[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MICHIGAN]

Synthesis of Potential Anticancer Agents. XII. Nitrogen Mustards from *p*-Aminobenzoic Acid Derivatives[1,2]

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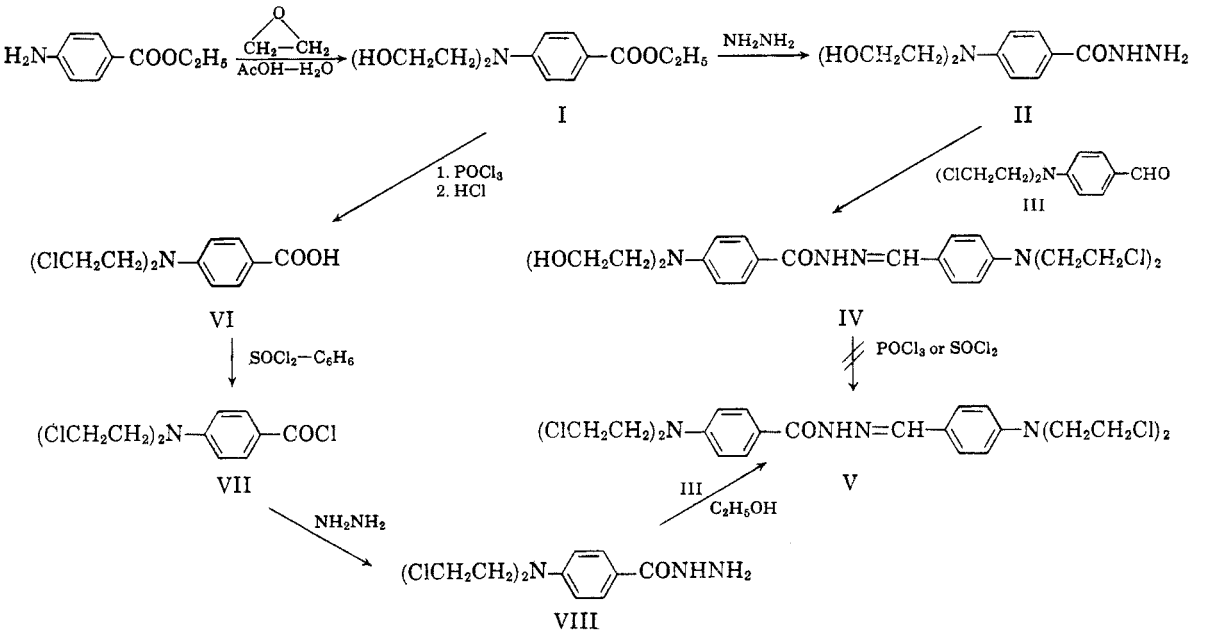
Received July 18, 1961

Synthesis of several nitrogen mustards derived from *p*-aminobenzoic acid is described.

Preliminary reports of activity of the *p*-[*N*,*N*-bis(2-chloroethyl)amino]benzylidenehydrazide of *p*-aminobenzoic acid[3,4] against the Dunning rat leukemia prompted an exploration of the synthesis of additional nitrogen mustards derived from *p*-aminobenzoic acid which we now report.

Reaction of ethyl *p*-aminobenzoate with ethylene oxide substantially as described by Pan and coworkers[5] gave ethyl *p*-[*N*,*N*-bis(2-hydroxyethyl)amino]benzoate (I) which on reaction with hydrazine gave the hydrazide (II). Condensation of II with *p*-[*N*,*N*-bis(2-chloroethyl)amino]benzaldehyde (III) gave the benzylidenehydrazide (IV). However synthesis of V was accomplished by the following reaction sequence. Treatment of I with phosphorus oxychloride followed by hydrolysis of the ester with concentrated hydrochloric acid[5] gave *p*-[*N*,*N*-bis(2-chloroethyl)amino]benzoic acid (VI). Retention of the nitrogen mustard function during the hydrolysis of the ester is another instance of the relative lack of reactivity of aromatic nitrogen mustards as contrasted to their aliphatic counterparts. With thionyl chloride VI gave the acid chloride (VII). In our hands VII was relatively stable in air in contrast to previous reports.[6] Further, VII as thus prepared melted several degrees higher than reported.[6] Addition of VII to a large excess of hydrazine resulted in instant separation of the hydrazide (VIII). Again failure of the mustard function to react with hydrazine is noteworthy. Condensation of VIII with III then readily gave the desired hydrazide (V).



