Unnatural Amino Acids. I. 3-Carboxytyrosine Derivatives

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Received April 28, 1965

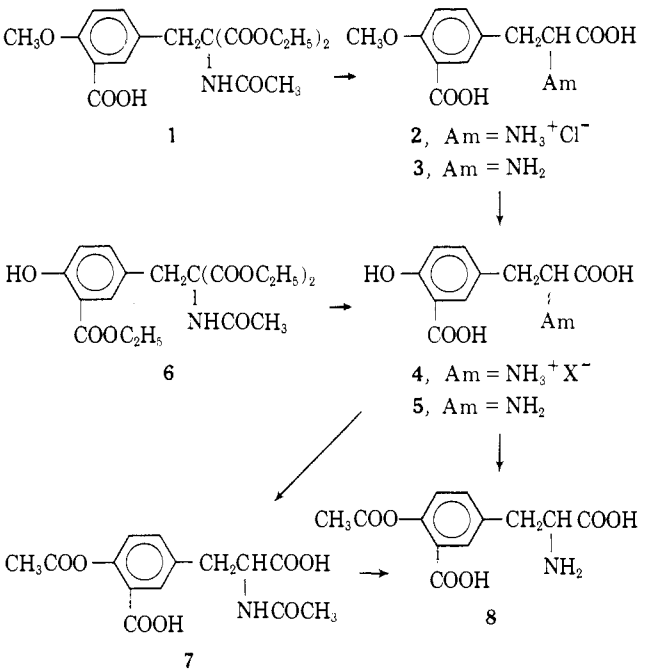
DL-4-Acetoxy-3-carboxyphenylalanine (aspirin-alanine, **8**) was synthesized to determine if the β-alanine moiety attached to a typical pharmacologically active group would function as a biological carrier and transport the aspirin grouping to tissue sites not reached by aspirin. The amino acid **8** was no better absorbed than aspirin and showed little analgesic activity in the rat-tail flick test but two of the intermediates in its synthesis, DL-3-carboxytyrosine (**5**) and DL-3-carboxy-4-methoxyphenylalanine (**3**), produced significant analgesic threshold elevations. One of these racemic amino acids (**3**) was found to possess clinical effectiveness comparable to aspirin but its use was limited by incomplete and erratic absorption.

Studies of the effects of amino acid analogs on the growth of selected strains of bacteria with specific amino acid requirements for normal growth have shown that variants of natural amino acids can penetrate biological membranes and inhibit the action of the related natural amino acids.[2] These results suggested that the β-alanine fragment may function as a biological carrier for a variety of R groups not normally found in biological systems. The implications of this conclusion did not escape several investigators but in the main they have not been explored.

Elliot, Fuller, and Harington[3] reasoned that penetration of microorganisms by drugs might be facilitated by an α-amino acid grouping and they therefore synthesized β-(aminoaryl)- and β-heterocyclic α-aminopropionic acids for antibacterial screening. Several of their compounds showed significant antibacterial effects *in vitro*. Burckhalter and Stevens[4] prepared β-alanine derivatives with large space-occupying groups such as are found in antimalarials and insecticides in the hope that the amino acid group would solubilize the compounds in biological fluids, allow absorption at the proper site, and possibly permit participation in peptide synthesis. The best example of the use of an amino acid as a biological carrier is found in the work of Bergel and Stock[5] and Larionov, *et al.*,[6] who combined the nitrogen mustard function with phenylalanine to form N-phenylalanine mustard (PAM). Blokhin, *et al.*,[7] found that oral administration of DL-PAM had a definite curative effect on patients with certain solid malignant tumors (seminoma, reticulum cell sarcoma, and Ewings tumor), while Bergsagel, *et al.*,[8] found that 33% of the patients with multiple myeloma in the group they studied showed significant improvement after oral therapy with L-PAM.

