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GAS CHROMATOGRAPHY – MASS SPECTROMETRY OF O-METHYLOXIME DERIVATIVES OF PROSTAGLANDINS

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O-methyloxime trimethylsilyl ether derivatives and O-methyloxime acetyl derivatives of prostaglandins containing a β-ketol system in the five membered ring and in some cases an additional keto group in the side chain have been found to be stable during gas chromatography GLC data obtained under different conditions are presented The mass spectrometric fragmentation of the compounds has been investigated using derivatives deuterated in the methyl ester group, the methoxime group, the trimethylsilyl ether groups or the acetyl groups The molecular ion can generally be seen Eliminations characteristic for trimethylsilyl ether and acetyl derivatives were observed and in addition β-cleavage at the methoxime group The latter reaction was in some cases accompanied by transfer of hydrogen to the charge retaining ion and loss of CH_3O from the methoxime group These reactions are of value for determination of location of keto groups and of the nature of substituents

The use of methoxime derivatives of steroid ketones in gas chromatography and mass spectrometry was introduced by Fales and Luukkainen in 1965[1]) These derivatives have subsequently been widely used in steroid work[2,3])

Prostaglandins of the PGE-type possess a β-ketol system, which is susceptible to degradation under conditions employed in gas chromatography This degradation can be prevented by conversion to a methoxime derivative The present report describes gas chromatographic and mass spectrometric studies of O-methyloxime (MO) derivatives of a number of prostaglandins as trimethylsilyl ethers (TMS) or acetates (Ac) These derivatives have recently been used extensively in our laboratory in studies of the biosynthesis[4]) and metabolism[5–7]) of prostaglandins

The abbreviations used are
MO – TMS derivative, O-methyloxime trimethylsilyl ether derivative
MO – Ac derivative, O-methyloxime acetyl derivative
Prostaglandin E_1, PGE_1, 11α, 15-dihydroxy-9-ketoprost-13-enoic acid,
Prostaglandin E_2, PGE_2, 11α, 15-dihydroxy-9-ketoprost-5,13-dienoic acid,
11-dehydro-$PGF_{1\alpha}$, 9α, 15-dihydroxy-11-ketoprost-13-enoic acid,
dihydro PGE_1, 11α, 15-dihydroxy-9-ketoprostanoic acid, dinor-PGE_1, 9α, 13-dihydroxy-7-ketodinorprost-11-enoic acid,

Materials and methods

Preparation of O-methyloxime trimethylsilyl ether derivatives

100 to 200 μg of the prostaglandins were dissolved in 0 3 ml of methanol and treated with diazomethane in ether After evaporation, the methyl ester was dissolved in 0 2 ml of pyridine containing 5 mg of methoxyamine-hydrochloride (Eastman Organic Chemicals, Rochester 3, New York) The solution was kept in a stoppered test tube in a desiccator overnight 20 μl of trimethylchlorosilane and 40 μl of hexamethyldisilazane were added and the mixture was left for an additional hour in the desiccator The sample was evaporated to dryness with an oil pump and the residue was dissolved in carbon disulfide before injection into the gas chromatograph

Preparation of O-methyloxime acetate derivatives

100 to 200 μg of methyl esters of the prostaglandins were dissolved in 0 2 ml pyridine containing 5 mg methoxyamine hydrochloride The reaction mixture was left at room temperature over night and evaporated to dryness The residue was extracted three times with 0 5 ml of ether and the ether phases were combined and evaporated to dryness The O-methyloxime methylester derivative was dissolved in 0 3 ml of pyridine and 0 3 ml of acetic anhydride was added The reaction mixture was left over night at room temperature After addition of ice, the solution was acidified and extracted with ether three times The ether solution was washed with small volumes of 0 2 N hydrochloric acid, 10% sodium bicarbonate and distilled water until neutral reaction and was then evaporated to dryness The residue was dissolved in carbon disulfide before injection into the gas chromatograph

Preparation of methyl-d_3-ester derivatives

The prostaglandin was treated with diazomethane and the methoxime derivative was prepared as described above for the MO—Ac derivatives The methyl ester O-methyloxime was treated with 0 25 ml of 0 5 N $NaOC^2H_3$ (prepared by treating tetradeuteromethanol, E Merck AG Darmstadt, with sodium) and left for 10 min at room temperature The solution was acidified with 1 ml of 0 3 N HCl and extracted with ether three times The ether phase was washed with water until neutral reaction and evaporated to dryness The residue was dissolved in 0 2 ml of dry pyridine and the Ac or TMS derivative was prepared according to the procedures described above

Preparation of O-methyl-d_3-oxime derivatives

MO– Ac and MO—TMS derivatives of the methyl ester of the prostaglandin were prepared using methoxyamine hydrochloride labelled with

deuterium in the methyl group The methoxyamine hydrochloride was prepared[7]) by treatment of potassium hydroxylamine disulfonate with deuterium labelled methyl iodide (Stohler Isotope Chemicals, 99 5% ^2H, Montreal)

Preparation of O-methyloxime trimethyl-d_9-silyl ether derivatives

The methyl ester MO-derivative was prepared as described above for MO -TMS derivatives To the pyridine solution was added 40 μl of trimethyl-d_9 chlorosilane (Merck, Sharp & Dohme, Montreal, Canada) and the test tube was allowed to stand for two hours at room temperature After evaporation to dryness the residue was dissolved in carbon disulfide before injection into the gas chromatograph

Preparation of O-methyloxime acetyl-d_3-derivatives

The standard procedure described above was followed using deuterium labelled acetic anhydride (Acetic Anhydride-d_6, Merck, Sharp & Dohme, Montreal, Canada)

Compounds

The MO—TMS derivatives of the methyl esters of PGE_1, PGE_2 and 11-dehydro-$PGF_{1\alpha}$ were prepared PGE_1 and PGE_2 were crystalline and only one spot appeared when the purity was checked on TLC using system AII[8]) 11-dehydro-$PGF_{1\alpha}$, an isomer of PGE_1[4]), gave a single spot on TLC

