for 24 hr. The excess aniline was removed by distillation at reduced pressure and the residue allowed to cool to room temperature. This residue was transferred to a Buchner funnel with the aid of ether, filtered and washed with the same solvent. The washed reaction product weighed 3.7 g. (35%) and melted at 157–160°. After recrystallization from water the compound melted at 159.5–160.5°.

Anal. Calcd. for $C_{13}H_{17}NO_2$: C, 71.20%; H, 7.82%; N, 6.39%. Found: C, 71.37%; H, 7.58%; N, 6.58%.

cis-N-(β-Phenylethyl)-3-hydroxycyclohexanecarboxamide. A mixture of 6.0 g. (0.048 mole) of the lactone of 3-hydroxycyclohexanecarboxylic acid and 13.0 g. (0.107 mole) of freshly distilled β-phenylethylamine was heated at 180° in an oil bath under reflux for 24 hr. The excess amine was removed by distillation at reduced pressure and the residue cooled to room temperature. The resulting product was washed with a small amount of ether, transferred to a Buchner funnel and filtered. The solid weighed 10.8 g. (92.5%) and melted at 113–116°. A sample after recrystallization from ether melted 119–120°.

Anal. Calcd. for $C_{15}H_{21}NO_2$: C, 72.84%; H, 8.56%; N, 5.66%. Found: C, 72.84%; H, 8.41%; N, 5.61%.

cis-N-(3-Hydroxycyclohexylmethyl)-β-phenylethylamine hydrochloride. A slurry, prepared by adding 75 ml. of dried tetrahydrofuran to 2.0 g. of lithium aluminum hydride, was contained in a 500 ml. round bottom flask equipped with a reflux condenser, dropping funnel and calcium chloride tube. To this slurry was added dropwise a solution of 2.5 g. (0.01 mole) of *cis-N-(β*-phenylethyl)-3-hydroxycyclohexanecarboxamide (XXII) in 150 ml. of dried tetrahydrofuran. The addition required approximately 20 min. After the solution was added, the reaction mixture was heated under reflux for 12 hr., and then 150 ml. of tetrahydrofuran was removed by distillation. The remaining mixture was cooled in an ice bath and 5 g. of chipped ice added in small pieces. When the reaction subsided, 100 ml. of 95% ethanol was cautiously added in small portions. The resulting mixture was removed from the ice-bath, heated to the boiling point on a steam bath and then allowed to cool to room temperature. The alcohol-tetrahydrofuran solution was decanted and the residue washed twice with 75-ml. portions of alcohol and the alcohol added to the supernatent liquid previously decanted. The alcohol and tetrahydrofuran were then evaporated on a steam bath, the residue cooled and extracted with 100 ml. portions of ether, which were combined and treated with dry hydrogen chloride. The hydrochloride precipitated and was removed by filtration. It weighed 1.6 g. (70%). After recrystallization from isopropyl alcohol it melted at 250–252° dec. after careful drying.

Anal. Calcd. for $C_{15}H_{24}ClNO \cdot \frac{1}{2}H_2O$: C, 64.61%; N, 9.04%; N, 5.02%. Found: C, 64.60%; H, 8.94%; N, 5.55%.

cis-N-(3-Acetoxycyclohexanemethyl)-β-phenylethylamine hydrochloride (IV). A solution of 1.5 g. of crude *cis-N-*(3-hydroxycyclohexylmethyl)-β-phenylethylamine in 9 ml. of glacial acetic acid was treated with 6 drops of concd. sulfuric acid and heated to reflux for 15 hr. The reaction mixture was cooled to 0° and an excess of 10% sodium hydroxide solution added with constant stirring. The alkaline mixture was extracted two times with 100-ml. portions of ether and the ethereal solutions were combined. After drying over sodium sulfate, the ethereal solution was filtered, cooled in an ice-bath and saturated with dry hydrogen chloride. The resulting precipitate (IV), 1.3 g. (75%), was filtered and after several recrystallizations from isopropyl alcohol melted at 201–202° dec.

Anal. Calcd. for $C_{17}H_{26}Cl\,NO_2$: C, 65.48%; H, 8.41%; N, 4.4%. Found: C, 65.65%; H, 8.17%; N, 4.48%.

