the aqueous solution was adjusted to pH 1.5 with *N* hydrochloric acid. After removal of the water *in vacuo*, a colorless solid was obtained, 2.4 g. (69%), m.p. 260–261°. The analytical sample was prepared by recrystallization from ethanol–ether, $[\alpha]^{24}D$ +15.1° (*c* 2.0, water).

(+)-N-Trifluoroacetyl-1-(4-benzamidophenyl)-2-propylamine.—To a cooled solution of 10 g. (0.035 mole) of (−)-N-trifluoroacetyl-1-(4-aminophenyl)-2-propylamine in 50 ml. of methylene chloride, was added dropwise with stirring 4.1 ml. (0.035 mole) of benzoyl chloride in 20 ml. of methylene chloride and 4.8 ml. (0.035 mole) of triethylamine in 20 ml. of methylene chloride. The mixture was stirred at 25° for 12 hr., water added, and the white solid that formed was removed by suction filtration. There was obtained 11.7 g. (96%), m.p. 219–220°. An analytical sample was prepared by recrystallization from ethanol–water, m.p. 220–221°, $[\alpha]^{23}D$ +15.7° (*c* 2.0, dimethylacetamide).

Anal. Calcd. for $C_{18}H_{17}F_3N_2O_2$: C, 61.71; H, 4.89; N, 8.00. Found: C, 61.77; H, 5.20; N, 7.97.

(+)-1-(4-Benzamidophenyl)-2-propylamine Hydrochloride.—A mixture of 3.0 g. (0.0086 mole) of (+)-N-trifluoroacetyl-1-(4-benzamidophenyl)-2-propylamine and 8.7 ml. of *N* sodium hydroxide in 25 ml. of ethyl alcohol was stirred at 25° for 16 hr. The alcohol was removed *in vacuo* and the aqueous solution acidified with *N* hydrochloric acid. The water was removed *in vacuo* and the residue recrystallized from ethanol–ether, 1.8 g. (70%), $[\alpha]^{24}D$ +17.8° (*c* 2.0, water).

Pharmacology. Rat.[30]—Food (Purina lab chow in spill-proof cups) was presented to mature Sprague–Dawley male rats for 1 hr. at the same time each day. *Ad libitum* watering was permitted. An aqueous solution of the compound was administered *via* stomach tube to randomly selected groups of 6 rats. Compounds were given twice weekly. Control groups received only water. Food intake was measured for 1 hr., beginning 1 hr. after the compound was administered. Compounds were active if there were significant (t-test, 5% level of confidence) reductions in food intake compared with controls. Compounds were tested at 30, 10, and 5 mg./kg. If inactive at one dose they were not tested at a lower dose.

For a selected group of compounds, potency determinations were made. A three-point parallel line bioassay design was superimposed on the usual testing procedure. Twelve rats were employed at each point for standard (usually (+)-amphetamine) and test compounds. The potencies were calculated after suitable statistical analysis.[19]

Dog.—Food (canned meat) was presented to mongrel dogs, weighing 2.74–5.48 kg., for 1 hr. at the same time each day. Each compound was administered by means of a capsule to randomly selected groups of 4 dogs. Compounds were given 2 or 3 times each week. Food intake was measured for 1 hr., beginning 1 hr. after the compound was administered. Compounds were considered to be active when food intake for each of the 4 treated dogs was at least 100 g. less than under control conditions. On control days, the same 4 dogs received only empty capsules. Borderline and procedurally suspect results were repeated.

Potency determinations for a selected group of compounds were made. The parallel line assays, employing groups of 12 dogs in crossover designs[19] were superimposed on the usual preliminary testing procedures.

(30) Recently, J. A. Gylys, J. J. D. Hart, and M. R. Warren, *J. Pharmacol. Expll. Therap.*, **137**, 365 (1962), reported that chlorphentermine causes anorexia in rats without any increase in motor activity.

The Synthesis of 8-D-Phenylalanine-, 8-*p*-Fluoro-L-phenylalanine-, and 8-*p*-Fluoro-D-phenylalanine-bradykinin

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Three new analogs of bradykinin have been prepared in which the penultimate amino acid phenylalanine has been replaced by D-phenylalanine, *p*-fluoro-L-phenylalanine, and *p*-fluoro-D-phenylalanine. The synthetic approaches used to obtain the three analogs were identical and consisted mainly of the stepwise lengthening of the peptide chain with the appropriate protected amino acid *p*-nitrophenyl ester.

The replacement of one amino acid by another of similar or diverse structure in a peptide which has been found to possess a profound biological effect has been a favored approach used by many investigators to obtain either compounds with enhanced activity, *e.g.*, desamino-oxytocin and 1-desamino-8-lysine-vasopressin[1] or inhibitors of the parent compound, *e.g.*, 3-homotyrosine-oxytocin.[2] Although this approach in the angiotensin area has not yielded analogs with enhanced, prolonged, or antagonistic properties, useful information has been obtained about structure–activity relationships.[3,4]

This report describes three new analogs of bradykinin in which phenylalanine in position 8 of the molecule has been replaced by D-phenylalanine, *p*-fluoro-L-phenylalanine, and *p*-fluoro-D-phenylalanine. The variation of a peptide structure by changing the optical configuration is a device that has not been greatly explored. Some interest in this direction has been reported[5,6] with the peptide antibiotics, the change being from D to L rather than L to D and an oxytocin analog containing a D-amino acid recently has been described.[7] The influence of such a change or of introducing *p*-fluorophenylalanine on the activity of a peptide was believed to be unpredictable, but recent experiences with angiotensin analogs[8] have indicated that in the bradykinin series activity might be retained.

