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1. *Studies on Uronic Acid Materials. Part VI.* The Variation in Composition and Properties of Gum Nodules from* Acacia seyal *Del.*

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As a preliminary to studies of the chemical structure of *Acacia seyal* gum, individual nodules of authenticated origin have been examined as crude gum and after purification by (i) precipitation, (ii) electrodialysis, and (iii) ion-exchange. Chemical composition and physical behaviour vary from nodule to nodule. The low natural nitrogen content is not decreased by any of the purification methods used. Passage through a column of diethylaminoethylcellulose shows that the purified gum from individual nodules is heterogeneous, two components being present. The proportion of component A in different nodules varies from 34% to 41%; components A and B contain 12·5% and 15·3%, respectively, of glucuronic acid.

As a result of specific immunological reactions [1] and electrophoresis studies,[2,3] it is now accepted [4-6] that gum arabic (*Acacia senegal* syn. *verek*) is a mixture of polysaccharides of similar composition; [1] no single over-all formula has significance,[5] and only general features can be indicated.[4] Early studies [7] have been criticised [5,6,8] on the grounds that composite commercial samples, inadequately authenticated, were used. Although it had been suspected,[1,9] despite some evidence to the contrary,[10] that different samples of certain plant gums varied in chemical constitution, the possible range of variation was not known until single nodules of *Combretum leonense* gum were studied.[11] The results implied that fine-structural differences exist from nodule to nodule, so that alcoholic precipitation of bulk material from an aqueous solution of many nodules produces a complex mixture of closely similar polymeric systems. Whenever sample size permits, it is therefore desirable to assess the extent of inter-nodule variation and to make structural studies on the simplest form of the polymer available, *i.e.*, that given by a single nodule, which itself may be polymolecular and/or polydisperse (terminology as in ref. 12).

Before studying the chemical structure of the components of *A. seyal* gum, we have investigated the extent to which a number of authenticated nodules vary in properties, in composition, and in heterogeneity (cf. ref. 6, p. 54).

* Part V, Anderson, Garbutt, and Smith, *Talanta*, 1962, **9**, 689.

[1] Heidelberger, Adams, and Dische, *J. Amer. Chem. Soc.*, 1956, **78**, 2853.
[2] Joubert, *J. S. African Chem. Inst.*, 1954, **7**, 107.
[3] Lewis and Smith, *J. Amer. Chem. Soc.*, 1957, **79**, 3929.
[4] Hirst, 4th Internat. Congress of Biochemistry, Vienna, 1958.
[5] Whistler, "Industrial Gums," Academic Press, New York, 1959.
[6] Smith and Montgomery, "The Chemistry of Plant Gums and Mucilages," Reinhold Publ., Inc., New York, 1959.
[7] *E.g.*, Thomas and Murray, *J. Phys. Chem.*, 1928, **32**, 676.
[8] Butler and Cretcher, *J. Amer. Chem. Soc.*, 1931, **53**, 4160.
[9] Anderson and Sands, *Adv. Carbohydrate Chem.*, 1945, **1**, 329.
[10] Hirst and Jones, *J.*, 1938, 1174.
[11] Anderson, Hirst, and King, *Talanta*, 1959, **3**, 118.
[12] Greenwood and Mathieson, *Chem. and Ind.*, 1956, 191.

Experimental

Collection and Origin of Specimens.—We are grateful to Mr. P. Vidal-Hall, Gum Research Officer to the Sudan Government, who collected suitable gum nodules from the red-barked *A. seyal* Del. (a close variant, *A. seyal* var. *fistula*, has a grey bark). *A. seyal* is not normally " tapped," and the nodules originated from " natural exudation." The nodules, taken only from trees which could be authenticated, were packed individually and despatched in sealed tins. Nodules I—VI were collected at Umm Ruaba Forest Reserve, Eastern Kordofan, on March 9th, 1960; nodules VII and VIII from El Ain Forest Reserve, Central Kordofan (700 miles distant from Umm Ruaba), on January 9th, 1961. Sample IX was a representative bulk sample of first quality commercial " gum talh " (*A. seyal*). Nodules I—VIII ranged in weight from 40 to 80 g.; their colour varied from pale yellow to dark brown. Nodules I—IV, VII, and VIII were clear and glassy, of spherical shape. Nodules V and VI were elongated and had a characteristic glazed appearance, which, we have since observed, results when nodules plasticise slightly at 90—100°. It therefore appears that nodules V and VI had been subjected to more vigorous natural drying conditions than the others; it is unlikely that they were products of an earlier season, since *A. seyal* nodules (unlike *A. senegal*) become brittle through dehydration and fall from the branches within a few months.