CHICAGO, ILL.

[CONTRIBUTION FROM THE DEPARTMENT OF MEDICINAL CHEMISTRY, GEIGY RESEARCH LABORATORIES AND THE DEPARTMENT OF PHARMACOLOGY, UNIVERSITY OF ILLINOIS COLLEGE OF MEDICINE]

Centrally Active 2-(Substituted phenyl)-β-alanines[1]

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Received January 6, 1961

Carbethoxylation of substituted phenylacetonitriles gave ethyl substituted phenylcyanoacetates which were reduced to the correspondingly substituted β-alanine esters. A number of the latter were reductively methylated. Hydrolysis of the esters proceeded easily to yield β-alanines. The pharmacological properties of these substances are briefly discussed.

γ-Aminobutyric acid (GABA), one of most abundant amino acids in the brain,[3] has been identified[4] as one of the active agents in the extracts (Factor I) of mammalian brain and spinal cord which inhibit impulse transmission in stretch receptor neurons of the crayfish, and block synaptic transmission in autonomic ganglia and monosynaptic spinal reflexes of mammals. These observations led to the suggestion that γ-aminobutyric acid may have an important function in the control of neurophysiological activity.[4] One other natural amino acid, β-alanine, possesses Factor I activity; it is however, only one-twentieth as active as γ-aminobutyric acid.[4] Purpura, Girado, and Gundfest studied the central inhibitory and excitatory actions of the homologous series of ω-amino acids from C_2 to C_8 by topical application of these substances to the cerebral cortex of cats.[5] β-Alanine was found to be slightly more potent that γ-aminobutyric acid as a cortical synaptic inhibitor in this test system.

None of the simple substitution products of γ-aminobutyric acid or of β-alanine have been studied

(1) Presented at the Frederick F. Blicke Symposium of the Division of Medicinal Chemistry at the 138th National Meeting of the American Chemical Society, New York, N. Y. September, 1960.

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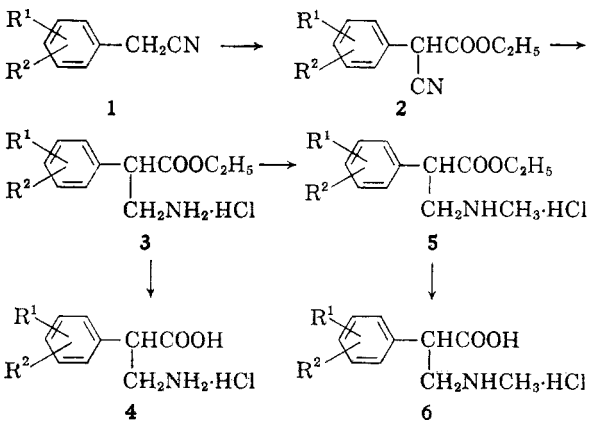
(3) J. Awapara, A. J. Landua, R. Fuerst, and B. Seale, *J. Biol. Chem.*, **187**, 35 (1950); E. Roberts and S. Frankel, *J. Biol. Chem.*, **187**, 55 (1950); S. Udenfriend, *J. Biol. Chem.*, **187**, 65 (1950).

(4) A. Bazemore, K. A. C. Elliott, and E. Florey, *Nature*, **178**, 1052 (1956).

(5) D. P. Purpura, M. Girado, and H. Grundfest, *Proc. Soc. Exptl. Biol. Med.*, **95**, 791 (1957).

pharmacologically. A program was, therefore, set up in these laboratories on the synthesis of analogs of both compounds. In this communication, the preparation and the biological properties are described of a number of 2-(substituted phenyl)-β-alanines, *N*-methyl-2-(substituted phenyl)-β-alanines, and their related ethyl esters.

