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SHORT COMMUNICATION

PLANT HORMONES—VII.

IDENTIFICATION AND ESTIMATION OF ABSCISIC ACID IN A CRUDE PLANT EXTRACT BY COMBINED GAS CHROMATOGRAPHY–MASS SPECTROMETRY*

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Abstract—By combined gas chromatography–mass spectrometry, abscisic acid and its *trans*-isomer were identified directly in a methylated crude acidic fraction from cider apple juice.

THE IDENTIFICATION and estimation of (±)-abscisic acid (I) in plant extracts has been described recently using a spectro polarimetric method either before[1] or after[2] dilution with (±)-abscisic acid. Following the successful application of combined gas chromatography–mass spectrometry (GLC–MS) to the identification of plant gibberellins,[3–6] we have explored the use of this method to the detection and estimation of abscisic acid in cider apple juice. Apple juice was chosen for this preliminary study because it had been shown by bioassay[7] to contain a relatively high concentration of abscisic acid.

Freshly pressed cider apple juice which had been treated with pectin esterase for 7 days was extracted as described by Pieniazek and Rudnicki.[7] The crude acidic fraction was methylated with diazomethane, then directly examined by gas chromatography (GLC) on columns of 2% SE-33 and 2% QF-1 supported on Gaschrom Q. The GLC trace obtained from an isothermal run at 160° on SE-33 is shown in Fig. 1*a*. The GLC traces for aged solutions of methyl abscisate and methyl phaseate[8] are shown in Fig. 1*b* and 1*c*. Fresh solutions of each of these methyl esters showed only the peaks of shorter retention times; the peaks of longer retention times in Fig. 1*b* and 1*c* are due respectively to the *trans*-isomers (III) and (IV), presumably formed by exposure of the solutions of methyl abscisate (I) and methyl phaseate (II) to light. GLC traces, similar to those shown in Fig. 1, were obtained with the 2% QF-1 column either isothermally at 183° or by temperature programming from 140–170° at 3° per min.

* This work was briefly mentioned in a paper by J. MacMillan and R. J. Pryce, delivered at a Symposium on Plant Growth Regulators held in London,8–9 January 1968, and organized by the Society of Chemical Industry in association with the Phytochemical Society.

[1] J. W. CORNFORTH, B. V. MILBORROW and G. RYBACK, *Nature* **210**, 627 (1967).
[2] B. V. MILBORROW, *Planta* **76**, 93 (1968).
[3] J. MACMILLAN, R. J. PRYCE, G. EGLINTON and A. MCCORMICK, *Tetrahedron Letters* 2241 (1967).
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[5] J. MACMILLAN and R. J. PRYCE, *Tetrahedron Letters* 4173 (1967).
[6] J. MACMILLAN and R. J. PRYCE, *Tetrahedron Letters* 1537 (1968).
[7] J. PIENIAZEK and R. RUDNICKI, *Bull. Acad. Polon. Sci.* **15**, 251 (1967).
[8] J. MACMILLAN and R. J. PRYCE, *Chem. Commun.* 124 (1968).

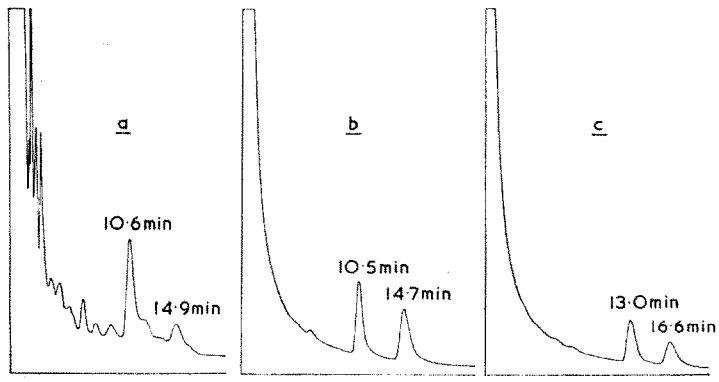
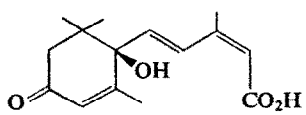
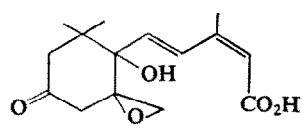


Fig. 1

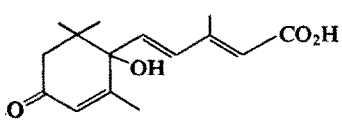
When scanned by GLC–MS, the peaks of shorter retention time in Fig. 1*a* and 1*b* gave identical mass spectra while the peaks of longer retention times gave mass spectra not significantly different from that of methyl abscisate. These results clearly show the presence of abscisic acid (I) and of the *trans*-isomer (III), also the absence of phaseic acid (II), in the crude acidic fraction from cider apple juice. The *trans*-isomer (III) is not an artefact of the work-up procedures; when pure (±)-abscisic acid was exposed to the conditions, used in the extraction of the apple juice, only traces of the *trans*-isomer (III) were detected by GLC.



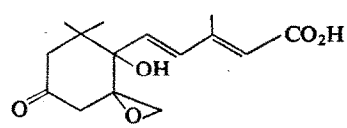
(I)



(II)



(III)



(IV)

By comparison with the peak areas from standard injections, the peak of methyl abscisate in Fig. 1*a* was found to represent 0·3 μg abscisic acid, corresponding to 30 μg per litre of apple juice and to 1 part in about 10^7 of fresh weight of apple fruit. This sensitivity could be increased by at least a factor of ten on the gas chromatogram instrument used. Using the wheat coleoptile bioassay, Pieniazek and Rudnicki[7] estimated the presence of 20 μg abscisic acid per litre of apple juice.

A gratifying feature of the GLC trace in Fig. 1*a* is the absence of significant amounts of other components in the crude extract with retention times close to those of methyl abscisate. This situation may not obtain in other plant extracts. Nevertheless the present investigation emphasizes the usefulness of GLC–MS in the study of plant growth substances.

EXPERIMENTAL

Gas chromatography (GLC) was performed on a Pye 104 Model 64 using silanized glass columns, 5 ft × ¼ in. o.d., packed with 2% QF-1 or 2% SE-33 on Gaschrom Q; the GLC traces shown in Fig. 1 were obtained

at an amplifier attenuation setting of 500. In combined gas chromatography–mass spectrometry (GLC–MS), using an L.K.B. 9000 instrument, the peaks were scanned from *m/e* 10–500 in 4 sec.

Extraction of Apple Juice

Freshly pressed apple juice (9 l.) which had been treated with pectin esterase was acidified to pH 2·5 with 2 N-HCl and extracted as described by Pieniazek and Rudnicki[7] including the lead acetate treatment. The recovered acids (67·6 mg) were dissolved in methanol (1·0 ml) and aliquots (0·1 ml) were prepared for GLC and GLC–MS by methylation with CH_2N_2.

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