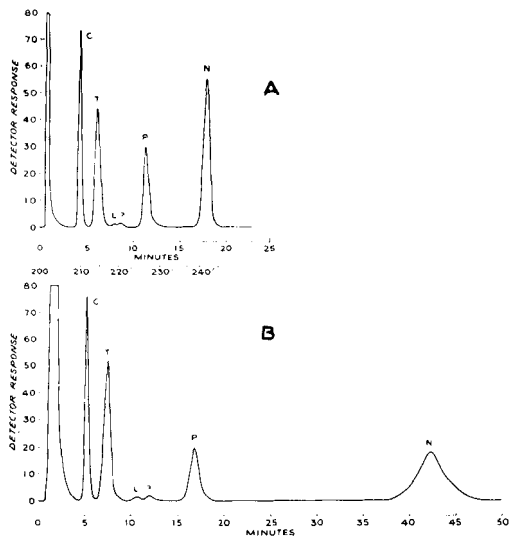

Fig. 5.—Gas chromatograms of the non-phenolic alkaloids of opium on silicone rubber SE-30, 1.15%. A, temperature programing; B, isothermal at 213°. C, codeine; T, thebaine; L, laudanosine; P, papaverine; N, noscapine. Column, 8 feet long and 3 mm. I.D.

operating conditions give two or even three peaks each. One of these appeared to represent the dehydrated alkaloid because the retention value of one of the peaks from atropine was identical with that given by apoatropine prepared according to Hesse (9). The degree of dehydration was found to be associated with the amount of glass wool placed on top of the column packing, and with the temperature of the flash heater. When a fairly large amount of glass wool was used, the degree of decomposition decreased as the flash heater temperature was reduced, but could never be entirely eliminated. When the amount of glass wool was reduced to a very small amount or removed completely, no decomposition took place even at flash heater temperatures of 350°. Similar dehydration of certain steroids has been described as a function of the flash heater temperature by Horning, *et al.* (10).

Hydrolysis and transesterification reactions were also sometimes noticed. Diacetylmorphine was eluted as a sharp peak when chromatographed alone. In mixtures with codeine, morphine or other alcoholic or phenolic substances, reactions taking place in the flash heater gave rise to several new esters not present in the original solution. Both 3, O-monoacetylmorphine (11) and 6, O-monoacetylmorphine[1] gave single peak chromatograms in the absence of glass wool. An apparent catalytic effect of glass wool resulted in peaks corresponding to morphine, monoacetyl- and diacetylmorphine.

REFERENCES

(1) Quin, L. D., *Nature*, **182**, 865(1958).
(2) Quin, L. D., *J. Org. Chem.*, **24**, 911(1959).
(3) Quin, L. D., *ibid.*, **24**, 914(1959).
(4) Lloyd, H. A., Fales, H. M., Highet, P. F., VandenHeuvel, W. J. A., and Wildman, W. C., *J. Am. Chem. Soc.*, **82**, 3791(1960).
(5) Bohemen, J., Langer, S. H., Perrett, R. H., and Purnell, J. H., *J. Chem. Soc.*, **1960**, 2444.
(6) Horning, E. C., Moscatelli, E. A., and Sweeley, C. C., *Chem. Ind. (London)*, **1959**, 751.
(7) Brochmann-Hanssen, E., *Medd. Norsk Farm. Selskap*, **17**, 76(1955).
(8) Langer, S. H., Pantages, P., and Wender, I., *Chem. Ind. (London)*, **1958**, 1664.
(9) Hesse, O., *Ann. Chem.*, **277**, 292(1893).
(10) Ziffer, H., VandenHeuvel, W. J. A., Haahti, E. O. A., and Horning, E. C., *J. Am. Chem. Soc.*, **82**, 6411(1960).
(11) Welsh, L. H., *J. Org. Chem.*, **19**, 1409(1954).

[1] We are indebted to Dr. E. L. Way for supplying us with a sample of this material.

