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ANALYTICAL PROCEDURES FOR STUDYING THE DEHYDRATION OF D-FRUCTOSE*

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ABSTRACT

In order to study the kinetics of the dehydration of D-fructose, procedures for the quantitation of fructose and its dehydration products, 5-hydroxymethyl-2-furaldehyde (HMF), levulinic acid, and "humin", were developed. For many reaction conditions, these compounds, together with soluble polymers (up to 15%) that are humin precursors, account for at least 98% of the amount of initial D-fructose. Fructose, HMF, and levulinic acid were determined by g.l.c. of their *O*-trimethylsilyl derivatives. U.v. absorption and titration could also be used for the determination of HMF and levulinic acid. Humin was determined gravimetrically.

INTRODUCTION

In a kinetic study of the dehydration of D-fructose, convenient analytical procedures for all the main components are desirable. Paper chromatography[1,2], which is time-consuming, and thin-layer chromatography[3,4] have been used, as have methods based on u.v. absorption and reducing power[5]. Some procedures for the isolation of 5-hydroxymethyl-2-furaldehyde[6–8] (HMF) and 4-oxopentanoic acid[9,10] (levulinic acid) have been described. We have used g.l.c. of *O*-trimethylsilyl (Me_3Si) derivatives, which is a relatively quick and reliable method that has already been reported for the determination of fructose[11–13] and HMF[14–16].

Measurement of the u.v. absorption has been the method most used for the determination of HMF[17–19], and levulinic acid can probably be determined by titration[1,20]. However, it is possible that other compounds will interfere with these determinations and hence they were checked by the g.l.c. procedure.

EXPERIMENTAL AND RESULTS

G.l.c. analyses. — For quantitative silylation, excess water must be removed from the samples. Although it was possible to silylate aqueous samples of HMF and

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*The Dehydration of D-Fructose (Formation of 5-Hydroxymethyl-2-furaldehyde and Levulinic Acid): Part I.

levulinic acid, such a procedure gave results with poor reproducibility. In the presence of HMF, all concentrating techniques resulted in polymerisation Polymerisation also occurred when vacuum evaporation and freeze-drying were carried out with samples containing only D-fructose and acids However, these samples could be concentrated in a closed system with air circulation, consisting of a sample tube at 20°, a cold trap at 0°, and an air pump

Better results for the fructose determinations were obtained when the samples were diluted with pyridine and dried with molecular sieves Because the Me_3Si derivatives of HMF and levulinic acid are not stable in pyridine, an extraction procedure was necessary HMF and levulinic acid were extracted from the sample with isobutyl methyl ketone, which has a much better distribution coefficient than benzene, butanone, or ethyl ether The extracts obtained with methyl, ethyl, or propyl acetate contained too much water

Analyses were made with a Pye PV 4000 gas chromatograph fitted with a hydrogen flame-ionisation detector and coiled columns (2 m × 2 mm i d) of stainless steel AISI 321 packed with (*a*) 3% or (*b*) 20% of PO-17 (Pierce Chem Co) on Chromosorb W (80–100 mesh) The carrier gas was nitrogen (dried over a molecular sieve) at 25 ml/min, and 0 2–1-μl samples were injected Injection and detection temperatures were 250 and 300°, respectively Peak areas were determined electronically Alkanes were used as internal standards

Silylation was effected with TRISIL-concentrate (Pierce Chem Co), or a 9.1 mixture of hexamethyldisilazane (HMDS) and trifluoroacetic acid[21] (TFA)

(*a*) D-*Fructose* Each sample (100 μl) was added to pyridine (1 ml) containing 0 2% of n-$C_{20}H_{42}$, and the solution was dried for 10 min with molecular sieve A-3 A portion (50 μl) of this solution was silylated by using ~100 μl of TRISIL-concentrate for 10 h or HMDS/TFA for 10 min

Quantitation was carried out by the conventional method using peak areas A set of calibration curves was necessary due to slight adsorption of fructose on the molecular sieves. The extent of the adsorption was dependent on the concentrations of acid catalyst, and formic and levulinic acids, and also on the concentration of fructose, but independent of the concentration of HMF n-Alkanes $>C_{10}$ were not adsorbed

