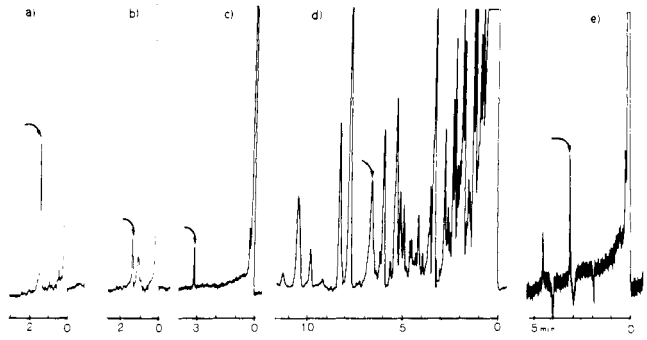

Figure 1. Glass capillary GLC chromatograms obtained from: (a) 10 ng of ETU in 1 μL of ethyl acetate, a 20-m Carbowax 20M column at 190 °C; (b) 1 ppb ETU in ethyl acetate, isolated from rats liver, ECD, a 20-m Carbowax 20M column at 190 °C; (c) 1 ng of ETU standard in 1 μL of ethyl acetate, a 30-m FFAP column at 180 °C, NPSD; (d) 2-benzylthio-1-pentafluorobenzoyl-2-imidazoline, isolated from plums, a 30-m OV-17 column at 170 °C, ECD. (e) 0.01 ng of 2-benzylthio-1-trifluoroacetyl-2-imidazoline in 1 μL of ethyl acetate, a 25-m OV-101 column at 180 °C, ECD.

and liquid scintillation counting experiments that most of the ETU was not in a free form detectable by GLC but possibly bounded with proteins or in a form of a metal complex having no retention in TLC with the solvent system used.

When the dried methanol extract was boiled in 50 mL of ethanol for 30 min, ETU was liberated, which could be also seen by TLC [R_f 0.48 for ETU using silica gel plates and EtOAc–MeOH–NH_4OH (90:6:6) solvent system]. When the ethanol solution was evaporated to dryness and the solid residue dissolved in 5 mL of ethyl acetate, the recovery of ETU was by glass capillary GLC found to be 80–90%. EBDC compounds can be decomposed to ETU in 80% yield (Marshall, 1977) and this can be used as a method to determine quantitatively the residues of EBDC compounds. The glass capillary GLC method could be successfully adapted also for this kind of analytical work. One milligram of Maneb was decomposed at 90 °C for 120 min in a water solution of pH 9. The water solution was evaporated in vacuum, the residue was dissolved in 5 mL of ethyl acetate, and ETU was determined with glass-capillary GLC with a recovery of 75%.

MATERIALS AND METHODS

ETU was synthesized from ethylenediamine and carbon disulfide (Allen et al., 1946), and the product (mp 198 °C) had, according to ^1H NMR and MS, a purity higher than 95%.

2-^{14}C-labeled ETU (sp act. 5.7 μCi/mmol) was synthesized from ^{14}C-labeled carbon disulfide (provided by Amersham, England), sp act. 62 mCi/mmol. 2-Benzylthio-1-trifluoroacetyl-2-imidazoline and 2-benzylthio-1-pentafluorobenzoyl-2-imidazoline were prepared according to the methods described by Newsome (1972) and Nash (1974).

In TLC experiments silica gel plates and a solvent system ethyl acetate–methanol–25% ammonium hydroxide (90:6:6) were used.

Measurements of radioactive ETU were carried out using a LKB Ultrobeta 1210 liquid scintillation counter. In the GLC experiments a Carlo Erba 2300 instrument was used.

The GLC glass capillary columns were prepared from a soda glass and had a length of 20–35 m and an inner diameter of 0.3 mm. The chromatograms were obtained isothermally at 170–200 °C. At the detection limits, the signal-to-noise ratio was 3:1.