Antiradiation Compounds III

N-2-Mercaptoethylpiperazines

By WILLIAM O. FOYE and DOUGLAS H. KAY

For the purpose of obtaining 2-mercaptoethylamine derivatives having an additional basic function located two carbons distant from the nitrogen, several N-2-mercaptoethylpiperazines have been synthesized. Procedures were employed that involved conversion of the 2-hydroxyethylpiperazines to the corresponding bromo compounds with subsequent thiolation, as well as those employing ethylene sulfide. Both 1-(2′-mercaptoethyl)piperazine and 1,4-bis(2′-mercaptoethyl)piperazine were found to give some protection to mice exposed to 575 roentgens of X-irradiation.

MERCAPTANS have been recognized as effective protective agents against the lethal effects of ionizing radiation, but all of the significantly successful compounds of this variety have so far had a basic function located two or three carbons distant from the sulfhydryl group. The most outstanding examples of the mercaptoamines are 2-mercaptoethylamine (1) and 2-mercaptoethylguanidine (2). Some question remains regarding the basicity of 2-acylthioethylamines, however, which were found to have appreciable antiradiation effects in mice (3), since the acyl groups shift to the nitrogen in neutral or alkaline media (3, 4). Otherwise, a fairly strongly basic group has been required in mercaptans for antiradiation properties, so it appeared desirable to prepare mercaptoamines having an additional basic group located two carbons from the parent amine.

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Previous experience with a series of dithiocarbamates having additional basic groups showed the two carbon distance to be of importance (5). 2-Diethylaminoethyldithiocarbamic acid had radiation-protective ability, while 3-dimethylaminopropyldithiocarbamic acid had none.

For the purpose of providing an additional basic function to the mercaptoamine type of molecule, N-2-mercaptoethylpiperazines have been prepared. In this connection, however, it should be mentioned that N,N-diethylaminoethyl mercaptan is less effective as a radiation-protective agent than 2-mercaptoethylamine (2, 6).

DISCUSSION

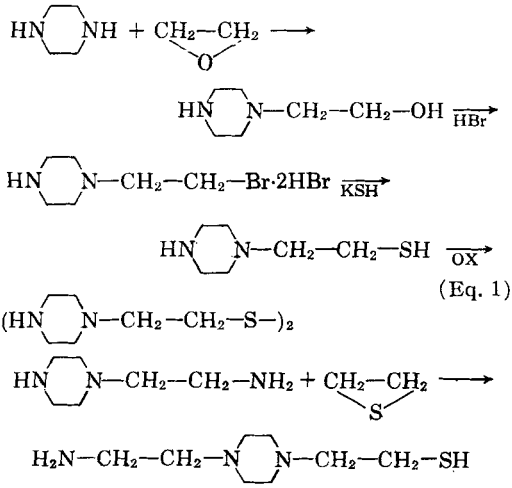
1-(2′-Mercaptoethyl)piperazine was prepared in a conventional manner from the corresponding alcohol. This compound was obtained from the reaction of anhydrous piperazine and ethylene oxide according to McElvain and Bannister (7). This procedure also gave small quantities of the 1,4-bis-alcohol. The latter compound was prepared in quantity, however, by the procedure of Pollard, *et al.* (8). The 1-(2′-hydroxyethyl)piperazine obtained was converted to the corresponding bromo compound by means of 48% hydrobromic acid, a much longer period of refluxing being required than that recommended by McElvain (7), however. Treatment of the 1-(2′-bromoethyl)piperazine dihydrobromide under pressure with a solution of potassium hydroxide saturated with hydrogen sulfide readily yielded the desired mercaptan. This compound was purified under nitrogen and isolated as the dihydrobromide. This reaction sequence is shown in Eq. 1.

