XXVI. STUDIES ON THE GUMS.

II. TRAGACANTHIN—THE SOLUBLE CONSTITUENT OF GUM TRAGACANTH.

By ARTHUR GEOFFREY NORMAN.

From the Biochemistry Department, University of Birmingham and the Fermentation Department, Rothamsted Experimental Station, Harpenden, Herts.

(Received December 1st, 1931.)

THE literature on the subject of gum tragacanth, like that of the gums in general, is fragmentary and confused. The first work of value is that of Ogle [1900] who stated that this gum is not a simple substance, but contains 36 % of a water-soluble carbohydrate constituent and 42 % of an insoluble one, which swells up in water. The soluble constituent was stated to give a product on precipitation with alcohol, different from arabin (acid gum arabic). Widstoe and Tollens [1900] treated gum tragacanth as a simple substance, from which they obtained on hydrolysis the sugars, fucose and arabinose, and from another sample, xylose and arabinose. In the same year Hilger and Dreyfus [1900] stated that this gum contained 15–22 % galactose, estimated as mucic acid, and 30–42 % arabinose. Tollens [1901], as a result of ultimate analyses, formed the opinion that this gum contained carboxyl groups, a view contrary to that held by Hilger and Dreyfus. O'Sullivan [1901] gave an account of some very detailed work on the composition of this gum. He opens with the statement that gum tragacanth had been held to be a mixture of "cellulose, starch, bassorin, a gum-like arabin, a little nitrogenous matter, sugar, and ash." The soluble constituent "gum-like arabin" he concludes to be a mixture of poly-arabinon-trigalactan-geddic acids and gives for it the formula

More recently von Fellenberg [1914] states that whereas the bassorin or insoluble portion is methoxylated to the extent of 5.38 %, the water-soluble constituent has no ester group of this type.

It seems, strangely enough, that a name has never been given to this constituent of gum tragacanth soluble in water, and it is proposed to term it tragacanthin.

EXPERIMENTAL.

Gum tragacanth on addition of water swells enormously, since the water-insoluble form, bassorin, constituting some 60–70 % of the gum, gives a very bulky jelly. O'Sullivan [1901] stated that he effected a separation of the soluble gum from the bassorin by means of fractional precipitation with alcohol. A solution of gum tragacanth in water was concentrated until a gelatinous scum began to form on the surface of the liquid. Dilute alcohol was added

gradually until a bulky and curdy precipitate came down, which was stated to be bassorin. From the alcoholic filtrate, on evaporation and addition of strong alcohol, a further precipitate was obtained, claimed to be the tragacanthin. This he fractionated several times, but nevertheless it cannot be held to be an ideal method of separation. Some more reliable method was sought for in this work, and many methods both physical and chemical were tried. For a time a stream-line filter works admirably with a suitable grade of paper, but unless it is very large it chokes before any considerable quantity has filtered. Finally, filtration on very large fluted filter-papers was adopted. This was an extremely laborious method since filtration was slow, and since a concentration of the gum higher than about 0.1 % could not be employed. The filter-papers have frequently to be changed; but nevertheless such a method makes it absolutely certain that the product obtained is tragacanthin only. The very dilute filtrate was concentrated under reduced pressure to a small bulk, and several volumes of 95 % alcohol were added, together with a small quantity of hydrochloric acid. A stringy precipitate formed, not unlike many pectin preparations in appearance. This was filtered off, dissolved in water, and again precipitated with acid alcohol. After several such reprecipitations the product was dried in absolute alcohol and finally in a desiccator over phosphorus pentoxide.

A satisfactory separation was also effected in the following manner. The solution of the gum was first made alkaline and then just slightly acid by the addition of acetic acid, whereupon the physical condition of the bassorin is somewhat altered, and it appears as large gelatinous flocks. These were removed by running the liquid through a super-centrifuge. The effluent from the centrifuge was allowed to stand and the clear supernatant liquid finally filtered through filter-paper as before. The final product in each case was a fine white powder very readily soluble in cold water.

A portion was hydrolysed by boiling for one hour with sulphuric acid, and the solution examined for sugars after the removal of the acid by baryta. Three volumes of hot alcohol were added to the hydrolysate to remove incompletely hydrolysed gum and the filtrate concentrated under reduced pressure to a thin syrup which was then utilised for the tests. Contrary to the observation of O'Sullivan [1901] galactose was not found, for only a trace of mucic acid remained on oxidation with nitric acid. The only sugar that could be detected was arabinose, which was present in some quantity. This was identified by means of its diphenylhydrazone.

The yield of carbon dioxide as a measure of uronic acid content was determined in the usual way.

<i>Tragacanthin.</i> Sample I	...	...	...	...	Ash	0.87 %
Yield of CO ₂ on ash-free basis	...	...	...	...	(i)	12.65 %
					(ii)	12.75 %

Mean value 12.70 % corresponding to 50.8 % uronic acid anhydride.

It is not possible to be definite as to the nature of the uronic acid, though there is some evidence for supposing that it is galacturonic.

The yield of furfuraldehyde was next determined so that a figure could be obtained by calculation for the pentose present in the molecule.

