[Chem. Pharm. Bull.
27(12)3039—3048(1979)]

UDC 615.273.5.011.5.015.11 : 547.595.2.04.09

Medicinal Chemical Studies on Antiplasmin Drugs. V.[1] 4-Aminomethylcyclohexanecarboxylic Acid Derivatives having a Methyl Group at C_2 or C_3

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(Received May 30, 1979)

Four isomers of 4-aminomethyl-2-methylcyclohexanecarboxylic acid (**9**) were synthesized from 4-cyano-*o*-toluic acid (**2**) or *cis* or *trans* 2-methyl-4-oxocyclohexanecarboxylic acid (**5**), and the isomers of 4-aminomethyl-3-methylcyclohexanecarboxylic acid (**23**) were also synthesized from 4-cyano-*m*-toluic acid (**13**) or ethyl *cis* or *trans* 7-methyl-1,4-dioxaspiro[4.5]decane-8-carboxylate (**15**). The configurations of these isomers were determined on the basis of those of the starting materials and by leading them to dimethyl 2-methylcyclohexane-1,4-dicarboxylate (**12**), which was compared with **12** obtained from 5-methylbicyclo[2.2.2]oct-2-ene (**10**). The preferred conformations of the isomers in aqueous solution were deduced from the nuclear magnetic resonance spectra. The antiplasmin activity of *t*-4-aminomethyl-*c*-3-methyl-*r*-1-cyclohexanecarboxylic acid (**23B**), which is thought to exist in the 1-*e*, 3-*e*, 4-*e* form in aqueous solution, and which was the most active among the compounds tested in this study, was only about 0.64 times that of *trans* 4-aminomethylcyclohexanecarboxylic acid (**1B**).

Keywords——antiplasmin activity; 4-aminomethylcyclohexanecarboxylic acid; methyl substituent; conformation; configuration; stereoisomer

It has already been shown[3] that replacement of the hydrogen at C_1, C_4 or the side chain of 4-aminomethylcyclohexanecarboxylic acid (AMCHA, **1**) by a methyl group reduced the antiplasmin activity, compared with *trans* AMCHA (**1B**). The presence of the methyl group at C_1 or C_4 may result in a conformational equilibrium different from that of **1B**, which exists predominantly in the aminomethyl- and carboxyl-equatorial form in aqueous solution. A methyl group at the side chain may hinder the interaction between the amino group of the drug and the receptor site.

The presence of a methyl group at C_2 or C_3 of **1** is expected to affect the interaction between the drug and the receptor site less than a methyl group at the side chain, and three among four possible isomers are expected to exist in the aminomethyl-equatorial form, which is assumed to be essential for a cyclohexane derivative to have potent antiplasmin activity. This paper deals with the synthesis, separation, assignment of configuration, and favored conformation in aqueous solution of each of the four isomers of 4-aminomethyl-2-methylcyclohexanecarboxylic acid (**9**) and 4-aminomethyl-3-methylcyclohexanecarboxylic acid (**23**).

4-Aminomethyl-2-methylcyclohexanecarboxylic Acid (9)

Hydrogenation of 4-cyano-*o*-toluic acid (**2**)[4] over Raney Ni afforded 4-aminomethyl-*o*-toluic acid (**3**) as its monohydrate in 85% yield. The structure of **3** was confirmed by its infrared (IR) spectrum, in which amino acid bands appeared at 2900—2130 and 1625 cm^{-1}. Hydrogenation of **3** over PtO_2 in 0.25 N HCl gave a mixture of **9**, from which *c*-4-aminomethyl-*c*-2-methyl-*r*-1-cyclohexanecarboxylic acid (**9A**), mp 270—272° (dec.), was isolated upon

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[1] Part IV: A. Okano, M. Inaoka, S Funabashi, M. Iwamoto, S. Isoda, R. Moroi, Y. Abiko, and M. Hirata, *J. Med. Chem.*, **15**, 247 (1972).

[2] Location: *Minamifunabori-cho, Edogawa-ku, Tokyo 132, Japan.*

[3] S. Isoda and M. Hirata, *Chem. Pharm. Bull.* (Tokyo), **27**, 2735 (1979).

[4] D. Peltier, *Bull. Soc. Sci. Bretagne*, 31, 7 (1956) [*C.A.*, **52**, 9017 (1958)].

