Gas Chromatographic Determination of the Absolute Configuration of Secondary Alcohols and Ketones Having 7-Carboxybicyclo[2.2.1]heptane Skeleton

Yoshimitsu YAMAZAKI and Hidekatsu MAEDA

*Fermentation Research Institute, Agency of Industrial Science and Technology,
Ministry of International Trade and Industry,
Yatabe-machi Higashi-1-1-3, Tsukuba,
Ibaraki 305, Japan*

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Stereoisomers of 7-carboxy- and/or 7-methoxycarbonylbicyclo[2.2.1]heptan-2-ol, heptan-2-one, hept-5-en-2-ol, and hept-5-en-2-one were prepared as the racemic and enantiomerically pure forms. Gas chromatographic enantiomer separation of these compounds was investigated for their diastereomeric derivatives prepared with eight chiral reagents. Among them, (*R*)-(-)-1-(1-naphthyl)ethyl isocyanate and (*R*)-(+)- α -methylbenzylamine were the most effective reagents for the hydroxy esters and keto acids, respectively, from the point of complete resolution of the enantiomers on OV-1 capillary columns. The peak components were assigned including the absolute configuration.

Chiral 2-hydroxy- or 2-oxobicyclo[2.2.1]-heptane-7-carboxylic acid, hept-5-ene-7-carboxylic acid, and their methyl esters are potentially useful synthons for optically active cyclopentanoids such as (-)-methyl jasmonate and natural prostaglandins.¹⁾ We have reported that the chiral hydroxy compounds were produced by the stereoselective microbiological hydroxylation of the prochiral precursors, 7-carboxy- or 7-methoxycarbonylbicyclo[2.2.1]heptane (**1**) and heptene (**2**), and in some cases the chiral oxo compounds were produced by the succeeding biological oxidation.¹⁾

The problem we encountered in the screening of microorganisms was to determine the absolute configuration and enantiomeric purity of the microbial products, as simply as possible and with a small amount of the sample, for convenience in rapid screening. The following methods can be applied to small-scale determination of absolute configuration, if at least one optically active specimen of known absolute configuration is available as a standard for each compound: (1) NMR

measurements;²⁾ (2) chromatographic resolution on chiral stationary phases;^{3a,b)} and (3) chromatography on achiral stationary phases, but for diastereomeric derivatives of the sample with a chiral derivatizing reagent.^{3b,c)} The NMR methods require relatively pure samples and the chiral phases for gas and high-performance liquid chromatography are usually hard to obtain. In method 3 coupled with gas chromatography, the amount of sample required is very small (<0.1 mg), purification of the sample is usually unnecessary, and various columns or stationary phases are easily available. This method with gas chromatography was used in our previous work¹⁾ to study the stereochemistry of microbial products. In this paper, we report details of the synthesis of authentic specimens, derivatization of these specimens to diastereomers with various chiral reagents, and gas chromatographic separation of these diastereomers.

The microbial products were the stereoisomers of the hydroxy or keto acids and methyl esters (**3**~**11**). Among them, some compounds (($\pm$)-**3a**,⁴⁾ ($\pm$)-**4a,b**,^{4,5)} ($\pm$)-

5a,b,^{4,5,6)} ($\pm$)-**6b**,⁴⁾ ($\pm$)-**7a,b**,^{4,7)} (+)-**7a**,^{8a)} ($\pm$)-**8a,b**,⁴⁾ (+)-**8b**,^{8b)} and (–)-**8b**,^{8a)} and ($\pm$)-**11a**,⁹⁾ have been reported earlier, but others including the necessary key compounds, (1*R*)- and/or (1*S*)-**5** and **11**, were not found

in the literature. Thus, racemic and chiral specimens for these compounds were prepared in a stereocontrolled way from a common precursor, tricyclo keto acid **12**.

Addition of hydrobromic acid to the cyclopropane ring of **12** gave the *syn* bromo keto acid **13**, as reported by Beeley *et al.*⁶⁾ The bromide was reduced with zinc powder to yield the *anti* keto acid **5a**. The dextrorotatory tricyclo acid (+)-**12** was similarly converted to the optically active keto acid (+)-**5a**. The dextrorotatory acid (+)-**12** was the synthetic precursor for natural prostaglandins.⁹⁾ The absolute configuration of (+)-**5a** is, therefore, 1*R*, 4*R* and 7*R*. The keto acid **5a** was methylated with diazomethane to the keto ester **5b**, which was reduced with sodium borohydride in isopropyl alcohol to give a mixture of the *endo anti* hydroxy ester **3b** and *exo anti* hydroxy ester **4b** in a ratio of 9:1. The ratio was estimated by gas chromatography and the *endo* and *exo* assignment was made by comparing the retention time of the peaks with that of another stereoselectively synthesized **4b** (see below) under the same gas chromatographic conditions.

The predominancy of *endo* hydroxy configuration in the sodium borohydride reduction has also been reported for other bicyclo-[2.2.1]heptanes.¹⁰⁾ Chiral specimens for **3b** and **4b** were prepared from (1*R*)-(+)-**5a** and (1*S*)-(–)-**5a**. The latter acid was obtained from (–)-**12**.

