Synthesis and Biological Activities of Demethylcycloheximides

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(+)-Demethylcycloheximides (**2~4**) and ($\pm$)-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*,¹⁾ has powerful antimicrobial activities against a number of phytopathogens.²⁾ It is known that **1** inhibits the protein synthesis in a eukaryotic cell,³⁾ 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,⁴⁾ 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.^{5,6)} Here, we have synthesized the racemic demethylcycloheximides (**2~5**) by an aldol condensation of the cyclohexanones (**6**) with 4-(2-oxoethyl)-2,6-piperidinedione (**8**)⁴⁾ to elucidate their stereochemistry and their biological activity.

Firstly, a stereoisomeric mixture of dide-methylcycloheximides (**2**) was synthesized by the reaction of the lithium enolate of cyclohexanone (**7**, M = Li, R¹ = R² = R³ = H) with the aldehyde **8**. The composition of the isomers (**2a, b**) was analyzed by high-pressure liquid chromatography (HPLC) to give the results shown in Table I. The mixture was separated into each component by thin-layer

chromatography (TLC) 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*,2'-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 (after D₂O

FIG. 1.

FIG. 2.

TABLE I. COMPOSITION OF DEMETHYLCYCLOHEXIMIDE ISOMERS^a

Ketone	Enolate 7 (M=)	Compound No.				Yield (%)
		2a	2b			
6a	Li	79	21			42
	Ph ₃ Sn	60	40			35
	(iPrO) ₃ Ti	41	59			42
		3a	3b	3c	3d	
6b	Li	38	6	41	16	41
	Ph ₃ Sn	29	10	42	19	39
	(iPrO) ₃ Ti	12	14	26	48	46
		4a	4b	4c	4d	
6c	Li	23	9	48	20	57
	(iPrO) ₃ Ti	12	13	29	47	49
		5a	5b			
6d	Li	100				51
	(iPrO) ₃ Ti	33	67			42

^a % composition of each isomer in the aldol condensation products.

exchange). On the other hand, cycloheximide (1) and α -epiisocycloheximide, 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.^{5,6} 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 triisopropoxy-

TABLE II. ¹H-NMR DATA OF CYCLOHEXIMIDE ANALOGS

Compound no.	Chemical shift ppm (J Hz)	
	Methyl-proton	2'-Proton ^a
2a		3.8 (m)
2b		4.17 (ddd, 10.8, 2.5, 2.5)
3a	1.02 (6.4)	3.9 (m)
3b	1.01 (6.3)	4.18 (ddd, 10.8, 2.7, 2.7)
3c	1.17 (6.8)	3.8 (m)
3d	1.17 (7.1)	4.13 (ddd, 10.5, 2.9, 2.9)
4a	1.02 (6.1)	3.8 (m)
4b	1.03 (6.1)	4.17 (ddd, 10.5, 2.7, 2.7)
4c	1.18 (6.8)	3.8 (m)
4d	1.18 (6.8)	4.18 (ddd, 10.5, 2.8, 2.8)
5a	0.86 (6.4), 0.89 (6.4), ^b 1.12 (5.4)	3.75 (dd, 11.4, 2.6)
5b	0.94 (6.8), 0.97 (5.9), ^b 0.79 (6.1)	4.06 (dd, 12.2, 6.8)

^a After D₂O exchange.

^b Isopropyl-methyl.

TABLE III. ¹³C-NMR DATA OF CYCLOHEXIMIDE ANALOGS

Compound no.	Chemical shift, ppm		
	3*-Methyl	5*-Methyl	2'-C
2a			69.0
2b			66.6
3a	14.2		69.0
3b	14.2		66.6
3c	16.4		68.9
3d	16.5		66.7
4a		21.2	68.7
4b		21.4	66.5
4c		18.6	69.1
4d		18.4	66.6
5a ^a			67.3
5b ^b			66.2
1	14.2 (<i>eq</i>)	18.4 (<i>ax</i>)	66.5 (<i>syn</i>)
Naramycin B ^c	17.6 (<i>ax</i>)	21.5 (<i>eq</i>)	68.7 (<i>anti</i>)

^a Isopropyl-methyl, 20.2, 21.6 ppm; 6*-methyl, 18.8 ppm.

