Our attention has been called to the danger of denaturation which accompanies the lyophilization of proteins in the presence of zinc and other metal ions,[10] and to the fact that the use of ion-exchange columns for the removal of zinc before lyophilization has been a standard practice in the Harvard laboratories for several years.[11,12] It is possible that some of the purification achieved in the refractionation is due to the denaturation of components other than albumin, but in general the elimination of any denaturation would be desirable, and advantages to be gained by such a modification of the procedure will be reported on in the future.

The lyophilized material was dissolved in a minimal volume of 0.2 *M* acetate buffer, *p*H 4.5, and dialyzed against 4 changes of the same buffer, followed by eight changes of distilled water. Since the zinc was not entirely removed by this procedure, the solution was then passed through a column of Dowex-50 in the hydrogen or sodium cycle. It is important for the success of refractionation that all of the zinc be removed, as indicated by a test with dithizone. The salt-free, zinc-free material was then lyophilized for storage.

Refractionation was carried out by dissolving 500 mg. of fraction V in 5 ml. of water, cooling to 0°, adding 25 ml. of a solution containing 230 ml. of 95% EtOH per liter, with stirring, and finally 0.5 ml. of solution 2. The additions were made in the −5° bath, and after one hour the precipitate was removed by centrifugation for 30 minutes at 4000 r.p.m. The alcohol solution must contain dissolved CO_2. When freshly distilled water is used, the *p*H of the 21.9% ethanol solution is adjusted to 5.4–5.5 at 25° by bubbling in CO_2.

LOS ANGELES, CALIFORNIA

(10) Personal communication from Prof. John T. Edsall.

(11) W. L. Hughes, Jr., H. G. Psyras and W. C. Starr, in "The Separation of the Formed Elements, the Protein, Carbohydrate, Lipid, Steroid, Peptide and Other Components of Plasma," University Laboratory of Physical Chemistry, Harvard University, 1950.

(12) W. L. Hughes, *et al.*, in "Conference on New Mechanized Equipment for the Collection and Processing of Human Blood," University Laboratory of Physical Chemistry, Harvard University, 1951.

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

Analogs of Pteroylglutamic Acid. X. N^1-(Pteridyl-2)-sulfanilamides[1]

BY MARVIN J. FAHRENBACH, KENNETH H. COLLINS, MARTIN E. HULTQUIST AND JAMES M. SMITH, JR.

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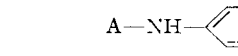
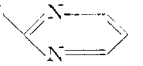
A number of N^4-acetyl-N^1-(substituted-pteridyl-2)-sulfanilamides have been prepared from N^4-acetyl-N^1-(4,5-diamino-6-hydroxypyrimidyl-2)-sulfanilamide by reaction with 1,2-diketones, diethyl oxalate and dichloroacetic acid. The N^4-acetyl group was hydrolyzed to yield the N^1-(substituted-pteridyl-2)-sulfanilamides.

It has been established that the sulfonamide drugs are antimetabolites for *p*-aminobenzoic acid (PABA) for many bacteria, and owe their therapeutic effectiveness to this fact.[3] The *p*-aminobenzoic acid moiety occurs in pteroylglutamic acid (I)[4] and it was logical to expect that the introduction of a *p*-aminobenzenesulfonamide grouping into I in the place of PABA might give interesting substances for biochemical studies, and possibly useful as antimetabolites of I. The first compound of this type to be reported was 4-(2-amino-4-hydroxypteridyl-6-methylamino)-benzenesulfonylglutamic acid (II), which was synthesized by Viscontini and Meier[5] by methods analogous to those used for I. Forrest and Walker subsequently prepared II also.[6] A somewhat similar compound, N^4-(2-amino-4-hydroxypteridyl-6-methyl)-sulfanilamide (III) was reported by Martin and Avakian,[7] and also by Sato, *et al.*[8] The same compound in pure form also was described by Forrest and Walker,[6] who were concerned with a theory of sulfonamide bacteriostasis which involved incorporation of sulfanilamide groups into biologically inert analogs of pteroic acid and pteroylglutamic acid. By the reaction of reductone with sulfanilamide, *p*-methylsulfonylaniline, sulfanilylglycine, diethyl sulfanilylglutamate and sulfadiazine, they obtained "reductone-anils" which were subsequently condensed with 2,4,5-triamino-6-hydroxypyrimidine to give pteridines of the general structures II–VI.