Analytical Methods.—The standard methods,[11] were used, namely: paper partition chromatography; determination of sugars liberated on hydrolysis; autohydrolysis; electrophoresis; and viscosity experiments. The suspended-level dilution viscometer had a water flow-time of 218 sec. at 25°. Methoxyl contents were found by the vapour-phase infrared method,[13] which distinguishes yields of methyl iodide from other volatile products arising from solvent retention, decomposition, etc. Results were corrected for moisture content. Optical rotations were found for 1% aqueous solutions.

Studies on Crude Material.—The nodules, individually crushed to pass a 30-mesh sieve, gave the results shown in Table 1.

Table 1.

Determinations on crude samples.

	I	II	III	IV	V	VI	VII	VIII	IX
Moisture (%)	13·5	13·6	13·3	14·3	11·0	11·0	16·1	15·9	11·4
	13·6	13·6	13·2	14·4	11·0	11·2	16·0	16·0	11·4
Ash (%)	3·42	2·81	2·89	3·31	2·04	1·94	2·90	2·70	3·55
	3·38	2·98	2·94	3·33	2·10	2·10	2·94	2·80	3·61
Nitrogen (%)	0·14	0·09	0·14	0·12	0·09	0·10	0·18	0·17	0·19
	0·15	0·10	0·14	0·13	0·10	0·10	0·19	0·18	0·19
Uronic anhydride (%)	12·4	12·2	12·1	11·2	9·0	9·2	16·4	12·1	11·6
	12·7	12·4	12·0	11·4	9·1	9·2	16·8	11·9	11·9
Methoxyl (%)	n.d.	n.d.	0·60	0·72	1·36	1·53	1·00	0·82	0·55
Limiting flow-time no.	8·7	8·7	12·7	14·7	8·2	9·8	15·3	19·0	15·6
$[\alpha]_D^{20}$	+52°	n.d.	+56°	+51°	n.d.	+48°	n.d.	+50°	+44°

n.d. = Not determined.

Autohydrolysis at 85—90° of 1% solutions of nodules III, V, and VIII gave arabinose, together with traces of galactose and an oligosaccharide. As was observed for *C. leonense* gum,[11] the increase in reducing power (see Fig. 1) varies from nodule to nodule. The acidity of the autohydrolysis solutions (pH 4·6) did not increase appreciably with time of heating (cf. *C. leonense*,[11] which had an appreciable acetyl content) and decomposition of the liberated sugars was not extensive. The viscosity of the solutions fell rapidly during autohydrolysis.

Purification of Crude Gum.—A portion of each crushed nodule was shaken with cold distilled water, to give a 2% solution, which was filtered through acid-hardened filter-paper. The solutions were acidified (0·1N in hydrochloric acid); addition of acetone (4 volumes) gave a white curdy precipitate which was removed by centrifugation. Further precipitation did not occur when the clear supernatant liquid was poured into acetone. This purification process was carried out a further 3 times: the purified gum was then dialysed against distilled water and freeze-dried.

[13] Anderson and Duncan, *Talanta*, 1961, **8**, 241.

TABLE 2.

Determinations on samples purified by precipitation.

	I	II	III	IV	V	VI	VII	VIII	IX
Ash (%)	2·38	1·81	2·71	2·49	0·91	n.d.	n.d.	n.d.	2·48
	2·41	1·82	2·78	2·51	0·92				2·52
Nitrogen (%)	0·14	0·09	0·13	0·16	0·07	0·10	0·18	0·17	0·20
	0·15	0·10	0·13	0·17	0·07	0·10	0·19	0·18	0·21
Uronic anhydride (%)	13·1	12·8	12·5	12·8	10·4	10·9	16·6	12·9	12·4
	12·9	12·9	12·7	12·6	10·6	10·8	16·4	12·7	12·6
Methoxyl (%)	n.d.	1·1	1·3	0·70	1·1	n.d.	n.d.	1·0	0·94
Limiting flow-time no.	11·4	9·1	12·0	12·8	7·4	11·6	13·8	17·4	13·2
$[\alpha]_D^{20}$	+58°	n.d.	+59°	n.d.	n.d.	n.d.	n.d.	+64°	n.d.

