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PLANT HORMONES—VIII.

COMBINED GAS CHROMATOGRAPHY–MASS SPECTROMETRY OF THE METHYL ESTERS OF GIBBERELLINS A_1 TO A_{24} AND THEIR TRIMETHYLSILYL ETHERS

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Abstract—Line diagrams of GC–MS low resolution mass spectra are presented for the methyl esters of gibberellins A_1 to A_{24} and for the trimethylsilyl ethers of the methyl esters of the hydroxylated gibberellins A_1 to A_8, A_{10}, A_{13}, A_{14}, and A_{16} to A_{23}. These reference spectra allow conclusive identification of the presently known gibberellins without access to authentic specimens. The utility of the mass spectra of these derivatives in structural diagnosis of the gibberellins is discussed. High resolution measurement of the composition of fragment ions in selected trimethylsilyl ethers are included.

COMBINED gas chromatography–mass spectrometry (GC–MS) has been shown to be an invaluable method of identifying known[1–3] and new[4] gibberellins in plant extracts. The method is sensitive and can be applied to crude extracts[1] although more detailed information is obtained[2,3] on partially purified fractions. A further advantage of the method is that the twenty-four known gibberellins can be conclusively identified without access to authentic specimens of the scarce gibberellins, provided reference mass spectra are available These reference spectra are presented in Figs. 1 and 2 for the methyl esters of gibberellins A_1 to A_{24} (MeA_1 to MeA_{24}) and for the trimethyl silyl ethers (TMSi ethers) of the methyl esters of the hydroxylated gibberellins A_1 to A_8, A_{10}, A_{13}, A_{14}, and A_{16} to A_{23}; the utility of the mass spectra of these derivatives in structural diagnosis of the gibberellins is presented below. For convenience the numbering of the gibbane nucleus is shown in (I).

Methyl Ester Trimethylsilyl Ethers (*Fig.* 1)

The spectra are distinct and characteristic for all hydroxylated gibberellins including those of isomeric composition. From a comparison with corresponding methyl esters the number of hydroxyl groups can be determined. Molecular ions (M^+) are observed in all the spectra and show the computed isotope peaks for the presence of one, two, or three silicon atoms. Other common features include fragment ions at M-31/32 and M-59/60 associated with the methoxycarbonyl group and M-15, M-89/90, *m/e* 73, and *m/e* 75, associated with the TMSi

[1] J. MACMILLAN, R. J. PRYCE, G. EGLINTON and A. MCCORMICK, *Tetrahedron Letters* 2241 (1967).
[2] R. J. PRYCE, J. MACMILLAN and A. MCCORMICK, *Tetrahedron Letters* 5009 (1967).
[3] J. MACMILLAN and R. J. PRYCE, *Tetrahedron Letters* 1537 (1968).
[4] R. J. PRYCE and J. MACMILLAN, *Tetrahedron Letters* 4173 (1967).

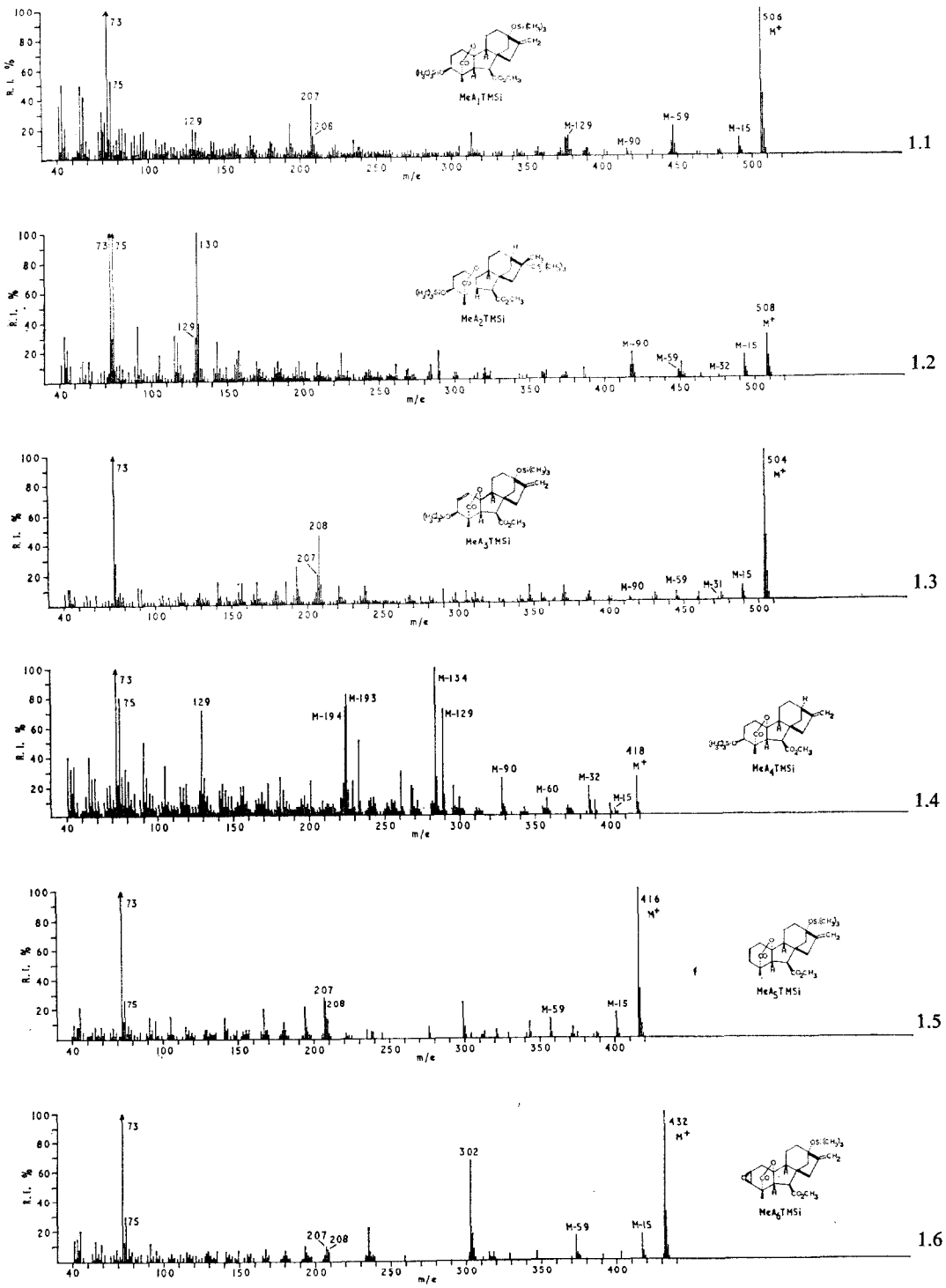
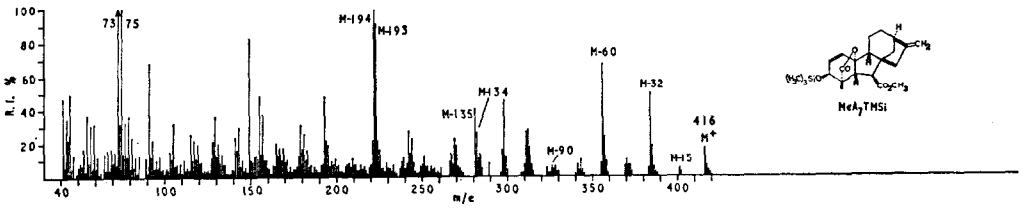


