acetate–petroleum ether (90–100°)–methanol–water (4:1:1:2) system, and the main fraction (hold-back volumes 6-7) yielded a glass weighing 320 mg. This material was rechromatographed on Celite with an ethyl acetate–petroleum ether (90–100°)–methanol–water (8:1:2:4) system, and the product collected from hold-back volumes 3–4 by evaporation to dryness under reduced pressure was a glass, $[\alpha]^{25}D$ −62° (0.8% in methanol), $\lambda_{max}^{MeOH}$ 274 mμ with an ε value of 18,100, indicating[18] *ca.* 96% 9-substituted purine having a molecular weight corresponding to II.

*Anal.* Calcd. for $C_{12}H_{18}N_6O_3$: C, 49.0; H, 6.12; N, 28.6. Found: C, 50.0; H, 7.16; N, 27.9.

**9-(2-Acetamido-2-deoxy-3,5-di-*O*-acetyl-D-ribopyranosyl)-6-chloropurine.**—To a suspension of 1.8 g. of crystalline 2-acetamido-tri-*O*-acetate A in 9 cc. of acetyl chloride was added 165 cc. of saturated ethereal hydrogen chloride. The solution was kept at −5° for 10 days, then evaporated to dryness under reduced pressure at a bath temperature not exceeding 40°. The evaporation was repeated twice with 50-cc. portions of benzene. The residue was dissolved in 140 cc. of ethylene dichloride and added to an azeotropically dried suspension of 4.6 g. of chloromercuri-6-chloropurine[17] in 120 cc. of ethylene dichloride. The mixture was stirred and refluxed for 20 hours, then filtered hot and the solids washed with hot chloroform. The filtrate and washings were combined and evaporated to dryness under reduced pressure. The residual gum was dissolved in a mixture of 150 cc. of chloroform and 100 cc. of 30% potassium iodide solution. The organic layer was separated, washed with 50 cc. of 30% potassium iodide solution, and dried over magnesium sulfate. Evaporation of the chloroform solution to dryness under reduced pressure left a brown gum weighing 1.57 g. (67%), ultraviolet absorption spectra of which indicated[18] it to be approximately 80% 9-substituted purine.

**9-(2-Acetamido-2-deoxy-D-ribopyranosyl)-6-dimethylaminopurine (XIX).**—To a solution of 1.57 g. of crude 9-(2-acetamido-2-deoxy-3,5-di-*O*-acetyl-D-ribopyranosyl)-6-chloropurine in 18 cc. of methanol was added 3.6 cc. of anhydrous dimethylamine in 5 cc. of methanol.[17] The solution was heated in an autoclave at 100° for 2 hours, then evaporated to dryness under reduced pressure. The residue was dissolved in 60 cc. of 50% aqueous methanol and stirred with 12 g. of Amberlite IRA-400 ion exchange resin for 30 minutes. The resin was removed by filtration and the solvent removed under reduced pressure. The residue was evaporated to dryness with ethanol several times under reduced pressure leaving an amber glass weighing 1.05 g. (82%). By ultraviolet absorption spectra, the glass was about 78% 9-substituted purine.[18] The crude material was partition chromatograped on Celite with an ethyl acetate saturated with water system.[34] The main peak at hold-back volumes 8–10, on evaporation to dryness under reduced pressure, yielded 210 mg. of glass. Re-chromatography of this material with the same system or with an ethyl acetate–petroleum ether (90-100°)–methanol–water (8:1:4:2) system afforded no further purification; $[\alpha]^{25}D$ +53.0° (0.85% in ethanol), $\lambda_{max}^{MeOH}$ 274 mμ (ε 17,700). This product consumed 0.7 molar equivalent of periodate. It is probably mainly the α-pyranoside XIX.

*Anal.* Calcd. for $C_{14}H_{20}N_6O_4$: C, 50.0; H, 5.95 N, 25.0. Found: C, 48.9; H, 6.55; N, 23.0

PEARL RIVER, N. Y.

