

Gas-liquid chromatography of 2-chloroethyl esters using a detector specific for halogens

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ABSTRACT The gas-liquid chromatography of 2-chloroethyl derivatives of short- and long-chain monocarboxylic acids, and of short-chain dicarboxylic acids and aldehydes has been studied with a dual-flame ionization detector that provided simultaneous records of the concentrations of chlorine and of total organic material in the column effluent. The chloroethyl derivatives of dicarboxylic acids and aldehydes could be distinguished from the chloroethyl esters of monocarboxylic acids on the basis of different ratios of responses of the two detectors. Analysis of a mixture of chloroethyl derivatives prepared from a natural product revealed that several compounds that might ordinarily have been identified as unsaturated or branched-chain fatty acid esters on the basis of their retention times had the higher chlorine content characteristic of chloroethyl derivatives of aldehydes or dicarboxylic acids.

KEY WORDS gas-liquid chromatography · chloroethyl derivatives · mono- and dicarboxylic acids · aldehydes · flame ionization detector specific for halogens

GAS-LIQUID CHROMATOGRAPHY (GLC) of short-chain fatty acids as esters of 2-chloroethanol has been described by Oette and Ahrens (1). They pointed out that chloroethyl esters of short-chain acids were detected with more uniform sensitivity by an argon ionization detector than were methyl or ethyl esters, and, since they were less volatile, could more easily be concentrated from a dilute solution for injection into a GLC column. Derivatives of chloroethanol offer another advantage if a detector specific for halogen is used, for the response of the detector will then be directly proportional to the number of carboxyl or other groups that can react with chloroethanol. A sensitive dual-flame ionization detector has been developed (2) which supplies two simul-

taneous outputs, one that is a measure specifically of the concentration of halogen or phosphorus in the flame gas, the other being the usual output of a flame ionization detector. This paper describes the GLC of chloroethyl derivatives with this detector. The primary objective of this study was to determine the utility of the qualitative as well as quantitative information provided by these simultaneous outputs in analyses of complex mixtures.

MATERIALS AND METHODS

Dual-Flame Detector

The flame ionization detector specific for halogens has been described in detail previously (2). A platinum wire mesh screen that had been dipped into a solution of rubidium chloride was mounted above the flame of a hydrogen flame ionization detector. The presence of halogens or phosphorus in the flame gases increased the rate of volatilization of the rubidium from the screen. The vapor pressure of the rubidium was monitored by flame ionization in a second flame mounted just above the heated area of the screen. While the electrical conductivity of the lower flame was a function of the concentration of organic material in the flame gases, the electrical conductivity of the upper flame, which received no unburned organic material, changed only in response to a change in the concentration of rubidium and therefore only to a change in the concentration of halogen or phosphorus. The electrical conductivity of each flame was monitored in the conventional way, and both were recorded simultaneously by a two-pen, strip chart recorder.

Columns

The GLC columns used were straight glass ones, 144 cm \times 4 mm I.D., and contained either ethylene glycol adipate polyester (EGA) (15% by weight) or General

Abbreviations: GLC, gas-liquid chromatography; EGA, ethylene glycol adipate polyester. Fatty acids are designated by number of carbon atoms: number of double bonds.

Electric Company SE-33 silicone gum (1% by weight) on Chromosorb W (Analabs, Inc., Hamden, Conn.).

2-Chloroethanol

The chloroethanol as supplied (Eastman Chemical Products, Inc., Rochester, N.Y.) contained appreciable quantities of a halogen-containing impurity with a retention time on the EGA column similar to that of chloroethyl butyrate. Oette and Ahrens identified this compound as β,β' -dichlorodiethyl ether that had been formed by condensation of the chloroethanol (1). In the esterification procedure, this compound was extracted into the hydrocarbon phase and concentrated along with the chloroethyl esters, so that it interfered in the analysis. Redistillation of the chloroethanol at atmospheric pressure increased rather than decreased its concentration. The impurity could be removed by repeated extraction of the chloroethanol with hexane (extraction ten times with an equal volume of hexane was the most effective procedure). Since the concentration of this impurity increased with time, only several days' supply of chloroethanol was prepared at one time.

