Synthesis and Biological Activities of Demethylcycloheximides

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(+)-Demethylcycloheximides (2 ~ 4) and (±)-4-[2-(3-isopropyl-6-methyl-2-oxocyclohexyl)-2-hydroxyethyl]-2,6-piperidinedione (5) were synthesized by an aldol condensation of the respective ketones (6) with 4-(2-oxo-ethyl)-2,6-piperidinedione (8). Their biological activities were examined against fungi, rice seedlings and lettuce seeds, but only some of them showed weak activity.

Cycloheximide (1), which was isolated from a cultured broth of Streptomyces griseus, has powerful antimicrobial activities against a number of phytopathogens. It is known that 1 inhibits the protein synthesis in a eukaryotic cell, and 1 is widely used as a reagent for biochemical studies. However, the toxicity of 1 makes it unsuitable as an agricultural fungicide, and a study of the structure-biological activity relationship for 1 is therefore important and interesting. Egawa et al. synthesized analogous compounds of 1 and examined their microbial activities, but the stereochemistry of these compounds was not clarified. Kudo et al. synthesized the chiral cis- and trans-cycloheximide isomers by an aldol condensation and established the stereochemistry of each stereoisomer. Here, we have synthesized the racemic demethylcycloheximides (2 ~ 5) by an aldol condensation of the cyclohexanones (6) with 4-(2-oxo-ethyl)-2,6-piperidinedione (8) to elucidate their stereochemistry and their biological activity.

Firstly, a stereoisomeric mixture of dideemethylcycloheximides (2) was synthesized by the reaction of the lithium enolate of cyclohexanone (7, M=Li, R=R=H) with the aldehyde 8. The composition of the isomers (2a, b) was analyzed by high-pressure liquid chromatography (HPLC) and recrystallization. The structure of each isomer was elucidated by 1H-NMR and 13C-NMR spectra (Tables II and III). Naramycin B and isocycloheximide, which had an anti-configuration at the 1'-position, showed 2'-carbon signals at 68.7 and 68.9 ppm from 13C-NMR, respectively, and 2'-protons and at 3.8 ppm (multiplet) from 1H-NMR.
Table I. Composition of Demethylcycloheximide Isomers

<table>
<thead>
<tr>
<th>Ketone Enolate</th>
<th>Compound No.</th>
<th>Yield (%)</th>
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<tbody>
<tr>
<td></td>
<td>7 (M=)</td>
<td></td>
</tr>
<tr>
<td>6a Li</td>
<td>20</td>
<td>46</td>
</tr>
<tr>
<td>6a Ph3Sn</td>
<td>20</td>
<td>46</td>
</tr>
<tr>
<td>6a (iPrO)3Ti</td>
<td>20</td>
<td>46</td>
</tr>
<tr>
<td>3a Li</td>
<td>20</td>
<td>46</td>
</tr>
<tr>
<td>3a Ph3Sn</td>
<td>20</td>
<td>46</td>
</tr>
<tr>
<td>3a (iPrO)3Ti</td>
<td>20</td>
<td>46</td>
</tr>
<tr>
<td>4a Li</td>
<td>20</td>
<td>46</td>
</tr>
<tr>
<td>4a Ph3Sn</td>
<td>20</td>
<td>46</td>
</tr>
<tr>
<td>4a (iPrO)3Ti</td>
<td>20</td>
<td>46</td>
</tr>
<tr>
<td>5a Li</td>
<td>20</td>
<td>46</td>
</tr>
<tr>
<td>5a Ph3Sn</td>
<td>20</td>
<td>46</td>
</tr>
<tr>
<td>5a (iPrO)3Ti</td>
<td>20</td>
<td>46</td>
</tr>
</tbody>
</table>

* % composition of each isomer in the aldol condensation products.

On the other hand, cycloheximide (1) and α-episocycloheximide, which had a syn-configuration at the 1”, 2”-position, showed 2’-carbon signals at 66.5 ppm from 13C-NMR and 2’-proton signals at 4.2 ppm (ddd) from 1H-NMR.56 Thus, by a comparison of their NMR data, it was deduced that 2a had an anti-configuration and that 2b had a syn-configuration.

Since the anti-aldol was produced predominantly by the reaction using lithium enolate, we next used triphenyltin and triisopropoxytitanium enolates (7, M = Ph3Sn and (iso-propoxy)3Ti) to obtain the syn-aldol as a major product.78 The results are summarized.
Synthesis of Demethylcycloheximides

in Table I.

