The Synthesis of 6-Hydroxymethylpteridines

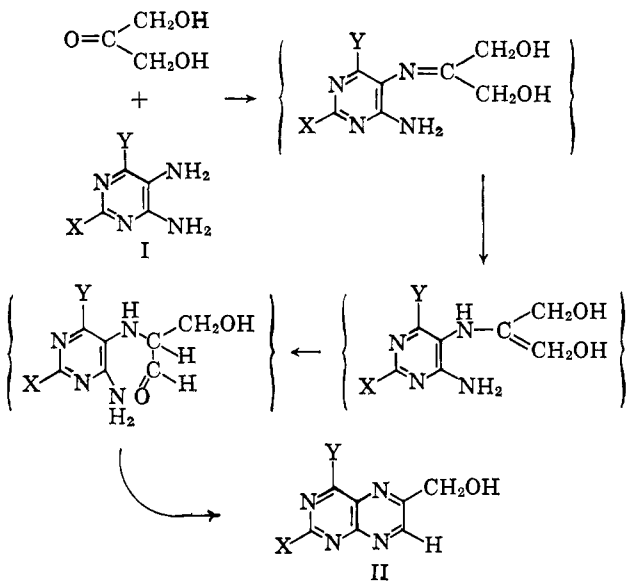
CHARLES M. BAUGH AND ELLIOTT SHAW

Department of Biochemistry, Tulane University School of Medicine, New Orleans, Louisiana

Received August 3, 1964

The condensation of dihydroxyacetone with 2,6-disubstituted 4,5-diaminopyrimidines in sodium acetate containing 1 equiv. of cysteine leads to good yields of the corresponding 6-hydroxymethylpteridines.

Recent evidence from *in vitro* studies indicates that 2-amino-4-hydroxy-6-hydroxymethylpteridine (IIa), probably in a reduced and phosphorylated form, is the pteridine precursor of folic acid.[1,2] In this laboratory we have recently isolated Ia and IIa from natural sources.[3,4] An available supply of this 6-hydroxymethylpteridine (IIa) by synthesis would facilitate biosynthetic studies and consequently preparative methods were investigated.



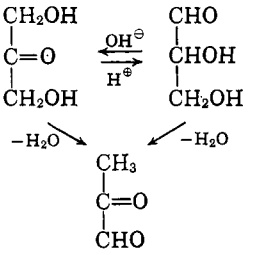
a, X = NH_2; Y = OH
b, X = NH_2; Y = NH_2
c, X = OH; Y = NH_2

2-amino-4-hydroxy-6-hydroxymethylpteridine (IIa) has previously been obtained by a variety of methods which have the disadvantages of using folic acid as a starting material,[5] of providing low yields, or of involving condensations that lead to mixtures difficult to separate.

Waller, *et al.*,[5] used a sulfurous acid cleavage of folic acid to obtain low yields of the 6-carboxaldehyde which was then treated with alkali to form equimolar amounts of IIa and the corresponding acid. More recently reduction of the aldehyde with sodium borohydride has been employed.[6]

The condensation of 6-hydroxy-2,4,5-triaminopyrimidine (Ia) with dihydroxyacetone has been the subject of considerable study.[7,8] The main difficulties with this reaction as a synthesis of IIa are a lack of control over the orientation of the condensation with resultant formation of 6- and 7-substituted pteridines and a tendency to form methyl as well as hydroxymethyl derivatives. However, 2-amino-4-hydroxy-6-hydroxymethylpteridine has been obtained from a reaction carried out in the presence of hydrazine followed by fractional crystallization of the diacetates.[7] Variations thus far reported do not appear to be promising.[9] Forrest and Walker reported the isolation of IIa from the condensation of Ia with dihydroxyacetone in the presence of sodium acetate, boric acid, and hydrazine. This condensation was carried out at 100° in an atmosphere of nitrogen.[10]

The formation of methylpteridines from glyceraldehyde[7,11] or dihydroxyacetone[7] when condensed with a diaminopyrimidine has been interpreted as due to dehydration of an intermediate hydroxymethyldihydropteridine. However, an alternative explanation, considered by us to be more likely, is that the reaction conditions, in the case of these carbonyl compounds, influence the equilibrium of a Lobry De Bruyn–Van Ekenstein transformation[12] between glyceraldehyde and dihydroxyacetone and the extent of a side reaction[13] leading to methylglyoxal by dehydration—all prior to condensation. This latter intermediate would be the source of the methylpteridines.



