

solution was instilled into the conjunctival sac of the left eye by means of a pipet (28 drops per ml.). The right eye was maintained as a control. The solution was allowed to remain in contact with the surface of the eyeball for 2 minutes by gently pressing the lids together. The time of abolishment of the wink reflex was noted and the duration of anesthesia was tested at 2-minute intervals.

### RESULTS

None of the compounds gave any evidence of any deleterious action on the eye. The eyes were carefully checked for corneal pitting, excessive lacrimation, and hyperemia. None of these was present. Pharmacological data are presented in Table II.

### SUMMARY

Several dialkylaminoalkyl esters of two indole carboxylic acids were prepared. All of the

compounds were effective as topical anesthetics, but had no action on unbroken skin. Nitration of indole-3-carboxylic acid reduced the duration of activity of the esters. The esters of indole-2-carboxylic acid showed a marked decrease in duration of local anesthetic activity.

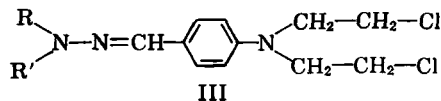
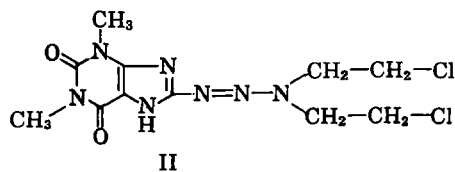
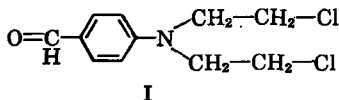
### REFERENCES

- (1) Moore, M. B., *THIS JOURNAL*, **33**, 194(1944).
- (2) Blicke, F. F., and Jenner, E. L., *J. Am. Chem. Soc.*, **64**, 1721(1942).
- (3) Blicke, F. F., and Blake, E. S., *ibid.*, **53**, 1015(1931).
- (4) Burtner, R. R., and Lehmann, G., *ibid.*, **62**, 527(1940).
- (5) Burtner, R. R., and Cusic, J. W., *ibid.*, **65**, 262(1943).
- (6) Majima, R., and Kotake, M., *Ber.*, **55**, 3861(1922).
- (7) Majima, R., and Kotake, M., *ibid.*, **63**, 2240(1930).
- (8) Doyle, F. P., *et al.*, *J. Chem. Soc.*, **1956**, 2855.
- (9) DiCarlo, F. J., *J. Am. Chem. Soc.*, **66**, 1420(1944).
- (10) Kermach, W. O., *et al.*, *J. Chem. Soc.*, **119**, 1602(1921).
- (11) Shirley, D. A., "Preparation of Organic Intermediates," John Wiley & Sons, Inc., New York 16, N. Y., 1951, pp. 116-117.
- (12) Slotta, K. H., and Behnisch, R., *Ber.*, **68**, 754(1935).

## Synthesis of Some Hydrazone Derivatives of *p*-[N,N-Bis( $\beta$ -chloroethyl)amino]benzaldehyde

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G. DOYLE DAVES, Jr., and C. C. CHENG

THE CONDENSATION of *p*-[N,N-bis( $\beta$ -chloroethyl)amino]benzaldehyde (1, 2) (I, benzaldehyde nitrogen mustard) with active methylene groups, (2, 3, 4) hydrazides (2) and amines (5, 6) have been reported. Recent discovery of the activity of some anil derivatives against Dunning leukemia in rats (6) and the inhibitory effect of 8-[bis( $\beta$ -chloroethyl)triazeno]theophylline (II) (7) on spontaneous tumor (8) prompted the synthesis of some benzalhydrazone derivatives (III) for biological evaluation.



The preparation of III, together with the starting materials used, is described in the experimental section.

### EXPERIMENTAL<sup>1</sup>

**Synthesis of Hydrazopyrimidines.**—To a mixture of 35 Gm. of chloropyrimidine in 70 ml. of absolute ethanol was added, with stirring, 35 ml. of anhydrous hydrazine at such a rate that the reaction temperature remained at 65-75° (occasional cooling in an ice bath may be necessary). After the addition was complete, the resulting solution was stirred at room temperature for 30 minutes, during

<sup>1</sup> All melting points were taken on the Thomas-Hoover melting point apparatus.