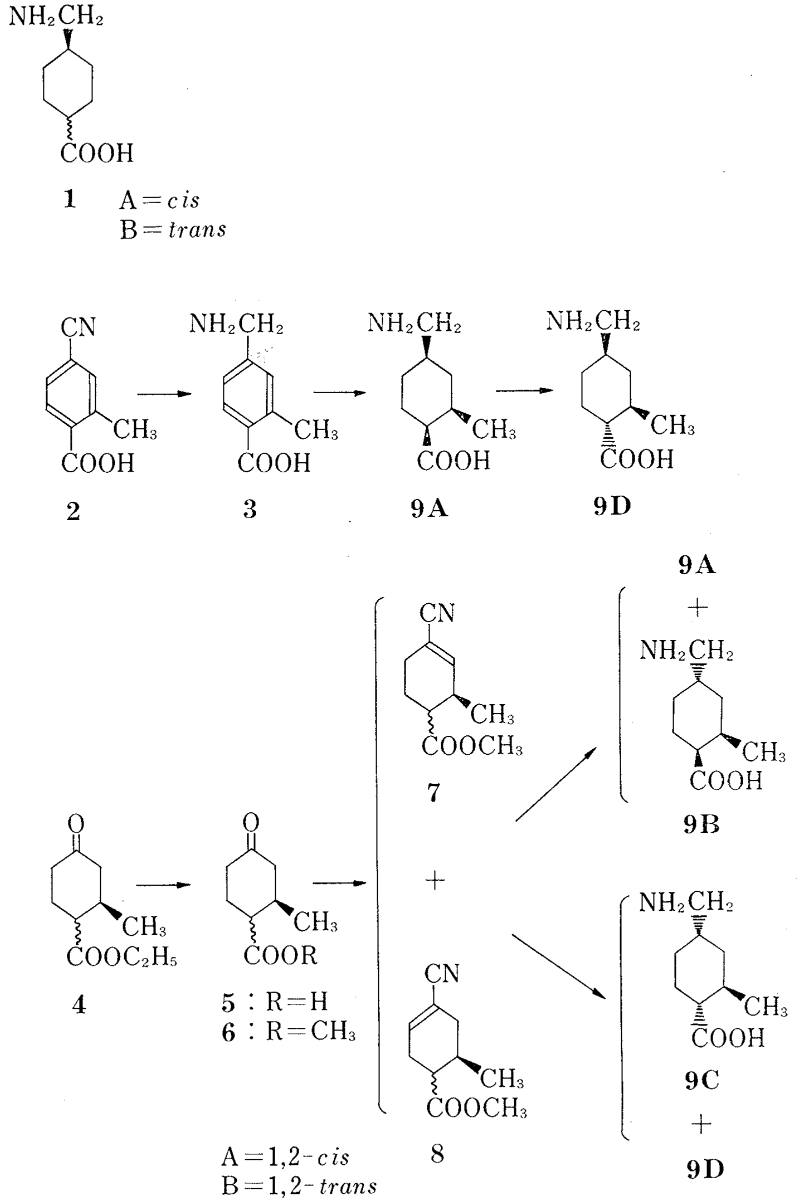

Chart 1

recrystallization from water in 60% yield. Isomerization of **9A** in 1 N NaOH at 200° gave a mixture, from which *t*-4-aminomethyl-*t*-2-methyl-*r*-1-cyclohexanecarboxylic acid (**9D**) was isolated as its *p*-tosylate, mp 259—262°. Decomposition of **9D** *p*-tosylate with ion exchange resin gave **9D**, mp 277—281° (dec.). The other two isomers, *t*-4-aminomethyl-*c*-2-methyl-*r*-1-cyclohexanecarboxylic acid (**9B**) and *c*-4-aminomethyl-*t*-2-methyl-*r*-1-cyclohexanecarboxylic acid (**9C**), could not be obtained in reasonable yield by these procedures. Next, two sets of isomers, **9A** and **9B**, and **9C** and **9D**, were obtained as follows.

Solidification and recrystallization of the hydrolysate of ethyl 2-methyl-4-oxocyclohexanecarboxylate (**4**)[5] gave pure *cis* 2-methyl-4-oxocyclohexanecarboxylic acid (**5A**). The *trans* isomer **5B** was obtained according to the procedure of Crenshaw *et al.*[5] After esterification with diazomethane, their purities were checked by gas chromatography (GC) of the methyl esters, **6A** and **6B**. Dehydration of **6A** cyanohydrin with $POCl_3$–pyridine afforded a liquid

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5) R.R. Crenshaw, G.M. Luke, T.A. Jenks, R.A. Partyka, G. Bialy, and M.E. Bierwagen, *J. Med. Chem.*, **16**, 813 (1973).

which was a mixture of **7A** and **8A**; two C_2-methyl signals appeared in its nuclear magnetic resonance (NMR) spectrum at 0.90 and 0.94 ppm, in a ratio of about 2: 3. Similarly, dehydration of **6B** cyanohydrin afforded a mixture of **7B** and **8B**; the NMR spectrum showed two C_2-methyl signals at 0.97 and 1.05 ppm and two olefinic proton signals at 6.42 and 6.56 ppm, in a ratio of about 1: 1. GC analysis offered no evidence of isomerization at C_1 during these reactions.

The mixture of **7A** and **8A** was hydrogenated over Raney Ni, and the product was hydrolyzed with 2 N NaOH at room temperature to give a mixture of **9A** and **9B**. Repeated recrystallization of the mixture as its *p*-tosylate gave **9A** *p*-tosylate, mp 184.5—185.5°, and **9B** *p*-tosylate, mp 257—261°, from which **9A**, mp 270—272° (dec.), and **9B**, mp 268—275° (dec.), were obtained, respectively. The lower melting isomer **9A** was identical with that already described by comparison of their IR spectra. A mixture of **9C** and **9D** was obtained from the mixture of **7B** and **8B**. Fractional recrystallization gave **9D**, mp 277—281° (dec.), which was identical with that already described by comparison of their IR spectra. From the mother liquor **9C**, mp 249—255° (dec.) was obtained after isolation as its 3,5-dinitrobenzoate, mp 212—220° (dec.).

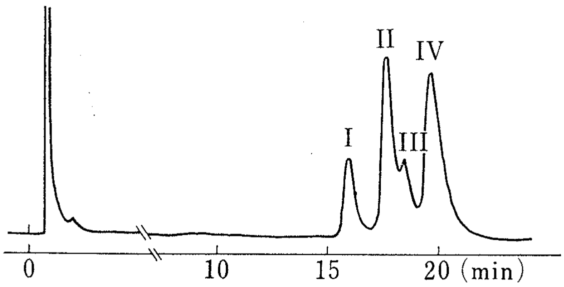

Fig. 1. Gas Chromatogram of Dimethyl 2-Methylcyclohexane-1,4-dicarboxylate (**12**)

Condition b9)
I: **12C** from **9c** or **23C** or **10**.
II: **12D** from **9D** or **23B**.
III: **12A** from **9A** or **23A** or **10**.
IV: **12B** from **9B** or **23D**.

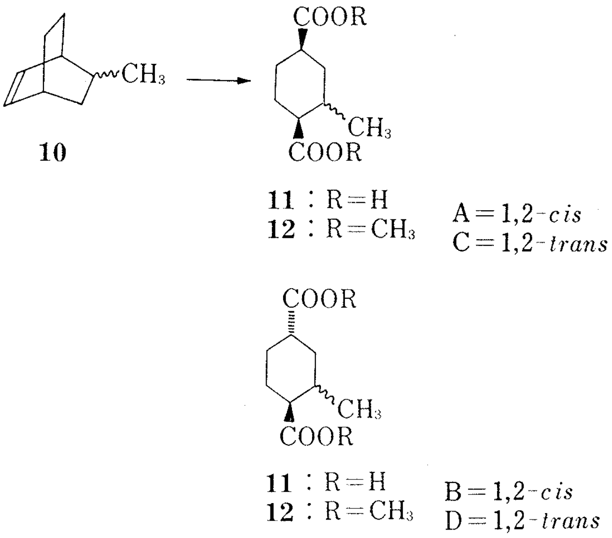

Chart 2

These four isomers were oxidized with $KMnO_4$, and the resulting diacids were esterified with diazomethane. GC analysis of these esters showed that: 1) each of the four isomers was pure, 2) esters obtained from **9A** and **9C** were identical with dimethyl *c*- and *t*-2-methylcyclohexane-*c*-4-*r*-1-dicarboxylate (**12A** and **12C**), respectively, obtained from a mixture of 5-*endo*- and 5-*exo*-methylbicyclo[2.2.2]oct-2-ene (**10**)6) as shown in Chart 2. Thus, the configurations of **9A**—**9D** were determined. Table I shows their physical properties, and Fig. 1 shows a gas chromatogram of the mixture of **12**.

On the basis of the NMR spectra, the favored conformations of **9A**—**9D** in aqueous solution were assumed to be as follows (Fig. 2).

It is thought that **9D** exists predominantly in the all-equatorial form rather than in the all-axial form. The similarities of the methyl signals of the 1,2-*trans* forms, **9C** and **9D**, suggest that **9C** exists in the carboxyl- and methyl-equatorial form, like **9D**. On the other hand, the dissimilarities of those of the 1,2-*cis* forms, **9A** and **9B**, suggest that one of them exists in the carboxyl-axial and methyl-equatorial form and the other in the carboxyl-equatorial and methyl-axial form. The side chain methylene signal of **9C** at 3.16 ppm, which is

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6) M. Tichy, A. Orahovats, and J. Sicher, *Collect. Czech. Chem. Commun.*, **35**, 459 (1970).

