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Medicinal Chemical Studies on Antiplasmin Drugs. VIII.[1] 4-Aminomethylcyclohexanecarboxylic Acid Derivatives having a Carboxyl or Carboxymethyl Group at C_2

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Four isomers of 4-aminomethyl-1,2-cyclohexanedicarboxylic acid (**4**) were synthesized from dimethyl 4-cyanophthalate (**2**) *via* 4-aminomethylphthalic acid (**3**), and the isomers of 4-aminomethyl-2-carboxymethylcyclohexanecarboxylic acid (**14**) were also synthesized from 5-aminophthalide (**10**). The configurations and preferred conformations in aqueous solution of the former isomers were determined on the basis of the nuclear magnetic resonance spectra and by converting the compounds to trimethyl 1,2,4-cyclohexanetricarboxylates (**5**), which were compared with **5** obtained from methyl bicyclo[2.2.2]-5-octene-2-carboxylate (**8**), trimethyl trimellitate (**6**), and trimellitic acid (**9**). Those of the latter isomers were deduced from the nuclear magnetic resonance spectra and the relationships of the isomerization products of **14**. The compound *t*-4-aminomethyl-*r*-1,*c*-2-cyclohexanedicarboxylic acid (**4C**), which is thought to exist in the 1-e, 2-a, 4-e form in aqueous solution, showed more potent antiplasmin activity than *trans*-4-aminomethylcyclohexanecarboxylic acid (**1A**).

Keywords——antiplasmin drug; 4-aminomethylcyclohexanecarboxylic acid; structure-activity relationship; 4-aminomethyl-1,2-cyclohexanedicarboxylic acid; 4-aminomethyl-2-carboxymethylcyclohexanecarboxylic acid; stereo isomer; conformation

The antiplasmin activity of a basic amino acid, 5-aminomethylpyridine-2-carboxylic acid, was shown to be higher than that of the corresponding neutral 4-aminomethylbenzoic acid (PAMBA).[3] Acidic amino acids, 4,4′-[iminobis(methylene)]bisbenzoic acid and others, were also reported to have activity.[4] According to Mangyo,[5] for amino acids to possess high antiplasmin activity it is necessary that they have no substituents which might alter the p*K*a of the unsubstituted compound. He also pointed out that the distance between the aminomethyl and carboxyl groups in a molecule is of importance.

In our extended investigation on structure-activity relationships of antiplasmin drugs, we have become interested in 4-aminomethylcyclohexanecarboxylic acid (AMCHA, **1**) derivatives with another carboxyl or carboxymethyl group at C_2; studies of these compounds should provide information about the effect of the acidity and molecular shape of the compounds on the activity. In this paper, we describe the synthesis, separation, assignment of configurations, favored conformation in aqueous solution, and antiplasmin activity of the four isomers of 4-aminomethyl-1,2-cyclohexanedicarboxylic acid (**4**) and those of 4-aminomethyl-2-carboxymethylcyclohexanecarboxylic acid (**14**).

4-Aminomethyl-1,2-cyclohexanedicarboxylic Acid (4)

Hydrogenation of dimethyl 4-cyanophthalate (**2**)[6] over Raney Ni followed by alkaline hydrolysis afforded 4-aminomethylphthalic acid (**3**). Hydrogenation of **3** over platinum in

1) Part VII: S. Isoda, H. Yamaguchi, Y. Satoh, and M. Hirata, *Chem. Pharm. Bull.*, **28**, 2329 (1980).
2) Location: *1-16-13, Kitakasai, Edogawa-ku, Tokyo 132, Japan.*
3) S. Isoda, H. Yamaguchi, Y. Satoh, T. Miki, and M. Hirata, *Chem. Pharm. Bull.*, **28**, 1408 (1980).
4) M. Hirata, Y. Ogawa, and Y. Abiko, Japan Patent Kokai 72-16429 (1972) [*C.A.*, **77**, 151680 (1972)].
5) M. Mangyo, *Seikagaku*, **36**, 735 (1964).
6) J. Gut, *Chem. Listy*, **50**, 1498 (1956).

