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COMPOSITION OF GUM EXUDATES FROM *ANACARDIUM OCCIDENTALE**

DOUGLAS M. W. ANDERSON, PHILIP C. BELL and JOHN R. A. MILLAR
Chemistry Department, The University, Edinburgh EH9 3JJ

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Abstract—The composition and solution properties of Indian and Papuan specimens of the gum from *Anacardium occidentale* have been studied and found to be closely similar. Contrary to earlier reports by Indian workers, this gum does not contain galacturonic acid. It does, however, contain glucose; this appears to be the first report of the presence of this sugar in a plant gum exudate. A freeze-dried sample of the gum was examined at intervals over a period of 2 months; its weight-average MW increased by a factor of three in that time, and molecular-sieve chromatography showed that self-association occurred with the formation of a small proportion of a very high MW component.

INTRODUCTION

RECENT STUDIES[2-4] of gum exudates from some species of the genus *Lannea* indicated that they could be of some commercial interest; studies have therefore been extended to other genera within the family Anacardiaceae. This communication reports the analytical data obtained for two specimens of the gum exudate from *Anacardium occidentale*, obtained from widely different geographical locations. This tree, known commonly as the Cashew tree and a native of tropical America, is widely cultivated and naturalized throughout the tropics, chiefly in coastal districts. Cashew nut shell polysaccharides have been investigated.[5,6]

The gum from *A. occidentale* was found[7] to have a positive rotation and to contain[7,8] arabinose, galactose and rhamnose; later it was also reported[9] to contain xylose. In 1966, Biswas and Bose[10] published the result of a preliminary investigation of the gum; they found no nitrogen or methoxyl content, but much galactose, a very low pentosan content (arabinose), traces of rhamnose, and galacturonic acid. Subsequently, the same workers published[11] essentially the same data, augmented by an extensive study of the structure

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of an aldobiouronic acid which they identified as 6-*O*-(D-galactopyranosyluronic acid)-D-galactopyranose. Their paper also cited evidence from precipitin reactions with specific antisera, carried out by Professor Heidelberger in New York; these indicated, in marked contrast, that the gum carried D-glucuronic acid or its 4-*O*-methyl derivative as an end-group, rather than galacturonic acid. However, this evidence was confused at the time by the anomalous precipitin reaction given in this respect by gum karaya; we now show that Professor Heidelberger's evidence for the absence of galacturonic acid was correct.

RESULTS AND DISCUSSION

The data presented in Table 1 show that the Indian and Papuan specimens of gum are remarkably similar. The results are in broad agreement with those of some earlier work which showed that *A. occidentale* gum had a positive rotation[7] (+18°),[10] and a high neutralization equivalent (5630)[10] and hence a low uronic acid content (4·3%).[11] The presence of small amounts of rhamnose[8,10] is confirmed, but traces of xylose[9] occur in the Indian specimen only. In addition, traces of mannose were detected together with small but significant amounts of glucose.

TABLE 1. ANALYTICAL DATA FOR TWO SPECIMENS OF THE GUM FROM *Anacardium occidentale*

	Specimen 1 (Indian)	Specimen 2 (Papuan)
Moisture (%)	9·5	7·9
Ash (%)*	1·3	1·1
N (%)*	0·45	0·16
Hence protein (%) (N × 6·25)*	2·8	1·0
MeO (%)†	0·31	0·21
$[\alpha]_D$ in H_2O (degrees)†	+24·2	+23·6
$[\alpha]_D$ in 7 M urea (degrees)†	+26·5	n.d.
Intrinsic viscosity $[\eta]$ (ml g^{-1})*‖	6·3	9·4
MW (MW × 10^4)*	26	18
Equivalent weight†	2814	3089
Hence uronic anhydride (%)†‡	6.2	5.7
Sugar composition† after hydrolysis (%)		
4-*O*-Methylglucuronic acid§	1·9	1·2
Glucuronic acid	4·3	4·5
Galactose	61	63
Arabinose	14	15
Rhamnose	7	7
Xylose	2	—
Mannose	2	1
Glucose	8	9

* Corrected for moisture content.
† Corrected for moisture and protein content.
‡ If all acidity arises from uronic acids.
§ If all methoxyl groups located in this acid.
‖ In M-NaCl.

This is, so far as we are aware, the first report of the presence of glucose in a plant gum exudate. After initial detection on chromatograms by means of a specific glucose oxidase spray, five separate gum nodules were carefully cleaned and washed to remove all traces

of bark and other surface contamination. They were then analysed separately and each was found to give glucose after hydrolysis. The complete separation of glucose from galactose by chromatography is difficult; an enriched glucose fraction containing some galactose was obtained in which the presence of glucose was demonstrated by (a) X-ray diffraction, (b) specific reaction with glucose oxidase, (c) m.p. and specific rotation evidence that corresponded to the values given by a synthetic glucose/galactose mixture. Final confirmation was obtained during a structural study[12] of the gum, when hydrolysis of the methylated gum gave isolatable amounts of 2,3,4,6-tetra-*O*-methyl-D-glucose. Tests were also made to ascertain that the glucose did not arise as a degradation artifact. A sugar mixture containing the proportions of galactose, arabinose, xylose, mannose, rhamnose and glucuronic acid found in the gum was prepared; after hydrolysis and chromatographic analysis under the same conditions as used for the gum, treatment with the glucose oxidase spray gave a negative result.

