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Studies of Alicyclic α-Amino Acids. III.[1] Synthesis and Biological Evaluation of 3-Amino-1,2,3,4-tetrahydrocarbazole-3-carboxylic Acids[2]

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3-Amino-1,2,3,4-tetrahydrocarbazole-3-carboxylic acid (IIa) and its 6-methoxy, benzyloxy and hydroxy derivatives (IIb—d), a new type of cyclic tryptophan analogue, were prepared. The optical resolution of acetamido-(−)-menthyl ester of IIa (XIIc) was achieved. It was found that IIa inhibited α-chymotrypsin competitively and trypsin noncompetitively.

Since Connors, *et al.*[4] observed the antitumor activity of 1-aminocyclopentane-1-carboxylic acid, increasing attentions[5–8] have been focused on alicyclic α-amino acids from the viewpoint of their biological interest. It has been shown that some of unnatural amino acids reveal inhibition of amino acid metabolism because of their structural resemblance to natural amino acids, *e.g.*, α-methyltryptophan[9–12] (Ia) and 5-hydroxy-α-methyltryptophan[13–16] (Ib), have been extensively studied in relation to the tryptophan metabolism.

In view of current interest in Ia, b, the synthesis of 3-amino-1,2,3,4-tetrahydrocarbazole-3-carboxylic acid derivatives (IIa—d), a new type of tryptophan analogue in which the position of α-amino function relative to the planar indole nucleus is fixed within small limits, was undertaken. It was hoped that these compounds might possess a similar biological profile to Ia, b.

We record the synthesis of IIa—d, the optical resolution of menthyl 3-acetamido-1,2,3,4-tetrahydrocarbazole-3-carboxylate (XIIc) and some results of biological evaluation of IIa.

The tetrahydrocarbazole system was constructed by the Fisher indole cyclization employing 4-benzoyloxycyclohexanone (III) and derivatives of phenylhydrazine (IVa—c); III was treated with IVa—c in refluxing acetic acid to give 3-benzoyloxy-1,2,3,4-tetrahydrocarbazoles (Va—c) without isolation of the intermediate hydrazones. 3-Benzoyloxy-6-unsubstituted-

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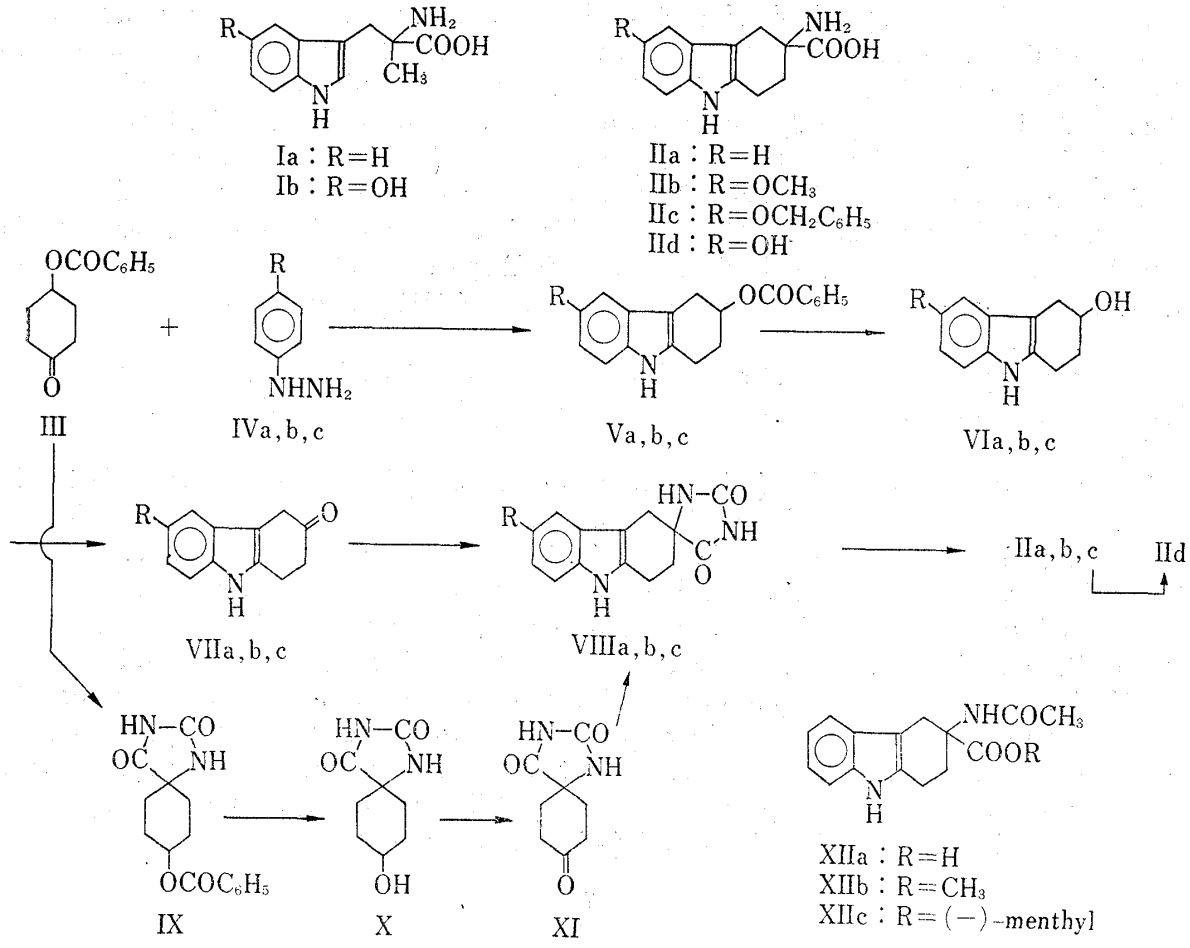