Esterification

Chloroethyl esters of short- and long-chain monocarboxylic acids and of short-chain dicarboxylic acids, and chloroethyl acetals of short-chain aldehydes were prepared by heating 10-mg mixtures of either the free fatty acids or their methyl esters in 1 ml of chloroethanol-sulfuric acid 98:2 for 2 hr at 60°C in a gas-tight, screw-cap tube with a Teflon liner in the cap. 1 ml of water was then added, and the chloroethyl esters were extracted three times with 2-ml aliquots of petroleum ether. The combined extracts were then washed three times with 4 ml of water, dried by the addition of sodium sulfate, and reduced in volume by evaporation in a stream of nitrogen, and aliquots were injected into the column. The time course and completeness of the reaction were determined from the record made by the conventional hydrogen flame detector. The yields of chloroethyl derivatives of different compounds were compared after differences in the responses of the flame detector to different organic molecules had been corrected for by a procedure based on the work of Sternberg, Gallaway, and Jones (3), as follows.

Correction of Response of Hydrogen Flame Detector

It was assumed that each carbon in each molecule, except the carboxyl carbon, was detected with equal sensitivity; that the carboxyl carbon was not detected at all; and that the chlorine had no disturbing effect. The weight of each chloroethyl ester, $C_nH_{2n-1}COOC_2H_4Cl$, in the sample was divided by its molecular

weight and multiplied by $n + 2$. Each observed peak area was divided by the value thus obtained and the resulting quotients were compared.

RESULTS

Synthesis of Chloroethyl Derivatives

Although the rates of reaction of different classes of compounds with chloroethanol differed, all the reactions were complete after 2 hr at 60°C. Esterification of both short-chain (2:0-8:0) and long-chain (12:0-18:0) monocarboxylic acids was complete after 1 hr; esterification of free dicarboxylic acids and formation of dichloroethyl acetals of aldehydes (6:0, 7:0, and 8:0) required 2 hr, as did the synthesis of chloroethyl esters from long-chain acids and from methyl esters by transesterification. The quantities of derivatives formed from these compounds remained constant for at least an additional 3 hr. A reaction time of 2 hr was therefore used. Although the amount of product did not decrease during this time period, the solutions (particularly of aldehydes) discolored slightly after 2 hr at 60°C and increasingly thereafter. At 80°C, all solutions became markedly discolored after 2 hr. There was no detectable formation of β,β' -dichloroethyl ether after 4 hr at 60°C, and only trace quantities after overnight heating at this temperature. At 80°C or higher, an appreciable amount of the ether was formed in 1 hr.

Comparison of the response of the hydrogen flame detector to equal quantities of hexadecane, added to the chloroethanol immediately before extraction as an internal standard, and of chloroethyl esters of long-chain fatty acids indicated that the yield of the esters was quantitative (Table 1).

TABLE 1 COMPARISON OF HYDROGEN FLAME IONIZATION DETECTOR RESPONSE TO HEXADECANE AND TO CHLOROETHYL ESTERS

Compound	Peak Area (Uncorrected)
Hexadecane	626
Chloroethyl ester of	
12:0*	613
14:0	661
16:0	669
18:0	672

The mixture was prepared by reaction of equal weights of each of the four acids in chloroethanol- H_2SO_4 , addition of an equal weight of hexadecane, and extraction of the esters. The sample injected into the GLC column contained about 10 μ g of each compound and was recorded at 10^{-8} ampere = 250 mm (full scale) with a chart speed of 25.4 mm/min.

* Fatty acids are designated as chain length : number of double bonds.