A = 2-amino-4-hydroxypteridyl-6-methyl-
I, R = $-CONHCH(COOH)CH_2CH_2COOH$
II, R = $-SO_2NHCH(COOH)CH_2CH_2COOH$
III, R = $-SO_2NH_2$
IV, R = $-SO_2CH_3$
V, R = $-SO_2NHCH_2COOH$
VI, R = $-SO_2NH$



These compounds (II–VI) were tested as antimetabolites of I for a strain of *Streptococcus lactis;* II and V inhibited growth and were reversed by I. All (II–VI) were inactive for the Walker rat carcinoma.

Three other related substances have been reported, namely, N^4-(2,4-diaminopteridyl-6-methyl)-sulfanilamide,[8] 4-(2,4-dihydroxypteridyl-6-methylamino)-benzenesulfonic acid[9] and sodium 4-(4-hydroxypteridyl-6-methylamino)-benzenesulfonate.[10] A number of derivatives of I bearing benzenesulfonyl groups on the 10-nitrogen have been synthesized by Weisblat and co-workers.[11]

Our work was undertaken with the purpose of preparing N^1-pteridylsulfanilamides analogous to Sulfadiazine and other chemotherapeutically important N^1-heterocyclic sulfanilamides. Compounds of the general structure VIII have been prepared.

(1) For the preceding paper in this series, see THIS JOURNAL, **75**, 4675 (1953).

(2) To whom inquiries regarding this paper should be addressed.

(3) For a review, see R. O. Roblin, Jr., *Chem. Revs.*, **38**, 263 (1946).

(4) R. B. Angier, *et al.*, *Science*, **103**, 667 (1946).

(5) M. Viscontini and J. Meier, *Helv. Chim. Acta*, **32**, 877 (1949).

(6) H. Forrest and J. Walker, *J. Chem. Soc.*, 2002 (1949).

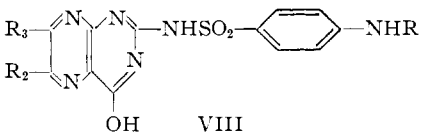
(7) G. J. Martin and S. Avakian, U. S. Patent 2,476,557, July 19, 1949.

(8) Hideo Sato, *et al.*, *J. Chem. Soc. Japan*, **72**, 866 (1951); *C. A.*, **47**, 5946 (1953).

(9) H. Urist and G. J. Martin, U. S. Patent 2,504,471, April 8, 1950.

(10) D. J. Brown, *J. Chem. Soc.*, 1644 (1953).

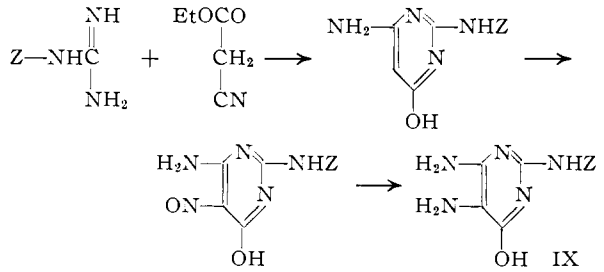
(11) D. Weisblat, *et al.*, THIS JOURNAL, **75**, 3625 (1953).



VIII

R_1 = H and CH_3CO (see Tables I and II)
R_2, R_3 may be = H, alkyl, aryl, OH

The essential intermediates were synthesized from N^4-acetylsulfaguanidine by condensation with cyanoacetic ester, followed by nitrosation and reduction to N^4-acetyl-N^1-(4,5-diamino-6-hydroxypyrimidyl-2)-sulfanilamide (IX).