Table I. 4-Aminomethyl-2- or 3-methylcyclohexanecarboxylic Acid (**9** and **23**)

No.	mp (°C) (dec.)	Analysis (%) Found			*Rf* value			NMR (in D_2O) δ			IR ν_{max}^{KBr} cm^{-1}
		C Calcd. for $C_9H_{17}NO_2$ 63.12	H 10.10	N 8.18	TLC a)	TLC b)	PPC a)	CH_3	$-\overset{\mid}{N}CH_2$	CH_2, CH (Ring)	
9A	270—272	62.84	9.90	7.84	0.32	0.17	0.49	1.02 (d, *J*=5)	2.95 (d, *J*=6)	1.15—2.5	2900, 2550, 2180, 1620, 1550, 1500, 1400, 1330, 1280, 1025, 730.
9B	268—275	63.12	9.73	8.16	0.21	0.09	0.35	0.90 (d, *J*=7)	2.89 (d, *J*=5)	1.3 —2.5	2950, 2580, 2120, 1660, 1635, 1530, 1400, 1330, 1290, 1190, 1015, 755.
9C	249—255	63.26	10.16	7.99	0.29	0.14	0.42	0.91 (d, *J*=4)	3.16 (d, *J*=7)	1.3 —2.5	2900, 2570, 2180, 1620, 1515, 1385, 1270, 1225, 720.
9D	277—281	63.01	9.98	8.22	0.28	0.11	0.39	0.92 (d, *J*=5)	2.91 (d, *J*=7)	1.3 —2.1	2900, 2550, 2200, 1620, 1550, 1505, 1400, 1290, 1230, 1160, 1030, 730.
23A	258—262	62.90	10.03	8.07	0.30	0.17	0.42	1.00 (d, *J*=7)	3.14 (d, *J*=7)	1.25—2.5	3400, 2930, 2570, 2460, 2150, 1630, 1550, 1520, 1455, 1400, 1285, 1165.
23B	252—255	62.91	10.03	7.97	0.25	0.14	0.39	1.05 (bs)	2.6—3.45 (m)	1.2 —2.3	3400, 2920, 2580, 2150, 1595, 1530, 1450, 1395, 1270, 970, 770, 705.
23C	251—255	62.78	10.03	8.04	0.30	0.22	0.45	1.03 (d, *J*=5)	2.8—3.4 (m)	1.3 —2.2 2.55	3350, 2920, 2520, 2180, 1635, 1540, 1440, 1390, 1280, 1200, 1020, 895.
23D	245—253	62.84	9.93	8.32	0.24	0.16	0.38	0.93 (d, *J*=7)	2.94 (d, *J*=7)	1.35—2.4	3400, 2910, 2590, 2320, 2180, 1630, 1520, 1450, 1300, 970, 925, 700.
1A					0.32[c)] 0.28[d)]	0.14[c)] 0.16[d)]	0.37				
1B					0.27[c)] 0.24[d)]	0.10[c)] 0.15[d)]	0.33				

a) Solvent: isoPrOH–H_2O (7:3).
b) Solvent: *tert*-BuOH–H_2O (3:1).
c) This value corresponds to **9**.
d) This value corresponds to **23**.