a : R=H, b : R=OCH_3, c : R=$OCH_2C_6H_5$, d : R=OH

Chart 1

1,2,3,4-tetrahydrocarbazole (Va) has been previously prepared in 52% yield[17] in an almost similar manner to our procedure. The improved yield of Va (93%) in the Fisher indole cyclization was obtained by addition of boron trifluoride etherate to the reaction mixture.

Hydrolysis of Va—c with sodium hydroxide in aqueous ethanol gave the corresponding 3-hydroxy-1,2,3,4-tetrahydrocarbazoles (VIa—c) in good yields. Despite many failures,[18] oxidation of VIa—c to 3-oxo-1,2,3,4-tetrahydrocarbazoles (VIIa—c) was achieved by means of the Oppenauer oxidation with aluminum isopropoxide and cyclohexanone in refluxing toluene (yields 60—70%).

The 3-ketones VIIa—c were easily converted into the corresponding hydantoins (VIIIa—c) under the Bucherer's conditions in excellent yields.

The hydantoin, VIIIa, was also prepared by an alternative route as follows; A mixture of *cis*- and *trans*-4-benzoyloxycyclohexane-1-spiro-5′-hydantoins (IX)[1] obtained from III by the Bucherer hydantoin synthesis was hydrolyzed to a mixture of *cis*- and *trans*-4-hydroxycyclohexane-1-spiro-5′-hydantoins (X). Chromic acid oxidation of X gave ketone (XI) which was subjected to the Fisher indole cyclization under the conditions mentioned above. The tetrahydrocarbazole-spiro-hydantoin thus obtained was identical with VIIIa in every respect. This route, however, is unfavorable because of low yields in the hydrolysis and oxidation steps (IX→X→XI).

17) J.H. Mason and E.H. Pavri, *J. Chem. Soc.*, **1963**, 2504.
18) Recently, similar observations in the oxidation of the 3-hydroxy-1,2,3,4-tetrahydrocarbazole system have been reported. (G.E.A. Coombes, O.J. Harvey, and S.T. Raid, *J. Chem. Soc. (C)*, **1970**, 325).

Although the spiro-hydantoin ring of VIIIa—c showed the resistance to hydrolysis, employment of drastic conditions (heating with 20% barium hydroxide in a sealed tube at 160° for 15 hr) made it possible to convert into the corresponding amino acids IIa—c in moderate yields. 3-Amino-6-hydroxy-1,2,3,4-tetrahydrocarbazole-3-carboxylic acid (IId) was obtained by catalytic hydrogenation of 6-benzyloxy amino acid (IIc) over paladium-carbon.

The structure of the amino acid IIa thus prepared was confirmed by its conversion to acetamido-methyl ester (XIIb). All amino acids, IIa—d, showed the characteristic bands assignable to amino acid function in their infrared (IR) spectra, and gave satisfactory elemental analyses, and consonant nuclear magnetic resonance (NMR) and mass spectra for their structures.

