Journal of Medicinal and Pharmaceutical Chemistry

VOL. 2 No. 6 (1960)

The Synthesis of Substituted Arylalkylaminopropionamide Analogues of Lysergic Acid Diethylamide and their Effect upon Isolated Cholinesterase Systems*

ANDREW LASSLO,† PAULINE D. WALLER, ANNE L. MEYER and B. V. RAMA SASTRY, *Department of Pharmacology, Division of Basic Health Sciences, Emory University, Atlanta 22, Georgia*

Introduction

The discovery of the psychotomimetic effects of (+)-lysergic acid diethylamide (LSD)[1] triggered off voluminous amounts of research activity ranging from a re-examination of relationships between cortical, autonomic and peripheral functions[2] to attempts at correlating chemical structure with psychopharmacological responses.[3,4] In the course of an investigation of the effect of gradual changes in the molecular constitution of synthetic entities upon biological response, we became interested in compounds related to LSD. Interestingly, LSD may be looked upon as a derivative of moieties which also happen to be components of other molecules known to induce central nervous stimulation. For example, LSD may be viewed as a derivative of the partly unsaturated 1-methyl-3-(*N*,*N*-diethylcarboxamido)piperidine component of its molecule, and several other piperidinecarboxylic acid derivatives (cocaine,[5a] meperidine,[5b] etc.) are known to effect psychic disturbances; in the first phase of our work we have undertaken the synthesis and the study of a series of piperidinecarboxamides[6-8] which is still in progress. Similarly, LSD could be visualized as a derivative of phenethylamine (see Fig. 1), and a number of other phenethylamine derivatives (mescaline,[5c] amphetamine,[5d] methamphetamine,[5e] trimethoxyamphetamine,[9]

* This investigation is supported by U.S. Public Health Service Research Grant No. M-2072 from the National Institute of Mental Health.

† Present Address: Department of Pharmaceutical and Medicinal Chemistry, University of Tennessee College of Pharmacy, Memphis 3, Tennessee.