Yield of furfuraldehyde on ash-free basis...	...	(i)	31.26 %
		(ii)	31.42 %
Mean value			31.34 %

Since uronic acids themselves yield 16.66 % of furfuraldehyde, the 50.8 % uronic acid anhydride present in the molecule is responsible for the yield of 8.48 % furfuraldehyde, leaving the balance 22.88 % as due to pentose. This, calculated as anhydroarabinose, is 43.12 %.

Since no sugar other than arabinose could be detected in the hydrolysis liquid, it seems likely that tragacanthin consists solely of uronic acid and arabinose, though it is only possible to account for 94 % of the molecule in this way. There is a great divergence between this conclusion and that of O'Sullivan [1901], who considered tragacanthin to be a complex poly-arabinon-trigalactan-geddic acid, yielding on hydrolysis, arabinose, galactose, and geddic acid, an isomer of arabic acid also obtained by him from certain constituents of gedda gum. It seems likely that the preparation obtained by O'Sullivan contained some bassorin, which would account for the presence of galactan units.

To throw further light on the constitution of this gum several hydrolyses were carried out, and an examination of the hydrolysis products was undertaken. A portion of tragacanthin was dissolved in 3 % sulphuric acid and boiled under reflux for the period stated. It was then nearly neutralised with barium carbonate while still hot, filtered and poured into hot alcohol. A precipitate rapidly settled out and the supernatant liquid was poured off before it cooled. The precipitate was redissolved, filtered and reprecipitated with hot alcohol as before. This process was repeated several times in each case to ensure the removal of all free sugar. Finally it was dried with absolute alcohol and over phosphorus pentoxide.

The following analytical figures were obtained for the various preparations:

Table I. *Hydrolysis of tragacanthin with 3 % sulphuric acid.*

Preparation	Tragacanthin sample I %	30 minutes' hydrolysis %	60 minutes' hydrolysis %	90 minutes' hydrolysis %
Ash	0.87	1.04	2.87	3.70
CO ₂ yield	12.70	18.83	19.27	20.28
Furfuraldehyde yield	31.34	23.76	23.18	22.20
Uronic acid anhydride	50.80	75.32	77.08	81.12
Furfuraldehyde from uronic acid	8.46	12.55	12.84	13.50
Furfuraldehyde from pentose	22.88	11.21	10.34	8.70
Anhydroarabinose	43.10	20.89	19.27	16.25

The composition of the hydrolysis products is illustrated in Fig. 1. It will be noted that during the initial period of hydrolysis the arabinose content

diminishes rapidly, and the uronic acid increases proportionately. After 30 minutes, however, very little more arabinose is removed during a further period of 30 minutes, and even up to 90 minutes the product remains of much the same order. After this period hydrolysis soon becomes complete. These

Fig. 1. Hydrolysis of tragacanthin with 3 % H_2SO_4 .

results throw a considerable amount of light on the constitution of the tragacanthin molecule. It is clear that the uronic acid and a portion of the arabinose form a more resistant nucleus acid, probably linked in the form of a ring. Attached to this by a glucosidic linkage must be the remaining arabinose, which is thus easily removed on hydrolysis. A simple arrangement agreeing fairly closely with the figures experimentally obtained is a nucleus acid consisting of three molecules of uronic acid linked with one of arabinose to form a four-membered ring, attached to which is a side chain of two molecules of arabinose, as illustrated diagrammatically below.

Such a nucleus would have a composition of approximately 20.0 % anhydroarabinose and 80.0 % uronic acid anhydride, which is not dissimilar from that corresponding with the figures obtained after hydrolysis for a period of 1 hour. It is, of course, not claimed that the constitution of this gum is

represented above, but the evidence obtained from a study of the course of hydrolysis strongly suggests this general type of arrangement. A study of gum arabic [Norman, 1929] on similar lines led to the conclusion that it also contains a nucleus acid, resistant to mild hydrolysis, to which are attached arabinose units by a type of linkage easily broken. A similar type of arrangement has just been described for mesquite gum by Anderson and Otis [1930]. In this substance they find a resistant nucleus containing three units of galactose and one of methoxyglycuronic acid, to which are attached through a dicarbonyl linkage, four units of arabinose. It is probable therefore that this general arrangement may be found to hold for other members of this group of substances. Bassorin, which is very much more complex in structure than tragacanthin seems to be similarly constituted.

SUMMARY.

1. Tragacanthin, the soluble constituent in gum tragacanth, may be separated by ordinary filtration in extreme dilution.
2. Uronic acid units are found to be present and to constitute about one-half of the molecule. Arabinose was the only sugar found; no galactose could be detected.
3. Hydrolysis products were prepared, the analytical figures for which give rise to the suggestion that a portion of the arabinose is united to the uronic acid to form a resistant nucleus, and the residue attached by glucosidic linkage, and therefore easily removable.

REFERENCES.

- Anderson and Otis (1930). *J. Amer. Chem. Soc.* **52**, 4461.
von Fellenberg (1914). *Chem. Zentr.* ii, 943.
Hilger and Dreyfus (1900). *Ber. deutsch. chem. Ges.* **33**, 1178.
Norman (1929). *Biochem. J.* **23**, 524.
Ogle (1900). *Pharm. J.* (3), **20**, 3 T.
O'Sullivan (1901). *J. Chem. Soc.* **79**, 1164.
Tollens (1901). *Ber. deutsch. chem. Ges.* **34**, 1434.
Widstoe and Tollens (1900). *Ber. deutsch. chem. Ges.* **33**, 132.