** *derived from bakers' yeast reduction product* **2**. δ: 0.95 (s, 3 × 3/5H), 1.06 (s, 3 × 2/5H), 2.0–2.5 (m, 6H), 3.51 (d, *J* = 1.0 Hz, 3 × 3/5H), 3.53 (d, *J* = 1.0 Hz, 3 × 2/5H), 3.87 (s, 3 × 3/5H), 3.88 (s, 3 × 2/5H), 3.89 (s, 3 × 2/5H), the other 3.89 (s, 3 × 3/5H), 5.39 (br. t, *J* = 4.0 Hz, 2/5H), 5.54 (t, *J* = 6.0 Hz, 3/5H), 5.79 (ddd, *J* = 7.0, 8.0, and 15.5 Hz, 2/5H), 5.91 (dt, *J* = 8.0 and 16.0 Hz, 3/5H), 6.24 (d, *J* = 15.5 Hz, 2/5H), 6.36 (d, *J* = 16.0 Hz, 3/5H), 6.80 (m, 3 × 3/5H), 6.87 (m, 3 × 2/5H), 7.40 (m, 3H), 7.52 (m, 2H).

Determination of the absolute configuration of **2s** *and* **2a**.

(1) *Application of a modification of Moshor's method.*[7]

(−)-MTPA esters **4′s** and **4′a** were prepared from **2s** and **2a** with (*R*)-(−)-MTPA chloride in the same manner as that already described. (+)-MTPA ester **4a** was also prepared from **2a** with (*S*)-(+)-MTPA chloride. The results are shown in Fig. 2.

(2) *Chemical conversion into known optically active lactone* **5**.

syn-Hydroxyketone **2s** was converted into **5** in accordance with the procedure described by Brooks *et al.*,[3,4] and the crude product gave colorless crystals from hexane–EtOAc without chromatographic purification; mp 97–98.5°C (*lit.*[3,4] mp 96°C), $[\alpha]_D^{24}$ 94.6° (*c* 0.23, $CHCl_3$) {*lit.*[3,4,8] $[\alpha]_D^{23}$ 94.7° (*c* 0.17, $CHCl_3$) and $[\alpha]_D^{25}$ 95° (*c* 1, $CHCl_3$)}. ^{1}H-NMR δ: 1.27 (s, 3H), 2.23 (m, 1H), 2.48 (m, 3H), 2.53 (d, *J* = 18.0 Hz, 1H), 2.83 (d, *J* = 18.0 Hz, 1H), 4.83 (dd, *J* = 1.0 and 4.5 Hz, 1H). These NMR data are practically identical with those of Brooks *et al.* FDMS *m/z*: 154 (M^+). *Anal.* Observed: C, 62.54; H, 6.65%. Calcd. for $C_8H_{10}O_3$: C, 62.32; H, 6.54%.

(3) *Chemical correlation between* **2s** *and* **2a**.

(*2S,3S*)-*syn-3,5-Dinitrobenzoate* (**6s**). To a stirred solution of **2s** (131 mg) in pyridine (2.6 ml) in an ice bath, a solution of 3,5-dinitrobenzoyl chloride (114 mg) in dry dichloromethane (1.3 ml) was added dropwise. After stirring for 2 h, the mixture was poured into water and extracted twice with ether. The extracts were combined, washed successively with aq. $CuSO_4$ (× 2), water (× 1), aq. $NaHCO_3$ (× 1) and brine (× 1), dried over sodium sulfate, and evaporated *in vacuo* to give a yellow crystalline residue (173 mg), which was purified by preparative TLC to give **6s** as a yellow powder (79 mg) after recrystallization from hexane–EtOAc; mp 151.4–151.9°C, $[\alpha]_D^{24}$ 132.2° (*c* 1.0, CH_2Cl_2). ν_{max} (Nujol) cm^{-1}: 1728, 1630, 1600, 1543, 1516. ^{1}H-NMR δ: 1.23 (s, 3H), 2.55 (dddd, *J* = 3.0, 4.0, 9.0, and 14.0 Hz, 1H), 2.48 (ddd, *J* = 5.0, 9.0, and 9.0 Hz, 1H), 2.51 (ddd, *J* = 5.0, 9.0, and 14.0 Hz, 1H), 2.59 (dd, *J* = 9.0 and 19.5 Hz, 1H), 2.61 (dd, *J* = 9.0 and 15.5 Hz, 1H), 2.66 (dd, *J* = 9.0 and 19.5 Hz, 1H), 3.77 (s, 3H), 3.83 (s, 3H), 5.69 (dd, *J* = 3.0 and 4.0 Hz, 1H), 5.87 (ddd, *J* = 6.0, 9.0, and 15.5 Hz, 1H), 6.09 (d, *J* = 15.5 Hz, 1H), 6.52 (d, *J* = 2.0 Hz, 1H), 6.54 (dd, *J* = 2.0 and 8.0 Hz, 1H), 6.60 (d, *J* = 8.0 Hz, 1H), 8.94 (d, *J* = 2.0 Hz, 2H), 9.30 (t, *J* = 2.0 Hz, 1H). MS *m/z*: 484 (M^+). HRMS *m/z*: 484.1441 (observed); 484.1482 (theoretical ion distribution for $C_{24}H_{24}N_2O_9$). *Anal.*

