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Note

Studies on *Acacia nilotica* gum exudates. Structural variation due to different habitats

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Acacia trees of different varieties found in India produce gum exudates that are of potential economical interest. We present herein these varieties and compare some of their analytical characterization and properties with those of the most known compounds from African countries. Six samples of Indian gums (*Acacia nilotica*) were obtained from different locations in Maharashtra, Andhra Pradesh (AP), Madhya Pradesh (MP) and Uttar Pradesh (UP), and two samples of African gums from Sudan and Nigeria. The eigth samples were analyzed for optical rotation, component content, viscosity behavior, and ^{13}C-n.m.r. spectra. Some interesting and important observations were noticed in the sugar analysis of the different gums (Table I). Indian gums showed negligible amount of rhamnose, lower amount of uronic acid, and higher amount of arabinose than the gum from Sudan. For instance, rhamnose is present as a trace in the

TABLE I

Constituent sugar analysis of acacia gum exudates[a]

Sample	*Protein (%)*	*Constituent sugar (%)*			
		Rhamnose	*Arabinose*	*Galactose*	*Uronic acid*
1	1.8	2.0	53.5	36.6	7.9
2	2.1	2.4	45.9	30.6	14.1
3	2.5	3.4	49.4	33.7	12.4
4	2.2	[b]	65.7	24.2	8.2
5	1.6	[b]	62.6	23.1	13.2
6	1.3	[b]	53.6	32.7	11.9
7	2.0	13.5	33.0	37.0	16.0
8	1.9	2.7	44.5	34.1	14.3

[a] By g.l.c. [b] Trace.

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TABLE II

Preliminary analysis of acacia gum exudates

Sample	*Color*	*Total ash (%)*	*Acid insoluble ash (%)*	*Residue insoluble in acid (%)*	*Moisture (%)*	*Methoxyl (%)*	$[\alpha]_{589}^{20}$ *(degrees)*	$[\alpha]_{365}^{20}$ *(degrees)*
1	Light-brown	1.98	0.42	9.45	8.31	0.41	+41	+131
2	Yellowish brown	·2.31	0.61	11.88	6.20	0.46	+13	+56
3	Dark-yellow	2.11	0.63	13.55	7.52	0.47	+49	+162
4	Dark-brown	2.48	0.72	7.83	8.11	0.39	+73	+233
5	Brown	2.37	0.69	6.65	7.22	0.82	+75	+237
6	Brown	2.25	0.73	5.83	7.89	0.63	+81	+253
7	Light-yellow	1.49	0.22	7.14	8.4	0.81	−28	−74
8	Yellowish light-brown	1.53	0.31	7.37	6.79	0.83	+48	+158

U.P. gum samples, whereas it amounts to 2.0–3.4% in the other Indian gums. This value is still much lower than that of the Sudan gum which contains 13.5% of rhamnose, in agreement with the reported[1] value of 13%. Nigerian gum also contains a lower amount of rhamnose, *i.e.*, 2.7%. Indian gums contain almost twice as much arabinose as Sudan and Nigeria gums. One of the Indian gums (4) contains 66% of arabinose, whereas Sudan gum has only 33%. Otherwise, arabinose content varied from 46 to 66%. Another important difference between Indian and African gums was the proportion of uronic acid[2,3]. Indian gums have 8–14% of uronic acid, whereas Sudan and Nigerian gums showed values of 16 and 14.3%, respectively. A part of the uronic acid was characterized as 4-*O*-methylglucuronic acid by the formation of 4-*O*-methylglucose after reduction *via* the carbodiimide derivative according to Taylor and Conrad[4].