No examples have been reported of the use of α-amino acid residues to transport pharmacologically active groups and a study was therefore launched in these laboratories to explore this area not only for its potential as a rational approach to the facilitation of drug transport across cell membranes, particularly the blood–brain barrier, but also for the information it would yield on the absorption of amino acids. Our first target compound, 4-acetoxy-3-carboxyphenylalanine (aspirin-alanine, **8**) possessed the necessary dual functionality for our study since it is a derivative of both aspirin and tyrosine. It was conveniently prepared by the method illustrated in Chart I. Alkylation of diethyl acetamidomalonate with 5-chloromethyl-2-methoxybenzoic acid in the presence of 2 equiv. of sodium ethoxide gave diethyl acetamido(3-carboxy-4-methoxybenzyl)malonate (**1**) which was hydrolyzed with 1.2 *N* HCl to DL-3-carboxy-4-methoxyphenylalanine hydrochloride (**2**) from which the free amino acid (**3**) was liberated at pH 3.2–3.5. Cleavage of the methoxyl group of **2** or **3** was best accomplished with 48% HBr and somewhat less effectively with concentrated HCl with the formation of the corresponding salts (**4**, X = Br^- or Cl^-) of DL-3-carboxytyrosine which at pH 3.2–

CHART I
PREPARATION OF ASPIRIN-ALANINE



(1) Psychopharmacology Service Center, National Institute of Mental Health, Bethesda, Md. 20014.

(2) G. J. Martin, "Biological Antagonism," The Blakeston Co., New York, N. Y., 1951; W. Shive and C. G. Skinner, *Ann. Rev. Biochem.*, **27**, 643 (1958).

(3) D. F. Elliot, A. T. Foller, and C. R. Harington, *J. Chem. Soc.*, 85 (1948).

(4) J. H. Burckhalter and V. C. Stevens, *J. Am. Chem. Soc.*, **73**, 56 (1951).

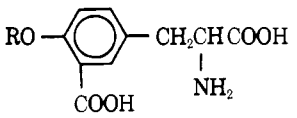
(5) F. Bergel and J. A. Stock, *J. Chem. Soc.*, 2409 (1954); F. Bergel, V. C. E. Burnop, and J. A. Stock, *ibid.*, 1223 (1955).

(6) L. F. Larionov, E. N. Shkodinskaya, V. I. Troosheikina, A. S. Khokhlov, O. S. Vasina, and M. A. Movikova, *Lancet*, **269**, 169 (1955).

(7) N. Blokhin, L. Larionov, N. Perevodchikova, L. Chebotareva, and N. Merkulova, *Ann. N. Y. Acad. Sci.*, **68**, 1128 (1958).

(8) D. E. Bergsagel, C. C. Sprague, C. Austin, and K. M. Griffith, *Cancer Chemotherapy Rept.*, **21**, 87 (1962).

Table I: Preliminary Pharmacological Data



Compd.	R	LD50 Species	LD50 Route	LD50 mg./kg.	Analgesic activity Test	Route	Dose, mg./kg.	Av. thres. elev., %
2	CH_3	Mouse	I.v.	683	Tail flick[a]	I.p.	150	+22
2	CH_3	Mouse	Oral	5000	Tail flick	I.p.	300	+81
3	CH_3	Rat	Oral	5000	Tooth pulp[b]	I.v.	50	+35
3	CH_3				Tooth pulp	I.v.	100	+41
2	CH_3				Tail flick	Oral	500	+29
2	CH_3				Tail flick	Oral	1000	+36
4[c]	H	Mouse	I.v.	481	Tail flick	I.p.	150	−2
4[c]	H	Mouse	Oral	5000	Tail flick	I.p.	300	+42
5	H	Rat	Oral	5000	Tooth pulp	I.v.	50	+36
5	H				Tooth pulp	I.v.	100	+46
4[c]	H				Tail flick	Oral	500	+13
4[c]	H				Tail flick	Oral	1000	+19
8	CH_3CO	Mouse	I.p.	1500	Tail flick	S.c.	300	+7
Aspirin					Tail flick	Oral	300	+4

[a] The time required by mice under the influence of the test compounds to respond to a hot light focused on their tails was determined. This reaction time was compared with that shown by the mice when not under the effect of a drug. The increases in reaction time, expressed as threshold elevations, were averaged for observations made at 15-min. intervals. [b] The time required by control rabbits and those under the influence of the test compounds to respond to an electrical stimulus administered by electrical probes implanted in the tooth pulp of the incisors was compared. Reaction to the current was shown when the animals lifted their heads sharply and tension in the neck muscles increased markedly. [c] X = Cl^-.

