was treated with 180 ml. (2.10 moles) of isopropylamine. The solution was refluxed for 132 hr. During this time the temperature of the solution rose from 59 to 75° and there precipitated 59.5 g. (62%) of white plates of isopropylamine hydrochloride, m.p. 155–158°; reported[14] 148–150°. The solution was evaporated to give a tan crystalline precipitate which was dissolved in 120 ml. of concentrated hydrochloric acid and reprecipitated into 2 l. of water containing 120 g. of sodium hydroxide. The tan granular precipitate was separated by filtration, washed with water and dried to give 108 g. (62%) of product, m.p. 107–110°; **picrate,** m.p. 174–176°.

3-Anilino-6-isopropylaminopyridazine.—A solution of 85.5 g. (0.500 mole) of 3-chloro-6-isopropylaminopyridazine in 300 ml. of reagent xylene was treated with 93 g. (1.00 mole) of purified aniline and the solution refluxed for 18 hr. The dark solution so obtained was treated with 100 ml. of concentrated hydrochloric acid to give a dense tan precipitate of amine hydrochlorides. The amine salts were separated from the xylene by filtration and extracted three times with mixtures of 250 ml. of chloroform and 250 ml. of 5% sodium hydroxide. The combined chloroform extracts were evaporated to give a dark semisolid residue of unreacted aniline and 3-chloro-6-isopropylaminopyridazine. The residue from the extraction was dried to give 90 g. (79%) of light tan granular solid, m.p. 177–178°. This was recrystallized from 750 ml. of 95% ethanol to give a first crop of 57.3 g. (50%) of material as shining yellow plates, m.p. 175.0–175.6°.

Evaporation of the mother liquor gave a residue which was recrystallized from 180 ml. of 95% ethanol to give a second crop, weight 17.5 g., m.p. 173.7–174.7°. The total yield was 74.8 g. (66%).

The infrared spectrum showed peaks for two types of N–H bond (3330 and 3190 cm.$^{-1}$), aryl hydrogen (3050 cm.$^{-1}$), alkyl hydrogen (2980 and 2920 cm.$^{-1}$), 3,6-disubstituted pyridazine (855 and 838 cm.$^{-1}$), and monosubstituted benzene (754 and 693 cm.$^{-1}$), as well as a complex of C–N and C–C stretching peaks in the 1700–1300 cm.$^{-1}$ region.

3,6-Bis(2-benzothiazolylthio)pyridazine.—Sodium ethoxide was prepared by dissolving 11.5 g. (0.500 g.-atom) of sodium in 500 ml. of absolute ethanol. To the ethoxide solution was added 83.5 g. (0.500 mole) of recrystallized 2-mercaptobenzothiazole. The resulting clear yellow solution was treated with 37.3 g. (0.250 mole) of 3,6-dichloropyridazine in 250 ml. of absolute ethanol and the mixture refluxed for 36 hr. After cooling the mixture to room temperature, the white precipitate was separated by filtration. Washing with two 200-ml. portions of ethanol and two 200-ml. portions of water followed by drying gave 56.2 g. (55%) of product, m.p. 176–178°.

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Nitrogen Substituted Phenoxazines

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Received June 3, 1963

Only a few 10-substituted phenoxazines were described in the literature[2] prior to the publication of Gilman's[3] facile preparation of phenoxazine in 1957. We wish to report a number of 10-substituted phenoxazines, which, it was hoped, would have interesting biological properties due to their analogy to the phenothiazines and which have not been reported elsewhere.[4]

From 1-bromo-3-chloropropane and phenoxazine in a solution of sodium amide we prepared 10-(3-chloropropyl)phenoxazine[4m] (**1**) which we treated with diallylamine, N-(2-hydroxyethyl)piperazine, and N-(2-aminoethyl)morpholine to form compounds **2**, **3**, and **4**, respectively. Compound **5** was prepared from phenoxazine and N,N-diallyl 2-chloroacetamide. Two more phenoxazine derivatives (**6** and **7**) were prepared from the dimethylamino alkylation of 2-acetylphenoxazine.[4a,c,n]

Phenoxazine was acylated with chloroacetyl chloride and 2-chloropropionyl chloride to give compounds **8** and **9**, respectively. We refluxed various amines with the appropriate chloroacylphenoxazine; the basic products were converted then to the hydrochlorides and the methyl halides (**10–15**). By a similar method, **16** was prepared from 2-acetylphenoxazine.