Although our results agree with those of earlier workers in the respects mentioned above, there are several ways in which we consider the previously published data to be incorrect. The gum contains easily detectable amounts of nitrogen and methoxyl groups (both earlier stated[10,11] to be absent), and the galactose/arabinose ratio reported[11] (17/1) appears to be much too high. Even if allowance is made for the fact that incomplete resolution of the glucose would increase the proportion of galactose found, it does not appear possible for the galactose/arabinose ratio to be greater than about 5:1 which is, nevertheless, a high value; the highest value found[13] so far is *ca* 7:1.

More important, however, is the incorrect identification[10,11] of galacturonic acid, despite the strong evidence from Heidelberger's sensitive and specific precipitin reactions[11] in support of the presence of glucuronic acid and the absence of galacturonic acid. *A. occidentale* gum contains glucuronic acid and, in smaller amounts, its 4-*O*-methyl derivative; the aldobiouronic acids involved have been investigated and shown[12] to be 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose (major component) and 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose. It is of interest that the gums from *Lannea* species (Anacardiaceae) contain galacturonic acid[2,3,4] in addition to glucuronic acid and its 4-*O*-methyl derivative. An aldobiouronic acid containing galacturonic acid is not easily separated[3] from the major aldobiouronic acid identified in *A. occidentale* gum, and, moreover, since this gum contains only a relatively small proportion of acidic residues, the identification of a component containing galacturonic acid could be difficult experimentally. We conclude, however, on the basis of careful tests with the present samples, that the gum from *A. occidentale* does not contain galacturonic acid, and that the combination of galacturonic acid, glucuronic acid and 4-*O*-methylglucuronic acid found in gums from the genus *Lannea* is not a general feature of the family *Anacardiaceae*.

As a consequence of the testing of individual gum nodules for the presence of glucose, several freeze-dried specimens of the gum were isolated. As a check on the accuracy of some of the experimental results found initially, the MW of some samples was re-determined, and found to be considerably higher than had been the case several weeks earlier.

This curious behaviour had been encountered on a number of previous occasions with other gum samples; after storage for several months, the intrinsic viscosity of two specimens of *Acacia senegal* gum increased[14] by *ca* 50% and molecular-sieve chromat-

[12] ANDERSON, D. M. W. and BELL, P. C. In preparation.
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ography showed[15] that high MW components developed during the storage of freeze-dried *Acacia* gums. The opportunity was therefore taken to investigate the behaviour of *A. occidentale* gum in this respect.

Samples of the gum from five separate nodules of the gum were dyed[16,17] with Procion red M-2B; after clean-up by passage through a column of Sephadex G15 to remove excess of the dyestuff, a portion of each solution was examined on a Bio-gel A-15M column. In each case, a single peak was given within the molecular-sieve range of the column (elution volume about 56 ml). The MW was also determined. After freeze-drying, the gum samples were examined immediately on the Bio-gel A-15M column, and again single peaks were recorded. As little as 2 days later, however, a small peak corresponding to material of very high MW was given at the void volume of the column (24·5 ml), and this peak continued to increase over a period of 2 months, during which time the MW, determined by light scattering, increased from $1{\cdot}53 \times 10^5$ to $5{\cdot}0 \times 10^5$. It is clear, therefore, that the self-aggregation of freeze-dried gum samples is not confined to *Acacia* species and, moreover, occurs in samples of low uronic acid content.

EXPERIMENTAL

Origin of specimens. Specimen 1 was collected on 13 October 1969, by Mr T. P. Baskaradoss, Forest Utilization Officer, Forests Department, Tamilnadu, Madras, India. Specimen 2 was collected by Mr S. J. Colwell, Forest Products Research Centre, Boroko, Papua, after wounding the bark and sapwood of a tree at Laloki Agricultural Quarantine Station, Port Moresby, T.P.N.G., on 4 June 1971.

Preparation of samples for analysis. Both gum specimens dissolved completely in cold H_2O within 2 days to give *ca* 10% solns. After filtration through Whatman No. 41, 1 and finally No. 42 papers, the solns were dialyzed against H_2O for 24 hr, and against dist. H_2O for 2×24 hr. The solns were again filtered through No. 42 papers, then freeze-dried: the recovery of gum on a dry wt basis was *ca* 80% for both specimens.

Analytical methods. The standard analytical methods used have been described in earlier parts of this series.[18] The organic phase of the following chromatographic solvent systems were used: (a) C_6H_6–BuOH–C_5H_5N–H_2O (1:5:3:3); (b) HOAc–EtOAc–HCOOH–H_2O (3:18:1:4); (c) HOAc–EtOAc–HCOOH–H_2O (8:18:3:9); (d) BuOH–EtOH–0·1 M HCl (1:10:5).

