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TRIMETHYLSILYLATION REACTION OF PROSTAGLANDIN-E METHYL ESTER WITH VARIOUS TRIMETHYLSILYLATING REAGENTS

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SUMMARY

Gas chromatographic investigation of the time course of the trimethylsilylation reaction of prostaglandin-E methyl ester (PGE-Me) with trimethylsilylimidazole–piperidine (PIP), N,O-bis(trimethylsilyl)trifluoroacetamide-PIP and other silylating reagents revealed that these reagents do not always give a single product. The reaction products were characterized by gas chromatography–mass spectrometry as the trimethylsilyl derivatives of PGA-Me, PGB-Me, PGE-Me, 9-enol-PGE-Me and 11-piperidyl-PGA-Me.

INTRODUCTION

As part of histochemical studies on marginal periodontal disease, we have examined the situation and extent of localization of prostaglandins (PGs) in human inflammed gingiva with periodontal disease and also the application of gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) in this field[1].

Trimethylsilyl (TMS) ethers are widely used in GC and GC–MS analyses of PGs. Recent studies have demonstrated that the reaction of prostaglandin-E (PGE) and its methyl ester (PGE-Me) with certain silylating reagents gave rise to a single peak in the gas chromatogram[2–4].

Nicosia and Galli[2] described a method for the simultaneous dehydration and silylation of PGE-Me to TMS-PGB-Me with a mixture of trimethylsilylimidazole (TMSI) and piperidine (PIP) (1:1). Roselló and co-workers[3,4] also reported a method for the simultaneous one-step derivatization of PGE to TMS-9-enol-PGE with a mixture of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and PIP (1:1). (Fig. 1). We found that these two methods do not always give a single peak in the gas chromatogram. We therefore re-examined the reaction of PGE-Me with TMSI–PIP, BSTFA–PIP and various other silylating reagents and identified the reaction products by GC–MS.

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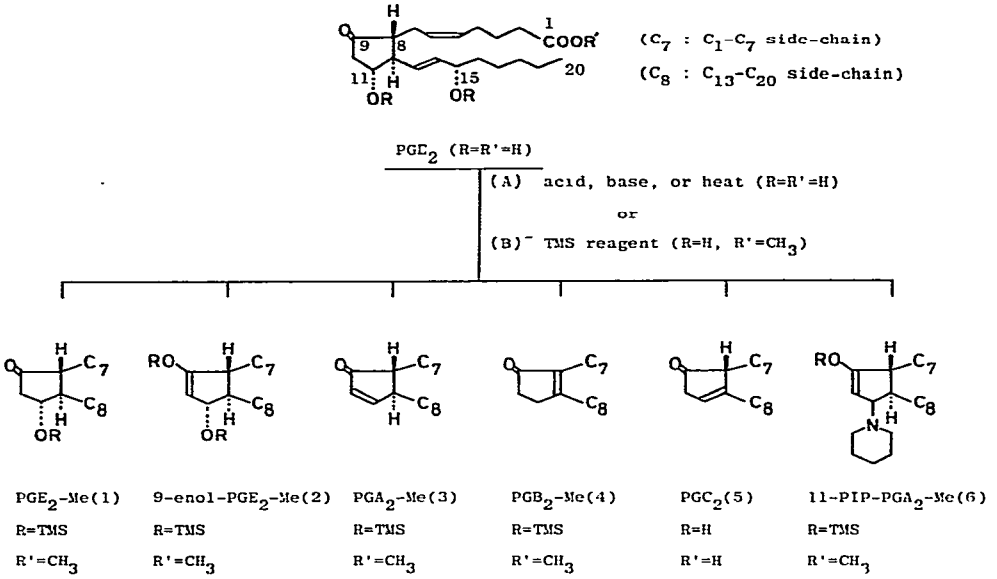

Fig. 1. Derivatization of PGE_2-Me.

EXPERIMENTAL

Reagents

BSTFA (Pierce, Rockford, IL, U.S.A.), TMSI, N,O-bis(trimethylsilyl)acetamide (BSA) (Gasukuro Kogyo, Osaka, Japan), pyridine, PIP, hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS), acetonitrile (Tokyo Kasei Kogyo, Tokyo, Japan), diethyl ether, methanol, *p*-toluenesulphonyl-N-methyl-N-nitrosoamide (Ishizu Junyaku, Osaka, Japan), PGA_1, PGA_2, PGB_1 and PGB_2 (Sigma, St. Louis, MO, U.S.A.) were commercially available and of standard grade. PGE_1, PGE_2, PGF_1 and PGF_2 were generously donated by Ono Pharmaceutical Co. (Osaka, Japan).

