The Synthesis of (+)-Marmelo Lactones A and B and the Assignment of the Absolute Configurations of Marmelo Lactones

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(+)-Marmelo Lactones **A** (**VA**) and **B** (**VB**) were synthesized from *erythro*- γ -methyl-L-glutamic acid (**IA**) and *threo* acid (**IB**), respectively. The absolute configurations of natural marmelo lactones were thus determined to be (2*R*, 4*S*) for (+)-marmelo lactone **A** and (2*R*, 4*R*) for (–)-marmelo lactone **B**.

In 1980 Tsuneya *et al.* isolated two novel terpene lactones from the fruit of a quince (*Cydonia oblonga* MILL, marmelo) as the main flavor components of the fruit.¹⁾ They were identified as 2,4-*cis* or *trans* stereoisomers of 2,7-dimethyl-5*E*, 7-octadien-4-olide and were named (+)-marmelo lactone **A** ($[\alpha]_D = +103^\circ$) and (–)-marmelo lactone **B** ($[\alpha]_D = -20^\circ$).¹⁾ The absolute configurations of the chiral centers at C2 and C4 were first proposed to be (2*R*, 4*R*) for (+)-**A** and (2*R*, 4*S*) for (–)-**B** on the basis of NMR and circular dichroism (CD).²⁾ However, the stereochemistry has not previously been synthetically established.

In order to confirm their configurations, we attempted to derive the two optically active marmelo lactones from compounds with known configurations. In our previous com-

munication,³⁾ we outlined the synthesis and revised the absolute configurations as (+)-lactone **A** having a (2*R*, 4*S*) configuration and (–)-lactone **B** having a (2*R*, 4*R*) configuration. In this report, we describe the results in detail with the experiments.

The synthetic routes to (4*S*)-marmelo lactones are depicted in Figs. 2 and 3. The stereochemistries of the key starting materials, *erythro*- γ -methyl-L-glutamic acid (**IA**) and *threo* acid (**IB**) have already been established

FIG. 1.

FIG. 2. Enzymatic Resolution of γ -Methyl-D,L-glutamic Acid.

since **IA** is known to occur in plants.^{4~7)} There have been some reports for the preparation of the acids:^{5~7)} they have been derived from the naturally occurring γ -methylene-L-glutamic acid⁵⁾ and then from the synthetic mixture of D,L- γ -methyl glutamic acid *via* the enzymatic resolution of the carbobenzoxy amide with papain.⁷⁾ In this report, we developed the enzymatic resolution with papain for the preparative purpose. The procedure was almost the same as the resolution of *N*-carbobenzoxy-D,L-glutamic acid.⁸⁾ The oily deposits of *N*-carbobenzoxy- γ -methyl-L-glutamic acid anilide ($[\alpha]_D^{23} = -15^\circ$) was separated from the D-acid and then deprotected to give a mixture of **IA** and **IB** as a white powder. ($[\alpha]_D^{23} = +27^\circ$, mp=167°C). An anion exchange column chromatography gave an *erythro* rich fraction (**IA**+ca. 20% **IB**, $[\alpha]_D^{23} +25.1^\circ$ in 6N HCl) and a *threo* rich fraction (**IB**+ca. 10% **IA**, $[\alpha]_D^{23} +43.5^\circ$ in 6N HCl). The minor

diastereomers were estimated roughly by the ^{13}C -NMR signal of the γ -methyl group in D_2O : **IA**=20.2 ppm and **IB**=20.0 ppm. The assignment of these fractions as *erythro* and *threo* was carried out on the basis of their $[\alpha]_D$ values and the order of elution, since the *threo* acid has been reported as more dextrotatory⁶⁾ and faster eluting than the *erythro* acid.⁵⁾ The *erythro* rich fraction (**IA**) and *threo* rich one (**IB**) were used as the starting materials without further purification, since (a) all the reactions were known to proceed with retention of the configurations,¹²⁾ (b) the minor diastereomer could be checked by NMR and/or HPLC during the reaction steps, (c) the two diastereomers were expected to react in almost the same yield, and (d) the isolation of the final compounds **VA** and **VB** was possible by the chromatography.

Treatment of **IA** with NaNO_2 and H_2SO_4 gave the lactone (**IIA**) as a waxy solid in a

FIG. 3. Preparation of (+)-Marmelo Lactone A (**VA**), and (+)-Marmelo Lactone B (**VB**).

