2-Amino-4-hydroxy-6-methyl-7,8-dihydropteridine as a Model for Dihydrofolate*

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ABSTRACT: Studies on the structure of dihydrofolic acid are rendered difficult by its lability and because important chemical and physical properties associated with the pteridine nucleus are frequently obscured by groups in the remainder of the molecule. To overcome these difficulties, 2-amino-4-hydroxy-6-methyl-7,8-dihydropteridine has been utilized as a model for dihydrofolic acid. The dihydropteridine was synthesized: (a) by a modification of the procedure of Boon and Leigh in which the two extra hydrogen atoms at positions 7 and 8 were introduced prior to closure of the pyrazine ring; and (b) by reduction of the oxidized pteridine with hydrosulfite or with zinc in basic solution. The 7,8-dihydro structure of the model pteridine was confirmed by measurement of the proton magnetic resonance spectrum. The ultraviolet spectrum of an equimolal mixture of the 7,8-dihydropteridine and *N*-methyl-*p*-aminobenzoylglutamic acid was shown to be identical with the spectrum of dihydrofolate at pH 7 and 13 but not at pH 1; reasons for the latter discrepancy are discussed.

p*K* values were determined for prototropic groups in the oxidized, dihydro, and tetrahydro forms of the pteridine.

There are three possible tautomeric structures for dihydrofolate (Figure 1) involving the position of the two hydrogen atoms in the pyrazine ring, and two additional "quinonoid" structures proposed by Kaufman (1963). The latter forms, however, have been invoked only as possibilities for a transient intermediate (λ_{max} at 302–304 mμ) that appears during the hydroxylation of phenylalanine or when tetrahydrofolate is oxidized by 2,6-dichlorophenolindophenol. Earlier work by Mathews and Huennekens (1960) had shown that "standard" dihydrofolate (*i.e.*,, the material prepared by reduction of folate with hydrosulfite) cannot be the 5,6 isomer but the decision between the other two forms proved to be more difficult. Zakrzewski (1963) and Smith *et al.* (1963) initially favored the 5,8 structure but more recent work by Pastore *et al.* (1963), Fu and Chinoperos (1963), Hillcoat and Blakley (1964), and Scrimgeour and Vitols (1966), using tracer techniques, ultraviolet absorption spectra and proton magnetic resonance (pmr) measurements, have provided strong support for the identity of "standard" dihydrofolate with the 7,8 form.

Despite the reasonable certainty of the above conclusion, dihydrofolate is not particularly well suited for structure determinations because it is difficult to obtain in crystalline form with the correct elemental analysis, owing most probably to its lability to air oxidation and its susceptibility to cleavage at the C^9–N^{10} bond. Even more important, the glutamate carboxyls in dihydrofolate tend to obscure the p*K* values of functional groups in the pyrazine ring in the pH range of 3 to 5 and the two CH_2 groups in the glutamate residue complicate the search (by proton magnetic resonance spectra) for a similar methylene carbon in dihydrofolate. Esterification of the carboxyl groups overcomes the first of these difficulties but does not lead to a pure, crystalline material. It seemed preferable, therefore, to carry out structure studies on an authentic crystalline 7,8-dihydropteridine, containing a methyl group at position 6 instead of the *p*-aminobenzoylglutamate moiety, and to relate the properties of the model compound to those of dihydrofolate itself.

2-Amino-4-hydroxy-6-methylpteridine was prepared by several routes, summarized in Figure 2: (a) reductive cleavage of folic acid at the C^9–N^{10} linkage; (b) hydrolysis of 2,4-diamino-6-methylpteridine with NaOH in a nitrogen atmosphere; and (c) condensation of 2,4,5-triamino-6-hydroxypyrimidine with *N*-(2-formyl-2-bromoethyl)pyridinium bromide in the presence of KI to yield *N*-(2-amino-4-hydroxy-6-pteridyl)methylpyridinium iodide, followed by conversion of the latter compound to the desired pteridine. Reduction of 2-amino-4-hydroxy-6-methylpteridine with hydrosulfite or with zinc in NaOH yielded, after chromatography on Dowex 50 (H^+ form), a dihydropteridine which was subsequently crystallized. The same dihydropteridine was prepared by direct condensation of 2-amino-4-hydroxy-5-phenylazo-6-chloropyrimidine with aminoacetone semicarbazone hydrochloride, followed by reductive ring closure according to the general proce-

