Anal. Calcd. for $C_{10}H_8$: C, 93.71; H, 6.29. Found: C, 93.84; H, 6.34.

Repeated experiments gave essentially the same results. Use of commercial sodium amide in this procedure gave a mixture of II and III in about a two to one ratio. The yield was 46%.

o-(1,2-Dibromoethyl)-(1,2-dibromovinyl)benzene (IV). Excess bromine was added to an ethereal solution of 0.5 g. (3.9×10^{-3} mole) of 1-ethynyl-2-vinylbenzene cooled in an ice bath. The red solution was allowed to warm to room temperature and after 1 hr. was poured into an aqueous sodium thiosulfate solution. Separation of the layers and washing of the colorless ether layer with water was followed by drying over magnesium sulfate. Removal of the ether provided an oil which crystallized on standing. Recrystallization from an ether-petroleum ether (b.p. 30–60°) mixture gave 0.7 g. (40% yield) of the tetrabromo derivative (IV), m.p. 84–86°.

Anal. Calcd. for $C_{10}H_8Br_4$: C, 26.82; H, 1.80; Br, 71.38. Found: C, 26.91; H, 1.98; Br, 71.89.

ROHM & HAAS CO.
REDSTONE ARSENAL RESEARCH DIVISION
HUNTSVILLE, ALA.

Alkaloids of *Balfourodendron riedelianum*. The O^4-Ethyl Analog of Balfourolone

HENRY RAPOPORT AND KENNETH G. HOLDEN[1]

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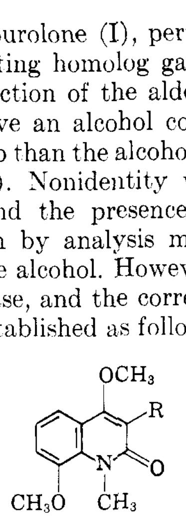
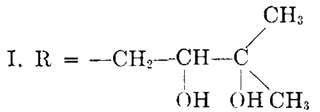
In previous publications[2,3] the isolation of alkaloids from *Balfourodendron riedelianum*, a Rutaceous plant indigenous to Brazil and Argentina, was described, and the structures of seven alkaloids were assigned. Balfourolone, m.p. 99–100°, the compound isolated in largest amount, was shown to have structure I. In a subsequent large scale isolation of alkaloids, the balfourolone fraction melted over a wide range, 88–108°. Repeated crystallization gave material whose melting point, 137°, did not change on further recrystallization. The ultraviolet spectrum of this higher melting material was identical with that of balfourolone (I), but its infrared spectrum showed some minor differences, and analysis established the presence of an additional methylene group. The identity of the ultraviolet spectra showed that the aromatic nucleus was the same as in balfourolone (I) and indicated that the additional carbon atom was probably contained in the side chain. An *O*- or *N*-ethyl group would also satisfy the data but seemed unlikely since all the other alkaloids from this plant source, and from Rutaceous plants in general,[4] have only *O*- and *N*-methyl groups.

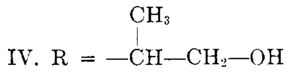
As with balfourolone (I), periodate oxidation of this higher melting homolog gave acetone and an aldehyde. Reduction of the aldehyde with sodium borohydride gave an alcohol containing one more methylene group than the alcohol, II, obtained from balfourolone (I). Nonidentity with the synthetic alcohol III,[3] and the presence of one C-methyl group as shown by analysis made IV a possible structure for the alcohol. However, this was shown not to be the case, and the correct structure of the homolog was established as follows:



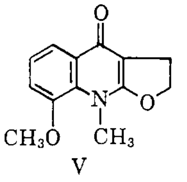

$$\text{I. R} = -CH_2-\underset{OH}{CH}-\underset{OH}{C}\begin{smallmatrix}CH_3\\ CH_3\end{smallmatrix}$$

II. R = $-CH_2-CH_2-OH$

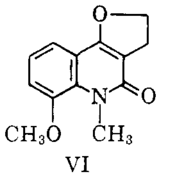
III. R = $-CH_2-CH_2-CH_2-OH$


$$\text{IV. R} = -\overset{CH_3}{CH}-CH_2-OH$$

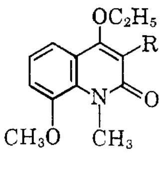
Acid catalyzed ring closure of the alcohol, II, obtained from balfourolone (I) has been shown[2] to give a mixture of the linear and angular dihydrofuroquinolines, V and VI. Refluxing the acetyl derivative of the alcohol obtained from the balfourolone homolog with 6*N* hydrochloric acid gave the same two products, identical in all respects with authentic samples of V and VI. From this it must be concluded that the alcohol obtained from the homolog of balfourolone has structure VII, and the homolog is then VIII.