FIG. 4. HPLC Pattern of the Mixture of Four Isomers of Marmelo Lactones Obtained from the **IA** Rich Compound.

Column, porasil (SS05), 4.5 mm ϕ $\times$ 250 mm; solvent, isooctane–isopropyl alcohol = 40:1; flow rate, 2.5 ml/min; chart speed, 10 nm/min; UV detector at 230 nm. (a) 5Z Marmelo Lactone **B**; (b) 5Z Marmelo Lactone **A**; (c) Marmelo Lactone **A** (**VA**); (d) Marmelo Lactone **B** (**VB**).

70~80% yield.^{9,10} The acid chloride (**IIIA**), which was quantitatively obtained as a syrup by treating **IIA** with SOCl_2 under reflux,¹¹ was converted to the aldehyde (**IVA**) by Rosenmund reduction.^{12~14} Finally, a Wittig reaction of **IVA** with triphenylphosphonium methallylid¹⁶ gave a mixture of the major **VA**, its 5Z and minor **VB** isomers. The HPLC analysis showed that the initial ratio of **IA** and **IB** was almost maintained to the final step (Fig. 4). From the mixture, **VA** was isolated by silica gel column chromatography in a total yield of 10~30%. **VA**: $[\alpha]_D^{23} + 93.6^\circ$ in methanol, lit.²⁾ $+103^\circ$. The ^1H -NMR, ^{13}C -NMR and mass spectral data of **VA** agreed with those of (+)-marmelo lactone **A**. Here, the 5E and 5Z isomers of **VA** and **VB** were readily characterized from the $J_{\text{H5,H6}}$ values of ^1H -NMR: the 5Z isomer¹⁷ gave lower values (9.52 Hz) than the 5E isomer (15.6 Hz).

In a similar way, **VB** was prepared from the **IB** rich fraction in a 10~30% yield. **VB**: $[\alpha]_D^{23} + 21.8^\circ$ in methanol, lit.²⁾ -20° for (–)-lactone **B**. The other spectral data of **VB** agreed with those of (–)-marmelo lactone **B**. The optical purities of **VA** and **VB** could be estimated to be more than 90% from their

values of optical rotation, although this was not checked by any other method.

Since all the reactions described above were known to proceed with retention of configurations, the absolute configurations of natural (+)-marmelo lactone **A** and (–)-marmelo lactone **B** should be (2R, 4S) and (2R, 4R), respectively.

In conclusion, (+)-marmelo lactones **A** and **B** were synthesized from *erythro*- and *threo*- γ -methyl-L-glutamic acid, respectively, in a moderate yield. The absolute configurations of marmelo lactone **A** and **B** were thus established synthetically and revised^{2,3} to be (2R, 4S) for (+)-lactone **A** and (2R, 4R) for (–)-lactone **B**.

The syntheses of (–)-marmelo lactones **A** and **B** from D-glutamic acid have been reported in other papers.¹⁵

EXPERIMENTAL

All melting points and boiling points were uncorrected. IR spectra were recorded on a Jasco A-202 spectrometer. NMR spectra were measured on a JEOL JNM-FX 100 Fourier transform spectrometer in CDCl_3 with TMS as an internal standard, unless otherwise stated. Direct mass spectra were recorded on a Hitachi M-52 spectrometer and CD spectra on a Dichrograph Mark III-J. Specific

rotations were measured on a Jasco ORD/UV-5 at 589 nm. HPLC was performed with a Jasco TRIOTAR equipped with a Jasco UV/DEC-100-II. Kiesel gel 60 GF₂₅₄ (Merck) was used for the analytical and preparative TLC.

Enzymatic resolution of N-carbobenzoxy-γ-methyl-D,L-glutamic acid. The mixture of diastereomers of carbobenzoxy-γ-methyl-D,L-glutamic acid obtained as a crude syrup by the method of Done and Fowden⁴⁾ was used for the resolution. Papain (1:350, digestive powder) was purchased from Wako Chemical Industries, Ltd.

