381. *Anthraquinone Colouring Matters: Galiosin; Rubiadin Primveroside.*

By Robert Hill and Derek Richter.

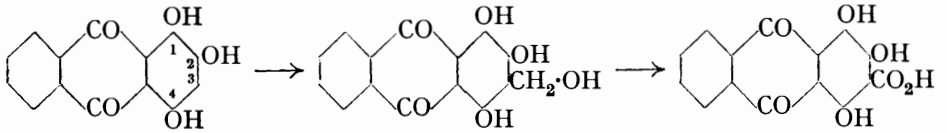
Several species of the tribe *Galieæ* of the *Rubiaceæ* contain coloured anthraquinone derivatives, which are present as glycosides in the roots. The dyers' madder plant, *Rubia tinctorum*, contains ruberythric acid, a glycoside of alizarin, and rubiadin-3-glycoside, which was first isolated by Schunck and Marschlewski (J., 1893, **63**, 969, 1137). It has recently been shown that galiosin, a glycoside of purpurincarboxylic acid, also is present in fresh madder root in relatively large amounts, and a new *glycoside* of rubiadin has been isolated from two closely related species (Hill and Richter, *Proc. Roy. Soc.*, 1936, *B*, in the press). Jones and Robertson (J., 1930, 1699) have established the constitution of the rubiadin-3-glucoside of Schunck and Marschlewski. The constitutions of the other three glycosides have not hitherto been determined.

It has now been found that galiosin is a primveroside of purpurin-3-carboxylic acid (III). The constitution of the sugar has been shown by hydrolysing it to glucose and a pentose, identified as *d*(+)-xylose by oxidation to *d*(+)-xylonic acid and preparation of the cadmium bromoxylonate.

The constitution of the aglycone was shown by synthesis: purpurin (I) was condensed with formaldehyde to give 3-*hydroxymethylpurpurin* (II), which was oxidised by nitroso-sulphuric acid in the presence of boric acid to purpurin-3-carboxylic acid (III).

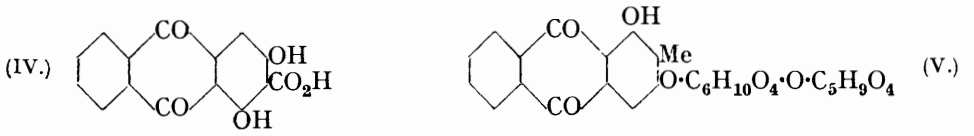
Galiosin differed from the other known hydroxyanthraquinone glycosides in being extremely unstable: it was hydrolysed by dilute acids and alkalis in the cold, or on boiling

for a few hours with water. The hydrolysis was accompanied by a colour change, since the glycoside is yellow and the aglycone is bright red.



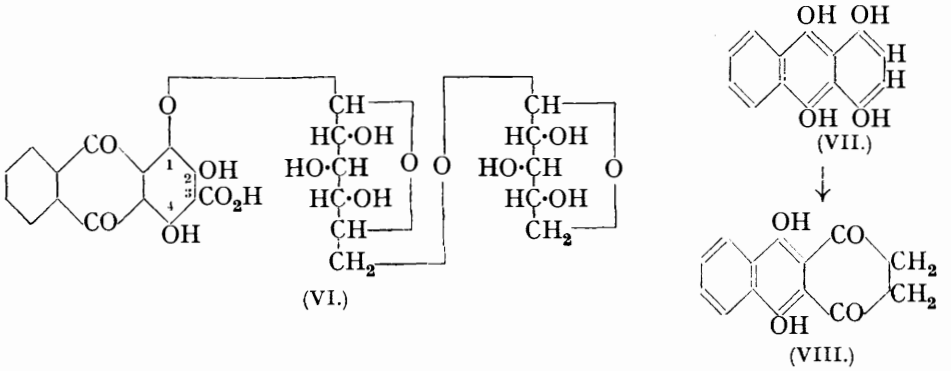
(I.) (II.) (III.)

Galiosin also possessed the unusual property of being hydrolysed in cold dilute aqueous solution by treatment with colloidal palladium and hydrogen. The aglycone was simultaneously reduced to munjistin (IV). An indication of the probable mechanism of this



(IV.) (V.)

reaction is given by the work of Zahn and Ochwat (*Annalen*, 1928, **462**, 72), who showed that leucoquinizarin has the constitution (VIII), being formed by a rapid rearrangement of the isomeric primary reduction product (VII). A similar rearrangement in the case of galiosin would involve the elimination of a sugar residue on the 1-hydroxyl group. From this reaction and from the colour reactions of galiosin it is concluded that the sugar residue is in the 1-position (VI).



