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Note

Marine sterols

VI*. Use of the thermostable liquid phase PZ-176 for the gas chromatographic–mass spectrometric analysis of marine sterols

JAMES A. BALLANTINE and KEVIN WILLIAMS

Department of Chemistry, Institute of Marine Sciences, University College of Swansea, Swansea (Great Britain)

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In a previous report[1] of an evaluation of low-bleed liquid phases for the gas chromatographic–mass spectrometric (GC–MS) analysis of complex mixtures of marine sterol trimethylsilyl (TMS) ethers, we recommended the use of the moderately polar phase Silar 5CP in conjunction with Dexsil 300GC. A major advantage of Silar 5CP was the large separation factors observed for sterol geometrical isomers (*e.g.*, fucosterol and isofucosterol)[1].

However our Silar-packed columns (4 mm I.D.) could not be used for GC–MS operation above 220° without appreciable substrate bleed, although the maximum recommended working temperature was 35° above this temperature, and continued use at 220° eventually led to a considerable deterioration in column performance. We have therefore investigated the performance of a more stable polar phase, PZ-176, for marine sterol analysis.

PZ-176 is a polyphenyl ether sulphone phase of high thermal stability[2], developed by Mathews *et al.*[3] and which had already been observed to separate a synthetic mixture of standard sterol TMS ethers, both in packed columns[3] and in glass open-tubular capillary columns[4,5]. We have extended this study to the use of this material for the separation of the complex mixtures of sterol TMS ethers obtained from various marine sources and wish to report that the separation characteristics of PZ-176 are almost identical to those of Silar 5CP with a similar aptitude for the separation of sterol geometrical isomers but with the added advantage of being considerably more thermostable. The PZ-176 packed columns have been used continuously at 255° with negligible substrate bleed into the mass spectrometer and with little observable deterioration in efficiency.

Here we report on the performance of PZ-176 columns for the separation of two mixtures of sterol TMS derivatives obtained from marine animals.

* Part V, see ref. 7.

EXPERIMENTAL

PZ-176 (which was generously supplied by Dr. R. D. Schwartz, Pennzoil Co., Shreveport, La., U.S.A.) was dissolved in tetrahydrofuran and was applied as a 1.2% coating on to Chromosorb G AW DMCS (100–120 mesh) by the sorption technique*. The packing was inserted into a coiled glass column (2.5 m × 4 mm I.D.) and conditioned at 300°. Analyses of the marine sterol TMS ethers were carried out at 255° in a Pye 104 gas chromatograph interfaced via a silicone rubber membrane separator into a modified AEI Model MS-9 mass spectrometer as previously described[1,6].

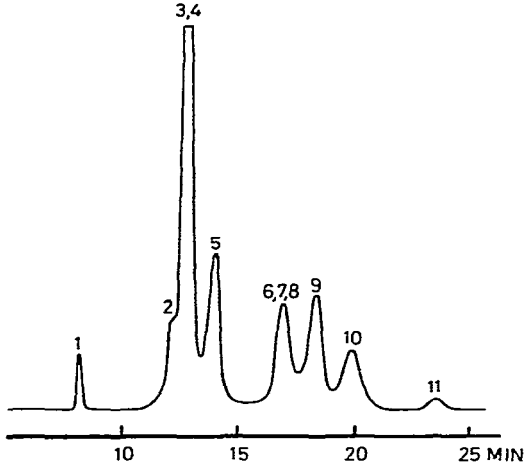

Fig. 1. *Cerastoderma edule* sterols (TMS) on PZ-176. For details see text and Table I.

RESULTS

Separation of cockle sterol TMS ethers on PZ-176

The GC profile of the sterol TMS ethers obtained after extraction of the cockle *Cerastoderma edule*[1], is shown in Fig. 1, and in Table I the data for this separation are compared with a separation on Silar 5CP.

Separation of tunicate sterol TMS ethers on PZ-176

The GC profile of the sterol TMS ethers obtained after extraction of the tunicate *Ascidia mentula* is shown in Fig. 2, and in Table II the data for this separation are compared with a separation on Silar 5CP which has been described previously[7].

* The "sorption technique" used was to pour a solution of PZ-176 in tetrahydrofuran (1.46% w/w) through a bed of Chromosorb G held in a tall glass funnel with a sinter in the base. The residual liquid was removed by suction and the perculation process repeated five times without agitation. The adsorbent was dried by an upwards current of warm nitrogen so as to just fluidise the bed without too much abrasion. When almost dry the adsorbent was spread out on a tray and dried in an oven at 200° before packing into the column. The liquid loading was calculated by weighing the residual PZ 176 in the mother liquors.