FIG. 1. MASS SPECTRA OF THE GIBBERELLIN METHYL ESTER TMSi ETHERS.



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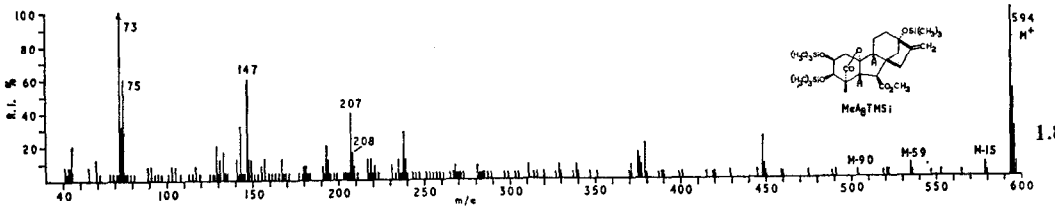

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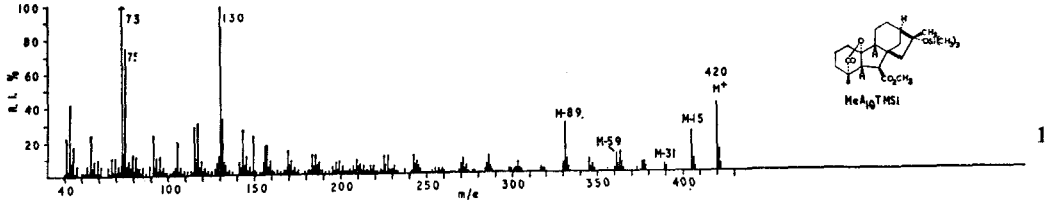

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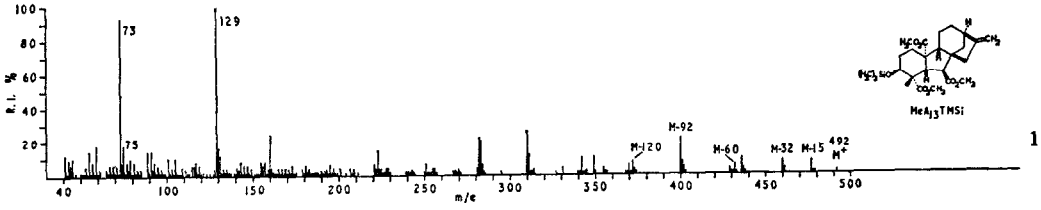

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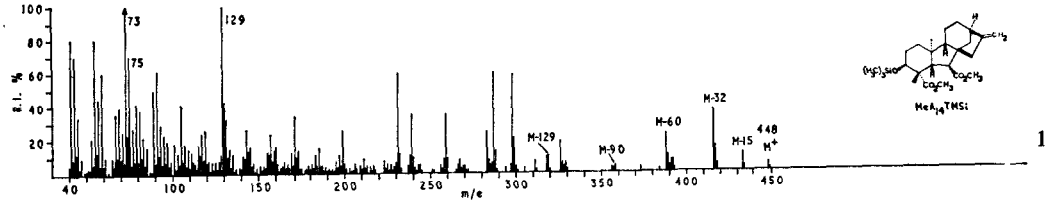

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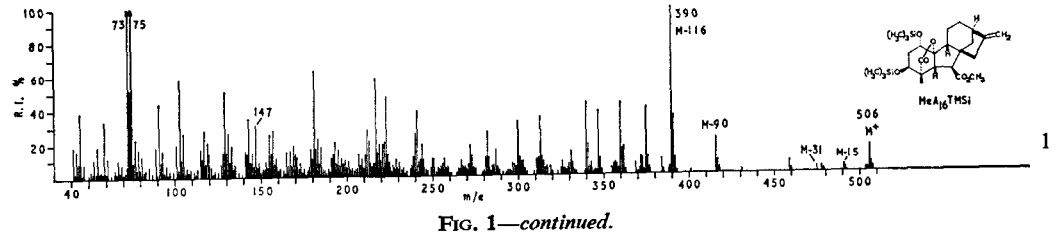

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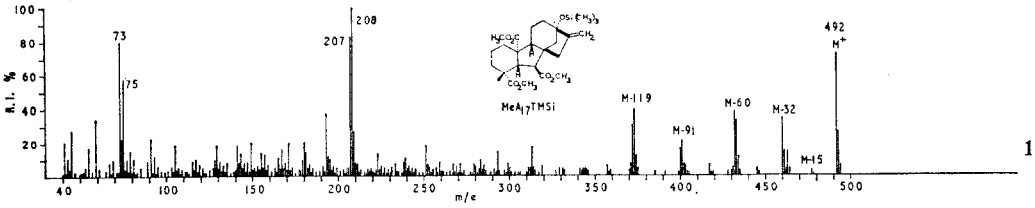
FIG. 1—*continued.*



