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GAS CHROMATOGRAPHIC SEPARATION OF MERCAPTANS AS DERIVATIVES

JÓZEF KOROLCZUK*, MAREK DANIEWSKI** and ZBIGNIEW MIELNICZUK
Institute of Food and Nutrition, ul. Powsińska 61/63, Warsaw (Poland)

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SUMMARY

Normal and branched mercaptans containing up to eight carbon atoms and their 2,4- and 2,5-dinitrophenylalkylthioether and thioalkylbenzoate derivatives have been analyzed by gas–liquid chromatography (GLC). The most suitable derivatives for GLC separation appeared to be the thioalkylbenzoates, which enabled all of the normal and branched mercaptans studied to be resolved on an SE-30 column.

Two methods of preparation of thioalkylbenzoates with benzoyl chloride were compared: (1) derivatization with pyridine under anhydrous conditions and (2) derivatization in aqueous solution with sodium hydroxide as acceptor of hydrochloric acid. The method was faster and more effective when aqueous alkaline solution was used.

INTRODUCTION

Mercaptans are important flavour constituents of many natural and processed food products. They are very active and most of them have an olfactory threshold as low as a few parts per billion[1], which is their usual concentration in foods. Because of this low concentration, before studying their composition in food products, mercaptans have to be isolated and pre-concentrated. Different types of distillation[2–5] can be used in order to separate mercaptans from food products. As distillates contain other volatile components in addition to mercaptans, a further step of preliminary fractionation and pre-concentration is usually needed, *e.g.*, by using typical group reagents[6–8]. The most selective group reagents used to separate mercaptans are heavy metal ions[9]. The disadvantage of these reagents, however, is the formation of sulphides and disulphides during the precipitation of the heavy metal derivatives and their subsequent decomposition by mineral acids[9].

Another means of identifying and studying the composition of mercaptans is the preparation of other derivatives with a blocked –SH group. This can be achieved

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* Present address: Food Machinery Institute, 1b Otwocka Street, 03-759 Warsaw, Poland.
** Present address: Chemistry Department, University of Halifax, Halifax, Nova Scotia, Canada.

by forming thioethers, sulphones or thioesters. The best known are 2,4-dinitrophenylalkylthioethers (2,4-DNPT), 2,4-dinitrophenylalkylsulphones (2,4-DNPS) and 3,5-dinitrothiobenzoates, which form crystals that are easy to identify by determining their melting points[10]. However, this method is not very useful for food products, as it is very difficult or impossible to prepare samples of single derivatives separated from the material being investigated that are large enough for the melting points to be determined. Because the amount of sample usually available for preparation is in the range of a few micrograms, the only suitable method that is sensitive enough to separate and detect the concentrations of the single mercaptans is gas–liquid chromatography (GLC).

Some of the derivatives of four *n*-mercaptans were studied by GLC by Gasco and Barrera[11].

In the present work, we tried to separate some other normal and branched mercaptans and some of their derivatives by GLC.

MATERIALS AND METHODS

Reagents

Mercaptans were synthesized in our laboratory from alkyl bromides[10] and purified by double distillation. The purity was checked by GLC. 1-Chloro-2,4-dinitrophenol and 1-chloro-2,5-dinitrophenol were of analytical grade (Merck, Darmstadt, G.F.R.). Benzoyl chloride was prepared from analytical grade benzoic acid and thionyl chloride[10]. Other reagents (pyridine, diethyl ether, *n*-hexane, ethyl acetate, methanol, acetone, sodium hydroxide, hydrochloric acid and sodium chloride) were commercial analytical grade reagents.

Preparation of 2,4-dinitrophenylalkylthioethers and 2,5-dinitrophenylalkylthioethers

A 4-μl volume of each mercaptan was injected into a test-tube together with 1 ml of a 10% solution of sodium hydroxide in methanol, then 1 ml of a 10% solution of 1-chloro-2,4-dinitrophenol or 1-chloro-2,5-dinitrophenol in methanol was added. The test-tube was closed with an ether-tight stopper, shaken vigorously and allowed to stand for 15 min. Then the sample was diluted with 10 ml of water and 2,4- or 2,5-DNPT was separated by three-fold extraction with diethyl ether. The ether layers were combined and evaporated to dryness. The mercaptan derivatives were then solubilized in 1 ml of ethyl acetate and used for GLC.

