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GAS CHROMATOGRAPHIC ANALYSIS OF ALIPHATIC AND AROMATIC ALDEHYDES AS TRIMETHYLSILYLATED DITHIOACETALS OF 2-MERCAPTOETHANOL

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SUMMARY

A new general method has been devised for the gas chromatographic analysis of aldehydes as their trimethylsilylated dithioacetal derivatives of 2-mercaptoethanol. The sequential derivatization reactions of mercaptalation and trimethylsilylation allow rapid and convenient analysis of both aliphatic and aromatic aldehydes with high accuracy and precision. A few applications of this method to products of enzymatic reactions are presented.

INTRODUCTION

Direct gas chromatography (GC) may be unprofitable for the analysis of aldehydes because of tailing of peaks and instability of samples. Recently we reported a convenient method for gas chromatographic analysis of monosaccharides[1,2] and conjugated aldehydes in products of periodate oxidation of carbohydrates[3,4] as their trimethylsilylated diethyl dithioacetals. This method gives a single peak for each aldehyde, unlike the hydrazone and oxime methods[5] which give dual peaks of steric isomers, and the derivatization procedure is simple and rapid. Further, the flame photometric detector is selectively sensitive to the dithioacetal derivatives. However, this method is not suitable for the analysis of aldehydes of small molecules, since the derivatives are extremely volatile, and ethanethiol has a pungent odour. This paper describes a new general method for the analysis of aliphatic and aromatic aldehydes by use of 2-mercaptoethanol, and odourless mercaptan.

EXPERIMENTAL

Materials

An extra pure sample of 2-mercaptoethanol was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). The authentic sample of the dithioacetal of *n*-octanal was prepared by mercaptalation of *n*-octanal with 2-mercaptoethanol in the presence of trifluoroacetic acid, followed by purification of the crude product on a column of silica gel with diethyl ether–*n*-hexane (1:1, v/v). Proton magnetic resonance (PMR)

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data ($CDCl_3$,δ) in ppm: 0.90 (triplet-like, $-CH_3$), 1.3 (multiplet, $C-CH_2-C \times 6$), 2.55 (broadened singlet, $OH \times 2$), 2.83 (triplet, $-S-CH_2-$), 2.87 (triplet, $-S-CH_2-$), 3.80 (triplet, $-CH_2-OH \times 2$), 3.8 (triplet-like, $-C-CH-S$). The refined sample as its trimethylsilyl derivative gave a single peak on GC. All other chemicals, solvents and samples of aldehydes were of the highest grade commercially available. The specimens of alcohol dehydrogenase (yeast, 322 U/mg) and monoamine oxidase (beef plasma, 25 U/mg) were obtained from Sigma (St. Louis, MO, U.S.A.) and Miles Labs. (Elkhart, IN, U.S.A.), respectively.

Apparatus

Gas chromatography was performed on a Shimadzu 4BMPF instrument equipped with a flame ionization detector (FID). The flow-rate of the carrier gas (nitrogen) was 50 ml/min.

Recommended procedure for the analysis of aldehydes

Dissolve a sample of an aldehyde or a mixture of aldehydes (total amount, 10^{-8}–10^{-6} mole) in 1,2-dichloroethane (50 μl). Add a 4:1 (v/v) mixture (20 μl) of 2-mercaptoethanol and trifluoroacetic acid, and keep the mixture for 30 min at 25°C. Add a pyridine solution (50 μl) of D-glucitol (internal standard), hexamethyldisilazane (100 μl) and chlorotrimethylsilane (50 μl) in this order, and incubate the mixture for 30 min at 50°C with occasional shaking. Centrigufe the mixture, and analyze the aldehydes by injecting the supernatant (1–5 μl) to the gas chromatography column. Standard column conditions are as follows: column, 3% OV-1 on Chromosorb W (2 m, glass); column temperature, 180°C (aliphatic aldehydes) or 210°C (aromatic aldehydes); flow-rate of carrier gas (nitrogen), 50 ml/min; detector FID (240°C).

