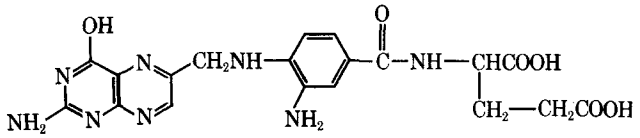
Synthesis of 3′-Aminopteroic Acid

J. N. WELLS and M. S. STRAHL

Abstract □ The synthesis of 3′-aminopteroic acid by three alternative routes is reported. The title compound was designed for possible folic acid antagonism but instead showed slight positive growth activity.

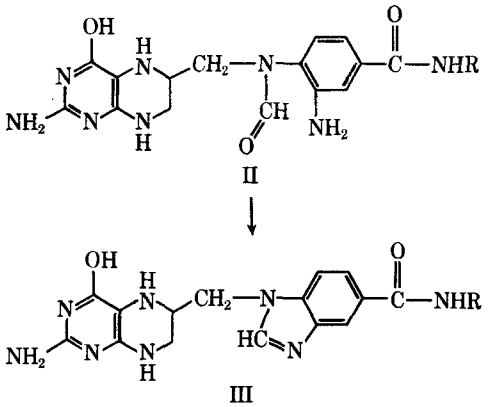
Keyphrases □ 3′-Aminopteroic acid—synthesis □ Folic acid activity—3′-aminopteroic acid effect □ Mass spectroscopy—identification, structure □ UV spectrophotometry—identification □ IR spectrophotometry—identification

The existence of ^{10}N-formyltetrahydrofolic acid as the source of the C_2 atom of the purine skeleton (1–3) suggested that 3′-aminofolic acid (I) could be a folic



I

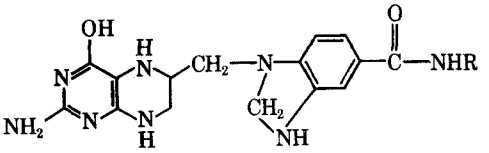
acid antagonist. 3′-Aminofolic acid could form ^{10}N-formyl-3′-aminotetrahydrofolic acid (II) through the same pathways that folic acid forms ^{10}N-formyltetrahydrofolic acid (Scheme I). If the formyl group, by



Scheme I

Schiff base formation with the 3′-amino function, could then be incorporated in the benzimidazole ring of III, the ^{10}N-carbon would be firmly bound and could not be readily transferred to an acceptor molecule. This would make III a likely competitive inhibitor of ^{10}N-formyltetrahydrofolic acid, since III would be unable to be utilized as a coenzyme and could block the active site of the transformylase enzyme (4) that mediates the C_1 transfer for purine synthesis. Similarly, III might also prevent ^{10}N-formyltetrahydrofolic acid from being transformed to $^{5}N,^{10}N$-anhydroformyltetrahydrofolic acid, the immediate source of the C_8 atom of the purine (1–3), by competing for the active site of the enzyme that effects that reaction.

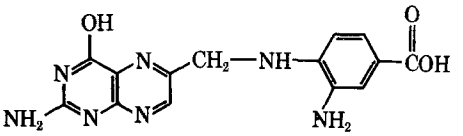
Another possibility for the production of an antimetabolite from II is that ^{10}N-hydroxymethyl-3′-aminotetrahydrofolic acid which might be formed through the serine-glycine interchange (5) may condense intramolecularly to form IV, since *N*-hydroxy-



IV

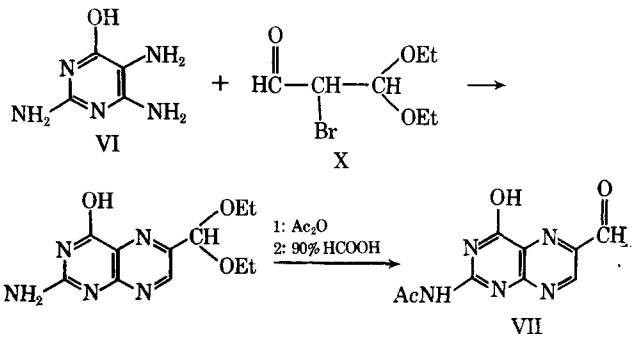
methylamines have a tendency to combine with another ligand (6). Compound IV could then be a competitive inhibitor of ^{10}N-hydroxymethyltetrahydrofolic acid by blocking the active site of cyclohydrolase (2). This would inhibit the formation of both $^{5}N,^{10}N$-methylenetetrahydrofolic acid used for thymine synthesis (7, 8) and $^{5}N,^{10}N$-anhydroformyltetrahydrofolic acid used to form the imidazole portion of the purine ring.