General procedure for the trimethylsilylation of PGA-Me, PGB-Me, PGE-Me and PGF-Me

The prostaglandins (100 μg) were dissolved in methanol (10 μl) and subjected to reaction with ethereal diazomethane (400 μl) prepared from *p*-toluenesulphonyl-N-methyl-N-nitrosoamide (2.14 g) and diethyl ether (30 ml). After evaporation *in vacuo*, the methyl ester was treated with the trimethylsilylating reagent (200 μl). An aliquot of the solution (1–2 μl) was injected into the gas chromatograph with a 5-μl Hamilton microsyringe.

Gas chromatography–mass spectrometry

A Shimadzu GCMS-7000 gas chromatograph–mass spectrometer equipped with a High-Speed MID-PM 9060S multi-ion detector was used under the following conditions: column, glass, 1 m × 3 mm I.D.; packing, 1.0% OV-17 on 60–80-mesh Chromosorb W AW, DMCS (Nishio Kogyo, Osaka, Japan) and 1.5% OV-210 on

80–100-mesh Shimalite W AW, DMCS (Shimadzu Seisakusho, Kyoto, Japan); carrier gas, helium at 30 ml/min; column temperature, 220°C for the OV-17 and 215°C for the OV-210 column; injection port temperature, 260°C; separator temperature, 270°C; ion source temperature, 290°C; ionizing voltage, 20 eV; trap current, 70 μA; accelerating voltage, 3 kV; recorder range, 2 mV; chart speed, 1 cm/min.

Mass spectrometry

Mass spectra were obtained with a Shimadzu GCMS-7000 gas chromatograph–mass spectrometer at 70 eV. The Visigraph was run at 10 cm/min (*ca. m/e* 700).

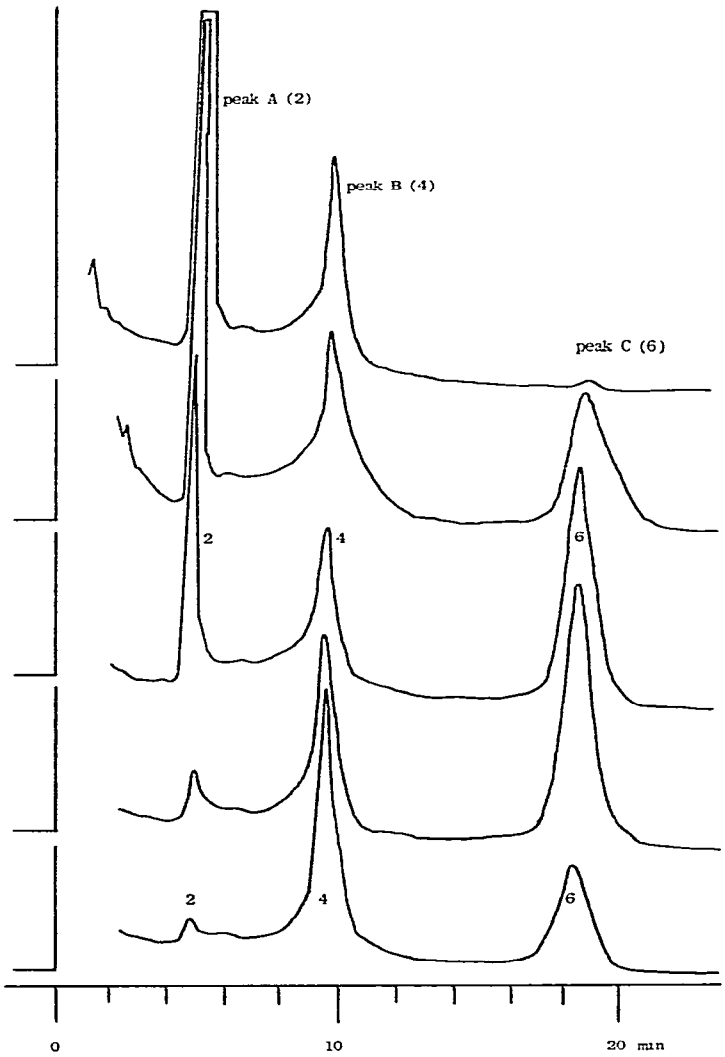

Fig. 2. Time course of the reaction of PGE_2-Me with PIP–TMSI reagent on an OV-17 column (1 min, 20 min, 50 min, 2 h and 5 days, reading downwards).