The 1,4-bis(2′-hydroxyethyl)piperazine was converted to the bis-mercaptan by the same reaction scheme. The corresponding dibromo compound had been previously reported by McElvain (7), but he listed a bromide analysis that varied from theoretical by several per cent and a melting range of 240–360°. Our product was found analytically acceptable after extraction with hot alcohol. The dibromo compound was converted to the desired bis-mercaptan by treatment under pressure with potassium hydroxide saturated with hydrogen sulfide. It was again necessary to convert the product to the dihydrobromide before isolation was successful.[1]

Ethylene sulfide was employed in an attempt to prepare mercaptoethylpiperazines more conveniently and in better yield. Reaction with anhydrous piperazine in 95% ethanol, however, led only to ethylene sulfide polymer. Reaction in chloroform gave an impure oil that reacted with carbon disulfide to give a solid melting at 55–60°. A satisfactory carbon-hydrogen analysis for the suspected 1-(2′-mercaptoethyl)piperazine-4-carbodithioic acid was not obtained.

Treatment of 1-ethylpiperazine with ethylene sulfide yielded a characterizable product, however. Analysis indicated that the monomercaptoethyl derivative was formed. A lower melting product, from which practically identical analytical values were obtained, was also isolated; this was assumed to be the corresponding disulfide. The low yields obtained may be explained by formation of ethylene sulfide polymer as generally found by Reynolds, *et al.* (10), from reactions with ethylene sulfide.

Reaction of 1-(2′-aminoethyl)piperazine with ethylene sulfide in chloroform also produced a mercaptoethyl derivative for which the analytical results were satisfactory. Infrared absorption showed the presence of a primary amine at 1620 cm.$^{-1}$ so the mercaptoethylation apparently took place on the piperazine nitrogen to give 1-(2′-aminoethyl) - 4 - (2′ - mercaptoethyl)piperazine (see Eq. 2). This was isolated as the trihydrochloride.



(Eq. 1)

(Eq. 2)

Some question remained regarding the structure of the 1-(2′-mercaptoethyl)piperazine. Since this reduced iodine rather slowly, it was suspected that the compound might possibly be the disulfide. However, oxidation of the dihydrochloride of this compound with hydrogen peroxide gave a product of different melting point and with approximately the same analytical values. The latter compound did not reduce iodine and accordingly should be the disulfide.

Biological Activities.—Tests for the ability of three of these compounds to protect mice against X-irradiation have been carried out at the Walter Reed Army Institute of Research under the direction of Dr. D. P. Jacobus. 1,4-Bis(2′-mercaptoethyl) piperazine afforded significant protection to mice when administered 30 minutes prior to exposure to 575 Roentgens. An increase of 65% in 30-day survival over that of the control animals was observed. 1-(2′-Mercaptoethyl)piperazine, however, provided only a 30% increase in 30-day survival. This much lower protective ability may be related to the relative slowness of the compound to reduce iodine and undergo mixed disulfide formation with glutathione (11) in accordance with the mixed disulfide hypothesis of radioprotective action (12). 1 - (2′ - Aminoethyl) - 4 - (2′ - mercaptoethyl)-piperazine, however, provided no protection at the same drug level. None of these compounds provided any protection for mice given a dosage of 800 *r*. A summary of the testing data is given in Table I.

[1] The free base, 1,4-bis-(2′-mercaptoethyl)piperazine, was recently reported by Reynolds, *et al.* (9), from the reaction between piperazine and ethyl 2-mercaptoethylcarbonate.

TABLE I.—ANTIRADIATION PROPERTIES OF N-(2-MERCAPTOETHYL)PIPERAZINES

Compound[a]	Drug Level, mg./Kg.	Adm. Time	Dose, r	30-Day Survival, % Treated	30-Day Survival, % Controls
1-(2′-Mercaptoethyl)piperazine	50	15 min. prior	575	60	30
1,4-Bis-(2′-mercaptoethyl)piperazine	60	30 min. prior	575	95	30

[a] Aqueous solutions of the dihydrobromides were brought to a pH of 7.2 before injection in mice.

EXPERIMENTAL

1-(2′-Hydroxyethyl)piperazine.—Using the procedure of McElvain and Bannister (7), we obtained a 52% yield of product, b.p. 110°(2 mm.). An 8% yield of 1,4-bis(2′-hydroxyethyl)piperazine was also recovered; m.p. 135–136°, which agrees with the reported value (7).

