ide, 20 ml., was added to a cooled (0°), stirred solution of 6.50 g. (17.9 mmoles) of the amine IVb in 25 ml. of glacial acetic acid. The mixture was allowed to warm to room temperature and was stirred for 18 hr., then was concentrated *in vacuo* to half its volume. The solution was diluted with 20 ml. of dichloromethane and extracted with 20 ml. of saturated aqueous $NaHCO_3$. The aqueous layer was extracted with two 50-ml. portions of dichloromethane, and the extracts were combined with the original dichloromethane solution. The dried organic solution was diluted with 12 ml. of absolute ethanol, cooled to 10°, and saturated with HCl. Dry ether was added to the cloud point and the solution was chilled, affording 6.68 g. (76%) of a white solid, m.p. 172–175°. The analytical sample, recrystallized from absolute ethanol, had m.p. 177–178°; λ_{max}^{Nujol} 2.90 and 6.60 (OH, NH), 5.72 (ester C=O), 6.00 μ (amide C=O).

Anal. Calcd. for $C_{22}H_{31}N_3O_7 \cdot HCl$: C, 54.4; H, 6.60; Cl, 7.22; N, 8.64. Found: C, 54.2; H, 6.77; Cl, 7.50; N, 8.48.

Ethyl α-Acetamido-α-carbethoxy-β-[7-bis(2-hydroxyethyl)-amino-3-indolyl]propionate (Vc).—Ethylene oxide, 3 ml., was added to a chilled (0°), stirred solution of 0.69 g. (1.90 mmoles) of the amine IVc in 9 ml. of 50% aqueous acetic acid. The solution was stirred at 0° for 1 hr., then allowed to reach room temperature, when stirring was continued for 20 hr. more. The solution was poured into 30 ml. of water, then adjusted to pH 7 with solid $NaHCO_3$ and extracted with three 20-ml. portions of ethyl acetate. The dried extract was treated with decolorizing carbon, then filtered and evaporated, yielding 0.87 g. of oily residue. Crystallization from 100 ml. of water afforded 0.60 g. (70%) of white crystals, m.p. 64–69°, collected in two crops. The analytical sample, recrystallized from water, had m.p. 67–69°; λ_{max}^{Nujol} 2.95, 3.08 and 6.55 (OH, NH), 5.69 and 5.75 (ester C=O), 6.00 μ (amide C=O).

Anal. Calcd. for $C_{22}H_{31}N_3O_7$: C, 58.8; H, 6.95; N, 9.35. Found: C, 59.1; H, 7.01; N, 9.16.

Ethyl α-Acetamido-α-carbethoxy-β-[6-bis(2-chloroethyl)-amino-3-indolyl]propionate (VIb).—A solution of 3.0 g. (6.2 mmoles) of Vb hydrochloride in 15 ml. of ethyl acetate was cooled to 10°, then treated with 20 ml. of cold saturated aqueous $NaHCO_3$. The organic layer was washed with 5 ml. of cold water, dried, and evaporated *in vacuo* to give Vb as a colorless foam.

To a chilled (0°), stirred solution of the free base in 24 ml. of dry pyridine was added dropwise 1.40 ml. (18.1 mmoles) of methanesulfonyl chloride. The reaction was stirred at room temperature for 18 hr., then was heated at 55–60° for 1.5–2 hr. The mixture was cooled to room temperature, then poured into 100 ml. of ice water. The oil which separated slowly crystallized yielding 1.65 g. (55%) of product, m.p. 135–142°. One recrystallization from 95% ethanol gave 1.33 g. of solid, m.p. 153–155°. The analytical sample had m.p. 157–158°; λ_{max}^{Nujol} 2.98, 3.07, and 6.59 (NH and possibly alcohol OH), 5.73 (ester C=O), 6.02 μ (amide C=O).

Anal. Calcd. for $C_{22}H_{29}Cl_2N_3O_5 \cdot 0.5C_2H_5OH$: C, 54.2; H, 6.28; Cl, 14.0; N, 8.25. Found: C, 54.3; H, 6.26; Cl, 13.8; N, 8.32.

This reaction could not be scaled up satisfactorily.

