THE GAS–LIQUID CHROMATOGRAPHIC BEHAVIOR OF STEROL SULFONATES

EFFECT OF STRUCTURE UPON THE NATURE OF THEIR ELIMINATION REACTION

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INTRODUCTION

It has recently been demonstrated that the methanesulfonates and *p*-toluenesulfonates of sterols quantitatively undergo an elimination reaction when applied to a gas–liquid chromatography (GLC) column to yield olefinic products[1]. The type of elimination reaction and the pattern of resulting olefins is dependent upon the nature of the parent hydroxyl group and its molecular environment. For example, the methanesulfonate and *p*-toluenesulfonate of the simple sterol cholestanol yield 2-cholestene, whereas the ester group is eliminated from the homoallylic alcohol cholesterol (equatorial hydroxyl) *via* a so-called non-classical resonance stabilized carbonium ion to produce 3,5-cyclo-6-cholestene, 3,5-cholestadiene and a smaller amount of a second C_{27} diene, possibly 2,5-cholestadiene. Because of stereochemical factors, however, the sulfonate of epicholesterol (axial hydroxyl) does not react *via* the "*i*-steroid rearrangement"[1,2], and gives mainly 3,5-cholestadiene, with but a trace of the *i*-steroid hydrocarbon. Although the elimination reaction which occurs with sterol sulfonates may appear to limit their usefulness as derivatives in steroid GLC, the olefin patterns are indicative of the structure of the steroid esters. Additional studies have now been carried out to ascertain the influence of unsaturation and alkyl substituents upon the GLC behavior of these "reactive derivatives".

EXPERIMENTAL

The sterol derivatives were prepared using methanesulfonyl chloride (pyridine catalyst; ethyl acetate solvent) as previously described[1]. Methanesulfonates of cholestanol, cholesterol, pregnenolone, stigmasterol and dihydrolanosterol were isolated and had satisfactory elementary analyses or melting points. Other methanesulfonates were prepared on a submilligram scale; excess reagent was removed in a stream of nitrogen, and the residue taken up in solvent and used directly for chromatography. 4β-Methylcholesteryl *p*-toluenesulfonate was a gift from Dr. R. M. MORIARTY. The GLC packing materials were prepared from 80–100 mesh Gas-Chrom P by established procedures[3]. Column (6 ft. × 4 mm U-tubes) conditions were as follows: 2 % SE-30

(General Electric Company); 230°; 16 p.s.i.; 1 % EGSS-Z[4] (Applied Science Laboratories, Inc.); 205°; 12 p.s.i.

RESULTS AND DISCUSSION

In order to determine whether the results observed for cholesterol are common to simple 3β-ol-Δ^5 sterols the methanesulfonates of a number of other so substituted compounds (desmosterol, dihydrobrassicasterol and stigmasterol) were also subjected to GLC analysis. The retention times (relative to cholestane) of the resulting olefins are presented in Table I, and it is clear that the sulfonates of each of the homoallylic sterols exhibit a triplet olefin pattern with both stationary phases. Fig. 1 illustrates the similarity in olefin pattern observed with SE-30 for three homoallylic methane-

