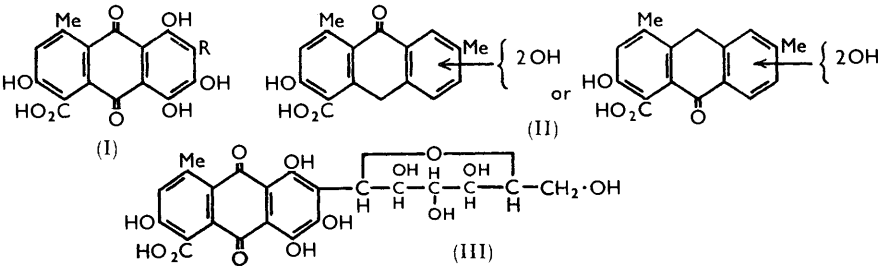
210. *C-Glycosyl Compounds. Part III.* Carminic Acid.*

By M. A. Ali and L. J. Haynes.

Carminic acid is shown to be the *C*-glucopyranosyl compound (III) by ozonolysis to give arabinose and by periodate oxidation of methyl carminate tetramethyl ether.

Carminic acid was given the structure (I; R = $C_6H_{11}O_5$) by Dimroth and Kamerer.[1] This paper is concerned with the structure of the side chain R.

Earlier workers [1,2] had shown by acetylation that this side chain contains four hydroxyl groups. In 1926, Miyagawa [3] showed that ozonolysis of carminic acid in water gave oxalic acid and a small quantity of a colourless optically active product. The latter with barium permanganate gave an amorphous optically active acid, probably $C_6H_{12}O_6$, which was reduced by sodium amalgam to a sugar ($C_6H_{12}O_5$) which was not identical with any known methylpentose. By analogy with the structure proposed for kermesic acid (I; R = Ac), Miyagawa suggested structure {I; R = CO·[CH(OH)]$_4$·CH$_3$} for carminic acid, but this was criticised by Todd [4] as being difficult to reconcile with the formation of coccinin (II) when carminic acid is fused with alkali. It was more likely that carminic acid was a *C*-glycosyl compound.[5]



Carminic acid with diazomethane gave methyl carminate tetramethyl ether (methylation of the four phenolic hydroxyl groups), which at 0° consumed 2·1 mol. of periodate with the formation of formic acid. This showed the presence of a C(OH)·CH(OH)·C(OH) system in the side chain and that Miyagawa's structure, which would require consumption of at least 3 mols. of oxidant, is incorrect.

Now we had shown [6] that oxidation of barbaloin with ferric chloride gave D-arabinose in good yield; further examination of this revealed that a second sugar is also formed, in much smaller amount, which gives a yellow colour with aniline oxalate and is identical on paper chromatography with glucose (see Table). We have not been able to identify this material rigidly by conversion into a crystalline derivative, probably because the amounts formed in the oxidation are so small, but there can be little doubt that it is D-glucose especially since barbaloin is known to be a glucose derivative. Similar oxidation of carminic acid gave a solution which on paper chromatography appeared to contain very small quantities of the same two sugars. Ozonolysis of carminic acid gave the same two sugars in rather better yield. They were separated by chromatography on thick paper

* The papers in *J.*, 1956, 3141, and *J.*, 1958, 2231, are regarded as Parts I and II of this series.

[1] Dimroth and Kamerer, *Ber.*, 1920, **53**, 471. For reviews of the work of Liebermann, von Miller, and Dimroth and their co-workers leading to this structure see Pollard and Cross in Thorpe's "Dictionary of Applied Chemistry," 4th edn., 1939, Vol. III, p. 226, and Thomson, "Naturally Occurring Quinones," Butterworths, London, 1957, pp. 222—227.

[2] von Miller and Rohde, *Ber.*, 1897, **30**, 1759.

[3] Miyagawa, *Mem. Coll. Eng. Kyushu Imp. Univ.* (*Japan*), 1924, **4**, 99; *Chem. Abs.*, 1927, **21**, 1127.

[4] Todd, *Ann. Reports*, 1941, **38**, 214.

[5] See, *e.g.*, Fieser and Fieser, "Textbook of Organic Chemistry," Heath, Boston, 1944, 857.

[6] Hay and Haynes, *J.*, 1956, 3141.

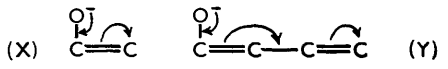
and one was rigidly identified as arabinose, but an attempt to confirm the identification of the other as its *p*-nitroaniline derivative was unsuccessful. Paper chromatography showed that the same two sugars were also formed on ozonolysis of barbaloin.

These results show that the side chain in carminic acid is glucopyranosyl. Unfortunately, from our degradation experiments we had insufficient arabinose to be able to determine its rotation, but since, following a suggestion of the Referees, we have found that L-arabinose benzoylhydrazone depresses the m. p. of the D-compound, the arabinose must be D-arabinose and there can be little doubt that carminic acid is the D-glucopyranosyl derivative (III).

Sugar	R_{xylose} in *A*	R_{xylose} in *B*	Colour with aniline oxalate
Arabinose	0·80; 0·82	0·82	Pink
Glucose	0·71; 0·69	0·50	Yellow
Sugars from barbaloin by $FeCl_3$	0·80	0·82	Pink
	0·68	0·52	Yellow
Sugars from carminic acid by $FeCl_3$	0·79; 0·82	0·83	Pink
	0·66; 0·69	0·54	Yellow
Sugars from barbaloin by O_3	0·82	—	Pink
	0·70	—	Yellow
Sugars from carminic acid by O_3	0·81	—	Pink
	0·69	—	Yellow

A = Butan-1-ol–pyridine–water (10 : 3 : 3). *B* = Butan-1-ol–acetic acid–water (2 : 1 : 1).