Ethyl α-acetamido-α-carbethoxy-β-[7-bis(2-chloroethyl)-amino-3-indolyl]propionate (VIc) was prepared from Vc essentially as described for the preparation of VIb except that the reaction mixture was not heated before it was poured into ice water. The crude yield of material with m.p. 135–140° was 69%. The analytical sample was obtained by two recrystallizations from benzene–petroleum ether (30–60°) and had m.p. 139–141°; λ_{max}^{Nujol} 2.96, 3.01, and 6.56 (NH), 5.70 and 5.77 (ester C=O), 6.03 μ (amide C=O).

Anal. Calcd. for $C_{22}H_{29}Cl_2N_3O_5$: C, 54.2; H, 6.01; Cl, 14.6; N, 8.64. Found: C, 54.4; H, 5.88; Cl, 14.5; N, 8.30, 8.37.

6-Bis(2-chloroethyl)amino-DL-tryptophan (IIIb).—A mixture of 2.0 g. of the blocked mustard VIb in 20 ml. of concentrated HCl was heated in an oil bath maintained at 120–130° for 2 hr., then was cooled to 10° and treated carefully with saturated aqueous sodium acetate. A yellow solid separated at pH 4–5. The solid was filtered, washed with ice water, then stirred with ice water that contained 5% acetic acid. After drying at room temperature, the product weighed 1.11 g. (67% yield); $\lambda_{max}^{cast\ film}$ 2.95 (NH), 3.1–3.6, 6.11, 6.50 (NH_3^+), 5.91 (acetic acid C=O), 6.40 and 7.10 μ (CO_2^-).

Anal. Calcd. for $C_{15}H_{19}Cl_2N_3O_2 \cdot HC_2H_3O_2$: C, 50.6; H, 5.70; Cl, 17.5; N, 10.4. Found: C, 50.5; H, 5.71; Cl, 17.5; N, 10.6.

In an earlier run in which aqueous $NaHCO_3$ was used to neutralize the hydrolytic solution, a 57% yield of hydrated free base was obtained; the infrared spectrum was very similar to that of the acetic acid containing product but lacked the band at 5.91 μ.

Anal. Calcd. for $C_{15}H_{19}Cl_2N_3O_2 \cdot 1.25H_2O$: C, 49.2; H, 5.74; Cl, 19.4; N, 11.5. Found: C, 49.1; H, 5.87; Cl, 19.7; N, 11.1.

7-Bis(2-chloroethyl)amino-DL-tryptophan (IIIc).—A solution of 4.0 g. (8.2 mmoles) of the blocked mustard VIc in 40 ml. of concentrated HCl was heated at reflux, under nitrogen, for 1.75 hr., then evaporated *in vacuo*, leaving a glassy residue (3.8 g.). A portion of this residue (2.69 g.) was dissolved in 55 ml. of dry pyridine. The solution was filtered to remove a small amount of insoluble material and the filtrate was treated with 150 ml. of dry ether. The precipitate was isolated by centrifugation and was thoroughly washed with dry ether three times using centrifugation to isolate the solid. The product, 1.92 g. (96%), carefully protected from atmospheric moisture, was dried *in vacuo* at 80° over P_2O_5; λ_{max}^{Nujol} 2.93 (NH), 3.6–4.0 and 6.21 (NH_3^+), 6.35 and 6.92 μ (CO_2^-).

Anal. Calcd. for $C_{15}H_{19}Cl_2N_3O_2 \cdot 0.25H_2O$: C, 51.7; H, 5.66; Cl, 20.0; N, 12.1. Found: C, 51.6; H, 5.63; Cl, 20.4; N, 11.4, 11.8.

6-Amino-L-tryptophan (VII) was prepared from 0.30 g. (1.2 mmoles) of 6-nitro-L-tryptophan[5] over 0.20 g. of platinum oxide in 30 ml. of water in the manner described for the preparation of 5-amino-DL-tryptophan.[3] The product, recrystallized from water, was a white crystalline solid, 0.25 g. (98%), m.p. 208–211°. The analytical sample had m.p. 210–212°; $[\alpha]^{24}D$ +10° (1% in 1 *N* HCl); λ_{max}^{Nujol} 2.80, 2.95, and 3.1 (NH, NH_2), 3.65, 3.81, 6.12, and 6.50 (NH_3^+), 6.27 and 7.12 μ (CO_2^-).

