The Synthesis of 9-Citrulline, 9-Histidine, and 9-Desarginine Bradykinin

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The synthesis of three analogs of bradykinin is described, in which the C-terminal arginine is replaced by citrulline and histidine or removed for 9-desarginine bradykinin.

Structural changes at the C-terminal portion of angiotensin appear to produce more profound biological effects than comparable changes at the N-terminal side. It was of interest to determine if the bradykinin molecule was as sensitive to change at the C-terminal side.

Removal of the C-terminal arginine was reported quite early to yield an inactive peptide.[1] However, this octapeptide was synthesized (Chart I) as a potential precursor to other 9-substituted bradykinins. We were unable to use this octapeptide as an intermediate to 9-substituted bradykinins, which we prepared ultimately as shown in Chart II. With minor variations, the three analogs were synthesized by the stepwise nitrophenyl ester procedure. This route to 9-citrulline bradykinin is markedly similar to one reported recently.[2]

The biological activity of these analogs is presented in Table I.[3] The inactivity of the octapeptide agrees with previous enzymatic degradation studies of bradykinin.[1] A striking feature of 9-citrulline and 9-histidine bradykinin is the separation of broncoconstrictor and hypotensive activity in the guinea pig. Both are extremely potent hypotensive agents with negligible bronchoconstrictor action.

Table I

Biological Activity of Bradykinin Analogs

Peptide	Broncho-constrictor activity,[a] guinea pig	Hypotensive activity,[b] guinea pig
9-Desarginine bradykinin	$<1/2000$	$<1/2000$
9-Citrulline bradykinin	$<1/1000$	$1/20$
9-Histidine bradykinin	$<1/2000$	$1/20$
Bradykinin	1	1

[a] H. O. J. Collier, J. A. Holgate, M. Schacter, and P. G. Shorley, *Brit. J. Pharmacol.*, **15**, 290 (1960). [b] A brief lowering of blood pressure through vasodilation and increase of permeability of skin capillaries.

Experimental[4]

Carbobenzoxy-L-seryl-L-prolyl-L-phenylalanine Methyl Ester (I).—A solution of 13 g. (0.055 mole) of carbobenzoxy-L-seryl-hydrazide in 140 ml. of water, 16 ml. of glacial acetic acid, and 5 ml. of concentrated hydrochloric acid was cooled to −1° and a solution of 3.85 g. (0.055 mole) of sodium nitrite in 10 ml. of water was added at such a rate that the temperature did not exceed 1°. The mixture was extracted with 450 ml. of cold (−10°) ethyl acetate. The ethyl acetate solution was separated and washed quickly with saturated sodium bicarbonate solution and dried over anhydrous magnesium sulfate in the cold. A solution of 0.05 mole of L-prolyl-L-phenylalanine methyl ester[5] in 120 ml. of cold ethyl acetate was added to this azide. The mixture was kept at 4° overnight, and then washed with water, aqueous potassium carbonate, and dilute hydrochloric acid, and dried over anhydrous magnesium sulfate. The solution was concentrated on a steam bath to 150 ml. when needles began to separate. The mixture was diluted with ether to yield 14.2 g. (57%), m.p. 97–99°, $[\alpha]^{23}D$ −65.5° (*c* 1, methanol).

Anal. Calcd. for $C_{26}H_{31}N_3O_7$: C, 62.76; H, 6.28; N, 8.45. Found: C, 62.78; H, 6.37; N, 8.61.

Carbobenzoxy-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanine Methyl Ester (II).—L-Seryl-L-prolyl-L-phenylalanine methyl ester (11 g.) was prepared by hydrogenolysis of 14.5 g. (0.029 mole) of carbobenzoxy-L-seryl-L-prolyl-L-phenylalanine methyl ester with 20% Pd–C in methanol. The tripeptide was dissolved immediately in 75 ml. of warm ethyl acetate and was treated with 12.6 g. (0.03 mole) of carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester. The mixture was stirred overnight at 35° to yield 16.2 g. (87%) of colorless needles, m.p. 156–159°, $[\alpha]^{23}D$ −40.5° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{35}H_{40}N_4O_8$: C, 65.18; H, 6.25; N, 8.69. Found: C, 65.12; H, 6.03; N, 8.87.

Carbobenzoxyglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanine Methyl Ester (III).—Carbobenzoxy-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanine methyl ester (15.5 g., 0.024 mole) was hydrogenated in methanol in the presence of 20% Pd–C in the usual manner. The reaction mixture was filtered and evaporated *in vacuo*. The oil was dissolved in 75 ml. of warm ethyl acetate and 8.0 g. (0.024 mole) of carbobenzoxyglycine *p*-nitrophenyl ester was added. The mixture was stirred at 35° for 2 days; it was washed with sodium carbonate, dilute hydrochloric acid, and saturated sodium chloride. The solution was concentrated to *ca.* 60 ml. and ether was added to yield 13 g. (77%) of colorless solid, m.p. 105–108°. It was recrystallized from methanol–water, m.p. 112–114°, $[\alpha]^{23}D$ −32.4° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{31}H_{43}N_5O_9$: C, 63.31; H, 6.17; N, 9.98. Found: C, 63.19; H, 6.08; N, 10.13.

Carbobenzoxy-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanine Methyl Ester (IV).—Carbobenzoxyglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanine methyl ester (12 g., 0.017 mole) was hydrogenated in methanol in the presence of 20% Pd–C. The solid, which was obtained by filtering the catalyst and evaporating *in vacuo*, weighed 9.3 g. It was dissolved in 60 ml. of dimethylformamide and 6.5 g. (0.0175 mole) of carbobenzoxy-L-proline *p*-nitrophenyl ester was added. The mixture was warmed at 35° for 3 days. It was diluted with ethyl acetate, and washed successively with aqueous sodium carbonate, dilute hydrochloric acid, and saturated sodium chloride solutions. The solution was concentrated to *ca.* 60 ml. and diluted with ether to yield 10.3 g. (79%) of a granular solid, m.p. 147–150°. An analytical sample was recrystallized from acetonitrile–ether, m.p. 153–155°.

Anal. Calcd. for $C_{42}H_{50}N_6O_{10}$: C, 63.14; H, 6.31; N, 10.32. Found: C, 63.02; H, 6.54; N, 10.39.

Carbobenzoxynitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanine Methyl Ester (V).—Carbobenzoxy-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanine methyl ester (3 g., 0.00375 mole) was dissolved in 50 ml. of glacial acetic acid containing 10 g. of anhydrous hydrogen bromide. The mixture was kept at room temperature 1.5 hr. and poured into 600 ml. of dry ether. The hygroscopic solid was collected, washed with ether, and dried *in vacuo*. The hydrobromide (2.6 g.) was dissolved in 15 ml. of dimethylformamide. The solution was cooled to 5° and 1.4 ml. of triethylamine was added. The mixture was filtered and to the filtrate was added 1.6 g. (0.0035 mole) of carbobenzoxy nitro-L-

(1) D. F. Elliott, G. P. Lewis, and E. W. Horton, *Biochem. Biophys. Res. Commun.*, **3**, 87 (1960).

(2) M. A. Ondetti, *J. Med. Chem.*, **6**, 10 (1963).

(3) E. D. Nicolaides, H. A. DeWald, and M. C. Craft, *Ann. N. Y. Acad. Sci.*, **104** (1963).

(4) Melting points were taken using a Thomas-Hoover capillary melting point apparatus and are corrected.

(5) W. Rittel, B. Iselin, H. Kappeler, H. Rinker, and R. Schwyzer, *Helv. Chim. Acta*, **40**, 614 (1957).