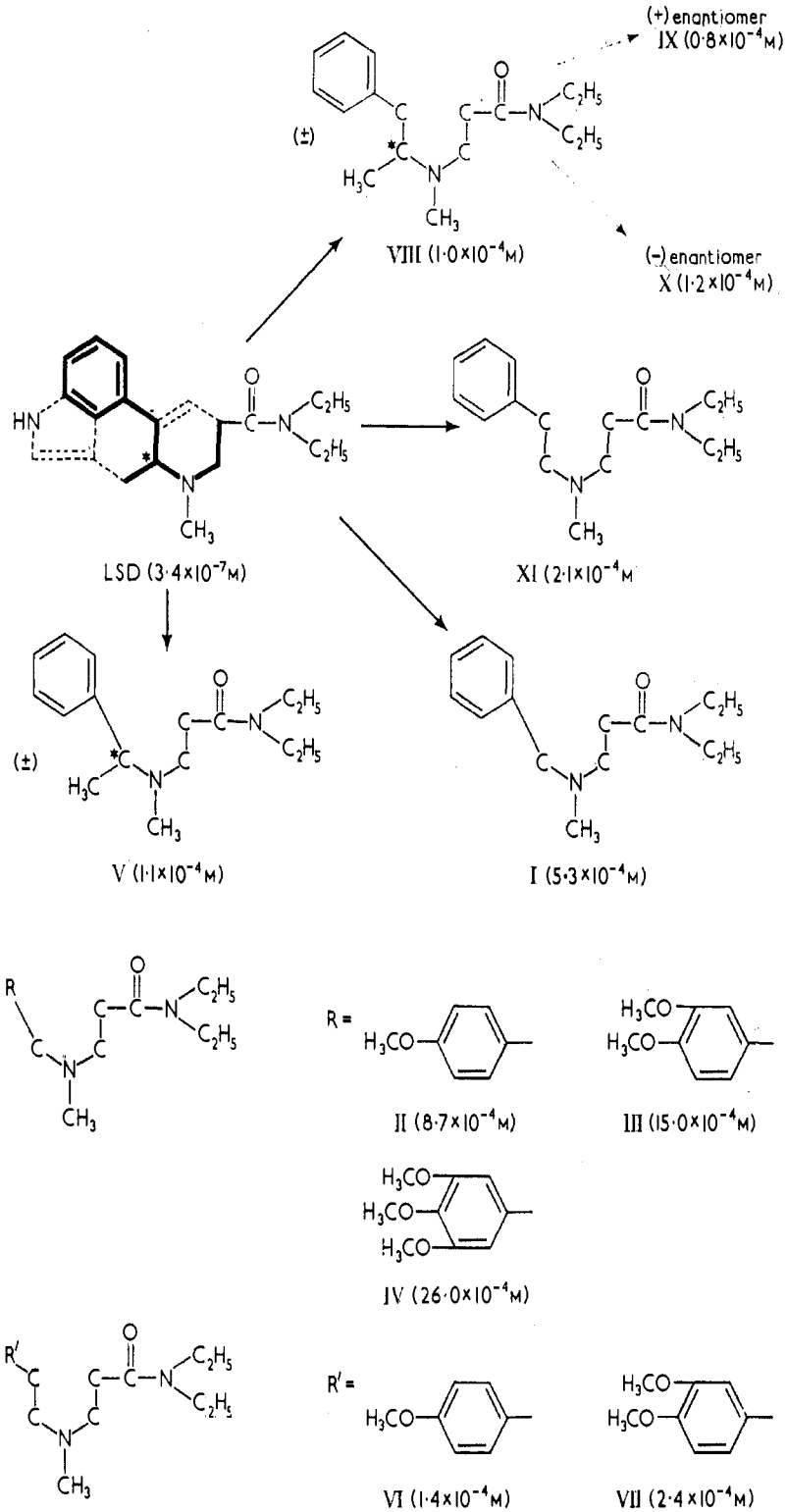


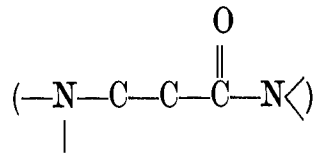
Fig. 1. Relationships between the molecular constitution of substituted arylalkylaminopropionamide analogues and their effects upon isolated human plasma cholinesterase. The figures in parentheses represent I_{50} concentrations (Procedure I). (Only the asymmetric carbon reproduced in the derivatives is marked in the parent molecule.)

etc.) have been found to possess psychotomimetic properties; at present, we are reporting on a series of compounds derived from the phenethylamine moiety.

The series was designed with three major considerations in mind: (1) the compounds are patterned after the LSD molecule (see Fig. 1) with gradual changes in their constitution or physical properties or both; (2) in addition, the compounds are derivatives either of molecules known to induce hallucination, e.g., VIII (*N*,*N*-disubstituted amphetamine or *N*-substituted methamphetamine), or of closely related analogues of molecules possessing such properties; and (3) the biological response of the series may be correlated with molecular constitution in terms of concepts which are fairly well established in contemporary theoretical chemistry (e.g., the comparative effect of variously substituted or unsubstituted benzyl and phenethyl radicals upon the tertiary amine, an important functional group common to all members of the series, etc.).

Since LSD was reported[10] to be: (1) a powerful inhibitor of 'pseudo'-cholinesterase in human serum and brain, (2) considerably less effective in inhibiting 'pseudo'-cholinesterase activity of rat, guinea-pig, rabbit, chicken and monkey brain, (3) the most potent inhibitor of 'pseudo'-cholinesterase among the several structurally related ergot alkaloids evaluated, and (4) without significant effect upon human brain and erythrocyte 'true' cholinesterase in concentrations which induced strong inhibition of corresponding 'pseudo'-cholinesterases, we felt that isolated cholinesterase systems might lend themselves rather well to the evaluation and study of our series of compounds.

The possibility that the β-aminopropionamide moiety



present in the parent molecule (LSD) as well as in the arylalkylaminopropionamide derivatives (see Fig. 1) and the earlier reported piperidinecarboxamide derivatives,[6,7] may be involved in the inhibitor–enzyme complex formation, in accordance with Bergmann, Wilson and Nachmansohn's,[11] Wilson's[12] and possibly Porter, Rydon and Schofield's[13] interpretation of the affinities of serum- and acetylcholinesterases,

cannot be excluded. With this thought in mind, we have attempted to: (1) influence the electron densities around the amino function and the carbonyl carbon which predominantly influence the affinities of the anionic and esteratic sites of the enzymes,[11,12] and (2) induce varying steric hindrance and other spatial limiting factors; the structural modifications around the carbonyl carbon and some of the spatial limiting factors have been realized in the piperidinecarboxamide series,[6,7] and their effects upon isolated cholinesterase systems will be discussed at a future date.

Experimental

*1. Synthetic Work**

The compounds listed in Table I were prepared by the following procedures.

