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N-SUBSTITUTED TRIMETHYLSILYLCARBAMATES AS SILYLATING AND/OR METHOXIME DERIVATIZING REAGENTS FOR GAS CHROMATOGRAPHIC ANALYSIS

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SUMMARY

N-Substituted trimethylsilylcarbamates were tested as derivatizing reagents for gas chromatographic analysis. N,N-Dimethyltrimethylsilylcarbamate was found to be a suitable agent for direct trimethylsilylation of the salts of alkaloids without previous liberation of the bases. N-Methoxy-N,O-bistrimethylsilylcarbamate was found to react simultaneously and quantitatively with the hydroxy and keto groups of steroids, resulting in the corresponding methoxime trimethylsilyl derivatives.

INTRODUCTION

The preparation of N-alkylcarbamic acid trimethylsilyl esters has been described in a previous pasper[1]. According to our experience, these compounds can advantageously be used for the silylation of alcohols[1], phenols[2], carboxylic acids and aromatic amines[3]. The silylation reaction is as follows:

$$R_1R_2NC(O)OSi(CH_3)_3 + HY \rightarrow YSi(CH_3)_3 + R_1R_2NH + CO_2$$

where R_1 = alkyl, R_2 = alkyl or H, Y = RO (R = alkyl or aryl), Y = RCO (R = alkyl or aryl) or Y = RR_xN (R = aryl, R_x = H, alkyl or aryl).

This reaction shows the following advantages: (1) the process is a non-equilibrium reaction, because carbon dioxide leaves the system; (2) the volatile by-products are eluted in early peaks during gas chromatography (GC), so contamination of the derivatives does not occur; and (3) the process is an autocatalytic reaction, because silylation is catalysed by the amine formed[4]. On the other hand, the amine can act as a proton acceptor.

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In this work, examples are given of the direct silylation of the salts of alkaloids, ephedrine, scopolamine and atropine, using N,N-dimethyltrimethylsilylcarbamate (DMCTMS) as a reagent. The GC determination of tropane-base alkaloids following silylation has already been reported[5,6]; ephedrine has been analysed mostly by means of N,O-acylation[7,8].

It should be noted that N-alkyl-substituted trimethylsilylcarbamates cannot be applied for the silylation of compounds containing oxo groups, because in this instance enamines or Schiff bases are formed, generating other transformations[9]. To overcome this drawback we synthesized a new type of silylcarbamate, N-methoxy-N,O-bistrimethylsilylcarbamate (BSMOC). In practice, the GC [or GC–mass spectrometric (GC–MS)] analysis of steroid hormones requires double derivatization in two reaction steps[10,11]: methoxime derivatization of keto groups and trimethylsilylation of hydroxy groups. This double derivatization of hydroxy ketosteroids has been studied by applying BSMOC.

EXPERIMENTAL

Materials

N-Substituted trimethylsilylcarbamates were synthesized in our laboratory. We used DMCTMS (b.p. 161°C/1013 mbar, 28°C/1.5 mbar; Kováts retention index on 5% OV-1 at 110°C, 944 ± 2) and BSMOC (b.p. 55–58°C/2.5 mbar; Kováts retention index on 5% OV-1 at 110°C, 1143 ± 3).

Derivatization

The derivatization experiments were carried out with 1–10 g/l solutions of the substrates in pyridine. Silylation of the salts of the alkaloids with DMCTMS were carried out with a varying excess of the DMCTMS, yielding maximum conversions (see Results and discussion). The simultaneous methoxime and trimethylsilyl derivatization reactions of steroid substrates were accomplished with a constant reagent (BSMOC)-to-substrate ratio (60) and varying reagent-to-acid catalyst (trifluoroacetic acid) molar ratios (10–1.5), generally at room temperature. The reactions were carried out in 1-ml vials, equipped with a tap and a rubber septum (the vials were thermostated, if necessary). The analysis of the reaction mixtures was carried out by GC. Samples (1 μl) were withdrawn through the septum, then injected into the GC apparatus. The reaction products were identified by GC–MS measurements.

