Synthesis and Properties of 7-Azaxanthopterin[1]

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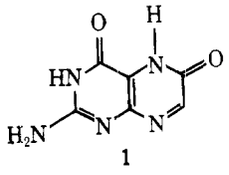
7-Azaxanthopterin (7-aminopyrimido[5,4-*e*]-*as*-triazine-3,5(2*H*,6*H*)-dione) (**2**) was prepared from 2-amino-4-hydrazino-6(1*H*)-pyrimidone (**4**) by acetylation to the 4-(2-acetylhydrazino) derivative **5**, Michael addition to diethyl azodicarboxylate to give 2-amino-4-(2-acetylhydrazino)-5-(1,2-dicarbethoxyhydrazino)-6(1*H*)-pyrimidone (**6**), and treatment with sodium ethoxide. 7-Azaxanthopterin (**2**) was also formed upon treatment of 2-amino-4-(1-methyl-2-acetyl(or formyl)hydrazino)-5-(1,2-dicarbethoxyhydrazino)-6(1*H*)-pyrimidone (**8a,b**) with sodium ethoxide; methyl transfer presumably occurs to ethoxide ion. 7-Azaxanthopterin (**2**) forms a stable trihydrate which reverts back to **2** in alkaline solution. UV spectroscopy shows that this transformation occurs in two discrete steps, suggesting the existence of both a covalent dihydrate and a covalent monohydrate of **2**.

Xanthopterin (**1**), one of several fluorescent pteridine pigments present in many species of insects, fish, and amphibia, holds a unique position as the first naturally-occurring pteridine to have been isolated.[2] Early biological studies indicated



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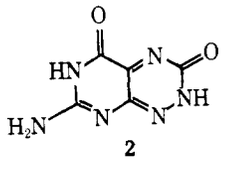

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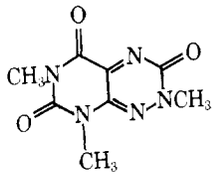

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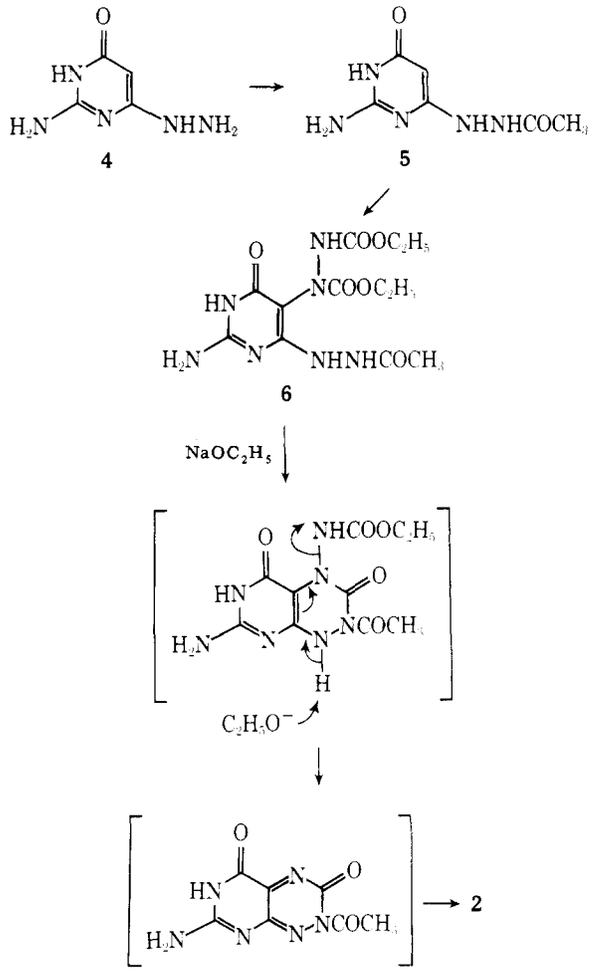
that xanthopterin was in some way related to nutritional anemia[3] and exhibited a growth inhibiting effect on malignant tumors in mice;[4] more recent studies have demonstrated its ability to stimulate renal mitosis[5] and have indicated antitumor activity.[6] As a consequence, there has been renewed interest in the development of efficient and unequivocal synthetic routes to this naturally-occurring insect pigment.[7-9] We thought it would be of considerable interest to examine the chemical, physical, and biological properties of 7-azaxanthopterin (**2**), not only because of its obvious structural similarity to xanthopterin itself, but because of its close relationship to the pyrimido[5,4-*e*]-*as*-triazine antibiotics, of which 2-methylfervenulone (MSD-92) (**3**) is the closest structural representative. The present paper describes a simple synthesis of 7-azaxanthopterin (**2**) and some of its unusual properties.

