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Metabolism of Prostaglandin $F_{2\alpha}$ in Guinea Pig Lung

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[9β-^{3}H]Prostaglandin $F_{2\alpha}$ was incubated with the particle free fraction of a homogenate of guinea pig lung. One less polar metabolite appeared, which was assigned the structure 9α,11α-dihydroxy-15-ketoprost-5-enoic acid. The identification was based on gas chromatographic and mass spectrometric analyses of the trimethylsilyl ether, acetate, methoxime-trimethylsilyl ether and methoxime-acetate derivatives of the methyl ester. The position of the double bond was established by oxidative ozonolysis of the methyl ester and acetate followed by gas chromatography-mass spectrometry.

Recently the metabolism of prostaglandins of the E type has been studied using homogenates of guinea pig lung [1–3]. The metabolites of prostaglandin E_1 were identified as 11α,15-dihydroxy-9-keto-prostanoic acid and 11α-hydroxy-9,15-diketo-prostanoic acid. Prostaglandin E_2 and E_3 were metabolized in an analogous way. The sequence of reactions for formation of the dihydroxy derivatives seems to be an initial oxidation of the alcohol group at C-15, reduction of the Δ^{13} double bond and subsequent reduction of the keto group at C-15 [4]. A purified 15-hydroxy-prostanoate dehydrogenase was found to catalyze the oxidation of a large number of prostaglandins, including prostaglandin F compounds [5]; however, the structures of metabolites formed from these compounds were not determined (*cf.* also [6]).

The present paper reports the structure of a metabolite formed from prostaglandin $F_{2\alpha}$ in homogenates of guinea pig lung.

EXPERIMENTAL PROCEDURE

Preparation of [9β-^{3}H]Prostaglandin $F_{2\alpha}$

This compound was prepared by reduction of prostaglandin E_2 with NaB^3H_4 as described earlier [7]. The product was diluted with unlabelled material to give a specific activity of 0.6 μCi/μmole.

Lung Preparation and Incubation

Lungs of male guinea pigs weighing about 500 g were rapidly excised and minced in ice-cold Bucher medium (tissue to buffer ratio, 1:3, w/v). Homogenization was performed with a Potter-Elvehjem homogenizer at +4°C. The homogenate was centrifuged at 8500 × *g* for 15 min and the supernatant at 105000 × *g* for 60 min. Labelled prostaglandin $F_{2\alpha}$ (1.5 μmole, dissolved in 0.05 ml of ethanol) was added to the final supernatant obtained from 6 g of guinea pig lung. The mixture was incubated aerobically at 37°C for 60 min with shaking. The incubation was terminated by the addition of 5 volumes of 96% ethanol. The protein precipitate was removed by filtration. The filtrate was evaporated to a small volume, diluted 10-fold with water, acidified to pH 3 and extracted three times with ether. The combined ether extracts were washed neutral with water, and the solvent was removed by evaporation at reduced pressure. The residue, containing about 95% of the added radioactivity, was purified using reversed phase partition chromatography and silicic acid chromatography.

Chromatographic Methods

Reversed phase partition chromatography was performed on columns of hydrophobic Hyflo Super-Cel as described earlier [8]. The system used was C-50: moving phase, methanol–water–acetic acid (150:150:2, by vol.); stationary phase, chloroform–iso-octanol (15:15, by vol.). The phases were equilibrated for 24 h at 23°C before use.

Silicic acid chromatography was performed on silicic acid (Mallinckrodt, 100–200 mesh) activated at 115°C. The columns were prepared with 1 g of silicic acid in ethyl acetate–benzene (10:90, v/v) and eluted with increasing concentrations of ethyl acetate in benzene. For purification of acetates the columns were instead prepared with 1 g of silicic acid in ether–hexane (20:80, v/v) and the chromatographies run with increasing concentrations of ether in hexane.

Gas-liquid chromatography was carried out on a Barber-Colman Gas Chromatograph (series 5000)

Trivial Names. Prostaglandin E_1, 11α,15-dihydroxy-9-ketoprost-13-enoic acid; prostaglandin E_2, 11α,15-dihydroxy-9-ketoprosta-5,13-dienoic acid; prostaglandin E_3, 11α,15-dihydroxy-9-ketoprosta-5,13,17-trienoic acid; prostaglandin $F_{2\alpha}$, 9α,11α,15-trihydroxyprosta-5,13-dienoic acid; prostaglandin A_2, 15-hydroxy-9-ketoprosta-5,10,13-trienoic acid.

with simultaneous registration of mass and radioactivity. The stationary phase used was 1% SE-30 on Gas Chrom P (100—120 mesh). The inner diameter of the column was 5 mm, and the carrier gas flow was 60 ml per minute. The temperature of the column was 230°C, of the flash heater 280°C and of the detector 255°C. Mixtures of methyl esters of normal saturated fatty acids were used as standards. The retention times of these standards were plotted on a logarithmic scale *versus* the number of carbon atoms of the carboxylic acid on the linear scale. These diagrams were used to convert observed retention times to C-values (*cf.* [9]).

