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Gas Chromatographic Analysis of Polyhydric Organic Compounds

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It is demonstrated that polyhydric organic compounds, which are often difficult or impossible to analyse, as such, by gas chromatography, may in a simple way quantitatively be converted into trimethylsilyl ethers, which, because of their greater volatility and stability, are better suited to separation by gas chromatographic methods.

The rapid development of high temperature gas chromatography during recent years has made possible the application of gas chromatographic methods to an increasing number of high boiling organic compounds. However, the tendency of many organic compounds to decompose at high temperatures has created a serious drawback. In this laboratory, we have now and then come across analytical problems, where the limited volatility and high temperature stability of polyhydric organic compounds, hindered the use of gas chromatography. On this account, it was considered to be of interest to try to convert the hydroxyl compounds into more volatile and more stable compounds. The method used for the conversion should be reasonably simple and rapid, and preferably universally applicable. These conditions are to a high degree fulfilled by an etherification method utilizing trimethylchlorosilane. This compound reacts with hydroxyl groups with the exchange of the trimethylsilyl group for hydrogen.

$$-\overset{|}{\underset{|}{C}}-OH + (CH_3)_3SiCl \rightarrow -\overset{|}{\underset{|}{C}}-OSi(CH_3)_3 + HCl$$

Under suitable conditions the reaction is quantitative, which means that not only a qualitative but also a quantitative analysis is within reach. In the following the practical performance of an analysis will be described and some results obtained with various polyhydric organic compounds will be discussed.

A method utilizing hexamethyldisilazane has been used in some instances for the trimethylsilylation of hydroxyl-containing organic compounds prior

to gas chromatography. Thus, Langer *et al.* separated monohydric phenols [1] and monohydric alcohols [2] after transforming them into their trimethylsilyl ethers. The same method was applied by Horning *et al.*[3] to some steroids. Hedgley and Overend [4] prepared trimethylsilyl ethers of methyl hexosides utilizing a method similar to the one reported in this work. However, the synthesis of the trimethylsilyl ethers was made at room temperature or under reflux and the ethers separated by distillation prior to gas chromatography. According to our experience this method does not lend itself to the quantitative determination of polyhydric organic compounds *via* their trimethylsilyl ethers. We believe that the present paper is the first one demonstrating the possibility of quantitative gas chromatographic analysis of polyhydric organic compounds in connection with trimethylsilylation.

EXPERIMENTAL

Etherification procedure. The sample (0.01 – 0.05 g) was weighed in a dry pyrex tets tube (1 × 10 cm) and trimethylchlorosilane and dry pyridine were added in 100 and 125 % excess of the calculated amounts. Pyridine serves to bind the hydrogen chloride formed and it also functions as a solvent. The amount of pyridine may be increased over that specified without any deleterious effect on the reaction. However, it is recommended not to fill the tube with the reactants to more than one third of its volume. The contents of the test tube were cooled by fastening the tube by means of a cork, in a short, wide tube containing crushed dry-ice. When cool, the tube with the reactants was sealed as near the mouth as possible, while still in the cooling bath, using a blast lamp. The ampoule was placed in a hot-oven in a vertical position at 100 – 150° for 10 – 15 h. The reaction temperature must be adjusted to the sample type. Many samples react quantitatively at 100°, others require a higher temperature. A general rule is that the temperature has to be increased when the number of hydroxyl groups increases. At 150°, all compounds tested in this laboratory reacted quantitatively. This temperature is, therefore, recommended, but for certain heat sensitive substances it may be too high. In that case, the temperature must be lowered. The heating time used here can often be reduced considerably. For convenience, we have chosen to heat the ampoules over night, but in many cases a couple of hours should be sufficient.

After heating, the ampoule was cooled and opened. If only a qualitative analysis was desired a sample of the clear liquid was withdrawn and chromatographed. In certain cases this procedure was also adequate for a quantitative analysis (*cf.* below). However, the general procedure when a quantitative analysis was desired was the following: the clear liquid over the hard cake of precipitated pyridine hydrochloride at the bottom of the ampoule was transferred to a dry test tube. Dry benzene (0.5 ml) was then added to the ampoule and the cake of pyridine hydrochloride crushed by means of a glass rod. The extraction of etherified sample from the precipitate was furthered by moving the rod up and down in the liquid and, at the same time, heating the ampoule slightly. The pyridine hydrochloride was knocked down using a centrifuge and the clear benzene solution transferred to the test tube with the pyridine solution of the bulk of the sample, by means of a dropping tube. Two further extractions of the pyridine hydrochloride were carried out. According to our experience, only traces of etherified sample are present in the pyridine hydrochloride after this threefold extraction. The benzene-pyridine solution was then chromatographed, either directly or after evaporation of some of the solvent to increase the concentration of the trimethylsilyl ethers.

