Note

Selective trimethylsilylation for the determination of 16(S)-amino-PGF$_{2\alpha}$ methyl ester by gas–liquid chromatography

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16(S)-Amino-PGF$_{2\alpha}$ methyl ester (compound I, see Fig. 1) is a prostaglandin derivative possessing high abortifacient activity[1]. Since it is one of the first prostanoids to bear an amino function the literature on quantitative silylation was surveyed both for F-type prostaglandins and compounds having similar structural parts, e.g., sphingosines. N,O-Bis(trimethylsilyl)acetamide (BSA) or N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) is the preferred silylating agent for quantitative gas chromatography (GC) of PGF methyl esters[2–4]. Using trimethylsilyldiethylamine (TMSDEA), the 11 and 15-hydroxyl groups are silylated selectively, while the 9-hydroxyl remains unreacted[5]. Hence an 11,15(OTMS)$_2$ derivative may be the by-product of incomplete silylation. In earlier studies, hexamethyldisilizane (HMDS)-based catalytic methods were also used both for analytical and preparative purposes[6–8]. For one-step silylation of PGF free acids, BSA or BSTFA with various catalysts are generally used[9–15].
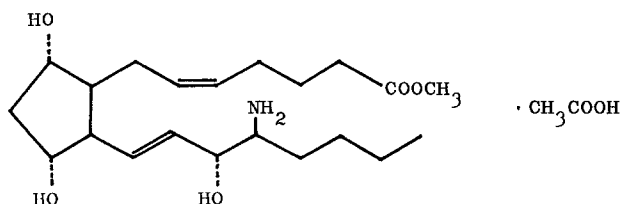

Fig. 1. Structural formula of 9α,11α,15(R)-trihydroxy-16(S)-amino-5(Z),13(E)-prostadienoic acid methyl ester acetate salt (compound I).

Sphingosines, resembling the C-13 to C-16 part of compound I, have widely been studied by gas–liquid chromatography (GLC) and mass spectrometry (MS). Silylation with HMDS–TMCS (trimethylchlorosilane) in pyridine yielded only O-silylated derivatives of free bases, as shown by their mass spectra[16].

EXPERIMENTAL

The materials used and their sources are given in Table I.

TABLE I

MATERIALS USED AND THEIR SOURCES

Material	Abbreviation	Source
16-Amino-PGF$_{2\alpha}$ methyl ester acetate salt	I	Institute for Drug Research, Budapest
Squalane used as internal standard	Sq	Carlo Erba, Milano
Hexamethyldisilazane	HMDS	Pierce Eurochemie
Trimethylchlorosilane*	TMCS	Pierce Eurochemie
Trimethylsilyldiethylamine	TMSDEA	Pierce Eurochemie
N,O-Bis(trimethylsilyl)acetamide	BSA	Pierce Eurochemie
N,O-Bis(trimethylsilyl)trifluoroacetamide	BSTFA	Pierce Eurochemie
Pyridine**	PY	Merck

    * A hydrochloric acid contaminant was precipitated with pyridine; the PY · HCl precipitate was filtered off on silanized glass wool.
    ** Freshly distilled and stored over KOH. Water content: maximum 0.05%, as determined by Karl Fischer titration.

Preparation of 16-amino-PGF$_{2\alpha}$ methyl ester-(OTMS)$_3$ (Compound II)
    A mixture of 25–45 μl of a solution of compound I (5 mg) in pyridine (1 ml) was added to 25 μl of a solution of squalane internal standard (2.5 mg) in pyridine (1 ml). The final volume was adjusted to 70 μl with PY. A 100-μl volume of HMDS and 5 μl of TMCS were added to this solution in a 1-ml Pierce vial equipped with a silicone rubber septum. After vigorous shaking, the reaction mixture was allowed to stand for 15 min at room temperature and then analysed by GC. Silylated samples in their original solutions were stable as indicated by the good reproducibility of the measurements: ± 3% within 72 h. To obtain fair quantitative results, direct injection was necessary.

Gas chromatography
    Gas chromatography was carried out by injections of 1-μl samples into a Hewlett-Packard 5720 A gas chromatograph equipped with a flame ionization detector. Silanized glass columns (2 m × 2 mm I.D.) packed with 3% OV-17, 3% OV-101 and 3% OV-210 on Gas-Chrom Q (80–100 mesh) were used. The column temperatures were in the range of 255–265°C (isothermal), and the injector and detector temperatures were 275°C. Nitrogen was used as the carrier gass at a flow-rate of 20 ml/min.