---

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMICAL PATHOLOGY, ST. MARY'S HOSPITAL MEDICAL SCHOOL]

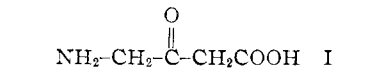
# The Synthesis and Properties of γ-Aminoacetoacetic Acid

BY J. B. NEILANDS,[1] A. NEUBERGER AND J. J. SCOTT

RECEIVED JULY 13, 1959

A method is described for the preparation of γ-aminoacetoacetic acid from acetonedicarboxylic acid *via* γ-oximinoacetoacetic acid.

The amino acid, γ-aminoacetoacetic acid (glycylacetic acid; 3-keto-4-aminobutyric acid (I)) is a substance which could arise biologically in a number of ways. Possible biosynthetic pathways leading to the formation of I would include the condensation of the Coenzyme A derivatives of glycine and acetic acid, the condensation of malonyl Coenzyme A and glycine (with concomitant loss of $CO_2$ from the glycine carboxyl), the reductive amination of oxaloacetic semialdehyde or finally, through a fatty acid-like oxidation of γ-aminobutyric acid.



I

Either I or its trimethylammonium analog might serve as an immediate precursor of *l*-carnitine, a physiologically important substance of unknown biogenesis.[2] Very recently, chromatographic evidence has been obtained that the reduced form of I, γ-amino-β-hydroxybutyric acid, first synthesized in 1923 by Tomita,[3] may occur in mammalian brain tissue where it is believed to act under certain circumstances as a natural regulator of the motor activity.[4,5] Direct decarboxylation of I, as contrasted to the oxidation of threonine,[6] would provide a second mechanism for the formation of aminoacetone. In addition, as the next lower homolog of δ-aminolevulinic acid, the effect of I on porphyrin metabolism would be worthy of investigation.

Since I is a β-keto acid as well as an α-keto amine, it was of interest to compare the stability and reactions of a compound of this type with those of a number of closely related substances previously studied in this Laboratory.[7] Also, by further reduction and N-trimethylation, I could easily be converted successively into optically inactive γ-amino-β-hydroxybutyric acid and carnitine.

Although the preparation of I might be attempted through a variety of alternative routes, the controlled nitrosation of free acetonedicarboxylic acid followed by reduction of the resultant γ-oximinoacetoacetic acid (II) appealed to the authors as a relatively direct and simple procedure.

(1) Fellow of the John Simon Guggenheim Foundation; on sabbatical leave from the University of California, Berkeley, 1958–1959.

(2) G. Fraenkel and S. Friedman, *Vitamins and Hormones*, **15**, 73 (1957).

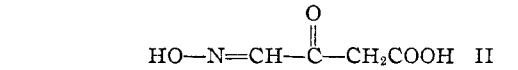
(3) M. Tomita, *Z. physiol. Chem.*, **124**, 253 (1923).

(4) T. Hayashi, *J. Physiol.*, **145**, 570 (1959).

(5) K. A. C. Elliott and H. H. Jasper, *Physiol. Rev.*, **39**, 383 (1959).

(6) W. H. Elliott, *Nature*, **183**, 1051 (1959).

(7) W. G. Laver, A. Neuberger and J. J. Scott, *J. Chem. Soc.*, 1474, 1483 (1959).



HO—N=CH—C(=O)—$CH_2$COOH II

When this nitrosation was attempted in ether–HCl by use of ethyl nitrite, there was no evidence of reaction and on removal of the solvent the starting material was obtained. In dilute HCl, on the other hand, a vigorous and instantaneous evolution of $CO_2$ occurred during the addition of $NaNO_2$. This was to be expected since dioximinoacetone is known to be formed under such circumstances.[8] Nevertheless, when equimolar quantities of $NaNO_2$ and acetonedicarboxylic acid were used, analysis of the reaction mixture by paper electrophoresis showed a considerable amount of ferric chloride-positive material to be present which *behaved as a monovalent acid at pH 5*. This substance was eventually isolated and characterized as γ-oximinoacetoacetic acid (II).

