Anal. Calcd. for $C_{13}H_{13}I_3N_2O_4$: I, 59.3; N, 4.36. Found: I, 59.3; N, 4.30.

3-Carbomethoxy-5-nitrobenzoyl Chloride (VI).—Hydrogen methyl 5-nitroisophthalate (II, 241 g., 1.07 mole) and phosphorus pentachloride (230 g., 1.07 mole) were mixed in toluene (100 ml.) at room temperature. The reaction proceeded slowly for 17.5 hr. after which heat (steam bath) was applied to complete solution in an additional 45 min. The toluene was evaporated under reduced pressure whereupon the crude product crystallized. Addition of carbon tetrachloride and evaporation under reduced pressure gave VI, m.p. 72.4–74°.

Anal. Calcd. for $C_9H_5ClNO_5$: neut. equiv., 244. Found: neut. equiv., 238 (determined by alcoholysis of the chloroformyl group and titration of the liberated hydrochloric acid).

Methyl N,N-Dimethyl-5-nitroisophthalamate (VII, $R_1 = R_2 = CH_3$).—One hundred grams of VI (0.41 mole) was added during 0.5 hr. to a well-stirred solution of dimethylamine (81 g. of a 25% aqueous solution, 0.45 mole) and sodium bicarbonate (69 g., 0.82 mole) in water (500 ml.), maintained at 0–5°. When the reaction appeared complete, the undissolved material was collected, slurried with sodium bicarbonate solution, and again collected, washed and air dried; yield of crude VII ($R_1 = R_2 = CH_3$), 90 g. (87%), m.p. 64.3–70.3°. A portion of the crude product was recrystallized twice from methanol, m.p. 89.3–91.5°.

N,N-Dimethyl-5-nitroisophthalamic Acid (III, $R_1 = R_2 = CH_3$).—Methanol (200 ml.) was added to 80 g. of crude methyl N,N-dimethyl-5-nitroisophthalamate and then 300 ml. water. Sodium carbonate (36.5 g.) was added in portions to the warmed mixture (pH 8–9). After filtration and acidification with hydrochloric acid, the resulting precipitate was collected and dried, yielding 54 g. of crude III ($R_1 = R_2 = CH_3$), m.p. 205.8–208.8°. This crude product was combined with 18 g. of similar material from another reaction, absolute ethanol (150 ml.) added, and the mixture digested for 10 min. Upon chilling N,N-dimethyl-5-nitroisophthalamic acid was precipitated, filtered and dried; yield, 64.3 g.

Methyl N-Amyl-5-nitroisophthalate (VII, $R_1 = H$; $R_2 = n\text{-}C_5H_{11}$).—3-Carbomethoxy-5-nitrobenzoyl chloride (55.6 g., 0.23 mole) in carbon tetrachloride (271 g.) was added slowly to a stirred mixture of *n*-amylamine (19.9 g., 0.23 mole), water (200 ml.), acetone (50 ml.), and sodium hydroxide (9.2 g.). The reaction mixture was kept alkaline at all times. The carbon tetrachloride and acetone were then removed by evaporation. The residual mixture was stirred under an air jet for 2.5 hr., cooled in an ice bath, and the aqueous layer decanted from the oily product. The crude product was utilized in the following preparation without further purification.

N-Amyl-5-nitroisophthalamic Acid (III, $R_1 = H$, $R_2 = n\text{-}C_5H_{11}$).—The crude methyl N-amyl-5-nitroisophthalamate was dissolved in the minimum amount of anhydrous alcohol and an equal volume of water added. After addition of sodium carbonate (pH 8), the mixture was heated for 0.5 hr. and allowed to stand for 2 days. Precipitation occurred during this period. The mixture was diluted with water and heated, whereupon most of the precipitate dissolved. The undissolved matter was filtered and the filtrate poured into an excess of dilute hydrochloric acid. The resulting precipitate was purified in the manner described for N-methyl-5-nitroisophthalamic acid and used directly in the synthesis of the desired 5-acylamino-2,4,6-triiodo-N-amylisophthalamic acids (Table III).