Studies on Samples Purified by Precipitation.—The results obtained are compared in Table 2. For the determinations of the limiting flow-time number, 4% saline was found to give adequate suppression of the electroviscous effect. Although the uronic anhydride content of each

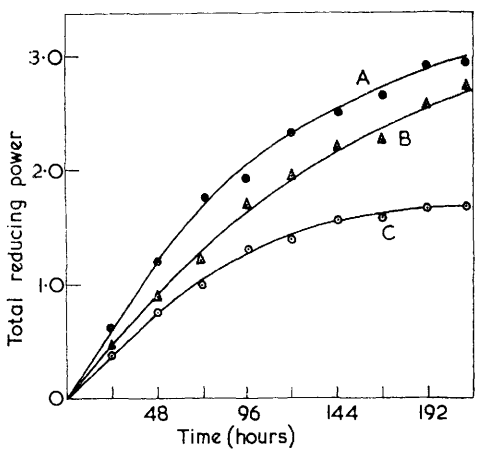

FIG. 1. Increase of reducing-power on autohydrolysis of (A) nodule (VII), (B) nodule III, and (C) nodule V. Reducing power is expressed as mg. of arabinose per 2 ml. of 1% solutions.

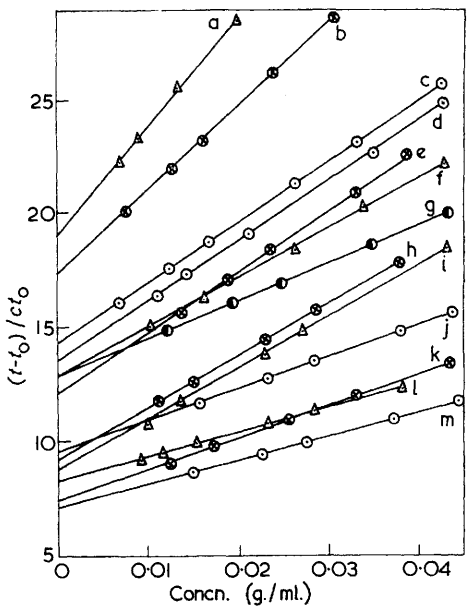

FIG. 2. Viscosity plots (in 4% aq. NaCl) of crude and purified samples. (a) VIII, crude. (b) VIII, pptd. (c) VIII, electrodialysed. (d) III, electrodialysed. (e) III, pptd. (f) III, crude. (g) III, ion-exchange. (h) II, pptd. (i) III, crude. (j) II, electrodialysed. (k) V, pptd. (l) V, crude. (m) V, electrodialysed.

sample was greater than that in the crude gum, indicating the elimination of some 5—10% of non-uronide contaminants, the precipitation processes had not reduced the nitrogen content, and the ash contents were not significantly reduced. Other purification methods were therefore investigated. It is well-known that bi- and ter-valent ions can cause gel-formation and cross-linking; [5] ash-free samples are therefore required for some analyses (cf. ref. 12).

Purification by Electrodialysis.—A portion of each crushed nodule was electrodialysed [7] (as 2% aqueous solution), ion-exchange membranes being used.[14] Cooling coils in the electrodialysis compartments prevented the temperature of the gum from rising above 30°. Trial experiments (on sample IX) showed that electrodialysis for 6 hr. (cf. ref. 7) was required to

[14] Anderson and Wylam, *Chem. and Ind.*, 1956, 191.

achieve the low ash values shown in Table 3. Since ash determinations at the 0·01% level consume relatively large amounts of material, determinations were not made on all samples.

After electrodialysis for several hours, the gum solutions separated into a clear, colourless upper layer and a viscous, slightly coloured lower layer. The upper layer was removed by pipette and found to contain only traces of gum. This effect may be worthy of further examination, since disintegration of a complex coacervate may be involved (cf. ref. 5).

Studies on Electrodialysed Samples.—The results obtained are compared in Table 3. Although the ash content had been effectively reduced, no elimination of nitrogen was achieved.

TABLE 3.

Determinations on electrodialysed samples.

	I	II	III	IV	V	VI	VII	VIII	IX
Ash (%)	0·02	0·01	n.d.	n.d.	0·02	0·02	0·03	n.d.	0·05
	0·02	0·02			0·03	0·03	0·04		0·05
Nitrogen (%)	0·15	0·11	0·16	0·17	0·10	0·11	0·19	0·17	0·17
	0·16	0·11	0·16	0·17	0·10	0·10	0·19	0·18	0·17
Uronic anhydride (%)	13·6	13·0	13·5	13·8	12·1	12·5	16·8	13·4	13·8
	13·7	13·3	13·6	13·6	12·2	12·3	16·6	13·5	13·8
Limiting flow-time no.	12·0	9·5	13·5	n.d.	7·0	12·4	13·6	14·2	n.d.