Procedure A

N-*Methyl-3,4,5-trimethoxybenzamide.* 3,4,5-Trimethoxybenzoic acid (25·0 g, 0·118 mole) was dispersed in anhydrous benzene (200 ml), the mixture was cooled to 10°, and thionyl chloride (140·3 g, 1·18 moles) was added; the reaction mixture was allowed to warm to room temperature, was subsequently heated at 45–50° for 45 min, and was finally refluxed at 67–70° for 1 h. The excess thionyl chloride and benzene were removed under reduced pressure (maximum pot temperature 40°). The residual thionyl chloride was removed by azeotropic distillation under reduced pressure with three 100-ml portions of anhydrous benzene. The acid chloride was dissolved in anhydrous ethyl ether (390 ml), and dry ethereal saturated methylamine solution (200 ml) was added to the cooled solution at a rate to maintain the temperature at 27–28°. Subsequently, the reaction mixture was saturated with methylamine by bubbling the gas into it until a sample of the reaction mixture indicated a pH of 10–11 on an indicator paper moistened with distilled water, and then refluxed for 2 h. Upon cooling, the solid component of the reaction mixture was filtered off and extracted for 2 h with boiling benzene (500 ml); the hot solution was suction-filtered through Celite (Johns-Manville filter aid). A total of 22·2 g (83 per cent yield) of pro-

* All melting points uncorrected. Analyses by Drs. G. Weiler and F. B. Strauss, Oxford, England.

duct crystallized out. The colourless crystals melted at 132·0–133·0°, in accordance with the literature.[14]

N-*Methyl-3,4,5-trimethoxybenzylamine.* To a hot solution of lithium aluminium hydride (32·6 g, 0·858 mole) in tetrahydrofuran* (400 ml), a hot solution of *N*-methyl-3,4,5-trimethoxybenzamide (88·0 g, 0·390 mole) in tetrahydrofuran (1100 ml) was added gradually, with mechanical agitation, in an atmosphere of nitrogen; the temperature of the mixture was maintained at 54–56° with a hot water bath. The reaction mixture was subsequently refluxed at 66·5° for 19 h.† The excess of lithium aluminium hydride and its addition products were decomposed by adding gradually aqueous 20 per cent sodium hydroxide (260 ml) while the temperature of the mixture was maintained at 24–29° during the addition. The semi-solid precipitate was filtered off on a Celite coated Buchner funnel and extracted first with two 300-ml portions of anhydrous ethyl ether, and then with two 300-ml portions of tetrahydrofuran.‡ The filtrate and extracts were dried over anhydrous sodium sulphate, filtered, the solvents were removed, and the combined residues were fractionated under reduced pressure. The compound distilled at 114·0–118·0°/0·08 mm, $n_D^{25·5}$ 1·5305. The compound was prepared twice with a maximum yield of 61 per cent.§ The benzilic acid salt, prepared as described in Procedure C and recrystallized from ethyl acetate, melted at 173·0–173·5°.

Anal. Calcd. for $C_{25}H_{29}NO_6$: C, 68·32; H, 6·65; N, 3·19. Found: C, 68·49; H, 6·65; N, 3·31.

β-(N-Methyl-3,4,5-trimethoxybenzylamino)-N′,N′-diethylpropionamide (IV). *N,N*-Diethyl-*β*-bromopropionamide (12·7 g, 0·061 mole) was added with stirring to *N*-methyl-3,4,5-trimethoxybenzylamine (26·0 g, 0·123 mole) cooled to 15°. The reaction mixture was heated on a silicone bath, maintained at 99–102° for 8 h, and after cooling treated with aqueous saturated potassium carbonate. The bases were extracted with benzene, the combined extracts

* Ether or tetrahydrofuran was used as solvent depending on the solubility of the amide.

† In some instances this reflux period was extended to a total of 70 h.

‡ In some instances tetrahydrofuran alone was used.

§ While the $LiAlH_4$ reduction of the benzyl methylamides proceeded with ease, the phenethyl methylamides yielded the corresponding methylamines in only very low yields by this procedure.

were dried over anhydrous sodium sulphate, filtered, and the benzene was removed under reduced pressure. The residue was fractionated, and the pure product (15·1 g, 73 per cent yield) distilled at 184·0–194·0°/0·095–0·10 mm, $n_D^{22·8}$ 1·5212. Five grams of the base was converted to the hydrochloride by treating it with hydrogen chloride in anhydrous ethyl ether, and the salt was recrystallized from ethyl acetate. The properties of the compound and its analysis are listed in Table I.

The N,N-*diethyl-β-bromopropionamide* was prepared as follows. To β-bromopropionyl chloride (144·0 g, 0·840 mole) dissolved in anhydrous ethyl ether (700 ml) and cooled to 0°, a solution of diethylamine (135·3 g, 1·85 moles) in anhydrous ether (400 ml) was added dropwise, with mechanical stirring, at a rate maintaining the temperature at 3–4°. The addition was completed within 1 h, and the reaction mixture was stirred for an additional 40 min. The precipitated diethylamine hydrochloride was removed by filtration and washed with two 100-ml portions of dry ethyl ether, the ethereal solutions were combined, and the solvent was evaporated on the steam bath. The reddish brown residual liquid (174·8 g) was fractionated under reduced pressure. The colourless liquid (127·8 g, 73 per cent yield), distilling at 84·0–94·0°/0·075–0·10 mm, $n_D^{26·5}$ 1·4812, was identified[15] as *N,N*-diethyl-β-bromopropionamide.