Gas chromatographic parameters

The gas chromatograph was a Hewlett-Packard 5720A with a 2 m × 2 mm I.D. silanized glass column packed with 3% SE-52 on Gas-Chrom Q (100–120 mesh) (for alkaloids) or 3% OV-210 or 3% SE-54 on Gas-Chrom Q (100–120 mesh) (for steroids). The oven temperature was 135–285°C (8°C/min) and 262°C or 272°C (two different isotherms). The flame ionization detector temperature was 275°C and the injector temperature was 275°C. The carrier gas was nitrogen at a flow-rate of 20 ml/min.

Gas chromatography–mass spectrometry

The instrumentation consisted of a VG Micromass 12F-1A with a Pye-Uni-

cam-104 GC oven. The electron energy was 70 eV. The GC parameters were as above, except that the oven temperature was 250°C and the carrier gas was helium at a flow-rate of 20 ml/min.

RESULTS AND DISCUSSION

The results indicated that with DMCTMS the salts of ephedrine, scopolamine and atropine can be silylated without previous liberation of the alkaloid bases in any solvent and also without a solvent. The acidic component of the salt is neutralized by the dimethylamine formed in the silylation reaction:

$$(H)B \cdot HA + (CH_3)_2NC(O)OSi(CH_3)_3 \rightarrow (CH_3)_3SiB + (CH_3)_2NH_2A + CO_2$$

where A = acid anion, (H)B = alkaloids and (H) = the protic hydrogen of alkaloids.

The relative molar responses (RMR) of the derivatives with respect to caffeine internal standard as a function of the reagent to substrate molar ratio is shown in Fig. 1.

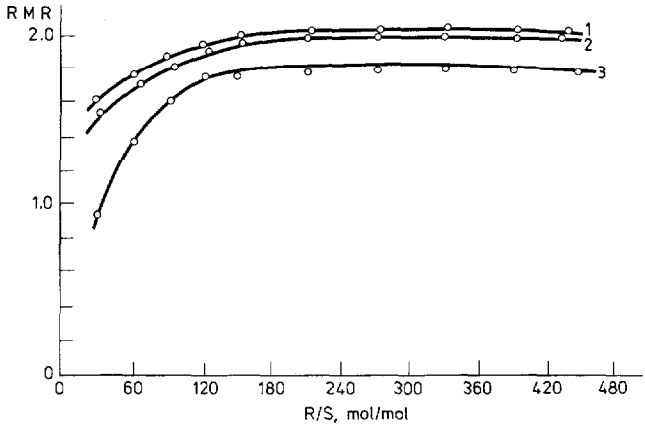


Fig. 1. RMR with respect to caffeine as internal standard *versus* reagent-to-substrate (R/S) ratios (mol/mol) for the silylation of the salts of ephedrine hydrochloride (1), scopolamine hydrochloride(2) and atropine sulphate (3) with DMCTMS. Analysis after a 5-min reaction time.

This type of silylation is a fast reaction, even at room temperature. An increase in RMR values could be detected only in the first 5 min. Above a relatively high molar ratio (> 120) the RMR is constant (with a standard deviation of 3.5%).

With ephedrine, N-silylation also takes place in addition to O-silylation, as shown by GC and GC–MS measurements (Fig. 2 and Table II). However, no interference effects are observed, as the RMR values corresponding to the sum of the intensities of the two signals are constant over a broad range of molar ratios (see Fig. 1).

The optimum parameters of the quantitative derivatization of the compounds studied (maximum RMR values and the corresponding required minimum reagent to substrate molar ratios) are given in Table I. The GC and GC–MS data for the derivatives (retention relative to caffeine; molecular ions) are summarized in Table II.

