292. *The Constitution of the Purine Nucleosides. Part VIII. Uric Acid Riboside.*

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Uric acid riboside, obtained from beef blood by Newton and Benedict, has now been isolated from liver. Comparisons of the ultra-violet absorption spectra of the riboside with those of the four monomethyluric acids show that the ribose radical is situated at position 9 of uric acid.

The red corpuscles of beef blood contain uric acid-*d*-riboside (Newton and Benedict, *J. Biol. Chem.*, 1922, **54**, 595; Newton and Davis, *ibid.*, p. 601), which occurs also in smaller amounts in the red corpuscles of human, horse, sheep, pig, dog, and chicken bloods (Newton and Davis, *ibid.*, p. 603). The same uric acid riboside has now been isolated from liver, and its identity with that of blood has been confirmed by comparison of their optical rotations and ultra-violet absorption spectra (Figs. 5 and 6).

Neither the position in the uric acid molecule to which the ribose is attached as a glycoside, nor the nature of the oxide ring of the sugar is known. The present communic-

ation solves the first problem, and it is hoped that the second will be the subject of a later paper; by analogy with the other purine nucleosides a furanose structure may be anticipated.

The method used in former cases has again been adopted (J., 1934, 1639; 1936, 765; 1937, 1912; 1938, 259, 692). The ultra-violet absorption spectra of uric acid riboside in water, acid, and alkali very closely resemble those of 9-methyluric acid (Fig. 4), and are quite unlike those of 1-, 3-, and 7-methyluric acids in the same solvents (Figs. 1—3). It is therefore clear that this nucleoside is uric acid-9-*d*-riboside, the sugar thus occupying the same position as in other purine nucleosides.

This observation emphasises afresh the need for the investigation of the source of uric acid riboside and of the oxidase which presumably produced it from a purine nucleoside or nucleotide, since Dixon and Lemberg (*Biochem. J.*, 1934, **28**, 2065) and Gulland and Macrae (J., 1933, 662) have shown that xanthine oxidase of milk is unable to effect this conversion.

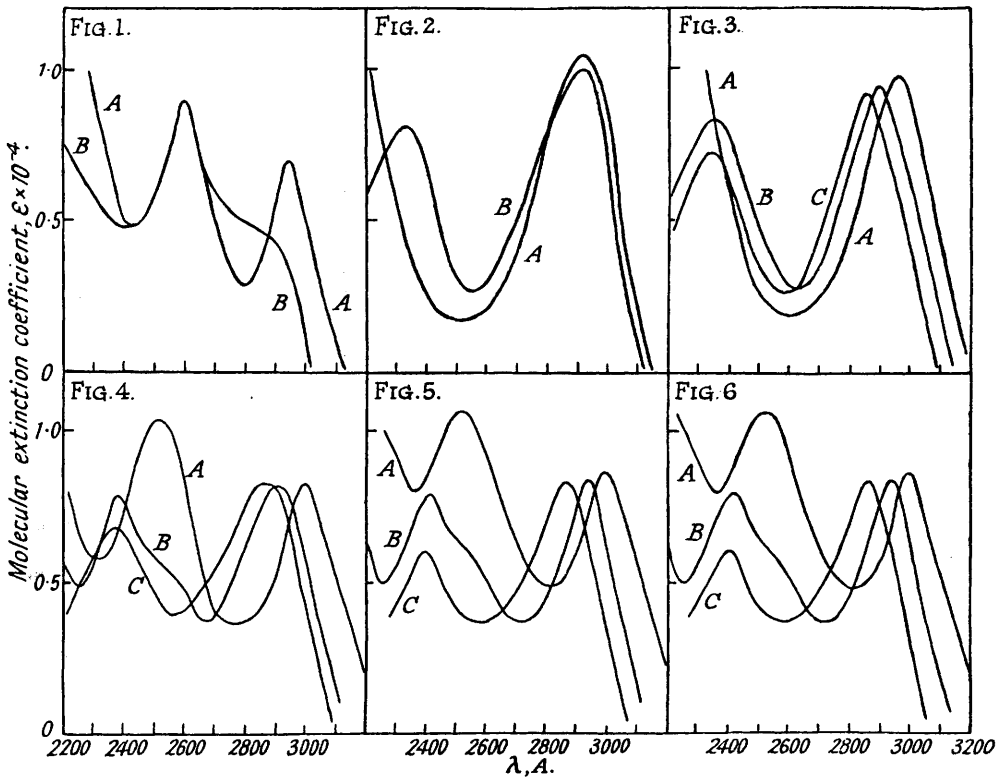

FIG. 1.—1-*Methyluric acid : A*, N/20-NaOH; *B, water and* N/20-HCl.
FIG. 2.—3-*Methyluric acid : A*, N/20-NaOH; *B, water and* N/20-HCl.
FIG. 3.—7-*Methyluric acid : A*, N/20-NaOH; *B, water ; C*, N/20-HCl.
FIG. 4.—9-*Methyluric acid : A*, N/20-NaOH; *B, water ; C*, N/20-HCl.
FIG. 5.—*Uric acid riboside from blood : A*, N/20-NaOH; *B, water ; C*, N/20-HCl.
FIG. 6.—*Uric acid riboside from liver : A*, N/20-NaOH; *B, water ; C*, N/20-HCl.

EXPERIMENTAL.

Uric Acid Riboside.—(i) The isolation from fresh beef blood followed the procedure of Davis, Newton, and Benedict (*loc. cit.*).

