Pyrimidine Derivatives VII

Some Condensed Derivatives of 2,4,5-Triamino-6-methylthiopyrimidine

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Three methods have been developed for the preparation of 2,4,5-triamino-6-methylthiopyrimidine: (*a*) by reduction of 2,4-diamino-6-methylthio-5-phenylazopyrimidine, (*b*) by reduction of 2,4-diamino-6-methylthio-5-nitrosopyrimidine, and (*c*) by methylation of 2,4,5-triamino-6-mercaptopyrimidine. Various cyclized derivatives have been prepared from the title compound. Condensation of the triaminomethylthio compound with 2,4-pentanedione failed to yield the desired diazepine derivative, but gave instead a product resulting from Schiff base formation at the 5-amino group of the pyrimidine.

THE SIGNIFICANT antitumor activity exhibited against transplantable mouse tumors by 6-thioguanine (1–3) and its *S*-methyl derivative, 2 - amino - 6 - methylthiopurine (VIII) (1,3) prompted the authors to attempt the preparation of various cyclic condensation products from 2,4,5-triamino-6-methylthiopyrimidine (IV), the synthesis of which has been fully explored in these laboratories (4). The synthesis of the triamine (IV) was accomplished *via* three alternate routes: (*a*) by reduction of the corresponding 5-phenylazo derivative, (*b*) by reduction of the 5-nitroso derivative, and (*c*) by methylation of the triaminomercapto compound.

DISCUSSION

Addition of dimethyl sulfate to an alkaline solution of 2,4-diamino-6-mercapto-5-phenylazopyrimidine (II) (5) afforded the methylthio derivative, 2,4 - diamino - 6 - methylthio - 5 - phenylazopyrimidine (III); *S*-methylation was indicated by the failure of III to discharge the color of Feigl's iodine-azide reagent (6, 7). Coupling of 2,4-diamino-6-methylthiopyrimidine (V) with benzenediazonium chloride in citric acid–disodium phosphate buffer at pH 5.0 proved to be a better method of preparation of III. Reduction of III with tin and hydrochloric acid afforded the triamine (IV) as an orange solid which became nearly colorless after purification.

IV is most easily obtained *via* the synthesis and reduction of 2,4-diamino-6-methylthio-5-nitrosopyrimidine (VI). Methylation of I with dimethyl sulfate in sodium hydroxide solution afforded V in 95% yield; methylation with methyl iodide in absolute methanol was also successful, but led to a lower yield of product. Nitrosation of V proceeded smoothly, affording VI in high yield as predicted by the generalization of Lythgoe and Todd (8, 9). Reduction of VI with sodium hydrosulfide and hydrogen sulfide afforded IV. Attempted reduction of the nitroso compound to IV with tin and hydrochloric acid led only to bright red-colored material. It was observed that IV, recovered from any solution containing acid, was always pale pink to orange in color, whereas from neutral or alkaline solution the product was colorless to slightly off-white.

The third route to IV involved direct methylation of 2,4,5-triamino-6-mercaptopyrimidine (VII). The reduction of II to VII in 37% yield by means of tin and hydrochloric acid was recently reported by these laboratories (5); reduction was successful only when highly purified phenylazo compound was used. It has now been found that *crude* phenylazo compound can be converted into VII in a yield of 53% by means of reductive cleavage with hydrogen sulfide and sodium hydrosulfide; this route represents a much simpler preparation of the mercaptotriamine. Methylation of VII at 0° with methyl iodide in alcoholic sodium methoxide gave IV in 81% yield. (Scheme I.)

During the course of this study, the instability of VII in alkaline solution, especially when exposed to light, was noted. Irradiated alkaline solutions of VII showed complete loss of pyrimidine character within 10 hr. and new compound formation[1] within 24 hr., as indicated by ultraviolet absorption spectra (Fig. 1). This rate of reaction was some fifteenfold more rapid than with solutions stored in the dark. Ring opening was suggested by the observation that no intermediate pyrimidine could be detected in the ultraviolet spectrum during the decomposition period. The ultraviolet spectra of VII taken immediately after dissolution showed $\lambda_{max.}^{pH10}$ 325 mμ (ε 13,720) and $\lambda_{max.}^{pH11.5}$ 318 mμ (ε 13,820); after

Received June 4, 1965, from The Children's Cancer Research Foundation and the Departments of Biological Chemistry and Pathology, Harvard Medical School at The Children's Hospital, Boston, Mass.

Accepted for publication July 27, 1965.