3.5 gave DL-3-carboxytyrosine (**5**).[9] Alkylation of diethyl acetamidomalonate with methyl 5-chloromethylsalicylate and hydrolysis of the alkylation product **6** was a more direct but less satisfactory route to **4** because of lower yields and greater experimental difficulties. Acetylation of **4** with a 1:1 mixture of acetic anhydride and acetic acid at reflux temperature gave the unwanted N,O-diacetate (**7**). Selective O-acetylation of **5** with the formation of aspirin-alanine (**8**) was accomplished with a mixture of acetic anhydride and acetic acid in perchloric acid by a modification of the procedure of Sakami and Toennies[10] for the O-acetylation of tyrosine.

Preliminary analgesic screening data (Table I) on the racemic amino acids **2**, **3**, **4** (X = Cl^-), **5**, and **8** showed that aspirin-alanine (**8**) was the least effective compound in the group while **2** and **4** (X = Cl^-) produced analgesia both by the oral and intraperitoneal route in several species of animals.

L-3-Carboxy-4-methoxyphenylalanine (prepared from the N-phthaloyl brucine salt) produced no pain threshold elevation in mice in the tail flick test. This observation suggests the unlikely possibility that D-**3** is responsible for all of the analgesic activity of the racemate. Unfortunately, all attempts to isolate the soluble N-phthaloyl brucinate precursor of D-**3** from the brucine salt mother liquors failed and it has not been possible to verify this conclusion by direct observation.[11]

DL-3-Carboxy-4-methoxyphenylalanine, which in preliminary clinical studies produced little gastric irritation, was somewhat more effective than aspirin (at one-third to the same milligram dosage) in patients with musculoskelatal aches and pains, acute respiratory infections, and headaches but was incompletely and erratically absorbed. Blood levels of DL-**3** in man after the administration of 700 mg. *p.o.* reached a maximum of 7.6 γ/ml. after 5 hr. Fecal studies established that from 25–45% of the administered dose of DL-**3** was not absorbed. Urinary excretion of DL-**3** ranged from a trace to 14%. In an extended series of studies with urine, stool, and blood from patients, a maximum of about 50% of the administered dose of DL-**3** could be accounted for. Studies on the absorption of DL-**3** from the perfused small intestine of the rat *in vitro* utilizing the method of Hogben, *et al.*,[12] gave results consistent with the poor absorption observed in patients. DL-**5** was somewhat less well absorbed from the rat small intestine than DL-**3**. Aspirin-alanine (**8**), the least active analgesic of the amino acids, was found to be as well absorbed as aspirin and much better absorbed than DL-**3** and DL-**5**. The observations suggest that there is no structure–activity correlation between these new amino acids and the salicylic acid–aspirin system and that carboxylation alone or together with alkylation or acylation of tyrosine yields products which differ too much from tyrosine to be absorbed efficiently by the tyrosine transport system and which are too polar to be well absorbed by passive diffusion.

Experimental Section[13]

5-Chloromethyl-2-methoxybenzoic Acid.—A suspension of 152 g. (1.0 mole) of *o*-methoxybenzoic acid in 75 ml. of concentrated

(9) After the completion of our studies, P. D. Larsen and A. Kjaer [*Acta Chem. Scand.*, **16**, 142 (1962)] reported the isolation of L-3-carboxytyrosine from the free amino acid fraction of seeds of *Reseda odorata* L. and its synthesis from L-tyrosine.

