STUDIES ON INDIAN PLANT GUMS: COMPOSITION AND GRADED HYDROLYSIS OF GUM KARAYA (*STERCULIA URENS* ROXB.)

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GUM karaya or Indian tragacanth is obtained from *Sterculia urens* Roxb. But the term is sometimes erroneously applied to the gum from *Cochlospermum gossypium* Kunth.[1] The gum is chiefly obtained from the deciduous forests of Northern and Central India, and finds extensive use in the textile, food and other industries; in fact it is a good substitute for gum tragacanth. In spite of its commercial importance and abundant availability, its chemistry does not seem to have been studied in any detail; only its physical properties have been recorded.[2] Hirst and co-workers have, however, referred to this gum as unpublished work in their publications on *Cochlospermum gossypium*[1] and *Sterculia setigera*,[3] which appeared a few years ago, but so far there has been no communication on the subject. As part of our investigations on Indian gums, the study of the gum from *Sterculia urens* has been carried out.

The material used in the present investigations was obtained from Madhya Pradesh. The impure gum occurs as the acetylated polysaccharide (acetyl content 6·72%; *cf. S. setigera* which contains 15·5%[3]). It was purified by precipitation from alkaline solution by means of acidified alcohol. During the course of this purification it gets deacetylated (*cf. S. setigera*[3]). Qualitative paper chromatographic analysis of the products of complete hydrolysis of the pure gum showed that only D-galactose, L-rhamnose and D-galacturonic acid were present. These were confirmed by the formation of suitable derivatives. It may be noted that in the related species, *S. setigera*, the gum has been found to contain D-tagatose also[3]; but in *S. urens* gum this ketose sugar could not be detected. The relative proportion of L-rhamnose and D-galactose was estimated by separating the two sugars on paper chromatogram and determining each sugar separately by alkaline oxidation with iodine,[4] and it was found to be 2:3. The uronic acid was estimated by the method of Dickson *et al.*[5] and it was 37·35 %. On this basis the composition of karaya gum works out to be L-rhamnose, D-galactose and D-galacturonic acid, present in the molecular proportion of 4:6:5.

The partial hydrolysis of the gum was brought about by refluxing with 5 % sulphuric acid for 150 minutes. The " aldobiuronic " acid portion was isolated as the barium salt. On complete hydrolysis the " aldobiuronic " acid gave rise to L-rhamnose, D-galactose and D-galacturonic acid, and its equivalent weight was found to be 337, indicating that it was an equimolecular mixture of two aldobiuronic acids composed of D-galacturonic acid and L-rhamnose on the one hand and the same acid and D-galactose on the other. If it were an aldotriuronic acid composed of L-rhamnose, D-galactose and D-galacturonic acid, the equivalent weight would have been 488.

A majority of the gums yield only one aldobiuronic acid on graded hydrolysis and the formation of two acids in *S. urens* gum leads to the suspicion that the original material might be a mixture of two closely allied polysaccharides but the gum did not appear to be heterogeneous. Further, there are a number of cases where two aldobiuronic acids have been indicated.[1, 3, 6–10] The occurrence of two aldobiuronic acids, therefore, seems to be more common than expected in the past.

Experimental

The gum used in the present investigation was in the form of pale yellow lumps, each weighing from 2 g. to 5 g. Its proximate analysis was carried out in the usual manner. Moisture, fat, fibre, ash and proteinous matter were estimated according to standard methods.[11] The uronic acid was determined according to the method of Dickson, Otterson and Link[5] by noting the amount of carbon dioxide liberated on boiling with 12% hydrochloric acid. The acetyl group determination was carried out by refluxing the gum in presence of absolute alcohol with *p*-toluene sulphonic acid and determining the amount of ethyl acetate so liberated.[12] The following results were obtained:—Moisture 16·84%, ash 5·92%, proteinous matter 0·63%, fat 0·28%, crude fibre 0·41%, acetyl group 6·72% and uronic acid 31·91%.

Purification of the Gum.—The powdered gum (80 mesh, 40 g.) was added slowly to 2 litres of water containing 40 g. of sodium hydroxide, and stirred well by means of an electric stirrer. The material gradually, but only partially, dissolved, and after 6 hours it formed a viscous brown mass. The mixture was carefully acidified with concentrated hydrochloric acid and then treated with 6 litres of alcohol, when the gum was precipitated as a pinkish stringy solid. After decanting off the supernatant liquid, the gum was squeezed in a cloth, broken up into small bits and again taken in 2 litres

of water containing 10 g. of sodium hydroxide, with continuous stirring for six hours as before. The solution, which was pale yellow and mucilaginous, was quite homogeneous now. It was centrifuged in order to remove the suspended impurities and from the centrifugate the gum was precipitated as before. The operations of dissolution, centrifuging and precipitation were repeated twice again when the gum was obtained in a pure state. It was then dried, first in air and then in an electric oven, kept at 50° C., for 24 hours and powdered. Yield: 25 g. The purified gum (obtained after the 4th precipitation) was pinkish white in colour and was somewhat hygroscopic, but was not freely soluble in water. It analysed as follows:— Ash 0·49%, uronic acid 37·35%, acetyl grouping nil, and $[\alpha]_D^{20}$ (in N-sodium hydroxide) + 53·21°.