The racemic *syn* keto acid **8a** was also synthesized from **12**. Treatment of **12** with sulfuric acid according to the method of Bindra *et al.*⁹⁾ yielded the keto lactone **14**, which was converted to the dithioketal lactone **15**. The dithioketal group was removed by reduction with Raney Ni. The resulting lactone **16** was hydrolyzed and oxidized with Jones reagent to give the *syn* keto acid **8a**. The dextrorotatory tricyclo keto acid (+)-**12** was similarly converted to the optically active *syn* keto acid (+)-**8a**, leading to the 1*R* absolute configuration of (+)-**8a**. Optically active **8a** and its methyl ester were also synthesized by Nakazaki *et al.*,⁸⁾ but by using bicyclo-

[2.2.1]hept-5-ene-2-*endo*-carboxylic acid as the starting material. Unfortunately, optical rotation for the acid has not been reported in the literature. Methylation and sodium borohydride reduction of **8a** (for both the racemic and optically active specimen) gave a mixture of the *endo syn* hydroxy ester **6b** and *exo syn* hydroxy ester **7b** in a ratio of 4:1. The minor product **7b** was identical in gas chromatography-mass spectrometry to another specimen of **7b** obtained from the hydrolyzate of **16**.

The unsaturated compounds **9**~**11** were also synthesized from the tricyclo keto acid **12**. Addition of hydroiodic acid to **12** gave the iodo keto acid **17a**,⁶⁾ which was converted to the methyl ester **17b** with diazomethane. The keto group of **17b** was protected as ethylene ketal. The iodo ketal ester **18** was treated with a base, 1,8-diazabicyclo[5.4.0]undec-7-ene, to afford the unsaturated ester ketal **19**. The protective groups in **19** were removed with an acid and base, respectively. The product, unsaturated keto acid **11a**, was purified by crystallization. Then, the acid was again methylated and reduced with sodium borohydride to give a mixture of the *endo* hydroxy ester **9b** and *exo* hydroxy ester **10b** in a ratio of 92:8. In the same way, the dextrorotatory tricyclo keto acid (+)-**12** was converted to the optically active unsaturated keto acid (–)-**11a**. This levorotatory acid (–)-**11a** was assigned 1*R* as the absolute configuration for the same reason as mentioned for (1*R*)-(+)-**5a**. Methylation and sodium borohydride reduction of (1*R*)-(–)-**11a** gave a mixture of (1*R*)-**9b** and (1*R*)-**10b**.

The *exo* hydroxy configuration of the minor product (**10b**) in the borohydride reduction of **11b** was confirmed by the fact that retention time and mass spectrum for the specimen of **10b** in gas chromatography-mass spectrometry were identical to those for another specimen of **10b**, which was obtained by the *exo*-selective hydroboration of a 7-substituted diene ester (**20**) with 9-borabicyclo[3.3.1]nonane. The reagent has been reported to form predominantly an *exo anti* alcohol from a 7-sub-

stituted norbornadiene.¹¹⁾ Catalytic hydrogenation of **10b** gave **4b**.

Gas chromatographic enantiomer separation of chiral alcohols and acids as their diastereomeric derivatives is an important analytical method for determining the absolute configuration and enantiomeric (optical) purity of the chiral compounds.^{3a,c)} The method has been widely studied for many kinds of substrates using various chiral derivatizing reagents such as (–)-menthyl chloroformate (**21**),¹²⁾ *N*-trifluoroacetyl-(*S*)-prolyl chloride (**22**),¹³⁾ (*R*)-(+)- α -methoxy- α -trifluoromethylphenylacetic acid chloride (**23**),¹⁴⁾ (*R*)-(+)- α -methylbenzyl isocyanate (**24**),¹⁵⁾ and (*R*)-(+)-*trans*-chrysanthemoyl chloride,^{13,16,17)} for alcohols; and chiral alcohols (e.g., (–)-menthol¹⁸⁾) and chiral amines (e.g., amino acid derivatives^{3c)}) and (*R*)-(+)- α -methylbenzylamine (**25**)¹⁹⁾ for acids. Earlier studies on the substrates of bicyclo[2.2.1]heptane skeleton have been done with trimethyl derivatives such as borneol,¹⁶⁾ isoborneol,¹⁶⁾ camphor²⁰⁾ and fenchol.¹⁶⁾ No work has been reported on a simpler unsubstituted or only monosubstituted norbornane derivative. Thus six species of diastereomeric deriva-

tives (**29**, **30**, **31**, **32**, **33** and **34**) were prepared by treating the present *anti* hydroxy

esters (as the mixture of **3b** and **4b**) with commercially available reagents **21**, **22**, **23**, (–)-camphanic acid chloride (**26**), **24** and (R)-(–)-1-(1-naphthyl)ethyl isocyanate (**27**), respectively. Also two species of derivatives (**35** and **36**) of the keto acid **5a** were prepared using (R)-(+)-1-phenylethanol (**28**) and the chiral amine **25**, respectively.