Mass spectrometry
    Mass spectra were recorded on a MAT SM-1 instrument under the following operating conditions: resolution, 1250; accelerating voltage, 8 kV; electron accelerating voltage, 70 or 12.5 V; electron current, 300 μA; ion-source temperature, 150°C; evaporation temperature (direct inlet), 120°C. High resolution mass measurements were made at resolution (R) = 10 000 (10% valley) using perfluorokerosene (PFK) as the reference standard. Measurements on metastable ion decomposition were made

by scanning the ion accelerating voltage. The electron energy of 12.5 eV was set to the ionization potential of oxygen.

Gas chromatography–mass spectrometry (GC–MS)

GC–MS measurements were carried out by using 3% OV-17 as stationary phase in a silanized glass column (2 m × 2 mm) and a Pye-104 gas chromatograph coupled with a Micromass MM. 12 F1A magnetic mass spectrometer. The column temperature was 260°C, and the carrier gas (helium) flow-rate was 20 ml/min. Mass spectra were recorded at an ion accelerating voltage of 3 kV, an ionizing energy of 70 eV and an electron current of 50 μA. The temperature of the ion source was kept at 200°C.

RESULTS AND DISCUSSION

According to our preliminary experiments, compound I always afforded a mixture of products with silylating reagents mostly used for F-type prostaglandins,
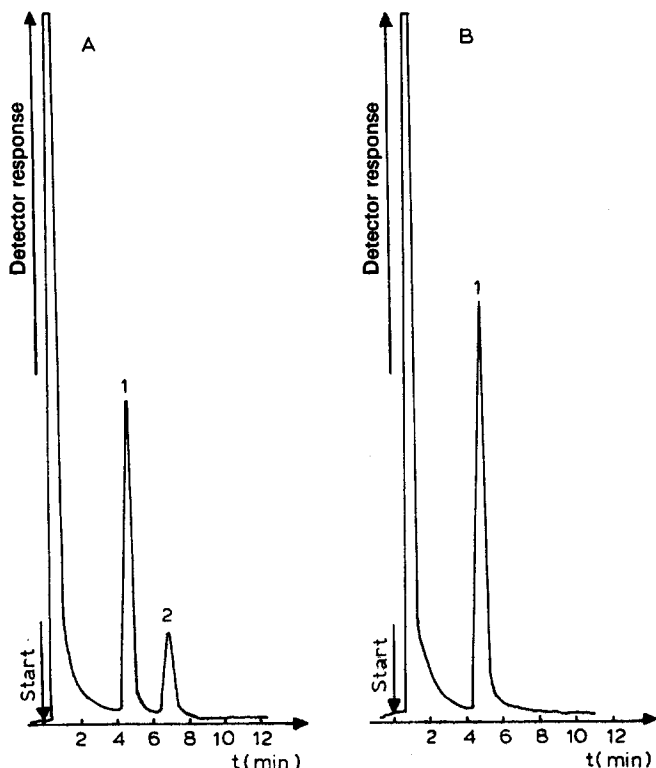

Fig. 2. Comparison of HMDS-based silylations of compound I in PY: A, without catalyst at 60°C, reaction time 100 min; B, with TMCS as catalyst at 25°C, reaction time 2 min. Peaks: 1 = totally O-silylated derivative (compound II); 2 = bis-O-silylated derivative (compound III). Reagent to substrate molar ratio: 1350:1. Substrate concentration: 1 mg/ml. Reagent to catalyst molar ratio: 20:1. GC conditions: 3% OV-17, otherwise see Experimental.

i.e., BSA or BSTFA with or without catalysts[2-4,9-15], owing to incomplete reaction of the primary amino function. Therefore we studied the applicability to compound I of the HMDS-based method described for sphingosines[16].