Potentiometric titrations showed that the ash-free gum behaved as a strong acid [7] (pH of a 1% aqueous solution = 2·9), and the values obtained for the neutralisation equivalent indicated that all the acidity arose from the uronic acid groups (*e.g.*, Found, for sample VIII: Neut. equiv., 1340; uronic anhydride = 13·5%. Required; Neut. equiv., 1300 if all acidity is due to uronic acid groups).

Purification by Ion-exchange.—A dilute aqueous solution of nodule III was filtered, then de-ionised [15] by passage through a column of the cation-exchange resin " ZeoKarb 225." Analysis of the freeze-dried eluate gave: ash 2·4%, nitrogen 0·14%, uronic anhydride 13·5%, $[\alpha]_D$ +59°. Viscosity determinations gave the plot shown in Fig. 2g. This ion-exchange method was not applied to the other samples since the purification achieved did not approach that given by electrodialysis.

Comparison of the Viscosity Behaviour of Samples before and after Purification.—Samples were examined carefully to assess the extent of inter-nodule variation and the effect on each nodule of the various purification procedures. The viscosity plots for the crude and the purified samples of nodules II, III, V, and VIII are shown in Fig. 2; these curves are typical and represent the range of behaviour observed.

Fractionation Experiments on Aqueous Solutions of the Gum.—(1) *Chemical precipitation methods.* No useful fractionation resulted from (*a*) graded addition of ethanol, (*b*) addition of iodine–potassium iodide reagent (cf. ref. 16), or (*c*) addition of cetyltrimethylammonium bromide [17] at pH 4, 7, or 9.

(2) *Electrophoresis.* Several experiments were made with glass-fibre paper in 2M-sodium hydroxide at 1000 v for 6—18 hr. Movements of several cm. resulted, but there was no distinct separation of components (cf. ref. 3).

(3) *Chromatography on diethylaminoethylcellulose.*[18] A solution of sample I (electrodialysed, 360 mg.) was treated on a column (40 × 5 mm.) of diethylaminoethylcellulose; gradient elution with phosphate buffer (pH 4·6, 0·05M ⟶ 0·25M) was used, followed by gradient elution with aqueous sodium hydroxide (0·1M ⟶ 0·5M). The average flow-rate was ~40 ml. per hr. Fractions (40 ml.) were screened by the phenol method.[19] Fig. 3 shows the elution pattern observed. The total recovery from the column was 331 mg.: component A (117 mg., 35%) and component B (214 mg., 65%) contained 12·5% and 15·4% of uronic anhydride, respectively.

Sample II (electrodialysed, 220 mg.) gave an elution pattern similar to that shown in Fig. 3. Component A (66 mg., 34%) and component B (126 mg., 66%) had uronic anhydride contents of 12·4% and 14·9%, respectively.

[15] Hamilton, Spriesterbach, and Smith, *J. Amer. Chem. Soc.*, 1957, **79**, 443.
[16] Whistler and Gaillard, *Arch. Biochem. Biophys.*, 1961, **93**, 332.
[17] Scott, *Chem. and Ind.*, 1955, 168.
[18] Neukom, Heri, Kundig, and Deuel, *Helv. Chim. Acta*, 1960, **43**, 64.
[19] Dubois, Gilles, Hamilton, Rebers, and Smith, *Analyt. Chem.*, 1956, **28**, 350.

Sample III (electrodialysed, 460 mg.) similarly gave 180 mg. (41%) of component A (uronic anhydride, 12·6%) and 258 mg. (59%) of component B (uronic anhydride, 15·2%).

Hydrolysis: Percentages of Sugars Present.—Treatment with 2N-sulphuric acid at 90—95° for 8 hr. completely hydrolysed the gum. Sample IX gave galactose 38%, arabinose 46%, rhamnose 3%, and glucuronic acid 12·5% (expressed as approximate percentages; cf. ref. 20). The method of determining the sugar ratios involves the separate stages of hydrolysis, neutralisation, reduction in volume, chromatographic separation, elution, and estimation of reducing

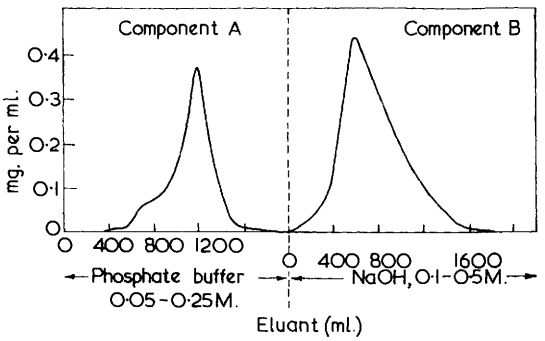

FIG. 3. Elution pattern from fractionation of a single nodule on diethylaminoethylcellulose. The broken line denotes change of solvent.

power: it is considered that the results cannot be more accurate than ±5—10% of the actual percentage present.