Observed: C, 59.43; H, 5.05; N, 5.57%. Calcd. for $C_{24}H_{24}N_2O_9$: C, 59.50; H, 4.99; N, 5.78%.

(2R,3S)-anti-3,5-Dinitrobenzoate (**6a**). A mixture of hydroxyketones (100 mg) enriched with **2a** was treated with 3,5-dinitrobenzoyl chloride (87 mg) in the same manner as that just described to give yellow crystals, which separated into a major and less polar band and a minor and more polar band on each preparative TLC plate. The former gave **6a** as a yellow powder (75 mg) after recrystallization from hexane–EtOAc; mp 196.5–197.0°C, $[\alpha]_D^{26}$ 27.0° (*c* 1.0, CH_2Cl_2). ν_{max} (Nujol) cm^{-1}: 3110, 1734, 1630, 1599, 1581, 1543, 1516. ^{1}H-NMR δ: 1.25 (s, 3H), 2.15 (dddd, J=7.5, 8.5, 9.5, and 13.0 Hz, 1H), 2.37 (ddd, J=1.0, 8.0, and 13.5 Hz, 1H), 2.39 (ddd, J=8.5, 9.0, and 19.0 Hz, 1H), 2.46 (ddd, J=1.0, 7.0, and 13.5 Hz, 1H), 2.54 (dddd, J=4.5, 6.0, 9.0, and 13.5 Hz, 1H), 2.64 (ddd, J=4.5, 9.5, and 19.0 Hz, 1H), 3.80 (s, 3H), 3.83 (s, 3H), 5.66 (dd, J=6.0 and 7.5 Hz, 1H), 5.97 (ddd, J=7.0, 8.0, and 16.0 Hz, 1H), 6.36 (br. d, J=16.0 Hz, 1H), 6.68 (d, 8.0 Hz, 1H), 6.70 (d, J=2.0 Hz, 1H), 6.78 (dd, J=2.0 and 8.0 Hz, 1H), 8.99 (d, J=2.0 Hz, 2H), 9.15 (t, J=2.0 Hz, 1H). FDMS *m/z*: 484 (M^+). HRMS *m/z*: 484.1503 (observed); 484.1482 (theoretical ion distribution for $C_{24}H_{24}N_2O_9$). *Anal.* Observed: C, 59.80; H, 5.01; N, 5.36%. Calcd. for $C_{24}H_{24}N_2O_9$: C, 59.50; H, 4.99; N, 5.78%.

(2R,3R)-syn-3,5-Dinitrobenzoate (**6's**). To a solution of the hydroxyketone mixture (150 mg) enriched with **2a**, 3,5-dinitrobenzoic acid (218 mg) and triphenylphosphine (270 mg) in dry THF (3 ml) in an ice bath, DEAD (160 ml) was added dropwise. The mixture was stirred at room temperature for 12 days and then at refluxing temperature overnight, and subsequently evaporated *in vacuo*. The residue thus obtained was extracted with dichloromethane. The extract was evaporated *in vacuo* to give a residue, which was chromatographed by preparative TLC, developed with hexane–ethyl acetate (1 : 1), to give **6's** (100 mg) as yellow crystals, which were recrystallized from hexane–EtOAc to give yellow crystals (65 mg), mp 151.8–152.0°C, $[\alpha]_D^{26}$ −135.4° (*c* 1.0, CH_2Cl_2). The ^{1}H-NMR spectrum of **6's** was identical to that of **6s**. HRMS *m/z*: 484.1482 (observed); 484.1482 (theoretical ion distribution for $C_{24}H_{24}N_2O_9$). *Anal.* observed: C, 59.98; H, 5.04; N, 5.40%. Calcd. for $C_{24}H_{24}N_2O_9$: C, 59.50; H, 4.99; N, 5.78%.

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