Acid conditions have been found to exert a directive influence on the course of pteridine synthesis through this condensation when keto acids or esters are the participating carbonyl compounds by favoring initial Schiff base formation at the 5-amino position[14] of the diaminopyrimidine. When dihydroxyacetone is the carbonyl compounds, however, acid conditions favor conversion to methylglyoxal. When the condensation of glyceraldehyde or dihydroxyacetone was carried out

(1) R. A. Weisman and G. Brown, *J. Biol. Chem.*, **239**, 326 (1964).
(2) T. Shiota, M. N. Disraely, and M. P. McCann, *Biochem. Biophys. Res. Commun.*, **7**, 194 (1962).
(3) C. M. Baugh and Elliott Shaw, *ibid.*, **10**, 28 (1963).
(4) C. L. Krumdieck, C. M. Baugh, and E. N. Shaw, *Biochem. Biophys. Acta.*, **90**, 573 (1964).
(5) C. W. Waller, A. A. Goldman, R. B. Angier, J. H. Boothe, B. L Hutchings, J. H. Mowat, and J. Semb, *J. Am. Chem. Soc.*, **72**, 4630 (1950).
(6) Procedure suggested by Dr. Robert B. Angier, Lederle Laboratories, Pearl River, N. Y.
(7) P. Karrer and R. Schwyzer, *Helv. Chim. Acta*, **30**, 1031 (1947).
(8) H. J. Backer and A. C. Houtman, *Rec. trav. chim.*, **70**, 725 (1951).
(9) F. Hoffman-LaRoche and Co. A. G., Swiss Patent 253,475 (Nov. 1, 1948); *Chem. Abstr.*, **44**, 669*f* (1950); American Cyanamid Co., British Patent 656,403 (Aug. 22, 1951).
(10) H. S. Forrest and J. Walker, *J. Chem. Soc.*, 2077 (1949).
(11) R. B. Angier, C. W. Waller, J. H. Boothe, J. H. Mowat, J. Semb, B. L. Hutchings, E. C. R. Stokstad, and Y. SubbaRow, *J. Am. Chem. Soc.*, **70**, 3029 (1948).
(12) H. O. L. Fischer, C. Taube, and E. Baer, *Ber.*, **60**, 480 (1927).
(13) J. C. Speck, Jr., *Advan. Carbohydrate Chem.*, **13**, 73 (1958).
(14) G. B. Elion and G. H. Hutchings, *J. Am. Chem. Soc.*, **69**, 2553 (1947); G. B. Elion, G. H. Hutchings, and P. B. Russell, *ibid.*, **72**, 78 (1950).

in 0.1 *N* HCl at 100°, good yields of 2-amino-4-hydroxy-7-methylpteridine were obtained. On the other hand, alkali enhances the conversion of glyceraldehyde to dihydroxyacetone,[12] but under these conditions diaminopyrimidines of type I are unstable.

We report here conditions that appear to be general for the formation of 6-hydroxymethylpteridines from dihydroxyacetone. The yields of recrystallized products range from 50–60% and, in further contrast to published methods, only 6-substituted pteridines are obtained.