Our synthetic route to **2** is outlined in Scheme I and illustrates still another exploitation of our previously described pyrimidine C-5 functionalization with diethyl azodicarboxylate[10] and the elaboration of the resulting Michael adducts to fused pyrimidotriazines such as fervenulone and 2-methylfervenulone (**3**).[11] Thus, 2-amino-4,6-dichloropyrimidine was hydrolyzed to 2-amino-4-chloro-6(1*H*)-pyrimidone,[12] which was treated with hydrazine to give 2-amino-4-hydrazino-6(1*H*)-pyrimidone (**4**). Attempted C-5 functionalization of **4** with diethyl azodicarboxylate gave only a black multicomponent mixture presumably because reaction of the terminal hydrazine nitrogen with the Michael acceptor was followed by decomposition of the resulting unstable tetrazene. However, prior acetylation of **4** with acetic anhydride in DMF followed by reaction with diethyl azodicarboxylate in DMF yielded **6** in 78% yield. Direct conversion of **4** to **6** could be

Scheme I



conveniently achieved by treatment of a solution of **4** in DMF at 100 °C first with acetic anhydride and then with diethyl azodicarboxylate. Subsequent treatment of **6** with sodium ethoxide in refluxing ethanol led to the separation of the orange gelatinous sodium salt of 7-azaxanthopterin; 7-azaxanthopterin (**2**) itself was obtained in 62% yield by neutralization of an aqueous solution of its sodium salt. The probable mechanism of the conversion of **6** to **2** is depicted in Scheme I.

We then examined a simple extension of this sequence of reactions for the preparation of N-methylated derivatives of **2**, which are of potential interest as analogues of the pyrimido[5,4-*e*]-*as*-triazine antibiotics. Treatment of 2-amino-4-chloro-6(1*H*)-pyrimidone with methylhydrazine gave the hydrazinopyrimidine **7**, whose structure was confirmed by its

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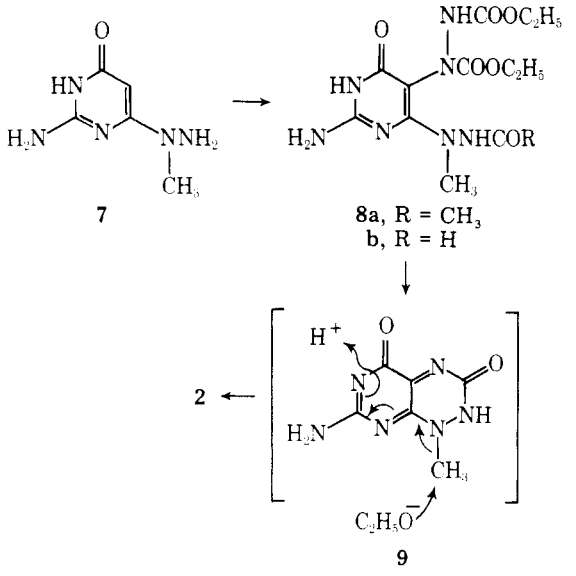
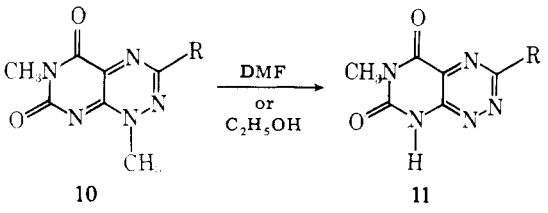
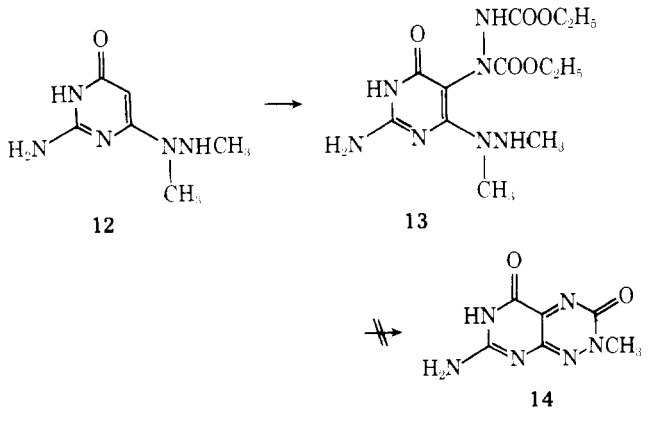
Scheme II