This investigation was supported in part by research grants CY3335 and C6516 and research career development award K3-CA-22,151 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

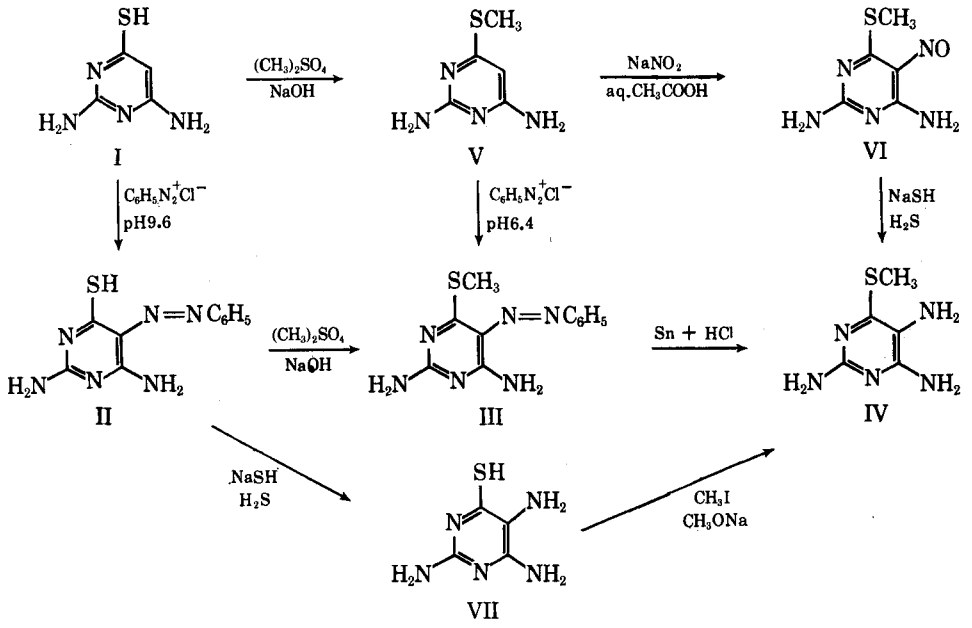
For paper VI in this series see *Reference 5*.

The technical assistance of Miss Dorothy H. Trites and Mr. Melvin M. Berman and the advice and assistance of Dr. Herbert N. Schlein and Dr. Siva Prasad Bhattacharya at various times during this investigation are acknowledged.

Infrared and ultraviolet spectra were obtained by Dr. James H. Gunnerson and Mr. Roger J. Cavallo.

Larger quantities of the following pyrimidines were obtained through the courtesy of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, and were prepared according to procedures developed in these laboratories: 2,4-diamino-6-mercaptopyrimidine (Aldrich Chemical Co., Milwaukee, Wis.) and 2,4,5-triamino-6-methylthiopyrimidine (Francis Earle Co., Peekskill, N. Y.).

[1] The structure of the rust-colored product formed during this reaction is presently under investigation.



Scheme I

1 hr. exposure to light, the pH 10 sample showed $\lambda_{max.}$ 318 mμ (ε 7630). This instability of VII may explain the discrepancy in our extinction coefficient values as compared with those of Elion *et al.* (10), who report for VII at pH 11 $\lambda_{max.}$ 320 mμ (ε 12,100).

Cyclization of IV to the corresponding purine (VIII) and triazole (IX) was accomplished in these laboratories (4) prior to the report of these compounds in the literature. Treatment of IV with ethyl orthoformate in acetic anhydride afforded 2-amino-6-methylthiopurine (VIII), which was characterized as the quarter-hydrate. VIII showed a strong propensity to retain water of crystallization, and anhydrous material could not be obtained even after protracted drying. Daves *et al.*, however, using essentially the same cyclization conditions, report the anhydrous product (11). This compound has also been prepared by several in-

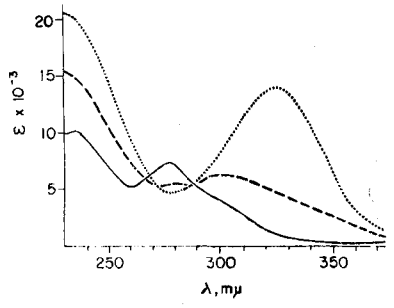
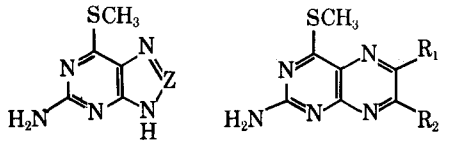
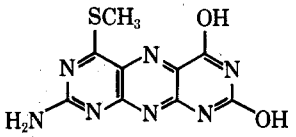


Fig. 1.—Light accelerated destruction of 2,4,5-triamino-6-mercaptopyrimidine (VII) in alkaline solution. Ultraviolet absorption spectra of a 5.36 × 10^{-5} *M* solution in pH 10 buffer. Key: . . . . ., freshly prepared solution; - - - - -, sample after exposure to light for 8 hr. or after dark storage for 7 days; ——, new compound formation within 24 hr. in the light or after 13 days in the dark. The product also exhibits a broad maximum in the visible spectrum at 470 mμ (ε 3400).



VIII, Z = CH
IX, Z = N

X, $R_1 = R_2 = H$
XI, $R_1 = R_2 = CH_3$
XII, $R_1 = R_2 = C_6H_5$
XIII*a*, R_1 = COOH, R_2 = OH
b, $R_1 = COOC_2H_5$, R_2 = OH



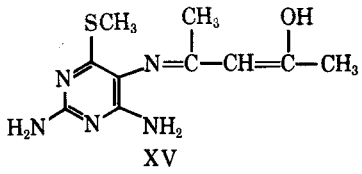
XIV

vestigators (12–14) by direct methylation of 2-amino-6-mercaptopurine, one of these groups (14) reporting the product as the monohydrate.