TABLE I

GAS CHROMATOGRAPHIC BEHAVIOR OF STEROLS, SULFONATE ESTERS AND REFERENCE COMPOUNDS

Compound	*Relative retention times*	
	2 % SE-30	*1 % EGSS-Z*
Cholestane	1.00[a]	1.00[b]
3,5-Cyclo-6-cholestene	0.80	0.99
3,5-Cholestadiene	1.10	1.63
Cholesterol	1.87	7.30
Cholesteryl methanesulfonate[c,d]	0.80, 1.02, 1.10	0.99, 1.43, 1.63
Epicholesterol	1.80	5.82
Epicholesteryl methanesulfonate[c,d]	1.02, 1.10	1.43, 1.63
Desmosterol	2.07	10.4
Desmosteryl methanesulfonate[d]	0.88, 1.12, 1.21	1.38, 1.99, 2.26
Dihydrobrassicasterol	2.50	9.83
Dihydrobrassicasteryl methanesulfonate[d]	1.04, 1.33, 1.43	1.33, 1.92, 2.17
Stigmasterol	2.68	10.9
Stigmasteryl methanesulfonate[d]	1.16, 1.46, 1.57	1.43, 2.09, 2.36
Ergosterol	2.33	11.6
Ergosteryl methanesulfonate[d]	0.89, 1.01, 1.29, 1.40	1.27, 1.55, 1.83, 2.63
4β-Methylcholesterol	2.38	8.14
4β-Methylcholesteryl *p*-toluenesulfonate[d]	1.40	2.04
4α-Methylcholesterol	2.31	8.11
4α-Methylcholesteryl methanesulfonate[d]	0.99, 1.42	1.11, 2.02
4-Methyl-4-cholesten-3β-ol[d]	1.22, 1.42	1.63, 2.03
4-Methyl-3,5-cholestadiene	1.40	2.03
Allocholesteryl *p*-toluenesulfonate[d]	1.00, 1.09	1.31, 1.63
Cholestanol	1.89	6.81
Cholestanyl methanesulfonate[c,d]	0.96	1.11
Lathosterol	2.11	9.05
Lathosteryl methanesulfonate[d]	1.11	1.53
8(14)-Cholesten-3β-ol	1.88	7.15
8(14)-Cholesten-3β-ol methanesulfonate[d]	0.97	1.25
Methostenol	2.52	9.58
Methostenyl methanesulfonate[d]	1.28, 1.49	1.71, 2.09
Dihydrolanosterol	2.91	8.95
Dihydrolanosteryl methanesulfonate[d]	1.00, 1.38, 1.55, 1.67	0.80, 1.48, 1.69, 1.84

[a] Absolute retention time, 10.8 min.
[b] Absolute retention time, 4.4 min.
[c] The *p*-toluenesulfonate behaves in an identical manner.
[d] Functional group eliminated.

sulfonates (parent sterols: cholesterol, C_{27}, dihydrobrassicasterol, C_{28} and stigmasterol C_{29}.

It was found that this type of GLC behavior could be expressed in a meaningful and useful fashion by employing the value obtained when the retention time of the olefin(s) is divided by the retention time of the parent sterol. When such an approach is used for these four sterols, the data seen in Table II make it clear that sulfonates of this structural type exhibit a characteristic set of values, and suggest that the same mechanism is operative in the elimination of the functional group of these homoallylic sterol derivatives*. Structural differences far removed from the site of the elimination do not affect the course of the reaction in these instances. The nature of the reaction is altered when a second double bond is introduced into the Δ^7 position, however, for the GLC behavior of ergosteryl methanesulfonate is considerably different from that of the closely related mono-unsaturated sterols (see Fig. 2 and Tables I and II). Gone

TABLE II

OLEFIN/STEROL FACTORS OBSERVED FOR SULFONATE ESTERS

Parent sterol	*Retention time ratios*[a]	
	SE-30	*EGSS-Z*
Cholesterol	0.43, 0.54, 0.59	0.13, 0.20, 0.22
Epicholesterol	0.57, 0.61	0.25, 0.28
Desmosterol[b]	0.42, 0.54, 0.59	0.13, 0.20, 0.23
Dihydrobrassicasterol	0.42, 0.54, 0.58	0.13, 0.20, 0.22
Stigmasterol	0.43, 0.54, 0.59	0.13, 0.19, 0.22
Ergosterol	0.38, 0.43, 0.55, 0.60	0.11, 0.13, 0.16, 0.23
4β-Methylcholesterol	0.59	0.25
4α-Methylcholesterol	0.43, 0.61	0.14, 0.25
Cholestanol	0.51	0.16
Lathosterol	0.53	0.17
8(14)-Cholesten-3β-ol	0.52	0.17
Methostenol	0.51, 0.59	0.18, 0.22
Dihydrolanosterol	0.34, 0.47, 0.53, 0.57	0.09, 0.16, 0.19, 0.21

[a] Determined from data in Table I.