Enzymatic oxidation of alcohols with dehydrogenase

A 0.015 *M* nicotine–adenine dinucleotide (1.5 ml), 0.05 *M* pyrophosphate buffer (pH 8.5, 1.3 ml) and a solution (0.1 ml) of alcohol dehydrogenase in 0.01 *M* phosphate buffer (pH 7.5) were added to a sample solution (0.1 ml) of ethanol or to a mixture of alcohols, and the reaction mixture was kept for 20 min at 25°C. Then the mixture was extracted with 1,2-dichloroethane (0.5 ml), and a 50 μl-portion of the organic layer was subjected to aldehyde analysis as described above.

Assay of the activity of monoamine oxidase

An aqueous solution (0.1 ml) of a mixture of substrate amines (each 10^{-5} mole) and 0.2 *M* phosphate buffer (pH 7.4, 2.80 ml) were added to a sample solution (0.1 ml) of monoamine oxidase, and the mixture was kept for 1 h at 25°C. Then the mixture was extracted with 1,2-dichloroethane (500 μl), and a 50 μl-portion of the organic layer was subjected to aldehyde analysis as described above.

RESULTS AND DISCUSSION

Table I gives the retention times of the trimethylsilylated dithioacetals of straight-chain aliphatic aldehydes, measured on various kinds of liquid phase. These derivatives were well separated on all the silicone phases at the given temperatures in *ca.* 1.5 h. The polyethylene glycol succinate column, however, gave

TABLE I

RETENTION TIMES OF THE DERIVATIVES OF STRAIGHT-CHAIN ALIPHATIC ALDEHYDES

Aldehyde	*Retention time (min)*			
	3% OV-1, 2 m, 180°C	*3% OV-17, 2 m, 200°C*	*10% SF-96, 2 m, 200°C*	*15% PEG succinate, 2 m, 145°C*
Methanal	—	7.56	—	—
Ethanal	6.81	7.69	7.35	11.48
Propanal	8.10	9.04	9.26	12.37
n-Butanal	10.57	11.26	11.72	14.37
n-Pentanal	14.47	14.89	15.54	18.36
n-Hexanal	19.94	20.87	21.41	24.14
n-Heptanal	29.24	28.33	29.53	33.13
n-Octanal	40.93	39.84	41.07	46.14
n-Nonanal	61.41	56.32	58.01	64.55
n-Decanal	88.00	79.44	81.98	90.32

broadened peaks, especially for the derivatives of higher aldehydes, and this tendency was common to other polar phases.

Fig. 1 shows the relationship between the number (n) of carbon atoms and the logarithm of the retention time (t'_R) adjusted for that of the solvent (1,2-dichloroethane). Good linearity was observed for all the liquid phases for n values between 4 and 10.

The dithioacetal derivatives of aromatic aldehydes were also well separated on silicone columns. Table II gives an example of separation of the derivatives of several substituted benzaldehydes, together with those of phenylacetaldehyde and cinnamaldehyde.

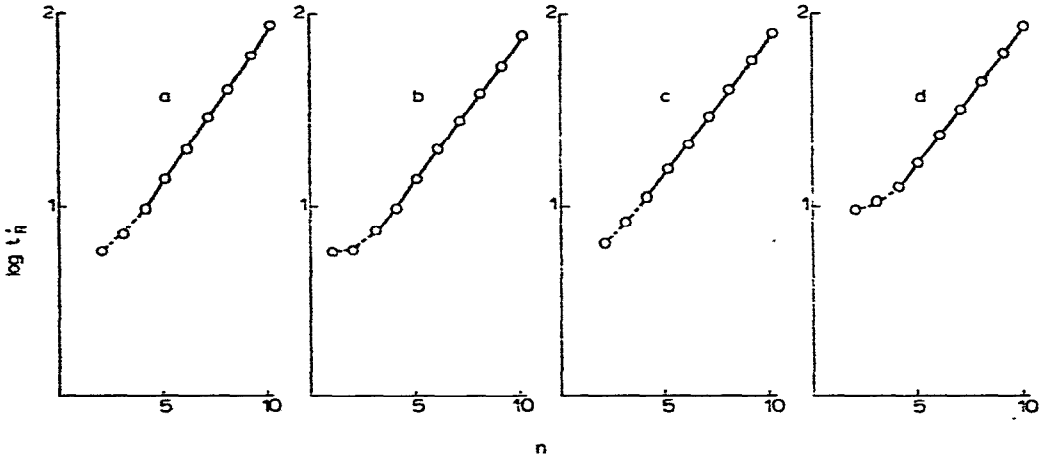
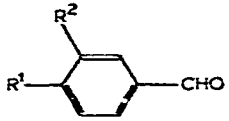