Most of the diastereomeric derivatives of **3b**+**4b** were separated on a capillary column (OV-1, 25 m), usually showing two pairs of peaks in each chromatogram (Fig. 1). It is reasonable to consider that the two (or one) smaller peak(s) of shorter retention time (35~55 min) represent the diastereomers of the minor component, *exo* alcohol **4b**, and the two larger peaks of longer retention time represent those of the major component, *endo* alcohol **3b**. Gas chromatography-mass spectrometry using the same column as above showed that all the larger peaks and some of the smaller peaks yielded the molecular ion and/or the ion of $M^+ - 31$ (OMe) expected for each derivative. The chromatograms in Fig. 1 were obtained in the conditions where the diastereomeric peaks were separated as far as possible within a practical retention time (<60 min). Thus, as is seen from Fig. 1, the gas chromatographic enantiomer separation of the *exo* alcohol **4b** was easily achieved by the derivatization with the chiral reagents except for **23** (Fig. 1c). On the other hand, resolution of the *endo* alcohol **3b** was satisfactorily achieved only when derivatized with **27** (Fig. 1f).

Gas chromatographic separation of all derivatives was further tested on more polar stationary phases (OV-1701 and BP-20). A substantial improvement was found for the derivative with **26** with an OV-1701 capillary column,*¹ where the diastereomers of the *endo* *anti* alcohol **3b** were completely separated. However, when diastereomeric derivatives of the *syn* alcohols (**6b**+**7b**) with **26** were sub-

FIG. 1. Gas Chromatograms for the Diastereomeric Derivatives of the *anti* Hydroxy Esters (**3b**+**4b**) with (–)-Menthyl Chloroformate (a), *N*-Trifluoroacetyl-(*S*)-prolyl Chloride (b), (R)-(+)- α -Methoxy- α -trifluoromethyl-phenylacetic Acid Chloride (c), (–)-Camphanic Acid Chloride (d), (R)-(+)- α -Methylbenzyl Isocyanate (e) and (R)-(–)-1-(1-Naphthyl)ethyl Isocyanate (f).

Chromatographic conditions: column, OV-1 capillary (25 m); inlet pressure and carrier flow rate, 1.25 kg/cm² and 75 ml/min; column temperature (°C) and flow rate in column (ml/min), 180 and 1.0 (a), 160 and 1.2 (b), 170 and 1.1 (c), 190 and 1.0 (d and e), and 230 and 0.83 (f). Relative retention time of the *exo* and *endo* diastereomers: a, 1.052 and 1.009; b, 1.068 and 1.009; c, 1.0 and 1.013; d, 1.041 and 1.017; e, 1.025 and 1.015; f, 1.032 and 1.022.

jected to this column, one peak (for one diastereomer of **6b**) was observed to overlap with the peak for one diastereomer of **4b**. This overlap could not be avoided either by changing the column temperature or by using a BP-20 column. Such an overlap did not occur in the chromatography for the derivatives with **27** on the OV-1 column.*² Therefore, we decided to use the chiral isocyanate **27** for derivatizing the present hydroxy esters for the gas chromatographic determination of the ab-

*¹ Data of retention time are given in EXPERIMENTAL. Chromatograms are available on request.

*² Peaks for the diastereomeric urethanes of (1*R*)-**7b** and (1*S*)-**4b** overlapped each other on the column (OV-1, 50 m) used in the screening work, but the overlap was not a serious problem.¹⁾

FIG. 2. Gas Chromatograms for the Diastereomeric Derivatives of the Racemic and Enantiomerically Pure Hydroxy Esters with (*R*)-(-)-1-(1-Naphthyl)ethyl Isocyanate.

Samples (hydroxy esters): a, ($\pm$)-**3b** + ($\pm$)-**4b**; b, ($\pm$)-**4b**; c, (*1R*)-**3b** + (*1R*)-**4b**; d, (*1S*)-**3b** + (*1S*)-**4b**. Chromatographic conditions: column, OV-1 capillary (50 m); inlet pressure and carrier flow rate, 1.75 kg/cm² and 105 ml/min; column temperature and flow rate in column, 250°C and 0.54 ml/min. Peak assignments, see the text.

solute stereochemistry.

Figure 2 shows the gas chromatograms with a 50-m OV-1 capillary column for the diastereomeric (*R*)-1-(1-naphthyl)ethyl urethanes of the racemic and enantiomerically pure *anti* hydroxy esters. Comparison of Fig. 2a (for the diastereomers of racemic **3b** and **4b**) with Fig. 2b (for the diastereomers of racemic **4b** prepared by hydroboration) confirms that the pair of smaller peaks of equal height (numbered 1 and 2) represents the diastereomers of the *exo* alcohol **4b** and consequently that the pair of larger peaks of equal height (numbered 3 and 4) represents those of the *endo* alcohol **3b**. The absolute configuration of each peak component was determined by comparing Fig. 2a with Figs. 2c and 2d, which are the chromatograms for the diastereomers of the *1R* and *1S* specimens, respectively. Thus, the peaks 1, 2, 3 and 4 represent the (*1S*)-

anti exo, (*1R*)-*anti-exo*, (*1R*)-*anti endo* and (*1S*)-*anti endo* isomers, respectively. Diastereomeric derivatives of the *syn* hydroxy esters (**6b** and **7b**) with **27** and those of the unsaturated hydroxy esters (**9b** and **10b**) with the same reagent also exhibited good separation between the members of the diastereomeric pair by gas chromatography (chromatograms not shown).^{*1} The peaks were assigned as described in EXPERIMENTAL, by comparing the chromatograms with those for the corresponding pure *exo* and pure *1R* specimens. The reagent, (*R*)-(-)-1-(1-naphthyl)ethyl isocyanate (**27**), was developed for resolution by high-performance liquid chromatography.^{3b)} This paper is the first to report application of this reagent to gas chromatography.

