Characterization.—For all 13 compounds, two-dimensional paper chromatograms were prepared, and the R_f values and fluorescence of each compound were ascertained (Table I). In addition, for eight of the compounds, melting points (uncorrected) were determined and the compounds subjected to elemental analysis. From the elemental analyses, the most probable empirical formulas have been calculated (Table III).

Identification of coumestrol, tricin, trifoliol, and salicylic acid was accomplished by comparison of their X-ray diffraction patterns and infrared spectra with those of authentic samples. In addition, mixed melting point determinations and two-dimensional paper chromatograms further confirmed their identity.

REFERENCES

(1) Bickoff, E. M., *et al.*, *Science*, 126, 969(1957).
(2) Fox, C. W., and Oldfield, J. E., *Proc. Western Sec., Am. Soc. Animal Prod.*, 13, (No. XLVII) (1962).
(3) Oldfield, J. E., Fox, C. W., and Bickoff, E. M., *J. Animal Sci.*, 19, 1281(1960).
(4) Matsushima, J., *Nebraska Univ. Agr. Exptl. Sta. Bull.*, 29 (April 17, 1959).
(5) Bickoff, E. M., *et al.*, *J. Agr. Food Chem.*, in press.
(6) Craig, L. C., and Post, O., *Anal. Chem.*, 21, 500(1949).
(7) Livingston, *et al.*, *Tetrahedron*, 20, 1963(1964).
(8) Livingston, A. L., *et al.*, *Anal. Chem.*, 32, 1620(1960).
(9) Fergerson, W. S., Ashworth, de B., and Terry, R. A., *Nature*, 163, 606(1949).

Synthesis of Some Hexamine Derivatives as Potential Antispasmodics

By GARY OMODT

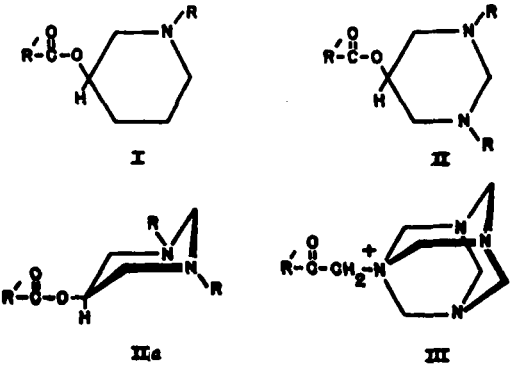
The activity of an antispasmodic of the atropine type appears to be related to the rigidity of the nitrogen-containing moiety. Hexamine contains a very rigid ring system, and it would seem that hexamine would furnish a promising starting point in the synthesis of a good antispasmodic. Some hexamine derivatives were synthesized by reacting the appropriate halomethyl amide or ketone with an excess of hexamine in refluxing chloroform. Preliminary pharmacological evaluation indicates that one of the hexamine derivatives possesses good antispasmodic activity.

THE LITERATURE indicates that a parasympatholytic antispasmodic containing a rigid nitrogen ring system is more active than when the nitrogen is not held in a conformation so rigid (1, 2). Perhaps the reason for this is due to a more or less fixed nitrogen-carbonyl distance *versus* a variable distance in the nonrigid compound. The foregoing hypothesis presumes that the fixed distance is optimum for combination with the potential acetylcholine receptor. Recent research has been directed along the lines of incorporating very rigid bridged nitrogen systems into synthetic antispasmodics (3–8).

Compounds with the general structure of I are on the market as useful antispasmodics (piperidolate, pipenzolate methylbromide, mepenzolate methylbromide) in which the R group is a small alkyl, and the R′ is a large, bulky blocking group. Considering only receptor combination and disregarding effects due to solubility and distribution, compounds relative to structure II might possibly possess activity equal to or better than compounds relative to structure I. This is the case because of the additional nitrogen located in an identical position (with relation to the carbonyl) to the first nitrogen, and thus providing an additional receptor combination site; *i.e.*, the chances of the compound combining with the potential acetylcholine receptor would be increased. This is illustrated better by structure IIa. This is actually structure II from a "45° side view," whereas structure II is a view from the "top."

In reference to compound III, three nitrogens of equal distance from the carbonyl are present, the distance being approximately equal to the nitrogen-carbonyl distance in structure I. In addition, structure III is very symmetrical with respect to the nitrogen ring system. Since all three nitrogens are of equal distance from the carbonyl (this distance being rigidly fixed) and the ring system is quite symmetrical, it would appear that a compound with a structure similar to that of III would be apt to combine with the potential acetylcholine receptor.

