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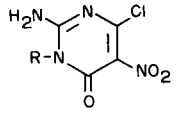
Synthesis of Some *N*-Methylated Pteridines Related to 7,8-Dihydroxanthopterin (1)

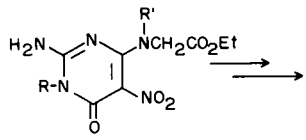
Helmut Zondler, H. S. Forrest and J. M. Lagowski

Seven methylated pteridines have been synthesized by condensation of a 4-chloro-5-nitropyrimidine with a β-ketoamine (or a blocked derivative), reduction of the nitro group, and subsequent ring closure. This scheme provides a new route to dihydroxanthopterin. Several cases are reported, however, in the uracil series where ring closure could not be effected.

In an attempt to establish unequivocally the identity of a naturally occurring pteridine as 7,8-dihydroxanthopterin, a number of methylated derivatives and some related methylpteridines were prepared. The presence of dihydroxanthopterin in freshly laid eggs of the hemipteran *Oncopeltus fasciatus* Dallas has been suggested on the basis of spectral evidence (2), but identification of an oxygen-sensitive compound occurring in small amounts (0.22 μg. per egg) requires an indirect approach. Our rationale was to release the contents of the eggs, including the unstable dihydroxanthopterin, in the presence of a methylating reagent and subsequently identify the pteridine as a more stable methylated derivative. Unfortunately, oxidation invariably preceded methylation and only methyl derivatives which could arise from either dihydroxanthopterin or xanthopterin were obtained.

All of the methylated compounds described were obtained by the same general procedure. An appropriate 6-chloro-5-nitropyrimidine was condensed with a β-ketoamine (or a blocked derivative), the nitro group was reduced, and ring closure was effected, without isolation of the 5,6-diaminopyrimidine, by short boiling in acid solution, which, in appropriate cases, also served to remove the group blocking the ketonic function. Thus, 2-amino-6-chloro-3,4-dihydro-3-methyl-5-nitro-4-oxopyrimidine condensed with the ethyl ester hydrochlorides of glycine and sarcosine to give pyrimidines I and I, R = R' = CH_3; reduction and subsequent ring closure gave 7,8-dihydro-3-methyl- (II) and 7,8-dihydro-3,8-dimethylxanthopterin (V), respectively. In analogous syntheses, 2-amino-6-chloro-3,4-dihydro-5-nitro-4-oxopyrimidine and the same two amino acid esters yielded pyrimidines VI and III, which in turn were converted into 7,8-dihydroxanthopterin (VII) (3) and 7,8-dihydro-8-methylxanthopterin (IV), respectively.

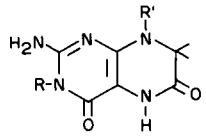




I R = CH_3, R' = H

III R = H, R' = CH_3

VI R,R' = H

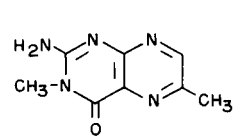


II R = CH_3, R' = H

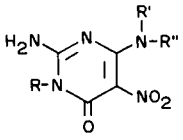
IV R = H, R' = CH_3

V R,R' = CH_3

VII R,R' = H

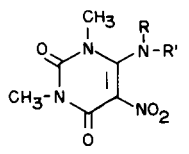


VIII



IX R = CH_3, R' = CH_2COOH, R'' = H

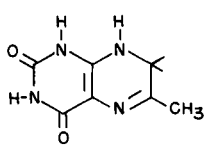
X R = CH_3, R' = $CH_2C(NOH)CH_3$, R'' = H