Diastereomeric esters of the keto acid **5a** with **28** were little separated by gas chromatography with the 25-m capillary column, but amides with (*R*)-(+)- α -methylbenzylamine (**25**) were well resolved.^{*1} Diastereomeric amides of the *syn* keto acid **8a** and the unsaturated keto acid **11a** with this chiral amine were also completely resolved by gas chromatography.^{*1} The absolute configuration of each peak component was determined using the amides of the optically active specimens.

In conclusion, the method has been established to determine the absolute stereochemistry of secondary alcohols and ketones with a 7-carboxybicyclo[2.2.1]heptane skeleton by gas chromatography. This method (for alcohols) is being applied in our screening,¹⁾ where crude extracts from fermentation broth containing 0.1~0.2 mg of the hydroxylated products are generally enough to be tested for the absolute configuration and enantiomeric purity of the products and all the procedures including gas chromatography can be completed within 24 hr. The present work will also provide useful information for the method of enantiomer separation by gas chromatography.

EXPERIMENTAL

Materials. The following reagents were purchased from commercial suppliers: 9-borabicyclo[3.3.1]nonane, (*S*)-(-)- and (*R*)-(+)- α -methylbenzylamine, (*R*)-(+)- α -methylbenzyl isocyanate, and (*R*)-(-)-1-(1-naphthyl)ethyl isocyanate, from Aldrich Chemical Co. (Milwaukee); (-)-camphanic acid chloride, from Fluka AG, Chemische Fabrik (Buchs, Switzerland); (-)-menthyl chloroformate/toluene and *N*-trifluoroacetyl-(*S*)-prolyl chloride/ CHCl_3 , from Regis Chemical Co. (Chicago); (*R*)-(+)- α -methoxy- α -trifluoromethylphenylacetic acid and TLC glass plates coated with silica gel F₂₅₄ (0.25 mm thick), from Merck Japan Ltd. (Tokyo); 1,8-diazabicyclo-[5.4.0]undec-7-ene and silica gel (Wako gel C-200) for column chromatography, from Wako Pure Chemical Co. (Tokyo); 7-chloronorbornadiene, from Frinon Laboratories (Vineland, U.S.A.); and (*R*)-(+)-1-phenylethanol, from Tokyo Chemical Industry Co., Ltd. (Tokyo). All other reagents were of the best grade commercially available.

Analysis. Melting points were measured in sealed capillaries on a hot stage and are uncorrected. Optical rotations were measured with a Union Giken PM-101 automatic polarimeter or an Atago Polax polarimeter. IR, PMR and MS spectra were taken with a Jasco IRA-1 infrared spectrometer, a JEOL GX-270 NMR spectrometer and a JEOL JMS-D300 mass spectrometer equipped with a gas chromatograph, respectively. Gas chromatography (GC) was carried out with Shimadzu GC-7A, GC-R1A and GC-9A gas chromatographs equipped with flame ionization detectors and data processors. When capillary columns were used, an inlet splitter (Gasukuro Kogyo) was attached to the chromatograph. Packing materials and capillary columns were obtained from Gasukuro Kogyo Inc. (Tokyo). Chromatographic conditions for packed columns were as follows: condition 1—with a glass column (3 mm i.d. $\times$ 1.5 m) packed with 5% SILAR-SCP on Chromosorb W AW (80–100 mesh) at a carrier (N_2) flow rate of 60 ml/min and a column temperature of 160°C; condition 2—similar to condition 1, except for the column diameter (2 mm i.d.), carrier (He) flow rate (15 ml/min) and column temperature (145°C); condition 3—with a glass column (2 mm i.d. $\times$ 1.5 m) packed with 5% SP-1000 on the same support as already mentioned at a He carrier flow rate of 18 ml/min and a column temperature of 170°C. Conditions for GC with capillary columns (silica capillaries with chemically bonded stationary phases of OV-1 (0.25 mm i.d. $\times$ 25 and 50 m), OV-1701 (0.25 mm i.d. $\times$ 25 m) and BP-20 (0.22 mm i.d. $\times$ 25 m)) are given in the latter section and the legends to Figs. 1 and 2. Helium was used as the carrier gas for all capillary columns. In the following sections, t_R represents retention time (min). Gas chromatography-mass spectrometry (GC-MS) was carried

out under GC condition 2, unless otherwise stated.

Synthesis

5-Oxotricyclo[2.2.1.0^{2,6}]heptane-3-carboxylic acid (12). The racemic acid ($\pm$)-**12** was prepared from norbornadiene according to Beeley *et al.*,⁶⁾ mp 143–144°C (lit.⁶⁾ mp 143–146°C). The racemic acid ($\pm$)-**12** was resolved by fractional crystallization of the salts with (-)- or (+)- α -methylbenzylamine to give the dextrorotatory acid (+)-**12** (mp 142–143°C, $[\alpha]_D^{25} + 91.7^\circ$ ($c = 1.0$, dioxane); lit.²¹⁾ mp 142.5–143.5°C, $[\alpha]_D^{25} + 81.8^\circ$ ($c = 1$, dioxane)) and levorotatory acid (-)-**12** (mp 140–142.5°C, $[\alpha]_D^{25} - 86.0^\circ$ ($c = 1.0$, dioxane). *Anal.* Found: C, 63.12; H, 5.31. Calcd. for $\text{C}_8\text{H}_8\text{O}_3$: C, 63.15; H, 5.30%.