Mass Spectrometry

Mass spectrometry was carried out in combination with gas chromatography using the LKB 9000 instrument. The column (1.5% SE-30 on Chromosorb P) was operated at 180—230°C. The electron energy was 22.5 eV and the trap current 60 μA.

Preparation of Derivatives for Gas-Liquid Chromatography and Mass Spectrometry

The compounds (300 to 500 μg) were dissolved in 1 ml of methanol and treated with diazomethane in ether. After evaporation of the solvent, the methyl ester was dissolved in 1 ml of pyridine. To 0.2 ml of this solution were added 50 μl of trimethylchlorosilane and 100 μl of hexamethyldisilazane. After 30 min at room temperature the sample was evaporated to dryness and was immediately dissolved in 0.1 ml of carbon disulfide.

Acetates were prepared by the addition of 0.3 ml of acetic anhydride to 0.3 ml of the pyridine solution of the methyl ester. The mixture was left at room temperature over night and was subsequently diluted with 1 ml of ice-cold water, acidified, and extracted three times with ether. The combined ether extracts were washed with small volumes of 0.2 N hydrochloric acid, 5% sodium bicarbonate and distilled water until neutral. After evaporation of the solvent the sample was purified on a silicic acid column. The radioactivity was found in one peak, eluted with ether—hexane (50:50, v/v). The material in this peak was collected, evaporated to dryness and dissolved in 0.1 ml of carbon disulfide prior to injection into the gas chromatograph.

The remaining 0.5 ml of the pyridine solution of the methyl ester was treated with 6 mg of methoxyamine hydrochloride (Eastman Organic Chemicals, Rochester 3, New York) to yield the corresponding *O*-methyloxime (methoxime) [10]. After 20 h at room temperature the reaction mixture was evaporated to dryness and extracted three times with 1 ml of ether. The combined ether phases were evaporated to dryness and the residue dissolved in 0.5 ml of pyridine. Of this solution of the methoxime-methyl ester 0.3 ml was subjected to acetylation and 0.2 ml to trimethylsilylation as described above.

Deuterated trimethylsilyl ether derivatives were prepared using trimethyl-d_9 chlorosilane (Merck, Sharp & Dohme, Montreal, Canada). The methyl ester or the methoxime-methyl ester of the compound (50 to 100 μg) was dissolved in 0.1 ml of pyridine, and to this solution was added 50 μl of trimethyl-d_9 chlorosilane. The mixture was kept at room temperature for 2 h and was subsequently evaporated to dryness. The sample was immediately dissolved in 0.1 ml of carbon disulfide.

Oxidative Ozonolysis

The methyl ester and acetate of the compound to be analyzed (100 to 200 μg) was dissolved in 5 ml chloroform and the solution was cooled to −15°C. A stream of ozone was passed through the solution for 4 min. The test tube was stoppered and the mixture allowed to reach room temperature spontaneously. The chloroform was removed under a stream of nitrogen, the sample was dissolved in 2 ml acetic acid, and 0.4 ml of hydrogen peroxide was added. The reaction mixture was kept at 50°C under argon over night, subsequently evaporated to dryness and esterified either by treatment with diazomethane or diazoethane. The sample was dissolved in carbon disulfide prior to gas chromatography, which was carried out as described above, but at a column temperature of 180°C.

Preparation of Methyl-9α,11α-dihydroxy-15-ketoprosta-5,13-dienoate

The methyl ester of [9β-^{3}H]prostaglandin $F_{2\alpha}$ (3 mg, 5.4 μCi) was dissolved in 12 ml of chloroform and stirred for 68 h with 30 mg of active MnO_2 [1]. The solvent was removed by evaporation, 12 ml of absolute ethanol was added, and the mixture centrifuged. The supernatant was extracted three times with ether after a 10-fold dilution with water. The ether extract contained about 95% of the radioactivity and was purified on a silicic acid column. The desired 15-keto compound was eluted with ethyl acetate—benzene (30:70, v/v), and the total yield was 85%. This material gave a single spot on thin layer chromatography with solvent system MI (benzene—dioxane, 50:40, v/v) [11], $R_F = 0.79$. The purity was also established by gas-liquid chromatography (C-value of the methyl ester and trimethylsilyl ether 24.2). Ultraviolet absorption gave λ_{max} in ethanol = 233 nm, $\varepsilon = 8550$. The infrared spectrum showed absorption at 5.78 μm (strong) (carbonyl of the carbomethoxy group), 5.99 μm (weak) (α,β-unsaturated keto group at C-15), 6.15 μm (medium) (Δ^{13} double bond conjugated with the keto group at C-15), and 10.2 μm (weak) (Δ^{13} *trans* double bond).

The mass spectrum of the trimethylsilyl ether showed ions at *m/e* 510 (M), 495 (M-15, loss of $\cdot CH_3$), 479 (M-31, loss of $\cdot OCH_3$), 439 (M-71, loss of the terminal pentyl group), 420 (M-90), and 330 (M-2×90). An ion of high intensity was found at *m/e* 394 (M-116) and was interpreted to be formed by elimination of $TMSiO-CH=CH_2$ from the five-membered ring. The base peak (*m/e* 253, M-(116 + 141)) was probably formed by the same elimination plus loss of the entire carboxyl side chain. α-Cleavage at C-15 gave rise to an ion at *m/e* 99, $[\cdot CO-(CH_2)_4-CH_3]^+$.