These speculations, plus the report that 3,4-diaminobenzoic acid has bacteriostatic activity antagonized by folic acid (9), prompted interest in the synthesis of 3′-aminopteroic acid (V).



V

2,4-Diamino-6-hydroxypyrimidine was nitrosated to give 2,4-diamino-5-nitroso-6-hydroxypyrimidine (10, 11). Reduction of this compound to 2,4,5-triamino-6-hydroxypyrimide (VI) (Scheme II) was effected in high



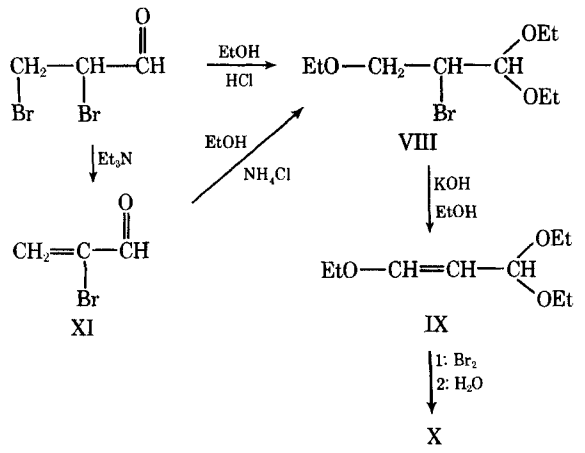
Scheme II

yield with either 44% ammonium sulfide (10) or by catalytic hydrogenation in aqueous sodium hydroxide (12). It was found in this study that appreciable 4-amino-2,6-dihydroxy-5-nitrosopyrimidine was formed by alkaline hydrolysis during the latter procedure. The slight modification of using 1 equivalent of base for solubilization minimized this hydrolysis.

The original synthesis of 2-bromo-3-ethoxypropanal diethyl acetal (VIII) (13), needed in the approach to the pteridine ring system, was described in a vague and ambiguous manner which is subject to many interpretations, all leading to low yields. Merely removing ethyl halide and water from the reaction mixture of 2,3-dibromopropanal in ethanol nearly doubles the published yield.

In the present study, the physical properties of 2-bromo-3-ethoxypropanal diethyl acetal (VIII) (b.p. 113–118°/14 mm., n_D^{25} 1.4440) did not correspond to the reported values [b.p. 103–104°/14 mm. (13), n_D^{25} 1.4593 (14)]. The confusion arising from this discrepancy and the importance of the intermediate prompted an independent synthesis of 2-bromo-3-ethoxypropanal diethyl acetal.

The reaction of 2,3-dibromopropanal (15) with triethylamine gave 2-bromopropenal (XI) (Scheme III).



Scheme III

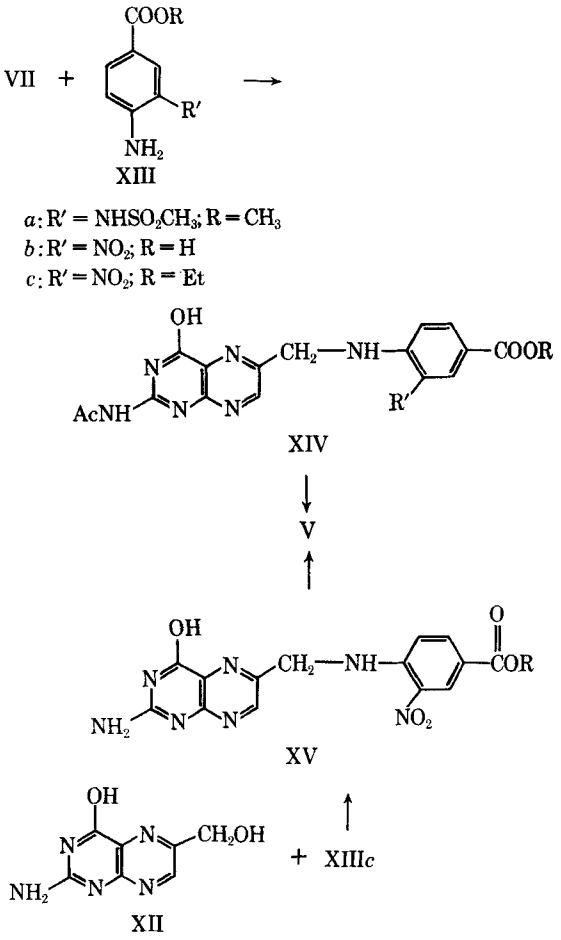
Reaction of XI with ethanol and ammonium chloride (16) gave 2-bromo-3-ethoxypropanal diethyl acetal (VIII), with physical properties and spectral characteristics identical to that obtained by the method of Fischer and Giebe (13).

