THE GAS–LIQUID CHROMATOGRAPHY OF DIMETHYLSILYL, TRIMETHYLSILYL AND CHLOROMETHYLDIMETHYLSILYL ETHERS OF STEROIDS

MECHANISM OF SILYL ETHER FORMATION AND EFFECT OF TRIMETHYLSILYLATION UPON DETECTOR RESPONSE

W. J. A. VANDENHEUVEL
Merck Sharp & Dohme Research Laboratories, Rahway, N.J. (U.S.A.)
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INTRODUCTION

Among the derivatives used in the gas–liquid chromatography (GLC) of steroids none seems to have been more widely accepted than the trimethylsilyl (TMSi) ether[1]. Not only is the separation of closely related steroids often greatly improved following conversion to this derivative, but with polar stationary phases the ethers are eluted much more rapidly than the parent compounds[1]. The use of TMSi ethers has also been shown to improve the quantitative aspects of steroid GLC[2]. Estrogens, 17-ketosteroids, sterols, bile acid methyl esters, C_{21} diols and triols, and corticoids have all been analyzed as TMSi derivatives[1–15]. The use of dimethylsilyl (DMSi) ethers has been proposed for the GLC of carbohydrates[16], and more recently THOMAS has suggested that chloromethyldimethylsilyl (CMDMSi) ethers may possess electron capture properties suitable for steroid GLC work at the nanogram level[17]. It thus seems timely to report the results of a study on the GLC behavior of a group of variously substituted hydroxysteroids, and their DMSi, TMSi and CMDMSi ethers. Comments on the mechanism of formation of silyl ethers, and on the effect of trimethylsilylation upon hydrogen flame and Lovelock argon ionization detector response, are also presented.

EXPERIMENTAL

Data were obtained with Barber-Colman Model 15 (argon ionization; 650 V) and 5000 (hydrogen flame ionization; 240 V, 38 p.s.i. air, 14 p.s.i. hydrogen) gas chromatographs. The column packings, prepared by established procedures[18], were 2 % SE-30 (a methylpolysiloxane; General Electric Company) and 1 % EGSS-Z (a copolymer prepared from ethyleneglycol, succinic anhydride and a methylphenylsilane monomer; Applied Science Laboratories) coated on acid-washed and silanized Gas-Chrom P (Applied Science Laboratories). Silyl ether derivatives for retention time comparisons were prepared by reaction of 0.5–2.0 mg of steroid at room temperature for three hours with 0.05 ml each of the appropriate reagents [tetramethyldisilazane (TMDS) and dimethylchlorosilane (DMCS) for dimethylsilylation, and hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS) for trimethyl-

silylation, all obtained from Applied Science Laboratories; 1,3-bis(chloromethyldimethyl)-1,1,3,3-tetramethyldisilazane [$(CM)_2$ TMDS] and chloromethyldimethylchlorosilane (CMDMCS) for chloromethyldimethylsilylation, supplied by Pierce Chemical Company] in redistilled tetrahydrofuran[3]. Cholesteryl TMSi and CMDMSi ethers, testosterone TMSi and CMDMSi ethers, the CMDMSi ether of 4-pregnen-20β-ol-3-one, and the di-DMSi, di-TMSi, and di-CMDMSi ethers of pregnanediol were isolated and possessed satisfactory elementary analyses. Other derivatives were prepared by a short procedure involving removal of excess reagents in a stream of nitrogen; the residue was taken up in redistilled carbon disulfide and chromatographed directly. Sample sizes were 0.2–1.0 μg per peak.

RESULTS AND DISCUSSION

The retention behavior of three pairs of epimerically-substituted hydroxysteroids and their DMSi, TMSi and CMDMSi ethers are presented in Table I. The stereochemical differences exemplified are often encountered in GLC work with biologically important steroids. It is clear that with the non-polar stationary phase

