composed into aminoacetone; the half-life appeared to be several weeks. Such solutions suffered less than 20% decomposition when allowed to stand for two days at room temperature.

A semi-quantitative determination of the decomposition rate of I–HCl was measured in 0.005 *N* HCl and in 0.1 *M* tris buffer, *p*H 7.8, by use of the ferric chloride reaction. A 1.3×10^{-3} *M* solution of I–HCl in the appropriate buffer was heated in a thermostat at 60°. At intervals a 1.0-ml. sample was withdrawn, diluted with 3.5 ml. of 0.005 *N* HCl and the *p*H adjusted to 2.3. Finally, 0.2 ml. of 3×10^{-2} *M* $FeCl_3 6H_2O$ was added and the volume diluted to 5.0 ml. The optical density of these solutions was measured at 500 mμ in a Unicam colorimeter against a blank containing all of the ingredients except the sample. From the results shown in Table I it is apparent that under both conditions tested the reaction closely approximates a first order curve. Preliminary experiments using similar methods of analysis indicate that the half-life at room temperature in 1.0 *N* NaOH is at least one hour. A solution which had been allowed to stand at *p*H 11.4 for 15 minutes gave the same titration curve as did a freshly dissolved sample. No attempt was made in the stability experiments to assess the effect of the buffer ions on the decomposition rate.

Compound I was only obtained as the hydrochloride; no effort was made to isolate the *zwitterion*.

Acknowledgments.—The authors are indebted to Mr. Tom Emery for certain of the physical measurements reported in this paper.

LONDON, W. 2, ENGLAND

[CONTRIBUTION FROM THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION]

The Structure of a Ribityl Pteridine Produced by the Yeast, *Eremothecium ashbyii*

BY WALTER S. MCNUTT[1]

RECEIVED MAY 2, 1959

The yeast, *Eremothecium ashbyii*, produces, in addition to large quantities of riboflavin, a ribityl pteridine, Compound A, which has a structure formally related to that of the vitamin. Compound A of high specific activity was obtained from cultures of this yeast fed guanine-U-C^{14}. One mole of Compound A consumes 3 moles of $NaIO_4$ and yields 1 mole of HCHO, 2 moles of HCOOH and a product having ultraviolet absorption spectra similar to those of Compound A. This last product upon reduction with $NaBH_4$ yields a substance identical with 6-methyl-8-(β-hydroxyethyl)-2,4,7-trioxohexahydropteridine. Comparison of 6-methyl-8-(1-D-ribityl)-2,4,7-trioxo-hexahydropteridine with Compound A in regard to their physical properties and the dilution of radioactive Compound A with the synthetic compound have established the identity of the two substances.

The C^{14} of uniformly labeled adenine is effectively incorporated into the isoalloxazine ring of riboflavin,[2] and the distribution of labeling among the carbon atoms of riboflavin suggests that the carbon atoms constituting the pyrimidine ring of adenine are incorporated as a unit into the pyrimidine portion of the isoalloxazine ring.[3] In a search for biochemical intermediates between adenine and riboflavin a substance, Compound A, was found which had a molar specific radioactivity almost identical with that of riboflavin. However, no evidence was adduced that Compound A is an actual intermediate in the biosynthesis of riboflavin. Neither was there evidence that it was derived from riboflavin through biological degradation.[4] Compound A was shown to be a 2,4-dioxo-pteridine with a substituent on the 8-position.[5] The Compound A which was isolated from cultures of *Eremothecium ashbyii* fed guanine-U-C^{14} had $^1/_4$ of its radioactivity in the ureido carbon atom,[6] as was true also of riboflavin obtained from cultures fed adenine-U-C^{14}.[2]

Thus from consideration of: (1) the comparative specific activities of riboflavin and Compound A from the same culture, (2) the distribution of C^{14} between the ureido carbon atom and the remainder of the molecule in each case and (3) the formal structural similarity of the two substances, it seems likely that the substances arise through certain biosynthetic steps common to both. An accounting of the radioactivity in the other carbon atoms of Compound A has yet to be made.

The experiments of Kuwada, *et al.*,[7] with crude enzyme preparations suggest that Compound A may arise in this yeast from a substance designated by Masuda[8] as Compound G. In cultures of this organism, 7 or more days old, only trace amounts of Compound G are present. Compound A is almost certainly identical with Compound V, from which Masuda, *et al.*,[9] obtained 6-methyl-2,4,7-trihydroxypteridine as a product of photodegradation, as well as the synthetic compound described by Plaut and Maley.[10] Its physical characteristics differ from those reported for these substances only in that it melts without decomposition and has an ultraviolet absorption spectrum differing somewhat from that described by Plaut and Maley.[10]

Evidence is presented in this article supporting the structure which these workers have proposed for this substance, together with the reasons for writing the structure as 6-methyl-8-(1-D-ribityl)-2,4,7-trioxo-hexahydropteridine.