Fig. 1. Relationship between the number (n) of carbon atoms and the adjusted retention time (t'_R) for straight-chain aliphatic aldehydes. Phases: a = 3% silicone OV-1; b = 3% silicone OV-17; c = 10% silicone SF-96; d = 15% polyethylene glycol succinate.

TABLE II

RETENTION TIMES OF THE DERIVATIVES OF SELECTED AROMATIC ALDEHYDES

Aldehyde		*Retention time (min) on 3% OV-1, 2 m, 210°C*
Benzaldehyde		8.21
Substituted benzaldehyde		
	$R^1 = OH, R^2 = H$	21.43
	$R^1 = R^2 = OH$	31.93
	$R^1 = OCH_3, R^2 = OH$	28.16
	$R^1 = R^2 = OCH_3$	24.54
Phenylacetaldehyde		10.65
Cinnamaldehyde		18.99

The derivatization procedure of the present method consists of the mercaptalation process with 2-mercaptoethanol and the subsequent trimethylsilylation process.

$$CH_3(CH_2)_6CHO \rightarrow CH_3(CH_2)_6CH(SCH_2CH_2OH)_2 \quad (1)$$

$$CH_3(CH_2)_6CH(SCH_2CH_2OH)_2 \rightarrow CH_3(CH_2)_6CH(SCH_2CH_2OTMS)_2 \quad (2)$$

The first process is affected by various factors. Therefore, an optimization study was performed by using *n*-octanal as the model aldehyde. The PMR spectrum of the product of mercaptalation gave a methine proton at *ca.* 3.8 ppm, indicative of the presence of a dithioacetal bond. An acetal bond would give its methine proton at lower field at *ca.* 5 ppm. This evidence confirms that the product was the dithioacetal, but not the acetal, of *n*-octanal. The methylene protons adjacent to the sulphur atom are split into a couple of two-proton triplets at 3.83 and 3.87 ppm, presumably due to restricted rotation of the S–CH_2 bond.

The dithioacetal formed gave a single GC peak of presumably the bis(trimethylsilyl) ether of the dithioacetal on treatment with chlorotrimethylsilane and hexamethyldisilazane in pyridine. Table III shows the influence of reaction solvent on mercaptalation, expressed as the yield of the trimethylsilylated dithioacetal

TABLE III

INFLUENCE OF REACTION SOLVENT ON THE MERCAPTALATION OF *n*-OCTANAL

Solvent	*Yield (%) of the dithioacetal derivative*
Diethyl ether	0.0
Diisopropyl ether	0.7
Ethyl acetate	25.1
Carbon tetrachloride	46.7
Toluene	47.2
Chloroform	47.8
Benzene	51.7
n-Hexane	88.6
1,2-Dichloroethane	100.6

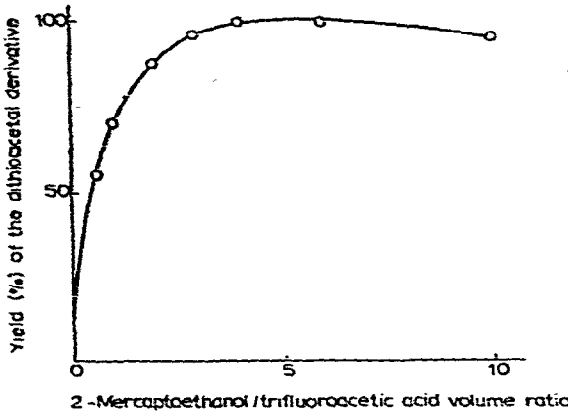


Fig. 2. Influence of the 2-mercaptoethanol: trifluoroacetic acid volume ratio on the yield of the derivative of *n*-octanal. Reaction temperature, 25°C; reaction time, 30 min.