TABLE I

RETENTION BEHAVIOR OF THREE PAIRS OF HYDROXY-STEROIDS AND THEIR SILYL ETHER DERIVATIVES

Steroid	*Relative retention time*	
	SE-30[a]	*EGSS-Z*[b]
Cholestane	1.00[c]	1.00[d]
Epicholesterol	1.81	5.81
Cholesterol	1.91	7.31
Epicholesteryl DMSi	1.64	1.71
Cholesteryl DMSi	2.06	2.26
Epicholesteryl TMSi	1.82	1.64
Cholesteryl TMSi	2.36	2.31
Epicholesteryl CMDMSi	4.03	6.12
Cholesteryl CMDMSi	5.33	8.94
Epitestosterone	0.57	10.3
Testosterone	0.56	10.9
Epitestosterone DMSi	0.48	2.17
Testosterone DMSi	0.56	2.66
Epitestosterone TMSi	0.54	2.11
Testosterone TMSi	0.64	2.62
Epitestosterone CMDMSi	1.25	9.58
Testosterone CMDMSi	1.52	11.6
4-Pregnen-20β-ol-3-one	0.88	13.7
4-Pregnen-20α-ol-3-one	0.95	16.1
4-Pregnene-20β-DMSi-3-one	0.97	4.64
4-Pregnene-20α-DMSi-3-one	1.04	5.14
4-Pregnene-20β-TMSi-3-one	1.14	4.92
4-Pregnene-20α-TMSi-3-one	1.21	5.32
4-Pregnene-20β-CMDMSi-3-one	2.58	20.6
4-Pregnene-20α-CMDMSi-3-one	2.81	23.1

[a] 235°, 16 p.s.i.
[b] 205°, 12 p.s.i.
[c] Absolute retention time, 9.0 min.
[d] Absolute retention time, 4.5 min.

SE-30 formation of the derivatives leads to a large increase in the separation observed for cholesterol (3β-ol-equatorial) and epicholesterol (3α-ol-axial), probably due to the fact that the introduction of these bulky substituents accentuates the differences in molecular shape between these two isomers. In line with this, the highest separation factor is found for the largest (in a steric sense) derivative, and for each pair of isomers the axial epimer is eluted more rapidly. The change in retention time with derivatization parallels the molecular weight of the substituent group, the greatest increase occurring with chloromethyldimethylsilylation. The equatorial derivatives all possess decreased volatility relative to the parent sterol, but with epicholesterol the TMSi ether is eluted at the same rate as the sterol, and its DMSi ether actually possesses increased volatility*. With the polar stationary phase EGSS-Z, the non-polar DMSi and TMSi ethers of both epimeric sterols are much more volatile (approximately three fold) than the parent compounds, and even the CMDMSi ethers do not exhibit greatly increased retention times. With this polar phase, as with SE-30, the volatility differences for the derivatives are DMSi $<$ TMSi $<$ CMDMSi, but in contrast to the results observed with the latter phase the increase in separation factor upon derivatization is far less. This results because the parent sterols themselves possess a large separation factor (1.26) with EGSS-Z, whereas with SE-30 this factor is much smaller (1.05). Polar phases have been called "selective" because of their ability to distinguish between compounds of close structural similarity, such as epimeric sterols[2,18]. The separation factor for cholesterol and epicholesterol on EGSS-Z, however, is large even for a selective phase, especially since their dihydro analogs, cholestanol and epicholestanol, exhibit a much smaller retention time difference with this phase (separation factor of 1.11). The derivatives of these saturated sterols exhibit epimer volatility differences very similar to those for the corresponding derivatives of cholesterol and epicholesterol—with EGSS-Z cholestanyl and epicholestanyl TMSi ethers exhibit a separation factor of 1.45, whereas with SE-30 it is 1.28 (separation factor for the parent alcohols of 1.02). The difference in GLC behavior with the polyester-phenylsiloxane phase caused by the presence of the 5:6 double bond is limited to the free sterols. A possible explanation is intramolecular hydrogen bonding between the axial hydroxyl group and the π-electrons of the double bond in epicholesterol (thus reducing the ability of this hydroxyl group to interact with the stationary phase) and the absence of this phenomenon, because of the different stereochemical orientation of the 3β-hydroxyl group, in cholesterol**. The fluoroalkyl polysiloxane, QF-1, exhibits a separation factor for cholestanol and epicholestanol approximately equal to that of EGSS-Z. Cholesterol and epicholesterol, however, are not as well separated with QF-1 as they are with SE-30[2], emphasizing the fact that retention behavior is very stationary phase-dependent.