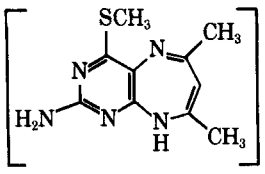
IV was converted into 5-amino-7-methylthio-1(3)*H*-*v*-triazolo[*d*]pyrimidine (IX) by the same procedure as that subsequently reported by Weiss *et al.* (15). Not previously noted is the ease of hydrolysis of the methylthio group of IX; 8-azaguanine (16)[2] is formed quantitatively when IX is merely warmed with dilute hydrochloric acid.

Condensation of IV with α-diketones proceeded smoothly and gave the corresponding pteridines. Thus, with diacetyl and benzil, 2-amino-6,7-dimethyl-4-methylthiopteridine (XI) and 2-amino-4-methylthio-6,7-diphenylpteridine (XII), respectively, were obtained; the reactions were accomplished in methanol–benzene solution. With gly-

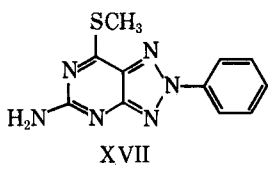
[2] An authentic sample of 8-azaguanine (NSC No. 749), supplied by the Cancer Chemotherapy National Service Center, was kindly made available to us for comparison by Dr. George E. Foley of this Foundation.



XV



XVI



XVII

oxal, the reaction was run in 50% aqueous ethanol solution containing an equivalent quantity of sodium bisulfite (17). This procedure led to relatively pure 2-amino-4-methylthiopteridine (X) in high yield, whereas the absence of sodium bisulfite gave crude material from which analytically pure product could not be obtained. X has recently been reported by McCormack and Mautner (18) in 30% yield *via* reaction of IV with glyoxal monohydrate in ethanol in the absence of sodium bisulfite.

Treatment of IV with alloxan monohydrate in glacial acetic acid in the presence of boron trifluoride-etherate afforded the tricyclic derivative, 2 - amino - 6,8 - dihydroxy - 4 - methylthiopyrimido-[5,6-*g*]pteridine (XIV). Alkaline degradation of XIV failed to yield 2-amino-7-hydroxy-4-methylthiopteridine-6-carboxylic acid (XIII*a*). Hydrolysis of the dihydroxypyrimidine moiety was accompanied by nucleophilic displacement of the methylthio group and gave, instead, isoxanthopterincarboxylic acid (2-amino-4,7-dihydroxypteridine-6-carboxylic acid), which was found to be identical with the product prepared by Taylor and Loux (19) *via* condensation of 2,4,5-triamino-6-hydroxypyrimidine and disodioketomalonate or alloxan in dilute sodium hydroxide. The desired pteridine was then obtained as the ethyl ester (XIII*b*) by treatment of IV with diethyl ketomalonate in glacial acetic acid in the presence of boron trifluoride. Attempts to hydrolyze the ester XIII*b* in either acid or base invariably led to hydrolysis of the methylthio function.

Treatment of IV with 2,4-pentanedione in refluxing methanol failed to give the corresponding 1,5-pyrimido[4,5-*b*]diazepine (XVI). Instead, the product was an off-white solid, the elemental analysis of which indicated the elimination of only one molecule of water during the reaction. The product of this reaction is believed to be 4-[(2,4-diamino - 6 - methylthio - 5 - pyrimidinyl)imino]-2-penten-2-ol (XV). The structure assignment is based upon a comparison of physical and spectral properties with those of the material obtained from the reaction of 2,4-pentanedione with 4,5-diaminopyrimidine, a compound which has been rigorously examined by the techniques of nuclear magnetic resonance spectrometry and mass spectrometry (20) and X-ray structure analysis (21). Interpretation of these data suggests that the product is an open chain compound resulting from condensation of one keto function with the 5-amino group of 4,5-diaminopyrimidine. The remaining oxygen function appears to lie at too great a distance from the 4-amino group of the resulting Schiff base, thus preventing cyclization to the diazepine.

Related to this series of cyclic derivatives of IV, but not a condensation product of the triaminomethylthiopyrimidine, is 5-amino-7-methylthio-2-phenyl-2*H*-*v*-triazolo[*d*]pyrimidine (XVII). This product was obtained from the phenylazo derivative (III) by controlled oxidation with cupric sulfate in aqueous pyridine following the procedure of Benson *et al.* (22).

Quantitative ultraviolet absorption spectra have been determined and are summarized in Table I.

EXPERIMENTAL[3,4]

2,4 - Diamino - 6 - methylthiopyrimidine (V).—A solution of 40.2 Gm. (0.21 mole) of 2,4-diamino-6-mercaptopyrimidine half-sulfate (5) dissolved in 2.3 L. of 1 *N* sodium hydroxide (steam bath temperature) was clarified with Darco.[5] The resulting clear yellow solution was cooled to 32° and to it was added, in aliquots, 32.3 ml. (0.34 mole) of freshly distilled dimethyl sulfate. The solution was stirred and warmed (45–50°) for 30 min. and then cooled to below room temperature. The solid was collected, washed with water, and dried; yield, 20.7 Gm. (63%), m.p. 196–202°. The pH of the clear filtrate was adjusted to 9.0 with dilute sulfuric acid and an additional yield of 4.0 Gm. (12%) was collected. The total yield of V free base was 24.7 Gm. (75%). This material is satisfactory for use in subsequent reactions without further purification. Analytically pure colorless prisms can be obtained by high vacuum (0.01 mm.) sublimation at a bath temperature of 150–175°, m.p. 200–203°. [Reported m.p. 202–204° uncorrected (11); 200–202° (24).]

