[Chem. Pharm. Bull. 27(9)2203—2206(1979)]

UDC 547.92.04.08 : 543.544.45.062

Quantitative Analysis of Steroidal Epoxides by Gas Liquid Chromatography[1]

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(Received January 10, 1979)

A new method is presented for the determination of some steroidal epoxides by gas liquid chromatography (GLC). Since such an epoxide undergoes decomposition, giving a complex chromatogram in GLC, the oxirane ring was cleaved stereoselectively to give the corresponding steroidal alcohol by refluxing with $LiAlH_4$ in ether. The alcohol was then derivatized to a trimethylsilyl or *tert*-butyldimethylsilyl ether and analyzed by quantitative GLC on SE-30 or OV-17, using 5α-cholestane as an internal standard.

Keywords——epoxide; 5α-cholestane; GLC; internal standard; stereoselective reduction; steroid; trimethylsilyl and *tert*-butyl dimethylsilyl ethers of steroidal alcohol

As has been shown previously,[3] the stereoselective β-epoxidation of cholesterol occurred in a single step in good yield when it was treated with ferric acetylacetonate and hydrogen peroxide under mild conditions in acetonitrile. The stereochemical nature of the epoxidation with this reagent system was found to be different from that with peracid.[4] Various yields of the epoxide mixtures were observed in the reactions of some steroidal olefins with this reagent in different solvents. Determination of the isomers in a mixture of steroidal epoxides is, however, generally troublesome due to the similarity of their physical properties. In nuclear magnetic resonance (NMR) spectrometry, the methine protons adjacent to the oxiranes often have similar chemical shifts, and the ratios of their peak areas cannot be determined precisely enough to permit quantitative analysis. The present paper describes a new method for the determination of some steroidal epoxides by gas liquid chromatography (GLC).

Steroidal epoxides undergo thermal decomposition, giving rise to a complex chromatogram in GLC. It was, therefore, necessary to design a simple and reliable method to transform such an epoxide into a stable and stereochemically corresponding compound. The oxirane ring was thus cleaved stereoselectively to the corresponding steroidal alcohol in axial configuration by refluxing the epoxide with lithium aluminum hydride in ether.[5] The alcohol obtained was then treated with N,O-bis(trimethylsilyl)acetamide (BSA) to give a trimethylsilyl (TMS) derivative or with *tert*-butyldimethylchlorosilane (TBDMSCl) in the presence of imidazole to give a TBDMS derivative[6] (Chart 1), which was subjected to GLC. The relative retention times of various silylated cholestanols were observed under different condi-

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[1] This paper constitutes Part XI of a series entitled "Metal Ion-Catalyzed Oxidation of Steroids;" Part X: H. Yamamoto, H. Takei, T. Yamamoto, and M. Kimura, *Chem. Pharm. Bull.* (Tokyo), **27**, 789 (1979).

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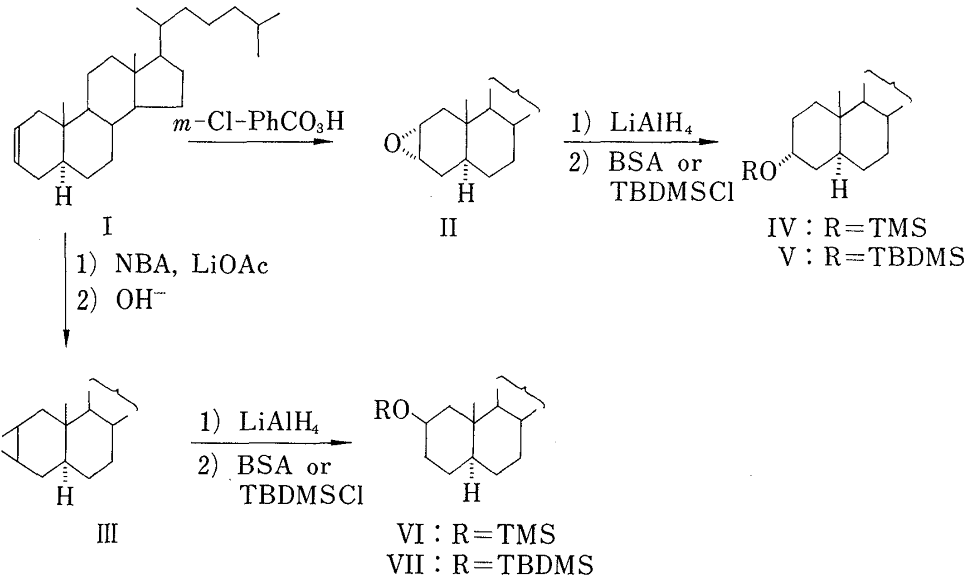

