Synthesis of Streptovitacin-C2 Isomers

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(2R*,4S*,6S*,aS*)- and (2R*,4R*,6R*,aS*)-Streptovitacin-C2 (STV-C2) (1a and 1b) were synthesized by an aldol condensation of (2R*,4S*)- or (2R*,4R*)-2,4-dimethyl-2-trimethylsiloxy-1-cyclohexanone (15a or 15b) with 4-(2-oxoethyl)-2,6-piperidinedione (16), which was followed by desilylation of the products. The stereochemistry of the synthesized STV-C2 isomers (1a and 1b) was elucidated by NMR. STV-C2 isomers (1a and 1b) did not show strong antimicrobial activity against Saccharomyces cerevisiae and Pyricularia oryzae.

STV-C2 (1), STV-B (2) and STV-A (3), the hydroxylated analogs of cycloheximide (4), are known as antitumor agents isolated from the cultured broth of Streptomyces griseus. Their chemical structures (Fig. 1) have been elucidated by Herr et al., but their stereochemistry was unresolved and their synthesis had not been achieved. In our previous reports, a marked difference of antimicrobial activity among the stereoisomers of cycloheximide was shown. In order to examine the effect of a hydroxyl group introduced on the 2,4-dimethyl-1-cyclohexanone (2,4-DMC) ring of 4 against antimicrobial activity, we synthesized the stereoisomers of STV-C2 (1). In this paper, we report the synthesis and
Table I. $^{13}$CNMR Data of the Synthetic Products

<table>
<thead>
<tr>
<th>Compound</th>
<th>C-2-Methyl carbon</th>
<th>C-4-Methyl carbon</th>
<th>C-α Carbon</th>
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<tbody>
<tr>
<td>(±)-10a</td>
<td>24.4</td>
<td>21.0</td>
<td>—</td>
</tr>
<tr>
<td>(+)-10b</td>
<td>25.9</td>
<td>21.2</td>
<td>—</td>
</tr>
<tr>
<td>11a*</td>
<td>14.7 (eq)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>11b*</td>
<td>16.8 (ax)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>12b</td>
<td>14.0 (eq)</td>
<td>21.1 (eq)</td>
<td>68.9 (threo)</td>
</tr>
<tr>
<td>13b</td>
<td>17.6 (ax)</td>
<td>21.5 (eq)</td>
<td>68.7 (threo)</td>
</tr>
<tr>
<td>(±)-17a</td>
<td>23.4</td>
<td>20.8</td>
<td>68.9</td>
</tr>
<tr>
<td>(−)-17b</td>
<td>24.1</td>
<td>20.5</td>
<td>69.0</td>
</tr>
<tr>
<td>(±)-1a</td>
<td>23.8</td>
<td>20.9</td>
<td>69.0</td>
</tr>
<tr>
<td>(±)-1b</td>
<td>25.9</td>
<td>21.2</td>
<td>66.3</td>
</tr>
</tbody>
</table>

* Reported data, see ref. 6.

** See refs. 3 and 4.

The antimicrobial activity of STV-C$_2$ isomers.

