(25 mg) was treated with Urushibara nickel A (about 0.8 g) as noted above for the preparation of bufalin by method C. By this means 4.6 mg of bufalin acetate (**3e**), mp 237–240°, was obtained.

The samples of bufalin acetate prepared by methods A–C were found identical[10] with an authentic specimen:[11] mass spectrum M^+ 462, 444 ($M^+ - H_2O$), 426 ($M^+ - HCl$), 402 ($M^+ - AcOH$), 384 ($M^+ - H_2O - AcOH$), 366 ($M^+ - AcOH - HCl$), and 348 ($M^+ - H_2O - AcOH - HCl$); uv λ_{max} 299 mμ (log ε 3.48 in methanol); ir ν_{max}^{KBr} 3550 (OH), 1730, 1696 (conjugated CO), 1632, 1540 (conjugated C=C), 1264, 1240, 1228 (CO), 950, 745 (C=C), and 720 cm^{-1} (Cl); pmr (in deuteriochloroform) δ 0.74 (18-methyl), 0.92 (19-methyl), 2.06 (s, 3β-acetoxyl), 2.47 (d, J = 3 Hz, 16-protons), 4.32 (d, J = 3 Hz, 15β-proton), 5.15 (s, 3α-proton), 6.35 (d, J = 10 Hz, 23-proton), 7.34 (d, J = 2.5 Hz, 21-proton), and 7.58 (q, J = 10 and 2.5 Hz, 22-proton).

Anal. Calcd for $C_{26}H_{35}O_5Cl$: C, 67.45; H, 7.62; Cl, 7.66. Found: C, 67.55; H, 7.65; Cl, 7.51.

Resibufogenin (2a). Method A. From Chlorohydrin 3d.—A solution prepared from chlorohydrin **3d** (22 mg) and freshly distilled α-collidine (2.5 ml) was heated at reflux for 4.5 hr. The crude product obtained by removal (under reduced pressure) of solvent was chromatographed on a column of silica gel. The fraction eluted with 5:1 ligroin–acetone was recrystallized from acetone–*n*-hexane to provide 15.8 mg of resibufogenin (**2a**) with a characteristic double melting point, 108–121 and 149–168°. An earlier[4a] specimen of resibufogenin prepared in our laboratory was found to melt at 110–121 and 148–168°. Both specimens were mutually identical.[10]

Method B. Hydrolysis of Resibufogenin Acetate (2b).—A 28-mg sample of resibufogenin acetate (**2b**) was hydrolyzed in ethanol (18 ml)–water (2 ml) with 0.3 g of Amberlite CG-120 (H^+ form) as summarized above for the hydrolysis of bufalin acetate. The preparative thin layer corresponding to R_f 0.42 was eluted and recrystallized from acetone–*n*-hexane to provide 18 mg of resibufogenin as plates melting at 109–122 and 147–167°. The products of methods A and B were mutually identical.[10]

Resibufogenin Acetate (2b).—Dehydrohalogenation of chlorohydrin acetate **3h** (20 mg) with α-collidine (2.4 ml) was performed as outlined above for the synthesis of resibufogenin. The product was chromatographed on a column of silica gel and the fraction eluted with 8:1 ligroin–acetone was recrystallized from acetone to afford 14.4 mg of resibufogenin acetate (**2b**), as needles melting at 226–228°, identical[10] with a specimen obtained by acetylating natural resibufogenin.

Registry No.—**1a**, 7439-77-2; **2a**, 465-39-4; **2b**, 4029-64-5; **3a**, 465-21-4; **3b**, 39707-10-3; **3c**, 39707-11-4; **3d**, 39707-12-5; **3e**, 4029-66-7; **3f**, 39707-14-7; **3g**, 39707-15-8; **3h**, 39707-16-9; **4b**, 24183-19-5.

Acknowledgment.—This investigation was supported by Public Health Research Grants CA-10612-04 and CA-10612-05 from the National Cancer Institute.

Mass Spectra of Prostaglandins. III. Trimethylsilyl and Alkyl Oxime–Trimethylsilyl Derivatives of Prostaglandins of the E Series[1]

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Received November 9, 1972

The mass spectra of the trimethylsilyl ester–trimethylsilyl ether derivatives of prostaglandins E_1 and E_2 and 8-isoprostaglandin E_2 and of their *O*-methyl oximes are reported and discussed. The high resolution spectra of these compounds are also considered. These spectra are compared with those of the analogous *O*-ethyl oximes and of the corresponding d_9-trimethylsilyl ether–d_9-trimethylsilyl ester and selectively labeled trimethylsilyl ester–d_9-trimethylsilyl ether derivatives. The 11-trimethylsilyloxy substituent had a strong fragmentation-directing influence on the molecular ion, and it was found that there was a marked stereochemical influence on fragmentation. Multiple origins were found for ions of several nominal masses; the most notable were those of *m/e* 199 and *m/e* 173. Ions of *m/e* 217 and *m/e* 204 were found to be formed by relatively long-range migrations of trimethylsilyl groups.

