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Note

N-Methoxy-N,O-bistrimethylsilyl carbamate as a derivatizing reagent for the gas chromatography of sitosterol degradation products

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According to present practice, double derivatization is required for the capillary gas chromatographic analysis of hydroxy-ketosteroids. The general procedure consists of transforming the keto groups into oxime methyl ether derivatives and silylation of the hydroxy groups[1,2].

N-Methoxy-N,O-bistrimethylsilyl carbamate (BSMOC) proved to be a suitable derivatizing agent for hydroxy-ketosteroids[3] and bile acids[4]. When acidic catalysts, *e.g.* trifluoroacetic acid, are used, simultaneous and quantitative silylation and methoximation take place. BSMOC was applied to the GC analysis of the microbial degradation products of sitosterol and some related compounds.

EXPERIMENTAL

The structures of the compounds investigated are shown in Fig. 1. Compounds 1–7 are known microbial degradation products of sitosterol[5–7]. Compound 8 was synthesized from compound 7 by elimination of the 9α-hydroxy group[8]. Compound 9 was obtained from compound 8 by microbial 1,2-dehydrogenation[8]. BSMOC (95% purity, b.p.. 55.0–58.0°C at 2.5 mbar, Kováts retention index on 5% OV-1 at 110°C, 1143 ± 3) was synthesized at the Department of General and Inorganic Chemistry, Eötvös Loránd University, Budapest[9]. Squalane internal standard was purchased from Fluka (Buchs, Switzerland).

The derivatization experiments were carried out with 7.5 g/l solutions of the substrates in pyridine [squalane (internal standard) concentration, 3.75 g/l]. The simultaneous methoxime and trimethylsilyl derivatization reactions of compounds 1–9 were accomplished with a constant molar ratio (120:1) of BSMOC to substrate and various molar ratios (from 5.6:1 to 1.8:1) of reagent to acid catalyst (trifluoroacetic acid) at

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* Squalane (internal standard) concentration, 3.75 g/l.

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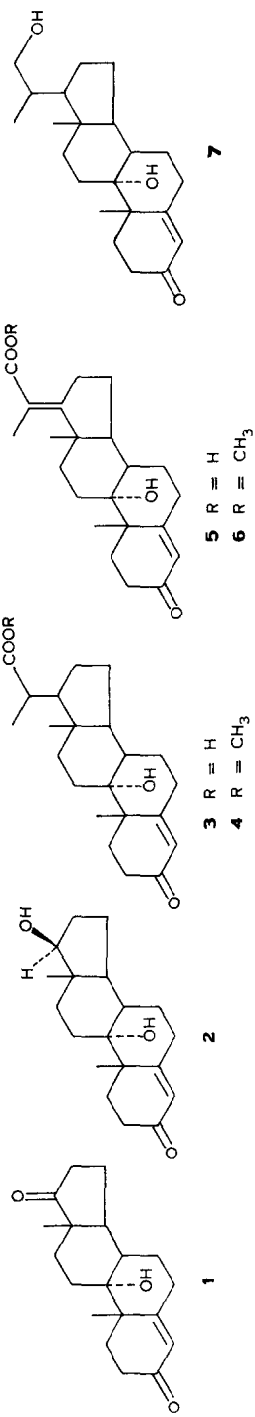

Fig. 1. Structures of the steroid substrates: 1 = 9α-hydroxy-4-androsten-3,17-dione; 9α,17β-dihydroxy-4-androsten-3-one; 3 = 9α-hydroxy-3-oxo-23,24-bisnorchol-4-en-22-oic acid; 4 = methyl ester of 3; 5 = 9α-hydroxy-3-oxo-23,24-bisnorchola-4,17(20)-dien-22-oic acid; 6 = methyl ester of 5; 7 = 9α,22-dihydroxy-23,24-bisnorchol-4-en-3-one; 8 = 22-hydroxy-23,24-bisnorchola-4,9(11)-dien-3-one; 9 = 22-hydroxy-23,24-bisnorchola-1,4,9(11)-trien-3-one.

room temperature. The optimum rates and product stabilities were found at a molar ratio of 2.8:1 for compounds 2–8 and 1.8:1 for compounds 1 and 9. The reactions were carried out in 1-ml vials, equipped with a rubber septum. Samples (1 μm) were withdrawn through the septum, then injected into the GC apparatus.

The gas chromatograph was a Hewlett-Packard 5880 A, equipped with a 25 m × 0.31 mm I.D. fused-silica capillary column, 5% phenyl-methylsilicone, cross-linked (film 0.17 μm, phase ratio 450:1). The oven temperature was programmed between 165°C and 285°C at 8°C/min. The flame ionization detector and the injector were at 255°C. Retention indices were determined at 275°C, with an accuracy of ±4 index units.

For GC–mass spectrometry (MS) we used a VG 12-250 mass spectrometer with a Hewlett-Packard 5790 gas chromatograph (column as specified above). A scan time of 4 s and an ionization energy of 70 eV were applied.

RESULTS AND DISCUSSION

The optimum parameters for the quantitative derivatization of the compounds studied were determined on the basis of the maximum relative molar response (RMR) values, relative to squalane. These data are summarized with the positions of the potential reactive groups in Table I.