2-Oxobicyclo[2.2.1]heptane-7-anti-carboxylic acid (5a) was prepared from **12** by the method of Beeley *et al.*,⁶⁾ in 93% yield, mp 103–105°C (lit.⁶⁾ mp 104–106°C). The optically active acids (+)-**5a** and (-)-**5a** were similarly prepared from (+)-**12** and (-)-**12**, respectively. (+)-**5a**: yield, 45%; mp 141–142.5°C, $[\alpha]_D^{25} + 18^\circ$ ($c = 0.87$, MeOH). *Anal.* Found: C, 62.30; H, 6.56. Calcd. for $\text{C}_8\text{H}_{10}\text{O}_3$: C, 62.33; H, 6.54%. (-)-**5a**: yield, 62%; mp 140–142°C, $[\alpha]_D^{25} - 18^\circ$ ($c = 0.83$, MeOH). *Anal.* Found: C, 62.38; H, 6.50. Calcd. for $\text{C}_8\text{H}_{10}\text{O}_3$: C, 62.33; H, 6.54%.

2-Endo-hydroxy- (3b) and 2-exo-hydroxybicyclo[2.2.1]heptane-7-anti-carboxylic acid methyl ester (4b). One milligram of **5a** was methylated with an excess amount of ethereal diazomethane. The solvent was changed to 0.1 ml isopropyl alcohol. To the solution was added about 1 mg of sodium borohydride. The mixture was allowed to stand at room temperature for 1 hr and then diluted with water (0.1 ml). After acidification with 2N HCl and saturation with NaCl, the product was extracted with ether (0.5 ml $\times$ 2). The extracts were worked up in the usual way*³ and then dissolved in 0.1 ml EtOAc. GC analysis (condition 1) for the EtOAc solution showed that the starting material ($t_R = 4.73$ for **5b**) had been completely converted to two hydroxy esters **3b** ($t_R = 6.70$) and **4b** ($t_R = 6.00$) in a ratio of 9:1. GC-MS m/z : 170 (M^+) for **3b** ($t_R = 18.2$) and m/z : 170 (M^+) for **4b** ($t_R = 16.0$). In the same way, (1*R*)-(+)-**5a** and (1*S*)-(-)-**5a** were converted to a mixture of (1*R*)-**3b** and (1*R*)-**4b** and a mixture of (1*S*)-**3b** and (1*S*)-**4b**, respectively.

Keto lactone 14 was prepared from **12** by the method of Bindra *et al.*,⁹⁾ in 46% yield, mp 193–194°C (lit.⁹⁾ mp 195–196°C). Similarly, (+)-**14** was obtained from (+)-**12** in 45% yield, mp 195–196°C, $[\alpha]_D^{24} + 231^\circ$ ($c = 0.31$, MeOH) (lit.⁹⁾ mp 195–197°C, $[\alpha]_D^{24} + 266^\circ$ ($c = 1$, MeOH)), MS m/z : 152 (M^+).

Dithioketal lactone (15). The keto lactone ($\pm$)-**14** (180 mg) was mixed with 0.5 ml of ethanedithiol and 0.5 ml of 47% BF_3 etherate. The mixture was allowed to stand at room temperature for 30 min. After addition of 10 ml MeOH, the mixture was concentrated by an evaporator to give a syrup, which was crystallized from MeOH as plates

*³ Washed with saturated brine, dried over Na_2SO_4 , and concentrated under vacuum or evaporated to dryness.

(70 mg). A remaining portion of the product in the mother liquor was isolated by column chromatography (silica gel, 1.5 × 15 cm, EtOAc/benzene). Fraction of 5% EtOAc/benzene (100 ml) was evaporated to dryness. The residue was crystallized from MeOH as plates (76 mg). The two crops were the same compound (**15**, 54% total yield) as evidenced by mp and IR; mp 165~166°C. *Anal.* Found: C, 52.61; H, 5.30; S, 28.06. Calcd. for $C_{10}H_{12}O_2S_2$: C, 52.61; H, 5.30; S, 28.08%.