A few examples[6,19] of optical resolution of cyclic α-amino acid *via* quinic acid or brucine salt have been reported. Attempts to resolute IIa *via* (−)-brucine salt did not give satisfactory results.

The resolution of acetamido-(−)-menthyl ester of IIa, (XIIc), however, was effected in a manner similar to that of α-amino acid *via* their menthyl esters[20a,b]; A mixture of N-acetate (XIIa) and (−)-menthol in toluene–benzene (3: 7) was refluxed with a catalytic amount of *p*-toluene sulfonic acid. A mixture of diastereoisomeric esters thus obtained was fractionally recrystallized from ethanol to give two diastereoisomers (α-isomer: mp 305—306°, $[\alpha]_D^{20}=+18.2°$ $c=0.7$ in EtOH. β-isomer: mp 277—278°, $[\alpha]_D^{20}=-119°$ $c=0.7$ in EtOH) in 9% and 4% yields, respectively. Gas chromatography of the mixture of diastereoisomeric menthyl esters prior to resolution revealed the presence of two peaks as shown in Fig. 1. High optical purity of diastereoisomers thus separated was demonstrated by the fact that the isomers showed a single peak in their gas chromatograms, respectively. (Fig. 1). Hydrolysis of α- and β-isomers to optically active amino acids, (+)- and (−)-IIa, was unsuccessful.

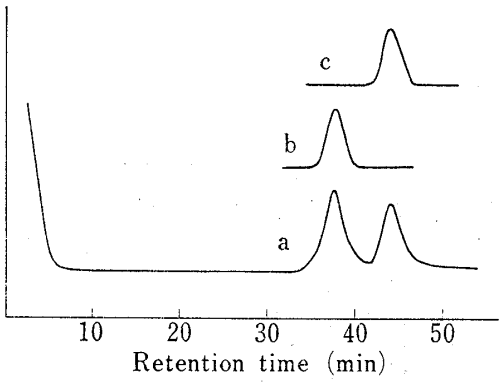

Fig. 1. Gas Chromatogram of Diastereoisomeric Menthyl 3-Acetamido-1,2,3,4-tetrahydrocarbazole-3-carboxylate(XIIc)

a: a mixture of α- and β-isomers
b: α-isomer $t_R=38.2$ min
c: β-isomer $t_R=45.8$ min
OV-17 chromosorb W, column length: 1.5 m×3 mm, column temperature: 220°, detection: FID, carrier gas: N_2, instrument: Shimadzu GC-3BF

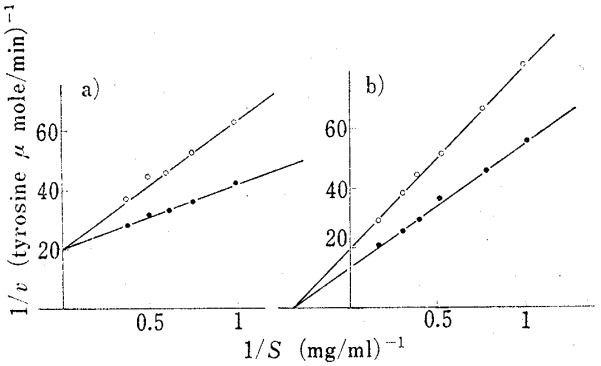

Fig. 2. Linewaver–Burk Plots for the Inhibition of Proteolytic Activities of α-Chymotrypsin and Trypsin

●: no IIa ○: 6.7×10^{-3}M of IIa
a) α-chymotrypsin (0.04 unit) b) trypsin (0.05 unit)
To a mixture containing of each 1 ml of casein (0.4—1.5 w/v%) solution (pH 8.0) and IIa solution, preheated at 37°, 1 ml of the enzyme solution was added and incubated for 30 min at 37°. The reaction was stopped by the addition of trichloroacetic acid (TCA-B solution). The products were measured by Folin-Ciocalteu's method.

It is well demonstrated that the specificity of α-chymotrypsin is directed toward the aromatic amino acid residues in natural and synthetic substrates. Therefore, enzymatic action of a cyclic analogue of tryptophan, IIa, to α-chymotrypsin was tested and compared with that to trypsin.

19) S-dim Fang, L-chüau Li, C-i Niu, and K-Fong Tseng, *Scientia Sinica*, **10**, 845 (1961).
20) *a*) K. Harada and T. Hayakawa, *Bull. Chem. Soc. Japan*, **47**, 192 (1964); *b*) S. Terashima, K. Achiwa, and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **13**, 1399 (1965) and cited references.