Compound A is a strong acid ($pK_a = 3.95$) and has a neutral equivalent corresponding to its molecular weight.[5] It does not give the test for *cis*-glycols employed by Makino[11] perhaps because of the buffering action of its sodium salt. Compound A is degraded by dilute NaOH to urea in

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(2) W. S. McNutt, *J. Biol. Chem.*, **210**, 511 (1954).

(3) W. S. McNutt, *ibid.*, **219**, 365 (1955).

(4) W. S. McNutt and H. S. Forrest, THIS JOURNAL, **80**, 951 (1958).

(5) H. S. Forest and W. S. McNutt, *ibid.*, **80**, 739 (1958).

(6) W. S. McNutt, *Federation Proc.*, **18**, 286 No. 1133 (1959).

(7) S. Kuwada, T. Masuda, T. Kiski and M. Asai, *Pharm. Bull. (Japan)*, **6**, 619 (1958).

(8) T. Masuda, *ibid.*, **5**, 136 (1956).

(9) T. Masuda, T. Kiski and M. Asai, *ibid.*, **6**, 291 (1958).

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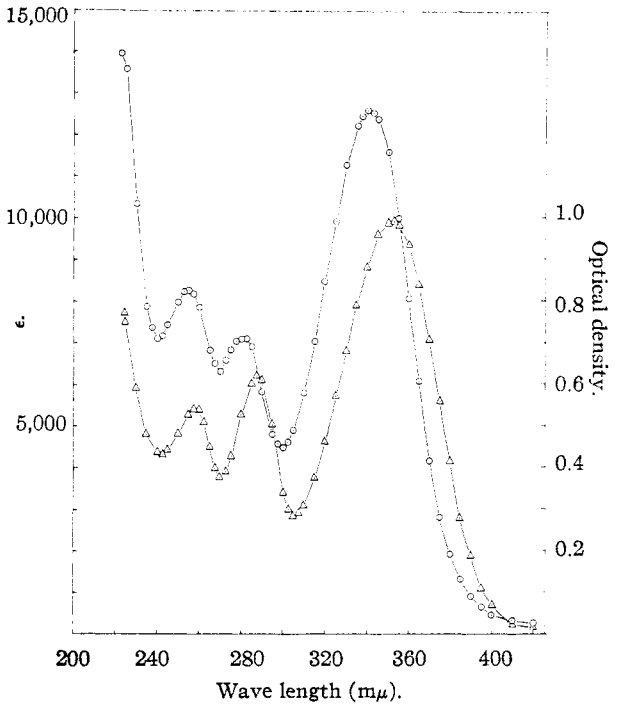
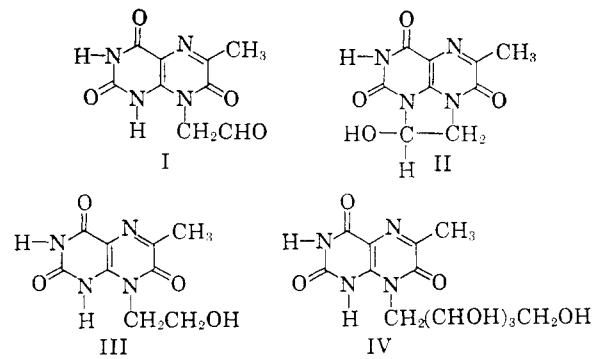


Fig. 1.—Ultraviolet absorption spectra of derivatives of Compound A: ⊙, ε of the periodate oxidation product of Compound A in 0.1 *N* NaOH; △, optical density of the product of $NaBH_4$ reduction of the periodate oxidation product in 0.1 *N* NaOH.

75% yield, which suggests that it is a 2,4-dioxopteridine with a substituent on the nitrogen in the 8-position.[5] One mole of Compound A consumes 3 moles of $NaIO_4$, not 2 moles as stated earlier,[5] and yields 1 mole of HCHO, 2 moles of HCOOH and a product, thought to be principally II, which has ultraviolet absorption spectra similar to those of Compound A but which show a shift toward shorter wave length (Fig. 1).



Reduction of this product with $NaBH_4$ yields a substance identical with 6-methyl-8-(β-hydroxyethyl)-2,4,7-trioxo-hexahydropteridine (III) as judged by cochromatography and ultraviolet spectrophotometry (Figs. 1 and 2).