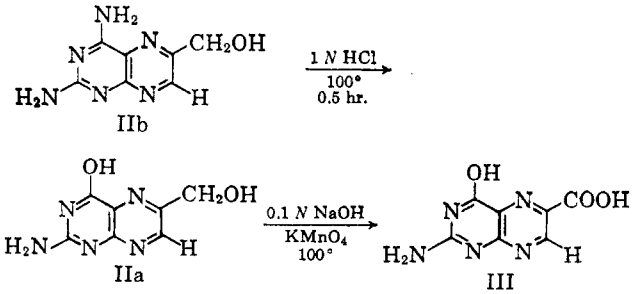
The conditions for these condensations are critical and were chosen to avoid extreme pH, to provide mild oxidizing conditions for aromatization of presumed reduced intermediates, and at the same time to prevent self-condensation of diaminopyrimidines to pyrimidopteridines.[15] It was observed that low concentrations (0.1%) of mercaptoethanol would fully protect Ia from self-condensation and that passing air through the reaction mixture supplied adequate oxidizing conditions to facilitate ring closure with pteridine formation. The volatility of mercaptoethanol and foaming of the reaction mixture due to the bubbling of air caused us to seek more suitable conditions. Accordingly, the nonvolatile reducing agent cysteine was substituted for mercaptoethanol and the reaction mixture was aerated by swirling of a rotary shaker.

The extension of these conditions to the condensation of dihydroxyacetone with other 5,6-diaminopyrimidines (Ib and Ic) was undertaken in order to demonstrate the general applicability of this synthesis, and also because of biochemical interest in the pteridine products (IIb and IIc).

These pteridines are analogs of a metabolic precursor of folic acid and as such one might expect them to inhibit organisms which synthesize folic acid.

The analytical data indicate that the formation of methylpteridines was not a conflicting side reaction in these condensations. However, these data do not bear on the question of what proportion of isomeric 6- and 7-hydroxymethyl compounds were being formed. Therefore careful attention was paid to the details of ultraviolet spectra and paper chromatography in order to clarify this point.

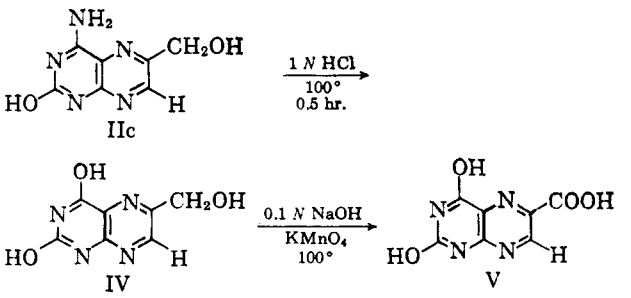
Additional support of the structure of IIa is the ratio of maxima in 0.1 *N* NaOH, ϵ 253 to ϵ 362 mμ, which we find to be 226:7.0 = 3.2. This is in exact agreement with the figure published by Petering and Schmitt for 2-amino-4-hydroxy-6-polyhydroxyalkylpteridines.[16]



Compound IIb was characterized by its deamination[17] to IIa as shown below and by subsequent oxidation of IIa to the carboxylic acid III.

This deamination of IIb may also be accomplished by heating in 0.1 *N* NaOH for 30 min. In both procedures the yields are quantitative and it appears that this deamination is a satisfactory synthesis for IIa.

Similarly compound IIc was characterized by deamination to IV. The 6-hydroxymethyllumazine (IV) was then oxidized to V.



As a chromatographic standard an authentic sample of lumazine-6-carboxylic acid (V) was synthesized by the method of Angier, *et al.*[18] No trace of the 7-isomer of V could be detected either on paper chromatograms or spectrally.[19]

For the detection of lumazine-7-carboxylic acid in the oxidation product of IIc the ratio of absorbancies, 265 to 245 mμ, in 0.1 *N* NaOH was considered to be a sensitive indicator. For the 6-isomer there is a maxima at 265 mμ and a minimum at 240 mμ, whereas the 7-isomer has a maxima at 245 mμ. Consequently for authentic lumazine-6-carboxylic acid the ratio of absorbancies, 265 to 245 mμ, is high (2.04); the value found from the acid prepared from IIc as described in this paper was 1.96, considered to be in satisfactory agreement.