Procedure B

(±)-N-*Formyl-α-methylbenzylamine.* A mixture of (±)-α-methylbenzylamine (24·7 g, 0·204 mole) and formamide (91·9 g, 2·04 moles) was heated at 125–130° for a total of 22 h.* The reaction mixture was fractionated under reduced pressure. The pure product (23·1 g, 76 per cent yield) distilled at 152·0–155·0°/1·5–1·7 mm, n_D^{26} 1·5406.

(±)-α,N-*Dimethylbenzylamine.* To a solution of lithium aluminium hydride (11·8 g, 0·310 mole) in dry ethyl ether† (600 ml), a solution of (±)-*N*-formyl-α-methylbenzylamine (23·1 g, 0·155 mole) in dry ethyl ether (180 ml) was added gradually, with mechanical agitation, in an atmosphere of nitrogen; the rate

* In some instances this reaction period was extended to a total of 90 h.

† Ether or tetrahydrofuran was used as solvent depending on the solubility of the amide.

Table I. Properties of substituted arylalkylaminopropionamide analogues

Compound no.	Procedure	Yield[a] %	Base: b.p., °C at mm Hg	Base: °C n_D	Base: °C $[\alpha]_D$	Salt: Acid component	Salt: m.p., °C	Salt: °C $[\alpha]_D$	Salt: Empirical formula	Analyses: Calcd. C	Calcd. H	Calcd. Cl	Calcd. N	Found C	Found H	Found Cl	Found N
I	A	81	127·0–129·5 (0·08)	25·5 1·5147	—	HCl[d]	165·0–165·5	—	$C_{15}H_{25}ClN_2O$	63·24	8·85	12·45	9·84	63·25	8·72	12·24	9·91
II	A	65	152·0–153·0 (0·07)	26·0 1·5171	—	HCl[d]	131·0–132·0	—	$C_{16}H_{27}ClN_2O_2$	61·04	8·65	11·26	8·90	61·27	8·68	11·26	8·90
III	A	68	174·0–175·0 (0·08)	27·5 1·5210	—	HCl[d]	137·0–138·0	—	$C_{17}H_{29}ClN_2O_3$	59·20	8·48	10·28	8·13	58·91	8·44	10·47	8·35
IV	A	73	184·0–194·0 (0·10)	22·8 1·5212	—	HCl[c]	114·0–115·0	—	$C_{18}H_{31}ClN_2O_4$	57·66	8·34	9·46	7·47	57·60	8·30	9·47	7·67
V	B	75	135·0–140·0 (0·08–0·30)	22·7 1·5150	—	HCl[c]	110·0–111·0	—	$C_{16}H_{27}ClN_2O$	64·30	9·11	11·86	9·38	64·2	9·07	11·96	9·15
VI	A	63	156·0–160·0 (0·08)	22·1 1·5162	—	Benzilic[c]	115·0–115·5	—	$C_{31}H_{40}N_2O_5$	71·51	7·74	—	5·38	71·35	7·78	—	5·40
VII	B	66	174·0–176·0 (0·08)	23·0 1·5182	—	HCl[c]	115·5–116·5[b]	—	$C_{18}H_{31}ClN_2O_3$	60·23	8·71	9·88	7·81	59·96	8·82	9·80	7·69
VIII	C	70	156·0–157·0 (0·08)	23·5 1·5122	—	Benzilic[f]	107·4–107·8	—	$C_{31}H_{40}N_2O_4$	73·78	7·99	—	5·55	73·64	7·94	—	5·57
IX	C	73	138·5–139·5 (0·07)	23·8 1·5114	27·5 +15·11° (c=5, in CH_3OH)	Benzilic[f]	119·2–119·6	23 +19·84° (c=5, in CH_3OH)	$C_{31}H_{40}N_2O_4$	73·78	7·99	—	5·55	73·77	8·06	—	5·65
X	C	77	144·0–148·0 (0·08–0·11)	25 1·5117	27 −14·81° (c=10, in CH_3OH)	Benzilic[f]	118·6–119·2	28 −18·78° (c=5, in CH_3OH)	$C_{31}H_{40}N_2O_4$	73·78	7·99	—	5·55	73·76	8·12	—	5·2
XI	B	62	151·0–153·0 (0·5)	27·5 1·5110	—	HCl[e]	93·5–94·5[b,g]	—	$C_{16}H_{27}ClN_2O$	64·30	9·11	11·86	9·38	64·13	9·20	11·64	9·3