IX

Z = p-$CH_3CONHC_6H_4SO_2$-

Subsequent condensation of IX with 1,2-diketones, diethyl oxalate or dichloroacetic acid yielded the compounds of type VIII, which are summarized in the tables. The N^4-acetyl compounds were hydrolyzed to the N^1-(pteridyl)-sulfanilamides by heating in aqueous sodium hydroxide solution (Table II). N^1-(4-Amino-6-hydroxypyrimidyl-2)-sulfanilamide was obtained from the N^4-acetyl compound by hydrolysis in alcoholic hydrogen chloride containing one mole of water. Purification of these pteridine derivatives was effected by repeated precipitations from aqueous alkali with acid. Both the N^4-acetyl compounds and the N^1-(pteridyl)-sulfanilamides were high-melting solids with very limited solubility in water or the common organic solvents.

N^4-Acetyl-N^1-(4-hydroxypteridyl-2)-sulfanilamide (X) (VIII, R_1 = CH_3CO, R_2 and R_3 = H) was obtained from IX and glyoxal, and it was hydrolyzed readily to N^1-(4-hydroxypteridyl-2)-sulfanilamide (XI) (VIII, R_1, R_2 and R_3 = H). Preparation of the xanthopterin analog (XII) (VIII, R = CH_3CO, R_2 = H, R_3 = OH) was accomplished in low yield by fusion of IX with dichloroacetic acid, and while the product was not obtained entirely pure the physical properties and ultraviolet absorption data indicated that the desired product had been obtained. Hydrolysis of the N^4-acetyl group was not attempted.

The N^4-acetylsulfanilyl derivative of leucopterin (XIII) (VIII, R_1 = CH_3CO, R_2 and R_3 = OH) was obtained by reaction of IX with diethyl oxalate in ethylene glycol in the presence of sodium methylate. The mixture became markedly more acidic during the reaction, due to the formation of the two new acidic groups. Reactions without sodium methylate were unsuccessful, and fusion of IX with oxalic acid gave products which were not identified. Hydrolysis of the N^4-acetyl group to XIV was easily accomplished.

6,7-Dialkyl derivatives were prepared from IX and 1,2-dialkyl-1,2-diketones in dilute aqueous ammonia. Reaction with 1,2-diaryl-1,2-diketones was accomplished by heating with IX in ethylene glycol. A sulfonamidopteridine was obtained similarly from 9,10-phenanthraquinone and IX.

Reaction of IX with nitrous acid yielded N^4-acetyl-N^1-(7-hydroxy-1-v-triazolo[d]pyrimidyl-5)-sulfanilamide, an 8-azaguanine derivative. It was purified and the N^4-acetyl group was hydrolyzed by methods similar to those used above.

Experimental

N^4-Acetyl-N^1-(4-amino-6-hydroxypyrimidyl-2)-sulfanilamide.—N^4-Acetylsulfaguanidine (129 g.), sodium methylate (54.8 g.) and ethyl cyanoacetate (113 g.) were mixed in 400 ml. of dry ethylene glycol, under a nitrogen atmosphere, and heated at 95° for 3 hours. A clear solution was obtained, which was poured into 2.5 l. of water keeping the temperature at 15–20°. The clarified solution was then acidified to pH 2.0–2.5 and the white precipitate filtered and washed. The wet cake was dissolved in 1.5 l. of water at 30° with ammonia, at pH 7.5–8.0. After clarification with 5 g. of activated carbon and 0.1 g. of sodium hydrosulfite, the solution was heated to 70° and acidified to pH 4–5 by gradual addition of 5 N hydrochloric acid; then, when crystallization occurred, the pH was adjusted to 1.5–2.0. The product was filtered at 10°, and reprecipitated a second time in the same manner. The yield of dry product was 121 g. (74.9%), m.p. 298–299°. A small sample was isolated as the calcium salt, which was dissolved in water, acidified, filtered, washed well and dried at 100° for four hours under vacuum. It then melted at 302.5–304.0°.

Anal. Calcd. for $C_{12}H_{13}N_5O_4S$: C, 44.57; H, 4.05; N, 21.66; S, 9.92. Found: C, 44.5; H, 4.11; N, 22.2; S, 9.92.