An unexpected finding was the small amount of acid apparent in the reaction mixture after Compound A had been oxidized with periodate. The reaction mixture, previously adjusted to *p*H 5 was titrated back to this same *p*H with standard NaOH. The amount titrated corresponded to

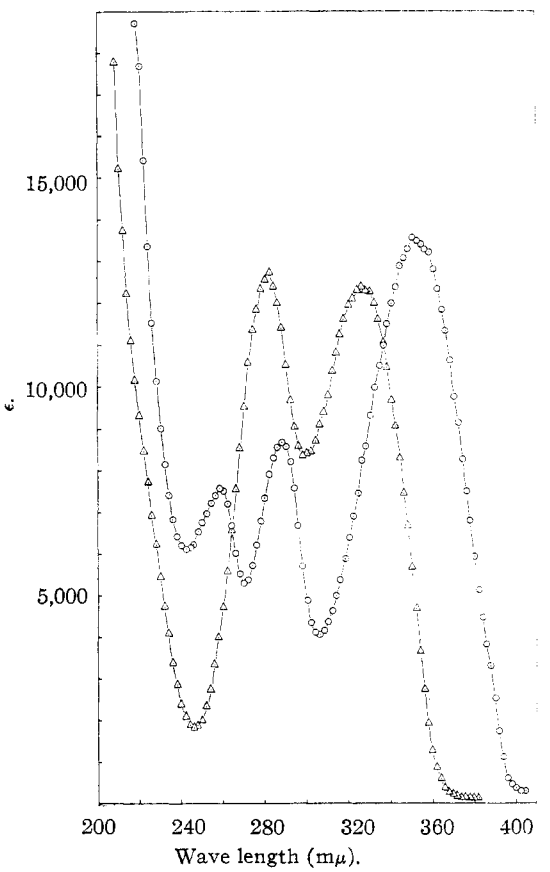


Fig. 2.—Ultraviolet absorption spectra of 6-methyl-8-(β-hydroxyethyl)-2,4,7-trioxo-hexahydropteridine: ⊙, in 0.1 *N* NaOH; △, in 0.1 *N* HCl.

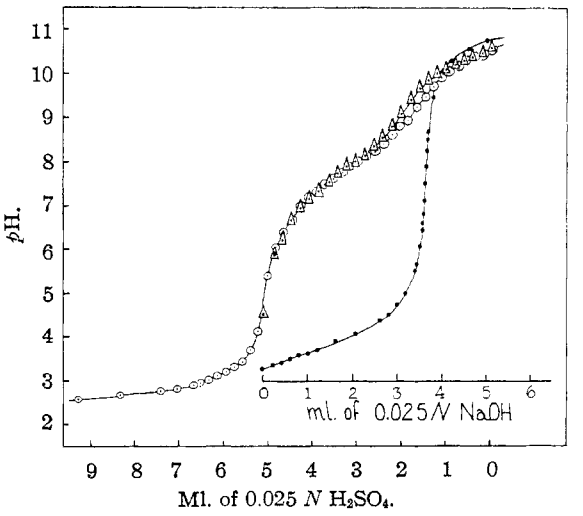


Fig. 3.—The potentiometric titration of the periodate oxidation product of Compound A and 6-methyl-8-(1-D-ribityl)-2,4,7-trioxo-hexahydropteridine: △, the periodate oxidation product of Compound A (20.0 mg. in 10 ml. of water) titrated with 0.025 *N* NaOH and, ⊙, back titrated with approximately 0.05 *N* H_2SO_4 (expressed as 0.025 *N*); ●, 6-methyl-8(1-D-ribityl)-2,4,7-trioxo-hexahydropteridine (30.1 mg. in 20 ml. of water) titrated with 0.025 *N* NaOH.

only 0.25 mole of acid per mole of compound. Riboflavin similarly treated gave the theoretical