* From the Department of Biochemistry, Scripps Clinic and Research Foundation, La Jolla, California 92038. *Received May 1, 1967.* Paper XIX in the series Folate Coenzymes and One-Carbon Metabolism. This investigation was supported by a grant (CA-6522) from the National Cancer Institute, National Institutes of Health, and by a contract (No. PH 43-65-14) from the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health.

dures of Boon and Leigh (1951) and Elion and Hitchings (1952).

Unlike dihydrofolate, in which the interpretation of proton magnetic resonance (pmr) measurements in the pteridine ring is complicated by the presence of aromatic ring and methylene protons of the *p*-aminobenzoylglutamate portion, the model dihydropteridine gave a very clean and simple spectrum (Figure 3). The characteristic resonance band at 8.92 ppm associated with a proton attached to a trigonal carbon in the fully oxidized pteridine (upper panel) was replaced in the dihydropteridine (lower panel) by a resonance band at 5.16 ppm which is characteristic of methylene protons in a pteridine nucleus (Viscontini *et al.*, 1963). Asahi (1964) has also reported values of 8.32 and 4.00 ppm in sodium deuterioxide for the oxidized and dihydro forms of 6-methylpteridine. Integration of the area under the resonance peaks confirmed the contribution of only one proton in the first case and of two protons in the second. The compound is thus a 7,8-dihydropteridine. Alternative structures for a dihydropteridine would lead to spectra different than that observed in b. If there were a proton at C-6, the methyl group at 2.72 ppm would be a doublet. If reduction had produced the 5,8 isomer, there would still have been a reasonance signal, occurring at a high parts per million value, corresponding to a single proton attached to a trigonal carbon atom (C-7). The present data, which establish conclusively that the simple model dihydropteridine must have the 7,8-dihydro structure, thereby support and extend the work of Pastore *et al.* (1963), who concluded from the more complex pmr spectra of dihydrofolate in D_2O that the material could only be the 7,8 tautomer.

The spectrum of dihydrofolate can be considered in terms of two chromophoric groups, namely the pteridine and the *p*-aminobenzoylglutamate residues. As a model for the latter, *N*-methyl-*p*-aminobenzoylglutamic acid was prepared by the method of Cosulich and Smith (1948) although, as reported by these workers, the free acid was difficult to crystallize; only the HBr complex of this material has been obtained in crystalline form (Fu *et al.*, 1965). Chromatography of the oily free acid on DEAE-cellulose yielded fractions with the correct absorption spectrum (Fu *et al.*, 1965). The concentration of this compound at pH 7.0 was determined by using ϵ 15.0 $\times$ 10^3 M^{-1} cm^{-1} as the extinction coefficient at 285 mμ. When a solution of *N*-methyl-*p*-aminobenzoylglutamate at pH 7.0 was added to a solution of 2-amino-4-hydroxy-6-methyl-7,8-dihydropteridine, the resultant spectrum of the mixture (each component at 3.4 $\times$ 10^{-5} M) was very similar to that of dihydrofolate at the same concentration (Figure 4). Likewise, the spectrum of dihydrofolate at pH 13 could be closely approximated by the mixed spectrum at the same pH. This was not true, however, when the comparison was made at pH 1 (Figure 5). In the case of the mixed spectrum, protonation of the secondary amino group (pK = 2.9; Osborn *et al.*, 1960) attached to the aromatic ring of *N*-methyl-*p*-aminobenzoylglutamate would be expected to suppress the absorption at 285 mμ. In the case of dihydrofolic acid, protonation of the N-10 position does not seem to have occurred at pH 1, as shown by the fact that the dihydrofolate spectrum is more closely approximated by combining the *neutral* spectrum of *N*-methyl-*p*-aminobenzoylglutamate to that of the dihydropteridine (Figure 5). Although not strictly comparable, it is of interest that the N-10 atom in tetrahydrofolate has an anomalously low pK (less than zero) which has been attributed to its spatial interaction with the protonated N-5 (Kallen and Jencks, 1966).