Dry reagents and dry equipment should be used. The reason for this is the fact that trimethylchlorosilane is very easily hydrolyzed and, the trimethylsilyl ethers formed are also sensitive to water, although more stable than the chlorosilane. The presence of water might result in the formation of partially etherified products, giving rise to additional peaks in the chromatogram. It is also recommended to chromatograph the sample as soon as possible, because on storage, even a small amount of moisture may have a deleterious effect.

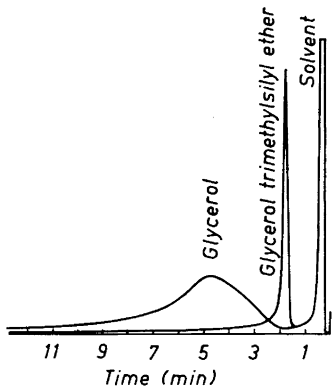

Fig. 1. Glycerol and glycerol trimethylsilyl ether. Column: silicone rubber gum, 1 m. Temperature 184°C, flow of He 75 ml/min.

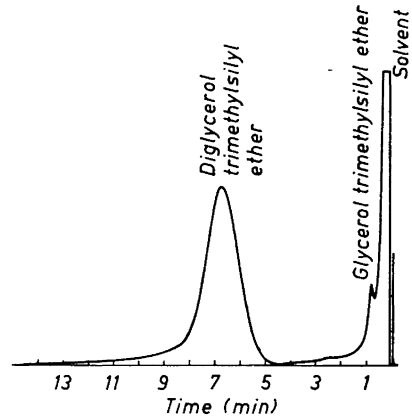

Fig. 2. Diglycerol trimethylsilyl ether. Column: Apiezon M, 1 m. Temperature 205°C, flow of He 75 ml/min.

The sample to be chromatographed contains, in addition to the etherified substance, benzene, pyridine, trimethylchlorosilane and some pyridine hydrochloride. Since the trimethylsilyl ethers investigated are high boiling substances, no interference is, as a rule, obtained from the other compounds in the mixture since they are eluted at an early stage (*cf.* Figs. 1—4).

Preparation of trimethylchlorosilane. Trimethylchlorosilane may be purchased, but is also easily prepared from hexamethyldisiloxane.

Hexamethyldisiloxane (0.5 mole) was dissolved in 150 ml icy conc. sulphuric acid in a 500 ml three-necked roundbottomed flask, equipped with reflux condenser and stirrer. Ammonium chloride (1.5 mole) was added in small doses with stirring and cooling of the reaction mixture in ice-water. The upper layer, consisting of trimethylchlorosilane, was separated and distilled with the exclusion of moisture. The pure trimethylchlorosilane (yield about 70 %) was collected at 58°C. It was stored in sealed glass ampoules in order to protect it from decomposition by moisture.

Most of the hexamethyldisiloxane, not converted to trimethylchlorosilane, may be recovered from the sulphuric acid by dropping out the acid into water and separating the upper layer which consists of hexamethyldisiloxane.

Gas chromatographic separations. The analyses were performed using a Perkin-Elmer Vapour Fractometer, Model 116 E, equipped with a thermistor detector. The columns used were made from aluminium tubing of 4 mm internal diameter. The stationary phases were applied in solution on to the solid support using a suitable low-boiling solvent. The solvent was evaporated and the dry powder then evenly packed in the column.

Two columns were used: one with silicone rubber gum (SE-30) and the other with Apiezon M as the stationary phase. The columns were of 1 ml length and the stationary phases (20 parts) were applied on Celite 545, 60—100 mesh (80 parts).

RESULTS AND DISCUSSION

Some results, demonstrating the application of the present method to the qualitative and quantitative analysis of polyhydric organic compounds, will be given below.

In Fig. 1, the advantage of the etherification method over regular gas chromatography for the analysis of glycerol is shown. A narrow peak was

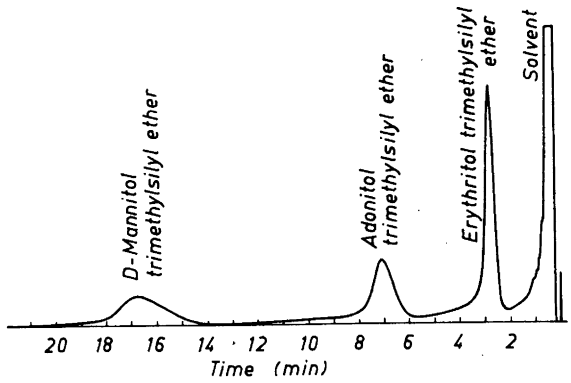

Fig. 3. Trimethylsilyl ethers of erythritol, adonitol and D-mannitol. Column: silicone rubber gum, 1 m. Temperature 210°C, flow of He 70 ml/min.

obtained for the trimethylsilyl ether of glycerol, while a broad one resulted in the regular chromatography of glycerol. Although it is not necessary to apply the etherification method in this case, its advantages, *e.g.* in determining trace amounts of glycerol, are obvious.