The synthesis of STV-C$_2$ isomers was performed by an aldol condensation of cis- and trans-dimethyl hydroxyketones (10a and 10b) with the aldehyde 16. The ketone 10a was synthesized from (+)-cw-2,4-DMC (5) as follows. Catalytic hydrogenation of 2,4-dimethylphenol (7) and subsequent oxidation of the resulting 2,4-dimethyl-1-cyclohexanols gave (+)-cis-2,4-DMC (5), which was then converted to its enol-acetate 8. Epoxidation of 8 with monoperphthalic acid, followed by alkaline hydrolysis, gave a stereoisomeric mixture of 2-hydroxy-2,4-DMC (9), which consisted of cis-dimethylhydroxyketone (10a, 7%) and trans-dimethylhydroxyketone (10b, 93%) by GLC analysis. The structure of these products was elucidated by their $^{13}$C-NMR data (Table I). The C-2-equatorial methyl carbons of cis-4-t-butyl-2-methyl-1-cyclohexanone (11a) and isocycloheximide (12) showed higher field shifts than those of the C-2-axial-methyl carbons of trans-4-t-butyl-2-methyl-1-cyclohexanone (11b) and naramycin B (13). Because the C-2 methyl carbon (24.4 ppm) of 10a shifted to a higher field than that of 10b (25.9 ppm), and the C-4-methyl carbons of 10a and 10b (21.0 and 21.2 ppm) were identical to the C-4-equatorial methyl carbons of isocycloheximide (12) and naramycin B (13) (21.1 and 21.5 ppm), it was concluded that 10a and 10b were the cis- and trans-dimethyl form respectively. Therefore, it became apparent that the synthesis of the hydroxyketones via epoxidation of the enol-acetate 8 gave the cis-dimethyl hydroxyketone 10a as the main product. Furthermore, trans-dimethyl hydroxyketone 10b was prepared from (R)-(+-)pulegone (6). Grignard reaction of 6 with methylimagnesium bromide gave the alcohol 14, whose ozonolysis gave the trans-dimethyl hydroxyketone 10b as an almost pure product. Treatment of each hydroxyketone (+)-10a and (+)-10b with N,O-bis(trimethylsilyl)-acetamide (BSA) gave the corresponding trimethylsilyl ethers (+)-15a and (+)-15b respectively. Lithium enolate 18a of (+)-15a was reacted with the aldehyde 16 to give a silylated threo-STV-C$_2$ isomer ((±)-17a) in almost pure form, and deprotection of the product gave a threo-(±)-STV-C$_2$ isomer (1a), mp 169~170°C. Condensation of lithium enolate 18b of another silyl-ether (+)-15b with the aldehyde 16 gave the silylated threo-STV-C$_2$ (−)-17b in pure form, and subsequent deprotection of the product gave the (+)-erythro-2STV-C$_2$ isomer (1b), [α]$_{D}$: + 6.2 x 10$^3$ (λ$_{max}$: 291 nm). The stereochemistry of the STV-C$_2$ isomers was elucidated by comparing their $^{13}$C-NMR data with those of cycloheximide isomers. Because the chemical shifts of methyl carbons of the hydroxyketone 10a (24.4 and 21.0 ppm) were very similar to those of the final product 1a (23.8 and 20.9 ppm), the configuration of the methyl groups of 10a and 1a was presumed to be the same. Similarly, the chemical shifts of the methyl carbons of the hydroxyketone 10b (25.9 and 21.2 ppm) were identical with those of 1b. In addition, the conformation of the side chains of 1a and 1b at C-6 was assumed to be equatorial rather than axial owing to steric repulsion between the C-2-axial substituents and C-6 side chain. C-α signals of the isomers (17a, 17b, 1a and 1b) were good indicators for the determination of relative stereochemistry between C-6 and C-α. In our previous reports,$^{3,4}$ it was revealed that the C-α methyl signals of the threo isomers...
appeared at near 69.0 ppm, and that those of the erythro isomers appeared at near 66.5 ppm. From these data (Table I), (±)-17a, (−)-17b and (±)-1a were concluded to be the threo form, and also (+)-1b to be the erythro form. 

The antimicrobial activity of the synthesized STV-C2 isomers against Saccharomyces cerevisiae and Pyricularia oryzae was examined to give the results shown in Table II. (±)-17a, C-2-hydroxylated isocycloheximide and (+)-1b, C-2-hydroxylated naramycin B, did not show strong antimicrobial activity. It was concluded that the introduction of a hydroxy group into the C-2 position of cycloheximide isomers decreased the antimicrobial activity.

### EXPERIMENTAL

All boiling points and melting points are uncorrected. 1H-NMR and 13C-NMR spectra were recorded on a JEOL JNM FX-100 spectrometer, and IR spectra were recorded on a JASCO IR-810 infrared spectrometer. Optical rotations were measured on a JASCO DIP-4 spectrometer, and circular dichroism (CD) spectra were measured on a Dichrograph Mark III-J spectrometer. Gas chromatographic analyses were performed on a JEOL JGC-1100 instrument with thermal conductance detector and stainless steel column (2 m x 3 mm) packed with 20% DEGS on Chromosorb W or 5% Lac 2R-446 on Chromosorb W. High pressure liquid chromatography (HPLC) was performed at 254 nm on a JASCO TRIROTAR instrument with a UV spectrometer, using a stainless steel column (4.6 mm x 250 mm) packed with silica-gel (SS-05) and a solvent system of methylene chloride/isopropyl alcohol=98/2 or 95/5 at a flow rate of 1 ml/min. MPLC was performed on the same instrument, using a glass column packed with a silica-gel (Lichroprep Si 60, 40 ~ 63 μm) and the same solvent system.