Complete Hydrolysis of the Gum.—After a number of preliminary experiments it was found that boiling the gum mildly under reflux with 5% sulphuric acid for 18 hours, taking 100 c.c. of the acid for every 5 g. of the gum, effected complete hydrolysis satisfactorily. The acid hydrolysate was neutralised with barium hydroxide using phenolphthalein as indicator. The precipitated barium sulphate was filtered off and the excess barium hydroxide was destroyed by the passage of carbon dioxide. After concentration to a small bulk (50 c.c.) in the presence of a little barium carbonate, the clear filtrate was treated with excess of alcohol (150 c.c.), when the barium salt of the uronic acid got precipitated. After leaving overnight the precipitated barium uronate was filtered and washed well with 70% alcohol in order to remove the adhering sugars. From the filtrate and the washings alcohol was distilled off and the volume made up to 200 c.c. The solution was analysed qualitatively and also quantitatively by paper chromatography for the constituent sugars. The barium uronate was purified by repeated precipitations from aqueous solution by means of alcohol. When macerated with hot methanol, it became quite crisp. This was examined separately.

Paper Chromatographic Examination of Sugar Solution.—Circular paper chromatography (method of Rao and Beri[13]) was used. Only D-galactose and L-rhamnose were detected and were confirmed by running mixed chromatograms by the method of Rao and Dickey.[14] The presence of D-galactose was further confirmed by the formation of mucic acid, m.p. 212° C., while the identity of L-rhamnose was confirmed by the formation of benzoyl hydrazone,[15] m.p. and mixed m.p. 180° C.

Quantitative Estimation of Sugars by Paper Chromatography.—The sugars were separated on filter-paper strips, using the solvent-descending

method,[16] extracted with water and finally estimated by oxidation with 0·1 N iodine solution in the presence of sodium carbonate-sodium bicarbonate buffer.[4] The sugars (L-rhamnose and D-galactose) were found to be in the molecular proportion of 2:3. The mixed sugar solution showed $[\alpha]_D^{20} = +51{\cdot}21°$ and this was in agreement with the above proportion of the sugars.

Identification of the Barium Uronate.—The barium salt of the uronic acid when examined by circular paper chromatography was found to be barium galacturonate.[17] Confirming this, an aqueous solution of the substance showed $[\alpha]_D^{20} = +24{\cdot}9°$ (barium galacturonate has $[\alpha]_D = +25{\cdot}1°$[18]), gave the characteristic brick-red precipitate with basic lead acetate[19] and on oxidation with nitric acid produced mucic acid, m.p. and mixed m.p. 212°.

Relative Proportion of the Constituent Sugars and Uronic Acid.—Uronic acid as already stated forms 37·35% of the gum. Hence the sugars constitute 62·65%. Since L-rhamnose and D-galactose are present in the molecular proportion of 2:3, the amount of L-rhamnose and D-galactose present works out to be 22·38% and 48·27% respectively. On the basis of this composition, L-rhamnose, D-galactose and D-galacturonic acid seem to be present in the gum molecule in the molecular ratio of 4:6:5.

Isolation of the "Aldobiuronic" Acid.—The purified gum (5 g.) was mildly boiled under reflux with 5% sulphuric acid (100 c.c.) with vigorous shaking to facilitate a rapid dissolution of the gum. In about 20 minutes the gum dissolved but the solution was somewhat turbid. It was, therefore, boiled for a while with a small amount of animal charcoal and filtered under suction. With the clear filtrate the boiling was continued. The course of hydrolysis was followed by a determination of the optical rotation at intervals of time. The rotation, when taken in a 10 cm. tube, assumed a constant value of +3·91° after boiling for about 120 minutes. After continuing the heating a little longer (150 minutes in all), the hydrolysate was cooled and then neutralised with barium hydroxide using phenolphthalein as indicator. The barium salt of the "aldobiuronic" acid was isolated in the usual manner by concentrating the neutralised hydrolysate to a small bulk and adding excess of alcohol. The precipitated salt was purified by repeated precipitations from aqueous solution by means of alcohol. After the 4th precipitation the substance was macerated with hot absolute methanol when it was obtained as a crisp powder. It was dried at 50° C. in an air-oven and finally in a vacuum desiccator at room temperature. Yield: 2·3 g. (Found: Ba, 16·68; uronic acid grouping, 47·94; an equimolecular mixture of the barium salts of D-galacturonosyl-D-

galactose, $C_{24}H_{38}O_{24}Ba$, and D-galacturonosyl-L-rhamnose, $C_{24}H_{38}O_{23}Ba$, requires Ba, 16·53; uronic acid grouping, 46·68%). In aqueous solution its specific rotation was found to be + 66·34° (C = 1%). From the aqueous solution the barium ion was carefully and exactly precipitated by means of dilute sulphuric acid and then the specific rotation of the aldobiuronic acid mixture was determined. It was found to be + 81·62° (C = 0·83%). The equivalent weight of the acid was determined by two methods: (1) from the barium content and (2) from the amount of carbon dioxide liberated when boiled with 12% hydrochloric acid and the two values obtained were respectively 344·2 and 337. The acid mixture underwent complete hydrolysis on boiling under reflux with 5% sulphuric acid for 18 hours and the hydrolysate contained L-rhamnose, D-galactose and D-galacturonic acid.

SUMMARY

Gum karaya (*Sterculia urens* Roxb.) is constituted from L-rhamnose, D-galactose and D-galacturonic acid which appear to be present in the molecular ratio of 4:6:5. D-tagatose, which has been reported to be present in other Sterculia gums, could not be detected in the sample of the karaya gum now examined. The original crude gum contains some acetyl groups also, which get detached during purification.

On graded hydrolysis with dilute sulphuric acid under controlled conditions, the gum yields L-rhamnose, D-galactose and a mixture of two aldobiuronic acids.

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