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Note

Characterization of thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ by combined gas chromatography and chemical-ionization mass spectrometry

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Prostaglandins are 20-carbon-atom fatty acids that have been implicated in numerous pharmacological, physiological and pathological processes[1]. Most of their diverse effects can be evoked by an extremely small amount and many assay methods have been developed for their quantitative and qualitative analysis, *e.g.*, thin-layer chromatography[2], enzyme assay[3], fluorescence[4], radioimmunoassay[5], mass spectrometry (MS) and bioassay using a smooth muscle preparation[6] or human platelet aggregation[7]. Of these methods, MS is the most accurate for the determination of prostaglandins and a number of studies have been concerned with their determination by this technique, especially electron-impact (EI) MS combined with gas chromatography (GC)[8,9].

The biological significance of thromboxanes and prostaglandin I_2 has recently become apparent. Thromboxane B_2 was first isolated and identified by Hamberg and Samuelsson[10] after incubation of [^{14}C]arachidonic acid with guinea-pig lung homogenates. The substance was later revealed to be an end metabolite of the labile compound thromboxane A_2, which has striking properties for inducing the aggregation of human platelets and vasocontraction[11]. Prostaglandin I_2 was recently shown by Moncada *et al.*[12] to be a labile substance generated by the arterial walls, with a striking capacity for the inhibition of platelet aggregation and vasodilation. Prostaglandin I_2 was shown to be rapidly decomposed non-enzymatically into 6-ketoprostaglandin $F_{1\alpha}$ in biological fluids[13].

The MS detection of thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ has been reported by Hamberg and Samuelsson[10] and Fenwick *et al.*[14], respectively. However, both groups failed to show a molecular ion as the base peak, as they used EI-MS, which generally gives many fragment ions with extremely low intensity in the high mass range.

This paper describes the use of ammonia chemical-ionization (CI) MS to identify the molecular weights of thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$.

EXPERIMENTAL

Materials

Thromboxane B_2 was kindly supplied by Upjohn (Kalamazoo, Mich., U.S.A.). 6-Ketoprostaglandin $F_{1\alpha}$ was a generous gift from Ono Pharmaceutical Co. (Osaka, Japan). N-Methyl-N^1-nitro-N-nitrosoguanidine and O-methylhydroxylamine hydrochloride were purchased from Wako (Osaka, Japan). Trimethylsilyl (TMS) imidazole and bis(TMS)trifluoroacetamide were purchased from Tokyo Kasei Industries (Tokyo, Japan).

Methods

Preparation of the derivatives for combined GC–MS. The following derivatives were prepared according to the methods described previously[15,16].

For methylation, diazomethane was freshly prepared from N-methyl-N^1-nitro-N-nitrosoguanidine by the method of Fales *et al.*[17]. A 0.9-ml volume of the ethereal diazomethane was added to the samples dissolved in 0.1 ml of absolute methanol, and the solution were then kept in the dark for 60 min. After this procedure, the solvents were evaporated under a stream of nitrogen.

For methoxime formation, 0.1 ml of a solution of O-methylhydroxylamine hydrochloride in pyridine (20 mg/ml) was added to each sample. The solution was mixed well and heated at 60° for 60 min. The pyridine was removed by evaporation with a stream of nitrogen.

For trimethylsilylation, 50 μl of a mixture of TMS imidazole and bis(TMS)-trifluoroacetamide (2:1, v/v) were added to each sample, and the reaction was allowed to proceed for 2 h at room temperature.

Combined GC–MS. A Shimadzu-LKB Model 9000 B gas chromatograph–mass spectrometer equipped with dual CI and EI sources was used. The data processing system included a GC–MS–PAC 300 DG, consisting of an OKITAC 4300 mini-computer with a 12K core and a magnetic disk. A glass column (1 m × 3 mm I.D.) packed with 3% OV-101 on Celite 545 (80–100 mesh) was used. The temperatures of the column, injection port and ionization chamber were maintained at 255°, 300° and 190°, respectively. The flow-rate of helium carrier gas was 30 ml/min. The CI mass spectra were obtained at an electron energy of 500 eV, an emission current of 500 μA and an accelerating voltage of 3.5 kV. Ammonia gas was used as the reagent gas at 1.2 Torr. The EI mass spectra were obtained at an electron energy of 25 eV, an emission current of 60 μA and an accelerating voltage of 3.5 kV.