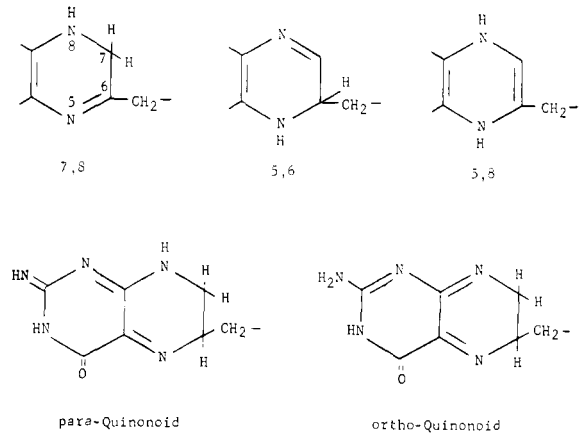

FIGURE 1: Tautomeric forms of dihydrofolate.

The results at both neutral and basic pH values, however, clearly support the conclusion that the spectrum of dihydrofolate is composed of two independently contributing species, namely the 7,8-dihydropteridine and *N*-alkyl-*p*-aminobenzoylglutamate. If it is assumed tht the N-10 position in dihydrofolate is not protonated until a very low pH (<1) is reached, then the above conclusion is valid for the entire pH range of 1–13.

Table I lists the pK values for 2-amino-4-hydroxy-6-methylpteridine and for its 7,8-dihydro and the 5,6,7,8-tetrahydro derivatives. The pK of 8.3 for the oxidized pteridine differs slightly from the figure of 8.4 recorded by Komenda (1959) for this compound but corresponds exactly to that given for folic acid (Albert, 1953). This pK is attributed to the hydroxyl group at the 4

TABLE I: pK_a Values of 2-Amino-4-hydroxy-6-methylpteridine and Reduced Products.

Form of Pteridine	pK_a Values	
Oxidized	8.3	2.8[a]
Dihydro	10.6	3.2[a]
Tetrahydro	10.5	5.4

[a] Estimated spectroscopically; all other values obtained titrimetrically.

