Synthesis of Peptides containing *cis*- or *trans*-3- or 4-Aminocyclohexanecarboxylic Acid Residues

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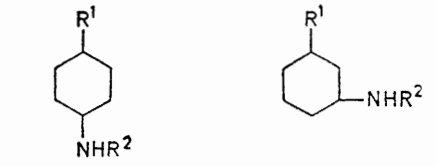
cis- and *trans*-3- and 4-Aminocyclohexanecarboxylic acids have been inserted into di- and tri-peptides containing L-phenylalanine and L-cysteine. Dipeptide derivatives containing L-phenylalanine and 3(4)-hydroxymethyl- or 3(4)-chloromethyl-cyclohexylamine are also described.

THE absolute stereochemistry of 2-aminocyclohexanecarboxylic acid derivatives has been investigated extensively.[1,2] *cis*- and *trans*-3-Aminocyclohexanecarboxylic acids have been prepared by a stereospecific method.[3] Selective coupling methods for the attachment of L-valine, glycine, and L-alanine derivatives to *cis*- or *trans*-4-aminocyclohexanecarboxylic acid have been described recently.[4] We wished to prepare di- and tri-peptides containing *cis*- or *trans*-3- or 4-aminocyclohexylcarbonyl, L-phenylalanyl, and L-cysteinyl residues.

Ethyl 4-aminocyclohexanecarboxylate[5] has been shown by t.l.c. and by spinning-band distillation to be a mixture of *trans*- and *cis*-isomers, separable only as *trans*- (I) and *cis*- (II) *N*-benzoyl derivatives. Attempted debenzoylation of compounds (I) and (II) by the reported procedure[5,6] gave only *N*-benzoyl-*trans*- (III) and -*cis*- (IV) 4-hydroxymethylcyclohexylamine. Hydrolysis of these benzamido-alcohols with hydrochloric acid afforded *trans*- (V) and *cis*- (VI) chloromethylcyclohexylamines as hydrochlorides. *cis*-3-Benzamidocyclohexanecarboxylate (VII), which was isolated in larger amount (see Table) than its *trans*-isomer (VIII), was used for the preparation of 3-hydroxymethyl- (IX) and 3-chloromethyl- (X) cyclohexylamines.



trans-	*cis*-	*cis*-	R¹	R²
(I)	(II)	(VII)	CO_2Et	Bz
(III)	(IV)	(IX)	CH_2OH	Bz
(V)	(VI)	(X)	CH_2Cl	H
(XI)	(XII)	(XIII)	CO_2Et	Boc-Phe
(XIV)		(XV)	CO_2H	Boc-Phe
(XVI)		(XVII)	(SBzl)CysOMe	Boc-Phe
(XVIII)	(XIX)		CH_2OH	Boc-Phe
(XX)	(XXI)	(XXII)	CH_2Cl	Boc-Phe
(XXIII)			CH_2Cl	Phe

Boc-Phe = t-butoxycarbonyl-L-phenylalanyl; (SBzl)Cys = *S*-benzyl-L-cysteinyl.

[1] W. L. F. Armarego and T. Kabayashi, *J. Chem. Soc.* (*C*), 1970, 1597.
[2] H. Nohira, K. Ehara, and A. Miyashita, *Bull. Chem. Soc. Japan*, 1970, **43**, 2230.
[3] F. R. Hewgill and P. R. Jefferies, *J. Chem. Soc.*, 1955, 2767.
[4] Wen-Yih Chen and R. K. Olsen, *J. Org. Chem.*, 1975, **40**, 350.
[5] W. Schneider and K. Lehman, *Tetrahedron Letters*, 1970, **49**, 4285.
[6] W. Schneider and A. Hütermann, *Arch. Pharm.*, 1965, **298**, 226.

The successful separations of the benzamidocyclohexanecarboxylates (I), (II), (VII), and (VIII) encouraged us to attempt the preparation and separation of stereoisomeric aminocyclohexanecarboxylic acid peptides. Thus, ethyl t-butoxycarbonyl-L-phenylalanyl-*trans*- (XI) and -*cis*- (XII) 4-aminocyclohexanecarboxylates were easily separated when the *N*-hydroxysuccinimido-ester of t-butoxycarbonyl-L-phenylalanine was coupled with a mixture of ethyl 4-aminocyclohexanecarboxylates. Similarly, the dipeptide (XIII) was obtained from a mixture of 3-amino-carboxylates. Finally, hydroxysuccinimido-esters of t-butoxycarbonyl-L-phenylalanyl-*trans*-4-amino- (XIV) and -*cis*-3-amino- (XV) cyclohexanecarboxylic acids were coupled with methyl esters of *S*-benzyl-L-cysteine to give *trans*-4- (XVI) and *cis*-3- (XVII) aminocyclohexanecarboxylic acid tripeptides.