Free acids showed significantly lower RMR values than the corresponding methyl esters, probably because of lower hydrolytic stability of the trimethylsilyl

TABLE I

OPTIMUM PARAMETERS OF THE QUANTITATIVE AND SELECTIVE* DERIVATIZATION OF COMPOUNDS 1–9 WITH BSMOC

Substrate concentration, 7.5 g/l; solvent, pyridine; reagent-to-substrate molar ratio, 120:1; catalyst, trifluoroacetic acid.

Substrate No. and potential reactive groups	*Reagent-to-catalyst molar ratio*	*Max. RMR with respect to squalane*	*Reaction time at max. RMR at room temperature (min)*
(1) 9α-OH, Δ^4-3-one, 17-one	1.8	1.170	75
(2) 9α-OH, 17β-OH, Δ^4-3-one,	2.8	1.167	55
(3) 9α-OH, Δ^4-3-one, COOH	2.8	0.633	60
(4) 9α-OH, Δ^4-3-one	2.8	1.154	60
(5) 9α-OH, Δ^4-3-one, COOH	2.8	0.605	60
(6) 9α-OH, Δ^4-3-one	2.8	1.188	60
(7) 9α-OH, 22-OH, Δ^4-3-one	2.8	1.115	60
(8) 22-OH, Δ^4-3-one	2.8	1.135	55
(9) 22-OH, $\Delta^{1,4}$-3-one	1.8	1.060	70

* 9α-OH did not react.

TABLE II

CHARACTERISTIC MS DATA AND GC RETENTION INDICES OF THE METHOXIDE-TRIMETHYL SILYL DERIVATIVES OF COMPOUNDS 1–9

Substrate No.	*MS data of the derivatives at 70 eV*			*GC retention indices* at 275°C*
	m/z	*R.I.**(%)*	*M−X⁺*	
1	360	28	M	2986
	329	7	M−31	
2	405	25	M	2945
	374	6	M−31	
3	461	23	M	3363
	446	12	M−15	
	430	8	M−31	
4	403	17	M	3278
	372	8	M−31	
5	459	10	M	3437
	428	5	M−31	
6	401	23	M	3354
	370	8	M−31	
7	447	15	M	3302
	416	10	M−31	
8	429	15	M	3090***
	398	8	M−31	3099***
9	427	8	M	3112

* Accuracy, ±4 index units; column, 5% phenylmethylsilicone, 25 m × 0.31 mm I.D.
** R.I. = relative intensity.
*** *Syn/anti* isomeric pairs: not separated in GC–MS measurements.

esters. However, these values were reproducible for a reaction mixture, with a standard deviation of ±3.5 rel.%, within 2 days. Characteristic MS data and GC retention indices of the derivatives are listed in Table II.

The GC–MS measurements, illustrated in Fig. 2 for compound 6, showed that the products are the methoxime or the methoxime-trimethylsilyl derivatives, but the 9α-hydroxy group remained intact. GC–MS measurements made in the course of the reactions, starting with the addition of the trifluoroacetic acid catalyst, revealed that rapid (instantaneous) silylation took place at the unhindered, or moderately hindered 17- and 22-hydroxy groups, as well as at carboxylic functions. The rate-determining step was the oxime formation, which showed the following order of reactivities for the different steroidal oxo groups: Δ^4-3-one > $\Delta^{1,4}$-3-one > Δ^4-3,17-dione.

The 9α-hydroxy group of compounds 1–7 was not silylated under the specified conditions, owing to steric hindrance by the axial hydrogens at C-7 and C-14.

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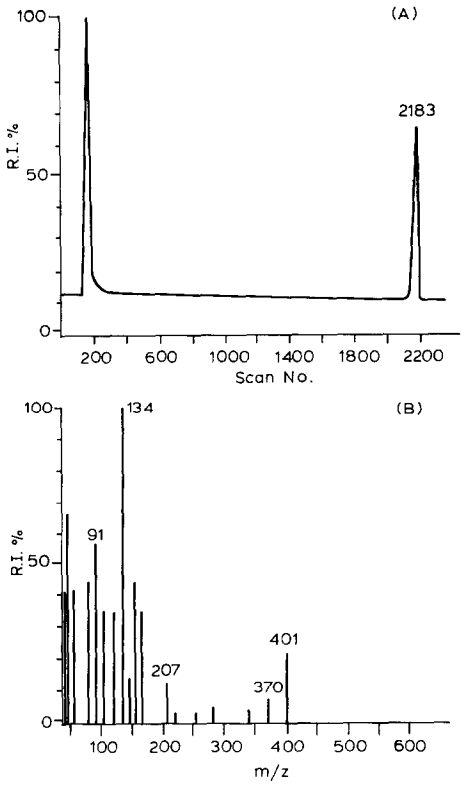

Fig. 2. (A) Total ion chromatogram and (B) mass spectrum of the methoxime derivative of compound 6, obtained with BSMOC reagent and trifluoroacetic acid catalyst in pyridine at room temperature. (For reaction and GC–MS parameters, see Experimental; molecular ion $M^+ = 401$).

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