Tetrahedron Letters No.13, pp. 1537-1542, 1968. Pergamon Press. Printed in Great Britain.

FURTHER INVESTIGATIONS OF GIBBERELLINS IN PHASEOLUS MULTIFLORUS BY COMBINED GAS CHROMATOGRAPHY-MASS SPECTROMETRY - THE OCCURRENCE OF GIBBERELLIN A_{20} (PHARBITIS GIBBERELLIN) AND THE STRUCTURE OF COMPOUND b

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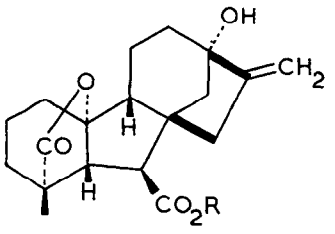
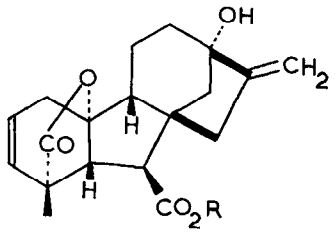
(Received in UK 12 December 1967)

The power of combined gas chromatography-mass spectrometry (g.c.-m.s.) in detecting and identifying plant gibberellins has been illustrated in previous communications by the identification[1,2] of the known gibberellins A_1, A_5, A_6, A_8 and A_{19} (bamboo gibberellin)*, and by the detection[3] of the previously unknown gibberellin A_{17}, in extracts of young seed of Phaseolus multiflorus. We now report the detection in these extracts of gibberellin A_{20} (Pharbitis gibberellin) (I, R=H) previously isolated from seed of Pharbitis nil by Takahashi et al.[4] and some observations on the structures of compounds a and b, previously isolated from seed of P. multiflorus by Jones.[5]



I



II

*The numbering system proposed by J. MacMillan and N. Takahashi, Nature, in press, is adopted.

Gibberellins A_5 (II, R=H) and A_{20} (I, R=H) are not well resolved by gas chromatography (g.c.) of their methyl esters or the trimethylsilyl ethers of the methyl esters.[6] The mass spectra of these derivatives are, however, sufficiently different to identify these gibberellins in an unresolved g.c. peak by g.c.-m.s. The total ion current trace shown in Fig. 1 is of a methylated aliquot of fraction 51, obtained as previously described[7] by elution with 60% acetone in water from a charcoal:celite column chromatogram of the crude acid fraction of immature seed of P. multiflorus. The main peak, originally assigned[7] to gibberellin A_5 methyl ester (II, R=Me) by g.c. alone before the g.c. characteristics of gibberellin A_{20} methyl ester (I, R=Me) had been determined, was scanned ($^m/e$ 0-500 in 2 sec.) at the points indicated. Line diagrams of the significant peaks in scans 1 to 4 between $^m/e$ 280 and 360 are compared in Fig. 2 with the corresponding portions of the mass spectra of authentic gibberellin A_5 and gibberellin A_{20} methyl esters. The data clearly show that the scanned peak of Fig. 1 is an unresolved mixture of gibberellin A_5 and A_{20} methyl esters whose respective maxima occur between scans 2 and 3 and between scans 1 and 2. This conclusion was confirmed, and the exact positions of these two gibberellin methyl esters was determined, by the method of accelerating voltage alternation (a.v.a.).[8] The a.v.a. trace of the molecular ions, $^m/e$ 346 (A_{20} methyl ester) and $^m/e$ 344 (A_5 methyl ester) is shown in Fig. 3. In the a.v.a. trace an ion ($^m/e$ 344) was also detected which corresponded to the small peak A in the total ion current trace (Fig. 1) of methylated fraction 51. Investigation of this component is in hand.