The differences in retention time for the epimeric hydroxy-steroids testosterone and epitestosterone are small with both stationary phases. Derivatization leads to a

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* The use of models indicates that the introduction of a bulky substituent such as a TMSiO group at the 3α position of cholestane or 5-cholestene (a change which, while increasing the molecular weight considerably, does not increase the retention time with SE-30) disrupts the planar nature of the α-side of these tetracyclic systems. This change is not unlike that effected when cholestane is epimerized to the A/B *cis*-coprostane, a stereochemical alteration which leads to an increase in volatility[2].

** Intramolecular hydrogen bonding in epicholesterol has been demonstrated by SCHLEYER *et al.*[10] using infrared spectroscopy techniques.

significant improvement in separation, and for reliable GLC analysis (especially quantitative) of mixtures of these two biologically important steroids[20], the formation of derivatives is a necessity. The epimer separation factors observed at the 17-position for the three kinds of silyl ethers are rather similar (see Table I), and are somewhat smaller in magnitude than those observed for substitution at the 3-position. Although epimeric substituents at C-17 are part of a rigid ring system and their stereochemical differences are well defined, there is not as sharp a difference in steric orientation as the equatorial–axial difference present at C-3, and for this reason it is not surprising that the epimers at C-17 show smaller differences in volatility. Dimethylsilylation and trimethylsilylation again lead to large decreases in retention time with EGSS-Z but there is little change in retention time following chloromethyldimethylsilylation, the decrease in polarity resulting from etherification being offset by the increase in molecular weight.

The epimeric 20α- and 20β-hydroxy-4-pregnen-3-ones possess sizable separation factors, especially with EGSS-Z. No improvement is observed following silyl ether formation, the separation factors for the derivatives actually being reduced in all cases except one (see Table I). The 20α-hydroxyl clearly leads to a lower volatility with these stationary phases (especially EGSS-Z) than does its epimer, which may be explained on the basis of differences in steric orientation[21] leading to greater availability of the 20α group for bonding with the stationary phase. Derivatization eliminates the bonding properties at C_{20}, and the epimer separation is then dependent upon differences in molecular shape. Not being part of a rigid cyclic system such steric differences do not influence volatility differences as much as they do at C-3 or even C-17. The effect of derivatization upon the retention behavior of closely related steroids thus depends not only upon the nature of the derivative employed and the stationary phase, but also upon the position of substitution on the steroid skeleton.

Pregnane-3α,20α-diol is capable of forming di-silyl ether derivatives, and the retention behavior of its di-DMSi, di-TMSi and di-CMDMSi ethers is presented in Table II. The preparation of mixed di-silyl ether derivatives of pregnanediol is also possible. Samples of pregnanediol were allowed to react with mixtures of silylating reagents* to form the mixed derivatives pregnanediol DMSi, TMSi di-ether, pregnanediol DMSi, CMDMSi di-ether and pregnanediol TMSi, CMDMSi di-ether. Table II presents the retention data for the peaks obtained from the three mixtures. With SE-30 each sample gave three peaks, two of which (the fastest and the slowest) were identifiable as the expected homogeneous di-derivatives, and the third peak, presumably the mixed di-ether, possessed an intermediate retention time. With EGSS-Z the dimethylsilylation–chloromethyldimethylsilylation and trimethylsilylation–chloromethyldimethylsilylation mixtures also yielded three peaks, but the mixed silyl peaks (intermediate retention times) both possessed shoulders. This inhomogeneity is not totally unexpected, since the two hydroxyl groups in pregnanediol are not equivalent, and positional isomerism is possible. The dimethylsilylation–trimethylsilylation reaction product, in contrast to its behavior with SE-30, gave only one peak. The di-DMSi and di-TMSi ethers, with this polar phase, are much more similar in volatility

* The three mixtures contained the following reagents: tetramethyldisilazane, dimethylchlorosilane, hexamethyldisilazane and trimethylchlorosilane; tetramethyldisilazane, dimethylchlorosilane, 1,3-bis(chloromethyldimethyl)-1,1,3,3-tetramethyldisilazane, and chloromethyldimethylchlorosilane; hexamethyldisilazane, trimethylchlorosilane, 1,3-bis(chloromethyldimethyl)-1,1,3,3-tetramethyldisilazane and chloromethyldimethylchlorosilane.