An additional 19% of crude product can be recovered as the half-sulfate by acidification of the original filtrate to pH 3.9 with dilute sulfuric acid. The entire yield of V may be obtained as the half-sulfate if the pH of the reaction mixture is adjusted to 4 with dilute sulfuric acid immediately after methylation. Two crystallizations of this material from water, the first with the aid of Darco, afforded pure V half-sulfate, m.p. 239–240°.

2,4 - Diamino - 6 - methylthio - 5 - phenylazopyrimidine (III).—*Method A. From V.*—A solution containing 1.23 Gm. (0.006 mole) of 2,4-diamino-6-methylthiopyrimidine half-sulfate in 250 ml. of pH 6.4 citric acid (0.1 *M*)–disodium phosphate (0.2 *M*) buffer was cooled to approximately 8°. To this was added a solution of benzenediazonium

[3] Ultraviolet absorption spectra were measured at pH 1 (0.1 *N* hydrochloric acid) and at pH 10 (0.05 *M* sodium carbonate–sodium borate buffer) with a Cary model 11 spectrophotometer. Infrared spectra were determined with a Perkin-Elmer model 137B spectrophotometer by the potassium bromide disk technique. Melting points are corrected and were taken by the capillary method at a rate of heating of 2°/min. in a modified Wagner-Meyer melting point apparatus (23). Decomposition points are not reproducible unless conditions are rigidly controlled. If not otherwise specified, drying of analytical samples was carried out at 70–100° for 17 hr. *in vacuo* over phosphorus pentoxide.

[4] Analyses were performed by the Scandinavian Microanalytical Laboratory, Herlev, Denmark; Dr. Carol K. Fitz, Needham Heights, Mass.; and Drs. Weiler and Strauss, Oxford, England.

[5] Darco G-60 activated carbon, Atlas Chemical Industries, Inc., Wilmington, Del.

TABLE I.—ULTRAVIOLET ABSORPTION SPECTRA

Compd.	pH 1 λmax., mμ	pH 1 ε × 10⁻³	pH 10 λmax., mμ	pH 10 ε × 10⁻³
III	238	17.1	232	16.1
	296	17.1	285	15.6
	377	22.8	383	24.2
IV	235	14.4	230	15.5
	310	8.5	310	8.3
V[a]	225	17.6	229	20.9
	289	14.1	287	11.2
VI	233	9.7		
	248	9.2	[b]	
	279	10.4		
	330	11.2		
IX[c]	298	16.5	223	21.9
			265	9.3
			321	9.7
X	253[d]	6.0	233	17.1
	323	10.8	269	14.2
	340[d]	11.0	311	3.9
	355	12.8	380	9.0
	370[d]	10.2		
XI	261	7.2	239	18.4
	314	9.8	268	14.3
	358	15.9	304	3.3
	374	14.0	377	10.7
XII	224	31.8	290	23.0
	285	14.4	407	13.9
	393	18.2		
XIII[b]			233	32.9
			283	16.5
			377	17.5
XIV[e]	291	10.6	287	13.9
	345	11.5	326	4.4
	394	24.9	423	23.4
XV	235	13.9	230	19.3
	310	8.1	302	25.0
XVII[f]	216	19.1		
	240	8.2		
	309	15.7		
	352	23.3		

[a] *Reference 24:* pH 2, 225 (18.2), 289 (14.1); pH 4, 222 (16.6), 286 (11.0). [b] Insoluble. [c] *Reference 15:* pH 1, 298 (19.1); pH 11, 265 (10.0), 321 (10.7). [d] Inflection. [e] Sample dissolved in dimethyl sulfoxide and diluted with pH 1 and pH 10 buffer. No spectral values recorded below 275 mμ because of solvent interference. [f] Ethanol, 217 (19.7), 240 (14.8), 248 (14.7), 285 (11.7), 366 (18.2).

chloride, prepared at 0–3° from 0.74 Gm. (0.006 mole) of aniline hydrochloride, 10 ml. of 2 *N* hydrochloric acid, and 0.35 Gm. (0.006 mole) of sodium nitrite, in which the excess nitrous acid had been destroyed by the addition of urea until the starch-iodide test became negative. The diazotized aniline solution was poured into the stirred buffered pyrimidine solution (the pH of the resulting mixture was 5.0) and the reaction was allowed to stir overnight with gradual warming to room temperature (final pH 5.0). The yellow solid was collected and washed thoroughly with water. The still moist product was crystallized directly from ethyl acetate; yield 0.523 Gm. (39%), m.p. 180–182°. This material is sufficiently pure for use in subsequent reactions.

