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COMPARISON OF FOURTEEN SUBSTITUTED SILYL DERIVATIVES FOR THE CHARACTERIZATION OF ALCOHOLS, STEROIDS AND CANNABINOIDS BY COMBINED GAS–LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY

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SUMMARY

The gas–liquid chromatographic (GLC) retention times and mass spectral (MS) properties of fourteen substituted silyl derivatives of a series of alcohols, steroids and cannabinoids are reported and discussed. Most of the derivatives were stable and gave well shaped GLC peaks. Their differing retention increment values could be exploited to effect separation between mono- and polyhydroxy compounds in complex mixtures and the retention increments themselves could be used to determine the number of derivatized functions in a molecule. Dimethylsilyl derivatives produced the most extensive MS fragmentation, larger derivatives tended to form abundant $[M-R]^+$ ions with fewer diagnostic fragment ions. The high ion currents carried by the $[M-R]^+$ ions were useful for single-ion studies.

INTRODUCTION

In addition to the widely used trimethylsilyl (TMS) derivatives[1], a number of other substituted silyl derivatives have been introduced in recent years for the characterization of biological molecules by gas phase analytical methods. Such derivatives have been synthesized for various reasons such as to introduce halogens for electron capture work or to increase stability. Examples of these derivatives are the alkyldimethylsilyl ethers, RMe_2Si- where R = hydrogen[2,3], ethyl[4], propyl[4], *tert.*-butyl[5–10], allyl[10,11], chloromethyl[12–15], bromomethyl[13,16,17], iodomethyl[13,18,19], trifluoropropyl[20], heptafluoropentyl[20], or pentafluorophenyl[20–23] and the trialkylsilyl ethers, R_3Si- where R = ethyl, *n*-propyl, *n*-butyl or *n*-hexyl[24–26]. The latter compounds were used for introducing large retention increment shifts in compounds containing two hydroxyl groups so that they could be separated from monohydroxy compounds where these occurred together in cannabis and nutmeg extracts. However, the large retention increment shifts produced by these derivatives presented difficulties when applied to more highly hydroxylated cannabinoids such as are found as metabolites of Δ^1-tetrahydrocannabinol (Δ^1-THC) in liver extracts[27], and we have, therefore, investigated

the utility of a range of substituted silyl derivatives for achieving separation of these compounds. For characterization by gas chromatography–mass spectrometry (GC–MS), the type of MS fragmentation produced by the various derivatives is also important as in many cases this can be altered considerably by changing the nature of the substituents[6,7,22,24]. This paper presents comparative GC–MS data for fourteen substituted silyl derivatives, several of them new, of a number of alcohols, steroids and cannabinoids in order to evaluate their usefulness as GC–MS derivatives.

EXPERIMENTAL

Preparation of derivatives

TMS derivatives. These were prepared by adding 10 μl of a mixture of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 2 parts), trimethylchlorosilane (TMCS, 1 part) and acetonitrile (2 parts) to 10 μg of the sample and heating for to 60° for 10 min.

Dimethylsilyl (DMS) and chloromethyldimethylsilyl (CDMS) derivatives. These were prepared as above using tetramethyldisilazane (TMDS) or di(chloromethyl)tetramethyldisilazane (CMTMDS) and either dimethylchlorosilane (DMCS) or chloromethyldimethylchlorosilane (CMDMCS) in place of the BSTFA and TMCS, respectively.

tert.-Butyldimethylsilyl (TBDMS) derivatives. A 10-μl portion of a mixture of *tert.*-butyldimethylchlorosilane and imidazole in dimethylformamide (Applied Science Labs., State College, Pa., U.S.A.) was added to the sample (10 μg) and the mixture was heated at 60° for 10 min.

Remaining derivatives (Table I). Pyridine, acetonitrile, or dimethylformamide (4 parts), the appropriate chlorosilane (2 parts) and either diethylamine (1 part) or imidazole (1 part) were mixed, cooled and centrifuged to remove the precipitate. 10 μl of these reagents were added to the samples (10 μg) and the mixtures were heated at 60° for 10 min.

Gas–liquid chromatography

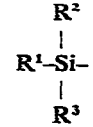
Methylene unit values were recorded using a Varian 2400 gas chromatograph fitted with dual flame ionization detectors and two 2 m × 2 mm glass columns packed with 3% SE-30 on Gas-Chrom Q (Applied Science Labs.). The column oven was temperature programmed from 150° at 4°/min. Nitrogen at 30 ml/min was used as the carrier gas and the injector and detector temperatures were set at 270° and 300°, respectively.

