Use of Dimethylsilyl Ether Derivatives in Gas Chromatography

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Abstract

The results show the use of dimethylsilyl (DMS) derivatives to be valuable for: 1) decreasing retention times of samples being analyzed by gas chromatography; 2) conducting analyses at lower temperatures; 3) analyses of mixtures which cannot be separated as trimethylsilyl (TMS) derivatives; and 4) analyses of mixtures of isomers on one column as both DMS and TMS derivatives rather than using two columns with one derivative. The work reported here is only of a preliminary nature and does not include a study of the kinetics of the reaction. It is hoped that this paper will stimulate more work in this area.

Introduction

THE USE OF TRIMETHYLSILYL ether derivatives has extended gas chromatographic analysis to many compounds which could not be analyzed in their natural form either because of extremely long elution times from the chromatographic column or because of poor peak symmetry. Examples include amines (1), amino acids (8), bile acids (5), carbohydrates (7,10), glycols (10), inositols (4), and sterols (3,6). Wells, Sweeley and Bentley (11) have described the preparation of dimethylsilyl ether derivatives of glucose, mannose and galactose and have suggested additional study. The purpose of this paper is to show that the use of dimethylsilyl ether derivatives can further extend the area of chromatographic analysis by decreasing still more the time required to elute the samples. In addition, it may be possible to obtain separations of components which could not be separated as trimethylsilyl ethers since the relative retention times for dimethylsilyl ethers differ in some cases from those of the trimethylsilyl ethers.

Experimental

All of the gas chromatographic analyses were performed on a Barber-Colman model 5000 chromatograph equipped with an argon ionization detector. The columns were glass U-tubes, 6 ft × 4 mm I.D. filled with one of the following packings: (a) 3% SE-30 on 100/120 Gas-Chrom Q, (b) 4% OV-17 (a phenyl methylsilicone) on 60/80 Gas-Chrom Q, (c) 3% JXR on 100/120 Gas-Chrom Q, and (d) 15% Apiezon L on 100/120 Gas-Chrom P.

These packings were prepared by a combination of the filtration (9) and fluidization techniques (2). The reagents used for the preparation of trimethylsilyl ethers were trimethylchlorosilane (TMCS), hexamethyldisilazane (HMDS) and pyridine (Baker "Analyzed" Reagent Grade) in the following volumetric ratio: 1/3/9. The reagents for the dimethylsilyl ethers were dimethylmonochlorosilane (DMMCS) (*not* dimethyldichlorosilane), tetramethyldisilazane (TMDS) and pyridine, also in the ratio 1/3/9. We have tried other ratios successfully but have not studied the effect in detail. Most of the above materials and reagents used were those normally offered for sale by Applied Science Laboratories and were not materials made specifically for research on this project. The only exceptions were the pyridine solvents. Both reagents were used in a similar manner. Approximately 50 mg of sample was added to 1 ml of reagent, mixed thoroughly and allowed to stand for 10-30 min. In some cases the excess reagent was then evaporated with a stream of nitrogen and the sample redissolved in a suitable solvent and injected into the chromatograph. When the dimethylsilyl ethers were prepared, spurious peaks sometimes appeared in the chromatogram if the sample remained in presence of excess reagent overnight. We did not determine the optimum reaction time but this determination should be made.

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TABLE I
Alcohols

	Relative retention times			t_{DMS}/t_{TMS}
	Alcohol	DMS	TMS	
Dodecanol	0.17	0.19	0.19	0.86
Tetradecanol	0.42	0.43	0.44	0.84
Hexadecanol	1.00 (14.0 min)	1.00 (8.9 min)	1.00 (10.4 min)	0.86
Octadecanol	......	2.29	2.28	0.86

Column: 6 ft × 4 mm I.D., 15% Apiezon L on 100/120 Gas-Chrom P, column temperature 120C, nitrogen flow 60 ml/min.