than on the non-polar phase, and the presence in the mixture of a component of intermediate volatility leads to non-resolution. GLC analysis of a sample of pregnanediol treated with a mixture of all the silylating reagents yielded three peaks with EGSS-Z, but the six (not including the positional isomers possible with the mixed silyls) theoretically possible compounds are observed with SE-30 (see Fig. 1), a phase more sensitivity to differences in molecular weight than the polar EGSS-Z. The use of mixed

TABLE II

RETENTION BEHAVIOR OF PREGNANEDIOL AND SIX DI-SILYL ETHER DERIVATIVES[a]

Steroid	*Relative retention time*	
	SE-30	*EGSS-Z*
Cholestane	1.00	1.00
Pregnane-3α,20α-diol	0.65	7.24
Pregnanediol $(DMSi)_2$	0.72	0.75
Pregnanediol DMSi, TMSi	0.71, 0.81, 0.94	0.79
Pregnanediol $(TMSi)_2$	0.94	0.81
Pregnanediol DMSi, CMDMSi	0.71, 1.76, 4.70	0.76, 2.81[b], 12.3
Pregnanediol TMSi, CMDMSi	0.95, 2.09, 4.71	0.80, 2.96[c], 12.2
Pregnanediol $(CMDMSi)_2$	4.72	12.3

[a] Same column conditions as Table I.
[b] Shoulder at 3.16.
[c] Shoulder at 3.07.

reagents to form multiple derivatives and the employment of an appropriate stationary phase for their separation thus allows a six-way characterization of pregnanediol in a single run. The formation of such derivative patterns, a rather non-classical approach to the identification of steroids, should be successful with other types of reagents, such as acid anhydrides[22].

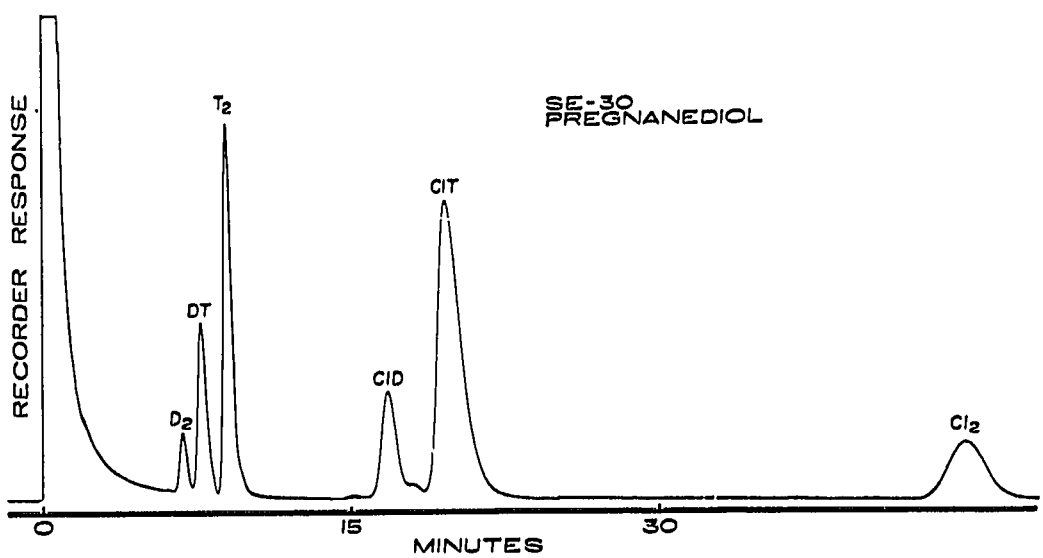

Fig. 1. Gas–liquid chromatographic analysis of the mixture of products obtained when a sample of pregnane-3α,20α-diol is treated with a combination of the six silylating reagents (listed in the Experimental section). The compounds are pregnanediol di-DMSi ether (D_2), pregnanediol DMSi, TMSi di-ether (DT), pregnanediol di-TMSi ether (T_2), pregnanediol DMSi, CMDMSi di-ether (CID), pregnanediol TMSi, CMDMSi di-ether (CIT), and pregnanediol di-CMDMSi ether (Cl_2). Column conditions given in the Experimental section.

