379. *Anthraquinone Colouring Matters: Ruberythric Acid.*

By Derek Richter.

THE presence of a glycoside of alizarin in madder was first shown in 1847 by Schunck, who obtained an amorphous preparation which he called "rubian" (*Annalen*, 1847, **66**, 176). Crystalline ruberythric acid was isolated a few years later by Rochleder, and was one of the first glycosides to be obtained pure (*Annalen*, 1851, **80**, 324). Glycosides of alizarin are also present in a number of common British species, including *Galium mollugo* and *G. verum* (Hill and Richter, in the press).

The investigations of Graebe and Liebermann (*Annalen*, 1869, Suppl. 7, 296) and of Liebermann and Bergami (*Ber.*, 1887, **20**, 2741) led to the conclusion that ruberythric acid was probably a diglucoside, as it gave on treatment with acids, alkalis, or hydrolytic enzymes two molecules of a dextrorotatory sugar which they believed to be glucose:

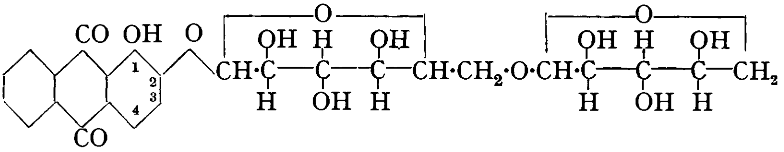
$$C_{26}H_{28}O_{14} + 2H_2O \longrightarrow 2C_6H_{12}O_6 + C_{14}H_8O_4$$

Schunck and Marschlewski prepared a number of derivatives of ruberythric acid which gave evidence that the two sugar residues are attached together in the form of a disaccharide instead of being on separate hydroxyl groups of the anthraquinone nucleus; but their work did not settle the formula (J., 1894, **65**, 187).

More recently the question of the constitution of ruberythric acid has been attacked from a new angle by Glaser and Kahler (*Ber.*, 1927, **60**, 1349), Zemplén and Müller (*Ber.*, 1929, **62**, 2107), and Robertson (J., 1930, 1136), who synthesised alizarin-1 : 2-*O*-diglucoside, alizarin-2-*O*-maltoside, and alizarin-2-*O*-cellobioside; but none of these compounds was identical with ruberythric acid. Jones and Robertson then found that the sugar from ruberythric acid gave the qualitative test for a pentose or a methyl pentose (J., 1933, 1167). They further synthesised an acetyl derivative of alizarin-2-*O*-β-primveroside,* but their product was not identical with hepta-acetylruberythric acid.

The sugar of ruberythric acid has now been obtained by enzymatic hydrolysis and identified as primverose. Since primverose does not melt sharply enough for a mixed melting point to be of value, the identification was completed by examining the crystallographic properties of the sugar.

The position of the sugar residue in ruberythric acid was determined by methylating the acid with silver oxide and methyl iodide, removing the methylated sugar residue by acid hydrolysis, and isolating alizarin 1-methyl ether, which was obtained in good yield.



This showed that the sugar residue must occupy the 2-hydroxyl group. Ruberythric acid was readily hydrolysed in 2 hours at 37° by the enzymes of *Primula officinalis* and *P. vulgaris*, which have been shown to belong to the hetero-β-glucosidases (Oppenheimer, " Die Fermente und ihre Wirkungen," 1935, VIII, 291); the attachment of the sugar to the aglycone is therefore of the normal β-type (cf. Jones and Robertson, *loc. cit.*).

Since ruberythric acid is a primveroside, the enzyme erythrozym obtained by Schunck from madder root must belong to the same class as the " primverosidases " (*J. pr. Chem.*, 1851, **63**, 222). The fact that the sugar residue in ruberythric acid occupies the 2-hydroxyl group suggests that a 1-methyl ether of ruberythric acid may be present in Chay root, from which Perkin and Hummel isolated alizarin 1-methyl ether (J., 1893, **63**, 1160).

EXPERIMENTAL.

Properties of Ruberythric Acid.—The acid was prepared from fresh madder root and separated from the other glycosides by fractional precipitation of their barium salts in 50% alcohol (Hill and Richter, *loc. cit.*); 600 g. of fresh root gave 1·0 g. of ruberythric acid, m. p. 257°. The microanalysis agreed with a pentosidohexoside monohydrate rather than with a dihexoside [Found: C, 54·2; H, 5·5. Rochleder found: C, 54·5; H, 5·2. Schunck found: C, 55·5, 55·6; H, 5·4, 5·2. Liebermann and Bergami found: C, 54·96, 55·23; H, 5·07, 5·20. $C_{25}H_{28}O_{14}$ (pentosidohexoside,H_2O) requires C, 54·3; H, 5·11%. $C_{26}H_{28}O_{14}$ (dihexoside) requires C, 55·32; H, 4·97%].

Enzymatic Hydrolysis of Ruberythric Acid.—The enzyme was prepared by grinding xylem (1 g.), dissected from the fresh root of *Rubia tinctorum*, in a mortar. Water was added, and the solid filtered off and washed well before extensive hydrolysis of the glycosides present had occurred. Ruberythric acid (100 mg.) was added to a suspension of the enzyme in water (200 c.c.) and the mixture was incubated at 37° with occasional shaking. Spectroscopic examination of the mixture in alkali showed that after 14 hours the hydrolysis was nearly complete. The alizarin and enzyme were filtered off, and the sugar solution extracted with butyl alcohol to remove undecomposed ruberythric acid, and evaporated under reduced pressure to 0·2 c.c. The solution was then filtered again and evaporated nearly to dryness; the residual gum in contact with 0·6 c.c. of methyl alcohol slowly became crystalline. The sugar was recrystallised twice from methyl alcohol. Yield 30 mg., decomp. 195°.