RESULTS AND DISCUSSION

It is well known that PGE, having an unstable β-hydroxy ketone ring structure, is readily dehydrated to the corresponding PGA, PGB and PGC (R = R′ = H in Fig. 1) by acid, base or heat. The problem arose, especially with seminal fluids, of whether PGA, PGB, 19-hydroxy-PGA and 19-hydroxy-PGB are natural compounds or artefacts[5–7]. Thermal degradation of PGE might be expected under much more severe GC conditions. In general, the ketone function of PGE is protected by the heat-stable alkyloxime formation and a variety of PG oximes have been reported. However, the formation of two geometric isomers of the *syn/anti* type is not desirable from an analytical point of view.

Nicosia *et al.* and Roselló and co-workers reported that the one-step reaction

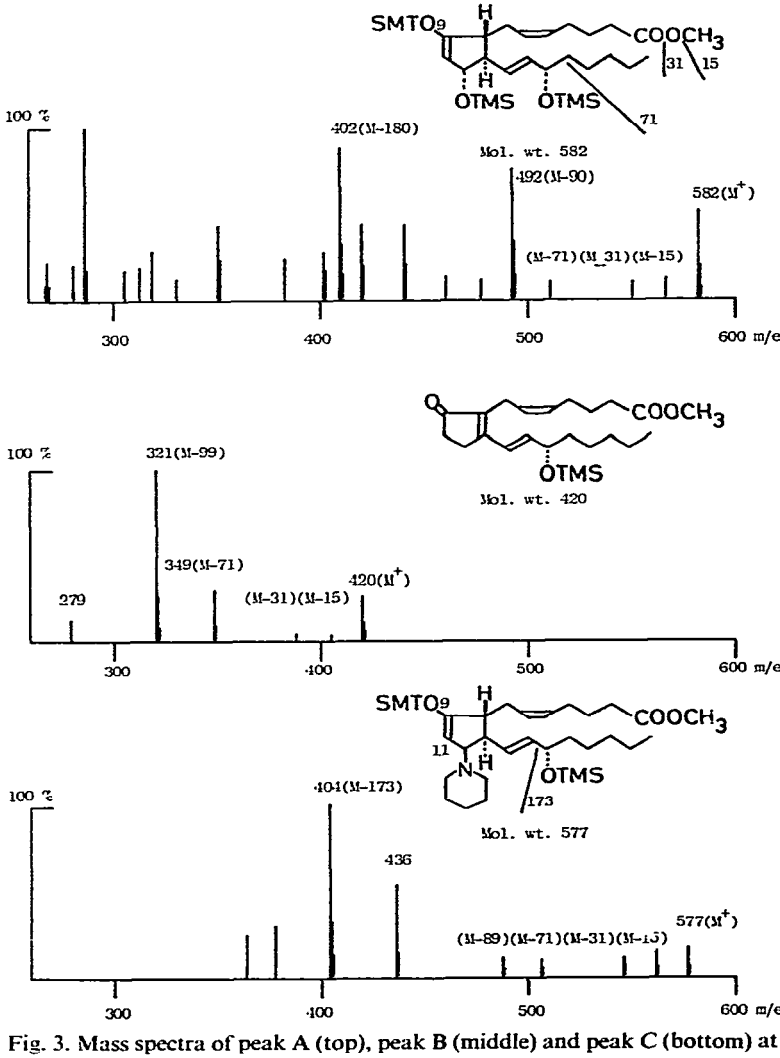

Fig. 3. Mass spectra of peak A (top), peak B (middle) and peak C (bottom) at 70 eV obtained by GC–MS.

of PGE and PGE-Me with TMSI–PIP (1:1) and BSTFA–PIP (1:1) could give a single peak in the gas chromatogram.

We first examined the reaction of PGE_2-Me with TMSI–PIP (1:1) according to the method of Nicosia *et al.* GC–MS analysis of the reaction mixture showed two additional peaks, in addition to TMS-PGB_2-Me (4) described by Nicosia *et al.*

The time course of the reaction is shown in Fig. 2. After 1 min at room temperature, three peaks (A, B and C) with a peak-height ratio of 75:21:1 were observed in the chromatogram. This ratio shifted to 40:15:10 after 20 min, 12:6:8 after 50 min, 2:6:9 after 2 h and 2:20:7 after 5 days. On the other hand, after 5 min at 85°C, the ratio was 2:20:7.