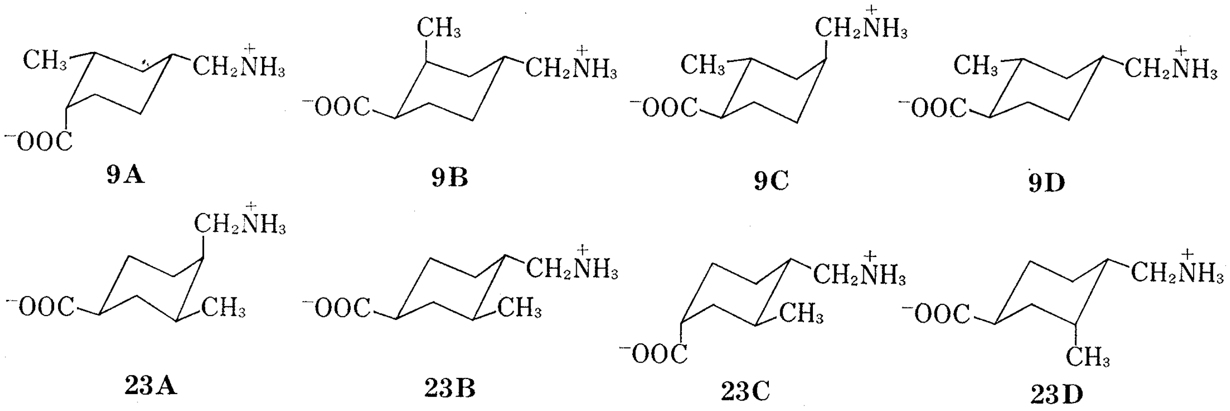

Fig. 2. Preferred Conformations of **9** and **23** in Aqueous Solution

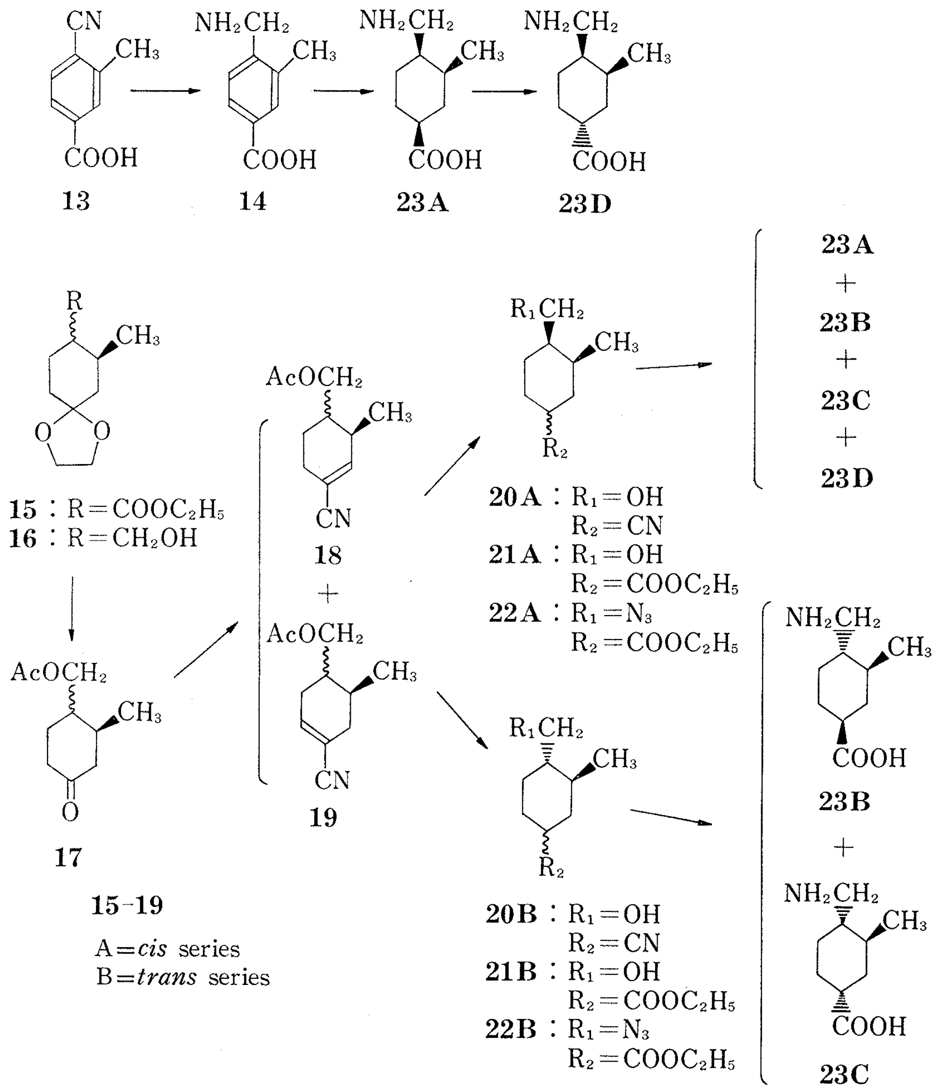

Chart 3

at 0.21—0.27 ppm lower field than those of the other compounds, indicates that only **9C** exists in the aminomethyl-axial form[3] and the others are in the aminomethyl-equatorial forms.

4-Aminomethyl-3-methylcyclohexanecarboxylic Acid (23)

Hydrogenation of 4-cyano-*m*-toluic acid (**13**)[7] over Raney Ni afforded 4-aminomethyl-*m*-toluic acid (**14**) in 53% yield. The structure of **14** was confirmed by its IR spectrum, in which amino acid bands appeared at 2850—2100 and 1620 cm^{-1}. Hydrogenation of **14** over PtO_2 in 1 N HCl gave a mixture of **23**, from which *c*-4-aminomethyl-*c*-3-methyl-*r*-1-cyclohexanecarboxylic acid (**23A**), mp 258—262° (dec.), was isolated as its 3,5-dinitrobenzoate, mp 205—217° (dec.), in 56% yield. Isomerization of **23A** in 0.4 N NaOH at 200° gave a mixture of products, from which *t*-4-aminomethyl-*t*-3-methyl-*r*-1-cyclohexanecarboxylic acid (**23D**), mp 245—253° (dec.), was isolated as its *p*-tosylate, mp 265—269°. The two isomers, **23A** and **23D**, were oxidized with $KMnO_4$, and the resulting diacids were esterified with diazomethane. GC analysis showed that the esters obtained from **23A** and **23D** were identical with the esters from **9A** and **9B**, respectively. The other two isomers, *t*-4-aminomethyl-*c*-3-methyl-*r*-1-cyclohexanecarboxylic acid (**23B**) and *c*-4-aminomethyl-*t*-3-methyl-*r*-1-cyclohexanecarboxylic acid (**23C**), could not be obtained in satisfactory yield by these procedures, but were obtained as follows.

Ethyl *t*-7-methyl-1,4-dioxaspiro[4.5]decane-*r*-8-carboxylate (**15B**),[5] about 92% trans form, was reduced with $LiAlH_4$ in tetrahydrofuran (THF) to give a hydroxymethyl derivative (**16B**) in 89% yield; in turn, **16B** was heated with AcOH to give (*t*-2-methyl-4-oxo-*r*-1-cyclohexyl)methyl acetate (**17B**) in 79% yield. Its trans purity was shown by GC to be about 95%. Dehydration of **17B** cyanohydrin with $POCl_3$–pyridine afforded a mixture of **18B** and **19B** in 89% yield. The mixture gave only two peaks on GC in a ratio of about 65: 35. In the NMR spectrum two olefinic proton signals appeared at 6.45 and 6.61 ppm in a ratio of about 3: 2. Thus, in this step the *trans* isomers **18B** and **19B** were separated from the *cis* isomers, **18A** and **19A**. Reduction of the mixture of **18B** and **19B** with Mg–CH_3OH[8] gave the 3,4-*trans* form of 4-hydroxymethyl-3-methylcyclohexanecarbonitrile (**20B**) in good yield. A solution of **20B** in 17% HCl–EtOH was allowed to stand at room temperature, and addition of water to the resulting iminoether gave the 3,4-*trans* form of ethyl 4-hydroxymethyl-3-methylcyclohexanecarboxylate (**21B**) in 37% yield. The formation of higher boiling material, the structure of which remained undetermined, was responsible for the low yield. Compound **22B**, the 3,4-*trans* form of ethyl 4-azidomethyl-3-methylcyclohexanecarboxylate, was prepared from **21B** *via* **21B** tosylate in a conventional manner. Two methylene (ester) signals at 4.11 and 4.14 ppm in the NMR spectrum of **22B** indicated the presence of isomers at C_1. Hydrogenation of **22B** over Pd–carbon followed by hydrolysis with 2 N HCl afforded a mixture of **23B** and **23C**, from which **23B**, mp 252—255° (dec.), was isolated as its *p*-tosylate. From the mother liquor, **23C**, mp 251—255° (dec.), was isolated as its 3,5-dinitrobenzoate. These two isomers were oxidized with $KMnO_4$ and the resulting diacids were esterified with diazomethane. GC analysis showed that the esters obtained from **23B** and **23C** were identical with the esters from **9D** and **9C**, respectively. Thus, the configurations of **23A**—**23D** were determined. Table I shows the physical properties of **23A**—**23D**.

Next, ethyl *c*-7-methyl-1,4-dioxaspiro[4.5]decane-*r*-8-carboxylate (**15A**),[5] about 95% *cis* form, was allowed to react in the same way as the *trans* form to give a mixture of **18A** and **19A**, in which **18B** and **19B** were absent. The amino acid **23**, which was obtained from the mixture of **18A** and **19A**, gave four peaks on gas chromatography, corresponding to the presence of **23A**—**23D** in a ratio of 31: 6: 7: 56. Isomerization at C_1 occurred to some extent during the stages after **18A** and **19A**.

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[7] Fr. Fichter and G. Shetty, *Helv. Chim. Acta*, **20**, 563 (1937).

[8] J.A. Profitt and D.S. Watt, *J. Org. Chem.*, **40**, 127 (1975).

On the basis of the NMR spectra, the favored conformations of **23A**—**23D** in aqueous solution were assumed to be as follows (Fig. 2). It is thought that **23B** exists in the all-equatorial form rather than in the all-axial form. The similarities of the side chain methylene signals of the 3,4-*trans* forms, **23B** and **23C**, suggest that **23C** exists in the aminomethyl- and methyl-equatorial form, like **23B**. The C_1-hydrogen signal of **23C** at 2.55 ppm, which could not be observed for other isomers due to overlapping with ring methylene signals, indicates that **23C** exists in the carboxyl-axial form. The side chain methylene signal of **23A** at 3.14 ppm, being at 0.20 ppm lower field than that of **23D**, indicates that **23A** exists in the aminomethyl-axial and methyl-equatorial form, and **23D** in the aminomethyl-equatorial and methyl-axial form.