The analytical sample was prepared as follows. The moist crude product was dried at 42° *in vacuo* overnight. A 0.500-Gm. sample of this solid was dissolved in 120 ml. of absolute ethanol. Addition of 360 ml. of water precipitated pale yellow material; after overnight refrigeration, the crystals were collected, washed with water, and dried at 45° *in vacuo* for 6 hr. The solid was precipitated from ethanol–water a second time and then crystallized from ethyl acetate. After refrigeration for 3 days, the crystals were collected, washed with ethyl acetate, and air dried. The thin yellow prismatic plates were dried at 45° *in vacuo* for 17 hr. and submitted for analysis, m.p. 180.5–182°.

Anal.—Calcd. for $C_{11}H_{12}N_6S$: C, 50.75; H, 4.65; N, 32.29; S, 12.32. Found: C, 50.76; H, 4.80; N, 32.09; S, 12.23.

Method B. Methylation of II.—A solution of purified 2,4-diamino-6-mercapto-5-phenylazopyrimidine (0.100 Gm., 0.4 mmole) in 180 ml. of 1 *N* sodium hydroxide was prepared by warming on the steam bath and filtered free of insoluble impurity. The clear orange solution was cooled to 33°, and freshly distilled dimethyl sulfate (0.4 ml., 4 mmoles) was added. The resulting suspension was warmed (45–50°) for 30 min. and then refrigerated overnight. The pale yellow solid was separated, washed with cold water, and dried; yield, 0.040 Gm. (39%). The solid was crystallized from ethyl acetate as before (45% recovery). The identity of this product with that obtained by *Method A* was confirmed by comparison of quantitative ultraviolet absorption spectra and by an undepressed mixed melting point.

2,4 - Diamino - 6 - methylthio - 5 - nitrosopyrimidine (VI).—A solution containing 49.0 Gm. (0.314 mole) of 2,4-diamino-6-methylthiopyrimidine in 1690 ml. of 25% aqueous acetic acid was cooled to 18° and to it was added, with stirring, a solution of 23.8 Gm. (0.345 mole) of sodium nitrite in 63 ml. of water. The resulting dark violet suspension was stirred at room temperature for 2 hr. After overnight refrigeration, the violet solid was separated and washed thoroughly with water until the wash water was neutral. The product was not dried but was used while still moist for reduction to IV.

Analytically pure material was obtained by repeating this nitrosation on a small scale with II which had been purified by vacuum sublimation. The solid was collected and washed as above and then dried; yield, 88%, m.p. dec. above 260°.

Anal.—Calcd. for $C_5H_7N_5OS$: C, 32.42; H, 3.81; N, 37.82. Found: C, 32.52; H, 4.06; N, 37.50.

2,4,5-Triamino-6-mercaptopyrimidine (VII) Dihydrochloride.—Crude 2,4-diamino-6-mercapto-5-phenylazopyrimidine (II) was prepared as previously described (5) and was used without further purification. II (18.5 Gm., 0.075 mole) was pulverized and suspended in 1.5 L. of 50% ethanol. To this was added 375 ml. of 1 *N* aqueous sodium hydrosulfide, which had been presaturated with hydrogen sulfide for 15 min. (to pH 8.1). The mixture was stirred at 65–67° for 3 hr. while hydrogen sulfide gas was continuously added. The suspension was brought to 25° and, with external cooling, the pH of the supernatant was adjusted to approximately 3 by addition of 97 ml. of 6 *N* hydrochloric acid. The reaction vessel was left open overnight to allow the remaining hydrogen sulfide to escape. After removal of sulfur by gravity filtration, the mother liquor was evaporated at 45–58° to approximately 1/5 of the original volume. The dark yellow solid that had formed was collected and dried (22.6 Gm.). The crude solid was extracted with three 75-ml. portions of absolute methanol and the undissolved material (17.3 Gm.) was crystallized from 2 *N* hydrochloric acid, as previously described (5); yield, 9.1 Gm. (53%). This material is identical

with samples of VII previously obtained in these laboratories *via* tin and hydrochloric acid reduction of II (5).

2,4,5-Triamino-6-methylthiopyrimidine (IV).—*Method A. By Reduction of III.*—2,4-Diamino-6-methylthio-5-phenylazopyrimidine (0.5 Gm., 1.9 mmoles) was suspended in 60 ml. of water containing 2 ml. of concentrated hydrochloric acid. With stirring, 0.2 Gm. of granulated tin was added and the temperature of the reaction was raised to 70–80° and maintained there for 90 min. At the end of this time, the ultraviolet absorption spectrum of the reaction solution indicated essentially complete conversion to IV. The solution was cooled to room temperature and saturated with hydrogen sulfide until no more precipitate formed. Removal of the precipitated sulfides afforded a colorless filtrate which was warmed on a steam bath (50–60°) to expel hydrogen sulfide. The pH of the solution was adjusted to 8.0–8.5 with 8 ml. of 5 *N* sodium hydroxide. Evaporation under vacuum to $^1/_6$ of the original volume resulted in the formation of crystals which, after overnight refrigeration, were collected, washed with cold water, and dried; yield, 0.2 Gm. (61%). One crystallization from water afforded IV as pale golden, elongated prismatic plates, m.p. 189–190° dec. [Reported (11) m.p. 191–192°.]