The MO- Ac derivatives were prepared from the methyl esters of dihydro-PGE_1, PGE_1 PGE_2, 11-dehydro-$PGF_{1\alpha}$, dinor-PGE_1, 11α-hydroxy-9,15-diketo-prostanoic acid, 9α-hydroxy-7,13-diketo-dinorprostanoic acid, 7α-hydroxy-5,11-diketo-tetranorprostanoic acid Dihydro-PGE_1 was prepared by catalytic hydrogenation of PGE_1 and was then purified on reversed phase partition chromatography[9]) The compound gave one spot when tested on TLC using plates impregnated with silver nitrate system AII[8]) The 11α-hydroxy-9, 15-diketo-prostanoic acid was synthesized by oxidizing PGE_1 with manganese dioxide in chloroform and subsequent catalytic hydrogenation[10]) The compound was purified by reversed phase partition chromatography and silicic acid chromatography The dinor and tetranor homologues were prepared by incubating 11α-hydroxy-9,15-diketo-prostanoic acid with a preparation of rat liver mitochondria and were isolated as previously described[5]) The three diketo compounds gave each one spot on TLC using plates impregnated with silver nitrate and solvent system, ethyl acetate, benzene, acetic acid 2,2,4-trimethyl pentane, water, 80 30 10 30 100

Dinor-PGE_1 was prepared by incubating PGE_1 in a rat liver homogenate It was purified by reversed phase partition chromatography and silicic acid

chromatography[5]) and gave one single spot on TLC using system AII[8])

Solvents

The ether, (Anhydrous ether, Analytical reagent Mallinckrodt), carbon disulfide and methanol, (Pro analysi, Merck, Darmstadt) were used without further purification The pyridine (Analytical reagent, Mallinckrodt) was distilled from and stored over KOH pellets Acetic anhydride (Pro analysi, Merck Darmstadt) was distilled before use

Gas chromatography

An F & M Biomedical Gas chromatograph model 400 was used The carrier gas (nitrogen) flow was 60 ml per minute and the inner diameter of the columns was three mm The column packings with 1% NGS, 2% Epon 1001 and 1% OV-1 as stationary phases were purchased ready for use from Applied Science Laboratories, Inc, State College Pa, while the Se-30 (F & M Scientific corporation, Avondale, Pa) and OV-17 (Applied Science Lab Inc) were applied on silanized Gas Chrom P (100–120 mesh) as described by Horning *et al*[11]) The flash heater and detector were kept at a temperature 20–30°C above that of the column

Mixtures of normal saturated fatty acid methyl esters were used as standards and diagrams were constructed by plotting retention times on a logarithmic scale versus the number of carbon atoms of the acid (C-values) on the linear scale The retention times of the derivatives were converted into "C-values" using these diagrams (cf [9])

Mass spectrometry–mass spectra were obtained with the combined gas chromatograph–mass spectrometer, LKB 9000, equipped with a 1% SE-30 column The electron energy was 22 5 eV and the trap current 60 μA

Results and discussion

Gas-liquid chromatography

Gas chromatographic data on the MO—TMS and MO—Ac derivatives of some prostaglandins are listed in table 1 A typical run is shown in fig 1 All mono-methoximes, except the MO—Ac derivative of 11-dehydro-$PGF_{1\alpha}$, gave rise to two well separated peaks That the appearance of two peaks in the gas chromatogram was not due to impurities was indicated by the finding that PGE_1 gave the same proportions of isomers (1 3) before and after recrystallization six times Furthermore, the mass spectra recorded on the two GLC peaks of the derivatives investigated mainly show intensity differences It is therefore assumed that the two peaks observed are due to syn and anti isomers (cf [2,3])

TABLE 1

"C-values" obtained for methoxime derivatives of prostaglandins

Compound	Column				
	1 % OV-1 190 C 6 ft	1 % OV-17 200 C 4 ft	1 % SE-30 195 C 6 ft	1 % NGS 210 C 6 ft	2 % Epon-1001 220 C 6 ft
MO - TMS derivatives					
PGE_1	24 4 25 0	24 5 25 1	24 5 25 0	24 6 25 1	25 8 26 2**
PGE_2	24 2 24 7	24 4 25 0	24 0 24 6	24 5 25 1	25 4 25 8**
11-dehydro-$PGF_{1\alpha}$	24 5 24 9	24 7 25 1	24 5 24 9	24 5 24 9	25 6 25 9**
MO—Ac derivatives					
dihydro PGE_1	25 5 26 0	27 5 28 0	25 7 26 1	31 3 31 8	30 1 30 6
PGE_1	25 5 25 9	27 7 28 1	25 5 25 9		30 1 30 6
PGE_2	25 3 25 7	27 7 28 2	25 2 25 6		29 8 30 3
11-dehydro-$PGF_{1\alpha}$	25 2	28 5	26 0		31 1
dinor-PGE_1	23 8 24 1	25 7 26 1	24 0 24 4		28 3 28 7
11α-hydroxy-9,15 diketoprostanoic acid	25 1* 25 5*	26 9 27 4 27 1 27 5	24 9 25 3* 25 0	29 8* 30 3*	29 0 29 4 29 1 29 5
9α-hydroxy-7,13-diketo-dinor-prostanoic acid	23 2* 23 6*	25 0 25 5 25 1 25 6	23 0 23 5* 23 1	27 7* 28 2*	27 0 27 4** 27 1 27 6
7α-hydroxy-5,11-diketo-tetranor-prostanoic acid			21 1 21 6 21 2 21 7	25 6* 26 1*	

* Indicates assymetric peak

** Column temperature 200 C

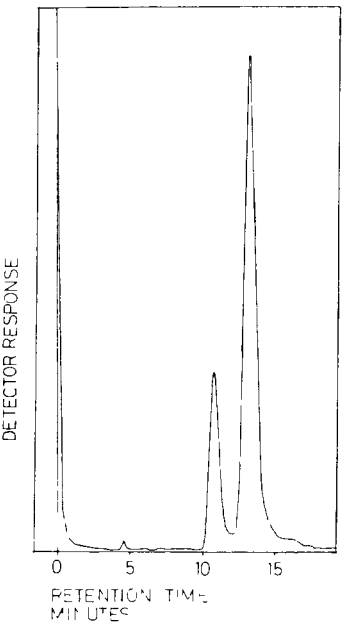

Fig 1 Gas chromatogram of the MO -TMS derivative of the methyl ester of PGE_2 obtained on a 1 % SE-30 column at 185 C

When the derivatives containing two O-methyloxime groups were chromatographed on a OV-17 or Epon 1001 column, all four isomers tended to separate (table 1), but it was not possible to obtain complete separation between the two isomers in the first and second pair Two well separated but assymmetric peaks were obtained when the same derivatives were run on OV-1 and NGS columns Separation of geometric isomers was similar on an SE-30 column (see table 1) When the MO—Ac derivatives of PGE_1, PGE_2, 11-dehydro-$PGF_{1\alpha}$ and dinor-PGE_1 were chromatographed on a 1% NGS column they gave broad and distorted peaks indicating degradation on the column

