

CHROM. 14,712

GAS CHROMATOGRAPHIC DETERMINATION OF LOWER FATTY ACIDS IN GASEOUS SAMPLES VIA CONVENTIONAL *IN SITU* DERIVATIZATION OF THE STRONTIUM SALTS CATALYSED BY POLY(CROWN ETHER)

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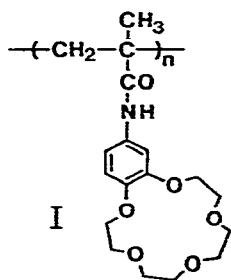
(Received December 7th, 1981)

SUMMARY

Poly(crown ether)-catalysed derivatization of lower fatty acids with *p*-bromophenacyl bromide or pentafluorobenzyl bromide has been used for their determination in gaseous samples by gas chromatography (employing either flame-ionization or electron-capture detection). Strontium hydroxide was employed as a base for the derivatization, taking advantage of the fact that a column packed with strontium hydroxide-coated glass beads is very suitable for preconcentration of the fatty acids from ambient air. *In situ* derivatization of lower fatty acids, preconcentrated on the glass beads as their strontium salts, proceeded nearly quantitatively in acetonitrile. Traces of lower fatty acids in artificial sample gases could be determined successfully using this conventional derivatization followed by direct injection of the reaction mixture into a gas chromatograph.

INTRODUCTION

Air pollution caused by traces of lower fatty acids has been well documented. High-sensitivity procedures for their determination are necessary, as the fatty acids generally have very low threshold values for perception. A popular method of determining fatty acids in the environment is chromatography including both gas and liquid chromatography, which must be preceded by preconcentration and chemical derivatization¹. However, the derivatization reaction does not necessarily proceed rapidly or quantitatively. One of the most notable successes in this field was the use of crown ethers as phase-transfer catalysts of the derivatization reactions²⁻⁴. Although the derivatization techniques were greatly improved by the use of crown ethers, some problems still remained, namely interference of the crown ethers themselves on the gas chromatogram and their toxicity. A polymer containing a crown ether in the side chain, *i.e.* a poly(crown ether) such as I, which is non-volatile and much less toxic than low-molecular-weight crown ethers, has been tested for its usefulness as a catalyst for the derivatization of fatty acids to their *p*-bromophenacyl esters⁵. The poly(crown ether) did not interfere with the gas chromatogram of the fatty acid



esters, unlike the volatile crown ethers which did so severely. By this method trace amounts of fatty acids and phenols in environmental aqueous samples have been successfully determined⁶.

Another important factor in the derivatization of acids contained in the atmosphere, which normally contains a very low level of lower fatty acids, is effective preconcentration prior to analysis. Alkali metal hydroxides can be employed for this purpose, but the hydroxides are usually very hygroscopic, thus making it difficult to preconcentrate traces of gaseous fatty acids from a large quantity of sample gas. Weak bases such as sodium furancarboxylate do not absorb them very fast. Thus, a column packed with glass beads coated with strontium hydroxide⁷ has been developed as a convenient tool for the preconcentration of lower fatty acids in sample gases.

Crown ethers could also be expected to catalyse reactions between strontium salts of fatty acids and derivatizing reagents, as they are known to form complexes with the strontium cation. This prompted us to combine the preconcentration of lower fatty acids by the column containing strontium hydroxide-coated glass beads with the poly(crown ether)-catalysed derivatization of the acids to their esters. This paper concerns the poly(crown ether)-catalysed derivatization of the strontium salts of lower fatty acids with *p*-bromophenacyl bromide or pentafluorobenzyl bromide prior to their determination by gas chromatography [employing either flame-ionization detection (FID) or electron-capture detection (ECD)], and the *in situ* derivatization of fatty acids preconcentrated by strontium hydroxide-coated glass beads from artificial sample gases.

EXPERIMENTAL

Chemicals

The poly(crown ether) (I) was synthesized according to the procedure described elsewhere⁸. Internal standards for gas chromatography, *p*-bromophenacyl isocaproate and pentafluorobenzyl isocaproate, were prepared by esterification of potassium salt of isocaproic acid with the corresponding derivatizing reagents⁹. *p*-Bromophenacyl bromide was recrystallized from light petroleum. Pentafluorobenzyl bromide, acetonitrile, and various fatty acids (acetic, propionic, *n*- and isobutyric and *n*- and isovaleric acids) were purified by distillation. Strontium hydroxide was of analytical-reagent grade.