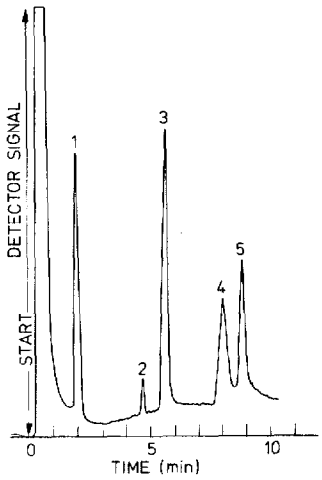


Fig. 2. Gas chromatogram of the trimethylsilyl (TMS) derivatives of alkaloids produced in the reaction of their salts with DMCTMS (3% SE-52, 135–285°C, 8°C/min). Peaks: 1 = ephedrine-O-TMS, 2 = ephedrine-N,O-TMS, 3 = caffeine, 4 = atropine-O-TMS, and 5 = scopolamine-O-TMS.

TABLE I

SILYLATION OF ALKALOID SALTS

Substrate	*Acidic component of the addition salt (HA)*	*Maximum RMR with respect to caffeine*	*Molar ratio of DMCTMS to substrate*
Atropine	H_2SO_4	1.75	120
Scopolamine	HCl	1.98	150
Ephedrine	HCl	2.05*	150

* With ephedrine, two derivatives, O-TMS and O,N-$(TMS)_2$, were observed. The RMR was calculated by addition of the two intensities.

TABLE II

IDENTIFICATION OF ALKALOID DERIVATIVES BY GC–MS

Substrate	*Derivative*	*MS molecular ions (70 eV) (M^+), m/e*	*Retention time relative to caffeine (3% SE-52, 135–295°C, 8°C/min)*
Atropine	O-TMS	361	3.05
Scopolamine	O-TMS	375	3.70
Ephedrine	(a) O-TMS	237	0.41
	(b) O,N-$(TMS)_2$	309	0.88

A further aim of these studies was to examine the derivatization reactions of BSMOC with some hydroxy ketosteroids. These reactions can be catalysed by organic acids, *e.g.*, trifluoroacetic acid. The reaction parameters of the experiments and the positions of the reactive groups of the substrates are summarized in Table III.

TABLE III

STEROID SUBSTRATES USED IN THE DERIVATIZATION EXPERIMENTS WITH BSMOC

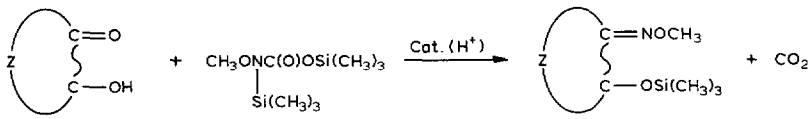
Substrate concentration, 10 g/l; solvent, pyridine; reagent-to-substrate molar ratio, 60; catalyst, trifluoroacetic acid.

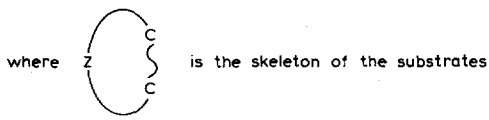
Substrate	*Reactive groups*	*Reagent to catalyst molar ratio*	*Reaction time (at 25°C) at total conversion (min)*
5-Dehydroepiandrosterone	3β-ol, 17-one	4.5	170
Androsterone	3β-ol, 17-one	4.5	25
Estrone	3-ol, 17-one	4.5	140
19-Nortestosterone	17β-ol, 3-one	4.5	10
Testosterone	17β-ol, 3-one	4.5	20
Δ^1-17α-Methyltestosterone	17α-ol, 3-one	4.5	170
Progesterone	3,20-dione	4.5	15
11α-Hydroxyprogesterone	11α-ol, 3,20-dione	2.0	20
11β-Hydroxyprogesterone	11β-ol, 3,20-dione	2.0	60
21-Hydroxyprogesterone	21-ol, 3,20-dione	4.0	30
17α-Hydroxyprogesterone	17α-ol, 3,20-dione	4.0	90*
Estradiol	3,17β-ol	4.0	Instantaneous

* At 90°C.