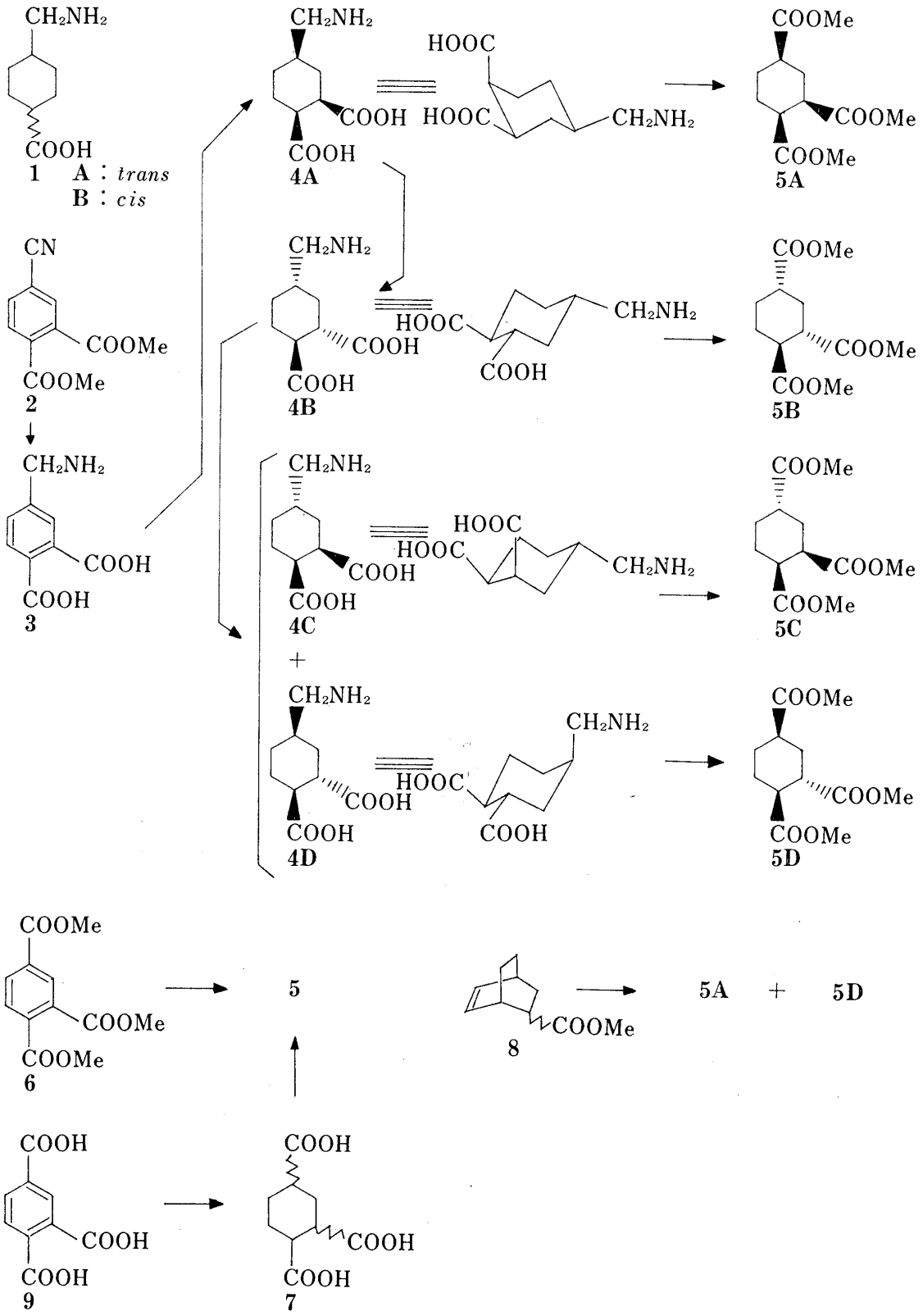

Chart 1

Only the 1*S* forms are shown.

0.5 N hydrochloric acid gave a mixture of **4**, from which *c*-4-aminomethyl-*r*-1,*c*-2-cyclohexanedicarboxylic acid (**4A**), mp 209—210° (dec.), was isolated in 61% yield upon recrystallization from water–ethanol. Isomerization of **4A** in 1 N sodium hydroxide at 180—200° gave a mixture of products, from which *t*-4-aminomethyl-*r*-1,*t*-2-cyclohexanedicarboxylic acid (**4B**), mp 254—255° (dec.), was isolated upon recrystallization from water. Further, isomerization of **4B** in 2 N sodium hydroxide at 180—200° gave a mixture of **4**. After separation of **4B** by means of fractional recrystallization, the mixture was chromatographed on Avicel to isolate *t*-4-aminomethyl-*r*-1,*c*-2-cyclohexanedicarboxylic acid (**4C**), mp 261—263° (dec.), **4A**, *c*-4-aminomethyl-*r*-1,*t*-2-cyclohexanedicarboxylic acid (**4D**), mp 256—259° (dec.), and **4B**. The purities of **4A**, **4B**, **4C**, and **4D** were established by thin-layer chromatography (TLC) and infrared (IR) spectroscopy.

The configuration of each isomer was determined in the following manner. Hydrogenation of trimethyl trimellitate (**6**)[7] over Raney Ni at 150—170° under 85 kg/cm² hydrogen pressure afforded a mixture of trimethyl 1,2,4-cyclohexanetricarboxylate (**5**), which gave, as shown in Fig. 1, four peaks on gas-chromatography (GC). The retention times of the four peaks were 21.9 (peak I), 23.2 (peak II), 28.6 (peak III), and 33.1 min (peak IV). The ratio of the peak areas did not alter on treatment of the mixture of **5** with 1 N potassium hydroxide at room temperature, followed by reesterification of the mixture of 1,2,4-cyclohexanetricarboxylic acid (**7**) with diazomethane. Oxidation of a mixture of methyl bicyclo[2.2.2]-5-octene-2-*endo*- and 2-*exo*-carboxylate (**8**), which was prepared from cyclohexa-1,3-diene and methyl acrylate according to the method of Tichy *et al.*,[8] with potassium permanganate ($KMnO_4$) and esterification of the resulting acids afforded a mixture of **5**, which gave two peaks, peak I and peak IV, on GC in a ratio of about 1:4. Taking into account the ratio of 2-*endo* and 2-*exo* forms of **8**, which was reported to be 82.5:17.5 by Tichy *et al.*,[8] and the fact that under these reaction conditions no isomerization occurs, as described above, peak I and peak IV are considered to arise from the 2-*exo* form of **8** and the 2-*endo* form of **8**, respectively. Namely, peak I corresponds to trimethyl *r*-1,*t*-2,*c*-4-cyclohexanetricarboxylate (**5D**) and peak IV corresponds to trimethyl *r*-1,*c*-2,*c*-4-cyclohexanetricarboxylate (**5A**). Hydrogenation of trimellitic acid (**9**) in acetic acid over platinum, followed by methylation with diazomethane afforded a mixture of **5**, which gave two peaks, peak II and peak IV, on GC in a ratio of 7:93. Bendel *et al.*[9] reported that the hydrogenation products of **9** over platinum under ordinary conditions are a mixture of 1,2-*cis* forms of **7**. Therefore, it was concluded that peak II corresponds to trimethyl *r*-1,*c*-2,*t*-4-cyclohexanetricarboxylate (**5C**) and peak III corresponds to trimethyl *r*-1,*t*-2,*t*-4-cyclohexanetricarboxylate (**5B**). Although Bendel *et al.*[9] distinguished two 1,2-*cis* forms of **5** from two 1,2-*trans* forms of **5**, the gas chromatogram reported is different from

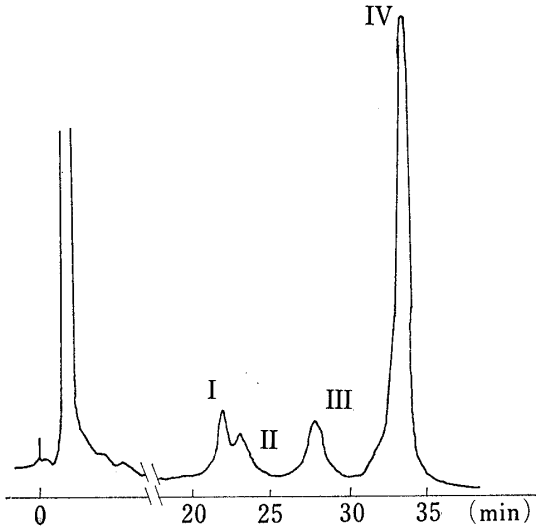

Fig. 1. Gas Chromatogram of Trimethyl 1,2,4-Cyclohexanetricarboxylate (**5**) derived from Trimethyl Trimellitate (**6**)

Condition A
I: **5D**, II: **5C**, III: **5B**, IV: **5A**.

