MARINE STEROLS

IX. EVALUATION OF A C$_{87}$ HYDROCARBON STATIONARY PHASE FOR THE ANALYSIS OF MARINE STEROLS IN GLASS OPEN-TUBULAR CAPILLARY COLUMNS

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SUMMARY

The recently introduced C$_{87}$-hydrocarbon thermostable stationary phase has been evaluated for its potential in separating the complex mixtures of sterols which are commonly found in the marine environment. In glass open-tubular capillary columns it has proved to be a very useful phase with excellent separating efficiency, particularly for the difficult 5α-stanol–Δ5-sterol trimethylsilyl ether pairs which have been a common feature of some of these mixtures.

INTRODUCTION

As part of our investigations of complex sterol mixtures from marine invertebrates we are currently interested in gas chromatography (GC) stationary phases which have the necessary properties of thermostability and selectivity for the separation of complex sterol mixtures[1,2]. Inherent in our studies was the realisation that columns of greater resolution were required in order to separate effectively the more complex sterol mixtures that we have encounted, for example in the jellyfish *Periphylla periphylla*[3].

This report is concerned with the high-resolution GC separations observed using a new thermostable non-polar C$_{87}$-hydrocarbon stationary phase developed by Kováts and co-workers[4,5] which has been proposed as a standard phase over a wide temperature range. Chromatograms are shown for two sterol trimethylsilyl (TMS) ether mixtures obtained from marine animals[1,6].

EXPERIMENTAL

Glass capillary columns were coated in our laboratory by a reproducible

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double dynamic coating procedure[7] employing modifications of methods developed by German and co-workers[8,9] and Schomburg and co-workers[10,11].

After silanization of the glass[11] and sonication of the first coating solution, the column (49 m × 0.4 mm I.D.) was coated by forcing a 2-cm^3 plug of the C_{87}-hydrocarbon and Silanox 101 in tetrachloromethane (0.047 g stationary phase and 0.21 g Silanox 101 in 10 cm^3 tetrachloromethane) through the column in front of a 15-cm mercury plug under nitrogen pressure at a plug velocity of 2 cm/sec. The remaining solvent was removed by nitrogen flow for 3 h, and the mercury plug coating procedure was repeated using a 2-cm^3 plug of the C_{87}-hydrocarbon in tetrachloromethane (0.26 g in 10 cm^3) at a plug velocity of 2 cm/sec. During the coating steps a dummy column of length slightly greater than the coating plug was attached to the end of the column by means of a shrink PTFE link.

The column was installed in a slightly modified Pye 104 gas chromatograph using a home-made dry injector device similar to that reported by Van den Berg and Cox[12] and with nitrogen make-up gas to the flame-ionisation detector. The column was conditioned for 72 h at 250° with a low flow-rate of helium carrier gas.

The chromatographic conditions employed for all of the analyses were as follows. Column temperature, 250°; injector temperature, 290–300°; detector temperature, 280°; helium carrier gas flow-rate, 4 cm^3/min; nitrogen make-up gas flow-rate, 20 cm^3/min. The effeciency of the SCOT column, 70,000 plates, was measured in terms of theoretical plates for cholesterol TMS ether (*i.e.* 1428 plates/m).
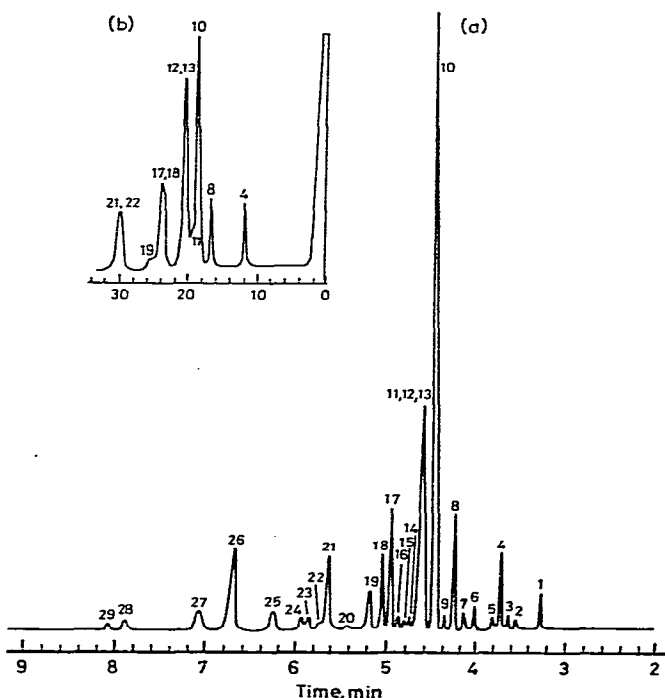

Fig. 1. (a) Chromatogram of cockle sterol TMS ethers on C_{87}-hydrocarbon SCOT column (b.) Identical sample on a De sil 300 GC packed column (2.5 m × 4 mm I.D.).

