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Study of Saponins and Oligosaccharides from *Acacia concinna* D. C. Pods

I. P. VARSHNEY and RAJ PAL

Department of Chemistry, G. S. Institute of Technology and Science, Indore-452003

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Acacia concinna D. C. pods have been found to contain three saponins named as acacinin C, D and E and a homologous series of oligosaccharides. The oligosaccharides have been identified as sucrose, raffinose, stachyose and verbascose. The traces of glucose and fructose have also been noticed. The acacinin D is a glycoside of acacic acid with six sugars viz. glucose, arabinose, xylose, fucose, rhamnose and an unidentified sugar, present in the molar ratio of 2:1:3:2:3:2 as determined by GLC of their alditol acetates. The partial structure has been assigned to acacinin D.

ACACIA concinna D. C. commonly known as 'Shikakai' in Hindi is a member of family *Leguminoseae*, sub-family *Mimoseae*. The sapogenin from the seeds and pods of this plant has earlier been studied by Varshney *et al.*[1,2,3] and is a new triterpenic acid, acacic acid. Recently Varshney *et al.* [4,5,6] isolated two new saponins, acacinin A and acacinin B, a homologous series of oligosaccharides and two polysaccharides from the seeds. As no work seems to have been done on the saponin and free sugar contents of pods, therefore, this work was taken up.

The beans of this plant obtained from the local market after the removal of seeds were defatted and then exhausted with 80% ethanol and the ethanol extract on usual treatment gave a light yellow coloured powder which gave all the tests for saponin. On TlC it showed the presence of four spots. Therefore it was purified by column chromatography on silica gel (cf. Table 1). This gave three pure saponins which have been named as acacinin E (R_f* 0.180), acacinin D (R_f 0.30) and acacinin C (R_f 0.480) respectively. The fourth substance is a mixture of sugars.

TABLE 1

Weight of silica gel 150g Length of column 100cm
Weight of substance 3g Diameter of column 2.5 cm
Volume of each fraction 20ml

Sr. No.	Fraction	Eluant	Substance
1.	1-8	Chloroform-methanol-water (65 : 35 : 10)	Impurities
2.	9-19	—do—	Acacinin C
3.	20-31	—do—	Acacinin C and D
4.	32-45	—do—	Acacinin D
5.	46-57	—do—	Acacinin D and E
6.	58-71	—do—	Acacinin E with traces of D
7.	72-79	—do—	Mixture of free sugar and acacinin E and D
8.	80-95	80% of methanol	Free sugars

Study of Acacinin D: It was obtained from fractions (32-45) as light yellowish powder m.p. 216°-17°, acetate m.p. 162°-64°. Acacinin D on acid hydrolysis gave an acid genin identified as acacic acid (3β, 16β, 21β-trihydroxy olean-12-en-18 α-H, 28 oic acid) by TLC. m.p., and IR spectrum with authentic sample. The hydrolysate after neutralization showed the presence of only five sugars identified as glucose, arabinose, xylose, fucose and rhamnose by co-chromatography with the authentic samples. The sugar mixture after transformation into alditol acetates on GLC showed the presence of six sugars viz, Glucose, arabinose, xylose, fucose, rhamnose and an unidentified sugar present in the molar ratio of 2 : 1 : 3 : 2 : 3 : 2 (Fig. 1).