Mass spectrometry

MO—TMS derivatives

The mass spectra of the MO—TMS derivatives of PGE_1, PGE_2 and 11-dehydro$PGF_{1\alpha}$ are shown in fig 2, 3 and 4 respectively Some of the mass spectrometric data have been summarized in table 2 Derivatives of PGE_1 and PGE_2 perdeuterated in the methyl ester group, the methoxime group

TABLE 2

Mass spectrometric data on MO—TMS derivatives of methyl esters of prostaglandins
A = side chain attached to C-8 B = side chain attached to C-12

Origin of mass spectrum		M	M-15	M-31	M-71	M-90	M-(90 + 31)	M-(90 + 71)	M-(A-1 + 31)	M-(A + 90)	M-(B + 45)	*m/e* 199	*m/e* 173
Peak I	PGE_1	541	526	510	470	451	420	380	368	308	297	199	173
Peak II	PGE_1	541	526	510	470	451	420	–	368	308	297	199	–
Peak I	11-dehydro-$PGF_{1\alpha}$	541	526	510	470	451	420	380	–	–	297	199	173
Peak II	11-dehydro-$PGF_{1\alpha}$	541	526	510	470	451	420	380	–	–	297	199	173
Peak I	PGE_2	539	524	508	468	449	418	378	368	308	295	199	173
Peak II	PGE_2	539	524	508	468	449	418	378	368	308	295	199	173

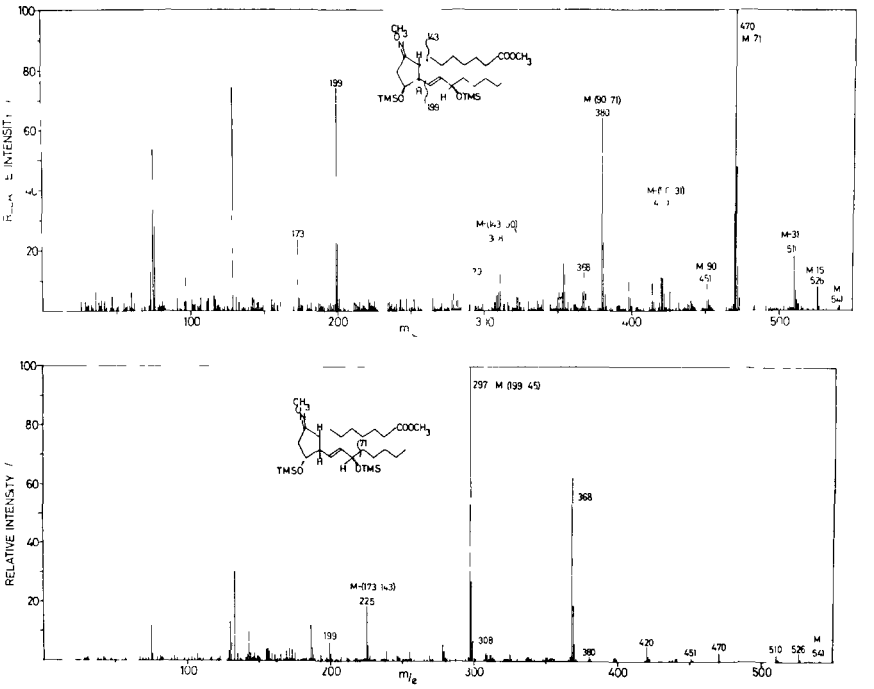

Fig 2 Mass spectra recorded on the first (upper spectrum) and second (lower spectrum) GLC peak of the MO—TMS-derivative of the methyl ester of PGE_1

and in the TMS groups, were prepared in order to facilitate the interpretation of the mass spectra

In the mass spectra of the derivative of PGE_1 (fig 2) the molecular ion (*m/e* 541) and ions due to eliminations of CH_3 (M-15), OCH_3 (M-31), $(CH_2)_4$ -CH_3 (M-71), TMSOH (M-90) and combinations of these i e M-(90+31) and M-(90+71) are seen The experiments with deuterium labelled derivatives showed that the elimination of 31 is due to loss of OCH_3 from the methyl ester or the methoxime groups However this is not the case with the ion appearing at *m/e* 420 (M-(90 + 31)) where 31 is derived exclusively from the methoxime group

The ion appearing at *m/e* 368 (M-173) is due to two types of eliminations The experiments with deuterated derivatives showed that this ion is formed either with retention or elimination of OCH_3 in the ester group, and one TMSOH group On the other hand the methoxy group of the methoxime was always lost The ion at *m/e* 368 might therefore be formed partly by elimination of the side chain attached to C-8 and OCH_3 from the methoxime group in a Mc Lafferty type of rearrangement with transfer of hydrogen

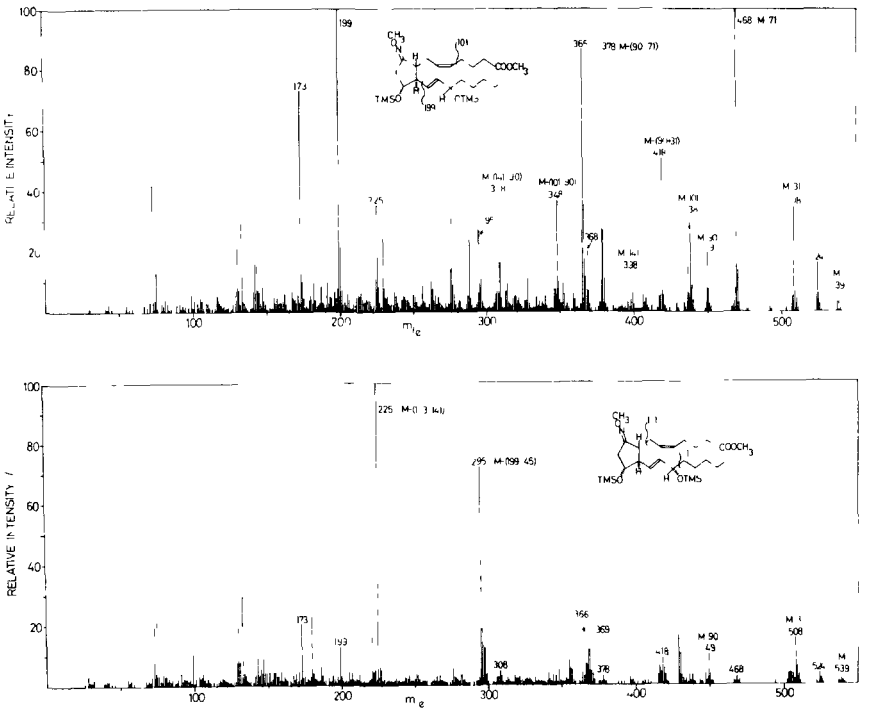

Fig 3 Mass spectra recorded on the first (upper spectrum) and second (lower spectrum) GLC peak of the MO—TMS-derivative of the methyl ester of PGE_2