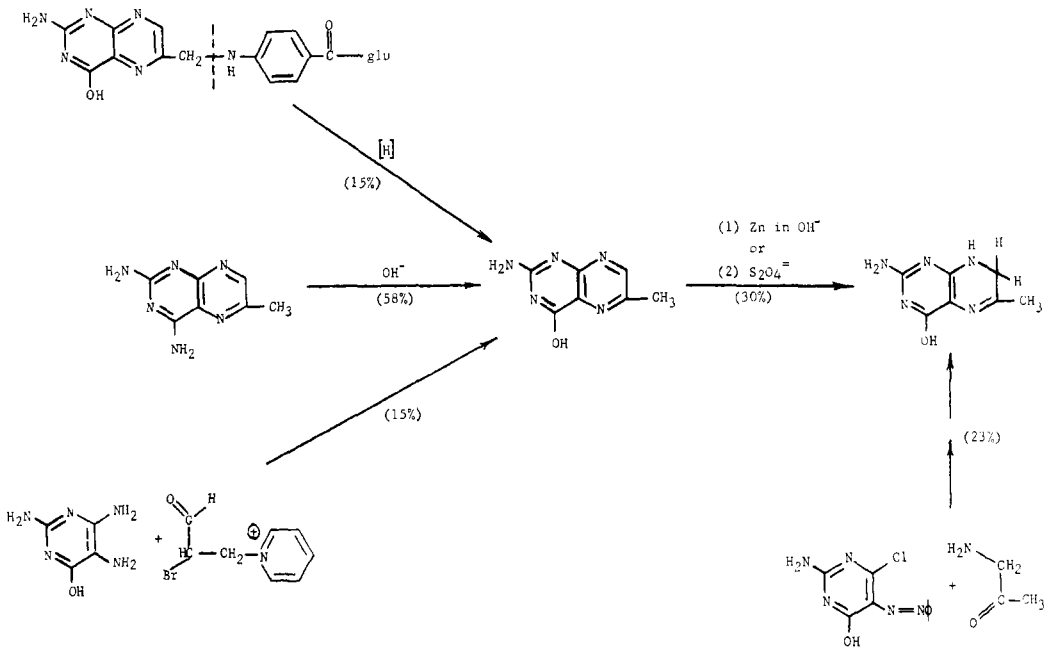

FIGURE 2: Routes for preparation of 2-amino-4-hydroxy-6-methylpteridine and the corresponding 7,8-dihydropteridine.

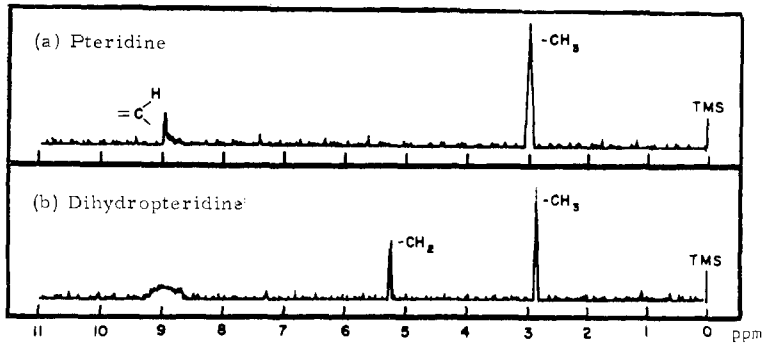

FIGURE 3: Proton magnetic resonance spectra: (a) 2-amino-4-hydroxy-6-methylpteridine and (b) 2-amino-4-hydroxy-6-methyl-7,8-dihydropteridine. Both spectra were taken in trifluoroacetic acid at 25°. TMS represents trimethylsilane.

position. The pK of 2.8, which may be compared to Komenda's value of 2.6, probably represents protonation of either the ring nitrogen atom at the 1 position or of the 2-amino group. The 2-amino group shows basic characteristics since it can be readily acetylated with acetic anhydride using a procedure similar to that described by Seeger *et al.* (1949) for the acetylation of 2,4-diamino-6-methylpteridine. The site of acylation at the 2-amino position was deduced from the similarity of the ultraviolet spectra of the acetamidopteridine and that of 2-amino-4-hydroxy-6-methylpteridine from pH 7–13 (*cf.* Experimental Section). Since a positive charge resulting from protonation on N-1 can be delocalized more effectively than a charge on the 2-amino group, it seems more likely that the pK of 2.8 is associated with the former group; a similar conclusion has been reached by Dieffenbacher *et al.* (1966) for 2-aminopteridines.

The pK values given in Table I for the tetrahydropteridine compare favorably with those determined by Pohland *et al.* (1951) for the related 2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine. Thus, 10.5 is the pK of the hydroxyl group at the 4 position and 5.4 probably represents the pK of the N-5 position. The ease of formylation of the 6-methyltetrahydropteridine at the 5 position (Cosulich *et al.*, 1952) and the ready susceptibility of N-5 in tetrahydrofolate toward alkylation (Gupta and Huennekens, 1967; Gupta *et al.*, 1967) provide further evidence for the basicity of this position.