Preparation of benzoyl thioesters

Two methods of preparation were applied.

Method I. A 4-μl volume of each mercaptan was injected into a mixture of 0.3 ml of benzoyl chloride and 0.2 ml of pyridine, the tube was sealed, shaken vigorously and placed in a boiling water bath for 20 min. After cooling the sample under a tap, 10 ml of water were added and thiobenzoates were separated by three-fold extraction with diethyl ether. The combined ether layers were evaporated to dryness and the thiobenzoates were solubilized in 1 ml of ethyl acetate and used for GLC.

Method II. A 4-μl volume of each mercaptan and 0.1 ml of benzoyl chloride were injected into 1 ml of an aqueous solution of sodium hydroxide with a concentration between 0.1 and 2.5 *N*, then 2 ml of acetone were added in order to solubilize benzoyl chloride. Reaction was carried out in test-tubes closed with ether-tight stoppers at room temperature or 100° for 10–90 min. Then the samples were cooled under a tap, 10 ml of water were added and the thiobenzoates were extracted as described above.

Equipment

The apparatus used was a JGC-810 gas chromatograph (Jeol, Tokyo, Japan) with a dual column system, flame ionization detectors and an SRG recorder (Sargent-Welch, Skokie, Ill., U.S.A.) with a disc integrator. The operating conditions are listed in Table I.

TABLE I

CONDITIONS FOR THE GLC SEPARATION OF MERCAPTANS AND THEIR DERIVATIVES

Parameter	*Mercaptans*	*Benzoates*	*2,4- and 2,5-DNPT*
Chromatographic columns (length × I.D.)	Glass, 2 m × 3 mm	Stainless steel, 3 m × 3 mm	Stainless steel, 3 m × 3 mm
Stationary phase	Chromosorb W, AW, DMCS, 80–100 mesh	Chromosorb W, HP, AW, DMCS, 100–120 mesh	Chromosorb, W HP, AW, DMCS, 100–120 mesh
Liquid phase	5% PEG 1000	4% SE-30	4% SE-30
N_2 flow-rate (ml/min)	30	50	50
H_2 flow-rate (ml/min)	30	30	30
Injection block temperature (°C)	200	300	300
Detector temperature (°C)	240	330	330
Initial column temperature (°C)	40	150	200
Temperature programme rate (°C/min)	10	10	10
Final column temperature (°C)	130	250	300

RESULTS AND DISCUSSION

Typical chromatograms of mercaptans, thiobenzoates and 2,4-DNPT are shown in Figs. 1, 2 and 3, respectively. Under the conditions described above, it is possible to separate several *n*-mercaptans with up to eight carbon atoms or their derivatives in less then 10 min. The 2,4-DNPT derivatives of some branched, secondary and tertiary mercaptans were not separated from the normal mercaptan derivatives and so *tert.*-butyl-2,4-DNPT appeared in the position of methyl-2,4-DNPT, and isopropyl-2,4-DNPT in the position of ethyl-2,4-DNPT. The same order and very similar retention times were observed for 2,5-DNPT derivatives. However, the 2,5-DNPT derivatives are obtained in 2–3 times higher yields than the 2,4-DNPT derivatives under the conditions of the reaction described above. Because some 2,4- and 2,5-DNPT derivatives give poor or no separations on SE-30 columns, they do not seem to be very useful for studying the composition of multi-component mixtures of normal and branched mercaptans.

The separation of the normal and branched mercaptans studied and their benzoates was much better (Figs. 1 and 2). For mercaptans, when a low-boiling solvent was used (*e.g.*, diethyl ether or acetone), the peaks of the methyl and *tert.*-butyl-