1,4-Bis-(2′-hydroxyethyl)piperazine.—The procedure of Pollard, *et al.* (8), was used, giving an 89% yield of product which melted at 135–136°.

1-(2′-Bromoethyl)piperazine Dihydrobromide.—Twenty grams (0.154 mole) of 1-(2′-hydroxyethyl)-piperazine was cooled in an ice bath and 75 ml. of 48% hydrobromic acid was added slowly, with shaking. The resulting solution was distilled slowly for 30 minutes and the reaction mixture was then refluxed for 8 days.

After a 24-hour reflux period, the presence of 1-(2′-hydroxyethyl)piperazine dihydrobromide was detected by removing a portion of the solution and rubbing it in acetone. A white crystalline solid was obtained which melted at 208–210°. A Volhard bromide determination gave the following result.

Anal.—Calcd. for $C_6H_{16}Br_2N_2O$: Br, 54.79. Found: Br, 54.36.

After 8 days of refluxing, the reaction mixture was evaporated to dryness *in vacuo*. The residue was triturated in acetone to yield approximately 38 Gm. (70%) of product which decomposed at 242–243°, the reported value (13), after recrystallization from 95% ethanol.

1-(2′-Mercaptoethyl)piperazine Dihydrobromide.—A solution containing 2.8 Gm. of 85% potassium hydroxide, 1.5 ml. of distilled water, and 20 ml. of absolute ethanol was saturated with hydrogen sulfide. It was added to 5.0 Gm. (0.014 mole) of 1-(2′-bromoethyl)piperazine dihydrobromide in a 250-ml. pressure bottle which was capped immediately and heated in a water bath for 3 hours with occasional shaking.

After being cooled, the flask contents were evaporated to dryness *in vacuo*. The residue was extracted with 100 ml. of hot absolute ethanol, filtered to remove potassium bromide, and the filtrate was concentrated to about 70 ml. About 35 ml. of ethyl acetate was added to the cooled solution and dry hydrogen bromide was introduced. The solution was stirred and the walls of the flask scratched, and a white, crystalline solid was obtained. After extraction with 95% ethanol, 2.5 Gm. of 1-(2′-hydroxyethyl)piperazine dihydrobromide, m.p. 208–210°, remained. Chilling the ethanolic extract gave 1.3 Gm. (31.6%) of 1-(2′-mercaptoethyl)-piperazine dihydrobromide which decomposed at 220°, with previous softening at 175°.

Anal.—Calcd. for $C_6H_{16}Br_2N_2S$: C, 23.35; H, 5.23. Found: C, 23.50; H, 5.20.

1,4-Bis-(2′-bromoethyl)piperazine Dihydrobromide.—Twenty-five grams (0.144 mole) of 1,4-bis(2′-hydroxyethyl)piperazine was cooled in an ice bath and 100 ml. of 48% hydrobromic acid was added slowly, with shaking. The resulting solution was distilled slowly for 1 hour and was then refluxed for 3 days.

After 24 hours, the reaction mixture was concentrated at 70° (20–30 mm.) and refluxing was resumed. The presence of 1,4-bis(2′-hydroxyethyl)-piperazine dihydrobromide was demonstrated at this time by removing a portion of the solution and rubbing it in acetone. A white, crystalline solid was obtained which melted at 170–185°. A Volhard bromide determination gave the following result.

Anal.—Calcd. for $C_8H_{20}Br_2N_2O_2$: Br, 47.61. Found: Br, 47.20.

After 3 days, the reaction mixture was evaporated *in vacuo* to a syrup. This was triturated in acetone to yield a gummy material; more was obtained by adding ethyl acetate to the acetone extract. The gummy product was extracted with 400 ml. of hot 95% ethanol, and 41 Gm. (62%) of white, crystalline product was obtained which was insoluble in the hot alcohol. The solid decomposed at 230°, which agrees with the reported value (7) for 1,4-bis(2′-bromoethyl)piperazine dihydrobromide.