[a] Yield of pure product, based on final condensation. [b] In sealed tube. Crystallized from: [c] ethyl acetate, [d] ethyl acetate–ethanol, [e] ethyl acetate–ether, [f] ethyl acetate–ligroin. [g] Norris and Blicke[17] reported the melting point of the hydrobromide as 69–73°.

of addition was adjusted to produce gentle refluxing. Subsequently, the reaction mixture was refluxed for 12 h (water-bath).* The excess of lithium aluminium hydride and its addition products were decomposed by gradually adding 94 ml of aqueous 20 per cent sodium hydroxide. The ethereal layer was decanted, and the remainder of the reaction mixture was extracted with two 200-ml portions of ethyl ether. The ethereal solutions were combined, filtered through Celite, and dried over anhydrous sodium sulphate. The dried solution was filtered, the ether was removed on the steam bath, and the residue was fractionated under reduced pressure. The pure product (14·6 g, 70 per cent yield) distilled at 84·0–87·0°/25 mm, $n_D^{25\cdot7}$ 1·5091, in accordance with the literature.[16] The base was converted to the hydrochloride by treating it with anhydrous hydrogen chloride in anhydrous ethyl ether. Upon recrystallization from ethanol–ethyl acetate, the salt melted at 173·5–174·5°, in accordance with the literature.[16]

(±)-β-(α,N-*Dimethylbenzylamino*)-N′,N′-*diethylpropionamide* (*V*) was prepared from (±)-α,*N*-dimethylbenzylamine (48·9 g, 0·362 mole) and *N*,*N*-diethyl-β-bromopropionamide (37·6 g, 0·181 mole) according to the method described in the preparation of IV (Procedure A). The pure product (35·6 g, 75 per cent yield) distilled at 135·0–140·0°/0·08–0·30 mm, $n_D^{22\cdot7}$ 1·5150. Five grams of the base was converted to the hydrochloride in the manner described above, and the salt was recrystallized from ethyl acetate. The properties of the compound and its analysis are listed in Table I.

Procedure C

(+)-α,N-*Dimethylphenethylamine.* (+)-α,*N*-Dimethylphenethylamine hydrochloride (95 g, 0·512 mole) was dispersed in ethyl ether and treated with aqueous saturated potassium carbonate. The combined ether extracts of the base were dried over anhydrous sodium sulphate, filtered, and the ether was removed on the steam bath. The base was obtained in a quantitative yield, n_D^{25} 1·5027, $[\alpha]_D^{27\cdot5}$ +5·14° (*c* = 5, in CH_3OH).

(+)-β-(α,N-*Dimethylphenethylamino*)-N′,N′-*diethylpropionamide* (*IX*) was prepared by the condensation of (+)-α,*N*-dimethylphenethylamine (70·1 g, 0·470 mole) and *N*,*N*-diethyl-β-bromo-

* In some instances this reflux period was extended to a total of 60 h.

propionamide (49·0 g, 0·235 mole) according to the method described in the preparation of IV (Procedure A). The benzilic acid salt was prepared in the following manner: to an ice-cooled solution of benzilic acid (2·4 g, 0·010 mole) in anhydrous ethyl ether (55 ml), a solution of the base (2·9 g, 0·010 mole) in anhydrous ethyl ether (20 ml) was added. The resulting white crystalline material was recrystallized twice by dissolving it in hot ethyl acetate, charcoaling the solution, filtering it through Celite, and adding just enough ligroin (b.p. 35–60°) to produce faint cloudiness; the solution was seeded, and crystallization ensued upon refrigeration. The properties of the compound and its analysis are recorded in Table I.

The hitherto unreported intermediates had the following characteristics: N-*Methyl-4-methoxybenzamide*, m.p. 119·5–120·5°. N-*Methyl-4-methoxybenzylamine*, b.p. 65·0–68·0°/0·08 mm, $n_D^{27.5}$ 1·5250; *benzilate salt*, m.p. 189·2–189·8°.

Anal. Calcd. for $C_{25}H_{35}NO_4$: C, 72·80; H, 6·64; N, 3·69. Found: C, 72·71; H, 6·61; N, 3·76.

N-*Methyl-3,4-dimethoxybenzamide*, m.p. 129·0–129·2°. N-*Methyl-3,4-dimethoxybenzylamine*, b.p. 97·0–115·0°/0·1 (–1·4) mm, $n_D^{26.1}$ 1·5330; *benzilate salt*, m.p. 152·5–153·0°.

Anal. Calcd. for $C_{24}H_{27}NO_5$: C, 70·40; H, 6·65; N, 3·42. Found: C, 70·25; H, 6·52; N, 3·32.

N-*Methyl-4-methoxyphenylacetamide*, m.p. 96·5–97·5°.

