EXAMINATION OF THE CONSTITUENTS OF JUTE SEEDS

Part III.—Some New Glycosides of Jute Seeds

M. Qudrat-i-Khuda, A. Khalique and D.C. Das

Natural Products Division, East Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Dacca

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From the alcoholic extract of jute seed powder, besides strophanthidin and raffinose, two bitter glycosides have been isolated. They have been named corchoside A and corchoside B. Corchoside A has strophanthidin as a glycone combined with a bisaccharose as the sugar moiety. The bisaccharide appears to consist of a glucose unit with a new monosaccharide, the constitution of which is under investigation. Corchoside B is another glucoside of strophanthidin where the sugar moiety appears to be raffinose.

In Part II[1] of this series of papers, the constitution of a sterol glucoside has been described. In the course of work with the different constituents of jute seed, two characteristic glycosides have been isolated, which have been named "corchoside A" and "corchoside B". Reichstein[2] has described a glycoside corchoroside A, but these two glycosides appear to be different from Reichstein's.

The isolation of the glycosides was effected with considerable difficulty. When the alcoholic extract of jute seed, containing the entire quantity of the bitters, was examined carefully, it was noticed that the extremely bitter solution contained a fairly large quantity of raffinose. The separation of the bitter from the sweetening agent was effected with several solvents.

When the sterol glucoside had been separated as described in Part II of this series, the solution was freed from alcohol and the mother liquor was diluted and treated with enough lead acetate to precipitate small quantities of a lead compound and then the excess lead from solution was removed as sulphide. The remaining clear mother liquor as aqueous solution was extracted first with ether, and then with chloroform, and finally with chloroform alcohol mixture. Ether removed practically the whole of strophanthidin but chloroform alcohol mixture gave gummy products which on careful chromatographic separation from the admixed small quantities of strophanthidin yielded two glycosides: corchoside A and corchoside B. When pure, one of these, corchoside A, melts at 216-220 °C. The melting point was however, somewhat variable depending upon the medium of crystallisation. From the methanol a solid melting at 188-192 °C. was obtained, but ethanol gave crystals melting at 198-200°C., while *n*-butanol yielded crystals melting at 216-220°C. These crystals were extremely hygroscopic and on standing in the open, absorbed moisture very rapidly and the m.p. changed considerably.

This compound was hydrolysed with acids when strophanthidin was isolated as the aglycone with a sugar mixture. By paper chromatography, one of these sugars has been established as glucose, while the other one appears to be a new monosaccharide. Thus it is very clear that the glucoside A is a glycoside of strophanthidin and a bisaccharose, the identity of which is yet to be established.

A careful examination of this glycoside was undertaken in order to see if it resembled the monosaccharide of strophanthidin described by Reichstein.[2] It was acetylated when a pentacetate of the product was obtained, which definitely proves that the sugar moiety is not a monosaccharide. Further, the estimation of the quantity of sugar separated on hydrolysis proved that practically a disaccharose equivalent of sugar was eliminated. These are evidences in favour of the glycoside being a disaccharide and of not possessing monosaccharide configuration.

The second glycoside that was separated by the chromatographic process melted at 137-140 °C. and is also very hygroscopic. This has been called corchoside B. Due to its hygroscopic nature it also caused difficulty in the determination of its melting point, which was determined after very careful drying, and in a sealed tube. Analysis of this compound tends to suggest that it is a glycoside of strophanthidin and a trisaccharide. Hydrolysis of the glycoside has given some strophanthidin and a mixture of three mono-saccharoses, glucose, fructose and galactose. The conclusion was therefore drawn that a molecule of raffinose has actually combined with strophanthidin to give corchoside B. The other conclusion that the three mono-saccharoses have reacted with the three hydroxyl group was not quite normal, and attempts are still being continued to isolate the tri-saccharide as such. Hydrolysis actually produced a sugar mixture which on paper chromatography showed spots for four

sugars, glucose, fructose, galactose and as well as raffinose, which thereby confirms the conclusion that the raffinose, after being released from its combination with strophanthidin, was almost completely hydrolysed but remained in small quantities undecomposed to show a spot on paper chromatogram.

Corchoside B was also acetylated when an acetyl derivative was formed which appeared to be a deca-acetyl compound.

From these results it can now be stated that a mono-glycoside, like corchoroside A of Reichstein, could not be isolated from jute seeds. More work is still in progress to identify and characterise the disaccharide which forms the glycoside, corchoside A. It is expected that when corchoside A is prepared in quantity, it will not be difficult to characterise that sugar, and particularly the mono-saccharide that produces a characteristic new spot on the paper chromatogram.