For formation of these *C*-glycosyl compounds in Nature it may be assumed that the "aglycone" is formed first: removal or separation of a proton from the aglycone then gives a system of the type (X) as in carminic acid and bergenin,[7] or (Y) as in barbaloin,



so that the glycosyl residue may be introduced on oxygen to give a glycoside or on carbon to give a *C*-glycosyl compound. In unpublished work we have shown that resorcinol readily forms a *C*-glycopyranosyl derivative and it seems likely that there exist in Nature *C*-glycosyl isomers of many of the glycosides of 1 : 3-dihydric phenols.

Experimental

Carminic acid (12·1 g.), isolated [8] from dried *Dactylopius coccus* Costa (250 g., "silver grain"), darkened at 120° and had R_F 0·17 in propan-1-ol–ammonia (*d* 0·880)–water (6 : 3 : 1) and 0·12 in butan-1-ol–pyridine–water (3 : 1 : 1), and $\nu_{max.}$ in Nujol 1708s, 1693s, 1677m, 1648m, 1632m, 1606s, 1566s, 1509 $cm.^{-1}$. With N-sulphuric acid at 100° for 4 hr. or with emulsin in acetate buffer pH 5 for 4 days it gave no sugar (paper chromatography). In periodate oxidation for 4 hr. at 0° there was a consumption of 6·2 mol.

Methyl Tetra-O-methylcarminate.—Carminic acid (2 g.) in anhydrous methanol (150 ml.) was cooled to 5° and diazomethane (*ca.* 2·5 g.) in dry ether (100 ml.) slowly added. There was a vigorous evolution of nitrogen and some carminic acid was precipitated. The mixture was kept at room temperature. After 16 hr. the solution was pale brown-red and most of the precipitated acid had redissolved. The mixture was warmed (60—65°) to remove excess of diazomethane and ether, cooled, filtered, and evaporated under reduced pressure (nitrogen). The residual, dark brown-red oil was chromatographed on magnesium carbonate (12 × 2·1 cm.), benzene being used as solvent and eluant; carminic acid and other impurities remained as a top, broad red band. The product separated as a brown-yellow band which on elution gave a deep yellow solution which was concentrated to *ca.* 10 ml. and rechromatographed. The bright yellow eluate was concentrated to a solution which was brownish-red by reflected and yellow by transmitted light. Removal of solvent under reduced pressure (nitrogen) gave a yellow glass. Slow evaporation (2 weeks) of a solution of this glass in benzene–light petroleum (b. p. 40—60°)

[7] Hay and Haynes, *J.*, 1958, 2231.
[8] Schunk and Marchlewski, *Ber.*, 1894, **27**, 2979; Dimroth and Scheurer, *Annalen*, 1913, **399**, 43.

gave *methyl tetra*-O-*methylcarminate* as yellow needles m. p. 185—188° [Found: C, 57·0; H, 5·4; OMe, 27·8, 28·1. $C_{22}H_{15}O_8(OMe)_5$ requires C, 57·6; H, 5·3; OMe, 27·6%].

Periodate Oxidation.—An aqueous solution of the methylation product (25·1 mg.) and 0·5M-sodium metaperiodate (10 ml.) were mixed, the whole was made up to 20 ml. with distilled water, and the solution set aside at 0°. The oxidation was followed by the titration of aliquot parts (2 ml.): it was complete in 2 hr., 2·1 mol. of oxidant being consumed and the solution becoming turbid. In another experiment, the solution was steam-distilled when the oxidation was complete, and formic acid was detected in the steam-distillate by Feigl's method.[9]

Ozonolysis of Carminic Acid.—2% Ozonised oxygen was passed through a cooled solution of carminic acid (3 g.) in water (60 ml.) until the colour changed from red to brownish-yellow (*ca.* 3 hr.). The solution was then steam-distilled: the distillate gave a positive test for formaldehyde with chromotropic acid. The aqueous residue, which became dark brown during the distillation, was extracted with ether (3 × 200 ml.), and the aqueous phase evaporated to dryness under reduced pressure. The residue, a dark brown syrup, was redissolved in water (200 ml.), and saturated aqueous lead acetate was added: a yellow-grey solid which was precipitated was removed. Excess of lead was removed from the filtrate by two treatments with hydrogen sulphide. The final aqueous solution was evaporated to dryness, giving pale yellow syrup (310 mg.); paper chromatography showed the presence of two sugars (see Table).

D-*Arabinose Benzoylhydrazone.*—The above syrup was chromatographed on several pieces of Whatman 3 mm. paper which had previously been continuously extracted with benzene–ethanol. The appropriate sections of the papers were eluted with water to give the two sugars as pale yellow syrups. On treatment with benzoylhydrazine in ethanol the syrup believed to be arabinose gave D-arabinose benzoylhydrazone, m. p. 184—187° (decomp.), undepressed on admixture with the authentic hydrazone,[10] m. p. 184—188° (decomp.).

We thank the Government of Pakistan for a scholarship and the Distillers Company Limited for a grant (to M. A. A.), T. and H. Smith Limited, Edinburgh, for gifts of *D. coccus*, and Dr. J. Evelyn Hay for advice and assistance.

UNIVERSITY COLLEGE OF THE WEST INDIES,
KINGSTON 7, JAMAICA. [*Received, October 9th*, 1958.]

[9] Feigl, " Spot Tests," English translation by R. E. Oesper, Elsevier, Amsterdam, 1954, p. 246.
[10] Hirst, Jones, and Woods, *J.*, 1947, 1048.