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FURTHER STUDIES ON THE GAS–LIQUID CHROMATOGRAPHIC BEHAVIOR OF SULFONATE ESTERS

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SUMMARY

The GLC behavior of the methanesulfonates of a number of hydroxysteroids has been studied. For 3-substituted steroids the nature of the elimination reaction, and the number of olefinic products, is determined by the stereochemistry at the C-3 and C-5 positions, the presence or absence of unsaturation at the 5:6 position, and the alkyl group substitution at the positions adjacent to C-3. The GLC elimination reactions of certain of the esters appear to parallel the results noted under solvolysis conditions. Thus cholesterol methanesulfonate and 2,2-dimethylcholesterol methanesulfonate yield *i*- or 3,5-cyclosteroid products, whereas pseudocholesterol methanesulfonate does not. Combined GLC–mass spectrometry has proven to be most helpful in the characterization of the olefinic products.

The gas–liquid chromatographic (GLC) behavior of sulfonate esters* of hydroxysteroids has been reported in several recent publications[1–3]. These compounds "reactive derivatives" undergo a thermal elimination reaction when applied to a GLC column and yield olefinic products, the structures of which are dependent upon the nature of the parent hydroxyl group. The homoallylic 3β-ol-Δ^5 system has been discovered to give a triplet of peaks, the two major products being an unsaturated 3,5-cyclo or *i*-steroid and a diene (for example, cholesterol methanesulfonate yields 3,5-cyclo-6-cholestene and 3,5-cholestadiene); the reaction is held to proceed via a resonance stabilized non-classical carbonium ion[1]. If the double bond is absent, however, a simple elimination reaction occurs. The methanesulfonate of the A/B *trans* sterol dihydrocholesterol (cholestanol) gives only one product, 2-cholestene[1]. The stereochemistry at the C-5 position appears to influence the direction of the elimination reaction, for it has been observed that the methanesulfonate of methyl lithocholate (A/B *cis*) gives two olefins[3]. In order to investigate the influence of stereochemistry at C-3 and C-5 upon olefin patterns observed for the 3-substituted sulfonate esters, we have employed the four readily available diastereoisomers 5α-androstan-3β- and 3α-ol-17-ones and 5β-androstan-3β- and 3α-ol-17-ones as model compounds.

* Methanesulfonate and *p*-toluenesulfonate esters behave in an identical fashion.

The methanesulfonates of the A/B *trans* compounds 5α-androstan-3β- and 3α-ol-17-ones each gave the same (by retention time) single peak when subjected to GLC with two stationary phases of widely different selectivity—SE-30 and EGSS-Z. The mass spectra of the two olefins (obtained by combined GLC–mass spectrometry with the LKB Model 9000) were identical and virtually indistinguishable from the mass spectrum of authentic 2-5α-androsten-17-one, indicating that the ester elimination had proceeded with loss of a proton from C-2. The retention behavior for the elimination product is of no value for assignment of structure with SE-30, and with EGSS-Z no assignment can be made, as the retention time is intermediate to that of 3-5α-androsten-17-one and the Δ^2-isomer. Fortunately, the mass spectra of the isomeric olefins differ significantly, making the choice a simple one, and this illustrates the great value of the combined technique*.