Method B. By Reduction of VI.—Moist 2,4-diamino-6-methylthio-5-nitrosopyrimidine (58.2 Gm., equivalent to 0.314 mole) was suspended in 940 ml. of 0.7 *N* sodium hydrosulfide solution. The suspension was stirred at 65–69° (internal temperature) while hydrogen sulfide gas was continuously added. A clear solution was obtained after 30 min. and the reaction was continued for 1 hr. longer; at the end of this time, the ultraviolet absorption spectrum indicated complete reduction to IV. The dark solution was clarified with Darco and, after filtration, was refrigerated for 1 hr. The off-white crystals were collected, washed with cold water, and dried; yield 20.3 Gm. (38%), m.p. 190–195°. The clear mother liquor was reduced to 200 ml. under vacuum and, after overnight refrigeration, deposited an additional 5.8 Gm. (11%) of product.

Two crystallizations of this material from warm (90°) 0.1 *N* sodium hydroxide afforded colorless prismatic plates which, after being dried at 45° for 17 hr. *in vacuo*, melted at 189–190° dec. There was no depression of melting point on admixture with material prepared according to *Method A*. Crystallization of IV from water alone led to yellow colored product; this coloration could be avoided by crystallizing from 0.1 *N* sodium hydroxide or from water containing a small quantity of sodium hydrosulfite.

An additional 29% of crude material was recovered as the half-sulfate by acidification of the original mother liquor to pH 3 with dilute sulfuric acid. Recrystallization of the crude half-sulfate salt from 10% ethanol, with Darco, afforded colorless prismatic rods, m.p. 219–222° dec. A drop of dilute sulfuric acid was added to the solvent in the final crystallization in order to prevent the development of a pink coloration in the crystallized material.

Method C. By Methylation of VII.—A cold solution of 0.400 Gm. (17.4 mmoles) of sodium in 80 ml. of absolute methanol was added to a stirred solution of 0.400 Gm. (1.74 mmoles) of 2,4,5-triamino-6-mercaptopyrimidine dihydrochloride in 240 ml. of absolute methanol stored in a salt-ice bath. Cold methyl iodide (10 ml.) was added, and the reaction mixture was stirred for 15 min. at 0°. The pH was then adjusted to 6 by the addition of 2.5 ml. of 3 *N* sulfuric acid. The resulting solid (mostly inorganic) was separated by suction filtration and extracted with 130 ml. of hot absolute methanol. The original filtrate and the methanol extract were combined and evaporated to dryness *in vacuo*. The dark yellow solid was crystallized from 5 ml. of 0.1 *N* sodium hydroxide (Darco). After overnight refrigeration, the crystals were collected, washed with a small volume of cold water, and dried; yield, 0.240 Gm. (81%). A second crystallization from 0.1 *N* sodium hydroxide afforded thin prismatic plates, m.p. 191–193°, which were identical with samples of IV prepared by *Methods A* and *B*.

2-Amino-6-methylthiopurine (VIII).—This procedure followed the general directions of Montgomery (25) and is similar to the preparation reported by Daves and co-workers (11) after the completion of this work. After cyclization of VII in ethyl orthoformate and acetic anhydride, the evaporated reaction mixture residue was hydrolyzed in 3 *N* sodium hydroxide at steam bath temperature for 20 min., clarified with Darco, and neutralized with acetic acid (88% yield). Repeated crystallization from water gave colorless prismatic needles. The quarter-hydrate, m.p. 227–229°, was obtained on vacuum drying for 8 hr. at 110°.

Anal.—Calcd. for $C_6H_7N_5S \cdot {}^1/_4\ H_2O$: C, 38.80; H, 4.07; N, 37.71; S, 17.27; C/N, 1.03. Found: C, 38.88; H, 4.31; N, 37.41; S, 17.20; C/N, 1.04.

It was observed that colorless crystals of VIII have a tendency to become pale yellow upon drying, even when protected from light, and that the compound demonstrates a strong affinity to hold water of crystallization. Intensive drying under different conditions failed to give anhydrous material.

5 - Amino - 7 - methylthio - 1(3)H-v-triazolo[d]-pyrimidine (IX).—This compound was prepared by a procedure essentially the same as that described by Weiss *et al.* (15).

Hydrolysis of the methylthio group is easily effected by warm dilute hydrochloric acid. The ultraviolet absorption spectrum of the crystalline solid recovered, after warming IX with 1 *N* hydrochloric acid at 90° for 25 min., was identical with that of 8-azaguanine (16) (λ_{max}^{pH1} 250 mμ, inflection 261 mμ).

2-Amino-4-methylthiopteridine (X).—To a solution of 1.71 Gm. (0.01 mole) of 2,4,5-triamino-6-methylthiopyrimidine in 50 ml. of 50% ethanol containing 1.09 Gm. (0.0105 mole) of sodium bisulfite was added 2.03 ml. (0.0105 mole, 5% excess) of 30% glyoxal, and the mixture was warmed for 90 min. at 50–60° (steam bath). After refrigeration for 1 hr., a small quantity of brown solid was separated. The dark yellow mother liquor was refrigerated overnight and the yellow crystalline solid that had formed was collected, washed with dilute aqueous ethanol, and dried; yield, 1.61 Gm. (84%). Part of this solid, 0.7 Gm., was dissolved in 100 ml. of absolute methanol and filtered free of a small quantity of impurity. The volume of the orange filtrate was reduced to 15 ml. After overnight refrigeration, the solid was collected, washed with absolute

methanol, and dried. The dark yellow prismatic rods (0.42 Gm., 60% recovery) melted at 221–222°. Two further crystallizations from absolute methanol, exactly as above, afforded analytically pure, dark yellow long pointed prismatic rods, m.p. 221–222°. [Reported (18) m.p. 215–218°.]