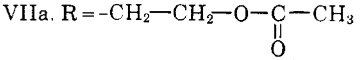
V

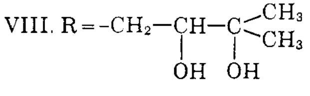


VI



VII. R = -CH_2-CH_2-OH


$$\text{VIIa. R} = -CH_2-CH_2-O-\underset{O}{\overset{}{\underset{\|}{C}}}-CH_3$$


$$\text{VIII. R} = -CH_2-\underset{OH}{CH}-\underset{OH}{C}\begin{smallmatrix}CH_3\\ CH_3\end{smallmatrix}$$

Synthesis of the homolog VIII, the O^4-ethyl analog of balfourolone, was easily accomplished by a route similar to that previously[1] used for balfourolone (I). Treatment of balfourodine (IX)[1,2] with ethyl iodide gave O^4-ethylbalfourodinium iodide (X) which, on treatment with alkali, was converted to VIII. The ultraviolet and infrared spectra, and melting point of VIII were identical with those of the high melting component isolated from the balfourolone fraction of plant extract.

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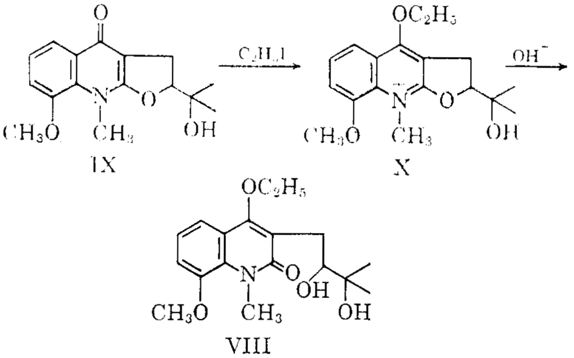
(1) Public Health Service Predoctoral Research Fellow of the National Heart Institute.

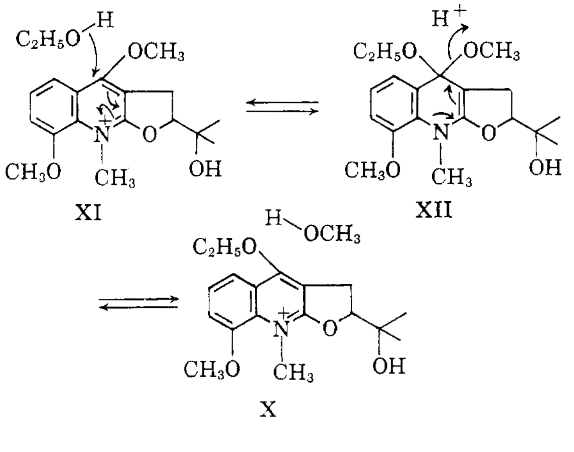
(2) H. Rapoport and K. G. Holden, *J. Am. Chem. Soc.*, **81**, 3738 (1959).

(3) H. Rapoport and K. G. Holden, *J. Am. Chem. Soc.*, **82**, 4395 (1960).

(4) J. R. Price in *Progress in the Chemistry of Organic Natural Products*, Vol. XIII, Springer-Verlag, Vienna, 1956, pp. 317–320.



Isolation of the O^4-ethyl analog (VIII) of balfourolone (I) must be explained by the occurrence of an O^4-ethylbalfourodinium salt (X) in the plant extract. The same evidence[1] which proved that balfourolone (I) was an artifact of the isolation procedure, being derived from an O^4-methylbalfourodinium salt (XI), pertains for the O^4-ethyl analog (VIII). In this case, however, the O^4-ethylbalfourodinium salt itself may be an artifact. Since extraction of plant material was carried out with boiling ethanol and the extract then remained in ethanolic solution for long periods of time before further extraction, the possibility of exchange with the solvent must be considered. Such a process might proceed by ethanol attack at the 4-position of an O^4-methylbalfourodinium salt (XI) to give the intermediate XII, which could then lose methanol to give an O^4-ethylbalfourodinium salt (X).



That the O^4-ethylbalfourodinium salt is naturally occurring is certainly possible but seems unlikely since all the other alkaloids isolated in this study, and from other Rutaceae, have only methoxyl functions. Furthermore, the lability of substituents at the 2- and 4- positions of 2,4-dialkoxyquinolines, as shown[2] by their facile rearrangements, makes the displacement by ethanol at the 4-position quite conceivable.

EXPERIMENTAL

Isolation of high-melting component (VIII) *from balfourolone fraction.* The balfourolone obtained by recrystallization of fraction C[1] from a large scale alkaloid isolation, while white and highly crystalline, melted over a wide range (88–108°). By eleven crystallizations from acetone-hexane the melting point was raised to a constant value of 137°.

Anal. Calcd. for $C_{18}H_{25}O_5N$: C, 64.5, H, 7.5, N, 4.2. Found: C, 64.3, H, 7.3, N, 4.3.

Periodate oxidation of the O^4-ethyl analog (VIII) *of balfourolone.* The oxidation and isolation of acetone and the aldehyde fraction were carried out in the same manner as for balfourolone.[1] From 1 g. (2.98 mmoles) of the diol VIII were obtained 370 mg. (1.92 mmoles, 64%) of the *p*-nitrophenylhydrazone of acetone, m.p. 149° (mixed m.p. 149°) and 800 mg. (2.91 mmoles, 98%) of aldehyde, infrared absorption in chloroform: λ_{max} 3.52 (w), 3.64 (w), 5.84 (s).