Propyl 5-Acetamido-2,4,6-triiodoisophthalamate (VIII, $R_1 = R_2 = H$; $R_3 = CH_3$, $R' = C_3H_7$).—5-Acetamido-2,4,6-triiodoisophthalamic acid (Table III, 110 g., 0.18 mole) was added to a stirred solution of sodium ethoxide, prepared by dissolving sodium (4.23 g., 0.18 mole) in absolute ethanol (500 ml.). After a few minutes *n*-propyl iodide (34.4 g., 0.20 mole) was added and the mixture heated and stirred under gentle reflux for 3 hr. A portion of the solvent was evaporated, and water added to the remaining solution to precipitate the ester. The white precipitate was collected, slurried with sodium bicarbonate solution and filtered. Recrystallization from 50% aqueous dimethylformamide gave propyl 5-acetamido-2,4,6-triiodoisophthalamate, 40 g. (34%), m.p. 271.0–271.5°.

Anal. Calcd. for $C_{14}H_{13}I_3N_2O_4$: I, 59.3. Found: I, 58.2.

Toxicity Determinations.—The acute toxicity studies were carried out by Drs. T. W. Tusing and J. K. Kodama, Hazleton Laboratories, Falls Church, Virginia. The methods employed in these studies are described in reference 2a.

The Synthesis of Arylalkylaminopropionamide Analogs of Lysergic Acid Diethylamide and their Effect upon Isolated Cholinesterase Systems. II[1]

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A series of α-arylalkylaminopropionic acid diethylamides, patterned after a component of the LSD molecule, was synthesized. Their effect upon isolated human plasma "pseudo"-cholinesterase systems was studied, and relationships between molecular constitution and biochemical response were explored.

In the preceding paper,[2] we suggested that the substituted (>N-C-C-CON<), β-aminopropionamide moiety present in (+)-lysergic acid diethylamide (LSD) as well as in the β-(arylalkylamino)propionamide[2] and piperidinecarboxamide[3–6] compounds derived from the corresponding components of the parent LSD molecule, might be involved in the inhibitor–enzyme complex formation in human plasma "pseudo"-cholinesterase systems. In order to complement our findings we prepared and evaluated the β-(methylamino), β-(dimethylamino) and β-(trimethylammonium) substituted diethylpropionamides (Fig. 1). Next, the pronounced difference between inductive effects elicited by some substituents on α- and β-carbons of aliphatic acids,[7] prompted us to examine the biochemical responses of the α-(arylalkylamino) substituted analogs (Fig. 2) of the previously studied β-(arylalkylamino)propionic acid diethylamides[2]; the fact that these two series of compounds also could be viewed as

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TABLE I

PROPERTIES OF SUBSTITUTED ARYLALKYLAMINOPROPIONAMIDE ANALOGS, II

Compound	Yield,[a] %	Base B.p. °C	Base B.p. mm.	Base n^t_D	Base $[\alpha]^{27.5}_D$	Hydrochloride[b] M. p., °C	Hydrochloride[b] $[\alpha]^{27.5}_D$	Empirical formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Chlorine Calcd.	Chlorine Found	Nitrogen Calcd.	Nitrogen Found
I	82	116.0–127.0	0.15–0.50	$1.5132^{24.9}$	...	181.5–182.0	...	$C_{15}H_{25}ClN_2O$	63.24	63.41	8.85	8.88	12.45	12.56	9.84	9.78
II	73	125.0–128.0	0.20	$1.5106^{27.2}$	...	163.0–164.0	...	$C_{16}H_{27}ClN_2O$	64.30	64.11	9.10	9.12	11.86	11.76	9.38	9.20
III	73	143.0–149.0	0.70–0.75	$1.5098^{24.0}$	...	209.0–210.0	...	$C_{17}H_{29}ClN_2O$	65.26	65.23	9.34	9.35	11.33	11.26	8.96	9.07
IV	79	132.0–134.0	0.08	$1.5101^{23.8}$	+32.85[c]	190.0–191.0	+37.16[d]	$C_{17}H_{29}ClN_2O$	65.26	65.41	9.34	9.35	11.33	11.38	8.96	8.86
V	77	131.0–142.0	0.10–0.15	$1.5103^{22.8}$	−34.72[c]	190.0–192.0	−41.12[d]	$C_{17}H_{29}ClN_2O$	65.26	65.30	9.34	9.32	11.33	11.36	8.96	8.96
VI	71	122.0–127.0	0.09–0.20	$1.5090^{23.0}$	...	121.5–122.0	...	$C_{16}H_{27}ClN_2O$	64.30	64.13	9.11	9.05	11.86	11.70	9.38	9.33