Experimental[20]

General Synthetic Procedure.—The following procedure was followed in the condensation of either Ia, Ib, or Ic with dihydroxyacetone.

The diaminopyrimidine sulfate (10 mmoles) was taken up in 40 ml. of water and $BaCl_2$ (10 mmoles) in 10 ml. of water was added. This mixture was placed in a boiling water bath for 10 min. After cooling, the $BaSO_4$ was removed by filtration and the solid was washed on the filter with about 10 ml. of water. The combined washings and filtrate were made up to 150 ml. with water. This was added to a solution of 150 ml. of 4 *M* NaOAc containing dihydroxyacetone (30 mmoles) and cysteine hydrochloride monohydrate (10 mmoles) in a 1-l. erlenmeyer flask and was placed on a rotary shaker at room temperature for 24 hr. After this period the flask was placed in the cold for several hours, the precipitate was then collected by suction filtration and washed with cold water. The precipitate was then resuspended in 100 ml. of water and heated to boiling; where necessary, drops of 1 *N* NaOH were added to effect solution. Norit (0.5 g.) was added and the hot solution, thoroughly mixed, was filtered while hot through a heated funnel. After cooling to room temperature the pH was adjusted to 6.0 with 1 *N* HCl if necessary, and the flask was placed in the cold for several hours.

(15) E. A. Falco and G. H. Hutchings, "Ciba Foundation Symposium, Chemistry and Biology of Pteridines," G. E. W. Wolstenholme, Ed., Little, Brown and Co., Boston, Mass., 1954, p. 183.

(16) H. G. Petering and J. A. Schmitt, *J. Am. Chem. Soc.*, **71**, 3977 (1949).

(17) E. C. Taylor and C. K. Cain, *ibid.*, **71**, 2538 (1949).

(18) R. B. Angier, J. H. Boothe, J. H. Mowat, C. W. Waller, and J. Semb, *ibid.*, **74**, 408 (1952).

(19) C. K. Cain, M. F. Mollette, and E. C. Taylor, Jr., *ibid.*, **70**, 3026 (1948).

(20) Microanalysis was by Alfred Bernhardt, Mülheim, Germany.

The precipitate was collected by filtration, washed with cold water, ethanol, (50:50) ethanol–ether, and finally with ether, then dried *in vacuo*.

The yield for various pteridines formed, based on dry weights, after an additional recrystallization from water, was 50–60%.

2-Amino-4-hydroxy-6-hydroxymethylpteridine (IIa),—was characterized by its infrared spectra. For comparison, an authentic sample was obtained by the $NaBH_4$ reduction[6] of an authentic sample of 2-amino-4-hydroxy-6-pteridinecarboxaldehyde.[21] A pure sample of IIa was oxidized to 2-amino-4-hydroxy-6-pteridinecarboxylic acid (III) with $KMnO_4$ in alkali.[22] Quantitative conversion to the acid III was obtained as judged by the change in spectra.[23] No trace of the 7-acid was seen either spectrally[24] or on paper chromatography in the solvent system of Weygand, *et al.*[25] The absence of the 7-carboxylic acid was judged by the lack of absorption at 400 mμ in 0.1 *N* NaOH where this isomer has a maxima as well as by the correspondence of the spectrum of the oxidation product to that of the 6-carboxylic acid III in all details.[23]

Spectral properties of IIa: in 0.1 *N* HCl, λ_{max} 247 mμ (ε 10,340), λ_{max} 322 mμ (ε 7690), λ_{min} 272 mμ (ε 1760); in 0.1 *N* NaOH, λ_{max} 253 mμ (ε 22,600), λ_{max} 362 mμ (ε 7000), λ_{min} 230 mμ (ε 8400), λ_{min} 300 mμ (ε 1000).

Anal. Calcd. for $C_7H_7N_5O_2$: C, 43.52; H, 3.65; N, 36.26; O, 16.57. Found: C, 43.26; H, 3.94; N, 34.97; O, 17.67.