These peaks were finally identified as TMS-9-enol-PGE_2-Me (2), TMS-PGB_2-Me (4) and TMS-11-piperidyl-PGA_2-Me (TMS-11-PIP-PGA_2-Me) (6) by comparison with authentic samples and data from the literature (Fig. 3). This is the first case in which PGE_2-Me has been converted into a PGA_2-Me derivative(TMS-11-PIP-PGA_2-Me) (6) by TMSI–PIP reagent, although PGA has been converted into TMS-11-PIP-PGA by BSTFA–PIP reagent[5].

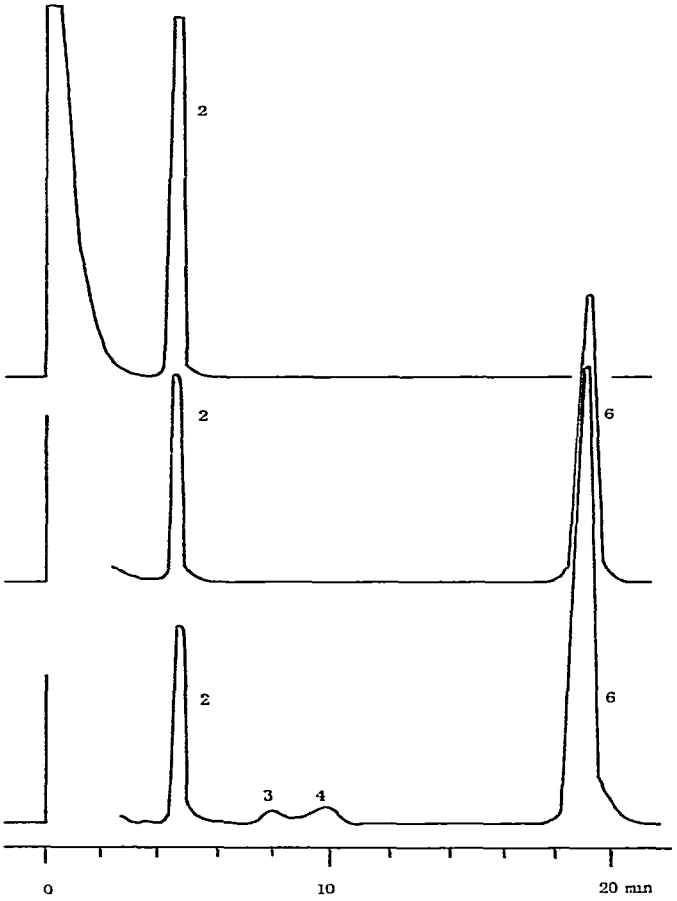

Fig. 4. Time course of the reaction of PGE_2-Me with PIP–BSTFA reagent on an OV-17 column (2 h, 2 weeks and 1 month, reading downwards).

Next, we examined the reaction of PGE_2-Me with BSTFA–PIP (1:1) according to the method of Roselló and co-workers.

The time course of the reaction is shown in Fig. 4. A single peak of TMS-9-enol-PGE_2-Me (2) was observed and reaction product was stable for at least 48 h, as described by Roselló and co-workers. However, an additional peak, which was identified as TMS-11-PIP-PGA_2-Me (6) by GC–MS, appeared after 2–3 weeks. Moreover, two other peaks, identified as TMS-PGA_2-Me (3) and TMS-PGB_2-Me (4), appeared in small amounts after 1 month. The same phenomena were observed with PGE_1-Me derivatives.

Lastly, the reaction of PGE_2-Me with various trimethylsilylating reagents was examined by GC–MS using an OV-210 column.

The results are shown in Table I and Fig. 5. The gas chromatogram of TMS-PGF_1-Me and TMS-PGF_2-Me is also shown in Fig. 5 in order to compare their retention times with those of TMS-PGE_2-Me derivatives. All of the reaction products were identified by comparing their retention times and mass spectra with those of authentic samples and data from the literature.

TABLE I

REACTION PRODUCTS OF PGE_2-Me WITH TMS REAGENTS

Reagent	*Composition*	*Conditions*	*Products**
1	Pyridine–HMDS-TMCS (10:2:1)	1 h at room temp.	1, 2, 3, u
2	Pyridine–BSA (1:1)	1 h at room temp.	1, 2, 3, 4, u
3	Pyridine–acetonitrile–BSA (100:25:75)	1 h at room temp.	1, 2, 3, 4, u
4	Pyridine–BSTFA (1:1)	1 h at room temp.	1, 2, 3, 4, u
5	Pyridine–TMSI (1:1)	1 h at room temp.	1, 2, 3, 4, u
6	PIP–TMSI (1:1)	2 months at room temp.	2, 3, 4, 6
7	PIP–BSTFA (1:1)	2 months at room temp.	2, 3, 4, 6

* u = unknown.