By a similar procedure, 5*-monodemethylcycloheximides (3a~d) and 3*-monodemethylcycloheximides (4a~d) were synthesized from 2- and 4-methylcyclohexanone (6b and 6c). The composition of each isomer is shown in Table I and their NMR data are summarized in Tables II and III. By a comparison of their chemical shifts and the splitting patterns of the signals of the 2'-position from NMR, it was deduced that 3a, 3c, 4a and 4c were anti-aldols, and that 3b, 3d, 4b and 4d were syn-aldols. Similarly, cycloheximide analogs (5a and 5b) were prepared from (+)-menthone (6d). The splitting patterns of the signals of 5a and 5b were different from the other products, but it was deduced that 5a was an anti-aldol and that 5b was a syn-aldol from the chemical shifts of the signals of the 2'-position and the reaction conditions. The differences among the chemical shifts of the methyl carbons afforded important information for an assignment of the stereochemistry of the cyclohexanone moiety. The 3*-methyl carbon signal (14.2 ppm for 1) with an equatorial orientation appeared in a higher field than that (17.6 ppm for naramycin B) with an axial orientation. Thus, it was deduced that the 3*-methyl carbons of 3a and 3b (14.2 ppm) were of an equatorial orientation, and that those of 3c (16.4 ppm) and 3d (16.5 ppm) were of an axial orientation. Similarly, as the 5*-methyl carbon signal (21.5 ppm for naramycin B) with an equatorial orientation appeared in a lower field than that (18.4 ppm for 1) with an axial orientation, it was deduced that 4a (21.1 ppm) and 4b (21.4 ppm) had an equatorial 5*-methyl group and that 4c (18.6 ppm) and 4d (18.4 ppm) had an axial one. The 3*-isopropyl and 5*-methyl groups were in a trans relation in 5a and 5b because the isopropyl and methyl group of menthone have a trans relation, and reaction was carried out under kinetic conditions. As shown in Table I,1*,3*-trans (3c + 3d) and 1*,5*-trans-monodemethylcycloheximides (4c + 4d) were major products by the 1,3-steric interaction of the reaction intermediates, and it was assumed that the stereochemical relationship between the 1*-substituent group and 3*-isopropyl group of 5 may have been trans from the 1,3-interaction.

The biological activities of 2~5 were tested on fungi (Saccharomyces cerevisiae, Aspergillus niger and Cochliobolus miyabeanus), rice seedlings and lettuce seeds. The results of antimicrobial tests are shown in Table IV, and although the syn-aldols (2b, 3b and 4d) showed very weak activities, the other compounds had no activities. In rice seedlings tests, only 3b showed weak activity, 67% growth at 100 ppm (control: 100%; 1: 0% at 100 ppm, 24% at 50 ppm and 53% at 10 ppm). In lettuce seed tests, the syn-aldols (3b, 3d and 4d) and 5a inhibited the germination. From these results and previous data,5,6) it was deduced that the presence of two methyl groups at the 3* and 5* positions on the cyclohex-

### Table IV. Antimicrobial Activities of Cycloheximide Analogs

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conc. (µg/disc)</th>
<th>S. cerevisiae</th>
<th>A. niger</th>
<th>C. miyabeanus</th>
</tr>
</thead>
<tbody>
<tr>
<td>2b</td>
<td>100</td>
<td>−</td>
<td>−</td>
<td>+&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3b</td>
<td>100</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4d</td>
<td>100</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100</td>
<td>40</td>
<td>15</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>20</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

<sup>a</sup> Inhibited zone <10mm.

<sup>b</sup> Authentic sample, purchased from Nakarai Chemical Co., Ltd.
anone ring and their stereochemistry are very important factors for the biological activities of cycloheximide analogs, and that the syn-aldehyds have relatively strong activities compared with those of the anti-aldehyds. Compound 3b, which had a cis relationship at the 1* and 3* positions and a syn-configuration with similar stereochemistry to 1, showed relatively strong activities. Two methyl groups at the 3* and 5* positions on 1 are considered to be essential for biological activities, and the analogs without dimethyl groups at the 3* and 5* positions may show very weak activities.

**EXPERIMENTAL**

All melting points (mp) 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. High-pressure liquid chromatography (HPLC) was performed on a JASCO TRIROTAR instrument with a UV spectrometer (at 254nm) and stainless steel column (4.6mm x 250mm) packed with silica gel (SS-05 or Finesil-S), using a solvent system of methylene chloride/isopropyl alcohol = 20/1. Physical data of the cycloheximide analogs.

a) **Aldol condensation of the lithium enolates of cyclohexanones (6)** with the aldehyde 8. To a stirred solution of LDA in 20ml of dry tetrahydrofuran, that had been prepared from 2.3 mmol of n-butyllithium and 2.3 mmol of diisopropylamine, was added 2.1 mmol of the ketone at -70°C. After stirring for 15 min, 300mg (1.9 mmol) of 8 was added to the reaction mixture at -70°C. After further stirring for 30 min at -70°C, the mixture was poured into ice-cold 5% aq. acetic acid, extracted and similarly purified.

