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A Comparative Study of Laboratory Methods for the Preparation of Arabic Acid

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SUMMARY

Different methods of precipitation of arabic acid from natural gum arabic are compared in terms of yield and molecular weight derived from gel chromatography experiments. All of the precipitation methods used gave products which were closely similar in terms of $\bar{M}_n$, *but precipitation with HCl/acetone and HAc/ethanol gave low yields (25 and 3%, respectively).*

INTRODUCTION

Over the years fundamental studies of gum arabic by different researchers have been based on laboratory scale preparations of arabic acid from solutions of natural gum by means of various precipitation procedures.

As gum arabic is well known to contain gum molecules having a broad molecular mass distribution, it appeared to be desirable to carry out a number of comparative precipitations to discover if significant differences between the methods exist in terms of subfractionation effects causing differences in the yields and average molecular mass of the resulting product.

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MATERIALS AND METHODS

Source of materials

Authenticated gum exudates from *Acacia senegal* were kindly supplied by the Gum Research Station, El Obeid, as the first collection of the 1978 gum season.

Preparation of gum acid

The bulk homogenised gum used as starting material for each of the separate experiments was prepared by filtration and freeze-drying of an aqueous solution (5%, w/v) of natural gum arabic.

Aliquot portions of an aqueous solution (2%, w/v) of the freeze-dried gum were acidified (pH 4) with 0·1 M acetic or hydrochloric acids, or treated with sodium chloride (0·4 M) and then precipitated with 4 volumes of ethanol or acetone. Dialysis against distilled water or centrifugation were used when necessary, and all precipitates obtained were dissolved in water and freeze-dried.

Chromatographic column fractionation

Solutions of the starting material (60–150 mg in 1 ml) were applied to columns (26×2; 60×2·5; 35×2·5 cm) of diethylaminoethyl-cellulose (DEAE-cellulose; Whatman Chromedia DE11) and eluted with: sodium acetate (pH 4·1, 0·02 M); sodium acetate (0·02 M) followed by gradient elution with sodium chloride in sodium acetate (Jermyn, 1962); sodium phosphate (0·05–0·25 M), followed by gradient elution with sodium hydroxide (0·1–0·5 M) (Anderson & Herbich, 1963); ammonium carbonate (0·01 M), followed by gradient elution with sodium chloride in ammonium carbonate (0·1–0·2 M).

Another series of fractionations of the homogenised material (30–50 mg in 2 ml solvent) was carried out on a column (Sephadex G-200, 45×2·6 cm) using as eluent: distilled water; tris buffer (1 M tris (hydroxyl)methylamine, pH 7·5 – Pronal, Budapest) (Cameron *et al.*, 1972); ammonium bicarbonate (0·2 M); ammonium carbonate (0·2 M).

Gel chromatography was carried out on a column (60×2·6 cm) of Sephadex G-200 (1 M tris buffer, pH 9, as eluent; flow-rate 10–12 ml/h). The columns were calibrated with dextran fractions (Pharmacia, Uppsala) of known number-average molecular mass. Calculations of $\bar{M}_n$ were made according to Determann (1968). Fractions were screened with anthrone (Whistler & Wolfram, 1962) and, when necessary, by the

phenol-sulphuric acid method (Dubois *et al.*, 1956). Spectral measurements were carried out with Perkin Elmer 137 and Fek-M spectrophotometers.

RESULTS AND DISCUSSION

Gel chromatography (DEAE-cellulose and Sephadex G-200) of portions of the same homogenised material, carried out with different eluents, showed different elution profiles and confirmed the heteropolymolecularity (Anderson & Stoddart, 1966) of the gum. Of the eluents used, the best resolution was achieved with ammonium carbonate ($k_{av} = 0$; 0·55; 0·77; 0·97).

The results shown in Table 1 indicate that the different methods used to prepare arabic acid from a bulk sample of natural gum arabic gave closely similar yields (40–54%), except in the case of precipitation with HCl/acetone and HAc/ethanol. So far as possible, all precipitations were conducted in an identical manner to give a basis for comparison of the yields obtained.

Comparative estimations of the average molecular mass of the arabic acid preparations were made by gel chromatography. As the values obtained for all preparations were closely similar to that of the starting

TABLE 1
Yield and Gel Chromatography Data for Natural Gum Arabic and Arabic Acids Prepared by Different Methods

Gum sample	*Method, reference*	*Yield (%)*	$\bar{M}_n \times 10^5$
Natural gum arabic			1·7
Arabic acid precipitated with:			
HCl/ethanol	Thomas & Murray, 1928	44·0	1·9
HCl/acetone	Anderson & Herbich, 1963	25·0	1·9
NaCl/ethanol	Swenson *et al.*, 1968	44·0	1·8
NaCl/acetone	Swenson *et al.*, 1968	54·0	1·8
HAc/ethanol	Walder, 1949	3·0	1·7
HAc/acetone	Walder, 1949	40·0	1·7

material ($1{\cdot}7$–$1{\cdot}9 \times 10^5$), it was concluded that the precipitation methods studied did not cause sub-fractionation effects with gum arabic.

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