XI R = $C(CH_3)_2COCH_3$, R' = H

XII R = $CH_2CH(OEt)_2$, R' = H

XIII R = $CH_2CH(OEt)_2$, R' = CH_3



XIV

In a similar series of reactions, 2-amino-6-chloro-3,4-dihydro-3-methyl-5-nitro-4-oxopyrimidine condensed with 2,2-ethylenedioxypropylamine (4) to give, after hydrolysis, 6-acetonylamino-2-amino-3,4-dihydro-3-methyl-5-nitro-4-oxopyrimidine; reduction, ring closure, and oxidation of the product yielded 2-amino-3,4-dihydro-3,6-dimethyl-4-oxopteridine (VIII). The diamine obtained on reduction of the condensation product (IX) of the same chloropyrimidine with glycine, however, did not undergo the expected ring closure to give a pteridine. Similarly, the diamine derived from the condensation product (X) of 2-amino-6-chloro-3,4-dihydro-3-methyl-5-nitro-4-oxopyrimidine and 2-hydroxyiminopropylamine hydrochloride (5) could not be converted into a pteridine. The reason for these failures is not clear, although presumably they are due to formation of imidazo[1,2-*c*]pyrimidines as discussed by Zondler and Pfleiderer (6).

In the uracil series, a number of derivatives obtained in the same general way could not be converted into pteridines. Thus, 1,3-dimethyl-4-(1',1'-dimethylacetonyl)amino-5-nitrouracil (XI), 6-(2',2'-diethoxyethyl)amino-1,3-dimethyl-5-nitrouracil (XII), and 6-[*N*-(2',2'-diethoxyethyl)-*N*-methyl]amino-1,3-dimethyl-5-nitrouracil (XIII) (obtained by condensation of 4-chloro-1,3-dimethyluracil with 1-amino-1,1-dimethylacetone (7), 2,2-diethoxyethylamine (8), and *N*-methyl-2,2-diethoxyethylamine, respectively, followed by nitration) could be reduced to 5,6-diamino derivatives in a normal manner, but pteridines were not obtained on heating the resulting diamines in acid.

Finally, 6-methyllumazine and its dihydro derivative (XIV) were synthesized by the same procedure in which 4-chlorouracil was condensed with 2,2-ethylenedioxypropylamine followed by nitration of the product and final reduction of the nitro compound. Ring closure was readily effected in this case, and the resulting dihydro compound was oxidized with hydrogen peroxide to 6-methyllumazine.

The absorption spectra of the new compounds described above are given in Table I.

TABLE I

Ultraviolet Absorption Spectra (a)

	λ max, mμ (log ε)	
	*p*H 1	*p*H 13
2-Amino-3,4-dihydro-5-nitro-4-oxopyrimidine	354 (3.75), 286 (3.81), 245 (4.06), 221 (4.29)	374 (3.80), 320 sh (3.64), 273 (3.72), 218 (4.24)
6-(*N*-Ethoxycarbonylmethyl-*N*-methyl)amino (III)	333 (4.20), 290 sh (3.66), 233 sh (4.17), 213 (4.35)	342 (4.22)
6-Ethoxycarbonylmethylamino-3-methyl (I)	332 (4.20), 282 sh (3.64), 233 (4.20)	345 (4.24)
6-Ethoxycarbonylmethylamino (VI)	356 (3.88), 303 (3.74), 224 (4.38)	358 (4.09), 310 sh (3.82), 227 (4.37)
6-(*N*-Carboxymethyl-*N*-methyl)amino-3-methyl	333 (4.16), 290 sh (3.66), 232 sh (4.16), 214 (4.32)	342 (4.22), 235 sh (3.95)
6-Acetonylamino-3-methyl	333 (4.17), 290 sh (3.62), 241 sh (4.14) 213 (4.32)	342 (4.20)
6-Carboxymethylamino-3-methyl (IX)	334 (4.18), 290 sh (3.66), 233 (4.18), 214 (4.33)	343 (4.24), 237 sh (3.98)
6-(2'-Hydroxyiminopropyl)amino-3-methyl (X)		
5-Nitrouracil	335 (2.72), 226 (4.04)	311 (3.97), 220 sh (3.93)
1,3-Dimethyl-6-(1',1'-dimethylacetonyl)amino (XI)	333 (3.92), 242 (4.19)	338 (4.01), 275 (3.40), 220 (4.17)
6-(2',2'-Diethoxyethyl)amino-1,3-dimethyl (XII)	360 (3.38), 266 (4.01)	291 (3.91), 225 (4.11)
6-[*N*-(2',2'-Diethoxyethyl)-*N*-methyl]amino-1,3-dimethyl (XIII)		
7,8-Dihydroxanthopterin	300 sh, 278	281
3-Methyl (b) (II)	311 (3.93), 281 (4.04), 229 (4.20)	283 (4.03), 226 (4.27)
8-Methyl (IV)	308 (3.99), 283 (4.08), 229 (4.19), 213 (4.22)	312 sh (3.88), 283 (4.04), 230 (4.48)
3,8-Dimethyl (V)	325 (3.88), 246 sh (3.98), 229 (4.17)	358 (3.68), 274 (4.13), 240 (4.14)
2-Amino-3,4-dihydro-3,6-dimethyl-4-oxopteridine (VIII)	339 (3.54), 267 (3.84), 230 (3.88)	375 sh (3.16), 325 (3.48), 273 (3.85), 256 sh (3.76), 226 (4.07)
7,8-Dihydro-6-methyllumazine (XIV)	332 (3.93), 240 sh (4.05), 229 (4.11)	368 (3.80), 253 (4.26)
6-Methyllumazine		