Radioactivity Assay

A Packard Tri-Carb model 3375 Liquid Scintillation Spectrometer was used for the assay of radioactivity.

Infrared Spectrometry

Infrared spectra were recorded with a Perkin-Elmer model 257 Grating Infrared Spectrometer on carbon tetrachloride solutions (25 μg per μl) in a microcell (sodium chloride window, path length 0.1 mm).

Solvents

Methanol, chloroform, carbon disulfide (reagent grade, Merck, Darmstadt), toluene (reagent grade, Fisher Scientific Company, Fair Lawn, New Jersey), and ether (ethyl ether anhydrous, analytical reagent, Mallinckrodt) were used without further purification. Ethyl acetate, hexane, benzene and acetic anhydride (reagent grade, Merck, Darmstadt) were distilled before use. Pyridine (analytical reagent, Mallinckrodt) was distilled from and stored over KOH pellets.

RESULTS

The residue obtained after evaporation of the ether extract of the incubation mixture was subjected to reversed phase partition chromatography, solvent system C-50. Two peaks of radioactivity were found, compound I and compound II (72—105 ml and 215—270 ml, respectively, on a 4.5 g column, Fig. 1). Compound I contained about 18% of the radioactivity applied to the column, and compound II about 77%. 5% were found in the stationary phase.

The elution volume of compound I was similar to that of prostaglandin $F_{2\alpha}$ in the same solvent system. In a control experiment where prostaglandin $F_{2\alpha}$ was incubated with boiled supernatant, all the radioactivity appeared in peak I. The identity of this compound with prostaglandin $F_{2\alpha}$ was established with gas-liquid chromatography and mass spectrometry (C-values of some derivatives on 1% SE-30 are listed in the Table).

The Structure of Compound II

Compound II was treated with diazomethane and the methyl ester was further purified using silicic acid chromatography. Essentially all of the radioactivity applied to the column emerged as one single peak, being eluted with ethyl acetate—benzene (30:70, v/v). The methyl ester was subsequently converted into four derivatives: trimethylsilyl ether, methoxime-trimethylsilyl ether, acetate, and methoxime-acetate as described above. These derivatives were subjected to gas-liquid chromatography and mass spectrometry. The C-values found on 1% SE-30 are given in the Table.

Fig. 2 shows the gas chromatogram of the methyl ester-methoxime-trimethylsilyl ether of compound II, and the mass spectrum of the derivative is given in

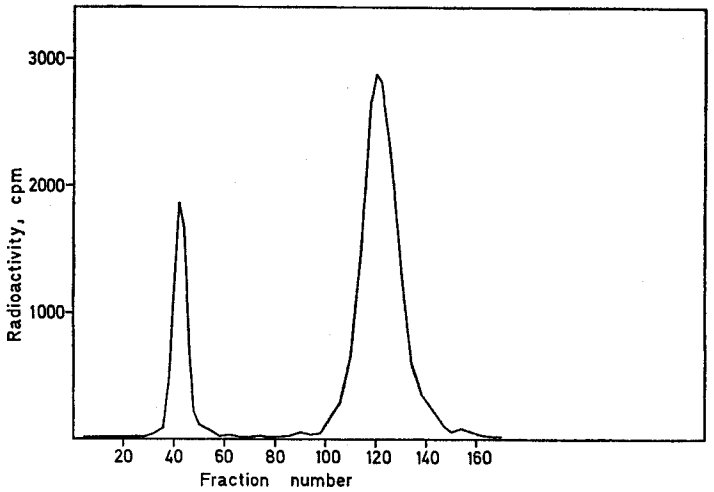

Fig. 1. *Reversed phase partition chromatography of the ether extract from an incubation of [9β-³H]prostaglandin $F_{2\alpha}$ with a homogenate of guinea pig lung.* Column, 4.5 g of hydrophobic Hyflo-Super Cel; solvent system, C-50; fraction volume, 2.8 ml. 10 μl were assayed for radioactivity

Table. *C-values found on gas chromatography*
Column, 1% SE-30. Column temperature, 230°C. Derivatives: Me = methyl ester, TMSi = trimethylsilyl ether, MO = methoxime, Ac = acetate

Compound	C-value
Prostaglandin $F_{2\alpha}$-Me-TMSi	24.1
Prostaglandin $F_{2\alpha}$-Me-Ac	26.0
Compound I-Me-TMSi	24.1
Compound I-Me-Ac	26.0
Compound II-Me-TMSi	24.2
Compound II-Me-MO-TMSi	24.2
Compound II-Me-Ac	25.6
Compound II-Me-MO-Ac	25.6