DISCUSSION

A high-resolution glass capillary GLC was proved to be a very sensitive, simple, and time sparing way of detecting ETU. Precleaning of the sample or the extract is not necessarily needed and a few picograms could be detected. For higher sensitivities, the preparation of fluoronated derivative is recommended.

The GLC columns described in this work can be constructed according to the instructions in the literature cited and commercial columns are available as well.

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Enzymatic and High-Pressure Liquid Chromatographic Estimation of Glucose, Fructose, and Sucrose in Powders from Stored Onions

The enzymatic estimate of sucrose was always higher than that from high-pressure liquid chromatography because of the presence of oligosaccharides. For glucose and fructose, there was no significant difference between the results of both methods.

During enzymatic analysis of glucose, fructose, and sucrose in stored onions, we obtained some evidence that the specificity of sucrose estimation could be affected by the presence of soluble oligosaccharides (Bacon, 1957;

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Table I. Analysis of Glucose, Fructose, and Sucrose, Enzymatically and with LC, in Powders Originated from Onions Hyduro, Harvest 1976 (Values Are Mass Fractions Expressed in g/100 g)[a]

date of sampling (1976/77)	glucose				fructose				sucrose			
	enzymatic		LC		enzymatic		LC		enzymatic		LC	
	$\bar{x}_1$	s_1	$\bar{x}_2$	s_2	$\bar{x}_1$	s_1	$\bar{x}_2$	s_2	$\bar{x}_1$	s_1	$\bar{x}_2$	s_2
09-27	14.72	0.16	15.86	0.42	6.32	1.21	6.56	0.32	18.16	0.30	8.55	0.16
10-29	13.68	0.20	14.44	0.21	8.43	0.15	8.87	0.10	24.18	0.76	8.26	0.24
11-22	13.42	0.27	12.23	0.11	10.00	0.23	10.37	0.22	19.10	0.80	9.97	0.17
12-20	11.74	0.41	12.03	0.22	11.84	0.47	10.53	0.27	19.69	0.39	10.91	0.33
02-14	11.86	0.10	12.31	0.38	14.29	0.82	13.61	0.24	21.59	0.41	13.75	0.15
03-14	12.89	0.13	14.51	0.14	13.96	0.37	14.66	0.24	18.40	0.78	14.59	0.31
04-11	11.46	0.06	13.18	0.23	15.95	0.75	16.26	0.23	18.30	0.70	12.66	0.37
05-04	11.70	0.17	12.44	0.53	14.75	0.25	15.05	0.17	15.70	1.30	11.01	0.08
06-06	9.82	0.34	10.20	0.09	14.37	0.31	14.04	0.31	17.25	0.31	11.11	0.10

[a] $\bar{x}_1$ and $\bar{x}_2$ are average of triplicate analysis; $s_1 = s_2 = (\Sigma d^2/n - 1)^{1/2}$.

Miniac, 1970). β-Fructofuranosidase (EC 3.2.1.26) can hydrolyze oligosaccharides (Bergmeyer and Bernt, 1974) and the subsequent estimated glucose is not exclusively from sucrose but also from soluble oligosaccharides, leading to too high a value for sucrose.

Sucrose from oligosaccharides can be separated by high-pressure liquid chromatography (LC) (Palmer, 1975).

Glucose, fructose, and sucrose in powders originating from stored onions, season 1976–1977, were analyzed by both methods to see whether there was a difference between the methods.

MATERIALS AND METHODS

Onions (*Allium cepa*, cv. Hyduro) were stored as reported by Gorin and Honkoop (1978). After different times, bulbs (not treated with maleic hydrazide) were removed to prepare several classes of samples. The one for analysis of glucose, fructose, and sucrose consisted of 40 bulbs. Rotten onions were discarded but sprouted onions were included.