The quaternized nitrogen in compounds possessing type III structure may affect the ability to



I II IIa III

Received August 7, 1963, from the Department of Pharmaceutical Chemistry, College of Pharmacy, South Dakota State University, Brookings.
Accepted for publication June 22, 1964.

bind with the receptor but should not adversely affect the distribution in the body since quaternization invariably increases antispasmodic activity in similar compounds; in fact, pipenzolate methylbromide and mepenzolate methylbromide are both quaternized compounds. This paper is concerned with the synthesis and pharmacological evaluation of compounds relating to structure III.

The compounds that were used to react with hexamine were phenacyl bromide, 10-bromoacetyl phenothiazine, 9-bromoacetyl carbazole, *N*-bromoacetyl diphenylamine, and chloromethyl benzohydryl ketone.[1] All of the compounds possess large bulky groups that correspond to R′ in structure III, except phenacyl bromide where R′ would be phenyl. Apparently only three of the above compounds (phenacyl bromide, 10-bromoacetyl phenothiazine, and 9-bromoacetyl carbazole) reacted with hexamine to give the expected products. The other two (*N* bromoacetyl diphenylamine and chloromethyl benzohydryl ketone), when reacted with hexamine in refluxing chloroform, yielded crystalline material that analyzed for what was apparently the mono hydrohalide salts of hexamine. The reaction by which these hydrohalide salts were formed is in question.

The hexamine derivatives were unstable in the presence of active hydrogen containing polar solvents. This might be expected when one considers the Dele'pine (Sommelet) reaction, where a hexamine derivative is decomposed by heating with acidic alcohol or water to give an amine or aldehyde. It is also stated that compounds of this type are virtually impossible to recrystallize (9). Because of this, the compounds were purified by washing with chloroform, pulverization, washing with hot benzene, then drying at 100° *in vacuo*.

Methods of Preparation

The halomethyl amides were synthesized by modifying slightly the methods of Dahlbom and Ekstrand (10, 11).

The halomethyl ketone was synthesized by reacting diphenylacetyl chloride with diazomethane and treating the product with dry hydrogen chloride according to the method for the preparation of benzyl chloromethyl ketone (12). The diphenylacetyl chloride was prepared by heating diphenylacetic acid with thionyl chloride as in the synthesis of *n*-butyryl chloride (13). A general method was used in the preparation of diazomethane (14).

The hexamine derivatives were prepared according to the method of Mackie and Misra (15) with some modification of reaction conditions and amounts of reactants. The general procedure consisted in dissolving hexamine (7.0 Gm., 0.05 mole) in 85 ml. of refluxing chloroform in a three-necked, round-bottom flask equipped with a stirrer, dropping funnel, condenser, and drying tube. The halomethyl compound (0.025 mole) was dissolved in 40 ml. of chloroform and added dropwise over a period of 1.5 hours to the vigorously stirred, refluxing solution. After complete addition of the halomethyl compound, the mixture was stirred and refluxed for 1 hour, then allowed to cool to room temperature. The crystals were removed by suction filtration and washed two times with 50-ml. portions of chloroform. The crystals were then air-dried, pulverized, and placed in 100 ml. of boiling benzene for 30 minutes. The hot benzene was removed by suction filtration and the crystals air-dried. The crystals were further dried for 6 hours *in vacuo* at 100°. Different amounts of reactants were used at times, and these are specified under the separate headings with corresponding per cent yields

[1] This compound was never isolated in a pure form and did not yield the expected hexamine derivative; it is possible that it was never obtained.

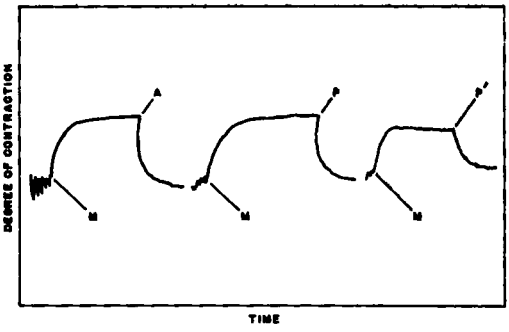


Fig. 1.—Pharmacological evaluation of 10-hexaminoacetyl phenothiazine bromide. Key: M, methacholine chloride solution; A, atropine sulfate solution; P, 10-hexaminoacetyl phenothiazine bromide solution; P′, 10-hexaminoacetyl phenothiazine bromide solution after standing 30 minutes.