Mechanism of silyl ether formation

Their ease of preparation, as well as their excellent GLC properties, is another reason for the widespread use of TMSi ethers in GLC analysis. Quantitative derivatization by treatment of the steroid with a mixture of HMDS and an equal or smaller amount of TMCS (sometimes referred to as a catalyst) can be carried out in a wide variety of solvents—tetrahydrofuran, hexane, acetone, pyridine, chloroform, dimethylformamide[1–15]. Following the work with pregnanediol, preliminary experiments were carried out using mixed sets of reagents (for example, HMDS and CMDMCS) with monohydroxysteroids, and these suggested that both types of compounds, the disilazanes and the chlorosilanes, function directly in the etherification reaction. The experiments described below were therefore undertaken to obtain more information concerning the nature of silyl ether formation.

Following the method of silylation described in the Experimental section, 600 μg of cholesterol dissolved in 200 μl tetrahydrofuran and 10 μl pyridine were treated with 30 μl HMDS. Reaction for three hours at room temperature led to less than 10 % conversion to the TMSi ether, but when the same experiment was carried out with the addition of 10 μl of TMCS a quantitative conversion was effected. It appeared, then, that a catalytic effect had indeed been observed. When the HMDS was absent from the reaction mixture, however, the TMCS was able to effect a greater than 90 % conversion of the sterol. (Similar results are seen when the chlorosilanes and disilazanes for chloromethyldimethylsilylation and dimethylsilylation are employed. Although large molar excesses are used, this has been the normal procedure[1–15], as removal of excess reagents is a relatively simple matter. It proved impossible to obtain the DMSi ether free of sterol, however.) The addition of the TMCS to the HMDS-containing solution brings the reaction to completion, but its function under these conditions appears to be as a silylation agent *per se*, rather than only as a "catalyst" for the reaction of the HMDS. This point is illustrated in Fig. 2. A solution of cholesterol in tetrahydrofuran and pyridine (identical to those described above) was treated with 30 μl of HMDS and 10 μl CMDMCS. Since this amount of HMDS alone causes less than 10% reaction, it is clear that the CMDMCS does effect an improvement in the conversion to the TMSi ether; however, approximately the same amount of cholesterol is converted to the CMDMSi ether, indicating that the chlorosilane also functions as a reagent in its own right. When DMCS and HMDS are used in such an experiment, a somewhat similar result is obtained.

In contrast to the results found in the presence of pyridine, without this base not only do the disilazanes by themselves fail to bring about appreciable silylation, but the chlorosilanes are also ineffective. In the case of a non-phenolic alcohol the reaction with a chlorosilane (for example, TMCS) is complicated by the fact that an equilibrium is established, *e.g.*,

$$(CH_3)_3SiCl + ROH \leftrightharpoons (CH_3)_3SiOR + HCl$$

Pyridine destroys the equilibrium by reacting with the HCl and allows the reaction to proceed to the right[23]. A combination of a disilazane and its corresponding chlorosilane (3:1), however, even in the absence of pyridine, leads to a quantitative conversion in three hours at room temperature. (This is true for trimethylsilylation