7) R. Wegschneider, H.F. Perndanner, and O. Auspitzer, *Monatsch. Chem.*, **31**, 1253 (1910) [*C.A.*, **5**, 1775 (1911)].
8) M. Tichy, A. Orahovats, and J. Sicher, *Collect. Czech. Chem. Commun.*, **35**, 459 (1970).
9) E. Bendel, W. Meltzow, and V. Vogt, *J. Chromatogr.*, **38**, 136 (1968).

Fig. 1. This may be because of the difference of the GC liquid phase.

Using the method described in an earlier paper,[10] the four isomers (**4A**, **4B**, **4C**, and **4D**) were oxidized with $KMnO_4$ and the resulting tricarboxylic acids were esterified with diazomethane. GC analysis of these esters showed that **4A** affords peak IV, **4B** affords peak III, **4C** affords peak II, and **4D** affords peak I. Thus, the configurations of **4A**, **4B**, **4C**, and **4D** were established. Table I lists the physical properties of **4A**, **4B**, **4C**, and **4D**.

Table I. 4-Aminomethyl-1,2-cyclohexanedicarboxylic Acid (4)

No.	mp (°C) (dec.)	Formula	Analysis (%) Calcd (Found) C	H	N	*Rf* value TLC[a]	NMR (in D_2O) δ $-NCH_2-$	$-CHCOO-$	CH_2, CH (ring)	IR ν_{max}^{KBr} cm^{-1}
4A	209—210	$C_9H_{15}NO_4$	53.72 (53.41	7.51 7.50	6.96 6.51)	0.17	2.97 (d, $J=6$)	2.35—2.8 3.07—3.40	0.9—2.35	3350, 3070, 2900, 2850, 2330, 1685, 1630, 1555, 1510, 1440, 1390
4B	254—255	$C_9H_{15}NO_4$	53.72 (54.00	7.51 7.63	6.96 7.09)	0.05	2.98 (d, $J=6$)	2.4 —2.7	0.9—2.4	3420, 3060, 2930, 2850, 1700, 1630, 1500, 1455, 1440, 1400, 1310
4C	261—263	$C_9H_{15}NO_4$	53.72 (54.02	7.51 7.51	6.96 6.22)	0.21	2.95 (d, $J=6$)	2.3 —2.7 3.15—3.45	0.9—2.3	3400, 3050, 2920, 2850, 2300, 1710, 1635, 1605, 1510, 1455, 1435, 1390
4D	256—259	$C_9H_{15}NO_4$	53.72 (53.63	7.51 7.19	6.96 6.53)	0.07	3.11 (d, $J=7$)	2.55—2.9	1.5—2.4	3420, 3050, 2920, 2850, 2320, 1700, 1630, 1500, 1450, 1390

a) Solvent: iso PrOH–H_2O (4: 1).

On the basis of the nuclear magnetic resonance (NMR) spectra, the favored conformations of **4A**, **4B**, **4C**, and **4D** in aqueous solution were assumed to be as follows (Chart 1). The side chain methylene signals of **4D** at 3.11 ppm, which is at 0.13—0.16 ppm lower field than those of the other isomers, indicate that only **4D** exists in the aminomethyl-axial form[10] and the other isomers are in the aminomethyl-equatorial form. Two kinds of C_1- or C_2-hydrogen signals, one of which appears at 2.3—2.8 ppm and the other at 3.1—3.45 ppm, of **4A** and **4C** suggest that each compound has one axial carboxyl group and one equatorial carboxyl group. On the other hand, the C_1- and C_2-hydrogen signals of **4B** and **4D** appear at higher field, 2.4—2.9 ppm, indicating that **4B** and **4D** both have no axial carboxyl group but two equatorial carboxyl groups.

4-Aminomethyl-2-carboxymethylcyclohexanecarboxylic Acid (14)

Treatment of 5-aminophthalide (**10**)[11] with potassium cyanide in dimethyl sulfoxide (DMSO) followed by alkaline hydrolysis afforded 4-amino-2-carboxymethylbenzoic acid, which was esterified with methanol-sulfuric acid to give methyl 4-amino-2-methoxycarbonylmethylbenzoate (**11**). The Sandmeyer reaction of **11** gave methyl 4-cyano-2-methoxycarbonylmethylbenzoate (**12**), which was hydrogenated in the presence of Raney Ni. The hydrogenated product was hydrolyzed with 4 N sodium hydroxide to give 4-aminomethyl-2-carboxymethylbenzoic acid (**13**).

10) S. Isoda and M. Hirata, *Chem. Pharm. Bull.*, **27**, 2735 (1979).
11) J. Tirouflet, *Bull. Soc. Sci. Bretagne Spec.*, **1951**, 35 [*C.A.*, **47**, 8693 (1953)].

