TABLE II

ANALYSES OF OXIDIZED METHYL OLEATE[a] FRACTIONS AFTER COUNTERCURRENT DISTRIBUTION

	Tube number								
	0	1	2-3	4-5	6-7	8-9	10	11-12	26-27
Peroxide value	3500		3070		3140	4010			140
Hydrogen no.		297	269	279	211	202	202	197	300
Hydroxyl, %			4.0			3.6			
Mole peroxide / Mole ester	0.58		0.51		0.52	0.66			0.02
Mole H_2 absorbed / Mole ester		1.11	1.22	1.18	1.56	1.63	1.63	1.67	0.99
Mole hydroxyl / Mole ester			0.74			0.67			
Acid no.			56.8			17.6			
Mol. wt.		318	324			322	327		303

[a] 0.854 mole oxygen absorbed per mole ester. Peroxide value of original sample = 2520 or 0.408 mole peroxide/mole methyl oleate.

that the lower numbered tubes are made up of a mixture of monomeric diperoxide and C_9 acids, 23% by weight of the acids would account for the lowering of the molecular weight of the material in these tubes.

The molecular weight determinations are of particular interest in that they give no evidence of polymer formation in the oxidation of methyl oleate.

Another oxidation and countercurrent distribution experiment is presented in Fig. 2 (and in Table II). It was similar to the one just described except that the oxidation was carried to 0.854 mole oxygen per mole ester. The results obtained are in general agreement. More of the ester was, of course, found to be oxidized. The divergence between weight of the oxidized fraction predicted on a simple monoperoxide basis and the actual weight was greater than before; whereas, 85.4% is calculated to have been oxidized on a simple monoperoxide reaction basis, only 58.0% was found to be oxidized experimentally. The average level of oxidation of the low number tubes calculated in a manner similar to that described above was 2.45 moles oxygen per mole ester.

Chief differences between the results of these experiments came in the increased amounts of material in the oxidized parts of the curve, particularly the peak in tube no. 1. The evidence that the third component is relatively increased over the second at the higher oxidation level is interpreted as showing that after the monohydroperoxide is formed, the subsequent attack of oxygen in either gaseous or peroxidic form is at the ethylenic bond.

Acknowledgment.—The authors are grateful to J. C. Cowan for his interest and encouragement throughout the course of the work.

PEORIA 5, ILL. RECEIVED NOVEMBER 2, 1950

[CONTRIBUTION FROM THE PHARMACEUTICAL RESEARCH SECTION, CALCO CHEMICAL DIVISION, AMERICAN CYANAMID COMPANY]

Analogs of Pteroylglutamic Acid. VII. 2-Alkylamino Derivatives

BY BARBARA ROTH, JAMES M. SMITH, JR., AND MARTIN E. HULTQUIST

2-Alkylamino analogs of pteroylglutamic acid and its 4-amino derivative have been synthesized by the reaction of the appropriate 5,6-diaminopyrimidines with 2,3-dibromopropanal and *p*-aminobenzoylglutamic acid. A number of new 2-alkylaminopyrimidines and pteridines are described.

The synthesis of a number of analogs of pteroylglutamic acid (I)[1] has been described in earlier communications from this Laboratory,[2] and since some of these are potent antagonists[3] for I it seemed advisable to investigate further modifications in the molecule.

The toxicity of N-[4-(2,4-diamino-6-pteridylmethyl)-aminobenzoyl]-glutamic acid (II) ("Aminopterin," "4-aminopteroylglutamic acid") was greatly reduced by the substitution of alkyl radicals on the 4-amino group.[4] The present paper describes the preparation of some analogs of I, and other pteridines, substituted in the 2-position by alkylamino groups.

2-Dimethylaminopyrimidines were synthesized by the condensation of 1,1-dimethylguanidine with cyanoacetic ester and malononitrile, to give 2-dimethylamino-4-hydroxy-6-aminopyrimidine and 2-dimethylamino - 4,6 - diaminopyrimidine, respectively. These compounds were readily nitrosated in the 5-position and reduced to 5,6-diaminopyrimidines which reacted with diacetyl to produce 2-dimethylamino-4-hydroxy- and 2-dimethylamino-

(1) C. W. Waller, *et al.*, THIS JOURNAL, **70**, 19 (1948); R. B. Angier *et al.*, *Science*, **103**, 667 (1946).

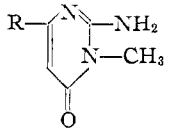
(2) D. B. Cosulich, *et al.*, THIS JOURNAL, **73**, 2554 (1951).