Without a catalyst, no reaction occurred at room temperature and the reaction was slow even at 60°C. At 60°C in the first 25 min the partially O-silylated, $(OTMS)_2$, derivative was the main product; this was slowly converted into the $(OTMS)_3$ derivative (see Figs. 2a and 4). The rôle of TMCS as catalyst was examined by systematically increasing its concentration in the HMDS–PY reaction mixture, at reagent to catalyst molar ratios of 750:1–1:1. The reagent to substrate molar ratio was 1350:1 in each case. Fig. 3 shows the responses relative to the internal standard, f_A, observed after 5 min of reaction *vs.* the actual reagent to catalyst molar ratios. No catalytic effect could be observed at reagent to catalyst molar ratios of 700:1–110:1. In this range the extent of conversion is low and the selectivity is poor, as shown by the appearance of additional peaks on the chromatogram (see curves x_3 and x_4 in Fig. 3).

At molar ratios of 50:1–1:1 the catalytic effect is pronounced and constant, the selectivity towards compound II being nearly 100% as shown by GC–MS. The position of the equilibrium for the catalysed reaction is practically the same as that at the end of the non-catalysed one. In Fig. 4 kinetic data (extent of conversion *vs.* time)
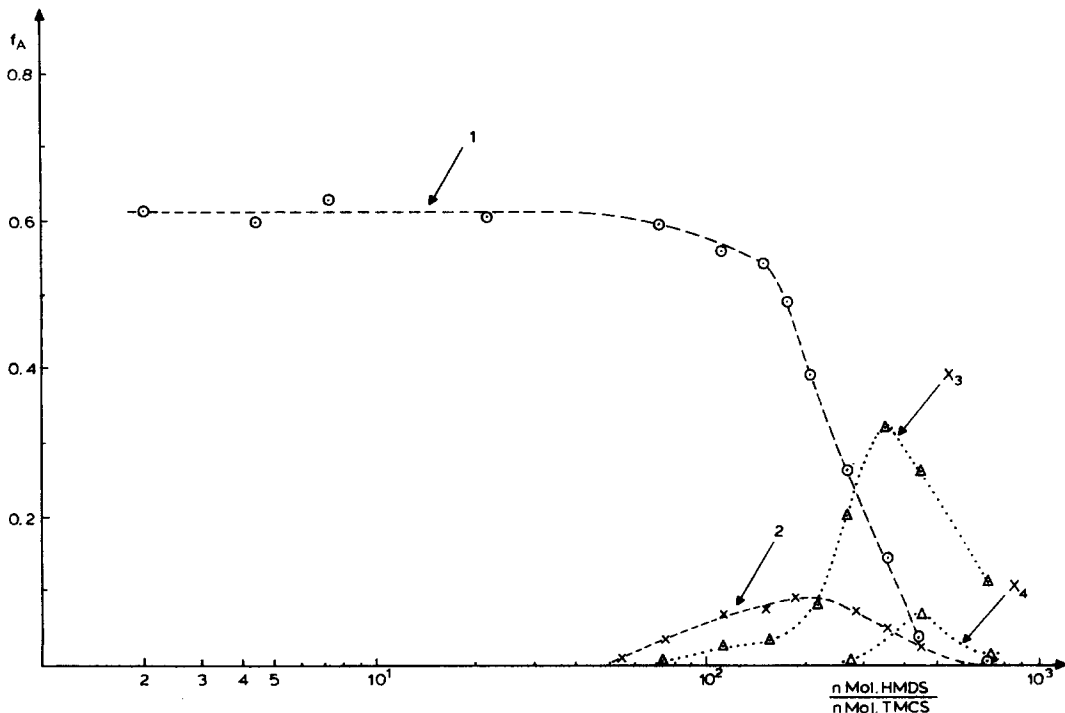