Mass spectrometry

Low resolution mass spectra were recorded at 25 eV with a V.G. Micromass 12B mass spectrometer interfaced to a V.G. 2040 data system. Sample inlet was via a chromatographic system similar to that described above and interfaced to the spectrometer by a glass jet separator. Helium was used as the carrier gas and the inlet and separator temperatures were maintained at 270° and 230°, respectively. The mass spectrometer was operated with an accelerating voltage of 2.5 kV and spectra were acquired by repetitive scanning at 3 sec per decade.

TABLE I

STRUCTURES AND SOME PROPERTIES OF THE ALKYLSILYL DERIVATIVES



Derivative	*Abbreviation*	R^1	R^2	R^3	*M.W. increment**	*Min. M.U.***
Trimethylsilyl	TMS	CH_3	CH_3	CH_3	72	8****
Dimethylsilyl	DMS	H	CH_3	CH_3	58	10****
Allyldimethylsilyl	ADMS	$CH_2 = CHCH_3$	CH_3	CH_3	98	14
Propyldimethylsilyl	PDMS	C_3H_7	CH_3	CH_3	100	13
tert.-Butyldimethylsilyl	TBDMS	*tert.*-C_4H_9	CH_3	CH_3	114	17****
Chloromethyldimethylsilyl	CMDMS	$ClCH_2$	CH_3	CH_3	106	14****
Dichloromethyldimethylsilyl	DCMDMS	Cl_2CH	CH_3	CH_3	140	18
Di(chloromethyl)methylsilyl§	DCMMS	$ClCH_2$	$ClCH_2$	CH_3	140	20
Phenyldimethylsilyl	PhDMS	C_6H_5	CH_3	CH_3	134	22
Benzyldimethylsilyl	BzDMS	C_6H_5–CH_2	CH_3	CH_3	148	28
Triethylsilyl	TES	C_2H_5	C_2H_5	C_2H_5	114	14
Tri-*n*-propylsilyl	TPS	C_3H_7	C_3H_7	C_3H_7	156	17
Tri-*n*-butylsilyl	TBS	C_4H_9	C_4H_9	C_4H_9	198	22
Tri-*n*-hexylsilyl	THS	C_6H_{13}	C_6H_{13}	C_6H_{13}	282	30

* Increase in molecular weight for each derivatized function.

** Minimum usable methylene unit value before reagent peaks interfere with sample (see text). Derivatives were prepared by the diethylamine–acetonitrile method unless indicated by ***.

*** Derivatives not prepared by diethylamine–acetonitrile method (see ** and Experimental).

§ Unstable.

RESULTS AND DISCUSSION

The fourteen silyl derivatives investigated are listed in Table I. Derivatives were formed from eight substrates, octadecanol, heptane-1,7-diol, four cannabinoids and two steroids (Table II). With the exception of the di(chloromethyl)methylsilyl (DCMMS) ethers which decomposed on the column with loss of HCl, all the derivatives were stable and gave good GC peak shapes. TMS, DMS, CMDMS and TBDMS derivatives were prepared by standard procedures (see Experimental) but several methods were used for the remaining derivatives. The use of imidazole in dimethylformamide to effect reaction of the chlorosilane gave rather inconsistent results with the production, in some cases, of two-phase systems. Imidazole in acetonitrile was better as a general method but precipitates tended to form in the sample mixtures and the syringe needles. No attempt was made to purify the derivatives as been described by some authors[7,9] because, for the study of complex mixtures, we wished to keep chemical "clean-up" procedures to a minimum to avoid losses. Better results were obtained using diethylamine as the base and acetonitrile as solvent. Pyridine worked in all cases, but the reagent mixtures were less stable and became very dark on standing.

Because of the rather high molecular weight of some of the silyl reagents, peaks derived from these reagents appeared in the chromatograms at moderate

TABLE II

METHYLENE UNIT VALUES FOR THE SILYL DERIVATIVES OF EIGHT SUBSTRATES ON 3% SE-30

For abbreviations of derivatives, see Table I. Δ^1-THC = Δ^1-tetrahydrocannabinol; CBN = cannabinol; CBD = cannabidiol.