Experimental

Isolation of Corchoside A and B.—As has already been reported,[3] finely powdered seeds of *Corchorus capsularis*, Linn. (10 kg.) were defatted by light petroleum ether (b.p. 60-80 °C.) and then extracted with ethanol (95%) which was freed from alcohol and dissolved in water (3 l.), and treated with an excess of lead acetate solution as described earlier.

The mother liquor on being extracted with different solvents gave several compounds as shown in Table 1.

Of these, strophanthidin has already been described earlier[3] and corchoside A and B are described here.

The separation of corchoside A and corchoside B from the chloroform-alcohol extracts were carried out on a column of alumina (neutral, Brockmann) and the elution was followed by Raymond test.[4] Fractions of 25 cc. of the solution were examined. The results obtained are given in Tables 2 and 3.

Purification and Characterization of Corchoside A.—The chloroform extract, on being crystallised from dilute ethanol gave strophanthidin (5 g.), m.p. 175-176 °C. The crude chloroform-alcohol extract (9:1, 4 g.) was chromatographed (Table 2) from benzene on neutral alumina (Brockmann, 40 g.); development with benzene-alcohol (88:12) eluted material gave strophanthidin; further elution with the same solvent mixture (80:20)

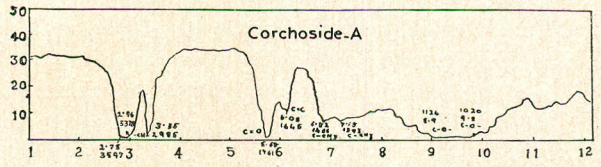

Fig. 1—I.R. curve of corchoside A.

yielded a hygroscopic solid (0.32 g.), corchoside A, m.p. 183-186 °C. and from methanol it gave semi-crystalline powder, m.p. 188-192°, $[\alpha]^{29}_D$ + 1.7° + 0.2° (c 4.7 in EtOH), λmax. (in EtOH) 303 mμ and 217 mμ, γmax. (in KBr) 3509 (broad, OH), 1761 (lactone), 1739(C=O), 1645(C=C), 1458, 1393 (C-CH_3), 1179, 1039 (C-o) Fig. 1. This substance gave a positive Legal colour reaction[5] and a strong Raymond test.[4] (Found: C, 57.2; H, 7.6; O, 34.7; Mol. wt. 714 (Rast); $C_{35}H_{52}O_{14}$, $2H_2O$; requires C, 57.4; H, 7.7; O, 34.9; Mol. wt. 732.8).

Crystallisation and Melting Point of Corchoside A.—The substance is hygroscopic, its cyrstallising power is very poor, and it was obtained only as a semi-crystalline mass; its m.p. depends on the time of exposure to the atmosphere and on the manner of heating; crystallised from ethanol into semi-crystalline powder, m.p. 198-200 °C. (freshly dried) but when crystallised from *n*-butanol, it melted at 216-220°C.; and when crystallised from methanol, it had a m.p. 190-192 °C. The pure and dry sample was analysed.

Water of Hydration in Corchoside A.—A sample, 45.9 mg. when dried in vacuum at 100°C. over P_2O_5 lost 2.2 mg., as water showing that it has about 4.8% of water. Calculated for $C_{35}H_{52}O_{14}$, $2H_2O$ water, 4.9%

Acetate of Corchoside A.—A sample (0.1980 g., m.p. 190-192°C.) dissolved in pyridine (4 cc.) was allowed to react with acetic anhydride (3 cc.) at 40°C. for 24 hours, at the end of which water (20 cc.) was added, and this was allowed to stand for 3 hours, and the precipitate (0.2249 g.) filtered and crystallised from dilute methanol, when it melted at 230-232°C. and had $(\alpha)_D^{30}$ + 3.1° (*c* 1.1 in methanol), γmax. (in

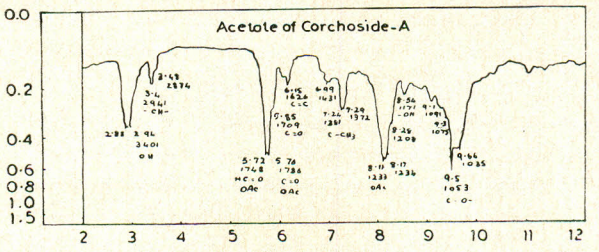

Fig. 2.—I. R. curve of acetate of corchoside A.