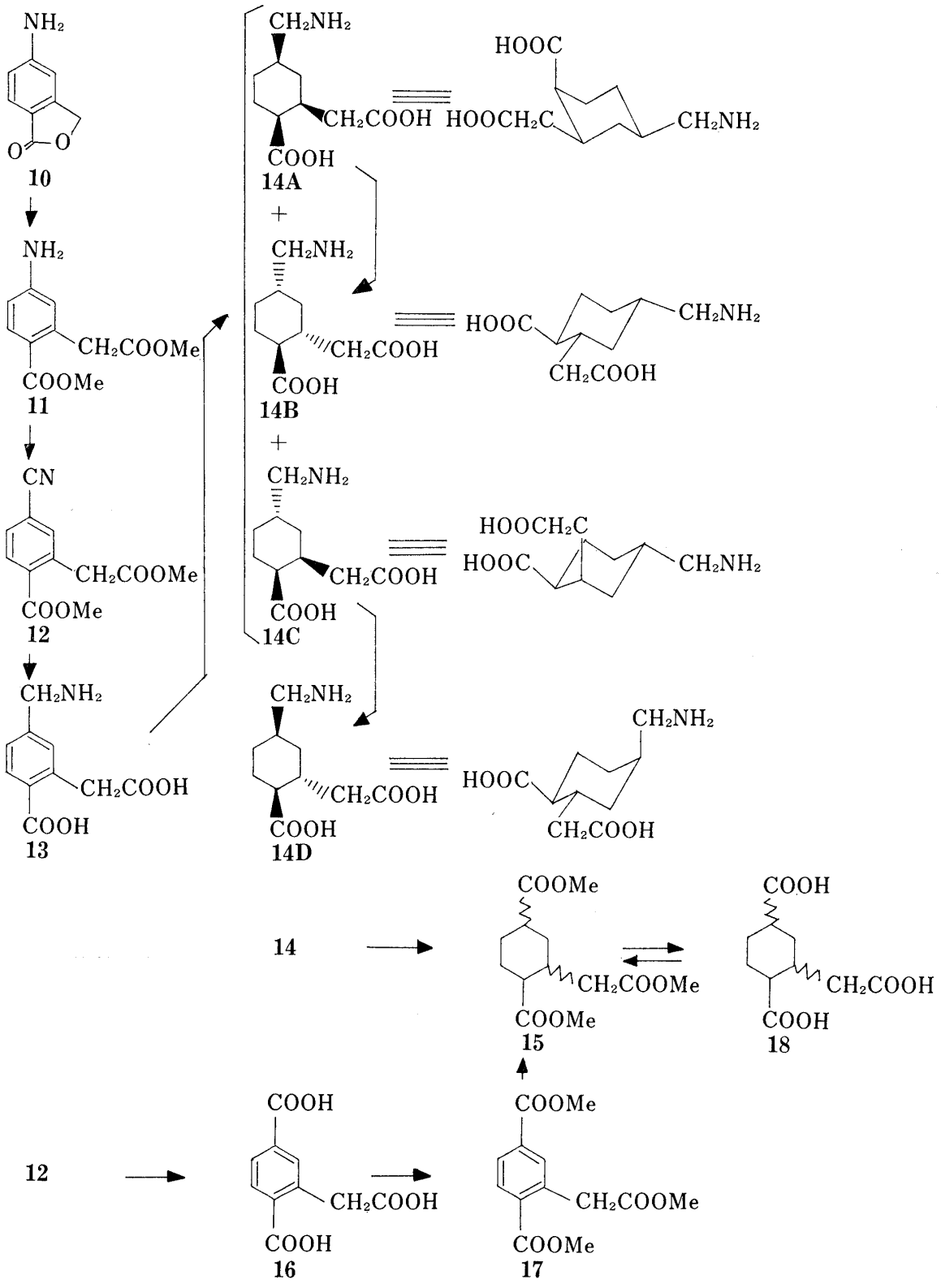

Chart 2

Only the 1*S* forms are shown.

Hydrogenation of **13** over platinum in 0.5 N hydrochloric acid gave a mixture of **14**. After separation of *c*-4-aminomethyl-*c*-2-carboxymethyl-*r*-1-cyclohexanecarboxylic acid (**14A**), mp 253—256° (dec.), by means of fractional recrystallization, the remaining mixture was chromatographed on Avicel to isolate **14A**, *t*-4-aminomethyl-*t*-2-carboxymethyl-*r*-1-cyclohexanecarboxylic acid (**14B**), mp 258—260° (dec.), and *t*-4-aminomethyl-*c*-2-carboxymethyl-*r*-1-cyclohexanecarboxylic acid (**14C**), mp 264—269° (dec.). The purity of each compound (**14A**, **14B**, and **14C**) was established by IR spectroscopy. Isomerization of the fractions which contained **14C** in 1 N sodium hydroxide at 180° gave a mixture of products, from which *c*-4-aminomethyl-*t*-2-carboxymethyl-*r*-1-cyclohexanecarboxylic acid (**14D**) was isolated as a syrup by means of Avicel chromatography. Attempts to crystallize **14D** were unsuccessful. Further, isomerization of **14A** in 0.3 N sodium hydroxide at 180—200° gave a mixture of products, from which **14B** was isolated in 72% yield.

Hydrolysis of **12** under alkaline conditions gave 2-carboxymethylterephthalic acid (**16**), mp 246—251° (dec.), which was esterified with diazomethane to afford dimethyl 2-methoxycarbonylmethylterephthalate (**17**), mp 67—68°. Although the melting points of **16** and **17** were lower than the reported values [mp 275—280° (dec.) for **16** and mp 84—85° for **17**[12]], their structures were confirmed by IR and NMR spectroscopy. Hydrogenation of **17** in acetic acid over platinum afforded a mixture of dimethyl 2-methoxycarbonylmethylcyclohexane-1,4-dicarboxylate isomers (**15**), the main component of which was presumed to be dimethyl *c*-2-methoxycarbonylmethyl-*r*-1,*c*-4-cyclohexanedicarboxylate. Alkaline isomerization of the mixture, followed by reesterification with diazomethane afforded another mixture of **15**, the main component of which was presumed to be dimethyl *t*-2-methoxycarbonylmethyl-*r*-1,*t*-4-cyclohexanedicarboxylate. In contrast to the case of **5**, no difference between these two mixtures was found on GC. Thus the configuration of each isomer (**14A**, **14B**, **14C**, and **14D**) could not be determined by the method described for **4**.