Preparation of Onion Powders (Table I). Forty bulbs (ca. 2 kg) were sliced over a container with liquid nitrogen and dropped directly into it. As cutting was done above the vapor of the liquid nitrogen, the effect of the lachrymatory factor was not perceived. The slices were immediately freeze-dried and kept under nitrogen at −80 °C. Later they were ground in a cross-beater mill (F. Kurt Retsch Type SK I; Haan, Rheinl., West Germany) in the early hours of a morning when relative humidity was not higher than 70%.

The sieve with pores 1.0 mm in diameter gave the most homogeneous powder. Other sizes of pores (8.0, 4.0, 2.0, 0.8, 0.5, and 0.25 mm) were not suitable. With pores larger than 1.0 mm, samples were heterogeneous and with pores smaller than 1.0 mm the scales were excluded.

The resulting onion powder was kept under nitrogen at −80 °C. The mass fraction of moisture in the powders was 0.85–1.90%, determined as in Gorin (1973).

Buffered methanol was freshly prepared from absolute methanol (Merck, art. 6009), volume fraction 0.8, and phosphate buffer (75 mmol/L, pH 6.8), volume fraction 0.2.

Enzymatic Analysis of Glucose, Fructose, and Sucrose (Boehringer Mannheim GmbH, Biochemica, 1976/1977a). Onion powder (1.0 g) was suspended in buffered methanol (100 mL) for 15 min at 55 °C. After decantation, an aliquot of the liquid phase (5.0 mL, pH 6.5, yellow) was diluted to 50 mL with distilled water. This solution was then colorless and was immediately poured into the cuvette for enzymatic estimation of glucose, fructose, and sucrose with a Beckmann DB-GT spectrophotometer, wavelength 340 nm, temperature 25 °C.

Respective solutions of pure commercial glucose, fructose, and sucrose (instead of powder) in buffered methanol (100 mL) gave satisfactory recoveries: 95–102%. The same recoveries were obtained by the "admixture technique" (Boehringer Mannheim GmbH, Biochemica, 1976/77b). It seems that the final concentration of methanol in the cuvette (63 mmol/L) did not affect the activity of the commercial enzymes.

Use of buffered methanol was based on the work of Drawert et al. (1969) and had the advantage of immediately inactivating the natural enzymes in the onions, making a pasteurization step (Gorin, 1973) unnecessary. The pH of the suspension or extract never dropped below 6.0, avoiding possible hydrolysis of sucrose (Bergmeyer and Bernt, 1974).

Analysis of Sugars by LC. High-pressure liquid chromatography (LC) was carried out at the Netherlands Institute for Dairy Research, NIZO, Ede, by the slightly modified method of Palmer (1975).

Onion powder (1.0 g) was suspended in buffered methanol (100 mL) for 15 min at 55 °C. Afterwards the suspension was sonically treated and left for one night at 20 °C before decantation. Aliquots of the upper phase (15 or 20 μL) were injected into a high-pressure liquid chromatograph Waters Associates: injector U6K, solvent delivery system 6000 A, Carbohydrate Analysis Column, detector Differential Refractometer R 401. Areas of the peaks of the samples were calculated and expressed relative to areas with standard solutions.

RESULTS AND DISCUSSION

Table I records the data of glucose, fructose, and sucrose in powders estimated enzymatically and by LC. The enzymatic values were the same after storage of the powder for 6 months under nitrogen at −80 °C. So the powder fixed the substrate for later analysis of metabolites.

For glucose and fructose, Student's t test for the difference between paired observations (De Jonge, 1964) suggested no significant difference between the averages obtained by the two methods. However, for sucrose there was a significant difference; values obtained enzymatically were always higher than those by LC.

That the difference was brought about by the reaction of β-fructofuranosidase with the oligosaccharides was confirmed by the following qualitative experiment.

Oligosaccharides fractions recovered from the LC after sucrose were bulked. They gave a positive reaction for the enzymatic test for sucrose.

The specificity of the enzymatic analysis of sucrose in onions is thus affected by the presence of oligosaccharides.

