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Note

Simple mass chromatographic procedure for the detection and identification of sterols using derivative correlations

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The characterization of sterols from plant and animal sources presents problems caused by the structural similarity of the compounds and the common occurrence of mixtures dominated by one or two major components. Extracts are normally subjected to one or more time-consuming pre-fractionation steps in order to concentrate the trace sterols and separate them from the major ones.

Such procedures, which take up most of the analysis time, are discussed in depth in a recent article[1]. The combined technique of gas chromatography–mass spectrometry (GC–MS) is the most powerful method of gaining structural information from the trace compounds[2] and application of various types of capillary columns is further improving work in this field[3–5]. Computerized techniques have been developed to reduce analysis times by absorbing some of the work load, *e.g.*, by detecting peaks which maximize together and subtracting the remainder, enabling a partial resolution of multi-component peaks to be accomplished[6].

The present communication describes a correlation technique to simplify the searching of scanned spectral series, stored on magnetic disk, for the presence of Δ^5-3β-hydroxy-sterols, using open tubular capillary columns.

EXPERIMENTAL

Materials

Sterols were either purchased from Steraloids (Wilton, N.H., U.S.A.), Serva (Heidelberg, G.F.R.) or Sigma (St. Louis, Mo., U.S.A.), or were kindly supplied by Drs. N. Ikekawa, A. Kanazawa and B.A. Knights. Systematic names: *cis*-22-dehydrocholesterol = 5,22(*Z*)-cholestadien-3β-ol; *trans*-22-dehydrocholesterol = 5,22(*E*)-cholestadien-3β-ol; cholesterol = 5-cholesten-3β-ol; desmosterol = 5,24-cholestadien-3β-ol; brassicasterol = (24*R*)-24-methyl-5,22-cholestadien-3β-ol; 24-methylenecholesterol = 24-methylene-5-cholesten-3β-ol; campesterol = (24*R*)-24-methyl-5-cholesten-3β-ol; stigmasterol = (24*S*)-24-ethyl-5,22-cholestadien-3β-ol; fucosterol = 24-ethyl-5,24(28) (*E*)-cholestadien-3β-ol; 28-isofucosterol = 24-ethyl-5,24(28)(*Z*)-cholestadien-3β-ol.

Derivatization

Trimethylsilyl (TMS) ether derivatives were prepared as described by Makita

and Wells[7] and *tert.*-butyldimethylsilyl (TBDMS) ethers according to the method of Kelly and Taylor[8]. Reagents were obtained from E. Merck (Darmstadt, G.F.R.) and Applied Science Labs. (State College, Pa., U.S.A.), and derivatives were dissolved in *n*-hexane prior to GC–MS.

GC–MS

GC–MS was carried out using an LKB 9000S instrument equipped with either a 25 m open tubular capillary column (LKB, Stockholm, Sweden) coated with OV-101 and having an internal diameter of 0.35 mm or coated with OV-17 (50 m × 0.35 mm I.D.). The use of a glass "falling needle" splitless injector[9] ensured that no solvent entered the column and ion source. Conditions: helium carrier gas flow-rate, 2 ml/min, made up to 30 ml/min before the double stage jet separator; column temperature, 260°; separator temperature, 270°; ion source temperature, 290°; ionizing voltage, 22.5 eV; trap current, 60 μA. Scanned mass spectra were recorded on magnetic tape or disk via an LKB 2130 data system. Typically, 300 spectra were recorded at 5-sec intervals (TMS ethers) or 10-sec intervals (TBDMS ethers), each spectrum being assigned a time by the computer.

RESULTS AND DISCUSSION

Fig. 1 shows the total ion current curve obtained upon injection of a mixture of steryl TBDMS ethers containing pairs fairly difficult to resolve using conventional packed columns. Each peak corresponds to 200–300 ng sterol. The improved resolution is apparent and the narrowness and height of the peaks provide cleaner mass spectra of trace compounds because the ratio of peak to background noise is much improved.