The mode of inhibition was determined by Lineweaver–Burk plots as shown in Fig. 2. The compound, IIa, was found to inhibit α-chymotrypsin competitively and trypsin noncompetitively, indicating that it can be bound to the active center of α-chymotrypsin and not to the site of trypsin. At the concentration of 6×10^{-3}M, IIa caused 32% and 16% inhibition to α-chymotryptic hydrolysis of casein and N-acetamido-L-tyrosine ethyl ester (ATEE), and 30% and 20% inhibition to tryptic digest of casein and N-tosyl-DL-arginine methyl ester (TAME), respectively.

Thus, the present observation suggests that the specificities in both enzymatic actions are quite different in spite of resemblance of the primary structure of α-chymotrypsin and trypsin.

Other biological evaluations are now in progress and will be reported in the near future.

Experimental[21]

3-Benzoyloxy-1,2,3,4-tetrahydrocarbazole (Va)——A mixture of 4-benzoyloxycyclohexanone (III) (10 g) and phenylhydrazine (IVa) (5.71 g) in AcOH (75 ml) was refluxed with boron trifluoride etherate (4 g) for 1 hr. After the reaction mixture was cooled, the resulting precipitate was collected by filtration and recrystallized from EtOH to give Va (12 g) as colorless crystals, mp 196° (lit.[17] mp 196—198°). *Anal.* Calcd. for $C_{19}H_{17}O_2N$: C, 78.33; H, 5.88; N, 4.81. Found: C, 78.57; H, 6.09; N, 4.81. IR cm^{-1} (KBr): 3350 (NH), 1700 (CO).

3-Benzoyloxy-6-methoxy-1,2,3,4-tetrahydrocarbazole (Vb)——A mixture of III (11 g) and 4-methoxyphenylhydrazine (IVb) hydrochloride (8.16 g) was refluxed in AcOH (100 ml) with anhyd. sodium acetate (4 g) for 1 hr. After the reaction mixture was cooled, the resulting precipitate was collected and recrystallized from EtOH to give Vb (14 g) as colorless needles, mp 145—146.° *Anal.* Calcd. for $C_{20}H_{19}O_3N$: C, 79.18; H, 5.65; N, 4.62. Found: C, 79.01; H, 5.63; N, 4.73.

3-Benzoyloxy-6-benzyloxy-1,2,3,4-tetrahydrocarbazole (Vc)——A mixture of III (6.5 g) and 4-benzyloxyphenylhydrazine (IVc) hydrochloride (7.5 g) in AcOH (70 ml) was refluxed with anhyd. sodium acetate (2.5 g) for 1 hr. The reaction mixture was diluted with H_2O and extracted repeatedly with ether. The ether extract was washed successively with 50% NaOH and H_2O, and dried over anhyd. Na_2SO_4. After removal of ether, the residue was recrystallized from EtOH to give Vc (7.2 g) as colorless needles, mp 164—165. *Anal.* Calcd. for $C_{21}H_{21}O_3N$: C, 78.57; H, 5.83; N, 3.52. Found: C, 78.19; H, 5.89; N, 3.32. IR cm^{-1} (KBr): 3350 (NH), 1690 (CO).

3-Hydroxy-1,2,3,4-tetrahydrocarbazole (VIa), and Its 6-Methoxy and Benzyloxy Derivatives (VIb,c)——A solution of Va (11 g) in EtOH (600 ml) containing 1N NaOH (200 ml) was heated under reflux for 45 min. After removal of EtOH, the remaining reaction mixture was diluted with H_2O and allowed to stand overnight to deposit a crystalline solid. The solid was recrystallized from EtOH to give VIa (6 g) as colorless needles mp 153° (lit.[18] mp 152—154). *Anal.* Calcd. for $C_{12}H_{13}ON$: C, 76.97; H, 7.00; N, 7.48. Found: C, 76.57; H, 6.97; N, 7.61. IR cm^{-1} (KBr): 3350, 3150 (NH, OH).

3-Hydroxy-6-methoxy- and 3-hydroxy-6-benzyloxy-1,2,3,4-tetrahydrocarbazoles (VIb,c) were obtained in a manner similar to the case of VIa. VIb: mp103—104°. *Anal.* Calcd. for $C_{13}H_{15}O_2N$: C, 71.86; H, 6.96; N, 6.45. Found: C, 71.56; H, 6.87; N, 6.32. IR cm^{-1} (KBr): 3350, 3300 (NH, OH). VIc: mp 141—142°. *Anal.* Calcd. for $C_{19}H_{19}O_2N$: C, 78.10; H, 6.53; N, 4.77. Found: C, 77.91; H, 6.40; N, 6.38. IR cm^{-1} (KBr): 3500, 3400 (NH, OH).