However treatment of IV with either phosphorus oxychloride or thionyl chloride failed to produce the desired bismustard (V).

Results of tests of these compounds against experimental animal tumors will be reported elsewhere.

(1) For the preceeding paper in this series see R. C. Elderfield and R. N. Prasad, *J. Org. Chem.*, **26**, 3863 (1961).

(2) This work was supported by Research Grant CY-2961 from the National Cancer Institute to the University of Michigan.

(3) Private communication from Dr. Ralph Jones, Jr. of the Jackson Memorial Hospital of the University of Miami, Miami, Fla.

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(5) P. C. Pan, Y. F. Jen, Y. Y. Wang, and Y. S. Kao, *Acta Chim. Sinica*, **25**, No. 6, 390 (1959).

(6) W. C. J. Ross, G. P. Warwick, and J. J. Roberts, *J. Chem. Soc.*, 3110 (1955).

EXPERIMENTAL[7,8]

Ethyl p-[N,N-bis(2-hydroxyethyl)amino]benzoate (I). This was prepared by a modification of the procedure of Pan and co-workers.[5] To a chilled suspension of 80 g. (0.48 mole) of ethyl *p*-aminobenzoate in 640 ml. of 25% acetic acid, 100 g. of ethylene oxide was added with swirling. The mixture was stirred at room temperature for 20–25 hr. after which the clear solution was made slightly basic with sodium bicarbonate and extracted with ethyl acetate. After drying over anhydrous calcium sulfate and removing most of the solvent under reduced pressure, the product separated as white crystals on chilling. Recrystallization from benzene gave 68 g. (56%) of white leaflets, m.p. 93–94°.

p-[N,N-Bis(2-hydroxyethyl)amino]benzhydrazide (II). A mixture of 5 g. (0.02 mole) of I, 10 g. of 95% hydrazine, and 50 ml. of absolute ethanol was refluxed for 7 hr. On concentration of the solution the hydrazide (4.5 g., 95%) separated. After recrystallization from 95% ethanol, it melted at 141–142°.

Anal. Calcd. for $C_{11}H_{17}N_3O_3$: C, 55.23; H, 7.11; N, 17.57. Found: C, 54.84; H, 7.30; N, 17.66.

4-[N,N-Bis(2-hydroxyethyl)amino]benz-[4'-bis(2-chloroethyl)amino]benzylidene hydrazide (IV). A mixture of 4.5 g. (0.019 mole) of II and 4.6 g. (0.019 mole) of III[4] in 40 ml. of absolute ethanol was refluxed for 30 min. Separation of IV began within 10 min., and the yield was quantitative. Recrystallization from dimethylformamide–absolute ethanol gave yellow needles, m.p. 209–210°.

Anal. Calcd. for $C_{22}H_{28}Cl_2N_4O_3$: C, 56.58; H, 5.99; N, 11.99; Cl, 15.20. Found: C, 56.56; H, 6.04; N, 11.82; Cl, 15.34.