CHART I

Cbz = carbobenzoxy; DCCI = Dicyclohexylcarbodiimide

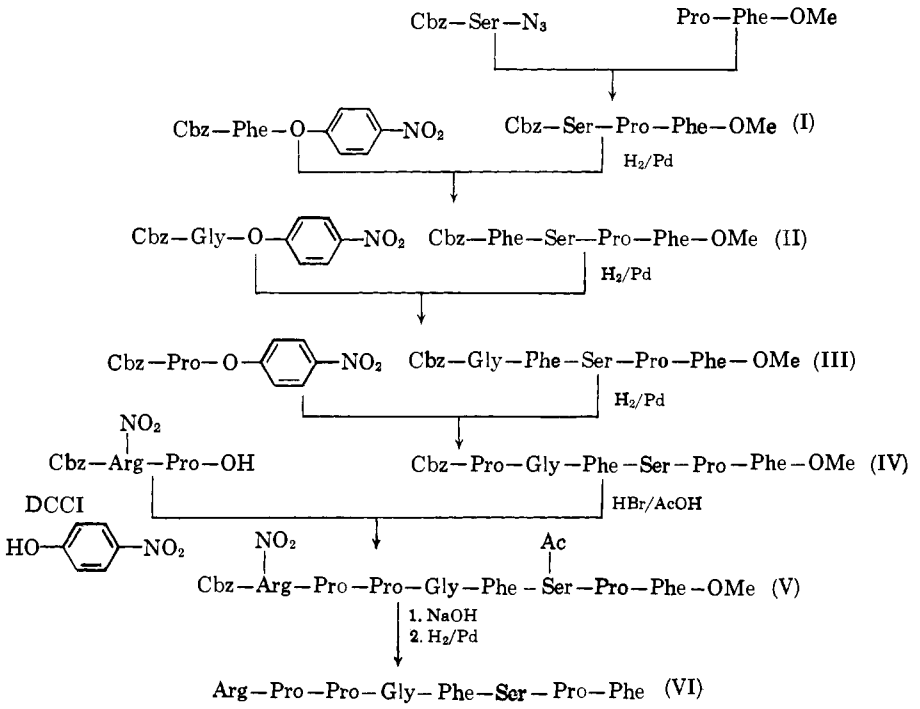
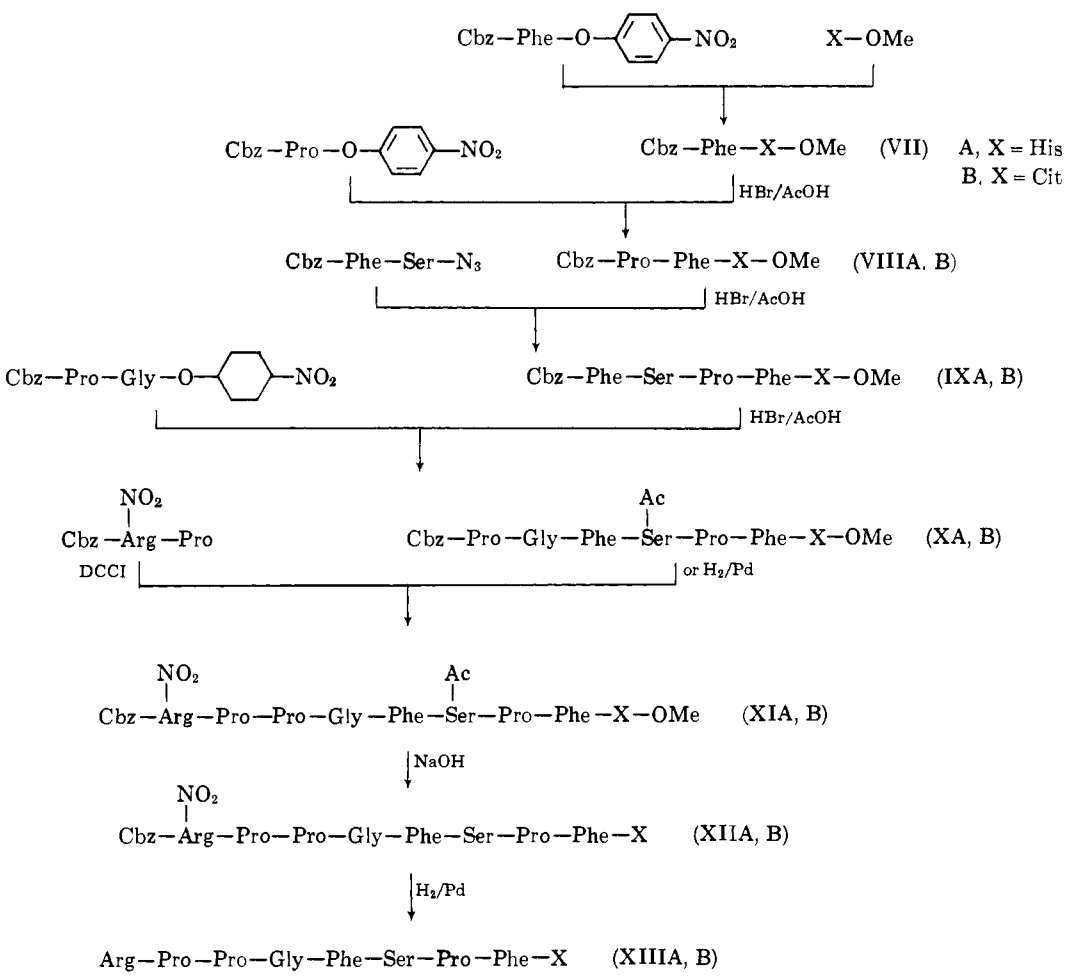