In Fig. 2 a chromatogram from a run of etherified diglycerol, $[HOCH_2CH(OH)CH_2]_2O$, is given. This compound is, among other things, used as a stationary phase in gas chromatography, *e.g.* for the analysis of aqueous mixtures [5]. It boils at atmospheric pressure between 420 and 450°C. The chromatogram shows also the presence of a small amount of glycerol, which is to be expected since glycerol is the starting material for the preparation of diglycerol [6]. The amount of glycerol present could easily be determined after adjusting the conditions to increase the separation between the glycerol ether and the solvent. For diglycerol, one peak only is shown in Fig. 2, but there is reason to believe that diglycerol is a mixture of several compounds of the same or similar molecular weight. This question will be further investigated.

To put the present etherification method to a more difficult test a mixture of three sugar alcohols, one tetrahydric (erythritol, mesoform), one pentahydric (adonitol) and one hexahydric (D-mannitol), was etherified and chromatographed. The results are shown in Fig. 3. It is obvious that the etherification

Table 1. Analysis values of erythritol.

Experiment No.	True value (g)	Observed (g)	% Error
1	0.0377	0.0364	3.4
2	0.0511	0.0511	0
3	0.0347	0.0347	0

Table 2. Analysis values of mixtures of adonitol and D-mannitol.

Experiment No.	Adonitol		% Error
	True value (wt %)	Observed (wt %)	
1	31.7	30.7	3.2
2	46.8	49.6	6.0
3	27.0	26.2	3.0
4	35.3	33.0	6.5
5	67.0	65.7	1.9

of the various alcohols is complete. It was decided also to study the possibilities of obtaining quantitative results using the etherification method.

In the first case erythritol was determined employing the internal standard method. Diphenyl proved to be a suitable standard substance. Known amounts of erythritol were etherified and the pyridine hydrochloride separated. To the solutions of erythritol ether were then added known amounts of diphenyl. The solutions were chromatographed and the relation between the peak heights plotted against the relation between the amounts of the two compounds. The best straight line through the origin was drawn. On the basis of the calibration curve some samples of erythritol were analyzed. The results are listed in Table 1. The agreement between the weighed and found amounts is astonishingly good, considering the fact that a micro synthesis is involved in the analytical procedure.

A mixture of adonitol and D-mannitol was then analyzed using the internal normalization method. Mixtures containing known amounts of the two

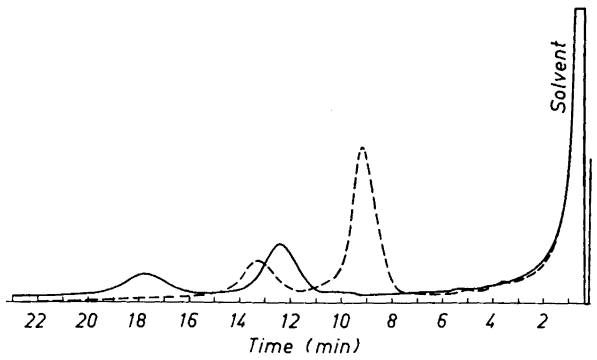

Fig. 4. Trimethylsilyl ethers of D-glucose (———) and D-mannose (- - - -). Column: silicone rubber gum, 1 m. Temperature 215°C, flow of He 70 ml/min.

compounds were etherified and chromatographed. The areas under the peaks were obtained by multiplying the top height by the band width at half the top height. The percentage area under the adonitol peak was calculated and plotted against the percentage weight of adonitol in the mixture and the best straight line through the origin was drawn. On the basis of the calibration curve, some mixtures of adonitol and D-mannitol were analyzed giving the results shown in Table 2. The first two analyses were made using the extraction method described in the experimental section. In experiments Nos. 3—5, no extraction was made. Instead the clear solution above the precipitate of pyridine hydrochloride was chromatographed directly. It is seen that, when using the internal normalization method, this procedure was not less accurate than that utilizing extraction. However, a necessary assumption is that, the amounts of the components retained in the precipitate of pyridine hydrochloride, are equivalent to their percentages in the mixture.

It has been shown that sugars of various kinds may be separated by gas chromatography after methylation [7-9]. Although, in this case, the method described in this paper may not have any advantage over methylation it was considered to be of interest to study its application to some sugars. The results obtained with D-glucose and D-mannose are shown in Fig. 4. In each case two peaks resulted. These peaks are believed to be due to the fully trimethylsilylated α- and β-forms of the two sugars. However, no attempts have been made to identify the compounds responsible for the peaks. In the case of sugars, there is a certain risk that isomerisation may take place in the hot pyridine solution and also that the hydrogen chloride formed may cause decomposition [10]. Experiments with some other sugars indicated that the transformations specified took place to a certain extent.

To summarize: the experiences made with the trimethylsilylation method indicate that it is fairly generally applicable in the conversion of polyhydric organic compounds to their trimethylsilyl ethers. However, the possibility of subsidiary reactions, taking place during the etherification process, must be taken into account. It is our opinion that the method should be of special value in the qualitative and quantitative analyses of polyhydric alcohols.

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