Biological Results

A pharmacological evaluation was run on each compound. Hexaminomethyl phenyl ketone bromide showed no activity, and 9-hexaminoacetyl carbazole bromide showed a slight decrease in methacholine chloride-induced spasm. Definite antispasmodic activity was evidenced by 10-hexaminoacetyl phenothiazine bromide; the activity compared favorably to the atropine sulfate control (see Fig. 1). The solutions of the test compounds were very unstable and upon standing at room temperature for a few minutes became cloudy. Even after standing for 30 minutes at room temperature and becoming quite cloudy, 10-hexaminoacetyl phenothiazine bromide exhibited considerable antispasmodic activity (see P′, Fig. 1).

EXPERIMENTAL

All melting points recorded in this paper were taken with the Fisher-Johns melting point apparatus. The melting points are all uncorrected. The halide analyses (Volhard) and the nitrogen analyses (semimicro Kjeldahl) were performed in these laboratories.

10-Bromoacetyl Phenothiazine.—Phenothiazine (10.0 Gm., 0.05 mole) and bromoacetyl bromide (10.1 Gm., 0.05 mole) were refluxed in 100 ml. of anhydrous benzene for 2 hours. The hot solution was filtered and concentrated to 50 ml. Petroleum ether (30–60°, 25 ml.) was carefully layered on top

of the solution, and crystallization was allowed to take place at room temperature. After crystallization had appeared to cease, the mixture was placed in the refrigerator for 2 hours. The crystals were then removed by suction filtration and air-dried. The yield was 12.3 Gm. (81.5%) of light tan crystals, softening up to 119°, m.p. 119–121°.

Anal.—Calcd. for $C_{14}H_{10}BrNOS$: N, 4.38. Found: N, 4.27.

An additional crop of crystals was obtained by concentrating the filtrate to 25 ml. and repeating the crystallization procedure. The yield was 1.9 Gm. (12.6%) of brown crystals softening up to 98°, m.p. 98–100°.

9-Bromoacetyl Carbazole.—Carbazole (8.4 Gm., 0.05 mole) and bromoacetyl bromide (10.1 Gm., 0.05 mole) were refluxed in 100 ml. of anhydrous benzene for 2 hours. Upon admixture of the reactants, a precipitate formed. The precipitate slowly disappeared during reflux; a clear solution resulted after the reflux period. Crystallization was carried out as under *10-Bromoacetyl Phenothiazine*. The first crop of light tan crystals weighed 9.2 Gm. (68.1% yield), softening up to 83°, m.p. 83–85°.

Anal.—Calcd. for $C_{14}H_{10}BrNO$: N, 4.86. Found: N, 4.83.

The second crop of crystals (light tan) weighed 3.7 Gm. (27.4% yield), softening up to 83°, m.p. 83–85°.

N-Bromoacetyl Diphenylamine.—Diphenylamine (8.5 Gm., 0.05 mole) and bromoacetyl bromide (10.1 Gm., 0.05 mole) were refluxed in 100 ml. of anhydrous benzene for 2 hours. Upon admixture of the reactants, a precipitate formed. The precipitate slowly disappeared during reflux; a clear solution resulted after the reflux period. Crystallization of the product was carried out as under *10-Bromoacetyl Phenothiazine*. The first crop of white crystals slowly turned light blue on exposure to the atmosphere and weighed 11.5 Gm. (84.6% yield), softening up to 118°, m.p. 118–120°.

Anal.—Calcd. for $C_{14}H_{12}BrNO$: N, 4.83. Found: N, 4.60.

The second crop of crystals slowly turned dark blue on exposure to the atmosphere and weighed 1.1 Gm. (8.1% yield), softening up to 116°, m.p. 116–118°.

Chloromethyl Benzohydryl Ketone.—Diazomethane was prepared from 21.5 Gm. (0.10 mole) of *p*-tolysulfonylmethyl nitrosamide. The total distillate from this preparation (assumed to contain 2.7 Gm. of diazomethane based on a 64% yield) was transferred to a three-necked, round-bottom, 1000-ml. flask equipped with a magnetic stirrer, a dropping funnel, and a reflux condenser with a drying tube. To the stirred solution was added, over a period of 15 minutes, a solution of 6.9 Gm. (0.03 mole) of diphenylacetyl chloride (b.p. 168–171°/15 mm.). The final ether solution, after washing with two 50-ml. portions of 5% sodium carbonate solution, was dried with anhydrous calcium chloride and the solvent removed on a rotating vacuum evaporator. The residue weighed 5.5 Gm. (75.3% yield). The residue was used without purification.

Hexaminomethyl Phenyl Ketone Bromide.—Phenacyl bromide (5.0 Gm., 0.025 mole) was reacted with hexamine (7.0 Gm., 0.05 mole) by following the general procedure. The yield of light pink crystals was 7.8 Gm. (92.0%), m.p. 163–165° dec.