b) **Aldol condensation of the triisopropoxytitanium enolates of ketones (6)** with 8. The lithium enolates of the ketones, that were prepared by the same method as that already described, were quenched with chlorotitanium triisopropoxide (1m solution in hexane) and stirred for 30 min at -70°C. Compound 8 was then added to the reaction mixture, and after stirring for 1 hr, the reaction mixture was quenched with saturated aq. ammonium fluoride, extracted and similarly purified.

c) **Aldol condensation of the trisopropoxytitanium enolates of ketones (6)** with 8. The lithium enolates of the ketones, that were prepared by the same method as that already described, were quenched with chlorotitanium triisopropoxide (1m solution in hexane) and stirred for 30 min at -70°C. The reaction mixture was quenched with saturated aq. ammonium fluoride, extracted and similarly purified.

### General procedure.

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### Physical data of the cycloheximide analogs.

a) **4-[2-(2-Oxocyclohexyl)-2-hydroxyethylj-2,6-piperidinedione (2a, b).** 2a: mp 106.0~106.5°C; IR $v_{max}$ (KBr) cm$^{-1}$: 3400, 3280, 3100, 1710, 1695, 1275, 1150, 1005; 13C-NMR (CDCl$_3$) $\delta$: 24.9, 27.0, 27.7, 30.6, 37.1, 38.4, 38.7, 42.8, 56.3, 69.0, 172.3, 217.6. *Anal. Found: C, 61.43; H, 7.54; N, 5.48. Calcd. for C$_{14}$H$_{21}$O$_4$N: C, 61.64; H, 7.56; N, 5.53%. HPLC: $t_R$ = 5.8 min.**

b) **4-[2-(3-Methyl-2-oxocyclohexyl)-2-hydroxyethylj-2,6-piperidinedione (3a~d).** 3a: mp 112.0~112.6°C; IR $v_{max}$ (KBr) cm$^{-1}$: 3430, 3230, 3100, 1700, 1280, 1265, 1150, 1015; 13C-NMR (CDCl$_3$) $\delta$: 14.2, 25.0, 27.1, 31.6, 37.1, 38.6, 38.7, 46.2, 56.5, 69.0, 172.6, 172.7, 216.0. *Anal. Found: C, 62.72; H, 7.93; N, 5.25. Calcd. for C$_{14}$H$_{21}$O$_4$N-C$_{14}$H$_{21}$O$_4$N: C, 62.90; H, 7.92; N, 5.24%. HPLC: $t_R$ = 6.4 min.**

c) **4-[2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethylj-2,6-piperidinedione (4a~d).** 4a: mp 107.0~108.0°C; IR $v_{max}$ (KBr) cm$^{-1}$: 3460, 3280, 3100, 1700, 1375, 1250, 1150, 1005; 13C-NMR (CDCl$_3$) $\delta$: 24.9, 27.0, 27.7, 30.6, 37.1, 38.4, 38.7, 42.8, 56.3, 69.0, 172.3, 217.6. *Anal. Found: C, 61.43; H, 7.54; N, 5.48. Calcd. for C$_{14}$H$_{21}$O$_4$N: C, 61.64; H, 7.56; N, 5.53%. HPLC: $t_R$ = 5.8 min.**

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c) **4-[2-(3,5-Dimethyl-2-oxocyclohexyl)-2-hydroxyethylj-2,6-piperidinedione (4a~d).** 4a: mp 107.0~108.0°C; IR $v_{max}$ (KBr) cm$^{-1}$: 3460, 3280, 3100, 1700, 1375, 1250, 1150, 1005; 13C-NMR (CDCl$_3$) $\delta$: 24.9, 27.0, 27.7, 30.6, 37.1, 38.4, 38.7, 42.8, 56.3, 69.0, 172.3, 217.6. *Anal. Found: C, 61.43; H, 7.54; N, 5.48. Calcd. for C$_{14}$H$_{21}$O$_4$N: C, 61.64; H, 7.56; N, 5.53%. HPLC: $t_R$ = 5.8 min.**
Synthesis of Demethylcycloheximides

5, 1.2 ml/min flow rate): t_R = 6.9 min. 4b: mp 153.0~154.0°C; IR v_max (KBr) cm⁻¹: 3460, 3260, 3220, 1705, 1380, 1255, 1200, 1105, 830; ¹³C-NMR (CDCl₃) δ: 21.4, 27.6, 31.7, 34.7, 35.5, 37.3, 37.7, 38.5, 41.7, 54.2, 66.5, 172.0, 172.2, 214.4. Anal. Found: C, 62.41; H, 7.91; N, 5.82. Calcd. for C₁₄H₂₁O₄N: C, 62.90; H, 7.92; N, 5.24%.