Prostaglandins of the E series are widely distributed in body fluids of man and of many other animals.[3] The 1,3-ketol moiety of the ring of these compounds is particularly unstable. The trimethylsilyl (TMS) derivatives were found to undergo partial decomposition during gas chromatography (gc),[4] but "clean" spectra of these derivatives could be obtained by combined gas chromatography–mass spectrometry (gc–ms). The oxime–TMS derivatives[5] were much more stable. In continuation of our studies of prostaglandin mass spectrometry,[1,6] we now report on the mass spectra of these derivatives of prostaglandins of the E series. All elemental compositions were compatible with high resolution data.

(1) For paper II, see B. S. Middleditch and D. M. Desiderio, *Prostaglandins*, in press.

(2) Fellow of the Intra-Science Research Foundation, 1971–1975.

(3) B. Samuelsson in "Lipid Metabolism," S. J. Wakil, Ed., Academic Press, New York, N. Y., 1970, p 107.

(4) B. S. Middleditch and D. M. Desiderio, *Prostaglandins*, **2**, 115 (1972).

(5) F. Vane and M. G. Horning, *Anal. Lett.*, **2**, 357 (1969).

(6) Paper I, B. S. Middleditch and D. M. Desiderio, *Lipids*, in press.

Results and Discussion

As was the case for the oxime–TMS derivatives of prostaglandins of the A series,[6] it was found that those of the E series gave two peaks on gc, presumably the syn and anti isomers. It was reported that such derivatives of the synthetic 8-isoprostaglandins of the E series gave only one peak on gc.[5] We have, however, found that two peaks are obtained, although they are less well separated than those of the naturally occurring E series.[4] As before,[1,6] we have examined the spectra of methyl oxime (MO)–TMS and ethyl oxime (EO)–TMS derivatives, as well as those of the corresponding TMS-d_0 derivatives and of the selectively labeled TMS ester–d_9-TMS ether analogs. Because of space limitations, the spectra of the EO–TMS derivatives are not illustrated here, but have been submitted to the Mass Spectrometry Data Centre, A. W. R. E., Aldermaston, Berks, England.

The spectra of TMS derivatives of prostaglandins E_1 (I) and E_2 (II) and of 8-isoprostaglandin E_2 (III) are shown in Figures 1–3, respectively. The spectra

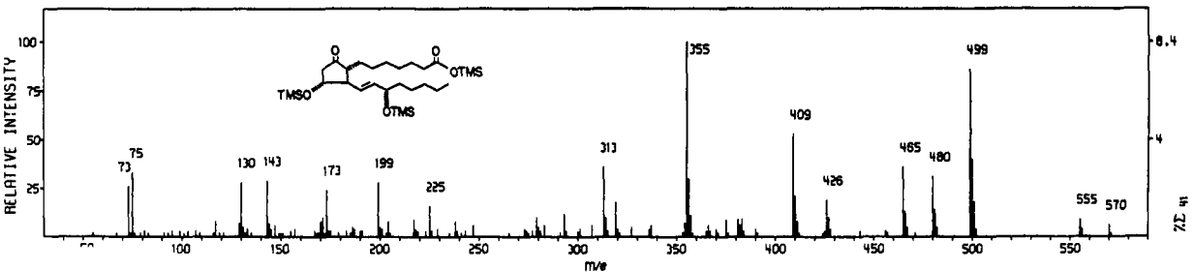


Figure 1.—Mass spectrum (22.5 eV) of TMS derivative of prostaglandin E_1 (I).

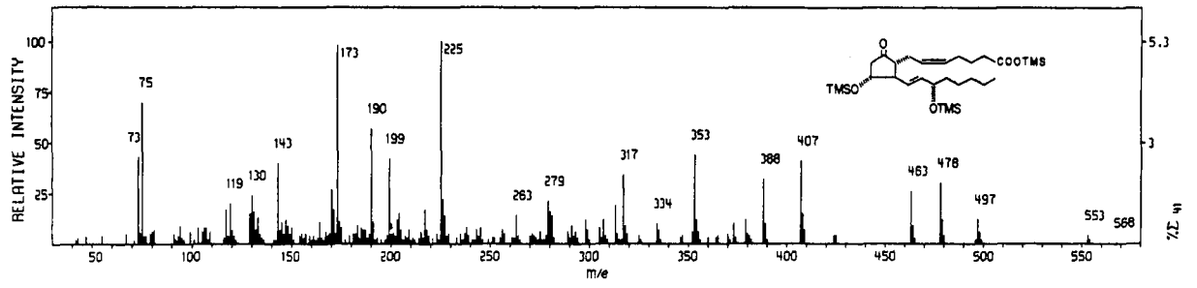


Figure 2.—Mass spectrum (22.5 eV) of TMS derivative of prostaglandin E_2 (II).