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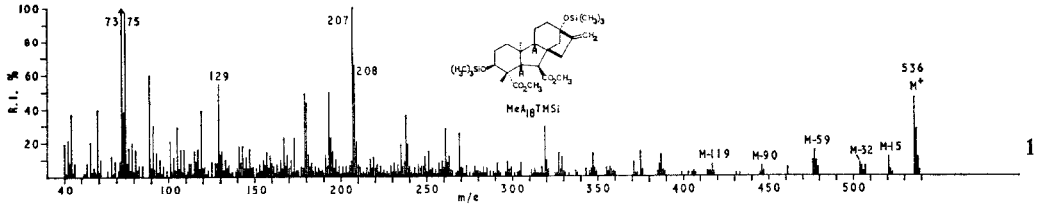

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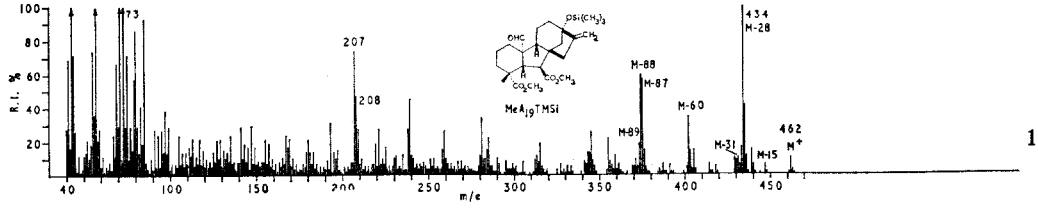

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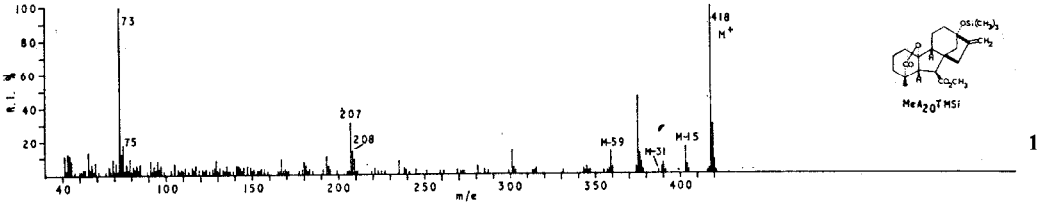

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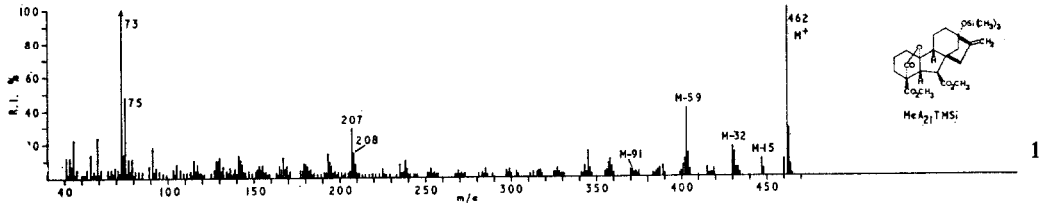

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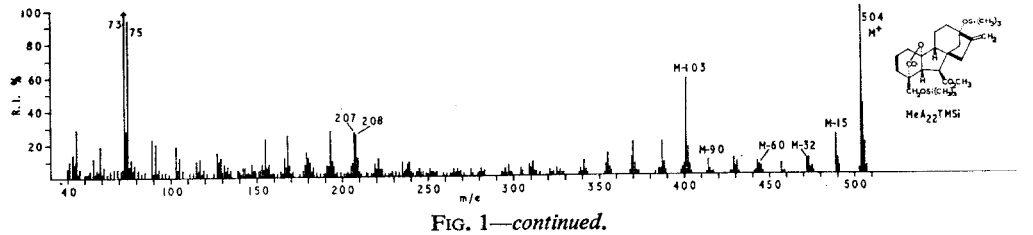

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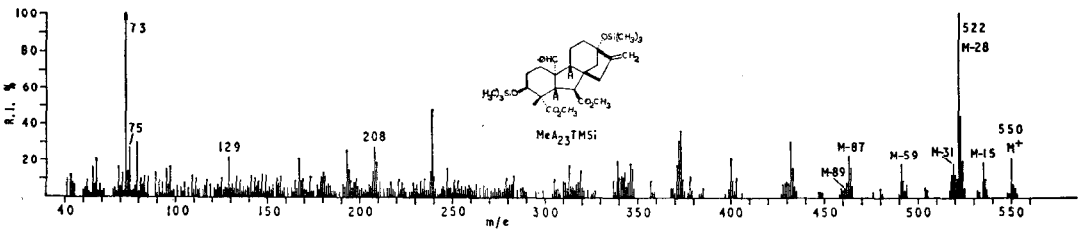
FIG. 1—*continued.*

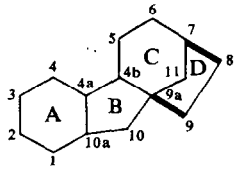


1.19

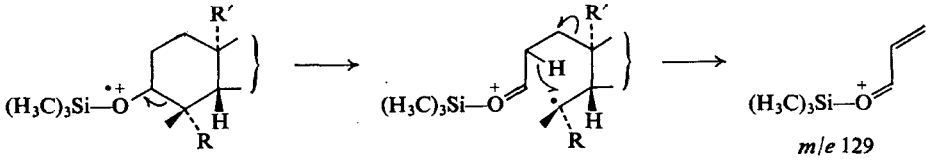
FIG. 1—*continued.*

ether group. In the spectra of the methyl ester TMSi ethers of the C_{20}-gibberellins and gibberellin A_{21} prominent peaks at M-90/91/92 are probably due to fragmentation of two methoxycarbonyl groups [M-(50/60+31/32)]. The ion at *m/e* 73 is very intense in all spectra and was not taken as the base peak for any of the line diagrams.