Chart 1

Table I. Relative Retention Times of Cholestanols on Various Stationary Liquids

	1.5% SE-30[a]	1.5% SE-52[a]	1.5% OV-17[a]	1.5% OV-210[b]
5 α-Cholestane	1.00(5.82)	1.00(4.56)	1.00(4.21)	1.00(2.42)
5 α-Cholestan-2β-ol	1.66	1.79	2.26	2.28
TMS ether (VI)	1.76	1.71	1.53	1.50
TBDMS ether (VII)	3.37	3.36	2.98	2.78
5 α-Cholestan-3α-ol	1.67	1.80	2.31	2.41
TMS ether (IV)	1.65	1.61	1.45	1.42
TBDMS ether	2.82	2.80	2.40	2.31
5 α-Cholestan-4β-ol	1.63	1.74	2.20	2.12
TMS ether	1.77	1.74	1.56	1.54
5 β-Cholestan-2α-ol				
TMS ether	1.23	1.18	1.11	1.06
5 β-Cholestan-3β-ol	1.57	1.66	2.10	2.24
TMS ether	1.63	1.60	1.41	1.42
TBDMS ether	3.08	3.13	2.57	2.52
5 β-Cholestan-4α-ol	1.46	1.55	1.86	1.80
TMS ether	1.21	1.18	1.06	1.13
TBDMS ether	2.19	2.18	1.98	1.93

Retention times (min) are given in parentheses.
Column temperature, *a*) 260°, *b*) 240°.

tions, and the results are summarized in Table I, indicating that SE-30 and OV-17 are suitable in the present case. The TMS compounds so far examined gave retention times sufficiently different for analysis, except for those (IV and VI) originating from the epoxides (II and III) of 5α-cholest-2-ene (I). The alternative TBDMS derivatives (V and VII) were found to be suitable for the analysis of II and III.

The standard procedure developed for the determination of epoxysteroids is shown schematically in Chart 2. With 5α-cholestane as an internal standard, the relative responses of the silyl ethers obtained from several epoxysteroids are summarized in Table II. The differences between these values of relative response may be ascribed in part to the fact that in this standard procedure the epoxides undergo two preliminary chemical treatments, reduction and silylation, before the gas chromatographic operation. The responses were adequate for our purposes; 2α,3α-epoxy- and 3β,4β-epoxy-5α-cholestanes, for example, could be determined with standard deviations of ±2.3 and 4.3%, respectively. Each epoxide gave a linear calibration curve over a range from 0.1 to 5 mg, which passed through the origin.

These findings suggest that the amount of epoxide (W_E mg) can be calculated as follows,

$$W_E = \frac{C_E \times W_S}{C_S \times R_E}$$

where C_E and C_S are the integrated counts for the peaks of the steroidal alcohol (relative response: R_E) derived from the epoxide and of the previously added internal standard (W_S mg), respectively.

epoxide mixture (0.1—5 mg)
↓ 1) $LiAlH_4$ (2 mg) in ether, refluxed for 2 hr
2) water (a few drops)
3) 5α-cholestane (0.2 mg)
cholestanol mixture
↓ BSA (0.1 ml), trimethylchlorosilane (2 drops) in acetonitrile (0.1 ml)
or
TBDMSCl (5—10 mg), imidazole (5—10 mg) in pyridine (0.2 ml)
GLC
column temp. 260°, N_2: 60 ml/min, 1.5% OV-17 or 1.5% SE-30

