365. *The Constitution of Purine Nucleosides. Part IX. Crotonoside.*

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Crotonoside, the nucleoside of the seeds of *Croton tiglium* L., is a *d*-riboside of *iso*guanine. By measurements of ultra-violet absorption spectra it has been shown that the sugar occupies the 9-position of the purine and it has been confirmed that the aglycone is *iso*guanine.

Cherbuliez and Bernhard (*Helv. Chim. Acta*, 1932, **15**, 464, 978) isolated from the seeds of *Croton tiglium* L. a nucleoside which they named crotonoside. This closely resembled guanosine; hydrolysis with hot dilute sulphuric acid yielded *d*-ribose and an aminohydroxypurine, which was isomeric with guanine and whose properties corresponded with those of *iso*guanine, 2-hydroxy-6-aminopurine (Fischer, *Ber.*, 1897, **30**, 2245). The absence of a substituent at C_8 of the aglucone was shown by effecting coupling in alkaline solution with diazotised 2 : 4-dichloroaniline, and it was concluded that crotonoside was *iso*guanine-*d*-riboside.

Spies and Drake (*J. Amer. Chem. Soc.*, 1935, **57**, 774) and Spies (*ibid.*, 1939, **61**, 350) also have isolated crotonoside and confirmed its composition by the isolation of crystalline *d*-ribose and *iso*guanine from the products of hydrolysis.

The point of attachment of the ribose to the *iso*guanine has now been determined by the method of comparison of the ultra-violet absorption spectra of nucleoside and methylpurines which has been used in the case of other nucleosides (for literature see Gulland, J., 1938, 1722; Falconer and Gulland, this vol., p. 1369).

Crotonoside was extracted from croton seeds by a simple method which avoided the long methyl alcohol extraction used by Cherbuliez and Bernhard.

9-Methyl*iso*guanine was prepared from 2 : 6-dichloro-9-methylpurine (Gulland and Story, J., 1938, 692) through the stages *2-chloro-6-amino-9-methylpurine* and *6-amino-2-ethoxy-9-methylpurine*.

7-Methyl*iso*guanine could not be obtained from 2 : 6-dichloro-7-methylpurine (Fischer, *Ber.*, 1897, **30**, 2400) by a corresponding series of stages; an isomeric change occurred during the treatment of 2-chloro-6-amino-7-methylpurine (Fischer, *Ber.*, 1898, **31**, 117) with sodium ethoxide and 7-methylguanine was formed. Fischer (*Ber.*, 1898, **31**, 542; 1899, **32**, 480) observed this isomeric change, and we have confirmed that it takes place by showing that the product of the action of alcoholic sodium ethoxide (from 99·8% alcohol) on 2-chloro-6-amino-7-methylpurine contained no ethoxyl (Zeisel), and by measuring the ultra-violet absorption spectra of this product, which were identical with those of authentic 7-methylguanine (Gulland and Story, J., 1938, 692).

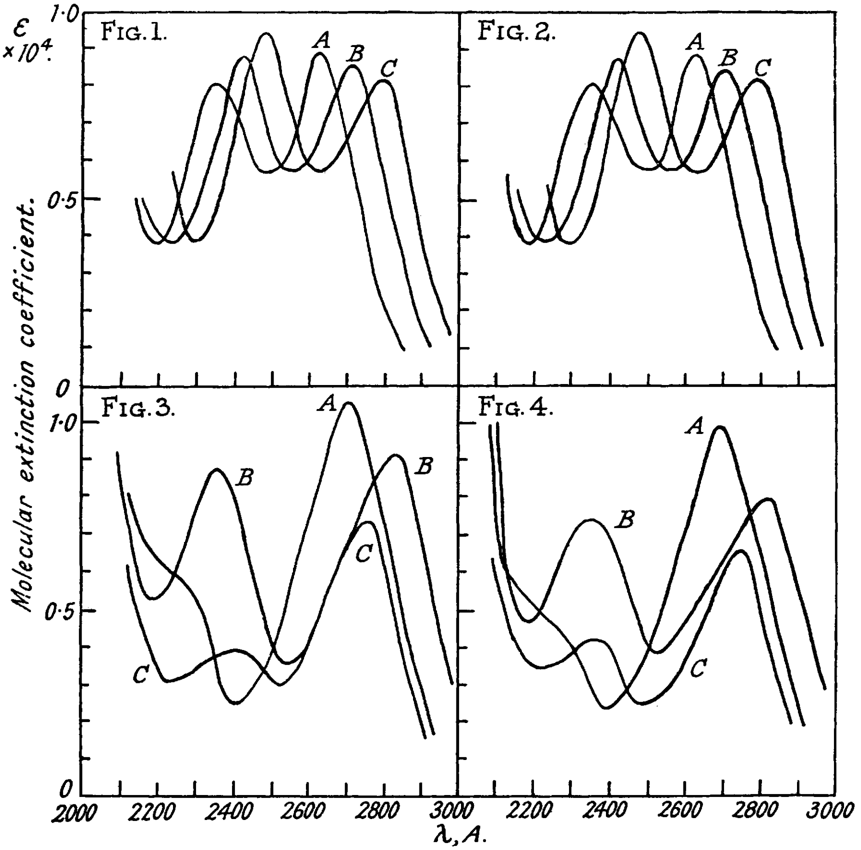
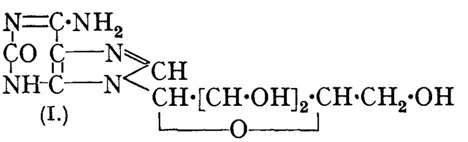