[a] Yield of pure product, based on final condensation. [b] All hydrochlorides recrystallized from ethyl acetate–ethanol. [c] *c*, 10 (in CH_3OH). [d] *c*, 2.5 (in CH_3OH).

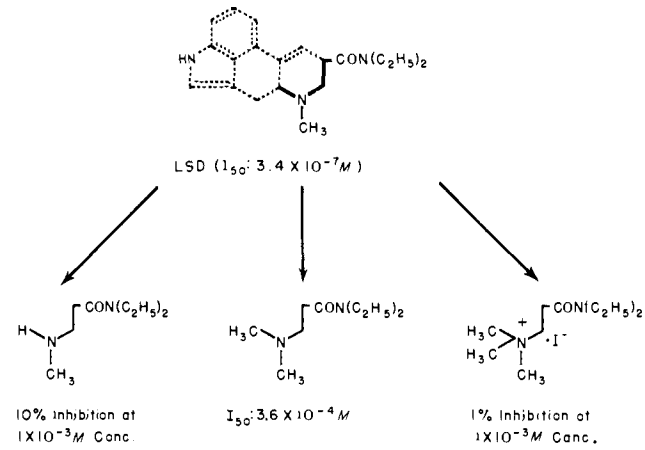

Fig. 1.—Relationships between the molecular constitution of alkylaminopropionamide analogs and their effects upon isolated human plasma "pseudo"-cholinesterase.

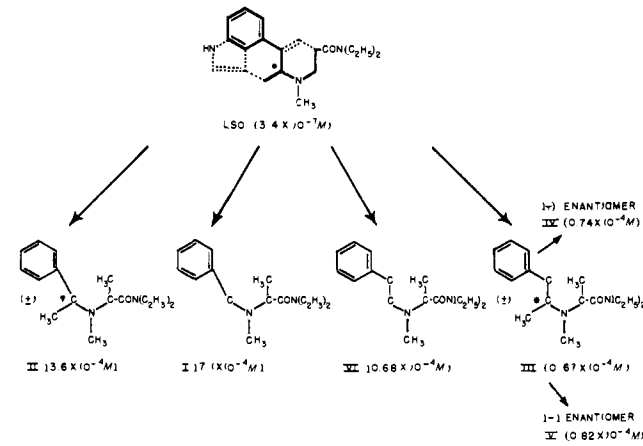

Fig. 2.—Relationships between the molecular constitution of α-(arylalkylamino)propionamide analogs and their effects upon isolated human plasma "pseudo"-cholinesterase. The figures in parentheses represent I_{50} concentrations. (Only the asymmetric carbon common to the parent molecule and the derivatives is marked.)

derivatives of α-alanine and β-alanine, respectively, lent additional emphasis to the study of these entities.

The evaluation of the β-(methylamino)-, β-(dimethylamino)- and β-(trimethylammonium)propionic acid diethylamides yielded interesting information (Fig. 1). The strikingly selective affinity of the enzyme for the tertiary-amino analog of this series appears to support our contention[2] that the substituted β-aminopropionamide moiety may be implicated in the reaction between the enzyme and these series of compounds.