Biological assay showed that the presence of a methyl group at C_2 of **1B** lowers the antiplasmin activity of **1B** more than a methyl group at C_3, and the activity of **23B**, which was the highest among the compounds tested in this study, was only about 0.64 times the activity of **1B**.

Experimental[9]

General Procedure for Separating the Amino Acid——Method A: The solution was applied to a column of Diaion SK#1 or Amberlite IR-120B (H^+ type). The column was washed with H_2O, and the amino acid was eluted with 1.5 N NH_4OH. The eluted solution was evaporated to dryness *in vacuo*.

Method B: The solution was passed through a column of Amberlite IR-45 (OH^- type) and the column was washed with H_2O. The solution was evaporated to dryness *in vacuo*.

4-Aminomethyl-*o*-toluic Acid (3)——A solution of 2[4] (1.61 g, 10 mmol) in 3% NH_4OH (50 ml) was hydrogenated in the presence of Raney Ni (1.0 ml) at room temperature and atmospheric pressure. The hydrogenation was completed in 4 hr. The catalyst was filtered off, and the filtrate was concentrated to half the original volume *in vacuo*. The solution was worked up according to method A, and the residue was recrystallized from H_2O to give 3, (1.41 g, 85%), mp 259—265° (dec.). *Anal.* Calcd. for $C_9H_{11}NO_2 \cdot H_2O$: C, 59.00; H, 7.15; N, 7.65. Found: C, 58.94; H, 7.14; N, 7.51. IR ν_{max}^{KBr} cm^{-1}: 3400, 2900—2100, 1620, 1580, 1530.

***cis* 2-Methyl-4-oxocyclohexanecarboxylic Acid (5A)**——An ice cooled solution of 4[5] (55.4 g, 0.30 mol) in MeOH (160 ml) was treated with 2.75 N KOH (160 ml, 0.44 mol), and the mixture was stirred at room temperature for 2 hr. The solvent was evaporated off *in vacuo* at room temperature, then 6 N HCl (80 ml, 0.48 mol) was added to the ice-cooled residue and the mixture was extracted with $CHCl_3$. The extract was washed with H_2O, dried over Na_2SO_4, and concentrated. The residue was distilled under reduced pressure to give **5A** as a colorless oil (28.9 g, 62%), bp 130—135° (0.5 mmHg) (reported[5] bp 128—131° (0.01 mmHg)), which solidified on standing. Recrystallization from iso-Pr ether gave **5A** as colorless prisms (22.6 g, 48%), mp 50.5—59°. *Anal.* Calcd. for $C_8H_{12}O_3$: C, 61.52; H, 7.75. Found: C, 61.77; H, 7.76. IR ν_{max}^{KBr} cm^{-1}: 3400, 2940, 1700, 1410, 1220, 1060. NMR ($CDCl_3$) δ: 1.02 (3H, d, $J=7$), 2.0—2.6 (7H, m), 2.9 (1H, m), 8.6 (1H, m).

Methyl 2-Methyl-4-oxocyclohexanecarboxylate (6)——*cis* Isomer (**6A**): Compound **5A** (22.0 g) was treated with excess CH_2N_2 in ether. The solvent was evaporated off, and the residue was distilled under reduced pressure to give **6A** as a colorless oil (21.4 g, 89%), bp 112° (5 mmHg). *Anal.* Calcd. for $C_9H_{14}O_3$: C, 63.51; H, 8.29. Found: C, 63.18; H, 8.37. IR ν_{max}^{neat} cm^{-1}: 2950, 1725, 1710, 1430, 1210, 1155. NMR ($CDCl_3$) δ: 0.96 (3H, d, $J=7$), 1.85—2.65 (7H, m), 2.85 (1H, m), 3.72 (3H, s). GC: t_R 36.4 (condition a).

trans Isomer (**6B**): Similarly, **5B**[5] (18.0 g) gave **6B** (19.1 g, 97%), bp 118° (5 mmHg). *Anal.* Calcd. for $C_9H_{14}O_3$: C, 63.51; H, 8.29. Found: C, 63.50; H, 8.16. IR ν_{max}^{neat} cm^{-1}: 2950, 1720, 1430, 1210, 1160. NMR ($CDCl_3$) δ: 0.99 (3H, d, $J=5$), 1.85—2.65 (8H, m), 3.73 (3H, s). GC: t_R 32.9 (condition a).

Methyl 4-Cyano-*c*-2-methyl-3-cyclohexene-*r*-1-carboxylate (7A) and Methyl 4-Cyano-*c*-2-methyl-4-cyclohexene-*r*-1-carboxylate (8A)——A mixture of **6A** (17.0 g, 0.10 mol), acetone cyanohydrin (10.2 g, 0.12 mol) and triethylamine (1.5 g, 0.015 mol) was allowed to stand in an ice box for 3 days. The reaction mixture

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9) Melting points, which were measured with a Yanagimoto melting point apparatus, and boiling points are uncorrected. The IR spectra were measured with a Hitachi 285 spectrophotometer. The NMR spectra were obtained on a Hitachi R-20B spectrometer using tetramethylsilane (TMS) as an internal standard or TMS in CCl_4 as an external standard when D_2O was used as a solvent. Thin-layer chromatography (TLC) was performed on Silica Rider (Daiichi Kagaku) plates, paper chromatography (PPC) was performed on Toyo Roshi No. 50 filter papers, and amino acids were detected by ninhydrin coloration. GC was carried out on a Hitachi K-53 instrument. The conditions were as follows: a) 45 m stainless steel Golay column coated with PEG 20M, column temperature 135°; b) 45 m Golay column coated with BDS, at 130°.

was concentrated under reduced pressure at 30°. $POCl_3$ (16.8 g, 0.11 mol) was added to an ice-cooled solution of this residue in pyridine (27.7 g, 0.35 mol) over a 1.0 hr period. The mixture was stirred at 0—5° for 0.5 hr, warmed at 70° for 0.5 hr, poured into ice-water, and extracted with iso-Pr ether. The extract was washed successively with 5% $NaHCO_3$ and saturated brine, dried over Na_2SO_4, and concentrated. The residue was distilled under reduced pressure to give a mixture of **7A** and **8A** as a colorless oil (13.5 g, 75%), bp 119—122° (1 mmHg). IR ν_{max}^{neat} cm^{-1}: 2950, 2200, 1730, 1635, 1430, 1370. NMR ($CDCl_3$) δ: 0.90 (d, $J=7$), 0.94 (*d*, $J=7$), 1.70—2.90 (6H, m), 3.69 (3H, s), 6.56 (1H, m). GC: t_R 75.6 (condition b).

Methyl 4-Cyano-*t*-2-methyl-3-cyclohexene-*r*-1-carboxylate (7B) and Methyl 4-Cyano-*t*-2-methyl-4-cyclohexene-*r*-1-carboxylate (8B)——Similarly, **6B** (17.0 g) gave a mixture of **7B** and **8B** (9.32 g, 52%), bp 125—130° (1 mmHg). IR ν_{max}^{neat} cm^{-1}: 2950, 2200, 1730, 1635, 1430, 1370. NMR ($CDCl_3$) δ: 0.97 (d, $J=7$), 1.05 (d, $J=7$), 1.7—2.7 (6H, m), 3.70 (3H, s), 6.42 (m), 6.56 (m). GC: t_R 59.0, 67.4 (condition b).