Thermal degradation of X occurred so readily that even distillation at 45°/0.4 mm. [lit. (17) b.p. 65–70°/4 mm.] afforded pure material in yields not greater than 25%. In this study the yield of X was increased to an apparent 95%, based on pteridine formation, by using the material directly after removal of the solvents. Satisfactory results also were obtained by using the dibromo precursor of X directly.

2-Amino-4-hydroxy-6-diethoxymethylpteridine was prepared according to published methods (17) from X and VI. Acetylation followed by treatment with 90% formic acid gave 2-acetamido-4-hydroxy-6-formylpteridine (VII).

Three syntheses of 3′-aminopteroic acid were developed. Two of these involved the reaction of alternative precursors of 3,4-diaminobenzoic acid with VII. The third was the reaction of ethyl 4-amino-3-nitrobenzoate (18) with 2-amino-4-hydroxy-6-hydroxymethylpteridine (XII) (19).

Methyl 4-amino-3-*N*-mesylaminobenzoate (XIII*a*) (Scheme IV) was first prepared by catalytic hydrogena-



Scheme IV

tion of methyl 3-*N*-mesylamino-4-nitrobenzoate in methanol. Isolation of XIII*a* resulted in some decomposition since the compound is sensitive to oxygen. To minimize this decomposition, the reduction was thereafter run in ethylene glycol monoethyl ether and this solution was used directly in the reaction with VII.

Compounds VII and XIII*a* were heated to reflux in ethylene glycol monoethyl ether containing *p*-thiocresol. The product (XIV*a*) was demesylated with HBr in acetic acid (20) and then hydrolyzed in aqueous sodium hydroxide. The mass spectrum of the resulting 3′-aminopteroic acid (V), obtained by placing the sample directly in the electron beam, exhibited, among others, ions at *m/e* 309 (M-18) and *m/e* 294 (M-33).

4-Amino-3-nitrobenzoic acid (XIII*b*) and VII were refluxed in ethylene glycol monoethyl ether containing *p*-thiocresol to give XIV*b*. This was then hydrolyzed in base and catalytically reduced to form 3′-aminopteroic acid (V). The mass spectrum of this product was run at 400°, which caused some decomposition and decarboxylation. Fragments at *m/e* 324 (M-3), 280 (M-3-CO_2), 162 (2-amino-4-hydroxypteridine ion), and 44 (CO_2) were apparent and, except for CO_2, were of low

abundance. The mass spectra of known pteridines run at 300–350° showed that loss of H_2 is a facile process.

Ethyl 4-amino-3-nitrobenzoate (XIII*c*) and XII were refluxed in anhydrous formic acid (21). The material isolated from this reaction was hydrolyzed in base. TLC utilizing silica gel H adsorbant and *sec*-butyl alcohol–acetic acid–water (8:2:5) as the solvent system (19) showed the presence of precursor plus a new pteridine (XV) under long wavelength UV. No new pteridine could be detected, and only XII was recovered when XIII*c* was omitted from this reaction.

Catalytic hydrogenation of the alkaline solution of XV gave a compound identical to the 3′-aminopteroic acid prepared by the other methods described. The mass spectrum of this compound, obtained by placing the sample directly in the electron beam, exhibited ions at *m/e* 309 and 294. The conventional mass spectrum run at 400° exhibited a spectrum identical to the one already described for these conditions.

Compound V did not burn completely during its elemental analysis and, therefore, gave somewhat low carbon and hydrogen values with much nitrogenous residue, a characteristic of pteridines (22, 23).