p-[N,N-Bis(2-chloroethyl)amino]benzoic acid. (VI). This was prepared essentially according to Pan and co-workers.[5] In view of the relative inaccessability of the original, details are given. Fifty milliliters of chilled phosphorus oxychloride was added slowly with swirling to 56 g. (0.221 mole) of I which was previously chilled in ice. The viscous mixture was heated on the steam bath until no further evolution of hydrogen chloride occurred. Excess phosphorus oxychloride was removed under reduced pressure, and the brown residue was refluxed with 200 ml. of concd. hydrochloric acid for 2 hr. After standing at room temperature overnight, the precipitate was collected and thoroughly washed with 50% ethanol. Recrystallization from 95% ethanol gave 33 g. (57%) of VI, m.p. 172–173°.

p-[N,N-Bis(2-chloroethyl)amino]benzhydrazide. (VIII). Twenty milliliters of purified thionyl chloride was added all at once to a solution of 9.6 g. (0.037 mole) of VI in 30 ml. of benzene, and the mixture was refluxed for 1 hr. After removal of the solvent at reduced pressure a quantitative yield of the acid chloride (VII), m.p. 88–94°, was obtained, reported m.p. 83–84°.[6] Recrystallization from benzene raised the m.p. to 94–96°.

A solution of crude VII in the minimum amount of dioxane was added dropwise to a solution of 20 g. of 95% hydrazine in 200 ml. of water. A white precipitate of VIII formed instantly and was collected. The yield was 10 g. (98%), m.p. 149–151.5°, after recrystallization from 95% ethanol.

Anal. Calcd. for $C_{11}H_{15}Cl_2N_3O$: C, 47.82; H, 5.43; N, 15.21; Cl, 25.72. Found: C, 47.82; H, 5.47; N, 15.22; Cl, 25.76.

4-[Bis(2-chloroethyl)amino]benz-[4'-bis(2-chloroethyl)-amino]benzylidene hydrazide. (V). A mixture of 4.1 g. (0.015 mole) of VIII and 4 g. (0.010 mole) of III in 100 ml. of 95% ethanol was heated on the steam bath for 20 min. A pale yellow precipitate appeared within 10 min. The crude product (6.3 g.), m.p. 225–227°, was collected and recrystallized from dimethylformamide–absolute alcohol.

Anal. Calcd. for $C_{22}H_{26}Cl_4N_4O$: C, 52.38; H, 5.15; N, 11.11; Cl, 28.18. Found: C, 52.30; H, 5.03; N, 10.98; Cl, 28.08.

Acknowledgment. We acknowledge the valuable assistance of James Hudson in the preparation of certain intermediates.

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(7) Melting points are uncorrected for stem exposure.

(8) Microanalyses by Spang Microanalytical Laboratory, Ann Arbor, Mich.

[CONTRIBUTION FROM THE VEGETABLE LABORATORY, UNITED STATES DEPARTMENT OF AGRICULTURE]

Isolation of (+)*S*-Methyl-L-cysteine Sulfoxide and of (+)*S*-*n*-Propyl-L-cysteine Sulfoxide from Onions as their *N*-2,4-Dinitrophenyl Derivatives

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Received May 24, 1961

(+)*S*-Methyl-L-cysteine sulfoxide and (+)*S*-*n*-propyl-L-cysteine sulfoxide have been isolated from onions as the *N*-2,4-dinitrophenyl derivatives. Configurational identity was established by infrared spectra and by optical rotation. *N*-Dinitrophenyl derivatives of several *S*-alkyl cysteines and of the corresponding sulfoxides have been prepared and their optical rotations recorded.

Virtanen and Matikkala[2] have recently isolated *S*-methylcysteine sulfoxide and *S*-*n*-propylcysteine sulfoxide from Finnish onions, *Allium cepa*, without, however, establishing their configurations. By analogy with the occurrence of (+)*S*-methyl-L-cysteine sulfoxide[3] in *Brassica* species and of (+)*S*-allyl-L-cysteine sulfoxide[4] in the closely related garlic, *Allium sativum*, the two amino acids from the onion would be presumed to be the dextrorotatory sulfoxides derived from L-cysteine.

The present work confirms the presence of these two amino acids in commercial American onions

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