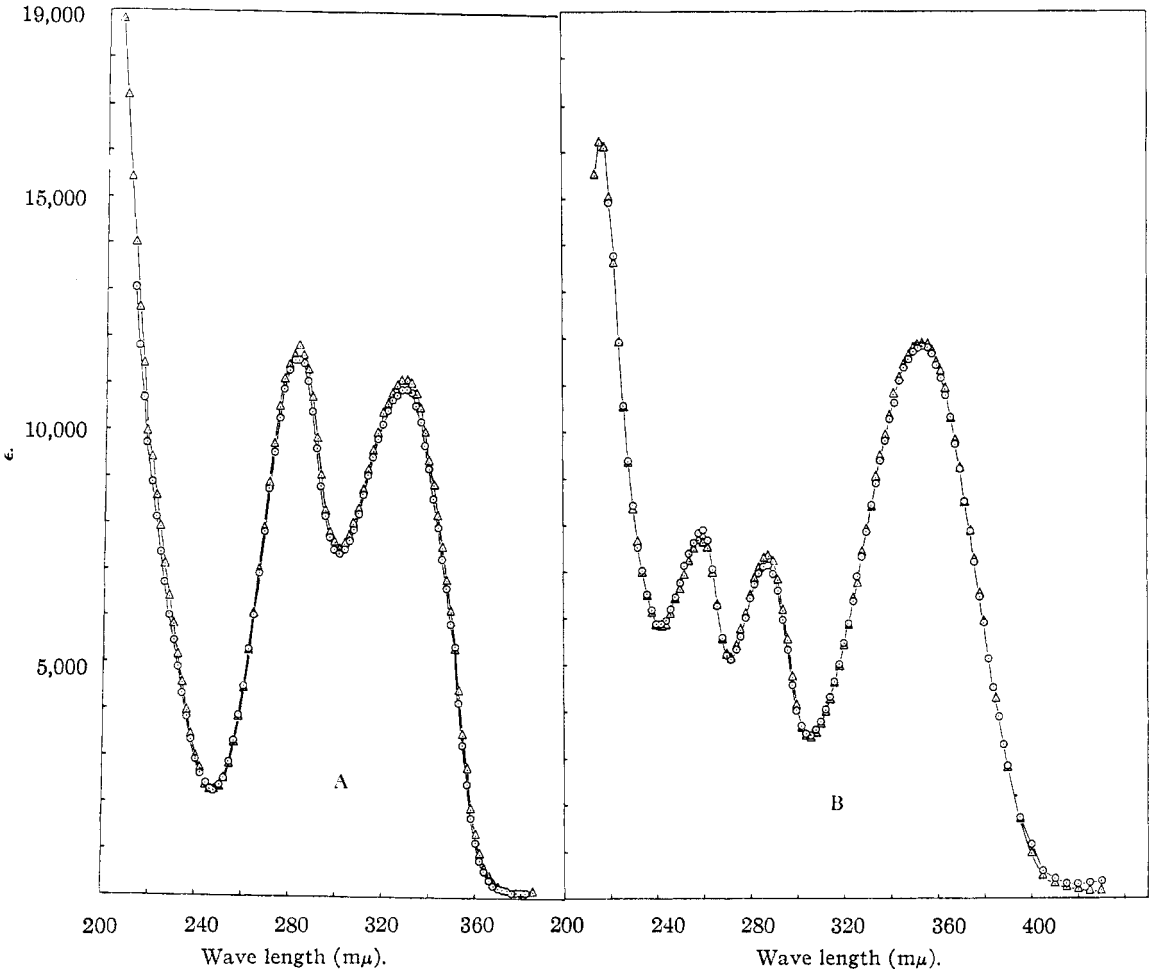


Fig. 4.—Ultraviolet absorption spectra of Compound A and 6-methyl-8-(1-D-ribityl)-2,4,7-trioxo-hexahydropteridine: ⊙, 6-methyl-8-(1-D-ribityl)-2,4,7-trioxo-hexahydropteridine; △, Compound A, in 0.1 *N* HCl (A) and 0.1 *N* NaOH (B).

yield (1.98 moles of acid per mole). However, 1.5 moles of acid per mole of Compound A were isolated by distillation from the periodate oxidation mixture, and this acid was characterized as HCOOH by conversion to its hydroxamide. A possible explanation for this discrepancy is that an intramolecular reaction involving the aldehydic group of I and its acidic group to give II, results in the apparent loss of one molecular equivalent of acid from the periodate oxidation mixture when titrated to *p*H 5. The oxidation product of m.p. 213–215°, although having a greatly reduced acidity and a potentiometric titration curve (Fig. 3) as might be expected of the monosodium salt of I, gave no alkaline ash upon ignition and had a sodium content of only 0.12%. The *p*H of the half-titrated substance was 7.6 as compared with 3.95 for Compound A, whereas I would be expected to have an acidity slightly greater than III or IV.