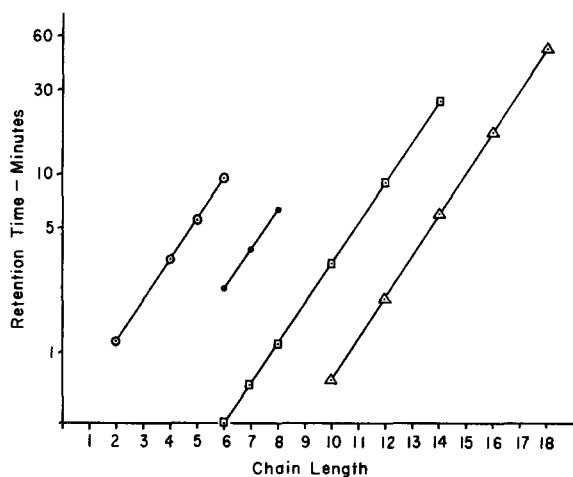


FIG. 1. Retention times of chloroethyl esters of monocarboxylic acids (\square), dicarboxylic acids (\circ), and chloroethyl acetals (\bullet), and methyl esters of monocarboxylic acids (\triangle) on a column of 1% SE-33 on Chromosorb W at 130°C. Retention time (logarithmic scale) vs. chain length of the acid or aldehyde.

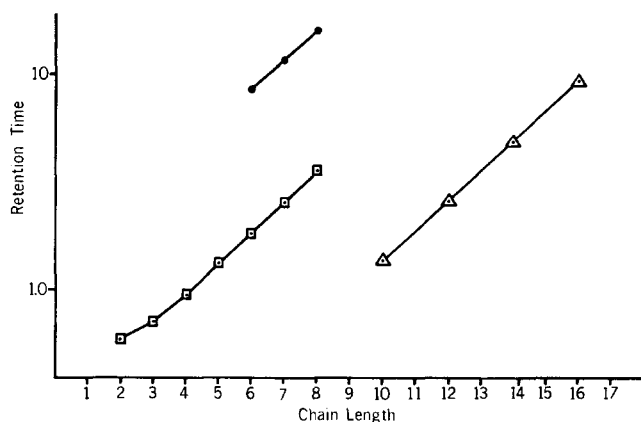


FIG. 2. Retention times of chloroethyl esters of fatty acids (\square), chloroethyl acetals (\bullet), and methyl esters of fatty acids (\triangle) on a column of 14% EGA polyester on Chromosorb W at 190°C.

Retention Times

The retention times for various derivatives on the two kinds of columns are plotted on Figs. 1 and 2, in which the retention times of fatty acid methyl esters are also plotted for comparison.

Flame Detector Responses to Chloroethyl Derivatives

The responses of the lower (conventional) flame detector per unit weight of short-chain monocarboxylic acid esters, corrected as described above, are given in Table 2. The responses to acetic, propionic, and butyric esters were consistently lower than those to the longer-chain esters (5:0–8:0), which were all the same. Fig. 3 shows the double GLC tracing.

In these analyses, the response of the halogen detector (upper flame) was, as predicted, proportional to the

number of moles of acid present. The response of both flames to chloroethyl propionate was low (Table 3) which indicated the presence of a smaller quantity than expected from the amount of propionic acid used. The ratio of the response per unit weight of the upper, halogen-sensitive flame to that of the lower flame decreased with increase in the chain length of the ester. The larger molecules, with more methylene groups per carboxyl, and therefore per chlorine, evoked a greater response per unit weight in the lower flame and a lesser response in the upper flame.

The predicted response to dicarboxylic acids was also observed. The ratio of responses of the two flames (upper: lower) to di-(chloroethyl)succinate was 0.8, compared to

TABLE 2 YIELDS OF CHLOROETHYL ESTERS OF SHORT-CHAIN MONOCARBOXYLIC ACIDS AFTER DIFFERENT REACTION PERIODS JUDGED FROM THE CORRECTED HYDROGEN FLAME DETECTOR RESPONSES

Chloroethyl Ester of	Relative Peak Areas			
	Hours of Incubation:			
	1	2	4	16
2:0	4.9	4.5	4.8	2.3
3:0	7.7	7.7	8.2	6.0
4:0	8.4	9.7	10.3	8.3
5:0	10.5	10.3	11.0	9.8
6:0	11.2	10.9	11.0	10.6
7:0	11.6	10.9	11.6	11.3
8:0	11.2	10.9	11.0	11.3

The chloroethyl esters were prepared by reaction of a mixture containing equal weights of each acid with chloroethanol at 60°C for the time specified. The values were derived from the areas of the peaks produced by the lower (conventional) flame detector as follows: the same quantity of methyl laurate was added to each esterification mixture, just before extraction, as an internal standard, and the areas of the peaks of the esters on each record were divided by the area of the methyl laurate peak. Each quotient was then divided by a factor proportional to the product of the number of moles of acid present in the original mixture and the number of detectable carbon atoms per mole.

