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Synthesis and Antimicrobial Activity of Optically Active *trans*-Cycloheximide Isomers

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Optically active *trans*-cycloheximide isomers such as cycloheximide [(2*S*,4*S*,6*R*,α*R*)-form (**1**)], naramycin B[(2*S*,4*S*,6*R*,α*R*)-form (**4**)], and new stereoisomers (2*S*,4*S*,6*S*,α*S*)-form (**8**) and (2*S*,4*S*,6*R*,α*S*)-form (**9**) were synthesized by an aldol condensation of *trans*-2,4-dimethyl-1-cyclohexanone (**5b**), with 4-(2-oxoethyl)-2,6-piperidinedione (**6**). The antimicrobial activity of *trans*-cycloheximide isomers (**1**, **4**, **8**, and **9**) was examined against *S. cerevisiae* and *P. oryzae*. The stereoisomers **1** and **4** exhibited marked antimicrobial activity against both microorganisms as compared with their C- α-epimers **8** and **9**.

Cycloheximide (**1**) is a powerful antimicrobial agent isolated from the cultured broth of *Streptomyces griseus*.[1] The stereochemistry of **1** has been elucidated by Johnson *et al.*[2] and confirmed by X-ray analysis.[3] Among the stereoisomers of **1**, isocycloheximide [(2*R*,4*S*,6*R*,α*R*)-form (**2**)], α-epi-isocycloheximide [(2*R*,4*S*,6*R*,α*S*)-form (**3**)] and naramycin B (**4**) are known in nature. Antimicrobial activity of cycloheximide isomers is unknown except for natural ones. Based on the stereochemical relationship of the 2,4-dimethyl-1-cyclohexanone (2,4-DMC) moiety, the stereoisomers of **1** are classified into two groups: *cis*-cycloheximide isomers containing *cis*-2,4-DMC and *trans*-cycloheximide isomers containing *trans*-2,4-DMC. Synthesis of *cis*-cycloheximide isomers (**2** and **3**)[4,5] was easily accomplished by an aldol condensation (Nielsen condensation) of the magnesium enolate **7a** prepared from *cis*-2,4-DMC (**5a**) with the aldehyde **6**. The synthesis of *trans*-cycloheximide isomers (**1** and **4**) was more difficult. Johnson *et al.*[6] have synthesized **1** through condensation of the enamine, prepared from *cis*-2,4-DMC (**5a**), with the aldehyde **6** in a low yield. Okuda *et al.*[4] have obtained α-epi-isocycloheximide (**3**) mainly by a Nielsen condensation of (+)-*trans*-2,4-DMC (**5b**) with **6**, but did not obtain *trans*-cycloheximide isomers. This result was explained by the epimerization of C-2 position during preparation of the enolate **7a** with *N*-methylanilino magnesium bromide. Nevertheless, aldol condensation still seems to be most promising method for the synthesis of *trans*-cycloheximide isomers, because it is the shortest route to synthesize isomers of **1**, and many modifications of the reaction with high stereoselectivity are known at the present time. House *et al.*[7] have reported a lithium-mediated *threo* selective aldol condensation of cyclic ketones, and Kuwajima *et al.* have reported a titanium-[8] and tin-[9] mediated *erythro* selective aldol reaction of cyclic ketones. Therefore, we reinvestigated the synthesis of *trasns*-cycloheximide isomers by a revised aldol condensation using these useful metal reagents.

Firstly, aldol condensation of the lithium enolate **7b**, prepared from the (+)-*trans*-ketone **5b**,[10] with the aldehyde **6** was investigated. The lithium enolate **7b**, generated from the ketone **5b** and lithium diisopropylamide (LDA), was reacted with the aldehyde **6**[5] at −70°C to give a stereoisomeric mixture of *trans*-cycloheximides (**1**, **4**, **8** and **9**) (58.0% yield). The mixture was separated into each