(3) (a) J. B. Thiersch and F. S. Philips, *Am. J. Med. Sciences*, **217**, 575 (1949); (b) J. M. Smith, Jr., *New Jersey J. Pharm.*, **22**, No. 8, 15 (1949).

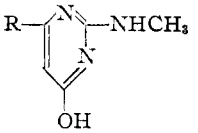
(4) B. Roth, J. M. Smith, Jr., and M. E. Hultquist, THIS JOURNAL, **72**, 1914 (1950).

4-amino-6,7-dimethylpteridines. The 2-dimethylamino analogs of I and II were prepared by modifications of the Waller reaction,[1] using 2,3-dibromopropanal, *p*-aminobenzoylglutamic acid, and the appropriate 2-dimethylamino-5,6-diaminopyrimidines. Purification was accomplished by methods similar to those previously described.

Majima[5] reported that the reaction of methylguanidine with ethyl acetoacetate gave two isomeric pyrimidine derivatives (III and IV, R = CH_3), and the structures were proved by hydrolysis of the amino groups to yield known hydroxy derivatives. With allylguanidine the results were similar. On the other hand, only the 2-methylamino isomers were obtained with acetylacetone or diethyl 2,2-



III

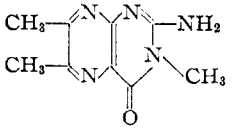


IV

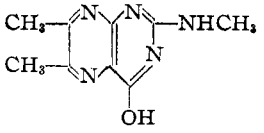
diethylmalonate. The 2-alkylamino isomers were reported to have lower melting points and to be more soluble in water and in alcohol than the 3-alkylated compounds. It has been found in this Laboratory that two pyrimidines can be separated from the products of the reaction of ethyl cyanoacetate with methylguanidine, and to the lower melting and more soluble substance has been assigned the 2-methylamino structure (IV, R = NH_2). It gives an orange nitroso derivative, while the higher melting substance, believed to be the 3(or 1)-methyl isomer (III, R = NH_2), yields a deep bluish-red nitroso derivative.

Attempts were made to synthesize 2-methylamino-4-hydroxy-6-aminopyrimidine by another route, *i.e.*, from 2-methylmercapto-4-hydroxy-6-aminopyrimidine[6] and methylamine. Although no reaction took place in alcoholic medium at 200°, the molecule was almost completely destroyed at 150–200° in water, and at 120° a mixture resulted; it is believed that in addition to replacement of the methylmercapto group by methylamino, replacement of the 6-amino group also took place to a considerable extent. At 100° there was obtained a substance similar to the lower-melting isomer (IV, R = NH_2) from the methylguanidine–cyanoacetic ester condensation. It gave an orange nitroso derivative, but could not be obtained entirely pure.

Both III and IV (R = NH_2) were nitrosated and reduced to the 5,6-diamines, which upon treatment with diacetyl produced pteridines of quite different properties. The 2-methylamino compound (VI) melted at 277–281°, while the isomer (V) did not melt below 370°. A consideration of the infrared data indicated that the assigned structures for V and VI, and hence III and IV, are in accord with the evidence.



V



VI

(5) R. Majima, *Ber.*, **41**, 176 (1908).

(6) C. O. Johns and E. J. Baumann, *J. Biol. Chem.*, **14**, 384 (1913).

The rocksalt region infrared spectrum of V shows medium strength primary amino absorptions at 3405 and 3225 cm.$^{-1}$ and weak hydroxyl absorption at 3395 cm.$^{-1}$. A strong ketonic carbonyl band is present at 1680 cm.$^{-1}$. Bands at 1658 and 1522 cm.$^{-1}$ indicate the presence of the >C=NH grouping. This infrared evidence shows that V is a keto–enol mixture, probably 2-amino-3,6,7-trimethyl-4-(3H)-pteridinone and 2,3-dihydro-2-imino-4-hydroxy-3,6,7-trimethylpteridine with the former present in predominant amount. The complete spectrum of V is given in Table I. The infrared spectrum of VI shows secondary amino absorptions at 3175 and 1556 cm.$^{-1}$ and an hydroxyl band at 3310 cm.$^{-1}$. A strong carbonyl absorption is present at 1682 cm.$^{-1}$ and a medium >C=N—C band at 1635 cm.$^{-1}$. This evidence reveals VI as a keto–enol mixture of 2-methylamino-4-hydroxy-6,7-dimethylpteridine and 2-methylamino-6,7-dimethyl-4-(3H)pteridinone. The complete spectrum of VI is given in Table II.