(a) Spectra were measured with a Cary Model 14 spectrophotometer. (b) Values of log ε were not determined because of the instability of II in solution.

EXPERIMENTAL

2-Amino-6-ethoxycarbonylmethylamino-3,4-dihydro-3-methyl-5-nitro-4-oxopyrimidine (I).

2-Amino-6-chloro-3,4-dihydro-3-methyl-5-nitro-4-oxopyrimidine (2.04 g.) and glycine ethyl ester hydrochloride (2.0 g.) were dissolved in dimethylformamide (10 ml.) by gentle warming. On addition of triethylamine (3 ml.), an exothermic reaction occurred and a solid precipitated. The solid was redissolved by addition of ethanol with heating. Upon cooling, the clear solution deposited crystalline I, which was collected, washed with ethanol, and dried. A further quantity of I was obtained by evaporation of the mother liquors and crystallization of the residue from ethanol. The total yield was 2.5 g. (92%). An analytical sample was prepared by recrystallization from water, m.p. 192°.

Anal. Calcd. for $C_9H_{13}N_5O_5$: C, 39.9; H, 4.8; N, 25.8. Found: C, 40.0; H, 4.9; N, 25.7.

7,8-Dihydro-3-methylxanthopterin Hydrochloride (II·HCl).

A methanolic solution of I (1.35 g.) was reduced at atmospheric pressure using 10% palladium on charcoal (130 mg.) as catalyst. After theoretical uptake of hydrogen, sufficient hydrochloric acid was added, with gentle heating, to dissolve the precipitated reduction product. The catalyst was then removed by filtration, and II·HCl (830 mg., 72%) crystallized from the filtrate upon cooling. An analytical sample was prepared by recrystallization from *N* hydrochloric acid.

Anal. Calcd. for $C_7H_9N_5O_2 \cdot HCl$: C, 36.3; H, 4.3; N, 30.2. Found: C, 36.2; H, 4.8; N, 30.1.

2-Amino-6-(*N*-ethoxycarbonylmethyl-*N*-methyl)amino-3,4-dihydro-5-nitro-4-oxopyrimidine (III).

In a reaction similar to that described above, a solution of 2-amino-6-chloro-3,4-dihydro-5-nitro-4-oxopyrimidine (950 mg.) and sarcosine ethyl ester hydrochloride (1.0 g.) in dimethylformamide (4.0 ml.) was treated with triethylamine (1.5 ml.). After acidification (acetic acid) with warming, filtering, and cooling, the reaction mixture yielded a yellow product (830 mg., 62%) which was collected and recrystallized from water, m.p. 203-204°.

Anal. Calcd. for $C_8H_{13}N_5O_5$: C, 39.9; H, 4.8; N, 25.8. Found: C, 39.6; H, 4.8; N, 25.7.