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Determination of Pentachlorophenol in Urine: The Importance of Hydrolysis

A gas chromatographic method for more reliable determination of pentachlorophenol (PCP) in urine has been developed. After hydrolysis and extraction the sample was reacted with diazomethane to produce the methyl ether of PCP prior to analysis by electron-capture gas chromatography. An acid alumina column clean-up system was developed to remove interferences from the sample extracts and allow detectability of 1 ppb PCP. Average recoveries of greater than 90% were obtained from urine fortified with known amounts of PCP. The importance of hydrolysis and comparisons of present methodologies will also be presented.

Pentachlorophenol (PCP) is a well-known, extensively used pesticide and a discussion on its uses, toxicity, and fate in the environment is found in the literature (Bevenue and Beckman, 1967). The occurrence of PCP in human urine is well documented (Bevenue et al., 1967; Wyllie et al., 1975).

Several methods for the determination of PCP in urine have been reported in the literature. Bevenue et al. (1966) and Rivers (1972) acidified urine, extracted with organic solvent, and methylated the urine extract with diazomethane prior to electron-capture gas chromatography (EC-GC). Cranmer and Freal (1970) partitioned possible interfering compounds into base prior to acidification and extraction of PCP into organic solvent. The urine extract was methylated with diazomethane and analyzed by EC-GC. Shafik et al. (1973) determined PCP and other halo- and nitrophenols in urine by acid reflux, extraction with ethyl ether, derivatization with diazoethane, and separation on a silica gel column prior to EC-GC analysis.

Methodology in the literature does not include a hydrolysis procedure for the determination of PCP in urine. In our laboratory we found that hydrolysis gave a much higher level for biologically incorporated PCP than when other methods not employing hydrolysis were used. Because of this finding a highly selective and more quantitative method for the determination of PCP in urine at low parts per billion levels will be described.

MATERIALS AND METHODS

Apparatus. Tracor, MT-220, gas chromatograph equipped with a nickel-63 electron-capture detector was operated in the pulsed linearized mode. A Borosilicate glass column (1.8 m × 4 mm i.d.) was packed with 80/100 mesh Gas-Chrom Q coated with 5% OV-210. The column was operated at 160 °C with 5% methane in argon at a flow rate of 40 mL/min. Detector, inlet, and transfer line temperatures were 300, 235, and 220 °C, respectively. At 5×10^{-11} amps full scale, PCP methyl ether gave a half-scale deflection at 10 pg and has a relative retention ratio to aldrin of 0.49.

Reagents and Materials. Anhydrous, granular sodium sulfate was Soxhlet extracted for 4 h with hexane and oven-dried at 130 °C.

Acid alumina, Brockman Activity I (Fisher Scientific), was dried for 24 h at 130 °C and stored in a desiccator.

Potassium hydroxide and hydrochloric acid were reagent grade.

N-Methyl-*N'*-nitro-*N*-nitrosoguanidine and pentachlorophenol (99+%) were obtained from Aldrich Chemical Co., Milwaukee, Wis. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine should be handled carefully since it is a known carcinogen.

All solvents were pesticide quality or equivalent.

Methylating Reagent. Potassium hydroxide (2.3 g) was dissolved in 2.3 mL of distilled water in a 125-mL Erlenmeyer flask and cooled to room temperature. Twenty-five milliliters of ethyl ether was then added and the flask was cooled in a refrigerator. The following step was carried out in a glovebox or a high-draft hood. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (1.5 g) was added in small portions to the flask with vigorous shaking. The ether layer was decanted into a scintillation vial and stored in a freezer (Stanley, 1966).

Preparation of Standard Solutions. An analytical standard of pentachlorophenol was prepared in acetone and stored at −15 °C in a brown glass bottle. A solution of PCP in acetone was methylated by adding 5 mL of diazomethane reagent in a high-draft hood (Howard and Yip, 1971). (CAUTION: Diazomethane is toxic and may be explosive under certain conditions.) The standard was

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