3-Oxo-1,2,3,4-tetrahydrocarbazole (VIIa), and Its 6-Methoxy and Benzyloxy Derivatives (VIIb,c)——To a boiling solution of VIa in freshly distilled toluene (400 ml) and cyclohexanone (200 ml) was added dropwise aluminium isopropoxide (12 g) in toluene (60 ml) at a rate equal to that at which toluene was distilled off. Distillation of the toluene was continued until an initial volume of the reaction mixture was reduced to about 150 ml. After addition of a saturated solution of sodium potassium tartarate in H_2O (80 ml), the reaction mixture was steam distilled. The remaining reaction mixture was extracted with $CHCl_3$, and the $CHCl_3$ extract was washed with H_2O and dried over anhyd. Na_2SO_4. After removal of $CHCl_3$, the residue was chromatographed on silica gel using $CHCl_3$ as eluant. The solid thus obtained was recrystallized from benzene to give VIIa (9.3 g) as colorless crystals, mp 154—156°. *Anal.* Calcd. for $C_{12}H_{11}ON$: C, 77.81; H, 5.99; N, 7.13. Found: C, 77.95; H, 5.94: N, 7.56. IR cm^{-1} (KBr): 3350 (NH), 1710 (CO). NMR ($CDCl_3$) τ: −0.9 (1H, broad, NH), 2.5—3.2 (4H, multiplet, aromatic protons), 4.5—7.6 (6H, multiplet, ring protons).

21) All melting points are uncorrected. Gas chromatographic analyses were performed on a Shimadzu GC-3BF instrument using a 1.5 m×3 mm stainless steel column packed with OV-17 at 200°. IR and NMR spectra were obtained with a Hitachi-215 spectrophotometer and with a Hitachi R-20B spectrometer using TMS as an internal standard.

Similarly, 3-oxo-6-methoxy- and benzyloxy -1,2,3,4-tetrahydrocarbazoles (VIIb, c) were prepared by the Oppenauer oxidation of the corresponding 3-hydroxy derivatives (VIb,c). VIIb: mp 149—151. *Anal.* Calcd. for $C_{13}H_{13}O_2N$: C, 72.64; H, 6.05; N, 6.60. Found: C, 72.54; H, 6.09; N, 6.51. IR cm^{-1} (KBr): 3300 (NH), 1700 (CO). NMR ($CDCl_3$) τ: −0.60 (1H, broad, NH), 2.7—3.5 (3H, multiplet, aromatic protons), 6.33 (3H, singlet, OCH_3). VIIc: mp 182°. *Anal.* Calcd. for $C_{19}H_{17}O_2N$: C, 78.33; H, 5.88; N, 4.81. Found: C, 78.55; H, 5.94; N, 4.92. IR cm^{-1} (KBr): 3200 (NH), 1700 (CO). NMR ($CDCl_3$) τ: −0.55 (1H, broad, NH), 2.4—3.2 (8H, multiplet, aromatic protons), 4.8 (2H, singlet, $-OCH_2-$).

1,2,3,4-Tetrahydrocarbazole-3-spiro-5′-hydantoin (VIIIa), and Its 6-Methoxy and Benzyloxy Derivatives (VIIIb,c)——A mixture of VIIa (1 g), KCN (0.75 g) and $(NH_4)_2CO_3$ (2 g) in 60% aq. EtOH (50 ml) was heated at 80—90° in a sealed tube for 20 hr. After addition of some water and then cooling, the reaction mixture deposited a crystalline solid, which is collected, washed with H_2O and dried. Recrystallization of the solid from AcOH afforded VIIIa (1.2 g) as colorless needles, mp 320°. *Anal.* Calcd. for $C_{14}H_{13}O_2N_3$: C, 65.87; H, 5.13; N, 16.28. Found: C, 66.01; H, 5.11; N, 16.28. IR cm^{-1} (KBr): 3250, 3200 (NH), 1760, 1710 (CO). NMR (DMSO-d_6) τ: −0.55 (1H, broad, NH), 1.7 (1H, broad, NH), 2.55—3.5 (4H, multiplet, aromatic protons).