GLC of the epimeric A/B *cis* sulfonates of 5β-androstan-3β- and 3α-ol-17-ones gave two peaks with both stationary phases. Unfortunately, the unsaturated 5β-androsten-17-ones were not available for comparison purposes. The GLC data indicated that the epimeric esters yield the same two olefins, and this was confirmed by combined GLC–mass spectrometry. One would assume that one of the olefins is 2-5β-androsten-17-one, and the other 3-5β-androsten-17-one. The earlier eluted component of these two mixtures exhibits a mass spectrum similar to, but not identical with, that of 3-5α-androsten-17-one, whereas the mass spectrum of the later eluted component rather closely resembles that of 2-5α-androsten-17-one. It is perhaps somewhat hazardous to draw conclusions relating to the position of the double bond in these two A/B *cis* (5β-H) olefins from a comparison of their mass spectra with the mass spectra of the Δ^2 and Δ^3 A/B *trans* (5α-H) olefins. The mass spectra of epimeric compounds may differ only slightly**, (for example, with our instrument, the LKB Model 9000, the mass spectra of coprostane (5α-H) and cholestane (5α-H) differ only in the relative abundancy of the peak at m/e 151), whereas differences between double bond isomers are usually more pronounced[4]. There is some basis for optimism, then, in using these compounds as reference standards, and tentative assignment of 3-5β-androsten-17-one is thus made for the more rapidly eluted elimination product, and for the olefin with the greater retention time the structure 2-5β-androsten-17-one is suggested. Formation of the Δ^2 olefin from the A/B *trans* sulfonates is not unexpected, as a double bond opposite an A/B *trans* fused ring junction is thermodynamically more stable than the isomeric Δ^3 system. Further, formolysis of the *p*-toluenesulfonate ester of 5α-androstan-3α-ol-17-one has been shown to produce a 9:1 mixture of the Δ^2 and Δ^3 isomers[5]. Under the GLC conditions employed in this study, the thermodynamic relationships may be such that with the 5β-androstane esters there is no predominant pathway for olefin formation, and hence the observed mixture of olefins.

Fig. 1 illustrates the GLC behavior of the methanesulfonates of 5α-androstan-3β-ol-17-one, 5β-androstan-3β-ol-17-one, and 5-androsten-3β-ol-17-one, which is included to show the effect of the double bond on the olefin pattern (note the triplet of peaks).

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* The GLC of 'reactive derivatives' combined with mass spectrometry is perhaps the ultimate in 'gas phase analytical chemistry'. Both the derivatization and the 'on column' elimination reaction can be carried out on the microgram scale, and the resulting olefinic products are characterized simultaneously by their GLC retention behavior and mass spectra.

** GLC behavior in some cases can be a better means of distinguishing between epimers[4].

The nature of the GLC elimination reaction is thus determined by several factors, including stereochemistry and the presence or absence of unsaturation. Alkyl group substitution in proximity to the reaction site can also influence the course and complexity of the elimination reaction. 4β-Methylcholesterol methanesulfonate undergoes simple elimination to yield 4-methyl-3,5-cholestadiene, whereas 4α-methylcholesterol tosylate yields the diene and 3,5-cyclo product; these results parallel solvolysis studies[2,6]. Solvolysis of 4,4-dimethylcholesterol tosylate has been shown to yield no *i*-steroid product, but rather a mixture of ring contracted dienes plus lesser amounts of A-nor alcohol and parent alcohol[7]. The reaction was held to proceed in a fashion similar to the dehydration of 3β-hydroxy-4,4-dimethyl triterpenes, with 3-isopropylidene-A-nor-5-cholestene suggested as one of the dienes. GLC of the methanesulfonate of 4,4-dimethylcholesterol yielded a multi-component chromatogram (see Fig. 2); the major component of the mixture was shown by combined GLC–mass spectrometry to be a C_{29} diene or its equivalent (molecular ion 396). Unfortunately, an authentic sample of 3-isopropylidene-A-nor-5-cholestene could not be obtained for comparison purposes.