G.c.-m.s. also revealed the presence of gibberellin A_{20} in much smaller amount in fraction 53, eluted with 62% acetone as previously described,[7] by scanning the low retention side of the gibberellin A_5 methyl ester peak. From g.c.-m.s. of the unfractionated crude extract, after methylation and after methylation followed by trimethylsilylation, we previously reported the detection of gibberellin A_5 accompanied in each case by an unresolved component which we identified as gibberellin A_4 (as the methyl ester and its trimethylsilyl ether). On re-examination this identification has proved to be erroneous, this second component being gibberellin A_{20}. We have no evidence for the occurrence of gibberellin A_4 in seed of P. multiflorus.

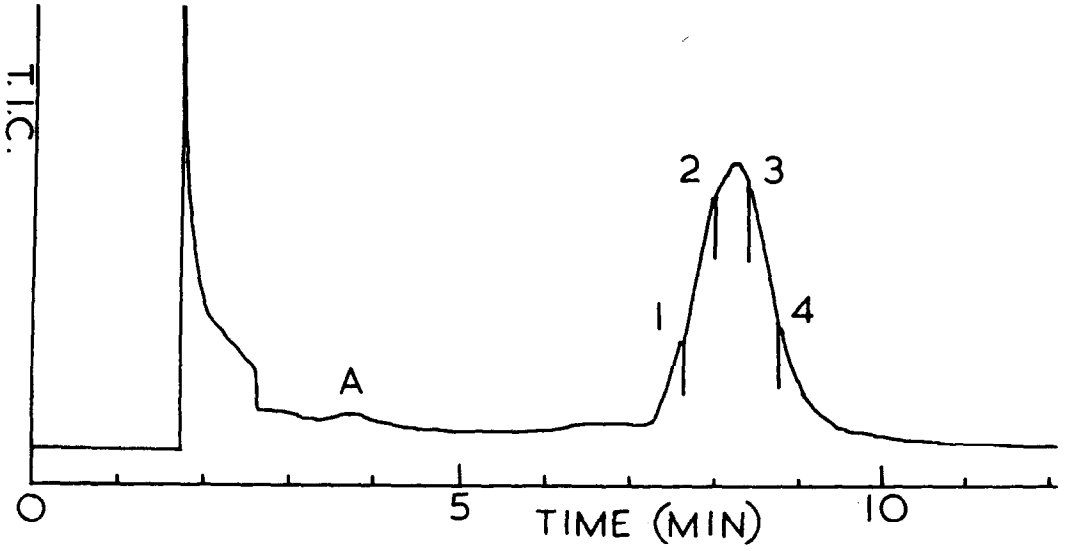


FIG. 1

Total ion current trace of methylated fraction 51 (7% QF-1 column, 1 m. x 1.5 mm. i.d.; helium carrier gas 16 ml./min.; isothermal at 205°).