At the start of this program, 2-phenyl-β-alanine was the only known 2-aryl-β-alanine. It had been prepared by (a) addition of hydroxylamine to atropic acid,[6] (b) treatment of phenylmalonic acid with formaldehyde and ammonia,[7] (c) treatment of 2-chlorotropic acid with aqueous ammonia and subsequent reduction with red phosphorus and hydriodic acid of the intermediate 2-hydroxy-2-phenyl-β-alanine,[8] and (d) conversion of phenylsuccinic acid to the anhydride, ammoniation of the anhydride to phenylsuccinamic acid, and Hofmann rearrangement of the succinamic acid.[9] We found that 2-(substituted phenyl)-β-alanines can be prepared conveniently in three steps from the requisite phenylacetonitriles (**1**). Carbethoxylation of **1** gave the cyanoacetates (**2**) which were reduced catalytically over palladium on carbon to the β-alanine esters (**3**). Acid hydrolysis of **3** afforded, in satisfactory yield, the desired β-alanines (**4**). Several of the primary amino esters (**3**) were reductively methylated and the methylation products (**5**) hydrolyzed to the related methyl-β-alanines (**6**). In general, reductive



methylation was difficult or impossible with the alanines or alanine esters in this series which bore strong electron releasing substituents on the benzene ring. *N*-Methylation products were obtained in very low yield from the *o*- and *p*-methoxyphenyl-β-alanine esters. All attempts to reductively methylate α-(3,4-methylenedioxy-, or 3,4-dimethoxyphenyl)-β-alanine or the corresponding ethyl esters met with failure.

Methylaminomethylation of *p*-nitrophenylacetic acid with formaldehyde and methylamine gave *N*-methyl-2-(*p*-nitrophenyl)-β-alanine[10] which was reduced catalytically to 2-(*p*-aminophenyl)-*N*-methyl-β-alanine. The latter was esterified with ethanolic hydrogen chloride at room temperature.

After the completion of our chemical work, E. Testa *et al.*[11] reported the preparation of 2-phenyl-β-alanine hydrochloride and its ethyl ester by the same method as that which we employed. F. F. Blicke and W. A. Gould[12] obtained ethyl 2-cyclohexyl-β-aminopropionate by the same general method and 2-phenyl-β-alanine and its *N*-methyl derivative by addition of ammonia and methylamine, respectively, to atropic acid.

Pharmacology. The pharmacological properties of the compounds in Table I were determined employing standard techniques. The median lethal dose for mice upon intraperitoneal administration ranged from 80–800 mg./kg. The acids were all distinctly less toxic (LD_{50} >400–800 mg./kg.) than the corresponding esters (LD_{50} = 80–400 mg./kg.). In sublethal doses in mice and rabbits the esters produced (a) signs of central nervous system excitation such as increased activity, hyperreactivity, tremors, and clonus; (b) muscle relaxation; or (c) a combination of excitation combined with partial muscle weakness. The most potent compound in the entire series, ethyl 3-amino-2-(2,4-dimethylphenyl)propionate hydrochloride (compound **22**), produced hypomotility and sedation in mice but hyperactivity culminating in clonic seizures in rabbits (15 mg./kg.).

Intravenous administration to anesthetized dogs of 10 mg./kg. of compounds **3** and **6** resulted in a definite increase and prolongation of the cardiovascular actions of epinephrine and nor-epinephrine without producing any appreciable alterations in the responses to acetylcholine, peripheral vagal or sciatic nerve stimulation. Larger doses (16 mg./kg.) of **1**, **25**, and **27** produced only equivocal potentiation of epinephrine.

Many of the esters in doses of 10–20 mg./kg. intraperitoneally doubled the sleeping time of mice receiving a standard dose of pentobarbital (**1**, **3**, **6**, **19**, and **27**). Some of these same compounds (**1**, **6**, **11**, **15**, **19**, **22**, and **27**) in doses of 10–20 mg./kg. intravenously produced short-lasting antagonism of the depressant effects of chlorpromazine (5 mg./kg. intravenously) or reserpine (2 mg./kg. intramuscularly) in rabbits.

In addition, the esters demonstrated local anesthetic activity upon intra-dermal administration; most of the derivatives were equipotent with pro-

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(9) M. R. Natarajan and S. Swaminathan, *J. Am. Chem. Soc.*, **69**, 2560 (1947).

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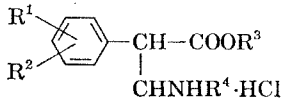
(12) F. F. Blicke and W. A. Gould, *J. Org. Chem.*, **23**, 1102 (1958).