1) Preparation of (±)-(2R*,4S*)-2,4-dimethyl-2-hydroxy-1-cyclohexanone (10a). A mixture of (±)-cis-2,4-DMC (5, 40 g), acetic anhydride (60 ml) and a catalytic amount of p-toluenesulfonic acid was refluxed for 10 hr. The reaction mixture was poured into aq. NaHCO₃ and extracted with n-hexane. The organic layer was washed with brine, and dried over anhyd. MgSO₄. Evaporation of the solvent gave 44 g (an 81.5% yield) of (±)-2,4-dimethyl-1-cyclohexen-1-yl acetate (8), which almost consisted of a single product (tR: 26.5 min) by GLC analysis (column, 20% DEGS on Chromosorb W; temp., 85°C; He flow rate, 15 ml/min). 8: bp 83 ~ 86°C (15 mmHg); IR νmax cm⁻¹: 2870, 1760 (C=O), 1450, 1370, 1230, 1210, 1120. To an ether solution (50 ml) of the enol acetate (8, 15 g), 73% of monoperphthalic acid in ether (300 ml) was added and the mixture stirred at room temperature for 18 hr. The reaction mixture was successively washed with 20% aq.
NaHSO₃, aq. NaHCO₃ and brine, and then dried over anhyd. MgSO₄. Evaporation of the solvent, followed by distillation under reduced pressure, gave a product (10.1 g, 61% yield), bp 110~120°C (15 mmHg); IR ν₂max cm⁻¹: 3400 (OH), 2920, 1720 (C=O), 1460, 1380, 1250, 1170, 1100, 1060, 990, 830, 840, 750; ¹H-NMR (CDCl₃) δ: 0.91 (9H, s, CH₃), 0.92 (3H, d, J=6.6 Hz, CH₃), 1.27 (3H, s, CH₃); ¹³C-NMR (CDCl₃) δ: 1.99, 20.9, 23.7, 27.0, 36.2, 37.1, 51.4, 75.8, 212.4. To a stirred solution of LDA (lithium diisopropylamide) in 50 ml of dry tetrahydrofuran, which was prepared from 2.2 mmol of n-butyl lithium and 2.2 mmol of diisopropylamine, were added 473 mg (2.2 mmol) of the silylated ketone 15 and then 350 mg (2.2 mmol) of the aldehyde 16 at -70°C. After stirring for 1.5 hr, the mixture was poured into ice-cooled 2% aq. acetic acid and extracted with CH₂Cl₂. The extract was washed with aq. NaHCO₃ and brine, and then dried over anhyd. MgSO₄. Evaporation of the solvent gave 512 mg of an oil, which was fractionated by MPLC (solvent system, CH₂Cl₂/iso-PrOH=98/2; flow rate, 2 ml/min) to give an aldol fraction (197 mg). The fragment mainly consisted of 17a by HPLC analysis (solvent system, same as MPLC; flow rate, 1 ml/min; τR: 8.8 min). Fractionation by MPLC gave the pure aldol 17a (89 mg, 11% yield); IR ν₂max cm⁻¹: 3500 (OH), 3220, 3110, 2950, 2925, 2875, 1700 (C=O), 1380, 1250, 1170, 1100, 980, 950, 890, 840; ¹H-NMR (CDCl₃) δ: 0.12 (9H, s), 0.91 (3H, d, J=6.6 Hz, CH₃), 1.27 (3H, s, CH₃); ¹³C-NMR (CDCl₃) δ: 2.0, 20.8 (q), 23.7, 27.0, 36.2, 37.1, 38.6, 39.4, 50.4, 51.4, 68.9 (d), 78.3, 172.5, 216.0. A solution of 17a in dry tetrahydrofuran (16) at 80°C for 24 hr, the reaction mixture was poured into ice-cooled 2% aq. acetic acid and extracted with CH₂Cl₂. The extract was washed with aq. NaHCO₃ and brine, and then dried over anhyd. MgSO₄. Evaporation of the solvent gave 512 mg of an oil, which was fractionated by MPLC (solvent system, CH₂Cl₂/iso-PrOH=98/2; flow rate, 2 ml/min) to give an aldol fraction (197 mg). The fragment mainly consisted of 17a by HPLC analysis (solvent system, same as MPLC; flow rate, 1 ml/min; τR: 