A possible interpretation of the titration curve (Fig. 3) is that around *p*H 9 in the titration with alkali the hydrogen at the 3-position of II is neutralized and that around *p*H 6 to 3 in the back titration with acid this "aldehyde ammonia" rapidly hydrolyzes to free the H at the 1-position. This interpretation implies a very rapid hydrolysis of the "aldehyde ammonia" in weak acid, because a slow reaction would have appeared as an inflexion differing greatly in slope from that characteristic of IV (Fig. 3). It is to be expected that II, a hemiacetal, would be less stable in acid than in alkali, and its similarity to a 2-deoxyfuranosyl compound suggests that it would be particularly labile.

A further investigation of this product is underway.

The foregoing facts suggest that Compound A is 6-methyl-8-(1-D-ribityl)-2,4,7-trioxo-hexahydropteridine (IV). Accordingly, Compound IV was synthesized and was found to agree with the natural product with respect to melting point, mixed melting point, pK_a, neutral equivalent, ultraviolet absorption spectra in acid and base (Fig. 4), and the infrared absorption spectrum (Fig. 5). The two substances, cochromatographed in two solvents, gave a single symmetrical spot, and radioactive Compound A was diluted with IV according to expectation. These findings are hence a confirmation of the work of Masuda, *et al.*,[9] and Plaut and Maley.[10]

In an earlier report[4] the empirical formula of the compound was incorrectly assigned 8 oxygen atoms rather than 7, and the amount of $NaIO_4$

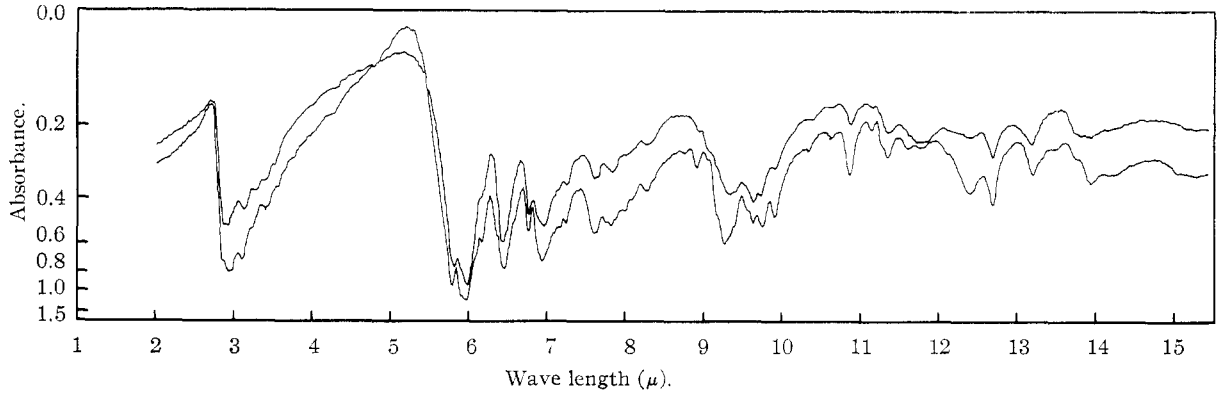


Fig. 5.—Infrared absorption spectra of Compound A and 6-methyl-8-(1-D-ribityl)-2,4,7-trioxo-hexahydropteridine: 0.2% in KBr pellets, 1–2 mm. thick, uncorrected for the absorption of the KBr. The spectrum of Compound A is the upper curve.

stated to be consumed per mole of compound was in error. The acidity of the compound which was thought to be due to a carboxyl group now appears to arise from an imino group.

These structures have been written in the triketo form. Elion and Hitchings[12] have shown that the condensation of ethyl oxomalonate with a 4-alkylamino-6-hydroxy-2,5-diaminopyrimidine yields a 7-oxo- and not a 7-hydroxypteridine. Evidence for the tri-keto form of the pteridines described here is found in the similarity of their ultraviolet absorption spectra to those of 2,4,7-trioxo-pteridines which have been synthesized by Pfleiderer.[13] Compound G[8] which presumably has a system of conjugated double bonds like that in riboflavin is also a colored compound, while compound A, when pure, is colorless as are the other pteridines here described.

Different substituents on the nitrogen in the 8-position have an effect upon the extinction coefficient in the region of 260 mμ (0.1 *N* NaOH) which is probably related to an interaction between the substituent and the hydrogen at the 1-position. As the substance which Plaut and Maley[10] described was prepared by a procedure which should give IV, the difference in the ultraviolet absorption spectra of these substances at the shorter wave lengths remains unexplained.

