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## Note

# Uronic acid determination*

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The colorimetric assays used for the quantitative determination of uronic acids based on carbazole[1], orcinol[2], and anthrone[3], and their modifications [4,5], suffer from the disadvantage that the response is influenced by the nature of the uronic acid and its molecular environment. Analysis of uronic acids based on decarboxylation is therefore preferable when sufficient material is available. The literature on the analysis of uronic acids has been critically reviewed by Anderson[6]; more-recent methods have also been described[7–9]. In 1958, Barker *et al.*[10] described a modification of the apparatus of McCready, Swensen, and Maclay[11], which enabled uronic acid determinations to be made on samples as small as 20 mg, and we now report a gravimetric version of this procedure.

Alterations shown in Fig. 1 were introduced in the previously described apparatus[10] and essentially consisted of the replacement of the sodium hydroxide absorption assembly[10] with a concentrated sulphuric acid trap attached to the standard carbon dioxide absorption tubes as are used in the combustion set-up. No alterations were introduced in the decarboxylation procedure, which involved heating the sample with 19% hydrochloric acid and absorbing and weighing the carbon dioxide released. The handling and weighing of the absorption tube was in accordance with the standard micro-method of determining carbon and hydrogen[12], and hence the method is especially recommended for analysts familiar with such operations.

The absence of errors attributable to improper filling of absorption tubes, poor ground-glass or rubber connections, or improper scavenging of reaction products is indicated by negligible blank values. It is essential that a newly assembled apparatus be conditioned, at least once, by running a determination on a 5-mg sample of D-glucurono-6,3-lactone. This conditioning is repeated every time the contents of the zinc or concentrated sulphuric acid traps or the absorption tubes are renewed.

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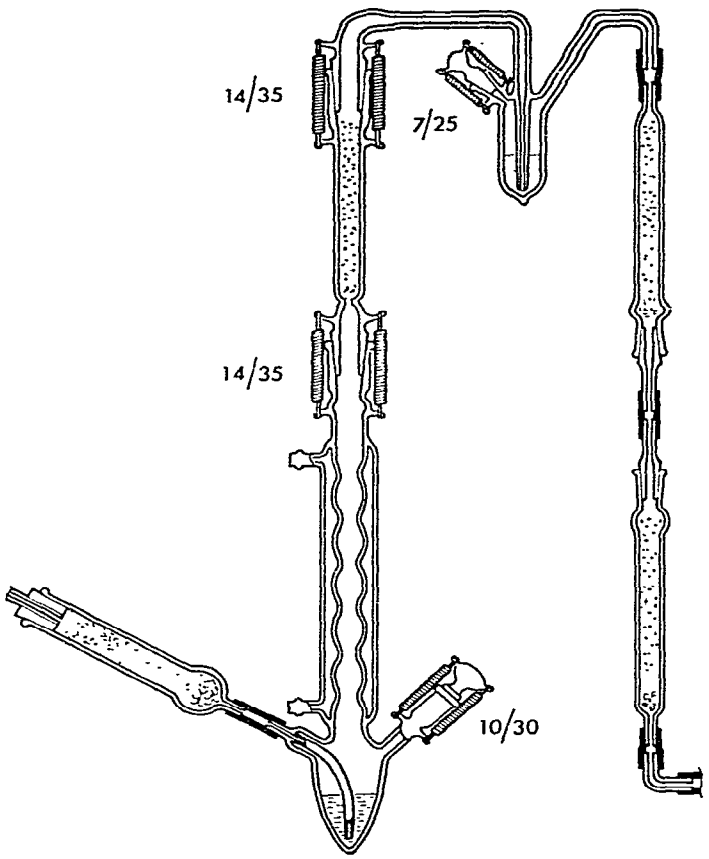


Fig. 1.

TABLE I

OPTIMAL TIME AND TEMPERATURE FOR DECARBOXYLATION OF D-GLUCURONO-6,3-LACTONE

| *Temperature (degrees)* | *Time (h)* | *Sample weight (mg)* | *$CO_2$ (mg)* | *Uronic acid (%)* |
|---|---|---|---|---|
| 145 | 0.5 | 5.955 | 0.555 | 37.2 |
| | 1 | 11.265 | 2.000 | 71.0 |
| | 1.5 | 12.060 | 3.007 | 99.4 |
| | 2 | 5.390 | 1.345 | 99.85 |
| | 2 | 11.550 | 2.906 | 100.6 |
| | 2.5 | 6.426 | 1.618 | 100.7 |
| | 2.5 | 9.862 | 2.478 | 100.5 |
| 115 | 2 | 9.330 | 1.850 | 79.4 |
| | 2 | 10.640 | 2.340 | 87.97 |
| | 3 | 14.750 | 3.380 | 91.6 |
| | 3.5 | 13.000 | 3.145 | 96.8 |

In view of our previous[10] and present results (Table I), and those recently reported by Whyte *et al.*[7], a reaction period of 3.5 h is insufficient for complete decarboxylation at lower temperatures (~115°). Anderson's recommendation[6] of a reaction time of 2.5 h at 108–109° was not applicable to the present method. In view of the large number of variables both in the apparatus design and the reaction conditions, a direct comparison between the two methods is not possible. A decarboxylation period of 2 h at 145° appeared to be optimal for our method and was therefore used in subsequent analyses.