^b Isopropyl-methyl, 20.8, 21.0 ppm; 6*-methyl, 14.6 ppm.

^c Reported data, see refs. 4 and 5.

titanium enolates (7, M=Ph₃Sn and (isopropoxy)₃Ti) to obtain the *syn*-aldol as a major product.^{7,8} The results are summarized

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 ($\pm$)-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 ap-

peared 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

Compound	Conc. (μ g/disc)	Inhibited zone (mm)		
		<i>S. cerevisiae</i>	<i>A. niger</i>	<i>C. miyabeanus</i>
2b	100	—	—	+ ^a
3b	100	+	+	+
4d	100	—	—	+
1^b	100	40	15	32
	10	20	+	+
	2	+	—	—

^a + inhibited zone <10 mm.

^b 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*-aldols have relatively strong activities compared with those of the *anti*-aldols. 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. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ 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 254 nm) and stainless steel column (4.6 mm $\times$ 250 mm) packed with silica gel (SS-05 or Finesil-5), using a solvent system of methylene chloride/isopropyl alcohol = 30/1.

General procedure.

a) *Aldol condensation of the lithium enolates of cyclohexanones (6) with the aldehyde 8.* To a stirred solution of LDA in 20 ml 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, 300 mg (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 and extracted with methylene chloride. The organic layer was washed with water, aq. NaHCO_3 and again water, and dried over anhyd. MgSO_4 . After evaporating *in vacuo*, an oily residue was purified by silica-gel column chromatography (methylene chloride/isopropyl alcohol = 20/1) to give a mixture of aldols. The composition of the isomers was analyzed by HPLC, the mixture being separated into each component by preparative TLC on silica-gel (40 $\times$ 20 cm, methylene chloride/isopropyl alcohol = 15/1) and recrystallization from ethyl acetate-hexane.

b) *Aldol condensation of the triphenyltin enolates of ketones (6) with 8.* The lithium enolates of the ketones, which were prepared by the same method as that already described, were quenched with triphenyltin chloride and stirred for 30 min at -70°C . Compound **8** was then added to the reaction mixture, and after stirring for 1 hr at -70°C , the reaction mixture was quenched with 5% aq.

acetic acid, extracted and similarly purified.

c) *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 (1 M 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.

Physical data of the cycloheximide analogs.

a) 4-[2-(2-Oxocyclohexyl)-2-hydroxyethyl]-2,6-piperidinedione (**2a, b**). **2a**: mp $106.0 \sim 106.5^\circ\text{C}$; IR ν_{max} (KBr) cm^{-1} : 3400, 3280, 3100, 1710, 1695, 1275, 1150, 1005; $^{13}\text{C-NMR}$ (CDCl_3) δ : 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 $\text{C}_{13}\text{H}_{19}\text{O}_4\text{N}$: C, 61.64; H, 7.56; N, 5.53%. HPLC (silica-gel SS-05, 2.0 ml/min flow rate): $t_R = 5.8$ min. **2b**: mp $142.5 \sim 143.5^\circ\text{C}$; IR ν_{max} (KBr) cm^{-1} : 3520, 3180, 3100, 1725, 1700, 1380, 1260, 1150, 1105; $^{13}\text{C-NMR}$ (CDCl_3) δ : 24.9, 26.7, 27.7, 37.3, 37.8, 38.6, 42.6, 55.5, 66.6, 172.1, 214.3. *Anal.* Found: C, 61.33; H, 7.76; N, 5.41. Calcd. for $\text{C}_{13}\text{H}_{19}\text{O}_4\text{N}$: C, 61.64; H, 7.56; N, 5.53%. HPLC t_R : 6.6 min.