One may wonder whether pteridines which have a substituent on the N in the 8-position might not be of importance in the biochemistry of pteridines in general. Just as many enzymatic reactions having to do with the biosynthesis of purines and pyrimidines involve not the substances themselves but their ribosyl and 2-deoxyribosyl derivatives, also in the biogenesis of pteridines analogous derivatives might be involved. Such a possibility was appreciated by Forrest, *et al.*,[14] who synthesized glycosyl substituted pteridines. Pfleiderer, also, in thinking about the analogies of purines and pteridines was led to prepare 2,4-dioxypteridines before any of them had been found in nature. The very compound which Masuda, *et al.*,[9] isolated as a photochemical degradation product of Compound V was one of the 2,4-dioxypteridines which Pfleiderer had synthesized.[13]

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(14) H. S. Forrest, R. Hull, H. J. Rodda and A. R. Todd, *J. Chem. Soc.*, 3 (1951).

Experimental

The Amount of Radioactivity on the Ureido Carbon Atom of C^{14}-Compound A.—Cultures of *Eremothecium ashbyii* were grown in the presence of guanine-U-C^{14} (Schwarz Laboratories, Mount Vernon, N.Y.); the C^{14}-Compound A was isolated as previously described[4] and was diluted with a carrier amount of Compound A and recrystallized from methanol–water–HCl.[5] Its specific activity was 239 c.p.m. per 10^{-6} mole (mol. wt., 328). The material was degraded with dilute NaOH[6] and the urea thus isolated as its dixanthydryl derivative[15,16] after recrystallization from pyridine–water melted at 285–287° dec. and had a specific activity of 59 c.p.m. per 10^{-6} mole.

Periodate Oxidation of Compound A.—Compound A, 6.56 mg. (20 μ moles), in 2 ml. of water at 24° was treated with 81 μ moles of $NaIO_4$ (0.2 *M*). At various times the moles of $NaIO_4$ consumed[17] per mole of Compound A was determined: 2 min., 2.7 moles; 4 min., 2.8 moles; 10 min., 2.8 moles; 60 min., 2.8 moles. No further increase occurred after 3 hr.

A solution of 26.7 μmoles of Compound A in water was brought to *p*H 4.7 with NaOH and treated at 24° with 4 molar equivalents of $NaIO_4$ at *p*H 4.7 in a final volume of 4.2 ml. The change in *p*H with time was as follows: 1 min., 4.3; 8 min., 4.1; 25 min., 3.9; 60 min., 3.8. After 90 min., at which time no further change had occurred, the solution was titrated with 0.02604 *N* NaOH to *p*H 4.7, 0.26 ml. being required. Thus, no more than 6.7 μmoles of acid (0.25 mole per mole of Compound A) were apparent in the reaction mixture. Riboflavin similarly treated gave 1.9 moles of acid per mole of riboflavin.

One hundred μmoles of Compound A was oxidized with 400 μmoles of $NaIO_4$ in a volume of 12 ml. at 23°. After 15 minutes, 2 g. of activated charcoal was added and the filtrate and washings treated with 60 mg. of dimedon in water. The *formaldehyde* dimedon derivative separated in a yield of 25.4 mg.; theory for 1 mole requires 29.2 mg. Its melting point, 189–191°, was not depressed when the product was mixed with an authentic sample. The filtrate from the dimedon derivative was treated with an excess of $FeSO_4$, acidified with H_2SO_4 and the volatile acid was quantitatively distilled. The distillate, 600 ml., was made alkaline, evaporated to 25 ml., acidified with H_2SO_4 and distilled as before. The amount of acid (pK_a, 4.0) obtained was 152 μmoles. The acid was identified as *formic acid* by conversion to its hydroxamide which was chromatographed in *n*-butanol–acetic acid–water. The R_f value observed was 0.51, that of an authentic sample was 0.50. The amount was estimated from the optical density of its iron complex at 485 mμ.[18] Of 200 μmoles of the

(15) R. Fosse, *Ann. Chim.*, [9] **6**, 35 (1916).

(16) R. E. Phillips and B. M. Pitt, THIS JOURNAL, **65**, 1355 (1943).

(17) E. L. Jackson, *Organic Reactions*, **2**, 361 (1944).

(18) R. J. Block, E. L. Durrum and G. Zweig, "A Manual of Paper Chromatography and Paper Electrophoresis," Academic Press, New York, N. Y., 1955, pp. 164–166.

acid measured by titration of the distillate, 194 μmoles of formic acid was found by the colorimetric test.

