CLV. THE PREPARATION OF GALACTURONIC ACID FROM PLANT MATERIALS, WITH A NOTE ON SOME OF ITS DERIVATIVES.

By HOMI RUTTONJI NANJI.

From the Biochemical Department, Imperial College of Science and Technology, South Kensington, S.W. 7.

(Received July 3rd, 1933.)

So far, the most satisfactory source of galacturonic acid has been a commercial "lemon pectic acid" of American origin. It was from this material that Link and Dickson [1930] obtained the acid in good yield. The commercial product is not, however, readily obtained in this country, and for this reason attempts were made to discover a suitable plant material, readily available, which could be used in place thereof.

A plant material with a high pectin content and relatively free from other polysaccharides would be ideal for the purpose. Suarez [1917] used expressed lemon pulp but was unable to obtain the acid in crystalline form. Ehrlich and Schubert [1929] used the so-called "hydrato-pectin" from beetroot, but their method was tedious and the yield poor. No other attempt seems to have been made to use plant tissues directly for the preparation of the acid.

Three materials were used in the attempt to prepare the acid:

- (1) sugar beet residues;
- (2) apple pomace;
- (3) lemon peel.

Of these, lemon peel was found by far the most satisfactory. Crude galacturonic acid was obtained from apple pomace, but it could not be separated from an unknown laevorotatory substance.

EXPERIMENTAL.

The lemon peel was cut into small pieces and boiled with 60 % alcohol for a short time, dried, and coarsely powdered. Portions of 100 g. were hydrolysed with 2000 cc. of 3 % H_2SO_4 for 20 hours, preliminary experiments having shown that a maximum yield of galacturonate was obtained under these conditions. After hydrolysis, the bulk of the sulphuric acid was removed by barium hydroxide, which was added with vigorous stirring, and finally by shaking with barium carbonate. The filtrate was evaporated to 400 cc. at a temperature below 40°, twice decolorised by shaking with norite, and from the solution the barium galacturonate was precipitated by pouring into 3 volumes of alcohol. The precipitate was well washed with warm alcohol and dried *in vacuo*. The amount obtained from 100 g. of the dry peel was 30 g., with an ash content equivalent to 26.48 % Ba. The barium salt was decomposed with sulphuric acid in the manner described by Link and Nedden [1932]. It was found that if the syrup did not crystallise within 2 days in a vacuum desiccator it was best to granulate

it by means of dry acetone; a considerable amount of brown colouring matter remained in the acetone. 10 g. of barium salt gave a yield of 3 g. of free galacturonic acid as a white crystalline powder. This could then be recrystallised from a mixture of dilute alcohol and acetone or glacial acetic acid and ether; in either case the acid was dissolved in the minimum quantity of the solvent in which it was soluble and then acetone or ether added till the solution was just turbid and allowed to crystallise. The acid was thus obtained as white crystals, M.P. 159°.

Derivatives of d-galacturonic acid.

Despite the widespread occurrence of galacturonic acid among natural products, little information had been published, at the time this work was undertaken, regarding any of its characteristic derivatives. At the suggestion of Dr H. W. Buston, the author commenced the study of certain derivatives of the acid, in order to extend the existing information in this direction. The appearance of a paper [1933] by Niemann *et al.*, in which they describe a number of hexuronic acid derivatives, rendered a continuance of the investigation unnecessary. It may be worth while, however, to record details of the preparation of the two derivatives which, in the opinion of the author, are the most useful for the purpose of characterising *d*-galacturonic acid, and which had been prepared and studied before the publication of the paper of Niemann *et al.*

(a) *Cinchonine d-galacturonate*. This is the most useful salt for identifying galacturonic acid, when it occurs as a constituent of plant products. It has a special advantage over some other derivatives in that it may be prepared directly from the barium salt of galacturonic acid, thereby rendering it unnecessary to obtain the acid in the free solid condition.

Of the methods of preparation tried, the following gave the most satisfactory results. 1 g. of dry barium galacturonate is suspended in 100 cc. absolute alcohol and 1.3 g. cinchonine sulphate (slightly less than the calculated quantity) dissolved in alcohol are added. The mixture is then mechanically shaken for 4–5 hours, warmed at 40–50° for an hour and filtered when the solution is still warm, to remove the precipitate of barium sulphate and any unchanged barium galacturonate. The precipitate is then washed once or twice with warm alcohol. The mixed filtrate is evaporated almost to dryness *in vacuo* at 50°, when cinchonine galacturonate readily crystallises out. It is triturated two or three times with small quantities of absolute alcohol to remove any unchanged cinchonine sulphate. Recrystallisation is best effected by dissolving the salt in the minimum quantity of hot alcohol, filtering and adding excess of ether or acetone until a distinct turbidity appears. The solution is then made clear again by gentle warming and allowed to crystallise in a refrigerator. The salt crystallises out in rosettes of tiny white needles in a few hours.

Properties. The salt melts at 173° (with decomposition)¹. (Found: C, 61.4; H, 6.7; N, 5.7 %. $C_6H_{10}O_7$, $C_{18}H_{22}N_2O$ requires C, 61.4; H, 6.6; N, 5.7 %.)

It is very sparingly soluble in cold, but dissolves readily in warm alcohol. It is quite insoluble in other organic solvents such as benzene, chloroform, and ether.

(b) *Phenylhydrazone of phenylhydrazine d-galacturonate*. Ehrlich was unable to prepare any phenylhydrazine derivatives from *d*-galacturonic acid, probably for reasons to be stated later. Niemann *et al.* [1933] report the preparation of a

¹ Ehrlich records two different melting-points, 156 and 178°. Also it is to be noted that his analysis shows a molecule of water of crystallisation in the salt. The variation of the physical properties of the cinchonine salts of hexuronic acids is commented upon by Niemann *et al.* [1933].

number of derivatives of phenylhydrazine, including the phenylhydrazone of phenylhydrazine *d*-galacturonate. This, in the experience of the author, is an extremely characteristic derivative; its preparation, however, necessitates the isolation of the acid in a free solid state and hence it is a less convenient substance to prepare than the cinchonine salt.

Niemann *et al.* report a 60 % yield of the derivative. By the following method the yield exceeds 90 %. A 5 % aqueous solution of the acid is treated with excess of phenylhydrazine acetate (1 g. acid, 1.2 g. phenylhydrazine, 1 g. 50 % acetic acid) and warmed at 40–50° for not more than 5 minutes. On cooling, the phenylhydrazone of phenylhydrazine galacturonate crystallises in rosettes of very flat elongated prisms of a pale yellow colour. More prolonged heating decomposes the derivative, with the production of brown oily substances; probably this explains the failure of Ehrlich to obtain any phenylhydrazine compounds from the acid. It is best recrystallised by suspending in boiling chloroform and adding hot methyl alcohol drop by drop until the solid almost dissolves. The solution is filtered and allowed to crystallise. The m.p. 131° (uncorr.) with decomposition was in agreement with that of Niemann *et al.* (Found: C, 55.2; H, 6.1; N, 14.2 % (Schoeller). $C_{18}H_{22}O_5N_4$, H_2O requires C, 55.1; H, 6.2; N, 14.3 %.)

REFERENCES.

- Ehrlich and Schubert (1929). *Ber. deutsch. chem. Ges.* **62**, 1974.
Link and Dickson (1930). *J. Biol. Chem.* **86**, 491.
— and Nedden (1932). *J. Biol. Chem.* **94**, 307.
Niemann, Schoeffel and Link (1933). *J. Biol. Chem.* **101**, 337.
Suarez (1917). *Chem. Ztg.* **41**, 87.