(10) W. Sakami and G. Toennies, *J. Biol. Chem.*, **144**, 203 (1942).

(11) The D-isomer possibly could have been separated from the racemate by an enzymatic method. Collateral developments in the program suggested, however, that further effort along this line would not be justified.

(12) C. A. Hogben, D. J. Tocco, B. B. Brodie, and L. M. Schanker, *J. Pharmacol. Exptl. Therap.*, **125**, 275 (1959).

(13) Microanalyses were performed by Schwarzkopf Laboratories, Woodside, N. Y. Melting points were determined on a Thomas–Hoover melting point apparatus and are corrected. *o*-Methoxybenzoic acid, methyl salicylate, phthalic acid, and brucine were purchased from Distillation Products Industries, Inc. Diethyl acetamidomalonate was obtained from Wintlirop Laboratories. The formaldehyde, acetic anhydride, and perchloric acid used in this study were Baker and Adamson laboratory reagent grade chemicals.

HCl was treated first with 87.5 g. (1.08 moles) of 37% formaldehyde and then with gaseous HCl until saturated (both operations at 0–15°). The reaction flask was stoppered and set aside at room temperature for 2 days. The crude product was filtered off, pressed dry on the filter funnel, and recrystallized from benzene; yield 120 g. (60%), m.p. 97–98°. The melting point was not changed by a second recrystallization.

Anal. Calcd. for $C_9H_9ClO_3$: C, 53.72; H, 4.50. Found: C, 53.53; H, 5.05.

Proof of Structure of 5-Chloromethyl-2-methoxybenzoic Acid.—5-Chloromethyl-2-methoxybenzoic acid (6 g.) was added to a solution of 2.4 g. of NaOH in 100 ml. of water. The mixture was warmed at 60–70°, treated with a solution of 7 g. of $KMnO_4$ in 150 ml. of water, and then was stirred and refluxed for 40 min. Manganese dioxide was filtered off, and the filtrate was cooled and acidified to pH 3–4. The white crystalline compound which precipitated melted at 255–257° crude and at 259–260° after recrystallization from methanol. The melting point data are in good agreement with the melting point of 4-methylisophthalic acid obtained by Schall[14] (m.p. 261°) from the oxidation of 4-methoxy-*m*-tolnic acid and by Fosdick and Fancher[15] (m.p. 255–256°) from the oxidation of 4-methoxy-*m*-xylene and establish the location of the chloromethyl group in our chloromethylation product as *para* to the methoxy group.

Diethyl Acetamido(3-carboxy-4-methoxybenzyl)malonate.—A solution of 26.2 g. (0.4 mole) of sodium ethoxide in absolute alcohol was prepared by dissolving 9.2 g. (0.4 g.-atom) of Na in 175 ml. of alcohol. The solution was cooled to 60° and 43.3 g. (0.2 mole) of diethyl acetamidomalonate dissolved in 80 ml. of ethanol was added. The mixture was stirred for 15 min. at 60° and then a solution of 41.4 g. (0.2 mole) of 5-chloromethyl-2-methoxybenzoic acid, dissolved in 100 ml. of alcohol, was added all at once. The mixture was stirred and refluxed for 12 hr. and allowed to stand overnight. Alcohol was removed *in vacuo*, the residue was dissolved in 250 ml. of water, and the solution was adjusted to pH 6.5. The acidified solution was extracted with ether, and the extracts were dried (Na_2SO_4), filtered, and concentrated to a heavy syrup which was dissolved in saturated $NaHCO_3$ and extracted with benzene to remove unreacted diethyl acetamidomalonate. Acidification of the bicarbonate solution with dilute HCl to pH 3 gave a precipitate of the desired product which melted at 106–107° after recrystallization from benzene; yield 65 g. (85%). A second recrystallization from benzene raised the melting point to 116–117°.

Anal. Calcd. for $C_{18}H_{24}NO_8$: C, 56.84; H, 5.52; N, 3.68. Found: C, 56.46; H, 5.82; N, 3.75.