2,4-Diamino-6-hydroxymethylpteridine (IIb) was characterized by deamination[17] to IIa and the $KMnO_4$ oxidation of IIa to III. The deamination to IIa was quantitative, no trace of the 7-isomer was detected. The selective removal of the 5-amino group in IIb was also accomplished by heating in a boiling water bath for 30 min. in 0.1 *N* NaOH.

Spectral properties of IIb: in 0.1 *N* HCl, λ_{max} 243 mμ (ε 15,730), 283 (4620), 336.5 (9840), λ_{min} 228 mμ (10,670), 265 (3520), 296 (4290); in 0.1 *N* NaOH, λ_{max} 227 mμ (ε 11,300), 257 (21,000), 368 (7200), λ_{min} 235 mμ (ε 10,600), 305 (1200).

Anal. Calcd. for $C_7H_8N_6O$: C, 43.74; H, 4.19; N, 43.74. Found: C, 43.63; H, 4.78; N, 43.78.

2-Hydroxy-4-amino-6-hydroxymethylpteridine (IIc) was characterized by its deamination to 2,4-dihydroxy-6-hydroxymethylpteridine (IV). This was accomplished by heating IIc in 0.1 *N* HCl in a boiling water bath for 30 min. This deamination also proceeds readily in 0.1 *N* NaOH at 100°. The lumazine IV was oxidized to the corresponding carboxylic acid V in alkaline permanganate. As a reference standard lumazine-6-carboxylic acid (V) was prepared by the method of Angier, *et al.*[18] No trace of the lumazine-7-carboxylic acid could be detected spectrally.[19]

Spectral properties of IIc: in 0.1 *N* HCl, λ_{max} 275 mμ (ε 5290), λ_{max} 342 mμ (ε 5170), λ_{min} 266 mμ (ε 4860), λ_{min} 300 mμ (ε 2860); in 0.1 *N* NaOH, λ_{max} 257.5 mμ (ε 13,840), λ_{max} 376 mμ (ε 4200), λ_{min} 236 mμ (ε 9600), λ_{min} 315 mμ (ε 1000).

Anal. Calcd. for $C_7H_9N_5O_3 \cdot H_2O$: C, 39.80; H, 4.29; N, 33.16. Found: C, 39.45; H, 4.29; N, 33.97.

Chromatographic data in four solvent systems for the synthetic pteridines and their oxidation products are given in Table I.

TABLE I

R_f VALUES IN FOUR SOLVENT SYSTEMS[a]

Pteridine	*i*-PrOH–NH_4OH–H_2O (7:1:2)	*n*-BuOH–EtOH–H_2O (100:35:72)	*n*-BuOH–HOAc–H_2O (4:1:5)	*sec*-BuOH–HOAc–H_2O (8:2:5)
IIa	0.30	0.37	0.38	0.52
IIb	0.36	0.39	0.35	0.59
IIc	0.24	0.26	0.40	0.58
III	0.10	0.17	0.22	0.44
V	0.14	0.19	0.20	0.47

[a] Paper chromatography was carried out on 10 × 16 in. sheets of Whatman No. 3 MM paper in closed jars in the dark. The ascending method was employed. Spots were detected by observing the completely dried papers in the dark with a Mineralite ultraviolet source, output maxima at 254 mμ.

Acknowledgment.—We express our appreciation to Dr. Carlos Krumdieck for his interest and encouragement and to Miss Lynn Capel for her technical assistance. We are also indebted to the American Cancer Society (T-67) and to the U. S. Public Health Service (CA-05294 of the National Cancer Institute) for support of this work.

(21) A gift from Dr. Robert B. Angier, Lederle Laboratories, Pearl River, N. Y.

(22) F. Weygand and G. Shaefer, *Chem. Ber.*, **85**, 307 (1952).

(23) E. L. R. Stokstad, B. L. Hutchings, J. H. Mowat, J. H. Boothe, C. W. Waller, R. B. Angier, J. Semb, and Y. SubbaRow, *J. Am. Chem. Soc.*, **70**, 5 (1948).