In the present analytical scheme, samples derivatized as TMS ether and TBDMS ethers are injected consecutively and the repetitively scanned spectra recorded on the same disk cartridge (TMS and TBDMS profiles). This allows both profiles to be examined at the same time via the interactive display of the data system and spectra compared. Retention times of the peaks in the TBDMS profile relative to cholesteryl TBDMS are calculated and these ratios are used to predict the corresponding retention times of the TMS peaks in the TMS profile.

In Table I are listed the retention times and predicted values for two experiments carried out with OV-101 and OV-17 columns and a number of sterols. The retention times of the sterol peaks in the corresponding TMS ether chromatograms were predicted by applying the formula

$$t_{xTMS} = \frac{t_{xTBDMS}}{t_{cTBDMS}} \cdot t_{cTMS} = R_{cxTBDMS} \cdot t_{cTMS}$$

where the retention time of cholesteryl TMS ether is known, t_{xTMS} is the predicted retention time of sterol "x" TMS ether, t_{xTBDMS}, t_{cTBDMS} and t_{cTMS} are the retention times of sterol "x" TBDMS ether, cholesteryl TBDMS ether and cholesteryl TMS ether, respectively, and $R_{cxTBDMS}$ is the retention time of sterol "x" TBDMS ether relative to that of cholesteryl TBDMS ether. Predicted and actual TMS ether times are also shown in Table I and for those sterols examined so far a reasonable agreement is obtained.

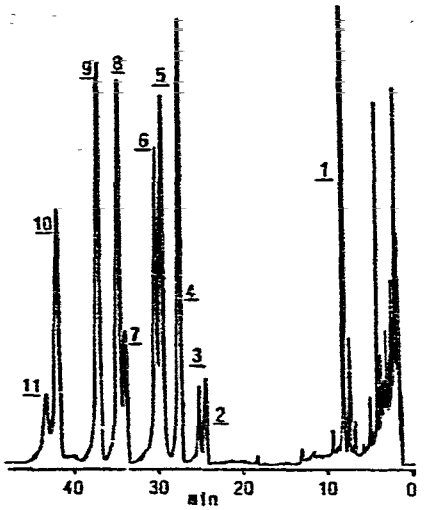

Fig. 1. Total ion current chromatogram of sterol *tert.*-butyldimethylsilyl ethers obtained using an open tubular capillary column (OV-101) at 260° connected to an LKB 9000S mass spectrometer. Peaks: 1 = 5α-cholestane; 2 = *cis*-22-dehydrocholesterol; 3 = *trans*-22-dehydrocholesterol; 4 = cholesterol; 5 = desmosterol; 6 = brassicasterol; 7 = 24-methylenecholesterol; 8 = campesterol; 9 = stigmasterol; 10 = fucosterol; 11 = 28-isofucosterol.

Searching spectra series via an interactive display is a difficult procedure, especially when trace compounds are of interest. These may lie in the background noise, no significant peaks being seen in the total ion current chromatogram or they

TABLE I

CORRELATION OF TMS AND TBDMS CHROMATOGRAMS

Retention times (t in sec) of some sterol TBDMS ether derivatives together with predicted and actual TMS ether times. Open tubular capillary columns (OV-101 and OV-17) at 260°.

Sterol	*OV-101 (25 m)*				*OV-17 (50 m)*			
	t_{TBDMS}*	R_{cTBDMS}**	t_{TMS}*** *predicted*	t_{TMS}§ *actual*	t_{TBDMS}*	R_{cTBDMS}**	t_{TMS}*** *predicted*	t_{TMS}§ *actual*
cis-22-Dehydrocholesterol	1400	0.89	774	775	2050	0.89	1189	1233
trans-22-Dehydrocholesterol	1450	0.92	800	795	2099	0.91	1217	1265
Cholesterol	*1580*	*1.00*	—	*870*	2304	*1.00*	—	*1337*
Desmosterol	1700	1.08	940	935	2625	1.14	1524	1560
Brassicasterol	1740	1.10	957	950	2495	1.08	1443	1523
24-Methylenecholesterol	1950	1.23	1070	1065	2912	1.26	1684	1721
Campesterol	2000	1.27	1105	1090	2934	1.27	1698	1689
Stigmasterol	2140	1.35	1175	1165	3189	1.38	1845	1820
Fucosterol	2420	1.53	1331	1305	3833	1.66	2219	2131
Sitosterol	2420	1.53	1331	1305	3603	1.56	2085	2046
28-Isofucosterol	2500	1.58	1375	1355	3939	1.71	2285	2214