3-Carboxy-4-methoxyphenylalanine Hydrochloride.—Diethyl acetamido(3-carboxy-4-methoxybenzyl)malonate (64 g., 0.168 mole) was refluxed with a mixture of 53 ml. of concentrated HCl and 475 ml. of water for 12 hr. The reaction mixture was concentrated to dryness *in vacuo* and the crystalline solid was recrystallized to constant melting point from isopropyl alcohol; yield 34 g. (73%), m.p. 215–216° dec.

Anal. Calcd. for $C_{11}H_{13}NO_5 \cdot HCl$: C, 48.98; H, 5.09; N, 5.09. Found: C, 48.08; H, 5.40; N, 5.33.

3-Carboxy-4-methoxyphenylalanine.—3-Carboxy-4-methoxyphenylalanine hydrochloride (5 g., 0.0181 mole) was dissolved in 100 ml. of water, the pH of the solution was adjusted to 3.45 (meter) with 20 ml. of 1.0 *N* NaOH, and the mixture was placed in a refrigerator overnight. The crystalline precipitate (m.p. 235°) was filtered off, washed with ice-cold water until the washings gave no more than a faint test for Cl^-, and then recrystallized from 250 ml. of hot water. The crystalline product was filtered off, washed with water, and dried at 65° *in vacuo*; yield 3.5 g. (81%), m.p. 242–244°.

Anal. Calcd. for $C_{11}H_{13}NO_5$: C, 55.22; H, 5.48; N, 5.86. Found: C, 55.28; H, 5.50; N, 5.75.

Methyl 5-Chloromethylsalicylate.—The preparation of methyl 5-chloromethylsalicylate, originally described by Bauer and Buchler,[16] was simplified as follows. A mixture of methyl salicylate (304 g., 2 moles), 2.0 l. of concentrated HCl, and 240 g. (3 moles) of chloromethyl ether was stirred for 18 hr. at room temperature during which time methyl 5-chloromethylsalicylate gradually precipitated. The crystalline product was filtered off and dried over KOH and $CaCl_2$ for 24 hr. After recrystallization from hexane, the product melted at 66–68°, yield 255 g. (63%), lit.[16] m.p. 68°.

Diethyl Acetamido(3-carbomethoxy-4-hydroxybenzyl)malonate.—Sodium metal (11.5 g., 0.5 g.-atom) was dissolved in 300 ml. of absolute ethanol. To this solution was added 108.5 g. (0.5 mole) of diethyl acetamidomalonate. The mixture was cooled in an ice bath and was then treated dropwise during 0.5 hr. with a solution of 100.3 g. (0.5 mole) of methyl 5-chloromethylsalicylate in 200 ml. of benzene. The mixture was stirred at room temperature for 40 hr., refluxed for 28 hr. with stirring, cooled, and acidified with 20 ml. of acetic acid. The acidified reaction mixture was filtered hot, concentrated to half volume, and poured onto 1 kg. of ice. After 3 hr., the precipitate was filtered, washed with water, and crystallized from approximately 500 ml. of 2-propanol; m.p. 129–130°. A second recrystallization from 300 ml. of 2-propanol gave 95.5 g. (50.2%) of the desired product, m.p. 131–135°.

Anal. Calcd. for $C_{18}H_{23}NO_8$: C, 56.69; H, 6.08; N, 3.67. Found: C, 56.81; H, 5.75; N, 3.47.

3-Carboxytyrosine Hydrochloride (4). A. By Hydrolysis of 3-Carboxy-4-methoxyphenylalanine Hydrochloride.—3-Carboxy-4-methoxyphenylalanine hydrochloride (40 g. 0.145 mole) was refluxed with 400 ml. of concentrated HCl for 14 hr. The reaction mixture was concentrated *in vacuo*, and the residue was recrystallized from a mixture of ethanol and methanol; yield 35 g. (91.5% of **4**, m.p. 260–261°.

