Journal of Chromatography, 116 (1976) 207–210
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CHROM. 8695

Note

Chromatography of saturated steroid hydrocarbons (steranes) on alumina

GEORGE RYBACK

Shell Research Limited, Milstead Laboratory, Sittingbourne Research Centre, Sittingbourne, Kent, ME9 8AG (Great Britain)

(Received August 11th, 1975)

The isolation of pure components from the complex mixtures of steranes obtained from petroleum and rock extracts is a formidable problem. The differences in their physical properties are small, and chemical derivatives cannot be made. Clathrate formation with thiourea and preparative gas–liquid chromatography (GLC) are widely used, but even this powerful combination fails to separate some of the mixtures encountered. We have found adsorption chromatography on active alumina to be a promising additional technique (*cf.* ref. 1) and report here its application to a range of isomeric and homologous steranes, including epimeric pairs of 24-alkyl-5α-cholestanes, which are inseparable by other methods.

EXPERIMENTAL

The apparatus consisted of two jacketed columns in series, each 1.4 m × 5 mm I.D., connected via a flow refractometer (Waters Assoc., Model R403) to a fraction collector, and filled under pentane with aluminium oxide (Woelm, activity I, 60 g). The columns and refractometer were thermostatted at 15° or 18°. Steranes (1–10 mg) were applied in a few drops of pentane and were eluted with pentane under *ca.* 0.5 atm pressure (flow-rate 30 ml/h). (Hexane has also been used, with similar results.) 2,6,10,14-Tetramethylpentadecane (pristane) was sometimes added to the steranes as a marker. The columns could be used for many consecutive runs without cross-contamination or appreciable loss of resolution.

RESULTS AND DISCUSSION

Results for a series of synthetic reference compounds are presented in Table I and Fig. 1 (A–C). All the compounds produced positive peaks (refractive index of eluates higher than that of pentane). The response to unit amounts of the different compounds appeared to vary somewhat, though this has not been quantified. Analysis of the collected fractions confirmed the separations indicated by the refractometer traces. The observed relative retention volumes of steranes are structure dependent, flatness of the carbon skeleton and length of the side chain being the major factors. Comparison of these values with relative retention volumes on a common GLC stationary phase such as SE-30 (Table I) shows that some steranes which are not

TABLE I

RELATIVE RETENTION VOLUMES OF STERANES AND TRITERPANES ON GAS–LIQUID AND ON LIQUID–SOLID CHROMATOGRAPHY COLUMNS

Compound	*Formula*	*Relative retention volume*	
		*GLC**	*Adsorption***
5α-Androstane	$C_{19}H_{32}$	0.14	0.55–0.57
5β-Androstane	$C_{19}H_{32}$	0.13	
5β-Cholestane	$C_{27}H_{48}$	0.92	
5α,14β-Cholestane	$C_{27}H_{48}$	0.87	
5α,17β(H)-Cholestane	$C_{27}H_{48}$	0.73	
(20*S*)-5α,17β(H)-Cholestane	$C_{27}H_{48}$	0.80	
(24*R*)-24-Methyl-5β-cholestane	$C_{28}H_{50}$	1.16	
(24*S*)-24-Methyl-5β-cholestane	$C_{28}H_{50}$	1.16	
Dammarane***	$C_{30}H_{54}$	1.49 1.55	0.63
5α-Pregnane	$C_{21}H_{36}$	0.23	0.68–0.69
20-Metnyl-5α-pregnane	$C_{22}H_{38}$	0.30	
5α,8α,14β-Cholestane	$C_{27}H_{48}$	0.98	
24,24-Dimethyl-5α-cholane	$C_{26}H_{46}$	0.75	0.72–0.76
(20*S*)-5α-Cholestane	$C_{27}H_{48}$	0.91	
Lupane	$C_{30}H_{52}$	1.83	
23-Methyl-5α-cholane	$C_{25}H_{4}$	0.57	0.86–0.89
24,24-Diethyl-5α-cholane	$C_{28}H_{50}$	1.29	
(24*R*)-24-Methyl-5α-cholestane	$C_{28}H_{50}$	1.29	
(24*R*)-24-Ethyl-5α-cholestane	$C_{29}H_{52}$	1.64	
(24*S*)-24-Ethyl-5α-cholestane	$C_{29}H_{52}$	1.64	
5α-Cholestane	$C_{27}H_{48}$	1.00	1.00
4α-Methyl-5α-cholestane	$C_{28}H_{50}$	1.17	1.03–1.07
4β-Methyl-5α-cholestane	$C_{28}H_{50}$	1.27	
(24*S*)-24-Methyl-5α-cholestane	$C_{28}H_{50}$	1.29	

* 1.8 m × 3.2 mm, 5% SE-30 on Chromosorb W, 250°.

** 2.8 m × 5 mm, aluminium oxide, pentane eluent, conditions described in text. Retention volumes measured from time of application of sample.

*** 1:1 Mixture of (20*R*)- and (20*S*)-epimers.

separated by preparative GLC are separated on alumina, and *vice versa*. Chromatography on alumina may also prove useful for isolating triterpanes, two of which are included in Table I.