CHART II



arginyl-L-proline,[6] 0.75 g. of dicyclohexylcarbodiimide, and 0.5 g. *p*-nitrophenol. The solution was kept at 5° overnight and then warmed at 45° for 40 hr. The reaction mixture was filtered, diluted with ethyl acetate, and washed successively with aqueous sodium carbonate, dilute hydrochloric acid, and saturated sodium chloride solution. The ethyl acetate solution was evaporated *in vacuo* to yield 3.5 g. of amorphous solid, m.p. 85–90°.

Anal. Calcd. for $C_{55}H_{71}N_{12}O_{15}$: C, 57.83; H, 6.27. Found: C, 58.08; H, 7.20.

Carbobenzoxynitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanine (IV).—The protected octapeptide above (2.7 g. 0.025 mole) was dissolved in 15 ml. of methanol and treated with 5 ml. of *N* sodium hydroxide for 2 hr. at room temperature. The solution was evaporated *in vacuo*, the residue was triturated with 70 ml. of water and filtered from 1.7 g. of insoluble material. The filtrate was acidified to precipitate a gum which solidified on standing to yield 0.65 g., m.p. 75–78°, $[\alpha]^{23}D$ −46.7° (*c* 0.52, dimethylformamide).

Anal. Calcd. for $C_{52}H_{67}N_{12}O_{14}\cdot H_2O$: C, 56.70; H, 6.23; N, 15.26. Found: C, 56.26; H, 6.31; N, 15.26.

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanine (VI).—The aforesaid octapeptide (450 mg.) was hydrogenated in acetic acid–methanol over platinum black in the usual manner to yield 350 mg. of water-soluble solid $[\alpha]^{24}D$ −80.5° (*c* 1, *N* acetic acid). It was shown to be homogeneous by paper electrophoresis.

Anal. Calcd. for $C_{44}H_{61}N_{11}O_{10}\cdot CH_3COOH\cdot 2H_2O$: C, 55.24; H, 6.95; N, 15.41. Found: C, 55.04; H, 6.67; N, 15.71.

Carbobenzoxy-L-phenylalanyl-L-histidine Methyl Ester[7] (VIIA).—To a solution of 25 g. (0.06 mole) of carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester in 70 ml. of dimethylformamide was added 14.5 g. (0.06 mole) of L-histidine methyl ester dihydrochloride and 17 ml. of triethylamine. The mixture was stirred at room temperature for 20 hr. and filtered. The filtrate was diluted with 250 ml. of ethyl acetate and washed with water, aqueous sodium carbonate, and aqueous saturated sodium chloride. The ethyl acetate solution was dried over anhydrous magnesium sulfate and concentrated to *ca.* 75 ml. Dilution with petroleum ether precipitated the crude dipeptide. It was recrystallized from chloroform–petroleum ether, 24.8 g. (92%), m.p. 121–124°, $[\alpha]^{23}D$ −19.4° (*c* 2, dimethylformamide).

Anal. Calcd. for $C_{24}H_{26}N_4O_4$: C, 63.99; H, 5.82; N, 12.44. Found: C, 64.04; H, 5.70; N, 12.58.

Carbobenzoxy-L-phenylalanyl-L-citrulline methyl ester (VIIB) was prepared in the same manner from carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester and L-citrulline methyl ester, hydrochloride in 60% yield, m.p. 170–171°, $[\alpha]^{26}D$ −10.9°, −12.4°.

Anal. Calcd. for $C_{24}H_{30}N_4O_6$: C, 61.27; H, 6.42; N, 11.91. Found: C, 61.44; H, 6.34; N, 12.08.

