and mixed melting point were identical with the high melting material isolated from the balfourolone fraction of plant extract.

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Nitrogen Mustard Derivatives of Phenothiazine and Phenoxazine

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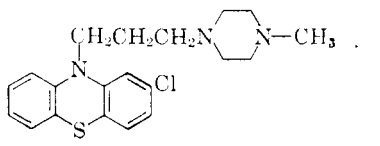
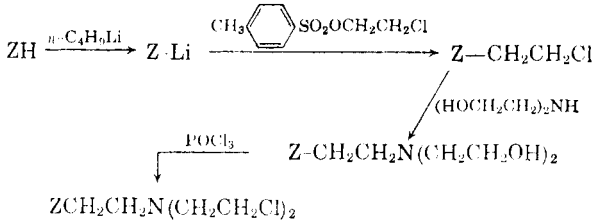
The intense search for effective anticancer chemotherapeutic agents has involved much attention to synthesis, animal and clinical evaluation, and even a significant level of routine medical application of a family of compounds known as "nitrogen mustards."[1] Most of the compounds contain the 2-chloroethylamino or the bis(2-chloroethyl)amino-groupings.

The heterocycle phenothiazine is the parent type of a significant list of medically useful derivatives, most of which involve effects on the central nervous system. A recent report[2] has indicated anticancer activity in prochlorperazine ("Compazine"), a phenothiazine type (I).



I

We wish to report the synthesis for anticancer evaluation of 10-{2-[bis(2-chloroethyl)amino]-ethyl}phenothiazine and the corresponding derivative of phenoxazine. The synthetic route chosen is indicated below. The symbol Z represents the 10-phenothiazinyl or 10-phenoxazinyl group.



The 2-chloroethyl and bis(2-chloroethyl)amino-ethyl derivatives of phenothiazine and phenoxazine have been submitted to the National Cancer Institute for anticancer tests. Significant results of the tests will be reported elsewhere.

EXPERIMENTAL[3]

10-(2-Chloroethyl)phenoxazine. A solution (45 ml.) of 0.063 mole of *n*-butyllithium in mixed pentane-heptane solvent (Foote Mineral Co. product) was added gradually (nitrogen atmosphere) to a solution of 11.5 g. (0.063 mole) of phenoxazine[4] in 75 ml. of dry benzene. The mixture was stirred for 30 min. during which time a yellow-red precipitate of *N*-lithiophenoxazine appeared. The resulting slurry was added to a solution of 15 g. (0.063 mole) of 2-chloroethyl *p*-toluenesulfonate in 90 ml. of benzene. The reaction mixture was stirred at reflux temperature for 16 hr., and then treated with excess water and washed several times with water. Evaporation of the benzene solution gave a brown oily residue. The oil was dissolved in ligroin (b.p. 100–115°) and the solution placed on an alumina (Alcoa F-20) chromatographic column. Elution with 1:1 mixture of benzene and the ligroin gave in the first fraction 9.3 g. (60%) of colorless solid product, m.p. 62°, after one crystallization from methanol.

Anal. Calcd. for $C_{14}H_{12}ClNO$: C, 68.43; H, 4.89; N, 5.70. Found: C, 68.49, 68.21; H, 4.90, 5.00; N, 5.91.

10-{2-[Bis(2-hydroxyethyl)amino]ethyl}phenoxazine. A solution of 9.8 g. (0.40 mole) of 10-(2-chloroethyl)phenoxazine in 170 ml. of diethanolamine was heated at 130–140° for 18 hr. After cooling the reaction mixture, 200 ml. of water was added and the precipitated oil extracted with benzene (two times) and chloroform (three times). The combined solutions were evaporated and the residual oil was triturated with petroleum ether (b.p. 65–75°) to yield 12 g. (95%) of white solid. After crystallization from benzene–petroleum ether, the product melted at 84°.

Anal. Calcd for $C_{18}H_{22}N_2O_3$: C, 68.79; H, 7.00; N, 8.92. Found: C, 68.51, 68.58; H, 7.12, 7.20; N, 8.72, 8.99.