The synthetic scheme used to prepare the three nonapeptides was identical with that used in the preparation of bradykinin[9] and is shown in Chart I.

The *p*-fluorophenylalanine was resolved into its optical antipodes using purified carboxypeptidase on

(1) D. R. Hope, V. V. S. Marti, and V. du Vigneaud, *J. Biol. Chem.*, **237**, 1563 (1962); W. Y. Chan and V. du Vigneaud, *Endocrinology*, **71**, 977 (1962).

(2) S. Guttmann, P. A. Jaquenoud, and R. A. Boissonnas, *Naturwissenschaften*, **44**, 632 (1957).

(3) R. Schwyzer, *Helv. Chim. Acta*, **44**, 667 (1961).

(4) F. M. Bumpus, P. A. Khairallah, K. Arakawa, I. H. Page, and R. S. Sueby, *Biochim. Biophys. Acta*, **46**, 38 (1961).

(5) B. F. Erlanger, W. V. Curran, and N. Kokowsky, *J. Am. Chem. Soc.*, **81**, 3055 (1959).

(6) E. D. Nicolaides and M. E. Munk, to be published.

(7) C. H. Schneider and V. du Vigneaud, *J. Am. Chem. Soc.*, **84**, 3003 (1962).

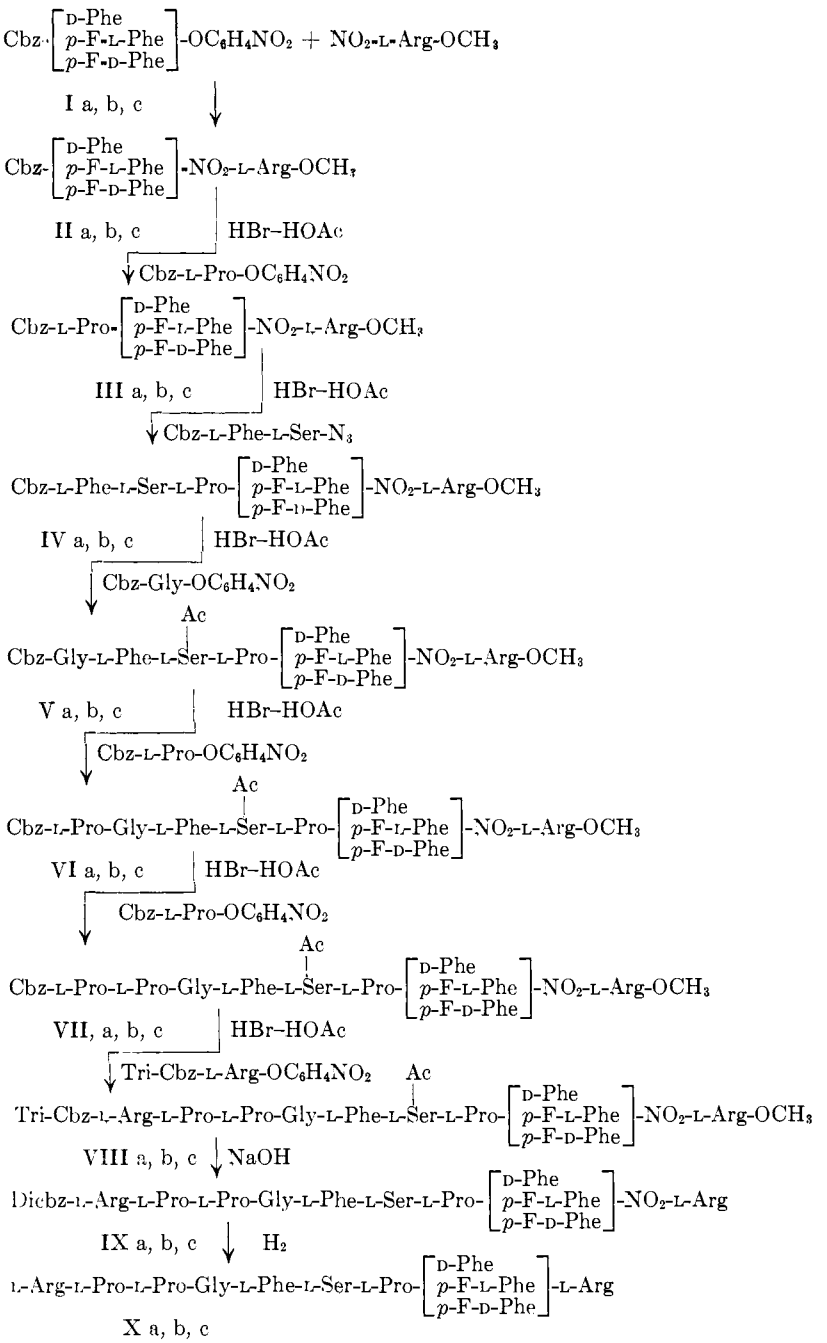
(8) E. D. Nicolaides, M. E. Munk, H. A. DeWald, and D. F. Hylander, unpublished results.

(9) E. D. Nicolaides and H. A. DeWald, *J. Org. Chem.*, **26**, 3872 (1961).