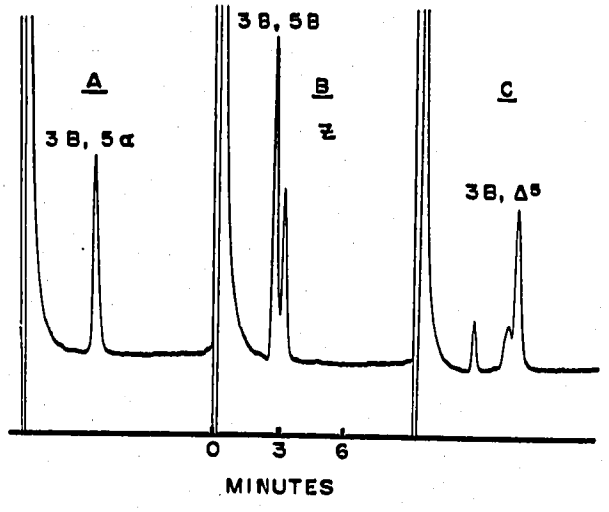

Fig. 1. GLC behavior on EGSS-Z of the methanesulfonates of 5α-androstan-3β-ol-17-one (panel A), 5β-androstan-3β-ol-17-one (panel B) and 5-androsten-3β-ol-17-one (panel C). Column conditions given in Table I.

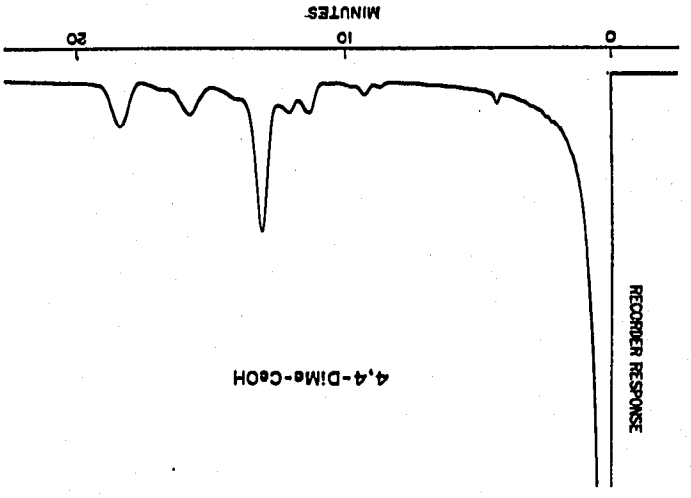

Fig. 2. GLC behavior on SE-30 of the methanesulfonate of 4,4-dimethylcholesterol. Column conditions given in Table I.

2,2-Dimethylcholesterol methanesulfonate has been reported to give an *i*-sterol, 2,2-dimethyl-3,5-cyclocholestan-6β-ol, when subjected to solvolysis; it was suggested that the reaction proceeded via a 3,5-cyclo cationic species similar to that participating in the solvolysis of cholesterol sulfonates[8]. If this parallel were to hold for the GLC of 2,2-dimethylcholesterol methanesulfonate, one would expect to observe an olefin peak corresponding to 2,2-dimethyl-3,5-cyclo-6-cholestene, for cholesterol methanesulfonate yields 3,5-cyclo-6-cholestene. The major component of the mixture of olefins resulting from the GLC of 2,2-dimethylcholesterol methanesulfonate has indeed been identified by retention time data and combined GLC–mass spectrometry as 2,2-dimethyl-3,5-cyclo-6-cholestene (see Fig. 3), strongly suggesting that the *i*-steroid intermediate does

play a role in this reaction. Formation of the *i*-olefin is more favored in this case than with cholesterol methanesulfonate, where somewhat more 3,5-cholestadiene than 3,5-cyclo-6-cholestene is observed.

Attempts to prepare 5,7-cyclo steroids by solvolysis of the sulfonates of pseudocholesterol and epipseudocholesterol have not proven successful[9–12]. A major product from both esters is 4,6-cholestadiene, indicating that the resemblance between the pseudocholesterol homoallylic system (4-en-7β-ol) and that of cholesterol (5-en-3β-ol) with respect to *i*-steroid formation is only superficial. Epipseudocholesterol methanesulfonate would not be expected to produce *i*-steroid, since, if anything, it more closely resembles epicholesterol (3α-ol, known not to produce an *i*-steroid[1,13]) than cholesterol. Considering the close parallel that has been noted between solvolysis reactions and eliminations on a GLC column, it would be predicted that pseudocholesterol and epipseudocholesterol methanesulfonates should yield mainly 4,6-cholestadiene and very little, if any 5,7-cyclo steroid. Fig. 4 shows a comparison of the chromatograms