The configuration and favored conformation in aqueous solution of each isomer was deduced to be as follows (Chart 2). Hydrogenation of **13** under ordinary conditions afforded the 1,2-*cis* and 1,4-*cis* form of **14**, **14A**, as a main product. The NMR spectrum of **14A** shows

Table II. 4-Aminomethyl-2-carboxymethylcyclohexanecarboxylic Acid (**14**)

No.	mp (°C) (dec.)	Formula	Analysis (%) Calcd (Found) C	H	N	*Rf* value TLC[a]	NMR (in D_2O) δ –NC<u>H</u>$_2$–	–C<u>H</u>COO–	C<u>H</u>$_2$, C<u>H</u>	IR ν_{max}^{KBr} cm^{-1}
14A	253—256	$C_{10}H_{17}NO_4$	55.80 (55.80	7.96 7.89	6.51 6.43)	0.35	2.95 (d, J=6)	2.7—2.9	1.15—2.55	3400, 2910, 2850, 2680, 2630, 2320, 1720, 1700, 1620, 1520, 1405, 1290
14B	258—260	$C_{10}H_{17}NO_4$	55.80 (55.66	7.96 7.93	6.51 6.43)	0.28	2.92 (d, J=6)		0.95—2.45	3380, 3180, 3060, 2900, 2830, 2550, 1710, 1620, 1570, 1490, 1370, 1330
14C	264—269	$C_{10}H_{17}NO_4$	55.80 (55.65	7.96 7.94	6.51 6.26)	0.28	2.95 (d, J=6)	2.6—2.8	0.85—2.55	2950, 2880, 2620, 2180, 1685, 1535, 1435, 1395, 1370, 1310
14D	—					0.28	3.12 (d, J=7)		1.5 —2.7	3400, 2900, 2650, 2330, 1690, 1520, 1440, 1385

a) Solvent: iso PrOH–H_2O (7: 3).

12) Z. Kumazawa, J. Iwasawa, and M. Nakajima, *Agr. Biol. Chem.* (Tokyo), **25**, 798 (1961).

that it has the equatorial-aminomethyl group (2.95 ppm) and the equatorial C_1-hydrogen atom (2.7—2.9 ppm); hence the carboxyl group takes the axial form and the carboxymethyl group takes the equatorial form. The isomerization product of **14A**, **14B**, exists in the all-equatorial form, since isomerization under alkaline conditions occurrs at C_1, and the NMR spectrum of **14B** shows that it has the equatorial aminomethyl group (2.92 ppm) and lacks equatorial C_1-hydrogen. The side chain methylene signals of **14D** (3.12 ppm) and lack of equatorial C_1-hydrogen indicate that **14D** exists in the aminomethyl-axial and carboxyl- and carboxymethyl-equatorial form. Therefore the C_1-isomer of **14D**, **14C**, exists in the amino-methyl- and carboxyl-equatorial and carboxymethyl-axial form. Table II shows the physical properties of **14A**, **14B**, **14C**, and **14D**.

Table III shows the antiplasmin activities of compounds obtained in this work, All 1,4-*trans* isomers, which are thought to exist in the 1-e and 4-e forms, showed more potent activity than the corresponding 1,4-*cis* isomers, which are thought to exist in the 1-e and 4-a forms or 1-a and 4-e forms (**4B**, **4C** and **4A**, **4D**; **14B**, **14C** and **14A**, **14D**). This relationship is in agreement with that of **1A** and **1B**. Each of the 1,2-*cis* and 1,4-*trans* forms, which are thought to exist in the 1-e, 2-a, and 4-e forms, showed more potent activity than the 1,2-*trans* and 1,4-*trans* form (**4C** and **4B**; **14C** and **14B**). Among the AMCHA derivatives tested in this study **4C** was the most potent, and its activity is 1.87 times that of **1A**.

Table III. Antifibrinolytic Activity[a] of AMCHA (**1**) and PAMBA Derivatives

Compound No.	Relative activity[b]
1A	1.0
4A	3.5×10^{-2}
4B	8.3×10^{-1}
4C	1.87
4D	3.8×10^{-2}
14A	1.5×10^{-2}
14B	2.5×10^{-1}
14C	5.9×10^{-1}
14D	1.1×10^{-1}
PAMBA	3.6×10^{-1}
3	6.3×10^{-1}
13	2.2×10^{-1}

a) The inhibitory effects on fibrin clot lysis were determined according to the method of Okamoto.[c]
b) Relative activities are assigned on a molar basis, taking the activity of **1A** as 1.0.[d]
c) S. Okamoto and U. Okamoto, *Keio J. Med.*, **11**, 105 (1962).
d) A. Okano, M. Inaoka, S. Funabashi, M. Iwamoto, S. Isoda, R. Moroi, Y. Abiko, and M. Hirata, *J. Med. Chem.*, **15**, 247 (1972).

It can therefore be presumed that a binding site for the C_2-axial carboxyl group exists on the drug receptor site apart from those for the amino group and the C_1-equatorial carboxyl group. The reason for the less potent activity of **14C** than **4C** may be that the longer distance between the cyclohexane ring and the carboxyl group at the C_2-side chain reduces the possibility of interaction between the C_2-side chain carboxyl group and the receptor site. These assumptions can account for the relative activities of PAMBA, **3**, and **13**.

Experimental

The following instruments were used. Melting points, a Yanagimoto MP-1 melting point apparatus; IR spectra, a Hitachi 285 spectrometer; NMR spectra, a Hitachi Perkin-Elmer R-20B spectrometer with tetramethylsilane (TMS) as an internal standard, or with TMS in CCl_4 as an external standard when D_2O

was used as a solvent; GC, a Hitachi K-53 instrument with a 4 m stainless steel column packed with 5% PEG succinate on Anakrom ABS at 190° (condition A) or with a 1 m stainless steel column packed with 10% XE-60 on Anakrom ABS at 200° (condition B). TLC was performed on silica gel plates (Merck), and amino acids were detected by ninhydrin coloration. All melting points and boiling points are uncorrected.

General Procedure for Separating the Amino Acid——Method (A): The solution was applied to a column of 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 worked up according to method (A), and the residual syrup was applied to a column of Amberlite IRA-400 (OH^- type). The column was washed with H_2O, and the amino acid was eluted with 1 N AcOH. The eluted solution was evaporated to dryness *in vacuo*.