TABLE 1.

Solvent	Amount	Spot on paper
Ether	5 g. crystalline strophanthidin.	A pink-blue spot, R_f. 0.87.
Chloroform	6.7 g. gum containing strophanthidin.	A pink-blue spot, R_f. 0.87.
Chloroform alcohol (9:1)	49 g. gum containing strophanthidin and Corchoside A.	Two pink-blue spots, R_f. 0.87 and 0.68.
Chloroform alcohol (4:1)	66.7 g. gum containing strophanthidin and Corchoside A and B.	Three pink-blue spots, R_f. 0.87, 0.68 and 0.31.

TABLE 2.—SEPARATION OF CORCHOSIDE A.

Chloroform alcohol extract	Amount of alumina	Fraction	Elution with benzene-alcohol	Products obtained
proportion of 9:1, 4 g.	40 g.	I — XX	88:12 500 cc.	1.5 g. of a gum yielding 0.7 g. crystalline strophanthidin
		XXI — IVL	80:20, 1.4 l.	0.6 g. of a gum containing corchoside A

The gum was dissolved in alcohol and treated with activated charcoal when 0.32 g. of corchoside A in pure form could be separated.

TABLE 3.—SEPARATION OF CORCHOSIDE B.

Chloroform alcohol extract	Amount of alumina	Fraction	Elution with benzene-alcohol	Products obtained
Proportion 4:1, 10 g.	60 g.	I to XX	88:12 500 cc.	0.7 g. of a gum giving 0.5 g. crystalline strophanthidin
		XXI to XL	50: 50, 500 cc.	1 g. of a gum providing 0.6 g. of corchoside A.
		XLI to CI	50-50, 1.5l.	0.6 g. of a gum yielding 0.23 g. of corchoside B.

The gummy products were further purified by crystallisation with alcohol. The alcoholic extracts were charcoaled and the corchoside A and B were separated in quantities mentioned in the last column.

KBr) 3401(OH), 1748, 1736 (OAc), shoulder at 1709 (C = O), 1650, 1626 (C = C), 1381, 1372 (C-CH_3) 1253 (OAc), 1171 (OH), 1053, 1031 (C-O-)cm.-1 (Fig. 2). Found: C, 58.2; H, 7.2; O, 34.3; OAc, 20.9. $C_{45}H_{62}O_{19},H_2O$; requries: C, 58.4, H, 7.0; O, 34.6; OAc 23.3%.

Water of Crystallisation.—A sample of the above acetate (31.1 mg., m.p. 230-232°C.) dried over P_2O_5 at 110°C. under vacuum lost water (0.6 mg., 1.90%). Calculated for $C_{45}H_{62}O_{19}$, H_2O, 1.95%.

Attempt to Hydrolyse Corchoside A by Gastric Juice.—It was not very successful. A sample (0.33 g., m.p. 188-192°) dissolved in methanol (15 cc.) was allowed to react with artificial gastric juice[6] (5 cc.) at normal temperature. At the end of 18 hours, the test revealed that only a very small amount was hydrolysed.

Hydrolysis of Corchoside A by Mineral Acids.—The sample (0.2014 g., m.p. 183-186°C.) dissolved in methanol (10 cc.) was refluxed on a steam bath with sulphuric acid (0.1 N, 10 cc.) until the hydrolysis was complete (1 hr.) as indicated on a paper chromatogram.[7] Most of the methanol was then removed on a water bath under reduced pressure, the reaction mixture was diluted with water (30 cc.), and extracted with chloroform (50 cc. × 5); the chloroform extract was washed with water (50 cc.) and washings were transferred to the main bulk of water, and on removal of the chloroform these gave crystals (0.1029 g.), m.p. 162-166°C. which when crystallised from ethanol, had m.p. 175-176°C., $[\alpha]_D^{30}$ + 38.5° (*c* 0.83 in EtOH), λmax. 303 mμ and 217 mμ. It gives a positive Legal colour reaction and a strong Raymond test,[5] and it was paper chromatographically identical with strophanthidin.

The aqueous mother liquor was neutralised with $BaCO_3$, filtered, and passed through ion-exchanger (mixed ion-exchanger V, Merck). This, on removal of solvent, left a syrup (0.08 g.) which forms needles when kept over P_2O_5. It is highly hygroscopic and sweet to taste. It readily reduces Fehling's solution, and gives a strong Keller-Kiliani test[8] (blue) and Dische reaction[9] (blue), $[\alpha]_D^{30}$—10.2° (c 0.59 in water).