(ii) Minced liver, adjusted to p_H5 by addition of dilute sulphuric acid and allowed to autolyse for 4 hours, was coagulated by being rapidly heated to 85°. The mixture was filtered while still warm, the filtrate concentrated under reduced pressure at 50° to one-tenth of its volume, cooled, saturated with ammonium sulphate, and the resulting precipitate centrifuged and dis-

carded. The liquid, when left in the refrigerator for several days, slowly deposited a buff-coloured precipitate which was collected, washed with ice water, and dissolved in the minimum of hot water. The semi-crystalline precipitate (180 mg.) which slowly separated when this solution was kept in the refrigerator for some days was dissolved in water (1 l.), and the solution acidified to $p_H 5$ with acetic acid and mixed with mercuric acetate solution until precipitation ceased. After 24 hours the precipitate was removed by filtration and discarded, and crystalline sodium acetate (100 g.) was added to the filtrate in amount sufficient to make a 10% solution. After two days the supernatant liquid was removed with a syphon, and the precipitate centrifuged, washed many times with water, suspended in warm water and decomposed with hydrogen sulphide. The clear colourless filtrate from mercuric sulphide was concentrated under reduced pressure at 50° until crystals began to form and was left in the refrigerator until separation was complete.

Uric acid riboside crystallised from water in colourless needles, or square or rectangular plates, which did not melt at a high temperature, and had in 0·1N-sodium hydroxide $[\alpha]_D^{20°} = -40·8°$ (blood, $c = 1·25$) and $[\alpha]_D^{20°} = -40·6°$ (liver, $c = 1·13$) (Found, in material dried in vacuum over phosphoric oxide at 110° : N, 18·6. Calc. for $C_{10}H_{12}O_7N_4$: N, 18·7%). Newton and Benedict state that uric acid riboside forms colourless square plates or flat needles which do not melt above 300°, and that the optical acitivity of " the sodium salt (prepared only in solution) " is $[\alpha]_D^{20°} = +20·42°$, c not being recorded. It gave a positive pentose test with phloroglucinol and hydrochloric acid, and when steam-distilled in 10% sulphuric acid, the distillate gave a positive furfural test with aniline acetate. It gave a positive murexide reaction, and a blue colour with the Folin–Marenzi uric acid reagent (Folin, *J. Biol. Chem.*, 1933, **101**, 111), but the intensity of colour was only 12·2% of that developed by uric acid.

A solution of uric acid riboside (30 mg.) was heated under reflux at 100° for 6 hours with 16% sulphuric acid (5 c.c.), and then cooled in the refrigerator. The rectangular needles which separated were dissolved in lithium carbonate solution (charcoal), from which excess of acid precipitated rod-shaped crystals of uric acid (14 mg.) (Found, in material dried at 110° : N, 33·1. Calc. for $C_5H_4O_3N$: N, 33·3%). When estimated colorimetrically by the Folin–Marenzi method, this sample developed the same colour intensity as pure uric acid.

Methyluric Acids.—1-Methyluric acid was prepared from theobromine through the stages of methylalloxan (Biltz, *Ber.*, 1912, **45**, 3674), 1-methyluramil, and 1-methyl-ψ-uric acid (Fischer and Clemm, *Ber.*, 1897, **30**, 3091) (Found : N, 30·4. Calc. for $C_6H_6O_3N_4$: N, 30·7%).

3-Methyluric acid was obtained from 4 : 5-diamino-2 : 6-dihydroxy-3-methylpyrimidine (Traube, *Ber.*, 1900, **33**, 3051) (Found : N, 30·4%).

7-Methyluric acid was made from alloxan by way of 7-methyl-ψ-uric acid (Fischer, *Ber.*, 1897, **30**, 561) (Found : N, 30·4%). 9-Methyluric acid was made by Fischer (*Ber.* 1897, **30**, 2220) and by Biltz and Heyn (*Annalen*, 1917, **413**, 87; *Ber.*, 1919, **52**, 768, 784), but the following simple preparation has been described only for the corresponding ethyl derivative (Armstrong, *Ber.*, 1900, **33**, 2310). Freshly prepared methyl *iso*cyanate (3 g.) (Schroeter, *Ber.*, 1909, **42**, 3356) was added in small portions during 1 hr. to a solution of uramil (5 g.) (" Organic Syntheses," 1932, Vol. XII, 84) in N-potassium hydroxide (75 c.c.). The resulting deep purple solution was acidified with hydrochloric acid after 10—15 minutes, and the precipitate collected, washed with cold water, and extracted with a mixture of water (100 c.c.) and ammonia (d 0·88; 5 c.c.). Acidification of the extract precipitated 9-methyl-ψ-uric acid, which was purified by solution in dilute ammonia (charcoal) and reprecipitation by acid. A suspension of this material in 15 times its weight of hydrochloric acid (d 1·19) was boiled gently for about ½ hour and mixed with an equal volume of water. 9-Methyluric acid, which had separated during the heating, was purified by solution in lithium carbonate, reprecipitation by acid, and crystallisation from water in colourless needles (Found : N, 30·6%).

Absorption Spectra.—Measurements were made with a Bellingham and Stanley quartz spectrograph No. 2 and photometer, the light source being a condensed spark between tungsten-steel electrodes. The solutions, prepared from dried materials and made to concentrations of M/40,000, were examined immediately against controls.

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