CHART I

Series:

a = D-Phenylalanine
b = *p*-Fluoro-L-phenylalanine
c = *p*-Fluoro-D-phenylalanine



the N-chloroacetyl derivative.[10] The *p*-nitrophenyl esters of carbobenzoxy-D-phenylalanine and the two *p*-fluoro derivatives were prepared in the usual manner using *p*-nitrophenol and dicyclohexylcarbodiimide and were obtained in a pure, crystalline state. All of the intermediate peptides following the pentapeptide stage and up to the hydrolysis of the methyl ester function with alkali were found to have O-acetyl groups on the serine hydroxyl function.[11] In a few cases it was found that the peptide was only partially acetylated. These products were found to be difficult to crystallize and had wide-range low melting points. Subsequent reactions occasionally gave the fully acetylated crystalline intermediates with considerably higher melting points.

The purity of the final products obtained after the catalytic hydrogenation step was determined by microanalysis, paper chromatography, and paper electrophoresis. These methods indicated the analogs to be pure, single components. The results of the biological comparison of the three analogs to synthetic bradykinin are given in Table I.[12] The high activity of the 8-*p*-fluoro-L-phenylalanine analog was unexpected. That this was true kinin-like activity is indicated by the various tests in which it was more active than bradykinin and also by the fact that the guinea pig bronchoconstriction was antagonized by aspirin. The reduction in activity of the 8-*p*-fluoro-D-phenylalanine analog parallels that seen between the 8-D-phenylalanine analog and bradykinin. None of the three analogs appeared to antagonize the action of brady-

(10) This resolution was kindly carried out by Dr. R. D. Westland of these Laboratories using the procedure described for phenylalanine: J. B. Gilbert, V. E. Price, and J. P. Greenstein, *J. Biol. Chem.*, **180**, 473 (1949).

(11) E. D. Nicolaides and H. A. DeWald, *J. Org. Chem.*, **28**, 1926 (1963).

(12) We are indebted to Dr. D. A. McCarthy, Dr. H. O. J. Collier, Miss P. G. Shorley, and Miss R. A. Hamilton for the results of these assays.

Table I

Biological Activity of Bradykinin Analogs

Peptide	Bronchoconstriction (guinea pig)[a] Antagonized by aspirin	Vasodilation Guinea pig	Vasodilation Dog[b]
8-D-Phenylalanine bradykinin	1/20	+	1/4	1/5
8-*p*-Fluoro-L-phenylalanine bradykinin	1/4	+	1/5	1/5
8-*p*-Fluoro-D-phenylalanine bradykinin	1/100	+	1/3	
Bradykinin	1	+	1	1

[a] See ref. 13. [b] See ref. 14.

kinin in the dog or guinea pig lung or blood pressure.

Experimental[15]

Carbobenzoxy-*p*-fluoro-L-phenylalanine.—To a cold (10°) solution of 16 g. (0.0875 mole) of *p*-fluoro-L-phenylalanine[16] {$[\alpha]^{23}D$ −23° (*c* 2, water)} in 45 ml. of 2 *N* sodium hydroxide was added dropwise 17 g. (0.1 mole) of carbobenzoxy chloride and 50 ml. of 2 *N* sodium hydroxide over 1 hr. The mixture was stirred an additional 1.5 hr. at 10°, washed 3 times with ether, and the aqueous layer was acidified with cold, concentrated hydrochloric acid. The solid which separated was extracted with ethyl acetate and the ethyl acetate solution was washed with water, dried over magnesium sulfate, and evaporated to *ca.* 100 ml. Petroleum ether was added giving white needles, 25 g. (93%), m.p. 102–104°, $[\alpha]^{23}D$ −6.8° (*c* 2, methanol).

Anal. Calcd. for $C_{17}H_{16}FNO_4$: C, 64.35; H, 5.08; N, 4.42; F, 5.99. Found: C, 64.14; H, 5.35; N, 4.39; F, 6.08.

Carbobenzoxy-*p*-fluoro-D-phenylalanine.—A reaction identical with the one described above was carried out on 21 g. (0.115 mole) of *p*-fluoro-D-phenylalanine, $[\alpha]^{23}D$ +21.6° (*c* 2, water), reported[16] $[\alpha]^{20}D$ +24° (*c* 2, water), giving 32 g. (88%) of white needles, m.p. 103–104°, $[\alpha]^{23}D$ +8.8° (*c* 2, methanol).

Anal. Found: C, 64.14; H, 5.12; N, 4.34; F, 5.91.

Carbobenzoxy-D-phenylalanine *p*-Nitrophenyl Ester (Ia).—To a cold (5°) solution of 32 g. (0.107 mole) of carbobenzoxy-D-phenylalanine in 250 ml. of dimethylformamide was added 14.8 g. (0.107 mole) of *p*-nitrophenol followed by 22.1 g. (0.107 mole) of dicyclohexylcarbodiimide. The mixture was kept at 5° overnight, the precipitate was removed, and the filtrate was evaporated to 100 ml. The solution was diluted with 400 ml. of ethyl acetate and was washed 3 times with water. The ethyl acetate solution was dried over magnesium sulfate, evaporated to 100 ml., and ether was added, giving a faint yellow solid which was recrystallized from ethyl acetate–ether, yield 34 g. (76%), m.p. 119–121°, $[\alpha]^{23}D$ +23.9° (*c* 2, dimethylformamide).