conversion to a benzylidene derivative with benzaldehyde. Reaction of **7** in hot DMF first with acetic anhydride and then with diethyl azodicarboxylate gave the protected Michael adduct **8a.** By analogy with the successful conversion of **6** to **2**, it was fully expected that treatment of **8a** with sodium ethoxide in hot ethanol would lead to the formation of **9** by cyclization, deacetylation, and aromatization by loss of ethyl carbamate. In the event, this sequence of reactions apparently did take place, but the actual product isolated from this reaction mixture was 7-azaxanthopterin (**2**) rather than its 1-methyl derivative **9**; i.e., demethylation had taken place under the reaction conditions (see Scheme II). This demethylation reaction has ample precedent, however, in other pyrimido[5,4-*e*]-*as*-triazines. 1-Methyltoxoflavins (**10**), for example, are effective methylating reagents even with weak nucleophiles, and are converted either with DMF or with ethanol into the demethylated derivatives **11**.[13] Treatment of the *N*-formyl Michael adduct **8b** with a smaller amount of sodium ethoxide under milder conditions likewise led directly to **2**.

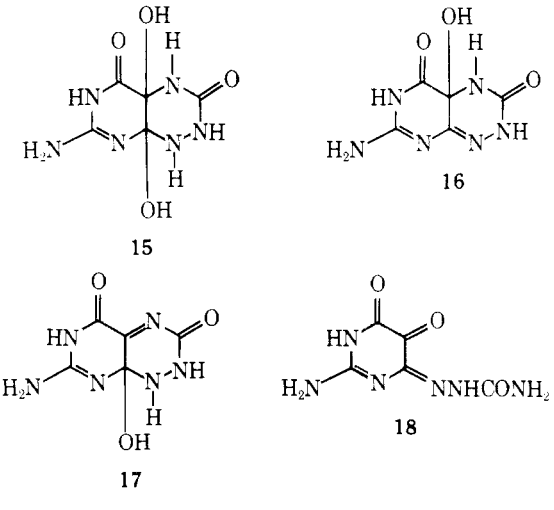


It appeared that advantage might be taken of this facile demethylation for the preparation of 7-methyl-7-azaxanthopterin (**14**), and our projected synthesis of this interesting hybrid molecule [possessing the pyrimidine substitution pattern of xanthopterin (**1**), and the triazine substitution pattern of 2-methylfervenulone (**3**)] thus involved treatment of 2-amino-4-chloro-6(1*H*)-pyrimidone with 1,2-dimethylhydrazine to give **12**, followed by reaction with diethyl azodicarboxylate to yield the Michael adduct **13**. It was anticipated that treatment of **13** with base would result in cyclization, loss of ethyl carbamate, and demethylation at position 8 to give the target compound **14**, but all attempts to effect this transformation failed; only extremely complex, multicomponent reaction mixtures were obtained.



7-Azaxanthopterin (**2**) is a red-orange amorphous powder, insoluble in all of the common organic solvents (as are most pterin derivatives and analogues), which does not melt or decompose below 300 °C. Its ultraviolet absorption spectrum in 0.1 N NaOH [257 nm (log ϵ 4.38) and 429 (3.70)] closely resembles that of xanthopterin (**1**), which exhibits maxima in 0.1 N NaOH at 255 nm (log ϵ 4.23) and 390 (3.48).[14] 7-Azaxanthopterin analyzes as a hemihydrate, while xanthopterin is a monohydrate.