4-Aminomethylphthalic Acid (3)——A solution of **2**[6] (8.80 g, 40 mmol) in conc. NH_4OH–MeOH (10 ml—300 ml) was hydrogenated in the presence of Raney Ni (8 ml) at room temperature and atmospheric pressure. The hydrogenation was completed in 12 hr. The catalyst was filtered off, and the filtrate was concentrated *in vacuo*. A solution of the residue in 1 N NaOH (120 ml) was refluxed for 1 hr. The insoluble material was filtered off, and the filtrate was worked up according to method (A). Treatment of the residual syrup with 1 N HCl to give pH 4.0 precipitated 3 (4.27 g, 55%) as a white powder on standing. Recrystallization from H_2O–EtOH gave **3** as a crystalline powder, mp above 300°. *Anal.* Calcd for $C_9H_9NO_4$: C, 55.39; H, 4.65; N, 7.18. Found: C, 55.51; H, 4.72; N, 7.32. IR ν_{max}^{KBr} cm^{-1}: 3050—2300, 1680, 1620, 1570, 1540, 1480, 1440. NMR (20% DCl) δ: 4.54 (2H, s, $NC\underline{H}_2C$), 7.98 (2H, s, arom.$\underline{H}$), 8.08 (1H, s, arom.$\underline{H}$).

***c*-4-Aminomethyl-*r*-1,*c*-2-cyclohexanedicarboxylic Acid (4A)**——A solution of **3** (14.85 g, 0.076 mol) in 0.5 N HCl (200 ml, 0.10 mol) was hydrogenated in the presence of PtO_2 (1.5 g) at 30—40° and atmospheric pressure. The hydrogenation was completed in 20 hr. The catalyst was filtered off, and the filtrate was worked up according to method (A). The residue was recrystallized from H_2O–EtOH to give **4A** (9.32 g, 61%) as a crystalline powder, mp 209—210° (dec.).

***t*-4-Aminomethyl-*r*-1,*t*-2-cyclohexanedicarboxylic Acid (4B)**——A solution of **4A** (1.35 g, 6.7 mmol) in 1 N NaOH (20 ml, 20 mmol) was heated in an autoclave at 180—200° for 16 hr. The resulting solution was worked up according to method (A), and the residue was recrystallized from H_2O to give **4B** (0.22 g, (16%) as colorless prisms, mp 254—255° (dec.).

***t*-4-Aminomethyl-*r*-1,*c*-2-cyclohexanedicarboxylic Acid (4C) and *c*-4-Aminomethyl-*r*-1,*t*-2-cyclohexanedicarboxylic Acid (4D)**——A solution of **4B** (1.28 g, 6.34 mmol) in 2 N NaOH (10 ml, 20 mmol) was heated in an autoclave at 180—200° for 60 hr. The resulting solution was worked up according to method (A). Recrystallization of the residue from H_2O–EtOH gave **4B** (687 mg, 54%), which was identical with an authentic sample (TLC and IR spectrum). The mother liquor was concentrated *in vacuo*, and the residue (660 mg) was chromatographed on Avicel (200 g) using iso PrOH–H_2O (4: 1) and subsequently (from tube No. 295) iso PrOH–H_2O (1: 1). Fractions of 5 ml were collected and analyzed by TLC. Frac. 1, tubes 51—73, was concentrated *in vacuo*, and the residue was worked up according to method (B). Recrystallization of the residue from H_2O–EtOH afforded **4C** (11 mg, 0.9%) as a colorless crystalline powder, mp 261—263° (dec.). Frac. 2, tubes 84—96, gave **4A**, which was identified by TLC. Frac. 3, tubes 106—226, gave **4D** (111 mg, 8.7%) as a crystalline powder, mp 256—259° (dec.). Frac. 4, tubes 237—367, gave **4B** (179 mg, 14%), which was identical with an authentic sample (TLC and IR spectrum).

Trimethyl 1,2,4-Cyclohexanetricarboxylate (5A—5D)——a) Using the method described in an earlier paper,[10] the amino acids **4A**—**4D** were each oxidized with $KMnO_4$ and the resulting triacids were esterified with CH_2N_2.

b) A solution of **6**[7] (25.2 g, 0.10 mol) in MeOH (80 ml) was hydrogenated in the presence of Raney Ni (5 ml) in an autoclave at 150—170°. The initial pressure of H_2 was 85 kg/cm^2. After 8 hr, Raney Ni (5 ml) was added to the mixture and it was hydrogenated for a further 8 hr. The catalyst was filtered off, and the filtrate was concentrated *in vacuo*. The residue was distilled under reduced pressure to give **5** (23.55 g, 93%) as a colorless liquid, bp 138—143° (2 mmHg). IR ν_{max}^{neat} cm^{-1}: 2955, 1730, 1435, 1265, 1200, 1170. NMR ($CDCl_3$) δ: 1.35—2.60(m), 3.20(m), 3.66(s), 3.67(s). GC: t_R 21.9, 23.2, 28.6, 33.1 (condition A).

c) A suspension of **5** (0.26 g, 1 mmol), which was obtained by the method described above, in 1 N KOH (4.0 ml, 4 mmol) was stirred at room temperature for 18 hr. After dilution with H_2O, the solution was freed from cations by passage down a column of Diaion SK#1 (H^+ type, 10 ml). The solution was concentrated *in vacuo* below 40°, and the residue was methylated with excess CH_2N_2 in ether. The solvent was removed and the residue was gas-chromatographed to give a chromatogram identical with that of the starting material.

Trimethyl *r*-1,*c*-2,*c*-4-Cyclohexanetricarboxylate (5A) and Trimethyl *r*-1,*c*-2,*t*-4-Cyclohexanetricarboxylate (5C) from 6——A solution of **6** (1.05 g, 5 mmol) in AcOH (30 ml) was hydrogenated in the presence of PtO_2 (0.35 g) at room temperature and atmospheric pressure. The catalyst was filtered off and the filtrate was concentrated *in vacuo*. The small portion of the residue was methylated with excess CH_2N_2 in ether. The solvent was removed, and the residue was gas-chromatographed. GC: t_R 23.2, 33.1 (condition A). The rest of the filtrate was concentrated *in vacuo*, and the residue was recrystallized from H_2O to give *r*-1,*c*-2,*c*-4-cyclohexanetricarboxylic acid (**7A**) (0.50 g, 48%) as colorless prisms, mp 168—172°. *Anal.* Calcd for $C_9H_{12}O_6$: C, 50.00; H, 5.60. Found: C, 49.32; H, 5.73.