2-Hydroxygibberellins are characterized in the mass spectra of their methyl ester TMSi ethers by a prominent ion at *m/e* 129, shown to be $C_6H_{13}OSi$ in the case of MeA_4 TMSi. This ion, accompanied in some cases by an M-129 peak, presumably has the structure (II) arising from ring A by the process (III) → (IV) → (II) which is typical of such TMSi ethers.[5]



(I)



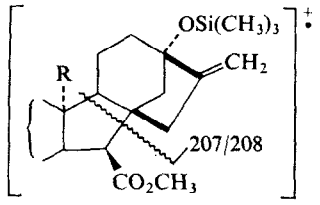
(III) (IV) (II)

The TMSi ethers of 7-hydroxygibberellin methyl esters are distinguished by an intense molecular ion which is the base peak in all the 7-TMSi ethers except those of gibberellins A_{17}, A_{18}, A_{19}, and A_{23}. In contrast, the molecular ions of the TMSi ethers of the 7-deoxygibberellins are of much lower intensity. This correlation is clearly illustrated by comparison of the isomeric TMSi ethers of gibberellins A_{13} (Fig. 1.10) and A_{17} (Fig. 1.13) methyl esters. This comparison also suggests a further distinction between these isomeric 2- and 7-monohydroxy-C_{20}-gibberellins, namely that the M-119/120 peak (see discussion of methyl ester spectra below) is stronger in the absence of the 2-TMSi ether grouping. Significant peaks at *m/e* 207/208, shown to be $C_{12}H_{19/20}OSi$ in the case of MeA_5 TMSi, are characteristic of the 7-TMSi ethers; they must contain rings C and D and probably arise by the cleavage shown in (V) with or without additional loss of hydrogen.

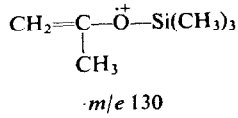
The methyl ester TMSi ethers of gibberellins A_2 (Fig. 1.2) and A_{10} (Fig. 1.9) have intense base peaks at *m/e* 130. This ion, shown to have the composition $C_6H_{14}OSi$ in the case of

[5] J. DIEKMAN and C. DJERASSI, *J. Org. Chem.* **32**, 1005 (1967).

MeA_{10} TMSi, is probably (VI) from cleavage of ring D and, therefore, diagnostic of an 8-TMSi ether.



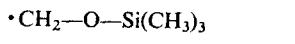
(V)



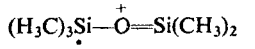
m/e 130

(VI)

The TMSi ether of the 1-hydroxymethyl group in gibberellin A_{22} is characterized by a peak at M-103 (Fig. 1.18); this ion corresponds to the loss of the fragment (VII).



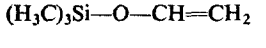
(VII)



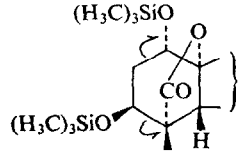
m/e 147

(VIII)

The vicinal 2,3-bis-TMSi ether of MeA_8 (Fig. 1.8) gives rise to an ion *m/e* 147 corresponding to (VIII); since this ion is observed in a spectrum obtained by GC–MS, it must come from rearrangement of the vicinal TMSi ether and cannot be the artefact from hexamethyldisilazane or trimethylsilyl chloride described by Budzikiewicz *et al.*[6] The bis-TMSi ether of MeA_{16} (Fig. 1.12) is unique in showing a base peak at M-116 ($C_{21}H_{30}O_5Si$) which could arise by loss of the fragment (IX) from ring A by the process suggested in (X).



(IX)



(X)

The TMSi ethers of MeA_{19} and MeA_{23} (Figs. 1.15 and 1.19 respectively) show an intense (base) peak at M-28 corresponding to the loss of CO from the aldehyde function. These two TMSi ethers also show prominent peaks at M-87/88 which are probably due to M-(28+59/60). Peaks of low intensity at M-28/29 are also observed in the spectra of the TMSi ethers of several non-aldehydic gibberellins but their origin is unknown.

The spectra of MeA_4 TMSi (Fig. 1.4) and MeA_7 TMSi (Fig. 1.7) are distinguished by intense peaks at M-134/135 (M-134 was shown to be $C_{15}H_{28}O_3Si$ in the case of MeA_4 TMSi and $C_{15}H_{26}O_3Si$ in the case of MeA_7 TMSi; M-135 was shown to be $C_{15}H_{25}O_3Si$ in the case of MeA_7 TMSi), and at M-193/194 ($C_{13}H_{24/25}OSi$ in the case of MeA_4 TMSi and $C_{23}H_{22/23}OSi$ in the case of MeA_7 TMSi). These fragment ions must contain the A rings of MeA_4 and MeA_7 TMSi ethers. Further, the M-134/135 and M-193/194 ions differ in composition by $C_2H_3O_2$ which could correspond to loss of a methoxycarbonyl group and suggests that the M-134/135 ions also contain the C-10 and the 10-methoxycarbonyl group.

[6] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Mass Spectrometry of Organic Compounds*, p. 476, Holden-Day, New York (1967).

High resolution measurements on the methyl ester TMSi ethers were carried out on an MS9 instrument by direct insertion of a portion of each reaction mixture of gibberellin methyl ester TMSi ether together with the silylating reagent (hexamethyldisilazane, trimethylsilyl chloride, and pyridine). During this work intense peaks arising from the silylating reagent were observed at *m/e* 130 ($C_4H_{12}NSi_2$) and *m/e* 147 ($C_5H_{15}OSi_2$). Only the latter of these two peaks has previously been noted[6] as being derived from the silylating reagent.

Methyl Esters (*Fig.* 2)

The mass spectra of alkyl esters of several C_{19}-gibberellins have been reported by two groups. Wulfson *et al.*[7,8] have discussed the low resolution spectra of the methyl and ethyl esters of gibberellins A_1, A_3, A_4, and A_7 while the high resolution spectra of the methyl