from C-6 to the nitrogen and partly by elimination of carbons 9, 10 and 11 with the methoxime group and one TMSO group The ion at *m/e* 308 (M-233) is presumably formed by elimination of the C-8 side chain (143) and TMSOH while the ion at *m/e* 297 most likely results from loss of the side chain attached to C-12 (199) and the methoxime group (45) Formation of the ions at *m/e* 225, 199 and 173 involves loss of the methyl group of the methyl ester, the methoxime group and one TMS group as shown by experiments with deuterated derivatives The ions at *m/e* 199 and 173 seem to be due to the C-12 side chain and $(TMSO\text{-}CH\text{-}(CH_2)_4\text{-}CH_3)^+$ respectively

The spectra of the derivative of PGE_2 (fig 3) show the molecular ion (*m/e* 539) and ions due to elimination of 15, 31, 71, 90, (90+31), (90+71) as discussed above Elimination of $(CH_2)_3\text{-}COOCH_3$ (101) and the complete C-8 side chain (141) gives ions at *m/e* 438 and *m/e* 398 respectively and additional loss of 90 gives ions at *m/e* 348 and 308 The spectra recorded on the deuterium labelled derivatives indicate that elimination of the C-8 side chain minus one hydrogen (140) and OCH_3 (31) from the methoxime group

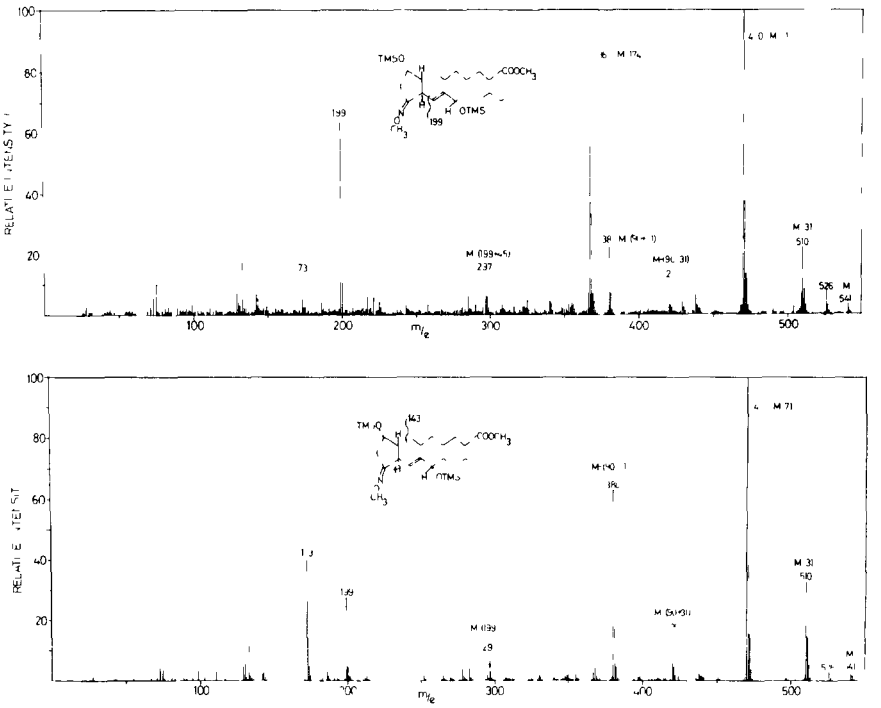

Fig 4 Mass spectra recorded on the first (upper spectrum) and second (lower spectrum) GLC peak of the MO -TMS-derivative of the methyl ester of 11-dehydro-$PGF_{1\alpha}$

gives the ion at *m/e* 368 The ion at *m/e* 366 (M-173) might partly be due to elimination of carbons 9-11 as discussed above for the derivative of PGE_1 However, in the upper spectrum (fig 3) an ion at *m/e* 365 (M-174) is also seen The deuterium experiments show elimination of CH_3 from the methoxime group and one TMS group The elimination of 244 (*m/e* 295) and the ions at *m/e* 225, 199, and 173 have been discussed above

The postulated losses of the C-8 side chain are supported by the appearance of the resulting ions at the same *m/e* value for the derivatives of both PGE_1 and PGE_2 (cf table 2)

The mass spectra of the MO-TMS derivative of 11-dehydro-$PGF_{1\alpha}$ are shown in fig 4 Both isomers show a molecular ion at *m/e* 541 and ions formed by elimination of 15 (*m/e* 526), 31 (*m/e* 510), 71 (*m/e* 470), 90 (*m/e* 451), 90 + 31 (*m/e* 420), 90 + 71 (*m/e* 380), 199 = 45 (*m/e* 297) and ions at *m/e* 199 and *m/e* 173 all of which have been discussed above The ion at *m/e* 367 (M-174) might be due to elimination of the C-8 side chain (143) and OCH_3 from the methoxime group

MO-Ac derivatives

The MO-Ac derivatives were prepared from the methyl esters of dihydro-PGE_1, PGE_1, PGE_2, 11-dehydro-$PGF_{1\alpha}$, dinor-PGE_1, 11α-hydroxy-9,15-diketo-prostanoic acid, 9α-hydroxy-7,13-diketo-dinorprostanoic acid and 7α-hydroxy-5,11-diketo-tetranorprostanoic acid The mass spectra recorded on these derivatives are shown in figs 5–12 and some mass spectrometric data are summarized in table 3 In order to facilitate the interpretation of the mass spectra, derivatives of PGE_1 and PGE_2 deuterated in the methyl ester group, the methoxime group or in the acetyl groups were prepared