For samples I—VIII, however, the results, particularly for the rhamnose content, varied by amounts which are considered to be outside the possible experimental error. The two most widely differing nodules were samples V and VII. Sample V gave glucuronic acid 11%, galactose 42%, arabinose 47% and rhamnose 1%: sample VII gave glucuronic acid 16%, galactose 34%, arabinose 42%, and rhamnose 8%.

DISCUSSION

The gum from *A. seyal* is similar to the *Acacia* gums previously studied in containing glucuronic acid, galactose, arabinose, and rhamnose. The presence of acid-labile residues and the marked decrease in viscosity detected on mild hydrolysis indicate that *A. seyal* probably further resembles other *Acacia* gums in having a main chain, resistant to hydrolysis, to which is attached acid-labile side-chains. Of the *Acacia* gums studied to date, all have given negative optical rotations with the exception of *A. karroo*,[20] to which must now be added *A. seyal*. The methoxyl content of the *A. seyal* nodules examined varied from 0·5% to 1·5%; only *A. mollissima* [21] has previously been reported to have a methoxyl content (0·35%). A methoxyl content of 1% has been found [22] to be significant in *Khaya grandifolia* gum.

The results presented in Tables 1—3 indicate that the inter-nodule variation in composition is greater than can be explained on the basis of possible analytical error. The variation is similar in extent to that previously found [11] for nodules of C. *leonense* gum.

The nodules examined were collected and authenticated by an expert on the identification of *Acacia* species. It may otherwise have been suggested that nodules V and VI (from their appearance), nodule VII (uronic acid content), and nodule VIII (viscosity) originated from some species other than *A. seyal*. However, the data for each nodule, taken as a whole, leave little basis for doubting the authenticity of the samples. Taken

[20] Charlson, Nunn, and Stephen, *J.*, 1955, 1428.
[21] Stephen, *J.*, 1951, 646.
[22] Aspinall, Hirst, and Mathieson, *J.*, 1956, 989.

jointly, the nitrogen content and the optical rotation of an *Acacia* gum are strongly indicative of its species: preliminary studies of other Sudanese *Acacia* species such as *A. arabica*, *A. laeta*, *A. dealbata*, *A. drepanolobium*, and *A. campylacantha* (which have not been studied previously) have shown that the nitrogen content of *A. seyal* is characteristically low, and, moreover, is not reduced by any of the methods of purification used. The mechanism of gum formation is still far from clear,[6] and further knowledge of the nature of the nitrogen content in plant gums would be of value in assessing the relative importance of the enzymic polymerisation theory [5] in relation to the alternative theories [6,23] that gum formation results from (*a*) normal plant metabolism or (*b*) pathological reactions to resist invading micro-organisms or to avoid loss of moisture.[5]

Although it has been reported that the ash content of some species of gum can be eliminated [10,22] by precipitation methods, our experiments with *Acacia* species have shown that their ash content cannot be reduced by more than about 50%, even after 4 re-precipitations. The results reported for *A. seyal* are typical in this respect. Electrodialysis is the most effective method of reducing the ash content to a very low value; as shown in Fig. 2, the most viscous nodule (VIII) showed a marked decrease in viscosity on purification, although the other nodules were not affected to a comparable extent. In general, the purification methods studied do not appear to alter significantly the physical properties of the gum.

Fractionation of *A. seyal* gum on diethylaminoethylcellulose gave two components having uronic anhydride contents of 12·5% and 15·3%, respectively; the close similarity of the elution patterns suggested that different nodules contained the same two components in slightly varying proportions. Conclusive evidence of heterogeneity is often difficult to achieve. Indeed, conflicting results may be given by different techniques; trypsin is electrophoretically heterogeneous, although only one component was evident on examination by the ultracentrifuge.[24] For gum arabic,[1] chemical fractionation has been less successful than immunochemical experiments. Our failure to separate the components of *A. seyal* by electrophoresis (cf. ref. 3) may therefore be explained by the fact that, in single nodules, the two components do not differ sufficiently in uronic acid content, upon which electrophoretic movement must depend to a large extent.[25] Studies of the chemical structure of the two components of this gum are in progress.

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Edinburgh, 9. [*Received, March 9th*, 1962.]

[23] Jones and Smith, *Adv. Carbohydrate Chem.*, 1949, **4**, 243.
[24] Perrone, Disitzer, and Dormont, *Nature*, 1959, **183**, 605.
[25] Cf. Colvin, Cook, and Adams, *Canad. J. Chem.*, 1952, **30**, 603.