RESULTS AND DISCUSSION

The methoxime–TMS and methyl ester–methoxime–TMS ether derivatives of thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ were successfully separated from each other by GC, the retention times of the methoxime–TMS derivatives of thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ being 2.7 and 3.0 min, respectively (Fig. 1). The retention times of the methyl ester–methoxime–TMS ether derivatives of thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ were 2.5 and 2.8 min, respectively.

Fitzpatrick *et al.*[18] demonstrated that the methyl ester–pentafluorobenzyl oxime–TMS ether derivatives of thromboxane B_2 gave two peaks on a gas chromato-

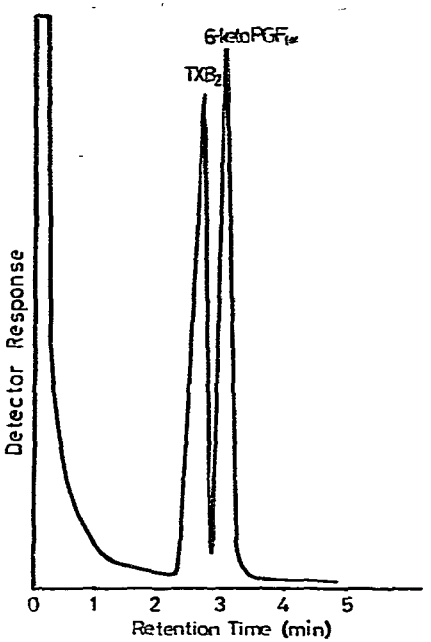

Fig. 1. Typical gas chromatograms of the methoxime–TMS derivatives of thromboxane B_2 (TXB_2) and 6-ketoprostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$).

gram, which were established to be associated with the *syn*- and *anti*-forms. As mentioned above, the methoxime–TMS derivative of thromboxane B_2 gave a single peak under our column condition[5]. This fact is useful for the quantitative determination of thromboxane B_2.

Fig. 2a and b shows the EI and CI mass spectra, respectively, of the methoxime–TMS derivative of thromboxane B_2. In the EI mass spectrometer, the mass spectrum of the derivative of thromboxane B_2 gave many fragment ions, such as the fragments at *m/e* 211 and 301, which were presumably due to $[O{-}N{=}CH{-}CH_2{-}CHOTMS{-}CH{=}CH{-}CH{=}CH]^+$ and $[OTMS{=}CH{-}CH{=}CH{-}CHOTMS{-}(CH_2)_4{-}CH_3]^+$. In the EI mass spectrum, the fragment ions in the high mass range were of an extremely low intensity and the molecular ion at *m/e* 687 was absent. On the other hand, the CI mass spectrum was generally characterized by the presence of only a few minor fragment ions such as the fragments at *m/e* 508 and 301, which were due to $[M + 1 - 2(TMSOH)]^+$ and $[OTMS{=}CH{-}CH{=}CH{-}CHOTMS{-}(CH_2)_4{-}CH_3]^+$. In our study, the derivative of thromboxane B_2 gave two main fragments in the high mass range, at *m/e* 598 and 688, which were presumably due to $[M + 1 - TMSOH]^+$ and $[M + 1]^+$; the fragment at *m/e* 598 was the base peak.