Derivative	*C_{16}-OH*	*C_7-Diol*	*Δ^1-THC*	*CBN*	*CBD*	*7-OH-Δ^1-THC*	*5α-A-3α-OH-17-one**	*5α-P-3β,20β-diol***
TMS	21.65	14.55	23.50	24.30	22.70	26.50	24.39	28.15
DMS	21.17	—	23.24	23.89	22.32	25.61	24.00	27.12
ADMS	23.55	18.11	25.14	26.00	25.65	29.73	26.50	32.57
PDMS	23.53	18.12	25.08	25.92	25.46	29.16	26.31	32.45
TBDMS	23.97	18.79	25.60	26.60	26.70	30.50	26.72	33.99
CMDMS	24.15	19.23	25.80	26.60	27.16	30.96	27.36	34.49
DCMDMS	25.52	—	26.93	27.73	29.32	—	28.90	—
DCMMS***	26.44	—	27.90	28.68	—	—	30.34	—
PhDMS	27.05	24.90	28.27	29.26	31.62	35.77	30.47	—
BzDMS	27.96	26.99	29.61	30.57	34.52	—	31.61	—
TES	24.85	20.80	26.35	27.25	27.80	32.20	28.01	36.07
TPS	26.70	24.40	27.60	28.55	29.80	34.40	29.60	—
TBS	28.90	28.75	29.55	30.55	33.25	—	31.82	—
THS	33.70	—	34.05	35.00	—	—	—	—

* 3α-Hydroxy-5α-androstan-17-one.

** 5α-Pregnane-3β,20β-diol.

*** Unstable.

methylene unit values thus limiting their usefulness to the derivatization of larger compounds. Table I lists the minimum methylene unit value at which each type of derivative could be used before reagent peaks interfered with the chromatogram. However, in only four cases was this value over 20 and most derivatives were found to be suitable for a wide range of compounds.

Methylene unit values are listed in Table II. The retention increments produced by each derivative varied with the type of substrate. For example, the derivatives of the phenolic group present in the cannabinoids produced a slightly lower increment than those of the aliphatic hydroxyl groups of octadecanol and heptane-1,7-diol whereas the increments for the steroid derivatives tended to be higher. The derivatives of the diols, in all cases, produced increments slightly less than twice those of the related monohydroxy compounds.

The retention increments introduced by each derivative increased with both the molecular weight of the derivative and with the distribution around the silicon atom. Concentration of most of the atoms in one of the silyl substituents such as in the TBDMS or DCMDMS derivatives produced lower increments than the isomeric derivatives (TES and DCMMS, respectively) where the distribution was more even about the silicon. The choice of a derivative suitable for any particular separation can be made with the help of Table II. Thus TBDMS derivatives would be unsuitable for the separation of the major cannabinoids because of the similar methylene unit values of cannabidiol and cannabinol.

The molecular weight increases attending derivative formation are listed in Table I. Most are in the range 80–150 and thus the derivatives are suitable for the study of polyfunctional compounds by MS. The high increments produced by the TBS derivatives limited their use for MS purposes, but they have been found to be the only R_3Si-type derivatives capable of separating all of the dihydroxy cannabinoids from their monohydroxy analogues[24].

Of the fourteen derivatives studied, the DMS derivatives produced the most extensive MS fragmentation. Molecular ions were usually more abundant than those produced by the TMS derivatives, but in general, fragmentation patterns were similar. The preference for DMS derivatives of long-chain alcohols to fragment by cleavage of the alcohol chain rather than by eliminating a methyl group from the silyl moiety has been discussed previously[28] as have various other aspects of the fragmentation of DMS derivatives[29,30]. Cannabinoid DMS derivatives gave spectra which were very similar to their TMS counterparts.

The spectra of the alkyl-DMS derivatives were, in most cases dominated by very abundant ions produced by elimination of the largest alkyl group. Phenyldimethylsilyl derivatives were the exception to this rule as they eliminated a methyl rather than a phenyl radical from the molecular ion. The formation and subsequent decomposition of the $[M-R]^+$ ions produced most of the major ions in the alcohol and steroid spectra and as loss of the large alkyl group led, in many cases to the same dimethylsiliconium fragment ion, the spectra of all the derivatives of a particular compound were similar[10,15]. Ions produced by loss of dimethylsilanol from $[M-R]^+$ were prominent in the spectra of the RMe_2Si derivatives of the androstanes in contrast to the abundant ions produced by trimethylsilanol loss observed in the spectra of the TMS derivatives. The use of the high ion currents carried by the $[M-R]^+$ ions, particularly in the TBDMS spectra, has been well documented both in the context

of single ion monitoring studies[6,10] and for obtaining abundant ions in the high-mass region of the spectra of 20-hydroxypregnanes. In contrast to the differing behaviour of the alkyl-DMS and TMS derivatives of the alcohols and steroids, the corresponding derivatives of the cannabinoids behaved similarly in the mass spectrometer as fragmentation was not usually associated with the silyl substituent. Abundant losses of the *tert.*-butyl, allyl, or benzyl groups were, however, observed in the spectra of the TBDMS, ADMS, and BzDMS derivatives. A useful feature of the derivatives containing an unsaturated alkyl substituent (ADMS and BzDMS derivatives) was their ability to stabilize the molecular ions. Similar stabilization has been reported with pentafluorophenyldimethylsilyl derivatives[22].