[b] The sample of desmosterol contained a small amount (5–10%) of a more volatile compound which yields the same olefin/sterol factors, and on the basis of this evidence may be said to also possess the 3β-ol-Δ^5 system.

is the familiar triplet of peaks, and in its place are two doublets. The olefin/sterol factors for the second and largest peak (0.43 and 0.13 with SE-30 and EGSS-Z, respectively) correspond exactly with the values for the *i*-hydrocarbons from the 3β-ol-Δ^5 systems. It is a characteristic of steroids possessing the 3,5-cyclo system to exhibit retention times considerably smaller than those for related compounds not containing the three-membered ring[1,3]. Indeed, the marked volatility of the earliest eluted peak resulting from the application of cholesteryl methanesulfonate to the GLC column was the first clue to the fact that the elimination reaction was occurring

* Except for the case of cholesterol, the appropriate reference compounds are not readily available, but by analogy with the results obtained for cholesterol this is a reasonable assumption: the olefin corresponding to the 0.42–0.43 (SE-30) and 0.13 (EGSS-Z) values possesses the 3,5-cyclo-Δ^6-system, and the 0.58–0.59 (SE-30) and 0.22–0.23 (EGSS-Z) values are due to the 3,5-diene.

via the *i*-steroid rearrangement[1]. The elimination of *p*-toluenesulfonic acid from ergosteryl *p*-toluenesulfonate in the absence of hydroxylic solvents (a condition in some ways not unlike the inert atmosphere of the GLC column) has been shown by FIESER, ROSEN AND FIESER[5] to lead to 3,5-cyclo-6,8(14),22-ergostatriene plus a lesser amount of another hydrocarbon of undetermined structure, and NES AND STEELE[6] have reported that, in addition to the above-mentioned 3,5-cyclo-triene, the elimination reaction leads to the production of a small amount of a contaminant hydrocarbon, possibly the isomeric *i*-steroid 3,5-cyclo-6,8,22-ergostatriene. The finding of at least one component from ergosteryl methanesulfonate with GLC behavior which suggests 3,5-cyclo structure is thus not surprising*.

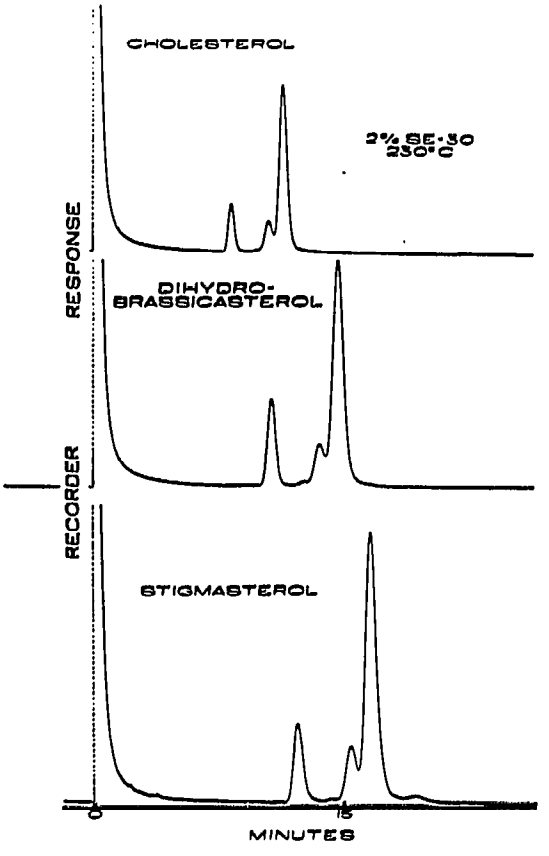

Fig. 1. Comparison of the gas–liquid chromatographic behavior of the methanesulfonates of cholesterol, dihydrobrassicasterol and stigmasterol with a 2% SE-30 packing. Column conditions: 6 ft. × 4 mm glass U-tube; 230°; 16 p.s.i.