(VI.) (VII.) (VIII.)

The new glycoside of rubiadin was also found to be a primveroside (V). It was readily converted into the rubiadin-3-glucoside of Schunck and Marschlewski by boiling with dilute acid, which removed the terminal pentose residue.

In both galiosin and rubiadin primveroside the sugar residues are attached to the aglycones by linkages of the normal β-glucosidic type, since they were hydrolysed by the enzymes of *Primula officinalis* and *P. vulgaris*, which have been shown to belong to the hetero-β-glucosidase group of enzymes (Oppenheimer, "Die Fermente und ihre Wirkungen," 1935, VIII, 282, 288, 291, gives a summary of the literature).

Experimental.

Properties of Galiosin.—Galiosin (0·7 g.) was isolated from fresh madder root (1245 g.) by the butyl alcohol extraction method (Hill and Richter, *loc. cit.*). It was easily decomposed by heating with solvents, but could be crystallised from water at 50° by cooling the bright yellow solution in the ice-chest, separating in yellow needles which decomposed above 100°. It was much more soluble in water than most of the known hydroxyanthraquinone glycosides: 0·1497 c.c. dissolved 1·93 mg. at 17·3°, which gives a solubility of 1·29%. It was easily salted out from aqueous solution by sodium chloride.

The partition coefficient between water and butyl alcohol was measured by shaking 0·5 mg. with water and butyl alcohol, removing samples with a micropipette, and estimating the aglycone colorimetrically in toluene solution after hydrolysis with dilute hydrochloric acid. A solution containing 1·0% of galiosin in the aqueous layer contained 0·034% in the butyl alcohol layer, indicating a partition coefficient of 0·034 at 17·6°.

Galiosin behaved as an acid in that it decomposed sodium bicarbonate and lithium carbonate. It dissolved in dilute alkalis to give deep orange-coloured solutions. On drying in a vacuum or at 50° in the air, crystalline galiosin formed an amorphous red product, which gave crystalline galiosin again on addition of water [Found (micro): C, 44·7; H, 6·3; loss on drying at room temperature in a vacuum desiccator, 17·5. Calc. for $C_{26}H_{26}O_{16},6H_2O$: C, 44·4; H, 5·5; H_2O, 18·2%].

Hydrolysis. Galiosin was completely hydrolysed by *N*/5-hydrochloric acid at 50° in 10 minutes, or by 5% sodium carbonate solution at 19° in 4·5 hours. It was partly hydrolysed by boiling for 4 hours in water.

The Aglycone of Galiosin.—Galiosin (20 mg.) was warmed with *N*/10-hydrochloric acid (5 c.c.) at 40°. The aglycone, which separated in bright red needles, was recrystallised from chloroform, separating in red plates with a green metallic lustre: yield 8 mg., m. p. 218—220° (decomp.) (all m. p.'s given are uncorr.). It was insoluble in alcohol, slightly soluble in hot water, and dissolved in toluene to give a red solution having a characteristic absorption spectrum; the bands were visible at a dilution of 1/200 mg. per c.c. in a layer 1·5 cm. thick. The aglycone was identified as purpurin-3-carboxylic acid by mixed m. p. with the synthetic product and by comparison of their absorption spectra and solubilities in solvents. Both substances showed absorption bands at 5650, 5320, and 4950 A. in toluene, at 5500 and 5100 A. in 8% sodium hydroxide solution, and at 5600, 5200, and 4850 A. in sulphuric acid containing 5% of boric acid. Both substances also reacted with 5% aqueous ammonia at 50° to give a violet-coloured product which showed a well-defined absorption band at 5600 A. in toluene solution [Found (micro): C, 60·4; H, 2·8. Calc. for $C_{15}H_8O_7$: C, 60·0; H, 2·7%].

Purpurin-3-carboxylic acid was also isolated from the wild madder plant, *Rubia peregrina*, and from *Galium verum* and *G. Mollugo*. It could be obtained from commercial madder (100 g.) by shaking an acidified aqueous suspension of the dried root with toluene and extracting the toluene solution with aqueous sodium bicarbonate, which gave the sodium salt. The purpurin-3-carboxylic acid (0·5 g.) was then precipitated by acid.