***c*-4-Aminomethyl-*c*-2-methyl-*r*-1-cyclohexanecarboxylic Acid (9A) and *t*-4-Aminomethyl-*c*-2-methyl-*r*-1-cyclohexanecarboxylic Acid (9B)**——A solution of a mixture of **7A** and **8A** (13.5 g, 79 mmol) and conc. NH_4OH (5 ml) in EtOH (100 ml) was hydrogenated in the presence of Raney Ni (5 ml) in an autoclave at room temperature for 12 hr. The initial pressure of H_2 was 120 kg/cm². The catalyst was filtered off, and the filtrate was concentrated *in vacuo*. A solution of this residue in 2 N NaOH (100 ml) was stirred at room temperature for 20 hr. The solution was worked up according to method A to give a mixture of **9A** and **9B** as a white powder (8.41 g, 62%).

Separation of 9A and 9B——A solution of a mixture of **9A** and **9B** (8.41 g) in H_2O (100 ml) was treated with *p*-TsOH·H_2O (9.3 g). The mixture was concentrated *in vacuo*, and fractional recrystallization from H_2O gave **9A**·*p*-TsOH (8.03 g, 45%), mp 184.5—188.5°, and **9B**·*p*-TsOH (0.88 g, 5%), mp 257—261°. A solution of **9A**·*p*-TsOH (8.03 g) in H_2O (200 ml) was worked up according to method A to give a white powder. Repeated recrystallization from H_2O gave **9A** as a white powder (0.68 g, 8%), mp 270—272° (dec.). Similarly, **9B**·*p*-TsOH (0.88 g) gave **9B** as a white powder (0.22 g, 2.6%), mp 268—275° (dec.).

9A from 3——A solution of 3 (0.17 g, 1 mmol) in 0.25 N HCl (20 ml) was hydrogenated in the presence of PtO_2 (0.05 g) at room temperature and atmospheric pressure. The hydrogenation was completed in 6.5 hr. The catalyst was filtered off, the filtrate was worked up according to method B. The residue was recrystallized from H_2O to give **9A** (0.10 g, 60%), mp 270—272° (dec.).

***c*-4-Aminomethyl-*t*-2-methyl-*r*-1-cyclohexanecarboxylic Acid (9C) and *t*-4-Aminomethyl-*t*-2-methyl-*r*-1-cyclohexanecarboxylic Acid (9D)**——Using the procedure described for **9A** and **9B**, a mixture of **7B** and **8B** (9.0 g) gave a mixture of **9C** and **9D** as a white powder (5.49 g, 62%).

Separation of 9C and 9D——A mixture of **9C** and **9D** (5.34 g) was recrystallized twice from H_2O–EtOH to give **9D** as white prisms (1.23 g, 23%), mp 277—281° (dec.). The mother liquor was concentrated *in vacuo*. A part of this residue (2.53 g) was dissolved in H_2O (10 ml), and 3,5-dinitrobenzoic acid (3.14 g) was added. The precipitate was collected and recrystallized twice from 50% EtOH to give **9C** 3,5-dinitrobenzoate, mp 212—220° (dec.). A mixture of the salt (2.50 g) in 1 N HCl (15 ml) was stirred at room temperature for 2 hr. The precipitate was filtered off, and the filtrate was worked up according to method A. The residue was recrystallized from H_2O–EtOH to give **9C** as a white powder (0.85 g, 26%), mp 249—255° (dec.).

9D from 9A——A solution of **9A** (5.0 g) in 1 N NaOH (45 ml) was heated at 200° for 18 hr in an autoclave. The reaction mixture was passed down a column of Diaion SK$\#^1$ (NH_4^+ type) and the column was washed with H_2O. The eluted solution was concentrated *in vacuo*. Next, *p*-TsOH·H_2O (5.7 g) was added to a solution of the residue in H_2O (300 ml). The mixture was evaporated to about one-third of its original volume. The precipitate was collected and recrystallized from H_2O to give **9D**·*p*-TsOH as colorless needles, (2.0 g), mp 259—262°. *Anal.* Calcd. for $C_{16}H_{25}NO_5S$: C, 55.95; H, 7.34; N, 4.08. Found: C, 55.88; H, 7.35; N, 4.22. Using the procedure described for **9A**, **9D**·*p*-TsOH gave **9D** as white prisms in almost quantitative yield.

4-Aminomethyl-*m*-toluic Acid (14)——A solution of 13[7] (11.94 g, 74 mmol) in 2 N NH_4OH (50 ml) was hydrogenated in the presence of Raney Ni (3 ml) in an autoclave at room temperature. The initial pressure of H_2 was 100 kg/cm² and the hydrogenation was completed in 8 hr. The catalyst was filtered off, then the filtrate was concentrated *in vacuo* and worked up according to method A. The residue was recrystallized from H_2O to afford **14** (6.50 g, 53%), mp 281—285° (dec.). *Anal.* Calcd. for $C_9H_{11}NO_2$: C, 65.43; H, 6.71; N, 8.48. Found: C, 65.39; H, 6.75; N, 8.74. IR ν_{max}^{KBr} cm^{-1}: 2850—2150, 1620, 1600, 1580, 1540.

***c*-4-Aminomethyl-*c*-3-methyl-*r*-1-cyclohexanecarboxylic Acid (23A)**——A solution of **14** (3.30 g, 20 mmol) in 1 N HCl (40 ml) was hydrogenated in the presence of PtO_2 (0.40 g) at room temperature and atmospheric pressure; the hydrogenation was completed in 16 hr. The catalyst was filtered off, and the filtrate was worked up according to method A to give a mixture of **23** (3.14 g, 91%). A solution of this residue (2.76g, 16 mmol) in H_2O (18 ml) was treated with a solution of 3,5-dinitrobenzoic acid (3.42 g, 16 mmol) in EtOH (18 ml). The precipitate was collected and recrystallized from 50% EtOH to give **23A** 3,5-dinitrobenzoate as white prisms (3.87 g), mp 205—217° (dec.). *Anal.* Calcd. for $C_{16}H_{21}N_3O_8$: C, 50.13; H, 5.52; N, 10.96. Found: C, 50.10; H, 5.52; N, 11.00. The salt was worked up according to the procedure described for **9C**, and recrystallization from H_2O–EtOH–acetone gave **23A** as white prisms (1.55 g, 56%), mp 258—262° (dec.).