Trimethyl *r*-1,*c*-2,*c*-4-Cyclohexanetricarboxylate (5A) and Trimethyl *r*-1,*t*-2,*c*-4-Cyclohexanetricarboxylate (5D) from 8——A solution of **8**[8] (200 mg, 1.2 mmol) in acetone (6 ml) and 0.26 N Na_2CO_3 (2 ml) was treated with $KMnO_4$ (700 mg, 4.43 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 Amberlite IR-120B (H^+ type, 30 ml). The column was washed with H_2O (150 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. GC: t_R 21.9, 33.1 (condition A).

Methyl 4-Amino-2-methoxycarbonylmethylbenzoate (11)——A solution of **10** (10.0 g, 67 mmol) and KCN (5.0 g, 77 mmol) in DMSO (50 ml) was heated at 170—175° for 8 hr. The solution was concentrated *in vacuo* and the residue was treated with 4 N NaOH (50 ml, 200 mmol). The mixture was refluxed for 5 hr and worked up according to method (A). A mixture of the residue, MeOH (150 ml) and conc. H_2SO_4 (15 ml) was refluxed for 5 hr and concentrated *in vacuo*. The residue was poured into ice-water, neutralized with Na_2CO_3, and extracted with $CHCl_3$. The extract was washed with H_2O, dried over Na_2SO_4 and concentrated *in vacuo*. Recrystallization of the residue from MeOH-IPE gave **11** (5.45 g, 37%) as pale brown prisms, mp 98—101°. *Anal.* Calcd for $C_{11}H_{13}NO_4$: C, 59.19; H, 5.87; N, 6.27. Found: C, 59.21; H, 6.12; N, 6.39. IR ν_{max}^{KBr} cm^{-1}: 3450, 3360, 3240, 1720, 1690, 1640, 1600, 1565, 1435, 1410, 1330. NMR ($CDCl_3$) δ: 3.70 (3H, s, $CH_2COOC\underline{H}_3$), 3.80 (3H, s, $COOC\underline{H}_3$), 3.92 (2H, s, $C\underline{H}_2COO$), 4.05 (2H, bs, $N\underline{H}_2$), 6.43 (1H, d, $J=3$, C_3-$\underline{H}$), 6.60 (1H, dd, $J=7.5$, $J=3$, C_5-$\underline{H}$), 7.87 (1H, d, $J=7.5$, C_6-$\underline{H}$).

Methyl 4-Cyano-2-methoxycarbonylmethylbenzoate (12)——A diazonium salt solution, which was prepared from **11** (4.46 g, 20 mmol) in 1.5 N HCl (32 ml, 48 mmol) and $NaNO_2$ (1.50 g, 22 mmol) in H_2O (5 ml), then neutralized to pH 7.0 by adding Na_2CO_3, was slowly added to a stirred solution of CuCN, which was prepared from $CuSO_4$ $5H_2O$ (7.0 g, 28 mmol) in H_2O (32 ml) and KCN (6.1 g, 92 mmol) in H_2O (16 ml), over a 15 min period with cooling (3—5°). After stirring for 5 hr at 0—5°, for 2 hr at room temperature and for 0.5 hr at 50—60°, the mixture was extracted with $CHCl_3$. The extract was washed with H_2O, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was distilled under reduced pressure, bp 140—142° (0.65 mmHg), and the distillate was recrystallized from C_6H_6-IPE to give **12** (2.79 g, 60%) as pale yellow prisms, mp 92—95°. *Anal.* Calcd for $C_{12}H_{11}NO_4$: C, 61.80; H, 4.75; N, 6.01. Found: C, 61.74; H, 4.76; N, 6.02. IR ν_{max}^{KBr} cm^{-1}: 2220, 1730, 1715, 1430, 1345, 1290, 1255. NMR ($CDCl_3$) δ: 3.70 (3H, s, $CH_2COOC\underline{H}_3$), 3.90 (3H, s, $COOC\underline{H}_3$), 4.04 (2H, s, $C\underline{H}_2COO$), 7.56 (1H, s, C_3-$\underline{H}$), 7.65 (1H, dd, $J=8$, $J=2$, C_5-$\underline{H}$), 8.09 (1H, d, $J=8$, C_6-$\underline{H}$).

4-Aminomethyl-2-carboxymethylbenzoic Acid (13)——A solution of **12** (26.9 g, 0.115 mol) in conc. NH_4OH–MeOH (20 ml—400 ml) was hydrogenated in the presence of Raney Ni (20 ml) at room temperature and atmospheric pressure. The hydrogenation was completed in 9 hr. The catalyst was filtered off, and the filtrate was concentrated *in vacuo*. A solution of this residue in 4 N NaOH (95 ml, 0.38 mol) was refluxed for 2 hr. The insoluble material was filtered off, and the filtrate was worked up according to method (B). The effluent was evaporated to dryness *in vacuo* to give **13** (16.61 g, 69%). Recrystallization from H_2O gave **13** as colorless prisms, mp above 300°. *Anal.* Calcd for $C_{10}H_{11}NO_4$: C, 57.41; H, 5.30; N, 6.70. Found: C, 56.77; H, 5.44; N, 6.53. IR ν_{max}^{KBr} cm^{-1}: 3300, 3000—2050, 1610, 1520, 1420, 1360. NMR (D_2O–CF_3COOH) δ: 4.04 (2H, s, $C\underline{H}_2COO$), 4.26 (2H, s, $NC\underline{H}_2C$), 7.40 (1H, s, C_3-$\underline{H}$), 7.51 (1H, d, $J=8$, C_5-$\underline{H}$), 8.06 (1H, d, $J=8$, C_6-$\underline{H}$).