10-{2-[Bis(2-chloroethyl)amino]ethyl}phenoxazine A solution of 5.0 g. (0.016 mole) of the above hydroxyethyl derivative in 15 ml. of phosphorus oxychloride[5] was heated on a steam bath for 1 hr. The excess phosphorus oxychloride was removed by distillation under reduced pressure and the resulting solid dissolved in chloroform. The chloroform solution was washed with cold water and the chloroform evaporated. The residue was suspended in benzene and the suspension was stirred with aqueous sodium carbonate solution. The resulting benzene solution was separated and the aqueous layer extracted two times with benzene. The combined benzene solutions were evaporated, and the residual yellow oil was chromatographed over 60–100 mesh "Florisil" adsorbent using benzene as the eluting solvent. The first fraction coming from the column was a yellow oil.

Anal. Calcd. for $C_{18}H_{20}Cl_2N_2O$: C, 61.54; H, 5.70; N, 7.98. Found: C, 61.71, 61.21; H, 5.91, 5.88; N, 7.84, 7.92.

Conversion of the above oil to the *hydrochloride* gave, after crystallization from ethanol, a 52% over-all yield of white crystalline product, m.p. 148°.

Anal. Calcd. for $C_{18}H_{21}Cl_3N_2O$: C, 55.74; H, 5.42; N, 7.23. Found: C, 55.95, 55.93; H, 5.66, 5.22; N, 7.30, 7.18.

10-{2-[Bis(2-hydroxyethyl)amino]ethyl}phenothiazine. 10-(2-Chloroethyl)phenothiazine[6] (6.0 g., 0.023 mole) was allowed to react with diethanolamine with reaction conditions and work-up essentially as described in the correspond-

(1) R. B. Ross and P. E. Swartzentruber, *Literature Survey of Nitrogen Mustards*, Cancer Chemotherapy National Service Center, Bethesda, Md., 1960.

(2) B. B. O'Malley, R. Willheim, and P. Fluss, Abstracts of Papers, 138th Meeting of the American Chemical Society, September 1960, p. 66C.

(3) Elemental microanalyses by Weiler and Strauss, Oxford, England.

(4) H. Gilman and L. O. Moore, *J. Am. Chem. Soc.*, 79, 3485 (1957).

(5) T. L. Fletcher and W. H. Wetzel, *J. Org. Chem.*, 25, 1354 (1960), use this reagent for a similar job and in our hands it was much superior to thionyl chloride or phosphorus pentachloride.

(6) H. Gilman and D. A. Shirley, *J. Am. Chem. Soc.*, 66, 888 (1944).

ing reaction of the phenoxazine type described above. The oily product was chromatographed over 60–100 mesh "Florisil." Elution with benzene brought off unchanged 10-(2-chloroethyl)phenothiazine and 95% benzene–5% acetone eluted the product, an oil weighing 5.95 g. (79%).

Anal. Calcd. for $C_{18}H_{22}N_2O_2S$: C, 65.42; H, 6.72; N, 8.47. Found: C, 65.52; H, 6.84; N, 8.42.

The *hydrochloride* melted at 143–144°.

Anal. Calcd. for $C_{18}H_{23}ClN_2O_2S$: C, 58.93; H, 6.05; N; 7.63. Found: C, 59.01, 58.85; H, 6.02, 5.87; N, 7.94, 7.72.

10-{2-[Bis(2-chloroethyl)amino]ethyl}phenothiazine. Prepared essentially in accordance with the procedure described above for the phenoxazine type, the product was a white crystalline solid (from petroleum ether, b.p. 65–75°), m.p. 54.5–55.5°. The yield was 55%.

Anal. Calcd. for $C_{18}H_{20}Cl_2N_2S$: C, 58.85; H, 5.50; N, 7.62. Found: C, 58.99, 58.74; H, 5.44, 5.38; N, 7.53, 7.58.

The *hydrochloride* melted with decomposition at 126–131° at atmospheric pressure but in an evacuated capillary tube at 132–133°.

Anal. Calcd. for $C_{18}H_{21}Cl_3N_2S$: C, 53.54; H, 5.24; N, 6.93. Found: C, 53.30, 53.30; H, 4.81, 4.99; N, 6.54, 6.59.

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The Preparation of 1,2-(α-Ketotetramethylene)ferrocene

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In connection with some other work we had need of a considerable quantity of 1,2-(α-ketotetramethylene)ferrocene. This compound has been previously reported by Rinehart,[2] who prepared it from ferrocene and succinic anhydride[3] by a modification of the normal α-tetralone synthesis. Although the initial Friedel-Crafts reaction of ferrocene and succinic anhydride proceeded smoothly in the manner reported, the hydrogenation of β-ferrocenoylpropionic acid to γ-ferrocenylbutyric acid failed repeatedly in our hands.[4] In an effort to circumvent the hydrogenation an attempt was made to reduce the keto acid by either the Clemmensen or Wolff-Kishner method. Both these reactions resulted in extensive decomposition of the material, and failed to yield any identifiable substances.