Anal. Calcd. for $C_{23}H_{20}N_2O_6$: C, 65.71; H, 4.80; N, 6.66. Found: C, 65.82; H, 5.03; N, 6.62.

Carbobenzoxy-*p*-fluoro-L-phenylalanine *p*-Nitrophenyl Ester (Ib).—The procedure used above gave in 88% yield faint yellow needles, 24 g., m.p. 137–138°, $[\alpha]^{23}D$ −29.6° (*c* 1, methanol).

Anal. Calcd. for $C_{23}H_{19}FN_2O_6$: C, 63.01; H, 4.48; N, 6.40. Found: C, 62.94; H, 4.39; N, 6.54.

Carbobenzoxy-*p*-fluoro-D-phenylalanine *p*-Nitrophenyl Ester (Ic).—From 32 g. of carbobenzoxy-*p*-fluoro-D-phenylalanine was obtained 42 g. (95%) of an off-white solid, m.p. 139–140°, $[\alpha]^{23}D$ +28.3° (*c* 0.5, methanol).

Anal. Found: C, 63.25; H, 4.52; N, 6.55.

Carbobenzoxy-D-phenylalanylnitro-L-arginine Methyl Ester (IIa).—To a cold solution (5°) of 12.8 g. (0.0475 mole) of nitro-L-arginine methyl ester hydrochloride in 200 ml. of dimethylformamide was added a cold (5°) solution of 4.8 g. (0.048 mole) triethylamine. The precipitate was removed and to the filtrate was added 20 g. (0.0475 mole) of carbobenzoxy-D-phenylalanine *p*-nitrophenyl ester. The solution was allowed to stand 4 days at room temperature. It was evaporated to 150 ml., diluted with 400 ml. of ethyl acetate, and washed twice with water, 3 times with dilute ammonium hydroxide, twice again with water, and finally with dilute hydrochloric acid. The ethyl acetate solution was dried over anhydrous magnesium sulfate and evaporated to an oil which gradually turned to a white solid on the addition of petroleum ether. The product was crystallized from ethyl acetate–petroleum ether, yield 20.4 g. (84%), m.p. 90–92°, $[\alpha]^{23}D$ −5° (*c* 2, dimethylformamide).

Anal. Calcd. for $C_{24}H_{30}N_6O_7$: C, 56.03; H, 5.87; N, 16.33. Found: C, 56.27; H, 5.97; N, 16.17.

Carbobenzoxy-*p*-fluoro-L-phenylalanylnitro-L-arginine Methyl Ester (IIb).—The reaction of nitro-L-arginine methyl ester with the *p*-nitrophenyl ester of carbobenzoxy-*p*-fluoro-L-phenylalanine gave the dipeptide in 83% yield as a white solid, 22 g., m.p. 94–97°, $[\alpha]^{23}D$ −11° (*c* 1, methanol).

Anal. Calcd. for $C_{24}H_{29}FN_6O_7$: C, 54.13; H, 5.48; N, 15.78. Found: C, 53.99; H, 5.71; N, 15.61.

Carbobenzoxy-*p*-fluoro-D-phenylalanylnitro-L-arginine Methyl Ester (IIc).—This dipeptide was obtained in 88% yield as a white solid; 44 g., m.p. 90–95°, $[\alpha]^{23}D$ −6.7° (*c* 1.1, methanol).

Anal. Found: C, 53.85; H, 5.39; N, 15.77.

Carbobenzoxy-L-prolyl-D-phenylalanylnitro-L-arginine Methyl Ester (IIIa).—Into a cooled (10°) solution of 20 g. (0.039 mole) of carbobenzoxy-D-phenylalanylnitro-L-arginine methyl ester in 250 ml. of glacial acetic acid was bubbled 20 g. (0.25 mole) of dry hydrogen bromide. The solution was allowed to stand at room temperature for 2 hr. with occasional swirling. It was added rapidly to 2 l. of vigorously stirred dry ether, the white precipitate which formed was allowed to settle and the supernatant liquid decanted. The solid was washed several times with dry ether and collected on a sintered glass funnel. The product was dried overnight in a vacuum desiccator; yield of cream-colored solid, 22 g. The solid was dissolved in 150 ml. of dimethylformamide and cooled to 0° along with 7 g. (0.070 mole) of triethylamine. The solutions were combined and after 10 min. the white precipitate was removed. To the filtrate was added 14.8 g. (0.040 mole) of carbobenzoxy-L-proline *p*-nitrophenyl ester. The solution was stirred at room temperature for 2 days, evaporated to 100 ml., and diluted with 500 ml. of ethyl acetate. The ethyl acetate solution was washed twice with water, 5 times with dilute ammonium hydroxide, twice again with water, and once with dilute hydrochloric acid. The ethyl acetate solution was dried over anhydrous magnesium sulfate and concentrated to 50 ml. A white precipitate formed upon the addition of petroleum ether and was crystallized from methanol–ether as a white solid; yield 18 g. (75%), m.p. 115–117°, $[\alpha]^{23}D$ −35.1° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{29}H_{37}N_7O_8$: C, 56.94; H, 6.10; N, 16.03. Found: C, 57.05; H, 6.13; N, 16.01.