Fig. 3. Extent of conversion of compound I in its reaction with HMDS in PY *vs.* the reagent to TMCS catalyst molar ratio. The reaction mixtures were analysed after 5 min of reaction at 25°C. For GC conditions see Fig. 2. Curves: 1, compound II; 2, compound III; x_3, x_4, partially silylated, unidentified compounds.
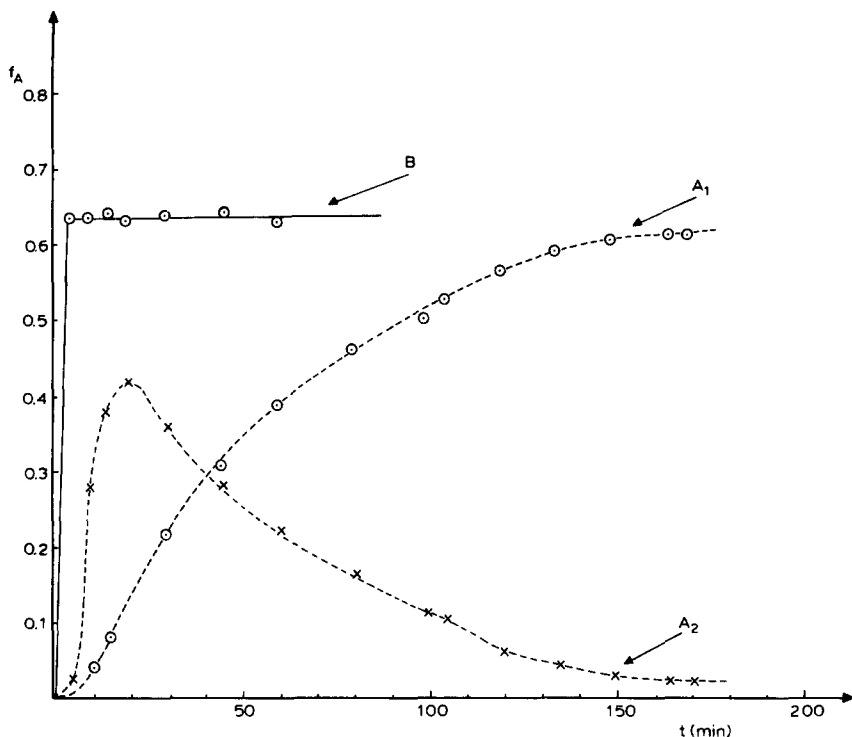
Fig. 4. Catalysis by TMCS of the HMDS-based silylation of compound I in Py. Curves: A_1, A_2, non-catalysed reaction at 60°C for compounds II and III respectively; B, catalysed reaction at 25° for compound II. $f_A = A_i/A_{st} \cdot m_{st}/m_i$ where A_i = peak area of the compound investigated, A_{st} = peak area of the internal standard and m_i, m_{st} = weights of the compound and of the internal standard in the reaction mixture. For molar ratios and substrate concentrations see Fig. 2.

are shown both for the non-catalysed (curves A) and catalysed (curve B) reactions. For routine determinations we used a molar ratio of 20:1, *i.e.*, 100 μl HMDS and 5 μl TMCS (see Fig. 2b).

Structure identification

The structures of the derivatives produced in the silylation reactions discussed above were confirmed by MS and GC–MS.

MS. Data for the characteristic ions in the 70 and 12.5 eV mass spectra of compound II are given in Table II. The fragmentation of compound II confirming its structure is depicted in Fig. 5.

As is well known for aliphatic amines, direct α-cleavage at the amino group gives rise to the base peak of the spectrum. The ion at m/z 86 in the spectrum of compound II represents about 55% of the total ion current at 70 eV. At 12.5 eV this proportion is reduced to 31.5%, while the relative abundance of the ion at m/z 424 is greatly increased, indicating that this ion is formed in a rearrangement process. Analogous hydrogen transfers between remote groupings are very frequent in MS fragmentation of prostaglandins[17]. The large increase in the abundance of the ion at

TABLE II

ABUNDANCE DATA (RELATIVE INTENSITIES, %) FOR CHARACTERISTIC IONS IN THE MASS SPECTRA OF COMPOUNDS II AND III OBTAINED BY MS (70 AND 12.5 eV) AND GC–MS (70 eV), RESPECTIVELY

m/z	Compound II			m/z	Compound III GC–MS 70 eV
	MS		GC–MS 70 eV		
	70 eV	12.5 eV			
599	1.6	16	0.2	512	0.9
584	1.7	2	0.2	424	1.2
568	0.4	2	–	352	6.5
513	0.5	4	0.1	334	1.9
424	9.5	61	10	211	2.8
334	2	12	3.8	126	3.6
283	2.5	5.6	3.6	86	100
158	2.5	14	2.8	73	12
86	100	100	100		

m/z 158 in the 12.5-eV spectrum of compound II indicates that this ion is also formed in a rearrangement process, namely in the transfer of the trimethylsilyl group to the amino group, which presumably proceeds in parallel with the process leading to the formation of the ion at m/z 424.