Commercial madder also contained purpurin, though no appreciable amount was present in the fresh root. Purpurin-3-carboxylic acid (in the amorphous form), when kept in dilute acid or heated with a solvent, was easily decarboxylated to give purpurin. Similarly, galiosin gave purpurin by hydrolysis and decarboxylation. This observation would appear to explain the presence of purpurin in commercial madder and the failure of other investigators to find a glycoside of purpurin in fresh madder root.

Synthesis of Purpurin-3-carboxylic Acid.—The presence of a carboxylic acid of purpurin in madder was shown by Liebermann and Plath (*Ber.*, 1877, **10**, 1618). Perkin and Cope synthesised purpurin-6 (or 7)-carboxylic acid; but this was not identical with the natural product from madder. It is stated in D.R.-P. 260,765 that purpurin-3-carboxylic acid has similar properties to the natural product; but the actual identity of the two compounds appeared to require confirmation. The method of preparing purpurin-3-carboxylic acid given in D.R.-PP. 260,765 and 272,301 involves the use of materials that are not easily accessible. Mitter and Biswas (*J. Indian Chem. Soc.*, 1927, **4**, 535) have given a further synthesis of purpurin-3-carboxylic acid, but were unable to obtain a pure product.

3-Hydroxymethylpurpurin. Formaldehyde (10 c.c., 35%) was added to a solution of purpurin (2·5 g.) in sulphuric acid (100 c.c.). The mixture was kept at 20° until on spectroscopic examination the bands of purpurin were seen to have disappeared and a strongly marked band at 5250 A. had developed. It was then poured into water (500 c.c.) and the product filtered off, washed, and crystallised from pyridine, with which it formed a complex. This was decomposed with dilute hydrochloric acid, and the resulting *3-hydroxymethylpurpurin* recrystallised from alcohol and then from chloroform, from which it separated in fine orange-red needles (1·5 g.), m. p. above 300°, slightly soluble in hot water, easily soluble in alcohol, chloroform, and pyridine. With sodium hydroxide it gave an insoluble violet sodium salt. In sulphuric acid it showed absorption bands at 5250 and 4900 A. (Found: C, 62·7; H, 3·3. $C_{15}H_{10}O_6$ requires C, 62·9; H, 3·5%).

Purpurin-3-carboxylic acid. A mixture containing hydroxymethylpurpurin (0·35 g.), boric acid (2 g.), and sodium nitrite (1 g.) in sulphuric acid (50 c.c.) was heated at 145° until a sample

showed the absorption bands at 5600, 5200, and 4850 A. of purpurin-3-carboxylic acid. The mixture was cooled and poured on ice, and the precipitate washed with water and crystallised from chloroform. Yield 0·22 g., m. p. 218—220° (decomp.) (Found: C, 60·0; H, 2·7. Calc. for $C_{15}H_8O_7$: C, 60·0; H, 2·7%).

Sugar Residue of Galiosin.—Disaccharide. On quantitative hydrolysis, 1·849 mg. of galiosin gave 0·876 mg., or 47%, of aglycone. This corresponds to two monose residues for each molecule of purpurin-3-carboxylic acid (a dihexoside would contain 48% of aglycone).

By hydrolysis under very mild conditions with dilute acid it was found possible to hydrolyse the sugar–aglycone linkage and obtain a disaccharide. Galiosin (50·8 mg.) was warmed at 50° with *N*/10-sulphuric acid (20 c.c.) until the yellow colour of the glycoside had disappeared. The aglycone was filtered off, and the sulphuric acid removed as barium sulphate by addition of the calculated quantity of barium hydroxide. On evaporation of the filtrate under reduced pressure a sugar (19 mg.) was obtained which crystallised from 90% acetic acid in acute-angled plates. A solution containing 19 mg. in 0·75 c.c. gave $[\alpha]_D^{18°}$ — 2° 6′ in a micropolarimeter. An estimation of the reducing power of the sugar by the Hagedorn–Jensen micro-method (*Biochem. Z.*, 1923, **135**, 46) gave that 0·59 mg. was equivalent to 0·30 mg. of glucose, which is in agreement with a disaccharide containing one free aldehyde group.