The inhibitory properties of the α-(arylalkylamino)-propionamide analogs are summarized in Fig. 2. While the difference between these and the inhibitory effects determined for the corresponding β-(arylalkylamino)-propionamides, do not seem very prominent, it is rather interesting to note that the general trend observed in the evaluation of the β-substituted series[2] is reflected in the biochemical response of the α-substituted derivatives. The current data actually further substantiate some of the findings reported in our preceding communication: *e.g.*, (1) the α-methylbenzyl derivatives are more potent inhibitors than the benzyl substituted ones, (2) the benzyl derivatives effect weaker inhibition than the phenethyl substituted ones, (3) neither of the en-

antiomers of the (±)-α-methylphenethyl analog revealed any pronounced specificity in either the α- or β-aminopropionamide series.

Perhaps most conspicuous is the fact that in the α-aminopropionamide series the phenethyl substituted analog VI is more than ten times stronger in its inhibitory effect than the benzyl substituted one (I), while in the β-aminopropionamide series the corresponding phenethyl compound is less than three times as potent an inhibitor as the respective benzyl derivative. Work toward a comprehensive interpretation, incorporating the present as well as our earlier findings, is currently in progress.

Experimental

Synthetic Work[8]

β-(Methylamino)-N′,N′-diethylpropionamide.—A solution of N,N-diethyl-β-bromopropionamide[2] (25.0 g., 0.120 mole) prepared in 200 ml. of anhydrous benzene was cooled to 15°, and—with cooling maintained—100 ml. of anhydrous benzene saturated with methylamine was added. Subsequently, methylamine was bubbled into the mixture (already basic to moistened phenolphthalein paper) at room temperature for 3 hr. The mixture was stirred for 3 additional hr. and then refluxed for 7 hr. Upon cooling, the reaction mixture was treated with aqueous saturated potassium carbonate. The bases were extracted with benzene, the combined extracts were dried over anhydrous sodium sulfate, filtered, and the benzene was removed under reduced pressure. Upon fractionation, the pure product (11.6 g., 61% yield) distilled at 74.0–79.0° (0.08 mm.); $n^{24.8}$D 1.4597. Five grams of the base was converted to the *hydrochloride* by treating it with hydrogen chloride in anhydrous ethyl ether. After 3 recrystallizations from ethyl acetate, the very hygroscopic salt melted at 86.5–87.0° (sealed tube).

Anal. Calcd. for $C_8H_{19}ClN_2O$: C, 49.34; H, 9.84; Cl, 18.21; N, 14.39. Found: C, 49.34; H, 9.91; Cl, 18.29; N, 14.44.

β-(Dimethylamino)-N′,N′-diethylpropionamide.—A solution of N,N-diethyl-β-bromopropionamide (25.0 g., 0.120 mole) in 200 ml. of anhydrous benzene was cooled to 12°, and—with cooling maintained—a solution of 10.8 g. (0.240 mole) of dimethylamine in 100 ml. of anhydrous benzene was added. The reaction mixture was stirred at room temperature for 1.5 hr., and then an additional 10.8 g. (0.240 mole) of dimethylamine was added. Subsequently, the reaction mixture was treated in the same manner as in the preparation of the preceding compound. The pure base (13.0 g., 63% yield) distilled at 78.0–84.0° (0.20–0.35 mm.); $n^{25.5}$D 1.4560. Five grams of the base was converted to the *hydrochloride* according to the procedure described in the preceding experiment; after 3 recrystallizations from ethanol–ethyl acetate, the very hygroscopic salt melted at 139.0–139.5° (sealed tube).

Anal. Calcd. for $C_9H_{21}ClN_2O$: C, 51.78; H, 10.14; Cl, 16.99; N, 13.42. Found: C, 51.71; H, 10.15; Cl, 17.05; N, 13.49.

β-(Trimethylammonium)-N′,N′-diethylpropionamide Iodide.—To a solution of 6.3 g. (0.044 mole) of iodomethane in 50 ml. of anhydrous benzene, a solution of 5.0 g. (0.029 mole) of β-(dimethylamino)-N′,N′-diethylpropionamide in 100 ml. of anhydrous benzene was added at room temperature; a fine white precipitate crystallized immediately. The reaction mixture was refluxed for 3.5 hr. After standing in the dark for 7 days, the crystalline component was filtered off, and after vacuum drying, the product weighed 8.0 g. (86% yield). After two recrystallizations from ethanol–ethyl acetate, the salt melted at 198.0–198.5°.