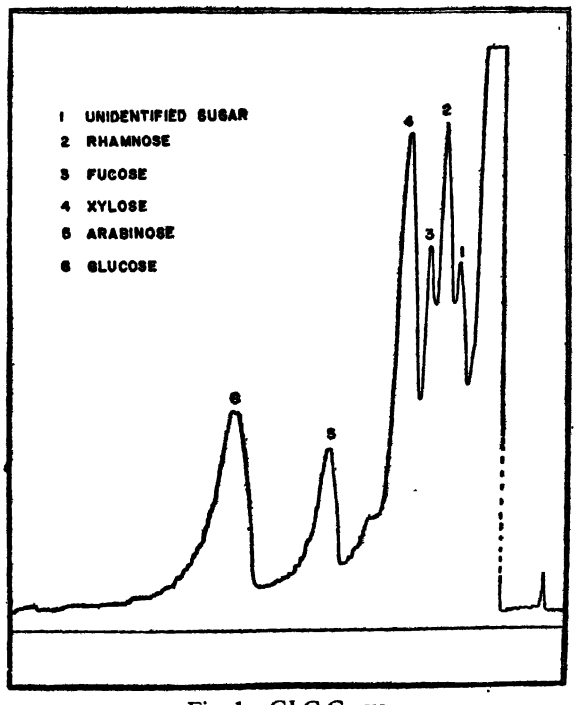
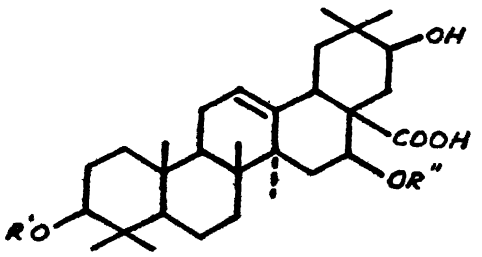


Fig. 1. GLC Curve

* R_f values were calculated on TLC using chloroform-methanol-water (65:35:10) as solvent.

The possibility of attachment of these sugars (13 moles) to the acacic acid may be at four places i. e. on C-3 OH, C-16 OH, C-21 OH, C-28 COOH. In order to find out the position of attachment of these sugars the acacinin D was treated with 5% methanolic sodium hydroxide. After the reaction the acacinin D was recovered unchanged indicating the absence of ester linkage i. e. no sugar or sugar chain is attached at C-28 COOH group. Further the IR spectrum of acacinin D showed no absorption in 1770 cm^{-1} region while the acacinin D acetate showed a sharp absorption at 1770 cm^{-1} indicating the presence of γ-lactone which must have been formed due to easy lactonisation of OH group at C-21 and COOH group at C-28 in the same manner as in case of acacic acid acetate[2]. This showed that no sugar or sugar chain was attached at C-21 OH and C-28 COOH groups. Now there remained two positions i. e. C-3 OH and C-16 OH groups for the attachment of these sugars, but all attempts in cleaving the sugars selectively from either of the hydroxyl groups failed. Therefore it was concluded that these sugars are attached on either or on both the hydroxyl groups.

On the basis of above evidences the following partial structure has been assigned for acacinin D (I).



(I)

R', R" = glucose:arabinose:xylose : fucose : rhamnose:unidentified sugar (2:1:3:2:3:2)

Study of free sugars : Fractions (80-95) on paper chromatography showed the presence of seven spots. Out of these, six were identified as verbascose, stachyose, raffinose. sucrose, glucose and fructose using similar procedure as described[6]. The presence of a homologous series of these oligosaccharides was confirmed by correlating the structure with papergram mobility[7]. When logarithm of partition function α' ($R_f/1\text{-}R_f$) for each different oligosaccharide (verbascose, stachyose, raffinose and sucrose) was plotted against molecular size (Fig. 2), a straight line was obtained. R_f values were calculated by running the paper (Whatman No. 1) in n-butanol:pyridine:water (6 : 4 : 3). After six descents, the sugars were located by spraying the paper with *p*-anisidine hydrochloride[8] and p-anisidine phosphate[9]. Stachyose, raffinose and sucrose were present nearly in equal amounts while verbascose, glucose and fructose were present in trace amounts. The seventh compound has not yet been identified completely but it gives all the tests for amino acid.

