failed. A cold water soluble polysaccharide complex could be isolated from the soil in question; by means of chromatographic analysis the same aldoses as those reported by Duff could be identified in the polysaccharide hydrolysate, but also in this case no ketoses could be detected.

On the other hand it was possible to identify fructose and other free sugars in a cold water extract. For identification of the chromatographically separated fructose a simple X-ray method has been devised [5]. The various sugars eluted from the paper chromatograms were also determined quantitatively according to Giri and Nigam [6]: Glucose 0.22, arabinose 0.04, fructose 0.035, xylose 0.03_5, galactose 0.02_5, and ribose <0.001, expressed as a percentage of dry (105°C) soil organic matter.

Experimental. 700 g samples of air-dry soil were first extracted with ether and then with water, both extractions carried out at room temperature (20°C). The water extract was concentrated *in vacuo* (maximum 40°C), and after precipitation of polysaccharides by means of ethanol the further concentrated filtrate was de-ionized with ion exchangers [7] and subjected to chromatographic analysis. Circular chromatograms on Whatman filter paper No. 1, obtained by the multisector and multiple development techniques described by Giri and Nigam [6], revealed several sugar bands. One of these bands reacted to colour tests in the following manner: Positive triphenyl tetrazolium bromide and α-naphthylamine phosphoric acid tests [6], negative aniline hydrogen phthalate test [8]. These reactions indicate the presence of ketoses and the absence of aldoses. The distance moved on the chromatogram corresponded to authentic fructose spotted on the same paper. As the R_F values of natural ketohexoses are very nearly identical, additional information is necessary for identification. It was not possible to obtain the eluted sugar in a crystalline state. Nor was it possible to analyze fructose derivatives precipitated direct from the water extract by the usual methods, because they were contaminated by considerable amounts of "humic matter". A larger sample was therefore prepared by means of a cellulose column [9]. The column eluates were examined by paper chromatography; one of the fractions revealed only one band, and this band reacted positively to ketose and negatively to aldose colour tests. A micro method for preparation of the osazone was successful, and the osazone could be identified by means of its powder diffraction pattern [5]. The sensitivity of this method also proved sufficient for identification of fructose eluted from paper chromatograms.

The other sugars mentioned could be completely separated on paper, and identified by colour reactions [6,8] combined with the distances moved as compared with those of authentic sugars on the same chromatogram. These distances were identical in three different developers (*n*-butanol-pyridine-water [6], ethyl acetate-pyridine-water [10], ethyl acetate-acetic acid-water [11]).

The authors wish to express their thanks to *Norges Almenvitenskapelige Forskningsråd* for a grant.

1. Haworth, W. N., Pinkard, F. W. and Stacey, M. *Nature* **158** (1946) 836.
2. Forsyth, W. G. C. *Chemistry & Industry* **1948** 515.
3. Duff, R. B. *J. Sci. Food Agr.* **3** (1952) 140.
4. Duff, R. B. *Chem. & Ind. London* **1952** 1104.
5. Michelsen, K. and Alvsaker, E. *Acta Chem. Scand.* **11** (1957) 1795.
6. Giri, K. V. and Nigam, V. N. *J. Indian Inst. Sci.* **36** (1954) 49.
7. Williams, K. T. and Bevenue, A. *J. Assoc. Offic. Agr. Chemists* **36** (1953) 969.
8. Partridge, S. M. *Nature* **164** (1949) 443.
9. Hough, L., Jones, J. K. N. and Wadman, W. H. *J. Chem. Soc.* **1949** 2511.
10. Fisher, F. G. and Dörfel, H. *Z. physiol. Chem. Hoppe-Seyler's* **297** (1954) 164.
11. Jermyn, M. A. and Isherwood, F. A. *Biochem. J.* **44** (1949) 402.

Received November 18, 1957.

A Simple X-Ray Method for Identification of Chromatographically Separated Fructose

K. MICHELSEN and E. ALVSAKER

Chemical Institute, University of Bergen, Bergen, Norway

Minute amounts of crystalline fructose may be identified by means of the X-ray diffraction pattern [1]. It may, however, be difficult to obtain small sugar amounts in a crystalline state, and we therefore considered it an advantage if it would be possible to convert the sugar into a derivative that could easily be prepared and was suitable for X-ray analysis. The osazone acted up to these requirements.

Experimental. Approximately 50 μg fructose dissolved in 15 μl water is placed on a watch glass (diameter 4 cm) by means of a micro pipette. 4 μl phenylhydrazine reagent (phenylhydrazine, acetic acid, water 3:3:10 by volume) and 2 drops acetic acid are added. The watch glass is placed between two larger, matched watch glasses, and heated on a boiling water bath. The two larger watch glasses have been preheated, and the undermost has been wetted with a few drops of water in order to reduce evaporation. After 3—5 min the small glass is withdrawn and examined under the microscope. It is neither necessary nor suitable to the purpose to recrystallize or otherwise purify the product. The tough mass in which the crystals are imbedded is non-crystalline, and acts as an excellent adhesive when the specimen is to be mounted on the glass fiber. The mounting is easily done in the following manner: A small quantity of the material is collected with a spatula to a globe and then formed between thumb and forefinger with a light squeeze. The glass fiber is brought into contact with the specimen, and by rolling between the fingers the desired magnitude and shape are readily obtained.

For X-ray powder diffraction data (Table 1) nickel-filtered copper-radiation and a camera 11.46 cm in diameter have been used. The intensities have been visually estimated. Identical diffraction pattern is obtained for fructosazone prepared by the usual macro method. The osazones of fructose, glucose, and mannose being identical, additional information is necessary to distinguish between these sugars (chromatographic separation, colour reactions).

Table 1. X-Ray powder diffraction data for fructosazone.

d, Å	I/I_1	d, Å	I/I_1
17.02	0.37	3.22	0.37
8.86	0.50	3.12	0.08
6.10	0.08	3.04	0.08
5.78	0.25	2.94	0.30
5.16	0.25	2.84	0.20
4.94	0.63	2.72	0.20
4.52	1.00	2.66	0.25
4.40	0.15	2.60	0.08
4.20	0.50	2.58	0.08
3.96	0.37	2.42	0.05
3.72	0.30	2.38	0.15
3.60	0.50	2.32	0.02
3.40	0.50	2.28	0.10
3.32	0.25		

The method proved successful for fructose quantities as small as 18 μg; this figure, however, probably does not represent the lower limit for the practical applicability of the method. The sensitivity is sufficient for identification of fructose eluted from paper chromatograms [2].

The fructosazone prepared by the micro method has an appearance that differs from the usual crystal aggregates obtained by macro methods: The crystal needles are always arranged radially, thus forming spherulites about 0.05 mm in diameter.

1. Werner, I. *Mikrochemie ver. Mikrochim. Acta* **39** (1952) 133.
2. Alvsaker, E. and Michelsen, K. *Acta Chem. Scand.* **11** (1957) 1794.

Received November 18, 1957.