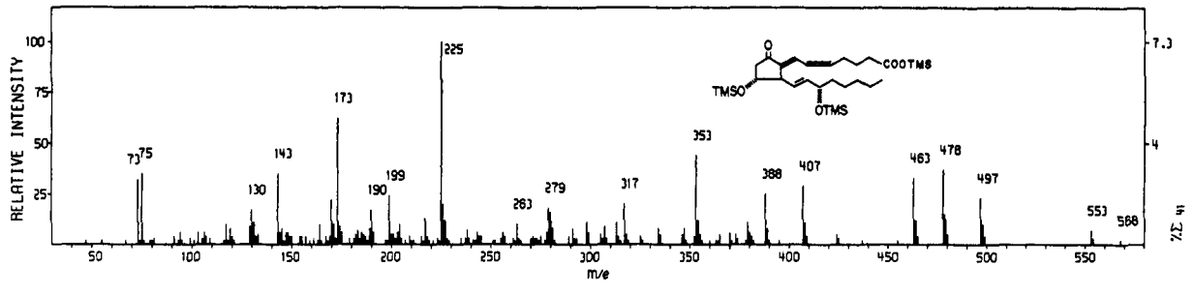
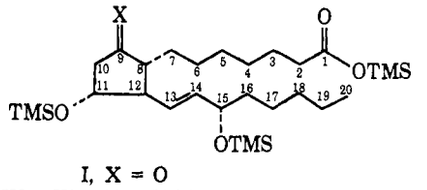


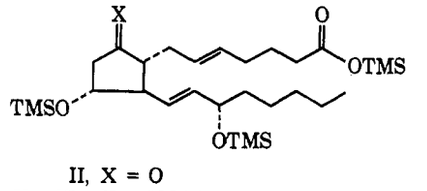
Figure 3.—Mass spectrum (22.5 eV) of TMS derivative of 8-isoprostaglandin E_2 (III).

of the corresponding MO–TMS derivatives (IV–VI) are shown in Figures 4–6. The syn and anti isomers are referred to in the same manner as those of the A series.[6] For example, the first isomer eluted during gas chromatography on SE-30 of the MO–TMS derivatives of prostaglandin E_1 is designated IVa (and the spectrum shown in Figure 4, top) and the second, IVb (Figure 4, bottom).

There is little difference between the spectra of the TMS derivative of the naturally occurring prostaglandin E_2 and that of the corresponding synthetic 8-isoprostaglandin E_2. Also, when the spectra of the MO–TMS derivatives of the various isomeric prostaglandins of the E_2 series are compared, it is found that the spectrum of Vb is somewhat similar to that of VIa. Quantitatively, the spectrum of Va is quite different from that of VIb. Nevertheless, the spectra of the MO–TMS derivatives of E_1 prostaglandins are qualitatively alike, as are those of the E_2 prostaglandins. For the sake of brevity in the ensuing discussion, only the spectra of IVa (Figure 4, top) and Va (Figure 5, top) will be considered in detail, although significant quantitative differences in the spectra will not be ignored.



I, X = O
IVa, IVb, X = NOMe (syn-anti isomers)



II, X = O
Va, Vb, X = NOMe (syn-anti isomers)

The molecular ions of neither the TMS derivatives nor the MO–TMS derivatives are very intense. Because of the low relative intensities of the ions involved, it is difficult to ascertain the origins of the methyl radicals lost in the formation of $[M - 15]^+$ ions in the spectra of the TMS derivatives, although d_9-TMS labeling shows that they all originate from TMS groups. In the case of the MO–TMS derivatives, however, it is found that IVa fragments by loss of a methyl radical from an ether TMS group, whereas Va loses methyl radicals mainly (80%) from the ester TMS group.

The molecules of trimethylsilanol eliminated from the molecular ions in the formation of $[M - 90]\cdot^+$ peaks have various origins. They are lost exclusively from ether TMS groups of I, but 10% are from the ester