A remarkable chemical difference between **1** and **2** was revealed as follows. When a dilute aqueous solution of the sodium salt of 7-azaxanthopterin (**2**) was warmed to 70 °C, acidified with acetic acid, and then cooled slowly to room temperature, amber needles separated over the course of 24 h. This material, both before and after recrystallization from water, proved to be a trihydrate of 7-azaxanthopterin. When a concentrated solution of this trihydrate in 0.1 N NaOH was allowed to stand for several hours and then acidified with acetic acid, 7-azaxanthopterin (**2**) separated; this proved to be the best method for its purification. The spectrum of the trihydrate in water consisted initially of two absorption bands at 230 and 260 nm, both of which increased proportionately with time until, after 2 h, maximum extinction coefficients were observed (log ϵ 3.84 and 4.02, respectively). It appears that this "hyperchromic effect" may be due to slow dissociation by solvation of associated molecules of the solute; a similar effect has been observed in DNA and is due to "unstacking" of the nucleotide bases by solvation.[15] Addition of aqueous NaOH (to give a 0.1 N solution) to the above aqueous solution of 7-azaxanthopterin trihydrate caused the 230-nm peak to disappear instantaneously; the 260-nm peak began to increase, and a weak band at 355 nm appeared from the baseline. After 15 min, the latter had reached maximum intensity, and at that time the spectrum consisted of two absorption maxima at 262 nm (log ϵ 4.23) and 355 (3.36). From that point on, the 355-nm band decreased again in intensity while the 262-nm peak continued to increase in intensity, and a band at 429 nm arose from the baseline. After 2 h, the ul-



traviolet spectrum of the basic solution was identical with that of 7-azaxanthopterin (**2**) itself.

It thus appears that 7-azaxanthopterin trihydrate reverts back to 7-azaxanthopterin in two discrete steps, the intermediate being observable by its ultraviolet absorption spectrum. This observation suggests that the trihydrate probably possesses at least two *covalently* bound water molecules; although no definitive structural information is available, a structure (**15**) involving addition of water to both bridgehead positions appears attractive. The intermediate between the trihydrate and **2** itself, which is detectable by its ultraviolet absorption spectrum, would therefore be the monocovalent hydrate **16** or **17** (ring-opened structures such as **18** cannot be excluded).

There is an apparent similarity between the avidity with which 7-azaxanthopterin (**2**) forms covalent hydrates and the suggested covalent addition of nucleophiles (e.g., benzoin, thiols) to flavins.[16,17] The possible role of **2** as an in vitro dehydrogenating agent, and as an in vivo inhibitor of flavin-linked dehydrogenases, would appear to deserve future attention.

Experimental Section

2-Amino-4-hydrazino-6(1*H*)-pyrimidone (4). To a stirred suspension of 15.0 g (103 mmol) of 2-amino-4-chloro-6(1*H*)-pyrimidone[12] in 250 mL of water was added 12 g (375 mmol) of anhydrous hydrazine, and the mixture was heated under reflux for 3 h. The resulting clear solution was cooled, and the precipitate which separated was collected by filtration, washed with water followed by ethanol, and dried to give 10.8 g (74%) of colorless crystals of **4**: mp 314–315 °C dec; IR (KBr) ν_{max} 3440, 3300, 1675, 1615 cm^{-1}.

Anal. Calcd for $C_4H_7N_5O$: C, 34.04; H, 5.00; N, 49.63. Found: C, 34.29; H, 5.16; N, 49.34.

2-Amino-4-(2-acetylhydrazino)-6(1*H*)-pyrimidone (5). To a stirred suspension of 700 mg (4.96 mmol) of 2-amino-4-hydrazino-6(1*H*)-pyrimidone in 35 mL of dimethylformamide was added 600 mg (5.88 mmol) of acetic anhydride, and the mixture was heated under reflux until all suspended material dissolved (1 h). Cooling resulted in the separation of colorless crystals which were collected by filtration, washed with ethanol, and dried: yield 900 mg (99%); mp 254–256 °C. Recrystallization from acetic acid yielded colorless crystals of **5** containing one molecule of acetic acid of crystallization: mp 295–297 °C dec; NMR (Me_2SO-d_6) δ 1.75, 1.78 (2s, 6 H, $-COCH_3$, CH_3COOH), 4.63 (s, 1 H, C-5 H), 6.4 (br s, 2 H, NH_2).

Anal. Calcd for $C_6H_9N_5O_2 \cdot CH_3COOH$: C, 39.50; H, 5.39; N, 28.80. Found: C, 39.50; H, 5.39; N, 28.93.