Anal. Calcd. for $C_{10}H_{10}NO_5 \cdot HCl$: C, 45.91; H, 4.62; N, 5.36. Found: C, 46.14; H, 4.77; N, 5.59.

B. By Hydrolysis of Diethyl Acetamido(3-carboxy-4-hydroxybenzyl)malonate.—Diethyl acetamido(3-carbomethoxy-4-hydroxybenzyl)malonate (94.5 g., 0.248 mole) was refluxed with 1.0 l. of 6 *N* HCl for 15 hr. The white crystalline precipitate was filtered off, sucked as dry as possible on the funnel, and dried in a vacuum desiccator ($CaCl_2$ and NaOH) overnight; yield 61.5 g. (95%), m.p. 275–276°.

Anal. Calcd. for $C_{10}H_{10}NO_5 \cdot HCl$: C, 45.90; H, 4.62; Cl, 13.55; N, 5.36. Found: C, 44.79; H, 4.45; Cl, 14.23; N, 5.22.

Recrystallization of 26 g. of the crude hydrochloride from water depressed the melting point to 270–271° and resulted in formation of a mixture of the amino acid hydrochloride and the free amino acid as shown by the low chlorine content.

Anal. Found: Cl, 9.25.

Recrystallization of the water-crystallized material from 2 *N* HCl gave a product that melted at 274–275°, yield 21.0 g.

Anal. Found: C, 45.74; H, 4.87; Cl, 13.61; N, 5.14.

3-Carboxytyrosine (5). A. Liberation from 3-Carboxytyrosine Hydrochloride.—3-Carboxytyrosine hydrochloride (30.0 g., 0.115 mole) was dissolved in approximately 130 ml. of water at 50–60°. After cooling to about 40°, the pH of the solution was adjusted to 3.2 by addition of approximately 12 ml. of 10 *N* NaOH. The free amino acid precipitated immediately. After refrigeration for 2 hr., the mixture was filtered and washed until almost free of Cl^-. Crude **5** melted at 289–290°. Leaching with boiling water did not change the melting point; yield 26.0 g. (99.5%).

Anal. Calcd. for $C_{10}H_{11}NO_5$: C, 53.32; H, 4.92; N, 6.22. Found: C, 52.95; H, 4.88; N, 6.20.

B. Liberation from 3-Carboxytyrosine Hydrobromide.—3-Carboxy-4-methoxyphenylalanine hydrochloride (5 g., 0.0182 mole) was refluxed with 50 ml. of 48% HBr for 5 hr. After 20 min. of reflux all of the amino acid had dissolved. Shortly thereafter, 3-carboxytyrosine hydrobromide started to crystallize from solution. The cooled mixture was filtered, the crystalline hydrobromide was taken up in water, and the pH was adjusted to 3.2. 3-Carboxytyrosine was filtered off after several hours of refrigeration; yield 2.4 g. (58%), m.p. 287–288°.

2-Acetamido-3-(4-acetyloxy-3-carboxyphenyl)propionic Acid.—3-Carboxytyrosine hydrochloride (1.0 g., 0.0039 mole) was dissolved in a boiling mixture (reflux) of 1:1 acetic anhydride–acetic acid (*ca.* 1 hr. for complete dissolution). The mixture was then warmed at 60° for 3 hr. and concentrated *in vacuo*. The syrupy residue crystallized on trituration under ether and was recrystallized from a mixture of ethyl acetate and isopropyl alcohol; yield 0.5 g. (40.4%), m.p. 90°.

Anal. Calcd. for $C_{13}H_{13}NO_7$: C, 54.41; H, 4.85; N, 4.52. Found: C, 54.66; H, 5.26; N, 4.12.