Carbobenzoxy-L-prolyl-L-phenylalanyl-L-histidine Methyl Ester (VIIIA).—Carbobenzoxy-L-phenylalanyl-L-histidine methyl ester (27 g. 0.06 mole) was dissolved in 250 ml. of glacial acetic acid containing 30 g. of dry hydrogen bromide. After 1 hr., the solution was poured into 1 l. of dry ether. The precipitate was collected, washed with ether, and dried *in vacuo*. The product (29 g.) was dissolved in 100 ml. of dimethylformamide and cooled to 4°. Triethylamine (17 ml.) was added and after 15 min., the mixture was filtered and 22 g. (0.06 mole) of carbobenzoxy-L-proline *p*-nitrophenyl ester was added. The yellow solution was stirred at 30° for one day and then diluted with 250 ml. of ethyl acetate. The solution was washed with water and aqueous potassium carbonate, as a voluminous precipitate formed in the organic layer. The mixture was filtered, washed with water, and the product air-dried. It was recrystallized from acetonitrile–ether to yield 23.2 g. (70%) of colorless needles, m.p. 160–162°, $[\alpha]^{23}D$ −38.2° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{29}H_{33}N_5O_6$: C, 63.59; H, 6.07; N, 12.79. Found: C, 63.27; H, 5.80; N, 12.66.

Carbobenzoxy-L-prolyl-L-phenylalanyl-L-citrulline methyl ester (VIIIB) was prepared in the same manner. From 21.6 g. of carbobenzoxy-L-proline *p*-nitrophenyl ester and 27.4 g. of carbobenzoxy-L-phenylalanyl-L-citrulline methyl ester there was obtained 26 g. (80%), m.p. 187–190°, $[\alpha]^{26}D$ −43° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{29}H_{37}N_5O_7$: C, 61.35; H, 6.57; N, 12.31. Found: C, 61.50; H, 6.56; N, 12.19.

Carbobenzoxy-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-histidine Methyl Ester (IXA).—Carbobenzoxy-L-prolyl-L-phenylalanyl-L-histidine methyl ester (7.6 g., 0.014 mole) was dissolved in 100 ml. of glacial acetic acid containing 15 g. of dry hydrogen bromide. After 1.5 hr., the mixture was diluted with 1 l. of dry ether to precipitate 8.7 g. of hygroscopic solid. This product was dissolved in 40 ml. of dimethylformamide, cooled to 4°, and treated with 7 ml. of triethylamine. Meanwhile carbobenzoxy-L-phenylalanyl-L-serine azide was prepared[8] from 6 g. of the hydrazide in 150 ml. of cold ethyl acetate. This dipeptide azide was added to the filtered solution of L-prolyl-L-phenylalanyl-L-histidine methyl ester and kept overnight at 5°. The mixture was washed with water, aqueous sodium bicarbonate, and dilute hydrochloric acid; the ethyl acetate solution was dried over magnesium sulfate and evaporated *in vacuo* to yield 6.0 g. of a glass. The pentapeptide was not crystallized.

Carbobenzoxy-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-citrulline methyl ester (IXB) was prepared in the same manner from 6 g. of carbobenzoxy-L-phenylalanyl-L-serine hydrazide and 7.5 g. of carbobenzoxyl-L-prolyl-L-phenylalanyl-L-citrulline methyl ester to yield 4.6 g. (39%), m.p. 149–152° from methanol, $[\alpha]^{23}D$ −41.8° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{41}H_{51}N_7O_{10}$: C, 61.40; H, 6.41; N, 12.23. Found: C, 61.22; H, 6.50; N, 12.05.

Carbobenzoxy-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-L-histidine Methyl Ester (XA).—Carbobenzoxy-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-histidine methyl ester (5.4 g., 0.0066 mole) was dissolved in 75 ml. of glacial acetic acid containing 15 g. of dry hydrogen bromide. The solution was kept at 25° for 1.5 hr. and then poured into 500 ml. of dry ether. The solid was collected, washed with dry ether, and dried *in vacuo*. The product (5.0 g.) was dissolved in 25 ml. of dimethylformamide, cooled to 4°, and treated with 2.8 ml. of triethylamine. The mixture was filtered and to the filtrate was added 2.9 g. (0.0068 mole) of carbobenzoxy-L-prolylglycine *p*-nitrophenyl ester. The mixture was stirred at 40–45° for two days and then diluted with ethyl acetate. The solution was washed with aqueous potassium carbonate and saturated sodium chloride solutions, dried over magnesium sulfate, and evaporated *in vacuo*. The solid was crystallized from methanol–water to yield 4.3 g. (62%) of colorless solid, m.p. 132–134°, $[\alpha]^{23}D$ −55.8° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{50}H_{59}N_9O_{12}\cdot H_2O$: C, 60.30; H, 6.17; N, 12.65, O-acetyl, 4.3. Found: C, 60.58; H, 6.21; N, 12.78; O-acetyl, 4.2.