Hydrolysis of the disaccharide. The disaccharide (13·4 mg.) was heated for 10½ hours on a boiling water-bath with *N*/5-sulphuric acid (1 c.c.): the rotation changed to $[\alpha]_D^{18°}$ + 30° 30′. In the resulting sugar mixture it was found by the Hagedorn–Jensen method that 0·45 mg. was equivalent to 0·42 mg. of glucose, which showed that two molecules of aldose had been formed by hydrolysis. The hydrolysed sugar product (1·8 mg.) gave a mixture of two phenylosazones, one of which crystallised from hot water, on cooling, in long branching needles, and the other crystallised from water at 100° in the characteristic habit of glucosazone. The hydrolysed sugar mixture gave the qualitative tests for pentose.

Identification of the pentose. An estimation by McCance's micro-method gave 20% of pentose in galiosin (4·9 mg.) (*Biochem. J.*, 1926, **20**, 1111). A pentosidoglucoside of purpurin-3-carboxylic acid would require 21%. (This method gave a negative result with the *methyl* pentose, rhamnose.)

The hydrolysed sugar mixture (containing 15 mg. of pentose) was then oxidised with bromine (1·3 c.c. of saturated aqueous solution) in the presence of suspended cadmium carbonate (50 mg.) for 2 hours at 40°, being shaken from time to time. The excess of cadmium carbonate was centrifuged off, and the clear solution evaporated nearly to dryness. After 12 hours, characteristic whetstone-shaped crystals of cadmium bromoxylonate, sparingly soluble in alcohol, separated; the pentose was therefore *d*(+)-xylose.

Identification of the disaccharide. The only naturally occurring pentosidoglucose which has a specific rotation near — 2° 6′ is primverose (or 6-β-*d*-xylosido-*d*-glucose), the rotation of which is given variously in the literature as — 2° 3′, — 3° 17′, and — 3° 30′ (Goris, Mascre, and Vischniac, *Bull. Sci. Pharmacol.*, 1912, **19**, 577, 648; Bridel, *Compt. rend.*, 1924, **179**, 780). Primverose yields on hydrolysis a mixture of glucose (+ 52°) and *d*(+)-xylose (+ 19°) giving a mean rotation of + 35° 30′, which agrees approximately with the value + 30° 30′ obtained (the low value may be due to the formation of furfural from the pentose during hydrolysis).

Like primverose, the disaccharide did not give a sharp melting point; but it crystallised from 90% acetic acid in acute-angled parallel-sided plates resembling in habit crystals prepared in a similar manner from a specimen of primverose kindly given by Prof. Charaux.

Position of the Sugar Residue in Galiosin.—Ruberythric acid, rubiadin glucoside, and other hydroxyanthraquinone glycosides in which the sugar residues are on a 2-hydroxyl group are hydrolysed only slowly or not at all by boiling with sodium hydroxide solution. Galiosin, on the other hand, was hydrolysed readily by warming to 50° with dilute sodium carbonate solution. In this respect it resembled the relatively unstable hydroxyanthraquinone 1-ethers and glycosides, which are known to be easily hydrolysed by alkalis (Gardner and Demaree, *J. Amer. Chem. Soc.*, 1936, **58**, 757).

In its colour reactions with alkalis and on mordanted silk galiosin differed markedly from alizarin, purpurin, and quinizarin, but closely resembled xanthopurpurin and munjistin. This can be simply explained if the sugar residue in galiosin is in the 1-position.

Galiosin also resembled munjistin in the strong yellow colour of the free substance, and in that its solutions in alkalis showed general absorption without giving sharp absorption bands. Further evidence obtained by studying the reduction of galiosin supports the view that the sugar residue is on the 1-hydroxyl group.

Galiosin had no reducing properties and did not react with phenylhydrazine, from which

it may be concluded that the primverose is coupled with the aglycone in the normal way through the aldehyde group of the sugar. Galiosin was rapidly hydrolysed by the enzymes present in *Primula officinalis*, *P. vulgaris*, and *P. sinensis*, which hydrolyse β-primverosides. Hydrolysis was shown by the colour change from yellow to red and by observing the absorption bands of the aglycone at 5650, 5320, and 4950 A. after its extraction in toluene.

Colour Reactions of Hydroxyanthraquinones.