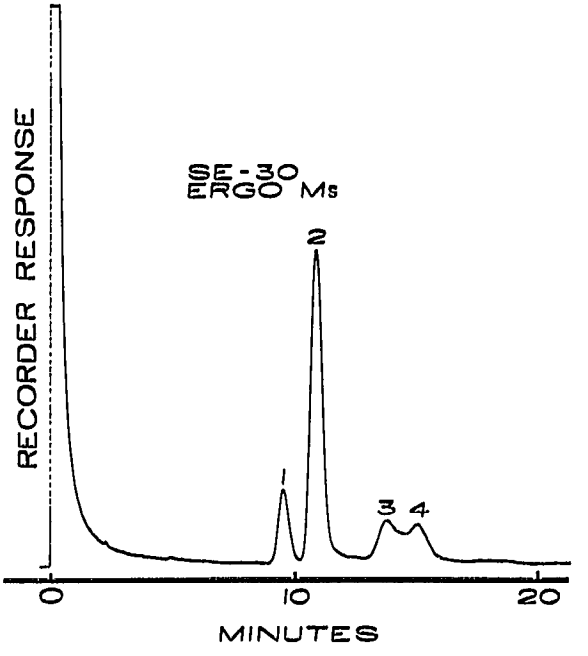

Fig. 2. Gas–liquid chromatographic behavior of ergosteryl methanesulfonate. Column conditions as in Fig. 1.

The introduction of a methyl group into the 4β position of a 3β-ol-Δ^5 system also leads to a change in the GLC behavior of the corresponding sulfonate. 4β-Methylcholesteryl *p*-toluenesulfonate yields only the product of simple elimination, 4-methyl-

* A sample of the hydrocarbon product from the *i*-steroid-forming dehydration of ergosterol with *p*-toluenesulfonyl chloride in pyridine (kindly provided by Professor L. F. FIESER) gave these two early peaks (exact correspondence with both stationary phases); with this sample the second peak was four to five times larger than the first.

3,5-cholestadiene, and a more rapidly eluted compound which could be ascribed to a 3,5-cyclosteroid is not observed. This is in accord with the report of MORIARTY AND DESOUSA[7] that, because of steric and thermodynamic factors, this ester does not undergo solvolysis to yield *i*-steroid product, but instead gives mainly diene. In contrast, these authors report that 4α-methylcholesteryl *p*-toluenesulfonate undergoes solvolysis in a manner analogous to that of cholesterol *p*-toluenesulfonate, and thus 3,5-cyclo-4α-methylcholestan-6β-ol is observed as a product, the reaction proceding through the homoallylic carbonium ion. One would therefore expect the 4α-methyl sulfonate to differ also from its 4β-isomer in GLC behavior, and behave in a fashion similar to the cholesterol ester. Two peaks are observed for the 4α-methyl sulfonate: 4-methyl-3,5-cholestadiene, and a faster eluted compound which with both stationary phases possesses an olefin/sterol factor (see Table II) completely compatible with *i*-steroid structure (the 4α-methyl analog of the 3,5-cyclo-6-cholestene from cholesterol). This lack of the early peak with 4β-methylcholesteryl *p*-toluenesulfonate, and its appearance with 4α-methylcholesteryl methanesulfonate, is the very same difference as that observed for cholesteryl *p*-toluenesulfonate and epicholesteryl methanesulfonate, and the basis for the differences is undoubtedly the same—the intermediacy of the homoallylic carbonium ion in the one case, and its absence in the other.

Although the early proposals that substances of the complexity and molecular weight of steroids could undergo passage through a heated (200–250°) tube and still retain their structural integrity were met with a considerable amount of skepticism, the past few years have witnessed the successful GLC analysis of a large number of naturally occurring and synthetic steroids substituted in a variety of ways[8*]. Steroidal allylic alcohols are dehydrated under the conditions of GLC analysis; the results observed for 4-methyl-4-cholesten-3β-ol are presented in Table I. No peak with the expected retention time for such a sterol is observed, but two early peaks, of roughly equal area, are obtained. The less volatile of these possesses a retention time equal to that of 4-methyl-3,5-cholestadiene, but on neither column does the earlier peak correspond to the early peak (presumably the *i*-steroid) found for 4α-methylcholesteryl methanesulfonate. When the allylic methanesulfonate is applied to the column the same two peaks are observed, but under these conditions the relative peak areas are changed, and the second peak is several fold larger. Allocholesterol (4-cholesten-3β-ol) is dehydrated upon application to the column, and its ester also yields two peaks (see Table I), neither one of which corresponds to 3,5-cyclo-6-cholestene.