TABLE I[a]

INFRARED SPECTRUM OF V

3405 cm.$^{-1}$	1233 s.	1680 s.	1002 s.
3395 w.	1197	1658	951 w.
3225	1188	1612	833
2925	1102	1589	817
2840 w.	1096	1522	797 v.w.
2765 w.	1044	1487	762 w.
2718	1014	1387 s.	747
1363 s.	732	1359	717 w.
1313 s.	691 v.w.	1272 w.	684 v.w.
1243 s.			

TABLE II[a]

INFRARED SPECTRUM OF VI

3310 cm.$^{-1}$	1153	1682 s.	857
3175	1115 w.	1635	845
3025	1094 w.	1600	821
2925	1035	1556	815
2858	998 s.	1496	792 w.
2735	979	1387 s.	765
1365 s.	754 v.w.	1318	723
1288	718 w.	1273 s.	694 v.w.
1266 s.	692 v.w.	1214	684 v.w.
1174			

[a] Infrared spectra of both V and VI were obtained as Nujol mulls and also as perfluoro–kerosene mulls in the rocksalt region. All spectra were run on a Perkin–Elmer Model 12A Spectrometer.

The ultraviolet absorption spectra of V and VI are shown in Fig. 1 (curves D and B) and maxima for VI occur at intermediate points between those of 2-amino-4-hydroxy-6,7-dimethylpteridine (A) and 2-dimethylamino-4-hydroxy-6,7-dimethylpteridine (C) as expected. The curve for V is very different.

The 2-methylamino-4-hydroxy-5,6-diaminopyrimidine also reacted with benzil to form a 6,7-diphenylpteridine, which was chlorinated and treated with ammonia in an attempt to prepare the known 2-methylamino-4-amino-6,7-diphenylpteridine[7] which has been synthesized by a different method. A mixture was obtained consisting largely of 2,4-diamino-6,7-diphenylpteridine. This type of reaction, *i.e.*, exchange of amino groups with the reactant amine or ammonia, has been observed

(7) C. K. Cain and E. C. Taylor, Jr., 116th Meeting, American Chemical Society, Atlantic City, N. J., September 18, 1949.

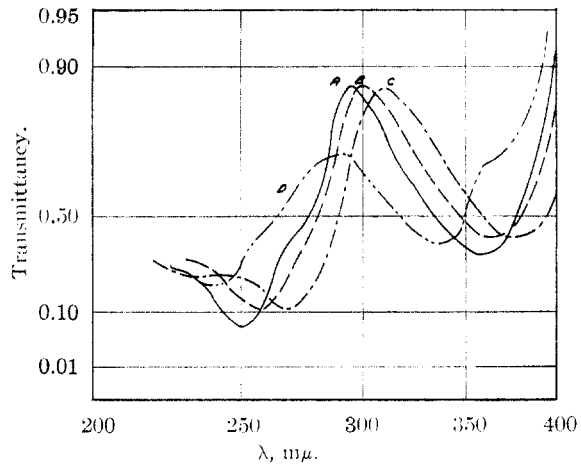


Fig. 1.—Ultraviolet absorption spectrum (10 mg./liter) in 0.1 *N* sodium hydroxide of: A, 2-amino-4-hydroxy-6,7-dimethylpteridine; B, 2-methylamino-4-hydroxy-6,7-dimethylpteridine; C, 2-dimethylamino-4-hydroxy-6,7-dimethylpteridine; D, 2-amino-3,6,7-trimethyl-4(3H)-pteridinone.

consistently in this Laboratory in treating chloro-, thio- or methylmercaptoaminopyrimidines with an excess of a primary amine or ammonia at higher temperatures.

N-[4-(2-Methylamino-4-hydroxy-6-pteridylmethyl)-aminobenzoyl]-glutamic acid resulted from the reaction of 2-methylamino-4-hydroxy-5,6-diaminopyrimidine, 2,3-dibromopropanal and *p*-aminobenzoylglutamic acid.[1] It was purified to 76%, as indicated by chemical assay.[8]

The biological activity of these compounds has been examined by Dr. B. L. Hutchings and Mr. A. C. Dornbush of the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York, and the details of this work will be reported elsewhere. The 2-alkylamino analogs have an antagonist activity much lower than II for *Streptococcus faecalis* R, as did the 4-alkylamino compounds.[4]

Experimental

2-Dimethylamino-4-hydroxy-6-aminopyrimidine.—To a solution of 25.8 g. (0.21 mole) of 1,1-dimethylguanidine hydrochloride[9] in 50 ml. of anhydrous methanol was added a solution of 22.7 g. (0.42 mole) of sodium methylate in 60 ml. of methanol. This mixture was heated under reflux and 20.7 g. (0.21 mole) of methyl cyanoacetate added dropwise over a ten-minute period. The mixture was then refluxed for three hours, filtered from sodium chloride, and neutralized with hydrochloric acid. A white precipitate was obtained, which was filtered off after cooling. After drying, this weighed 26.0 g., representing an 81% yield of 2-dimethylamino-4-hydroxy-6-aminopyrimidine. After recrystallization from water it melted at 290.5–292.5°.

