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FLOPHEMESYL DERIVATIVES OF ALCOHOLS, PHENOLS, AMINES AND CARBOXYLIC ACIDS AND THEIR USE IN GAS CHROMATOGRAPHY WITH ELECTRON-CAPTURE DETECTION

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SUMMARY

Flophemesyl (pentafluorophenyldimethylsilyl) derivatives of alcohols, phenols, carboxylic acids and amines have been prepared and their suitability for determining trace amounts of these compounds by gas chromatography assessed. They are very sensitive to electron-capture detection (ECD) down to the picogram to femtogram range and have advantages over presently used ECD derivatives particularly for lower-molecular-weight compounds.

INTRODUCTION

In an investigation of fluorine-containing silylating reagents suitable for gas chromatography (GC) of sterols with electron-capture detection (ECD), we discovered the usefulness of pentafluorophenyldimethylsilyl (for convenience contracted to flophemesyl, Fig. 1) reagents[1]. By the use of flophemesylamine, the diethylamine, and flophemesyl chloride, sterol hydroxyl groups of various degrees of steric hindrance could be silylated and rendered suitably volatile for gas chromatography and detectable in picogram quantities[2]. Because the flophemesyl group affects the fragmentation of the sterol ethers in mass spectrometry, causing the formation of much stronger, high-mass hydrocarbon ions than is found with trimethylsilyl ethers, these derivatives are also very useful for the determination of sterol structure by GC–mass spectrometry[1,3], and for determination by single or multiple ion monitoring. Cholesterol heptafluorobutyrate is unstable to gas chromatography, eliminating heptafluorobutyric acid[4], whereas the flophemesyl ether of cholesterol is stable, with good peak shape and volatility[1].

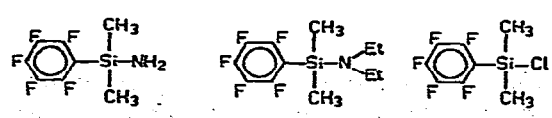

Fig. 1. Flophemesyl reagents.

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In a brief communication, we described the extension of flophemesyl reagents to the preparation of simple alcohol ethers[5]. We now describe in detail the preparation and properties of flophemesyl derivatives of alcohols, phenols, amines and carboxylic acids.

EXPERIMENTAL

Flophemesylamine (density 1.32 g cm^{-3}) flophemesyldiethylamine (1.16 g cm^{-3}) and flophemesyl chloride (1.30 g cm^{-3}) were obtained from Lancaster Synthesis Ltd. (St. Leonard Gate, Lancaster, Great Britain). Small quantities for immediate use were transferred from the sealed glass vials and stored at 0° in Hypo-vials (Pierce and Warriner, Chester, Great Britain), sealed with rubber septa. Reactions were carried out in 1-cm^3 Reacti-vials (Pierce and Warriner) sealed with PTFE coated discs and screw caps. At suitable times, 1-μl samples were withdrawn for GC. Analysis was performed on a Pye Series 104 gas chromatograph fitted with injection heaters, detector oven and flame ionization and electron-capture detectors. Two 5 ft. × 0.25 in. O.D. glass columns were used, the first filled with 10% SE-30 on Chromosorb P AW DMCS and the second with 3% OV-101 on Chromosorb W AW DMCS. Nitrogen carrier gas was used at 60 cm^3 min^{-1}. The ^{63}Ni electron-capture detector was operated with a pulse space of 150 μsec, pulse width 0.75 μsec and pulse height 47–60 V, at 300°. A pulse space of 200 μsec gave greatest sensitivity for detecting flophemesyl ethers but produced high background noise and negative solvent peaks. The nitrogen purge gas was turned off about 30 min before the detector was used.

General method of derivative formation

The compound (10 mg) was dissolved in toluene (30 μl) to which flophemesylamine (30 μl, 165 μmoles) was added slowly. If the compound was sparingly soluble in toluene or if reaction was slow, the mixture was heated to 60° in the closed Reacti-vial.

Phenol ethers were prepared by the general method, or at a faster rate by a catalysed reaction as follows. Flophemesylamine (15 μl, 82 μmoles) and flophemesyl chloride (15 μl, 75 μmoles) were added to a solution of phenol (10 μl, 110 μmoles) in diethyl ether (30 μl). The mixture was centrifuged before withdrawing samples. Reaction was complete in 5 min.

Esters were prepared by the general method or that used for phenols.

