Estimation of glycerol and diglycerol in the presence of each other

Polymerisation of glycerol results in a series of compounds containing two or more glycerol residues. Commercial diglycerols contain usually, beside a small quantity of glycerol, substantial amounts of dimeric polymer and varying amounts of higher polymers. These mixtures may be analysed by periodate oxidation combined with the determination of hydroxyl values but as in most indirect methods the errors may be considerable. Further complications arise from the possible existence of three isomers of diglycerol *i.e.* α,α'-, α,β'- and β,β'-diglycerols which consume 2, 1 and 0 moles of periodate respectively, although an appreciable formation of the β,β'-isomer is unlikely. When faced with the need of estimating diglycerol in the presence of glycerol, the amount of available material precluding the application of indirect chemical methods, a chromatographic separation of the two compounds was attempted. Paper chromatography with solvent systems such as butanol–acetic acid–water, chloroform–ethanol and others failed to give satisfactory separation but gas–liquid chromatography proved feasible. The present communication reports the identification and quantitative determination of glycerol and diglycerol in the form of acetates using an argon ionisation detector.

Experimental

Materials. Commercial glycerol triacetate was purified by distillation *in vacuo*. Diglycerol tetra-acetate was prepared by acetylation of a commercial diglycerol which contained on the basis of periodate analysis approximately 53 % of α,α'-dimer, 45 % of α,β'-dimer and 2 % of glycerol. The acetylation was carried out by refluxing 1 g of diglycerol with 25 g of acetic anhydride for 2 h and removing the excess of the acetylating agent *in vacuo* at 100°. The product was dissolved in ethyl ether and washed with water. The ethereal solution was dried with anhydrous sodium sulphate and the ether was evaporated *in vacuo*. The product was purified by passing 0.2 g lots through a preparative chromatograph (Wilkens Aerograph Model A-700) using a column packed with celite containing 20 % Apiezon L. Subsequent chromatographic analyses showed it to be free of glycerol triacetate.

A fractionally distilled and repeatedly crystallised methyl stearate m.p. 39.5–41° was used as reference compound for the determination of the relative retention times of glycerol and diglycerol acetates.

Apparatus. A chromatograph constructed in this Laboratory and fitted with an Argon ionising detector[1] and operating at 207° was used in this work. The columns were 240 cm long and had 6.5 mm I.D. The selection of a suitable packing required prolonged experimenting. Liquid phases of high polarity such as polydiethyleneglycol adipate on celite adsorbed diglycerol tetraacetate entirely. Columns with 5–10 % Apiezon L on celite effected a good separation of glycerol and diglycerol acetates but the dimer was partly adsorbed. Satisfactory results were obtained with silicone high vacuum grease and silicone rubber gum SE-30 on glass beads. Glass beads of 0.177 mm diameter (A.S.T.M. Grade 80) were coated with 0.25 % of silicone high vacuum grease purified as described by NELSON AND MILUN[2]. The same grade of glass beads was coated with 0.5 % of silicone rubber gum SE-30.

Analysis of synthetic mixtures and relative retention times. The separation of three known mixtures of glycerol triacetate and diglycerol tetraacetate is shown in Fig. 1