Anal.—Calcd. for $C_{14}H_{19}BrN_4O$: Br, 23.56; N, 16.52. Found: Br, 23.92; N, 16.66.

10-Hexaminoacetyl Phenothiazine Bromide.[2]—10-Bromoacetyl phenothiazine (7.6 Gm., 0.025 mole) was reacted with hexamine (7.0 Gm., 0.05 mole) by following the general procedure. The yield of white crystals was 8.8 Gm. (79.3%), m.p. 195–200° dec.

Anal.—Calcd. for $C_{20}H_{22}BrN_5OS$: Br, 17.46; N, 15.21. Found: Br, 17.36; N, 15.18.

9-Hexaminoacetyl Carbazole Bromide.—9-Bromoacetyl carbazole (6.8 Gm., 0.025 mole) was reacted with hexamine (7.0 Gm., 0.05 mole) by following the general procedure. The yield of white crystals was 8.6 Gm. (83.5%), m.p. 165–168° dec.

Anal.—Calcd. for $C_{20}H_{22}BrN_5O$: Br, 18.66; N, 16.35. Found: Br, 18.52; N, 16.27.

Pharmacological Evaluation

The pharmacological evaluation was carried out by utilizing a constant-temperature muscle bath and kymograph. Locke's solution (200 ml.) was used to bathe the muscle strip. When the temperature of the Locke's solution had reached 38°, a flow of oxygen was bubbled through the solution; a 5–7-cm. section of freshly excised rabbit ileum was attached to the muscle hooks so that the muscle section was completely submerged in the warm Locke's solution. The kymograph drum was slowly rotated, and 0.5 ml. of a 6.67×10^{-3} *M* solution of methacholine chloride was added to the Locke's solution. After the muscle strip had responded to the methacholine chloride, 0.5 ml. of a 6.67×10^{-3} *M* solution of atropine sulfate[3] was added. The same procedure was employed with each of the test compounds,[4] utilizing a fresh strip of ileum and 200 ml. of fresh Locke's solution each time. All test solutions were prepared immediately before use by dissolving the compounds in cold Locke's solution to inhibit decomposition. The results of the evaluation are shown in Fig. 1.

REFERENCES

(1) Gyermek, L., and N'ador, K., *J. Pharm. Pharmacol.*, **9**, 209(1957).
(2) Cusic, J. W., and Robinson, R. A., *J. Org. Chem.*, **16**, 1921(1951).
(3) Randall, L. O., Benson, W. M., and Stefko, P. L., *J. Pharmacol.*, **104**, 284(1952).
(4) Rhodes, H. J., and Soine, T. O., THIS JOURNAL, **45**, 746(1956).
(5) Omodt, G., and Gisvold, O., *ibid.*, **49**, 153(1960).
(6) Counsell, R. E., and Soine, T. O., *ibid.*, **49**, 289(1960).
(7) Mertes, M. P., and Gisvold, O., *ibid.*, **50**, 475(1961).
(8) Martell, J. M., and Soine, T. O., *ibid.*, **52**, 331(1963).
(9) Angyal, S. J., in "Organic Reactions," Vol. 8, John Wiley and Sons, Inc., New York, N. Y., 1954, p. 206.
(10) Dahlbom, R., and Ekstrand, T., *Acta Chem. Scand.*, **5**, 102(1951); through *Chem. Abstr.*, **45**, 8016(1951).
(11) Ekstrand, T., Swedish pat. 127,566 (March 14, 1950); through *Chem. Abstr.*, **45**, 188(1951).
(12) McPhee, W. D., and Klingsberg, E., *Org. Syn.*, **26**, 13 (1946).
(13) Hickinbottom, W. J., "Reactions of Organic Compounds," Longmans, Green and Co., Inc., New York, N. Y., 1948, p. 226.
(14) DeBoer, Th. J., and Backer, H. J., *Org. Syn.*, **36**, 16 (1956).
(15) Mackie, A., and Misra, A., *J. Chem. Soc.*, **1955**, 1281.

[2] 10-Hexaminoacetyl phenothiazine chloride has previously been prepared by Mackie and Misra for use as an anthelmintic agent (15).

[3] The concentration of the atropine sulfate solution should have been 3.33×10^{-3} to insure comparison on a molecular basis.

[4] When preparing solutions of the test compounds, 10-hexaminoacetyl phenothiazine bromide and 9-hexaminoacetyl carbazole bromide would not dissolve completely. Therefore, 6.67×10^{-3} does not indicate the correct molar concentrations for these compounds.