We prepared phenoxazine-10-carbonyl chloride[4f,o] (**17**) by reacting phenoxazine with phosgene at atmospheric pressure. This was then converted to the ethyl ester **18** and a basic ester **19** which was isolated as the methiodide. In addition, the unsubstituted hydrazide **20** was formed.

Compounds **10**, **12**, and **13** were studied for anticholinergic and spasmolytic activity *in vitro* using acetylcholine ($1–5 \times 10^{-7}$) and histamine ($1–12.5 \times 10^{-6}$) induced spasms in the isolated guinea pig ileum preparation, and $BaCl_2$ ($1–10^{-3}$) induced spasms in the isolated rabbit ileum preparation. Compound **10** in a concentration of $1–5 \times 10^{-5}$ inhibited $BaCl_2$-induced spasms by 19% and in a concentration of $1–2 \times 10^{-7}$ inhibited acetylcholine-induced spasms by 10%. Compound **12** was not active against acetylcholine-induced spasms in a concentration of $1–2 \times 10^{-7}$, but inhibited $BaCl_2$-induced spasms by 30% in a concentration of $1–2.5 \times 10^{-7}$. Compound **13** inhibited $BaCl_2$-induced spasm by 40% in a concentration of $1–2.5 \times 10^{-7}$, acetylcholine-induced spasm by 20% in a concentration of $1–2.5 \times 10^{-8}$, and histamine-induced spasm by 30% at $1–2 \times 10^{-7}$.

The effects of compound **10**, **12**, and **13** on the mean arterial blood pressure of dogs anesthetized with pentobarbital (35 mg./kg. i.v.) was studied by means of a mercury manometer following intravenous administration of 10 mg./kg. Compound **10** produced a 70% decrease in mean arterial pressure, **12** produced a 60% decrease, and **13** produced a 36% decrease. The hypotensive activity of all three compounds was of short duration, returning to control level in less than 15 min.

Experimental

10-(3-Chloropropyl)phenoxazine (1).—To a solution of sodium amide in liquid ammonia, prepared from 3.9 g. (0.17 g.-atom) of

(1) Hazleton Laboratories, Falls Church, Virginia.

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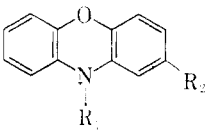
TABLE I

DERIVATIVES OF PHENOXAZINE



No.	R_1	R_2	Formula	M.p., °C.[a]	Nitrogen, Calcd.	Nitrogen, Found
1	$CH_2CH_2CH_2Cl$	H	$C_{15}H_{13}NO\cdot HCl$	50–51	5.39	5.47
2	$CH_2CH_2CH_2N(CH_2CH{=}CH_2)_2\cdot HCl$	H	$C_{21}H_{23}N_2O\cdot HCl$	95–96	7.55	7.77
3	$CH_2CH_2CH_2N$(piperazine)NCH_2CH_2OH[b]	H	$C_{21}H_{27}N_3O_2$	106–107	11.89	11.81
4	$CH_2CH_2CH_2NHCH_2CH_2N$(morpholine)$O\cdot 2HCl$	H	$C_{21}H_{27}N_3O_2\cdot 2HCl$	209–211	9.89	9.60
5	$CH_2CON(CH_2CH{=}CH_2)_2$	H	$C_{23}H_{24}N_2O_2$	144–147	8.74	8.63
6	$CH_2CH_2N(CH_3)_2\cdot HCl$	$COCH_3$	$C_{18}H_{20}N_2O_2\cdot HCl$	232–234	8.42	8.13
7	$CH_2CH_2CH_2N(CH_3)_2\cdot HCl$	$COCH_3$	$C_{19}H_{22}N_2O_2\cdot HCl$	246–247	8.05	7.88
8	$COCH_2Cl$[c]	H	$C_{14}H_{10}ClNO_2$	144–145	5.39	5.36
9	$COCH_2(CH_3)Cl$	H	$C_{15}H_{12}ClNO_2$	131–132	5.12	5.01
10	$COCH_2N$(piperidine)$\cdot HCl$	H	$C_{18}H_{18}N_2O_2\cdot HCl$	182–183	8.47	8.09
11	$COCH_2N$(piperidine)$\cdot CH_3I$	H	$C_{19}H_{21}IN_2O_2$	218–219	6.42	6.03
12	$COCH(CH_3)N(CH_3)_2\cdot HCl$	H	$C_{17}H_{18}N_2O_2\cdot HCl$	216–217	8.79	8.54
13	$COCH(CH_3)N(CH_3)_2\cdot CH_3I$[d]	H	$C_{18}H_{21}IN_2O_2$	221–222	6.60	6.31
14	$COCH(CH_3)N$(pyrrolidine)$\cdot HCl$	H	$C_{19}H_{19}N_2O_2\cdot HCl$	204–205	8.12	7.81
15	$COCH(CH_3)N$(pyrrolidine)$\cdot CH_3Br$[e]	H	$C_{20}H_{23}BrN_2O_2$	228–229	6.95	6.97
16	$COCH(CH_3)N(CH_3)_2\cdot HCl$	$COCH_3$	$C_{19}H_{20}N_2O_3\cdot HCl$	140–141	7.64	7.76
17	$COCl$	H	$C_{13}H_8ClNO_2$	142–143	14.43	14.26
18	$COOC_2H_5$	H	$C_{15}H_{13}NO_3$	74–75	5.49	5.51
19	$COOCH_2CH_2N[CH(CH_3)_2]_2\cdot CH_3I$	H	$C_{22}H_{29}IN_2O_3$	167–169	5.64	5.36
20	$CONHNH_2$	H	$C_{13}H_{11}N_3O_2$	156–157	17.42	17.06