Fig. 3a and b shows the EI and CI mass spectra, respectively, of the methoxime–TMS derivative of 6-ketoprostaglandin $F_{1\alpha}$. In the EI mass spectrometer, the derivative of 6-ketoprostaglandin $F_{1\alpha}$ produced many fragment ions, but those in the high mass range and the molecular ions were of a low intensity. On the other hand, in the CI mass spectrum the ion at *m/e* 688, $[M + 1]^+$, is the base peak. The other fragment ions recorded at *m/e* 598, 508 and 418 were presumably due to successive

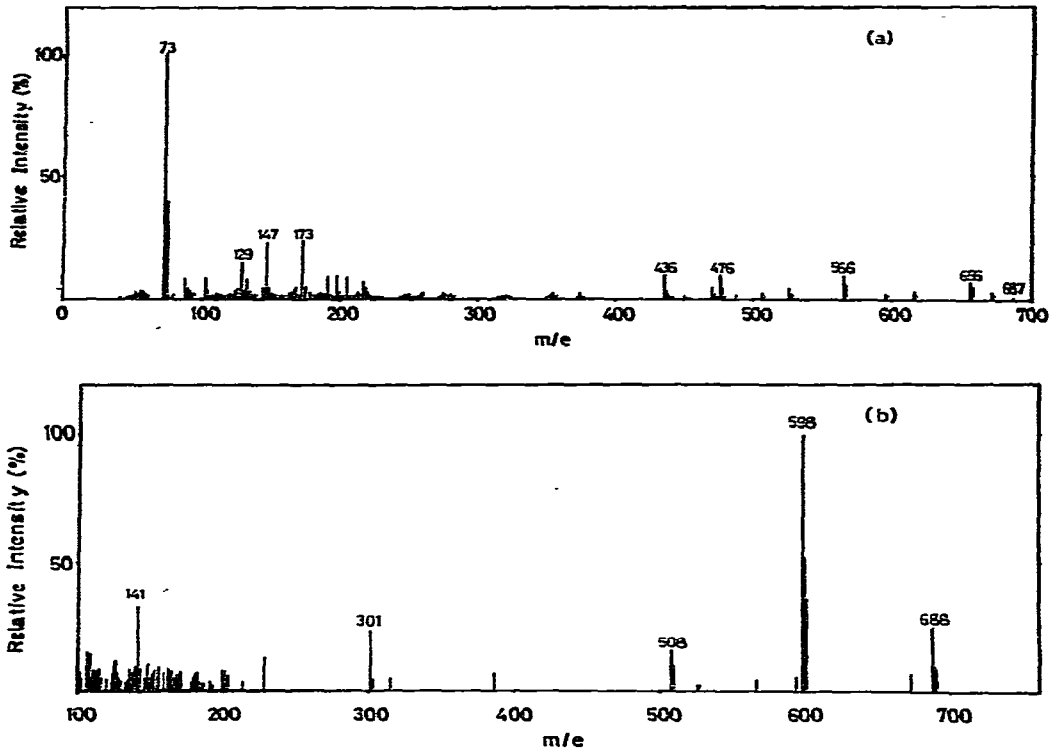

Fig. 2. Mass spectra of the methoxime–TMS derivative of thromboxane B_2. (a) EI mass spectrum; (b) ammonia CI mass spectrum.

eliminations of trimethylsilanol, *i.e.*, $[M + 1 - TMSOH]^+$, $[M + 1 - 2(TMSOH)]^+$ and $[M + 1 - 3(TMSOH)]^+$, respectively.

Recently, Cottee *et al.*[19] reported the CI mass spectrum of the methyl ester–methoxime–TMS ether derivative of 6-ketoprostaglandin $F_{1\alpha}$. However, they failed to record the molecular ion as the base peak; the relative intensity of the molecular ion was less than 5%. The discrepancy between their and our result depended on the different reagent gases used: they used methane whereas we used ammonia. Our preliminary experiments on the CI-MS of various prostaglandins and thromboxane B_2, in which three different reagent gases (methane, isobutane and ammonia) were compared, showed the superiority of ammonia gas. Therefore, in CI-MS, ammonia is probably the most useful reagent gas for the structural and quantitative analysis of both thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$.