TABLE II
Phenols

	Relative retention times		t_{DMS}/t_{TMS}
	DMS	TMS	
Phenol	1.00 (8.5 min)	1.00 (11.2 min)	0.76
o-cresol	1.73	1.74	0.76
m-cresol	1.85	1.78	0.79
p-cresol	1.98	1.98	0.76

Column: 6 ft × 4 mm I.D., 15% Apiezon L on 100/120 Gas-Chrom P, column temperature 120C, nitrogen flow 60 ml/min.

Results

Fatty Alcohols

Table I includes data on four alcohols, showing the retention times of the free alcohols and the DMS and TMS derivatives. The last column is the ratio of absolute retention times of the two types of derivatives. For a homologous series this should be a constant except for the smaller molecules in the series, for which small differences in functional groups, etc., usually have a more noticeable effect than for the larger molecule. In the case of the homologous series, the relative retention times for the two types of derivatives are essentially the same.

Phenols

The data in Table II show the DMS derivatives to be eluted approximately 25% faster than the corresponding TMS derivatives. Note that the *ortho* and *meta* isomers are better separated as DMS derivatives

TABLE III
Glycols

	Relative retention times		t_{DMS}/t_{TMS}
	DMS	TMS	
1,3-Propanediol	0.48	0.49	0.56
1,4-Butanediol	1.00 (9.0 min)	1.00 (15.8 min)	0.57
1,5 Pentanediol	1.95	1.89	0.59
Neopentyl glycol	0.63	0.57	0.63

Column: 6 ft × 4 mm I.D.; 15% Apiezon L on 100/120 Gas-Chrom P, column temperature 120C, nitrogen flow 60 ml/min.

TABLE IV
Carbohydrates

	Relative retention times		t_{DMS}
	DMS	TMS	t_{TMS}
Glucose	1.00 (5.10 min)	1.00 (11.4 min)	0.45
2-Deoxy-D-ribose	0.165	0.113	0.65
Arabinose	0.281	0.205	0.26
Xylitol	0.308	0.327	0.42
Fucose	0.314	0.256	0.55
Arabinitol	0.317	0.332	0.43
Phloroglucinol	0.317	0.200	0.71
Xylose	0.338	0.291	0.52
Ribitol	0.356	0.338	0.47
2-Deoxy-D-glucose	0.488	0.426	0.51
Mannose	0.548	0.475	0.52
Sorbitol	0.663	0.852	0.35
Galactose	0.690	0.590	0.52
Inositol	1.296	1.525	0.38

Column: 3% JXR on 100/120 Gas-Chrom Q, column temperature 175C, argon flow 40 ml/min at 17 psig.

than as TMS. It would be possible with an efficient column to analyze quantitatively all three isomers by doing two analyses, one with DMS derivatives and one with TMS derivatives rather than by doing the analyses on two different columns. This is an example of possible major use of these derivatives and should not be overlooked.

Glycols

Table III shows some data obtained for both DMS and TMS derivatives for several glycols. With two hydroxyl groups per molecule the retention times for the DMS derivatives are slightly more than half those for the corresponding TMS derivatives. Note from the third column of data that, as the size of the molecule increases, the ratio of absolute retention times DMS to TMS increases.

Carbohydrates

While there appears to be considerable variation in the ratio of absolute retention times in Table IV, closer examination reveals considerable consistency among similar compounds. Note that the retention time ratio for all of the anomeric forms (DMS to TMS) is approximately 0.52. The two main advantages of the use of DMS derivatives are illustrated in these data:

1) Use of DMS derivatives will permit faster analysis of carbohydrates than of corresponding TMS derivatives, and it will be possible to analyze considerably higher molecular weight carbohydrates as a result of the greatly reduced retention times.

2) Materials which cannot be separated as TMS derivatives possibly could be separated as DMS derivatives. As an example note the data for arabinitol and ribitol. Of course it is possible that the converse will also be true and that materials well separated as TMS derivatives might not be separated as DMS derivatives.