Few data exist for pK values of dihydropteridines related to the present model compound and hence the

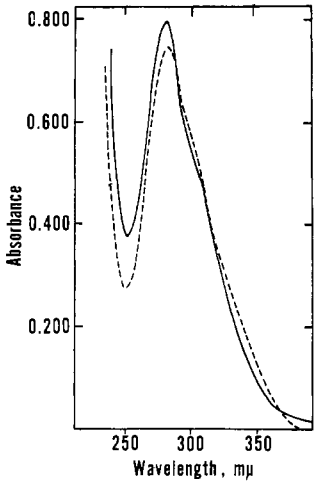

FIGURE 4: Spectra at pH 7.0 of dihydrofolate (– – – –) and equimolal mixture (———) of 2-amino-4-hydroxy-6-methyl-7,8-dihydropteridine plus *N*-methyl-*p*-aminobenzoylglutamate. Each compound was present at 3.4×10^{-5} M.

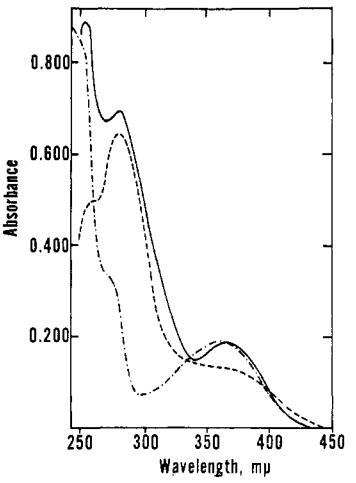

FIGURE 5: Spectra of dihydrofolate at pH 1.0 (– – – –), equimolal mixture at pH 1.0 (–·–·–·) of 2-amino-4-hydroxy-6-methyl-7,8-dihydropteridine plus *N*-methyl-*p*-aminobenzoylglutamate, and (———) spectrum of dihydropteridine at pH 1.0 added to spectrum of *N*-methyl-*p*-aminobenzoylglutamate at pH 7.0. Each compound was present at 3.4×10^{-5} M.

assignment of prototropic groups in the latter is somewhat arbitrary. The similarity of the high p*K* value (10.6) of the dihydropteridine to that found for tetrahydropteridine suggests that this figure again represents the hydroxyl group at the 4 position. The lower p*K* value of 3.2, determined spectroscopically because of difficulties with precipitation at the concentrations used in the titrimetric method, may well correspond to the N-8 position.

Experimental Section

Methods

Absorption spectra in the visible and ultraviolet regions were obtained with a Cary recording spectrophotometer, Model 14. Proton magnetic resonance spectra were determined with a Varian A-60 instrument through the courtesy of Dr. Earl Wadsworth. Elemental analyses were performed by the Spang Microanalytical Laboratory, Ann Arbor. Ascending paper chromatography was carried out on Whatman No. 1 paper using the following solvent systems: (A) ethanol–water (70:30), (B) ethanol–0.05 N HCl (70:30), and (C) ethanol–0.1 N HCl (70:30). Compounds were located as fluorescent or quenching spots under ultraviolet light (Mineralite).

Titrimetric p*K* determinations on 10-ml solutions (0.01–0.02 M) of the compounds were carried out at 22° with a Radiometer titrator, Model II, equipped with a SBR 2c recorder; in order to prevent precipitation of the pteridine compounds, the customary inert electrolyte was omitted. Prior to titration, the sample of 2-amino-4-hydroxy-6-methyl-5,6,7,8-tetrahydropteridine was heated at 78° (0.4 mm) for 24 hr over KOH in order to eliminate acetate present in the reduced pteridine.

For spectrophotometric p*K* titrations, constant amounts of the pteridine were added to the same volume of a series of buffer solutions in the pH range 2–5 and having an approximate ionic strength of 0.1. Measurements of pH at 22° were obtained with a Radiometer pH meter, Model 25. Ionization constants were obtained from the inflection points when absorbance at a given wavelength was plotted against pH. Results are reported in terms of the negative logarithm of the apparent acid dissociation constant.