Dawson *et al.*[20] reported the generation of thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ during the perfusion of sensitized guinea-pig lung with [^{14}C]-arachidonic acid. We also found these two substances to be formed from arachidonic acid by the homogenates of rat carrageenin granuloma[15,21]. Dawson *et al.* also found some chemotactic activity in thromboxane B_2 and a selective response to respiratory smooth muscle for 6-ketoprostaglandin $F_{1\alpha}$. Recently, we demonstrated that thromboxane B_2 was produced by activated macrophages[22] and that thromboxane B_2 had

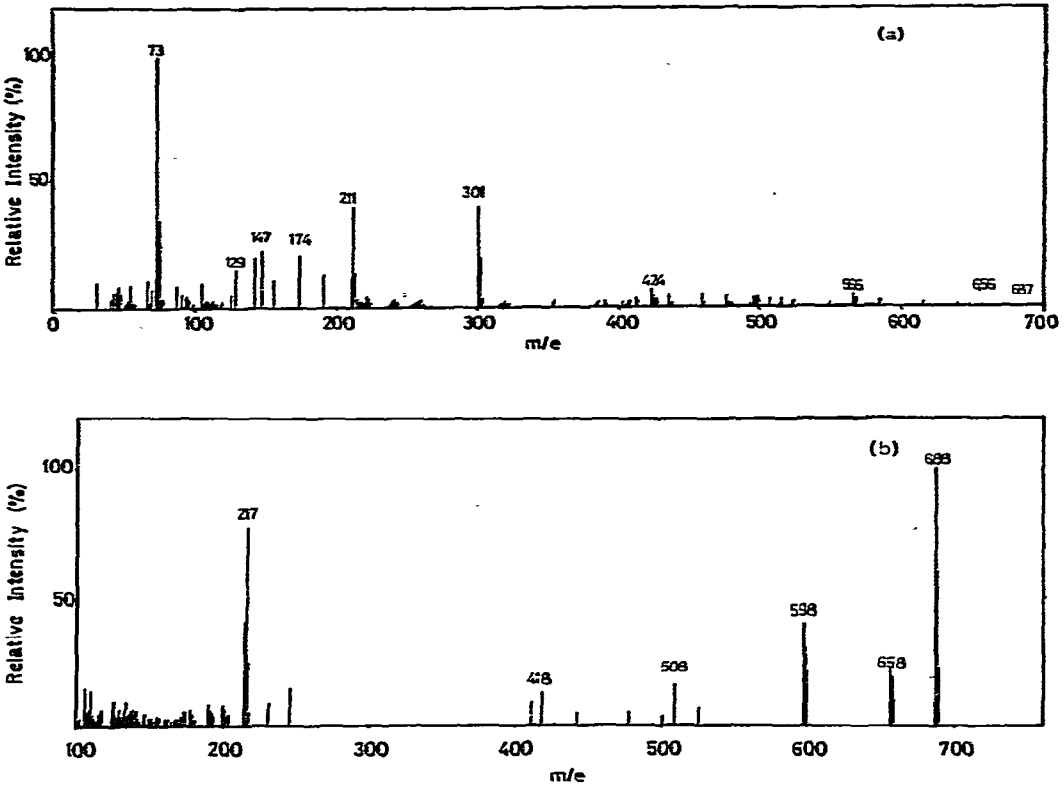

Fig. 3. Mass spectra of the methoxime–TMS derivative of 6-ketoprostaglandin $F_{1\alpha}$. (a) EI mass spectrum; (b) ammonia CI mass spectrum.

a significant effect on the synthesis of DNA, RNA and hexosamine-containing substances in cultured fibroblasts[23]. The biosynthesis of 6-ketoprostaglandin $F_{1\alpha}$ appears to be ubiquitous and has been demonstrated in rat stomach fundus[24], bovine seminal vesicle[16], rabbit heart[25], rat uterus[14], etc. These facts suggest that thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ (including their labile precursors such as thromboxane A_2 and prostaglandin I_2, G_2 and H_2) have considerable relevance for certain biological responses, such as immunological reactions and inflammation.

The studies described here contribute the first step towards the quantitative determination of thromboxane B_2 and 6-ketoprostaglandin $F_{1\alpha}$ in the normal and pathological fluid of various tissues. Such work is currently under way in our laboratories.

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