8.8 min). Fractionation by MPLC gave the pure aldol 17a (89 mg, 11% yield); IR ν₂max cm⁻¹: 3500 (OH), 3220, 3110, 2950, 2925, 2875, 1700 (C=O), 1380, 1250, 1170, 1100, 980, 950, 890, 840; ¹H-NMR (CDCl₃) δ: 0.12 (9H, s), 0.91 (3H, d, J=6.6 Hz, CH₃), 1.27 (3H, s, CH₃); ¹³C-NMR (CDCl₃) δ: 2.0, 20.8 (q), 23.7, 27.0, 36.2, 37.1, 38.6, 39.4, 50.4, 51.4, 68.9 (d), 78.3, 172.5, 216.0. After heating the aldol 17a in 5 ml of 30% aq. acetic acid-tetrahydrofuran (1:1) at 80°C for 24 hr, the reaction mixture was poured into ice-cooled 2% aq. acetic acid and extracted with CH₂Cl₂. The extract was washed with aq. NaHCO₃ and brine, and then dried over anhyd. MgSO₄. Evaporation of the solvent gave 512 mg of an oil, which was fractionated by MPLC (solvent system, CH₂Cl₂/iso-PrOH=98/2; flow rate, 2 ml/min) to give an aldol fraction (197 mg). The fragment mainly consisted of 17a by HPLC analysis (solvent system, same as MPLC; flow rate, 1 ml/min; τR: 8.8 min). Fractionation by MPLC gave the pure aldol 17a (89 mg, 11% yield); IR ν₂max cm⁻¹: 3500 (OH), 3220, 3110, 2950, 2925, 2875, 1700 (C=O), 1380, 1250, 1170, 1100, 980, 950, 890, 840; ¹H-NMR (CDCl₃) δ: 0.12 (9H, s), 0.91 (3H, d, J=6.6 Hz, CH₃), 1.27 (3H, s, CH₃); ¹³C-NMR (CDCl₃) δ: 2.0, 20.8 (q), 23.7, 27.0, 36.2, 37.1, 38.6, 39.4, 50.4, 51.4, 68.9 (d), 78.3, 172.5, 216.0. After heating the aldol 17a in 5 ml of 30% aq. acetic acid-tetrahydrofuran (1:1) at 80°C for 24 hr, the reaction mixture was poured into ice-cooled 2% aq. acetic acid and extracted with CH₂Cl₂. The extract was washed with aq. NaHCO₃ and brine, and then dried over anhyd. MgSO₄. Evaporation of the solvent gave 512 mg of an oil, which was fractionated by MPLC (solvent system, CH₂Cl₂/iso-PrOH=98/2; flow rate, 2 ml/min) to give an aldol fraction (197 mg). The fragment mainly consisted of 17a by HPLC analysis (solvent system, same as MPLC; flow rate, 1 ml/min; τR: 8.8 min). Fractionation by MPLC gave the pure aldol 17a (89 mg, 11% yield); IR ν₂max cm⁻¹: 3500 (OH), 3220, 3110, 2950, 2925, 2875, 1700 (C=O), 1380, 1250, 1170, 1100, 980, 950, 890, 840; ¹H-NMR (CDCl₃) δ: 0.12 (9H, s), 0.91 (3H, d, J=6.6 Hz, CH₃), 1.27 (3H, s, CH₃); ¹³C-NMR (CDCl₃) δ: 2.0, 20.8 (q), 23.7, 27.0, 36.2, 37.1, 38.6, 39.4, 50.4, 51.4, 68.9 (d), 78.3, 172.5, 216.0. After heating the aldol 17a in 5 ml of 30% aq. acetic acid-tetrahydrofuran (1:1) at 80°C for 24 hr, the reaction mixture was poured into ice-cooled 2% aq. acetic acid and extracted with CH₂Cl₂. The extract was washed with aq. NaHCO₃ and brine, and then dried over anhyd. MgSO₄. Evaporation of the solvent gave 512 mg of an oil, which was fractionated by MPLC (solvent system, CH₂Cl₂/iso-PrOH=98/2; flow rate, 2 ml/min) to give an aldol fraction (197 mg). The fragment mainly consisted of 17a by HPLC analysis (solvent system, same as MPLC; flow rate, 1 ml/min; τR: 8.8 min).
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27.1, 37.1, 38.5, 38.7, 39.1, 49.7, 50.6, 69.0, 75.8, 172.5, 172.6, 215.8; Anal. Found: C, 60.2; H, 8.52; N, 4.62. Calcd. for C15H23O4N: C, 60.58; H, 7.69; N, 4.71%.