The mass spectra of the derivative of PGE_1 (fig 5) showed the molecular ion (*m/e* 481) and ions due to elimination of 31 (*m/e* 450), 60 (*m/e* 421) and

TABLE 3

Mass spectrometric data on MO—Ac derivatives of methyl esters of prostaglandins A = the side chain acetoxy group in this chain C the methyl end side chain

Origin of mass spectrum		M	M-31	M-60	M-(60 + 31)	M-2 60	M-(120 + 31)	M-(A-1 + 60)
Peak I	Dihydro PGE_1	483	452	423	392	363	332	281
Peak II	Dihydro PGE_1	483	452	423	392	363	332	281
Peak I	PGE_1	481	450	421	390	361	330	279
Peak II	PGE_1	481	450	421	390	361	330	279
Peak	11-dehydro-$PGF_{1\alpha}$	481	450	421	390	–	330	–
Peak I	PGE_2	479	448	419	388	359	328	279
Peak II	PGE_2	479	448	419	388	359	328	279
Peak I	dinor PGE_1	453	422	393	362	333	302	279
Peak II	dinor PGE_1	453	422	393	362	333	302	279
Peak I	11α-hydroxy-9,15-diketoprostanoic acid	468	437	408	377	–	–	–
Peak II	11α-hydroxy-9,15-diketoprostanoic acid	468	437	408	377	–	–	–
Peak I	9α-hydroxy-7,13-diketodinorprostanoic acid	440	409	380	349	–	–	–
Peak II	9α-hydroxy-7,13-diketodinorprostanoic acid	–	409	380	349	–	–	–
Peak I	7α-hydroxy-5 11 diketotetranor-prostanoic acid	412	381	352	321	–	–	–
Peak II	7α-hydroxy-5,11-diketotetranor-prostanoic acid	412	381	352	321	–	–	–

combinations of these, viz $31+60$ (m/e 390), 2×60 (m/e 361) and $2\times60+31$ (m/e 330) The loss of 31 is due to loss of OCH_3 from either the methyl ester or the methoxime groups, and the loss of 60 is due to elimination of acetic acid The ion at m/e 362, is formed by elimination of acetic acid (60) and an acetyl radical (59) Ions, which probably are formed by loss of the C-8 side chain minus one hydrogen (142) are seen at m/e 279 (M-(142+60)) and m/e 219 (M-(142+2×60)) The ion at m/e 188 is most likely due to additional elimination of OCH_3 (31) from the methoxime group Two metastable ions at m/e about 172 and 161 support this fragmentation sequence The ions at m/e 279, 219 and 188 are also seen in the mass spectrum of PGE_2 whereas corresponding ions of dihydro-PGE_1 appears at m/e 281, 221 and 190 The C-8 side chain minus one hydrogen (142) and the C-12 side chain (169)

containing the carbomethoxy group B = the methyl end side chain of derivatives containing one of derivatives containing one methoxime group in this chain

M-(A+60)	M-(A-1+120)	M-(A+120)	M-(A-1+120+31)	M-(A-1+B)	M-(56+31)	M-(31+71+45)	M-(A-1+31)	M-(C+31+15)	M-(C+60)	M-(A-1+60+31)
–	221	–	190	170						
–	221	–	190	170						
–	219	–	188	170						
–	–	–	188	170						
278	–	–	–	–						
–	219	218	188	170						
–	219	218	188	170						
–	219	–	188	170						
–	219	–	188	170						
–	–	–	–	–	381	321	295	266	252	235
–	–	–	–	–	381	321	295	266	252	235
–	–	–	–	–	353	293	295	238	224	235
–	–	–	–	–	353	293	295	238	224	235
–	–	–	–	–	325	265	–	210	196	–
–	–	–	–	–	325	265	–	210	196	–

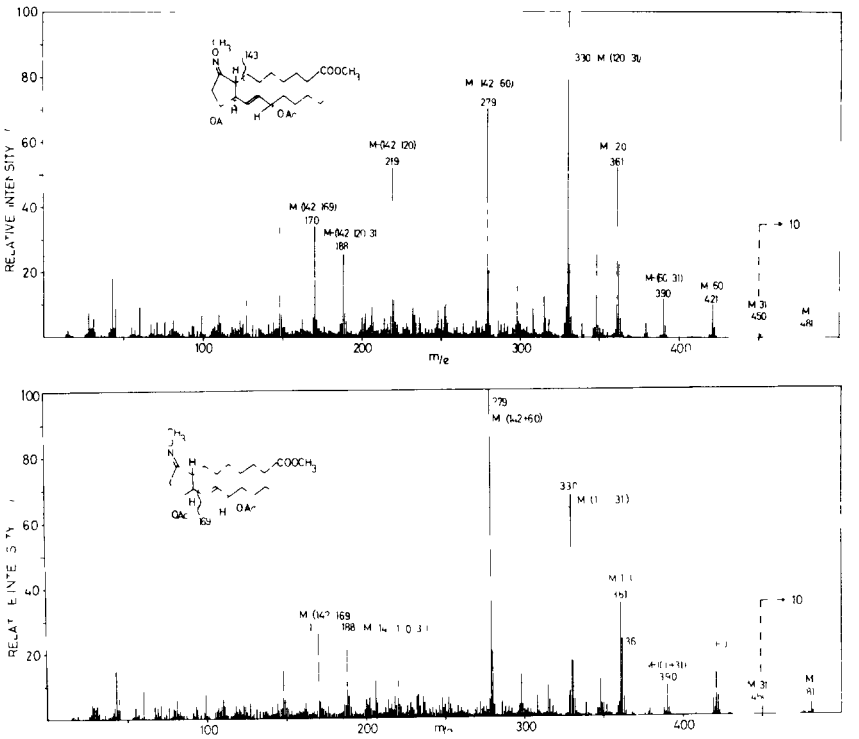

Fig 5 Mass spectra recorded on the first (upper) and second (lower) GLC peak of the MO—Ac derivative of the methyl ester of PGE_1 The scale is enlarged ten times above *m/e* 449

are probably both eliminated during the formation of the ion at *m/e* 170 since this ion is common for all the monomethoxime acetates in this study except the derivative of 11-dehydro-$PGF_{1\alpha}$

The derivative of PGE_2 (fig 6) shows a fragmentation pattern similar to that of PGE_1 The ions appearing at *m/e* 279, *m/e* 219, *m/e* 188 and *m/e* 170 are common for the derivatives of PGE_1 and PGE_2 indicating elimination of the C-8 side chain as discussed above The ion at *m/e* 218 is probably formed by elimination of the intact C-8 side chain and two molecules of acetic acid