Anal. Calcd. for $C_6H_{10}N_4O$: C, 46.7; H, 6.54; N, 36.3. Found: C, 46.6; H, 6.40; N, 36.6.

2-Dimethylamino-4-hydroxy-5-nitroso-6-aminopyrimidine.—Two grams of 2-dimethylamino-4-hydroxy-6-aminopyrimidine in 20 ml. of water was warmed and acidified with dilute sulfuric acid until all was in solution. The *p*H was adjusted to 4 with sodium acetate, and 0.74 g. of sodium nitrite in 2 ml. of water was added slowly at 80°. A dark purple solution was formed which yielded a red precipitate on cooling. Upon recrystallization from water, it melted with decomposition at 259°.

Anal. Calcd. for $C_6H_9N_5O_2$: C, 39.3; H, 4.84; N, 38.2. Found: C, 39.3; H, 4.84; N, 38.2.

2-Dimethylamino-4-hydroxy-5,6-diaminopyrimidine Sulfite.—Five grams of 2-dimethylamino-4-hydroxy-5-nitroso-6-aminopyrimidine was dissolved in 30 ml. of water with the minimum amount of dilute sodium hydroxide. Sodium dithionite was then added to this solution at 50° until the red color of the mixture had disappeared. This required approximately 10 g. On cooling, a yellow precipitate was formed, which was isolated; dry weight, 3.5 g. It was recrystallized from water in the presence of a trace of sodium dithionite and dried *in vacuo*.

Anal. Calcd. for $(C_6H_{11}N_5O)_2 \cdot H_2SO_3 \cdot H_2O$: C, 32.9; H, 5.98; N, 32.0; S, 7.31. Found: C, 33.4; H, 5.97; N, 32.6; S, 6.86.

N-[4-(2-Dimethylamino-4-hydroxy-6-pteridylmethyl)-aminobenzoyl]-glutamic Acid ("N^2,N^2-Dimethylpteroylglutamic Acid") (VII).—A solution of 20 g. of 2-dimethylamino-4-hydroxy-5,6-diaminopyrimidine sulfite in 330 ml. of water was acidified with hydrochloric acid, and the sulfur dioxide which was formed was removed by warming under vacuum. To the resultant solution was added 10.7 g. of *p*-aminobenzoylglutamic acid, and the *p*H was adjusted to 3.0 with sodium hydroxide. To this mixture at 45° were added dropwise, and simultaneously, solutions of 3.98 g. of sodium dichromate in 23 ml. of water, and 17.3 g. of 2,3-dibromopropanal in 16 ml. of glacial acetic acid, over a 20-minute period. The *p*H was maintained at 3 during this period. After an additional 20 minutes at 45°, the mixture was cooled to 10°, and the product filtered off. It weighed 21.3 g. and had a chemical assay[8] of 24.8% as VII. The crude material was purified as follows: 20 g. was mixed with 1600 ml. of water, heated to 80° and 37 ml. of 5 *N* sodium hydroxide added. After 15 minutes, a solution of 5.1 g. of calcium chloride in 14 ml. of water was added, and the mixture was filtered with the aid of Hyflo Super-Cel. The filtrate was adjusted with 10% zinc chloride to *p*H 10.8 and filtered again. It was then neutralized to *p*H 3, cooled to 5°, and filtered with the aid of Hyflo Super-Cel. The cake was taken up in dilute alkali and reprecipitated at *p*H 3 again. The resultant cake was then mixed with 375 ml. of water plus 4.5 g. of lime, heated at 80° for 15 minutes, and filtered. The filtrate was adjusted with 10% zinc chloride to *p*H 10.8, filtered again, and the solution neutralized to *p*H 3 with hydrochloric acid, cooled, and the precipitate filtered. The precipitate was mixed with 450 ml. of water and 0.25 g. of magnesium oxide added. After 15 minutes at 60°, 0.2 g. of Darco G-60 was added. The mixture was clarified, and the product precipitated at *p*H 3 again. It was then recrystallized twice from 180 volumes of 0.1 *N* hydrochloric acid, yielding a microcrystalline solid with a chemical assay[8] of 85.1% as VII. In 0.1 *N* sodium hydroxide VII exhibited maxima at 278 and 387 mμ, and minima at 247 and 343 mμ. In 0.1 *N* hydrochloric acid maxima were at 245 and 293 mμ, and minima at 234 and 266 mμ.