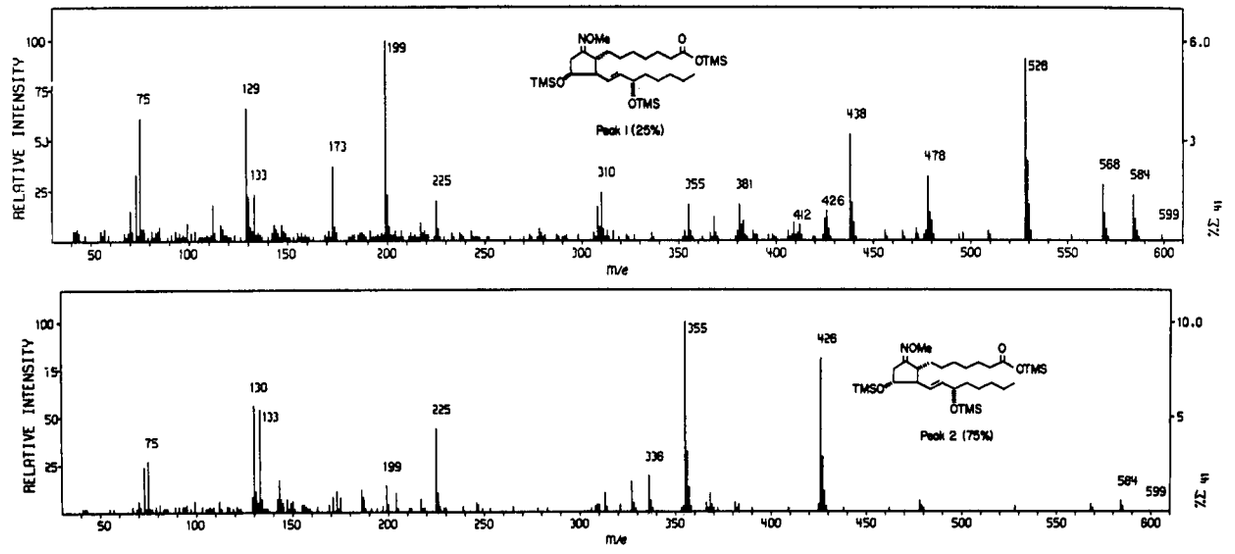


Figure 4.—Mass spectra (22.5 eV) of MO–TMS derivatives of prostaglandin E_1: first (IVa) and second (IVb) gc peaks.

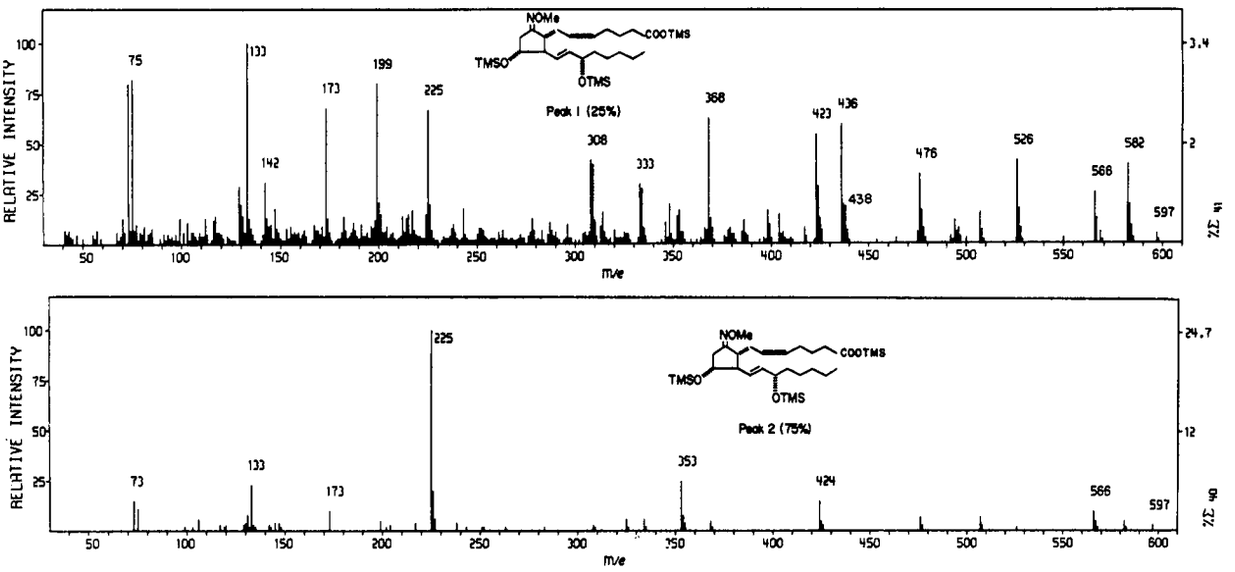


Figure 5.—Mass spectra (22.5 eV) of MO-TMS derivatives of prostaglandin E_2: first (Va) and second (Vb) gc peaks.

