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Note

Dimethoxymethylsilyl ethers: stable silyl derivatives for gas chromatography and mass spectrometry

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Although trimethylsilyl (TMS) derivatives have proved to be the most widely used derivatives for gas–liquid chromatography (GLC), mass spectrometry (MS) and GLC–MS applications[1,2], they suffer from the disadvantage of being very sensitive to water. *tert.*-Butyldimethylsilyl (TBDMS) ethers, in which the bulky *tert.*-butyl group shields the silicon from attack by water, are very much more stable[3–7]. The mass spectra of the TBDMS derivatives differ considerably from those of the corresponding TMS derivatives because of the pronounced tendency for the molecular ion to fragment by elimination of a *tert.*-butyl radical. Although this is advantageous for selected ion monitoring studies and for simplification of spectra, much structural information is lost since the decomposition of the molecular ion via the $[M - R]^+$ ion reflects the nature of the derivative and not the derivatised molecule. A derivative was therefore required which would combine the stability of the TBDMS ethers with the fragmentation behaviour of the TMS ethers. Dimethoxymethylsilyl ethers were found to be satisfactory for this purpose. The two methoxy groups stabilised the silicon atom towards attack by water by a combination of steric and electronic effects and the methyl group allowed retention of fragmentation pathways involving loss of a methyl radical from the silyl group. Steric effects caused the dimethoxymethylsilyl reagents to be less reactive than the TMS reagents, but this effect could be utilised for the selective silylation of equatoriai hydroxyl groups; the axial hydroxyl groups were much less reactive. This paper describes the GLC–MS properties of the dimethoxymethylsilyl derivatives of a number of steroids.

EXPERIMENTAL

Dimethoxymethylchlorosilane was obtained from Cambrian Chemicals (Croydon, Great Britain).

Preparation of derivatives

Acetonitrile (200 μl), pyridine (10 μl), diethylamine (50 μl), and dimethoxymethylchlorosilane (50 μl) were well mixed, warmed at 60°C for 5 min, cooled and centrifuged. A 10-μl volume of the above reagent mixture was added to the steroid sample (10 μg) and the mixture was heated at 60°C for 10 min, or a 1-mg amount of imidazole was added to the mixture prepared as described above and the resulting mixture was heated for 10 min at 60°C.

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Gas chromatography

Methylene unit values were recorded with a Varian 2440 gas–liquid chromatograph fitted with two flame ionization detectors and two 2 m × 2 mm I.D. glass columns packed with either 3% SE-30 or 3% OV-17 on 100–120 mesh Gas-Chrom Q (Applied Science Labs., State College, PA, U.S.A.). Nitrogen at 30 ml/min was used as the carrier gas, the injector and detector temperatures were 300°C and the column oven was temperature programmed from 150°–300°C at 4°/min.

Gas chromatography–mass spectrometry

GLC–MS data were recorded with a V. G. Micromass 12B mass spectrometer interfaced via a glass jet separator to a Varian 2440 gas–liquid chromatograph which was fitted with a 2 m × 2 mm I.D. glass column packed with 3% SE-30 on 100–120 mesh Gas-Chrom Q. Operating conditions: accelerating voltage, 2.5 kV; electron energy, 25 eV, trap current, 100 μA; injector, separator and ion source temperatures, 300°, 280° and 240°C respectively; gas–liquid chromatograph oven in the range 200–280°C to give a retention time of about 5 min per sample; scan, 3 sec/decade, exponential, down. Data were recorded and processed with a V.G. 2040 data system.

RESULTS AND DISCUSSION

All of the derivatives shown in Table I gave single GLC peaks when prepared as described above. The dimethoxydiethylaminosilane reagent was less reactieve than other alkyl-diethylaminosilyl reagents prepared by analogous methods. Thus steroids containing axial hydroxy groups (*e.g.* 3α-hydroxy-5α- and 3β-hydroxy-5β-androstan-17-one) were not silylated at room temperature or at 60°C whereas those containing equatorial hydroxy groups reacted fairly rapidly. For example, at room temperature (23°C) the reaction with the equatorial hydroxy group in 3β-hydroxy-5α-androstan-17-one was complete in about 1 h; the equivalent reaction using TMS-diethylamine gave the TMS derivative in 18 min with acetonitrile and pyridine as solvents. Reactivity differences towards TMS-diethylamine of this type have previously been reported by Weisz *et al.*[8].