In order to resolve the difficulties cited, β-ferrocenoylpropionic acid was reduced with sodium borohydride to γ-ferrocenylbutyrolactone, which with no unusual purification was smoothly hydrogenated to γ-ferrocenylbutyric acid.

The overall yield for these two steps, based on crude β-ferrocenoylpropionic acid is 71%, which compares favorably with the 93% reported by Rinehart[3] for the direct reduction if one takes into account the loss of β-ferrocenoylpropionic acid in purification.

The cyclization of γ-ferrocenylbutyric acid to 1,2-(α-ketotetramethylene)ferrocene was carried out with polyphosphoric acid as mentioned by Rinehart.[2]

EXPERIMENTAL[5]

β-Ferrocenoylpropionic acid. This material was prepared from succinic anhydride and ferrocene, using essentially the procedure of Reinhart *et al.*[3]

From 20.0 g. of ferrocene and 7.08 g. of succinic anhydride, in the presence of 15.6 g. of aluminum chloride, 18.5 g. (91%) of crude product, m.p. 167–169° was obtained. The melting point was not increased significantly by recrystallization. Concentration of the nonacidic fraction of the reaction mixture to dryness afforded 8.70 g. of recovered ferrocene.

γ-Ferrocenylbutyrolactone. To a solution of 13.6 g. of β-ferrocenoylpropionic acid in 635 ml. of isopropyl alcohol was added 32.0 g. of sodium borohydride. The mixture was heated under reflux 2 hr., cooled, diluted with water, filtered through Celite, and cautiously acidified with dilute hydrochloric acid. The orange solution was extracted with chloroform, the extracts washed with water, 5% sodium carbonate, and dried over magnesium sulfate. Removal of the chloroform *in vacuo* gave 12.7 g. of crude lactone. Recrystallization from cyclohexane–ethyl acetate gave 9.3 g. (77%) of yellow crystals m.p. 130–132°. The analytical sample was crystallized from cyclohexane, m.p. 132–133°.

Anal. Calcd. for $C_{14}H_{14}FeO_2$: C, 62.26; H, 5.22. Found: C, 62.59; H, 5.47.

The infrared spectrum of this compound (Nujol) showed a single carbonyl band at 5.66 μ.

γ-Ferrocenylbutyric acid. A solution of 10.6 g. of γ-ferrocenylbutyrolactone in 218 ml. of acetic acid was hydrogenated 16 hr. at 50 p.s.i. and room temperature, using 2.0 g. of platinum oxide catalyst. After filtering the reaction mixture through Celite to remove the spent catalyst and dilution with water, sufficient ascorbic acid was added to discharge the green color caused by the presence of a small quantity of ferrocinium ion. The resulting pale orange solution was extracted with chloroform, washed thoroughly with water, and extracted with 10% sodium carbonate. Acidification of the basic extracts gave 9.8 g. (92%) of product, m.p. 115–117°. This material was sufficiently pure for conversion to α-ketotetramethyleneferrocene.

1,2-(α-Ketotetramethylene)ferrocene. A mixture of 9.8 g. of γ-ferrocenylbutyric acid and 150 g. of polyphosphoric acid was allowed to stand at room temperature, with occasional stirring for 2.5 hr. The reaction mixture was poured into water, allowed to stand several hours, and extracted with chloroform. The extracts were washed with water, 5% sodium carbonate, dried, and the solvent removed at reduced pressure affording 4.04 g. (44%) of ketotetramethylene

(1) Department of Chemistry and Geology, Clemson College, Clemson, S. C., to whom all inquiries concerning this work should be directed.

(2) K. L. Rinehart and R. J. Curby, *J. Am. Chem. Soc.*, **79**, 3290 (1957).

(3) K. L. Rinehart, R. J. Curby, and P. E. Sokol, *J. Am. Chem. Soc.*, **79**, 3420 (1957).

(4) We were in one instance able to successfully carry out the hydrogenation on an exhaustively purified sample of the acid. However, this reduced drastically the overall yield for the sequence.

(5) Analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Infrared spectra were carried out in carbon tetrachloride solution, or as Nujol mulls on a Perkin-Elmer Model 137 spectrophotometer. Melting points were determined on a Fisher-Johns block and are uncorrected.