Anal.—Calcd. for $C_8H_{18}Br_4N_2$: C, 20.81; H, 3.92. Found: C, 21.07; H, 4.05.

1,4-Bis-(2′-mercaptoethyl)piperazine Dihydrobromide.—A solution containing 2.3 Gm. of 85% potassium hydroxide, 1.5 ml. of distilled water, and 15 ml. of absolute ethanol was saturated with hydrogen sulfide. It was added to 4.0 Gm. (0.008 mole) of 1,4-bis-(2′-bromoethyl)piperazine dihydrobromide in a 250-ml. pressure bottle which was capped immediately and heated in a water bath for 3 hours with occasional shaking.

After being cooled, the flask contents were evaporated almost to dryness *in vacuo*. The residue was extracted with 175 ml. of hot absolute ethanol, filtered, and the filtrate was concentrated to about 125 ml. About 30 ml. of ethyl acetate was added and anhydrous hydrogen bromide was introduced into the chilled solution. The solution was chilled and the vessel scratched, and a white, crystalline solid was obtained. The solid was washed with cold ethyl acetate and extracted five times with 200-ml. portions of hot 95% ethanol About 5.0 Gm. (52%) of 1,4-bis-(2′-mercaptoethyl)piperazine dihydrobromide was obtained which was insoluble in the hot alcohol. The compound decomposed at 260°.

Anal.—Calcd. for $C_8H_{20}Br_2N_2S_2$: C, 26.09; H, 5.47. Found: C, 26.24; H, 5.37.

1-Ethyl-4-(2′-mercaptoethyl)piperazine Dihydrochloride.—Ten grams (0.087 mole) of 1-ethylpiperazine (14) was cooled in an ice bath and treated with 5.26 Gm. (0.087 mole) of ethylene sulfide (15) slowly, with shaking, during 20 minutes. The ice bath was removed, and the mixture was allowed to stand overnight at room temperature under nitrogen. It was then refluxed gently for 2 hours and again allowed to stand overnight under

nitrogen. The mixture was evaporated at 60° (20–30 mm.) under nitrogen, and a semisolid was obtained after cooling. This was taken up in 30 ml. of absolute ethanol and cooled, and the solution was saturated with anhydrous hydrogen chloride. A white solid precipitated and after extraction with absolute ethanol, 0.5 Gm. remained which melted at 240–250°, with decomposition beginning at about 210°. The compound contained 11.52% sulfur. From the chilled extract was obtained 0.65 Gm. of solid which melted at 205–207°. This contained 11.55% sulfur. Neither product formed an addition compound with carbon disulfide, and both reduced iodine T.S.

Anal.—Calcd. for $C_8H_{20}Cl_2N_2S$: C, 38.86; H, 8.09; S, 12.95. Found: C, 38.57; H, 8.34; S, 11.52.

1-(2′-Aminoethyl)-4-(2′-mercaptoethyl)-piperazine Trihydrochloride.—A solution of 4.6 Gm. (0.036 mole) of 1-(2′-aminoethyl)piperazine in 30 ml. of chloroform was cooled in an ice bath and treated with ethylene sulfide (4.3 Gm., 0.072 mole) slowly, with shaking, during 30 minutes. The mixture was allowed to stand for 3 days at room temperature under nitrogen, and was then distilled under nitrogen at 40° (20–30 mm.). The residual oil was extracted with two 50-ml. portions of ether, and 125 ml. of absolute ethanol was added to the ether-insoluble material. The alcohol solution was chilled and saturated with anhydrous hydrogen chloride; a solid was obtained which was extracted with 100 ml. of hot acetone in two portions and quickly transferred to a desiccator. About 2.5 Gm. (24%) of ivory-colored product was obtained which melted at 165–170°.

Anal.—Calcd. for $C_8H_{22}Cl_3N_3S$: C, 32.15; H, 7.42; S, 10.71. Found: C, 32.52; H, 7.72; S, 10.94.