***t*-4-Aminomethyl-*t*-3-methyl-*r*-1-cyclohexanecarboxylic Acid (23D)**——A solution of **23A** (0.34 g, 2 mmol) in 0.4 N NaOH (10 ml, 4 mmol) was heated in an autoclave at 200° for 15 hr. The resulting solution was worked up according to method A. A solution of the residue in H_2O (5 ml) was treated with *p*-TsOH·H_2O (0.38 g). The precipitate was collected and recrystallized from H_2O to give **23D**·*p*-TsOH as white prisms (0.39 g), mp 265—269°. This salt was freed from *p*-TsOH according to method B. Recrystallization from H_2O–EtOH gave **23D** as white needles (0.14 g, 41%), mp 245—253° (dec.).

(7-Methyl-1,4-dioxaspiro[4,5]decane-*r*-8-yl)methanol (16)——*trans* Isomer (**16B**): A solution of **15B**[5] (108.4 g, 0.48 mol) in THF (100 ml) was added dropwise to a suspension of $LiAlH_4$ (14.4 g, 0.38 mol) in THF (500 ml) at room temperature. The solution was refluxed for 0.5 hr and worked up in the usual way to give **16B** as a colorless oil (78.5 g, 89%), bp 123—130° (3 mmHg). IR $\nu_{\max}^{\text{neat}}$ cm^{-1}: 3450, 2920, 2860, 1440, 1140, 1110, 1075. NMR ($CDCl_3$) δ: 0.92 (3H, d, $J=5$), 1.1—1.9 (9H, m), 3.3—3.7 (2H, m), 3.93 (4H, s).

cis Isomer (**16A**): Similarly, **15A**[5] (7.2 g, 31.5 mmol) gave **16A** as a colorless oil (4.98 g, 85%), bp 125—130° (3 mmHg). IR $\nu_{\max}^{\text{neat}}$ cm^{-1}: 3400, 2920, 2860, 1450, 1110, 1070. NMR ($CDCl_3$) δ: 0.93 (3H, d, $J=6$), 1.2—2.1 (9H, m), 3.6—3.8 (2H, m), 3.92 (4H, s).

(2-Methyl-4-oxocyclohexyl)methyl Acetate (17)——*trans* Isomer (**17B**): A solution of **16B** (77 g, 0.41 mol) in AcOH (700 ml) was refluxed for 8 hr. The reaction mixture was distilled under reduced pressure to give **17B** as a colorless oil (60.4 g, 79%), bp 125—126° (6 mmHg). IR $\nu_{\max}^{\text{neat}}$ cm^{-1}: 2950, 1720, 1450, 1365, 1220, 1030. NMR ($CDCl_3$) δ: 1.01 (3H, d, $J=5$), 1.1—2.5 (8H, m), 2.06 (3H, s), 3.9—4.4 (2H, m).

cis Isomer (**17A**): Similarly, **16A** (4.3 g) gave **17A** as a colorless oil (3.7 g, 87%), bp 131—135° (10 mmHg). IR $\nu_{\max}^{\text{neat}}$ cm^{-1}: 2950, 1730, 1710, 1450, 1420, 1370, 1230, 1030. NMR ($CDCl_3$) δ: 0.88 (3H, d, $J=7$), 1.6—2.6 (8H, m), 2.08 (3H, s), 4.07 (2H, d, $J=6$).

(4-Cyano-*t*-2-methyl-3-cyclohexene-*r*-1-yl)methyl Acetate (18B) and (4-Cyano-*t*-2-methyl-4-cyclohexene-*r*-1-yl)methyl Acetate (19B)——A mixture of **17B** (27.6 g, 0.15 mol), acetonecyanohydrin (16.6 g, 0.195 mol) and triethylamine (6.1 g, 0.06 mol) was allowed to stand at room temperature for 5 days. The reaction mixture was concentrated under reduced pressure at 40°. $POCl_3$ (29.9 g, 0.195 mol) was added to an ice-cooled solution of this residue in pyridine (47.5 g, 0.60 mol) over a 0.5 hr period. The mixture was stirred at 0—5° for 0.5 hr, warmed at 70° for 0.5 hr, poured into ice water, and extracted with iso-Pr ether. The extract was washed with 1 N HCl, H_2O, 5% $NaHCO_3$ and H_2O successively, dried over Na_2SO_4, and concentrated. The residue was distilled under reduced pressure to give a mixture of **18B** and **19B** as a colorless oil (25.5 g, 89%), bp 137—139° (4 mmHg). IR $\nu_{\max}^{\text{neat}}$ cm^{-1}: 2950, 2200, 1740, 1635, 1230, 1025. NMR ($CDCl_3$) δ: 1.11 (3H, d, $J=7$), 1.4—2.4 (5H, m), 2.06 (3H, s), 4.1 (2H, m), 6.45 (0.5H, m), 6.61 (0.5H, m). GC: t_R 62.5, 74.4 (condition a).

(4-Cyano-*c*-2-methyl-3-cyclohexene-*r*-1-yl)methyl Acetate (18A) and (4-Cyano-*c*-2-methyl-4-cyclohexene-*r*-1-yl)methyl Acetate (19A)——A colorless oil, bp 147—150° (8 mmHg). IR $\nu_{\max}^{\text{neat}}$ cm^{-1}: 2950, 2210, 1740, 1635, 1230, 1030. NMR ($CDCl_3$) δ: 0.94 (d, $J=7$), 0.95 (d, $J=7$), 1.4—2.6 (m), 2.05 (s), 4.00 (d, $J=8$), 6.60 (m). GC: t_R 77.8 (condition a).

***t*-4-Hydroxymethyl-*c*-3-methyl-*r*-1-cyclohexanecarbonitrile and *c*-4-Hydroxymethyl-*t*-3-methyl-*r*-1-cyclohexanecarbonitrile (20B)**——Mg (12.2 g, 0.50 gatm) was added portionwise to a stirred solution of a mixture of **18B** and **19B** (19.3 g, 0.10 mol) in MeOH (1.0 l) under ice cooling. The temperature of the solution was maintained below 12°. When all the Mg was dissolved, the solution was cooled to 5° and more Mg (12.2 g, 0.50 gatm) was added. The same procedure was repeated once more. The reaction mixture was allowed to stand at room temperature overnight, then the solvent was removed under reduced pressure. Next, iso-Pr ether (1.0 l) and conc. HCl (300 ml) were added to the residue under ice cooling. The iso-Pr ether layer was separated and the water layer was extracted continuously with iso-Pr ether. All the iso-Pr ether was combined and dried over Na_2SO_4. The solvent was removed and the residue was distilled under reduced pressure to give **20B** as a colorless oil (13.3 g, 93%), bp 140—145° (5—7 mmHg). IR $\nu_{\max}^{\text{neat}}$ cm^{-1}: 3400, 2920, 2850, 2230, 1445, 1045. NMR ($CDCl_3$) δ: 0.96 (d, $J=6$), 1.0—3.0 (m), 3.65 (m).

***c*-4-Hydroxymethyl-*c*-3-methyl-*r*-1-cyclohexanecarbonitrile and *t*-4-Hydroxymethyl-*t*-3-methyl-*r*-1-cyclohexanecarbonitrile (20A)**——A colorless oil, bp 135° (3 mmHg). IR $\nu_{\max}^{\text{neat}}$ cm^{-1}: 3350, 2920, 2850, 2220, 1440, 1030. NMR ($CDCl_3$) δ: 0.83 (d, $J=7$), 1.01 (d, $J=6$), 1.4—2.6 (m), 3.4—3.7 (m).