N^1-(4-Amino-6-hydroxypyrimidyl-2)-sulfanilamide.—A mixture of 32.3 g. of N^4-acetyl-N^1-(4-amino-6-hydroxypyrimidyl-2)-sulfanilamide, 500 ml. of 20% hydrogen chloride in anhydrous 2B ethanol, and 2.82 g. of concentrated hydrochloric acid was stirred for 17 hours. The solid which had precipitated was filtered, washed with ethanol, and then dissolved in 400 ml. of water with ammonia at pH 7.5–8.0. The solution was clarified with 1 g. of activated carbon and acidified to pH 2.8–3.0. The product precipitated as hexagonal plates; it was filtered, washed with water and acetone and dried to yield 19.2 g., m.p. 266–268°. It was reprecipitated once more without changing the m.p.; the yield was 18.1 g. (62.4%).

For analysis the compound was dried at 100° for ten hours and 132° for four hours in vacuum.

Anal. Calcd. for $C_{10}H_{11}N_5O_3S$: C, 42.8; H, 3.95; N, 24.9; S, 11.4. Found: C, 42.4; H, 4.42; N, 25.0; S, 11.1.

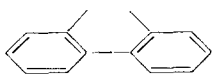
N^4-Acetyl-N^1-(4-amino-5-nitroso-6-hydroxypyrimidyl-2)-sulfanilamide.—A solution of 64.6 g. of N^4-acetyl-N^1-(4-amino-6-hydroxypyrimidyl-2)-sulfanilamide in 3 l. of water at 30° with ammonia added to give pH 8.0–8.5, and 15.2 g. of sodium nitrite, was clarified with a little activated carbon. Then while maintaining the temperature of the solution at 40°, the pH was adjusted to 1.8–2.0 by the addition of 5 N hydrochloric acid. The yellow precipitate was filtered at 10°, washed with water, and dissolved in 3 l. of water at 30° with ammonia to give pH 8.0–8.5. The solution was clarified with a little activated carbon, 0.5 g. of sodium nitrite added, and then 4 N hydrochloric acid added gradually. The product precipitated as yellow needles of the trihydrate, which were filtered at 10°, washed, and dried at 50°. The yield was 80.4 g. (99.2%), m.p. 219–221°. Runs with sodium hydroxide in place of ammonia gave similar results.

Anal. Calcd. for $C_{12}H_{12}N_6O_5S\cdot 3H_2O$: C, 35.5; H, 4.47; N, 20.7; S, 7.92. Found: C, 35.3; H, 4.21; N, 20.6; S, 8.17. Dried at 100° for seven hours in vacuum: Calcd. for $C_{12}H_{12}N_6O_5S$: C, 40.9; H, 3.44; N, 23.9; S, 9.12. Found: C, 40.5; H, 3.37; N, 24.1; S, 8.97.

N^4-Acetyl-N^1-(4,5-diamino-6-hydroxypyrimidyl-2)-sulfanilamide (IX).—Sixty and nine-tenths grams of N^4-acetyl-N^1-(4-amino-5-nitroso-6-hydroxypyrimidyl-2)-sulfanilamide was slurried in 900 ml. of water at 38° and dissolved with sufficient 5 N sodium hydroxide to obtain pH 8.0–8.5. Then 75 g. of sodium hydrosulfite was added over a five-

TABLE I

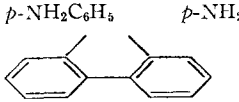
N^4-ACETYL DERIVATIVES (VIII, R_1 = CH_3CO)