The pteridine containing product obtained by oxidation with periodate and thought to be principally II was eluted from the charcoal with 30% pyridine. Its spectral characteristics are shown in Fig. 1. A crystalline product was obtained as follows: Compound A, 123 mg., was dissolved in 5 ml. of water and to it 260 mg. of $NaIO_4$ in 10 ml. of solution was added. The *p*H of the solution dropped to 2 and crystals began separating in about 20 min. They were washed with water and dried in a desiccator containing H_2SO_4 and NaOH; yield 67 mg. The substance partially melted at 213–215° to give a gum and started decomposing around 245°. The periodate oxidation product of the synthetic compound, IV, had the same characteristic m.p. as had also a mixture of the two substances. It did not give an alkaline ash (Na by flame photometer, 0.12%). In chromatograms on paper (*n*-butanol–water–acetic acid), the substance gave two fluorescent spots of R_f 0.43 and 0.39.

Anal. Calcd. for $C_9H_8N_4O_4 \cdot H_2O$: C, 42.5; H, 4.0; N, 22.4; neut. equiv., 254. Found: C, 42.6; H, 4.4; N, 20.5 (no ash); neut. equiv., 260. The ultraviolet absorption spectrum of the substance in 0.1 *N* HCl was as follows: $\epsilon_{min}^{243\ m\mu}$, 1,790; $\epsilon_{min}^{298\ m\mu}$, 7,480; $\epsilon_{max}^{278\ m\mu}$, 10,300; $\epsilon_{max}^{323\ m\mu}$, 10,600.

This spectrum shows a slight hypsochromic shift in comparison with that of Compound A. In alkaline solution this shift is more pronounced (Fig. 1).

The potentiometric titration of the substance which had been recrystallized from dilute acetic acid is shown in Fig. 3.

Conversion of the Periodate Oxidation Product of Compound A to 6-Methyl-8-(β-hydroxyethyl)-2,4,7-trioxo-hexahydropteridine.—The periodate oxidation product, 10 mg., was dissolved in 3 ml. of warm water. The solution was cooled and added to 1 ml. of water containing 10 mg. of $NaBH_4$. After standing at room temperature for 1/2 hr. the solution was adjusted to *p*H 1 with HCl and chromatographed on paper (ethanol, 40; water, 8; concd. NH_4OH, 4 ml.). Two fluorescent compounds were present (R_f, 0.48, 0.55) of which the second cochromatographed with III to give a single symmetrical spot in this solvent system, and also in *n*-propanol–water–NH_3 in which the R_f was 0.44. The absorption spectra of this product in acid and base (Fig. 1) agreed perfectly with those of III (Fig. 2).

6-(β-Hydroxyethylamino)-2,4-dioxypyrimidine.—1.49 g. of 6-chloro-2,4-dioxypyrimidine,[19] m.p. 305–307° dec., was suspended in 12 ml. of water and 1.2 g. (2 molecular equivalents) of β-hydroxyethylamine were added. The β-hydroxyethylamine salt of 6-chloro-2,4-dioxypyrimidine was formed, m.p. 194–196°. The mixture was sealed in a tube and heated at 120–130° for 7 hr. Upon cooling, 0.71 g. of 6-(β-hydroxyethylamino)-2,4-dioxypyrimidine separated and was recrystallized from hot water; m.p. 253–256°.

Anal. Calcd. for $C_6H_9N_3O_3$: C, 42.1; H, 5.3; N, 24.6. Found: C, 42.4; H, 5.1; N, 24.7. The ultraviolet absorption spectrum of the substance in 0.1 *N* HCl follows: $\epsilon_{min}^{237\ m\mu}$ 2,160, $\epsilon_{min}^{267\ m\mu}$ 23,620; in 0.1 *N* NaOH; $\epsilon_{min}^{242\ m\mu}$ 2,480, $\epsilon_{max}^{267 m\mu}$ 19,530.

5-Nitroso-6-(β-hydroxyethylamino)-2,4-dioxypyrimidine.—6-(β-Hydroxyethylamino)-2,4-dioxypyrimidine, 40 mg., was dissolved in 2 ml. of water. To the cooled solution, 40 mg. of $NaNO_2$ and 1 drop of glacial acetic acid were added. Deep red crystals of 5-nitroso-6-(β-hydroxyethylamino)-2,4-dioxypyrimidine separated; yield 32 mg., m.p. 255° dec.