In another experiment, the first *p*H 3 filtrate, which contained 720 γ/ml. of the product by chemical assay,[8] was allowed to stand in the ice-box two days, yielding a small precipitate which was filtered. The filtrate from this was allowed to stand for two days more, yielding a yellow precipitate, which was filtered and dried.

Anal. Calcd. for $C_{21}H_{23}N_7O_6 \cdot H_2O$: C, 51.7; H, 5.17; N, 20.1. Found: C, 51.8; H, 5.70; N, 20.0.

2-Dimethylamino-4-hydroxy-6,7-dimethylpteridine.—To a solution of 2.9 g. of 2-dimethylamino-4-hydroxy-5,6-diaminopyrimidine sulfite in 30 ml. of warm water was added 1 g. of diacetyl. The mixture was heated at 85° for 45 minutes, cooled and neutralized with ammonia, yielding an orange precipitate. This was recrystallized twice from alcohol and then melted with decomposition at 283–288°. The substance showed ultraviolet absorption maxima at 268 and 377 mμ in 0.1 *N* NaOH; in 0.1 *N* HCl, maxima were at 248, 295 and 326 mμ.

Anal. Calcd. for $C_{10}H_{18}N_6O$: C, 54.8; H, 5.98; N, 32.0. Found: C, 54.8; H, 6.04; N, 32.3.

2-Dimethylamino-4,6-diaminopyrimidine.—A mixture of 50 g. (0.595 mole) of dicyandiamide and 100 g. (1.23 moles) of dimethylamine hydrochloride was heated at 180° for three hours, and then poured into 600 ml. of absolute alcohol. The solution was cooled to 10° and filtered from a

(8) B. L. Hutchings, *et al.*, *J. Biol. Chem.*, **168**, 705 (1947).
(9) E. A. Werner and J. Bell, *J. Chem. Soc.*, **121**, 1790 (1922).

small amount of white precipitate. To the filtrate was then added 58.5 g. (1.08 moles) of sodium methylate. The mixture was heated under reflux, and 66.7 g. (1.01 moles) of malononitrile added dropwise over a 20-minute period. Refluxing was continued for two hours, after which the mixture was cooled and the product filtered and washed with ice-water to remove salt. Sixty-one grams (40% of theory) of a white product was obtained, which after recrystallization from dilute alcohol melted at 259–260°.

Anal. Calcd. for $C_6H_{11}N_5$: C, 47.0; H, 7.24; N, 45.7. Found: C, 47.3; H, 7.14; N, 45.8.

2-Dimethylamino-4,6-diamino-5-nitrosopyrimidine.—To a suspension of 10 g. of 2-dimethylamino-4,6-diaminopyrimidine in 200 ml. of water was added 5 *N* sulfuric acid, to obtain solution, and sodium acetate to adjust the *p*H to about 4. The solution was heated to 85° and a 25% solution of sodium nitrite added slowly until a permanent spot on starch–potassium iodide paper was reached. A red precipitate was formed, which was filtered off after cooling. The dry weight was 11.2 g.; m.p. 283° (dec.).

Anal. Calcd. for $C_6H_{10}N_6O$: C, 39.6; H, 5.53; N, 46.1. Found: C, 39.6; H, 5.57; N, 46.0.

2-Dimethylamino-4,5,6-triaminopyrimidine Sulfate.—To 42.9 g. of 2-dimethylamino-4,6-diamino-5-nitrosopyrimidine in 550 ml. of water was added 5 *N* hydrochloric acid to *p*H 2.5. Sodium dithionite (130 g.) was then added slowly at approximately 60° until all of the red color of the nitroso compound was gone. The mixture was heated to 70° to complete the reaction and then acidified to approximately *p*H 2 with dilute sulfuric acid. The product precipitated as the sulfate, and was filtered after cooling well; dry weight, 56 g. It was purified for analysis by reprecipitation from alkaline solution with sulfuric acid.

Anal. Calcd. for $C_6H_{12}N_6 \cdot H_2SO_4$: C, 27.1; H, 5.30; N, 31.6; S, 12.0. Found: C, 27.0; H, 5.46; N, 31.5; S, 12.0.