TABLE I
GAS CHROMATOGRAPHIC CONDITIONS

Detector	Temperature ($^{\circ}\text{C}$)			Carrier gas	Flow-rate (ml/min)
	Injection port	Column	Detector		
FID	250	200	250	N_2	30
ECD	200	130	250	He	30

Apparatus

The gas chromatographs employed were Shimadzu GC-3BF (with FID) and Yanaco G-2800 (with ECD) instruments. Glass columns, 2.1 m \times 3 mm, packed with 4% OV-17 on Gas-Chrom Q, and 2.0 m \times 3 mm, packed with 5% PEG-HT on Chromosorb W AW were employed for the FID and ECD chromatographs, respectively. The injection port, column, and detector temperatures are summarized in Table I, which includes details of carrier gas and flow-rate.

A glass column (70 \times 15 mm I.D.), packed with 2 g of strontium hydroxide-coated glass beads (1%; 20–30 mesh) was employed for preconcentration of the fatty acids in the sample gas.

Procedure

An aqueous solution (0.1 ml) of fatty acid [$3 \cdot 10^{-2}$ to $3 \cdot 10^{-5}$ M ($3 \cdot 10^{-4}$ to $3 \cdot 10^{-6}$ M for ECD system)] and strontium hydroxide ($5 \cdot 10^{-2}$ M) was carefully evaporated. To the residue was added 1 ml of acetonitrile solution containing *p*-bromophenacyl bromide [$(2 \cdot 10^{-2}$ M) ($1.2 \cdot 10^{-6}$ M pentafluorobenzyl isocaproate for ECD system)]. In this case of *in situ* derivatization of strontium salts of fatty acids on glass beads, the procedure was as follows. Traces of lower fatty acids were pre-concentrated by passing 20 l of sample gas through the column at a flow-rate of 1 l/min. To the glass beads was then added 1 ml of an acetonitrile solution containing *p*-bromophenacyl bromide, poly(crown ether) and the internal standard (the same solution as above). The mixture was allowed to reflux with vigorous stirring. An aliquot (2 μl for the FID system, 0.2 μl for the ECD system) of the reaction mixture was then injected directly into the gas chromatograph.

RESULTS AND DISCUSSION

Strontium salts of fatty acids have not often been utilized for crown ether-catalysed esterifications, unlike potassium salts. In order to determine the reactivity behaviour of the fatty acid strontium salts and to optimize the reaction conditions, derivatization of the isolated strontium salts with *p*-bromophenacyl bromide was carried out in the presence of a large excess of strontium hydroxide prior to determination of the fatty acids on the FID gas chromatograph. Acetic, propionic, *n*- and isobutyric and *n*- and isovaleric acids, which are very important in air pollution, were selected as fatty acids. Acetonitrile was employed as reaction solvent; this has often been found to be a good solvent for solid-liquid phase-transfer reactions catalyzed by crown ethers. The results of studies on the effect of reaction time and

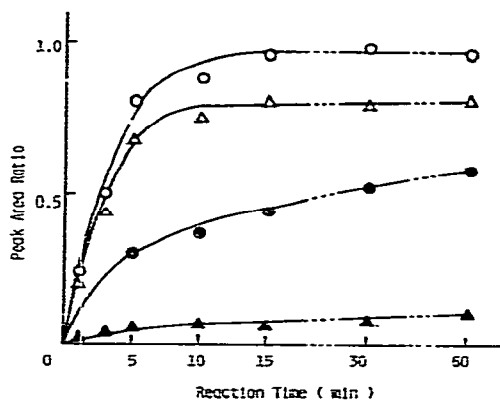


Fig. 1. Time dependence of peak-area ratio on derivatization of fatty acids with *p*-bromophenacyl bromide (FID system). [Acid] = $3 \cdot 10^{-3}$ M (0.1 ml). Acids: acetic (Δ); *n*-valeric (O); acetic, without crown ether (\blacktriangle); *n*-valeric, at room temperature (\bullet).

temperature on the ratio of peak areas of the fatty acid ester to the internal standard in the gas chromatogram are shown in Fig. 1 for the reactions of acetic and *n*-valeric acid strontium salts with *p*-bromophenacyl bromide. This esterification in acetonitrile seems to proceed rather fast under reflux, but very slowly at room temperature. A large excess of strontium hydroxide does not seem to interfere with the reaction. Since reaction hardly occurs in the absence of poly(crown ether), as anticipated, the crown ether should be present in the reaction system. In our previous work⁵, the potassium salts of fatty acids were found to be very reactive with *p*-bromophenacyl bromide in the presence of poly(crown ether), and the reaction proceeded almost quantitatively even at room temperature. This difference in reactivity between the strontium and potassium salts of fatty acids may be derived from the fact that the poly(crown ether) can complex with potassium cations more easily than with strontium cations irrespective of their similar ionic radii. In the poly(crown ether)-catalysed derivatization of