No.	R_2	R_3	Reaction medium	Temp., °C.	Yield, %	M.p., °C. dec.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found	Empirical formula
X	H	H	Dil. NH_4OH	70–75	73.8	287–289	46.7	46.3	3.36	3.36	23.3	23.0	8.91	9.04	$C_{14}H_{12}N_6O_4S$
XII	OH	H	Fusion with $Cl_2CHCOOH$[a]	85–90	Low	232–240	39.0	37.9	4.21	3.93	19.6	19.7	7.45	7.44	$C_{14}H_{12}N_6O_5S\cdot 3H_2O$
XIII	OH	OH	Ethylene glycol, $NaOCH_3$	120–125	66.4	305–315	39.3	39.4	3.76	4.01	19.6	19.6	7.50	7.81	$C_{14}H_{12}N_6O_5S\cdot 2H_2O$[b]
XV	CH_3	CH_3	Dil. NH_4OH	70–75	96.2	300–302	49.5	49.7	4.16	4.16	21.7	21.7	8.27	8.06	$C_{16}H_{16}N_6O_4S$
XVIIa	CH_3(or C_4H_9)	C_4H_9(or CH_3)	Dil. NH_4OH	80–85		241–243	53.0	52.9	5.15	5.21	19.5	19.7	7.45	7.51	$C_{19}H_{22}N_6O_4S$
XVIIb	C_4H_9(or CH_3)	CH_3(or C_4H_9)	Dil. NH_4OH	80–85											
XIX	C_6H_5	C_6H_5	Ethylene glycol	120–130	66.5	330–331	60.8	60.9	3.93	3.99	16.4	16.5	6.25	6.15	$C_{26}H_{20}N_6O_4S$
XXI	p-$NH_2C_6H_5$	p-$NH_2C_6H_5$	Ethylene glycol	110–112	83.8	240–245	55.6	55.2	4.28	5.05	20.0	20.0	5.72	5.52	$C_{26}H_{22}N_8O_4S\cdot H_2O$
XXIII			Ethylene glycol	120	66.2	369–371	61.1	61.1	3.55	3.48	16.47	16.5	6.28	6.38	$C_{23}H_{18}N_6O_4S$

[a] Subsequently heated with aqueous ammonia. [b] H_2O: calcd., 8.41; found, 8.3 (Karl Fischer), 8.58 (loss on drying 20 hours at 100° under vacuum).

TABLE II

N^1-(4-HYDROXYPTERIDYL-2)-SULFANILAMIDES (VIII, R_1 = H)

No.	R_2	R_3	Yield, %	M.p., °C., dec.	Empirical formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found
XI	H	H	91.7	307–312	$C_{12}H_{10}N_6O_3S$	45.3	44.9	3.23	3.2	26.5	26.3	10.11	9.92
XIV	OH	OH	47.3	375	$C_{12}H_{10}N_6O_5S$	41.2	41.1	2.88	2.58	24.0	24.3	9.17	8.8
XVI	CH_3	CH_3	87.1	311–313	$C_{14}H_{14}N_6O_3S$	48.54	48.41	4.07	4.37	24.3	24.8	9.26	9.21
XVIIIa	CH_3(or C_4H_9)	C_4H_9(or CH_3)	76.5	247–250	$C_{17}H_{20}N_6O_3S$	52.6	52.6	5.19	5.19	21.7	21.8	8.27	8.30
XVIIIb	C_4H_9(or CH_3)	CH_3(or C_4H_9)	74.0	201.5–202.7	$C_{17}H_{20}N_6O_3S$	52.6	52.5	5.19	5.27	21.7	21.9	8.27	8.49
XX	C_6H_5	C_6H_5	95.6	355–357[a]	$C_{24}H_{18}O_3N_6S\cdot H_2O$	59.1	59.8	4.12	4.21	17.2	16.7	6.57	6.56
XXII	p-$NH_2C_6H_5$	p-$NH_2C_6H_5$	94.5	365–367	$C_{24}H_{20}N_8O_3S$[b]	57.6	57.5	4.03	4.16	22.4	22.4	6.42	6.34
XXIV			77.8	358–361	$C_{24}H_{16}N_6O_3S$	61.3	61.2	3.44	3.42	17.9	18.2	6.84	6.63

[a] Put into bath at 300°; sintered at 321.5–323° (loss of H_2O?) and finally melted with decomposition at 355–357°. Dried at 180° for ten hours under vacuum. *Anal.* Calcd. for $C_{24}H_{18}N_6O_3S$: C, 61.2; H, 3.85; N, 17.86; S, 6.81. Found: C, 61.1; H, 4.13; N, 18.0; S, 6.83. [b] The material was ordinarily obtained as a hydrate which melted at 352–367° with decomposition. Drying at 180° in vacuum removed the water.