7,8-Dihydro-8-methylxanthopterin (IV).

A methanolic solution of III (1.35 g.) was hydrogenated as described above. Concentrated hydrochloric acid (10 ml.) was added to the reduction mixture, followed by sufficient hot water to effect solution of the product, and the whole was filtered. Upon cooling, 550 mg. (56%) of IV separated from the filtrate. An analytical sample, prepared by recrystallization from water, melted at 350°.

Anal. Calcd. for $C_7H_9N_5O_2$: C, 43.1; H, 4.6; N, 35.9. Found: C, 43.1; H, 4.8; N, 35.7.

2-Amino-6-(*N*-carboxymethyl-*N*-methyl)amino-3,4-dihydro-3-methyl-5-nitro-4-oxopyrimidine (I, R = R' = CH_3).

To 2-amino-6-chloro-3,4-dihydro-3-methyl-5-nitro-4-oxopyrimidine (2.04 g.) in dimethylformamide (5 ml.) was added a solution of sarcosine (2.0 g.) in dimethylformamide (5 ml.) and water (5 ml.). The mixture was heated briefly, cooled, and acidified (acetic acid). On the addition of water (40 ml.), the product (2.05 g., 80%) separated and was recrystallized from water, m.p. 219°.

Anal. Calcd. for $C_8H_{11}N_5O_5$: C, 37.4; H, 4.3; N, 27.2. Found: C, 37.7; H, 4.5; N, 27.2.

7,8-Dihydro-3,8-dimethylxanthopterin Hydrochloride (V·HCl).

The above 6-(*N*-carboxymethyl-*N*-methyl)aminopyrimidine (1.50 g.) was suspended in methanol (50 ml.) and hydrogenated over 10% palladium on charcoal. The suspension was treated with 4 *N* hydrochloric acid (75 ml.), the whole refluxed for 5 minutes and filtered. The product (1.20 g., 84%), which separated on cooling, was collected, washed with ethanol, and dried. An analytical sample was prepared by recrystallization from 2 *N* hydrochloric acid, m.p. 350°.

Anal. Calcd. for $C_8H_{11}N_5O_2 \cdot HCl$: C, 39.1; H, 4.9; Cl, 14.5; N, 28.5. Found: C, 39.1; H, 5.3; Cl, 14.5; N, 28.3.

2-Amino-6-ethoxycarbonylmethylamino-3,4-dihydro-5-nitro-4-oxopyrimidine (VI).

Treatment of a solution of 2-amino-6-chloro-3,4-dihydro-5-nitro-4-oxopyrimidine (2.65 g.) and glycine ethyl ester hydrochloride (2.50 g.) in dimethylformamide (10 ml.) and methanol (10 ml.) with triethylamine (3 ml.) yielded VI (2.6 g., 73%) which was recrystallized from ethanol, m.p. 280°.

Anal. Calcd. for $C_8H_{11}N_5O_5$: C, 37.4; H, 4.3. Found: C, 37.8; H, 5.2.

7,8-Dihydroxanthopterin Hydrochloride (VII·HCl).

Compound VI (2.05 g.) was hydrogenated in a mixture of water (40 ml.), *N* sodium hydroxide (15 ml.), and methanol (10 ml.) in the presence of Raney nickel. After theoretical hydrogen uptake (5 hours), the catalyst was removed by filtration, the filtrate treated with water (80 ml.), the whole boiled and filtered, and concentrated hydrochloric acid (10 ml.) added to the filtrate. After allowing the solution to cool, 1.3 g. (90%) of VII·HCl was obtained. If sulfuric acid was substituted for hydrochloric acid in this procedure, VII·0.5 H_2SO_4 (3), was obtained.

6-Acetonylamino-2-amino-3,4-dihydro-3-methyl-5-nitro-4-oxopyrimidine.