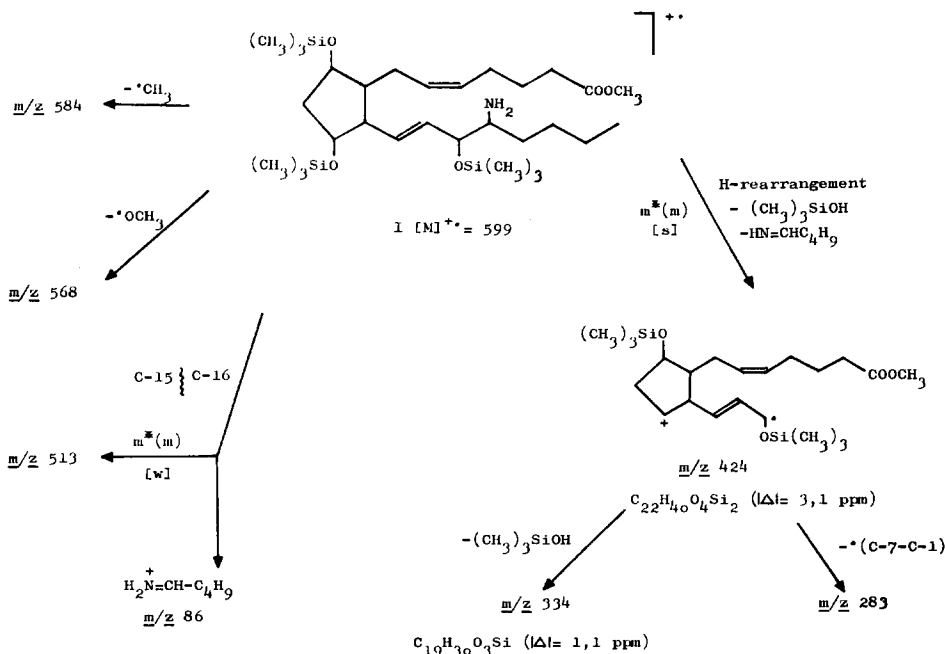*GC–MS*. The main peak (peak 1 in Fig. 2) occurring upon GC–MS analysis

Fig. 5. Mass spectral fragmentation of compound II. m*–(m) = Measured metastable ion; [w] = weak intensity of metastable peak; [s] = strong intensity of metastable peak; $| \Delta |$ = $| m_{calculated} - m_{measured} | / m_{calculated}$ (in ppm).

TABLE III

RETENTION DATA FOR COMPOUNDS II AND III

	3% OV-17		3% OV-101		3% OV-210	
	III	*II*	*III*	*II*	*III*	*II*
Retention to relative squalane (270°C)	3.14	2.07	2.58	1.44	4.83	2.83
Kováts retention index (270°C)	3080	2905	3020	2850	3350	3180

gave a similar mass spectrum to that recorded by direct MS measurement of compound II (see Fig. 5). No molecular ion could be observed in the mass spectrum of peak 2 occurring after the main peak on OV-17 (see Figs. 2a and 5). The highest mass number detected, m/z 512, can be interpreted in terms of the loss of a $\cdot CH_3$ radical from the molecular ion (m/z = 527). This molecular weight corresponds to an $(OTMS)_2$ derivative of compound I. The base peak at m/z 86 indicates the presence of a free amino group in the molecule, while the observed 18-Dalton difference between m/z 352 and 334 (water elimination) indicates a free OH group which is assumed to be at C-9[5]. Thus, the most probable structure for the compound (III) giving rise to peak 2 is 16-amino-$PGF_{2\alpha}$ methyl ester-11,15-$(OTMS)_2$.

Gas chromatography

Gas chromatographic characterization of the $(OTMS)_2$ and $(OTMS)_3$ derivatives of compound I has been done on the basis of Kováts retention indices as well as retentions relative to squalane as internal standard on three different stationary phases (OV-17, OV-101 and OV-210). The retention data are given in Table III.

Determination of compound I

The determination was carried out on the basis of a calibration curve of the peak height ratio *vs.* the concentration relative to the squalane internal standard. Five calibration points over the range 0.085–1.55 $\mu g/\mu l$ indicated a linear relationship. The accuracy of the method (the standard deviation of the measured x values) was found to be ± 1.5–2.5 relative per cent, depending mainly on the column conditions.

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