J. Indian Chem. Soc.,
Vol. L, August 1973, pp. 544-545

Structure of Gylcosides of Acacia Concinna D. C. Seeds

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Manuscript received 20 *October* 1972; *revised* 6 *June* 1973; *accepted* 20 *June* 1973

Re-examination of the seeds of Acacia concinna D.C. yielded two new saponins, acacinin A and acacinin B and a free sugar named as concinnin. Acacinin A on hydrolysis yields Acacic acid (3β, 16β, 21β-trihydroxy-olean-12-ene-18 α H-28-oic acid) and glucose, arabinose, xylose, fucose and rhamnose as sugar moieties, while on alkaline hydrolysis it yields a prosapogenin identical with acacinin B. The structure of acacinin A and B have been discussed.

THE Saponin from the seeds of *Acacia concinna* D.C. commonly known as "Shikakai" was earlier studied by Varshney and Sharma[1]. It is a trigly-coside of acacic acid (I)[2,3] containing glucose, arabinose and xylose as sugar moities. Present communication deals with the reinvestigation of the saponin of accacia concinna seeds which has been found to be a mixture of acacinin A, acacinin B and a free sugar concinnin.

The crude saponin obtained from the seeds of *Acacia concinna* D.C. was purified by chromatography on a column of silica gel and gave three compounds named as acacinin A, acacinin B and concinnin (a free sugar). The purity of acacinin A and acacinin B has been confirmed by TLC and Electrophoresis using borate buffer.

Acacinin A m.p. 170°–71° $[\alpha]_D^{33}$, $= -41{\cdot}2°$ on acetylation with pyridine and acetic anhydride in the cold gave an acetate m.p. 177°–78°. Acacinin A on 7% aq. sulphuric acid hydrolysis gave the genin found to be identical with acacic acid (I). The hydrolysate was neutralized with barium carbonate followed by deionization with Amberlite IRA 400 and IR 50 (H^+) ion exchange resins. The sugars thus obtained were identifie by TLC and paper chromatography as glucose, arabinose, xylose, fucose and rhamnose. Acacinin A on hydrolysis with 5% sodium hydroxide gave a prosapogenin identical with acacinin B in which the -COOH group is free indicating that acacinin A, has got two types of sugar linkages one attached to -COOH group and other attached to any of the three hydroxyl groups while acacinin B has one type of the linkage *i.e.*, attached to hydroxyl groups and the -COOH is free.

As the C-16 hydroxyl group is in hindered position, the attachment of sugar residue in this position is ruled out. Thus acacinin A should have a sugar linkage at C_{21} -OH and/or C_3 -OH, but normally the sugars are found to attached at C_3-OH group. Further work on the sequence and attachment of sugars is in progress.

Study of the free sugar concinnin :

The free sugar isolated from the Acacia concinna seeds was purified by column chromatography and gave a pure product m.p. 110°–114°, $(\alpha)_D^{33°} = +99{\cdot}4°$ on hydrolysis with 10% sulphuric acid it gave a mixture of three sugars galactose, glucose and fructose confirmed by co-chromatography with authentic samples by TLC and paper chromatography.

Experimental

Defatting : Well powdered seeds (200 g.) were exhaustively extracted with petroleum ether (60°–80°). The recovery of the solvent gave a greenish yellow oil.

Extraction : The defatted seed powder was exhaustively extracted with 95% enthanol. The extract after concentration gave a brown, syrupy mass. It was then successively extracted with petroleum ether,, carbon tetrachloride, chloroform and acetone to dissolve the impurities soluble in these solvents. The residue was dissolved in ethyl alcohol and precipitated by addition to a large quantity of ether/acetone. This process was repeated several times and finally treated with activated charcoal to give a colourless saponin powder (5 g.).

Purification of Saponin : The saponin powder thus obtained on TLC showed three spots (chloroform : methanol : water 65 : 35 : 10). These were separated by column chromatography on silica gel using different proportions of chloroform : methanol : water as eluent.