RESULTS

Separation of cockle sterol TMS ethers on C_{87}-hydrocarbon SCOT column

The GC profile of the sterol TMS ethers obtained after extraction of the cockle *Cerastoderma edule*[1] is shown in Fig. 1. This chromatogram is compared with the result obtained for a 1% Dexsil-300 GC packed column on the same sample[1] (Fig. 1b). An analysis of the sterol content of the sample for the capillary column in terms of Kováts[13] retention index values with the co-injection of n-C_{28} and n-C_{36} is given in Table I.

TABLE I

PERFORMANCE OF COCKLE STEROL TMS DERIVATIVES ON C_{87}-HYDROCARBON SCOT COLUMN

Cockle sterols	Sterol identity*	Kováts retention index[13] $I_{250°}$
C-1	**	—
C-2	**	—
C-3	**	2938
C-4	26C 5, 22E	2954
C-5	26C 22E***	2976
C-6	26C***	3033
C-7	Occelasterol	3069
C-8	27C 5, 22E	3087
C-9	27C 22E***	3108
C-10	27C 5	3128
C-11	27C	3148
C-12	27C 5, 24(28)	}3155
C-13	28C 5, 22E	}3155
C-14	28C 22E***	3176
C-15	**	3191
C-16	**	3202
C-17	28C 5, 24(28)	3216
C-18	28C 5	3224
C-19	29C 5, 22E	3242
C-20	29C 5, 24(28)E	3290
C-21	29C 5	3299
C-22	29C 5, 24(28)Z	3310
C-23	29C***	3320
C-24	29C 24(28)Z***	3331
C-25	**	3377
C-26	27C 5, (24 oxo)***	3408
C-27	31C?	3441
C-28	32C?	3500
C-29	32C?	3506

* The shorthand notation for the sterols refers to the number of carbon atoms followed by C, followed by the position of any double bonds and an indication of their geometrical isomerism. All the compounds are 3β-sterols and the additional carbon atoms are attached to C_{24}. Note that it is not possible to distinguish between C_{24} epimers by GC.
** Not identified.
*** Tentative identification only.

Separation of the tunicate sterol TMS ethers on C_{87}-hydrocarbon SCOT column

The GC profile of the sterol TMS ethers obtained after extraction of the tunicate *Ascidia mentula*[6] is shown in Fig. 2. This chromatogram is compared with the result obtained for a 1% Dexsil-300 GC packed column on the same sample[6] (Fig. 2b). An analysis of the sterol content of the sample for the capillary column is given in Table II. An analysis of the sterol content of the sample for the capillary column in terms of Kováts[13] retention index values with the co-injection of n-C_{28} and n-C_{36} is given in Table II.
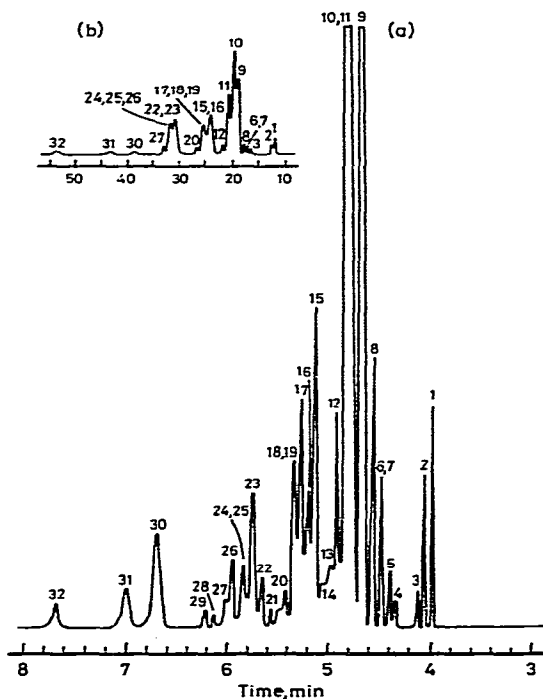

Fig. 2. (a) Chromatogram of tunicate sterol TMS ethers on C_{87}-hydrocarbon SCOT column. (b) Identical sample on a Dexsil 300 GC packed column (2.5 m × 4 mm I.D.).

The efficiency of this C_{87}-hydrocarbon SCOT column for marine sterol separations can be established by measuring the resolution[14] and separation factors[1,15] for pairs of sterol TMS ethers which have proved traditionally to be difficult to separate by other GC stationary phases. Table III contains this information in addition to measurements on sterol pairs which the C_{87}-phase is unable to separate.

DISCUSSION

With the advent of improved chromatographic methods, primarily GC, many marine sterol profiles have been found to be very complex. Although the potential of open-tubular capillary GC for the analysis of sterols had been realised some time