2-Amino-4-(2-acetylhydrazino)-5-(1,2-dicarbethoxyhydrazino)-6(1*H*)-pyrimidone (6). Method A. A suspension of 3.0 g (16.4 mmol) of 2-amino-4-(2-acetylhydrazino)-6(1*H*)-pyrimidone and 11 g (63.2 mmol) of diethyl azodicarboxylate in 150 mL of dimethylformamide was stirred for 45 min at 90 °C. The resulting yellow solution was concentrated under reduced pressure to dryness, the solid residue was triturated with ethyl acetate, and the light tan crystals were collected by filtration, washed with ether, and dried to give 4.6 g (79%) of crude **6**. Recrystallization from ethyl acetate gave colorless crystals of **6**: mp 173–175 °C; NMR (Me_2SO-d_6) δ 0.8–1.3 (m, 6 H), 1.8 (s, 3 H), 3.7–4.4 (m, 4 H), 6.6 (br s, 2 H, NH_2); IR (KBr) ν_{max} 1725, 1650, 1600, 1255 cm^{-1}.

Anal. Calcd for $C_{12}H_{19}N_7O_6$: C, 40.33; H, 5.36; N, 27.44. Found: C, 40.04; H, 5.50; N, 27.26.

Method B. A mixture of 350 mg (2.48 mmol) of 2-amino-4-hydrazino-6(1*H*)-pyrimidone and 270 mg (2.65 mmol) of acetic anhydride in 25 mL of dimethylformamide was stirred at 100 °C for 1 h, and then 1.7 g (9.8 mmol) of diethyl azodicarboxylate was added. Stirring was continued for an additional 45 min at 100 °C, and the hot solution was concentrated under reduced pressure and then worked up as described above to give 620 mg (70%) of **6**, identical in all respects with the material prepared by method A.

2-Amino-4-(1-methylhydrazino)-6(1*H*)-pyrimidone (7). To a stirred solution of 15.0 g (103 mmol) of 2-amino-4-chloro-6(1*H*)-pyrimidone[12] in 250 mL of water was added 16 g (348 mmol) of methylhydrazine, and the mixture was heated under reflux for 1 h. During this time, the initial suspension became clear and then a white solid precipitated. The reaction mixture was cooled and filtered, and the collected solid was washed thoroughly with water followed by ethanol and dried to give 11.8 g (70%) of the hemihydrate of **7**: mp 286–288 °C dec; NMR (Me_2SO-d_6) δ 3.10 (s, 3 H), 5.01 (s, 1 H, C-5 H), 6.13 (s, 2 H, NH_2); IR (KBr) ν_{max} 1690, 1665, 1600 cm^{-1}.

Anal. Calcd for $C_5H_9N_5O \cdot 0.5H_2O$: C, 36.58; H, 6.14; N, 42.66. Found: C, 36.39; H, 6.33; N, 42.86.

2-Amino-4-(1-methyl-2-acetylhydrazino)-5-(1,2-dicarbethoxyhydrazino)-6(1*H*)-pyrimidone (8a). To a stirred suspension of 310 mg (1.89 mmol) of 2-amino-4-(1-methylhydrazino)-6(1*H*)-pyrimidone hemihydrate in 10 mL of dimethylformamide was added 220 mg (2.16 mmol) of acetic anhydride, the mixture was heated at 70 °C for 30 min, and 700 mg (4.02 mmol) of diethyl azodicarboxylate was added. A copious precipitate separated immediately. The reaction mixture was heated at 70 °C for an additional 30 min and was then cooled and filtered, and the collected colorless crystals were washed with ethanol followed by ether, yield 590 mg (84%). An analytical sample, mp 275–276 °C, was prepared by recrystallization from aqueous acetic acid: NMR (Me_2SO-d_6) δ 0.9–1.3 (m, 6 H), 1.63 (s, 3 H, $COCH_3$), 3.20 (s, 3 H, $-NCH_3$), 3.7–4.3 (m, 4 H), 6.55 (br s, 2 H, NH_2), 9.38 (s, 1 H, NH), 10.50 (s, 1 H, NH), 10.79 (s, 1 H, NH); IR (KBr) ν_{max} 3480, 1725, 1600, 1570 cm^{-1}.

Anal. Calcd for $C_{13}H_{21}N_7O_6$: C, 42.05; H, 5.70; N, 26.40. Found: C, 41.99; H, 5.80; N, 26.53.