* Mass spectra obtained at 10-sec intervals.

** Times relative to cholesteryl TBDMS ether.

*** Predicted on basis of cholesteryl TMS time (870 sec) and R_{cTBDMS} values.

§ Retention time of most intense spectrum in peak.

are surrounded by contaminant peaks. The value of predicting the approximate TMS ether times in the way described here lies in the fact that the mass spectra of steryl TMS ethers in trace amounts are not readily seen because the ion current tends to be spread over a large number of fragment ions, particularly in the lower and less specific mass regions[10,11]. The mass spectra of some steroid and Δ^5-3β-hydroxy-sterol TBDMS ethers are, however, characterized by predominant M—57[8,12–15] or M—147[16,17] ions and these are very readily detected, even where no peak is apparent in the total ion current trace. This technique has been applied to an extract from the brown alga, *Sargassum vulgare*, and a very large number of potential sterols revealed[5]. Although the TMS spectra are not so readily found during a search through the recorded spectra, they do contain much more structural information. It is therefore possible using the scheme outlined here to perform a preliminary screening of sterol containing samples using TBDMS ether derivatives in order to locate the approximate region of occurrence of the TMS derivatives to within 26 sec for the present sterols using the 25 m OV-101 column and within *ca.* 80 sec for the 50 m OV-17 column. Considering the length of the latter column and the long retention times, these variations are acceptable, *e.g.*, for fucosterol the deviation from the predicted retention times was within 4% in the GC–MS combination. By forming simultaneous fragment ion plots (mass chromatograms) of possible M—57 ions a large variety of trace sterols may be revealed in one operation providing that isomers have different retention times on the phase employed. The TBDMS ether mass chromatogram for the mixture just described (OV-101) is shown in Fig. 2. Corresponding TMS ether spectra or partial spectra may then be located via the equation providing that similar conditions are employed and both derivatives have the same degree of substitution.

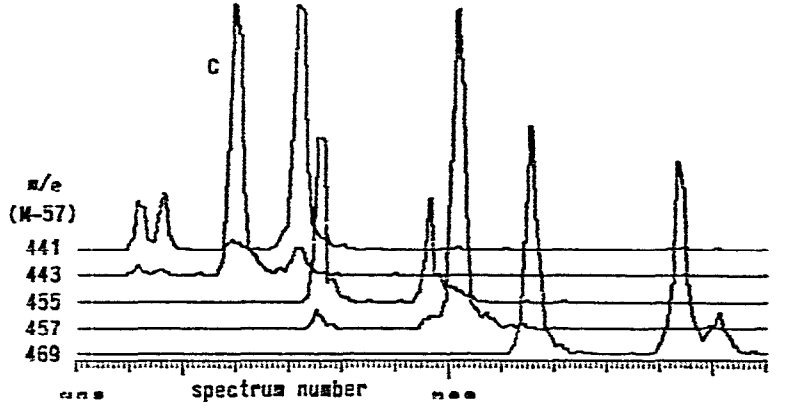

Fig. 2. TBDMS ether mass chromatogram for the sterol mixture shown in Fig. 1. Abundances of M—57 ions are plotted from the whole spectra recorded during GC–MS (C = cholesterol).

The sensitivity of the present system is *ca.* 1–10 ng injected depending upon the instrumental sensitivities of the various sterols. Application of multi-channel mass fragmentography will allow much greater sensitivity to be attained.

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