3′-Aminopteroic acid (V) was tested for growth inhibition against *Lactobacillus casei* (ATCC 7469) in yeast extract veal infusion 0.1% glucose broth. Aliquots of a 64 mcg./ml. solution of V were added to the test cultures, and the medium was incubated at 35° for 20 hr. Comparatively massive amounts of V resulted in only slight positive growth activity.

EXPERIMENTAL[1]

2-Bromopropenal—To 56 g. (1.0 mole) of acrolein in 200 ml. of anhydrous ether at 0–5° was added, with stirring, 160 g. (1.0 mole) of bromine. Triethylamine (101 g.) was added dropwise to the well-stirred mixture while the temperature was maintained at 0°. The solution was filtered and the solid was washed with anhydrous ether. The washings were combined with the filtrate, and the solvent was removed *in vacuo* at room temperature. The residue was distilled to give 61 g. of compound, b.p. 80–84° (123 mm.), $n_D^{26.5}$ 1.5029. Thermal degradation limited the yield to 45%, although the theoretical amount of triethylamine hydrobromide was recovered. IR (neat) 3.20 μ(=CH_2), 3.51 μ(C$\underline{H}$O), 5.85 μ(C=O), 6.21 μ(C=C); NMR (CCl_4) δ 6.84 (d of d, 2, CH_2), 9.28 (s, 1, C$\underline{H}$O). This compound was used without further purification.

2-Bromo-1,1,3-triethoxypropane—*Method A*—To 61 g. (0.452 mole) of 2-bromopropenal were added 74 ml. of absolute ethanol and 3.0 g. of NH_4Cl. The reaction mixture was treated as described by Alberti and Sollazzo (16) to yield 36 g. (31%) of product, b.p. 113–118° (14 mm.), $n_D^{28.5}$ 1.4439.

Method B—A modified procedure of Fischer and Giebe (13) was followed. To a stirred solution of 366 g. (6.55 moles) acrolein in 500 ml. anhydrous ether was added dropwise 1062 g. (6.64 moles) of bromine. The temperature was maintained at 0°. The ether was removed *in vacuo* to leave 1143 g. of residue. This was added to 2800 ml. of anhydrous ethanol containing 16 g. of dry HCl. The reaction mixture was refluxed for several hours; then the liquid distilling between 45–50° was removed until no more ethyl halide (b.p. 37°) was evolved. Benzene (1000 ml.) was added to the reaction mixture, and the tertiary azeotrope of water, benzene, and ethanol was collected in a Dean-Stark trap. The solution was cooled to room temperature, and solid sodium bicarbonate was added with stirring until the solution was alkaline. The solution was filtered, and the solvents were removed *in vacuo*. The residue was distilled to yield forerun plus the desired compound, b.p. 113–118° (14 mm.), n_D^{21} 1.4440. The forerun was recycled through the same procedure to yield a combined weight of 1290 g. (76%) of product. The IR, NMR, and mass spectra of the products formed by Methods A and B were identical: NMR (CCl_4) δ 1.32 (m, 9, CH_3), 3.70 (m, 9, CH_2, αH, βH), 4.52 (C_1H).

1,3,3-Triethoxypropene-1—A modified procedure of Price and Moos (24) was followed. A stirred solution of 111 g. (0.437 mole) of 2-bromo-1,1,3-triethoxypropane and 70 g. of potassium hydroxide in 226 ml. of absolute ethanol was refluxed under nitrogen for 2 hr. Most of the ethanol was removed *in vacuo* at a maximum temperature of 45°. Then 100 ml. of water was added to dissolve the solids, and the top layer was collected. The aqueous layer was extracted with ether, and the organic layers were combined. The ether was removed *in vacuo*, and the residue was distilled to yield 65.9 g. (86%) of product distilling at 78–84° (9 mm.), n_D^{25} 1.4225 [lit. (24) b.p. 94–96° (20 mm.), n_D^{25} 1.4220]. IR showed absorbance at 6.01 μ(C=C), and the NMR was consistent with the structure (IX).