Carbobenzoxy-L-prolyl-*p*-fluoro-L-phenylalanylnitro-L-arginine Methyl Ester (IIIb).—A reaction identical with the one above, but using carbobenzoxy-*p*-fluoro-L-phenylalanylnitro-L-arginine methyl ester gave 20 g. (85%) of white solid, m.p. 95–98° $[\alpha]^{23}D$ −54.7° (*c* 2.2, methanol).

Anal. Calcd. for $C_{29}H_{36}FN_7O_8$: C, 55.32; H, 5.76; N, 15.56. Found: C, 55.30; H, 5.68; N, 15.74.

Carbobenzoxy-L-prolyl-*p*-fluoro-D-phenylalanylnitro-L-arginine Methyl Ester (IIIc).—The tripeptide was obtained as a colorless solid, yield 42 g. (82%), m.p. 106–108°, starting with 42 g. of carbobenzoxy-*p*-fluoro-D-phenylalanylnitro-L-arginine methyl ester, $[\alpha]^{23}D$ −28.9° (*c* 2, methanol).

Anal. Found: C, 55.40; H, 5.83; N, 15.40.

Carbobenzoxy-L-phenylalanyl-L-seryl-L-prolyl-D-phenylalanylnitro-L-arginine Methyl Ester (IVa).—The carbobenzoxytripeptide methyl ester IIIa (16.4 g., 0.027 mole) was dissolved in 200 ml. of glacial acetic acid at 10° containing 20 g. (0.25 mole) of dry hydrogen bromide. The solution was kept at room temperature for 2 hr. and poured into 1.5 l. of dry ether. The precipitate was removed, washed thoroughly with dry ether, and dried *in vacuo*. The yield of crude product was 18.5 g. Twelve grams (0.03 mole) of carbobenzoxy-L-phenylalanyl-L-serine hydrazide

(13) H. O. J. Collier, J. A. Holgate, M. Schachter, and P. G. Shorley, *Brit. J. Pharmacol.*, **15**, 290 (1960).

(14) E. Beck, *Circulation*, **17**, 798 (1958).

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

(16) E. L. Bennett and C. Niemann, *J. Am. Chem. Soc.*, **72**, 1800 (1950).

was dissolved in 100 ml. of glacial acetic acid and 17 ml. of 2 *N* hydrochloric acid and the solution cooled to 5°. To the cold solution was added in portions 3.3 g. (0.056 mole) of sodium nitrite in 20 ml. of water. The solution was allowed to stand 5 min. and was then diluted with 500 ml. of ice-water. The precipitate was extracted with cold ethyl acetate, the organic layer was washed twice with ice-water, and then with cold, saturated sodium carbonate solution until the wash water was basic. The ethyl acetate solution was dried over anhydrous magnesium sulfate at 0°. To a freshly prepared solution of 14.8 g. (0.027 mole) of L-prolyl-D-phenylalanylnitro-L-arginine methyl ester hydrobromide in 100 ml. of dimethylformamide at 5° was added 6.8 g. (0.068 mole) of triethylamine. The mixture was filtered and the filtrate was added to the ethyl acetate solution containing the dipeptide azide. The solution was kept for 2 days at 5°, washed with water, aqueous 5% sodium bicarbonate solution, water, dilute hydrochloric acid, and was dried and evaporated to a small volume. A gummy solid which formed upon the addition of petroleum ether was crystallized from ethyl acetate–petroleum ether as a white solid; yield 17 g. (76%), m.p. 112–115°, $[\alpha]^{23}D$ −26.7° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{41}H_{51}N_9O_{11}$: C, 58.21; H, 6.08; N, 14.90. Found: C, 57.87; H, 6.08; N, 14.77.

Carbobenzoxy-L-phenylalanyl-L-seryl-L-prolyl-*p*-fluoro-L-phenylalanylnitro-L-arginine Methyl Ester (IVb).—The azide from 13 g. (0.033 mole) of carbobenzoxy-L-phenylalanyl-L-serine hydrazide was prepared and allowed to react with 16 g. (0.029 mole) of L-prolyl-*p*-fluoro-L-phenylalanylnitro-L-arginine methyl ester yielding after crystallization from methanol–ether 10 g. (40%) of white solid, m.p. 148–150°, $[\alpha]^{23}D$ −41.8° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{41}H_{50}FN_9O_{11}$: C, 57.00; H, 5.84; N, 14.61. Found: C, 56.54; H, 5.95; N, 14.80.

Carbobenzoxy-L-phenylalanyl-L-seryl-L-prolyl-*p*-fluoro-D-phenylalanylnitro-L-arginine Methyl Ester (IVc).—From 30 g. (0.0485 mole) of carbobenzoxy-L-prolyl-*p*-fluoro-D-phenylalanylnitro-L-arginine methyl ester (IIIc) *via* the azide procedure was obtained 30 g. (72%) of white solid, m.p. 130–133°, $[\alpha]^{23}D$ −26.5° (*c* 1, dimethylformamide).

Anal. Found: C, 57.03; H, 5.81; N, 14.77.