Amines were derivatized using the general method. Time for complete reaction was approximately 15 min. The addition of 30 μl of pyridine halved the reaction time.

Stability studies

The pure flophemesyl derivative (100 μl) was mixed with an equal volume of the test reagent in a 3-cm^3 stoppered glass centrifuge tube, thoroughly agitated and left at room temperature. From time to time 1-μl samples were withdrawn for analysis by GC.

Thin-layer chromatography

Separations were performed on 5 × 20 cm glass plates coated with a 0.3 mm layer of Kieselgel PF_{254} (Merck, Darmstadt, G.F.R.). Plates were activated at 120° for 1 h and cooled in a dry-box. Solvents were run 15 cm up the plates for development.

RESULTS

Flophemesyl reagents are very sensitive to moisture, and hydrolyse to flophemesyl disiloxane [1,3-bis(pentafluorophenyl)-1,1,3,3-tetramethyldisiloxane]. Some disiloxane is always produced as by-product of derivative formation; its presence does not interfere with the analysis of the products. To ensure complete silylation, a 25-fold excess of reagent was used. Flophemesyl ethers can be prepared from all the simpler alcohols by reaction with flophemesylamine in toluene. Only tertiary alcohols did not react immediately, *tert.*-butanol requiring 10 min at room temperature, or less at higher temperature. The addition of catalysts or polar solvents did not significantly alter reaction rates. Diols, such as ethylene glycol, butane-2,3-diol and triethylene glycol react normally to add two flophemesyl groups. The retention times and Kováts' indices of some representative simple alcohols are listed in Table I.

TABLE I

FLOPHEMESYL ETHERS OF SIMPLE ALCOHOLS, CHROMATOGRAPHED ON 10% SE-30 COLUMN

Flophemesyl disiloxane has a retention time of 10.0 min at 180° on 10% SE-30, and a Kováts' index of 1585.

Parent alcohol	*Column temperature (°C)*	*Retention time (min)*	*Kováts' index*
Methanol	132	3.9	1000
Ethanol	132	5.4	1065
1-Propanol	142	6.85	1180
2-Propanol	142	5.7	1140
1-Butanol	142	8.6	1230
2-Butanol	142	8.7	1235
Isobutanol	142	11.75	1270
tert.-Butanol	142	7.05	1185
Benzyl alcohol	180	8.4	1520
Cyclohexanol	180	7.6	1355
3-Octanol	180	8.9	1535
3-Nonanol	180	11.9	1640
Ethylene glycol*	210	10.1	1840

* Diflophemesyl ether.

Phenols are usually more difficult to silylate than alcohols. The reaction with flophemesylamine was slower without a catalyst: a 1:1 mixture of flophemesylamine and flophemesyl chloride was more effective than a 10:1 mixture of the same reagents. Some simple phenol ethers are listed in Table II. The silylation of *p*-nitrophenol appeared to proceed normally, but the derivative had a GC retention time lower than expected and the highest ion in the mass spectrum was 24 a.m.u. greater

TABLE II

FLOPHEMESYL DERIVATIVES OF PHENOLS, CARBOXYLIC ACIDS AND AMINES CHROMATOGRAPHED ON 10% SE-30 COLUMN

At 186°, flophemesyl disiloxane elutes between the phenol and *p*-cresol ethers, unresolved from *o*-cresol ether on this column.

Parent compound	*Column temperature (°C)*	*Retention time (min)*	*Kováts' index*
Phenol	186	4.1	1490
p-Cresol	186	5.2	1570
p-Chlorophenol	186	6.55	1690
p-Bromophenol	186	6.45	1685
Benzoic acid	186	6.6	1690
p-Toluic acid	228	7.5	1910
Dodecanoic acid	224	4.95	1700
Eicosanoic acid	300	11.55	—
Butylamine	164	5.65	1280
Diethylamine	126	2.05	580
Aniline	164	7.2	1360
Cyclohexylamine	110	4.05	875
2-Phenylethylamine	186	8.95	1890

than the expected molecular weight, even after repeated purification; no explanation for the anomaly has been found.

The conversion of carboxylic acids to silyl esters is a more difficult task. Complete reaction with flophemesylamine, with or without addition of flophemesyl chloride was achieved in approximately 10 min. No other catalyst was found effective, and non-polar solvents, *e.g.* toluene were preferred to pyridine, which caused some decomposition of the ester. When methyl 12-hydroxystereate was treated with flophemesylamine in pyridine, methyl 12-flophemesyloxystearate was formed quantitatively, and no transesterification was noted.