TABLE I

2-ARYL-β-ALANINES AND ESTERS

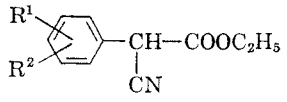


No.	R¹	R²	R³	R⁴	M.P.	Recryst. Solv.[a]	Molecular Formula	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
1	H	H	C_2H_5	H	160–161	E	$C_{11}H_{15}NO_2$·HCl	57.51	7.02	6.10	57.42	7.08	6.21
2	H	H	H	H	236–238	M-W	$C_9H_{11}NO_2$·HCl	53.48	6.00	6.94	53.59	6.68	6.83
3	H	H	C_2H_5	CH_3	145–146	I-E	$C_{12}H_{17}NO_2$·HCl	59.56	7.40	5.74	59.48	7.29	5.61
4	H	H	H	CH_3	174–175	I-Et	$C_{10}H_{13}NO_2$·HCl	55.91	6.53	6.49	56.41	6.38	6.01
5	H	H	CH_3	H	192–195	M	$C_{10}H_{13}NO_2$·HCl	55.91	6.53	5.49	55.53	6.68	6.63
6	3-Cl	H	C_2H_5	H	160–161	I	$C_{11}H_{14}ClNO_2$·HCl	50.00	5.72	5.29	49.91	5.81	5.38
7	3-Cl	H	H	H	255	I-E	$C_9H_{10}ClNO_2$·HCl	45.77	4.69	5.93	45.74	5.11	6.12
8	3-Cl	H	C_2H_5	CH_3	148–149	B	$C_{12}H_{17}ClNO_2$·HCl	51.62	6.49	5.03	51.77	6.38	5.58
9	4-Cl	H	C_2H_5	H	190	E	$C_{11}H_{14}ClNO_2$·HCl	50.01	5.78	5.38	49.42	5.51	5.03
10	4-Cl	H	H	H	199–200	I-Et	$C_9H_{10}ClNO_2$·HCl	45.88	4.69	5.93	46.28	4.73	5.61
11	4-Cl	H	C_2H_5	CH_3	175–177	I-E	$C_{12}H_{17}ClNO_2$·HCl	51.62	6.49	5.03	51.59	6.36	5.20
12	4-Cl	H	H	CH_3	195–196	D-I	$C_{10}H_{12}ClNO_2$·HCl	48.02	5.24	5.59	48.00	5.34	5.51
13	4-H_2N	H	H	CH_3	192–195	E-M	$C_{10}H_{13}N_2O_2$·HCl	52.06	6.56	12.18	52.06	6.14	12.06
14	4-H_2N	H	C_2H_5	CH_3	220–221	E	$C_{12}H_{19}N_2O_2$·2HCl	48.40	6.78	9.49	48.40	6.54	9.27
15	2-CH_3O	H	C_2H_5	H	115–117	E-Et	$C_{12}H_{17}NO_3$·HCl	55.49	6.98	5.39	55.76	7.04	4.91
16	2-CH_3O	H	H	H	206–207	E-A	$C_{10}H_{13}NO_3$·HCl	51.83	6.09	6.05	51.92	6.08	6.28
17	2-CH_3O	H	C_2H_5	CH_3	syrup	—	$C_{12}H_{19}NO_3$·HCl	57.02	7.36	5.12	57.34	7.37	5.08
18	2-CH_3O	H	H	CH_3	syrup	—	$C_{11}H_{15}NO_3$·HCl	53.76	6.56	5.70	53.36	6.80	6.10
19	4-CH_3O	H	C_2H_5	H	165–166	I	$C_{12}H_{17}NO_3$·HCl	55.49	6.98	5.39	55.60	7.16	5.53
20	4-CH_3O	H	H	H	290–293	I-A	$C_{10}H_{13}NO_3$·HCl	51.83	6.09	6.05	51.40	7.21	6.29
21	4-CH_3O	H	C_2H_5	CH_3	oil	—	$C_{12}H_{15}NO_3$	66.10	7.62	5.93	66.37	7.41	5.73
22	2-CH_3	4-CH_3	C_2H_5	H	131–132	B-P	$C_{13}H_{19}NO_2$·HCl	60.56	7.82	5.43	60.44	8.00	5.69
23	2-CH_3	4-CH_3	H	CH_3	200–201	B	$C_{12}H_{17}NO_2$·HCl	59.01	7.37	5.73	59.34	7.42	5.59
24	3-CH_3	4-CH_3	H	H	225–226	I-W	$C_{11}H_{15}NO_2$·HCl	57.64	6.55	6.11	57.17	6.92	5.83
25	3-CH_3O	4-CH_3O	C_2H_5	H	173–174	I-Ea	$C_{13}H_{19}NO_4$·HCl	53.88	6.96	4.84	54.00	7.08	4.98
26	3-CH_3O	4-CH_3O	H	H	243–244	E-Et	$C_{11}H_{15}NO_4$·HCl	50.29	6.52	5.33	50.25	6.29	5.21
27	3,4-OCH_2O		C_2H_5	H	175–176	E-Et	$C_{12}H_{15}NO_4$·HCl	52.55	5.84	5.10	52.25	6.04	5.42
28	3,4-OCH_2O		H	H	238–240	E-Et	$C_{10}H_{11}NO_4$·HCl	48.89	4.91	5.70	48.97	5.14	6.03