Once formed, these dimethoxymethylsilyl derivatives were much more stable than the corresponding TMS derivatives. For example, only about 10% hydrolysis was observed after the dimethoxymethylsilyl derivative of 3β-hydroxy-5α-androstan-17-one was heated at 60°C for 1 h in aqueous methanol. The corresponding TMS derivative was completely hydrolysed in less than 10 min. Hydrolysis of the dimethoxymethylsilyl ethers with recovery of the free steroid could, however, be achieved by heating the derivative in aqueous methanol in the presence of dilute hydrochloric acid or sodium hydroxide. The stability of these derivatives is presumably a combination of steric effects and the interaction of the lone pairs of electrons on the oxygen atoms with the empty d orbitals on the silicon; this would inhibit attack by water.

Gas–liquid chromatographic characteristics

Methylene unit values are listed in Table I. On SE-30 the monohydroxy steroids gave values of about 1.6 units higher than the corresponding TMS derivatives[7] and on OV-17 the values were about 2.4 units higher. The values were twice as large in the

TABLE I

GLC-MS DATA FOR THE DIMETHOXYMETHYLSILYL DERIVATIVES

M^+ data are given as *m/z*, numbers between parentheses indicate relative abundance.

Compound	*Methylene unit*		$M^{+\cdot}$	*MS data*	
	SE-30	*OV-17*		*Base*	$[M - (CH_3O)_2 CH_3OH]^+$
3α-Hydroxy-5α-androstan-17-one	26.05	29.23	394 (11)	272*	272 (100)
3β-Hydroxy-5α-androstan-17-one	26.93	30.43	394 (35)	272*	272 (100)
3α-Hydroxy-5β-androstan-17-one	26.00	29.45	394 (1)	272*	272 (100)
3β-Hydroxy-5β-androstan-17-one	26.00	29.22	394 (1)	272*	272 (100)
17β-Hydroxy-5α-androstan-3-one	27.06	30.60	394 (5)	161**	272 (40)
3β-Hydroxy-Δ^5-androstan-17-one	26.84	30.40	392 (2)	270	270 (100)
5α-Androstan-3β,17β-diol	29.35	31.83	500 (11)	161**	378 (42)
Δ^5-Androstan-3β,17β-diol	29.30	31.82	498 (3)	256***	376 (87)
3β-Hydroxy-Δ^5-pregnan-20-one	28.67	32.10	420 (2)	298*	298 (100)
5α-Pregnane-3β,20β-diol	31.16	33.74	528 (0)	149****	406 (1)

* $[M - (CH_3O)_2 CH_3SiOH]^{+\cdot}$.
** $[(CH_3O)_2 CH_3SiO^+ = CH\text{–}CH = CH_2]$.
*** $[M - 2\times (CH_3O)_2 CH_3SiOH]^{+\cdot}$.
**** $[(CH_3O)_2 CH_3SiO^+ = CH\text{–}CH_3]$.

case of the diols. No significant differences were observed between the separation of isomers over the results obtained from the TMS derivatives.

Mass spectrometric characteristics

The fragmentation patterns of the alkylsily derivatives of steroids can be classified into two types. In the first type, exemplified by the TMS derivatives, abundant diagnostic silicon-containing ions are present and the TMS groups are eliminated mainly as trimethylsilanol. In the second type of fragmentation, shown by most derivatives containing alkyl groups larger than methyl attached to the silicon atom, the major fragmentation route is initiated by elimination of an alkyl group. Fragmentation of this ion usually involves loss of dialkylsilanol (R_2SiOH_2) rather than trialkylsilanol (R_3SiOH). Diagnostic silicon-containing ions are usually absent. Thus, although the latter fragmentation pattern results in simple spectra containing abundant $[M - R]^+$ ions suitable for single ion monitoring studies, more informative spectra are produced by derivatives showing the first type of fragmentation. The dimethoxymethylsilyl derivatives fall into this class; other stable silyl derivatives containing bulky substituents such as the TBDMS ethers generally show the second type of fragmentation. Thus the spectra of the stable dimethoxymethylsilyl derivatives were more informative than those of the TBDMS ethers and, in addition, the molecular-weight increment attenting derivative formation was lower (104 U compared with 114 U).

Molecular ions from the dimethoxymethylsilyl derivatives were usually slightly less abundant than those in the spectra of the corresponding TMS derivatives, and the $[M - CH_3]^+$ ions were much less abundant reflecting the single methyl group bound to silicon. Very abundant $[M - (CH_3O)_2CH_3SiOH]^-$. (M — trialkylsilanol) ions were produced by most of the derivatives studied. Diagnostic silicon-containing ions were also abundant and were the base peaks in several of the spectra (Table I). Again the abundance of these ions reflected their counterparts in the spectra of the corresponding TMS derivatives.

Monoalkyoxydimethylsilyl derivatives were also investigated and will be the subject of another paper. They were prepared by the reaction of an alcohol with the diethylaminodimethylsilyl derivative of the steroid and showed similar GLC–MS characteristics to the dimethoxymethylsilyl ethers.

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