Anal. Calcd. for $C_{11}H_{13}N_3O_2 \cdot 0.125H_2O$: C, 59.5; H, 6.40; N, 18.9. Found: C, 59.8; H, 6.21; N, 18.8.

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Carcinogenic Activity of 2-N,N-Dimethylamino-5-phenylazopyridine[1]

ELLIS V. BROWN,[2] ANDREW F. SMETANA, AND ALI A. HAMDAN

Department of Chemistry, Seton Hall University, South Orange, New Jersey

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We have previously synthesized and tested *p*-dimethylaminophenylazopyridines for rat hepatocarcinogenic activity.[3] They can be considered as pyridine analogs of Butter yellow, a well known carcinogen. The 3-pyridine analog has a modest activity[4] while the 2-isomer has no activity and the 4-isomer has a high activity.[1] It seemed of interest to synthesize the 3-isomer in which the dimethylamino group is on the pyridine instead of the benzene ring in order to investigate its activity.

The first synthesis which we undertook involved the Mills' reaction[5] using 2-dimethylamino-5-aminopyridine and nitrosobenzene. This failed, possibly due to instability on the part of the diamino compound.[6] In the second and successful method, 2-

(1) This investigation was assisted in part by a United States Public Health Service grant (C-2219), National Institutes of Health.

(2) Chemistry Department, University of Kentucky, Lexington, Ky.; to whom inquiries should be directed.

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chloro-5-phenylazopyridine[5] was autoclaved with an alcoholic solution of dimethylamine.

Experimental

Mills Reaction with 2-Dimethylamino-5-aminopyridine.—2-Dimethylamino-5-nitropyridine[6] was reduced in a Parr apparatus with 5% palladium on carbon in 95% ethanol. The diamine was unstable to air and on treatment with nitrosobenzene in acetic acid gave no isolable azo compound.

2-N,N-Dimethylamino-5-phenylazopyridine.—2-Chloro-5-phenylazopyridine, once recrystallized from ethanol, was prepared in 94% yield by the method of Mills.[5] Six grams of this compound was treated with a solution of 10.2 g. of dimethylamine in 65 ml. of absolute ethanol in a sealed tube at 150° for 4 hr. The solvent and excess dimethylamine were removed *in vacuo* and the product was recrystallized twice from ethanol; m.p. 114.5–115.2°; the yield of yellow plates was 3.4 g. (54.5%).

Anal. Calcd. for $C_{13}H_{14}N_4$: C, 69.03; H, 6.20; N, 24.77. Found: C, 68.95; H, 6.32; N, 24.55.

Biological Testing and Results.—Young male rats of the Sprague–Dawley strain, approximately 8 weeks of age and weighing 150 to 200 g., were distributed as equally as possible by initial body weight into groups of 10 animals each. Each group was fed a diet, patterned after the "low protein, low riboflavine" diet of Miller, *et al.*,[7] to which had been added one of the azo compounds at a level of 0.06%. The composition of the basal diet on a kilogram basis was as follows: crude casein, 120 g.; cerelose, 770 g.; Osborne and Mendel salt mixture, 40 g.; corn oil, 50 g.; Vitab (rice bran concentrate, obtained from Charles Bowman Co.), 20 g.; riboflavine, 0.5 mg.; vitamin A palmitate, 67,500 IU (we are grateful to Charles Pfizer and Co., Inc., for a generous supply). The control group received only the basal diet. All the rats were kept individually in screen-bottomed cages and were offered food and water *ad libitum*. Laparotomies were performed at the indicated times and microscopic examinations were made whenever an animal died or at the end of the experiment. Feeding 2-N,N-dimethylamino-5-phenylazopyridine at 0.06% level produced no tumors in 12 months. The data are indicated in Table I.