Ethyl *c*-4-Hydroxymethyl-*t*-3-methyl-*r*-1-cyclohexanecarboxylate and Ethyl *t*-4-Hydroxymethyl-*c*-3-methyl-*r*-1-cyclohexanecarboxylate (21B)——A solution of **20B** (7.47 g, 52 mmol) in 17% HCl–EtOH (75 ml) was allowed to stand at room temperature for 5 days. The solvent was evaporated off *in vacuo*, H_2O (10 ml) was added to the ice-cooled residue and the solution was left overnight at room temperature. The reaction mixture was then extracted with $CHCl_3$. The extract was washed with saturated brine, dried over Na_2SO_4 and concentrated. The residue was distilled under reduced pressure to give **21B** as a colorless oil (3.85 g, 37%), bp 140—143° (5 mmHg). IR $\nu_{\max}^{\text{neat}}$ cm^{-1}: 3400, 2920, 2860, 1720, 1440, 1180. NMR ($CDCl_3$) δ: 0.93 (2H, d, $J=5$), 1.25 (3H, t, $J=8$), 1.0—2.7 (m), 3.15 (2H, m), 4.12 (q, $J=8$), 4.14 (q, $J=8$).

Ethyl *c*-4-Hydroxymethyl-*c*-3-methyl-*r*-1-cyclohexanecarboxylate and Ethyl *t*-4-Hydroxymethyl-*t*-3-methyl-*r*-1-cyclohexanecarboxylate (21A)——A colorless oil, bp 135—140° (0.4 mmHg). IR $\nu_{\max}^{\text{neat}}$ cm^{-1}: 3400, 2920, 2850, 1725, 1440, 1150, 1030. NMR ($CDCl_3$) δ: 0.85 (d, $J=7$), 0.95 (d, $J=6$), 1.23 (t, $J=7$), 1.4—2.6 (m), 3.4—3.7 (m), 4.10 (q, $J=7$).

Ethyl *t*-4-Azidomethyl-*c*-3-methyl-*r*-1-cyclohexanecarboxylate and Ethyl *c*-4-Azidomethyl-*t*-3-methyl-*r*-1-cyclohexanecarboxylate (22B)——To a stirred solution of **21B** (2.0 g, 10 mmol) in pyridine (8 ml), *p*-TsCl (2.5 g, 13 mmol) was added portionwise over a 15 min period with ice cooling. After stirring for 2 hr at

−5—0°, the mixture was allowed to stand in an ice box for 2 days, then poured onto ice and extracted with ether. The extract was washed with H_2O, 1 N HCl and H_2O, then dried over Na_2SO_4 and concentrated. The residue was dissolved in dimethylformamide (8 ml) and NaN_3 (0.85 g, 13 mmol) was added. The mixture was stirred at 100° for 3 hr, poured into ice-water and extracted with iso-Pr ether. The extract was washed with H_2O, dried over Na_2SO_4 and concentrated. The residue was distilled under reduced pressure to give **22B** as a colorless oil (1.67 g, 74%), bp 132—135° (4 mmHg). IR $\nu_{\max}^{\text{neat}}$ cm^{-1}: 2920, 2850, 2080, 1720, 1440, 1170, 1020. NMR ($CDCl_3$) δ: 0.93 (3H, d, $J=6$), 1.24 (3H, t, $J=8$), 1.0—2.6 (m), 3.4 (m), 4.11 (q, $J=8$), 4.14 (q, $J=8$).

Ethyl *c*-4-Azidomethyl-*c*-3-methyl-*r*-1-cyclohexanecarboxylate and Ethyl *t*-4-Azidomethyl-*t*-3-methyl-*r*-1-cyclohexanecarboxylate (22A)——A colorless oil. IR $\nu_{\max}^{\text{neat}}$ cm^{-1}: 2930, 2860, 2090, 1730, 1445, 1265, 1160. NMR ($CDCl_3$) δ: 0.86 (d, $J=7$), 0.93 (d, $J=6$), 1.11 (t, $J=7$), 1.23 (t, $J=7$), 0.8—2.7 (m), 3.1—3.4 (m), 3.55—3.85 (m), 4.12 (q, $J=7$).

***t*-4-Aminomethyl-*c*-3-methyl-*r*-1-cyclohexanecarboxylic Acid (23B) and *c*-4-Aminomethyl-*t*-3-methyl-*r*-1-cyclohexanecarboxylic Acid (23C)**——A solution of **22B** (2.25 g, 10 mmol) in EtOH (100 ml) was hydrogenated over 5% Pd–carbon (2.0 g) at room temperature and atmospheric pressure for 6 hr. The catalyst was filtered off, and the filtrate was concentrated *in vacuo*. The residue was treated with 2 N HCl (20 ml) and the mixture was refluxed for 5 hr. The insoluble material was filtered off and the filtrate was worked up according to method B to give a mixture of **23B** and **23C** as a colorless powder (1.20 g, 71%).

Separation of 23B and 23C——Compound **23B** was also isolated as its *p*-tosylate by the method described above. The salt was worked up according to method B to give **23B**, and recrystallization from H_2O–EtOH gave **23B** as a colorless powder (0.11 g, 9.2%), mp 252—255° (dec.). From the mother liquor, **23C** was isolated as its 3,5-dinitrobenzoate. The salt was worked up according to the procedure described for **9C**, and recrystallization from H_2O–EtOH gave **23C** as a colorless powder (0.20 g, 17%), mp 251—255° (dec.).

23A and 23D from 22A——Compound **22A** was hydrogenated and worked up by the method described above. The amino acid was oxidized without purification and gas-chromatographed.

Dimethyl *c*-2-Methylcyclohexane-*c*-4-*r*-1-dicarboxylate (12A) and Dimethyl *t*-2-Methylcyclohexane-*c*-4-*r*-1-dicarboxylate (12C)——A solution of **10**⁶⁾ (61 mg, 0.5 mmol) in acetone (3 ml) and 0.26 N Na_2CO_3 (0.85 ml) was treated with $KMnO_4$ (285 mg, 1.8 mmol), and the mixture was stirred at room temperature overnight. After addition of iso-PrOH, the precipitate was filtered off and the filtrate was freed from cations by passage down a column of Diaion SK#1 (H^+ type, 10 ml). The solution was evaporated to dryness *in vacuo* and the residue was methylated with excess CH_2N_2 in ether. The solvent was removed, and the residue was gas-chromatographed.

Dimethyl 2-Methylcyclohexane-1,4-dicarboxylate (12) from Amino Acid——Using the method described in an earlier paper,³⁾ the amino acids **9A**—**9D** and **23A**—**23D** were each oxidized with $KMnO_4$ and the resulting diacids were esterified with CH_2N_2.

Acknowledgement The author is grateful to Drs. Y. Abiko and M. Iwamoto for biological assay and to the staff of the analytical section of this Institute for elemental analyses.