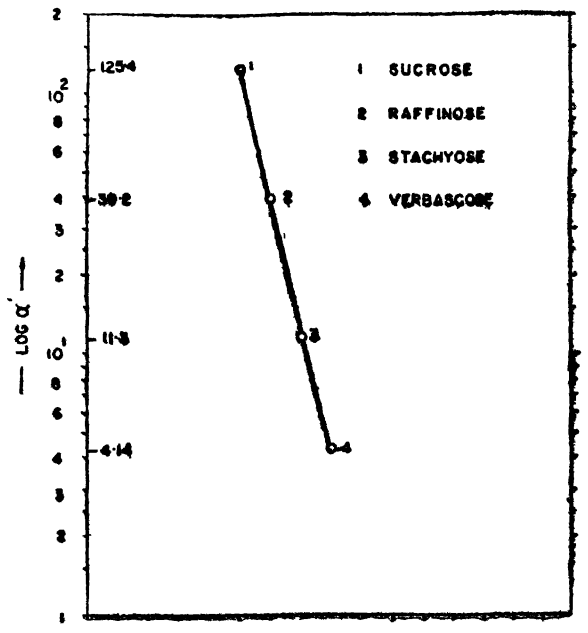


Fig. 2.

Experimental

Extraction : The well defatted pods (500 gm) were exhausted with ethanol (80%) and ethanol extract was concentrated and gave brownish gummy mass. It was extracted with various solvents, petroleum ether, ether, carbon tetrachloride, chloroform and acetone to remove the impurities soluble in these solvents and then dissolved in minimum quantity of methanol and was precipitated in large volumes of ether/acetone a number of times. This gave a light brown coloured powder (24 g) which gave all the tests for saponin.

Purification of the saponin : The saponin on silica gel TLC plates using chloroform:methanol:water (65 : 35 : 10) as solvent showed the presence of three brown spots and one big black spot on spraying with 5% aqueous sulphuric acid. These were separated by column chromatography on silica gel (cf. Table 1). The fractions giving the same spots on TLC were combined to yield three substances. Fractions (9-19) gave acacinin C and fractions (32-45) gave acacinin D. Fractions (58-71) after rechromatography gave acacinin E. Out of the three saponin acacinin D could be obtained in good quantity and therefore it was studied in detail.

Study of acacinin D : To ensure the purity, acacinin D was examined by electrophoresis on Whatman No. 1 paper using borate buffer (0.1*M*) at 400 V for 4 hr. The paper was dried and then sprayed with stannic chloride solution followed by heating at 100° for 5 min. It gave a single spot. It had m. p. 216°-17°.

Hydrolysis of acacinin D : Acacinin D (0.5 g) was suspended in 5 ml. of sulphuric acid 70% (w/v) for half an hr. at 5° and another half an hour at room temperature, it was then diluted to 2N sulphuric acid with distilled water and refluxed for 6 hr. The genin which separated was filtered, washed free of acid, dried and

purified by transforming into its sodium salt by refluxing with alcoholic sodium hydroxide (4.0%) for 4 hr. The sodium salt of the genin was then decomposed with hydrochloric acid. The free genin thus obtained was crystallised from methanol as colourless needles and identified as acacic acid by TLC, m. p., m. m. p and IR spectra.

Identification of sugars : The hydrolysate obtained after hydrolysis of acacinin D was neutralized with freshly precipitated barium carbonate and then deionised by passing through a column of ionexchange resins, Amberlite IRA 400 and Amberlite IR 120 (H^+). The sugar solution was then concentrated to a syrup under reduced pressure at 40°-50°. It was chromatographed on Whatman filter paper No. 1 in n-butanol:pyridine: water (6 : 4 : 3) alongside with authentic samples and showed the presence of five sugars identified as glucose, arabinose, xylose, fucose and rhamnose.

Gas-liquid chromatography of sugars : The mixture of the sugars (20 mg) was transformed into alditol acetates by reducing with sodium borohydride (40 mg) followed by acetylating with acetic anhydride (3 ml) and pyridine (3 ml). The mixture of alditol acetates was then injected into the column (3% ECNSS-M on chromosorb W) of GLC (Inj. temp. 200°, oven temp. 165° and FID temp. 198°) and gave six peaks corresponding to the alditol acetates of glucose, arabinose, xylose, fucose, rhamnose and an unidentified sugar respectively. From the GIC curve molar ratio of the sugars was determined as 2 : 1 : 3 : 2 : 3 : 2 (Fig. 1) using triangulation method.