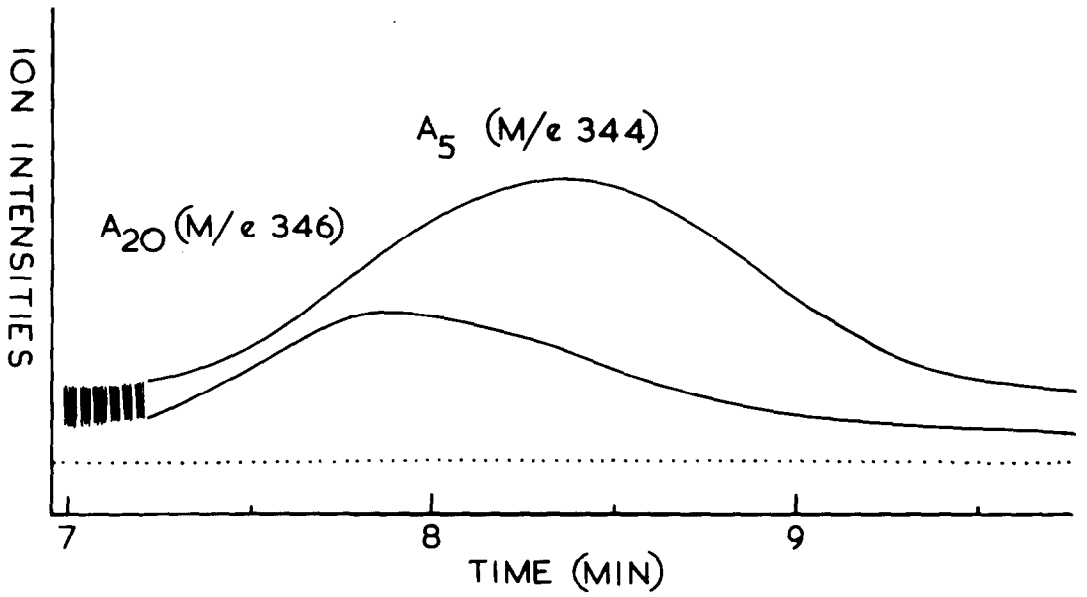


FIG. 3

A.v.a. trace obtained from methylated fraction 51.

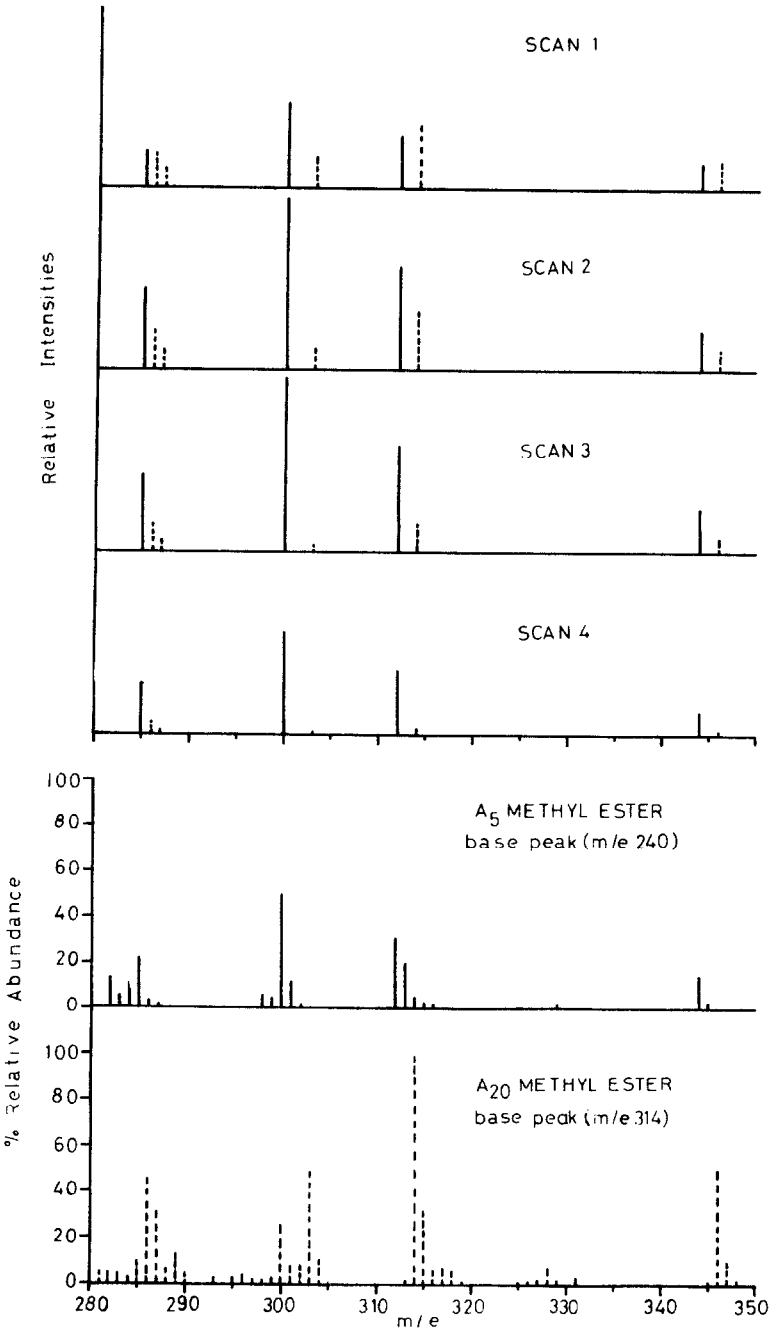
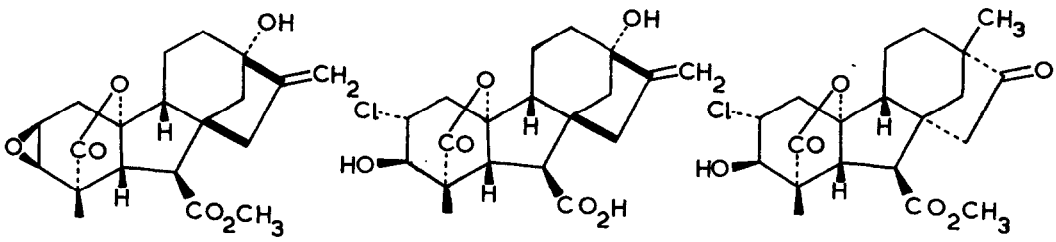