(24) J. H. Mowat, J. H. Boothe, B. L. Hutchings, E. L. R. Stokstad, C. W. Waller, R. B. Angier, J. Semb, D. B. Cosulich, and Y. SubbaRow, *ibid.*, **70**, 14 (1948).

(25) F. Weygand, A. Wacker, and V. Schmied-Koworzik, *Experientia*, **6**, 184 (1950).

5α-Androstano[3,2-*b*]pyrroles[1]

THEODORE C. MILLER AND ROBERT G. CHRISTIANSEN

Sterling-Winthrop Research Institute, Rensselaer, New York

Received July 28, 1964

5α-Androstano[3,2-*b*]pyrrol-17β-ol (**7a**) was synthesized by the following sequence of steps: 17β-hydroxy-5α-androstan-3-one (**1a**) → 17β-hydroxy-2-(hydroxymethylene)-5α-androstan-3-one (**2a**) → 2α-allyl-17β-hydroxy-5α-androstan-3-one acetate (**4b**) → 2α-(formylmethyl)-17β-hydroxy-5α-androstan-3-one acetate (**5a**) → 5α-androstano[3,2-*b*]-1′-benzylpyrrol-17β-ol acetate (**6a**) → **7a**. The 2α-allylation of **1a** was also accomplished by direct allylation of **1a** and by the allylation of the pyrrolidine enamine (**3**) of **1a**. A similar series of steps afforded 17-methyl-5α-androstano[3,2-*b*]pyrrol-17β-ol (**7b**) from 17β-hydroxy-17-methyl-5α-androstan-3-one (**1b**). A small amount of alkylation at the 4-position was observed in the direct allylation of **1a** and **1b**.

As part of a program to prepare analogs of the excellent anabolic agent, 17α-methyl-5α-androstano[3,2-*c*]-pyrazol-17β-ol (stanozolol, Winstrol®), and related steroid-fused pyrazoles[2a,b] and isoxazoles,[3a,b] the synthesis of the 5α-androstano[3,2-*b*]pyrroles (**7a** and **7b**) was undertaken. This paper reports the successful synthesis of **7a** and **7b**[4] by the sequence of steps shown in the flow chart: first, introduction of the allyl group into the 2α-position of the 5α-androstan-3-one (**1**) either directly, *via* the 2-(hydroxymethylene)-3-one

(1) Steroidal Heterocycles. XI. Paper X: J. H. Ackerman, G. O. Potts, A. L. Beyler, and R. O. Clinton, *J. Med. Chem.*, **7**, 238 (1964).

(2) (a) R. O. Clinton, A. J. Manson, F. W. Stonner, A. L. Beyler, G. O. Potts, and A. Arnold, *J. Am. Chem. Soc.*, **81**, 1513 (1959); (b) R. O. Clinton, A. J. Manson, F. W. Stonner, H. C. Neumann, R. G. Christiansen, R. L. Clarke, J. H. Ackerman, D. F. Page, J. W. Dean, W. B. Dickinson, and C. Carabateas, *ibid.*, **83**, 1478 (1961).

(3) (a) R. O. Clinton, A. J. Manson, F. W. Stonner, R. G. Christiansen; A. L. Beyler, G. O. Potts, and A. Arnold, *J. Org. Chem.*, **26**, 279 (1961); (b) A. J. Manson, F. W. Stonner, H. C. Neumann, R. G. Christiansen, R. L. Clarke, J. H. Ackerman, D. F. Page, J. W. Dean, D. K. Phillips, G. O. Potts, A. Arnold, A. L. Beyler, and R. O. Clinton, *J. Med. Chem.*, **6**, 1 (1963).

(4) A recent patent [J. C. Orr and A. Bowers, U. S. Patent 3,032,551 (May 1, 1962)] reported the synthesis of 5α-androstano[3,2-*b*]pyrroles by the reaction of 5α-androstan-3-ones with α-aminoaldehydes or α-amino ketones, but no physical data for the pyrroles were given.