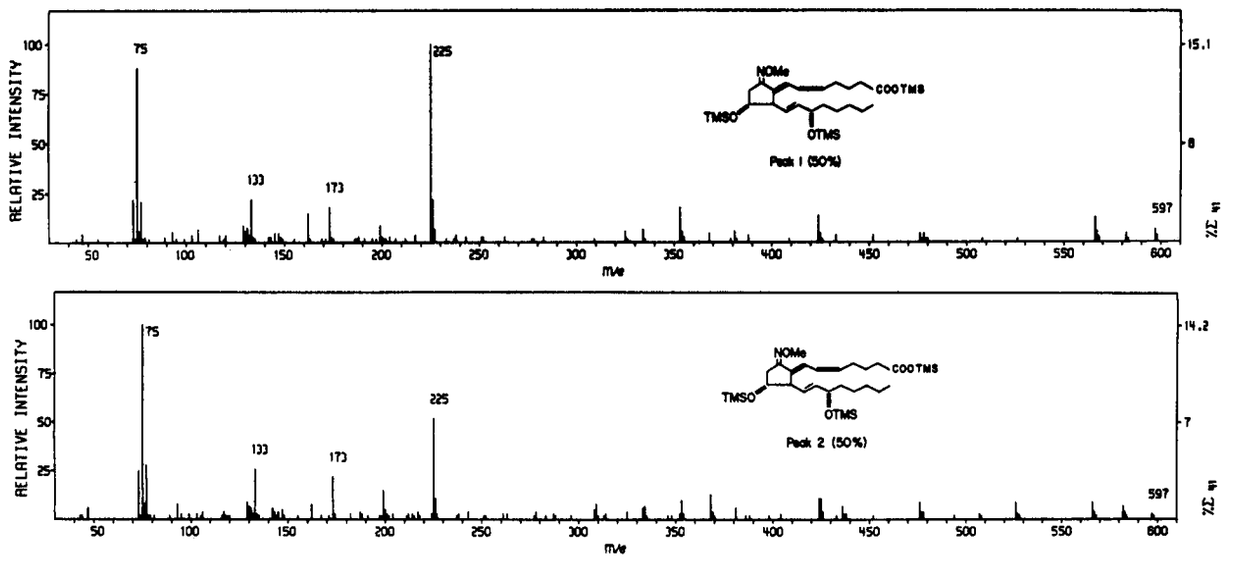
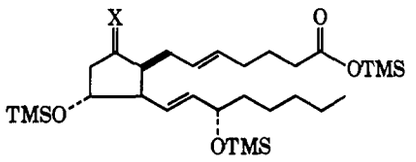
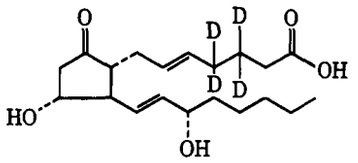


Figure 6.—Mass spectra (22.5 eV) of MO–TMS derivatives of 8-isoprostaglandin E_2: first (VIa) and second (VIb) gc peaks.

TMS group of II. Examination of the spectrum of the TMS derivative of *3,3,4,4-d*$_4$-prostaglandin E_2 (VII, kindly provided by U. Axen[7]) shows that, in



IV, X = O
VIa, VIb, X = NOMe (syn-anti isomers)



VII

the latter case, the hydrogen atom eliminated with the trimethylsilyloxy group originates from C-3 or C-4; Djerassi and co-workers have found (for TMS ethers of cyclohexyl derivatives) that trimethylsilanol may be eliminated *via* a 1,4 process.[8] The $[M - 90]\cdot^+$ ion is weak in the spectrum of IVa, but it can clearly be seen that, in the spectra of labeled analogs of IVa, it is formed by loss of trimethylsilanol from an ether TMS group. A second elimination of trimethylsilanol (also from an ether TMS group) gives rise to an abundant ion only in the spectrum of II (*m/e* 388, 32%). Only weak ions are formed by sequential eliminations of trimethylsilanol in other spectra; these are accompanied also by ions resulting from additional losses of methyl radicals.

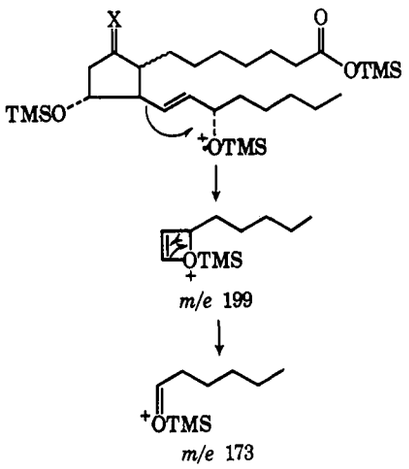
Further fragmentations of the C-1/7 and C-13/20 chains and the C-8/12 ring, and fragmentations directed by the *O*-methyl oxime group will be discussed in turn.

Fragmentations of the C-1/7 Chain.—No major fragment ions are formed by direct fragmentation of this chain. Weak ions are present in the spectra of all of the TMS derivatives which appear to be formed by scission of the C-7/8 bond of the $[M - 90]\cdot^+$ ion formed by loss of an ether TMS group (*m/e* 279). The ions of *m/e* 398 in the spectra of the MO–TMS derivatives appear also to be formed by scission of the C-7/8 bond. These ions were found to contain neither an ester TMS group nor any hydrogen atoms from C-3 or C-4. Further loss of trimethylsilanol leads to the formation of ions of *m/e* 308.