The mass spectrum of the derivative of 11-dehydro-$PGF_{1\alpha}$ (fig 7) shows eliminations similar to those of PGE_1 However, the ions appearing at *m/e* 350, *m/e* 290 and *m/e* 278 are unique for 11-dehydro-$PGF_{1\alpha}$ The first ion (*m/e* 350) is probably formed by loss of the pentyl group (71) of the C-12 side chain and acetic acid (60) and gives rise to the ion at *m/e* 290 by additional elimination of acetic acid The intact C-8 side chain (143) and one molecule

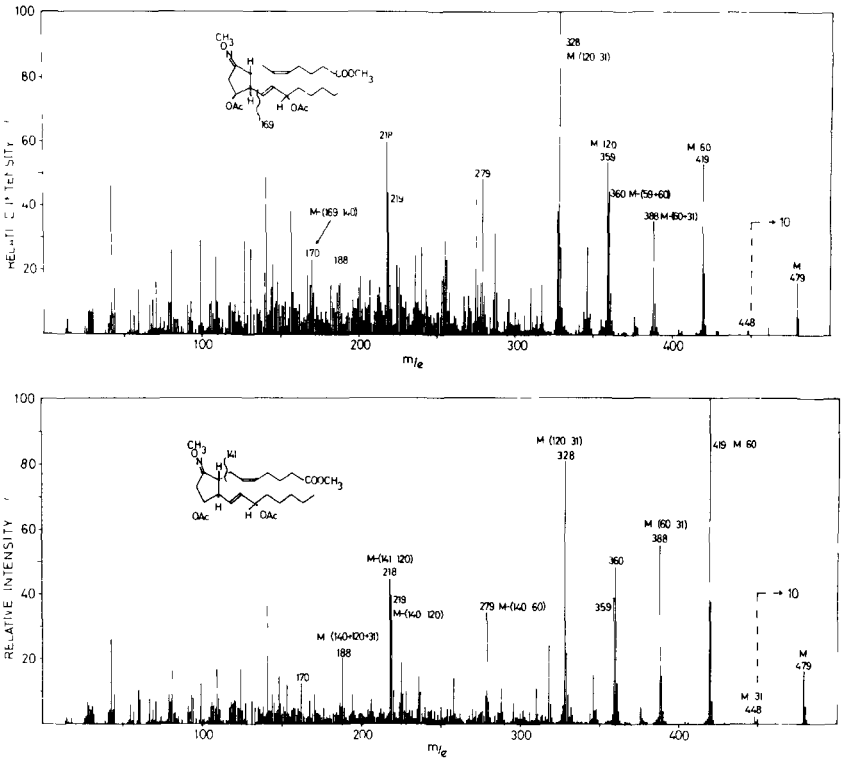

Fig 6 Mass spectra recorded on the first (upper) and second (lower) GLC peak of the MO Ac derivative of the methyl ester of PGE_2 The scale is enlarged ten times above m/e 449

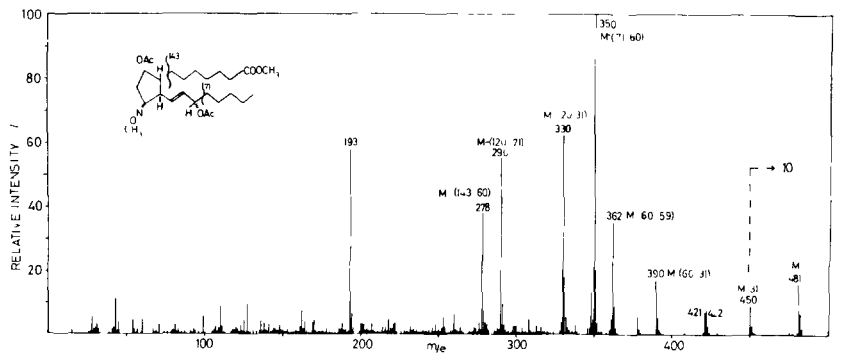

Fig 7 Mass spectrum recorded on the GLC peak of the MO—Ac derivative of the methyl ester of 11-dehydro-$PGF_{1\alpha}$ The scale is enlarged ten times above m/e 449

of acetic acid (60) are probably lost when the ion appearing at *m/e* 278 is formed

The mass spectra of the derivative of dihydro-PGE_1 (fig 8) are very similar to those of PGE_1 showing ions at *m/e* values two mass units above the corresponding ions in the spectra of PGE_1 However, the ion appearing at *m/e* 170 is common for PGE_1 and dihydro-PGE_1 as well as for PGE_2 indicating elimination of the C-12 and C-8 side chains as discussed above

The mass spectra of dinor PGE_1 are shown in fig 9 The fragmentation pattern of this compound is similar to that of PGE_1, with most of the ions appearing at *m/e* values which are 28 mass units lower However, the ions at *m/e* 279, *m/e* 219, *m/e* 188 and *m/e* 170 which are formed by elimination of the carbomethoxy side chain are common for the mono-methoxime acetates in this study except dihydro-PGE_1

The spectra of the derivative of 11α-hydroxy-9,15-diketo-prostanoic acid are shown in fig 10 The molecular ions (*m/e* 468) and ions due to elimination