TABLE I

GC DATA FOR COCKLE STEROL TMS DERIVATIVES

RRT: relative retention time. 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 of the compounds are 3β-sterols and the additional carbon atoms are attached to C24 unless otherwise stated. Note that it is not possible to distinguish C24 epimers by either GC or MS techniques, *e.g.*, 29C 5 represents (24ξ)-24-ethylcholest-5-en-3β-ol, *i.e.* either β-sitosterol or clionasterol.

Cockle sterol	*Identity*	*PZ-176 RRT (255°)*	*Silar 5CP RRT (220°)*
C-1	26C 5,22(E)	0.65	0.64
C-2	27C 5,22(E)	0.93	0.93
C-3	27C 5	1.00 (13 min)	1.00 (16 min)
C-4	27C	1.00	1.00
C-5	28C 5,22(E)	1.09	1.10
C-6	27C 5,24(25)	1.32	1.30
C-7	28C 5	1.32	1.30
C-8	29C 5,22(E)	1.32	1.45
C-9	28C 5,24(28)	1.43	1.45
C-10	29C 5	1.55	1.58
C-11	29C 5,24(28)Z	1.82	1.88

TABLE II

GC DATA FOR TUNICATE STEROL TMS DERIVATIVES

Tunicate sterol	*Identity**	*PZ-176 RRT (255°)*	*Silar 5CP[7] RRT (220°)*
AM-1	26C 5,22(E)	0.64	0.64
AM-2	26C 22(E)	0.64	0.64
AM-3	27C 5,22(E)**	0.90	0.87
AM-4	27C 22(E)***	0.90	0.87
AM-5	27C 5,22(E)	0.95	0.93
AM-6	27C 22(E)	0.95	0.93
AM-7	27C 5	1.00 (13 min)	1.00 (16 min)
AM-8	27C	1.00	1.00
AM-9	28C 5,22(E)	1.09	1.10
AM-10	28C 22(E)	1.09	1.10
AM-11	28C 5	1.32	1.30
AM-12	28C	1.32	1.30
AM-13	29C 5,22(E)	1.32	1.45
AM-14	29C 22(E)	1.32	1.45
AM-15	28C 5,24(28)	1.43	1.45
AM-16	28C 24(28)	1.43	1.45
AM-17	29C 5	1.55	1.58
AM-18	29C	1.55	1.58
AM-19	29C 5,24(28)E	1.70	1.79
AM-20	29C 24(28)E	1.70	1.79
AM-21	29C 5,24(28)Z	1.82	1.88
AM-22	29C 24(28)Z	1.82	1.88

* See text to Table I.

** This is the 27-nor compound occelasterol.

*** This is the 27-nor compound patinosterol.

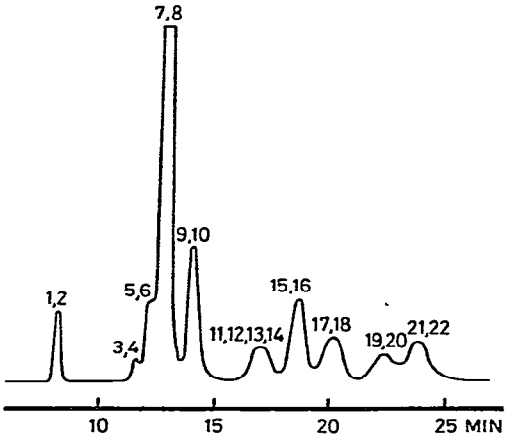

Fig. 2. *Ascidia mentula* sterols (TMS) on PZ-176. For details, see text and Table II.

DISCUSSION

Essentially, the sterol TMS separations obtained from the complex biological mixtures were very similar in both phases with only small variations in *RRT*. The only significant difference was the position of stigmasterol (29C, 5, 22E) which was retained longer by the Silar phase. No separation of the Δ5-sterol/5α-stanol pairs was achieved on either of these phases.

However, the sterol TMS peaks were sharper with PZ-176 than with Silar 5CP, more theoretical plates (2960 compared with 2291) being measured for the cholesterol TMS peak. In spite of the increased temperature and shorter retention times, the resolution of the compounds on the PZ-176 phase was better than that observed with the Silar phase.

Our results indicate that a combination of Dexsil 300GC, which is effective for the separation of Δ5-sterol/5α-stanol pairs[7], and PZ-176 would seem to be the best pair of liquid phases for the routine GC–MS analysis of complex marine sterol mixtures as their TMS derivatives. The results obtained from these two phases are complementary, and by using subtractive techniquee the components within each peak can be determined by MS.

Complete resolution of these extremely complex biological mixtures will necessitate the use of column systems with much greater separating powers. We are currently investigating the use of glass capillary PLOT columns containing these two phases for this purpose.

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