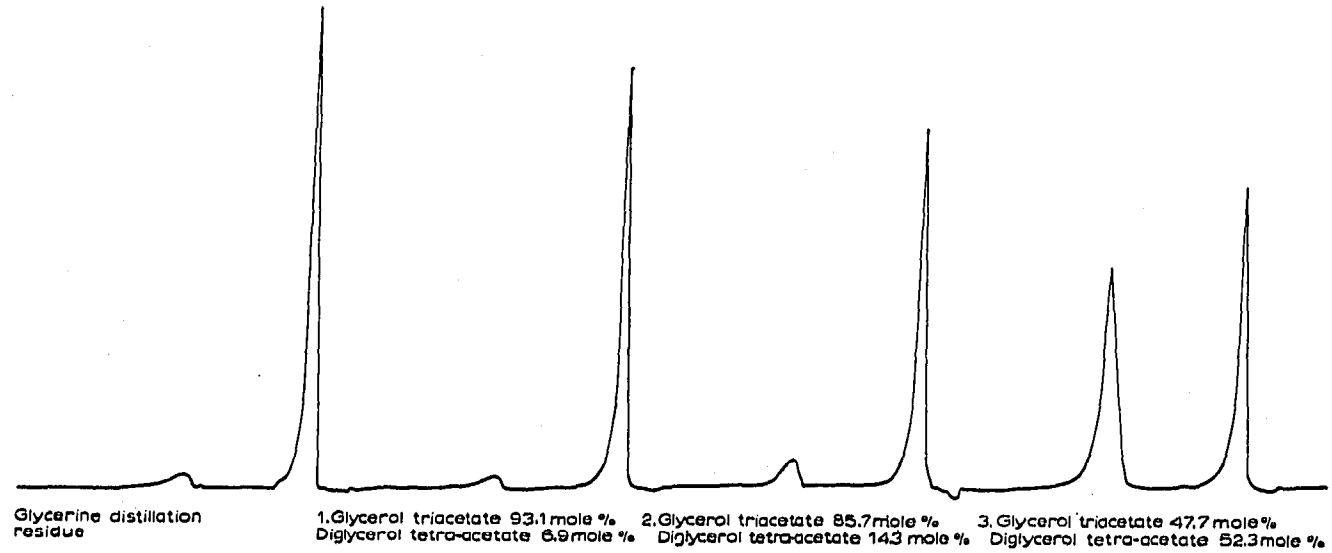

Fig. 1. Chromatograms of known binary mixtures of glycerol triacetate and diglycerol tetraacetate and of an acetylated glycerine distillation residue.

and the differences between the amounts weighed and found are listed in Table I. As could be expected, resolution of the two isomeric diglycerol tetraacetates undoubtedly present in the mixture did not take place.

The relative retention times of glycerol triacetate, diglycerol tetraacetate and methyl stearate are shown in Table II. The separation of methyl stearate from both acetates appeared excellent but the values for it, found in mixtures of known composition, were greatly in excess of the amounts taken, possibly owing to different molar responses.

Some applications of the method. A sample of glycerine residue from a commercial glycerine distilling plant—glycerine "foots"—containing approximately 12 % glycerol, 24 % sodium chloride and free alkali and 64 % water was analysed for its diglycerol content. After removing most of the water *in vacuo* the sample was taken up in acetic acid and filtered. Acetic acid was evaporated *in vacuo*, the residue was acetyl-

TABLE I

CHROMATOGRAPHIC DETERMINATION OF DIGLYCEROL TETRAACETATE IN KNOWN BINARY MIXTURES OF GLYCEROL AND DIGLYCEROL ACETATES AND IN GLYCERINE DISTILLATION RESIDUE

Glassbeads coated with 0.25 % silicone grease and 0.5 % SE-30 respectively.

Mixture	*Diglycerol tetraacetate (mole %)*		
	Taken	*Found**	
		Silicone grease	*SE-30*
1	6.9	5.9 ± 0.5	7.2 ± 0.9
2	14.3	13.8 ± 0.6	14.2 ± 1.3
3	52.3	51.9 ± 0.8	52.4 ± 0.8
Glycerine dist. residue	—	—	8.1 ± 0.7

* Based on four chromatograms in each case.

TABLE II

RELATIVE RETENTION TIME OF GLYCEROL TRIACETATE, DIGLYCEROL TETRAACETATE AND METHYL STEARATE

Compound	*Silicone grease*	*SE-30*
Glycerol triacetate	0.081	0.067
Diglycerol tetraacetate	0.516	0.486
Methyl stearate	1.000	1.000

ated and purified as described in the preparation of diglycerol tetraacetate and chromatographed (see Fig. 1 and Table I).

To estimate the diglycerol content in products such as commercial monoglycerides or other glyceride mixtures 0.1–0.5 g of the sample is saponified with ethanolic or methanolic potassium hydroxide and after removing most of the alcohol *in vacuo* the mixture is acidified with aqueous acetic acid and the liberated fatty acids are extracted with chloroform. The aqueous layer is heated *in vacuo* below 100° to evaporate most of the water and the residue acetylated and purified as previously described. By saponifying and re-acetylating a known mixture of glycerol triacetate and diglycerol tetraacetate it was found that the composition of the mixture remained unchanged, thus proving the stability of diglycerol under the above mentioned conditions.

A simultaneous estimation of glycerol, diglycerol and fatty acids in glyceride mixtures could be effected by applying a method suggested by HORROCKS AND CORNWELL[3] according to which glycerides are subjected to hydrogenolysis with lithium aluminium hydride followed by acetylation and gas–liquid chromatography. However, owing to the limited resolving power of the columns suited for diglycerol estimation this procedure is applicable only to simple glyceride mixtures containing essentially saturated fatty acids.

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Received April 23rd, 1964