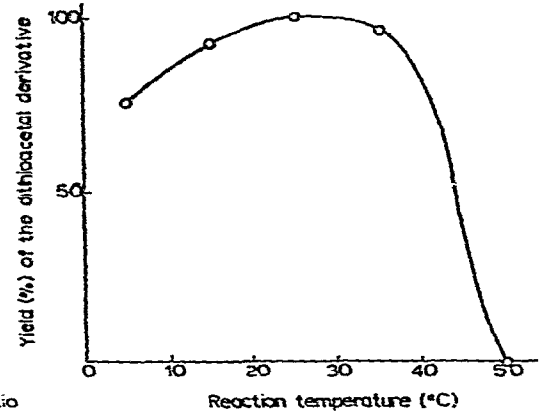


Fig. 3. Effect of reaction temperature on the yield of the dithioacetal derivative of *n*-octanal. Volume ratio of 2-mercaptoethanol to trifluoroacetic acid, 4; reaction time, 30 min.

derivative. 1,2-Dichloroethane gave almost quantitative yields of the dithioacetal derivative, whereas other solvents gave lower yields. The difference in yield among solvents is probably due to the difference in affinity of solvents toward the proton supplied by the acid catalyst. The most suitable catalyst proved to be trifluoroacetic acid; similar results were obtained for the determination of monosaccharides[1,2] and the aldehydes in products of periodate oxidation of carbohydrates[3,4]. The yield of the dithioacetal derivative increased with increasing volume ratio of 2-mercaptoethanol to trifluoroacetic acid, giving a quantitative yield between the volume ratios of 4 and 10, as shown in Fig. 2. Fig. 3 shows the effect of reaction temperature on mercaptalation. The maximal yield of the dithioacetal derivative was obtained at 25°C. Lower and higher temperatures were disadvantageous for dithioacetal formation. Fig. 4 shows the course of mercaptalation of *n*-octanal. The reaction was complete in 30 min at 25°C.

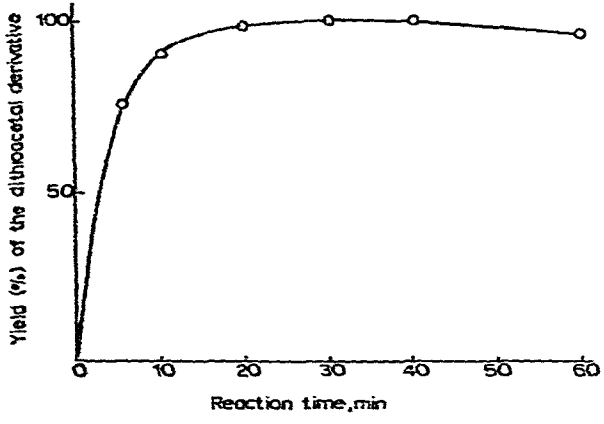


Fig. 4. Course of mercaptalation of *n*-octanal. Volume ratio of 2-mercaptoethanol to trifluoroacetic acid, 4; reaction temperature, 25°C.

On the basis of the results mentioned above, a recommended procedure was devised for the determination of aliphatic aldehydes and is described in the Experimental section. The analysis time was only 2 h, including the GC operation, significantly shorter than that required for the acetalation method[6]. Under the conditions described above, the calibration curve of *n*-octanal was linear for sample amounts ranging from 10^{-8} to 10^{-5} mole, and the coefficient of variation was 2.2% ($n = 10$) at the 10^{-7} mole level. Similar results were obtained for other aliphatic aldehydes. For aromatic aldehydes, the same reaction conditions were applicable, as substantiated by the average yield (99.6%) of the dithioacetal derivative of *p*-hydroxybenzaldehyde. Its calibration curve was also linear in the same range of sample amount.