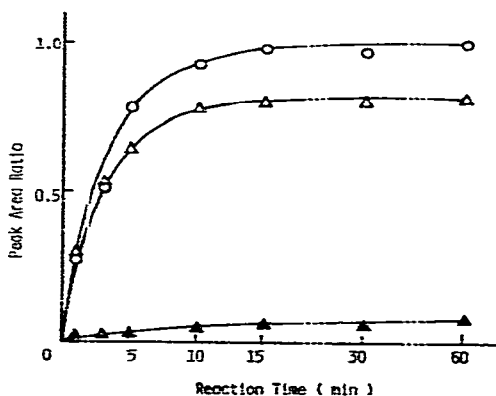


Fig. 2. Time dependence of peak area ratio on derivatization of fatty acids with pentafluorobenzyl bromide (ECD system). [Acid] = $3 \cdot 10^{-4}$ M (0.1 ml). Acids: acetic (Δ); *n*-valeric (O); acetic, without crown ether (\blacktriangle).

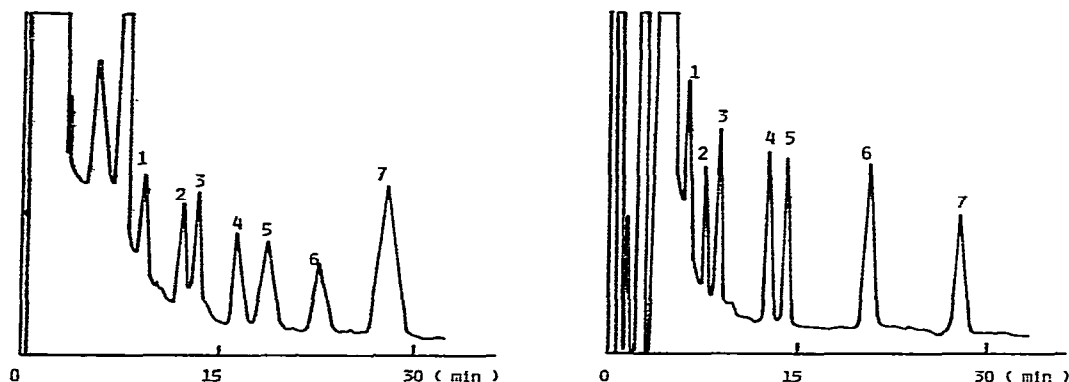


Fig. 3. Typical gas chromatogram of a mixture of fatty acid *p*-bromophenacyl esters (FID system). Acids: 1 = acetic; 2 = propionic; 3 = isobutyric; 4 = *n*-butyric; 5 = isovaleric; 6 = *n*-valeric; 7 = isocaproic (internal standard).

Fig. 4. Typical gas chromatogram of a mixture of fatty acid pentafluorobenzyl esters (ECD system). Acids: 1 = acetic; 2 = propionic; 3 = isobutyric; 4 = *n*-butyric; 5 = isovaleric; 6 = *n*-valeric; 7 = isocaproic (internal standard).

strontium salts of acetic and *n*-valeric acids, the peak area ratio in the gas chromatogram approached a constant value within 15 min under reflux conditions. The results for the other fatty acids employed in this study were essentially the same. It is, however, recommended that the reaction mixture is allowed to reflux for 30 min in order to achieve nearly quantitative derivatization of the fatty acids.

In order to detect traces of fatty acids as their esters with a higher sensitivity than FID, one may be forced to use ECD, and for this purpose the fatty acids should be derivatized with ECD-sensitive reagents such as pentafluorobenzyl bromide¹⁰. However, the derivatization of strontium salts of fatty acids with pentafluorobenzyl bromide should be carried out under much lower concentrations of fatty acid and derivatizing reagent than the above-mentioned FID systems because of the higher sensitivity of the ECD gas chromatograph. The lower fatty acids were nevertheless found to be derivatized with pentafluorobenzyl bromide almost quantitatively under the same conditions (reaction time and temperature) as employed for the *p*-bromophenacyl bromide system (Fig. 2).

Figs. 3 and 4 show typical chromatograms of a mixture of fatty acids which had been successfully derivatized with *p*-bromophenacyl bromide for the FID system and pentafluorobenzyl bromide for the ECD system. Excellent separation was obtained, and the calibration plots gave straight lines over a wide concentration range, the lowest detection limit of which was at the ng level for the *p*-bromophenacyl bromide system and at the pg level for the pentafluorobenzyl bromide systems (Figs. 5 and 6).

Attempts were made to carry out *in situ* derivatization of lower fatty acids preconcentrated by strontium hydroxide-coated glass beads and to apply this method to the determination of traces of acetic, propionic, *n*-butyric and *n*-valeric acids. Several sample gases having an acid concentration at the ppb* level were prepared by

* Throughout this article, the American billion (10^9) is meant.