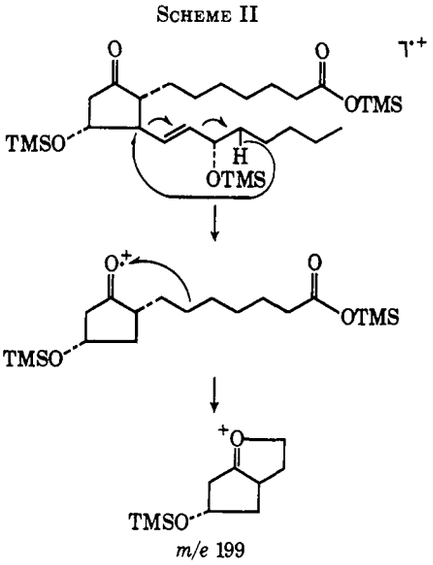
The ion of *m/e* 438 in the spectrum of Va was found not to contain an ester TMS group or hydrogen atoms from C-3 or C-4. It is probably formed by loss of C-1/4, with substituents, from the molecular ion. The rather weak ion of *m/e* 412 in the spectrum of IVa may be produced by α cleavage to the oxime group[9] of the C-6/7 bond.

It was found that, in the spectra of TMS and MO–TMS derivatives of prostaglandins of the A[6] and B[1] series, ions of *m/e* 199 and *m/e* 173 were formed, presumably as shown in Scheme I. Careful examination of the high resolution spectra of derivatives of prostaglandins of the E series, and of the low resolution spectra of labeled analogs, reveals that such ions are also formed by fragmentation of the C-1/7 chain of these compounds. In addition to the ion at *m/e* 199 of Scheme I, the spectra of TMS derivatives of all of the prostaglandins of the E series were found to contain ions of *m/e* 199 with elemental composition $C_{10}H_{19}O_2Si$. This ion in the spectra of the TMS derivatives of prostaglandins E_1 was found to contain an ether TMS group and may be formed as shown in Scheme II.

SCHEME I



SCHEME II

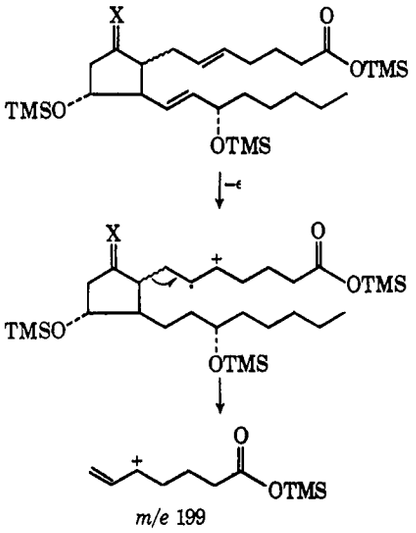


Those in the spectra of the TMS derivatives of prostaglandins E_2 and 8-isoprostaglandin E_2 were, however, found to contain an ester TMS group, and may be formed as in Scheme III. Also, ions of the MO–TMS derivatives of prostaglandins E_2 and 8-isoprostaglandin E_2 were observed which apparently comprise C-1/7 with substituents and are formed as shown in Scheme III.

It was found that the ions of *m/e* 173 in the spectra of TMS and MO–TMS derivatives of prostaglandins E_1 were doublets. The major component ($C_9H_{21}OSi$) was formed as in Scheme I, but the minor component ($C_8H_{17}O_2Si$) was found to contain an ester TMS group. The latter could be formed as shown in Scheme IV.

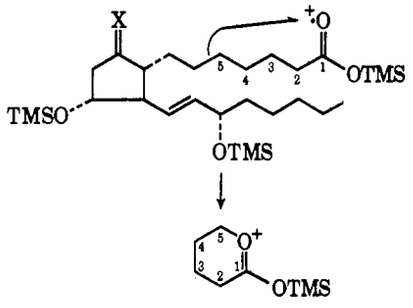
(7) U. Axen, K. Gréen, D. Hörlin, and B. Samuelsson, *Biochem. Biophys. Res. Comm.*, **45**, 519 (1971).
(8) P. D. Woodgate, R. T. Gray, and C. Djerassi, *Org. Mass Spectrom.*, **4**, 257 (1970).
(9) B. S. Middleditch and B. A. Knights, *Org. Mass Spectrom.*, **6**, 179 (1972).

SCHEME III



m/e 199

SCHEME IV



m/e 173

Fragmentations of the C-13/20 Chain.—Scission of the C-15/16 bond, directed by the 15-trimethylsilyloxy group, gives rise to moderately intense ions of type $[M - 71]^+$ in each of the spectra under consideration.[6] A series of ions is formed by successive eliminations of trimethylsilanol from the $[M - 71]^+$ ions.