Alcohol VII. Reduction of the crude aldehyde fraction (720 mg., 2.62 mmoles) obtained from the oxidation of the diol VIII with excess sodium borohydride gave 720 mg. (2.60 mmoles, 99%) of crude alcohol VII which was chromatographed on 15 g. of activity III alumina. Elution with benzene-chloroform (1:1) gave an oil, which was molecularly distilled at 150°/50 μ, but was not purified further.

Anal. Calcd. for $C_{15}H_{19}O_4N$: C, 65.0, H, 6.9, N, 5.1, OCH_3, 22.4, CCH_3, 5.4. Found: C, 63.5, H, 6.7, N, 5.1, OCH_3, 21.9, CCH_3, 5.1.

Acetyl derivative VIIa. A solution of the alcohol VII (180 mg., 0.65 mmole) in 2 ml. of pyridine and 2 ml. of acetic anhydride was allowed to stand at room temperature for 24 hr. The reaction mixture was then poured into 25 ml. of 1*N* hydrochloric acid and the resulting solution was extracted with chloroform (3 × 15 ml.), the chloroform extracts being washed with a 40-ml. portion of 1*M* aqueous sodium carbonate solution. Drying and evaporation of the combined derivative (VIa) which after recrystallization from hexane and methanol-water was sublimed at 90°/30 μ, m.p. 101–102°.

Anal. Calcd. for $C_{17}H_{21}O_5N$: C, 64.0, H, 6.6, CCH_3, 9.4. Found: C, 63.8, H, 6.5, CCH_3, 9.3.

Ring closure of the acetyl derivative VIIa. A solution of the acetyl derivative (VIIa) (50 mg., 0.19 mmole) in 5 ml. of 6*N* hydrochloric acid was boiled under nitrogen for 18 hr. The cooled reaction mixture was diluted with 10 ml. of water and extracted with chloroform (3 × 10 ml.). Drying and evaporation of the combined organic extracts gave 12 mg. of material which was chromatographed on 2 g. of activity III alumina. Elution with benzene and benzene-chloroform (3:1) gave 4 mg. (0.017 mmole, 9%) of *6-methoxy-5-methyl-4-oxo-2,3,4,5-tetrahydrofuro[3,2-c]quinoline* (VI) which after recrystallization from benzene-hexane, melted at 142° (mixed m.p. 143–144° with material melting at 144–145°[2]) and had ultraviolet and infrared spectra identical with those of an authentic sample.[2] When the reaction mixture was neutralized with sodium hydroxide and extracted with chloroform (3 × 10 ml.), 25 mg. (0.11 mmole, 58%) of *8-methoxy-9-methyl-4-oxo-2,3,4,9-tetrahydrofuro[2,3,-b]quinoline* (V) was obtained. After crystallization from acetone-hexane and methanol-water it melted at 171° (mixed m.p. 170° with material melting at 170–171°[5]) and had ultraviolet and infrared spectra identical with an authentic sample.[2]

Synthesis of the O^4-ethyl analog (VIII) *of balfourolone.* A solution of balfourodine (IX) (370 mg., 1.28 mmoles) in 1 ml. of absolute ethanol and 10 ml. of ethyl iodide was allowed to stand at room temperature for 48 hr. The precipitated *O^4-ethylbalfourodinium iodide* (X) (190 mg., 0.43 33%) was collected and 88 mg. (0.20 mmole) was dissolved in 15 ml. of 0.5*M* aqueous sodium hydroxide. After standing for 24 hr. at room temperature, the solution was extracted with ether (2 × 10 ml.) to give 55 mg. (0.16 mmole, 82%) of the O^4-ethyl analog (VIII) of balfourolone, m.p. 136–137°, whose ultraviolet and infrared spectra and melting point

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(5) This material, compound XI of ref. 2, was reported incorrectly therein as melting at 135–137°.

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

Department of Chemistry
University of California
Berkeley, Calif.

Nitrogen Mustard Derivatives of Phenothiazine and Phenoxazine

David A. Shirley, Kalyanmay Sen, and John C. Gilmer

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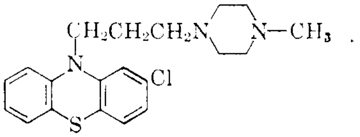
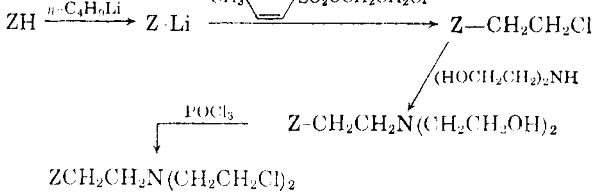
The intense search for effective anticancer chemotherapeutic agents has involved much attention to synthesis, animal and clinical evaluation, and even a significant level of routine medical application of a family of compounds known as "nitrogen mustards."[1] Most of the compounds contain the 2-chloroethylamino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

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(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).