To a solution of 2-amino-6-chloro-3,4-dihydro-3-methyl-5-nitro-4-oxopyrimidine (0.5 g.) in ethanol (10 ml.) containing triethylamine (0.3 ml.) was added 2,2-ethylenedioxypropylamine (0.4 ml.) (4), and the whole refluxed for 5 minutes. The crude product, which separated on cooling, was collected and dissolved in boiling 50% aqueous ethanol (100 ml.) containing concentrated hydrochloric acid (6 ml.). The solution was evaporated to dryness *in vacuo* and the residue crystallized from water, yield 0.38 g. (64%). An analytical sample was recrystallized from 1.5 *N* hydrochloric acid, m.p. 246° dec. (melting point apparatus preheated to 240°).

Anal. Calcd. for $C_8H_{11}N_5O_4$: C, 39.8; H, 4.6; N, 29.0. Found: C, 40.5; H, 4.8; N, 29.4.

2-Amino-3,4-dihydro-3,6-dimethyl-4-oxopteridine (VIII).

The 6-acetonylaminopyrimidine (2.85 g.) was hydrogenated in ethanol (50 ml.) containing hydrochloric acid (6 *N*; 10 ml.) using 10% palladium on charcoal as catalyst. After theoretical hydrogen uptake, the suspension was treated with sufficient hydrochloric acid to effect solution of the product using gentle heat. The catalyst was removed by filtration, and the filtrate was treated with hydrogen peroxide (30%; 2 ml.). After standing overnight at 4°, the reaction mixture was evaporated to *ca.* 50 ml.; the VIII·H_2O (0.4 g.) which precipitated was collected. Neutralization of the filtrate gave a further quantity of VIII·H_2O (0.72 g.). The combined product (1.12 g., 45%) was recrystallized twice from water and dried at 100°.

Anal. Calcd. for $C_8H_9N_5O \cdot H_2O$: C, 45.9; H, 5.3; N, 33.5. Found: C, 45.6; H, 5.1; N, 33.7.

2-Amino-6-carboxymethylamino-3,4-dihydro-3-methyl-5-nitro-4-oxopyrimidine (IX).

Condensation of 2-amino-6-chloro-3,4-dihydro-3-methyl-5-nitro-4-oxopyrimidine (0.5 g.) and glycine (0.4 g.) in 50% aqueous dimethylformamide (40 ml.) gave, on concentration of the reaction mixture and subsequent cooling, 0.35 g. (83%) of IX. An analytical sample was recrystallized twice from water, m.p. 280-285° dec.

Anal. Calcd. for $C_7H_9N_5O_5$: C, 34.6; H, 3.7; N, 28.8. Found: C, 34.6; H, 3.9; N, 28.7.

2-Amino-3,4-dihydro-6-(2'-hydroxyiminopropyl)amino-3-methyl-5-nitro-4-oxopyrimidine (X).

A solution of 2-amino-6-chloro-3,4-dihydro-3-methyl-5-nitro-4-oxopyrimidine (0.41 g.) in ethanol (5 ml.) was boiled with 2-hydroxyiminopropylamine hydrochloride (0.3 g.) (5) for a few minutes. Water (64 ml.) was added to the boiling suspension to give a clear solution, from which the product (0.42 g., 97%) separated on cooling. Two recrystallizations from aqueous ethanol gave pure X, m.p. 238-239° dec.

Anal. Calcd. for $C_8H_{12}N_6O_4$: C, 37.5; H, 4.7; N, 32.8. Found: C, 37.6; H, 4.8; N, 32.6.

1,3-Dimethyl-4-(1',1'-dimethylacetonyl)amino-5-nitrouracil (XI).

Fuming nitric acid (3.5 ml.) was added to a solution of 4-chloro-1,3-dimethyluracil (3.5 g.) in concentrated sulfuric acid (10 ml.), maintaining the temperature below 20°. The nitration mixture was poured onto ice and the whole extracted with chloroform. The chloroform extract was dried over magnesium sulfate and concentrated to *ca.* 5 ml. To the chloroform solution of the nitropyrimidine was added 1-amino-1,1-dimethylacetone hydrochloride (2.70 g.) (7) and triethylamine (4 ml.) in ethanol (7 ml.), and the whole was warmed to give a clear red solution. The solution was evaporated and the residue (2.05 g., 30%) crystallized from 50% aqueous ethanol, m.p. 159°.