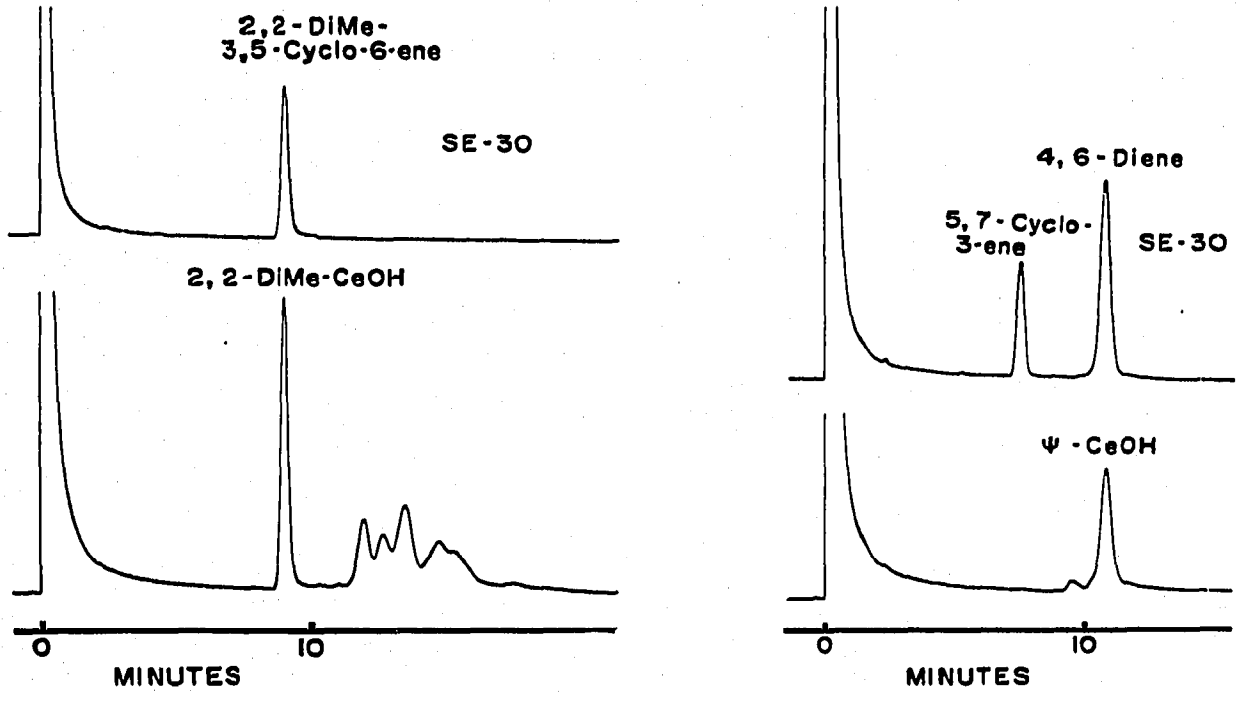

Fig. 3. GLC behavior on SE-30 of 2,2-dimethyl-3,5-cyclo-6-cholestene (upper chromatogram) and the methanesulfonate of 2,2-dimethylcholesterol (lower chromatogram). Column conditions given in Table I.

Fig. 4. GLC behavior of 5,7-cyclo-3-cholestene and 4,6-cholestadiene (upper chromatogram) and the methanesulfonate of pseudocholesterol (lower chromatogram). Column conditions given in Table I.

obtained from the methanesulfonate of pseudocholesterol and a mixture of two reference compounds, 5,7-cyclo-3-cholestene and 4,6-cholestadiene; essentially the same picture is seen for the methanesulfonate of epipseudocholesterol. Combined GLC–mass spectrometry has confirmed that the major product from both of these esters is 4,6-