HPLC: t_R = 8.5 min. 4c: mp 135.0~135.8°C; IR vmax (KBr) cm⁻¹: 3420, 3250, 3090, 1705, 1695, 1725, 1726, 2156. Anal. Found: C, 62.64; H, 7.89; N, 5.18. Calcd. for C₁₄H₂₁O₄N: C, 62.90; H, 7.92; N, 5.24%.

HPLC: t_R = 8.2 min. 4d: mp 101.0~103.0°C. IR vmax (KBr) cm⁻¹: 3450, 3220, 3095, 1700, 1375, 1265, 1150, 1070, 1005, 840; ¹³C-NMR (CDCl₃) δ: 18.6, 27.6, 31.7, 34.7, 35.5, 37.3, 37.7, 38.5, 52.1, 69.1, 172.5, 172.6, 215.6. Anal. Found: C, 62.18; H, 8.01; N, 5.28. Calcd. for C₁₄H₂₁O₄N: C, 62.90; H, 7.92; N, 5.24%.

HPLC: t_R = 8.9 min.

d) 4-[2-[(3)-Isopropyl-6-methyl-2-oxocyclohexyl]-2-hydroxyethyl]-2,6-piperidinedione (5a, b). 5a: mp 61.8~62.5°C; IR v_max (KBr) cm⁻¹: 3470, 3200, 2360, 3090, 1700, 1375, 1265, 1150, 1070, 1005, 840; ¹³C-NMR (CDCl₃) δ: 18.8, 20.2, 21.6, 26.0, 27.8, 30.8, 34.6, 37.2, 38.5, 38.9, 40.9, 58.6, 62.0, 67.3, 172.2, 172.4, 217.7. Anal. Found: C, 65.75; H, 8.99; N, 4.45. Calcd. for C₁₇H₂₇O₄N: C, 65.99; H, 8.80; N, 4.53%.

HPLC (Finesil-5, 1.5 ml/min flow rate): t_R = 4.0 min. 5b: mp 150.0~157.0°C; IR v_max (KBr) cm⁻¹: 3470, 3200, 3090, 1700, 1280, 1290, 1265, 1150, 1090, 1040, 820; ¹³C-NMR (CDCl₃) δ: 18.6, 27.6, 31.7, 34.7, 35.5, 37.3, 37.7, 38.5, 52.1, 69.1, 172.5, 172.6, 217.7. Anal. Found: C, 65.77; H, 8.81; N, 4.56. Calcd. for C₁₇H₂₇O₄N: C, 65.99; H, 8.80; N, 4.53%.

Antimicrobial assay of the cycloheximide analogs (2~5) against Saccharomyces cerevisiae, Aspergillus niger and Cochliobolus miyabeanus. The antimicrobial activity of the synthesized analogs was determined by the conventional paper disc method against S. cerevisiae, A. niger, and C. miyabeanus. S. cerevisiae was cultured in a liquid malt medium at 28°C for 48hr, and diluted 100 fold with 1.2% agar malt medium. A. niger and C. miyabeanus were cultured on a slant potato medium at 28°C for 1 week. After adding sterilized water and shaking, the supernatants were diluted with 1.2% agar potato medium. The cultured broth of each strain was layered on a Petri dish, and 4 paper discs (8 mm, thin) containing each test sample were placed in position. After 48 hr at 28°C, the growth inhibitory zones around the discs were measured to give the results shown in Table IV. Compounds 2a, 3a, 3c, 3d, 4a, 4b and 4c showed no activity at a concentration of 100 µg/disc, and 5a and 5b also showed no activity at a concentration of 200 µg/disc.

Germination inhibitory test against lettuce (Lactuca sativa L. var. Green Lake). Four lettuce seeds were germinated in a sample tube with a filter paper soaked in a test solution (containing 0.05% Tween 20) for 5 days at 28°C in the light (ca. 5000 lux). The number of germinated seeds was measured. The germination rates (control, 100%) were: 3b, 50%; 3d, 63%; 4d, 20%; 5a, 25% (each at 100 ppm); and 1, 0% at 100 ppm, 10% at 50 ppm and 40% at 10 ppm. The other compounds showed no activity.

Rice seedling test. Rice seedlings (Sasahishiki, an ordinary variety of Oryzae sativa L.), which were germinated in water for 2 days at 28°C, were grown in a sample solution containing 0.7% agar in a sample tube. After growing for 5 days at 28°C in the light (ca. 5000 lux), the length of the second leaf sheath was measured.

REFERENCES