Carbobenzoxyglycyl-L-phenylalalanyl-O-acetyl-L-seryl-L-prolyl-D-phenylalanylnitro-L-arginine Methyl Ester (Va).[17]—The carbobenzoxy group was removed from 15.5 g. (0.0187 mole) of the pentapeptide IV a in the usual way with hydrogen bromide–acetic acid giving 19.6 g. of crude product. The solid was dissolved in 150 ml. of dimethylformamide cooled to 0° and 7.5 g. (0.075 mole) of triethylamine (5°) added. The precipitate was removed by filtration and 7.6 g. (0.023 mole) of carbobenzoxyglycine *p*-nitrophenyl ester was added to the filtrate. The solution was allowed to stand 2 days at room temperature, evaporated to 75 ml., and diluted with 200 ml. of ethyl acetate. The solution was washed with water, saturated aqueous sodium carbonate until the yellow color was removed, water, and dilute hydrochloric acid and was dried and evaporated to an oil. The oil was crystallized from methanol–ether giving a white solid, yield, 12.5 g. (73.5%) m.p. 118–122°, $[\alpha]^{23}D$ −20.3° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{45}H_{56}N_{10}O_{13}$: C, 57.19; H, 5.97; N, 14.83; OAc, 4.56. Found: C, 57.11; H, 5.84; N, 15.46; OAc, 1.60.

Carbobenzoxyglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-*p*-fluoro-L-phenylalanylnitro-L-arginine Methyl Ester (Vb).—A reaction similar to the last one using 4.3 g. (0.013 mole) of carbobenzoxyglycine *p*-nitrophenyl ester and 10 g. (0.0118 mole) of the *p*-fluoro-L-phenylalanine pentapeptide IVb gave after crystallization from boiling methanol, 7.5 g. (69%), m.p. 226–228°, $[\alpha]^{23}D$ −50° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{45}H_{55}FN_{10}O_{13}$: C, 56.12; H, 5.77; N, 14.54; OAc, 4.48. Found: C, 55.98; H, 5.98; N, 14.71; OAc, 4.52.

Carbobenzoxyglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-*p*-fluoro-D-phenylalanylnitro-L-arginine Methyl Ester (Vc).—Twenty-eight grams (0.03 mole) of the *p*-fluoro-D-phenylalanine pentapeptide (IVc) yielded 24 g. (87%) of white solid, m.p. 110–120°, $[\alpha]^{23}D$ −20.4° (*c* 1, dimethylformamide).

Anal. Found: C, 55.93; H, 5.92; N, 15.27; OAc, 2.73.

(17) Microanalysis of this and several subsequent compounds indicated that only partial acetylation had occured during HBr–HOAc decarbobenzoxylation which resulted in a mixture of products being obtained. Analyses, however, have been calculated for the fully acetylated compounds.

Carbobenzoxy-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-D-phenylalanylnitro-L-arginine Methyl Ester (VIa).—The carbobenzoxyhexapeptide methyl ester Va (11 g., 0.0122 mole) was dissolved in 150 ml. of glacial acetic acid and 25 g. (0.31 mole) of hydrogen bromide was bubbled into the cooled (10°) solution. The solution was kept at room temperature for 2 hr. and poured into 2 l. of rapidly stirred dry ether. The precipitate was removed, washed thoroughly with dry ether, and dried *in vacuo* giving 13.8 g. of white solid (theory 10.4 g.). The solid was dissolved in 150 ml. of dimethylformamide and cooled along with 6 g. (0.06 mole) of triethylamine. The solutions were combined and the precipitate was removed by filtration. To the filtrate was added 5 g. (0.0135 mole) of carbobenzoxy-L-proline *p*-nitrophenyl ester and the resulting solution was stirred at room temperature for 3 days. The solution was concentrated to an oil and ether–ethyl acetate was added forming a cream-colored precipitate which was washed with ether, then water. The hygroscopic material was recrystallized three times from methanol–ethyl acetate–ether as a white solid, yield, 6 g. (50%), m.p. 121–126°, $[\alpha]^{23}D$ −37° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{50}H_{63}N_{11}O_{14}$: C, 57.63; H, 6.09; N, 14.79; OAc, 4.12. Found: C, 56.99; H, 6.24; N, 14.62; OAc, 2.35.

Carbobenzoxy-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-*p*-fluoro-L-phenylalanylnitro-L-arginine Methyl Ester (VIb).—Reaction of carbobenzoxy-L-proline *p*-nitrophenyl ester and the decarbobenzoxylated hexapeptide (Vb) on a 0.00815-mole scale gave 6.5 g. (78%) of white product, m.p. 195–197°, $[\alpha]^{23}D$ −58.7° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{50}H_{62}FN_{11}O_{14}$: C, 56.65; H, 5.90; N, 14.54; OAc, 4.05. Found: C, 56.49; H, 5.90; N, 14.97; OAc, 3.77.

Carbobenzoxy-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-*p*-fluoro-D-phenylalanylnitro-L-arginine Methyl Ester (VIc).—From 23 g. (0.0025 mole) of the carbobenzoxyhexapeptide (Vc) there was obtained, using the *p*-nitrophenyl ester synthesis 13 g. (51%) of VIc, m.p. 120–125°, $[\alpha]^{23}D$ −29.4° (*c* 1, dimethylformamide).

Anal. Calcd. for monohydrate: C, 55.65; H, 6.03; N, 14.87; OAc, 4.05. Found: C, 55.74; H, 6.06; N, 14.87; OAc, 0.52.

Carbobenzoxy-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-D-phenyalanylnitro-L-arginine Methyl Ester (VIIa).—Removal of the carbobenzoxy group from 5.2 g. (0.0051 mole) of the heptapeptide VIa was accomplished with hydrogen bromide acetic acid as previously described. The crude product amounted to 5.9 g.

