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PREPARATION AND ASSESSMENT OF FLUOROCARBONSILYL ETHERS AS GAS CHROMATOGRAPHY DERIVATIVES FOR STEROIDS

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SUMMARY

Trifluoropropyl, heptafluoropentyl, and pentafluorophenyl groups substituted into silyl ethers of sterols provide stable and volatile derivatives useful in the gas chromatography of sterols. The first two show low sensitivity toward electron capture detection (ECD), but the pentafluorophenyl group is more sensitive to ECD than the currently used chloromethyl group and is more thermally stable than the heptafluorobutyryl group.

INTRODUCTION

There is a continuing need to find derivatives of steroids which can be determined quantitatively at very low concentrations in biological fluids. One of the most sensitive methods available is vapour phase electron capture detection(ECD) from a gas chromatograph. The derivatives must then be sensitive to the detector, volatile in the chromatograph, and be capable of being detected in the presence of a large amount of other substances. This need is particularly strong in the case of insect moulting hormones of the ecdysone group, where determinations at the nanogram level are required. A further complication of the ecdysones is that they contain a ketone group which is partially converted to enol ethers by some silylating reagents[1].

One alternative is first to protect the ketone as its methoxime[2,3], but two isomers are produced in this way which are partially or completely resolved on the gas chromatographic (GC) column, giving double peaks which are less easy to quantify and to see at low concentrations. Ecdysones possess several hydroxyl groups which can be protected with acylating reagents such as acetic anhydride[4] and heptafluorobutyric anhydride[5], but it is extremely difficult to obtain quantitative conversion to a single GC peak. Miyazaki *et al.*[6] have recently described a method of converting phyto- and zoo-ecdysones to heptafluorobutyrates using an exchange reaction between the sterol trimethylsilyl ethers and a mixture of the required anhydride and heptafluorobutyrylimidazole, which gave partially selective exchange at C-2, but there was reaction at other positions, yielding secondary peaks. Moreover, at least one hormone —2-deoxycrustecdysone— has been isolated from arthropods[7] which does not possess the necessary 2-OH and would be missed by this method.

Of the various reagents available for introducing silyl ethers[8,9], trimethylsilylimidazole has two advantages: it does not affect enolizable ketones and it is the strongest silylating reagent known, being capable of complete reaction with hindered hydroxyl groups which do not react quantitatively with acylating reagents.

We have investigated the production of some silylating reagents containing a fluorocarbon group for possible use in the ecdysone field which would produce sterol derivatives which combine volatility and sensitivity to electron capture. We have also investigated the conversion of such reagents to the imidazole derivative for convenience of use.

EXPERIMENTAL

All reactions were carried out with anhydrous reagents under an atmosphere of dry nitrogen.

The preparation of 3,3,3-trifluoropropyldimethylchlorosilane (I) was carried out in a sealed tube by reaction of dimethylchlorosilane and 3,3,3-trifluoropropene in the presence of chloroplatinic acid[10]. The optimum reaction conditions are given in Table I. Similarly 3,3,4,4,5,5,5-heptafluoropentyldimethylchlorosilane (II) was prepared from dimethylchlorosilane and 3,3,4,4,5,5,5-heptafluoropent-1-ene (Table I).

Both I and II were converted to the corresponding disilazanes, by reaction with ammonia, according to the method used by Osthoff and Kantor[11] for producing hexamethyldisilazane. The disilazanes were reacted directly with imidazole[12] to produce trifluoropropyldimethylsilylimidazole (III) and heptafluoropentyldimethylsilylimidazole (IV), respectively.

TABLE I

EXPERIMENTAL CONDITIONS FOR THE PREPARATION OF FLUOROALKYLDIMETHYLCHLOROSILANES IN A SEALED TUBE OF 75-ml VOLUME

	Compound I	*Compound II*
Alkene, g	2.00 (gas)	3.00
Dimethylchlorosilane, g	2.00	1.68
0.1 *M* H_2PtCl_6 (isopropanol solvent), ml	0.25	0.20
Furnace temperature, °C	210	240
Reaction period, h	6	7
Yield, %	65	55

The preparation of pentafluorophenyldimethylsilane (V) from pentafluorobenzene and dimethylchlorosilane has been described by Oliver and Graham[13]. The product was chlorinated[14] in the dark, with the temperature maintained below 25° with an ice–salt bath, to give pentafluorophenyldimethylchlorosilane (VI). Ammonolysis of VI by a standard method[11] gave the silylamine (b.p. 52°/6 mmHg, yield 65%), and treatment of VI with hexamethyldisilazane in the presence of aluminium chloride gave 1,3-bis(pentafluorophenyl)-1,1,3,3-tetramethyldisilazane (b.p. 151°/125 mmHg, yield 55%)[15]. Attempts to convert the disilazane, silylamine or the silyldiethylamine into the corresponding silylimidazole, under a variety of conditions, were unsuccessful. Attempts to prepare[16] the N,O-bis(pentafluorophenyldimethylsilyl)acetamide also failed.