During the nitrosation in dilute HCl, a granular, microcrystalline solid appeared. This material was separated by filtration and shown by electrophoretic analysis to be a mixture of II and neutral compounds (*p*H 5), the latter being identified with the aid of authentic markers as mainly dioximinoacetone and perhaps a trace of mono-oximinoacetone. The filtrate contained these compounds and, in addition, a certain quantity of unreacted acetonedicarboxylic acid. The neutral compounds could be extracted with ether at *p*H 5 leaving, in the case of the precipitated fraction, relatively pure II and, in the soluble fraction, II contaminated with a small amount of residual acetonedicarboxylic acid. In the separation at *p*H 5 it was necessary to work quickly since II is unstable in aqueous solution and fairly rapidly decomposes into reddish-brown material(s).

It has thus far not been possible to devise a practical procedure for obtaining large, well-formed crystals of II from an anhydrous solvent. Such crystals have been obtained from aqueous HCl, the yield, however, is very small since extensive decomposition accompanies the crystallization process and the product is contaminated with the brownish material(s) mentioned above. For the routine preparation of I *via* II it appears preferable to remove the bulk of the oximinoacetones at *p*H 5 and proceed to the final product which may then be purified by recrystallization. In the working-up of that portion of II which did not crystallize out during nitrosation, an ether extraction after insertion of the amino group sufficed to remove most of the unreacted acetonedicarboxylic acid.

The behavior of I and II on titration was found to agree with that expected from consideration of their structure. In contrast to derivatives of α-amino-β-keto acids[7] loss of a proton from the $NH_3^+$ group is not accompanied by enolization as judged by change in the spectrum. In fact an enolic absorption does not appear below a *p*H substantially higher than the second *pK'*. The stability of I appears to be at least as great as that of the usual type of β-keto acid and hence it follows that the substance could exist as such within living cells.

(8) O. Touster, *Org. Reactions*, **VII**, 327 (1953).

## Experimental[9]

**Acetonedicarboxylic Acid.**—Sulfuric acid-free acetonedicarboxylic acid was prepared by recrystallization of the crude product[10,11] from ethyl acetate–chloroform.

**Synthesis of γ-Oximinoacetoacetic Acid (II).**—To 14.6 g. (0.1 mole) of acetonedicarboxylic acid in 70 ml. of distilled water is added 20 ml. of concentrated HCl. The solution is cooled to 5° in an ice-bath and the nitrite solution, containing 6.9 g. (0.1 mole) of $NaNO_2$ in 50 ml. of distilled water, is added dropwise with mechanical stirring over a one hour period. After all of the nitrite solution had been added the reaction mixture was allowed to stand at 5° for 10 minutes. The white, microcrystalline precipitate was removed by filtration and the solid washed with a small amount of dilute HCl. After drying the product weighed 5.6 g.

The filtrate as obtained above was immediately extracted with six portions of ethyl acetate, the extract was dried over solid $MgSO_4$, filtered, and the solvent cautiously removed under reduced pressure. The tan-colored solid weighed 4.2 g. after drying as above.

Electrophoretic analyses on paper were performed with the apparatus of Markham and Smith.[12] A potential of 10 v./cm. was applied for about 90 minutes. The buffer used was 0.025 *M* acetate, *p*H 5.0, and, after drying, the papers were sprayed with 1% $FeCl_3 \cdot 6H_2O$ in acetone. Under the conditions acetonedicarboxylic acid, which was used as a marker, appeared as a violet zone with an anodic migration rate approximately double that of the reddish-brown spot subsequently identified as II. The neutral substance(s) had shifted slightly to the cathode and appeared as a bluish-black spot several hours after application of the ferric chloride spray.

In this manner both solid and soluble fractions from the nitrosation reaction were shown to contain neutral substance(s), probably mainly dioximinoacetone together with a small amount of oximinoacetone, and II. The soluble fraction contained, in addition, a small amount of unreacted acetonedicarboxylic acid.