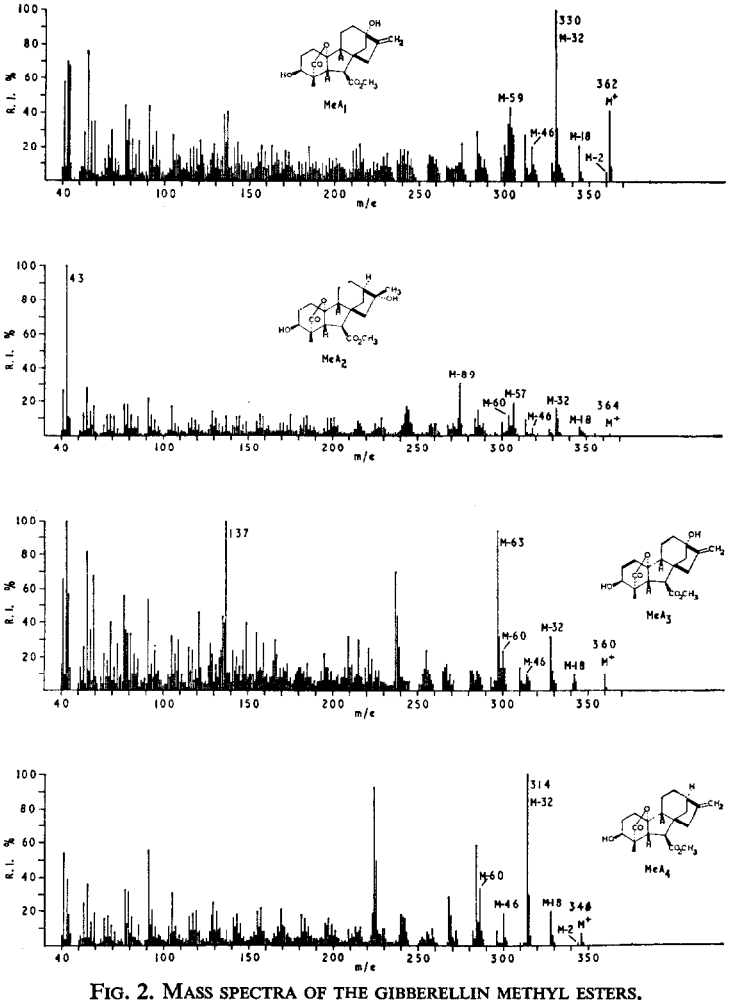


FIG. 2. MASS SPECTRA OF THE GIBBERELLIN METHYL ESTERS.

[7] N. S. WULFSON, V. I. ZARETSKII, I. B. PAPERNAJA, E. P. SEREBRYAKOV and V. F. KUCKEROV, *Tetrahedron Letters* 4209 (1965).

[8] V. I. ZORETSKII, N. S. WULFSON, I. B. PAPERNAJA, I. A. GURVICH, V. F. KUCHEROV, I. M. MILSTEIN, E. P. SEREBRYAKOV and A. V. SIMOLIN, *Tetrahedron* **24**, 2327 (1968).

esters of gibberellin A_1 to A_5, A_7 to A_9, and A_{20} have been discussed by Takahashi *et al.*[9] Some high resolution spectral data has been presented for the methyl esters of gibberellins A_{19},[10] A_{21},[11] A_{22},[11] and A_{24}[12] and there are some low resolution data available for gibberellin A_{23}[13] methyl ester. In general the present data confirm and extend the conclusions of the above workers although some modifications to their correlation of fragmentation patterns with structure are suggested.

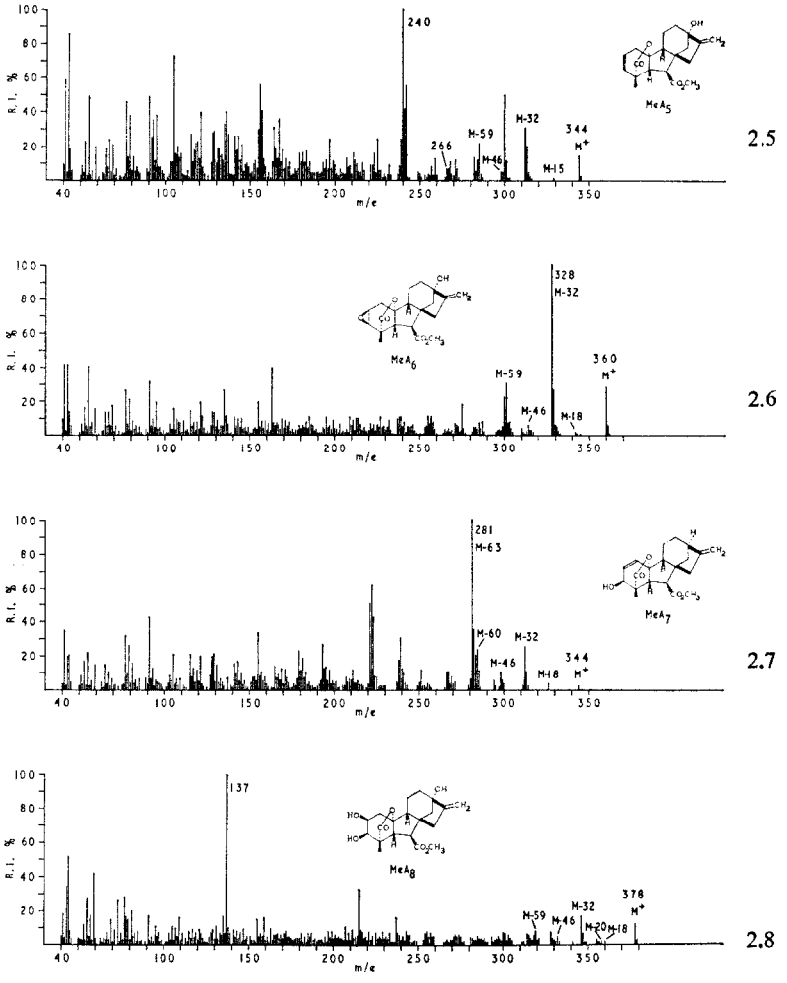


FIG. 2—*continued.*

[9] N. TAKAHASHI, N. MUROFUSHI, S. TAMURA, N. WASADA, H. HOSHINO, T. TSUCHIYA, T. AOYAMA and H. MORITA, *Tetrahedron Letters* 895 (1967).

[10] N. MUROFUSHI, S. IRUICHIJIMA, N. TAKAHASHI, S. TAMURA, J. KATO, Y. WADA, E. WATANABE and T. AOYAMA, *Agr. Biol. Chem. (Tokyo)* **30**, 917 (1966).

[11] N. TAKAHASHI, N. MUROFUSHI, T. YOKOTA, S. TAMURA, J. KATO and Y. SHIOTANI, *Tetrahedron Letters* 4861 (1967).

[12] D. M. HARRISON, J. MACMILLAN and R. H. B. GALT, *Tetrahedron Letters* 3137 (1968).

[13] K. KOSHIMIZU, H. FUKUI, M. INUI, Y. OGAWA and T. MITSUI, *Tetrahedron Letters* 1143 (1968).