4) Synthesis of (+)-(2R,4R,6R,aR)STV-C2 (1b). (+)-rafls-Dimethylhydroxyketone (10b, 1 g), which was prepared from (R)-( + )-pulegone (6) and N,S-bis(trimethylsilyl)acetamide (4.5ml), was dissolved in 20ml of dry dimethylformamide, and the solution was refluxed for 24hr. After treating the reaction mixture with the treatment for 15a, 846 mg of the trimethylsilyl derivative (+)-15b was obtained. (+)-15b: bp 101~103°C (15mmHg); IR ν^cm^-1: 2950, 2925, 1730 (C=O), 1460, 1370, 1250, 1190, 1140, 1060, 990, 940, 880, 800, 790, 750, 740, 700, 690, 600, 540; [α]D_2+112.7° (c=1.5, CHC13). ^-NMR (CDC13) S: 0.12 (9H, s), 0.98 (3H, d, J=5.9Hz, CH3), 1.41 (3H, s, CH3); 13C-NMR (CDC13) S: 2.7, 21.3, 26.9, 30.1, 35.5, 38.3, 52.0, 79.7, 211.5. To a stirred solution of LDA in 50ml of tetrahydrafuran, which was prepared from 1.2 mmol of«-butyllithium and 1.2 mmol of diisopropylamine, were added 264mg (1.2mmol) of (+)-15b and then 195mg (1.2mmol) of the aldehyde 16 at -70°C. After stirring for 1.5hr, the reaction mixture was poured into ice-cooled 2% aq. acetic acid and extracted with CH2C12. The extract was washed with aq. NaHCO3 and brine, and then dried over anhyd. MgSO4. Evaporation of the solvent gave 328 mg of an oil, which as fractionated by MPLC (solvent system, CH2Cl2/iso-PrOH =98/2; flow rate, 2 ml/min) to give the aldol fraction (72mg, 16.2% yield), which only consisted of 17b (tR: 10min) by HPLC analysis (solvent system, CH2Cl2/iso-PrOH =98/2; flow rate, 1 ml/min) to give the aldol fraction (4mg of (+)-(2R,4R,6R,aS)-STV-C2 (1b), for which the HPLC analysis (solvent system, CH2Cl2/iso-PrOH =95/5; flow rate, 1 ml/min) showed only one peak (t_R: 6min). (+)-1b: IR ν^lim cm^-1: 3425 (OH), 3300, 3125, 2950, 1720 (1=C=O, CO–N), 1400, 1280, 1160, 1120, 1040; ^-H-NMR (CDCl3) δ: 1.0 (3H, d, J = 5.9 Hz, CH3), 1.42 (3H, s, CH3), 4.2 (1H, d-d-d, J = 10.7, 2.4, 2.4 Hz); ^-C-NMR (CDCl3) δ: 21.2, 25.9, 27.6, 28.4, 35.0, 37.1, 38.1, 38.4, 50.3, 50.4, 66.1, 75.2, 171.9, 172.1, 216.8; CD (c =4×10^-4, MeOH) [α]_max: +6.2×10^3 (i_max: 291 nm); MS m/z: 298 (M^+ +1, 1%), 280 (1%), 251 (30%), 194 (100%), 193 (73%), 135 (22%), 112 (37%).

5) Antimicrobial assay of STV-C2 isomers (±)-la and (+)-1b against Saccharomyces cerevisiae (HUT 7099) and Pyricularia oryzae (Ken 62-89). The antimicrobial activity of (±)-la and (+)-1b was determined by the conventional paper disc method as has been described in the previous papers. Acknowledgments. We are gratefully appreciate Mr. Hisayuki Takahashi for his technical assistance. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

7) See refs. 3 and 4. The coupling constants of cycloheximide, the α-epimer of naramycin B and α-epi-cycloheximide were erroneously described, and the correct values are as follows. Cycloheximide: J = 10.2, 2.7, 2.7 Hz. The C-α-epimer of naramycin B: J = 10.5, 2.7, 2.7 Hz. α-Epicycloheximide: J = 10.8, 2.6, 2.6 Hz.