[a] Melting points are corrected and were taken on a Thomas-Hoover capillary melting point apparatus. [b] Prepared according to the method of preparation of compound 2; also prepared by Vanderhaeghe.[3g] [c] Footnote 4h. [d] Prepared from 10-(2-dimethylaminopropionyl)phenoxazine and methyl iodide; same method as used for compound 11. [e] Prepared from 10-(2-pyrrolidinopropionyl)-phenoxazine and methyl iodide.

sodium, 27.5 g. (0.15 mole) of phenoxazine was added with stirring. After 1 hr., 35 g. (0.23 mole) of 1-bromo-3-chloropropane was added over a period of 2 hr. The ammonia was allowed to evaporate and the residue was taken up in ether and filtered to remove precipitated sodium bromide. Evaporation of the ether left a solid which was dissolved in methanol and crystallized to yield 30 g. (77%) of product.

10-(3-Diallylaminopropyl)phenoxazine (2).—To a solution of 10 g. (0.039 mole) of 10-(3-chloropropyl)phenoxazine in 100 ml. of toluene, 15 g. (0.155 mole) of diallylamine and 0.5 g. of copper powder were added. The mixture were kept for 16 hr. at room temperature, then heated for 1 hr. on the steam bath, and followed by refluxing for 48 hr. The toluene and excess diallylamine were distilled off *in vacuo*. The residue was treated with a 10% sodium hydroxide solution, and the liberated bases were taken up in ether. After evaporation of the solvent the residue was distilled. The liquid boiled at 180–182° (1 mm.). The product was converted to the hydrochloride which crystallized from acetone–ether and weighed 5 g. (36%).

10-(Diallylcarbamoylmethyl)phenoxazine (5).—To a solution of sodium amide prepared from 2.53 g. (0.11 mole) of sodium in 150 ml. of liquid ammonia, was added with stirring 18.3 g. (0.1 mole) of phenoxazine. The stirring was continued 30 min. and then 20.6 g. (0.12 mole) of N,N-diallyl-2-chloroacetamide was added. The ammonia was allowed to evaporate and the residue was taken up in ethanol. On addition of water the compound precipitated. It was crystallized from ethanol to yield 18 g. (56.5%).

2-Acetyl-10-(dimethylaminoethyl)phenoxazine Hydrochloride (6). To a solution of sodium amide (see preparation of **5**), 22.5 g. (0.1 mole) of 2-acetylphenoxazine was added slowly with stirring. The stirring was continued 1 hr., and then 16.1 g. (0.15 mole) of 2-chloroethyldimethylamine was added slowly. The ammonia was allowed to evaporate at room temperature (72 hr.). The residue was treated with 10% hydrochloric acid and ether. From the ether solution, 10 g. (45%) of starting material could be isolated. The acid solution was made basic and extracted with ether. After evaporation of the ether, the residual product was converted to the hydrochloride in ethyl acetate. It crystallized from ethyl acetate and weighed 11 g. (34%).