TABLE III

ULTRAVIOLET ABSORPTION DATA[a]

No.	*N*/10 NaOH, 10 mg./l. Max. mμ	% Trans.	Min. mμ	% Trans.	*N*/10 HCl, 10 mg./l. Max., mμ	% Trans.	Min., mμ	% Trans.
X	262		31.25		265		299	
	364				324			
XI	265		315		254		285	
	368				322			
XII	258	22.5	345	94.2	264	5.18	235	27.3
	385	93.5			362	91.8	346	92.2
XIII	252	15.2	315	53.8	265	33.8	238	48.5
	342	49.0						
XIV	249	14.2	283	43.5	300	33.4	257	62.2
	291	43.0	322	50.4				
	343	45.4						
XV	257.5				270		299	
	362		310		328			
XVI	260		311		266		291	
	365				327			
XVIIa	259	13.2	312	86.9	330[b]	67.0	242	54.0
	364	59.5			269	37.1	300	80.1
XVIIIa	260				260			
	365				328			
XVIIIb	260				260			
	365				328			
XIX	256	23.0	270	25.0	277	30.5	331	70.1
	282	22.2	337	82.3	369	50.3		
	388	51.5						
XX	253	22.5	267	25.0	248	24.0	323	73.0
	285	20.4	337	80.8	365	42.0		
	389	49.6						
XXI	270	22.5	355	73.2	279[b]	24.1	232	29.5
					360	43.2	320	72.8
XXII	267	15.9	358	69.8	247[b]	25.0	235	28.0
					359	41.8	320	71.3
XXIII	263	9.2	287	30.0	Insoluble			
	303	22.0	350	84.2				
XXIV	252	9.2	257	9.8	Insoluble			
	263	8.7	284	26.0				
	303	19.0	350	82.5				

[a] In cases where values for transmittancy (T) are not given the curves are qualitative only, due to insolubility of the compounds. [b] Heated to 70–90° to aid solution; these values should be considered as qualitative only.

minute period, and the solution was clarified with 6 g. of activated carbon. The filtrate was acidified with 5 *N* sulfuric acid to *p*H 1.8–2.0, and the temperature was lowered to 10°. The solid was filtered and washed with ice-water. It was dissolved in 1500 ml. of water with ammonia at *p*H 8.0–8.5, clarified, and precipitated with 5 *N* sulfuric acid. The product was in the form of fine elongated needles. It was filtered, washed, and air-dried to give 52.1 g. (85.8%), m.p. 215–217° dec. Other samples melted with decomposition over the range 215–222°. For analysis a sample was reprecipitated several times from aqueous ammonia and aqueous sodium hydroxide solution. It darkened at 210° and fused with gradual decomposition above 230°.

Anal. Calcd. for $C_{12}H_{14}N_6O_4S \cdot {}^1/_2H_2SO_4 \cdot H_2O$: C, 35.55; H, 4.23; N, 20.73; S, 11.86. Found: C, 35.7; H, 4.37; N, 20.8; S, 11.8. Dried five hours at 100° in vacuum: Calcd. for $C_{12}H_{14}N_6O_4S \cdot {}^1/_2H_2SO_4$: C, 37.1; H, 3.91; N, 21.7; S, 12.4. Found: C, 37.0; H, 3.88; N, 21.7; S, 12.4.

N^4-Acetyl-N^1-(4-hydroxypteridyl-2)-sulfanilamide (X).—Twenty-eight and four-tenths grams of IX was dissolved in 700 ml. of water with 25 ml. of concentrated ammonium hydroxide. At 70–75°, 27.2 g. of commercial 30% glyoxal solution was added over a five-minute period, along with 10 ml. more concentrated ammonium hydroxide to maintain the *p*H 10.0–10.5. The solution was heated at 70–75° for 30 min., then acidified to *p*H 2.5–3.0 with hydrochloric acid and the solid filtered. It was slurried in 700 ml. of water at 30° and dissolved with ammonia, the solution was clarified with activated carbon, and acidified at 85–90°. The solid was precipitated once more in the same manner, then washed well with water and dried at 50°. The yield was 18.6 g. (73.8%) of material melting at 287–289° (dec.). For analysis a sample was reprecipitated several times as above, then finally dissolved in dilute sodium hydroxide and precipitated with acetic and hydrochloric acids.