2-Oxobicyclo[2.2.1]heptane-7-syn-carboxylic acid (8a). The dithioketal lactone **15** (140 mg) was mixed with a freshly prepared Raney Ni suspension (W-4, 3 g of wet weight in 6 ml EtOH). The mixture was refluxed for 30 min with stirring, and the catalyst was filtered off. The filtrate was concentrated by an evaporator, and the residual syrup (crude lactone **16**; GC-MS ($t_R=21.1$) m/z : 138 (M^+)) was dissolved in a mixture of 0.6 ml MeOH and 0.3 ml of 6 N NaOH. The solution was kept at 60°C for 1 hr and then diluted with 1 ml water. After acidification with 6 N HCl and saturation with NaCl, the aqueous solution was extracted with EtOAc (7 ml × 3). The combined organic phase was washed with 1 ml of saturated brine and then evaporated to dryness. The residue (crude *syn* hydroxy acid **7a**; GC-MS for the methyl ester ($t_R=11.0$) m/z : 170 (M^+)) was dissolved in 2 ml acetone and mixed with 0.3 ml of Jones reagent under cooling with ice. After 30 min, 0.6 ml of isopropyl alcohol and 1 ml water were added to the reaction mixture. The green solution was saturated with NaCl and then extracted with EtOAc (5 ml × 3). The extracts were worked up in the usual way to give a syrup, which was purified by silica gel column chromatography (EtOAc/benzene). The product ($\pm$)-**8a** was crystallized from benzene/hexane as leaflets (48 mg, 51% yield), mp 117~119°C (lit.⁴⁾ mp 122~123°C). *Anal.* Found: C, 62.34; H, 6.52. Calcd. for $C_8H_{10}O_3$: C, 62.33; H, 6.54%. IR ν_{max}^{KBr} cm^{-1} : 2800~2300, 1740 (C=O), and 1690 (COOH). In the same way, (1*R*)-(+)-**8a** was synthesized from (+)-**14** via dithioketal lactone (mp 157~158°C, $[\alpha]_D^{25} +60.9^\circ$ ($c=0.63$, MeOH)). *Anal.* Found: C, 52.57; H, 5.28; S, 28.19. Calcd. for $C_{10}H_{12}O_2S_2$: C, 52.61; H, 5.30; S, 28.08% in 41% yield as needles, mp 82~83°C, $[\alpha]_D^{24} +9.8^\circ$ ($c=0.39$, MeOH). *Anal.* Found: C, 62.20; H, 6.59. Calcd. for $C_8H_{10}O_3$: C, 62.33; H, 6.54%. MS m/z : 154 (M^+), 126, 108, 81, 80, 67, and 66. IR ν_{max}^{KBr} cm^{-1} : 3400, 2970, 2800~2300, 1740 (C=O), 1710 (COOH), 1420, 1300, 1220, 1200, 1150, 1090, and 710. PMR ($CDCl_3$) δ : 1.57 (2H, m), 1.83~2.05 (3H, complex m), 2.41 (1H, m), 2.80 (2H, m), 2.92 (1H, m), and 8.70 (1H, br.s, COOH).

2-Endo-hydroxy- (6b) and 2-exo-hydroxybicyclo[2.2.1]heptane-7-syn-carboxylic acid methyl ester (7b) were prepared as a mixture (**6b**:**7b**=4:1) from both ($\pm$)-**8a** and (1*R*)-(+)-**8a** in the same way as described for **5a**. GC-MS m/z : 170 (M^+) for **6b** ($t_R=16.2$) and m/z : 170 (M^+) for **7b** ($t_R=11.3$).

2-Iodo-5-oxobicyclo[2.2.1]heptane-7-syn-carboxylic acid (17a) was synthesized from **12** by the method of Beeley *et al.*,⁶ in 76% yield, dec 204~205°C (lit.⁶) mp 227~228°C),

MS m/z : 280 (M^+). The acid **17a** was methylated with ethereal diazomethane and the product **17b** was crystallized from EtOH as rods (90% yield), mp 105~106°C. *Anal.* Found: C, 36.59; H, 3.72; I, 43.33. Calcd. for $C_9H_{11}O_3I$: C, 36.76; H, 3.77; I, 43.15%.

Iodo ketal ester (18). To a solution of 1.85 g of the iodo keto ester **17b** in 14.5 ml benzene were added 3.5 g of freshly prepared 2-methyl-2-ethyl-1,3-dioxolane²²⁾ and 0.27 g of tosic acid monohydrate. The mixture was stirred at room temperature overnight. Then, the mixture was diluted with 10 ml benzene and successively washed with 10% $NaHCO_3$ (15 and 5 ml). The organic phase was worked up in the usual way and the product **18** was crystallized from ether/pentane as leaflets (1.93 g, 91% yield), mp 78~79°C. *Anal.* Found: C, 38.93; H, 4.48; I, 37.59. Calcd. for $C_{11}H_{15}O_4I$: C, 39.07; H, 4.47; I, 37.53%.