Received April 14, 1962, from Midwest Research Institute, 425 Volker Boulevard, Kansas City, Mo.  
Accepted for publication May 21, 1962.

This investigation was supported by research contract SA-43-ph-3025 from the Cancer Chemotherapy National Service Center, National Cancer Institute of the National Institutes of Health, Public Health Service.

The authors wish to express their appreciation to Professor Roland K. Robins of Arizona State University for helpful suggestions, and to Mr. Hal P. Van Fossen, Mrs. Margaret L. Rounds, Mrs. Carol R. Tuttle, and Mrs. Phyllis G. Lewis for their valuable assistance in performing analytical and instrumental measurements.

which time crystallization took place. The reaction mixture was diluted with 200 ml. of ethanol and filtered. The solid product was washed well with ethanol and ether. The resulting hydrazinopyrimidine was of sufficient purity to be used for the preparation of hydrazones.

5-Bromo-4,6-di(hydrazino)pyrimidine was thus obtained from 5-bromo-4,6-dichloropyrimidine (9) in 46% yield, m.p. 150° dec.

*Anal.*—Calcd. for  $C_4H_7BrN_6$ : N, 38.3 Found: N, 38.5.

Similarly 5-bromo-4-hydrazino-2-(methylthio)pyrimidine was prepared from 5-bromo-4-chloro-2-methylthiopyrimidine (10) in 35% yield, m.p. 148–150°.

*Anal.*—Calcd. for  $C_5H_7BrN_4S$ : N, 24.0. Found: N, 24.1.

**2-(Hydrazinomethyl)naphthimidazole.**—To 75 ml. of anhydrous hydrazine was added, portionwise, 30 Gm. of 2-chloromethylnaphthimidazole [prepared by the method of Bloom and Day (11), using 2,3-naphthalenediamine rather than *o*-phenylenediamine] at such a rate that the reaction temperature remained below 80°. Crystallization began after the addition was complete. The reaction mixture was allowed to stand at room temperature for 20 minutes, diluted with 200 ml. of ethanol and filtered. The product was washed with ethanol and ether, and recrystallized from a mixture of dimethylformamide and water to give 14 Gm. of light yellow crystals, m.p. 245° dec.

*Anal.*—Calcd. for  $C_{12}H_{12}N_4 \cdot H_2O$ : N, 24.3. Found: N, 24.0.

5-Uracilcarbohydrazide was similarly prepared from 60 ml. of anhydrous hydrazine and 35 Gm. of 5-carbethoxyuracil (12) to yield, after recrystallization from dilute hydrochloric acid, 13 Gm. of white prisms, m.p. 245° dec.

*Anal.*—Calcd. for  $C_5H_6N_4O_3 \cdot HCl$ : N, 27.2. Found: N, 27.0.

**General Method for the Preparation of *p*-[N,N-Bis( $\beta$ -chloroethyl)amino]benzaldehyde Derivatives (III).**—To 250 ml. of absolute ethanol was added 0.04 mole of substituted hydrazine. The mixture was heated till solution took place (for those hydrazines that are insoluble in absolute ethanol, 80% aqueous ethanol was substituted). The solution was treated with charcoal and filtered. To the filtrate, heated at 60°, 0.04 mole of *p*-[N,N-bis( $\beta$ -chloroethyl)amino]benzaldehyde (1, 2) dissolved in 250 ml. of absolute ethanol was added with stirring followed by two drops of concentrated hydrochloric acid. A precipitate appeared almost immediately. Stirring was continued for 5 minutes and the reaction mixture was filtered. The solid was washed with ethanol and ether, and dried *in vacuo* to give the desired product of analytical purity.

The following compounds were prepared:

2 - [*p* - [N,N - Bis( $\beta$  - chloroethyl)amino]benzalhydrazono]pyrimidine, m.p. 187–188°, was prepared from 2-(hydrazino)pyrimidine (13) in 52% yield.

*Anal.*—Calcd. for  $C_{15}H_{17}Cl_2N_5$ : C, 53.2; H, 5.2; N, 20.7. Found: C, 53.5; H, 5.2; N, 20.4.

4 - [*p* - [N,N - Bis( $\beta$  - chloroethyl)amino]benzalhydrazono] - 6 - chloropyrimidine, m.p. 295–297°, was prepared from 6-chloro-4-hydrazinopyrimidine (9) in 86% yield.