See Tables I and III for analytical and ultraviolet absorption data.

N^1-(4-Hydroxypteridyl-2)-sulfanilamide (XI).—A solution of 30 g. of N^4-acetyl-N^1-(4-hydroxypteridyl-2)-sulfanilamide and 20 g. of sodium hydroxide in 300 ml. of water was heated under reflux for three hours. The crude product was precipitated with acid and collected on the filter. It was slurried in 300 ml. of water, and ammonium hydroxide was added to adjust the solution to *p*H 9.5–10.0. Complete solution was obtained momentarily, then the ammonium salt precipitated. It was filtered, and then dissolved in 400 ml. of water with 5 *N* sodium hydroxide. The solution was heated to 60° and acidified, and the product was reprecipitated twice more from dilute sodium hydroxide

with acid. The yield was 24.3 g. (91.7%) of material melting at 300–310° dec. For the analytical data and ultraviolet absorption spectra see Tables II and III.

N^4-Acetyl-N^1-(4,6-dihydroxypteridyl-2)-sulfanilamide (XIII).—Fusion of IX with chloroacetic acid and subsequent heating in aqueous sodium bicarbonate gave low yields of XII. Fusion with dichloroacetic acid appeared to be more satisfactory, possibly because it should give the aromatized form rather than a dihydropteridine. Fusion of 8.72 g. of IX with 15.5 g. of dichloroacetic acid at 85–90° under vacuum, followed by dilution with water (200 ml.) and neutralization with ammonia, gave a solution which was warmed at 40–45° for three hours. On acidification an amorphous yellow product was obtained which was purified by repeated precipitations from dilute ammonia with acid, to yield 0.33 g. in the form of deep yellow spherulites, m.p. 232–240° (dec.). See Tables I and III.

N^4-Acetyl-N^1-(4,6,7-trihydroxypteridyl-2)-sulfanilamide (XIII).—A mixture of 65.4 g. of IX in 200 ml. of dried (heated to 145°) ethylene glycol was heated to 60° and a solution of 25 g. of sodium methylate in 50 ml. of dried ethylene glycol was added slowly until the reaction mixture gave a red spot on moist Brilliant Yellow test paper. The temperature was raised to 120°, and more of the sodium methylate solution added to maintain the red spot on Brilliant Yellow test paper. About 0.2 g. of sodium hydrosulfite was added to decolorize the solution. The total usage of the sodium methylate solution was 45 ml. or 22.5 g. of sodium methylate. Then 43.8 g. of diethyl oxalate was added over a 15-minute period; there was an immediate vigorous reaction and alcohol distilled out. The reaction mixture became acidic to moist congo red paper. About ten minutes after all the diethyl oxalate had been added a deep yellow solid began to precipitate. Reaction at 120–125° was continued one hour, then the mixture was allowed to cool to room temperature and poured into 1 l. of water at 50°, and adjusted to *p*H 3 with hydrochloric acid. The crude product was purified by repeated precipitation from dilute alkali with acid, the final one being at 95°. The cream-colored long needles were dried at 50°, and the yield was 42.7 g. See Tables I and III.

N^1-(4,6,7-Trihydroxypteridyl-2)-sulfanilamide (XIV).—This compound was prepared by hydrolysis of XIII in dilute sodium hydroxide. See Tables II and III.

Reactions of IX with 1,2-Diketones.—Condensation of IX with diacetyl took place rapidly in dilute aqueous ammonia, and the product XV was purified by repeated precipitation from ammonia or sodium hydroxide solution. Hydrolysis in sodium hydroxide yielded XVI. From the condensation of IX with the unsymmetrical diketone, heptane-2,3-dione, a mixture of the two possible 6(7)-methyl-7(6)-butyl isomers was obtained which was separated by fractional crystallization of the N^4-acetyl derivatives (XVIIb and a). Hydrolyses of the N^4-acetyl groups yielded the two compounds XVIIIa and b which had different melting points but the same ultraviolet absorption maxima and minima; see tables. These are believed to be the isomeric N^1-(6(7)-methyl-7(6)-butylpteridyl-2)-sulfanilamides.