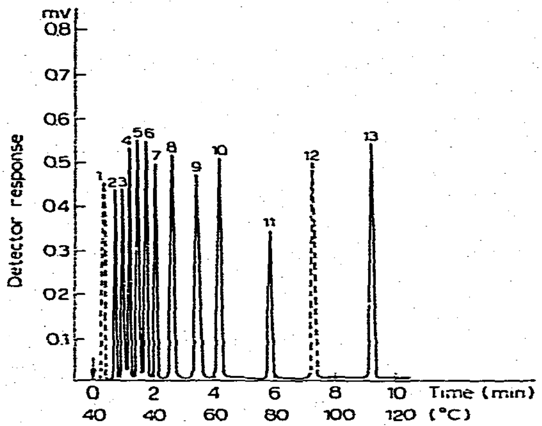

Fig. 1. Chromatogram of alkyl mercaptans: 1 = methyl; 2 = *tert.*-butyl; 3 = ethyl; 4 = isopropyl; 5 = *n*-propyl; 6 = *sec.*-butyl; 7 = isobutyl; 8 = *n*-butyl; 9 = isoamyl; 10 = *n*-amyl; 11 = *n*-hexyl; 12 = *n*-heptyl; 13 = *n*-octyl. Positions of peaks 1 and 12 were taken from Fig. 4. Conditions of separation are given in Table I.

mercaptans were covered by the solvent peak. When *n*-dodecane[9] or *n*-octanol was used as the solvent, it was possible to separate all of the above mercaptans with up to eight carbon atoms. The disadvantage of the direct separation of mercaptans on a PEG-1000 column is that thiols themselves are very labile. Also, the column has a low stability, especially when heavy metal ions are used for the precipitation and concentration of mercaptans from diluted samples of distillates.

On the contrary to free mercaptans, benzoylthioesters are stable derivatives and their separation on an SE-30 column is satisfactory. It is possible to resolve several of these derivatives formed from normal, secondary, tertiary and more highly branched mercaptans (Fig. 2). Gasco and Barrera[11] separated benzoates of normal C_1–C_4

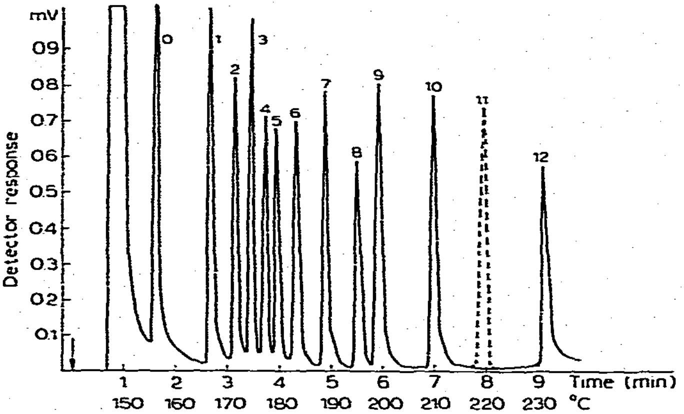

Fig. 2. Chromatogram of thioalkylbenzoates: 1 = methyl; 2 = ethyl; 3 = isopropyl; 4 = *n*-propyl; 5 = *tert.*-butyl; 6 = isobutyl; 7 = *n*-butyl; 8 = isoamyl; 9 = *n*-amyl; 10 = *n*-hexyl; 11 = *n*-heptyl; 12 = *n*-octyl; 0 = benzoyl chloride. Position of peak 11 was taken from Fig. 4. Conditions of separation are given in Table I.

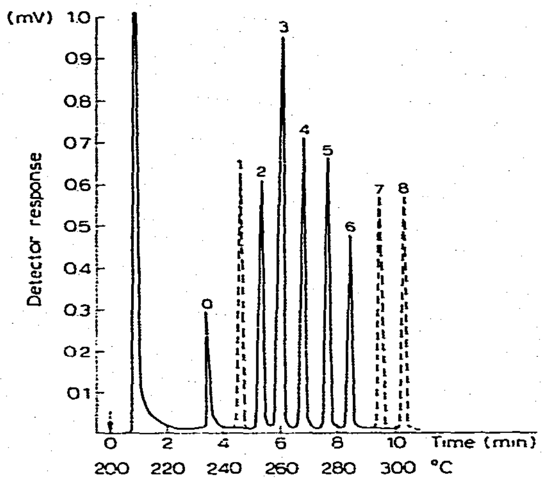

Fig. 3. Chromatogram of 2,4-dinitrophenylalkylthioethers: 1 = methyl + *tert.*-butyl; 2 = ethyl + isopropyl; 3 = *n*-propyl; 4 = *n*-butyl; 5 = *n*-amyl; 6 = *n*-hexyl; 7 = *n*-heptyl; 8 = *n*-octyl; 0 = 1-chloro-2,4-dinitrophenol. Positions of peaks 1, 7 and 8 were taken from Fig. 4. Conditions of separation are given in Table I.