Materials

Chemicals were obtained from the following sources: Dowex 1 and Dowex 50 resins from Bio-Rad Laboratories; DEAE-cellulose from Schleicher & Schuell; folic acid, purified by the method of Hutchings *et al.* (1948a,b), and *p*-aminobenzoylglutamic acid from the California Corp. for Biochemical Research; 2,4-diamino-6-methylpteridine (purified before use by the method of Seeger *et al.* (1949)), 2-amino-4-chloro-6-hydroxypyrimidine, and 2,4,5-triamino-6-hydroxypyrimidine sulfate from Cyclo Chemical Corp.; acrolein from Eastman Organic Chemicals; and bromine, pyridine, and aniline from the J. T. Baker Chemical Co.

N-Methyl-p-aminobenzoylglutamic Acid. *p*-Aminobenzoylglutamic acid (5.3 g, 20 mmoles) was converted to *p*-iodobenzoylglutamic acid (3.50 g, 9.3 mmoles, 45%) by the procedure of Cosulich and Smith (1948). Treatment of the latter compound (1.00 g, 2.6×10^{-3} mole) with methylamine at 130° in a sealed tube, followed by chromatography on DEAE-cellulose (gradient elution, 0.005–0.1 M ammonium acetate (pH 7); 1 l. each; followed by 0.1–1.0 M, 1 l. each), gave

N-methyl-*p*-aminobenzoylglutamic acid as an oil (0.25 g, 0.89 mmole, 34%), R_F 0.41 in system B.

2-Amino-4-hydroxy-6-methylpteridine. A. FROM FOLIC ACID. The following modification of the procedure of Hutchings *et al.* (1948b) was used. Folic acid (0.250 g, 0.56 mmole) was dissolved in 250 ml of 0.4 N sulfuric acid. To the resultant solution was added 5 g of zinc dust and the mixture was stirred for 30 min. Excess zinc was removed by filtration and 5 g of manganese dioxide was added to the stirred filtrate. After 10 min, the mixture was filtered and Zn^{2+} was precipitated as zinc phosphate by the addition of approximately 400 ml of 1 M K_2HPO_4. The precipitate was removed by filtration and the pH of the filtrate was adjusted to 6.0 by the addition of concentrated NH_4OH. A further precipitate was removed by filtration and the filtrate was allowed to stand at 0° for 12 hr. 2-Amino-4-hydroxy-6-methylpteridine precipitated from the solution and was recrystallized twice as the sodium salt from 5 N NaOH. Acidification to pH 6.0 yielded the product as a yellow amorphous solid (0.015 g, 0.085 mmole, 15%), R_F 0.49 in system A.

B. FROM 2,4-DIAMINO-6-METHYLPTERIDINE. 2,4-Diamino-6-methylpteridine (5.50 g, 31 mmoles) was converted to 2-amino-4-hydroxy-6-methylpteridine by the procedure of Seeger *et al.* (1949). Recrystallization from 5 N NaOH followed by neutralization with HCl gave the product as a yellow amorphous solid (3.20 g, 18 mmoles, 58%).

C. FROM 2,4,5-TRIAMINO-6-HYDROXYPYRIMIDINE. 2,4,5-Triamino-6-hydroxypyrimidine sulfate (23.9 g, 0.10 mole) was dissolved with slight warming in 80 ml of 2 N KOH and then an additional 20 ml of 5 N KOH was added in order to avoid rapid decomposition of the free pyrimidine that occurs at pH 7.0. An equivalent amount of $BaCl_2 \cdot 2H_2O$ (24.6 g, 0.1 mole) in 40 ml of water was added to precipitate sulfate. Concentrated HCl (30 ml) was added rapidly to the resulting suspension to give a pH of 2.0. $BaSO_4$ was removed by filtration and the amount of pyrimidine in the filtrate was estimated from the extinction coefficient of 9.9×10^3 M^{-1} cm^{-1} at 264 mμ (Cavalieri *et al.*, 1948) to be 97 mmoles in 220 ml. To this filtrate was added an aqueous solution of *N*-(2-formyl-2-bromo)ethylpyridinium bromide (Hultquist *et al.*, 1948), prepared from 2,3-dibromopropionaldehyde and dry pyridine. Following the procedure of Hultquist *et al.* (1948) a solution of KI (10 g, in 10 ml) was added to the reaction mixture. *N*-(2-Amino-4-hydroxy-6-pteridyl)-methylpyridinium iodide was obtained as yellow crystals (5.50 g, 14 mmoles, 15%), R_F 0.39 in system A. 2-Amino-4-hydroxy-6-methylpteridine was obtained from the iodide in 77% yield by the method of Boothe *et al.* (1948).