Anal. Calcd. for $C_{10}H_{13}NO_2$: C, 67·02; H, 7·31; N, 7·82. Found: C, 67·2; H, 7·44; N, 7·77.

N-*Methyl-4-methoxyphenethylamine*, b.p. 79·0–83·0°/0·075–0·20 mm, $n_D^{24.2}$ 1·5222; *benzilate salt*, m.p. 151·0–151·5°.

Anal. Calcd. for $C_{24}H_{27}NO_4$: C, 73·26; H, 6·92; N, 3·56. Found: C, 73·35; H, 7·08; N, 3·54.

N-*Methyl-3,4-dimethoxyphenethylamine*, b.p. 99·0–112·0°/0·075–0·12 mm, $n_D^{27.1}$ 1·5264; *benzilate salt*, m.p. 143·0–143·5°.

Anal. Calcd. for $C_{25}H_{29}NO_5$: C, 70·90; H, 6·90; N, 3·31. Found: C, 70·94; H, 6·84; N, 3·15.

2. *Biochemical Evaluation*

Procedure I. Cutter's cholase* (purified human plasma 'pseudo'-cholinesterase) with $8·22 \times 10^{-3}$ M acetylcholine iodide as substrate

* Cutter Laboratories, Berkeley, Calif.

was chosen for our initial experiments. The work was carried out on a GME–Lardy W–3 Warburg instrument, using 15-ml flasks. The Krebs-Ringer bicarbonate buffer, consisting of $2{\cdot}3\times10^{-2}$ M $NaHCO_3$, $7{\cdot}5\times10^{-2}$ M KCl, $7{\cdot}5\times10^{-2}$ M NaCl and 4×10^{-2} M $MgCl_2.6H_2O$ was prepared according to Cohen;[18a] the final reaction mixture was calculated[18b] to yield a pH of 7·6 with the indicated $NaHCO_3$ concentration, in a gas phase of 5 per cent CO_2 and 95 per cent N_2, at 37° and 740 mm Hg. The displacement of air in the reaction vessels with the above gas mixture was carried out by means of the gas exchange technique under reduced pressure[18c]. The reaction volume totalled 3·2 ml, with 0·60 ml substrate solution in the side arm and all other reaction components in the main compartment. The evaluants were introduced in 0·08 ml of aqueous solution appropriately concentrated to yield the desired dilution of the compounds in the final reaction mixture. Equilibration was initiated at −60 min with readings every 5 min beginning at −25 min. Dumping was effected at 0 min, with the side arm being washed twice with the reaction mixture. Each manometer was read at 10-min intervals during the reaction period of 0 to +60 min.

Procedure II. Some of the experiments required a change in the original arrangement (Procedure I) of the reaction components, and the following modification was introduced: the enzyme solution (0·40 ml) was placed in the side arm of the reaction vessel, and the main compartment contained the substrate and inhibitor solutions along with the buffer balance (combined volume in main compartment 2·80 ml). Otherwise the same procedure was followed.

We expressed the rate as

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

where V signifies μl CO_2/h evolved within the reaction interval of +10 to +30 min, during which the rate was linear in all instances. The percentages of inhibition were calculated as follows:

$$I = \left(\frac{V_c - V_i}{V_c}\right) \times 100,$$

where V_c represents the control rate and V_i the inhibited rate.

Procedure III. In experiments in which the reaction was not preceded by a preincubation period, the reactants were situated in accordance with the original arrangement (Procedure I); however, dumping was effected just prior to their exposure to the water bath. In this instance, the rate V was calculated from the reaction interval of +20 to +40 min, during which the rate was linear.

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 per cent inhibition) was graphically determined. Since several of our compounds have been characterized in the form of benzilic acid salts, we evaluated the effect of the benzilate ion (sodium benzilate*) on the enzyme system; a 3 per cent inhibition was computed at 1×10^{-3} M concentration.

Discussion

The human plasma 'pseudo'-cholinesterase system used in these experiments was characterized kinetically. A linear reciprocal Lineweaver–Burk plot[19] of the enzyme activity, at varying enzyme and substrate concentrations, was graphically evaluated as suggested by Dixon[20] and Reiner[21]; the Briggs–Haldane constants[21,22] (0·0068, 0·0073, 0·0076 and 0·0062) did not vary significantly with enzyme concentration and were within the range of variability for independent determinations in the system investigated.[23]

Since some enzyme preparations are subject to thermal inactivation, we have compared the activity of our systems in simultaneous runs with (Procedure I) and without (Procedure III) a 60-min preincubation period: the loss in activity due to preincubation was computed to be 3·4 per cent.