*Anal.*—Calcd. for  $C_{15}H_{16}Cl_3N_5$ : C, 48.3; H, 4.3; N, 18.7. Found: C, 48.5; H, 4.3; N, 18.9.

5 - [*p* - [N,N - Bis( $\beta$  - chloroethyl)amino]benzalhydrazono]uracil, m.p. 225° dec., was prepared from 5-hydrazinouracil hydrochloride (14) in cold water in 74% yield.

*Anal.*—Calcd. for  $C_{15}H_{17}Cl_2N_5O_2$ : C, 48.4; H, 4.6; N, 18.8. Found: C, 48.8; H, 4.7; N, 18.7.

5 - [*p* - [N,N - Bis( $\beta$  - chloroethyl)amino]benzalhydrazonocarbonyl]uracil, m.p. 290° dec., was prepared from 5-uracilcarbohydrazide hydrochloride in 56% yield.

*Anal.*—Calcd. for  $C_{15}H_{17}Cl_2N_5O_3$ : C, 48.2; H, 4.3; N, 17.6. Found: C, 48.3; H, 4.5; N, 17.6.

6 - [*p* - [N,N - Bis( $\beta$  - chloroethyl)amino]benzalhydrazonocarbonyl]uracil, m.p. 182° dec., was prepared from orotic acid hydrazide (15), using dimethylformamide as solvent, in 79% yield.

*Anal.*—Calcd. for  $C_{16}H_{17}Cl_2N_5O_3 \cdot H_2O$ : C, 46.1; H, 4.6; N, 16.8. Found: C, 46.0; H, 4.7; N, 16.4.

2 - [*p* - [N,N - Bis( $\beta$  - chloroethyl)amino]benzalhydrazono]benzothiazole, m.p. 197–199°, was prepared from 2-hydrazinobenzothiazole (16) in 43% yield as light yellow crystals.

*Anal.*—Calcd. for  $C_{18}H_{18}Cl_2N_4S$ : C, 55.0; H, 4.5; N, 14.2. Found: C, 55.3; H, 4.8; N, 13.9.

2 - [*p* - [N,N - Bis( $\beta$  - chloroethyl)amino]benzalhydrazonomethyl]naphthimidazole, m.p. 225° dec., was prepared from 2-(hydrazinomethyl)naphthimidazole in 56% yield as white crystals.

*Anal.*—Calcd. for  $C_{23}H_{23}Cl_2N_5$ : C, 62.7; H, 5.2; N, 15.9. Found: C, 62.3; N, 5.4; N, 16.3.

4 - [*p* - [N,N - Bis( $\beta$  - chloroethyl)amino]benzalhydrazonosulfonyl]toluene, m.p. 179–181°, was prepared from *p*-toluenesulfonyl hydrazide (17) in 75% yield as light orange crystals.

*Anal.*—Calcd. for  $C_{18}H_{21}Cl_2N_3O_3S$ : C, 52.2; H, 5.2; N, 10.2. Found: C, 52.2; H, 5.2; N, 10.0.

Di - [*p* - (N,N - bis( $\beta$  - chloroethyl)amino)benzal]oxalic carbohydrazone, m.p. 244–245°, was prepared from oxalyl hydrazide (18) in 77% yield as light yellow crystals.

*Anal.*—Calcd. for  $C_{22}H_{22}Cl_4N_6O_2$ : C, 50.2; H, 4.9; N, 14.6. Found: C, 50.3; H, 5.2; N, 14.2.

4 - [*p* - [N,N - Bis( $\beta$  - chloroethyl)amino]benzalhydrazono]benzoic acid, m.p. 226–228°, was prepared from *p*-(hydrazino)benzoic acid (19) in 74% yield.

*Anal.*—Calcd. for  $C_{18}H_{19}Cl_2N_3O_2$ : C, 56.9; H, 5.0; N, 11.0. Found: C, 57.1; H, 5.3; N, 10.8.

4 - [*p* - [N,N - Bis( $\beta$  - chloroethyl)amino]benzal]thiosemicarbazone, m.p. 189–191°, from thiosemicarbazide in 63% yield as light yellow crystals.

*Anal.*—Calcd. for  $C_{12}H_{16}Cl_2N_4S$ : C, 45.2; H, 5.0; N, 17.5. Found: C, 45.4; H, 5.1; N, 17.2.