**Purification of Crude II.**—One gram of the dried, crude product (which had separated as a solid during the nitrosation) was dissolved in 200 ml. of water and the *p*H adjusted to 5 with the addition of 2 *N* NaOH. The solution was shaken with ether until the extracts yielded a negative oximinoketone test (bright yellow color in dilute alkali). The aqueous phase was re-acidified by the addition of 20 ml. of concentrated HCl and, after addition of 50 g. of $(NH_4)_2SO_4$, extracted with ether until the extracts gave only a weak ferric chloride test. The ethereal solution obtained in the latter procedure was dried over $MgSO_4$ and evaporated under reduced pressure to yield 0.7 g. of white, microcrystalline, electrophoretically homogeneous II. The total yield of purified II was 0.7 × 5.6 or 3.9 g. (0.03 mole), 30%.

In the case of the crude II derived from the filtrate of the nitrosation reaction, a small amount of acetonedicarboxylic acid could be demonstrated to be present in the light tan colored product obtained after purification as above.

**Recrystallization of II.**—The purified, microcrystalline material (obtained from the solid portion of the nitrosation reaction) was dissolved in a 100-fold weight of methanol and an equal volume of water was added. The solution was cautiously evaporated under reduced pressure to one quarter of the original volume, 0.5 volume of concentrated HCl was added and the solution placed in the ice-box overnight. Next day the earth-colored crystals, consisting of stubby needles joined at the base, were collected by filtration, washed with dilute HCl and dried in the desiccator. The crystalline material melted at 142–143° with effervescent decomposition and left a reddish-brown residue. The mother liquor was very darkly colored and the yield of crystals was extremely poor.

**Characterization of II.**—Automatic electrometric titration[13] in water at 24° gave $pK_a'$ values of 3.5 and 8.7 and a

(9) Melting points are uncorrected. Microanalyses are by Weiler and Strauss, Microanalytical Laboratory, Oxford.

(10) D. M. MacDonald and S. F. MacDonald, *Canad. J. Chem.*, **33**, 573 (1955).

(11) "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1948, p. 10.

(12) R. Markham and J. D. Smith, *Biochem. J.*, **52**, 552 (1952).

(13) J. B. Neilands and M. D. Cannon, *Anal. Chem.*, **27**, 29 (1955).

neutral equivalent of 133 (to *p*H 6.1; theoretical 131.1). The substance is essentially insoluble in dilute HCl, sparingly soluble in water (giving a *p*H of about 2), moderately soluble in ethanol and ethyl acetate, and very soluble in methanol. It dissolves in alkali, giving a bright yellow color which is discharged on re-acidification. The ferric chloride reaction in water was brownish-violet.

When II was dissolved in water the initially colorless solution became reddish-brown within the course of a few hours. However, II was completely stable at room temperature either as the dry solid or as a solution in anhydrous ether. It was not hygroscopic.

In a quantitative microhydrogenation of II, a 51-mg. (0.39 mmole) sample was dissolved in 20 ml. of 10% HCl, 20 mg. of palladium charcoal were added and the mixture shaken in an atmosphere of hydrogen. The absorption of gas ceased after 12 minutes; a volume of 17 ml. (0.75 mmole) 96%, had been consumed.

*Anal.* Calcd. for $C_4H_5O_4N$: C, 36.64; H, 3.84; N, 10.69. Found: C, 36.92; H, 3.67; N, 10.78.