The fragmentation of the methyl esters is less clearly diagnostic of structure than in the TMSi ethers. The loss of 31 and 32 and 59 and 60 mass units from the molecular ion, observed by the previous workers, is characteristic of methoxycarbonyl groups and is not very informative. The methyl esters of C_{20}-gibberellins possess two or three methoxycarbonyl groups and

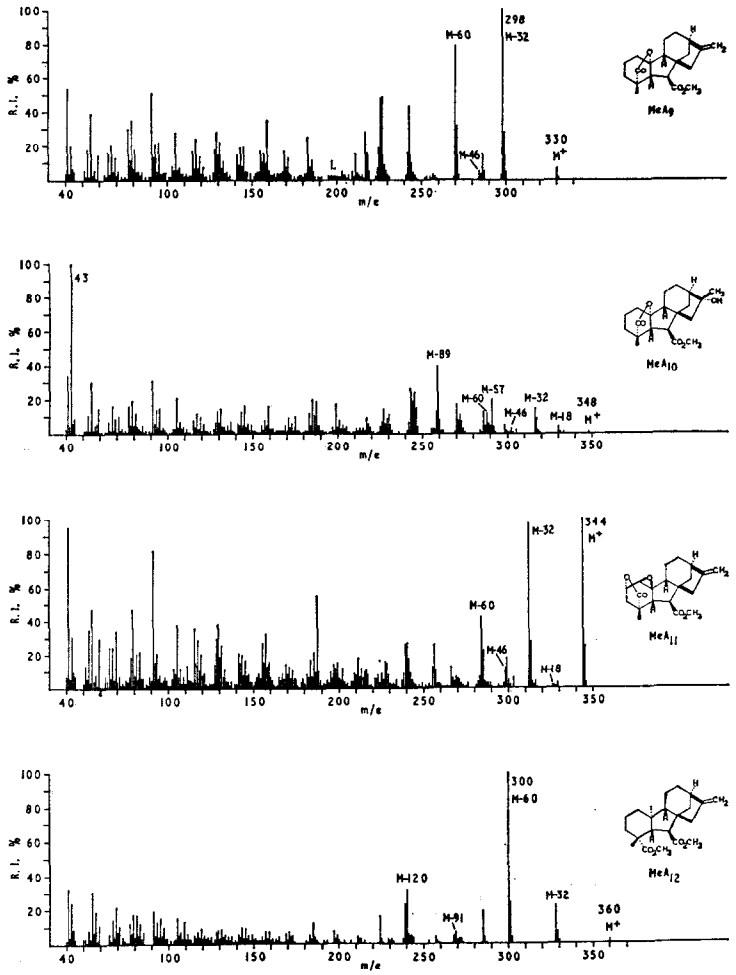


FIG. 2—*continued.*

can be distinguished from the C_{19}-gibberellins, with the exception of the dicarboxylic acid gibberellin A_{21}, by intense peaks at M-119/120 in addition to the M-59/60 peak. A 2-hydroxyl group appears to inhibit the loss of the second 60 mass units since MeA_{17} (Fig. 2.17) shows a more intense M-120 ion than MeA_{13} (Fig. 2.13). Similar relative intensities in the mass spectra of MeA_{17} TMSi and MeA_{13} TMSi (see discussion of MeTMSi spectra above and Figs. 1.13 and 1.10 respectively). While the methyl esters of all the C_{20}-gibberellins with two or three methoxycarbonyl groups all show distinct M-119/120 peaks only gibberellins A_{13}, A_{17}, and A_{18} have such peaks in the mass spectra of the MeTMSi/s. The mass spectra of the methyl

esters of these di- and tri-carboxylic acids (the C_{20}-gibberellins and gibberellin A_{21}) also show peaks at M-91/92 which presumably arise from loss of 59/60 and 31/32 mass units from two methoxycarbonyl groups. MeA_{23} (Fig. 2.23) shows an intense peak at M-64 presumably due to M-(2×32) from two methoxycarbonyl groups. The dimethyl esters of gibberellins

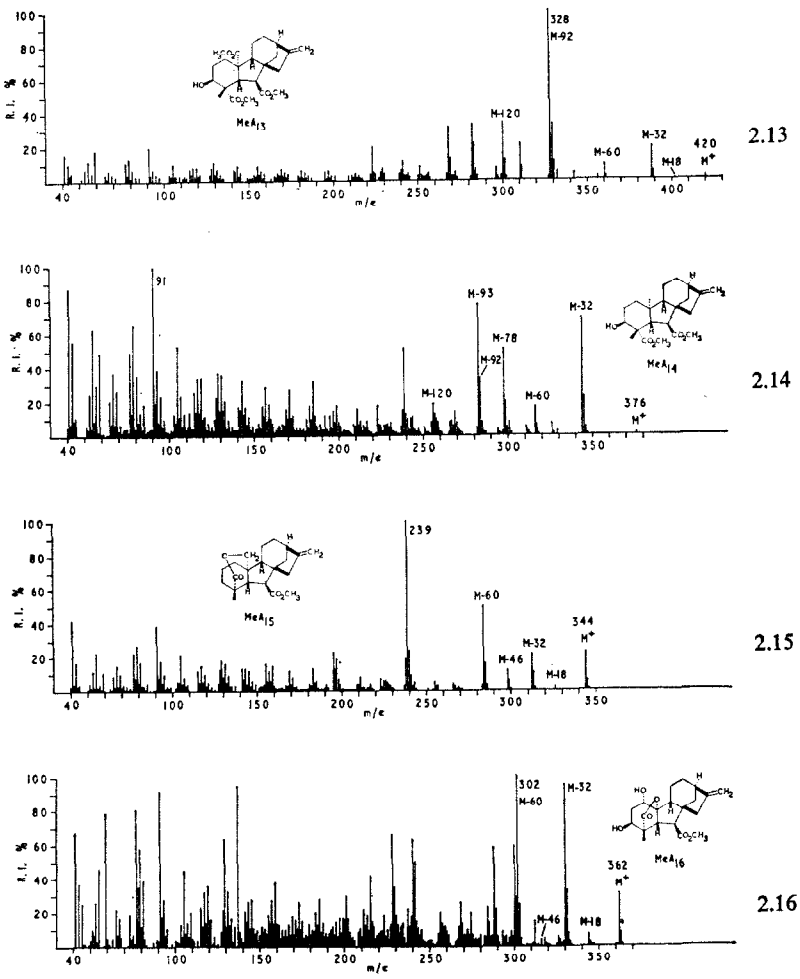


FIG. 2—*continued.*

A_{14} (Fig. 2.14) and A_{18} (Fig. 2.18) show very similar fragmentation patterns; the characteristic peaks at M-60, M-78, and M-93 possibly representing the successive losses of methyl formate, water, and a methyl group; in these two esters the M-93 peak is much more intense than the M-92 peak.