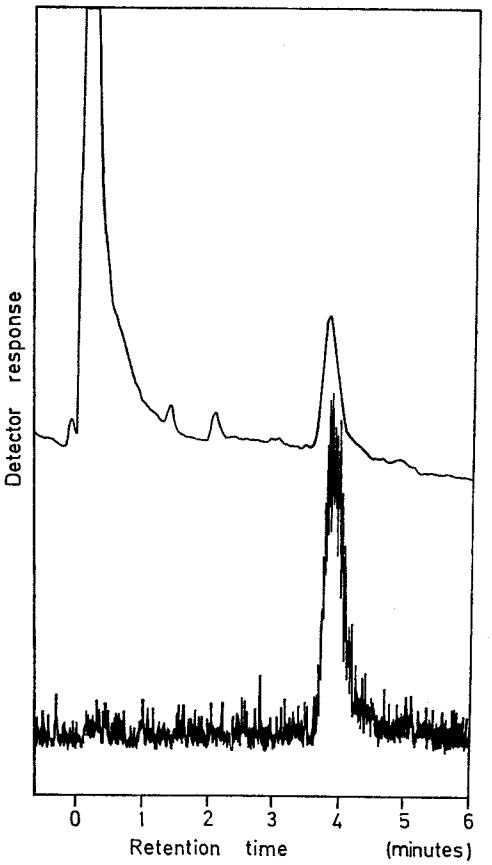

Fig. 2. *Gas-liquid chromatography of the methyl ester-methoxime-trimethylsilyl ether of compound II.* Column, 1% SE-30 on Gas Chrom P. Temperature, 230°C. Upper curve: mass. Lower curve: radioactivity

Fig. 3. The following prominent ions were found: *m/e* 541, which was interpreted as the molecular ion, M, *m/e* 526 (M-15), 510 (M-31), 470 (M-71), 451 (M-90), 420 (M-(90 + 31)), 330 (M-(2×90 + 31)), and 310 (M-(90 + 141), loss of the entire carboxyl side chain). This fragmentation pattern was in accordance with the methyl ester of a C-20 prostenoic acid, containing two trimethylsilyloxy groups and one methoxime group. The presence of two trimethylsilyloxy groups was supported by the mass spectrum of the corresponding derivative, labelled with nine deuterium atoms in each trimethylsilyl ether group (see Experimental Procedure). In this spectrum some of the ions had been shifted to *m/e* values 9 or 18 mass units higher than the corresponding ions in the spectrum of the non-labelled derivative. Thus, the molecular ion was found at *m/e* 559 (541 + 18), and other ions were found at *m/e* 541 (M−C^2H_3), 528 (510 + 18,) 488 (470 + 18), 460 (451 + 9, M−(C^2H_3)$_3$SiOH), 429 (429 + 9), and 330 (unchanged position, loss of both deuterium labelled trimethylsilyloxy groups).

The position of the methoxime group could not be unequivoqually established by these mass spectra. However, in the mass spectrum of the methyl ester-methoxime-acetate (Fig. 4). several fragments indicated that the methoxime group was in the C-15 position. Thus, ions of high intensities were found at *m/e* 481 (M), 450 (M-31), 425 (M-56, β-cleavage with respect to C-15 with transfer of hydrogen to the charged ion), 422 (M-59), 410 (M-71, α-cleavage with loss of the terminal pentyl group), 390 (M-(60 + 31)), 334 (M-(60 + 31 + 56)), 330 (M-(2×60 + 31)), 274 (M-(2×60 + 31 + 56)), 233 (M-(2×60 + 128), α-cleavage between C-14 and C-15), and 218 (M-(2×60 + 143), β-cleavage between C-13 and C-14). Some of the smaller fragments were also in accordance with the proposed position of the methoxime group, *e.g. m/e* 156 (the entire methyl side chain, containing the methoxime group), and *m/e* 128 (α-cleavage between C-14 and C-15, with the ion retaining the methoxime group).

Compound II was therefore likely to be 9α,11α-dihydroxy-15-ketoprost-5-enoic acid. This structure was also supported by the mass spectrometric analyses of the corresponding keto derivatives. In the mass spectrum of the methyl ester and trimethylsilyl ether of the compound (Fig. 5), the ion with the highest *m/e* value found was *m/e* 512 (M). Other ions were found at *m/e* 497 (M-15), 422 (M-90), 407 (M-(90 + 15)), and 332 (M-2×90). Apart from these nonspecific cleavages, several ions formed by α- and β-cleavages of the ketone at C-15 were seen, *e.g. m/e* 441 (M-71), 308 (M-(90 + 114)), 233 (M-(2×90 + 99)), and 218 (M-(2×90 + 114)).

Further evidence for the proposed structure of the compound was provided by the mass spectrum of the methyl ester and acetate (Fig. 6). The ion with the highest *m/e* value found was *m/e* 392, which was interpreted as being formed by loss of acetic acid, (M-60). Other ions were: *m/e* 332 (M-2×60), 301 (M-(2×60 + 31)), 276 (M-(2×60 + 56)), 233 (M-(2×60 + 99)), and 218 (M-(2×60 + 114)).