3-Methyl-6-(1'-methyl-2'-*p*-[N,N-bis( $\beta$ -chloroethyl)amino]benzal}hydrazono)-2-(methylthio)-4(3H)pyrimidinone, m.p. 218°, was prepared from 3-methyl-6-(1'-methylhydrazino)-2-(methylthio)-4(3H)-pyrimidinone (20) in 80% yield as light brown crystals.

*Anal.*—Calcd. for  $C_{18}H_{23}Cl_2N_5OS$ : C, 50.5; H, 5.4; N, 16.4. Found: C, 50.3; H, 5.6; N, 16.8.

3-Methyl-6-(1'-methyl-2'-*p*-[N,N-bis( $\beta$ -chloroethyl)amino]benzal}hydrazono)uracil, m.p. 239°, was prepared from 3-methyl-6-(1'-methylhydrazino)uracil (20) in 50% yield.

*Anal.*—Calcd. for  $C_{17}H_{21}Cl_2N_5O_2$ : C, 51.3; H, 5.3; N, 17.6. Found: C, 51.3; H, 5.5; N, 17.5.

3-Methyl-6-(1'-methyl-2'- $\{p$ -[N,N-bis( $\beta$ -chloroethyl)amino]benzal} - hydrazino) - 5 - (phenylazo) uracil, m.p. 204°, was obtained from 3-methyl-6-(1'-methylhydrazino)-5-(phenylazo)uracil (20) in 48% yield.

*Anal.*—Calcd. for  $C_{20}H_{26}Cl_2N_7O_2$ : C, 55.0; H, 5.0; N, 19.5. Found: C, 54.8; H, 4.9; N, 19.7.

The following compounds were prepared by essentially the same procedure except that 5 ml., rather than two drops, of concentrated hydrochloric acid was added to the reaction mixture. As a result, the desired product was isolated as its hydrochloride.

4 -  $\{p$  - [N,N - Bis( $\beta$  - chloroethyl)amino]benzalhydrazono} - 5 - bromo - 2 - methylthiopyrimidine hydrochloride, m.p. 210–213° dec., was prepared from 5 - bromo - 4 - hydrazino - 2 - methylthiopyrimidine in 56% yield.

*Anal.*—Calcd. for  $C_{16}H_{18}BrCl_2N_5S \cdot HCl$ : C, 38.5; H, 3.8; N, 14.1. Found: C, 38.7; H, 4.0; N, 14.5.

2,4 - Di -  $\{p$  - [N,N - bis( $\beta$  - chloroethyl)amino]benzalhydrazono}pyrimidine dihydrochloride, m.p. 180° dec., was prepared from 2,4-di(hydrazino)pyrimidine (21) in 40% yield.

*Anal.*—Calcd. for  $C_{22}H_{30}Cl_4N_8 \cdot 2HCl$ : C, 46.8; H, 4.8; N, 16.8. Found: C, 46.5; H, 4.7; N, 16.7.

2,4 - Di -  $\{p$  - [N,N - bis( $\beta$  - chloroethyl)amino]benzalhydrazono} - 6 - methylpyrimidine hydrochloride, m.p. 190° dec., was prepared from 2,4-di(hydrazino)-6-methylpyrimidine (22) in 67% yield.

*Anal.*—Calcd. for  $C_{27}H_{32}Cl_4N_8 \cdot HCl$ : C, 50.1; H, 5.1; N, 17.3. Found: C, 49.7; H, 5.4; N, 17.3.

5 - Bromo - 4,6 - di $\{p$  - [N,N - bis( $\beta$  - chloroethyl)amino]benzalhydrazono}pyrimidine hydrochloride,

m.p. 207° dec. was prepared from 5-bromo-4,6-di(hydrazino)pyrimidine in 79% yield.

*Anal.*—Calcd. for  $C_{26}H_{30}BrCl_4N_8 \cdot HCl$ : C, 43.8; H, 4.4; N, 15.8. Found: C, 43.4; H, 4.8; N, 15.8.

## SUMMARY

A number of hydrazono derivatives of  $p$ -[N,N-bis( $\beta$ -chloroethyl)amino]benzaldehyde have been synthesized. The compounds have been submitted for general screening at the Cancer Chemotherapy National Service Center.