The fractions giving the same spots on TLC were combined and purified again to yield three substances.

Acacinin A (m.p. 170°–71°) $(\alpha)_D^{33°} = -41{\cdot}2°$, acacinin B and a free sugar concinnin (m.p. 110° 114°, $(\alpha)_D^{33°} = +99{\cdot}4°$).

TABLE 1

Length of column = 60 cm
Diameter of column = 2·5 cm
Weight of column = 150 g.
Weight of substance = 2·0 g.
Volume of each fraction = 20 ml

No.	Fraction	Eluent	Substance
1.	1–5	Chloroform : methanol 65 : 35	Acacinin B
2.	6–8	Chloroform : methanol : water 65 : 35 : 10	Mixture of acacinin B and acanin A.
3.	9–17	Chloroform : methanol : water 65 : 35 : 10	Acacinin A.
4.	18–20	65 : 35 : 10	Mixture of acacinin A and free sugar.
5.	21–25	40 : 50 : 10	Free Sugar.
6.	26–35	Methanol	Free Sugar.

Acetylation : The acacinin A on acetylation with acetic anhydride and pyridine in the cold in the usual manner gave an acetate m.p. 177°–78°.

Hydrolysis of Acacinin A : Acacinin A (500 mg) was dissolved in water (200 ml) and hydrolysed with sulphuric acid 7% by refluxing for 4 hr. The sapogenin which separated was filtered, washed and dried (215 mg).

Purification of Sapogenin : The sapogenin was refluxed with alcoholic potassium hydroxide (10%) for an hour and the volume was reduced to half. The solution was then diluted with water and extracted with ether. The aqueous layer was then acidified with hydrochloric acid which precipitated the acid genin. The precipitated acid genin was filtered, washed and dried.

Identification of sugars : The hydrolysate obtained by hydrolysis of Acacinin A was neutralised with freshly precipitated barium carbonate and deionised by {IRA-400 and IR-50 (H^+)} ion-exchange reins in the usual manner. The neutralised sugar solution obtained was concentrated under reduced pressure and on TLC as well as paper chromatography on Whatman Filter paper No. 1, showed five spots identified as glucose, arabinose, xylose, fucose and rhamnose by co-chromatography with authentic samples.

Alkaline hydrolysis of acacinin A : Acacinin A when hydrolysed with sodium hydroxide (5%) in 50% ethanol by refluxing for 4 hr on a water bath. This gave a prosapogenin and a oligosaccharide, the prosapogenin was found to be identical with acacinin B by TLC. The prosapogenin on hydrolysis with aqueous sulphuric acid 7% gave for sugars which were identified as glucose, arabinose, xylose, and rhamnose by co-chromatography with authentic samples.

Acacinin B : Acacinin B was hydrolysed with sulphuric acid 7% by refluxing for 2 hr and the sugars obtained were neutralised in the usual manner. These were chromatographed on Whatman filter paper No. 1 and were identified as glucose, arabinose, xylose and rhamnose by co-chromatography with authentic samples.

The prosapogenin obtained by alkaline hydrolysis of acacinin A was found to be identical with acacinin B. On sulphuric acid hydrolysis, it also gave the same four sugars as obtained from acacinin B.

Hydrolysis of Concinnin (free sugar) : Concinnin m.p. 110°–114°, $(\alpha)_D^{33} = +99{\cdot}4°$ (H_2O) was purified by column chromatography on silica gel and then hydrolysed with sulphuric acid (10%). The hydrolysate obtained was neutralised with freshly precipitated barium carbonate and deionised by amberlite IRA-400 and IR-50 (H^+) ion-exchange resins. The neutralised solution obtained was concentrated under reduced pressure and on paper chromatography showed the presence of three sugars which were identified as galactose, glucose and fructose.

Acknowledgement

Thanks are due to the University Grants Commission and the Council of Scientific and Industrial Research, New Delhi for the award of a junior research fellowship to two of the authors (G.H. and R.P.).

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