**Synthesis of γ-Aminoacetoacetic Acid (I).**—A 0.7-g. (5.34 mmoles) quantity of purified II (not recrystallized) was dissolved in 75 ml. of methanol and 20 ml. of ice-cold water was added. After the addition of 2 ml. of concentrated HCl and 300 mg. of palladium charcoal[14] the mixture was shaken in an atmosphere of $H_2$. Uptake of gas proceeded very rapidly, the theoretical volume of 240 ml. being consumed in 45 minutes. Shaking was continued for an additional 5 minutes and the charcoal then removed by filtration through a double layer of Whatman No. 1 filter paper. The clear, colorless solution was cautiously evaporated under reduced pressure to yield crystals of I–HCl which were usually in the form of long, colorless, radial needles. The last traces of water and HCl were removed under vacuum in the presence of NaOH and $H_2SO_4$. The preparation was dissolved in the least amount of dry ethanol and a trace impurity removed by the addition of several volumes of dry ethyl acetate. The solvent was evaporated and the solid again dissolved in the least amount of dry ethanol and recrystallized by the addition of dry ether.

The yield was 0.66 g. (4.27 mmoles), 80%, making a total of 0.66 × 5.6 or 3.7 g. (24 mmoles).

In the case of the purified II obtained from the filtrate from the nitrosation reaction, the solution following hydrogenation was extracted with ether (after removal of the charcoal) in order to eliminate the unreacted acetonedicarboxylic acid. On evaporation to dryness a lightly yellow colored oil was obtained. This was dissolved in dry ethanol and purified as above. The yield was 0.15 g. of I–HCl per gram of crude II thus making a total of 0.15 × 4.2 or 0.63 g. (4.1 mmoles).

The total combined yield of recrystallized I–HCl from 0.1 mole of acetonedicarboxylic acid was 4.33 g. (0.028 mole), 28%.

**Characterization of I.**—Recrystallized I–HCl consists of small, white rosettes which melt at 110–112° with effervescence and decomposition. The product is very soluble in water and methanol, moderately soluble in ethanol and insoluble in ether. In contrast to aminoacetone–HCl, the hydrochloride of I is only slightly hygroscopic; it is however very retentive of water of crystallization (see below).

The purity was examined by paper electrophoresis at *p*H 2.5 in 0.025 *M* citrate buffer using authentic specimens of aminoacetone (prepared by reduction of oximinoacetone) and (+)-β-hydroxy-γ-aminobutyric acid (m.p. 214–215°, prepared from the N-benzoyl derivative[15]) as markers. After recrystallization a single, round ninhydrin-positive spot (spray: 0.1% ninhydrin in acetone) was found with a migration rate about one-half that of aminoacetone and only slightly less than that of β-hydroxy-γ-aminobutyric acid. Preparations of I prior to recrystallization contained trace amounts of aminoacetone.

The ninhydrin reaction of I was characteristic of α-aminoketones, *i.e.*, the initial bright yellow spot slowly transformed into a purple spot. The β-hydroxy-γ-aminobutyric acid, on the other hand, gave a purple color instantly. A paper electrophoretogram in which I had been applied as a narrow strip across the origin was set up and run in the usual way. After one hour at 500 v. d.c. the current was shut off, the paper was removed and dried and then cut longitudinally into three ribbons. One ribbon was sprayed with ninhydrin, another with ferric chloride and the third was sectioned and subjected to the Ehrlich reaction for α-ketoamines.[16] The Ehrlich reagent gave an intense red reaction and the ferric chloride spray a faint dark zone; both reactions were obtained only in that zone which was directly in line with the ninhydrin-positive material. The molar color yield for the Ehrlich reaction in solution was within 5% of that given by either aminoacetone or δ-aminolevulinic acid. The ferric chloride reaction of I–HCl in water was reddish-brown.

The $R_f$ of I in *n*-butanol, 4; acetic acid, 1; water, 5 was 0.19; in phenol saturated with water the $R_f$ was 0.45. With both solvent systems there was considerable streaking to the faster, aminoacetone region of the paper.

In dilute alkali I develops a moderately strong, acid-reversible absorption band with a maximum at 252 mμ. At *p*H 11.6 $\epsilon_{252}$ is of the order of 1000 and is still increasing with *p*H.