In several of the mass spectra, a loss of 141 could be seen. This was interpreted to be due to loss of the entire carboxyl side chain, provided that the double

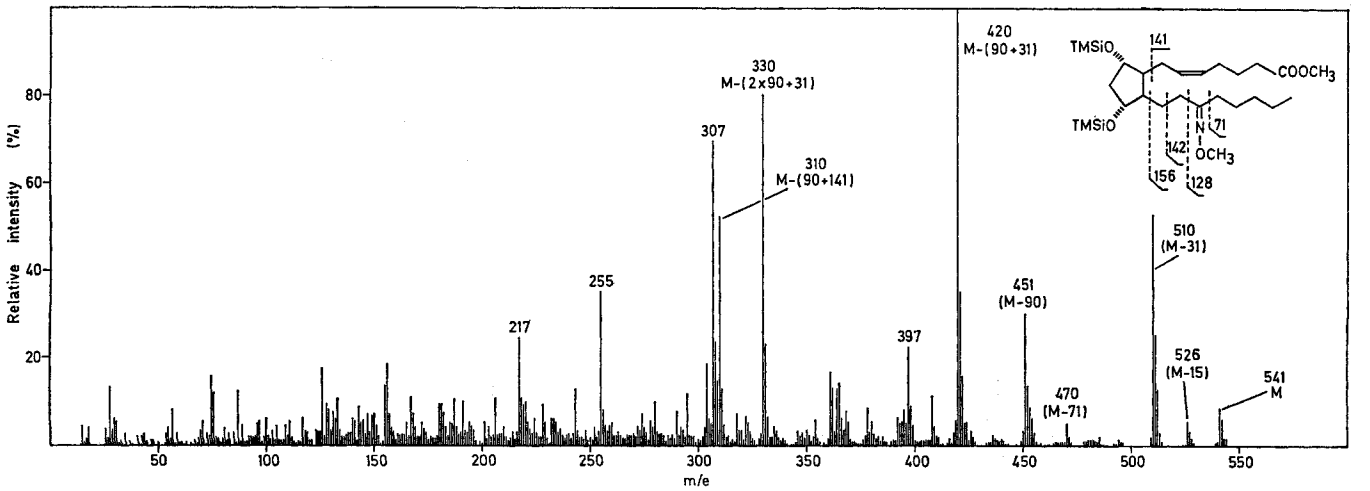

Fig. 3. *Mass spectrum of the methyl ester-methoxime-trimethyl silyl ether of compound II*