Properties of the disaccharide. A solution containing 29·6 mg. of the sugar in 0·75 c.c. of water gave $[\alpha]_D^{18^\circ}$ $-3^\circ\,30'$. An estimation of the reducing power of the sugar by the Hagedorn–Jensen micromethod gave that 0·71 mg. was equivalent to 0·37 mg. of glucose, which is in agreement with a reducing disaccharide with one free aldehyde group. With phenylhydrazine it gave a crystalline phenylosazone, yellow prisms, m. p. 203—205° (decomp.).

Hydrolysis of the disaccharide. The sugar was hydrolysed by heating it for 5 hours on a

* In a private communication Prof. Robertson has intimated that in view of more recent work the constitution given for the synthetic acetyl derivative cannot be regarded as certain.

boiling water-bath with 0·4*N*-sulphuric acid: the rotation changed to $[\alpha]_D^{18^\circ} + 31^\circ\ 30'$. An estimation of the reducing power of the sugar obtained by hydrolysis gave that 0·46 mg. was equivalent to 0·40 mg. of glucose, indicating that two molecules of aldose had been formed by hydrolysis.

A pentose estimation by McCance's method (*Biochem. J.*, 1926, **20**, 1111) with 4·3 mg. of ruberythric acid gave 26%. A pentosidohexoside of alizarin + H_2O requires 27% pentose. The pentose was identified by oxidising it to the corresponding acid : a solution of the pentose (6 mg.) in water (1 c.c.) was warmed with saturated bromine solution (1 c.c.) and cadmium carbonate (50 mg.) for 2 hours at 46°. The excess of cadmium carbonate was centrifuged off, and the solution evaporated nearly to dryness. Whetstone-shaped crystals of cadmium bromoxylonate, sparingly soluble in 80% alcohol, separated on standing, showing the pentose to be *d*(+)-xylose.

When 1·2 mg. of the hydrolysed sugar mixture were treated with phenylhydrazine, an insoluble osazone with the characteristic appearance of glucosazone crystallised from the solution at 100°. A solution containing 1·1 mg. of the aldohexose, when shaken with *Saccharomyces cerevisiae* and phosphate buffer mixture at p_H 5 in an atmosphere of nitrogen in a Warburg apparatus at 30°, was rapidly fermented. This confirmed that the aldohexose was glucose (*Saccharomyces cerevisiae* does not ferment pentoses).

Identification of the disaccharide. The chemical examination of the disaccharide obtained from ruberythric acid showed it to be made up of glucose and *d*(+)-xylose. The observed rotation of the sugar, $-\ 3^\circ\ 30'$, and of its hydrolysis products, $+\ 31^\circ\ 30'$, agreed with the corresponding values, $-\ 3^\circ\ 30'$ and $+\ 35^\circ\ 30'$, for primverose, or 6-β-*d*-xylosido-*d*-glucose. The decomposition temperature, 195—200°, also agreed with that of primverose.

A comparison was then made of the crystallographic properties of the disaccharide and a pure specimen of primverose kindly given by Prof. Charaux. Crystallisation of the disaccharide from 90% acetic acid was induced by seeding with primverose, and similarly the crystallisation of the primverose was induced by seeding with the disaccharide. Both sugars formed anhydrous crystals of parallel-sided tabular habit, showing similar double refraction and interference.

Disaccharide	$\alpha = 140^\circ$	$\beta = 115^\circ$	μ (maximum) = 1·565
Primverose	$\alpha = 142^\circ$	$\beta = 114^\circ$	,, = 1·563

Both crystals gave a slow ray vibrating parallel to their best developed faces. These measurements confirm the identity of the two sugars.

Position of the Sugar Residue.—Methylation. Ruberythric acid (200 mg.) was ground with silver oxide (6 g.), and the mixture taken up in 80 c.c. of acetone and boiled with methyl iodide (6 c.c.) under reflux for 6 hours. The silver compounds were then filtered off, and the solution evaporated to dryness. The product was washed with light petroleum and then with water, and crystallised from methyl alcohol, from which it separated in pale yellow needles. Yield 190 mg., m. p. 170—180°. It did not give a red colour with cold alcoholic sodium hydroxide, which showed that the hydroxyl group in the anthraquinone nucleus was methylated [Found : OMe, 25·2. $C_{25}H_{23}O_9(OMe)_5$ requires OMe, 25·0%].

Alizarin 1-*methyl ether.* The methylated ruberythric acid (150 mg.) was hydrolysed by boiling for 1½ hours with acetic acid (2 c.c.), concentrated hydrochloric acid (1 c.c.), and water (2 c.c.). The product, which separated on cooling, was taken up in chloroform and extracted from the chloroform with aqueous ammonia, and the solution treated with barium hydroxide solution. The filtered solution of the ammonium salt was acidified with acetic acid, and the crystalline precipitate separated and recrystallised from dilute acetic acid, forming clusters of pale yellow plates (40 mg.), m. p. 176°. It had all the properties of alizarin 1-methyl ether and differed from alizarin 2-methyl ether in giving an orange-red solution in alkalis and in being readily hydrolysed by heating with sodium hydroxide solution to give alizarin.

The author thanks Sir Frederick Hopkins and Dr. R. Hill for their interest and advice, Mr. H. I. Drever for his assistance with the crystallographic measurements, the Medical Research Council for a personal grant held while a part of the work was in progress, and the Chemical Society for a grant which defrayed a part of the cost.

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