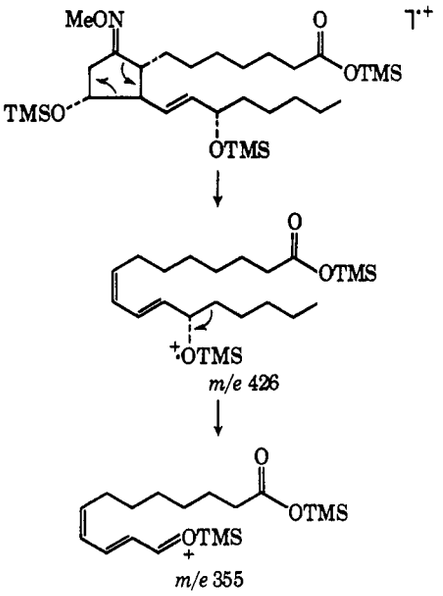
The genesis of ions of *m/e* 173 and *m/e* 199 has already been discussed.

It has been suggested that the ion of *m/e* 426 in the spectrum of the MO–TMS derivative of prostaglandin E_1 (IVa) is formed by loss of C-1/5, with substituents, and that this ion can further lose C_5H_{11} from C-16/20 to afford the ion of *m/e* 355.[5] However, high resolution mass measurement shows that the ion of *m/e* 426 has composition $C_{23}H_{46}O_3Si_2$ (calcd 426.2985, found 426.2986) and that of *m/e* 355 has composition $C_{18}H_{35}O_3Si_2$ (calcd 355.2125, found 355.2138). The corresponding pair of ions is not shifted in mass in the spectra of the EO–TMS derivative. It seems likely that the ion of *m/e* 426 is formed by cleavage of the ring and that the ion of *m/e* 355 is produced by subsequent loss of C_5H_{11} (corresponding to C-16/20) as depicted in Scheme V. A similar fragmentation mode was proposed for the MO–TMS derivative of the methyl ester of prostaglandin E_1.[10]

Fragmentations of the C-8/12 Ring.—Whereas the rings of derivatives of prostaglandins of the A and B series were relatively stable,[1,6] the 11-trimethylsilyloxy group in derivatives of the E series has a strong fragmentation-inducing influence.

(10) K. Gréen, *Chem. Phys. Lipids*, **3**, 254 (1969).

SCHEME V

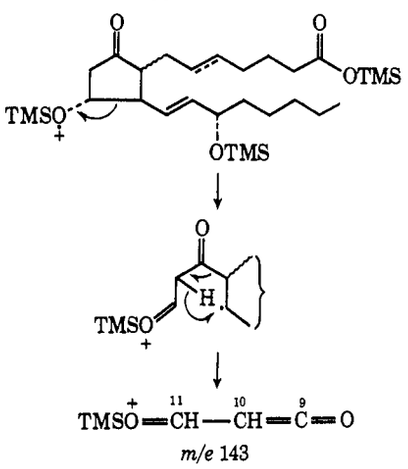


m/e 426

m/e 355

A number of ions are formed *via* initial cleavage of the C-11/12 bond. This bond is particularly weak by virtue of its being α to the 11-trimethylsilyloxy group, β to the C-13/14 bond, and (in the case of the MO–TMS derivatives) γ to the oxime moiety. Spectra of the TMS derivatives contain ions of *m/e* 143 which are partially due to fragmentation of the ring ($C_6H_{11}O_2Si$: calcd 143.0528, found 143.0530) as shown in Scheme VI and partialy due to minor components

SCHEME VI



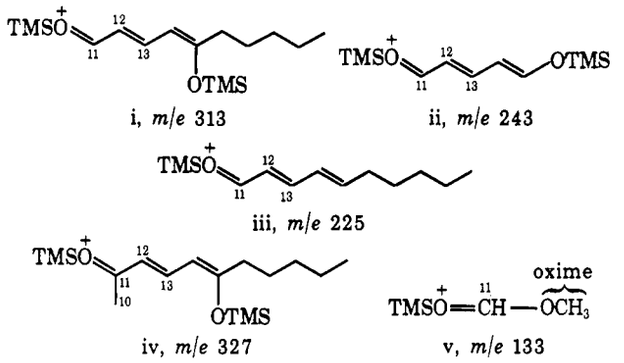
m/e 143

having composition $C_7H_{15}OSi$ (calcd 143.0892, found 143.0882) and containing an ether TMS group. Complementary ions, formed by loss of C-9/11, were observed at *m/e* 426 (E_1 series) or *m/e* 424 (E_2 series) in the spectra of TMS and MO–TMS derivatives (Scheme V).

Numerous fragment ions are formed by eliminations of trimethylsilanol from the ions of *m/e* 426 and 424 (at *m/e* 336, 334, 246, 244) and from the ions of *m/e* 355 and 353 formed by loss of C_5H_{11} (at *m/e* 265, 263).

A second category of ions formed by cleavage of the ring requires initial cleavage of the C-10/11 bond. The driving force for the formation of fragment ions in this manner is apparently the ease of

formation of highly conjugated ions promoted by juxtaposition of the 11- and 15-trimethylsilyloxy groups. The elemental compositions of ions i–iii are all compatible with the high resolution data and with the spectra of labeled analogs.