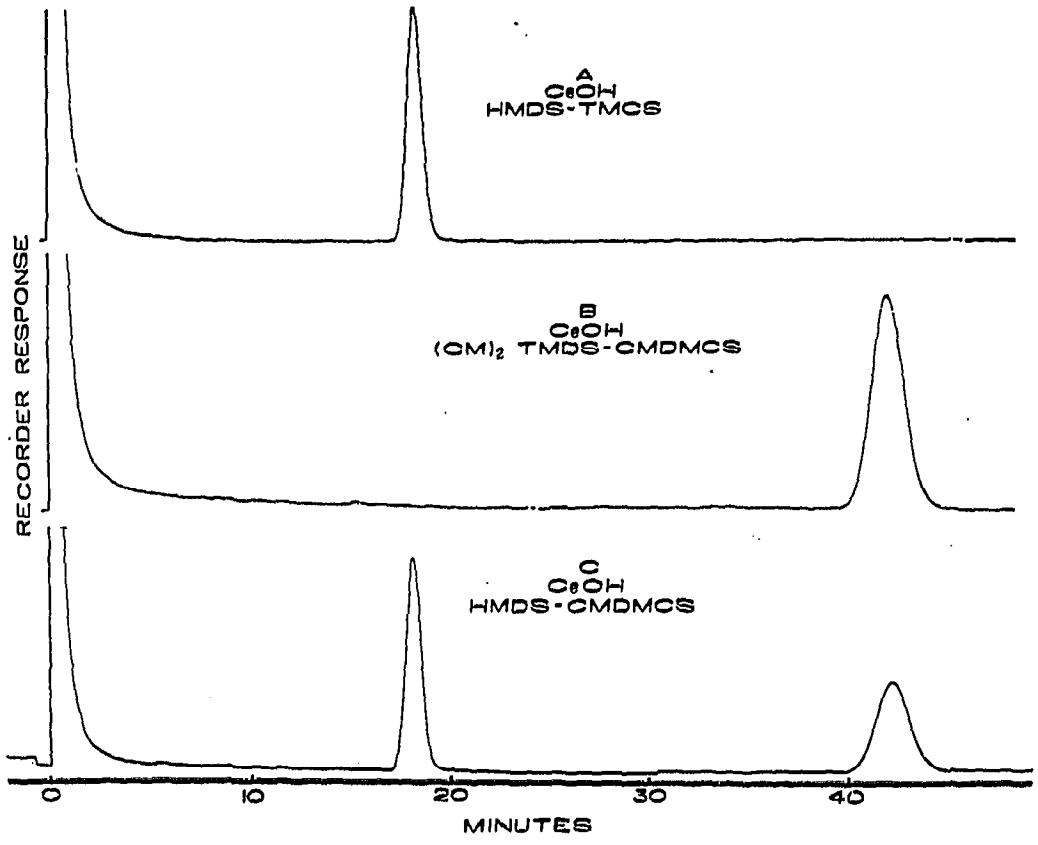

Fig. 2. Gas–liquid chromatographic analyses (with 2 % SE-30) of the reaction products obtained when cholesterol (CeOH) is allowed to react (pyridine catalyst) with HMDS and TMCS (A), $(CM)_2$TMDS and CMDMCS (B), and HMDS and CMDMCS (C). See the Experimental section for column conditions and abbreviations.

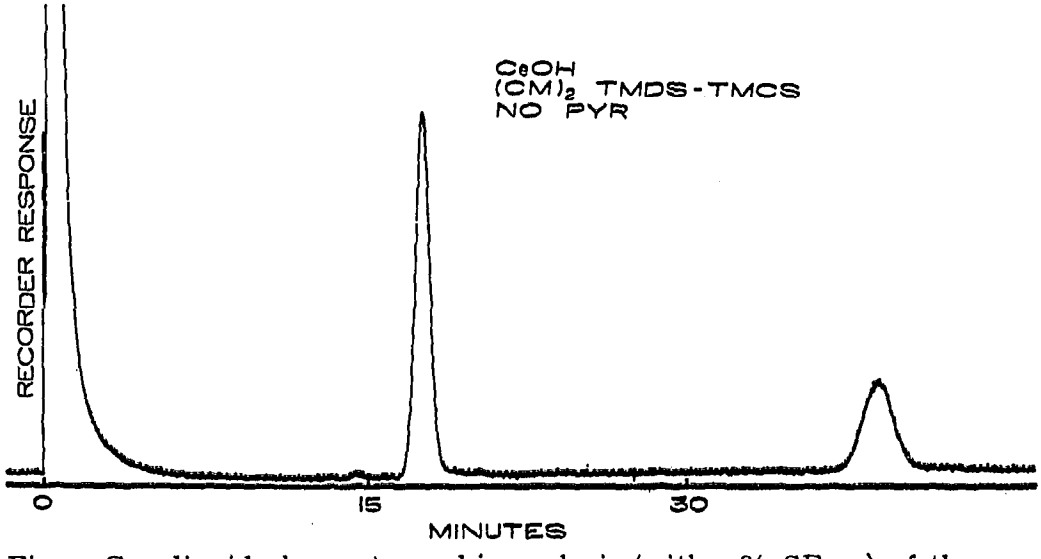

Fig. 3. Gas–liquid chromatographic analysis (with 2 % SE-30) of the reaction product obtained when cholesterol (CeOH) is treated with a combination of $(CM)_2$TMDS and TMCS in the absence of pyridine. See the Experimental section for column conditions and abbreviations.