Primary amines reacted vigorously with flophemesylamine catalysed by flophemesyl chloride, preferably in toluene. With flophemesylamine alone, the reaction is milder and requires about 15 min for completion. With a highly hindered amine such as di-isopropylamine, no reaction was observed in a variety of solvents, nor on warming to 100° for up to 2 h. No N,N-bisflophemesylamines were detected, presumably because of steric hindrance of the first entering group. Some amine derivatives are also listed in Table II.

The stability of flophemesyl derivatives has been studied under a number of conditions in assessing their usefulness for analysis. All the compounds prepared (even amines) have good GC properties, with good peak shape, and no evidence of decomposition. Alcohol and phenol ethers were successfully subjected to preparative GC. The technique was not used on esters or amines but no difficulty is forseen with it. The alcohol ethers were stable at 0° in a variety of neutral solvents (Table III) but were hydrolysed quite rapidly by acids or water, and more slowly by pyridine, regenerating the parent alcohol and flophemesyl disiloxane. The phenol ethers were also quite stable at 0° in anhydrous non-polar solvents but were more susceptible to mild hydrolytic conditions, such as moisture, acids or bases (Table IV). Some hydrolysis occurred even while washing the solutions of derivatives with acid

TABLE III

THE STABILITY OF 3-OCTYL FLOPHEMESYL ETHER MIXED WITH AN EQUAL VOLUME OF TEST REAGENT

In all the solutions analysed by GC the flophemesyl derivatives were hydrolysed back to their alcohols.

Test reagent	*Result*
n-Hexane	No decomposition after 72 h
Toluene	No decomposition after 72 h
Dichloromethane	No decomposition after 72 h
Diethyl ether	No decomposition after 72 h
Control	No decomposition after 72 h
Pyridine	30% decomposition in 8 h
Water	50% hydrolysis in 2 h
0.5 *N* HCl	60% decomposition in 90 min
Glacial acetic acid	50% hydrolysis in 10 min

TABLE IV

THE STABILITY OF PHENYL FLOPHEMESYL ETHER MIXED WITH AN EQUAL VOLUME OF TEST REAGENT

Test reagent	*Result*
Toluene	No decomposition after 72 h
n-Hexane	No decomposition after 72 h
Control	No decomposition after 72 h
Dichloromethane	10% decomposition in 72 h
$(NH_4)_2SO_4$ crystal	10% decomposition in 50 h
Pyridine	30% decomposition in 7 h
3% $NaHCO_3$	50% hydrolysis in 10 min
Water	60% hydrolysis in 15 min
0.5 *N* HCl	90% hydrolysis in 5 min
Glacial acetic acid	90% hydrolysis in 5 min

or alkali to remove excess reagents. The solutions were stable to washing with phosphate buffer (pH 6.0).

The flophemesyl ester of dodecanoic acid was stable in non-polar solvents but was readily decomposed by acids, bases, water or phosphate buffer. In pyridine 30% of the ester was hydrolysed in 1 h. N-Flophemesyl-N-(2-phenylethyl)amine was also unstable to washing in solution with aqueous reagents, and it was very sensitive to traces of moisture in organic solvents (20% decomposition in 8 h in diethyl ether), but was stable in the presence of excess flophemesylating reagents.

A mixture of flophemesyl ethers of benzyl alcohol, 3-octanol and phenol could be separated by thin-layer chromatography (TLC) on silica gel with diethyl ether–*n*-hexane (1:19). Some hydrolysis of the benzyl and phenyl ethers occurred during development. Partial hydrolysis of alcohol ethers often occurred, the extent depending upon the activity of the plates used. Spots were located by fluorescence quenching, neither the flophemesyl ethers nor flophemesyl disiloxane absorbed iodine vapour. TLC of flophemesyl esters and amines was not attempted. The TLC properties of some flophemesyl ethers are given in Table V.