FIG. 1.—*Deaminated crotonoside (? xanthosine)*, M/40,000-*solution*. A, N/20-HCl; B, *water*; C, N/20-NaOH.
FIG. 2.—*Xanthosine, ex guanosine*, M/40,000-*solution*. A, N/20-HCl; B, *water*; C, N/20-NaOH.
FIG. 3.—*Crotonoside*, M/50,000-*solution*. A, N/20-HCl; B, *water*; C, N/20-NaOH.
FIG. 4.—9-*Methyl*iso*guanine*, M/50,000-*solution*. A, N/20-HCl; B, *water*; C, N/20-NaOH.

Since 7-methyl*iso*guanine could not be obtained, crotonoside was deaminated with nitrous acid, and the ultra-violet absorption spectra of the deaminated product in acid, alkaline, and neutral aqueous media were compared with the corresponding spectra of xanthosine prepared by deamination of guanosine, in which the ribose has been shown to be attached to the 9-position of the purine (Gulland, Holiday, and Macrae, J., 1934, 1639; Gulland and Story, J., 1938, 692). The ultra-violet absorption spectra of the deaminated crotonoside (Fig. 1) are identical with those of authentic xanthosine (Fig. 2) and it was therefore concluded that the deaminated crotonoside, and consequently crotonoside itself, have the sugar in the 9-position (I), the furanose structure of the sugar being assumed to be the more probable in view of the identity of the optical rotations of crotonoside and guanosine on one hand and of deaminated crotonoside and xanthosine on the other.


(I.)

Comparisons of the ultra-violet absorption spectra of crotonoside (Fig. 3), 9-methyl*iso*guanine (Fig. 4), and guanosine (Gulland and Story, J., 1938, 692) confirm that crotonoside is a 9-substituted derivative, that it is not identical with guanosine, and that its aglycone is *iso*guanine.

EXPERIMENTAL.

Isolation of Crotonoside.—Finely ground, unshelled croton seeds (500 g.) were extracted twice with hot water (3 l. and 2 l.) at 80° for 20 minutes, and the extracts filtered whilst still hot. Neutral lead acetate (20% solution) was added to the combined filtrates until no further precipitation occurred, and the precipitate was removed and rejected. To the filtrate, neutral lead acetate solution and 5N-ammonia were added until precipitation was complete; when the precipitate had settled, the supernatant liquid was decanted, and the precipitate centrifuged and washed thoroughly with water. It was suspended in hot water (1 l.) and decomposed with hydrogen sulphide, and the lead sulphide filtered off. The filtrate was concentrated to about 50 c.c. under reduced pressure below 50° and left in the refrigerator for 24 hours. The precipitate which had separated was collected and recrystallised twice from hot water (norit). Crotonoside (0·53 g.) formed colourless needles, m. p. 243—245° (decomp.), which were insoluble in the usual organic solvents but dissolved readily in dilute alkalis and mineral acids and gave a strong pentose reaction (Found in material dried at 110° in a vacuum over phosphoric oxide : N, 24·6; amino-N, 4·4. Calc. for $C_{10}H_{13}O_5N_5$: N, 24·7; amino-N, 4·9%). It had $[\alpha]_D^{20^\circ}$ − 60·2° in 0·1N-sodium hydroxide (c = 1·035). Guanosine has $[\alpha]_D^{20^\circ}$ − 60·52° (Levene and Jacobs, *Ber.*, 1909, **42**, 2472).