Cholesterol silyl ethers were prepared by standard procedures using either direct reaction with the silyldiethylamine or the disilazane catalysed by the chlorosilane. To 50 μl of the chlorosilane in 200 μl of pure dry hexane was added 50 μl of diethylamine. The mixture was allowed to stand for 20 min and the solids settled by centrifugation. The supernatant liquid was added to 1–5 mg of cholesterol in screw-capped reacti-vials (Pierce, Rockford, Ill., U.S.A.), which were heated at 60° for 3 h. The solvent was removed by a stream of dry nitrogen and the silyl ether dissolved in ethyl acetate for flame ionization detection (FID) or hexane for ECD following GC. Complete conversion was achieved in all cases. Chloromethyldimethylsilyl ethers were prepared by published procedures[8].

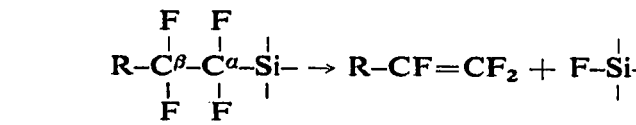
Gas–liquid chromatography (GLC) was carried out with a Pye Series 104 dual flame ionization and electron capture detector; the temperature of the injection port heater being kept at 30° higher than that of the column oven, and the detector oven temperature being kept at 300°. Nitrogen was used as carrier gas and the ECD was operated without purge. Mass spectra were obtained with an Hitachi Perkin-Elmer RMU6 spectrometer. The direct solid inlet temperature was 150–200°, the trap current 60 μA, the electron energy 80 eV, and the accelerating voltage 1.8 kV. For GC–mass spectrometry (MS) a Pye Series 104 gas chromatograph was used coupled to the mass spectrometer via a Watson-Bieman separator maintained at 250°, with a helium flow-rate of 18 ml min^{-1}, a source temperature of 250°, and a current, electron energy and voltage of 60 μA, 80 eV, and 1.8 kV, respectively.

For electron capture, hexane was specially purified and dried and an internal standard of known sensitivity was added at each injection to check the detector stability and the column absorption effects. In no case did the solvent peak overlap the derivative peak.

RESULTS AND DISCUSSION

No simple relationship has so far become apparent between molecular structure and electron capture sensitivity, and there is no short cut but to make and test possible derivatives. Among the halogens, ECD sensitivity increases with atomic weight, but GC volatility decreases. Polyfluorinated groups are the most promising in combining sensitivity and volatility.

Fluoroalkylsilanes with fluorine atoms α or β to silicon are thermally unstable at GC temperatures necessary for sterols, giving alkenes by fluorine migration and elimination[18].



We therefore prepared two silanes containing fluorine atoms at the γ and further positions from silicon, *viz.* 3,3,3-trifluoropropyldimethylchlorosilane (I) and 3,3,4,4,5,5,5-heptafluoropentyldimethylchlorosilane (II). Pentafluorophenyldimethylchlorosilane (VI) also had not been previously reported and was synthesized for evaluation.

The reagents were used to prepare the corresponding fluorosilyl ethers of cholesterol. The resulting derivatives had excellent thermal stability and high volatility on chromatography (Table II), the non-polar nature of the fluoroalkyl groups producing relatively shorter retention times on polar columns, in spite of the increased molecular weights.

TABLE II

RELATIVE RETENTION TIMES AND ELECTRON CAPTURE SENSITIVITY OF CHOLESTEROL SILYL ETHERS

Columns: (A) 3 ft. 1% OV-101 on Gas-Chrom Q, 75 ml min^{-1} N_2, 250°; retention time for cholesterol 1.58 min. (B) 3 ft. 1% Dexsil 300 GC on Gas-Chrom Q, 75 ml min^{-1} N_2, 290°; retention time for cholesterol 1.03 min. (C) 1.5 ft. 1.5% OV-101 plus 1.0% tetramethylcyclobutanediol adipate[24], 90 ml min^{-1} N_2, 250°; retention time for cholesterol 2.45 min.

Compound	*Relative retention time*			*Detection limit* expressed as ng of cholesterol*	*Pulse period (μsec)*
	A	*B*	*C*		
Cholesterol	1.00	1.00	1.00	—	—
Me_3Si cholesterol	1.17	0.92	0.69	—	—
$CF_3(CH_2)_2SiMe_2$-cholesterol	1.47	1.24	1.06	1500.	50
$CF_3(CF_2)_2(CH_2)_2SiMe_2$-cholesterol	1.60	0.87	0.68	115	15
$ClCH_2SiMe_2$-cholesterol	2.54	1.87	1.69	75.0**	5
$C_6F_5SiMe_2$-cholesterol	3.68	2.84	2.78	4.0	50

* Pulse width 0.75 ± 0.25 μsec, pulse height 47–60 V positive. Detection limit is defined as signal:noise ≥ 2.