2-Oxobicyclo[2.2.1]hept-5-ene-7-anti-carboxylic acid (11a). The iodo ketal ester **18** (1.2 g) was dissolved in a mixture of 12 ml toluene and 5.5 g of 1,8-diazabicyclo[5.4.0]undec-7-ene. The mixture was refluxed for 9 hr. After dilution with 50 ml benzene, the reaction mixture was successively washed with 10% citric acid (50 and 10 ml) and saturated brine (10 and 5 ml). The organic phase was evaporated to dryness. The residue (crude unsaturated ketal ester **19** (GC-MS ($t_R=10.8$, in GC condition 2, but column temperature=160°C) m/z : 210 (M^+)) was mixed with 10 ml of 30% AcOH/water and heated to 95°C for 2 hr. The acetic acid and water were removed under vacuum as an azeotropic mixture with toluene and EtOH, respectively. The residual liquid was chromatographed on a silica gel column (2 × 15 cm), eluting with 200-ml portions of 0~20% EtOAc/benzene mixtures. The eluate was divided into 20-ml fractions, and monitored by TLC for the product. Fractions containing the pure product (10% EtOAc/benzene) were combined and concentrated to give a syrup (unsaturated keto ester **11b**, GC-MS ($t_R=13.0$) m/z : 166 (M^+)), which was hydrolyzed in a mixture of 2 ml MeOH and 1 ml of 6 N NaOH at 40°C for 30 min. The solution was diluted with water (3 ml), acidified with 6 N HCl, saturated with NaCl, and extracted with EtOAc (20 ml × 3). The extracts were worked up in the usual way and the product ($\pm$)-**11a** was crystallized from benzene/hexane as leaflets (0.36 g, 67% yield from **18**), mp 126~127°C (lit.⁹) mp 120~122°C). *Anal.* Found: C, 63.12; H, 5.30. Calcd. for $C_8H_8O_3$: C, 63.15; H, 5.30%. IR ν_{max}^{KBr} cm^{-1} : 2600~2300, 1730 (C=O), and 1700 (COOH). PMR ($CDCl_3$) δ : 6.13 (1H, m) and 6.54 (1H, m).

(1*R*)-(-)-**2-Oxobicyclo[2.2.1]hept-5-ene-7-anti-carboxylic acid** ((1*R*)-(-)-**11a**) was synthesized from (+)-**12** (0.25 g) by the same sequential procedures already described. The intermediates (**17a**, **17b** and **18**) were not crystallized in this case, but checked by TLC with reference to the corresponding racemic specimen (*Rf* 0.49, 0.55, 0.35 and 0.39 for **17b**, **18**, **19** and **11b**, respectively, on silica gel F₂₅₄ developed with EtOAc/benzene (1:9)). The final product was crystallized from benzene/hexane as

leaflets (82 mg, 33% yield), mp 152~153°C, $[\alpha]_D^{25} -644^\circ$ ($c=0.30$, MeOH). *Anal.* Found: C, 63.41; H, 5.29. Calcd. for $C_8H_8O_3$: C, 63.15; H, 5.30%. *MS* m/z : 152 (M^+), 124, 110, 82, 79, and 66. *IR* $\nu_{\max}^{KBr}$ cm^{-1} : 3420, 3100, 3020, 2700~2300, 1730 (C=O), 1700 (COOH), 1410, 1320, 1250, 1220, 1120, 1080, 860, 780, 740, and 700. *PMR* ($CDCl_3$) δ : 2.09 (2H, m), 3.16 (1H, br.s), 3.39 (1H, m), 3.55 (1H, m), 6.13 (1H, m), 6.54 (1H, m), and 8.50 (1H, br.s, COOH).

2-Endo-hydroxy- (9b) and 2-exo-hydroxybicyclo[2.2.1]hept-5-ene-7-anti-carboxylic acid methyl ester (10b) were prepared as a mixture (**9b**:**10b**=92:8) from both ($\pm$)-**11a** and (1*R*)-(-)-**11a** in the same way as described for **5a**. GC-MS m/z : 168 (M^+) for **9b** ($t_R=11.8$ in GC condition 3) and m/z : 168 (M^+) for **10b** ($t_R=14.0$).

2-Exo-hydroxy esters 10b and 4b from 7-methoxycarbonylbicyclo[2.2.1]hepta-2,5-diene (20). Bicyclo[2.2.1]hepta-2,5-diene-7-carboxylic acid (mp 79~81°C, *MS* m/z : 136 (M^+); lit.²³) mp 84.5~86°C) was synthesized by the carboxylation of 7-chloronorbornadiene according to Stapersma *et al.*²³ The acid was methylated with diazomethane. The diene ester **20** (100 mg) was dissolved under N_2 in 1.4 ml dry tetrahydrofuran containing 90 mg 9-borabicyclo[3.3.1]nonane. After stirring at room temperature for 20 min, 0.32 ml of 6*N* NaOH and 0.30 ml of 30% H_2O_2 were added to the solution. The mixture was refluxed for 25 min. Then, the reaction mixture was diluted with water (3 ml), acidified with 6*N* HCl, saturated with NaCl, and extracted with ether (7 ml $\times$ 3). The organic phases were combined and mixed with an excess amount of ethereal diazomethane. The mixture was allowed to stand at room temperature for several hours and then evaporated to dryness. The residue was purified by silica gel column chromatography (EtOAc/benzene) and finally **10b** was obtained as a liquid (52 mg, 46% yield), GC-MS ($t_R=14.2$ in GC condition 3) m/z : 168 (M^+). This sample of **10b** (25 mg) was dissolved in 1.5 ml MeOH and hydrogenated over 10% Pd/C (10 mg) at atmospheric pressure for 2 hr. The catalyst was filtered off. Concentration of the filtrate gave **4b** as a liquid in an almost quantitative yield, GC-MS ($t_R=16.0$) m/z : 170 (M^+).