TABLE II

PERFORMANCE OF TUNICATE STEROL TMS DERIVATIVES ON C_{87}-HYDROCARBON SCOT COLUMN

Tunicate sterols	Sterol identity*	Kováts retention index[13] $I_{250°}$
AM-1	26C 5, 22E	2961
AM-2	26C 22E	2983
AM-3	**	3001
AM-4	**	3068
AM-5	Occelasterol	3073
AM-6	Patinosterol	3091
AM-7	27C 5, 22E	3091
AM-8	27C 22E	3110
AM-9	27C 5	3133
AM-10	27C	} 3153
AM-11	28C 5, 22E	} 3153
AM-12	28C 22E	3180
AM-13	**	3194
AM-14	**	3205
AM-15	28C 5, 24(28)	3216
AM-16	28C 5	3225
AM-17	28C 24(28)	3235
AM-18	28C	} 3245
AM-19	29C 5, 22E	} 3245
AM-20	29C 22E	3263
AM-21	**	3274
AM-22	29C 5, 24(28)E	3291
AM-23	29C 5	3300
AM-24	29C 24(28)E	} 3309
AM-25	29C 5, 24(28)Z	} 3309
AM-26	29C	3320
AM-27	29C 24(28)Z	3329
AM-28	30C?	3361
AM-29	30C?	3375
AM-30	27C 5, (24 oxo)	3411
AM-32	32C?	3505

* See Table I.
** Not identified.

ago[16,17] surprisingly few research groups[18-24] have actually employed open-tubular GC techniques for sterol analysis. Since sterols, as fairly non-polar molecules, are readily amenable to separation in glass SCOT columns, one of the reasons may well be that much of the literature relevent to open-tubular GC techniques seems to be written "by experts for experts", and here we would certainly agree with Bertsch et al.[25] in this respect. As newcomers to this area of research we have developed techniques for the easy and reproducible preparation of both polar and non-polar glass SCOT columns[7].

In our previous investigations we have encountered 5α-stanols in significant amounts in many samples[3,6,25]. We are therefore particularly interested in stationary phases which afford a separation of 5α-stanol–Δ^5-sterol pairs. The resolution (Table III) accomplished for the sterol–stanol pairs was far greater using the C_{87}-hydro-

TABLE III

RESOLUTION CHARACTERISTICS OF THE C_{87}-HYDROCARBON PHASE

Sterol pairs[*]		Data for C_{87} capillary		Data for Dexsil-300 GC packed column (2.5 m × 4 mm I.D.)	
		Resolution	Separation factor	Resolution	Separation factor
Well separated on C_{87}					
27C5, 22E	/27C5	6.0	0.89	1.36	0.89
27C5	/27C	2.2	0.97	0.68	0.96
28C5	/29C5, 22E	1.30	0.96	0.70[**]	0.95
28C5	/28C5, 24(28)	1.21	1.02	0.12[**]	1.00
29C5	/29C5, 24(28)E	1.18	1.02	0.06[**]	0.99
29C5	/29C5, 24(28)Z	1.12	0.98	0.67[**]	0.95
29C5, 24(28)E	/29C5, 24(28)Z	1.96	0.96	0.55[**]	0.96
Poorly separated on C_{87}					
27C	/27C5, 24(25)	0.47	0.98	4.6	0.94
27C	/28C5, 22E	0.47	0.98	4.6	0.94
28C	/29C5, 22E	0.12	1.0	1.2	0.99
Poorly separated on both systems					
28C5, 22E	/27C5, 24(25)	0	1.0	0[**]	1.0
27C, 22E[***]	/27C5, 22E	0	1.0	0[**]	1.0
29C 24(28)E	/29C5, 24(28)Z	0	1.0	0.5[**]	1.01

[*] See Table I.
[**] Well separated on PZ-176 (ref. 2).
[***] Patinosterol.

carbon phase than for any other stationary phase which we have employed in open-tubular capillary columns[7].

In our experience most phases useful for sterol–stanol pair separations, such as the non-polar Dexsil-300 GC and SE-30 ultraphase, do not give an adequate separation of the isomers of fucosterol TMS [29C5, 24(28) E], isofucosterol TMS [29C5, 24(28) Z] and β-sitosterol TMS (29C5), even in open-tubular capillary columns[7]. Separation of this triplet is usually afforded by more polar phases such as Silar-5CP (ref. 1) and PZ-1 76 (ref. 2). However the C_{87}-hydrocarbon phase affords a good separation of each of these three compounds (AM-22, 23 and 25) as can be seen from Fig. 2 and Table III.

The C_{87}-hydrocarbon phase is therefore an excellent thermostable phase for the separation of certain marine sterol pairs, however the separation of the sterol–stanol pairs on this phase (resolution = 2.2 for cholesterol–cholestanol) is so immense that a problem is created in that the stanol tends to coincide with a higher homologue; _i.e._ cholestanol (27C) coincides with brassicasterol (28C5, 22E; Table III). There are therefore limitations to the sole use of the C_{87}-hydrocarbon phase for the analysis of complex sterol mixtures, but when the retention data from this phase is considered in conjunction with the information from Dexsil-300 GC and PZ-176 columns a very good analytical result can be achieved.

The observation that the C_{87}-hydrocarbon phase generally has different separating characteristics from our standard phases has led us to consider using the Laub and Purnell window diagram technique[27,28] to calculate the best combination

of different in-series capillary columns for the efficient separation of these very complex marine sterol mixtures and work is proceeding along these lines.

The open-tubular GC method has allowed us to identify tentatively several sterols and stanols present in small amounts in the cockle sterol mixture which were undetected using our packed column GC–mass spectrometric techniques[1]. Also the increase in resolution enabled the relative amounts of each sterol to be determined more easily.

ACKNOWLEDGEMENTS

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