2-Bromo-3,3-diethoxypropanal—A modification of the procedure of Sletzinger *et al.* (17) was followed. To 1,3,3-triethoxypropene (30.6 g., 0.174 mole) dissolved in 100 ml. of dry ether was added 28 g. (0.174 mole) of bromine dropwise, with stirring at 0°. The ether was removed *in vacuo* at room temperature, and the residue was added dropwise to a stirred solution of 100 ml. water and 25 ml. acetone at 0°. The reaction mixture was stirred for 1 day at 0° and then extracted with chloroform. The chloroform extracts were dried over anhydrous sodium sulfate, and the solvent was removed *in vacuo* at room temperature. The residue was distilled to yield 8 g. (23%) of product, b.p. 46–47° (0.4 mm.), $n_D^{23.5}$ 1.4530. IR (neat) 5.77 μ(CHO); NMR (CCl_4) δ 1.28 (t, 6, CH_3), 3.82 (q, 4,CH_2), 4.25 (d of d, 1, αH), 4.96 (d, 1, βH), 9.68 (d, 1, C$\underline{H}$O).

Methyl 3-*N*-Mesylaminobenzoate—Methyl 3-nitrobenzoate (25) (46.8 g., 0.256 mole) was dissolved in 300 ml. of methanol and reduced in a Parr hydrogenator at an initial hydrogen pressure of 50 p.s.i., using 5 g. of Raney nickel as catalyst. After reduction was complete, the catalyst was removed by filtration and the methanol removed *in vacuo* at a temperature of 50°. The residue (methyl 3-aminobenzoate) was added to a solution of 27.1 g. triethylamine in 300 ml. of dry ether. To this was added, with stirring, a solution of 30.7 g. (0.268 mole) mesyl chloride in 50 ml. dry ether. The temperature was maintained at 0–5°. After the addition of mesyl chloride, the solution was stirred overnight at room temperature and then filtered; the solid was washed with dry ether. The white solid was stirred with 600 ml. of hot water and the mixture was filtered. The remaining solid was dried overnight at 60° (10 mm.) and then added to 500 ml. of refluxing methanol. The hot solution was filtered, and the remaining solid was washed with hot methanol. The combined methanol filtrates were concentrated to 250 ml. and chilled at 0° overnight. The precipitate was collected and recrystallized from methanol to yield 45.84 g. (78%) of product, m.p. 123°; IR (KBr) 5.92 μ(C=O), 7.55–7.90 μ and 8.75 μ(—SO_2—); mass spectrum *m/e* 229 (M), 198 (M-MeO), 150 (M-Ms), 79 (Ms). The M^+2 peak (about 4% of M) indicated one sulfur atom present in the molecule (26).

Anal.—Calcd. for $C_9H_{11}NO_4S$: C, 47.15; H, 4.84. Found: C, 46.64; H, 4.95.

Methyl 3-*N*-Mesylamino-4-nitrobenzoate—To a solution of 11.95 g. (52.2 mmoles) of methyl 3-*N*-mesylaminobenzoate in 100 ml. of acetic anhydride was added dropwise, with stirring, 8.0 ml. of 70% nitric acid. The reaction mixture was heated for 20 min. on a steam bath and then stirred overnight at room temperature. The solution was filtered, and the solid was recrystallized from methanol to yield 1.01 g. (7.0%) of yellow crystals, m.p. 168–169°. For analysis, the compound was sublimed at 130° (0.6 mm.), m.p. 168.5–169°; IR (KBr) 5.80 μ(C=O), 6.54 μ(—NO_2), 7.75–7.80 μ and 8.75 μ(—SO_2—); mass spectrum *m/e* 274 (M), 243 (M-MeO), 195 (M-Ms). The compound was hydrolyzed in 15% HCl (27) to yield 3-amino-4-nitrobenzoic acid (28), thus confirming the position of the nitro group.

[1] All melting and boiling points are reported uncorrected. Melting points were determined using a Büchi capillary melting-point apparatus. Manometers were calibrated with several compounds of confirmed boiling points to ensure correct pressure readings. A Perkin-Elmer model 21 IR spectrophotometer was used to obtain IR spectra. UV spectra were obtained by using a Bausch and Lomb model 505 recording spectrophotometer. NMR spectra were obtained using a Varian A-60-A spectrometer at sweep widths of 500 or 1000 Hz., with tetramethylsilane as internal standard. Solvents are specified. Mass spectra were obtained using a Hitachi RMU-6A mass spectrometer operating at 70 ev. unless otherwise indicated. Refractive indexes were obtained with a Bausch and Lomb Abbe refractometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN 37912, and Midwest Microlab, Inc., Indianapolis, IN 46226.

Anal.—Calcd. for $C_9H_{10}NO_6S$: C, 39.47; H, 3.68. Found: C, 39.24; H, 3.87.