Anal.—Calcd. for $C_7H_7N_5S$: C, 43.51; H, 3.65; N, 36.25; S, 16.59. Found: C, 43.48; H, 3.62; N, 36.28; S, 16.60.

2 - Amino - 6,7 - dimethyl - 4 - methylthiopteridine (XI).—A solution of 8.0 Gm. (0.047 mole) of 2,4,5-triamino-6-methylthiopyrimidine, 16 ml. of diacetyl, 220 ml. of absolute methanol, and 370 ml. of benzene was refluxed for 2 hr. and then allowed to stand at room temperature overnight. The solution was then taken to dryness under vacuum and the yellow residue crystallized directly from 1.45 L. of absolute methanol. After overnight refrigeration, the yellow prismatic needles were collected, washed with cold methanol, and dried; yield 9.1 Gm. (88%), m.p. 272–274° dec., with darkening above 255°.

Anal.—Calcd. for $C_9H_{11}N_5S$: C, 48.85; H, 5.01; N, 31.65. Found: C, 49.01; H, 5.27; N, 31.73.

2 - Amino - 4 - methylthio - 6,7 - diphenylpteridine (XII).—A solution containing 0.513 Gm. (3 mmoles) of 2,4,5-triamino-6-methylthiopyrimidine and 0.630 Gm. of benzil (3 mmoles) in 25 ml. of 3:2 methanol–benzene (v/v) was refluxed for 24 hr. The reaction mixture was reduced in volume to about 15 ml., cooled to room temperature, and refrigerated for 5 hr. The yellow solid (0.850 Gm.) was separated, washed with cold methanol, and air dried. Crystallization from 35 ml. of 1:1 methanol–benzene gave 0.700 Gm. of yellow prismatic needles (82%), m.p. 235–236°. For analysis, a portion of the above product was recrystallized from 1:1 methanol–benzene and dried under vacuum, first at 75° for 48 hr. and then at 95° for 5 hr. Above 95°, XII begins to sublime rapidly *in vacuo*.

Anal.—Calcd. for $C_{19}H_{15}N_5S$: C, 66.06; H, 4.38; N, 20.28. Found: C, 65.6; H, 4.6; N, 20.0.

Ethyl 2-Amino-7-hydroxy-4-methylthiopteridine-6-carboxylate (XIIIb).—A solution of 5.13 Gm. (0.03 mole) of 2,4,5-triamino-6-methylthiopyrimidine in 50 ml. of glacial acetic acid (prepared by warming on a steam bath) was added all at one time to a stirred solution of 5.22 Gm. (0.03 mole) of diethyl ketomalonate and 4.26 Gm. (0.03 mole) of boron trifluoride-etherate in 80 ml. of glacial acetic acid. The resulting pale yellow reaction mixture was warmed to 60° and, after a short while, turned dark red and began to deposit a bright yellow solid. The temperature was maintained at 60–65° for 45 min. The flask was cooled to room temperature and the solid collected. The yellow product was ground in a mortar under 95% ethanol, filtered, washed thoroughly with cold water, and dried; yield, 5.57 Gm. (66%). The analytical sample was obtained by high vacuum sublimation at 220–240°, m.p. 306.5–307°.

Anal.—Calcd. for $C_{10}H_{11}N_5O_3S$: C, 42.69; H, 3.95; N, 24.90; S, 11.40. Found: C, 42.62; H, 4.14; N, 24.73; S, 11.40.

2 - Amino - 6,8 - dihydroxy - 4 - methylthiopyrimido [5,6-g]pteridine (XIV).—To a solution of alloxan monohydrate (1.60 Gm., 0.01 mole) dissolved in 100 ml. of warm (35°) glacial acetic acid containing 1.42 Gm. of boron trifluoride-etherate (0.01 mole) was added a solution of 1.71 Gm. of 2,4,5-triamino-6-methylthiopyrimidine (0.01 mole) dissolved in 30 ml. of glacial acetic acid. Upon admixture, the reaction mixture turned deep purple and soon began to deposit yellow solid. The reaction mixture was stirred for 45 min. at 30–35° and was then permitted to cool. The yellow precipitate was separated and washed well with water; yield, 2.0 Gm. (72%). Purification was accomplished by precipitation from a 1:2 (v/v) dimethylsulfoxide–methanol mixture. The product fails to melt below 360°.

Anal.—Calcd. for $C_9H_7N_7O_2S$: C, 38.98; H, 2.55; N, 35.36; S, 11.57. Found: C, 39.08; H, 2.93; N, 35.08; S, 11.47.