	Positions of free hydroxyl groups.	Colour in Na_2CO_3.	Colour on alumina-mordanted silk.
Alizarin	1 : 2	Violet	Red
Quinizarin	1 : 4	Violet	Red
Purpurin	1 : 2 : 4	Purple	Red
Purpurin-3-carboxylic acid	1 : 2 : 4 (CO_2H)	Purple	Red
Xanthopurpurin	1 : 3	Orange	Yellow
Munjistin	1 : 3 (CO_2H)	Orange	Yellow
Galiosin	—	Orange	Yellow

Reduction of Galiosin.—Mitter and Biswas (*loc. cit.*) have shown that purpurin-3-carboxylic acid is reduced by alkaline sodium hyposulphite to munjistin, the 1-hydroxyl group being eliminated. This reaction also took place with galiosin, the sugar residue being removed in the process. The reduction could be made to take place under extremely mild conditions, as with colloidal palladium and hydrogen in neutral solution at 18°. Munjistin was formed quantitatively, and no munjistin glycoside could be found.

Reduction with hyposulphite. Galiosin (50 mg.) and sodium hydrogen carbonate (0·2 g.) in water (10 c.c.) were treated with sodium hyposulphite (0·1 g.) at 18°. After 15 minutes the mixture was shaken in the air, neutralised with dilute sulphuric acid, and extracted with chloroform. On evaporation to dryness a crystalline residue of munjistin (20 mg.) was left, m. p. 229—232° (decomp.).

Reduction with hydrogen and palladium. Colloidal palladium solution (1 c.c. containing 1 mg. of palladium, as prepared by Wohl and Mylo, *Ber.*, 1912, **45**, 340) was added to a solution of galiosin (25 mg.) in water (10 c.c.), and hydrogen passed in. After 30 minutes the colour had become much paler and a yellow precipitate had formed. The mixture was extracted with chloroform, from which munjistin (10 mg.) separated, on evaporation to dryness, in rectangular plates, m. p. 229—232° (decomp.), similar in properties to a specimen prepared by the method of Mitter and Biswas (*loc. cit.*). It was soluble in dilute sodium hydrogen carbonate solution, gave an insoluble red barium salt, and decomposed above its m. p., with loss of carbon dioxide, to give xanthopurpurin, m. p. 264°.

Rubiadin Primveroside (V).—This was isolated by the method of Hill and Richter (*loc. cit.*) from *Galium verum*; 500 g. of fresh roots gave 0·7 g. of the *glycoside*, which separated from 50% aqueous alcohol in pale yellow, parallel-sided plates, m. p. 248—250°. The crystals were almost insoluble in cold water, but dissolved on warming. When the aqueous solution was shaken with butyl alcohol, most of the glycoside passed into the butyl alcohol layer. It gave a red insoluble barium salt and a red lead salt, which was precipitated on treatment of the aqueous solution with normal lead acetate and ammonia.

The solution obtained by hydrolysing the glycoside with acid gave the qualitative reactions for a pentose. A micro-pentose estimation by McCance's method with 3·9 mg. of the glycoside gave 24% of pentose. A rubiadin pentosidohexoside would require 27% [Found (micro): C, 56·8; H, 5·3. $C_{26}H_{28}O_{13}$ requires C, 56·9; H, 5·2%].

Hydrolysis. When the glycoside (155 mg.) was boiled with 0·4*N*-sulphuric acid (15 c.c.) for 6 hours, a flocculent yellow precipitate separated. After several recrystallisations from 97% alcohol this formed yellow needles (50 mg.), m. p. 268°. It was found by mixed m. p. and comparison of solubilities to be identical with the rubiadin-3-glucoside of Schunck and Marschlewski. We are very grateful to Prof. A. Robertson for an authentic specimen of rubiadin-3-glucoside for comparison.

After the hydrolysis and removal of the rubiadin-3-glucoside a pentose was found in the solution. This was identified as *d*(+)-xylose by oxidising it with bromine and isolating cadmium bromoxylonate crystals.

The fact that the rubiadin glycoside gave red salts with alkalis showed that one hydroxyl group in the anthraquinone nucleus was free, and therefore the pentose was attached to the glucose as a disaccharide. Since primverose was isolated from galiosin, it is probable by analogy that this *d*(+)-xylosidoglucoside also was a primveroside. The glycoside was hydrolysed by

enzymes present in *Primula officinalis* and *P. vulgaris*, from which it follows that the glycosidic linkage was of the β-type.

Rubiadin primveroside has not yet been isolated from madder, but its presence in closely related species suggests that it may be a precursor of the rubiadin-3-glucoside of Schunck and Marschlewski, which was obtained by boiling madder extracts with acid.

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