Methyl 3-*N*-Mesylamino-4-aminobenzoate—Methyl 3-*N*-mesylamino-4-nitrobenzoate (500 mg., 1.89 mmoles) was dissolved in 50 ml. of methanol and catalytically hydrogenated at an initial pressure of 50 p.s.i. using 0.5 g. of Raney nickel. After the requisite amount of hydrogen had been taken up, the solution was filtered; the filtrate was evaporated *in vacuo* at room temperature to yield a white solid which darkened upon exposure to air. Mass spectrum *m/e* 244 (M), 213 (M-MeO), 165 (M-Ms). To minimize contact with atmospheric oxygen, this reduction was thereafter run in ethylene glycol monoethyl ether and the solution was used in the next step.

3′-Aminopteroic Acid—*Method A*—2-Acetamido-4-hydroxy-6-formylpteridine (17) (0.50 g., 2.14 mmoles), 3-nitro-4-aminobenzoic acid (0.50 g., 2.7 mmoles), and 1.87 g. of *p*-thiocresol were refluxed for 6 hr. in ethylene glycol in a nitrogen atmosphere. The product was isolated in the manner described by Sletzinger *et al.* (17) and then hydrolyzed in 100 ml. of 0.1 *N* NaOH (98°) for 1 hr. under nitrogen. This solution was catalytically hydrogenated for 2 days at a pressure of 60 p.s.i. using 5 g. of Raney nickel. After reduction was complete, the catalyst was removed by filtration; dilute HCl was added to the filtrate to precipitate the product. The solid was collected by centrifugation, washed with water and acetone, and dried at 100°/10 mm. to yield 37.3 mg. of product; m.p. > 300°. Satisfactory combustion analysis could not be attained due to the tendency of this compound to form a noncombustible residue. Some decarboxylation and decomposition occurred when the compound was heated to over 400°, necessary to obtain the mass spectrum due to the low volatility of V. Mass spectrum, *m/e* 324 (M-3), 280 (M-3)-CO_2, 162 and 44 (CO_2).

Method B—2-Amino-4-hydroxy-6-hydroxymethylpteridine (XII) (19) (0.193 g., 1 mmole) and ethyl 4-amino-3-nitrobenzoate (18) (0.420 g., 2 mmoles) were refluxed under nitrogen in a preformed solution of 20 g. 90% formic acid and 11.3 g. acetic anhydride for 15 hr. The reaction mixture was cooled, and the products were precipitated by addition of dry ether. The solid was dissolved in 50 ml. of 0.2 *N* NaOH and refluxed under nitrogen for 1 hr. TLC showed the presence of unreacted XII plus another pteridine. The alkaline solution containing the new pteridine was catalytically reduced for 10 hr. with hydrogen at 60 p.s.i. using 2 g. of Raney nickel. The solution was filtered, and the filtrate was brought to the pH of Congo red paper with dilute HCl. The residue was collected by filtration and dried at 130°/10 mm. for 8 hr. The mass spectrum of this residue, obtained by placing a sample directly in the electron beam, exhibited ions at *m/e* 309 (M-18) and 294 among others.

Method C—Methyl 3-*N*-mesylamino-4-nitrobenzoate (0.300 g., 1.23 mmoles) in 15 ml. of ethylene glycol monoethyl ether was hydrogenated overnight at a pressure of 50 p.s.i., using 5 g. of Raney nickel. The solution was filtered with suction, and 0.23 g. of 2-acetamido-4-hydroxy-6-formylpteridine (1 mmole) was added to the colorless filtrate. This reaction mixture was then refluxed for 12 hr. under nitrogen, the solution was cooled, and 3.5 g. of *p*-thiocresol was added. The solution was again refluxed for 4 hr. in a nitrogen atmosphere. The product was isolated after the method of Sletzinger *et al.* (17). This residue was allowed to stand in a solution of 0.5 g. of phenol in 3.0 ml. of 38% HBr in acetic acid for 2 days with intermittent shaking. The solution was filtered through glass wool into 50 ml. of water. Sodium hydroxide was added to make the solution just alkaline, and then 1.2 g. of sodium hydroxide was added and the solution was heated under nitrogen for 3 hr. on a steam bath. The solution was cooled and filtered, and the filtrate was acidified to the pH of Congo red paper. The precipitate was collected by filtration, washed with copious amounts of water, and then washed with acetone and ether. The solid was dried at 120°/10 mm. for 24 hr. to yield 22.4 mg. of compound whose mass spectrum exhibited ions at *m/e* 309 and 294 when a solid sample was placed directly in the electron beam. Again large amounts of uncombustible residue resulted upon combustion in an attempt to obtain an elemental analysis.