2-Amino-4-hydroxy-6-methyl-7,8-dihydropteridine. A. BY REDUCTION OF 2-AMINO-4-HYDROXY-6-METHYLPTERIDINE WITH HYDROSULFITE. 2-Amino-4-hydroxy-6-methylpteridine (0.100 g, 0.56 mmole) was dissolved in 2 ml of 1 N NaOH and the solution was diluted to 25 ml. To the stirred solution was added sodium hydrosulfite (1.00 g, 5.8 mmoles). After 4 hr, the pH had dropped to 4.5 and was further adjusted to 2.5 by the addition of dilute HCl. The precipitated product was recovered by filtration and taken up with warming into a minimum quantity of 0.1 N NaOH. The resultant solution was chromatographed on a 3 × 18 cm column of Dowex 50 (H^+ form) resin; 18-ml fractions were collected automatically. Gradient elution with HCl (2.0–4.0 N, 1 l. each) displaced the unreacted, oxidized pteridine from the column, followed by the dihydropteridine. After lyophilization, the dihydro compound was obtained as an amorphous solid (0.040 g, 0.17 mmole, based on the monohydrate hydrochloride, 30%). The product was recrystallized twice by dissolving it with gentle warming in the minimum quantity of 0.1 N NaOH and then adding concentrated HCl to a final concentration of 0.5 N. The resultant yellow, needle-shaped crystals were dried for 2 days at 60° (0.1 mm), λ_{max} 252 mμ in 0.1 N HCl (ϵ 20×10^3 M^{-1} cm^{-1}).

B. BY REDUCTION OF 2-AMINO-4-HYDROXY-6-METHYLPTERIDINE WITH ZINC–NaOH. 2-Amino-4-hydroxy-6-methylpteridine (1.00 g, 5.6 mmoles) was dissolved in 100 ml of 1 N NaOH and zinc dust (3 g) was added to the stirred solution. After 45 min the mixture was filtered and the filtrate was adjusted to pH 2.5 by the addition of concentrated HCl. The pale yellow precipitate was recovered by filtration and redissolved in 100 ml of 0.5 N NaOH. Zinc oxide was removed by filtration. The clear yellow solution was made 5 N by the addition of solid NaOH and the volume was reduced to 75 ml. A pale yellow, leafy precipitate of the sodium salt of 2-amino-4-hydroxy-6-methyldihydropteridine, which formed upon cooling, was collected by filtration and dissolved in 80 ml of water with gentle heating. Concentrated HCl was added to the solution to a final concentration of 0.5 N and, upon cooling, 2-amino-4-hydroxy-6-methyl-7,8-dihydropteridine monohydrate monohydrochloride appeared as yellow, needle-shaped crystals. After three recrystallizations by this procedure, the product was dried for 2 days at 60° (0.1 mm) (0.390 g, 1.7 mmoles, 30%), λ_{max} 252 mμ in 0.1 N HCl (ϵ 20.2×10^3 M^{-1} cm^{-1}). *Anal.* Calcd for $C_7H_9ON_5 \cdot H_2O \cdot HCl$: C, 36.1; H, 5.15; Cl, 15.2; N, 30.0. Found: C, 36.3; H, 5.18; Cl, 15.2; N, 29.8.