Chart 2

Table II. Relative Responses of Steroidal Epoxides as Their Corresponding Alcohol-TMS Derivatives

Epoxide	Corresponding alcohol	Relative response (R_E)
	5α-Cholestane[a]	1.00
2α,3α-Epoxy-5α-cholestane (II)	5α-Cholestan-3α-ol (IV)	0.87
2β,3β-Epoxy-5α-cholestane (III)	5α-Cholestan-2β-ol (VI)	0.90
3α,4α-Epoxy-5α-cholestane	5α-Cholestan-3α-ol	0.72
3β,4β-Epoxy-5α-cholestane	5α-Cholestan-4β-ol	0.70
2α,3α-Epoxy-5β-cholestane	5β-Cholestan-2α-ol	0.64
2β,3β-Epoxy-5β-cholestane	5β-Cholestan-3β-ol	0.88
3α,4α-Epoxy-5β-cholestane	5β-Cholestan-4α-ol	0.80
3β,4β-Epoxy-5β-cholestane	5β-Cholestan-3β-ol	0.76

a) 5α-Cholestane was used as an internal standard.

Further studies on the stereoselectivity of epoxidation using the ferric acetylacetonate-hydrogen peroxide system are now in progress, and the results obtained by the present GLC method will be reported in the near future.

Experimental

Apparatus——A Shimadzu GC-4BM gas chromatograph equipped with a glass coil column (2 m×3 mm i.d.) packed with stationary liquid on Shimalite W (60—80 mesh) (Table I), and a hydrogen flame ionization detector equipped with a Shimadzu ITG-4AX digital integrator were used.

Materials——5α-Cholestane (mp 77°) was prepared and purified as reported elsewhere.7) Authentic specimens of 2α,3α-epoxy-5α(mp 104—106° from EtOH; lit.8) mp 101—103°)-, 3α,4α-epoxy-5α (mp 118—120° from MeOH; lit.9) 118—120°)-, 2β,3β-epoxy-5β (mp 86—87° from EtOH; lit.10) mp 87—88.5°)-, and 3β,4β-epoxy-5β (mp 66—68° from EtOH; lit.11) mp 71°)-cholestanes were prepared by the general method using *m*-chloroperoxybenzoic acid12) and purified by repeated recrystallization. Authentic specimens of 2β,3β-epoxy-5α (mp 84—85.5° from acetone; lit.13) mp 86—88°)-, 3β,4β-epoxy-5α (mp 96—98° from EtOH–MeOH; lit.14) mp 98—99°)-, 2α,3α-epoxy-5β (mp 67—70° from acetone)-, and 3α,4α-epoxy-5β (mp 54.5—56° from aq. MeOH; lit.15) mp 55—56°)-cholestanes were prepared by the general method using the alkaline acetylhypobromite system16) and purified by repeated recrystallization. All melting points were measured with a hot-stage apparatus and are uncorrected.

Pretreatment of Epoxide for GLC——Epoxide mixture (0.1—5 mg) was refluxed in ether (5 ml) with $LiAlH_4$ (2 mg) for 2—3 hr. Excess reagent was then decomposed by adding a few drops of water. After adding the internal standard (5α-cholestane, 0.2 mg), the ether layer was separated and dried over anhydrous $MgSO_4$. Ether was evaporated off with a stream of N_2. The cholestanols obtained were silylated with *N*,*O*-bis(trimethylsilyl)acetamide (0.1 ml) and trimethylchlorosilane (2 drops) in acetonitrile (0.1 ml) or with *tert*-butyldimethylchlorosilane (5—10 mg) and imidazole (5—10 mg) in pyridine (0.2 ml).

Acknowledgement We wish to express our gratitude to Dr. T. Tomita, Hokkaido University School of Medicine, for stimulating discussions. A part of this work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

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