REFERENCES

(1) F. M. Huennekens, M. J. Osborn, and H. R. Whiteley, *Science*, **128,** 120(1958).

(2) F. M. Huennekens and M. J. Osborn, *Advan. Enzymol.*, **21,** 369(1959).

(3) E. M. Kosower, "Molecular Biochemistry," McGraw-Hill, New York, N. Y., 1962, p. 39.

(4) A. White, P. Handler, and E. L. Smith, "Principles of Biochemistry," 3rd ed., McGraw-Hill, New York, N. Y., 1963, p. 560.

(5) L. Jaenicke, in "Ciba Foundation Symposium on Chemistry and Biology of Pteridines," G. E. W. Wolstenholme and M. P. Cameron, Eds., Churchill, London, England, 1954, p. 219.

(6) J. R. Feldman and E. C. Wagner, *J. Org. Chem.*, **7,** 31 (1942).

(7) B. M. McDougall and R. L. Blakley, *J. Biol. Chem.*, **236,** 832(1961).

(8) A. Kornberg, *Harvey Lect., Ser.*, **53,** 83(1957–1958).

(9) J. L. Sirks, *J. Microbiol. Serol.*, **19,** 171(1953).

(10) W. Traube, *Chem. Ber.*, **33,** 1371(1900).

(11) J. A. VanAllan, *Org. Syn.*, coll. vol. IV, p. 245.

(12) British pat. 648,904, Merck and Co., Inc. (1951); through *Chem. Abstr.*, **45,** P8563(1951).

(13) E. Fischer and G. Giebe, *Chem. Ber.*, **30,** 3053(1897).

(14) S. M. McElvain and L. R. Morris, *J. Amer. Chem. Soc.*, **73,** 206(1951).

(15) R. H. Baker and S. W. Tinsley, Jr., *ibid.*, **72,** 393(1950).

(16) C. Alberti and R. Sollazzo, *Org. Syn.*, coll. vol. III, p. 372.

(17) M. Sletzinger, D. Reinhold, J. Grier, M. Beachem, and M. Tishler, *J. Amer. Chem. Soc.*, **77,** 6365(1955).

(18) J. A. Silk, *J. Chem. Soc.*, **1956,** 2058.

(19) C. Bough and E. Shaw, *J. Org. Chem.*, **29,** 3610(1964).

(20) D. I. Weisblat, B. J. Magerlein, A. R. Hange, D. R. Meyers, and S. T. Rolfson, *J. Amer. Chem. Soc.*, **75,** 3625(1953).

(21) Swiss pat. 258,141 (1949); through *Chem. Abstr.*, **44,** 1546h (1950).

(22) R. C. Elderfield and A. C. Mekta, in "Heterocyclic Compounds," vol. IX, R. C. Elderfield, Ed., Wiley, New York, N. Y., 1967, p. 1.

(23) J. A. Fildes, in "Pteridine Chemistry," W. Pfleiderer and E. C. Taylor, Eds., Macmillan, New York, N. Y., 1964, p. 507.

(24) R. W. Price and A. Moos, *J. Amer. Chem. Soc.*, **67,** 207 (1945).

(25) O. Kamm and J. B. Segur, *Org. Syn.*, coll. vol. I, p. 372.

(26) R. M. Silverstein and G. C. Bassler, "Spectrometric Identification of Organic Compounds," 2nd ed., Wiley, New York, N. Y., 1967, p. 4.

(27) S. Searles and N. Shago, *Chem. Rev.*, **59,** 1077(1959).

(28) S. I. Heilbron, "Dictionary of Organic Compounds," 4th ed., Oxford University Press, Oxford, England, 1965, p. 176.

ACKNOWLEDGMENTS AND ADDRESSES

Received August 31, 1970, from the *Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy, Purdue University, Lafayette, IN 47907*

Accepted for publication November 18, 1970.

The authors thank E. Ronald Wright, Department of Biological Sciences, Purdue University, for biological testing.