The spectra of the trialkylsilyl derivatives were similar to those of the alkyl-DMS derivatives. Spectra of the alcohol and steroid derivatives contained extremely abundant $[M-R]^+$ ions, in many cases carrying a larger percentage of the total ion current than the $[M-57]^+$ ions from the corresponding TBDMS derivatives. It is of interest that the TES derivatives which produced the same molecular weight increment as the TBDMS derivatives frequently gave rise to more abundant $[M-R]^+$ ions. Ion currents also tended to rise with increasing derivative size. Table III gives the percentage of the total ion current carried by the $[M-R]^+$ ions for several derivatives of a number of steroids. Methylene unit values are listed in Table IV. Detection limits in the order of 10 pg have been obtained during single ion monitoring studies of the TES and TBDMS derivatives of 3β-hydroxy-5α-androstan-17-one[10,31]. An additional feature of the spectra of the trialkylsilyl derivatives was the presence of ions produced by loss of C_nH_{2n} from one of the alkyl chains[31–35]. Molecular ions were weak or absent from the spectra of many of the trialkylsilyl derivatives of the alcohols and steroids and in common with the alkyl-DMS spectra, structurally diagnostic fragment ions were weak in all except the cannabinoid spectra. The nature of the derivative had little effect on the fragmentation of any of the cannabinoids. Thus the use of the differ-

TABLE III

PERCENTAGE OF THE TOTAL ION CURRENT* CARRIED BY THE $[M-R]^+$ IONS FROM SEVERAL DERIVATIVES OF THIRTEEN STEROIDS**

A = androstane; P = pregnane.

Steroid	*TMS*	*TBDMS*	*TES*	*TPS*	*TBS*
5α-A-3β-OH-17-one	16.0	32.9	38.6	35.0	40.2
5α-A-3α-OH-17-one	6.6	13.9	29.9	35.3	34.5
5β-A-3α-OH-17-one	1.8	13.2	25.8	33.4	33.7
5β-A-3α-OH-17-one	1.3	17.4	26.6	30.0	37.0
5α-A-3α,17β-diol	1.5	15.4	12.0	18.0	—
5α-A-3β,17β-diol	3.4	18.2	26.6	25.1	—
5β-A-3α,17β-diol	0.7	15.6	11.7	18.2	—
Δ^5-A-3β,17β-diol	0.9	18.1	16.6	18.5	—
5α-P-3β,20α-diol	0.8	19.9	21.7	22.6	—
5α-P-3β,20β-diol	0.0	24.4	18.6	21.6	—
5β-P-3α,20β-diol	0.8	9.7	16.6	23.6	—
Δ^5-P-3β,20α-diol	0.0	5.2	10.6	16.2	—
Δ^5-P-3β-OH-20-one	1.1	24.0	24.4	31.3	33.6

* % Total ion current above *m/e* 40.

** Values for the ADMS derivatives are given in ref. 10.

TABLE IV

METHYLENE UNIT VALUES (3% SE-30) FOR THE R_3Si DERIVATIVES OF THE STEROIDS LISTED IN TABLE III*,**

A = androstane, P = pregnane.

Steroid	*Derivative*		
	TES	*TPS*	*TBS*
5α-A-3β-OH-17-one	29.06	31.05	33.45
5β-A-3α-OH-17-one	28.00	29.72	31.93
5β-A-3β-OH-17-one	28.10	30.09	32.51
5α-A-3α,17β-diol	32.71	36.00	—
5α-A-3β,17β-diol	34.00	37.82	—
5β-A-3α,17β-diol	32.50	36.50	—
Δ^5-A-3β,17β-diol	33.77	37.70	—
5α-P-3β,20α-diol	36.27	—	—
5α-P-3β,20β-diol	36.07	—	—
5β-P-3α,20β-dol	34.35	37.10	—
Δ^5-P-3β,20α-diol	36.22	—	—
Δ^5-P-3β-OH-20-one	30.86	32.80	35.22

* For the methylene unit values of TMS, ADMS and TBDMS derivatives see ref. 10.
** See Table II for values for the derivatives of 5α-A-3α-OH-17-one.

ent retention increments provided by the various derivatives can be utilized fully for the study of these compounds without any subsequent loss of structural information from the mass spectra. Tabulated mass spectra for the compounds discussed in this paper have been sent to the Mass Spectrometry Data Centre, A.W.R.E., Aldermaston, Great Britain.

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