component by medium pressure liquid chromatography (MPLC) on silica-gel, and their structures were elucidated by ^{13}C- and ^{1}H-NMR spectra (Tables I and II). The spectral data of synthetic **1** were identical with those of natural cycloheximide (**1**).[12,14] The ^{1}H-NMR spectrum of **4** was also in good accordance with that of naramycin B (**4**).[11] Chemical shift differences among the methyl carbons afforded an important indication for assignment of the stereochemistry of the 2,4-DMC moiety on cycloheximides. As the chemical shifts of the methyl carbons (δ 14.0, 18.4 ppm) of **8** coincided well with those of **1** (δ 14.2, 18.4 ppm), it is clear that **8** has *equatorial* C-2 methyl and *axial* C-4-methyl groups. It is also apparent that **9** has *axial* C-2-methyl and *equatorial* C-4-methyl groups from the agreement of the methyl signals between **9** (δ 17.7, 21.6 ppm) and **4** (δ 17.6, 21.5 ppm). The splitting pattern of the C-α-proton was a good indicator to assign the C-α-stereochemistry of the stereoisomers (**1**, **4**, **8** and **9**) as shown in Table II. After exchanging a hydroxyl proton with D_2O, signals of a C-α-proton in **1** (*erythro* aldol) and **9** showed d-d-d coupling (**1**: $J=10.2$, 5.4, 5.4 Hz; **9**: $J=10.5$, 5.3, 5.3 Hz). This fact showed that **9** is *erythro* aldol. As the splitting

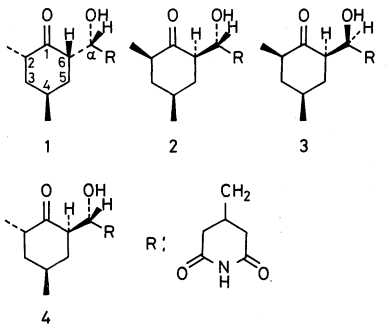

FIG. 1. Structure of Natural Cycloheximide Isomers.

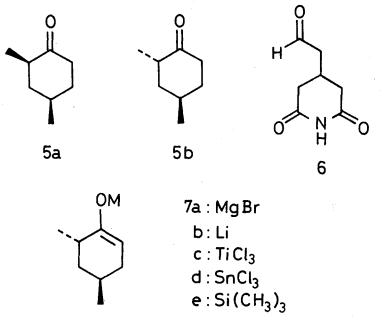

FIG. 2.

TABLE I. ^{13}C-NMR DATA FOR SYNTHETIC CYCLOHEXIMIDES

Compound	Chemical shifts[a] of methyl signals (ppm)	
	At C-2	At C-4
1	14.2	18.4
1[b]	14.2 (*eq*)	18.4 (*ax*)
4	17.6 (*ax*)	21.5 (*eq*)
8	14.0	18.4
9	17.7	21.6

[a] The known orientation of methyl groups is shown in brackets: *eq*, *equatorial*; *ax*, *axial*.
[b] Reported data, see ref. 12.

TABLE II. ^{1}H-NMR DATA FOR SYNTHETIC CYCLOHEXIMIDE ISOMERS

Compound	Chemical shifts, ppm (J Hz)	
	Methyl proton	C-α-Proton
1	0.99 (6.4), 1.25 (7.1)	4.2 (d-d-d, $J=10.2$, 5.4, 5.4)
1[a]	0.98 (6.1), 1.23 (6.7)	4.1[b]
4	0.99 (6.3), 1.22 (7.3)	3.8 (m)
4[a]	0.98 (5.9), 1.21 (7.4)	3.7[b]
8	0.99 (6.4), 1.26 (6.8)	3.8 (m)
9	1.00 (6.3), 1.22 (7.6)	4.2 (d-d-d, $J=10.5$, 5.3, 5.3)

[a] Data of natural products. See ref. 11. ^{1}H-NMR spectra of the authentic **1** (mp 115~116°C), obtained by recrystallization of commercially available reagent (purchased from Nakarai Chemical Co., Ltd.) agreed completely with that of synthetic **1**.
[b] Coupling constants are not described.

pattern of the C-α proton in **4** (*threo* aldol) and **8** is multiplet, **8** was deduced to be *threo* aldol.

Next, an aldol reaction of the trichlorotitanium enolate **7c** and trichlorotin enolate **7d** with the aldehyde **6** were individually examined. The enolates, **7c** and **7d** were prepared by reaction of the silyl enol ether **7e** with titanium tetrachloride and tin tetrachloride, respectively. Both enolates **7c** and **7d** were reacted with the aldehyde **6** at −45°C to give a mixture of *trans*-cycloheximide isomers in a low yield (19.2% and 8.2%). As shown in Table III, the reaction products from the enolate **7c** contained a higher content of *erythro* aldol (**1** and **9**) than those from the lithium enolate **7b**. In the case of enolate **7d**, the isomer **4** was produced as a main product.