TABLE I

CARCINOGENICITIES OF THE AZO COMPOUNDS

Code	—Incidence of liver tumors[a]— 4 months	7 months	12 months
Control (no dye)	0/10	0/10	0/10
N,N-Dimethyl-*p*-phenylazoaniline[b]	7/10	9/10	10/10
N,N-Dimethyl-*p*-(3-pyridylazo)-aniline	1/8	7/8	8/8
5-Phenylazo-2-N,N-dimethylpyridine	0/10	0/10	0/10

[a] Number of rats with tumors/number of rats in experiment. All azo compounds were fed at the 0.06 level. [b] Commonly called Butter yellow.

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Some 5-Fluoropyrimidines[1a]

G. J. DURR[1b]

Department of Chemistry and Physics, Central Michigan University, Mount Pleasant, Michigan

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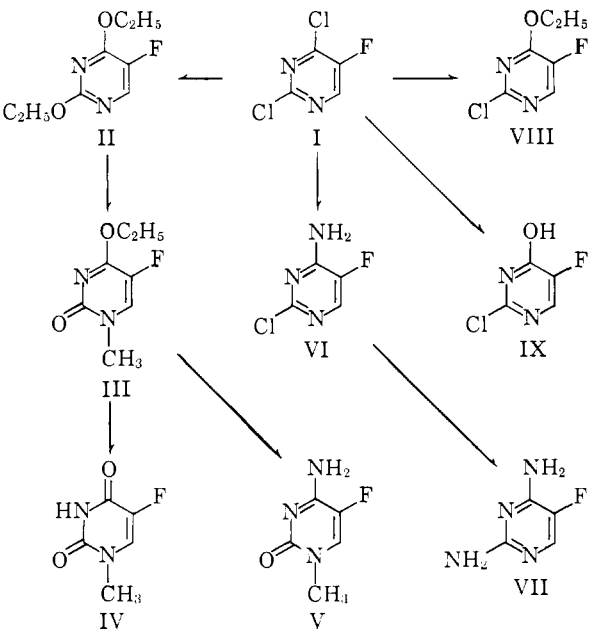
Since the synthesis of 5-fluorouracil by Duschinsky, Pleven, and Heidelberger[2] this compound has attacted a great deal of attention as an antimetabolite in the treatment of cancer.[3] Thus, it was desirable to prepare several derivatives of 5-fluoropyrimidine.

In this project the key intermediate was 2,4-dichloro-5-fluoropyrimidine (I).[4] The versatility of 2,4-dichloropyrimidines as intermediates in synthetic pyrimidine chemistry is well known[5] and is due to the reactive halogen atoms attached to the electrophilic pyrimidine ring. The conversion of I to 2,4-diethoxy-5-fluoropyrimidine (II) was effected with sodium ethoxide and is reported elsewhere.[6] Reaction of II with methyl iodide gave 4-ethoxy-5-fluoro-1-methyl-2(1H)-pyrimidone (III). The assignment of the methyl group to the N-1 position is based on the known reactions of 2,4-diethoxypyrimidines with alkyl halides and with O-acylglycosyl halides (Hilbert–Johnson synthesis of nucleosides) to yield N-1 alkyl derivatives and N-1 glycosyl nucleosides.[7] Hydrolysis and amminolysis of III resulted in the formation of 5-fluoro-1-methyluracil (IV) and 5-fluoro-1-methylcytosine (V), respectively.



Reaction of I with alcoholic sodium ethoxide (1 mole) or sodium hydroxide resulted in the formation of 2-chloro-4-ethoxy-5-fluoropyrimidine (VIII) and 2-chloro-5-fluoro-4-hydroxypyrimidine (IX), respectively.[8] Attempts to replace the chlorine atom of IX by amino or methoxy groups failed.

The partial structural relationship between the folic acid antagonist, aminopterin, and 2,4-diamino-5-

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(8) Compound IX was prepared independently by Dr. R. Duschinsky of Hofmann-La Roche, Inc., who has verified that substitution took place at the 4-position. Dr. Duschinsky has also prepared and identified 2-chloro-5-fluoro-4-methoxypyrimidine. Allocation of the ethoxy group to the 4-position of VIII is based on analogy to this work: private communication by Dr. R. Duschinsky.