Reagent 1 (pyridine–HMDS–TMCS, 10:2:1), the mildest reagent used, gave TMS-9-enol-PGE_2-Me (2), TMS-PGA_2-Me (3), TMS-PGE_2-Me (1) and an unknown peak (u) with a peak-height ratio of 8:3:4:2.5 (structures are shown in Fig. 1). Reagent 2 (pyridine–BSA, 1:1), reagent 3 (pyridine–acetonitrile–BSA, 100:25:75) and reagent 4 (pyridine–BSTFA, 1:1) showed almost the same peak pattern, consisting of 2, 3, 1, 4 and u with a peak-height ratio of 1:8:3:1:2.5. Reagent 5 (pyridine-TMSI, 1:1) gave a large amount of 2 and several small peaks. The peak-height ratio of 2, 3, 1, 4 and u was 9:3:1:1.2:1. In the reaction using piperidine as the catalyst, as described above, TMSI–PIP (reagent 6) gave small amounts of 2 and 3 and large amounts of 6 and 4, with a peak-height ratio of 0.2:0.2:1.4:2.2. Reagent 7 (BSTFA–PIP) gave overwhelmingly peaks of 6 and 2, with peaks of 3 and 4 as minor components, the peak-height ratio being 50:20:2:2.

These results suggest that the method of Roselló and co-workers is to be preferred for the determination of PGE by GC–MS as it gives a single peak of TMS-9-enol-PGE-Me. However, it is necessary to make it stable for about at least 2 weeks in order to analyse many samples, or to discriminate between the 9-enol peak and the PGF peak in the gas chromatogram.

Further studies are in progress.

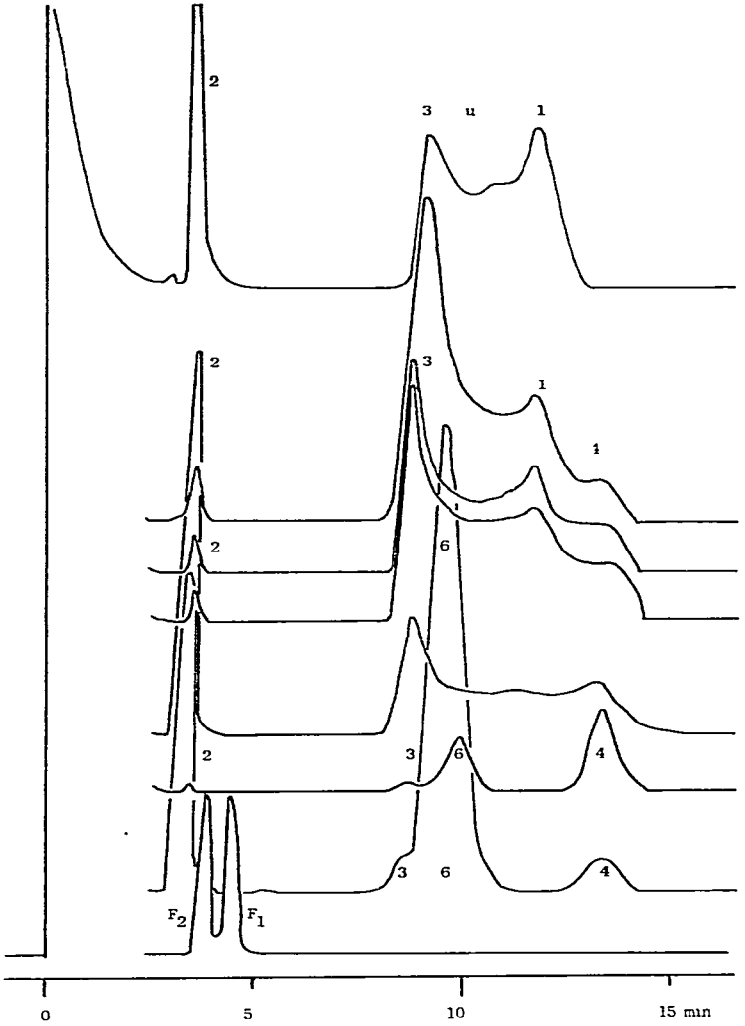

Fig. 5. Gas chromatograms of the reaction products of PGE_2-Me with various trimethylsilylating reagents on an OV-210 column (reagents 1–7, reading downwards).

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