All gums, except the Sudan gum, showed positive optical rotations[1] (Table II). Sudan gum has $[\alpha]_D$ −28° and the Nigerian gum +48°; whereas the gums from U.P. (4–6) showed rotation from +73 to +81°. The sample from M.P. showed a rotation of +49°, that from Maharashtra of +41°, and that from Andhra +13°. Since all the gums showed the analytical features of arabinogalactans (Table I), the observed variations in optical rotation values must be indicative of differences in interglycosidic linkage configurations. This assumption was confirmed by the patterns of the anomeric signals in the ^{13}C-n.m.r. spectra. The signals (Fig. 1) were partially assigned according to literature data[3,5,6]. Comparison of the different spectra led to the following observations. All the Indian gums gave spectra that were distinct from the spectrum obtained for the *Acacia senegal* gum. They were in general more complex with many more signals, in particular in the regions around δ 100 (region of anomeric carbon atoms) and 60 (region

of free hydroxymethyl groups). On this basis, the gums could be divided into four structurally different types: *Acacia senegal* gum (**7**) was clearly distinct from the other gums. Gums **8** and **1** showed a high degree of similarity, and gums **4–6** have obvious common structural features, as expected from their origin. Gums **2** and **3** belong to different types.

Viscosity measurements[7] were carried out on solutions at different concentrations. Solutions at a 20% concentrati[illegible]ıot show significant differences in viscosity (20–30 cP), whereas among those at [illegible]ɔ concentration the Nigeria gum had the highest viscosity (400 cP) followed by the Sudan gum (350 cP) and the UP samples (~300 cP). The same trend was observed for the solutions at 50% concentration. When these were made alkaline to pH 10.0, all the samples showed an increase in viscosity by ~200 cP.

This investigation showed that nondegradative methods, such as ^{13}C-n.m.r. spectroscopy, provide good "fingerprints" of all the gums, which take into account both analytical and structural characteristics. It is clear that all the gums studied had typical differences. As suggested by the various optical rotations, some particular physicochemical characteristics differentiate the Indian gums from the African gums and may explain the lower viscosity at a 40% concentration. The aforementioned observations on the chemistry of Indian Acacia gums are in agreement with earlier observations on other acacia gums (*A. senegal, A. arabica,* and *A. nilotica*)[1,8]. Positive optical rotations have been reported for the last two-named gums[9] and also a viscosity lower than that of *A. senegal* gum.

EXPERIMENTAL

General methods. — Optical rotations were equilibrium values at 20° as recorded with a Perkin–Elmer Automatic Polarimeter. Viscosities were determined with a Brookfield Synchro-Lectric Viscometer Model RVT and LVT at 20 and 30 r.p.m., respectively, using spindles No. 1, 2, and 3. Descending paper chromatography was performed on Whatman No. 1 sheets with (*a*) 4:1:5 butanol–acetic acid–water and (*b*) 18:3:1:4 ethyl acetate–acetic acid–formic acid–water and acid aniline phthalate as spray reagent. G.l.c. was performed on a Hewlett–Packard 5890 instrument, equipped with a flame-ionization detector and a macrobore column (25 m × 0.53 mm) of SP 2380, using a temperature program at 200° for 2 min and then an increase of 1.5°/min up to 225°. Quantitative analysis was performed with a Hewlett–Packard 3380 A integrator. Ion-exchange resin Amberlite IR-120 (H^+) was used for de-ionization. All evaporations were carried out under reduced pressure at 40°.

Origin and purification of acacia gums. — The six gums from India [*Acacia nilotica* (L) Willd.ex Del.subsp. *indica* (Benth., also considered by Brenan as the exact synonymous of *Acacia arabia* (Lam) Willd.] and the two gums from Africa were numbered as follows: (**1**) *Acacia nilotica* gum exudate from Maharashtra; (**2**) *A. nilotica* gum exudate from Andhra Pradesh; (**3**) *A. nilotica* gum exudate from Madhya Pradesh; (**4**) *A. nilotica* gum exudate from Uttar Pradesh I (Banthra Research Station); (**5**) *A. nilotica*