Alditols

An example of a shift in relative retention time is shown in Table V. Mannitol precedes sorbitol as a TMS derivative and is too close for good separation; as a DMS derivative mannitol is eluted afterward with good separation between the two. Again, the ability to change the *relative* retention times is proven to be of value for a difficult separation. There is essentially no change for the dulcitol.

TABLE V
Alditols

	Relative retention times	
	DMS	TMS
Sorbitol	1.00	1.00
Dulcitol	1.02	1.01
Mannitol	1.15	0.96
Inositol	1.95	1.85

3% SE-30 on 100/120 Gas-Chrom Q, 6 ft × 4 mm I.D., column temperature 160C.

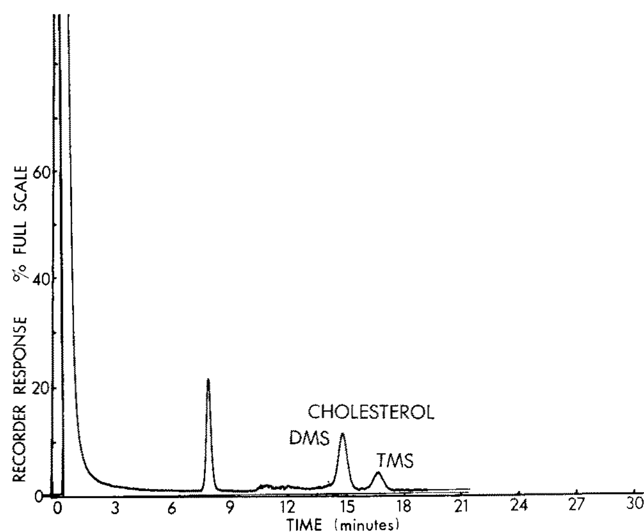

FIG. 1.

Sterols

For very large molecules with only one hydroxyl group there is not an appreciable difference in retention times. The data in Table VI show that the retention times of DMS derivatives are almost 95% of the values for the TMS ethers. However, a greater decrease would be expected for polyhydroxyl steroids.

It is interesting to note that the separation factors among the three sterols is essentially the same for their free forms and for both types of derivatives.

The chlorosilane used in the reagent for preparation of the silyl ethers is often referred to as the catalyst. This may well be, but it also enters into the reaction. Proof of this is shown in Fig. 1.

A sample containing cholestane and cholesterol was added to a mixture of pyridine, tetramethyldisilazane and trimethylchlorosilane. After 10 min the excess reagent was evaporated and the sample redissolved and analyzed on a column of 3% JXR on 100/120 Gas-Chrom Q at 247C. Note that both the dimethyl- and the trimethylsilyl ethers of cholesterol were formed with the latter being present in the smaller amount. When a similar experiment was performed with a reagent of pyridine, hexamethyldisilazane and dimethylchlorosilane, similar results were obtained but the ratio of DMS and TMS derivatives was reversed.

During the experimental work it become apparent that the preparation and use of DMS derivatives required somewhat more care than the TMS derivatives. In addition to the need for maintaining an anhydrous

TABLE VI
Sterols

	Relative retention times separation factors					
	Free	DMS	TMS	Free	DMS	TMS
Cholestane	1.00 (7.26 min)					
Cholesterol	2.55	1.89	1.99	2.55	1.89	1.99
7-Dehydro-cholesterol	3.01	2.21	2.33	1.18	1.17	1.17
Desmosterol	3.09	2.28	2.41	1.03	1.03	1.03

4% OV-17 60/80 Gas-Chrom Q, 6 ft × 4 mm I.D., column temperature 254C, flow 74 ml/min at 22 psig.

system prior to and during the derivative preparation, it was found that the DMS derivatives were readily hydrolyzed. It was also desirable to remove the excess reagent promptly; when the samples remained in contact with the reagent overnight, spurious peaks appeared.

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