Anal. Calcd. for $C_6H_8N_4O_4$: C, 36.0; H, 4.0; N, 28.0; neut. equiv., 200. Found: C, 36.5; H, 3.9; N, 29.5; neut. equiv., 200; pK_a, 4.5. The ultraviolet absorption spectrum of the substance in 0.1 *N* HCl was as follows: $\epsilon_{min}^{283\ m\mu}$ 4,740, $\epsilon_{inflexion}^{260\ m\mu}$ 7,087, $\epsilon_{max}^{227\ m\mu}$ 15,680, $\epsilon_{max}^{317\ m\mu}$ 9,970; in 0.1 *N* NaOH; $\epsilon_{min}^{237\ m\mu}$ 2,610 $\epsilon_{inflexion}^{260\ m\mu}$ 4,470, $\epsilon_{max}^{310\ m\mu}$ 18,020.

6-Methyl-8-(β-hydroxyethylamino)-2,4,7-trioxo-hexahydropteridine.—6-(β-Hydroxyethylamino)-2,4-dioxypyrimidine, 0.7 g., was converted to the nitroso compound as described above. It was suspended in 6 ml. of water and sodium hydrosulfite, 1.5 g., was added. The pale yellow solution was evaporated to 2 ml. at 50°, and the light yellow crystals which separated were washed with water, ethanol and benzene; m.p. > 315°. This compound, presumably 5-amino-6-(β-hydroxyethylamino)-2,4-dioxypyrimidine, gave a negative test for sulfur and hence is unlike the bisulfite compound reported by Masuda.[19] This result agrees with that reported by Birch and Moye.[20] The product which had assumed a greenish color upon standing in air overnight was taken up in 5 ml. of water. Glacial acetic acid, 5 drops, and ethyl pyruvate, 2 ml., were added and the solution was heated at 90° for 1 hr. Upon evaporation pale yellow crystals of 6-methyl-8-(β-hydroxyethyl)-2,4,7-trioxo-hexahydropteridine separated; yield 0.25 g.; m.p. > 315°. The compound was recrystallized from 50% acetic acid and dried *in vacuo* at 26°.

Anal. Calcd. for $C_9H_{10}N_4O_4 \cdot H_2O$: C, 42.2; H, 4.7; N, 21.9; neut. equiv., 256. Found: C, 42.3; H, 4.7; N, 22.4; neut. equiv., 247; pK_a, 4.2.

The ultraviolet absorption spectra of this compound (Fig. 2) have minima and maxima in agreement with those of Compound A, although the extinction coefficients at all of the maxima do not agree.

6-Methyl-8-(1-D-ribityl)-2,4,7-trioxo-hexahydropteridine.—This compound was synthesized by a procedure similar to that which has since been described by Plaut and Maley.[10,21] 6-(1-D-Ribitylamino)-2,4-dioxypyrimidine, m.p. 182–185°, sodium 5-nitroso-6-(1-D-ribitylamino)-2,4-dioxypyrimidine, m.p. 167–170° and sodium 6-methyl-8-(1-D-ribityl)-2,4,7-trioxo-hexahydropteridine, m.p. 280–282° crystallized without the need for chromatography. The acid, obtained from the sodium salt by crystallization from water–HCl–methanol, softened at 248° and melted at 259–262°.

Anal. Calcd. for $C_{12}H_{16}N_4O_7$: C, 43.9; H, 4.9; N, 17.1; neut. equiv., 328. Found: C, 43.7; H, 4.7; N, 16.9; neut. equiv., 332; pK_a, 3.95.

The ultraviolet and infrared absorption spectra are shown in Figs. 4 and 5. The melting point of a mixture of Compound A with this compound was not depressed (m.p., softened at 242°, melted at 259–262°). The two substances chromatographed as a single symmetrical spot in cochromatograms on paper with *n*-butanol–acetic acid–water and *n*-propanol–NH_3–water systems.[4]

Dilution of C^{14}-Compound A with 6-Methyl-8-(1-D-ribityl)-2,4,7-trioxo-hexahydropteridine-C^{14}.—Compound A (106 μg.; 27,900 c.p.m.) was diluted with 51 mg. of 6-methyl-8-(1-D-ribityl)-2,4,7-trioxo-hexahydropteridine. The material which recrystallized from HCl–water–methanol was washed well with methanol and dried over H_2SO_4. The crystals, 14.4 mg., contained 7,080 c.p.m. Calcd., 7,860 c.p.m.

Acknowledgments.—I am deeply indebted to Dr. Hugh S. Forrest, with whom I worked in the early part of these investigations, for his many constructive suggestions and for the benefit of his experience in the chemistry of the pteridines. I am grateful to Miss Jane Regan for her able assistance, Dr. W. Pfleiderer for his genuine interest and helpfulness, Dr. Karl Folkers for his gift of D-ribamine, and Mr. John P. Plank of the Perkin-Elmer Corporation, Norwalk, Connecticut, for determinations of the infrared absorption spectra.

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