Alkaline hydrolysis of acacinin D : Acacinin D (100 mg) was refluxed with methanolic sodium hydroxide (5%, 40 ml) for 6 hr. on water bath. The methanol was then evaporated on water bath by adding an equivalent amount of water and the product was extracted with n-butanol. The resulting product was identified by chromatography as acacinin D i. e. the starting material itself.

Acetylation of acacinin D : The dried acacinin D was dissolved in pyridine (10 ml) and acetic anhydride (10 ml) was added to it. It was then left for 16 hr. at room temperature. The reaction mixture was then poured drop by drop into iced water and the acetate formed was extracted with chloroform. The chloroform extract was washed with water and dried over anhydrous sodium sulphate and gave an acetate m. p. 162°-64°. The IR spectrum of acetate showed a sharp absorption at 1770 cm^{-1}.

Study of free sugars : Fractions (80-95) (cf. Table 1) were concentrated under reduced pressure to give a brown syrup. This was chromatographed on Whatman filter paper No. 1 in n-butanol:pyridine:water (6 : 4 : 3) and sprayed with *p*-anisidine hydrochloride showing the presence of seven spots ; out of these six were identified as verbascose, stachyose, raffinose, sucrose, glucose and fructose. The syrup was resolved into fractions by placing it in a narrow band near one edge of a number of Whatman filter paper No. 3 MM in n-butanol:pyridine:water (6 : 4 : 3). After 6 descents the strips were cut and eluted with 50% aqueous methanol using guide strips. It gave five fractions. Each fraction was crystallised from *n*-butanolacetic acid or 95% aqueous ethanol.

Fraction I had m. p. 220°, $[\alpha]_D$ +168°, R_f 0.039 and is verbascose (lit. m. p. 219°-20°). On hydrolysis it gave galactose, glucose, and fructose identified by co-chromatography with authentic samples.

Fractions II had m. p. 150°, $[\alpha]_D$ + 131°, R_f 0.102 and is stachyose (lit. m. p. 152°, $[\alpha]_D$ + 131.3°). This on hydrolysis gave galactose, glucose and fructose only. Fraction II on hydrolysis with invertase showed the presence of three sugars, two of them have been identified as manninotriose (O-α-D-Gal_p-(1→6)-O-α-D-Gal_p (1→6)-D-G_p) and fructose and the third which is present in traces could not be identified. The fraction II (stachyose) on methylation followed by hydrolysis gave four methylated sugars identified as 2, 3, 4, 6-tetra-O-methyl-D-fructofuranose (Rg 1.02), 2, 3, 4, 6-tetra-O-methyl-D-galactopyranose (Rg 0.88), 2,3,4-tri-O-methyl-D-glucopyranose (Rg 0.84) and 2, 3, 4-tri-O-methyl-D-galactopyranose (Rg 0.64)*.

Fraction III had m. p. 78°-80°, $[\alpha]_D$ + 104°, R_f 0.282 and is raffinose (lit. m. p. 80°, $[\alpha]_D$ + 105.2°). On hydrolysis this gave only galactose, glucose and fructose.

Fraction IV had m. p. 185°-87°, $[\alpha]_D$ + 66°, R_f 0.566 and is sucrose (lit. m. p. 184°-85°, $[\alpha]_D$ + 65.5°). On acetylation it gave an acetate m. p. 68°-70°, $[\alpha]_D$ + 59.6°. On hydrolysis it gave glucose and fructose only.

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* Rg values in case of methyl sugars refer to the movement relative to 2, 3, 4, 6-tetra-O-methyl-D-glucopyranose.

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