Reduction of the dipeptides (XI) and (XII) with lithium aluminium hydride gave the corresponding *trans*- (XVIII) and *cis*- (XIX) 4-hydroxymethylcyclohexylamine derivatives. The *trans*-4- (XX), *cis*-4- (XXI), and *cis*-3- (XXII) chloromethylcyclohexylamine dipeptides were prepared by reactions of the chloromethylcyclohexylamines (V), (VI), and (X) with the hydroxysuccinimido-ester of t-butoxycarbonyl-L-phenylalanine. *N*-Deprotection is exemplified by the hydrolysis with trifluoroacetic acid of the dipeptide (XX) to give L-phenylalanyl-*trans*-4-chloromethylcyclohexylamine (XXIII).

Stereoisomeric 3- and 4-aminocyclohexanecarboxylic acids and derivatives *

Compd.	M.p. (°C) [a]	Yield (%)	$[\alpha]_D$ (°) (*c*)
(I)	167—168	31.9 [b]	
(II)	98—99	49.4 [b]	
(III)	153—155	80.0	
(IV)	91—93	81.0	
(VII)	110—112	70.8 [b]	
(VIII)	85—87	6.2 [b]	
(IX)	142—144	86.0	
(XI)	157—159	39.0 [b]	+18.0 (1.2)
(XII)	68—70	45.1 [b]	+14.5 (1.25)
(XIII)	133—135	65.0 [b]	+10.9 (1.28)
(XIV)	173—175	84.2	+15.4 (0.52)
(XV)	131—133	96.7	+12.2 (0.19)
(XVI)	179—181	60.4	
(XVII)	143—145	70.0	−6.25 (0.96) [c]
(XVIII)	144—145	92.0	+13.0 (1.95)
(XIX)	foam	75.0	+12.0 (1.17)
(XX)	138—140	69.7	+17.0 (0.7)
(XXI)	105—107	70.0	+14.0 (0.78)
(XXII)	143—145	78.5	+14.8 (0.67)
(XXIII)	87—90 [d]	94.0	−39.7 (0.78) [e]

[a] Recrystallized from methylene chloride–ether–n-hexane. [b] Based on total amount of *trans–cis* aminocarboxylates. [c] In methylene chloride. [d] From ethyl acetate–n-hexane. [e] In ethyl acetate.

* Elemental analyses and i.r. data are available as Supplementary Publication No. SUP 21728 (2 pp.); for details of Supplementary Publications see Notice to Authors No. 7, *J.C.S. Perkin I*, 1975, Index issue.

EXPERIMENTAL

The same techniques and apparatus were used as described previously.[7] In addition, optical rotations were measured for solutions in anhydrous methanol at 21 °C (l = 1 dm) unless otherwise stated.

3(4)-*Aminocyclohexanecarboxylic Acids*.—Hydrogenation of *m*(*p*)-aminobenzoic acid (1.46 mmol) was carried out in 30% ethanol (18 ml) for 5 h, at 25 °C and 60 lb in^{-2}, with platinum oxide (50 mg) as catalyst. The *cis–trans*-mixture of 3-aminocarboxylic acids, m.p. 260—285°, was isolated in 76.5% yield from the *m*-isomer and the 4-aminocarboxylic acids, m.p. >300°, were obtained in 92% yield from the *p*-isomer.

Ethyl 3(4)-*Aminocyclohexanecarboxylates*.—The *trans–cis*-mixture of 3- or 4-aminocyclohexanecarboxylic acids (3.65 mmol) was refluxed in ethanolic 3% hydrochloric acid (25 ml) for 18 h; the solution was evaporated to dryness and the residue dissolved in water (20 ml) and treated with concentrated ammonia (to pH 10). A chloroform extract afforded a *cis–trans*-mixture of 3-aminocarboxylates separated (90%), R_F *ca.* 0.3 and 0.2 (CH_2Cl_2–MeOH, 4 : 1), $\nu_{max.}$ 1 724 cm^{-1} (ester C=O) or a mixture of 4-aminocarboxylates (96%), R_F *ca.* 0.2 and 0.3, $\nu_{max.}$ 1 724 cm^{-1} (ester C=O).

Ethyl 3(4)-*Benzamidocyclohexanecarboxylates*.—To the *cis–trans*-mixture of ethyl 3- or 4-aminocyclohexanecarboxylates (3 mmol) in anhydrous pyridine (8 ml), a solution of benzoyl chloride (3.2 mmol) in anhydrous pyridine (7 ml) was added. The mixture was stirred for 24 h at room temperature, then evaporated to dryness. The residue was dissolved in water–methylene chloride (20 and 30 ml), acidified with concentrated hydrochloric acid, and extracted into methylene chloride. The ethyl *trans–cis*-4-benzamidocarboxylates were obtained in 87.3% yield; R_F *ca.* 0.7 (methylene chloride–ether, 4 : 1); and the ethyl *cis–trans*-3-benzamidocarboxylates in 86.3% yield (R_F *ca.* 0.83).