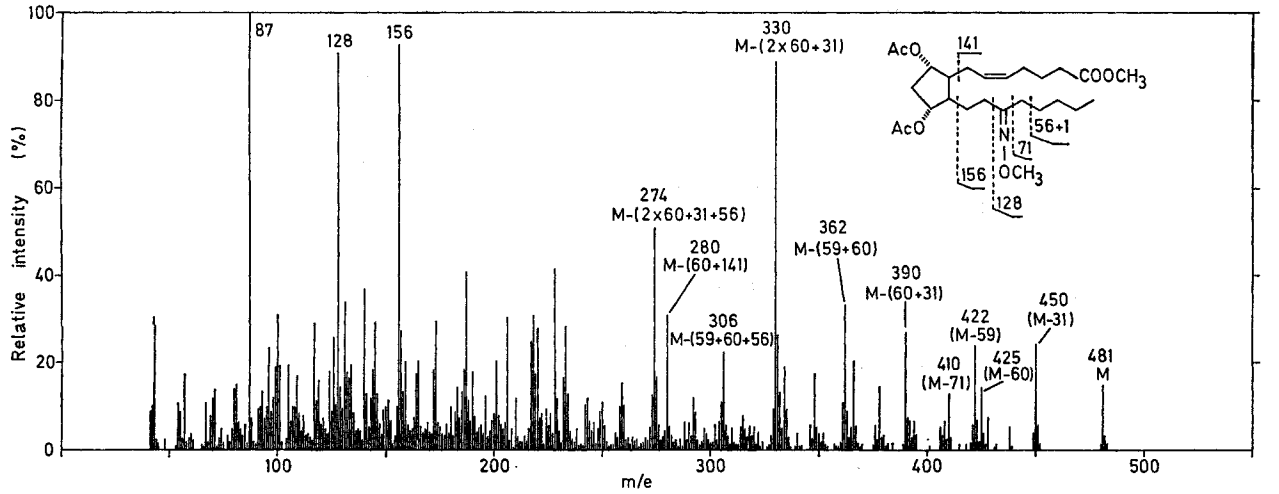

Fig. 4. *Mass spectrum of the methyl ester-methoxime-acetate of compound II*

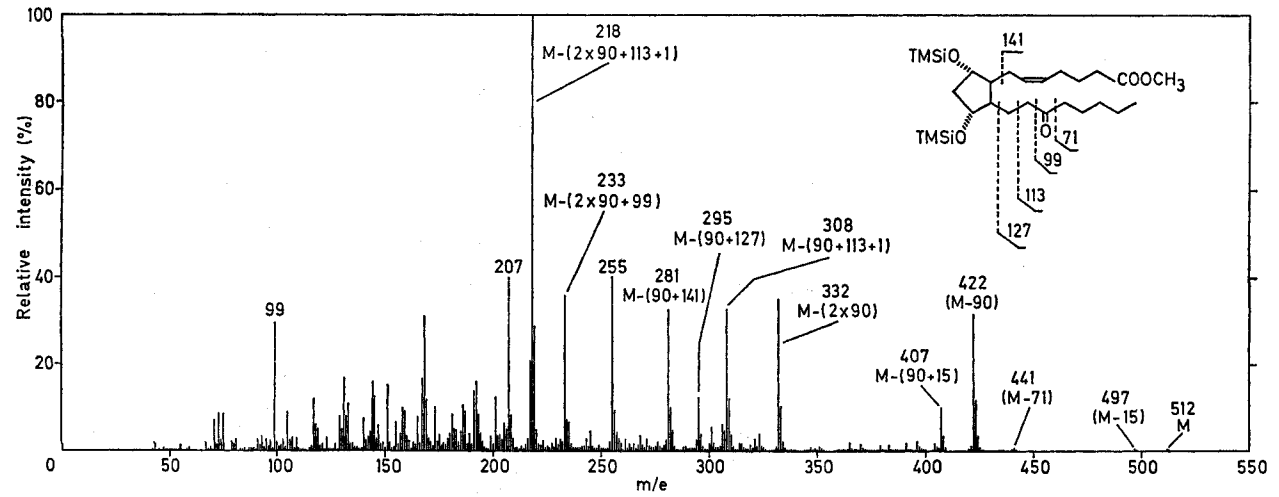

Fig. 5. *Mass spectrum of the methyl ester-trimethylsilyl ether of compound II*

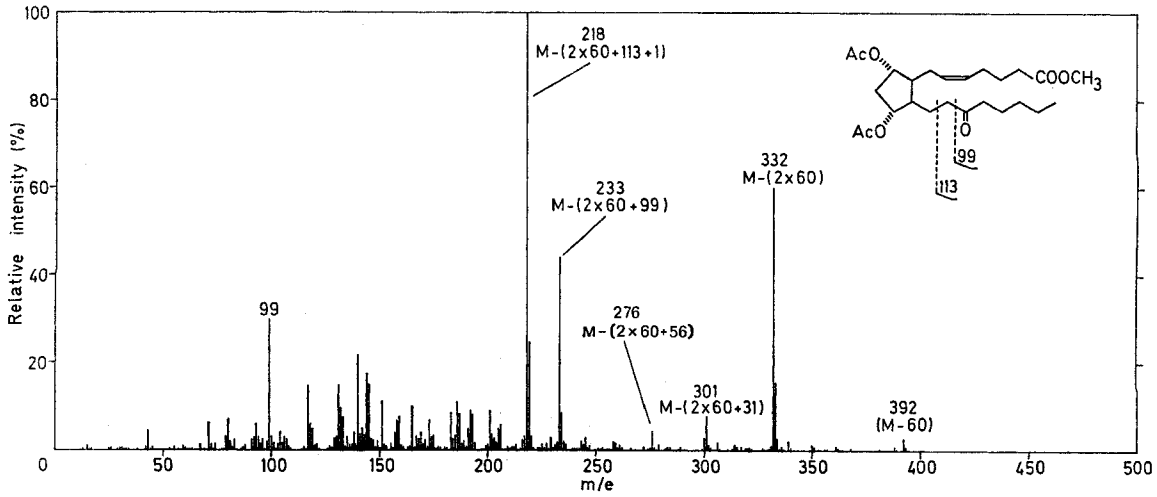

Fig. 6. *Mass spectrum of the methyl ester-acetate of compound II*

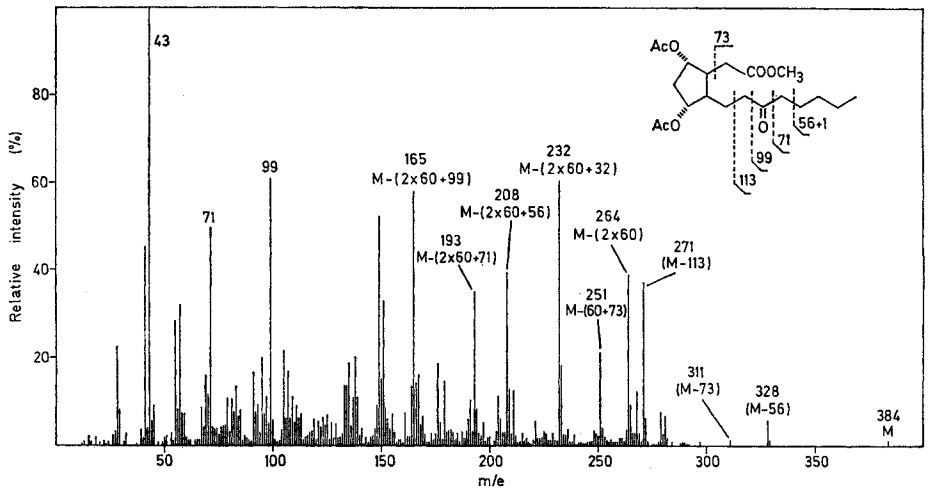

Fig. 7. *Mass spectrum of the product of the ozonolysis of compound II.* Derivative: methyl ester and acetate