To a solution of the crude racemate (14 g) in 2 N NaOH (25 ml) were added aniline (8 g), cysteine hydrochloride solution (0.6 g in 5 ml of water) and the enzyme solution (40 ml of citrate-NaOH buffer, pH=5.0). The reaction mixture was made up to 100 ml by the addition of water and incubated at 40°C. N-Carbobenzoxy-γ-methyl-L-glutamic acid anilide (CBZ anilide) soon started to precipitate as an oily product. After incubating for 12 hr at 40°C, the solution was cooled to 4°C and extracted with CHCl₃ (30 ml × 3). The extracts were combined, washed with sat. NaCl, dil. HCl and water, and then dried over Na₂SO₄. Evaporation of the solvent *in vacuo* gave CBZ anilide as a white powder, 7 g (80% of the theoretical yield), mp. 145~153°C. *Anal.* Found: C, 64.89; H, 6.00; N, 7.45. Calcd. for C₂₀H₂₂O₅N₂: C, 64.85; H, 5.98; N, 7.56. ¹H-NMR: 1.3 (3H, d), 1.7~2.2 (2H, m), 2.3~2.9 (1H, m), 4.5~4.8 (1H, m), 5.1 (2H, s), 6.0 (2H, broad), 7.0~7.6 (10H, m), 9.2~9.3 (1H, broad).

(+)-Erythro-γ-methyl-L-glutamic acid (IA) and (+)-threo-γ-methyl-L-glutamic acid (IB). The CBZ anilide (7 g) was dissolved in methanol (50 ml) containing Pd-black (100 ml) and hydrogenated under atmospheric pressure at room temperature, while the reaction was checked with TLC: CBZ anilide, *R*_f=0.84 (BuOH : AcOH : MeOH = 45:4:8, UV detection); γ-methyl-L-glutamic acid anilide, *R*_f=0.68 (BuOH : AcOH : H₂O = 4:1:1, ninhydrin detection). After completing the reaction (2~3 hr), the mixture was concentrated *in vacuo* to give a syrupy residue (4.8 g) of γ-methyl-L-glutamic acid anilide. The crude anilide (4 g) was dissolved in 4 N HCl (200 ml), the solution was refluxed for 12 hr and then cooled to room temperature. The solution was washed with benzene (20 ml × 3) to remove the aniline and concentrated under a reduced pressure to give a viscous pale yellow syrup of crude γ-methyl-L-glutamic acid (3 g), which was crystallized from water-acetone, 2.5 g (82% yield from the CBZ anilide). ¹³C-NMR showed that this solid was a mixture of IA and IB, mp 150~158°C, [α]_D²³ = +31.5° (c=0.5, 6 N HCl). ¹H-NMR δ_{D₂O}^{DSS}: 1.2 (3H, d), 1.8~2.3 (2H, m), 2.5~2.9 (1H, m), 3.8 (1H, t). TLC: *R*_f=0.33 (BuOH : AcOH : H₂O =

4:1:1, ninhydrin detection).

Separation of IA and IB by anion exchange column chromatography. The crude mixture of IA and IB (3 g) was mounted on an anion exchange column (Dowex 1, ×8, 100~200 mesh, acetate form^{*1}, 3 cmφ × 80 cm) and eluted with 0.2 N AcOH. The eluent was detected by TLC with BuOH-AcOH-H₂O (4:1:1) and the optical rotation at 300 nm. The faster moving fractions, 30~45, (one fraction = 15 g) were collected and evaporated *in vacuo* to give a white solid (700 mg) which was recrystallized from water-acetone to give a IB rich compound, 500 mg, mp 178°C, [α]_D²³ +43.5° (c=0.4, 6 N HCl), lit. +30.2° (c=0.24, 5 N HCl),⁶⁾ +22.2° (c=0.24, 5 N HCl).⁵⁾ ¹³C-NMR δ_{D₂O}^{DSS}: 20.0, 37.4, 40.2, 56.1, 177.2, 183.8. *Anal.* Found: C, 44.27; H, 7.12; N, 8.33. Calcd. for C₆H₁₁O₄N: C, 44.71; H, 6.87; N, 8.69. The slower fractions, 50~65, were collected and evaporated to give a IA rich compound as a syrup (600 mg), which was crystallized from water-acetone, mp 170°C, [α]_D²³ +25.1° (c=0.41, 6 N HCl), lit. +22.2° (c=0.66, 5 N HCl),⁶⁾ +19.2° (c=2.5, 6 N HCl).⁵⁾ ¹³C-NMR δ_{D₂O}^{DSS}: 20.2, 37.0, 39.9, 56.1, 177.2, 184.4. *Anal.* Found: C, 44.70; H, 7.05; N, 8.62. Calcd. for C₆H₁₁O₄N: C, 44.71; H, 6.87; N, 8.69.