Sugar analysis. Each specimen (50 mg) was hydrolyzed with N-H_2SO_4 for 7·5 hr at 100°. The solns were neutralized, deionized and concentrated. PC in solvents (a) and (b) indicated that four main sugar components were detectable, corresponding to galactose, arabinose, rhamnose and glucose. Small amounts of mannose were also observed in both specimens on chromatograms run in solvent (b); specimen 1 was also found to contain trace amounts of xylose. Another chromatogram, run in solvent (b) was sprayed with glucose oxidase: a purple/green spot was obtained for both *Anacardium* specimens as well as for a glucose standard. Chromatograms run in solvent (b) and solvent (c) indicated the presence of one main aldobiouronic acid [R_{gal} 0·28 solvent (b); 0·61 solvent (c)] and traces of another component [R_{gal} 0·62 solvent (b); 0·83 solvent (c)]. These acids have been previously characterized.[12] Each specimen (50 mg) was hydrolyzed with 2 N-H_2SO_4 for 7·5 hr at 100°. After neutralization, deionization and concentration, PC of the hydrolysates in solvent (d) indicated the neutral sugars found previously, together with a brown spot, (R_{gal} 0·75). The presence of D-glucuronic acid was carefully checked by running the 2 N-hydrolysate in solvent (d) against standard glucuronic acid (R_{gal} 0·75, brown spot) and galacturonic acid (R_{gal} 0·64, red-brown spot); the distinction was unambiguous, both in terms of the R_{gal} value and spot colour. The neutral sugar ratios were determined by running each hydrolysate in solvents (a) and (b). Solvent (a) allowed the calculation (R_{gal} values shown in brackets) of the ratios of galactose: glucose (R_{gal} 1·21); arabinose + mannose (1·42); xylose (1·73); rhamnose (2·25). Solvent (b) gave the ratios of galactose + glucose (R_{gal} 1·15); mannose (1·27); arabinose + xylose (1·45); rhamnose (2·07). By combination of these sets of ratios, the overall composition of the gum was calculated.

Extraction and identification of glucose. Purified gum (4·46 g, dry wt) was hydrolyzed with 1 N-H_2SO_4 (250 ml) for 8 hr at 100°. After cooling, the soln was neutralized with $BaCO_3$, filtered, deionized with Amberlite IR (120) H^+ resin, concentrated to a syrup (1–2 ml), and chromatographed on Whatman 3MM papers for 24 hr in solvent (a). Side-strips were cut and developed using a glucose oxidase spray, and the located glucose was eluted from the chromatograms. After conc to a syrup (1 ml), the glucose soln was re-chromatographed in solvent

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(a) for 36 hr; after location as before, the glucose was eluted and concentrated to a syrup (0·5–1·0 ml). Chromatography was then carried out in solvent (b) for 36 hr. The glucose was located and eluted as before, the soln conc to a thick syrup and EtOH (3 ml) added. An off-white ppt formed at this stage. The EtOH soln was refluxed at 60° for 30 min and cooled. The product was filtered and dried at 60°, giving a pale brown amorphous solid (61 mg). Some of the solid was dissolved in a drop of H_2O and chromatographed in solvent (b) for 24 hr. Development with aniline oxalate gave one large spot (R_{gal} 1·09) which gave a positive reaction with glucose oxidase. Since it is difficult to separate glucose completely from galactose, a mixture was suspected. Some of the product was dissolved in H_2O (*c* 0·66), and a trace of NH_4OH was added. Found: $[\alpha]_D$ +73·2°. Since the values of $[\alpha]_D$ for D-glucose and D-galactose are +52·8° and +81·7° respectively, the product contained *ca* 30% glucose. X-ray diffractograms of D-glucose, D-galactose, a 30:70 mixture of glucose and galactose, and the product extracted from *Anacardium occidentale* gum were obtained (Philips PW 1050 diffractometer, 34 kV, 20 mA). These showed clearly that the product was a mixture of galactose and glucose. Final confirmation of the presence of glucose in *A. occidentale* gum was obtained in a structural study,[12] when 2,3,4,6-tetra-*O*-methyl-D-glucose was obtained as one of the products after methylation and hydrolysis.

Self-aggregation of gum. By light-scattering, the MW of the first of the five single-nodule samples was found to be $1·53 \times 10^5$ on 6 June 1972. A check on the accuracy of this result, made 15 days later, showed the MW to be $4·50 \times 10^5$; 6 weeks later, this had increased to $5·0 \times 10^5$. A further five nodules were therefore purified separately, as described above. Portions of each nodular sample were dyed[16,17] with the reactive dyestuff Procion Red M-2B, and, after passage through a Sephadex G15 column to remove excess of the dyestuff, small portions (corresponding to 3 mg of the gum in 0·1 ml 1 M–NaCl) were examined on a column containing Bio-gel A-15M agarose gel.

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