b) 4-[2-(3-Methyl-2-oxocyclohexyl)-2-hydroxyethyl]-2,6-piperidinedione (**3a ~ d**). **3a**: mp $112.0 \sim 112.6^\circ\text{C}$; IR ν_{max} (KBr) cm^{-1} : 3430, 3230, 3100, 1700, 1380, 1280, 1265, 1150, 1015; $^{13}\text{C-NMR}$ (CDCl_3) δ : 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 $\text{C}_{14}\text{H}_{21}\text{O}_4\text{N}$: C, 62.90; H, 7.92; N, 5.24%. HPLC (Finesil-5, 1.2 ml/min flow rate) $t_R = 6.4$ min. **3b**: mp $147.5 \sim 148.0^\circ\text{C}$; IR ν_{max} (KBr) cm^{-1} : 3450, 3210, 3090, 1695, 1385, 1280, 1270, 1150, 1125, 1103, 830; $^{13}\text{C-NMR}$ (CDCl_3) δ : 14.2, 25.0, 27.6, 27.7, 37.1, 37.3, 38.1, 38.6, 46.0, 55.5, 66.6, 172.2, 172.4, 215.8. *Anal.* Found: C, 62.42; H, 7.90; N, 5.26. Calcd. for $\text{C}_{14}\text{H}_{21}\text{O}_4\text{N}$: C, 62.90; H, 7.92; N, 5.24%. HPLC: $t_R = 7.2$ min. **3c**: mp $143.5 \sim 144.3^\circ\text{C}$; IR ν_{max} (KBr) cm^{-1} : 3510, 3200, 3100, 1710, 1690, 1370, 1275, 1145, 1065, 1000; $^{13}\text{C-NMR}$ (CDCl_3) δ : 16.4, 20.0, 27.0, 29.7, 33.5, 37.1, 38.7, 38.8, 44.8, 53.1, 68.9, 172.5, 172.6, 218.2. *Anal.* Found: C, 62.69; H, 7.82; N, 5.17. Calcd. for $\text{C}_{14}\text{H}_{21}\text{O}_4\text{N}$: C, 62.90; H, 7.92; N, 5.24%. HPLC: $t_R = 8.1$ min. **3d**: mp $100.0 \sim 101.0^\circ\text{C}$. IR ν_{max} (KBr) cm^{-1} : 3440, 3220, 3095, 1700, 1380, 1268, 1150, 1070, 1000, 830; $^{13}\text{C-NMR}$ (CDCl_3) δ : 16.5, 19.8, 26.4, 27.6, 33.4, 37.3, 38.6, 44.8, 52.5, 66.7, 172.7, 172.8, 217.4. *Anal.* Found: C, 62.34; H, 7.99; N, 5.17. Calcd. for $\text{C}_{14}\text{H}_{21}\text{O}_4\text{N}$: C, 62.90; H, 7.92; N, 5.24%. HPLC: $t_R = 9.0$ min.

c) 4-[2-(5-Methyl-2-oxocyclohexyl)-2-hydroxyethyl]-2,6-peperidinedione (**4a ~ d**). **4a**: mp $107.0 \sim 108.0^\circ\text{C}$; IR ν_{max} (KBr) cm^{-1} : 3460, 3230, 3100, 1703, 1380, 1360, 1280, 1265, 1140; $^{13}\text{C-NMR}$ (CDCl_3) δ : 21.2, 26.9, 31.7, 35.5, 37.0, 38.2, 38.4, 38.6, 42.0, 55.2, 68.7, 172.6, 172.8, 215.3. *Anal.* Found: C, 62.76; H, 8.14; N, 5.27. Calcd. for $\text{C}_{14}\text{H}_{21}\text{O}_4\text{N}$: C, 62.90; H, 7.92; N, 5.24%. HPLC (Finesil-