TABLE I

GLC BEHAVIOR OF HYDROXYSTEROIDS, SULFONATE ESTERS AND REFERENCE COMPOUNDS

Compound	*Relative retention times*	
	2% SE-30[a]	*1% EGSS-Z*[b]
Cholestane	1.00	1.00
5-Androsten-3β-ol-17-one	0.41	5.55
5-Androsten-3β-ol-17-one Ms[c]	0.18, 0.23, 0.24	0.67, 1.05, 1.16
5-Androsten-3β-ol-16-one	0.41	5.68
5-Androsten-3β-ol-16-one Ms	0.18, 0.23, 0.24	0.69, 1.07, 1.18
5α-Androstan-3β-ol-17-one	0.42	5.10
5α-Androstan-3β-ol-17-one Ms	0.22	0.83
5α-Androstan-3α-ol-17-one	0.42	4.49
5α-Androstan-3α-ol-17-one Ms	0.22	0.83
2-5α-Androsten-17-one	0.22	0.82
3-5α-Androsten-17-one	0.22	0.84
5β-Androstan-3β-ol-17-one	0.38	4.06
5β-Androstan-3β-ol-17-one Ms	0.19, 0.21	0.69, 0.78
5β-Androstan-3α-ol-17-one	0.39	4.56
5β-Androstan-3α-ol-17-one Ms	0.19, 0.21	0.69, 0.78
5α-Androstan-3β-ol-16-one	0.42	5.42
5α-Androstan-3β-ol-16-one Ms	0.22	0.87
Pseudocholesterol	1.64	4.82
Pseudocholesterol Ms	0.96, 1.09[d]	1.14, 1.30, 1.57
Epipseudocholesterol	1.53	4.03
Epipseudocholesterol Ms	0.96, 1.08	1.14, 1.30, 1.56
5,7-Cyclo-3-cholestene	0.76	0.87
4,6-Cholestadiene	1.08	1.58
2,2-Dimethylcholesterol	2.48	7.50
2,2-Dimethylcholesterol Ms	0.90, 1.20, 1.27, 1.35	0.95, 1.32, 1.62, 1.95
2,2-Dimethyl-3,5-cyclo-6-cholestene	0.90	0.96
4,4-Dimethylcholesterol	2.71	8.43
4,4-Dimethylcholesterol Ms	1.30, 1.57, 1.83	1.18, 1.47, 2.04, 2.59
Cholanhydroxamic acid[e]	0.99, 2.27	—
Cholanhydroxamic acid Ms[f]	0.98	—
Methyl cholanate	1.08	—

[a] SE-30 (a non-polar or non-selective dimethylpolysiloxane; Supelco, Inc.). Column conditions: 6 ft. × 4 mm glass U-tube; 17 p.s.i.; 232°; cholestane time, 10.0 min.

[b] EGSS-Z (a polar or selective co-polymer of ethyleneglycol, succinic acid, and a methylphenylsiloxane; Applied Science Laboratories, Inc.). Column conditions: 6 ft. × 4 mm glass U-tube; 10 p.s.i.; 205°; cholestane time, 2.6 min.

[c] Methanesulfonates (Ms) of steroids were prepared by a submilligram procedure in ethyl acetate with methanesulfonyl chloride (pyridine catalyst) as previously reported[1-3]. The retention times are for the olefins resulting from elimination of this functional group.

[d] Underlining indicates major peak.

[e] Undergoes a partial conversion (Lossen rearrangement) to the corresponding isocyanate.

[f] Undergoes Lossen rearrangement to the isocyanate.

cholestadiene; no peaks with retention behavior corresponding to 5,7-cyclo-3-cholestene* are observed.