An M-18 peak is shown not only by the hydroxylated gibberellins but also by the two epoxides, MeA_6 (Fig. 2.6) and MeA_{11} (Fig. 2.11) and, unexpectedly by the MeA_{15} (Fig. 2.15). The relative intensities of the M-18 peaks in the isomeric gibberellins MeA_{13} and MeA_{17}

have been measured accurately both in GC-MS spectra obtained with an LKB 9000 instrument and in spectra obtained by direct insertion of the solids using an A.E.I. MS9 spectrometer, at an ionization voltage of 70 eV in both cases. MeA_{17} showed an M-18 peak with 30 and 80 per cent of the molecular ion in the respective instruments, compared with 20 and

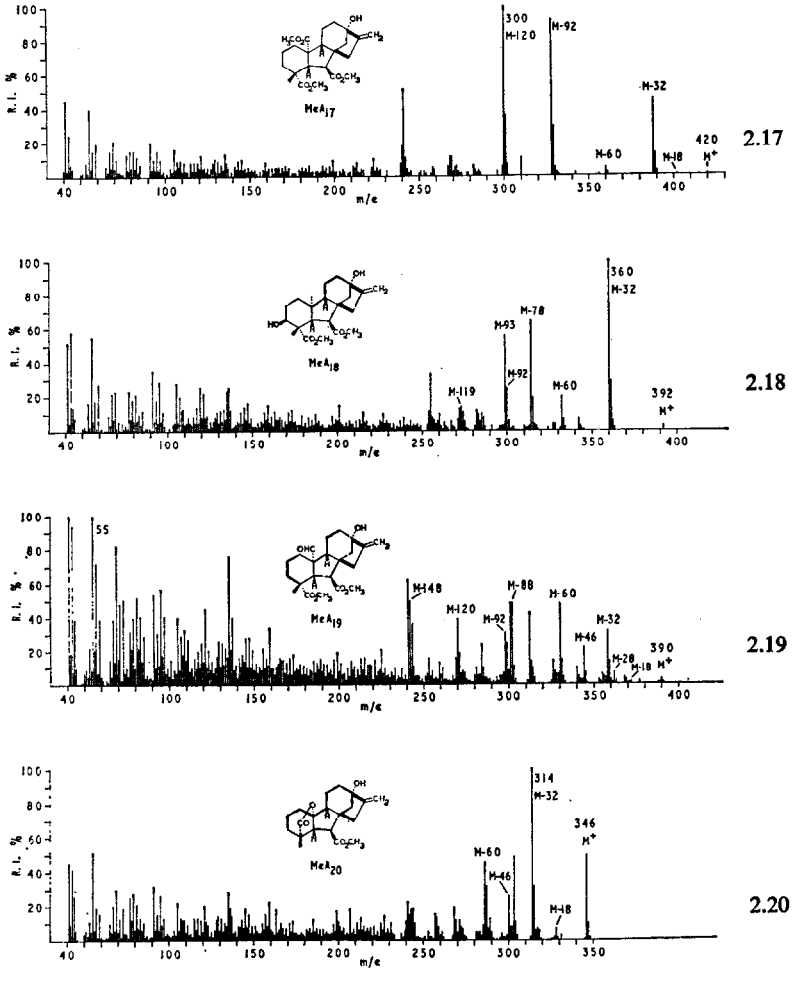


FIG. 2—*continued.*

17 per cent for MeA_{13}. These results contradict a previous suggestion[9] that 7-hydroxygibberellins can be distinguished from gibberellins with a 2-hydroxyl group by the lower intensity of the M-18 peak. The location of the hydroxyl group in gibberellins is most securely established from the spectra of the MeTMSi derivatives.

The methyl esters of gibberellins A_1 (Fig. 2.1) and A_4 (Fig. 2.4) show an M-2 peak which must be due to dehydrogenation of the 2-hydroxyl function and not to incomplete separation of MeA_3 from MeA_1 by GC or to the presence of MeA_4 and MeA_5 in gibberellin A_4 methyl ester. The spectrum of MeA_1 does not contain peaks at *m/e* 342 and 297 characteristic of

MeA_3 (Fig. 2.3); similarly the spectrum of MeA_4 does not contain peaks at *m/e* 281 (MeA_7, Fig. 2.7) or 266 (MeA_5, Fig. 2.5). The peak shown by MeA_8 (Fig. 2.8) at M-20 is possibly the loss of 18 mass units from an M-2 ion.

The M-46 peak, previously assigned[9] to the loss of CO_2 and H_2 from the C_{19}-gibberellins with a five-ring lactone, is also shown by MeA_{15} (Fig. 2.15) with a six-ring lactone. However, an M-46 peak is also shown by the methyl esters of the aldehydic gibberellins A_{19}