N-[4-(2-Dimethylamino-4-amino-6-pteridylmethyl)-aminobenzoyl]-glutamic Acid ("N^2,N^2-Dimethyl-4-amino-pteroylglutamic Acid") (VIII).—A mixture of 21.3 g. of 2-dimethylamino-4,5,6-triaminopyrimidine sulfate and 19.5 g. of barium chloride dihydrate in 330 ml. of water was heated to 60° for ten minutes, cooled to 45°, and 10.7 g. of *p*-aminobenzoylglutamic acid added. The *p*H was adjusted to 3, and solutions of 3.98 g. of sodium dichromate in 23 ml. of water and 17.6 g. of 2,3-dibromopropanal in 16 ml. of glacial acetic acid were added dropwise over a 20-minute period, maintaining the *p*H at 3 with sodium hydroxide during the addition. Heating at 45° was continued for 20 minutes longer, after which the mixture was cooled and the product filtered, washed with water and acetone, and dried. This weighed 40.6 g., and contained 5.73 g. of VIII by chemical assay.[8] It was purified as follows: 38 g. of the crude product was mixed with 1600 ml. of water, heated to 80°, and 37 ml. of 5 *N* sodium hydroxide added. After all was in solution, 5.1 g. of calcium chloride in 13.2 ml. of solution was added, and the mixture filtered with the aid of Hyflo Super-Cel. The filtrate was adjusted to *p*H 10.8 with 10% zinc chloride solution, clarified, and neutralized to *p*H 3.8 with hydrochloric acid. After cooling well, the precipitate was filtered and mixed with 820 ml. of water plus 7.4 g. of lime. This was heated to 80° for 15 minutes, filtered with Hyflo Super-Cel, and the filtrate treated with 10% zinc chloride to a *p*H of 10.8. The mixture was again clarified and neutralized to *p*H 3.8. The product, isolated by filtration after cooling, was then taken up in 650 ml. of water, heated to 80°, and 0.7 g. of magnesium oxide added. After treating this mixture with 1 g. of Darco G-60, it was clarified and the filtrate neutralized to *p*H 4. The precipitate was filtered and recrystallized twice from 0.1 *N* hydrochloric acid and then treated again with magnesium oxide–Darco as above described. There was obtained 1.4 g. of a yellow microcrystalline solid having a chemical assay[8] of 79.9% as VIII. In 0.1 *N* sodium hydroxide solution VIII exhibited maxima at 275 and 397 mμ, and minima at 252 and 340 mμ. In 0.1 *N* hydrochloric acid maxima were at 253, 293 and 350 mμ, and minima at 242, 268 and 327 mμ.

2-Dimethylamino-4-amino-6,7-dimethylpteridine.—A mixture of 5 g. of 2-dimethylamino-4,5,6-triaminopyrimidine sulfate and 4.59 g. of barium chloride in 50 ml. of water was heated on the steam-bath for ten minutes and filtered hot. To the filtrate was added 1.62 g. of diacetyl, and the resultant solution heated 30 minutes on the steam-bath. Upon neutralizing with ammonia, a yellow precipitate formed. This was recrystallized from dilute alcohol, followed by recrystallization from dilute hydrochloric acid, which resulted in a bright yellow product obtained as the hydrochloride.

Anal. Calcd. for $C_{10}H_{14}N_6 \cdot HCl \cdot 3H_2O$: C, 38.9; H, 6.86; N, 27.2; Cl, 11.5. Found: C, 38.7; H, 6.67; N, 27.2; Cl, 11.3.

The substance showed ultraviolet absorption maxima at 271 and 385 mμ in 0.1 *N* sodium hydroxide, with a small maximum at 242 and a slight irregularity at 251 mμ; in 0.1 *N* hydrochloric acid, maxima were at 251 and 345 mμ with a slight maximum at 290 mμ.

2-Methylamino-4-hydroxy-6-aminopyrimidine (IV, R = NH_2).—A mixture of 1 kg. (14.8 moles) of methylamine hydrochloride and 620 g. (7.4 moles) of dicyandiamide was heated at 180° for 3.5 hours. The melt was then cooled to 100° and poured into 6 l. of absolute alcohol. The alcoholic solution was cooled and filtered from a trace of insoluble material. To this solution was slowly added 1,440 g. (26.6 moles) of sodium methylate, followed by the addition of 1,280 g. (13.3 moles) of ethyl cyanoacetate over a half-hour period. The mixture was refluxed for four hours, filtered from salt, and neutralized to approximately *p*H 7 with dilute hydrochloric acid. The hot mixture was filtered, yielding a precipitate (a) of 550 g. which melted between 270–280° (see 3-methyl-2,6-diamino-4(3H)-pyrimidone below). On cooling the liquor, 20 g. of additional material was deposited. The liquor was concentrated to a volume of 2 l. On cooling, a heavy precipitate (b) formed; dry weight, 442 g. This melted between 210–240°. This material was recrystallized several times from alcohol and from water, and then melted at 227–229°.

Anal. of (b). Calcd. for $C_5H_8N_4O \cdot H_2O$: C, 38.0; H, 6.37; N, 35.4. Found: C, 37.8; H, 6.91; N, 35.5.