Table II shows results obtained from some neutral components, and its is clear that interferences from these compounds are negligible.

TABLE II

INTERFERENCE FROM NEUTRAL SUGARS

| *Sample* | *Sample weight (mg)* | *$CO_2$ (mg)* |
|---|---|---|
| D-Glucose | 28.730 | 0.030 |
| L-Arabinose | 6.650 | 0.000 |
| L-Rhamnose | 5.150 | 0.068 |

A series of twelve determinations on crystalline samples of D-glucurono-6,3-lactone and D-galacturonic acid, using a sample weight ranging between 4–18 mg, showed a percentage recovery of between 99–100.6. The results for various acidic polysaccharides are given in Table III. Comparison of some of these values with the reported results in the literature shows excellent agreement for the extracellular polysaccharide of *X. campestris* and reasonable agreement for that from *A. viscosus*.

TABLE III

URONIC ACID DETERMINATION (ACIDIC POLYSACCHARIDES)

| *Sample* | *Uronic acid (%)* |
|---|---|
| Acidic arabinogalactan[a] from rapeseed (*Brassica campestris*) meal[13] | 6.9 |
| Pectin[b] (citrus, grade II, Sigma Chemical Company) | 73.2,72.9 |
| Acidic polysaccharide[a] from *Tremella mesenterica* (Y-6158)[14] | 10.0[d] |
| Acidic polysaccharide[a] from *Tremella mesenterica* (Y-6151)[14] | 10.1[d] |
| Acidic polysaccharide[a] from *Cryptococcus laurentii* (Y-1401)[14] | 14.0[d] |
| Extracellular polysaccharide[a] from *Xanthomonas campestris* NRRL B-1459[15] | 21.6,22.2[e] |
| Extracellular polysaccharide[c] from *Anthrobacter viscosus* NRRL B-1973[16] | 21.5,22.5[f] |

[a]Contains glucuronic acid. [b]Contains galacturonic acid. [c]Contains mannuronic acid. [d]These determinations were carried out by the present method. The authors (ref. 14) regret the oversight in quoting the Anderson reference. [e]The borate–carbazole method[17] gave 22.0%. [f]The borate–carbazole method[17] gave 25.0%.

## EXPERIMENTAL

*Apparatus.* — The apparatus (Fig. 1) consisted of a pear-shaped flask with a sealed, water-cooled condenser and a side-arm inlet tube reaching to the bottom of the flask. A second Quickfit (10/30) neck provided for the introduction of the sample and the acid. The top of the condenser carried a Quickfit (14/35 joints) trap, containing closely packed granulated zinc held in position by glass-wool plugs. The top of the apparatus was connected by a bulb containing conc. sulphuric acid to the standard carbon dioxide absorption tubes. The top and bottom absorption tubes were filled, respectively, with anhydrone (magnesium perchlorate) and soda-asbestos kept in place by glass-wool plugs. The cleaning, filling, and sealing of absorption tubes was in accordance with the standard procedures used for carbon and hydrogen determination[12]. The entire apparatus was protected from atmospheric carbon dioxide by calcium chloride tubes. The nitrogen flow-rate was controlled by a micro needle-valve (not shown). All the Quickfit joints were lubricated with silicone grease and held together by springs. The apparatus was cleaned with warm chromic acid and dried prior to use.

*Method.* — Hydrochloric acid (3 ml; 51.3 ml of micro-analytical grade hydrochloric acid made up to 100 ml with distilled water) was introduced into the reaction flask by means of a pipette. Carbon dioxide-free nitrogen was bubbled through the apparatus (1 bubble/2 sec), and the oil-bath, preheated to 145°, was placed in position; the level of the oil was 0.2 cm below that of the liquid in the flask. After 10 min, the soda-asbestos tube and the oil bath were removed, and the tube was wiped with chamois, left to equilibrate for 10 min near the balance, then weighed, and replaced in position. The sample, weighed as a pellet or in a narrow tube (length, 1 cm), was dropped quickly into the reaction flask, and the bath (maintained at 145°) was placed in position. The flow-rate of nitrogen was adjusted to $\sim 1$ bubble/sec and heating at 145° was continued for 2 h. The absorption tube was then disconnected, wiped with chamois, left to equilibrate for 10 min near the balance, and finally weighed.

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