and chloromethyldimethylsilylation; DMCS and TMDS give only a 50 % conversion.) The treatment of 600 μg of cholesterol in 200 μl tetrahydrofuran with 30 μl $(CM)_2$-TMDS and 10 μl TMCS under the above conditions resulted in the product pattern observed in Fig. 3; the two theoretically possible ethers are formed, proving that both the chlorosilane and the disilazane serve as sources of silyl ether groups. The use of HMDS and CMDMCS in combination results in an even larger proportion of the sterol being transformed to the silyl ether derived from the chlorosilane. When cholesterol is treated with HMDS and DMCS (3:1) approximately equal amounts of the two silyls are formed; when the disilazane is TMDS and the chlorosilane is TMCS or CMDMCS, however, the main silyl ether product (two-thirds to three-quarters) is derived from the chlorosilane.

Returning to Fig. 3, it is evident that in the absence of pyridine the TMCS is acting as an acid catalyst[23] for the reaction of the $(CM)_2$TMDS, as well as serving as a true reagent and source of TMSi groups. The disilazane, a secondary amine, is functioning both as a base or source of base or "acid acceptor"[23] (ammonia), which is necessary for the reaction of the chlorosilane, and as a source of CMDMSi groups.

LANGER, CONNELL AND WENDER[23], who did pioneering work on the preparation and properties of TMSi ethers of lower molecular weight alcohols, proposed the following equation for the reaction of an alcohol with equivalent amounts of TMCS and HMDS:

$$3\ ROH + (CH_3)_3SiCl + (CH_3)_3SiNHSi(CH_3)_3 \rightarrow 3\ ROSi(CH_3)_3 + NH_4Cl$$

Although the present work was not designed to be a full kinetic study of the silylation reaction, it is clear that under our pyridine-free conditions (considerably greater amount of the disilazane than the chlorosilane) the chlorosilane appears to be a more ready source of silyl ether groups than the disilazane. In the presence of pyridine this amine (and possibly also the disilazane) functions as a base catalyst for the reaction of the chlorosilane with the alcohol, and the chlorosilane also acts as catalyst for the reaction of the disilazane.

Effect of trimethylsilylation upon detector response

The quantitative analysis of free (underivatized) sterols can be carried out successfully if packing free of adsorptive properties is employed[24]. The hydrocarbon-like TMSi ethers are less subject to selective irreversible adsorption ("loss on the column"), however, and for this reason these derivatives are often used in quantitative steroid GLC[2]. Since detector response has been reported to be determined by steroid structure *per se*[25], changes in peak area relationships accompanying functional group changes should not be ascribed solely to column effects. The response/mass factors for the TMSi ethers of the 17-ketosteroids with a hydrogen flame ionization detector, for example, have been reported to be greater than those for the parent compounds[5].

In order to study the change in observed detector response when a sterol is transformed to its TMSi ether, a sample of cholesterol was divided into three equal portions. The first was dissolved in sufficient solvent (carbon disulfide, 0.05 % in cholestane) to give a 0.1 % solution (weight/volume percentages in both cases). Quantitative analysis of this two-component mixture at the 1–2 μg cholesterol/peak level employing the SE-30 column with both Lovelock argon and hydrogen flame

ionization detectors resulted in the data presented in Table III. The second portion of cholesterol was trimethylsilylated, the excess reagents removed (nitrogen) and the residue dissolved in a volume of the cholestane solution (carbon disulfide possesses the ability to dissolve ammonium chloride[26]) equal to the amount used to dissolve the underivatized cholesterol. Quantitative analysis of aliquots of this mixture showed a

TABLE III

DETERMINATION OF RESPONSE FOR CHOLESTEROL AND CHOLESTERYL TMSi ETHER COMPARED WITH RESPONSE FOR CHOLESTANE

Mixture[a]	*Weight ratio*	*Molar ratio*	*Area*[b] *ratio*		*Area ratio*[c] *Weight ratio*		*Area ratio*[c] *Molar ratio*	
			A.D.[d]	*F.D.*[e]	*A.D.*	*F.D.*	*A.D.*	*F.D.*
Cholesterol	2.00	1.92	1.52	1.50	76	75	79	78
Cholesteryl TMSi	2.37	1.92	2.06	2.07	87	87	107	108
Cholesteryl TMSi	2.00	1.62	1.75	1.74	88	87	108	107