2-Methylamino-4-hydroxy-6-aminopyrimidine from 2-Methylmercapto-4-hydroxy-6-aminopyrimidine.—2-Methylmercapto-4-hydroxy-6-aminopyrimidine was prepared according to the directions of Johns and Baumann.[6] A number of reactions were carried out between this compound and methylamine in an effort to replace the 2-methylmercapto- by methylamino-. A preliminary experiment in alcohol with a large excess (11 moles) of methylamine at 200° indicated no reaction. A second reaction in water at 200° led to complete decomposition. At 150° in water partial decomposition took place. The reaction was then repeated by heating 10 g. of the pyrimidine with 40 ml. of 25% aqueous methylamine solution for five hours at 120°. A white crystalline product was obtained which melted at 245–247.5° after recrystallization from water. The analysis indicated that the 4-amino group had been partially replaced by methylamino.

Anal. Calcd. for $C_5H_8N_4O$: C, 42.9; H, 5.75; N, 40.0. Calcd. for $C_6H_{10}N_4O$: C, 46.7; H, 6.54; N, 36.3. Found: C, 45.1; H, 6.63; N, 37.7.

A sample of this was nitrosated by the usual procedure, giving an orange nitroso derivative with the following analysis.

Anal. Calcd. for $C_6H_9N_5O_2$: C, 39.3; H, 4.95; N, 38.2. Found: C, 39.3; H, 5.13; N, 38.4.

The reaction between the methylmercaptopyrimidine and methylamine was then repeated by heating at 100° for three hours. A product was obtained which after recrystallization from water and then from alcohol melted at 229–237°, and gave a mixed melting point of 224–227° with the pyrimidine prepared from methylguanidine (m.p. 227–229°). It gave an orange nitroso derivative.

The reaction was then repeated at 100° using just two equivalents of methylamine. Incomplete reaction occurred. With several equivalents of methylamine at 80°, reaction was also incomplete.

2-Methylamino-4-hydroxy-5-nitroso-6-aminopyrimidine.—Nine grams of 2-methylamino-4-hydroxy-6-aminopyrimidine was nitrosated in the usual manner to yield an orange precipitate which formed immediately; weight, 9.5 g. It did not melt below 360°.

Anal. Calcd. for $C_5H_7N_5O_2 \cdot H_2O$: C, 32.1; H, 4.85; N, 37.4. Found: C, 31.7; H, 4.63; N, 37.0.

2-Methylamino-4-hydroxy-5,6-diaminopyrimidine Sulfate.—Nine grams of the above described nitroso derivative was reduced in the customary way with 22 g. of sodium dithionite. The product was isolated as the sulfate and puri-

fied by reprecipitation from dilute ammonium hydroxide with sulfuric acid, and dried *in vacuo*.

Anal. Calcd. for $C_5H_9N_5O \cdot {}^1/_2H_2SO_4 \cdot {}^1/_2H_2O$: C, 28.2; H, 5.20; N, 32.9; S, 7.52. Found: C, 28.5; H, 5.90; N, 33.5; S, 7.57.

2-Methylamino-4-hydroxy-6,7-dimethylpteridine.—One gram of 2-methylamino-4-hydroxy-5,6-diaminopyrimidine sulfate in 100 ml. of water at 60° was treated with 1 g. of diacetyl. The mixture was heated 15 minutes at 60–70° and allowed to stand overnight. It was concentrated to a 25-ml. volume, made faintly alkaline with ammonia, and the product collected on the filter. After recrystallization from 25 ml. of water, 0.22 g. of fine light yellow needles was obtained, m.p. 277–281°.

Anal. Calcd. for $C_9H_{11}N_5O \cdot H_2O$: C, 50.7; H, 5.62; N, 32.7. Found: C, 51.3; H, 5.78; N, 32.7.

2-Methylamino-4-hydroxy-6,7-diphenylpteridine.—A mixture of 1 g. of 2-methylamino-4-hydroxy-5,6-diaminopyrimidine sulfate and 0.57 g. of barium chloride in 20 ml. of water was heated on the steam-bath a few minutes and filtered from barium sulfate. The filtrate was added to a solution of 1 g. of benzil in 25 ml. of alcohol and refluxed for two hours, yielding a yellow precipitate. The mixture was cooled, neutralized with ammonia, filtered, and washed well with alcohol to remove unreacted benzil. The precipitate was extracted with warm dilute sodium hydroxide and neutralized with acetic acid to yield a light yellow precipitate. This was recrystallized twice from dimethylformamide and then decomposed between 346–354°.