The olefin/sterol factors observed for cholestanyl methanesulfonate (0.51 with SE-30, and 0.16 with EGSS-Z) may be taken as typical of simple sterols which are saturated or which possess double bonds far enough removed from the ester group so that they play no significant role in the elimination reaction. In line with this, the methanesulfonate of lathosterol (7-cholesten-3β-ol) yields a single peak with olefin/sterol factors of 0.53 (SE-30) and 0.17 (EGSS-Z), with no indication of the kind of participation of the Δ^7 bond in the elimination of the ester group as is observed for the Δ^5 bond. The $\Delta^{8(14)}$ bond also appears to play no direct role in the ionization of the ester, for the methanesulfonate of 8(14)-cholesten-3β-ol produces a single product with olefin/sterol factor values of 0.52 (SE-30) and 0.17 (EGSS-Z). The introduction of a methyl group into the 4-position of lathosterol to give methostenol (4α-methyl-7-

* A few types of steroids, such as the vitamins D and molecules possessing the cortisone side chain, are transformed during the GLC process[8].

cholesten-3β-ol), however, leads to a more complex elimination reaction, for the methanesulfonate of this C_{28} sterol gives two products. This is not unexpected, for it is known that the presence of an alkyl group on the carbon atom adjacent to the site of the formation of a carbonium ion may increase the complexity of reaction and lead to multiple products[9]. The presence of a *gem*-dimethyl group α to an electron deficient carbon atom strongly favors carbonium ion rearrangement of the carbon skeleton of such molecules[10]. Dehydration of triterpene alcohols (containing an equatorial 3-hydroxyl-4,4-dimethyl system) results in Wagner rearrangement *via* a carbonium ion and leads to an alteration in the ring skeleton. The dehydration of lanostan-3β-ol with phosphorus oxychloride in pyridine gives a mixture of 2-lanostene and 3-isopropylidene-A-norlanostane[11], and dihydrolanosterol undergoes a carbonium ion rearrangement with phosphorus pentachloride to yield 3-isopropylidene-A-nor-8-lanostene[12]. Such reactions—the loss of water *via* a carbonium ion to yield olefinic product(s)—are in essence little different from the loss of methanesulfonic acid by sterol methanesulfonate to yield olefin. Application of the methanesulfonate of dihydrolanosterol to a GLC column results in the formation of at least four products (see Table I), indicating considerable molecular alteration; however, the pattern is distinct from the triplet of peaks found for 3β-ol-Δ^5 systems (see Table II). Indeed, this latter pattern is observed only for the cholesterol-like homoallylic systems, with one exception—the corresponding 3,5-cyclo-6-ols (*i*-sterols). Isolation of the 3,5-cyclocholestane-6-sulfonates has not proven possible[13,14], but when an aliquot of the methanesulfonation reaction mixture is applied directly to the column an elimination reaction may still be observed*. Under these conditions, for example, the esters of 3,5-cyclocholestan-6α-ol and 6β-ol yield the same olefins as cholesteryl methanesulfonate (derivatives of *i*-sterols and the corresponding 3β-ol-Δ^5-sterols react *via* the same hybrid carbonium ion[14], but there is relatively more 3,5-cyclo-6-cholestene from the *i*-sterol derivatives, and cholesteryl chloride (identified on three different stationary phases) may also be observed (see Fig. 3).

The normal, stable derivatives in widespread use in GLC (for example, trimethylsilyl ethers) are not only of great value in quantitative aspects of analysis[8], but are also helpful in qualitative work, since a "peak shift" or change in retention behavior following exposure of a sterol to trimethylsilylation conditions is an excellent indication that the steroid possesses an hydroxyl group. Although methanesulfonates and *p*-toluenesulfonates are not chromatographed intact a peak shift phenomenon of sorts is observed—the formation of olefinic products. In point of fact, the formation and elimination reaction of "reactive derivatives" such as the sulfonates not only indicate the presence of hydroxyl groups, but as has been made clear in this report, the olefin patterns observed also supply valuable information concerning the molecular environment of the parent hydroxyl group. The stereochemistry and the presence or absence of unsaturation or alkyl substituents in the vicinity of the hydroxyl group all contribute to the course of the elimination reaction. Although structural factors far removed from the site of the elimination reaction do not appear to affect its direction, the possibility exists that instances will be encountered where structural

* The possibility exists, of course, that the 3,5-cyclosteroid rearranges to the more thermodynamically stable Δ^5 system under the esterification conditions, and before application to the column. On the other hand, the *i*-sulfonate may form, but be too unstable to withstand the isolation procedure.

differences may, through "conformational transmission" or long range effects[15], affect the reaction (*e.g.*, determine which carbon adjacent to the ester-substituted carbon will supply the co-eliminated hydrogen)*.