6-Methoxy- and benzyloxy-1,2,3,4-tetrahydrocarbazole-3-spiro-5′-hydantoin (VIIIb,c) were also prepared from VIIb,c in a manner similar to the above case. VIIIb: mp 264—265°. Purification of this compound was not achieved. The IR spectrum of the crude VIIIb, however, showed characteristic bands assignable to a hydantoin moiety. IR cm^{-1} (KBr): 3300, 3200 (NH), 1760, 1710 (CO). VIIIc: mp 265—266°. *Anal.* Calcd. for $C_{21}H_{19}O_2N_3$: C, 69.79; H, 5.30; N, 11.63. Found: C, 69.83; H, 5.50; N, 11.67. IR cm^{-1} (KBr): 3450, 3300 (NH), 1780, 1740 (CO), NMR (DMSO-d_6) τ: −0.63 (1H, broad, NH), 1.70 (1H, broad, NH), 4.93 (2H, singlet, $-OCH_2-$), 2.57—3.40 (8H, multiplet, aromatic protons).

3-Amino-1,2,3,4-tetrahydrocarbazole-3-carboxylic Acid (IIa)——Hydantoin, VIIIa, (5 g) was heated in a sealed tube with $Ba(OH)_2$ (15 g) in H_2O (70 ml) at 150—160° for 15 hr. The content was boiled to remove ammonia generated during the reaction and filtered. The resulting clear solution was treated with excess of $(NH_4)_2CO_3$ until no further precipitation occurred. After removal of the precipitate by filtration, the filtrate was concentrated to dryness under reduced pressure. Recrystallization of the residue from H_2O–AcOH (1: 1) afforded amino acid IIa (3 g) as colorless crystals, mp above 320°. *Anal.* Calcd. for $C_{13}H_{14}O_2N$: C, 67.81; H, 6.13; N, 12.17. Found: C, 67.69; H, 6.01; N, 12.35. IR cm^{-1} (KBr): 3450 (NH), 3200—2500 (NH_3^+), 1680, 1620, 1580 (NH_3^+, COO^-).

A solution of IIa in dry pyridine and Ac_2O was allowed to stand overnight at room temperature. The reaction mixture was poured into ice water and the product precipitated was collected by filtration. When the filtrate was concentrated under reduced pressure, the second crop of the product was obtained. Recrystallization of the combined crude product from MeOH gave acetate, XIIa, (1 g) as colorless crystals, mp 275—276°. *Anal.* Calcd. for $C_{15}H_{16}O_3N_2$: C, 66.16; H, 5.92; N, 10.26. Found: C, 66.11; H, 5.87; N, 10.25. IR cm^{-1} (KBr): 3400 (NH), 3000—2400 (OH), 1720 (CO), 1640 ($NHCOCH_3$). NMR (DMSO-d_6) τ: −0.65 (1H, broad, NH), 2.07 (1H, broad, NH), 2.5—3.2 (4H, multiplet, aromatic protons), 8.12 (3H, singlet, $NHCOCH_3$).

A solution of XIIa (1 g) in ab. MeOH (20 ml) saturated with dry HCl gas was refluxed for 10 hr. The reaction mixture was concentrated under reduced pressure to dryness. Recrystallization of the residue from MeOH gave methyl 3-acetamido-1,2,3,4-tetrahydrocarbazole-3-carboxylate (XIIb) (0.9 g) as colorless crystals, mp 274—275°. *Anal.* Calcd. for $C_{16}H_{18}O_3N_2$: C, 67.11; H, 6.34; N, 9.78. Found: C, 66.94; H, 6.24; N, 9.95. IR cm^{-1} (KBr): 3350, 3320 (NH), 1720 (CO), 1650 ($NHCOCH_3$). NMR (DMSO-d_6) τ: −0.7 (1H, broad, NH), 1.9 (1H, broad, NH), 2.5—3.2 (4H, multiplet, aromatic protons), 6.48 (1H, singlet, $COOCH_3$), 8.2 (3H, singlet, $NHCOCH_3$). Mass Spectrum *m/e*: 286 (M^+).

