A Micro-Method for the Estimation of Uronic Acids

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The method of Tracey (1948) has been modified so as to allow the estimation of the order of 0·1 mg. of free or combined uronic acid. Tracey estimated the carbon dioxide, liberated by heating the sample with hydrochloric acid in a sealed bulb, in a Van Slyke-Neill apparatus; we have used a modification of the Conway (1947) diffusion technique.

METHOD

Preparation of bulbs. The bulbs (Fig. 1, *A*) are made from 11 mm. diameter Pyrex tube. Their external diameter is about 12 mm.; they are prolonged into necks 4–5 cm. long, of diameter 3–4 mm. and drawn as thin as possible. Before use, the bulbs are washed in dilute H_3PO_4 and dried in an oven, to remove combined or adsorbed CO_2.

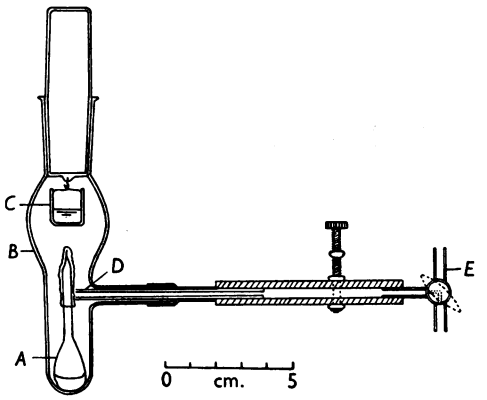

Fig. 1. Diffusion apparatus for CO_2. *A*, bulb; *B*, diffusion tube; *C*, bucket containing $Ba(OH)_2$; *D*, capillary tube; *E*, tap connecting with evacuated reservoir and soda-lime tube.

Treatment of samples with acid. The sample, containing up to 0·2 mg. of uronic acid in up to 0·4 ml., is pipetted into a bulb and 8N-HCl is added to give a final concentration of 3·3N. The bulb is sealed off with a small flame during evacuation on a water pump (to remove free CO_2), leaving a neck about 3 cm. long. The bulb is then heated for 5 hr. at 110–120° in an electric oven.

Diffusion of CO_2. This is carried out in a tube (Fig. 1, *B*), in which a bucket, *C*, containing $Ba(OH)_2$ can be suspended. This tube is washed with dil. H_3PO_4. The outside of the bulb is washed in dil. H_3PO_4 and dried, and a wisp of cotton wool is wrapped round its neck, to prevent glass splinters flying into the bucket. The bulb is placed in the tube, care being taken that there is no fluid in the neck. The stopper is put into the tube and a capillary tube, *D*, is fitted, with a rubber sleeve, into its side arm. The tube is then freed from CO_2 by connecting the capillary through a three-way tap, *E*, alternately to an evacuated reservoir and to an open-ended soda-lime tube. A glass bucket is filled with 0·1–0·25 ml. of approximately 0·01N-$Ba(OH)_2$ and hung, with as little delay as possible, from the platinum hook on the stopper of the diffusion tube. The stopper is put in again, with the bucket, and the tube is at once evacuated and filled with CO_2-free air another three times. The tube is then closed, by clipping the rubber tube attached to its capillary, and the neck of the bulb is broken by pressure from the inner end of the capillary. In order to accelerate the diffusion of CO_2 into the $Ba(OH)_2$, the bottom part of each tube is placed through a hole in a circular wooden board; the board covers an open tin within which is a 40W. lamp. The temperature of the lower part of the tube is thus maintained at 40–50° while its upper parts are cool. Under these conditions diffusion is complete in 1 hr.

Blank bulbs contain 3·3N-HCl only; they are not heated but are introduced at the diffusion stage.

Titration. This is performed with 0·01N-HCl, diluted by weight from constant-boiling HCl, in a 0·25 ml. Heatley (1939) microburette. Titration is done as quickly as possible, the solution in the bucket being stirred with a stream of CO_2-free nitrogen. Phenolphthalein is used as indicator. The $Ba(OH)_2$ is standardized each day. Each bucket is titrated immediately after its removal from the diffusion tube.