Carbobenzoxy-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-L-citrulline methyl ester (XB) was prepared in the foregoing manner from 2.3 g. (0.0054 mole) of carbobenzoxy-L-prolylglycine *p*-nitrophenyl ester and 4.2 g. of carbobenzoxy-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-citrulline methyl ester in 68% yield (3.5 g.), m.p. 210–212° from methanol-water, $[\alpha]^{23}D$ −59° (*c* 0.76, dimethylformamide); lit.[3] m.p. 214–216°, $[\alpha]^{26}D$ −47.7° (*c* 1.09, dimethyl sulfoxide).

Anal. Calcd. for $C_{50}H_{63}N_9O_{13}$: C, 60.16; H, 6.36; N, 12.63; O-Acetyl, 4.3. Found: C, 59.71; H, 6.19; N, 12.40; O-acetyl, 4.9.

Carbobenzoxynitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-L-histidine Methyl Ester (XIA).—A solution of 3.7 g. (0.004 mole) of carbobenzoxy-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-L-histidine methyl ester in methanol was hydrogenated over 20% Pd–C in the usual manner. The filtered solution was evaporated *in vacuo* to yield 3.1 g. of the free heptapeptide methyl ester. This solid was dissolved in 15 ml. of dimethylformamide and 1.8 g. (0.004 mole) of carbobenzoxynitro-L-arginyl-L-proline[5] was added. The solution was cooled to 5° and 1 g. of dicyclohexylcarbodiimide was added. The mixture was kept at 5° overnight, then filtered, and the filtrate was evaporated *in vacuo*. The residue was triturated with ether. The solid was washed thoroughly with sodium bicarbonate to yield 4.2 g. of amorphous powder, m.p. 85–90°, $[\alpha]^{23}D$ −32° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{61}H_{77}N_{15}O_{18}$: C, 57.40; H, 6.10; N, 16.46. Found: C, 57.64; H, 6.87; N, 15.97.

Carbobenzoxynitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-L-citrulline methyl ester (XIB) was prepared in the foregoing manner from 1.15 g. (0.0025 mole) of carbobenzoxynitro-L-arginyl-L-proline

(6) R. P. Boissonnas, St. Guttmann, and P-A. Jaquenoud, *Helv. Chim. Acta*, **43**, 1349 (1960).

(7) D. Theodoropoulos and L. C. Craig, *J. Org. Chem.*, **20**, 1169 (1955).

(8) E. D. Nicolaides and H. A. DeWald, *ibid.*, **26**, 3872 (1961).

and L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-L-citrulline methyl ester hydrobromide (2.2 g.). The crude solid solidified from acetonitrile to yield 1.4 g., m.p. 118–120°, $[\alpha]^{23}D$ −54.4° (*c* 0.5, dimethylformamide); lit.[2] m.p. 155–160° (sintering 115°), $[\alpha]^{20}D$ −61.3° (*c* 0.99, dimethylformamide).

Anal. Calcd. for $C_{61}H_{81}N_{15}O_{17}\cdot 3H_2O$: C, 54.25; H, 6.49; N, 15.56. Found: C, 54.32; H, 6.35; N, 15.02.

Carbobenzoxynitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-histidine (XIIA).—The protected nonapeptide methyl ester (2.4 g., 0.002 mole) was dissolved in 20 ml. of methanol and 2.5 ml. of *N* sodium hydroxide was added. The mixture was allowed to stand 2.5 hr. and was evaporated *in vacuo*. The residue was partitioned between 75 ml. of water and 75 ml. of ethyl acetate. The aqueous layer was separated and acidified with *N* hydrochloric acid to precipitate a small amount of oil which solidified, 0.3 g., m.p. 140° with preliminary softening.

Anal. Calcd. for $C_{58}H_{73}N_{15}O_{15}\cdot H_2O$: C, 56.27; H, 6.11; N, 16.97. Found: C, 56.02; H, 6.28; N, 16.94.

Carbobenzoxynitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-citrulline (XIIB) was prepared by hydrolysis of 1 g. of the protected methyl ester in methanolic sodium hydroxide to yield 0.65 g. of amorphous solid, m.p. 125–130°.

Anal. Calcd. for $C_{58}H_{77}N_{15}O_{16}\cdot H_2O$: C, 55.38; H, 6.31; N, 16.70. Found: C, 55.46; H, 6.62; N, 15.74.