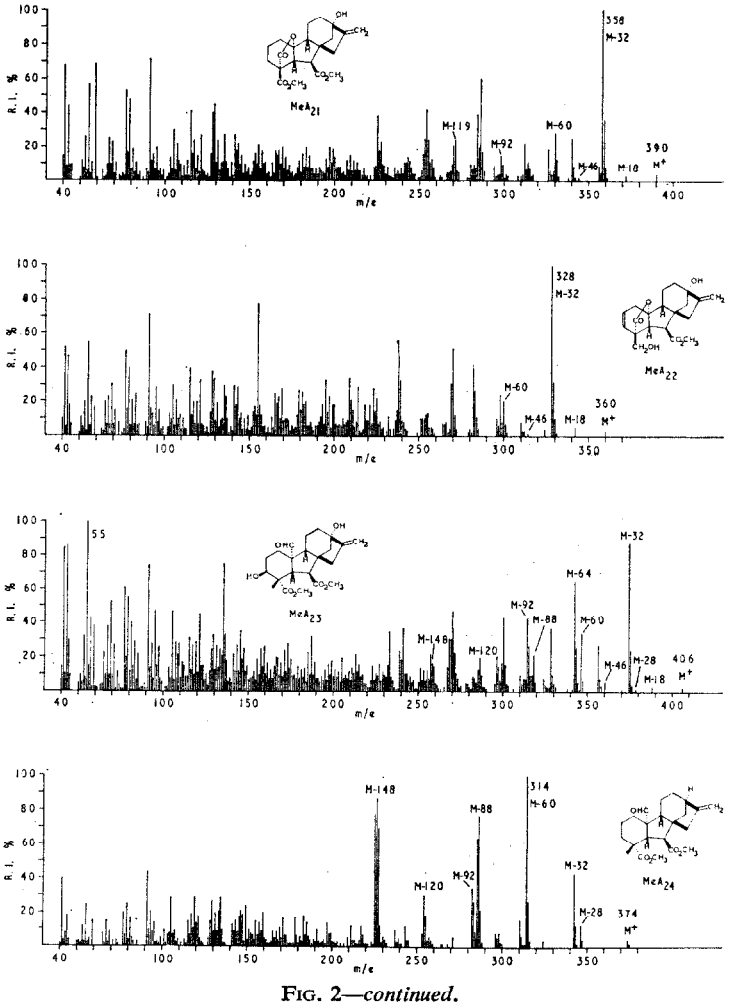
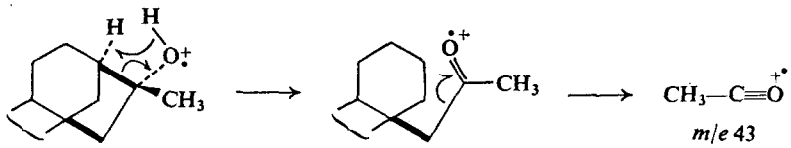


FIG. 2—*continued.*

(Fig. 2.19), A_{23} (Fig. 2.23), and A_{24} (only 0·5 per cent of base peak and not shown in Fig .2.24) and possibly represents the combined loss of 18 and 28 mass units. The methyl esters of these aldehydic gibberellins are characterized by the loss of 28 mass units from the molecular ion and from the M-32 (M-60), M-60 (M-88) and M-120 (M-148) ions.

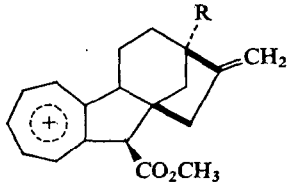
The methyl esters of gibberellins A_2 and A_{10} (Figs. 2.2 and 2.10 respectively) show identical fragmentation patterns with a base peak at *m/e* 43 which could rise by the fragmentation pathway (XI) → (XII) → (XIII). The peak at M-57 is considered to represent the cleavage



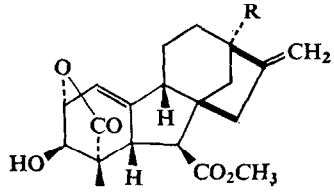
(XI) (XII) (XIII)

of ring D with the transfer of one hydrogen atom and the peak at M-89 to represent the same cleavage combined with loss of 32 mass units.

The mass spectra of MeA_3 (Fig. 2.3) and MeA_7 (Fig. 2.7) are characterized by a very intense peak at M-63. This peak probably represents the loss of H_2O, CO_2, and H from ring A presumably to give a tropyllium ion (XIV). This result is contrary to the findings of Wulfson *et al.*[8] who reported that MeA_3 and MeA_7 showed intense peaks at M-62 and could be



(XIV) R = OH in MeA_3
R = H in MeA_7



(XV) R = OH
(XVI) R = H

distinguished from their corresponding isomeric 1→3-lactones (XV) and (XVI) which showed intense M-63 peaks. It is our experience that the mass spectra of MeA_3 and its isomeric 1→3-lactone (XV), and of MeA_7 and its isomeric 1→3-lactone (XVI), are virtually indistinguishable.

Whilst we have often observed M + 14 peaks in mass spectra obtained by direct insertion of gibberellin methyl esters prepared with diazomethane, no such peaks were observed in the GC–MS mass spectra. Diazomethane is known to form a cyclopropyl derivative with the exocyclic double bond in gibberellin A_4.[14]

EXPERIMENTAL

Low resolution mass spectra were obtained with an LKB 9000 combined gas chromatograph–mass spectrometer operating at 70 eV. GC columns used were 6 ft × ⅛ in. i.d. (glass) packed with 1 or 2 per cent QF-1 on acid-washed and silanized Gas Chrom P or A respectively, and 10 ft × ¼ in. i.d. (glass) packed with 1 per cent SE-30 on acid-washed and silanized Gas Chrom P. For both types of GC columns operating temperatures were *ca.* 200° with a helium flow rate of *ca.* 30 ml/min. The flash heater and molecular separator were both kept at *ca.* 250° for all determinations. The scan time for each mass spectrum was between 4 and 6 sec. For each mass spectrum the background mass spectra of SE-30 and QF-1 were subtracted as appropriate.

Samples of the gibberellin methyl esters and methyl ester TMSi ethers were obtained as previously described.[15] Each mass spectrum was obtained from GC of *ca.* 5 μg of sample which represents a convenient, but not minimal, quantity facilitating subtraction of background spectra. GC-MS of the methyl esters was carried out exclusively on the QF-1 columns where decomposition was minimal,[15] and GC–MS of the methyl ester TMSi ethers was carried out using either QF-1 or SE-30 columns as convenient.

High resolution mass spectral data on the gibberellin methyl ester TMSi ethers were obtained with an A.E.I. MS9 instrument by direct insertion of the evaporated silylation mixture into the ion source (70 eV and

[14] D. C. ALDRIDGE, J. R. HANSON and T. P. C. MULHOLLAND, *J. Chem. Soc.* 3539 (1965).
[15] B. D. CAVELL, J. MACMILLAN, R. J. PRYCE and A. C. SHEPPARD, *Phytochem.* 6, 867 (1967).

110°). Two intense peaks derived from the silylating reagent (hexamethyldisilazane and trimethylsilyl chloride in pyridine) were observed at *m/e* 130·049 ($C_4H_{12}NSi_2$ requires 130·050) and *m/e* 147·065 ($C_5H_{15}OSi_2$ requires 147·066).

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