2-Amino-4-(1-methyl-2-formylhydrazino)-5-(1,2-dicarbethoxyhydrazino)-6(1*H*)-pyrimidone (8b). To a stirred suspension of 310 mg (1.89 mmol) of 2-amino-4-(1-methylhydrazino)-6(1*H*)-pyrimidone hemihydrate in 25 mL of dimethylformamide was added 220 mg (2.5 mmol) of formylacetic anhydride, and the mixture was stirred at room temperature for 5 min. The resulting clear solution was heated to 100 °C, 700 mg (4.02 mmol) of diethyl azodicarboxylate was added, and the resulting mixture was heated at 100 °C for 2 h. Evaporation to dryness under reduced pressure, trituration of the solid residue with ethanol/ether, and filtration gave 630 mg (93%) of a colorless solid which was recrystallized from aqueous acetic acid: mp 265–266 °C dec; NMR δ 0.9–1.3 (m, 6 H), 3.18 (s, 3 H, $-NCH_3$), 3.8–4.3 (m, 4 H), 6.5 (br s, 2 H, NH_2), 7.87 (s, 1 H, -NCHO); IR (KBr) ν_{max} 1725, 1650, 1600, 1570 cm^{-1}.

Anal. Calcd for $C_{12}H_{19}N_7O_6$: C, 40.34; H, 5.36; N, 27.44. Found: C, 40.62; H, 5.52; N, 27.30.

7-Aminopyrimido[5,4-*e*]-*as*-triazine-3,5(2*H*,6*H*)-dione (7-Azaxanthopterin) (2). Method A. To a solution of 340 mg (14.8 mmol) of sodium in 50 mL of anhydrous ethanol was added 1.75 g (4.72 mmol) of 2-amino-4-(2-acetylhydrazino)-5-(1,2-dicarbethoxyhydrazino)-6(1*H*)-pyrimidone, and the resulting orange solution was heated under reflux for 2 h. During this time, the gelatinous, orange sodium salt of **2** separated. The solvent was removed under reduced pressure, the residual orange solid was dissolved in 40 mL of water, several drops of 1 N sodium hydroxide were added (until a clear solution was obtained), and 1.5 mL of glacial acetic acid was then added all at once. The bright orange solid which separated was collected by filtration, washed thoroughly with water, ethanol, and then ether, and dried: yield 550 mg (62%); mp >360 °C dec. This material was best purified by conversion to its trihydrate (see below), followed by regeneration as follows. A 50-mg (0.21-mmol) amount of the trihydrate of **2** was dissolved in 5 mL of 0.2 N sodium hydroxide, and the resulting solution was allowed to stand at room temperature for 5 h. Addition of glacial acetic acid caused the separation of 30 mg of the hemihydrate of 7-azaxanthopterin (**2**) as a bright orange powder: mp >300 °C (74% recovery); IR (KBr) ν_{max} 1665, 1605, 1495, 1410 cm^{-1}; UV (0.1 N NaOH) λ_{max} nm (log ϵ) 257 (4.38), 429 (3.70).

Identical results were obtained when 2-amino-4-(2-formylhydrazino)-5-(1,2-dicarbethoxyhydrazino)-6(1*H*)-pyrimidone (**8b**) was substituted for **8a** in the above procedure.

Anal. Calcd for $C_5H_4N_6O_2 \cdot 0.5H_2O$: C, 31.75; H, 2.66; N, 44.44. Found: C, 31.80; H, 2.71; N, 44.76.

Method B. To a solution of 500 mg (21.7 mmol) of sodium in 50 mL of anhydrous ethanol was added 1.48 g (4.15 mmol) of 2-amino-4-(1-methyl-2-acetylhydrazino)-5-(1,2-dicarbethoxyhydrazino)-6(1*H*)-pyrimidone, and the resulting orange solution was heated under reflux for 18 h. Solvent was removed under reduced pressure, the residual green solid was dissolved in 25 mL of water, and the solution was acidified with 6 N hydrochloric acid. The dark orange solid which separated was collected by filtration, washed thoroughly with water followed by ethanol, and dried; yield 530 mg (67%) of **2**, identical with the material prepared by method A.

The **trihydrate** of 7-azaxanthopterin (**2**) was prepared as follows. To a fine suspension of 40 mg (2.12 mmol) of 7-azaxanthopterin (hemihydrate) in 20 mL of water was added 0.5 mL of 1 N sodium hydroxide, and the resulting solution was filtered, heated to 70 °C, and then acidified by the addition of 200 mg of glacial acetic acid. The resulting orange solution was allowed to cool slowly to room temperature; filtration after 24 h gave 35 mg (71%) of amber needles, mp

>300 °C dec (changes crystalline form at 180 °C).

Anal. Calcd for $C_5H_4N_6O_2{\cdot}3H_3O$: C, 25.64; H, 4.30; N, 35.89. Found: C, 25.38; H, 4.59; N, 35.63.