4-Acetyloxy-3-carboxyphenylalanine.—3-Carboxytyrosine (2.4 g., 0.00107 mole) and 12 g. of acetic anhydride were dissolved at 0° in 50 ml. of an acetic acid solution of 4.31 g. of 70% $HClO_4$ and 2.52 g. of 98.7% acetic anhydride. The mixture was stirred

(14) C. Schall, *Ber.*, **12**, 828 (1879).
(15) L. S. Fosdick and O. E. Fancher, *J. Am. Chem. Soc.*, **63**, 1277 (1941).
(16) K. H. Bauer and K. Buchler, *Arch. Pharm.*, **262**, 128 (1942).

at room temperature for 19 hr. and was then treated with 1 ml. of water, 3.48 g. of amylamine, and 500 ml. of ether. The gummy crystalline precipitate was washed with ether (decantation) and then recrystallized from ethanol; yield 2.5 g. (65%), m.p. 184–185°. The crystalline product gave a negative ferric chloride test for the free phenolic group.

Anal. Calcd. for $C_{12}H_{13}NO_6$: C, 53.92; H, 4.87; N, 5.25. Found: C, 53.65; H, 4.90; N, 5.47.

N-Phthalyl(3-carboxy-4-methoxyphenyl)alanine.—A mixture of 12.0 g. (0.046 mole) of 3-carboxy-4-methoxyphenylalanine, 7.5 g. (0.051 mole) of phthalic anhydride, and 75 ml. of dry pyridine was refluxed for 1.5 hr. with the exclusion of moisture and concentrated *in vacuo*, and the residue was dissolved in 30 ml. of acetic anhydride. The resulting solution was refluxed for 10 min., cooled, poured onto 300 g. of ice, and stored overnight in a refrigerator. The precipitate was filtered off and recrystallized from 75 ml. of ethyl acetate; yield 13.8 g. (69%). The purified product melted first at 170–171°, solidified, and remelted at 218–219°.

Anal. Calcd. for $C_{19}H_{15}NO_7$: C, 61.77; H, 4.09; N, 3.79. Found: C, 61.87; H, 4.41; N, 4.01.

L-N-Phthalyl-3-carboxy-4-methoxyphenylalanine Brucine Salt.—A solution of N-phthalyl-3-carboxy-4-methoxyphenylalanine brucine salt obtained by dissolving 50.2 g. (0.136 mole) of N-phthalyl-3-carboxy-4-methoxyphenylalanine and 53.8 g. (0.136 mole) of brucine in 735 ml. of near-boiling 60% ethanol was allowed to cool slowly to room temperature overnight. The precipitate of colorless needles was filtered off [yield 45 g. (87%), m.p. 167–172°, $[\alpha]^{25}D$ −86.3° (0.200 g. in 10 ml. of 60% ethanol)] and recrystallized from 250 ml. of 60% ethanol; yield 39 g. (74.4%), m.p. 167–172°, $[\alpha]^{25}D$ −114.3° (0.194 g. in 10.00 ml. of 60% ethanol).

Anal. Calcd. for $C_{42}H_{21}N_3O_{11}\cdot 2H_2O$: C, 63.04; H, 5.56; N, 5.25. Found: C, 63.05; H, 5.69; N, 5.47.

L-N-Phthalyl-3-carboxy-4-methoxyphenylalanine.—A suspension of 63.5 g. (0.0795 mole) of L-N-phthalyl-3-carboxy-4-methoxyphenylalanine brucine salt was made basic with 160 ml. of 1 *N* NaOH, stirred for 0.5 hr. at room temperature, and filtered. The filtrate was extracted 7 times with 150-ml. portions of chloroform until it gave a negative test for brucine and then acidified with 170 ml. of 1 *N* HCl. The precipitate (29.1 g., 94%) which formed in the mixture on standing overnight in the refrigerator melted at 133–134° and had $[\alpha]^{25}D$ −206° (0.0601 g. in 3.00 ml. of ethanol). It was recrystallized several times from a mixture of ethyl acetate and cyclohexane with no change in optical rotation.

Anal. Calcd. for $C_{19}H_{15}NO_7$: C, 61.77; H, 4.09; N, 3.79. Found: C, 62.77; H, 4.00; N, 4.50.