[a] A, acetone; B, benzene; D, dioxane; E, ethanol; Ea, ethyl acetate; Et, ether; I, isopropyl alcohol; M, methanol; P, petroleum ether (b.p. 35–80°); W, water.

TABLE II

CYANOACETATES



Compound	R¹	R²	B.P./Mm.	Yield, %	Molecular Formula	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
1	H	H	138–140/4[a]	68.0	$C_{11}H_{11}NO_2$	—	—	—	—	—	—
2	3-Cl	H	135–136/1	42.3	$C_{11}H_{10}ClNO_2$	—	—	6.24	—	—	6.11
3	4-Cl	H	141–142/1	95.0	$C_{11}H_{10}ClNO_2$	—	—	6.24	—	—	6.08
4	2-CH_3O	H	125–126/0.3	46.5	$C_{12}H_{13}NO_3$	65.76	5.98	6.39	65.78	5.99	6.12
5	4-CH_3O	H	145–148/0.5	71.0	$C_{12}H_{13}NO_3$	—	—	6.39	—	—	6.74
6	2-CH_3	4-CH_3	133–135/1	81.5	$C_{13}H_{15}NO_2$	71.88	6.99	6.45	72.47	7.08	7.16
7	3-CH_3	4-CH_3	158–160/2	84.1	$C_{13}H_{15}NO_2$	71.88	6.99	6.45	71.73	6.76	6.71
8	3-CH_3O	4-CH_3O	183–185/2	40.0	$C_{13}H_{15}NO_4$	62.65	6.02	—	62.51	6.50	—
9	3,4-OCH_2O		172–175/1.5	38.2	$C_{12}H_{11}NO_4$	61.80	4.71	—	61.62	4.89	—

[a] E. C. Horning and A. F. Finelli, ref. 17, reported b.p. 125–135/3.5 mm. and 70–78% yield.

caine whereas a few (6, 11, 22) were two to four times as active.

Definitive correlations among local anesthetic activity, potentiation of epinephrine, prolongation of pentobarbital sleeping time, and antagonism of reserpine and chlorpromazine, could not be established on the basis of these initial observations; however, it is hoped that further investigations will provide a clue to the mechanism of action of these compounds.

EXPERIMENTAL

Phenylacetonitrile was purchased from Matheson, Coleman and Bell. *m*-Chloro-, *p*-chloro-, and *p*-methoxyphenylacetonitrile were obtained from Aldrich Chemical Co. 2,4- and 3,4-Xylylacetonitrile were obtained through the courtesy of J. R. Geigy, S. A. Basle, Switzerland.