While it should be possible to improve the separations, using longer columns of microparticulate alumina, even the simple, low-resolution apparatus described here is of practical value. Thus, (20*S*)-5α-cholestane has been isolated[2] from a preparative GLC fraction derived from a crude oil and containing at least four other, as yet unidentified, steranes which have smaller retention volumes on alumina (Fig. 1D).

Of special significance is the ability of the alumina column to separate (24*R*)-24-methyl-5α-cholestane (5α-campestane) and (24*S*)-24-methyl-5α-cholestane (5α-ergostane), illustrated in Fig. 1E by the partial resolution of a pure 24-methyl-5α-

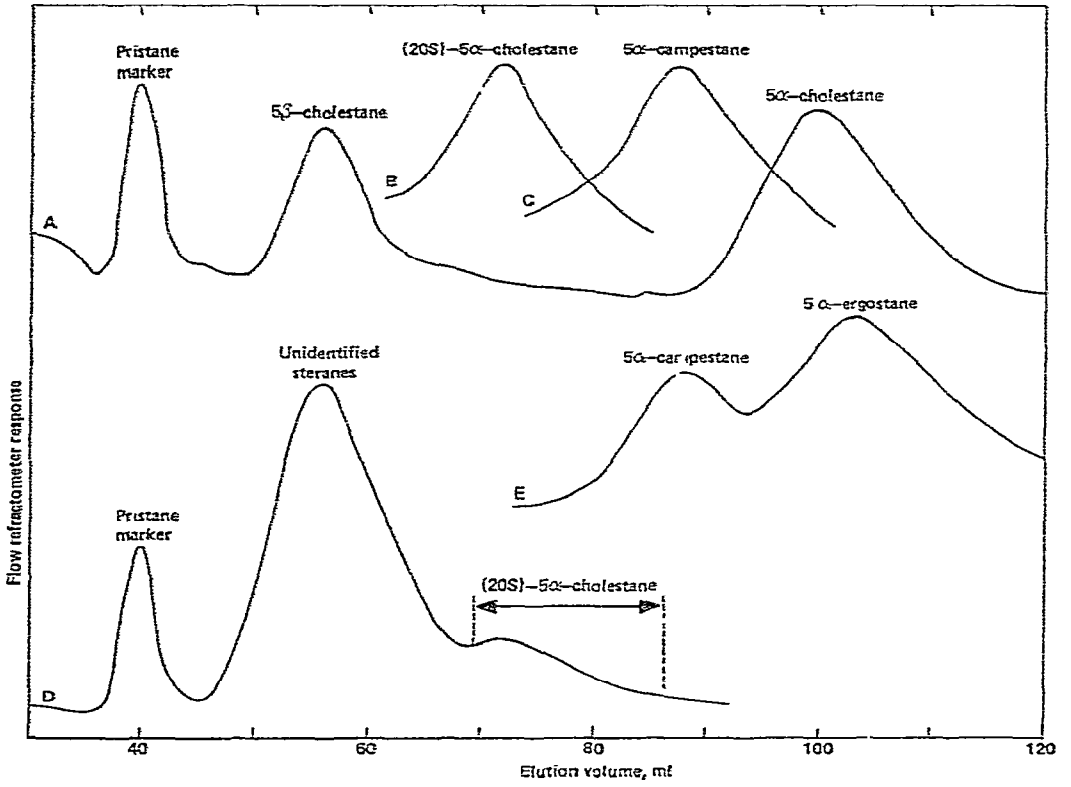

Fig. 1. Chromatography of steranes on alumina. Apparatus and conditions are described in text. A, B, and C, some reference steranes. D, sterane mixture obtained from Rozel Point, Utah, crude oil, the fractions which were combined to yield (20*S*)-5α-cholestane are indicated. E, the 24-methyl-5α-cholestane fraction obtained from Green River shale, Colorado.

cholestane fraction obtained from an oil shale into the two isomers. By re-chromatography of the middle fractions, the two components were obtained nearly free from each other, the relative quantities agreeing with the ratio 1:3 estimated by 220 MHz proton magnetic resonance analysis of the original mixture[2]. The corresponding 5β-steranes are not separated. A mixture of (24*R*)- and (24*S*)-24-ethyl-5α-cholestane (5α-stigmastane and 5α-poriferastane, respectively) gave a single peak on the refractometer trace, but proton magnetic resonance analysis revealed enrichment of the earlier fractions in the (*R*)-epimer, and of the later fractions in the (*S*)-epimer, indicating that some resolution had occurred. No chromatographic separations of (24*R*)- and (24*S*)-24-alkylcholestanes, nor of epimer pairs of the parent sterols, have previously been reported. The configuration at C-24 is important in studies of plant sterols[3,4]. Reduction of 24-methylsterols to hydrocarbons which can be separated and quantified provides an accurate method of determining the 24*R*:24*S* ratio in such sterols, which could be extended to the 24-ethyl compounds if alumina columns of higher resolution are used.

ACKNOWLEDGEMENTS

I thank Dr. L. J. Mulheirn for providing the synthetic steranes used in this study[4,5] and Mrs P. R. Jennings and Miss S. Drewitt for technical assistance.

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