2-Amino-4-(1,2-dimethylhydrazino)-6(1*H*)-pyrimidone (12). To a solution of 10 g (179 mmol) of potassium hydroxide in 175 mL of water was added 11.3 g (85 mmol) of *sym*-dimethylhydrazine dihydrochloride. To this solution was then added 5.75 g (39.5 mmol) of 2-amino-4-chloro-6(1*H*)-pyrimidone,[12] and the resulting mixture was heated to reflux. After 30 min, a clear solution was obtained, and shortly thereafter white crystals started to separate. Refluxing was continued for an additional 1.5 h, the reaction mixture was cooled, and the precipitate was collected by filtration: yield 5.65 g (85%); mp 300–303 °C; NMR (CF_3COOH) (Me_4Si as an external reference) δ 2.56 (s, 3 H, CH_3), 2.96 (s, 3 H, CH_3); IR (KBr) ν_{max} 3350, 3275 cm^{-1}.

Anal. Calcd for $C_6H_{11}N_5O$: C, 42.59; H, 6.55; N, 41.40. Found: C, 42.44; H, 6.75; N, 41.53.

2-Amino-4-(1,2-dimethylhydrazino)-5-(1,2-dicarbethoxyhydrazino)-6(1*H*)-pyrimidone (13). A suspension of 680 mg (4.02 mmol) of 2-amino-4-(1,2-dimethylhydrazino)-6(1*H*)-pyrimidone in 50 mL of dimethylformamide was heated to 100 °C, 775 mg (4.45 mmol) of diethyl azodicarboxylate was added, and the mixture was stirred at 100 °C for 15 min. The resulting orange solution was cooled to room temperature and filtered to remove a small amount of dark solid, and the filtrate was concentrated to dryness under reduced pressure. The gummy orange residue was dissolved in a small amount of ethyl acetate, and ether was added to induce crystallization. Filtration gave a cream-colored solid which was washed with ether, yield 1.12 g (81%). Recrystallization from ethyl acetate/ether gave colorless crystals, mp 192–194 °C dec; although microanalytical data for nitrogen were not satisfactory, the product was assigned structure **13** with confidence on the basis of its NMR spectrum in Me_2SO-d_6: δ 1.12 (t, 3 H), 1.16 (t, 3 H), 2.41 (s, 3 H, $-NHCH_3$), 3.21 (s, 3 H, C-4 NCH_3), 4.05 (2 superimposed quartets, 4 H); IR (KBr) ν_{max} 1750, 1705, 1635, 1595 cm^{-1}.

Anal. Calcd for $C_{12}H_{21}N_7O_5$: C, 41.98; H, 6.17; N, 28.56. Found: C, 41.65; H, 6.24; N, 27.50.

Registry No.—**2**, 68629-77-6; **4**, 6298-85-7; **5**, 6829-78-7; **6**, 68629-79-8; **7**, 67873-21-6; **8a**, 68629-80-1; **8b**, 68629-81-2; **12**, 68629-82-3; **13**, 68629-83-4; 2-amino-4-chloro-6(1*H*)-pyrimidone, 1194-21-4; hydrazine, 302-01-2; methylhydrazine, 60-34-4; *sym*-dimethylhydrazine dihydrochloride, 306-37-6; diethyl azodicarboxylate, 1972-28-7.