(2R, 4S)-4-Carboxy-2-methylbutan-4-olide (IIA). To a solution of the IA rich compound (1.26 g) in water (8.5 ml) were simultaneously added 2 N H₂SO₄ (5 ml) and NaNO₂ (720 mg in 5 ml of water) over 20 min. After stirring for 12 hr at room temperature, the solution was evaporated *in vacuo* to dryness and eluted with hot acetone (10 ml × 3). The acetone extracts were combined, dried over Na₂SO₄ and concentrated *in vacuo* to give a viscous syrup (1 g) of a crude mixture of IIA containing small amount of IIB. The crude syrup was distilled under a reduced pressure (8 mmHg, 180~190°C bath temp.) to give a white solid of a IIA rich compound, 700 mg, (69% from IA), mp 50~56°C. IR ν_{max}^{film} cm⁻¹: 3500 (w), 2950 (m), 2700~2500 (m), 1760 (s). ¹³C-NMR: 15.20, 34.03, 34.79, 73.75, 173.85, 178.76.

(2S, 4S)-4-Carboxy-2-methylbutan-4-olide (IIB). In a similar manner to the preparation of IIA, the IB rich compound (1.26 g) was converted to the IIB rich compound (760 mg, 74% yield from IB) as a viscous syrup, bp 180~190°C bath temperature/8 mmHg. IR ν_{max}^{film} cm⁻¹: 3500 (w), 2950 (m), 2700~2400 (m), 1760 (s). ¹³C-NMR: 15.41, 32.74, 73.51, 173.94, 178.76. ¹H-NMR: 1.3 (3H, d), 1.8~2.2 (1H, m), 2.6~3.0 (2H, m), 4.9 (1H, dd), 9.2 (1H, broad s).

(2R, 4S)-4-Chloroformyl-2-methylbutan-4-olide (IIIA). A mixture of IIA (700 mg) and thionyl chloride (1.3 g) was

*1 The acetate form resin was prepared from the Cl form (500 g) by several treatments with 1500 ml of 3 N AcONa, followed by thoroughly washing with water.

*2 NMR data of I~IV were obtained by subtracting the signals due to their minor isomers.

refluxed for 2 hr and allowed to stand at room temperature for 6 hr. After removal of the excess thionyl chloride, the residue was distilled under a reduced pressure to give an oily product of the **IIIA** rich compound containing a small amount of **IIIB** (700 mg, 98% yield from **IIA**). IR $\nu_{\text{max}}^{\text{film}} \text{ cm}^{-1}$: 2950 (m), 1780 (s), 1750~1700 (s). $^{13}\text{C-NMR}$: 15.22, 32.65, 33.94, 73.43, 174.87, 179.40. $^1\text{H-NMR}$: 1.3 (3H, d), 1.9~2.2 (1H, m), 2.2~2.5 (2H, m), 4.95 (1H, dd).

(2*S*, 4*S*)-4-Chloroformyl-2-methylbutan-4-olide (**IIIB**). In a similar way to the preparation of **IIIA**, the **IIIB** rich compound (700 mg) was converted to the **IIIB** rich one as an oily product (700 mg, 98% yield from **IIIB**). bp 120~130°C bath temperature/8 mmHg. IR $\nu_{\text{max}}^{\text{film}} \text{ cm}^{-1}$: 2950 (m), 1780 (s), 1750~1700 (s). $^1\text{H-NMR}$: 1.3 (3H, d), 2.0~2.2 (1H, m), 2.2~2.5 (2H, m), 4.6 (1H, dd). $^{13}\text{C-NMR}$: 15.39, 34.11, 34.70, 73.66, 174.28, 179.4.

(2*R*,4*S*)-4-Formyl-2-methylbutan-4-olide (**IVA**). To a suspension of 5% Pd/BaSO₄ (70 mg) in dry toluene (10 ml) was added a toluene solution (2 ml) of the freshly distilled **IIIA** (600 mg) and tetramethyl thiourea (0.2 mg). Into the solution was passed hydrogen with stirring at 65~70°C for 1~2 hr, while the reaction was checked with TLC (CHCl₃-CH₃OH=8:1): **IIIA**, *R_f*=0.8 (I₂ detection); **IVA**, *R_f*=0.5 (I₂ or 2,4-dinitrophenylhydrazine detection). After completing the reaction, the reaction mixture was rapidly filtered through a pad of celite to remove the catalyst and concentrated *in vacuo* to give a crude **IVA** rich syrup (500 mg) which was diluted with dry THF (50 ml) and used in the next reaction without isolation.