Chromatography of the ethyl *trans–cis*-4-benzamidocarboxylates (720 mg) on a silica gel (40 g) column and elution with a linear gradient (0.1—5%) of methanol in methylene chloride separated the cis-*isomer* (II), R_F *ca.* 0.4 (ten developments in methylene chloride), τ 4.21—3.70 (1 H, m, NH) and 2.90—2.20 (5 H, m, aromatic); and the trans-*isomer* (I), R_F *ca.* 0.3, τ 3.68 (1 H, d, NH) and 2.82—2.05 (5 H, m, aromatic). Similarly the cis- (VII), R_F *ca.* 0.30, τ 3.60 (1 H, d, NH) and 2.83—2.08 (5 H, m, aromatic); and the trans- (VIII) 3-*benzamido-isomer*, R_F *ca.* 0.25, τ 4.0—3.32 (1 H, m, NH) and 2.83—2.02 (5 H, m, aromatic), were obtained.

N-*Benzoyl*-trans- (III) *and* cis- (IV) 4-*hydroxymethylcyclohexylamines*.—Ethyl *trans*- (I) or *cis*-4-benzamidocyclohexanecarboxylate (II) (0.24 mmol) in anhydrous ether (30 ml) was added dropwise (1.5 h) to a stirred solution of lithium aluminium hydride (2.4 mmol) in anhydrous ether (30 ml). The mixture was refluxed for 20 h (*trans*-isomer) or 5 h (*cis*-isomer), then decomposed in the cold with water and N-sulphuric acid. From an ethereal extract a solid separated which was purified by preparative t.l.c.; the trans-*isomer* (III) showed τ (CD_3OD) 6.82—6.48 (2 H, m, CH_2O) and 2.80—1.76 (5 H, m, aromatic); and the cis-*isomer* (IV), τ 6.47 (2 H, d, CH_2O) and 2.91—2.08 (5 H, m, aromatic).

N-*Benzoyl*-cis-3-*hydroxymethylcyclohexylamine* (IX).—Ethyl *cis*-3-benzamidocyclohexanecarboxylate (VII) (110 mg, 0.4 mmol) in anhydrous tetrahydrofuran (15 ml) was added dropwise (1 h) to a suspension of lithium aluminium hydride (148 mg, 4.0 mmol) in anhydrous tetrahydrofuran

[7] V. Škarić, V. Turjak-Zebić, and D. Škarić, *J.C.S. Perkin I*, 1974, 1406.

(35 ml) and the mixture was stirred for 3 h at room temperature. The product, τ [$(CD_3)_2CO$] 6.61 (2 H, d, CH_2O) and 2.72—1.95 (5 H, m, aromatic), was extracted into methylene chloride and separated as described for (III) and (IV).

Hydrochlorides of trans-4- (V), cis-4- (VI), *and* cis-3- (X) *Chloromethylcyclohexylamines.*—A solution of *N*-benzoyl-*trans*-4- (III), *-cis*-4- (IV), or *-cis*-3- (IX) hydroxymethylcyclohexylamine (1.25 mmol) in hydrochloric acid (10 ml) was sealed in a tube and heated for 50 h at 110—115 °C, then diluted with water (20 ml), and partitioned with methylene chloride. The evaporated water layer yielded the appropriate hydrochloride of trans-4-*chloromethylcyclohexylamine* (V), m.p. 140—165° (87%), $\nu_{max.}$ 3 448, 2 924br, 2 532, 2 062, and 1 597 cm^{-1}; cis-4-*chloromethylcyclohexylamine* (VI), m.p. 125—135° (76%), $\nu_{max.}$ 3 484, 2 924br, 2 513, 1 996, and 1 608 cm^{-1}; or cis-3-*chloromethylcyclohexylamine* (X), m.p. 150—160° (80%), $\nu_{max.}$ 3 483, 2 941br, 2 532, 2 020, and 1 600 cm^{-1}.

