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IDENTIFICATION OF GIBBERELLINS IN CRUDE PLANT EXTRACTS BY COMBINED GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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In a previous paper(1) we described conditions for the gas-liquid chromatography (g.l.c. or g.c.) of gibberellin methyl esters and their trimethylsilyl (T.M.S.) ethers. Application of these g.l.c. methods to a crude acid extract, obtained from immature seed of Phaseolus multiflorus, showed the presence of gibberellins A_1, A_5, A_6 and A_8, previously(2,3) isolated from these seed, and the possible presence of gibberellins A_4 and A_{13}.

Continuing our search for rapid and definitive methods of identifying gibberellins in plants we have explored the use of combined gas chromatography-mass spectrometry (g.c.-m.s.) using an L.K.B.900 instrument. Fig. 1 shows the total ion current trace obtained with the methylated crude acid extract from immature seed of P. multiflorus. The peaks were scanned ($^m/e$ 10-500 in 4 sec.) at the points indicated by the numbers 1 to 8. Scan 1 is unidentified; scans 2 and 3 were identical and showed the presence of a compound of molecular weight 420 containing all the fragment ions of gibberellin A_{13} methyl ester but with different relative intensities. Scan 4 was essentially the same as scans 2 and 3 but included additional fragment ions. Scans 5 and 6 were similar and appeared to be mixtures of the methyl esters of gibberellins A_4 and A_5 together with an unknown component. Scans 7 and 8 were respectively identical to the mass spectra of the methyl esters of gibberellins A_5 and A_6.

The total ion current trace, obtained from the methylated and trimethylsilylated crude extract, is shown in Fig. 2 and scans 1 to 12 were taken at the points indicated.

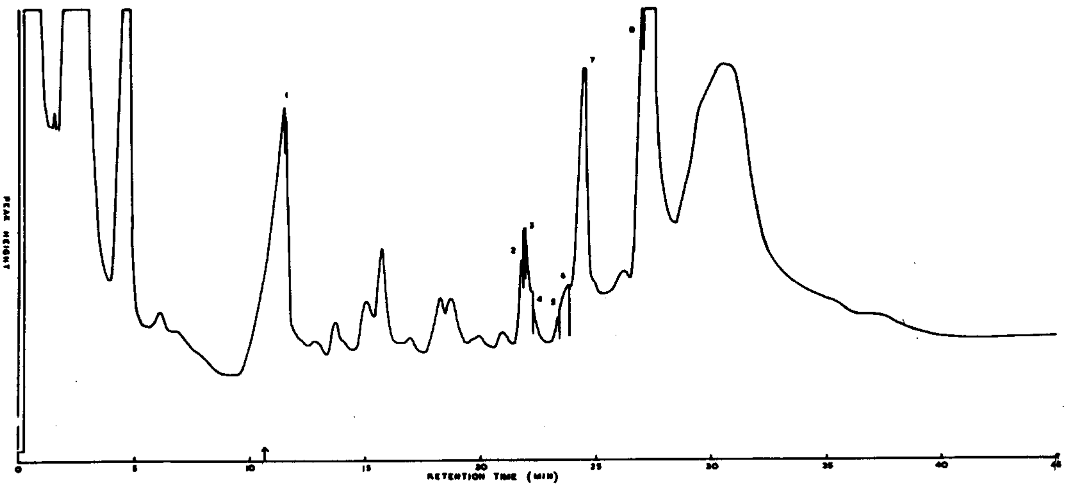

FIG. 1 Total ion current trace of methylated crude extract.

(1% QF-1 column, 6' x 1/8" i.d.; helium carrier gas at 30 ml./min.; initial temp. 140°, then programmed to 250° at 5°/min. from time indicated by arrow).

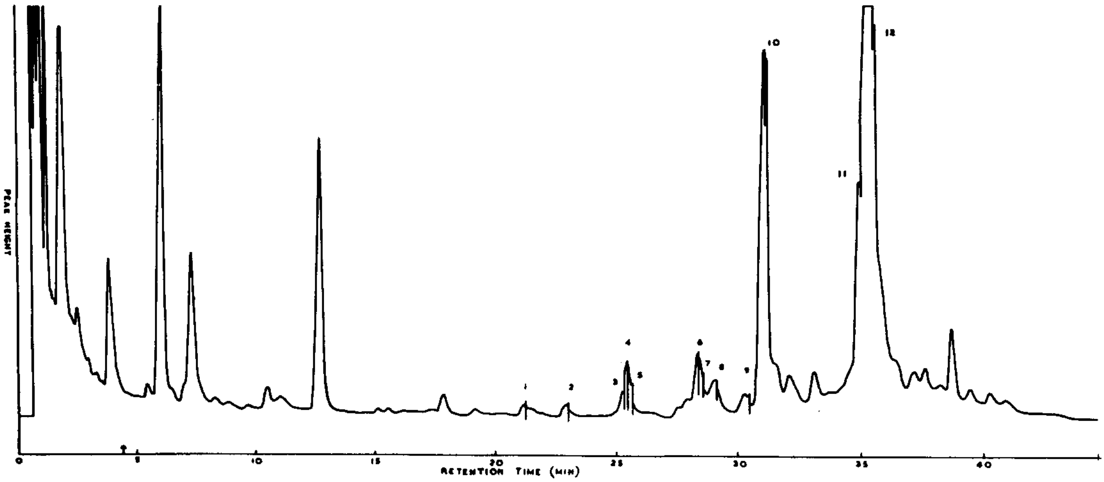

FIG. 2 Total ion current trace of trimethylsilylated, methylated crude extract.

(1% SE-30 column, 10' x 1/8" i.d.; helium carrier gas at 30 ml./min.; initial temp. 160° then programmed to 250° at 2.5°/min. from time indicated by arrow).

Scans 1,2,8 and 9 are unidentified. Scan 3 was identical to the mass spectrum of the T.M.S. ether of gibberellin A_5 methyl ester. Scans 4 and 5 indicated mixtures of the T.M.S. ethers of gibberellins A_4 and A_5 methyl esters. Scan 6 showed the presence of the T.M.S. ether of gibberellin A_6 methyl ester together with a component of molecular weight of 492. The latter compound was the sole component in scan 7 and was not identical to the T.M.S. ether of gibberellin A_{13} methyl ester. Scan 10 was identical to the mass spectrum of the authentic derivative of gibberellin A_1 and scans 11 and 12 were identical to that of the T.M.S. ether of gibberellin A_8 methyl ester.

Thus the previous identification(1) of gibberellins A_1, A_4, A_5, A_6 and A_8 by g.l.c. alone has been confirmed by g.c.-m.s. The peaks in the g.l.c. traces with the same retention times as gibberellin A_{13} methyl ester and its trimethyl silyl ether had the correct molecular weights and showed similar but different fragmentation patterns to the authentic derivatives. The identity of this "isomer" of gibberellin A_{13} is under investigation.

Mass spectra of the gibberellin methyl esters and their T.M.S. ethers will be published in the full paper. With such spectra available, g.c.-m.s. may provide a direct and certain method of identifying known gibberellins without the need for access to authentic specimens.

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