Amount of Strophanthidin and Sugar in Corchoside A.—Purified samples (m.p. 190-192°C.) dissolved in methanol (10 cc.) and hydrolysed with sulphuric acid (0.1 N, 10 cc.) in the manner described above, and in several experiments gave strophanthidin and sugar, in amounts as given in Table 4.

TABLE 4.

Sample, mg.	Strophanthidin		Sugar	
	Yield, mg.	%	Yield, mg.	%
1377	737	53·5	575	41.8
1045	573	54.9	419	40.1
2014	1029	51.2	800	39.7

Purification and Characterization of Corchoside B.—The crude chloroform-alcohol extract (4:1, 10 g.) was chromatographed from benzene on neutral alumina (Brockmann, 60 g.); development with benzene alcohol (88:12) eluted strophanthidin (0.5 g.); continued elution with benzene-alcohol (80:20) yielded a gum (1 g.) containing mostly corchoside A; on further development of the residue with the same solvent mixture in different proportion (50-50), eluted a highly hygroscopic corchoside B which as obtained from this mixed solvent, melted at 137-140° (in sealed tube) and has $[\alpha]_D^{30}$ — 9.6° (*c*.-1.2 in EtOH), λmax. (in

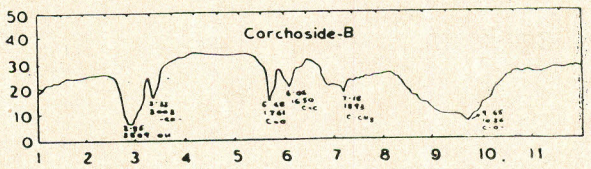

Fig. 3.—I.R.curve of corchoside B.

EtOH) 303 and 217 mμ, νmax. (in KBr) 3509 (broad, OH), 1761 (C=O, lactone) 1742 (C=O), 1650 (C=C), 1471, 1393 (C-CH_3), 1176, 1036 (C-O) (Fig. 3). The substance responded strongly to a Legal[5] and Raymond[4] test.

Acetate of Corchoside B.—A sample (228 mg. m.p. 137-140°C.) dissolved in dry pyridine (8 cc.) was allowed to react with acetic anhydride (5 cc.) at 40°C. for 24 hours. The solvent was removed under reduced pressure on a water bath, the residue triturated with water (20 cc.), and on filtration gave the acetate (272 g.) m.p. 112-120°C. crystallised from dilute methanol (charcoal), m.p. 136-142°C., $[\alpha]_D^{23}$ + 3.1° (c

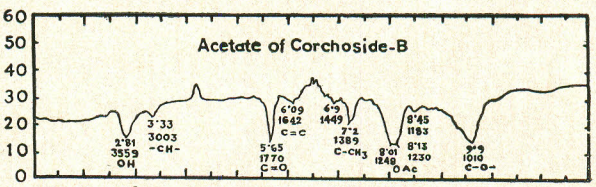

Fig. 4.—I.R. curve of acetate of corchoside B.

1.62 in methanol), γmax. (in KBr) 3610 (OH), 1786 (lactone), shoulder at 1742 (C=O), 1642 (C=C), 1399 (C-CH_3), 1252, 1229 (OAc), 1055 (C-O-) cm.$^{-1}$ (Fig. 4). It analysed for deca-acetate. Found: C, 55.87, H, 6.23, OAc, 30.92. $C_{61}H_{82}O_{31}$ requires: C, 55.89; H, 6.30; OAc, 32.8%.

Hydrolysis of Corchoside B.—A sample (0.1501 g., m.p. 138-140°C.) dissolved in methanol 10(cc.) when hydrolysed under reflux with aqueous sulphuric acid (0.1 N, 10 cc.) exactly in the same way as described for corchoside A, yielded a crystalline solid (0.065 g.), m.p. 160-165 °C., crystallised from ethanol m.p. 175-476°C., $[\alpha]\ ^{30}_{D} + 36.5°$ (c 1.2 in EtOH), λmax. 303 mμ and 217 mμ, gave a positive Legal[4] and Raymond[5] tests, and paper chromatographically identical and infrared superimposable upon an authentic strophanthidin, and mixed m.p. with a sample of strophanthidin of the same m.p. remained undepressed.

The aqueous mother liquor on working up gave a mixture of sugar in the form of syrup (0.07 g.). It readily reduces Fehling's solution and on paper chromatography gave four spots identical with raffinose, glucose, fructose, and galactose.

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