10-(Chloroacetyl)phenoxazine (8).—To a solution of 36.6 g. (0.2 mole) of phenoxazine in 400 ml. of benzene was added 28.3 g. (0.25 mole) of chloroacetyl chloride, and the mixture was refluxed for 20 hr. The solvent and excess acetyl chloride were removed under reduced pressure. The residue, crystallized from ethanol, weighed 39 g. (75%).

10-[1-Pyrrolidyl)acetyl]phenoxazine Hydrochloride (10).—A mixture of 19.5 g. (0.075 mole) of 10-(chloroacetyl)phenoxazine and 14.2 g. (0.2 mole) of pyrrolidine in 200 ml. of ethyl methyl ketone was refluxed for 24 hr. The reaction mixture was concentrated *in vacuo*. The residue was shaken in a mixture of 10% hydrochloric acid and ether. The acid solution was separated and made alkaline with 10% sodium hydroxide. The product was extracted with ether. After evaporation of the ether, the residue was dissolved in ethyl acetate and converted to the hydrochloride which was crystallized from ethyl acetate; yield, 13 g. (52.5%).

10-[(1-Pyrrolidyl)acetyl]phenoxazine Methiodide (11).—The iodide was obtained by refluxing for 6 hr. a solution of 8.8 g. (0.3 mole) of 10-[(1-pyrrolidyl)acetyl]phenoxazine in 50 ml. of ethyl acetate with 7.05 g. (0.05 mole) of methyl iodide. The

ethyl acetate was evaporated and the residue was crystallized from ethyl acetate–ether to give 10 g. (77%) of **11**, m.p. 213–214°.

10-(2-Dimethylaminopropionyl)phenoxazine Hydrochloride (12).—A suspension of 27.3 g. (0.1 mole) of 10-(2-chloropropionyl)phenoxazine and 1 g. of potassium iodide in 600 ml. of ethyl methyl ketone was saturated with 15 g. (0.25 mole) of dimethylamine at 5–10°. The suspension was kept in a pressure bottle 24 hr. at room temperature, then heated to 80° and kept at that temperature for 48 hr. The solution was cooled, filtered, and evaporated under reduced pressure. The residue was shaken with a mixture of 10% hydrochloric acid and ether. The acid solution was separated and made alkaline, and the base was extracted with ether. The ether was evaporated, and the residue was converted to the hydrochloride in acetone–ethyl acetate. The product, crystallized from ethyl acetate, weighed 24 g. (75%).

2-Acetyl-10-(2-dimethylaminopropionyl)phenoxazine Hydrochloride (16).—A mixture of 22.5 g. (0.1 mole) of 2-acetylphenoxazine, 250 ml. of benzene, and 17.8 g. (0.14 mole) of 2-chloropropionyl chloride was refluxed for 24 hr. The solvent and excess 2-chloropropionyl chloride were removed under reduced pressure. The resulting oil was dissolved in 150 ml. of dimethylformamide and 0.75 g. of potassium iodide was added. This solution was saturated with 15 g. of ethylamine at 5–10°. The suspension was kept in a pressure bottle 24 hr. at room temperature and then was heated to 60° and kept at that temperature for 48 hr. After being cooled and filtered, the solution was evaporated under reduced pressure. The residue was shaken with a mixture of 10% hydrochloric acid and ether. The acid solution was separated and made alkaline, and the base was extracted with ether. After evaporation of the ether, the residue was converted to the hydrochloride in acetone–ether. The hydrochloride, crystallized from ethyl acetate–ethanol and then recrystallized from butanol, weighed 9 g. (25%).

Phenoxazine-10-carbonyl Chloride (17). Method A.—To a solution of 36.6 g. (0.2 mole) of phenoxazine in 200 ml. of benzene was added a solution of 24.8 g. (0.25 mole) of phosgene in 100 ml. of toluene. The mixture was kept 3 hr. at room temperature, then heated gradually over a period of 2 hr. to the boiling point, and refluxed for 2 hr. The liquid was evaporated and the residue crystallized from benzene to yield 25.9 g. (52%).