L-3-Carboxy-4-methoxyphenylalanine.—A suspension of 24 g. (0.065 mole) of L-N-phthalyl-3-carboxy-4-methoxyphenylalanine in 1.68 l. of approximately 1 *N* HCl was refluxed for 24 hr. and then concentrated *in vacuo* to a small volume. *o*-Phthalic acid was filtered off, and the filtrate was concentrated *in vacuo*. The residue was dissolved in water and the solution again was concentrated to dryness *in vacuo*. This was repeated several times to ensure complete removal of excess HCl. The residue was dissolved in water and treated with 9 g. of Ag_2CO_3. The precipitate of AgCl was filtered off, the filtrate was treated with H_2S, filtered, and concentrated *in vacuo*. Benzene was added to the residue and the remaining water was removed by azeotropic distillation. The solid residue was leached several times with hot purified dioxane and dried; yield 12.06 g. (78%), m.p. 91° (partially melted), 236–238° dec., $[\alpha]^{25}D$ −17.9° (0.00983 g. in 1.00 ml. of water). After recrystallization from acetonitrile–water, the preparation had $[\alpha]^{25}D$ −19.1° (2.62 mg. in 1.00 ml. of water), $[\alpha]^{25}D$ −1.81° (2.21 mg. in 1 ml. of 1 *N* HCl), and the same melting point as the crude material. The more positive rotation of the product in 1 *N* HCl suggests an L-configuration in accordance with the rule of Lutz and Jirgensons.[17]

Anal. Calcd. for $C_{11}H_{13}NO_5$: C, 55.22; H, 5.48; N, 5.86. Found: C, 54.98; H, 5.59; N, 5.76.

Acknowledgment.—We wish to express our appreciation to Drs. F. Häfliger, M. Weiner, and P. Greengard, each of whom arranged some of the biological studies reported in this paper.

(17) O. Lutz and B. Jirgensons, *Ber.*, **63**, 448 (1930); **64**, 1221 (1931).

Quinazolines and 1,4-Benzodiazepines. XXV.[1] Structure–Activity Relationships of Aminoalkyl-Substituted 1,4-Benzodiazepin-2-ones

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Received June 16, 1965

The syntheses of a number of 1,3-dihydro- and 1,3,4,5-tetrahydro-2H-1,4-benzodiazepin-2-ones, having basic (aminoalkyl) side chains in positions 1 or 4, are described. The acute toxicities of these compounds were determined and the compounds were screened for sedative, muscle relaxant, taming, and anticonvulsant effects in mice, and for sedative and muscle relaxant activity in cats. Many of these benzodiazepinones showed central nervous system activity qualitatively similar to that of chlordiazepoxide. Optimal activity was observed in compounds having a 2-fluorophenyl substituent in position 5. The tetrahydrobenzodiazepinones showed significantly less CNS activity than the corresponding dihydro compounds.

In continuation of our studies of psychopharmacologically active 1,4-benzodiazepines, we have prepared the compounds listed in Tables I and II by four methods.

Alkylation of 1,3-dihydro-2H-1,4-benzodiazepin-2-ones with sodium methoxide and various aminoalkyl halides (method A) gave the 1-substituted benzodiazepin-2-ones listed in Table I. An alternative route (method B) was found useful in cases where the necessary aminoalkyl halide was not available. Alkylation of 1,4-benzodiazepin-2-ones with 1-bromo-3-chloropropane gave compounds of type II, which readily underwent nucleophilic replacement of chlorine by amines.[2]

The tetrahydrobenzodiazepinones (Table II) were made either by alkylation of 1,3,4,5-tetrahydrobenzo-

(1) Paper XXIV: W. Metlesics, R. Tavares, and L. H. Sternbach, *J. Org. Chem.*, **30**, 1311 (1965).

(2) H. L. Yale and F. Sowinski, *J. Am. Chem. Soc.*, **82**, 2039 (1960).