Anal. Calcd. for $C_{10}H_{23}IN_2O$: C, 38.22; H, 7.38; I, 40.39; N, 8.92. Found: C, 38.23; H, 7.43; I, 40.30; N, 8.82.

N,N-Diethyl-α-bromopropionamide.—To 100.0 g. (0.463 mole) of α-bromopropionyl bromide, dissolved in 700 ml. of anhydrous ethyl ether and cooled to 0°, a solution of 74.5 g. (1.019 moles) of diethylamine in 400 ml. of anhydrous ethyl ether was added dropwise, with mechanical stirring. During the addition the pot temperature was kept at 1–3°. The addition was completed within 45 min., and the reaction mixture was stirred in the cold for an additional 40 min. The precipitated hydrobromide was removed by filtration. The solvent was removed from the ethereal filtrate on the hot water bath. The residual liquid (89.6 g.) was fractionated under reduced pressure. The colorless liquid (74.2 g., 77% yield), distilling at 113.0–118.0° (17 mm.), n^{27}D 1.4825, was identified[9,10] as N,N-diethyl-α-bromopropionamide.

The α-(arylalkylamino)propionamide derivatives (Compounds I–VI, Fig. 2) were prepared by the *general procedure* illustrated in the description of the synthesis of I. The properties of the bases and their hydrochlorides, and the respective analyses are reported in Table I.

Representative Procedure. α-(N-Methylbenzylamino)-N′,N′-diethylpropionamide (I).—To 34.9 g. (0.288 mole) of N-methylbenzylamine cooled to 10°, 30.0 g. (0.144 mole) of N,N-diethyl-α-bromopropionamide was added, with stirring. The reaction mixture was heated in a silicone bath, maintained at 97–102°, for 8 hr. and after cooling treated with aqueous saturated potassium carbonate. The bases were extracted with benzene, the combined extracts were dried over anhydrous sodium sulfate, filtered, and the benzene was removed under reduced pressure. The residue was fractionated, and the pure product (29.5 g., 82% yield) distilled at 116.0–127.0° (0.15–0.50 mm.); $n^{24.9}$D 1.5132. The base was converted to the *hydrochloride* by treating it with hydrogen chloride in anhydrous ethyl ether. After three recrystallizations from an ethanol–ethyl acetate system, the salt melted at 181.5–182.0°.

Biochemical Evaluation.—The compounds discussed in this paper were evaluated under exactly the same conditions as those reported in the preceding communication (Procedure I).[2] We expressed the rate as

$$V = \left(\frac{(\mu\text{l. } CO_2 \text{ at 30 min.}) - (\mu\text{l. } CO_2 \text{ at 10 min.})}{20}\right) \times 60$$

where V signifies μl. CO_2/hr. evolved within the reaction interval of +10 through +30 min., during which the rate was linear in all instances. The percentages of inhibition were calculated by the equation

$$I = ((V_c - V_i)/V_c) \times 100$$

where V_c represents the control rate and V_i the inhibited rate. The effect of a given compound was evaluated at four appropriate concentrations, with at least two independent duplicate determinations for each concentration, and the I_{50} (molarity of compound effecting 50% inhibition) was graphically determined. The human plasma "pseudo"-cholinesterase[11] system used in these experiments was characterized kinetically. A linear reciprocal Lineweaver–Burk plot[12] of the enzyme activity, at varying enzyme and substrate concentrations, was graphically evaluated as suggested by Dixon[13] and Reiner[14]; the Briggs–Haldane constants[14,15] did not vary significantly with enzyme concentration and were within the range of variability for independent determinations in the system investigated.[16]

Acknowledgment.—We gratefully acknowledge the valuable suggestions of Dr. John M. Reiner.

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