1-(2′-Mercaptoethyl)piperazine Dihydrochloride.—The same reaction sequence was used as in the preparation of the corresponding dihydrobromide. A very small yield of hygroscopic product was obtained which melted in the range of 145–150°.

Anal.—Calcd. for $C_6H_{16}Cl_2N_2S_2 \cdot H_2O$: C, 30.37; H, 7.65; N, 11.81. Found: C, 30.15; H, 7.51; N, 10.49.

1-Piperazinoethyl Disulfide Tetrahydrochloride.—The previous product, 1.0 Gm., was dissolved in 5 ml. of 50% aqueous ethanol containing 5 ml. of 3% hydrogen peroxide solution. The solution was heated on a water bath for 30 minutes and allowed to stand overnight. The solution was evaporated *in vacuo* to a gum, treated with absolute ethanol, and again concentrated *in vacuo*. This process was repeated. The semicrystalline material was removed from the flask and triturated in absolute ethanol; an extremely hygroscopic product was isolated which did not decolorize iodine T.S. without remaining in solution for some time. The product, 0.7 Gm., melted at 95–100°.

Anal.—Calcd. for $C_{12}H_{30}Cl_4N_4S_2 \cdot 2H_2O$: C, 30.51; H, 7.25; N, 11.86. Found: C, 29.90; H, 7.19; N, 10.20.

REFERENCES

(1) Bacq, Z. M., Hervé, A., Fischer, P., Lecomte, J., Blavier, J., Deschamps, G., LeBihan, H., and Rayet, P., *Arch. Intern. Physiol.*, **59**, 442(1951).
(2) Doherty, D. G., Burnett, W. L., Jr., and Shapira, R., *Radiation Res.*, **7**, 13(1957).
(3) Foye, W. O., Duvall, R. N., and Mickles, J., THIS JOURNAL, **51**, 168(1962).
(4) Wieland, T., and Bokelmann, E., *Ann.*, **576**, 20(1952).
(5) Foye, W. O., and Mickles, J., *J. Med. Pharm. Chem.*, **5**, 846(1962).
(6) Langendorff, H., and Koch, R., *Strahlentherapie*, **99**, 567(1956).
(7) McElvain, S. M., and Bannister, L. W., *J. Am. Chem. Soc.*, **76**, 1126(1954).
(8) Adelson, D. E., MacDowell, L. G., and Pollard, C. B., *ibid.*, **57**, 1988(1935).
(9) Reynolds, D. D., Fields, D. L., and Johnson, D. L., *J. Org. Chem.*, **26**, 5125(1961).
(10) Reynolds, D. D., Fields, D. L., and Johnson, D. L., *ibid.*, **26**, 5111(1961).
(11) Jacobus, D. P., personal communication.
(12) Pihl, A., and Eldjarn, L., *Pharmacol. Rev.*, **10**, 437 (1958).
(13) Hromatka, O., and Engel, E., *Ber.*, **76**, 712(1943).
(14) Moore, L. S., Boyle, M., and Thorn, V. M., *J. Chem. Soc.*, **1929**, 39.
(15) Reynolds, D. D., *J. Am. Chem. Soc.*, **79**, 4951(1957).

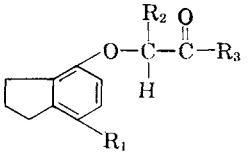
ERRATUM

In the paper titled "The Pharmacologic Effects of a New Analgesic α-4-dimethylamino-1,2-diphenyl-3-methyl-4-propionyloxybutane" (1), the analgesic should have been identified as α-4-dimethylamino-1,2-diphenyl-3-methyl-2-propionyloxybutane.

(1) Robbins, E. B., THIS JOURNAL, **44**, 497(1955).

ERRATUM

In the paper titled "Indanols V. Indanoxyacetic Acid Derivatives" (1), Table I should have included the structure heading



between compounds 41 and 42 to indicate that the second part of the Table referred to 7-substituted-4-indanols.

(1) Shapiro, S. L., Bazga, T., and Freedman, L., THIS JOURNAL, **51**, 582(1962).