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Gas Chromatography of C_{27}, C_{28}, and C_{29} Sterols*[1]

Since the gas chromatographic separation of steroids was reported by Horning and his co-workers,[1] this method has become an important technique in the study of steroids. A recent report on the gas-liquid chromatography of sterol methyl ethers[2] prompted publication of the present communication.

In order to obtain a correlation between the structure of steroids and retention time, and to identify and separate the naturally occuring steroids, especially algae sterols, which we have been studying in our laboratory for some time,[3,4] gas chromatography of a number

Table I. Relative Retention Time of Sterols[a]

Compound	Time	Position of double bond
C_{27}-Sterols		
Cholestane (I)[b]	1	
Cholesterol (II)	1.69	5
$\Delta^{8(14)}$-Cholestenol (III)	1.73	8(14)
Δ^{14}-Cholestenol (IV)	1.83	14
Δ^{7}-Cholestenol (V)	1.93	7
20-Iso-22-dehydrocholesterol (VI)[5]	1.35	5, 22
22-Dehydrocholesterol (VII)[3]	1.57	5, 22
$\Delta^{7,22}$-Cholestadien-3β-ol (VIII)[6]	1.87	7, 22
C_{28}-Sterols		
Δ^{22}-24β-Methylcholesterol (brassicasterol) (IX)[8]	1.91	5, 22
Δ^{22}-24-Methylcholesterol (24α,β (1:1) mixture) (X)[8]	1.91	5, 22
Δ^{22}-24-Methylcholesterol (24α,β (3:1) mixture) (XI)[8]	1.92	5, 22
24-Methylencholesterol (XII)[9]	2.16	5, 24(28)
$\Delta^{8(14)}$-Ergosten-3β-ol (XIII)	2.17	8(14)
5,6-Dihydroergosterol (XIV)	2.21	7, 22
Δ^{24}-24-Methylcholesterol (XV)[7]	2.47	5, 24
C_{29}-Sterols		
Stigmasterol (XVI)	2.38	5, 22
Δ^{22}-24-Ethylcholesterol (24α,β (1:1) mixture) (XVII)[8]	2.38	5, 22
Fucosterol (XVIII)[4]	2.76	5, 24(28)
Δ^{24}-24-Ethylcholesterol (XIX)[7]	2.98	5, 24
Fucostadienone (XX)	3.79	4, 24(28)
Δ^{24}-24-Ethylcholestenone (XXI)[7]	4.06	4, 24

a) Barber-Colman Model-10. Argon ionization detector, 9 ft. × 8 mm. i. d. column, pressure, 35 lb./in^2, temp. 220°, 1% SE-30 phase on Chromosorb W, 60~80 mesh. Flash temp. 290°. Cell temp. 170°.
b) Retention Time, 9.1 min.

*1 Steroid Studies, Part XXXIII.

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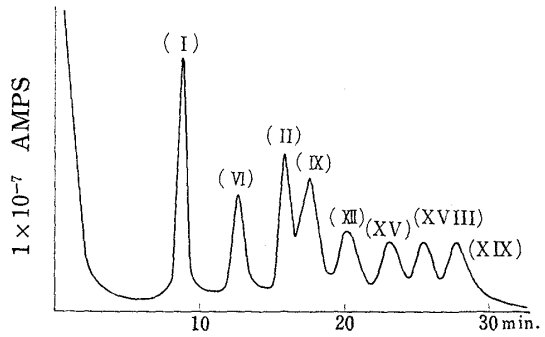

Fig. 1. Separation of a Mixture of Eight Sterols (I, II, VI, IX, XII, XV, XVIII and XIX) (For conditions see (a) and (b) under Table I)

of synthesized and natural sterols was carried out, using a column packed with 1% SE-30 on Chromosorb W. The relative retention times of the compounds are shown in Table I and the chromatogram in Fig. 1 illustrates the separation of a mixture consisting of eight sterols.

20-Iso-22-dehydrocholesterol (VI) was observed to possess a lower retention time than 22-dehydrocholesterol (VII). The effect of increasing methylene groups resulted in a difference of 0.34~0.6 in relative retention time for compounds possessing double bonds in the same positions (e.g. VII → IX → XVI). An increase in retention time was observed to occur in the order of $\Delta^5 < \Delta^{8(14)} < \Delta^{14} < \Delta^7$ for the double bonds in various positions of the cholesterol ring. In the case of Δ^{22}-cholestanol and Δ^{22}-24-methylcholestanol, the order was the same (VII, VIII, IX, XIV). Double bonds at C_{22} decrease the retention time[2] as shown in (II) → (VII) and (V) → (VIII). The order of the retention time of the compounds having a double bond in the side chain was $\Delta^{22} < \Delta^{24(28)} < \Delta^{24}$ in the C_{28} and C_{29} sterols.

A C_{24}-α and β mixture of Δ^{22}-24-methylcholesterol (X, XI) and a similar α and β mixture of Δ^{22}-24-ethylcholesterol (XVII) prepared by the Wittig reaction[8] from 1-iodo-2,3-dimethylbutane (racemate) and 1-iodo-2-ethyl-3-methylbutane (racemate), respectively, were not separated into the respective isomers by this method. Single peaks showing the same retention time as brassicasterol (IX) and stigmasterol (XVI) were observed for these mixtures respectively.

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