(*b*) *HMF and levulinic acid* (*aqueous samples*) Each sample (100 μl) was shaken with isobutyl methyl ketone (100 μl) containing 0 2% of n-$C_{14}H_{30}$ for 10 min at 20° A portion (25 μl) of the extract was silylated with TRISIL-concentrate (~50 μl) for 15 min, and acetone (100 μl) was added For samples containing <10% of water, each sample (25 μl, diluted when necessary with 25 μl of 1-propanol to obtain a homogeneous mixture) was added to acetone (100 μl) containing 0 2% of n-$C_{14}H_{30}$. The mixture was silylated with TRISIL-concentrate (50 μl) for 15 min at 20°

The addition of acetone appeared to stabilise the Me_3Si derivative and reduced tailing on the chromatograms Correction factors were necessary for the extraction procedure, because the concentration ratio for isobutyl methyl ketone/water (~1 06 for HMF and ~0.49 for levulinic acid) is dependent on the concentrations of the components in the mixture

A typical chromatogram for fructose analysis is given in Fig 1 The use of a column temperature of 230° allowed more-accurate analysis, because the different forms of fructose were not separated The main peak should[11-13] be the β-pyranoid form, and small quantities of the other forms are lost in the tail. In samples containing polyethyleneglycol-600 (which was used to decrease the water concentration in a reaction mixture) more of the furanoid form is present[22,23] (Fig 2) A chromato-

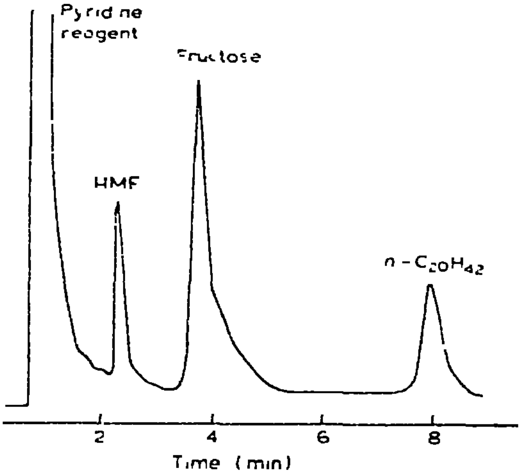

Fig 1 Chromatogram for fructose analyses, column b, 230°

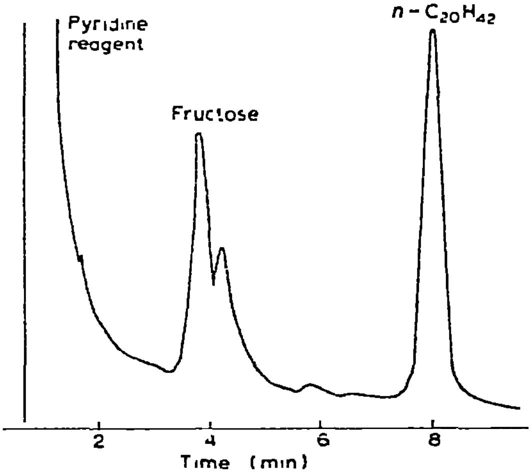

Fig 2 Chromatogram for fructose analyses (sample containing 70% of polyethyleneglycol), column b, 230°

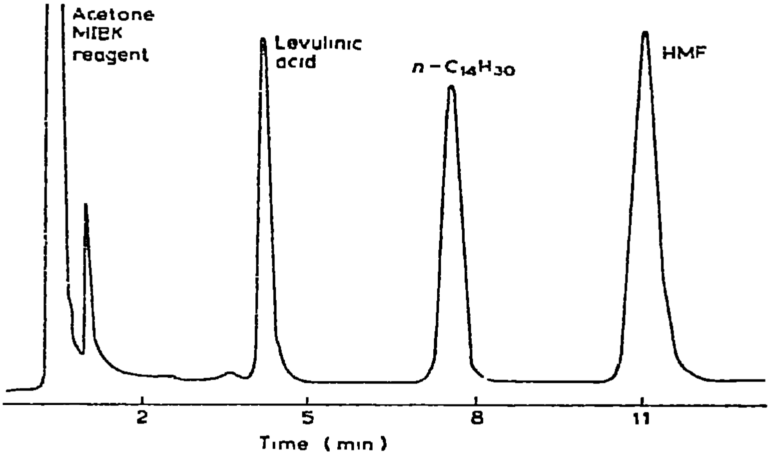

Fig 3 Chromatogram for HMF and levulinic acid analyses, column a, 130°.

gram for HMF and levulinic acid analyses is shown in Fig. 3. One peak for levulinic acid is obtained, which is most likely to be the Me_3Si derivative of the lactol form.