References and Notes

(1) We are grateful to the National Cancer Institute, National Institutes of Health (Grant No. CA 12876), and to Eli Lilly & Co. for financial support of this work.
(2) H. Wieland and C. Schopf, *Chem. Ber.*, **58**, 2178 (1925).
(3) R. Tschesche and H. L. Wolf, *Hoppe-Seyler's Z. Physiol. Chem.*, **240**, 127 (1936).
(4) M. L. Hesselbach and D. Burk, *Rec. Chem. Progr.*, **5**, 37 (1944).
(5) A. Haddow, W. C. J. Ross, and G. M. Timmis, *Perspect. Biol.*, **15**, 177 (1972).
(6) G. R. Pettit, L. E. Houghton, N. H. Rogers, R. M. Coomes, D. F. Berger, P. R. Reucroft, J. F. Day, J. L. Hartwell, and H. B. Wood, *Experientia*, **28**, 381 (1972).
(7) E. C. Taylor and P. A. Jacobi, *J. Am. Chem. Soc.*, **95**, 4455 (1973).
(8) E. C. Taylor, R. F. Abdulla, K. Tanaka, and P. A. Jacobi, *J. Org. Chem.*, **40**, 2341 (1975).
(9) E. C. Taylor and A. J. Cocuzza, *J. Org. Chem.*, **44**, 302 (1979).
(10) E. C. Taylor and F. Sowinski, *J. Org. Chem.*, **39**, 907 (1974).
(11) E. C. Taylor and F. Sowinski, *J. Org. Chem.*, **40**, 2321 (1975).
(12) H. S. Forrest, R. Hull, H. J. Rodda, and A. R. Todd, *J. Chem. Soc.*, 3 (1951).
(13) (a) F. Yoneda and T. Nagamatsu, *Tetrahedron Lett.*, 1577 (1973); (b) *J. Am. Chem. Soc.*, **95**, 5735 (1973); (c) F. Yoneda, T. Nagamatsu, and M. Ichiba, *J. Heterocycl. Chem.*, **11**, 83 (1974); (d) F. Yoneda and T. Nagamatsu, *ibid.*, **11**, 271 (1974).
(14) J. R. Totter, *J. Biol. Chem.*, **154**, 105 (1944).
(15) A. L. Lehninger, "Biochemistry", 2nd ed., Worth Publishers, Inc., New York, N.Y., 1975, p 874.
(16) T. C. Bruice and J. P. Taulane, *J. Am. Chem. Soc.*, **98**, 7769 (1976).
(17) (a) I. Yokoe and T. C. Bruice, *J. Am. Chem. Soc.*, **97**, 450 (1975); (b) E. L. Loechler and T. C. Hollocher, *ibid.*, **97**, 3235 (1975).

New Synthesis of 2-Amino-6-alkoxypyrazines from *N*-Nitrosobis(cyanomethyl)amine and Alkoxides

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Reaction of *N*-nitrosobis(cyanomethyl)amine with sodium alkoxides gives good yields of 2-amino-6-alkoxypyrazines in one step. Products were tentatively identified initially by spectral (IR, ^1H and ^{13}C NMR, UV, MS), polarographic, and elemental analytical methods. 2-Amino-6-methoxypyrazine was unequivocally identified by single-crystal X-ray examination.

During systematic studies on the analysis and anchimeric properties of *N*-nitrosamines by differential pulse polarography (DPP),[2-4] we examined *N*-nitrosobis(cyanomethyl)amine (**1**), $NCCH_2N(N{=}O)CH_2CN$, in neutral alcohol solutions and also in the presence of the corresponding sodium alkoxides. In neutral alcohols, **1** yields only a single 2e current–potential curve (E_p −1.20 V), but when sodium alkoxide was present as many as four curves were observed. The peak heights of these curves varied with time but finally one curve was obtained (E_p −1.94 V) whose height remained constant.

Since the final electroreducible product is stable, its preparation and identification were undertaken. Compound **1** was dissolved in methanol containing sodium methoxide and the solution was allowed to remain overnight at room temperature. Workup yielded a white, crystalline sublimate, mp 110–112 °C (**2**), in approximately 50% yield. The E_p of **2** was identical with that originally observed for the final stable product derived from **1**.

The product **2** was homogeneous on TLC in several systems. IR (KBr pellet) no longer showed C≡N absorption but a strong band was observed at 1650 cm^{-1}. UV (CH_3OH) showed two absorption bands at 324 nm (ϵ = 6420) and 242 nm (ϵ = 8360). The ^1H-NMR spectrum ($CDCl_3$) showed two sharp singlets at δ 7.41 (1 H) and 7.53 (1 H), a broad singlet centered at 4.3 (2 H), and sharp singlet at 3.8 (3 H). The two-proton signal at δ 4.3 disappeared immediately upon addition of D_2O. A ^{13}C-NMR proton-decoupled spectrum ($CDCl_3$–Me_2SO-d_6) showed five sharp signals. Mass spectral fragmentation of **2** gave a molecular ion peak at *m/e* 125. A chemical ionization mass spectrum of **2** in ammonia–methane showed only two peaks at *m/e* 126 and 58.[5] These values, coupled with elemental analyses and a value of 126 for the osmometric molecular weight, correspond to a molecular formula of $C_5H_7N_3O$ (**2**).

When **1** was similarly treated with ethanol–sodium ethoxide, a white, crystalline product (**3**), mp 77–79 °C, was obtained in about the same yield as **2**. The IR and UV spectra

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