[a] The second component in each mixture was a known amount of cholestane. Ratios are calculated with respect to the hydrocarbon.
[b] Measurements of area were height × width at half-height.
[c] × 100.
[d] Argon ionization detector.
[e] Flame ionization detector.

large increase in the peak area ratios over the values found for the free sterol. The entire increase cannot be ascribed to a greater detector response ("ion yield") for an ether group, however. Trimethylsilylation is a functional group change resulting in an increase in molecular weight from 386 to 458, and because of this change the weight or mass of the cholesteryl TMSi ether is 1.18 times that of the cholesterol in the first mixture. Since the TMSi ether was taken up in the same volume of solvent as the parent sterol, the weight percentage of the TMSi ether solution is 1.18 times greater, accounting for some of the increase in response. The third portion of the cholesterol was trimethylsilylated and dissolved in a volume of the cholestane solution 1.18 times greater than that used for the other two samples, so that it was 0.1 % (weight/volume) in cholesteryl TMSi. A comparison of the peak area ratios for the two ether-containing samples indicates that with both detection systems the values for the first trimethylsilylated sample are larger than those for the second derivatized sample by factors (1.18, argon; 1.19, flame) very close to the weight percentage composition factor of 1.18. The last four columns in Table III compare detector responses for cholesterol and its TMSi ether on weight (mass) and molar bases, and it can be seen that the TMSi ether does give greater response values, especially on a molar basis. On a weight (mass) basis the flame response for the TMSi ether is 116 % that of the parent sterol, this factor being similar to the value (113 %) observed when the weight response of testosterone TMSi was compared to that of testosterone under similar conditions[27]. With the argon detector, however, these latter two steroids exhibited identical responses on a mass basis[27], whereas Table III indicates that with this detection system the response/mass for cholesteryl TMSi ether is 1.15 times that of cholesterol.

In addition to the possibility that selective loss may exist for sterols when compared to less polar compounds*, another complicating issue in the discussion of quantitation is the variation of the response of a given detection system to a given compound with the geometry of the detector cell, the imposed voltage, the amount and nature of "column bleed", and, in the case of the hydrogen flame ionization detector, the temperature and composition of the flame[2,18,22]. Thus in new quantitative applications the relationship between structure and response ("ion yield") for the compounds under study should be determined for the column and detection system to be used. It is a questionable practice to rely implicitly on literature reports for detector characteristics, and to assume that these will be unchanged for a different chromatographic system and a detector which is sensitive to a variety of factors**. Rather, such reports should be used as guidelines for establishing one's own methodology. Many satisfactory quantitative GLC methods of high precision and accuracy have been established, testifying to the essential value of the technique[28].

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SUMMARY

The GLC behavior of three pairs of epimeric hydroxysteroids and their dimethylsilyl, trimethylsilyl and chloromethyldimethylsilyl ethers has been investigated through the use of two stationary phases of different partitioning properties. In general, stereochemical differences at C-3 result in greater retention differences than those at C-17, with derivative formation leading to improved separation; at C-20 the free hydroxyl groups lead to greater epimer differentiation than do derivatives. Chloromethyldimethylsilylation leads to increased retention time with "polar" and "non-polar" stationary phases, in contrast to dimethylsilylation and trimethylsilylation which effect a reduction in retention times with the former. The formation of multiple derivatives (homogeneous and heterogeneous) of polyhydroxy steroids through the use of mixtures of different silylating reagents enhances the ability to characterize such compounds. Studies on the mechanism of formation of silyl ethers have disclosed that the disilazane and the corresponding chlorosilane each function as sources of silyl ether groups in the etherification reaction. On both a molar basis and a weight basis the trimethylsilyl ether of cholesterol yields a greater detector (argon ionization and flame ionization) response than the parent sterol.

* The packing employed in the above reported experiments, however, was shown[24] not to exhibit selective loss for cholesterol at the sample levels used.

** Witness the conflicting results concerning response factors for work with sterol derivatives reported by MIETTINEN, AHRENS AND GRUNDY[10] and ROZANSKI[11].

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