TABLE V

TLC SEPARATION OF FLOPHEMESYL ETHERS ON SILICA GEL

Mobile phase	R_F *value*			
	benzyl	*3-octyl*	*phenyl*	*flophemesyl disiloxane*
Acetone–light petroleum (b.p. 60–80°) (3:7)	0.16			0.77
Acetone–carbon tetrachloride (1:4)	0.18			0.56
Toluene–hexane (1:9)	0.35	0.20	0.63	
Carbon tetrachloride–light petroleum (b.p. 60–80°) (1:20)	0.32	0.18	0.51	
Ethyl acetate–hexane (1:50)	0.46			
Ethyl acetate–hexane (1:50)		0.46		0.64
Diethyl ether–hexane (1:20)		0.35	0.25	0.46

The sensitivity of detection of flophemesyl ethers was determined with both the flame ionization detector and electron-capture detector. The limit of detection of *n*-butyl flophemesyl ether (Fig. 2) using the flame ionization detector of our Pye 104 GC system was 5 ng with a linear range of 10^5, *i.e.* up to 500 μg. While the limit of detection is comparable with that of a number of organic compounds, the upper limit at which linearity is lost, is lower. We have already shown that the electron-capturing process is dissociative with flophemesyl ethers[5] and therefore the detector is best operated at the highest temperature possible[6,7]. We found with our Pye 104 ECD system, operated at 300°, that neopentyl alcohol flophemesyl ether could be determined to 25 fg. In general practice we find this sensitivity difficult to maintain, because of the extreme problems of contamination of sample and detector at this level. With a less sensitive detector the limit for *n*-butyl flophemesyl ether was 5 pg with a linear range of 10^3 which is unusually large for this detector. The lower limit was chiefly determined by detector noise; the attenuator setting was at 10^3 and in favourable conditions can be set lower.

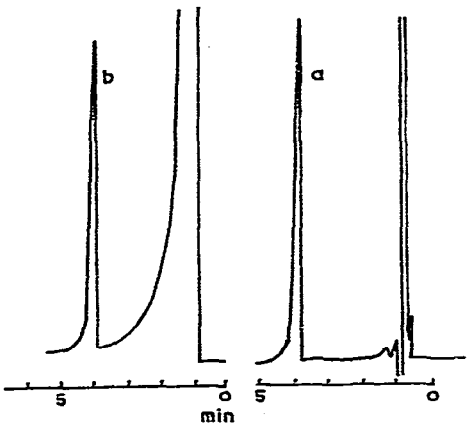

Fig. 2. GC traces of *n*-butylflophemesyl ether in toluene on 3% OV-101 column at 122° using (a) electron-capture detection and (b) flame ionization detection (not at the same concentration).

DISCUSSION

The great sensitivity and selectivity of the electron-capturing detector towards halogenated compounds (as well as to some other classes) makes this a very useful detector for trace analysis. Polar compounds not naturally electron-capturing can be made both volatile to GC and sensitive to ECD by the formation of halogen-containing derivatives[8,9]. In general, haloacyl esters and haloalkylsilyl ethers are the most widely employed for this purpose. Each type has certain disadvantages —either poor hydrolytic, chromatographic or thermal stability or low volatility. We have here attempted to assess the new group of flophemesyl derivatives which we have introduced, as general GC–ECD reagents. They show good chromatographic and thermal stability, high sensitivity to electron-capture detection, rapid and quantitative derivative formation, but like trimethylsilyl ethers are subject to hydrolysis under some conditions. The mass of the flophemesyl group ($C_8H_6F_5Si = 225$) increases retention times appreciably, making it very useful for attaching to small molecules, but unsuitable where several hydroxyl group occur, *e.g.* as sugar derivatives.

The extreme sensitivity of detection and relatively good stability of flophemesyl derivatives place them among the best ways of determining lower-molecular-weight alcohols, phenols, acids and amines.

The mass spectra of most flophemesyl derivatives are very simple, sharing a few high-intensity, high-mass ions. This makes them very suitable both for structure determination of the parent compound and also for determination by single or multiple ion monitoring. The mass spectra of simple alcohol and aliphatic amine flophemesyl derivatives are dominated by α-cleavage. Details of the mass spectra are discussed elsewhere[10].

Recently, higher alkyl groups have been introduced into silylating reagents to increase hydrolytic stability[11], *e.g. tert.*-butyl dimethylsilyl ethers have substantially greater hydrolytic stability compared to trimethylsilyl ethers. It would be interesting to study the properties of pentafluorophenylmethyl isopropyl silyl or pentafluorophenylsilolanyl reagents as more stable ECD protecting reagents for molecules where the increase in molecular weight and retention time would not be a disadvantage. An interesting application of pentafluorophenylmethyl isopropyl silyl reagents would be to the attempted separation of optically active compounds at trace levels.

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