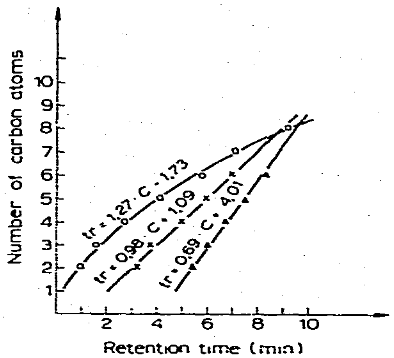

Fig. 4. Correlations between retention time and number of carbon atoms: ○, *n*-mercaptans; ×, *n*-thioalkylbenzoates; △, *n*-2,4-dinitrophenylalkylthioesters.

mercaptans. In our work, we were able to separate some other normal and branched mercaptan benzoates. The order of the peaks on the chromatogram for normal and branched mercaptan benzoates was the same as was found for normal and branched alcohol benzoates[11] and phenylhydrazones of carbonyls[12]. The retention times of secondary and isomercaptan benzoates were lower than those of normal mercaptans. Tertiary mercaptan benzoates showed the lowest retention times. These results can be explained in terms of the length of the carbon chain and molecular volume.

For normal mercaptans separated on a PEG 1000 column, the retention time was not a linear function of the number of carbon atoms in the mercaptan molecule (Fig. 4). It can be explained by chemical interaction between free –SH groups and the PEG liquid phase. For 2,4-DNPT and thiobenzoates, there was a linear correlation between the number of carbon atoms in the mercaptan chain and the retention time (Fig. 4). Possibly the same dependence could be found for branched mercaptan derivatives as was found for alcohols[11].

In the preparation of benzoylthioesters, it is possible to use either pyridine in an anhydrous medium as a catalyst or aqueous sodium hydroxide. Gasco and Barrera[11] prepared benzoates with pyridine in anhydrous benzene extracts of the volatile concentrate from distillates, which required about 20 h. In order to shorten the procedure, we tried both possibilities. To accelerate the reaction under anhydrous conditions, we kept the samples in sealed tubes on a boiling water bath for 15 min (ref. 10). This time was, however too short to decompose benzoyl anhydride, which appears on the chromatogram in almost the same position as *n*-heptyl thiobenzoate. Pyridine and an excess of benzoyl chloride do not affect the separation as they appear in front of the chromatogram, before methyl thiobenzoate.

Benzoyl anhydride was decomposed either by heating the sample for 5 min at 100° after adding water or by shaking for 1 min at room temperature with 0.1 *N* sodium hydroxide solution, added after completing the benzoylation reaction under anhydrous conditions. Under the above benzoylation conditions, the yield of the reaction was about three times lower for secondary than for normal mercaptans. When the reaction was carried out in sodium hydroxide solution at 100° for 15 min, the yield of the reaction was about twice as high for *n*-mercaptans and six times higher for secondary mercaptans than under anhydrous conditions. Benzoyl chloride and benzoyl anhydride were completely decomposed. The yield of the reaction in aqueous solution depended on the concentration of sodium hydroxide and increased when the concentration increased from 0.1 to 0.5 *N*, and then remained unchanged up to a concentration of 2.5 *N*.

While prolonging the time of reaction at 100° from 5 to 60 min, the yield in the benzoylation of the short-chain mercaptans slightly decreased, probably owing to decomposition of the mercaptans or their derivatives. Longer chain mercaptan derivatives were stable. When the benzoylation was carried out at the room temperature a reaction time between 5 and 90 min had no effect on the yield of benzoylation, which was the same for normal and secondary mercaptans and very similar to that obtained at 100°. The only difference was that benzoyl chloride and benzoyl anhydride were decomposed only after reaction for 60 min.

Summarizing our results, it can be concluded that benzoylthioesters could be suitable derivatives for the GLC separation and studies of the composition of the multi-component mercaptan mixtures. Rapid and simple benzoylation in aqueous solutions and complete solvent extraction of the derivatives enables sufficiently concentrated solutions for GLC and other analytical techniques to be prepared. The addition of an internal standard might make this method suitable for the quantitative determination of the composition of mercaptans in various materials, *e.g.*, food products.

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