These results show that an aldol condensation of the *trans*-ketone **5b** with the aldehyde **6** afforded *trans*-cycloheximide isomers as expected, although the reaction yield and content of cycloheximide (**1**) were low.

Finally, the antimicrobial activity of *trans*-cycloheximide isomers (**1**, **4**, **8** and **9**) was examined against *Saccharomyces cerevisiae* and *Pyricularia oryzae* (Table IV). The stereoisomers **1** and **4** showed marked antimicrobial activity against both microorganisms as compared with their C-α-epimers **8** and **9**. Especially, **8**, a C-α-epimer of cycloheximide, did not show growth inhibition against *S. cerevisiae*. Recently, Berg *et al.*[15] have reported that natural naramycin B (**4**) inhibited the growth of yeasts and some phyto-

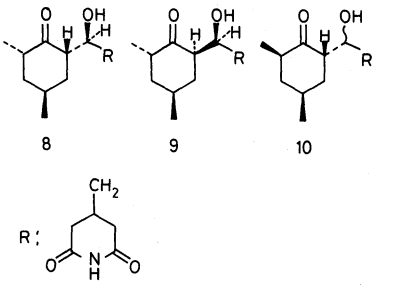

FIG. 3.

TABLE III. COMPOSITION OF *trans*-CYCLOHEXIMIDE ISOMERS

Enolate	Compound No.			
	1	**4**	**8**	**9**
7b	2.8[a]	37.5	50.3	9.4
7c	28.9	21.1	31.6	18.4
7d	9.5	69.7	—	20.8

[a] % composition of each isomer in *trans*-cycloheximide isomers obtained by aldol condensation.

TABLE IV. ANTIMICROBIAL ACTIVITY OF *trans*-CYCLOHEXIMIDE ISOMERS

Compounds	Concentration (μg/disc)	Inhibited zone (mm)			
		S. cerevisiae (HUT 7099)		*P. oryzae* (IFO 5279)	
		48 hr	72 hr	48 hr	72 hr
2*S*,4*S*,6*S*, α*R*-form (**1**)	1	27.0	25.0	19.0	17.0
	10	36.0	35.0	51.0	43.0
	100	43.0	40.0	59.0	58.0
2*S*,4*S*,6*R*, α*R*-form (**4**)	1	—	—	13.0	—
	10	20.0	20.0	17.0	13.0
	100	34.0	32.0	33.0	31.5
2*S*,4*S*,6*S*, α*S*-form (**8**)	100	—	—	14.5	14.0
2*S*,4*S*,6*R*, α*S*-form (**9**)	10	10.0	10.0	14.5	11.0
	100	17.0	17.0	19.0	17.5

pathogens. Our results reconfirmed the strong antimicrobial activity of **4**.

Further work is under way to synthesize the optically active *cis*-cycloheximide isomers from (+)- and (−)-*cis*-2,4-DMC (**5a**).[13]

EXPERIMENTAL

All boiling points and melting points are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL JNM FX-100 spectrometer. IR spectra were recorded on a JASCO IR-810 infrared spectrometer. Optical rotations were measured on a JASCO DIP-4 spectrometer. Circular dichroism (CD) spectra were measured on a Dichrograph Mark III-J spectrometer using a 1 cm cell in MeOH. Gas chromatographic analyses were performed on a JEOL JGC-1100 instrument with a thermal conductance detector and stainless steel column (2 m × 3 mm) packed with 20% DEGS on Chromosorb W (column temp, 95°C; He flow rate, 15 ml/min). High pressure liquid chromatography (HPLC) was performed on a JASCO TRIROTAR instrument with a UV spectrometer (at 254 nm) and stainless steel column (4.6 mm × 250 mm) packed with silica-gel (SS-05) (solvent system: methylene chloride/isopropyl alcohol=98/2; flow rate: 1 ml/min). MPLC were performed on the same instrument using a glass column packed with a silica-gel (Lichroprep Si 60, 40～63 μm) and using the same solvent system.

1) (+)-*trans-2,4-Dimethyl-1-cyclohexanone* (**5b**). Cycloheximide (**1**) (15 g) was degraded by heating at 220°C to give 2.61 g (38.9%) of the isomeric mixture of 2,4-DMC: 63～65°C (20 mmHg); $[\alpha]_D^{20}$ +58.3° (c=30.6, EtOH). It consisted of 89.6% of *trans*-2,4-DMC (**5b**) (t_R: 12.8 min) and 10.4% of *cis*-2,4-DMC (**5a**) (t_R: 10.8 min) by GLC analysis. The mixture was used in the following reactions.