bond remained in the Δ^5 position. To establish the number and position of double bonds more rigorously the methyl ester and acetate of compound II was subjected to oxidative ozonolysis. Before analysis with gas chromatography and mass spectrometry, the products of the ozonolysis were treated with either diazomethane or diazoethane. The C-value of the methyl ester of the labelled part of the molecule was 21.0 and that of the corresponding ethyl ester 21.6. The difference (C 0.6) indicated that this part of the molecule formed by the oxidative ozonolysis only contained one carboxyl group [12]. The C-value of the methyl ester also indicated that the original methyl ester-acetate of compound II (C-value 25.6) had been degraded by five carbon atoms, which was consistent with the presence of a Δ^5 double bond in compound II.

The mass spectrum of the product of the ozonolysis of compound II after treatment with diazomethane contained several ions that supported the proposed cleavage between C-5 and C-6 in the original compound (Fig. 7). The ion with the highest *m/e* value was found at *m/e* 384 (M) and other ions were found at *m/e* 328 (M-56), 271 (M-113), 268 (M-(60 + 56)), 264 (M-2 × 60), 232 (M-(2 × 60 + 32)), 208 (M-(2 × 60 + 56)), 193 (M-(2 × 60 + 71)), 165 (M-(2 × 60 + 99)), and 99. The interpretation of the mass spectrum was supported by the spectrum of the corresponding ethyl ester, where the corresponding ions were found at the following *m/e* values: 398 (384 + 14), 342 (328 + 14), 285 (271 + 14), 282 (268 + 14), 278 (264 + 14), 232, 222 (208 + 14), 207 (193 + 14), 179 (165 + 14) and 99.

Further support for the proposed structure of compound II, 9α,11α-dihydroxy-15-ketoprost-5-