Miscellaneous precautions. When they are not in use, the grease used for the stoppers and all rubber connexions are kept in a desiccator over soda lime; the desiccator and its contents are evacuated from time to time.

RESULTS

The method has been tested with a sample of citrus pectin, provided by Dr M. V. Tracey, who found that it yields 12·0 g. of CO_2/100 g. Thirteen determinations gave a mean titre of 0·135 ml., with a standard deviation of 0·006 ml. (standard deviation of mean 0·002 ml.) compared with a theoretical titre of 0·136 ml. It was also tested with a sample of acetone glucurone, provided by Dr P. W. Kent; thirteen determinations gave a mean of 0·143 ml., standard deviation 0·007 ml., compared with a theoretical yield of 0·145 ml. The mean of nine determinations of the blank was 0·008 with a standard deviation 0·003.

DISCUSSION

The accuracy of this method depends on keeping the values of the blanks low, that is, on avoiding contamination with atmospheric or other carbon dioxide

as far as possible. The precautions described above have been found to be necessary in order to achieve this.

It was thought at first that a certain amount of hydrogen chloride, as well as carbon dioxide, would diffuse into the alkali. Accordingly, 10N-phosphoric acid was tried instead of hydrochloric acid, but this did not liberate all the carbon dioxide, even after heating for 24 hr. at 150°. Sulphuric and benzenesulphonic acids were also tried, but both gave high and variable yields, probably through their acting as oxidants. Sodium hydroxide was tried in the buckets instead of barium hydroxide, the carbon dioxide being estimated by the difference of titres with acid to the phenolphthalein and methyl orange end points. Finally, by use of the titrimetric method of Sendroy (1937) to estimate chloride, it was found that no detectable amount of hydrochloric acid was reaching the barium hydroxide.

Diffusion for periods greater than 2 hr. was found to be undesirable, because of a gradual increase of the blank titre. In confirmation of the work of Tracey (1948), 5 hr. heating with acid was found to liberate all the carbon dioxide from citrus pectin, acetone glucurone and from hyaluronic acid. The yield from methyl galacturonide was incomplete in this period.

SUMMARY

A modification of the method of Tracey (1948) is described, by which uronic acids may be estimated in amounts of the order of 0·1 mg. The carbon dioxide evolved by heating with hydrochloric acid is collected in barium hydroxide and estimated by titration with acid.

We are grateful to Dr F. B. Strauss for advice; to Dr M. V. Tracey for the gift of a sample of citrus pectin; to Dr P. W. Kent for samples of acetone glucurone and of methyl galacturonide; and to the Medical Research Council for a Research Grant under which this work was carried out.

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The Reduction of Glutathione by Plant Tissues

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The close association between glutathione (GSSG) and ascorbic acid (AA) in plant tissues is well known. In germinating seeds and sprouting potato tubers ascorbic acid and glutathione appear at the same time (Pett, 1936; Hopkins & Morgan, 1943). Moreover, many plant tissues contain the enzyme dehydroascorbic acid reductase which, as Hopkins and his collaborators have shown, catalyses the transfer of hydrogen from reduced glutathione (GSH) to dehydroascorbic acid (DHA) (Hopkins & Morgan, 1936; Crook & Hopkins, 1938; Crook, 1941).

The presence of oxidative enzyme systems catalysing the oxidation of AA, such as ascorbic oxidase, polyphenol oxidase, or peroxidase, now well established, would indicate the possibility of hydrogen transfer from GSH to oxygen via the ascorbic-dehydroascorbic acid system in accordance with the following scheme:

$$2GSH + DHA \rightarrow AA + GSSG$$
$$AA + O_2 \rightarrow DHA + H_2O.$$

How far such a system represents part of a hydrogen transfer system analogous to that of the cytochrome system depends on the ability of plant tissues to reduce GSSG. That plant tissues do possess this power is indicated by the work of Kohman & Sanborn (1937) and Ganapathy (1938), who found that compounds containing —S—S— groups could be reduced by plant juice. Even more convincing was the work which has been carried out on seeds during the initial stages of germination. Firket & Comhaire (1929) noted that, while absent in

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