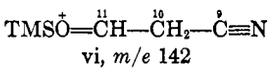


The ion of *m/e* 327 (iv) appears to be formed by loss of C-1/9 from the molecular ions.

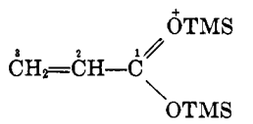
An interesting ion is present in the spectra of the MO–TMS derivatives at *m/e* 133 and of the EO–TMS derivatives at *m/e* 147. The former has composition $C_5H_{13}O_2Si$ (calcd 133.0685, found 133.0663). These data suggest that the ion is formed by migration of the oxime alkoxy group to C-11 in the formation of an ion of type v.

Fragmentations Directed by the *O*-Methyl Oxime Group.—As expected, the molecular ion may lose a methoxy radical from the oxime moiety to give an ion of type $[M - 31]^+$. This ion can undergo further fragmentation by, for example, elimination of molecules of trimethylsilanol. As in the case of the MO–TMS derivatives of prostaglandin A_1, losses of methanol as well as, or instead of, methoxy radicals sometimes take place in combination with other eliminations.[6]

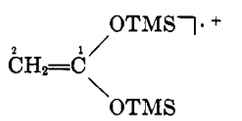
The ion of *m/e* 142 in the spectra of MO–TMS (and EO–TMS) derivatives has elemental composition $C_6H_{12}NOSi$ (calcd 142.0688, found 142.0716). It contains an ether TMS group, but none of the hydrogen atoms at C-3 or C-4. It is probably formed by fission of the ring and loss of the oxime alkoxy group, leading to a structure such as vi.



vi, *m/e* 142



vii, *m/e* 217



viii, *m/e* 204

Fragmentations Influenced by Remote Interactions of TMS Groups.—The influences of remote interactions of TMS groups on mass spectral fragmentation modes are well known.[11] None were observed in the spectra of derivatives of prostaglandins of the A and B series,[1,6] but two such ions are seen in many of the spectra of prostaglandins of the E series, the first at *m/e* 217, the second at *m/e* 204. d_9-TMS labeling shows that both contain two TMS groups and selective d_9-TMS labeling indicates that, in each case, one of the TMS groups derives from the ester moiety. In the spectra of the MO–TMS derivatives of *3,3,4,4-d*$_4$-prostaglandin E_2 (VII), the former ion is shifted to *m/e* 219, whereas the latter remains at *m/e* 204. These ions, then, probably have structures vii and viii.

(11) For leading references, see C. J. W. Brooks and B. S. Middleditch in "Modern Methods of Steroid Analysis," E. Heftmann, Ed., Academic Press, New York, N. Y., in press.

Conclusions

The mass spectra both of the TMS and of the MO–TMS derivatives of prostaglandins of the E series contain many relatively abundant fragment ions. Nevertheless, the majority have been interpreted satisfactorily by comparison with the spectra of labeled analogs and by high resolution measurement. We have found the technique of selective d_9-TMS labeling to be of particular value in these studies when it has been necessary to distinguish between ether and ester TMS groups in fragment ions. In this manner we have been able to recognize several ions formed by long-range TMS migrations.

This discussion would be incomplete without consideration of the effect of stereochemistry on fragmentation, although we have demonstrated that the various isomeric prostaglandin derivatives can be distinguished by gas chromatography.[4] The spectrum of the second gc peak of the MO–TMS derivative of prostaglandin E_1 (IVb, Figure 4, bottom) is dominated by ions of *m/e* 426 and *m/e* 355 (whereas this is not the case in IVa). It has been demonstrated that these ions arise by cleavage of the ring (Scheme V). It could be suggested that the rationale for the ease of fragmentation of the ring is the adjacency of the methoxy group with the side chain since only one of the syn–anti isomers fragments in this manner with such ease. It might then be argued that, on the mass spectrometric evidence, the order of elution of the syn–anti isomer pair is reversed for the 8 isomer. At this stage, however, we feel that such speculation is unwarranted because we are unable to identify unequivocally the syn and anti isomers.

Experimental Section

Prostaglandins were kindly provided by J. E. Pike and U. Axen of The Upjohn Co., Kalamazoo, Mich., and by K. Sano of Ono Pharmaceutical Company, Osaka, Japan. **Derivatives** were prepared as previously described.[6] **Mass spectrometry** was performed using LKB 9000 (low resolution, gc-ms) and CEC 21-110B (high resolution, direct insertion probe) instruments as previously described.[6]

Registry No.—I, 39003-19-5; II, 39003-20-8; III, 39003-21-9; IVa (9*Z* isomer), 39003-22-0; IVb (9*E* isomer), 39062-24-3; Va (9*Z* isomer), 39003-23-1; Vb (9*E* isomer), 39003-24-2; VIa (9*Z* isomer), 39003-25-3; VIb (9*E* isomer), 39003-26-4.

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