C. FROM 2-AMINO-4-CHLORO-6-HYDROXYPYRIMIDINE. Bromoacetone was converted to aminoacetone semicarbazone hydrochloride *via* phthalimidoacetone and aminoacetone hydrochloride (Albert and Matsuura, 1961). Following the procedure of Boon and Leigh (1951), aminoacetone semicarbazone hydrochloride (1.50 g, 9.0 mmoles) was condensed with 2-amino-4-chloro-5-phenylazo-6-hydroxypyrimidine (1.10 g, 4.4 mmoles), derived from 2-amino-4-chloro-6-hydroxypyrimidine by diazotization, to give 2-amino-4-(2′-oxopropylamino)-5-phenylazo-6-hydroxypyrimidine (0.600 g, 1.9 mmoles, 43%) as the bright orange hydrochloride. Reductive ring closure yielded 2-amino-4-hydroxy-6-methyl-7,8-dihydropteridine as the crystalline hydrochloride (0.100 g, 0.43 mmole, 23%), λ_{max} 252 mμ in 0.1 N HCl (ϵ 20×10^3 M^{-1} cm^{-1}).

2-Amino-4-hydroxy-6-methyl-5,6,7,8-tetrahydro-

pteridine. 2-Amino-4-hydroxy-6-methylpteridine (0.021 g, 0.12 mmole) suspended in 10 ml of glacial acetic acid was catalytically reduced in a microhydrogenation unit (Ogg and Cooper, 1949) at room temperature (22°) over platinum oxide (0.020 g) according to the method used by Hatefi *et al.* (1960) for the conversion of folate to tetrahydrofolate. The uptake of hydrogen, after correction to standard temperature and pressure, was 4.77 ml (0.22 mmole). The tetrahydropteridine, which was soluble in glacial acetic acid, was separated from the catalyst by filtration into a vessel immersed in a Dry Ice–methoxyethanol cooling bath and the solvent was then removed by lyophilization to yield 2-amino-4-hydroxy-6-methyl-5,6,7,8-tetrahydropteridine as a white powder (0.017 g). Titrimetric determinations showed the presence of 1 mole of acetic acid/mole of this compound, λ_{max} 265 mμ in 0.1 N HCl (ϵ 16.0 × 10^3 M^{-1} cm^{-1}); λ_{max} 303 mμ in 0.1 M phosphate buffer (pH 7.0) (ϵ 9.5 × 10^3 M^{-1} cm^{-1}); λ_{max} 297 mμ in 0.1 N NaOH (ϵ 7.5 × 10^3 M^{-1} cm^{-1}).

2-Acetamido-4-hydroxy-6-methylpteridine. 2-Amino-4-hydroxy-6-methylpteridine (0.500 g, 2.8 mmoles) was suspended in 20 ml of acetic anhydride and the mixture was heated under reflux for 12 hr. After cooling to 5° and standing for 24 hr, crystals separated from the dark solution; these were collected by filtration, washed with acetic anhydride and cold ethanol, taken up with warming in 10 ml of ethanol, and treated with charcoal. Filtration gave a pale yellow solution which, after concentration and standing for 24 hr at 5°, yielded 2-acetamido-4-hydroxy-6-methylpteridine as a pale yellow crystalline solid (0.200 g, 32.5%), mp 220° dec, R_F 0.78 in system A; λ_{max} 278 and 330 mμ in 0.1 N HCl (ϵ 10.9 × 10^3 and 7.5 × 10^3 M^{-1} cm^{-1}); λ_{max} 275 and 330 mμ in 0.1 M phosphate (pH 7.0) (ϵ 10.0 × 10^3 and 7.8 × 10^3 M^{-1} cm^{-1}); λ_{max} 252 and 347 mμ in 0.1 N NaOH (ϵ 22.2 × 10^3 and 7.0 × 10^3 M^{-1} cm^{-1}). After drying at 78° (0.4 mm) for 2 days the following data were obtained. *Anal.* Calcd for $C_9H_9O_2N_5$: C, 49.3; H, 4.11; N, 31.9. Found: C, 49.2; H, 4.10; N, 31.9.

Acknowledgments

The authors are indebted to Drs. D. J. Vonderschmitt and V. S. Gupta for helpful discussions of this problem.

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