## REFERENCES

- (1) Anker, R. M., and Cook, A. H., *J. Chem. Soc.*, **1944**, 489.
- (2) Elderfield, R. C., Covey, I. S., Geiduschek, J. B., Meyer, W. L., Ross, A. B., and Ross, J. H., *J. Org. Chem.*, **23**, 1749(1958).
- (3) Chou, H. L., Lin, H. C., and Yüan, C. Y., *Kó Hsüeh T'ung Pao*, **14**, 457(1959).
- (4) Popp, F. D., *J. Chem. Soc.*, **1960**, 5271.
- (5) Ross, W. C. J., Warwick, G. P., and Roberts, J. J., *J. Chem. Soc.*, **1955**, 3110.
- (6) (a) Popp, F. D., *J. Org. Chem.*, **26**, 1566(1961); (b) Popp, F. D., and Kirsch, W., *ibid.*, **26**, 3858(1961).
- (7) Usbeck, G. A., Jones, J. W., and Robins, R. K., *J. Am. Chem. Soc.*, **83**, 1113(1961).
- (8) Testing data to be published.
- (9) Chesterfield, J., McOmie, J. F. W., and Sayer, E. R., *J. Chem. Soc.*, **1955**, 3478.
- (10) Peters, E., Holland, J. F., Bryant, B., Minnemeyer, H., Hohenstein, C., and Tieckelmann, H., *Cancer Res.*, **19**, 729(1959).
- (11) Bloom, A., and Day, A. R., *J. Org. Chem.*, **4**, 14(1939).
- (12) Wheeler, H. L., Johnson, T. B., and Johns, C. O., *Am. Chem. J.*, **37**, 392(1907).
- (13) Sirakawa, K., Ban, S., and Yoneda, M., *J. Pharm. Soc. Japan*, **73**, 598(1953).
- (14) Behrend, R., and Ernert, P., *Ann.*, **258**, 359(1890).
- (15) Alberi, J. T., U. S. pat., 2,832,782 (April 29, 1958).
- (16) Colonna, M., and Andrisano, R., *Pubbl. Ist. Chim. Univ. Bologna*, No. 5, 3(1943).
- (17) Freudenberg, K., and Blümmel, F., *Ann.*, **440**, 45(1924).
- (18) Diels, O., and Kock, U., *ibid.*, **556**, 38(1944).
- (19) Takayanagi, G., *J. Chem. Soc. Japan*, **53**, 427(1932).
- (20) Daves, G. D., Jr., Robins, R. K., and Cheng, C. C., *J. Am. Chem. Soc.*, **84**, 1724(1962).
- (21) Boarland, M. P. V., McOmie, J. F. W., and Timms, R. N., *J. Chem. Soc.*, **1952**, 4691.
- (22) Andrisano, R., *Boll. Sci. Fac. Chim. Ind. Bologna*, **5**, 52(1944–1947).

# Isolation of Acetyldigitoxin

By OLE GISVOLD

A method for the convenient isolation of acetyldigitoxin from *Digitalis mertonensis* and *D. siberica* is described. The former yielded acetyldigitoxin in approximately twice the amount of digitoxin that could be isolated from *D. purpurea*.

PREVIOUS reports (1) on the nature of the chief desglucoglycosides of *Digitalis mertonensis*<sup>1</sup> yielded somewhat inconclusive results. In these investigations both fresh and dried leaves were used together with a number of different extrac-

tion conditions and solvents to prepare the primary extracts. Both acetyldigitoxin and digitoxin, when combined, represented the major desglucoglycosides. In one case it was chiefly digitoxin, in another, it was chiefly acetyldigitoxin. It now has been demonstrated that when a primary extract of fresh leaves of *D. mertonensis* is made with 35 per cent methanol and the subsequent purification steps carried out with care, acetyldigitoxin is the chief desglucoglycoside that can be detected and subsequently isolated in good yield. The leaves must be in a healthy condition and free from damaged or partially damaged leaves. Where any amounts of the

Received March 29, 1962, from University of Minnesota College of Pharmacy, Minneapolis.

Accepted for publication June 6, 1962.

This work was supported in part by Research Project Grant NE-06569 from The National Heart Institute of The United Public Health Service.

<sup>1</sup> See *New Phytologist*, **31**, 225(1932).