Anal. Calcd. for $C_{11}H_{16}N_4O_5$: C, 46.5; H, 5.7; N, 19.7. Found: C, 46.6; H, 5.7; N, 19.6.

6-(2',2'-Diethoxyethyl)amino-1,3-dimethyl-5-nitrouracil (XII).

After nitration of 4-chloro-1,3-dimethyluracil (5.23 g.) by the procedure given above, the chloroform solution of the nitro compound was evaporated to *ca.* 25 ml. and treated slowly with a mixture of 2,2-diethoxyethylamine (4.5 ml.) (8) and triethylamine (3.5 ml.) in chloroform (10 ml.). The syrup obtained after heating 10 minutes on a water bath and evaporation was triturated with acetone to cause precipitation of triethylamine hydrochloride, which was removed by filtration. The filtrate was again evaporated to a syrup and triturated with ether. The resulting solid XII (7.0 g., 62%) was collected and recrystallized twice from ethanol-ether, m.p. 96-97°.

Anal. Calcd. for $C_{12}H_{20}N_4O_6$: C, 45.6; H, 6.4; N, 17.7. Found: C, 45.7; H, 6.5; N, 17.9.

6-[*N*-(2',2'-Diethoxyethyl)-*N*-methyl]amino-1,3-dimethyl-5-nitrouracil (XIII).

In a similar preparation, substituting *N*-methyl-2,2-diethoxyethylamine for 2,2-diethoxyethylamine, 4-chloro-1,3-dimethyluracil (3.5 g.) yielded XIII (3.30 g., 42%) which was recrystallized twice from 50% aqueous ethanol, m.p. 112°.

Anal. Calcd. for $C_{13}H_{22}N_4O_6$: C, 47.3; H, 6.7; N, 17.0. Found: C, 47.7; H, 6.7; N, 17.3.

7,8-Dihydro-6-methyllumazine (XIV).

6-(2',2'-Ethylenedioxypropyl)amino-5-nitrouracil (2.53 g.), obtained as described above, was reduced in 0.2 *N* sodium hydroxide (50 ml.) using 10% palladium on charcoal as catalyst. At completion of reduction, concentrated hydrochloric acid (10 ml.) was added with heating to effect solution of the product, and the catalyst was removed by filtration. On cooling, XIV·HCl (1.15 g., 47%) separated from the filtrate and was recrystallized from 0.5 *N* hydrochloric acid.

Anal. Calcd. for $C_7H_8N_4O_2 \cdot H_2O \cdot HCl$: C, 35.8; H, 4.7; N, 23.9. Found: C, 36.1; H, 4.6; N, 23.7.

The free base (XIV) was obtained by recrystallization of XIV·HCl from water, m.p. 300° dec.

Anal. Calcd. for $C_7H_8N_4O_2$: C, 46.7; H, 4.5; N, 31.1. Found: C, 46.7; H, 4.4; N, 29.6.

6-Methyllumazine.

To a solution of XIV·HCl (260 mg.) in hot water (80 ml.) was added hydrogen peroxide (30%, 0.5 ml.). After standing overnight at 4°, the mixture was evaporated to about 12 ml.; the crystalline product began to separate. The suspension was heated to effect complete solution and allowed to cool slowly. After 12 hours, the crystals were collected, washed with water, and dried at 100° *in vacuo* over phosphorus pentoxide, yield 140 mg. (71%), m.p. 330-332°.

Anal. Calcd. for $C_7H_6N_4O_2$: C, 47.2; H, 3.4; N, 31.4. Found: C, 46.6; H, 3.2; N, 31.1.

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(1) This work was supported by U. S. Public Health Grants GM-12323, AM-09973, and career development award 5-K3-GM-22,684 (JML), and by the Robert A. Welch Foundation, Houston, Texas.

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