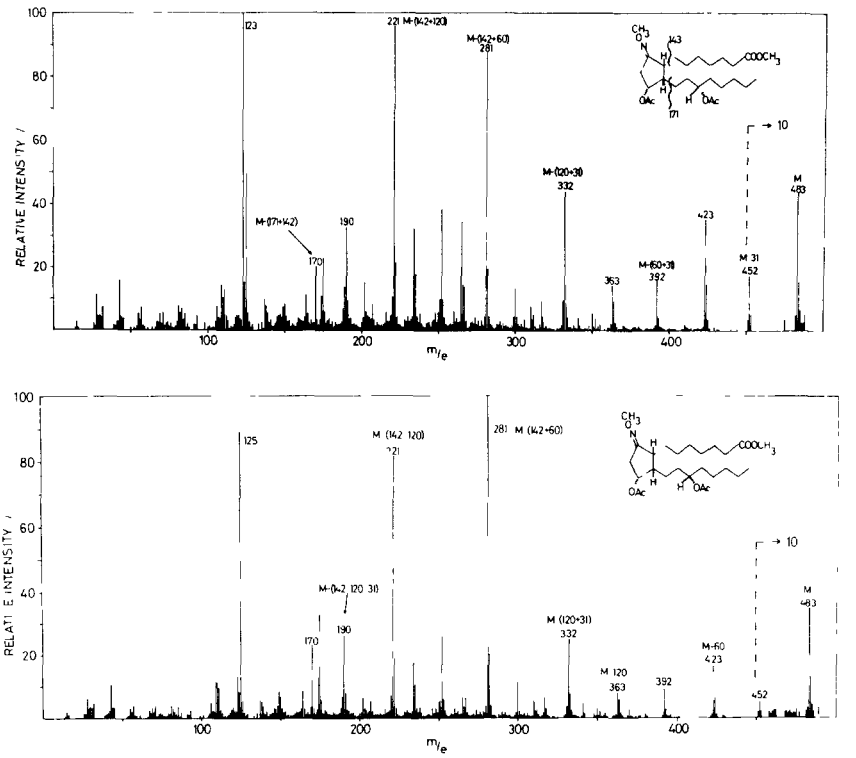

Fig 8 Mass spectra recorded on the first (upper) and second (lower) GLC peak of the MO—Ac derivative of the methyl ester of dihydro-PGE_1 The scale is enlarged ten times above *m/e* 449

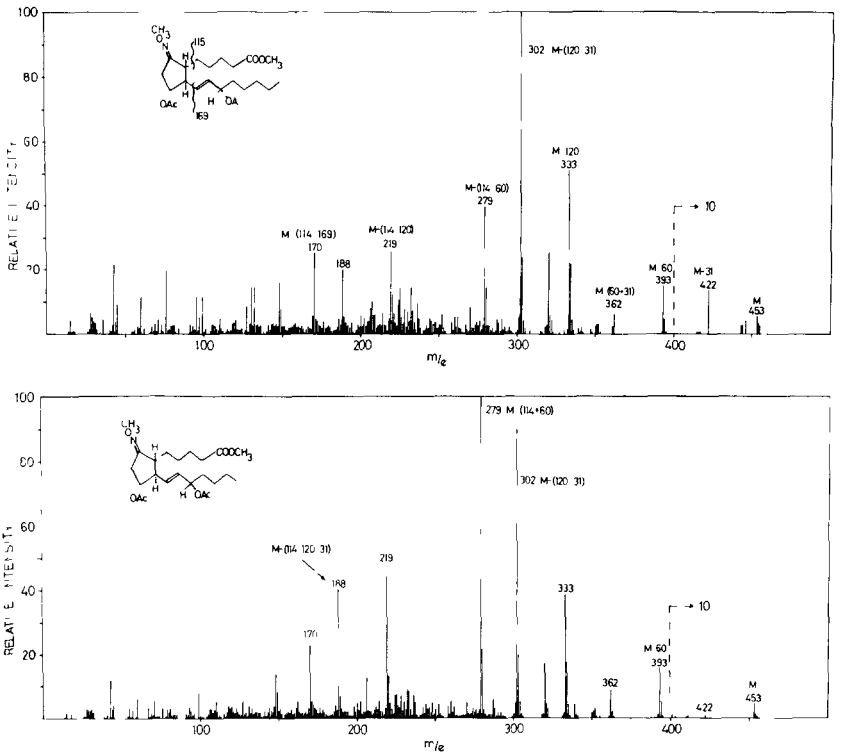

Fig 9 Mass spectra recorded on the first (upper) and second (lower) GLC peak of the MO—Ac derivative of the methyl ester of dinor-PGE_1 The scale is enlarged ten times above *m/e* 399

of 31 (*m/e* 437), 60 (*m/e* 408) and 60+31 (*m/e* 377) are present The ion at *m/e* 381 is probably formed by cleavage between C-16 and C-17 (β-cleavage) and transfer of hydrogen from C-18 (γ-transfer) to the nitrogen atom of the methoxime group and elimination of the OCH_3 group (31) from the methoxime This fragmentation which is seen for all di-methoximes in this study, is of great diagnostic value for locating a methoxime group The ion at *m/e* 321 is presumably formed by elimination of 31 (OCH_3), 71 ($(CH_2)_4$-CH_3) and one methoxime group (45) Loss of the C-8 side chain minus one hydrogen (142) and one methoxy group (31) gives rise to an ion at *m/e* 295 and additional loss of 60 forms the ion at *m/e* 235 The C-12 side chain (156) is most likely eliminated when the ions at *m/e* 266 (M-(156+31+15)) and *m/e* 252 (M-(156+60)) are formed The mass spectra of the derivatives of 9α-hydroxy-7,13,-diketo-dinorprostanoic acid and 7α-hydroxy-5,11-diketo-tetranorprostanoic acid are shown in figs 11 and 12 respectively and some pertinent data

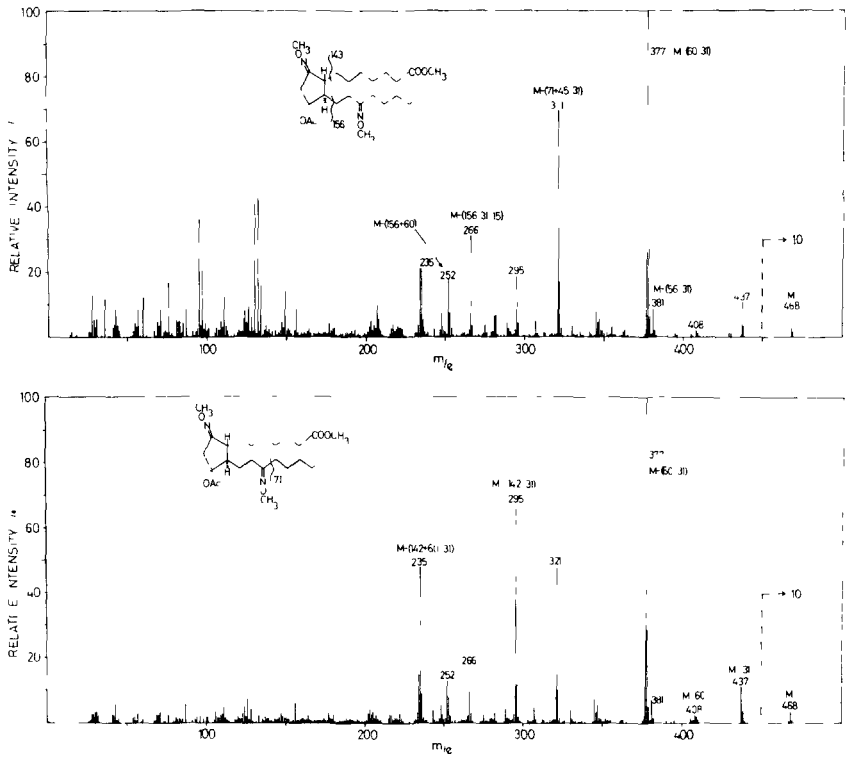

Fig 10 Mass spectra recorded on the first (upper) and second (lower) GLC peak of the MO - Ac derivative of the methyl ester of 11α-hydroxy-9,15-diketoprostanoic acid The scale is enlarged ten times above *m/e* 449