Preparation of diastereomeric derivatives and GC

Derivatives of the anti hydroxy esters (3b+4b). Two hundred and fifty milligrams of **5a** were converted to a mixture of **3b** and **4b** in an analogous manner to that already described, but using MeOH as the solvent in the borohydride reduction. The product (**3b**:**4b**=97:3) was dissolved in 2.5 ml toluene. An equal portion of the solution (0.5 ml) was added to the following mixtures: (a) 3 ml toluene containing 0.3 mmol **21** and 100 μ l pyridine; (b) 3 ml $CHCl_3$ containing 0.3 mmol **22** and 100 μ l pyridine; (c) 0.5 ml toluene containing an acid chloride freshly prepared from 200 mg **23** and 100 μ l pyridine; (d) 0.5 ml toluene containing 200 mg **26** and 100 μ l pyridine; (e) 10 mg *N,N*-dimethylethanolamine and 100 μ l **24**. The

mixtures of (c) and (d) were heated to 60°C for 1 hr, and the mixtures of (b) and (e) were heated to 90°C for 0.5 and 14 hr, respectively. The mixture of (a) was heated to 90°C for 0.5 hr and then stirred at room temperature overnight. Each mixture was diluted with EtOAc (15 ml) and successively washed with 5 ml of water, 5% $NaHCO_3$, 10% citric acid, and saturated brine. The EtOAc layers were dried over Na_2SO_4 , concentrated to approximately 1 ml, and subjected to GC. Derivatives of the *syn* hydroxy esters (**6b+7b**) with **26** were similarly prepared, but in a small scale and without the washing procedures.

Derivatives with the isocyanate 27. An EtOAc solution containing about 0.5 mg of the sample (one of the racemic and/or enantiomerically pure specimens of **3b+4b**, **4b**, **6b+7b**, **7b**, **9b+10b**, and **10b**) was placed in an ampoule and the solvent was removed under vacuum. To the residue was added a mixture of 17.8 μ l toluene, 0.2 μ l *N,N*-dimethylethanolamine (as a catalyst),²⁴ and 2 μ l **27**. The ampoule was sealed and heated to 95°C for 12 hr. The reaction mixture was diluted with 30~100 μ l MeOH before GC analysis. The reaction rate was more than 95% in the case of **3b+4b** as evidenced by GC for the unreacted substrates.

Derivatives of the keto acids. A portion of ($\pm$)-**5a** (about 0.5 mg) was mixed with 50 μ l thionyl chloride. The mixture was allowed to stand at room temperature for 1 hr. The excess thionyl chloride was removed under vacuum, and a trace amount of the reagent was purged with toluene. The residue was mixed with 20 μ l toluene, 2 μ l pyridine and 1 μ l **25**, and kept at room temperature for over 1 hr. This sample was adequately diluted with MeOH before GC. A similar procedure was repeated, but using (+)-**5a** and (-)-**5a**, ($\pm$)-**8a** and (+)-**8a**, and ($\pm$)-**11a** and (-)-**11a**. Also a reaction was carried out with **5a** and 2 μ l **28** instead of **25**. The reaction rate in the case of **5a** with **25** was more than 80% in the above coupling condition, as evidenced by GC for the remaining **5a** (after methylation with diazomethane).

GC on OV-1701 and BP-20 for the derivatives of 3b+4b (a) and 6b+7b (b) with 26. Retention times (min) on OV-1701 capillary column: a—42.20, 44.13, 49.25 and 50.42; b—34.78, 36.25, 44.54 and 45.60; conditions: inlet pressure, 1.25 kg/cm²; carrier flow rate at inlet, 70 ml/min; column temperature, 220°C; and flow rate in column, 0.83 ml/min. Retention times (min) on BP-20 capillary column: a—28.98, 30.90, 35.52 and 37.14; b—22.20, 23.70, 30.60 and 32.04; conditions: inlet pressure, 1.8 kg/cm²; carrier flow rate at inlet, 70 ml/min; column temperature, 200°C; and flow rate in column, 1.0 ml/min.

GC for the derivatives of 6b+7b with 27 on OV-1. The same conditions as those in Fig. 1f were used. Retention times (min): 33.28, 35.33, 40.68 and 41.20 (37.74, 38.89, 44.39 and 45.36 for the peaks in Fig. 1f).

GC for the diastereomeric urethanes of the hydroxy esters with 27 on the 50-m OV-1 capillary column. Chromatographic conditions were the same as those in Fig. 2. Retention time (min) and assignment (as hydroxy ester):

51.35, (1*S*)-**4b**; 52.68, (1*R*)-**4b**; 59.22, (1*R*)-**3b**; 60.30, (1*S*)-**3b**; 47.79, (1*S*)-**7b**; 50.13, (1*R*)-**7b**; 56.13, (1*R*)-**6b**; 56.79, (1*S*)-**6b**; 45.90, (1*S*)-**10b**; 47.07, (1*R*)-**10b**; 53.49, (1*R*)-**9b**; 54.32, (1*S*)-**9b**.

GC for the diastereomeric amides of the keto acids with **25**. Conditions: OV-1 capillary (25 m); inlet pressure and carrier flow rate, 1.25 kg/cm² and 75 ml/min; column temperature and flow rate in column, 180°C and 1.0 ml/min. Retention time (min) and assignment (as keto acid): 28.54, (1*R*)-**5a**; 29.46, (1*S*)-**5a**; 30.60, (1*S*)-**8a**; 32.52, (1*R*)-**8a**; 25.15, (1*R*)-**11a**; 26.13, (1*S*)-**11a**.

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