

The elution pattern of the methylated substance appearing in the oestradiol fraction closely followed the elution pattern of the 3-methyl ether of oestradiol-17 α .

Oestradiol fractions isolated according to the modified Stimmel procedure showed intense pink colour and green fluorescence when subjected to the test of Kägi and Miescher. An identical reaction was shown by oestradiol-17 α , while oestradiol-17 β showed only a faint colour reaction.

The absorption spectra of the methylated oestradiol fractions, using the modified Kober reagent, closely followed the one of oestradiol-17 α -3-methyl ether with the absorption maximum at 524 m μ .

The absorption spectra of the non-methylated oestradiol fractions, using fuming sulphuric acid as reagent, closely followed the one of oestradiol-17 α , with distinct maxima at 300 and 430 m μ .

The Kober reaction is known to be highly specific to the oestrogenic hormones. Using the Brown method for isolation of the oestrogens, both the β - and α -isomer of oestradiol will appear in the oestradiol fraction. No other Kober chromogen is known to appear in this fraction. The conformity between the absorption spectra of the methylated and non-methylated substance isolated in the oestradiol fractions, and the absorption spectra of oestradiol-17 α -3-methyl ether and oestradiol-17 α * respectively, and the results of the *cis-trans* test for the 17-hydroxy group, support the validity of the concept that the Kober chromogen isolated from meconium of calves is identical to oestradiol-17 α .

The quantities found is of the order of magnitude of 20 mg/kg in unconjugated, and 25–35 mg/kg in conjugated form, both figures representing free oestradiol-17 α . There seems to be no significant difference between the sexes as regards the amounts present. This would indicate that the substance is of maternal origin.

As far as the present author is aware, the only previous paper reporting the presence of oestrogens in similar material is the one presented by Kinsella *et al.*³, who in 1956 isolated oestriol from human meconium. No other oestrogen was present in this material.

Isolation of greater quantities of oestradiol-17 α from bovine meconium is being carried out. The work shows that the substance is easily crystallized from ethanol-water mixtures.

* Gift from Ciba, Basel.

A detailed report of the work in progress will be published later.

Added in proof: By use of infrared spectroscopy it has now been definitely established that the isolated substance is identical with oestradiol-17 α .

This investigation was financially supported by the Agricultural Research Council of Norway.

1. Velle, W. *To be published.*
2. Velle, W. *Acta Endocrinol.* **27** (1958) 64.
3. Brown, J. B. *Biochem. J.* **60** (1955) 185.
4. Stimmel, B. F. *J. Biol. Chem.* **162** (1946) 99.
5. Kakusjkina, E. A. and Orlova, V. G. *Biochimia (Moskva)* **21** (1956) 26.
6. Kägi, M. and Miescher, K. *Helv. Chim. Acta* **22** (1939) 683.
7. Bauld, W. S. *Biochem. J.* **63** (1956) 488.
8. Axelrod, L. R. *Recent Progr. in Hormone Research* **9** (1954) 69.
9. Kinsella, R. A., Francis, F. E., Thayer, S. A. and Doisy, E. A. *J. Biol. Chem.* **219** (1956) 265.

Received October 27, 1957.

Carbohydrates in a Cold Water Extract of a Pine Forest Soil

E. ALVSAKER and K. MICHELSEN

Chemical Institute, University of Bergen, Bergen, Norway

In a preliminary communication Hawthorth *et al.*¹ state that levans have been isolated from polysaccharides obtained by extraction of soil with buffer solutions. We have not, however, been able to find any information about the existence of free fructose in soil. Forsyth² mentions the occurrence of small amounts of free glucose, xylose, rhamnose, and galactose in water extracts of several types of soil, but no details are given about methods of identification. Hot (85°C) water soluble soil polysaccharides have been investigated by Duff^{3,4}. In the polysaccharide hydrolysates several aldoses could be identified by chromatographic methods.

An attempt was made to isolate levans from the uppermost layer (F-layer) of a pine forest soil from Western Norway, according to the experimental conditions described by Hawthorth, but the attempt

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 devised⁵. The various sugars eluted from the paper chromatograms were also determined quantitatively according to Giri and Nigam⁶: Glucose 0.22, arabinose 0.04, fructose 0.035, xylose 0.035, galactose 0.025, 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 exchangers⁷ 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 Nigam⁶, 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 tests⁸, negative aniline hydrogen phthalate test⁹. 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 column⁹. 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 pattern⁵. 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^{8,9} 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-water⁸, ethyl acetate-pyridine-water¹⁰, ethyl acetate-acetic acid-water¹¹).

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 pattern¹. 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.