The picrate, prepared in aqueous solution, formed yellow needles, m. p. 210—215° (decomp.); Cherbuliez and Bernhard record 210° (decomp.).

Deamination of Crotonoside.—A solution of crotonoside (0·4 g.) in warm 30% aqueous sodium nitrite (10 c.c.) was cooled to room temperature and mixed with glacial acetic acid (3 c.c.). After remaining at room temperature for 30 minutes, the mixture was warmed for 10 minutes at 60° and cooled, and 20% lead acetate solution and 5N-ammonia were added until precipitation ceased. The precipitate was centrifuged, washed with water, suspended in hot water (250 c.c.), and decomposed with hydrogen sulphide. The lead sulphide was collected and washed with hot water, and the filtrate was concentrated to about 5 c.c. under reduced pressure at 50° and left in the refrigerator. The precipitate was collected and twice recrystallised (norit) from hot water. Deaminated crotonoside (? xanthosine) (0·21 g.) formed needles which did not melt and gave a strong pentose reaction (Found in material dried at 110° in a vacuum over phosphoric oxide : N, 19·7. Calc. for $C_{10}H_{12}O_6N_4$: N, 19·7%). In N/10-sodium hydroxide (c = 1·029) it had $[\alpha]_D^{20^\circ}$ − 51·2°. Authentic xanthosine has $[\alpha]_D^{30^\circ}$ − 51·2° (Levene and Jacobs, *Ber.*, 1910, **43**, 3163).

2-*Chloro*-6-*amino*-9-*methylpurine.*—One part of 2 : 6-dichloro-9-methylpurine (Gulland and Story, J., 1938, 692) was heated with 40 parts of dry alcoholic ammonia (half-saturated at 20°) for 3 hours in a sealed tube at 85—90°. The contents of the tube were evaporated to dryness, and ammonium chloride extracted from the solid with warm water. The residue was dissolved in dilute hydrochloric acid (norit) and reprecipitated with ammonia. 2-*Chloro*-6-*amino*-9-*methylpurine* formed colourless needles (Found : N, 37·8. $C_6H_6N_5Cl$ requires N, 38·1%).

6-*Amino*-2-*ethoxy*-9-*methylpurine.*—Sodium (1·0 g.) was dissolved in absolute alcohol (20 c.c.), chloroaminomethylpurine (1·0 g.) added, and the mixture heated for 3 hours in a sealed tube at 130°. The alcohol was distilled off, the residue dissolved in a little water, and the *ethoxy*-compound, m. p. 252—254° (decomp.), precipitated with an excess of acetic acid. When allowed to separate from alcohol, it formed a gelatinous mass which became micro-crystalline when stirred (Found : N, 36·0. $C_8H_{11}ON_5$ requires N, 36·3%). It was readily soluble in hot alcohol but only slightly soluble in water and benzene.

9-*Methyl*iso*guanine.*—An excess of phosphonium iodide was added to the ethoxy-compound in 10 parts of hydriodic acid (d 1·95). The mixture was shaken at 30—40° until the initial rapid reaction had ceased and then heated to boiling and evaporated to dryness on the water-bath. The residue was dissolved in water, and the base precipitated with ammonia and purified by conversion into the sulphate; a solution in boiling 10% sulphuric acid was cooled, and the sulphate crystallised in minute rectangular plates. When these were dissolved in boiling water and the solution was made alkaline with ammonia and cooled, 9-*methyl*iso*guanine* separated in a semigelatinous condition; it became micro-crystalline on standing (Found: N, 41·8. $C_6H_7ON_5$ requires N, 42·4%).

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 the concentrations recorded on the Figs., were examined immediately in a layer thickness of 4 cm. against controls.

We are grateful to Mr. S. A. Berridge for preparing a specimen of crotonoside by the method of Cherbuliez and Bernhard, and to Mr. J. W. Albans for assistance.

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[*Received, September* 15*th*, 1939.]