It was found earlier that the retention behavior of the products resulting from the GLC of sterol methanesulfonates could be meaningfully expressed by dividing the retention times of the olefins by the retention time of the parent sterol to give a so-called olefin/sterol factor[2]. The methanesulfonates of cholesterol and other simple 3β-ol-Δ^5 steroids give values of approximately 0.43, 0.54, and 0.59 with SE-30, and 0.13, 0.20, 0.22 with EGSS-Z[2]. The smallest value is for the 3,5-cyclo structure, and the largest is for the 3,5-diene system. The value characteristic of the *i*-steroid olefin is roughly 3/4 that for the diene with SE-30, and about 6/10 that for the diene with EGSS-Z. Approximately the same values were found for the other simple 3β-ol-Δ^5 sterols investigated (*e.g.*, desmosterol, stigmasterol). Table II shows that dehydroepiandrosterone (5-androsten-3β-ol-17-one) also gives the same set of values. For a

TABLE II

OLEFIN/STEROL FACTORS FOR SULFONATE ESTERS

Parent sterol	*Retention time ratios*[a]	
	SE-30	*EGSS-Z*
5-Androsten-3β-ol-17-one	0.44, 0.56, 0.59	0.12, 0.19, 0.21
5-Androsten-3β-ol-16-one	0.44, 0.56, 0.59	0.12, 0.19, 0.21
5α-Androstan-3β-ol-17-one	0.52	0.16
5α-Androstan-3β-ol-16-one	0.52	0.16
5α-Androstan-3α-ol-17-one	0.52	0.19
5β-Androstan-3β-ol-17-one	0.51, 0.55	0.17, 0.19
5β-Androstan-3α-ol-17-one	0.50, 0.54	0.15, 0.17
Pseudocholesterol	0.59, 0.66[b]	0.24, 0.27, 0.33
Epipseudocholesterol	0.63, 0.71	0.29, 0.32, 0.39
2,2-Dimethylcholesterol	0.36, 0.48, 0.51, 0.54	0.13, 0.18, 0.22, 0.26
4,4-Dimethylcholesterol	0.48, 0.58, 0.68	0.14, 0.17, 0.24, 0.31

[a] Determined from data in Table I.
[b] Underlining indicates factor from major component.

homoallylic system which does not yield an *i*-steroid product, such as epicholesterol or 4β-methylcholesterol, the smaller value is absent. Neither pseudocholesterol nor epipseudocholesterol methanesulfonate gives an olefin/sterol factor 3/4 (SE-30) or 6/10 (EGSS-Z) those found for 4,6-cholestadiene and pseudocholesterol (olefin/sterol factors of 0.66, SE-30, and 0.33, EGSS-Z) or epipseudocholesterol (olefin/sterol factors

* This compound is a 5β,7β-cyclosteroid prepared photochemically from 4,6-cholestadiene[12]. Under similar conditions 3,5-cholestadiene yields a 3β,5β-cyclosteroid. The 3α,5α-cyclosteroids result from solvolytic reactions of 3β-ol-Δ^5 sulfonate esters. The 5β,7β-cyclo-3-cholestene may, therefore, not be the truly appropriate reference standard. The hallmark of *i*- or cyclo-steroids is their early elution relative to their non-cyclo isomers, and no early peak of any significant area relative to that of the diene is noted on either column. The methanesulfonate of *i*-pseudocholesterol (5α,7α-cyclocholestan-4ζ-ol) (ref. 11) did not yield any peak of significant magnitude in the retention time range expected for 5α,7α-cyclo-3-cholestene.

of 0.71, SE-30, and 0.39, EGSS-Z) (see Table II). The predicted *i*-olefin values would be 0.49 (SE-30) and 0.20 (EGSS-Z) for pseudocholesterol, and 0.53 (SE-30) and 0.23 (EGSS-Z) for epipseudocholesterol. This approach suggests that no *i*-steroid is formed. When the retention times of 5β,7β-cyclo-3-cholestene and these two sterols are employed to calculate hypothetical olefin/sterol factors, the values are 0.46 (SE-30) and 0.18 (EGSS-Z) for pseudocholesterol, and 0.49 (SE-30) and 0.22 (EGSS-Z) for epipseudocholesterol.