have been summarized in table 3 The mass spectra of the C_{18} and C_{16} homologues show several ions which appear at *m/e* values which are 28 and 56 mass units respectively below corresponding ions in the spectra of the C_{20} homologue These ions are due to eliminations involving a methoxy group, acetic acid, a methoxime group, the terminal five or four carbon atoms and the complete methyl end side chain (see table 3)

However, the ions at *m/e* 295 and 235 are common for both the C_{18} and C_{20} derivatives These ions are presumably formed by elimination of the side chain containing the carbomethoxy group except for one hydrogen which is transferred to the charge retaining part of the molecule The failure of the C_{16} derivative to undergo a similar rearrangement is presumably due to the proximity of the carbomethoxy group to the hydrogen to be transferred In the mass spectra of the C_{16} derivative an ion is seen at *m/e* 234 instead

The present work demonstrates the usefulness of methoxime derivatives

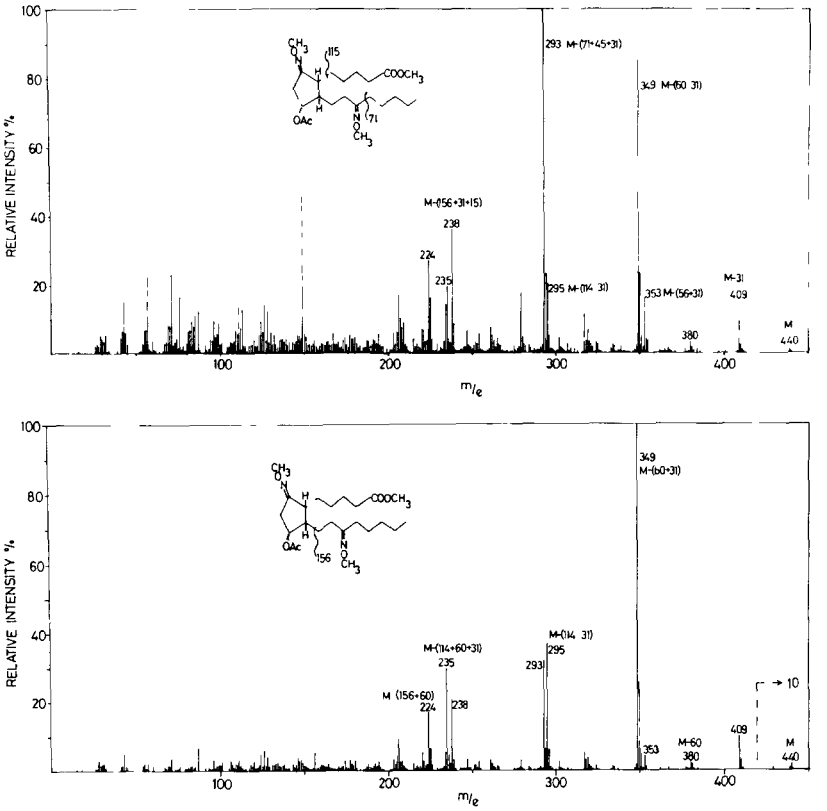

Fig 11 Mass spectra recorded on the first (upper) and second (lower) GLC peak of the MO – Ac derivative of the methyl ester of 9α-hydroxy-7,13-diketo-dinorprostanoic acid The scale is enlarged ten times above *m/e* 419 in the lower spectrum

for analysis by gas chromatography and mass spectrometry of prostaglandins containing keto groups With these derivatives extensive thermal degradation can be avoided and on electrone impact the molecular ion is generally seen The mass spectra provide information on the nature of functional groups, the location of the methoxime group(s) and the nature of substituents attached

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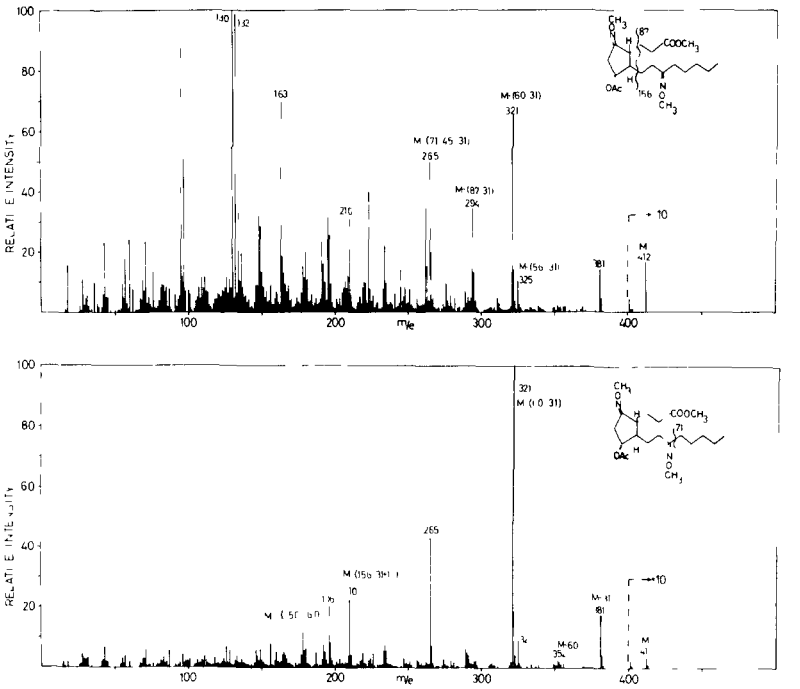

Fig 12 Mass spectra recorded on the first (upper) and second (lower) GLC peak of the MO—Ac derivative of the methyl ester of 7α-hydroxy-5,11-diketo-tetranorprostanoic acid The scale is enlarged ten times above *m/e* 399

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