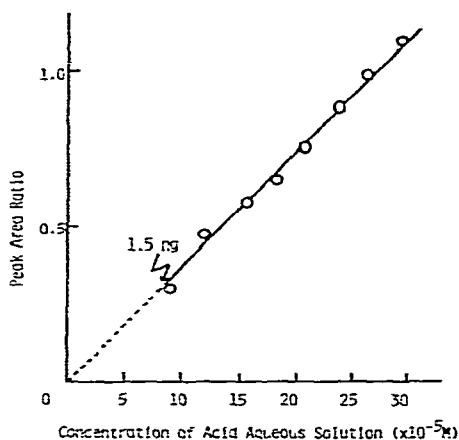


Fig. 5. Calibration plots for *n*-valeric acid-*p*-bromophenacyl bromide system (FID system) at the lowest concentration range.

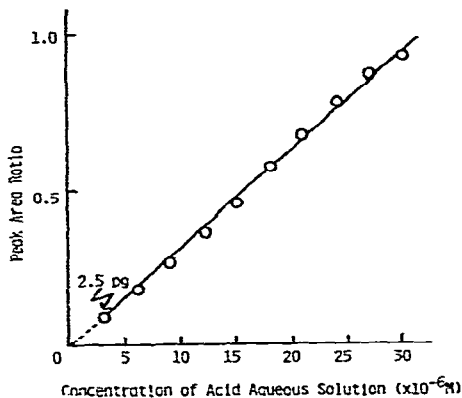


Fig. 6. Calibration plots for *n*-valeric acid-pentafluorobenzyl bromide system (ECD system) at the lowest concentration range.

injecting an aliquot of fatty acid into a polyethylene bag with a microsyringe, followed by shaking of the bag. Traces of fatty acid contained in a 20-l bag were preconcentrated by a column packed with strontium hydroxide-coated glass beads. The glass beads were then placed in an acetonitrile solution containing *p*-bromophenacyl bromide, poly(crown ether) and the internal standard. The mixture was refluxed with vigorous stirring for 30 min, and then the fatty acid in the sample gas was determined by direct injection of 2 μ l of the reaction mixture without further treatment. Table II shows the results of the *in situ* derivatization of some lower fatty acids followed by their gas chromatographic determination. The calculated and found amounts of fatty acid in the polyethylene bag are in good agreement with acceptable negative errors of only a few per cent, due to experimental errors in preparing the

TABLE II

DETERMINATION OF LOWER FATTY ACIDS IN ARTIFICIAL SAMPLE GASES

Acid	Calculated amount (ng)	Found amount (ng)	Error (%)
Acetic	72.0	71.1	-1.3
	36.0	34.9	-3.1
	14.4	13.6	-5.6
Propionic	88.8	86.9	-2.1
	44.4	42.0	-5.4
	17.8	16.1	-9.6
<i>n</i> -Butyric	106	105	-0.9
	52.8	49.2	-6.8
	21.1	19.3	-8.5
<i>n</i> -Valeric	122	119	-2.5
	61.2	58.3	-4.7
	24.5	22.9	-6.5

sample, *i.e.* incomplete vaporization of the fatty acid during injection into the bag. The table again suggests that the process of preconcentration of the fatty acids as their strontium salts on the strontium hydroxide-coated glass beads followed by their derivatization by *in situ* esterification is nearly quantitative. Let us consider the lowest *n*-valeric acid concentration in a sample gas determined in this study. For 25 ng of *n*-valeric acid contained in 20 l of sample gas, the acid concentration is 0.27 ppb, which is lower than its threshold value for perception (0.62 ppb). Thus, this *in situ* derivatization technique for fatty acids combined with their preconcentration using strontium hydroxide-coated glass beads can be utilized for practical purposes.

In our previous work⁵, the preconcentration of lower fatty acids in sample gases was achieved by bubbling the gas into concentrated solutions of potassium carbonate. A small amount of the solution was evaporated to dryness, and then the isolated potassium salts of the fatty acid were derivatized with *p*-bromophenacyl bromide via a poly(crown ether)-catalysed solid-liquid phase-transfer reaction. Isolation of the potassium salts is time consuming, and also requires precautions to prevent the solution from bumping during evaporation. In order to eliminate this process, attempts were made at a liquid-liquid phase-transfer reaction between the potassium carbonate solution containing the preconcentrated lower fatty acids and a water-immiscible organic solution containing the derivatizing reagent and the poly(crown ether); however these failed, probably because of the slowness of the liquid-liquid phase-transfer reaction. The *in situ* derivatization technique developed in this study does not require a tedious isolation process, and this is also a great advantage for the practical application of this technique.

ACKNOWLEDGEMENT

This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture and Science, Japan.

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