2) Aldol condensation of the lithium enolate **7b** *with the aldehyde* **6**. To a stirred solution of LDA in 50 ml of dry tetrahydrofuran, prepared from 5.7 mmol of *n*-butyllithium and 5.7 mmol of diisopropylamine, was added 720 mg (5.7 mmol) of the ketone **5b** and then 880 mg (5.7 mmol) of the aldehyde **6**[5] at −70°C. After stirring at −70°C for 30 min, the mixture was poured into ice-cooled dil. acetic acid and extracted with methylene chloride three times. The combined extracts were washed with aq. $NaHCO_3$, brine and dried over anhyd. $MgSO_4$. Evaporation of the solvent left 1.32 g of an oil, which was chromatographed on silica-gel with a solvent system of methylene chloride/isopropyl alcohol (98/2) to give the starting ketone (205 mg), optically active *trans*-cycloheximide isomers (**1**, **4**, **8** and **9**) (929 mg) and optically active neocycloheximide (**10**)[11] (131 mg). The optically active *trans*-cycloheximide isomers consisted of 50.3% of **8** (t_R: 14.0 min), 37.5% of **4** (t_R: 15.2 min), 2.8% of **1** (t_R: 17.0 min) and 9.4% of **9** (t_R: 18.0 min) by HPLC analysis. Repeated fractionation by MPLC gave pure hydroxy ketones, **8** (91 mg), **4** (99 mg), **1** (9 mg) and **9** (72 mg). The physical data are as follows. **8**: mp 106～107°C; IR ν_{max}^{KBr} cm^{-1}: 3350, 3190, 3010, 1705, 1375, 1280, 1270, 1120, 875, 820; $[\alpha]_D^{20}$ −23.5° (c=2.5, MeOH); ^{13}C-NMR ($CDCl_3$) δ: 14.0 (q), 18.4 (q), 27.0, 37.0, 37.2, 38.5, 38.7, 40.8, 42.6, 51.3, 69.0, 172.5, 172.6, 217.0. *Anal.* Found: C, 63.80; H, 8.23; N, 4.97, Calcd. for $C_{15}H_{23}O_4N$: C, 64.03; H, 8.24; N, 4.98%. **4**: mp 117.5～118.5°C; IR ν_{max}^{KBr} cm^{-1}: 3350, 3220, 3080, 1710, 1380, 1295, 1275, 1150, 1005, 820; $[\alpha]_D^{20}$ +85.6° (c=0.7, MeOH); ^{13}C-NMR ($CDCl_3$) δ: 17.6 (q), 21.5 (q), 25.9, 27.0, 37.0, 38.1, 38.3, 38.7, 41.2, 44.8, 51.0, 68.7, 172.5, 172.6, 219.2. *Anal.* Found: C, 63.64; H, 8.31; N, 4.97, Calcd. for $C_{15}H_{23}O_4N$: C, 64.03; H, 8.24; N, 4.98%. **1**: mp 110～111°C; IR ν_{max}^{KBr} cm^{-1}: 3520, 3470, 3200, 3090, 1720, 1685, 1380, 1280, 1270, 1250, 1150; CD ($c=2\times10^{-4}$, MeOH) $[\theta]_{max}$: -0.9×10^3 (λ_{max}: 295 nm) (authentic sample [14]): $[\theta]_{max}$: -1.0×10^3 (λ_{max}: 295 nm) at the same concentration); ^{13}C-NMR ($CDCl_3$) δ: 14.2 (q), 18.4 (q), 26.7, 27.6, 33.1, 37.3, 37.9, 38.5, 40.5, 42.6, 50.1, 66.6, 172.1, 172.2, 216.3. *Anal.* Found: C, 63.69; H, 8.28; N, 4.96, Calcd. for $C_{15}H_{23}O_4N$: C, 64.03; H, 8.24; N, 4.98%. **9**: mp 98.5～99.5°C; IR ν_{max}^{KBr} cm^{-1}: 3450, 3260, 1705, 1390, 1270, 1255, 1155, 1110, 830, 810; $[\alpha]_D^{20}$ +74.7° (c=0.55, MeOH); ^{13}C-NMR ($CDCl_3$) δ: 17.7 (q), 21.6 (q), 25.9, 27.6, 34.4, 37.2, 37.9, 38.5, 41.1, 44.5, 50.1, 66.6, 172.3, 172.5, 218.2. *Anal.* Found: C, 63.67; H, 8.28; N, 5.03, Calcd. for $C_{15}H_{23}O_4N$: C, 64.03; H, 8.24; N, 4.98%.