The GLC retention data and mass spectra prove that 2,2-dimethylcholesterol methanesulfonate yields the *i*-steroid in high yield. The olefin/sterol factors calculated from these two compounds are 0.36 for SE-30 and 0.13 for EGSS-Z; the latter value is very close to that for other *i*-steroid hydrocarbons, but the former is somewhat low. Presence of the geminal dimethyl group thus distorts the retention time relationships. Note, however, that a shift in position of the keto group of the androstanes from the 17-position to the 16-position does not change the olefin/sterol factors (see Table II).

Methanesulfonyl chloride can form 'reactive derivatives' from compounds other than hydroxysteroids. It has been demonstrated that when acetylated long chain

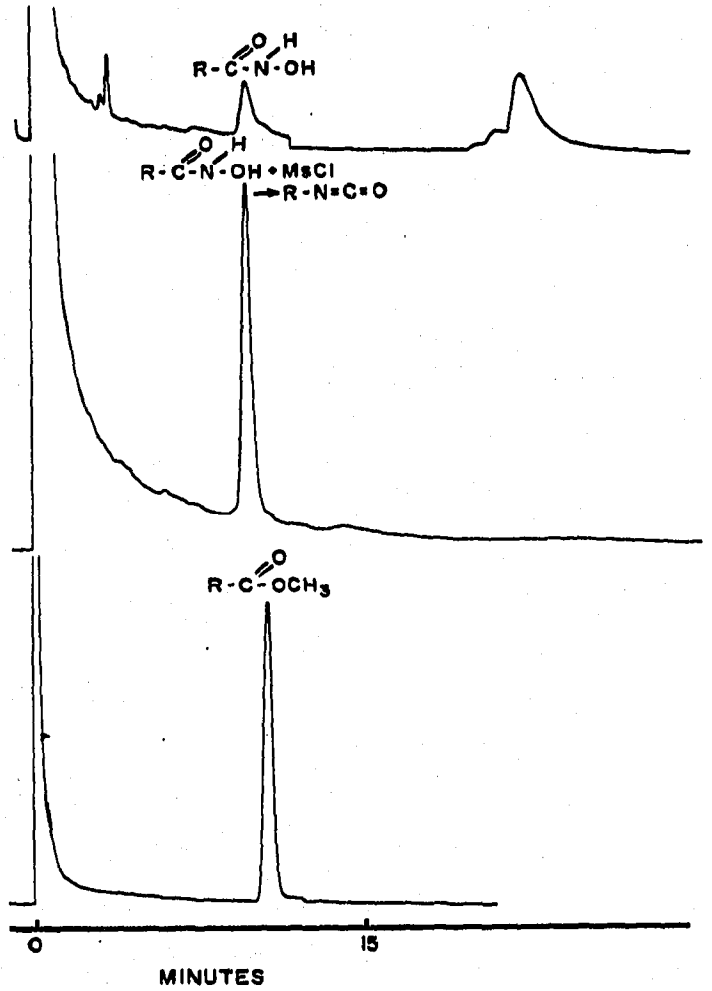

Fig. 5. GLC behavior on SE-30 of cholanhydroxamic acid (top chromatogram), the methanesulfonate of cholanhydroxamic acid (middle chromatogram), and methyl cholanate (bottom chromatogram).

hydroxamic acids are applied to a GLC column, they undergo a quantitative Lossen rearrangement to the corresponding isocyanate (the hydroxamic acid itself undergoes only a partial conversion)[14]. The methanesulfonyl derivative is also a candidate for this thermally-induced intramolecular rearrangement. Fig. 5 illustrates the GLC behavior of the hydroxamic acid analog of cholanic acid (cholanhydroxamic acid) and its methanesulfonyl chloride reaction product. The mass spectrum of the single peak (10 min) derived from the ester is compatible (for example, molecular ion 357) with that for the expected Lossen rearrangement product, the corresponding isocyanate (24-nor-cholanyl isocyanate, mol. wt. 357).

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