5, 1.2 ml/min flow rate): t_R = 6.9 min. **4b**: mp 153.0~154.0°C; IR $\nu_{\max}$ (KBr) cm^{-1} : 3460, 3260, 3220, 1705, 1380, 1355, 1260, 1105, 830; ^{13}C -NMR (CDCl_3) 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, 5.10. Calcd. for $\text{C}_{14}\text{H}_{21}\text{O}_4\text{N}$: C, 62.90; H, 7.92; N, 5.24%. HPLC: t_R = 7.5 min. **4c**: mp 135.0~135.8°C; IR $\nu_{\max}$ (KBr) cm^{-1} : 3420, 3250, 3090, 1705, 1695, 1380, 1280, 1265, 1150, 1120; ^{13}C -NMR (CDCl_3) δ : 18.6, 26.9, 27.0, 33.0, 36.1, 37.1, 38.4, 38.7, 52.1, 69.1, 172.5, 172.6, 215.6. *Anal.* Found: C, 62.64; H, 7.89; N, 5.18. Calcd. for $\text{C}_{14}\text{H}_{21}\text{O}_4\text{N}$: C, 62.90; H, 7.92; N, 5.24%. HPLC: t_R = 8.1 min. **4d**: mp 101.0~103.0°C. IR $\nu_{\max}$ (KBr) cm^{-1} : 3450, 3220, 3095, 1700, 1375, 1265, 1150, 1070, 1005, 840; ^{13}C -NMR (CDCl_3) δ : 18.4, 27.5, 32.2, 32.6, 37.1, 37.9, 38.1, 38.4, 50.8, 66.6, 171.8, 172.0, 214.7. *Anal.* Found: C, 62.18; H, 8.01; N, 5.28. Calcd. for $\text{C}_{14}\text{H}_{21}\text{O}_4\text{N}$: 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 $\nu_{\max}$ (KBr) cm^{-1} : 3470, 3200, 3090, 1700, 1380, 1290, 1265, 1145, 1090, 1040, 820; ^{13}C -NMR (CDCl_3) 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 $\text{C}_{17}\text{H}_{27}\text{O}_4\text{N}$: 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 156.0~157.0°C; IR $\nu_{\max}$ (KBr) cm^{-1} : 3270, 3205, 3090, 1700, 1385, 1285, 1270, 1150, 1070, 840; ^{13}C -NMR (CDCl_3) δ : 14.6, 20.8, 21.0, 24.8, 27.7, 27.8, 28.3, 33.7, 37.3, 38.7, 40.2, 56.6, 59.3, 66.2, 172.7, 217.0. *Anal.* Found: C, 65.77; H, 8.81; N, 4.56. Calcd. for $\text{C}_{17}\text{H}_{27}\text{O}_4\text{N}$: C, 65.99; H, 8.80; N, 4.53%. HPLC: t_R = 6.8 min.

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 48 hr, 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 $\mu\text{g}/\text{disc}$, and **5a** and **5b** also showed no activity at a concentration of 200 $\mu\text{g}/\text{disc}$.

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 (Sasanishiki, an ordinary variety of *Oryza 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

- 1) B. E. Leach, J. H. Ford and A. J. Whiffen, *J. Am. Chem. Soc.*, **69**, 474 (1947).
- 2) A. J. Whiffen, *J. Bacteriol.*, **56**, 283 (1948).
- 3) (a) M. R. Siegel and H. D. Sisler, *Biochim. Biophys. Acta*, **87**, 70 (1964); (b) M. R. Siegel and H. D. Sisler, *Biochim. Biophys. Acta*, **103**, 558 (1965); (c) L. Felicetti, B. Colombo and C. Baglioni, *Biochim. Biophys. Acta*, **119**, 120 (1966); (d) B. Colombo, L. Felicetti and C. Baglioni, *Biochem. Biophys. Res. Commun.*, **18**, 389 (1965).
- 4) Y. Egawa, M. Suzuki and T. Okuda, *Chem. Pharm. Bull.*, **11**, 582 (1963).
- 5) S. Kudo, T. Oritani and K. Yamashita, *Agric. Biol. Chem.*, **48**, 2315 (1984).
- 6) S. Kudo, T. Oritani and K. Yamashita, *Agric. Biol. Chem.*, **48**, 2739 (1984).
- 7) Y. Yamamoto, H. Yatagai and K. Murayama, *J. Chem. Soc., Chem. Commun.*, **1981**, 162.
- 8) M. T. Reetz and R. Peter, *Tetrahedron Lett.*, **1981**, 4691.