The formation of 3,5-cyclosteroids *via* the solvolysis in buffered media of sulfonate esters of 3β-ol-Δ^5-sterols, and the formation of isopropylidene-A-nor products *via* dehydration (phosphorus pentachloride) of 3β-ol-triterpenes, are transformations which have become diagnostic for these two types of hydroxyl-substituted systems[10]. The data reported in this paper indicate that the GLC behavior (olefin/sterol factors) of methanesulfonates of 3β-ol-Δ^5-sterols is highly characteristic of this system**, and it is conceivable that characteristic patterns may also be found for other systems, including the 3-equatorial hydroxyl-4,4-dimethyl system found in triterpenes. Diagnostic tests utilizing GLC would be very welcome, as the formation of "reactive derivatives" is easily accomplished, and both the elimination reaction leading to the olefinic products and the analysis of the products are effected simultaneous on a microgram scale.

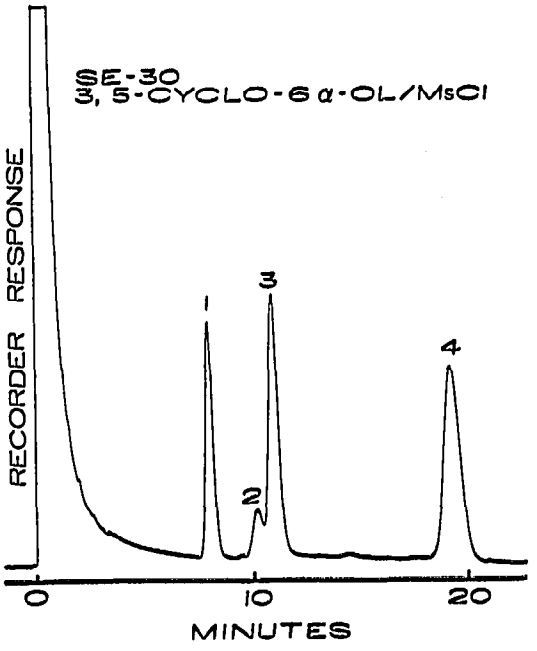

Fig. 3. Gas–liquid chromatographic analysis of an aliquot of the reaction mixture of 3,5-cyclocholestan-6α-ol and methanesulfonyl chloride (pyridine catalyst). Peaks 1 and 3 correspond to 3,5-cyclo-6-cholestene and 3,5-cholestadiene, respectively; peak 2 is a second C_{27} diene, and peak 4 is cholesteryl chloride. Column conditions as in Fig. 1.

* Since many of the interesting sterols are available only in very small quantities, the use of combined GLC–mass spectrometry[1,16–18] would be a very helpful means for determining the structure of the olefins. An effective preparative GLC system for the collection of submilligram quantities of steroids would also be of value. Such instrumentation was not available during the course of the presently reported work, but it is anticipated that these deficiencies will be corrected in the near future. There is no doubt, however, that GLC data alone are of great value, as is demonstrated in this paper.

** The presence of other functional groups does not alter the olefin/sterol factors; for example, the methanesulfonate of pregnenolone (5-pregnen-3β-ol-20-one) yields the factors expected for a 3β-ol-Δ^5-sterol (0.42, 0.54, 0.58 with SE-30, and 0.13, 0.19, 021 with EGSS-Z).

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SUMMARY

The gas–liquid chromatographic behavior of the methanesulfonates of a variety of sterols has been investigated. These compounds undergo an elimination reaction; the structure of the resulting olefin or olefins is dependent upon the stereochemistry of the hydroxyl group of the parent sterol and the presence or absence of unsaturation and alkyl substituents in its immediate molecular environment. The observed GLC behavior, which may be meaningfully presented as "olefin/sterol factors" (retention time of olefin relative to the retention time of the sterol), suggests that certain types of sterol systems may exhibit characteristic olefin peak patterns when "reactive derivatives" such as the sulfonate esters are analyzed by GLC. The 3β-ol-Δ^5 system, for example, yields a triplet of peaks which is not duplicated by any other naturally occurring sterol type so far investigated.

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