Anal. Calcd. for $C_{19}H_{14}N_5O$: N, 21.3. Found: N, 20.9.

Chlorination of 2-Methylamino-4-hydroxy-6,7-diphenylpteridine.—A mixture of 0.175 g. of 2-methylamino-4-hydroxy-6,7-diphenylpteridine, 10 ml. of phosphorus oxychloride, and 0.7 g. of phosphorus pentachloride was refluxed for two hours; hydrogen chloride was evolved, and after two hours the material was all in solution. The excess phosphorus oxychloride was distilled off, and the residue poured onto ice, yielding a sirup which soon hardened to a yellow solid. This was isolated and dried in the vacuum desiccator; weight, 0.21 g.

Attempted Preparation of 2-Methylamino-4-amino-6,7-diphenylpteridine.—One gram of the above chlorinated product was mixed with 20 ml. of methanol saturated with ammonia at 0°, and heated in a sealed tube at 155° for 16 hours. The product proved to be a mixture melting between 260–280°. On recrystallization, a fraction melting between 280–284° was obtained which did not depress the melting point of 2,4-diamino-6,7-diphenylpteridine.

N-[4-(2-Methylamino-4-hydroxy-6-pteridylmethyl)-aminobenzoyl]-glutamic Acid.—The reaction of 2-methylamino-4-hydroxy-5,6-diaminopyrimidine sulfate with *p*-aminobenzoylglutamic acid and 2,3-dibromopropanal was carried out in a manner identical with that described for the preparation of VIII. From a reaction using 8.4 g. of the pyrimidine, 15.8 g. of a crude light brown product was obtained having a chemical assay of 11.4%. This represents a 23.8% yield based on the *p*-aminobenzoylglutamic acid used. The product was purified by methods similar to those described above for VII and VIII to give a chemical assay of 76.6%.

3-Methyl-2,6-diamino-4(3H)-pyrimidone Sulfate.—One hundred grams of the high-melting isomer (a) from the condensation of methylguanidine with ethyl cyanoacetate was recrystallized twice from 4 l. of water containing about 100 ml. of alcohol. There was obtained 34 g. melting at 265–272°, depending upon the rate of heating.

Eight grams of the above was dissolved in 400 ml. of water at 50°, and sulfuric acid was added to *p*H 2. Darco G-60 was added and the solution was filtered, and the filtrate cooled in the ice-box overnight. The white precipitate was filtered, washed with water, and dried to give 7.2 g. of the sulfate.

Anal. Calcd. for $C_5H_8N_4O \cdot {}^1/_2H_2SO_4 \cdot H_2O$: N, 8.0. Found: N, 7.7.

3-Methyl-2,6-diamino-5-nitroso-4(3H)-pyrimidone.—To 25 g. of the base from above in 2,500 ml. of water was added dilute sodium hydroxide to effect solution, and then 28 g. of sodium nitrite. Acetic acid was slowly added, and a tan precipitate was obtained which was filtered, suspended in 3,000 ml. of fresh water, and heated to 100°. A bluish-red nitroso compound resulted. It was purified by dissolving in dilute ammonia, clarifying with Darco G-60, and precipitating with acetic acid. The yield was 10.2 g.

Anal. Calcd. for $C_5H_7N_5O_2 \cdot H_2O$: N, 37.4. Found: N, 37.4.

3-Methyl-2,5,6-triamino-4(3H)-pyrimidone Sulfate.—Ten grams of the above described nitroso compound was dissolved in 300 ml. of water with the minimum amount of dilute sodium hydroxide and then at 60° 20 g. of sodium dithionite was added. A pale yellow solution resulted and, on cooling, a light cream precipitate appeared. It was recrystallized once from 100 ml. of warm water, then dissolved in 75 ml. of water, and dilute sulfuric acid was added. On cooling, a white crystalline product was obtained (5.1 g.).

Anal. Calcd. for $C_5H_9N_5O \cdot H_2SO_4$: C, 23.7; H, 4.38; S, 12.7. Found: C, 23.7; H, 4.4; S, 12.1.

2-Amino-3,6,7-trimethyl-4(3H)-pteridinone (V).—Two grams of the above triaminopyrimidone in 75 ml. of water at 40° was treated with 1 g. of diacetyl. The intense yellow color which appeared soon faded and a light colored precipitate formed. The mixture was warmed at 50° for a few minutes and cooled to give 1.1 g. of product. For analysis, it was recrystallized from water, with a Darco G-60 treatment. It began to sublime at 350–360°, and did not melt below 370°.

Anal. Calcd. for $C_9H_{11}N_5O$: C, 52.7; H, 5.40; N, 34.1. Found: C, 52.6; H, 5.70; N, 34.1.

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