Reduction of I–HCl to the corresponding hydroxy compound was carried out by the method of Harington and Randall.[14] A 154 mg. (1.0 mmole) sample of I–HCl was dissolved in 10 ml. of water and 100 mg. of palladium charcoal, 40 mg. of $H_2PtCl_6 \cdot 6H_2O$ and 2 mg. of $FeCl_3 \cdot 6H_2O$ were added. The mixture was shaken in an atmosphere of $H_2$. The absorption of gas ceased after 45 minutes at which time 22 ml. had been taken up. After removal of the charcoal the solution was adjusted to *p*H 6 with dilute NaOH and the cations removed on a short column of Dowex 50 (hydrogen form). The amino acid was eluted with 1.0 *N* $NH_4OH$ and, after evaporation of the effluent in a desiccator overnight in the presence of $H_2SO_4$, the product crystallized and was recrystallized from water–ethanol. The yield of colorless prisms was 77 mg. (0.65 mmole), 65%, with m.p. 217–218°. The m.p. was not depressed by admixture with the authentic specimen referred to above. The $R_f$ of both the reduction product and the authentic compound in the *n*-butanol:acetic acid:water solvent was 0.32.

A 133 mg. (0.86 mmole) sample of I–HCl was heated for a few minutes at 120° in a closed vessel attached to a manometer. A volume of 18 ml. (0.80 mmole), 93%, of gas was collected.

On electrometric titration[13] of I hydrochloride in water at 24°, $pK_{a_2}'$ values of 2.9 and 8.3 were found. The neutral equivalent, based on $pK_{a_2}'$, was 155 (theoretical 153.6).

*Anal.* Calcd. for $C_4H_7O_3N \cdot HCl$: C, 31.3; H, 5.3; N, 9.1. Calcd. for $C_4H_7O_3N \cdot HCl \cdot H_2O$: C, 28.0; H, 5.9; N, 8.2.

Found in material dried *in vacuo* over NaOH–$H_2SO_4$ at room temperature: C, 27.8; H, 6.1. Another sample dried in a similar manner gave C, 29.1; H, 6.1. This sample lost 5.7% of its weight on further drying. Drying the material further at 35° *in vacuo* over $P_2O_5$ gave a higher carbon value. Found: C, 30.6; H, 5.9; N, 9.2. It appears that the crystals contain one molecule of water and this is only incompletely removed by prolonged drying.

**Stability.**—When stored in a vacuum desiccator I–HCl was stable for at least several months at room temperature. Dilute aqueous solutions of I–HCl held at 5° gradually de-

TABLE I

STABILITY OF γ-AMINOACETOACETIC ACID AT 60° IN 0.005 *N* HCl AND IN 0.1 *M* TRIS BUFFER *p*H 7.8[a]

| Incubation time, min. | Optical density, cm.$^{-1}$ at 500 mμ[b] 0.005 *N* HCl | 0.1 *M* tris *p*H 7.8 |
|---|---|---|
| 0 | 0.116 | 0.116 |
| 5 | ... | .082 |
| 15 | .092 | .047 |
| 30 | .072 | .016 |
| 60 | .044 | |
| 90 | .028 | |
| 120 | .014 | |
| Half-life | 44 min. | 11 min. |

[a] Experimental details given in the text. [b] Ferric complex.

(14) C. R. Harington and S. S. Randall, *Biochem. J.*, **25**, 1917 (1931).

(15) M. Bergmann, E. Brand and W. Weinmann, *Z. physiol. Chem.*, **131**, 1 (1923).

(16) D. Mauzerall and S. Granick, *J. Biol. Chem.*, **219**, 435 (1956).

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 $1.3 \times 10^{-3}$ *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 $3 \times 10^{-2}$ *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

(1) Department of Pharmacology, Tufts University, School of Medicine, Boston, Massachusetts.

(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).

(10) G. W. E. Plaut and G. F. Maley, *Arch. Biochem. Biophys.*, **80**, 219 (1959).

(11) K. M. Makino, *Biochem. Z.*, **282**, 263 (1935).