Characteristic MS data and GC retention indices of the derivatives are listed in Table IV.

The GC–MS measurements showed that the products of the reactions of the steroid substrates with BSMOC are the methoxime trimethylsilyl (MO-TMS) derivatives. Unreacted substrates or by-products could not be detected after completion of the reactions. It should be noted that with substrates containing only keto or hydroxy groups (*e.g.*, progesterone or estradiol) the products are the methoxime or the trimethylsilyl derivatives, respectively. The following reaction scheme can be derived, based on the analytical data, for the reaction of BSMOC with the hydroxy ketosteroid substrates:





This equation shows that two fragments of the reagent molecule are bound to the substrate. The reaction is catalysed by volatile organic acids such as trifluoroacetic acid and the process therefore takes place in a single operation, usually under mild conditions and within a short reaction time.

Significant differences were found in the reactivity of the compounds as a function of steric hindrance (*cf.*, Table III). Thus, testosterone could be derivatized in 20

TABLE IV

IDENTIFICATION OF STEROID DERIVATIVES

R.I. is relative intensity.

Substrate	*MS data for the derivatives (70 eV)*			*GC retention indices (262°C)*	
	$(M-X)^+$	*R.I. (%)*	*m/z*	*OV-210*	*SE-54*
5-Dehydro-epiandrosterone	M	22	389	2880	2700
	M−15	14	374		
	M−31	61	358		
	M−90	22	299		
Androsterone	M	4	391	2915	2735
	M−15	4	376		
	M−31	100	360		
	M−121	100	270		
Estrone	M	100	371	3000	2730
	M−15	10	356		
	M−31	78	340		
	M−88	17	283		
19-Nortestosterone	M	100	375	3000	2640
	M−15	6	360		
	M−31	10	344		
Testosterone	M	100	389	3040	2675
	M−15	7	374		
	M−30	8	359		
	M−31	10	358		
Δ^1-17α-Methyl-testosterone	M	100	401	3180	2800
	M−15	–	386		
	M−31	26	370		
Progesterone	M	100	372	3360	2970
	M−15	10	357		
	M−31	40	341		
11α-Hydroxy-progesterone	M	100	460	3535*	3200*
	M−15	5	445		
	M−31	45	429		
	M−90	45	370		
11β-Hydroxy-progesterone	M	100	460	3450*	3165*
	M−15	25	445		
	M−31	55	429		
	M−90	65	370		
21-Hydroxy-progesterone	M	100	460	3435*	3220*
	M−15	8	445		
	M−31	80	429		
	M−90	–	370		
17α-Hydroxy-progesterone	M	37	460	3380*	3150*
	M−15	7	445		
	M−31	100	429		
	M−90	3	370		
Estradiol	M	100	416	2850	2700
	M−15	7	401		

* At 272°C.

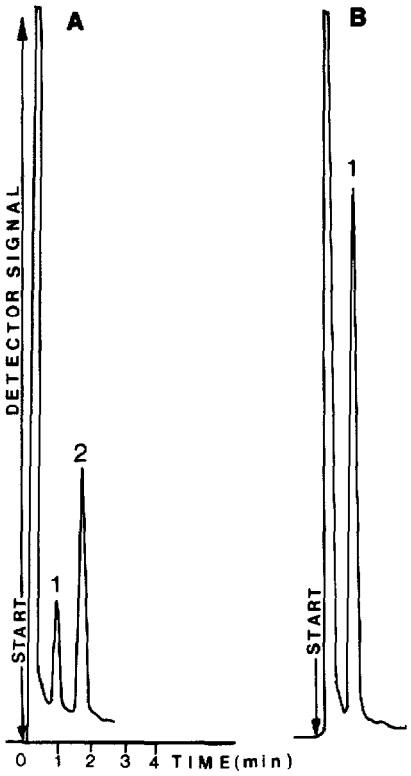


Fig. 3. Derivatization of androsterone with BSMOC with trifluoroacetic acid as catalyst in pyridine at 25°C. Chromatograms were recorded after reaction times of (A) 5 min and (B) 25 min (OV-210, 262°C). Peaks: 1 = -MO, -TMS derivative, and 2 = -TMS derivative.

min at 25°C, whereas Δ^1-methyltestosterone, containing a 17α-methyl group, required 170 min at 25°C. A considerable difference was observed between testosterone and 17α-hydroxyprogesterone (20 min at 25°C and 90 min at 90°C, respectively).