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-histidine (9-Histidine Bradykinin) (XIIIA).—Carbobenzoxynitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-histidine (250 mg.) was hydrogenated in glacial acetic acid–methanol over palladium black in the usual manner. The mixture was filtered and evaporated *in vacuo*. The residue was dissolved in water, filtered, shell frozen, and lyophilized to give 220 mg. of a solid which gave a single spot on electrophoresis in acetate buffer, pH 5.6, 3 hr. at 30 ma.

Anal. Calcd. for $C_{50}H_{68}N_{14}O_{11}\cdot CH_3COOH\cdot 4H_2O$: C, 52.20; H, 6.46; N, 17.75. Found: C, 52.59; H, 6.76; N, 15.77.

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-citrulline (9-Citrulline Bradykinin) (XIIIB).—Hydrogenation of 500 mg. of the protected citrulline nonapeptide in the same manner yielded 275 mg. of water-soluble solid, $[\alpha]^{22}D$ −79° (*c* 0.85, *N* acetic acid), lit.[2] $[\alpha]^{22}D$ −88.7° (*c* 1, *N* acetic acid).

Anal. Calcd. for $C_{50}H_{72}N_{14}O_{12}\cdot 2CH_3COOH\cdot 4H_2O$: C, 51.70; H, 7.08; N, 15.65. Found: C, 51.00; H, 6.8; N, 15.40.

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Thyromimetics. III. The Synthesis and Relative Thyromimetic Activities of Some 4′-Ethers of Iodinated Thyronines and Thyroalkanoic Acids

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4′-Methyl, ethyl, and β-diethylaminoethyl ethers of several thyroxine-like compounds were prepared by treating suitable intermediates with base and dialkyl sulfates or β-diethylaminoethyl chloride. Protecting groups were hydrolyzed to yield the desired ethers (IIa–f, Va–d, and VIa and b) which were screened for their ability to lower plasma cholesterol levels in rats fed a cholesterol–cholic acid diet. Interesting compounds were studied further for their ability to stimulate oxygen consumption and/or to increase heart weight. A comparison of these results with the values from the cholesterol-lowering screen gave some indication of whether the compounds had a desired separation of activities.

During the study in our Laboratories of various classes of thyroxine-like compounds for their thyromimetic activities, it was noted that by controlling the administered dose of the methyl ether of 3,3′,5-triiodothyropropionic acid a good separation between calorigenic and antigoitrogenic responses could be obtained in the rat.[1] This initial observation coupled with the report by Herman, Lee, and Parker[2] that 4′-methyl ethers of thyromimetic substances often possess a large separation between the minimum effective hypocholesteremic dose and the dose which causes weight loss in animals prompted us to prepare a few examples of methyl ethers in an earlier study.[3] These results were encouraging enough that several additional 4′-methoxy compounds have now been screened for their cholesterol-lowering activity. In addition, two 4′-ethyl ethers were prepared to determine what effects a larger alkyl group in the 4′-position would have on biological activity. Moreover, since the preparation of β-diethylaminoethyl esters of several iodinated thyroalkanoic acids resulted in compounds with good hypocholesteremic activity,[3] the cholesterol-lowering activity of some representative 4′-β-diethylaminoethyl ethers was determined. It was hoped that a study of these types of structures would reveal compounds that would prove to be potent hypocholesteremic agents in man at doses which would cause no calorigenic or cardiac distress.

The methyl and ethyl ethers of thyroalkanoic acids (IIa–f) used in this study were prepared by treating the requisite acids (I) with either dimethyl or diethyl sulfate in the presence of aqueous base.[3] Diethylaminoethyl ethers (IV) were prepared from the requisite thyroalkanoic acid ethyl esters (III) on treatment with sodium methoxide and β-diethylaminoethyl chloride. These intermediates (IV) were then converted to the desired ethers (V) upon hydrolysis with hydrochloric acid.

In some instances the intermediate esters (IV) were isolated, purified, and characterized as their hydrochlorides. In other instances they were hydrolyzed directly to V.

The methyl ethers VIa and b of 3,3′,5-triiodo-D and L-thyronine were prepared *via* their N-benzoyl methyl esters as indicated by Tomita, *et al.*[4]

(1) C. M. Greenberg, L. F. Mansor, C. A. Bocher, H. L. Saunders, and J. F. Kerwin, *Endocrinology*, **70**, 365 (1962).

(2) R. G. Herman, C. C. Lee, and R. Parker, *Arch. Intern. Pharmacodyn.*, **133**, 284 (1961).

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