3-Amino-6-methoxy- and benzyloxy-1,2,3,4-tetrahydrocarbazole-3-carboxylic acids (IIb,c) and their acetyl derivatives were obtained in a manner similar to the above case. IIb: mp 264—266°. *Anal.* Calcd. for $C_{14}H_{16}O_3N_2$: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.52; H, 6.11; N, 10.81. IR cm^{-1} (KBr): 3400 (NH), 3200—2500 (broad, NH_3^+), 1680, 1640, 1540 (NH_3^+, COO^-). Mass Spectrum *m/e*: 260 (M^+). Acetate: mp 266—267°. *Anal.* Calcd. for $C_{16}H_{18}O_4N_2$: C, 63.56; H, 6.00; N, 9.72. Found: C, 63.29; H, 6.04; N, 9.04. IR cm^{-1} (KBr): 3400, 3320 (NH), 1710 (CO), 1610 ($NHCOCH_3$). IIc: mp 256—257°. *Anal.* Calcd. for $C_{20}H_{20}O_3N_2 \cdot H_2O$: C, 67.78; H, 6.26; N, 7.91. Found: C, 67.74; H, 6.41; N, 7.97. IR cm^{-1} (KBr): 3450 (NH), 3200—2500 (NH_3^+), 1680, 1590, 1540 (NH_3^+, COO^-).

3-Amino-6-hydroxy-1,2,3,4-tetrahydrocarbazole-3-carboxylic Acid (IId)——To a solution of IIc (1 g) in ab. MeOH (50 ml) was added 10% palladium carbon (0.3 g). The mixture was shaken in hydrogen at room temperature and atomospheric pressure until uptake ceased. The reaction mixture was filtered and concentrated under reduced pressure. Recrystallization of the residue from aqueous MeOH gave IId (0.4 g) as silver grey crystals, mp 295—297°. *Anal.* Calcd. for $C_{13}H_{14}O_3N_2$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.15; H, 5.72; N, 11.10. IR cm^{-1} (KBr): 3400 (NH), 3200—2500 (NH_3^+, COO^-). NMR (DMSO-d_6) τ: −0.43 (1H, broad, NH), 2.8—3.6 (3H, multiplet, aromatic protons). Mass Spectrum *m/e*: 246 (M^+). IId thus obtained was treated with Ac_2O–pyridine to give 3-acetamido-6-acetoxy derivative in 90% yield. Colorless crystals (from MeOH), mp 273—274°. *Anal.* Calcd. for $C_{17}H_{18}O_5N_2$: C, 61.81; H, 5.49; N, 8.48. Found: C, 62.17; H, 5.50; N, 8.43. IR cm^{-1} (KBr): 3370, 3330 (NH), 2500 (broad, OH), 1750—1700 (broad,

CO), 1640 ($NHCOCH_3$). NMR (DMSO-d_6) τ: −0.85 (1H, broad, NH), 2.0 (1H, broad, NH), 2.6—3.5 (3H, multiplet, aromatic protons), 7.73 (3H, singlet, $OCOCH_3$), 8.12 (3H, singlet, $NHCOCH_3$).

4-Hydroxycyclohexane-1-spiro-5′-hydantoin (X)——A mixture of 4-benzoyloxycyclohexane-1-spiro-5′-hydantoin (IX) (5 g) and $Ba(OH)_2$ (10 g) in MeOH–H_2O (1: 1) (200 ml) was refluxed for 1 hr. The reaction mixture was filtered to remove insoluble materials and concentrated under reduced pressure. The aqueous solution thus obtained was acidified with conc. H_2SO_4 to deposit $BaSO_4$ and benzoic acid. After removal of these compounds by filtration, the filtrate was centrifuged to separate still remaining $BaSO_4$. To the resulting clear solution was added basic $PbCO_3$ to precipitate $PbSO_4$. After removal of $PbSO_4$, H_2S gas was bubbled through the solution to remove excess lead ion as PbS. The solution free from lead ion was extracted repeatedly with ether and the combined ether extracts were evaporated under reduced pressure to dryness. Recrystallization of the residue from H_2O give X (0.9 g) as colorless crystals, mp above 300°. *Anal.* Calcd. for $C_8H_{12}O_3N_2$: C, 52.16; H, 6.57; N, 15.21. Found: C, 52.27; H, 6.61; N, 15.47. IR cm^{-1} (KBr): 3300, 3100 (NH, OH), 1720 (CO).

4-Oxocyclohexane-1-spiro-5′-hydantoin (XI)——To a solution of X (2 g) in AcOH (10 ml) was added CrO_3 (2.8 g) in H_2O with vigorous stirring and on ice cooling. The reaction mixture was allowed to stand overnight at room temperature and then extracted with EtOAc. The EtOAc layer was washed with 5% NaOH and H_2O, and dried over anhyd. Na_2SO_4. After removal of EtOAc, the residue was recrystallized from EtOAc to give XI (0.4 g) as colorless crystals, mp 253—254.° *Anal.* Calcd. for $C_8H_{10}O_3N_2$: C, 52.74; H, 5.53; N, 15.38. Found: C, 52.70; H, 5.83; N, 15.08. IR cm^{-1} (KBr): 3200 (broad, NH), 1770, 1720 (CO).

