Note

The solubilisation of gum exudates by sodium borohydride*

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The preparation, extraction, and method of purification of any complex natural product all require careful consideration if the subsequent analyses are to be meaningful. When a new method of extraction is devised, it is important to check that the chemical composition and physical characteristics of the material have not been modified and that the molecules of polymeric species have not been degraded.

Some plant-gum exudates are not completely soluble in cold water; a comparative study[1] showed that the most effective solvent for such gums, particularly those that swell in water to form gels, is 1% aqueous sodium borohydride. Analytical and structural studies of the polysaccharides extracted in succession from *Acacia drepanolobium* gum by cold water (fraction *A*), by M sodium chloride (fraction *B*), and by sodium borohydride (fraction *C*) showed[1] that these three fractions were structurally very similar; the only significant analytical difference involved their molecular weights (*A*, 9.5×10^5; *B*, 10.2×10^5; and *C*, 22.0×10^5). A similar effect was observed[2] in studies of *Araucaria* species, and in a survey of the gum exudates from new *Acacia* species of the *Botryocephalae* group it was observed[3] that the water-soluble species of gum were of much lower molecular weight than the species that required treatment with sodium borohydride.

This note reports series of analyses made on *Acacia rubida* gum and *Entada africana* gum to establish if degradation resulted when they were extracted for longer periods of time and with more-concentrated solutions of sodium borohydride than had been used previously.

Acacia rubida gum was chosen for study because it is only ~25% soluble in cold water. The results of analyses of the water-soluble fraction are shown in Table I. The water-insoluble residue (8 g) was then added to 1% aqueous sodium borohydride (200 ml) and stirred gently. Aliquots (50 ml) were withdrawn after 1, 3, and 5 h. Sodium borohydride (0.5 g) was then added to the remaining 50 ml to increase its concentration to 2%, and the gum was left in this solution for a further 20 h. Each aliquot was filtered, dialysed against running tap water for 48 h and then against distilled water for 24 h, filtered through Whatman No. 1 paper, and freeze-dried. The analytical data obtained for *Acacia rubida* are shown in Table I.

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TABLE I

ANALYTICAL DATA[a] FOR THE GUM FROM *Acacia rubida*

	Number of hours contact with 1% (w/v) aqueous sodium borohydride				
	0	*1*	*3*	*5*	*25*[b]
Ash, %	1.71	1.60	1.54	1.30	2.10
Nitrogen, %	0.50	0.56	0.59	0.57	0.57
Specific rotation, degrees	−23	−23	−23	−24	−21
Intrinsic viscosity, $ml.g^{-1}$	10.0	11.5	15.0	16.0	13.4
Molecular weight, $\bar{M}_w \times 10^3$	255	294	322	351	306
Methoxyl, %	0.25	n.d.	n.d.	n.d.	n.d.
Equivalent weight	2890	2825	2750	2695	2165
Hence, uronic anhydride, %	6	6	6	6.5	8
Galactose, %	62	n.d.	n.d.	n.d.	n.d.
Arabinose, %	31				
Rhamnose, %	1				

[a]All data are corrected for moisture content. [b]Sodium borohydride concentration increased to 2 for the period 5–25 h.

Entada africana gum swells strongly in cold water, giving a viscous gel but not true solution. The gum (5 g) was stirred gently with a solution of sodium borohydri (1.5 g) in cold water (200 ml), and aliquots (50 ml) were removed after 24 h a 48 h. Borohydride (1 g) was then added to the remaining 100 ml, increasing the co centration to 1.75%; an aliquot (50 ml) was removed 24 h later, *i.e.* after contact wi borohydride for 3 days. More borohydride (1 g) was then added to the remaini 50 ml, giving a final concentration of 3.75%, and this solution was left for a furth

TABLE II

ANALYTICAL DATA[a] FOR THE GUM FROM *Entada africana*

	Number of days contact with aqueous sodium borohydride			
	1[b]	*2*[b]	*3*[c]	*6*[d]
Ash, %	2.80	2.92	3.03	3.20
Nitrogen, %	1.54	1.57	1.60	1.58
Specific rotation, degrees	−58	−58	−57	−56
Intrinsic viscosity, $ml.g^{-1}$	27.0	27.4	22.5	16.8
Molecular weight, $\bar{M}_w \times 10^3$	920	980	885	765
Equivalent weight	1175	1300	1330	1380
Hence, uronic anhydride, %	15	14	13	13
Galactose, %	25	n.d.	n.d.	n.d.
Arabinose, %	50			
Rhamnose, %	10			

[a]All data are corrected for moisture content. [b]Concentration of sodium borohydride, 0.75%. [c]Concentration of sodium borohydride, 0.75% for 2 days and 1.75% for 1 day. [d]As for [c], then 3.75% for the final 3 days.

3 days. (The concentrations noted are nominal and are calculated on the basis of the weights of sodium borohydride used; no estimate of the decrease in borohydride concentration with time was attempted.) The aliquots withdrawn after 1, 2, and 3 days, respectively, were worked up as a described above for *Acacia rubida*. The material treated with borohydride for a total of 6 days was dialysed against running tap water for 4 days and against distilled water for 2 × 24 h, and then freeze-dried. The analytical data obtained for the *Entada africana* extracts are shown in Table II.

The results substantiate the earlier indications[1–3] that treatment with 1% aqueous sodium borohydride for periods of a few hours does not cause decomposition or degradation, but leads to the recovery of material of higher molecular weight than is solubilised by cold water. This effect was certainly shown by *Acacia rubida* gum for periods of up to 5 h. Treatment with 2% borohydride for a further 20 h did result in analytical differences, but the changes are surprisingly small; the viscosity and molecular weight of this product are both higher than the values given by the water-soluble material. *Entada africana* gum appears to be even more resistant than *Acacia rubida* to degradation or decomposition in the presence of dilute sodium borohydride. Table II indicates that only after 2 days, and at a borohydride concentration of 1.75%, does any decrease in viscosity or molecular weight tend to occur, and even after 6 days the degradative effect cannot be regarded as drastic.

The effect of this remarkably efficient solvent will have to be evaluated on a much wider range of specimens before the exercise of caution can be in any way relaxed, but the present evidence indicates that, provided the solutions are as dilute as possible and are used for the minimal length of time necessary, aqueous sodium borohydride is a valid solvent for gum exudates that are not water-soluble.

EXPERIMENTAL

Origins of specimens. — The gum from *Acacia rubida* A. Cunn. was collected by Mr. A. N. Rodd, at Burbong, New South Wales, on April 25th, 1968 (reference voucher No. NSW 99697). *Entada africana* gum was collected at Shika Research Station, Zaria, Nigeria, by Mr. G. O. Magaji on March 25th, 1969.

The analytical methods were as described previously[2]; the methods for determining specific rotations and weight-average molecular weight (light-scattering) have also been described[4]. The determinations of equivalent weight were made on material that had been exhaustively electrodialysed until an ash content $<0.1\%$ was obtained.

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