o-Methoxybenzaldehyde, 3,4-dimethoxybenzaldehyde and piperonal were purchased from Distillation Products Ind. and converted to the desired nitriles in three steps via a crossed Cannizzaro reaction with formaldehyde, treatment of the alcohols with thionyl chloride and metathesis of the chlorides with sodium cyanide according to the general procedures for these conversions described by Shepard and Noth.[14] The nitriles so prepared had physical constants in good agreement with those reported in the literature as follows: (1) *o*-Methoxyphenylacetonitrile: b.p. found 97–99°/0.9 mm.; Pschorr *et al.*,[15] reported b.p. 141–143°/15 mm. (2) 3,4-Dimethoxyphenylacetonitrile: b.p. found 140–141°/1 mm.; Kaufmann and Muller[16] reported b.p. 171–178°/10 mm. (3) 3,4-methylenedioxyphenylacetonitrile: b.p. found 125–129°/0.2 mm; Shepard and Noth[14] found b.p. 134–142°/0.5 mm. Ethyl phenylcyanoacetate and the new cyanoacetates described in Table II were prepared by carbethoxylation of the related nitriles by the method of Horning and Finelli.[17]

(13) All melting points and boiling points are uncorrected. We are indebted to Mr. John Deonorine of these laboratories for microanalyses.

(14) E. R. Shepard and J. F. Noth, *J. Am. Chem. Soc.*, **72**, 4364 (1950).

(15) R. Pschorr, O. Wolfes, and W. Buckow, *Ber.*, **33**, 162 (1900).

(16) A. Kaufmann and H. Muller, *Ber.*, **51**, 123 (1918).

The following examples are typical of the procedures employed for the preparation of the new substituted β-alanines and the related esters that are described in Table I.

Ethyl 3-amino-2-phenylpropionate hydrochloride. Ethyl phenylcyanoacetate (10 g., 0.053 mole) was dissolved in 150 ml. of absolute alcohol. Concentrated hydrochloric acid (4.5 ml.) and 50 mg. of 10% palladium on carbon catalyst were added to the solution and the mixture hydrogenated in a Parr hydrogenation apparatus at 50 lbs. p.s.i. initial pressure. Absorption of hydrogen was complete after 2 hr. The catalyst was removed by filtration and the filtrate evaporated *in vacuo* to a crystalline residue which melted after two recrystallizations from ethanol at 160–161°; yield 6 g. (47.5%).

Ethyl 2-(p-chlorophenyl)-3-methylaminopropionate hydrochloride. A mixture of 11.2 g. (0.05 mole) of ethyl *p*-chlorophenylcyanoacetate, 150 ml. of absolute alcohol, 4.5 ml. of concd. hydrochloric acid, and 50 mg. of 10% palladium on carbon catalyst was shaken in a Paar hydrogenation apparatus at 50 p.s.i. initial pressure of hydrogen until the calculated quantity of hydrogen had been absorbed. The mixture was filtered, mixed with 5 g. (0.05 mole) of 37% formaldehyde and fresh catalyst, and shaken as before at 50 p.s.i. hydrogen pressure until hydrogenation was complete. The reaction mixture was filtered and concentrated *in vacuo* and the residue, a mixture of syrup and crystals, was recrystallized twice to constancy of melting point from a 3:1 isopropyl–ethyl alcohol mixture. Yield, 5 g. (36%).

2-(3,4-Methylenedioxyphenyl)-β-alanine hydrochloride. A solution of 7.7 g. (0.0282 mole) of ethyl 3-amino-2-(3,4-methylenedioxyphenyl)propionate hydrochloride in 50 ml. of 3*N* hydrochloric acid was refluxed for 1.5 hr. and then concentrated to dryness. The crystalline residue was recrystallized from a mixture of 95% ethanol and ether; yield 3.5 g. (50.5%).

Methyl 3-amino-2-phenylpropionate hydrochloride. A solution of 3 g. (0.015 mole) of 2-phenyl-β-alanine hydrochloride in 50 ml. of dry methanol was saturated with gaseous hydrogen chloride with cooling and set aside for 4 days. The mixture was concentrated *in vacuo* at 30–40° and the syrupy residue crystallized by trituration with ether. The crystalline product melted without change after two recrystallizations from methanol. Yield: 2 g. (62%).

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(17) E. C. Horning and A. F. Finelli, *Org. Syntheses*, **30**, 43 (1950).