TABLE 3 RESPONSE OF UPPER AND LOWER FLAME DETECTORS TO A MIXTURE OF CHLOROETHYL ESTERS OF EQUAL WEIGHTS OF SHORT-CHAIN ACIDS

Chloroethyl Ester of	Upper Flame Response per Mole	Lower Flame Response per Mole of Detectable Carbon
3:0	86 \pm 2	50 \pm 10
4:0	98 \pm 2	47 \pm 2
5:0	100 \pm 3	49 \pm 1
6:0	100 \pm 1	48 \pm 2
7:0	110 \pm 2	48 \pm 2
8:0	100 \pm 1	48 \pm 4

The areas of the peaks produced by the upper flame were each divided by a factor proportional to the number of moles of the corresponding acid present in the original mixture. The areas in the record produced by the lower flame were similarly normalized by dividing by a factor proportional to the number of moles of detectable carbon present in the original mixture.

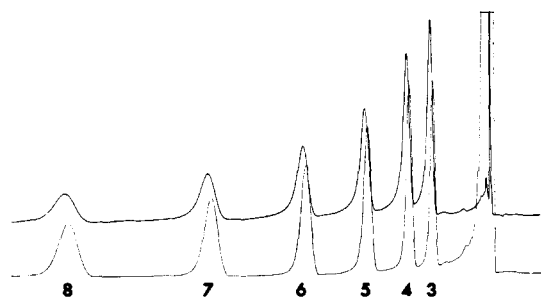


FIG. 3. GLC of chloroethyl esters of short-chain fatty acids prepared from a mixture containing approximately equal weights of the various acids (numbers represent chain length). The upper record shows the output of the upper, halogen-sensitive flame detector; the lower record shows the output of the lower, conventional flame detector. The full scale sensitivity was 3×10^{-8} amp on both records. The sample injected was derived from about $10 \mu\text{g}$ of each acid.

0.25 for chloroethyl decanoate. The expected ratio (upper:lower) for the succinate diester is proportional to the number of chlorine atoms, 2, divided by the number of detectable carbon atoms, 6, per molecule, or 1:3; the expected ratio for chloroethyl decanoate, derived similarly, is 1:11. The calculated ratio of upper:lower for succinate to upper:lower for decanoate is 3.67. The ratio found was 3.2.

In analyses of mixtures of long-chain fatty acid chloroethyl esters of known composition on the 15% EGA column at 195°C , the peak areas per unit weight for the oleate and stearate were consistently lower than those for the palmitate. The responses of both flames were equally affected. When the same sample was analyzed on a 1% SE-33 column at a lower column temperature (150°C), the peak areas per unit weight for the longer-chain esters were as high as those for the shorter-chain esters. The disproportionately low responses to the long-chain esters in the analyses on the EGA column were therefore attributed to losses on the column, which were probably related to the high temperature of the column and the relatively longer time for which the long-chain esters were exposed to the high temperature.

The retention times of chloroethyl derivatives of carboxylic acids and aldehydes corresponded to those of monocarboxylic esters of appreciably greater chain length. When they were all present in a given mixture, the derivatives of the aldehydes and dicarboxylic acids were easily distinguished on the dual flame record not only because of their higher halogen content, but also because of the relatively lower response of the lower flame.