Method B.—A slurry of 36.6 g. (0.2 mole) of phenoxazine, 15.8 g. (0.2 mole) of pyridine, and 80 ml. of toluene was added gradually at 5–10° to a solution of 24.8 g. (0.25 mole) of phosgene in 100 ml. of toluene. The mixture was stirred at room temperature for 72 hr. and then filtered. The precipitate was washed with water and recrystallized from benzene to yield 6 g. of product. The filtrate was washed with water, dried, and distilled under reduced pressure. The residue recrystallized from benzene yielded 20 g. The total yield was 52%.

10-Carbethoxyphenoxazine (18).—A solution of 24.5 g. (0.1 mole) of phenoxazine-10-carbonyl chloride in 300 ml. of ethanol was refluxed for 24 hr. The ethanol was evaporated partially, and the precipitated solid was collected. It weighed 20.5 g. (81%).

(2-Diisopropylaminoethyl)phenoxazine-10-carboxylate Methiodide (19).—To a solution of 9.8 g. (0.04 mole) of phenoxazine-10-carbonyl chloride in 100 ml. of dry benzene, 11.8 g. (0.08 mole) of 2-(diisopropylamine)ethanol was added. The mixture was refluxed for 12 hr., cooled, washed with water, and the benzene layer was separated and dried. After evaporation of the solvent, 40 g. (0.28 mole) of methyl iodide was added and the mixture was refluxed for 2 hr. The excess methyl iodide was evaporated. The residue was crystallized from ethanol to yield 4.5 g. (31%).

Phenoxazine-10-carboxhydrazide (20).[5]—Hydrazine (4.5 g., 0.14 mole, 95%) was added at 0° to a solution of 7.5 g. (0.03 mole) of phenoxazine-10-carbonyl chloride. The mixture was kept at 0° for 20 min., then brought to room temperature and filtered. The residue was washed with water and then crystallized from isopropyl alcohol to yield 5.4 g. (73%) of white plates.

Acknowledgment.—The authors are grateful to Mr. Sidney Alpert for organic analyses and to Dr. J. Morton Beiler for biological experimentation.

(5) Prepared by Mr. Walter W. Bennetts, Jr.

The Effect of Piperidinecarboxamide Derivatives on Isolated Human Plasma Cholinesterase[1]

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Received June 21, 1963

In two preceding papers[2,3] we suggested that the substituted β-aminopropionamide moiety (>N–C–C–CON<), present in (+)-lysergic acid diethylamide (LSD) as well as in β-(arylalkylamino)propionamide[2,3] and piperidinecarboxamide compounds (carbamoylpiperidine compounds),[4–6] 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. Since LSD may be looked upon as a derivative of the partially unsaturated 1-methyl-3-(N,N-diethylcarbamoyl)piperidine (N,N-diethyl-1-methyl-3-piperidinecarboxamide) component of its molecule, and since several other piperidinecarboxylic acid derivatives (cocaine,[7a] meperidine,[7b] etc.) are known to effect psychic disturbances, a study of relationships between the molecular constitution, physicochemical characteristics, and biochemical response of piperidinecarboxamides was undertaken.[4,5,8–10]

The member compounds of each series have been designed with gradual changes in their chemical structure or physical properties or both. Furthermore, they have been planned in such a manner that potential differences in the biochemical response effected by structural variation may permit a detailed study of the nature of the interaction between the member compounds of a given synthetic series and a given enzyme. The data reported for these specific compounds reflect the responses effected by (1) the nature and degree of alkyl substitution on the amido function, (2) the mono- and the corresponding bis(carbamoylpiperidino) substitution on the alkane homologs, (3) the number and arrangement of methylene units in the alkane component attached to the ring-nitrogen(s), and (4) unsaturation in the piperidine ring.

The study involving substituent variation on the amido function of 1-methyl-3-carbamoyl-1,2,5,6-tetrahydropyridine was inspired by the report of Bergmann, *et al.*,[11] suggesting relationships between the electrophilic character of the carbonyl carbon in nicotinic

(1) This investigation is being supported by grants from the National Institute of Mental Health (USPHS MY-2072/MH-04379) and the Geschickter Fund for Medical Research, Inc.

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