Hydrogenation of 13 and Separation of 4-Aminomethyl-2-carboxymethylcyclohexanecarboxylic Acid (14)——A solution of **13** (7.75 g, 37 mmol) in 0.5 N HCl (200 ml) was hydrogenated in the presence of PtO_2 (1.0 g) at 30—40° and atmospheric pressure. After 18 hr, further PtO_2 (0.5 g) was added to the solution and hydrogenation was continued for another 6 hr. The catalyst was then filtered off, and the filtrate was worked up according to method (B). The residue was recrystallized from H_2O–EtOH to give **14A** (4.57 g, 57%) as colorless prisms, mp 253—256° (dec.). The mother liquor was concentrated *in vacuo*, and the residue (3.11 g) was applied to a column of Avicel (200 g) and chromatographed using iso PrOH–H_2O (15: 1) followed by (from tube No. 120) iso PrOH–H_2O (4: 1). Fractions of 10 ml were collected and analyzed by TLC or IR spectroscopy. Frac. 1, tubes 186—210, was concentrated *in vacuo* and the residue was recrystallized from H_2O–EtOH to give **14A** (0.83 g, total 5.40 g, 68%), as colorless prisms, mp 252—258° (dec.). Frac. 2, tubes 226—236, was concentrated *in vacuo* and the residue was recrystallized from H_2O–EtOH to give **14B** (0.10 g, 1.3%) as colorless prisms, mp 258—260° (dec.). Frac. 3, tubes 237—286, was concentrated *in vacuo* and the residue was fractionally recrystallized from H_2O–EtOH to give **14B** (0.05 g, total 0.15 g, 2%) as colorless prisms, mp 258—260° (dec.) and **14C** (0.27 g, 3%) as colorless prisms, mp 264—269° (dec.).

***c*-4-Aminomethyl-*t*-2-carboxymethyl-*r*-1-cyclohexanecarboxylic Acid (14D)**——The mother liquor of recrystallization of frac. 2 and 3 was collected and evaporated to dryness *in vacuo*. The residue (0.65 g) was dissolved in 1 N NaOH (10 ml), and the solution was heated at 180° for 16 hr. The solution was worked up according to method (B), and the residue was applied to a column of Avicel (180 g), and chromatographed using iso PrOH–H_2O (10: 1) followed by (from tube No. 175) iso PrOH–H_2O (6: 1). Fractions of 10 ml were collected and analyzed by TLC and IR spectroscopy. Tubes 238—311 were combined and concentrated *in vacuo* and the residue was recrystallized from H_2O–EtOH to give **14B** (0.14 g). The mother liquor was rechromatographed on Avicel to give **14D** (19 mg) as a syrup.

***t*-4-Aminomethyl-*t*-2-carboxymethyl-*r*-1-cyclohexanecarboxylic Acid (14B) from 14A**——A solution of **14A** (215 mg, 1.0 mmol) in 0.3 N NaOH (11 ml, 3.3 mmol) was heated in an autoclave at 180—200° for 60 hr. The resulting solution was worked up according to method (B), and the residue was recrystallized from H_2O–EtOH to give **14B** (154 mg, 72%) as colorless prisms, mp 258—260° (dec.).

2-Carboxymethylterephthalic Acid (16)——A solution of **12** (466 mg, 2 mmol) in 8 N NaOH (10 ml) was refluxed for 12 hr. The insoluble material was filtered off, and the filtrate was treated with conc. HCl (10 ml), then the solution was left at room temperature overnight. The precipitates were collected by filtration. Recrystallization from 50% MeOH gave **16** (243 mg, 52%) as colorless prisms, mp 246—251° (dec.) (reported[12] mp 275—280° (dec.)). IR ν_{max}^{KBr} cm^{-1}: 3400, 3100—2500, 1680, 1560, 1490, 1405, 1270. NMR (CD_3OD) δ: 4.08 (2H, s, $C\underline{H}_2COO$), 7.9—8.1 (3H, m, arom.$\underline{H}$).

Dimethyl 2-Methoxycarbonylmethylterephthalate (17)——Compound **16** (50 mg, 0.22 mmol) was treated with a solution of excess CH_2N_2 in ether. The solvent was removed, and the residue was recrystallized from 50% MeOH to give **17** (35 mg, 59%) as colorless plates, mp 67—68° (reported[12] mp 84—85°). *Anal.* Calcd for $C_{13}H_{14}O_6$: C, 58.66; H, 5.30. Found: C, 58.71; H, 5.28. IR ν_{max}^{KBr} cm^{-1}: 1730, 1700, 1490, 1425, 1340, 1280, 1265. NMR ($CDCl_3$) δ: 3.67 (3H, s, $CH_2COOC\underline{H}_3$), 3.86, 3.90 (each 3H, s, $COOC\underline{H}_3$), 4.04 (2H, s, $C\underline{H}_2COO$), 7.85—8.10 (3H, m, arom.$\underline{H}$).

Dimethyl 2-Methoxycarbonylmethyl-1,4-cyclohexanedicarboxylate (15)——a) A solution of **17** (266 mg, 1 mmol) in AcOH (30 ml) was hydrogenated over PtO_2 (100 mg) at 40—50° and atmospheric pressure for 9 hr. The catalyst was filtered off, and the filtrate was concentrated *in vacuo* to give **15** (196 mg, 72%) as a pale yellow oil, which was gas-chromatographed without purification. NMR ($CDCl_3$) δ: 1.1—2.9 (11H, m), 3.65 (9H, s).

b) A solution of **15** (97 mg, 0.36 mmol) in 0.2 N NaOH (10 ml) was heated at 170° for 7 hr. The solution was treated with conc. HCl and concentrated *in vacuo*. The residue was treated with a solution of excess CH_2N_2 in ether. The insoluble material was filtered off, and the filtrate was concentrated *in vacuo* to give **15** as a pale yellow syrup, which was gas-chromatographed.

c) Using the method described in an earlier paper[19] the amino acids **14A**—**14C** were each oxidized with $KMnO_4$ and the resulting acids were esterified with CH_2N_2.

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