Next we compared the effect of the inhibitor preincubated (Procedure I) and not preincubated (Procedure II) with the enzyme preparation. The results are summarized in Table II. The parent compound (LSD†), as well as a representative of its

* Prepared from benzilic acid, Eastman Kodak Co., Rochester, N.Y.

† D-Lysergic acid diethylamide, Sandoz Pharmaceuticals, Hanover, N.J. (courtesy Mr. John M. Cole).

more potent derivatives (Compound V), exhibited about the same inhibitory properties in both procedures. In the case of our reference reagent (physostigmine),* however, preincubation increased its inhibitory power more than sevenfold.

Although LSD is a considerably more potent inhibitor than our series of derivatives, the arylalkylaminopropionamide analogues retained sufficient inhibitory activity to illustrate rather interesting relationships between chemical structure and biochemical

Table II. Comparative effect of inhibitor preincubated and not preincubated with human plasma cholinesterase

Inhibitor	Arrangement of reactants during the 60-min preincubation period at 37°			
	Main comp.: Enzyme and inhibitor vol.: 2·60 ml Side arm: Substrate vol.: 0·60 ml (Procedure I)		Main comp.: Substrate and inhibitor vol.: 2·80 ml Side arm: Enzyme vol.: 0·40 ml (Procedure II)	
	V_c	I_{50}	V_c	I_{50}
LSD	288	$3{\cdot}4 \times 10^{-7}$ M	308	$3{\cdot}0 \times 10^{-7}$ M
Compound V	280	$1{\cdot}1 \times 10^{-4}$ M	313	$0{\cdot}9 \times 10^{-4}$ M
Physostigmine	291	$0{\cdot}3 \times 10^{-7}$ M	295	$2{\cdot}2 \times 10^{-7}$ M

response in isolated human plasma 'pseudo'-cholinesterase systems (see Fig. 1).

The influence of aromatic methoxy substitution upon the inhibitory properties of the benzyl as compared to the phenethyl analogues is strikingly different. It is apparent that aromatic methoxy substitution substantially reduces the inhibitory effectiveness of the benzylaminopropionamides, while the potency of the phenethylamino series is practically unaffected. If one should adopt Bergmann, Wilson and Nachmansohn's,[11] Wilson's[12] and possibly Porter, Rydon and Schofield's[13] concept of the site of enzyme–inhibitor interaction, the data could be interpreted as follows: the methoxyl group, which has been found to be electron-releasing when attached to a benzene ring,[24] can relay its

* Physostigmine sulphate, USP, Merck & Co., Inc., Rahway, N.J.

effect through the benzyl radical and influence the charge of the tertiary amine; this effect, however, becomes barely appreciable after relay through the two intervening aliphatic carbons of the phenethyl moiety.[25] Accordingly, in the benzylamino series the electropositivity of the tertiary amine functions is diminished in proportion to the degree of methoxy substitution, and this in turn decreases correspondingly the enzyme's affinity for these derivatives; as anticipated, there is no equivalent effect among the phenethylaminopropionamide analogues.

α-Methyl substitution has a decided potentiating effect upon the inhibitory properties of the benzyl derivative (V), and the trend seems to be retained in the corresponding phenethyl analogue (VIII). The difference in the enzyme's affinity for the two enantiomers (IX and X) of compound VIII is not prominent; one could rationalize, however, that the functions affected by the asymmetric centre are not within the actual region of the inhibitor that is believed to be participating in the enzyme–inhibitor interaction.[11,12]

In accordance with the findings of Thompson's group,[10] the concentration of LSD ($3{\cdot}4 \times 10^{-7}$ M) effecting 50 per cent inhibition in isolated 'pseudo'-cholinesterase systems (human plasma) elicited only insignificant inhibition (2 per cent or less) in corresponding* isolated acetylcholinesterase systems (bovine erythrocyte†); and, remarkably, all our derivatives, in concentrations inhibiting 'pseudo'-cholinesterase activity 50 per cent, effected considerably lower to very low inhibitions in the acetylcholinesterase systems. Since our acetylcholinesterase systems do not meet the kinetic requirements of a linear Lineweaver–Burk plot (*loc. cit.*),‡ we are planning to augment the data obtained at $8{\cdot}22 \times 10^{-3}$ M acetylcholine concentration with determinations of inhibition at several additional substrate concentrations.

We are aware of the fact that the effectiveness of a psychotomimetic agent *in vitro* cannot be interpreted as evidence for the

* The determinations were carried out by means of Procedure I; with the exception of using acetylcholinesterase, the reaction conditions were the same as those in the 'pseudo'-cholinesterase systems, including the reaction rates (V_c at $8{\cdot}22 \times 10^{-3}$ M substrate concentration).

† Bovine erythrocyte cholinesterase, Winthrop Laboratories, New York, N.Y.