FIG. 2

Portions of the mass spectra obtained from scans in Fig. 1 together with relevant parts of the mass spectra of gibberellin A_5 and A_{20} methyl esters.



III IV V

We have also examined by g.c.-m.s. compounds a and b, obtained from immature seed of P. multiflorus by Jones[5] who kindly provided the remaining traces of his samples. The extant sample of compound a was found to be a complex mixture with no major peaks which when scanned by g.c.-m.s. as the methylated derivative, before and after trimethylsilylation, gave mass spectra ill-defined from the background; no useful information could be derived. Compound b, after methylation, revealed one major peak with retention times of 17.5 min. (QF-1) and 9.5 min. (SE-33) under the g.c. conditions previously described.[7] The mass spectrum obtained by scanning this peak was practically identical to that of gibberellin A_6 methyl ester (III). However, the mass spectrum of the major peak, obtained after methylation and trimethylsilylation, showed (i) a parent ion at $^{M}/e$ 540 with an $(M+2)^+$ ion of the correct intensity for the presence of one chlorine atom, and (ii) a chlorine containing fragment ion at $(M-59)^+$ which is typical of gibberellin A_6 methyl ester and its trimethylsilyl ether. The base peak was the molecular ion which is characteristically a strong peak in the mass spectra of 7-hydroxy gibberellins. These facts provide strong support for the structure (IV), originally suggested for compound b by Jones.[5]

Thermal loss of hydrogen chloride from the methyl ester of (IV) to regenerate gibberellin A_6 methyl ester (III) may be a general property of diaxial chlorohydrins. The chlorohydrin (V), obtained[9] from gibberellin A_6 with aqueous hydrochloric acid followed by methylation similarly gave no chlorine containing parent ion only that of the corresponding epoxide; this thermal elimination appears to occur in the ionisation chamber and not in the molecular separator of the LKB 9000 since the same result was obtained with an A.E.I. MS9 using direct insertion. As with the trimethyl-

silyl ether of Jones gibberellin b the mass spectrum (g.c.-m.s.) of the trimethylsilyl ether of the chlorohydrin (V) showed a chlorine-containing parent ion at $^{M}/e$ 468.

We are indebted to the S.R.C. for a Research Studentship (R.J.P.); to Professor N. Takahashi, University of Tokyo, for a specimen of gibberellin A_{20}; to LKB Instruments Ltd., Stockholm, for the use of an LKB 9000 instrument in their Applications Laboratory; and to Professor R. Ryhage, Karolinska Institutet, Stockholm, for the use of the a.v.a. modification to the LKB 9000 instrument.

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