** Negative deflection compared to other compounds.

The sensitivity of the derivatives using the ECD was assessed at increasing dilution and results are given in Table II. An increase from three to seven fluorine atoms increased sensitivity tenfold, but neither of the fluoroalkyl ethers were as sensitive as the chloromethyldimethylsilyl ether which is available from the commercial reagent. However the pentafluorophenyl group, though less volatile than the others tested, was much more sensitive to ECD and nearly twenty times more sensitive than the chloromethyl group. The pentafluorophenyl group is much more sensitive than the chloroacetates or trifluoroacetate and gives better peak shapes than the former[19]. It would have been interesting to make comparison with heptafluorobutyrylcholesterol but the compound is unstable[17].

Because of the complexity of biological materials some purification step is usually necessary before GC of sterols. Monoheptafluorobutyrates are usually stable to thin-layer chromatography (TLC) but some diheptafluorobutyrates (*e.g.* of testosterone and 20α-hydroxypregn-4-en-3-one) are degraded on silica[20]. The silyl ethers of cholesterol examined here were all stable to TLC on silica gel using a variety of solvents, and cholesterol and pentafluorophenyldimethylsilylcholesterol were easily separated on a neutral alumina column with quantitative recovery of the ether.

For sub-microgram quantities, GC–MS can be a great advantage in compound identification. A distinct fragmentogram with a strong molecular ion is a considerable advantage. The mass spectra of the fluoroalkyl and fluorophenyl ethers of cholesterol

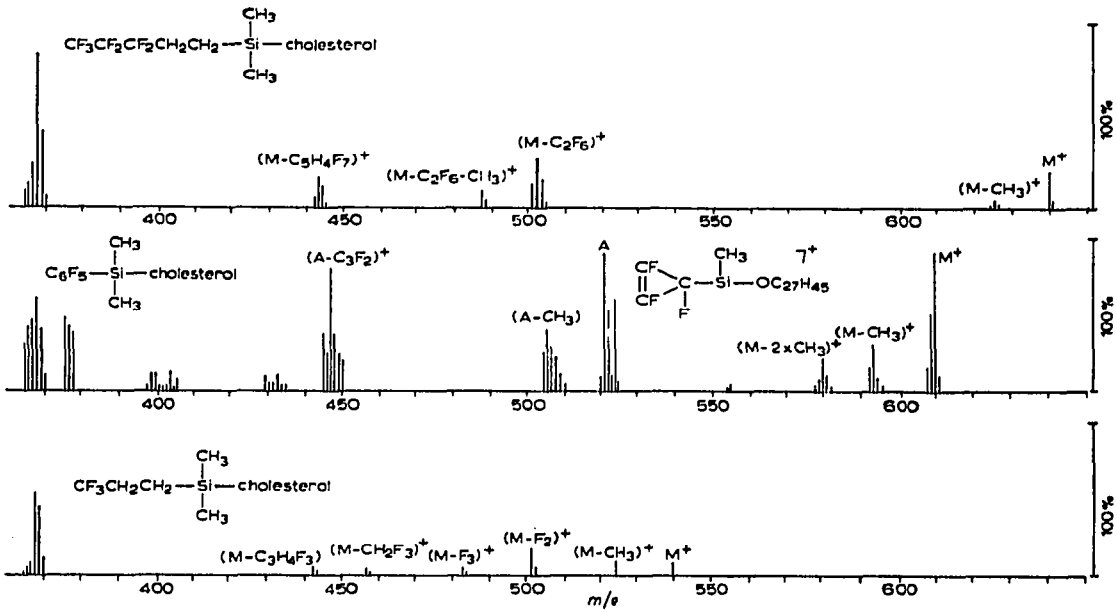


Fig. 1. Mass spectra of the fluoroalkyl and fluorophenylsilyl ethers of cholesterol for the region *m/e* 360–M^+.

are shown in Fig. 1 for the region *m/e* 360–M^+, together with assignment of their breakdown pathways. The fluorophenyl ether particularly gives a prominent molecular ion (*m/e* 610) which could be very useful in identification.

CONCLUSIONS

The fluoroalkylsilyl ethers provide stable, volatile derivatives of sterols, but their ECD response is poor. Pentafluorophenyldimethylsilyl ethers have the advantage of equal stability and greater sensitivity than chloromethyldimethylsilyl ethers and also greater stability to nucleophilic attack at silicon, which occurs with the chloromethyl group. The fluorophenyl ethers could find use in many fields such as sugar, amine, acid, and pesticide analysis, where chloromethyl ethers are currently used[21,22] and where substitution and elimination of the chloromethyl group leading to non-sensitive derivatives has been a problem[22,23]. The fluorophenyl ether of cholesterol produces a strong molecular ion which could be useful in GC–MS identification.

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