In an analysis of a mixture of chloroethyl derivatives prepared from a commercial facial soap, three kinds of compounds could be distinguished (Fig. 4). The sample was prepared by first converting the fatty acid salts to methyl esters and then heating the methyl esters with

chloroethanol- H_2SO_4 , as described above. One class of compounds, less than 1% of the total, consisted of unreacted methyl esters (labeled "m" in the figure), identified by their retention times as well as by their failure to elicit responses in the upper flame. The second class of compounds comprised the chloroethyl esters of the fatty acids, detected by both upper and lower flames. These esters were in the same relative proportions as the methyl esters in the original mixture. The third class of compounds (labeled "E" in the figure) had higher ratios of upper:lower flame responses than the chloroethyl esters and was possibly comprised of acetals or diesters of dicarboxylic acids. Several of these compounds would ordinarily have been identified as unsaturated or branched-chain esters on the basis of their retention times.

DISCUSSION

The 2-chloroethyl esters of monocarboxylic acids and of short-chain dicarboxylic acids, and 2-chloroethyl acetals of aldehydes can be simply and quantitatively made from either the free acid or the methyl ester. The synthesis requires somewhat more attention to detail than does the synthesis of methyl esters; 1-2 hr at 60°C is required for a complete reaction. Some charring occurs and significant quantities of chlorine-containing impurities form when chloroethanol is heated much longer or at higher temperatures. Because of these difficulties, the preparation of chloroethyl derivatives of fatty acids by direct transesterification of the fatty acids in triglycerides was not studied. Methyl esters can be prepared from these lipids easily and can then be converted to their chloroethyl counterparts.

Detection of fatty acid chloroethyl esters with a halogen detector yielded a response proportional to the number of carboxyl groups, a "molar" response in the case of mono-

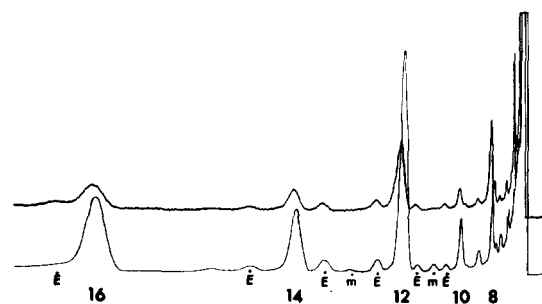


FIG. 4. GLC of a mixture of chloroethyl derivatives prepared from a sample of a commercial facial soap on a 14% EGA column. Above, the output of the halogen-sensitive detector; below, that of the conventional flame detector. Full scale sensitivity on the upper record was 10^{-9} and on the lower, 3×10^{-9} amp full scale. The peaks labeled 8, 10, 12, etc. were chloroethyl esters of the corresponding chain length; those labeled "m" were methyl esters; those labeled "E" contained more halogen per unit weight than did chloroethyl esters of monocarboxylic acids.

carboxylic acids. Although a number of methods of chlorine detection have now been described which could presumably be used for this purpose, none is as sensitive as the conventional hydrogen flame ionization detector. Oette and Ahrens (1) found that the electron capture detector was not very sensitive to chloroethyl esters. We have confirmed this observation. Since the response of the usual ionization detectors to the various fatty acids can generally be easily calibrated, one may reasonably question whether a detector that responds in proportion to the number of molecules rather than to their weight or to some other physical property would offer any significant practical advantage. On the other hand, the analysis shown in Fig. 4 demonstrates that simultaneous recording of two molecular properties can be helpful for qualitative identification of compounds in a GLC effluent as well as for their quantification. Since almost every GLC detector responds differently, many combinations of detectors can be and have been used. Results that are easiest to interpret are obtained when the two or more detectors have predictable responses for different compounds, rather than different responses for each individual compound, and when the response of each detector is specific and independent of that of the other. Although compounds are

identified with very high precision only by using detectors such as mass, UV and IR spectrometers that are so specific that chromatography is unnecessary, the recording of two distinct molecular properties represents a step in the direction of greater precision.

Compared to methyl or ethyl esters, the lesser volatility of the chloroethyl derivatives is helpful in analysis of short-chain compounds but is troublesome in the analysis of long-chain esters. The difficulties in the latter case may perhaps be resolved by the same techniques found useful in GLC analysis of steroids, including the use of lightly coated solid supports operated at relatively low temperatures.

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