Since the dithioacetal method thus developed is simple and rapid, it is widely applicable to both aliphatic and aromatic aldehydes, and suitable especially for those from biochemical sources. Fig. 5 shows an example of its application to the products of enzymatic reaction of alcohols with yeast alcohol dehydrogenase. Under the specified conditions, the product from a mixture of ethanol, *n*-propanol and *n*-butanol contained all the corresponding aldehydes, whose derivatives were well separated, as shown in Fig. 5a. The yield of each aldehyde increased, giving parabolic curves with increasing reaction time, and the yield increased with decrease in the number of carbon atoms (Fig. 5b). The calibration curve of ethanol obtained for the reaction time of 20 min was linear in the range of sample concentration, 0.1–1.5%. The use of a flame photometric detector will allow the determination of lower concentrations.

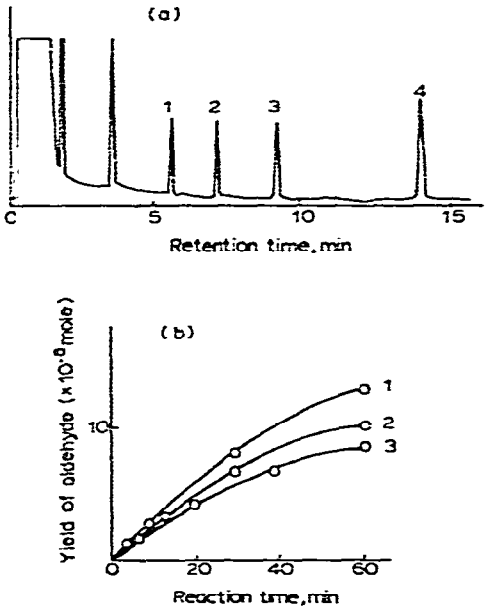


Fig. 5. a, Gas chromatogram for the products of enzymatic oxidation of a mixture of ethanol, *n*-propanol and *n*-butanol with yeast alcohol dehydrogenase. Peaks 1, 2, 3 and 4 are assigned to the trimethyl silylated dithioacetals of ethanal, propanal and *n*-butanal and trimethylsilylated D-glucitol (internal standard), respectively. b, Course of formation of aldehydes from an equimolar mixture of ethanol (1), *n*-propanol (2) and *n*-butanol (3).

Fig. 6 shows another example of application. Monoamine oxidase converts various bioamines into corresponding aldehydes. Based on substrate preferences, it has been claimed that there are two types of isozyme, monoamine oxidase A and B. The former type has a high affinity for serotonin and norepinephrine, while the latter is specific to benzylamine and phenylethylamine. Therefore, analysis of the products from a mixture of these amines will serve for classification of the type of isozyme and estimation of the enzyme activity. In the experiment reported herein, which used a mixture of these four bioamines as substrate, only the derivatives of benzaldehyde and phenylacetaldehyde were detected; no other aldehyde derivatives were found even at elevated temperatures. This evidence indicates that the enzyme used belonged to the B-type monoamine oxidase. The amounts of the aldehyde derivatives were also consistent with the activity described for this preparation.

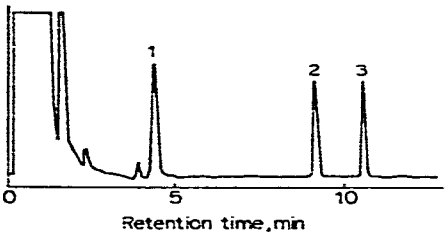


Fig. 6. Gas chromatogram for the products of enzymatic oxidation of a mixture of benzylamine, phenylethylamine, serotonin and norepinephrine with beef plasma monoamine oxidase. Peaks 1, 2 and 3 are assigned to trimethylsilylated D-glucitol (internal standard), the trimethylsilylated dithioacetal of benzaldehyde and the trimethylsilylated dithioacetal of phenylacetaldehyde, respectively.

The foregoing results demonstrate that the dithioacetal method described is useful for the determination of biochemical compounds related to aldehydes. Further applications of this method will be described elsewhere.

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