In a same manner as described previously, XI was subjected to the Fisher indolization. The product was identical with VIIIa prepared from VIIa in every respect.

Preparation and Resolution of Menthyl 3-Acetamido-1,2,3,4-tetrahydrocarbazole-3-carboxylate (XIIc)——A mixture of XIIa (1.5 g), (−)-menthol (1.5 g) and *p*-toluenesulfonic acid monohydrate (1.5 g) in 100 ml of a benzene–toluene mixture (7: 3) was refluxed with a Dean and Stark distillation apparatus in azeotropic distillation for 30 hr. The nascent water was then removed azeptropically. The insoluble materials were collected by filtration, and then extracted with EtOAc. The EtOAc extract was washed with 8% aq. $NaHCO_3$ and dried over anhyd. Na_2SO_4. After addition of EtOAc, the filtrate was also washed with 8% aq. $NaHCO_3$ and H_2O, and dried over anhyd. Na_2SO_4. The combined organic layer was concentrated to dryness and the residue was recrystallized from EtOAc to give a mixture of diastereoisomers, XIIc, (1 g) as colorless needles, mp 260—262°. *Anal.* Calcd. for $C_{25}H_{34}O_3N_2$: C, 73.14; H, 8.35; N, 6.82. Found: C, 72.94; H, 8.44; N, 6.96. Gas chromatography of the mixture of diastereoisomers thus obtained showed the presence of two peaks at t_R=38.2 min and t_R=45.8 min. Repeated fractional recrystallizations of the mixture from EtOH resulted in the complete resolution of isomeric α- and β-menthylesters. α-Isomer (less soluble in EtOH): 0.2 g, mp 277—278°. *Anal.* Calcd for $C_{25}H_{34}O_3N_2$: C, 73.14; H, 8.35; N, 6.82. Found: C, 73.17; H, 8.51; N, 6.88. IR cm^{-1} (KBr): 3350 (NH), 1715 (CO), 1650 ($NHCOCH_3$). NMR (DMSO-d_6) τ: −0.7 (1H, broad, NH), 1.9 (1H, broad, NH), 2.5—3.2 (4H, multiplet, aromatic protons), 9.0—9.5 (18H, multiplet, aliphatic protons). $[\alpha]_D^{20}$ −119° (c=0.7, EtOH). Gas chromatography: t_R=45.8 min. β-isomer (more soluble in EtOH): 0.08 g, mp 305—306°. *Anal.* Calcd. for $C_{25}H_{34}O_3N_2$: C, 73.14; H, 8.35; N, 6.82. Found: C, 72.94; H, 8.44; N, 6.96. IR cm^{-1} (KBr): 3400 (NH), 1715 (CO), 1650 ($NHCOCH_3$). NMR (DMSO-d_6) τ: −0.7 (1H, broad, NH), 1.92 (1H, broad, NH), 2.5—3.2 (4H, multiplet, aromatic protons), 8.95—9.5 (18H, multiplet, aliphatic protons). $[\alpha]_D^{20}$ +18.2° (c=0.7, EtOH). Gas chromatography: t_R=38.2 min.

Assay of Enzymatic Activity of 3-Amino-1,2,3,4-tetrahydrocarbazole-3-carboxylic Acid (IIa)——Materials: casein, TAME and ATEE were obtained from Merk, Nakarai Kagaku and Seikagaku Kogyo, respectively. Pure trypsin (trypsin from bovine pancreas, 2×crystallized) and α-chymotrypsin were purchased from Sigma Chemical and Worthington Biochemical, respectively.

Assay of Enzymatic Activity: Esterase activities of trypsin and α-chymotrypsin were determined by the spectrophotometric procedure[22] using TAME and ATEE as substrates. All assays were carried out at pH 8.0, 37 in 0.1M tris-HCl buffer. Proteolytic activity was assayed by Folin–Ciocalteu's method[23] using casein in 0.1M phosphate buffer under the same condition as mentioned above.

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23) O. Folin and V. Ciocalteu, *J. Biol. Chem.*, 73, 627 (1927).