The solid was dissolved in 100 ml. of dimethylformamide, the solution cooled to 5°, and 2.2 g. (0.022 mole) of triethylamine was added. After 10 min. the precipitate was removed by filtration and 2.2 g. (0.006 mole) of carbobenzoxy-L-proline *p*-nitrophenyl ester was added to the filtrate. The solution was stirred at room temperature for 4 days and evaporated to an oil. A yellowish gum formed on the addition of ether. The gum was washed with water and ether and recrystallized from methanol–ethyl acetate–ether 3 times, giving 2.8 g. (50%) of off-white solid, m.p. 123–126°.

Anal. Calcd. for $C_{55}H_{70}N_{12}O_{15}$: C, 57.99; H, 6.19; N, 14.76; OAc, 3.92. Found: C, 57.46; H, 6.14; N, 14.73; OAc, 2.00.

Carbobenzoxy-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-*p*-fluoro-L-phenylalanylnitro-L-arginine Methyl Ester (VIIb).—The *p*-nitrophenyl ester method gave 5 g. (76%) of the desired product from 6 g. of the carbobenzoxyheptapeptide (VIb). The white solid melted at 166–168°, $[\alpha]^{23}D$ −67.5° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{55}H_{69}FN_{12}O_{15}$: C, 57.08; H, 6.01; N, 14.53; OAc, 3.72. Found: C, 56.35; H, 6.11; N, 14.74; OAc, 3.49.

Carbobenzoxy-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-*p*-fluoro-D-phenylalanylnitro-L-arginine Methyl Ester (VIIc).—From 6 g. of the corresponding carbobenzoxyheptapeptide (VIc) was obtained 4 g. (60%) of the carbobenzoxyoctapeptide, m.p. 140–150°, $[\alpha]^{23}D$ −38.4° (*c* 1, dimethylformamide).

Anal. Found: C, 56.58; H, 6.21; N, 14.59; OAc, 2.02.

Tricarbobenzoxy-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-D-phenylalanylnitro-L-arginine Methyl Ester (VIIIa).—The carbobenzoxyoctapeptide methyl ester (VIIa) 2.8 g. (0.0025 mole) was dissolved in 75 ml. of glacial acetic acid and cooled to 10°. The solution was treated with 10 g. (0.125 mole) of dry hydrogen bromide and kept at room temperature for 1.5 hr. The solution was poured into ether, the solid was removed, washed well with ether, and dried *in vacuo*, giving 3.7 g. of white solid. The crude hydrobromide salt was dissolved in 75 ml. of dimethylformamide, cooled to 0°,

and 2 g. (0.02 mole) of triethylamine added. After 5 min. the precipitate was removed by filtration. To the filtrate was added 2.2 g. (0.00315 mole) of tricarbobenzoxy-L-arginine *p*-nitrophenyl ester. The solution was allowed to stand for 2 days at room temperature and was evaporated to an oil. The gum was washed repeatedly with water, ether, and ethyl acetate and was gradually solidified. The solid was crystallized from methanol–ethyl acetate; yield 2 g. (52%), m.p. 95–115°, $[\alpha]^{23}D$ −37° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{77}H_{94}N_{16}O_{20}$: C, 59.04; H, 6.05; N, 14.31; OAc, 2.75. Found: C, 58.47; H, 6.20; N, 14.45; OAc, 1.97.

Tricarbobenzoxy-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-*p*-fluoro-L-phenylalanylnitro-L-arginine Methyl Ester (VIIIb).—This compound was prepared according to the above described procedure. A 2.5-g. (0.00225 mole) run of the carbobenzoxyoctapeptide (VIIb) gave 3 g. (87%) of cream-colored solid, m.p. 145–147°, $[\alpha]^{23}D$ −47.5° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{77}H_{93}FN_{16}O_{20}\cdot 3H_2O$: C, 56.54; H, 6.10; N, 13.70; OAc, 2.72. Found: C, 56.20; H, 6.19; N, 13.57; OAc, 2.58.

Tricarbobenzoxy-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-seryl-*p*-fluoro-D-phenylalanylnitro-L-arginine Methyl Ester (VIIIc).—From 2.3 g. (0.0033 mole) of tricarbobenzoxy-L-arginine *p*-nitrophenyl ester and 3.5 g. (0.0031 mole) of the *p*-fluoro-D-phenylalanine octapeptide (VIIc) a cream solid amounting to 3.5 g. (74%) was obtained, m.p. 120–125°, $[\alpha]^{23}D$ −36.1° (*c* 1, methanol).

Anal. Found: C, 56.59; H, 6.00; N, 14.22; OAc, 2.21.

Dicarbobenzoxy-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-D-phenylalanylnitro-L-arginine (IXa).—To a solution of 1 g. (0.00066 mole) of the tricarbobenzoxynonapeptide methyl ester (VIIIa) in 30 ml. of methanol was added 1 ml. of 2 *N* sodium hydroxide. The solution was stirred at room temperature for 25 min., water was added, and the solution remained clear. The solution was filtered and 1.5 ml. of 2 *N* hydrochloric acid was added. The precipitate was removed by filtration and the solid was crystallized from methanol–ether, yield 625 mg. (79%), m.p. 170–175° $[\alpha]^{23}D$ −38° (*c* 1, methanol).

Anal. Calcd. for $C_{66}H_{84}N_{16}O_{17}\cdot 2H_2O$: C, 56.21; H, 6.29; N, 15.90. Found: C, 56.27; H, 6.39; N, 15.98.