3) Preparation of trimethylsilyl enol ether (*TMS ether*) **7e**. To a stirred solution of LDA in 50 ml of dry tetrahydrofuran, prepared from 13.4 mmol of *n*-butyllithium and 13.4 mmol of diisopropylamine, was added 1.41 g (11.2 mmol) of the ketone **5b** at −70°C. A few minutes later, 2.0 g of trimethylsilyl chloride was added in one portion. The temperature was maintained for 1 hr and then raised to room temperature. After stirring for 2 hr, the reaction mixture was poured into aq. $NaHCO_3$ and extracted with *n*-hexane. The extract was washed with brine and dried over anhyd. $MgSO_4$. Evaporation of the solvent, followed by distillation under reduced pressure gave an oily **7e** (1.2 g) (t_R: 3.4 min by GLC analysis): bp 85～86°C (15 mmHg); IR ν_{max}^{film} cm^{-1}: 2960, 2910, 1670; ^{1}H-NMR ($CDCl_3$) δ: 0.93 (3H, d, J=5.9 Hz), 1.05 (3H, d, J=6.8 Hz), 3.7 (1H).

4) Aldol condensation of the trichlorotitanium enolate **7c** *with the aldehyde* **6**. To solution of titanium tetrachloride (1 M, 1.5 ml) in melthylene chloride, 350 mg (1.7 mmol) of the TMS ether **7e** was added in one portion and stirred for 5 min at 20°C. After cooling to −45°C, 270 mg (1.7 mmol) of the aldehyde **6** in 2 ml of methylene chloride was added at once. The mixture was stirred for 5 min and then poured into aq. $NaHCO_3$. After acidification with dil. acetic acid, the mixture was extracted with

methylene chloride. The extract was washed with aq. $NaHCO_3$, brine and dried over anhyd. $\cdot MgSO_4$. Evaporation of the solvent gave an oil (255 mg), from which the fraction of *trans*-cycloheximide isomers (95 mg) was collected by MPLC. This consisted of 31.6% of **8** (t_R: 14.0 min), 21.1% of **4** (t_R: 15.2 min), 28.9% of **1** (t_R: 17.0 min) and 18.4% of **9** (t_R: 18.0 min) by HPLC analysis.

5) *Aldol condensation of the trichlorotin enolate* **7d** *with the aldehyde* **6**. To a solution of tin tetrachloride (1 M, 2.0 ml) in methylene chloride, 350 mg of the TMS ether **7e** (1.7 mmol) was added in one portion and the mixture stirred for 10 min at 20°C. After cooling to −45°C, 270 mg (1.7 mmol) of the aldehyde **6** in 2 ml of methylene chloride was added at once. The mixture was stirred for 5 min and then poured into aq. $NaHCO_3$. After acidification with dil. acetic acid, the mixture was extracted with methylene chloride. The extract was washed with aq. $NaHCO_3$, brine and dried over anhyd. $MgSO_4$. Evaporation of the solvent gave an oil (161 mg), from which the fraction of *trans*-cycloheximide isomers (41 mg) was collected by MPLC. This fraction consisted of 69.7% of **4** (t_R: 15.2 min), 9.5% of **1** (t_R: 17.0 min) and 20.8% of **9** (t_R: 18.0 min) by HPLC analysis.

6) *Antimicrobial assay of trans-cycloheximide isomers against Saccharomyces cerevisiae* (*HUT 7099*) *and Pyricularia oryzae* (*IFO 5279*). The antimicrobial activities of (−)-**1**, (+)-**4**, (−)-**8** and (+)-**9** were determined by the conventional paper disc method against *S. cerevisiae* and *P. oryzae*. Test strains were cultured in a medium containing 2% malt extract, 0.1% peptone, 2% glucose and 0.1% agar at 28°C for 48 hr, and diluted 120 fold with 1% agar medium. The cultured broth of each strain was layered on a Petri dish (diameter 80 mm) and paper discs (8 mm, thin) containing 1, 10 and 100 μg of the respective test samples were placed in position. After 48 and 72 hr at 25°C, the growth inhibitory zones around the discs were measured to give the results shown in Table IV.

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