The derivatization of androsterone, exhibiting no steric hindrance, is shown in Fig. 3 by gas chromatograms recorded after reaction times of 5 min and 25 min. In this instance, GC–MS showed that, after a rapid silylation, oxime formation takes place with a longer reaction time. The derivatization of 17α-hydroxyprogesterone is demonstrated in Fig. 4 by gas chromatograms recorded after various reaction times.

RMR values for some representative steroid substrates were measured with respect to cholesteryl propionate in order to determine the GC recoveries of the derivatives. These were 0.710 for progesterone, 0.605 for 11β-hydroxyprogesterone and 0.510 for 17α-hydroxyprogesterone, respectively (the standard deviations were 3.5%).

The derivatization method developed for the hydroxy ketosteroids with BSMOC has the following advantages over the process used earlier[11]:

(1) the formation of the methoxime and the trimethylsilylation, performed in a single step without intermediary treatment of the sample in the case of hydroxy ketosteroids;

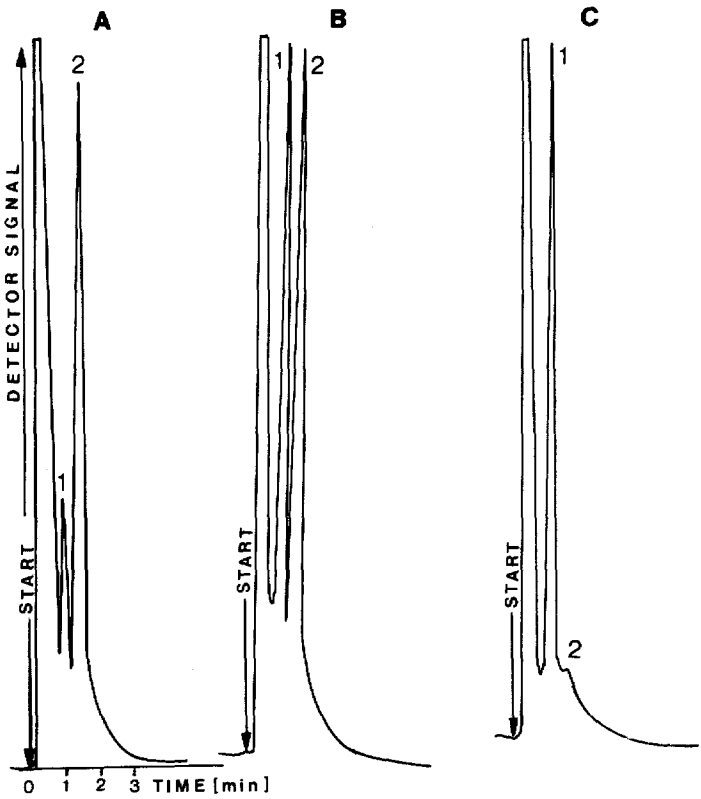


Fig. 4. Derivatization of 17α-hydroxyprogesterone with BSMOC with trifluoroacetic acid as catalyst in pyridine at 90°C. Chromatograms were recorded after reaction times of (A) 10 min, (B) 45 min and (C) 90 min. Peaks: 1 = 17α-OTMS, bismethoxime derivative; and 2 = 17α-OH, bismethoxime derivative.

(2) the method is also suitable for quantitative methoxime formation with ketosteroids or the quantitative trimethylsilylation of hydroxy steroids;

(3) the reaction usually takes place under mild conditions.

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