(2*S*, 4*S*)-4-Formyl-2-methylbutan-4-olide (**IVB**). In the same manner as the preparation of **IVA**, **IIIB** rich compound (600 mg) was converted to the crude aldehyde **IVB** (500 mg), which was used in the next reaction without isolation.

(2*R*, 4*S*, 5*E*)-2,7-Dimethyl-5,7-octadien-4-olide, ((+)-marmelo lactone A) (**VA**). To a suspension of Ph₃PCH₂C(=CH₂)CH₃Cl¹⁶⁾ in THF (20 ml) was added *n*-butyllithium (15% w/w in hexane, 1.8 ml). The mixture was stirred for 30 min at room temperature under N₂. To the mixture was added the crude aldehyde (**IVA**) (500 mg in 5 ml of THF) and the resulting mixture was vigorously shaken for 2 hr at room temperature. This mixture was poured into sat. NaCl and dried over Na₂SO₄. After removing the solvent *in vacuo*, the residue (300 mg) was chromatographed on a preparative TLC (20 cm × 20 cm, Kieselgel (7 g) + AgNO₃ (2.6 g)) developed with *n*-hexane-ethyl acetate (7:1, v/v). After developing twice with the solvent, the slower moving band was scratched off the plate and eluted with ethyl ether. The ethereal extract was passed through a short silica gel column (1 cmφ × 10 cm) and the eluent was concentrated to give an oily product of **VA** containing a small amount of **VB**. The mixture was mounted on a

silica gel column and eluted with *n*-hexane-ethyl acetate (8:1, v/v). The faster moving fractions were collected and evaporated to give an oily product of **VA** (120 mg in total of **VA** from 500 mg of crude **IVA**). $[\alpha]_D^{23} +93.6^\circ$ (*c*=0.09, CH₃OH). $^1\text{H-NMR}$: 1.30 (3H, d, *J*=7.1 Hz), 1.85 (3H, s), 2.0~2.30 (2H, m), 2.68 (1H, m), 5.04 (3H, m), 5.62 (1H, dd, *J*=6.1 and 15.6 Hz), 6.38 (1H, d, *J*=15.6 Hz). $^{13}\text{C-NMR}$: 15.5, 18.5, 33.7, 36.5, 77.9, 118.6, 126.6, 135.1, 140.6, 179.7. MS *m/z*: 166 (*M*⁺), 151 (*M*⁺ - CH₃), 138, 123, 109, 93, 81, 69, 68, 42, 41.

(2*S*, 4*S*, 5*E*)-2,7-Dimethyl-5,7-octadien-4-olide ((+)-marmelo lactone B) (**VB**). In a similar manner to the preparation of **VA**, the crude aldehyde (**IVB**) (400 mg) yielded a crude **VB** rich mixture (300 mg), which was mounted on a silica gel column eluted with *n*-hexane-ethyl acetate (8:1, v/v). The slower moving fractions were collected to give an oily product of **VB** (140 mg, 27% yield from **IVB**). The crude oil was purified by preparative TLC developed with ethyl ether-petroleum ether (1:5, v/v) to give **VB** as an oil, $[\alpha]_D^{23} +21.8^\circ$ (*c*=0.11, CH₃OH). $^1\text{H-NMR}$: 1.29 (3H, d, *J*=6.6 Hz), 1.68 (1H, dd, *J*=5.6 and 12.2 Hz), 1.86 (3H, s), 2.40~2.80 (2H, m), 4.80 (1H, m), 5.06 (2H, broad s), 5.63 (1H, dd, *J*=6.8 and 15.6 Hz), 6.42 (1H, d, *J*=15.6 Hz). $^{13}\text{C-NMR}$: 15.3, 18.5, 36.0, 38.2, 78.7, 118.8, 126.7, 136.3, 140.7, 179.0. MS *m/z*: 166 (*M*⁺), 151 (*M*⁺ - CH₃), 138, 123, 109, 93, 81, 69, 68, 42, 41.

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