Dicarbobenzoxy-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-*p*-fluoro-L-phenylalanylnitro-L-arginine (IXb).—Hydrolysis of 2 g. (0.0013 mole) of the tricarbobenzoxynonapeptide methyl ester (VIIIb) with 1.6 ml. (0.0032 mole) of 2 *N* sodium hydroxide gave 1.2 g. (66%) of white solid, m.p. 155–160°, $[\alpha]^{23}D$ −62.5° (*c* 1, methanol).

Anal. Calcd. for $C_{66}H_{83}FN_{16}O_{17}\cdot 3H_2O$: C, 54.71; H, 6.19; N, 15.47; F, 1.37. Found: C, 54.46; H, 6.19; N, 15.37; F, 1.48.

Dicarbobenzoxy-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-*p*-fluoro-D-phenylalanylnitro-L-arginine (IXc).—A similar hydrolysis of 2.5 g. of the corresponding tricarbobenzoxy *p*-fluoro-D-phenylalaninenonapeptide (VIIIc) gave 1.8 g. (80%) of tan solid, m.p. 160–165°, $[\alpha]^{23}D$ −31.3° (*c* 1, dimethylformamide).

Anal. Found: C, 54.24; H, 6.11; N, 15.84; F, 1.65.

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-D-phenylalanyl-L-arginine Triacetate Salt (Xa).—The dicarbobenzoxynonapeptide (IXa) (400 mg., 2.9×10^{-4} mole) was dissolved in 30 ml. of glacial acetic acid, and 200 mg. of palladium black catalyst and 20 ml. of methanol were added. The resulting mixture was hydrogenated for 24 hr. at room temperature and 2–3 lb. (0.14–0.21 kg./cm.^{2}) pressure. The catalyst was removed by filtration. The filtrate was evaporated *in vacuo*, the residue was dissolved in 50 ml. of water, the solution was filtered, shell frozen, and lyophilized leaving 333 mg. of white powder, m.p. 155–168°, $[\alpha]^{23}D$ −73° (*c* 0.89, water).

Anal. Calcd. for $C_{56}H_{85}N_{15}O_{17}\cdot H_2O$: C, 53.46; H, 6.97; N, 16.70: Found: C, 53.16; H, 7.07; N, 17.07.

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-*p*-fluoro-L-phenylalanyl-L-arginine Triacetate Salt (Xb).—The appropriate dicarbobenzoxynonapeptide IXb (200 mg.) was hydrogenated in the manner previously described. The catalyst was removed and lyophilization of the filtrate gave 194 mg. of fluffy white solid, $[\alpha]^{23}D$ −79° (*c* 0.696, water).

Anal. Calcd. for $C_{56}H_{84}FN_{15}O_{17}$: C, 53.45; H, 6.73; N, 16.70; F, 1.51. Found: C, 52.79; H, 6.79; N, 17.42; F, 1.61.

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-*p*-fluoro-D-phenylalanyl-L-arginine (Xc).—From 500 mg. of the dicarbobenzoxy 8-*p*-fluoro-D-phenylalanine nonapeptide (IXc) there was obtained after catalytic hydrogenation 470 mg. of light tan solid, $[\alpha]^{23}D$ −63.4° (*c* 1, water). The analytical sample was dried at 110° for 18 hr.

Anal. Found: C, 53.11; H, 6.64; N, 17.33; F, 1.19.

For the paper chromatography of the three bradykinin analogs, two different solvent systems were employed: (A) *t*-butyl alcohol–acetic acid–water (2:1:1) and (B) isopropyl alcohol–concentrated ammonium hydroxide–water (70:5:25). The spots were developed with bromophenol blue and Sakaguchi reagents. Single spots were obtained in each case. The R_f values obtained were: 8-D-phenylalanine bradykinin (A) 0.79, (B) 0.52; 8-*p*-fluoro-L-phenylalanine bradykinin (A) 0.72, (B) 0.49; 8-*p*-fluoro-D-phenylalanine bradykinin (A) 0.77, (B) 0.56. Paper electrophoresis of the three nonapeptides was carried out in acetate buffer, pH 5.6, using a constant current of 30 ma. for 3 hr. The mobilities of the analogs were found to be identical and could not be distinguished from bradykinin which moved a distance of 6.7 cm. from the point of origin.

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The Anticonvulsant Properties of Some Substituted Benzamides

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A number of substituted aromatic amides have been prepared and their anticonvulsant properties are reported. Two compounds, 4-amino-N-cyclopropyl-3,5-dichlorobenzamide and 4-amino-N-cyclopropyl-3,5-dibromobenzamide, were shown to be potent antagonists to the convulsant action of strychnine.

During a routine search for compounds which might possess muscle relaxant properties, it was found that 4-amino-N-cyclopropyl-3,5-dichlorobenzamide (8) was outstanding. The muscle relaxant properties of this amide have been reported earlier.[1]

It is interesting to note that although 4-amino-3,5-dichlorobenzoic acid was first prepared by Elion[2] in 1923, no simple derivatives other than the methyl ester[3] and the acid chloride[4] were ever prepared. Elion prepared the acid in low yield by chlorination of *p*-amino-

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(3) E. Müller and E. Tietz, *Chem. Ber.*, **74B**, 807 (1941).

(4) M. Schubert, *Ann.*, **558**, 31 (1947).