Reactions of IX with benzil, 4,4′-diaminobenzil and 9,10-phenanthroquinone were accomplished by heating at 120–125° in ethylene glycol with a catalytic amount of sodium methylate. Purification and hydrolysis were by the methods indicated above. Data on these compounds are given in the tables.

N^4-Acetyl-N^1-(7-hydroxy-1-*v*-triazolo[d]pyrimidyl-5)-sulfanilamide.—To a mixture of 202.8 g. of IX in 2 l. of water and 280 ml. of 5 *N* hydrochloric acid at 0–5° was added dropwise a solution of 41.4 g. of sodium nitrite in 300 ml. of water. After all the nitrite had been added, the slurry was stirred at 5° for 20 minutes, then heated to 80°. A solution was obtained momentarily, then the product precipitated. It was collected on the filter, and reprecipitated three times from large volumes of hot dilute alkali with acid, with a final precipitation from dilute ammonium hydroxide by means of acid. The slurry was cooled to 60°, the product then filtered, washed, and dried. The yield was 102.2 g. (58.5%) of the hemihydrate, in the form of rectangular plates; m.p. 264–267° (dec.).

Anal. Calcd. for $C_{12}H_{11}N_7O_4S \cdot {}^1/_2H_2O$: C, 39.22; H, 3.57; N, 26.69; S, 8.73. Found: C, 39.2; H, 3.71; N, 26.9; S, 8.74.

In 0.10 *N* sodium hydroxide solution at a concentration of 10 mg./l. the following ultraviolet absorption data were obtained: maximum 256 mμ, 20.8% transmittance; minimum 239 mμ, 29.8% transmittance. In 0.10 *N* hydrochloric acid at 10 mg./l.: maximum 265 mμ, 18.0% transmittance; minimum, 235 mμ, 40.1% transmittance.

The anhydrous material was obtained by isolation at a slightly higher temperature.

Anal. Calcd. for $C_{12}H_{11}N_7O_4S$: C, 41.26; H, 3.17; N, 28.07; S, 9.18. Found: C, 41.1; H, 3.33; N, 28.1; S, 9.00.

N^1-(7-Hydroxy-1-*v*-triazolo[d]pyrimidyl-5)-sulfanilamide.—Sixty grams of the N^4-acetyl compound above was heated with 41.2 g. of sodium hydroxide in 1800 ml. of water under reflux for four hours, then diluted with 4.2 l. of water and the product was precipitated with acid. The yield of dry crude product was 46.5 g. (88.1%) in two crops. It was purified by reprecipitation from dilute alkali with acid. The yield was 43.6 g. (82.9%) of the sesquihydrate, m.p. 216–220° dec.

Anal. Calcd. for $C_{10}H_9N_7O_3S \cdot 1{}^1/_2H_2O$: C, 35.9; H, 3.59; N, 29.4; S, 9.54. Found: C, 36.0; H, 4.24; N, 28.9; S, 9.44.

The anhydrous product, m.p. 208–215 dec., was obtained by drying the sesquihydrate seven hours at 100° under vacuum.

Anal. Calcd. for $C_{10}H_9N_7O_3S$: C, 39.08; H, 2.95; N, 31.92; S, 10.44. Found: C, 39.0; H, 2.99; N, 31.8; S, 10.5.

Ultraviolet absorption data: in 0.10 *N* NaOH, 10 mg./l.; maximum at 257 mμ, 18.1% transmittance; minimum 240 mμ, transmittance 29.5%. In 0.10 NHCl,[12] 10 mg./l.: maximum at 265 mμ, transmittance 32.0%; minimum 256 mμ, transmittance 32.5%.

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(12) Heated to boiling to aid solution.