‡ Our findings are in agreement with those reported by Augustinsson[26] and others.

involvement of the specific enzyme in the mechanism of psychotomimetic responses. Yet, even without conclusive causal connection, this approach may furnish valuable information on the nature of psychopharmacological agents. Furthermore, the experimental verification of the chemical reasoning which led to the development of a series of compounds designed to explore an active site of an enzyme, endorses the general validity of this approach for the study of enzymodynamic properties.

Summary. The synthesis of substituted arylalkylaminopropionamide analogues of lysergic acid diethylamide and their effect upon isolated cholinesterase systems was reported. Although LSD is a considerably more potent inhibitor than our series of derivatives, the arylalkylaminopropionamide analogues retained sufficient inhibitory activity to illustrate rather interesting relationships between chemical structure and biochemical response in isolated human plasma 'pseudo'-cholinesterase systems; furthermore, the new entities seem to have retained the inhibitory specificities of the parent compound.

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

(*Received 19 March, 1960*)

References

[1] Stoll, W. A. *Schweiz. Arch. Neurol. Psychiat.*, **60**, 279 (1947)
[2] Bircher, R. P. *Angiologia*, **10**, 81 (1959)
[3] Rothlin, E. *Ann. N.Y. Acad. Sci.*, **66**, 668 (1957)
[4] Gogerty, J. H. and Dille, J. M. *J. Pharmacol.*, **120**, 340 (1957)
[5] Goodman, L. S. and Gilman, A. *The Pharmacological Basis of Therapeutics*, Second Edition. 1955. New York, N.Y.; The Macmillan Co.: (a) p. 363; (b) p. 264; (c) p. 174; (d) p. 523; (e) p. 532.
[6] Lasslo, A., Marine, W. M. and Waller, P. D. *J. org. Chem.*, **21**, 958 (1956)
[7] Lasslo, A. and Waller, P. D. *J. org. Chem.*, **22**, 837 (1957)
[8] Jordan, S. E., Lasslo, A., Livingston, H. L., Alperin, H. and Gersing, A. *Arch. int. Pharmacodyn.*, **115**, 452 (1958)
[9] Peretz, D. I., Smythies, J. R. and Gibson, W. C. *J. ment. Sci.*, **101**, 317 (1955)
[10] Thompson, R. H. S., Tickner, A. and Webster, G. R. *Brit. J. Pharmacol.*, **10**, 61 (1955) (*cf.* Fried, G. H. and Antopol, W. *Fed. Proc.*, **16**, 357 (1957))
[11] Bergmann, F., Wilson, I. B., Nachmansohn, D. *J. biol. Chem.*, **186**, 693 (1950) (*cf.* Wilson, I. B. and Bergmann, F. *J. biol. Chem.*, **186**, 683 (1950))
[12] Wilson, I. B. *J. biol. Chem.*, **208**, 123 (1954)

[13] Porter, G. R., Rydon, H. N. and Schofield, J. A. *Nature, Lond.*, **182**, 928 (1958)
[14] Sonn, A. and Meyer, W. *Ber. dtsch. chem. Ges.*, **58**, 1101 (1925)
[15] Gearien, J. E. and Liska, K. J. *J. Amer. chem. Soc.*, **76**, 3554 (1954)
[16] Busch, M. and Leefhelm, M. *J. prakt. Chem.*, [2] **77**, 21 (1907)
[17] Norris, P. E. and Blicke, F. F. *J. Amer. pharm. Ass., Sci. Ed.*, **41**, 637 (1952)
[18] Umbreit, W. W. *et al.* *Manometric Techniques*, Second Edition. 1957. Minneapolis, Minn.; Burgess Publishing Co.; (a) p. 149; (b) p. 25; (c) p. 71.
[19] Lineweaver, H. and Burk, D. *J. Amer. chem. Soc.*, **56**, 658 (1934)
[20] Dixon, M. *Biochem. J.*, **55**, 170 (1953)
[21] Reiner, J. M. *Behaviour of the Enzyme Systems*, p. 28. 1959. Minneapolis, Minn.; Burgess Publishing Co.
[22] Briggs, G. E. and Haldane, J. B. S. *Biochem. J.*, **19**, 339 (1925)
[23] Reiner, J. M. Personal Communication
[24] Fieser, L. F. and Fieser, M. *Organic Chemistry*, Third Edition, pp. 566–567. 1956. New York, N.Y.; Reinhold Publishing Corp.
[25] Ingold, C. K. *Structure and Mechanism in Organic Chemistry*, pp. 231–232. 1953. Ithaca, N.Y.; Cornell University Press
[26] Augustinsson, K-B. *Acta physiol. scand.*, **15** (Supplementum 52), 102 (1948)