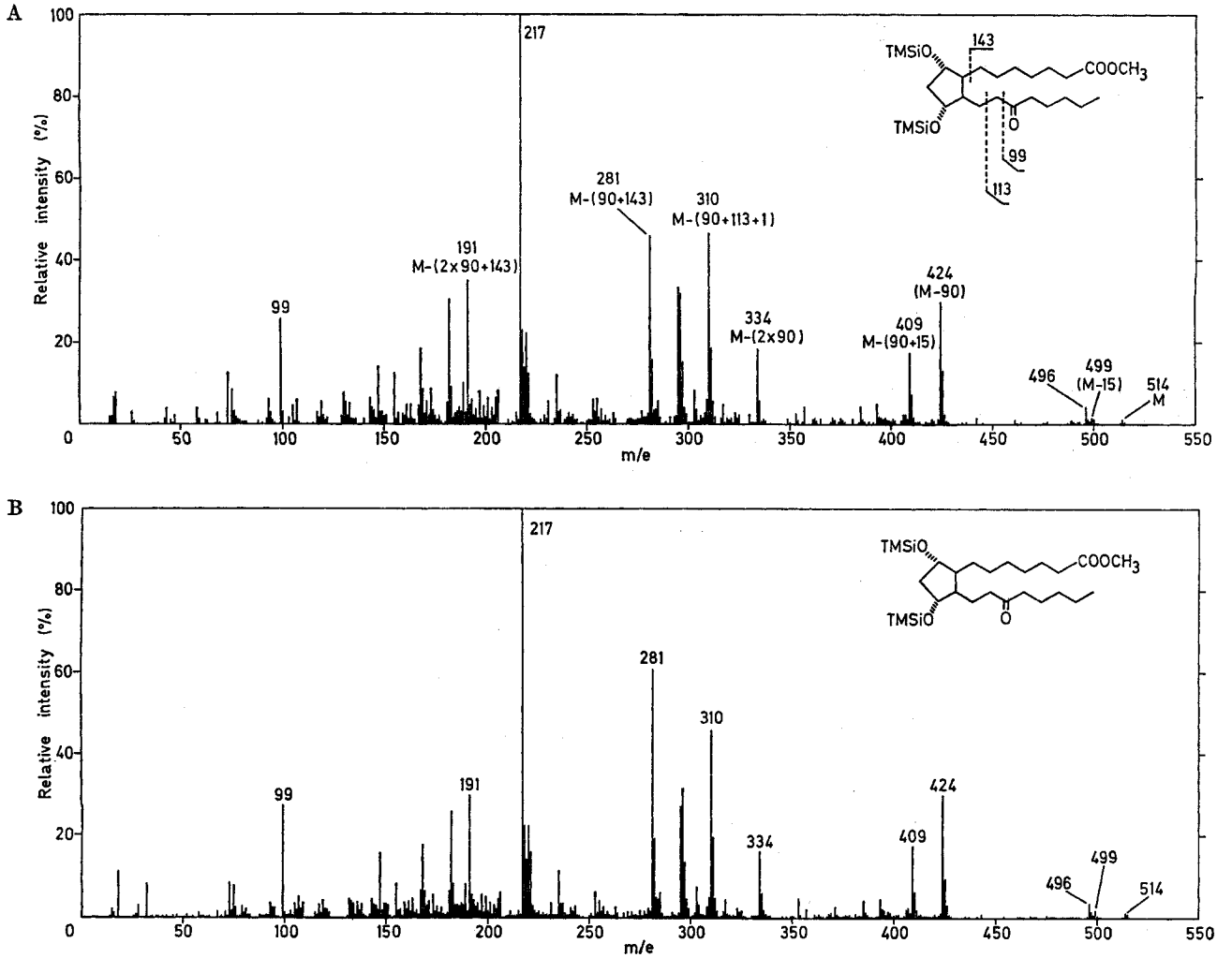

Fig. 8. *Mass spectrum of the hydrogenated product of (A) compound II. (Derivative: methyl ester and trimethylsilyl ether) and (B) methyl 9α,11α-dihydroxy-15-ketoprosta-5,13-dienoate (derivative: trimethylsilyl ether)*

enoic acid, could be obtained by comparison of the calculated C-values of various derivatives with those actually found. The C-value of methyl prostanoate on 1% SE-30 has been found to be 19.2 [13]. An additional trimethylsilyloxy group adds 1.9 to this value, and the contribution of a keto group or a methoxime group is 1.8. The C-value of the methyl ester and trimethylsilyl ether of 9α,11α-dihydroxy-15-ketoprostanoic acid on SE-30 would thus be 24.8 (found: 24.6, see below). The influence of a double bond depends on its position: the introduction of a Δ^{13} double bond results in a lowering of the C-value with 0.1—0.2 units. A Δ^{5} double bond, however, exerts a more pronounced influence and will generally lower the C-value of the corresponding saturated compound with 0.3—0.5 units (*cf.* [10]). Thus, the C-value of the methyl ester and trimethylsilyl ether of 9α,11α-dihydroxy-15-ketoprost-5-enoic acid would be 24.3—24.5 (found: 24.2). Similar calculations for the acetate or the methoxime-acetate of compound II (an additional acetoxy group will generally add 2.5 to the C-value of methyl prostanoate on SE-30) gave a C-value of 25.5—25.7 (found: 25.6).

Finally the methyl ester of compound II was subjected to catalytic hydrogenation, using palladium on carbon as catalyst [1]. Methyl 9α,11α-dihydroxy-15-ketoprosta-5,13-dienoate was treated similarly for use as reference. The two saturated compounds were trimethylsilylated and analysed by gas chromatography and mass spectrometry. The C-values on SE-30 were 24.6 for both derivatives. The two resulting mass spectra were practically identical (Fig. 8), and the mass spectrum of the product derived from compound II clearly indicated that two hydrogens had been incorporated. Thus, the molecular ion was found at *m/e* 514, and other ions were found at *m/e* 424 (M-90), 409 (M-(90+15)), and 334 (M-(2×90)).

DISCUSSION

Prostaglandin $F_{2\alpha}$ is metabolized by guinea pig lung into one less polar compound, which has been identified as 9α,11α-dihydroxy-15-ketoprost-5-enoic acid. The formation of this derivative from prostaglandin $F_{2\alpha}$ involves oxidation of the secondary alcohol group at C-15 and reduction of the Δ^{13} double bond. These reactions have previously been shown to take place with prostaglandins of the E type [1—3]. The oxidation seems to be of importance for the biological inactivation of the prostaglandins [15] and is catalyzed by a NAD^+-dependent dehydrogenase (15-hydroxy-prostanoate dehydrogenase). This enzyme has been purified from swine lung and was shown to catalyze the oxidation of the alcohol group at C-15 in prostaglandins of the E, F and A type [5]. No data are available at the present time concerning the Δ^{13}-reductase.

The inactivation of prostaglandins E_1, E_2, $F_{2\alpha}$ and A_2 by the lungs has been confirmed in perfusion studies with cat, dog, rat and guinea pig [16—21]. The inactivation is of enzymatic nature and probably due to the enzyme system described. Therefore, it is likely that the lungs possess a role in the biological inactivation of the prostaglandins.

A recent study of the metabolism *in vivo* of prostaglandin $F_{2\alpha}$ in the guinea pig showed that the main urinary metabolite was 5α,7α-dihydroxy-11-ketotetranor-prostanoic acid [7]. The formation of this product probably involves an initial conversion of prostaglandin $F_{2\alpha}$ into 9α,11α-dihydroxy-15-ketoprost-5-enoic acid by the lungs, and a subsequent degradation of this compound by two steps of β-oxidation in the liver prior to excretion into the urine.

The formation of 9α,11α-dihydroxy-15-ketoprost-5-enoic acid from prostaglandin $F_{2\alpha}$ has recently also been demonstrated in guinea pig liver and swine kidney [14, 22].

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