Ethyl t-Butoxycarbonyl-L-*phenylalanyl*-trans-4- (XI), cis-4- (XII), *and* cis-3- (XIII) *Aminocyclohexanecarboxylates.*—The hydrochlorides of the *trans–cis*-mixture of 3- or 4-aminocyclohexanecarboxylates (2.71 mmol), the hydroxysuccinimido-ester of t-butoxycarbonyl-L-phenylalanine (2.71 mmol), and triethylamine (1 ml) were dissolved in anhydrous 1,2-dimethoxyethane (25 ml). The mixture was stirred at room temperature for 4 h, diluted with water (80 ml), and partitioned with methylene chloride (20 ml). In the case of the 4-isomers an oil separated from the organic layer which partly crystallized (79.5%), R_F *ca.* 0.53 (methylene chloride). Chromatography on a silica gel (50 g) column [elution with methylene chloride–ether (97 : 3, 95 : 5, and 90 : 10)] separated crystalline and oily fractions. The crystalline fractions after rechromatography yielded the trans-4-*isomer* (XI) (39%), and the oily fractions the cis-4-*isomer* (XII) (45%).

The fraction of R_F *ca.* 0.53, obtained from the 3-amino-isomers was purified by preparative t.l.c. (87.5%) and then by fractional crystallization to give the cis-3-*isomer* (XIII) (65%), τ 8.60 (9 H, s, Bu^t) and 2.92—2.73 (5 H, s, aromatic).

t-Butoxycarbonyl-L-*phenylalanyl*-trans-4-*amino*- (XIV) *and* cis-3-*amino*- (XV) *cyclohexanecarboxylic Acids.*—A solution of the *trans*-4-amino- (XI) or the *cis*-3-aminocyclohexanecarboxylate (XII) (0.82 mmol) in methanol (3.5 ml) was treated with methanolic N-potassium hydroxide (3.5 ml). The mixture was refluxed for 5 h, and then evaporated to dryness. The residue was diluted with water (10 ml) and acidified with acetic acid to pH 3. The crystalline *precipitate* was filtered off, washed with water, and purified by preparative t.l.c.

t-Butoxycarbonyl-L-*phenylalanyl*-trans-4- (XVI) *and* cis-4- (XVII) *aminocyclohexylcarbonyl*-S-*benzyl*-L-*cysteine Methyl Esters.*—A solution of t-butoxycarbonyl-L-phenylalanyl-*trans*-4- (XIV) or *cis*-3-aminocyclohexanecarboxylic acid (XV) (0.26 mmol) and *NN'*-dicyclohexylcarbodi-imide (0.28 mmol) in anhydrous 1,2-dimethoxyethane (10 ml) was kept for 20 h at 0—5 °C. The precipitate was filtered off and the filtrate treated with *S*-benzyl-L-cysteine methyl ester (58 mg, 0.26 mmol) and kept for 16 h at room temperature. The mixture was then diluted with water (15 ml) and partitioned with methylene chloride. The residue from the organic layer was purified by chromatography on a silica gel (7 g) column with methylene chloride–ether (98 : 2, 97 : 3, and 95 : 5) as eluant and then by preparative t.l.c.; the *product* showed τ 8.60 (9 H, s, Bu^t), 6.32 (2 H, s, $S{\cdot}CH_2$), 6.29 (3 H, s, OCH_3), and 2.76 and 2.73 (2 × 5 H, s, aromatic).

t-Butoxycarbonyl-L-*phenylalanyl*-trans- (XVIII) *and* cis- (XIX) 4-*hydroxymethylcyclohexylamines.*—A solution of L-phenylalanyl-*trans*-4- (XV) or *-cis*-4-aminocyclohexanecarboxylate (XII) (0.24 mmol) in anhydrous tetrahydrofuran or ether (20 ml) was treated with lithium aluminium hydride (2.4 mmol) and the *product* was worked up as described for (III), (IV), and (IX); τ 8.60 (9 H, s, Bu^t), 2.92—2.73 (5 H, s, aromatic), and *ca.* 6.64 (2 H, d, CH_2O).

t-Butoxycarbonyl-L-*phenylalanyl*-trans-4- (XX), cis-4- (XXI), *and* cis-3- (XXII) *chloromethylcyclohexylamines.*—The hydrochloride of the *trans*-4- (V), *cis*-4- (VI), or *cis*-3-cyclohexylamine (X) (0.28 mmol), the hydroxysuccinimido-ester of t-butoxycarbonyl-L-phenylalanine (0.32 mmol), and triethylamine (0.2 ml) reacted together in anhydrous 1,2-dimethoxyethane (8 ml). The *product* was worked up as described for (XI) and (XII). The crude materials were purified by preparative t.l.c.; n.m.r. spectra were similar to those of (XVIII) and (XIX).

L-*Phenylalanyl*-trans-4-*chloromethylcyclohexylamine* (XXIII).—The 4-chloromethylcyclohexylamine (XXII) (100 mg, 0.26 mmol) was dissolved in trifluoroacetic acid (1.1 ml) at 0 °C, and kept at room temperature for 15 min. The solution was evaporated to dryness, and residue dissolved in methylene chloride. The solution was partitioned with aqueous 50% potassium carbonate. From the organic layer an *oil* separated (70 mg, 94%), which crystallized.

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