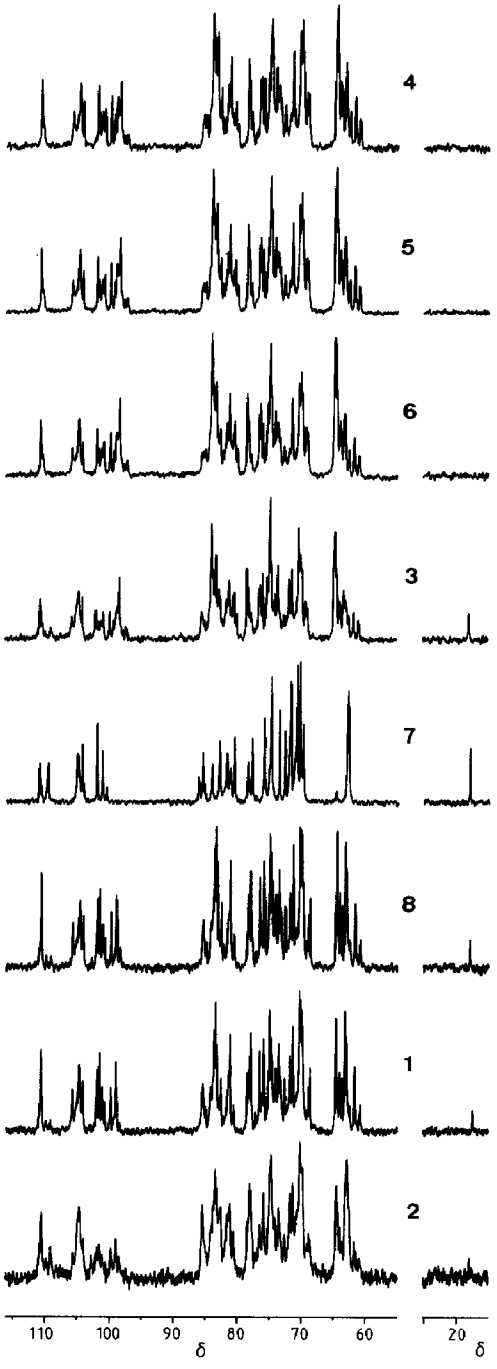

Fig. 1. Partial ^{13}C-n.m.r. spectra of *Acacia nilotica* gum exudates from: (**4**) Uttar Pradesh, (**5**) Uttar Pradesh, (**6**) Uttar Pradesh, and (**3**) Andhra Pradesh. Partial ^{33}C-n.m.r. spectra of gum arabic from: (**7**) *Acacia senegal* from Sudan, (**8**) *Acacia seyal* from Nigeria, (**1**) *Acacia nilotica* from Maharashtra and (**2**) *Acacia nilotica* from Madhya Pradesh.

gum exudate from Uttar Pradesh II (Kukrail Forest during winter); (**6**) *A. nilotica* gum exudate from Uttar Pradesh III (Kukrail Forest during summer); (**7**) gum arabic from *Acacia senegal* (Sudan); and (**8**) gum arabic from *Acacia seyal* (Nigeria).

The crude gums were extracted in a Soxhlet apparatus with ethanol for 8 h to remove resinous salts. The samples were powdered and dissolved in the minimum amount of water, and the solutions filtered through a sintered glass funnel under high vacuum and evaporated. The residual gums were again dissolved in water ($\sim$30% solution), precipitated with ethanol and acetone, and dried under vacuum at 40°.

Viscosity studies of acacia gums. — A weighted amount of gum was dissolved in the minimum quantity of water and methanol added to prevent lump formation. The suspension was transfered to a mixer with a measured quantity of water, diluted to the desired concentration, and agitated vigorously till the solution became homogeneous.

Total hydrolysis of acacia gums. — The gum (0.5 g) in 0.5M H_2SO_4 (75 ml) was boiled for 14 h. The acid was neutralized with $BaCO_3$, the suspension filtered, and the filtrate passed through a small column of ion-exchange resin and concentrated to a syrup. Paper chromatography of the hydrolyzate in solvents (*a*) and (*b*) revealed the presence of galactose, arabinose, rhamnose, and uronic acid. In some gum hydrolyzates, traces of glucose were also detected. The resulting monosaccharides were reduced with $NaBH_4$ and then acetylated to give alditol acetates that were subjected to g.l.c.

Carboxyl group reduction of uronic acids. — Uronic acids were determined colorimetrically with the 3-phenylphenol technique of Blumenkrantz and Asboe-Hansen[10] using D-glucuronic acid as the standard. Reduction of the uronic acid was performed by derivatization with 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-4-toluenesulfonate at pH 4.75, followed by reduction with $NaBH_4$ at pH 7.0 according to Taylor and Conrad[3].

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