A useful quantity is the ratio of the peak-area ratio and the weight ratio for the compound and internal standard. Theoretical values could be calculated from reported, relative, molar responses[24,25] and are 1.44 for fructose, 0.81 for HMF, and 0.78 for levulinic acid. For D-fructose, experimental values in the range 1.30–1.50 (average, 1.36) were found. Apparently, ~10% of the signal is lost due to incomplete silylation or breakdown of Me_3Si derivatives. The theoretical value of 1.44 is somewhat low. Values close to theoretical for HMF and levulinic acid were obtained when silylation was carried out in anhydrous solvents, but in the presence of even small amounts of water (2%), silylation is not quantitative and values in the range 0.55–0.75 for HMF and 0.60–0.75 for levulinic acid were obtained. According to Aronovic[26], one of the reaction intermediates, 3-deoxyglycosulose, should be detected as a Me_3Si derivative. We did not detect this compound, and its concentration was therefore <2% of the initial concentration of D-fructose.

U.v absorption — For twice-recrystallised[27] HMF and within 15 min after the preparation of the dilute solution, the values ε_{284} 17,800 and ε_{228} 2,400 were obtained. Reported values are $\varepsilon_{281-284}$ 14,300[17], 16,700[18] (the most-used value), and 17,900[19]. The variation in value is probably due to the instability of HMF in very dilute solutions[28].

Extinctions were measured at 284 nm with a Hitachi-101 spectrophotometer and a 0.097-mm flow-through cuvette. Dilutions of only 100 had to be made, and according to Beer's law, using ε_{284} 17,800, concentrations of HMF given by $0.58 \times \varepsilon_{284}$ were reproducible within 0.5%.

Titration — Rehydration of HMF gives formic and levulinic acids in equimolar amounts[20]. For dehydration experiments with D-fructose carried out at 95° and high acidity, the amount of acid formed was twice the amount of levulinic acid (as determined by g.l.c.) The ratio of levulinic and formic acids was checked by the separation of volatile and less-volatile acids with the use of air circulation in a closed system consisting of a sample tube (at 20°), a cold trap (at 0°), and an air pump. Separation was only successful in the absence of D-fructose and HMF. The non-volatile part contained as much acid as the amount of levulinic acid determined by g.l.c. The amount of acid in the distillate was the sum of the HCl used as the catalyst and the levulinic acid. The total amount of acids was determined titrimetrically with less than 1% deviation. Titration curves were recorded on a Titrigraph SBR-2c (Radiometer). Samples of 1 ml were titrated with 0.5–4 ml of M KOH in a total volume of 20 ml of water.

Humin analyses. — Humin was collected by filtration and dried to constant weight at 1 mmHg over silica gel (Found for humin from fructose: C, 61.5 ±1.0; H, 4.3 ±0.2%; for humin from HMF: C, 65.0 ±1.0; H, 4.0 ±0.2%). The analytical values are in good agreement with literature data[29-32]. The temperature of drying is very important[29], because after 180° for 2 h all humin has an approximate composition: C, 66.4; H, 3.9%; indicating a maximum loss of 4 molecules of water per hexose residue.

Colour measurement — Mass balances made during a reaction showed a maximum deficit of 15%, decreasing to 2% at the end of the dehydration reaction. This is probably due to the presence of soluble polymers, which are humin precursors and give the reaction mixture a brown colour. The colour of the solution (after filtration of humin) was proportional to this deficit The ratios of absorbances at 350, 400, and 450 nm were 5:2·1. From the colour and the deficit, an absorbance coefficient for the unknown colourant(s) of 3–10 l/g cm at 400 nm (pH < 1) was estimated

DISCUSSION

The analytical procedures described here were used in the kinetic studies on the dehydration of D-fructose described in the following papers Several thousands of samples were analysed, and the following conclusions were drawn. (*1*) The g.l.c. procedures for fructose, HMF, and levulinic acid are relatively quick and reliable, as compared with previous procedures[1-5]. (*2*) In many cases, u.v. absorption can be used for the determination of HMF, but high values are obtained with non-aqueous solvents, due to the formation of furan ethers Samples from reactions carried out at pH >4 showed a high absorption, whereas no HMF was present according to the g.l.c. procedure. (*3*) When only levulinic and formic acids are formed in equimolar amounts, titration can be used for their determination. However, this condition was satisfied only for reactions at high acidity (above 0.5M HCl) and < 100° (*4*) Determination of the amount of humin and measurement of the colour of the solution presented a valuable check on the accuracy of the analyses

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