4 - [(2,4 - Diamino - 6 - methylthio - 5 - pyrimidinyl)imino]-2-penten-2-ol (XV).—A mixture of 8.06 Gm. (0.05 mole) of 2,4,5-triamino-6-methylthiopyrimidine and 5.5 Gm. (0.055 mole) of 2,4-pentanedione in 400 ml. of absolute methanol was stirred and refluxed for 8 hr. The volume of the reaction mixture was reduced on a rotary evaporator until solid began to deposit. The product was collected, washed with a small volume of methanol, and air dried; yield, 9.86 Gm. (84%). Recrystallization of 1.0 Gm. of product from methanol returned 0.82 Gm. of off-white crystalline solid, m.p. 225.5–226.5°.

Anal.—Calcd. for $C_{10}H_{15}N_5OS$: C, 47.4; H, 5.97; N, 27.7. Found: C, 47.2; H, 5.7; N, 27.2.

5 - Amino - 7 - methylthio - 2 - phenyl - 2H - v-triazolo[d]pyrimidine (XVII).—A mixture of 2,4-diamino - 6 - methylthio - 5 - phenylazopyrimidine (0.250 Gm., 0.001 mole), cupric sulfate pentahydrate (1.41 Gm., 0.006 mole), pyridine (50 ml.), and water (50 ml.) was placed in a flask fitted with a reflux condenser and a gas inlet tube. The yellow-green mixture was refluxed for 19 hr., during which time it turned a deep blue in color; a slow stream of oxygen was continuously bubbled through the reaction during the entire reflux period. The hot mixture was poured into 250 ml. of ice water, and the resulting suspension was refrigerated overnight to insure complete precipitation. The yellow product was separated by suction filtration and washed, first with sodium tartrate solution to remove copper salts, and then with water. After overnight drying, the product was recrystallized several times from 95% ethanol; yield, 0.100 Gm. (40%), m.p. 206–208°.

Anal.—Calcd. for $C_{11}H_{10}N_6S$: C, 51.14; H, 3.90; N, 32.54; S, 12.42. Found: C, 51.40; H, 4.12; N, 32.75; S, 12.45.

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Pharmacological Evaluation of a Series of 3,6-Substituted Pyridazine Derivatives

By BARRY DUBINSKY, WILLIAM J. KINNARD, and JOSEPH P. BUCKLEY

A series of 3,6-substituted pyridazine derivatives were tested for central pharmacologic activity. On the basis of their effects on gross behavior, spontaneous, and forced motor activity of albino mice, the alkoxy-pyridazine derivatives were characterized as CNS depressants, while the alkoxy-2-dimethylaminoethoxy-pyridazine derivatives produced initial stimulation followed by a depression of spontaneous activity. The latter compounds also shortened hexobarbital sleeping time in mice and induced a low-voltage fast-wave activity in the electrocorticogram of the cat. None of the compounds produced any alteration of avoidance escape responding in mice or rats or caused a significant inhibition of convulsions produced by chemo- or electrostimulation in rats. A substudy indicated that there was little or no species difference in the learning ability or the response to chlorpromazine of mice and rats in the conditioned avoidance response test (pole climbing).

PYRIDAZINE, pyrimidine, and pyrazine are six-membered ring structures, which include two nitrogen atoms in the *ortho*, *meta*, and *para* positions, respectively. Pyrazine and, in particular, pyrimidine furnish a number of important derivatives useful in medicine; however, as noted by Wilson (1), fewer derivatives of pyridazine have been found to be useful. The pharmacological spectrum of pyridazine derivatives is very broad, ranging from sulfa drugs having antibacterial action (2), to drugs having neuromuscular blocking activity (3), central nervous system stimulating activity (4), and sedative activity (5). Thus, while a number of compounds with therapeutic potential have been synthesized and investigated, it is still not possible to describe with certainty the unique contribution of the pyridazine ring to a series of its derivatives.

The major purpose of the current investigation was to study the pharmacology of pyridazine and a series of closely related pyridazine derivatives synthesized by Drs. P. Coad and R. A. Coad, Department of Chemistry, Chapman College, Orange, Calif.[1]

The testing procedure for evaluation of psychopharmacologic agents involves the use of both mice and rats; and, while these species are closely related phylogenetically, there may be differences in behavior which are neglected in the transition from the use of mice in preliminary tests to the use of rats in the more sophisticated studies of learned behavior. It appeared then that there was a need to compare acquisition of a learned behavior and drug effects on an on-going behavior in the two species. Such a comparison could serve to bridge the gap between the use of mice in preliminary screening of centrally acting compounds and the use of rats in more intensive tests.

METHODS

Pyridazine and 15 of its derivatives (Table I) were examined for potential central nervous system activity. Pyridazine (I), the parent compound, and the other compounds were grouped according to Coad *et al.* (6). The compounds of group III had an alkoxy substituent as R and a chloro substituent. The compounds of group IV are either bis-alkoxy or bis-dimethylaminoethoxy derivatives, and the compounds of group V have an alkoxy substituent

Received June 4, 1965, from the Department of Pharmacology, School of Pharmacy, University of Pittsburgh, Pittsburgh, Pa.
Accepted for publication August 19, 1965.
Presented to the Scientific Section, A.PH.A., Detroit meeting, March 1965.
This investigation was supported in part by research grant MH-03029 from the National Institute of Mental Health, U. S. Public Health Service, Bethesda, Md.

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