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IDENTIFICATION OF ABSCISIC ACID GLUCOSE ESTER, INDOLE-3-ACETIC ACID, ZEATIN AND ZEATIN RIBOSIDE IN RECEPTACLES OF PEAR

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Key Word Index—*Pyrus communis*; Rosaceae; abscisic acid; zeatin; high performance liquid chromatography; mass spectrometry.

Abstract—The identity of abscisic acid glucose ester, indole acetic acid, zeatin, and its riboside in pear receptacles was revealed by use of chromatographic, ultraviolet and mass spectral analysis.

INTRODUCTION

In a previous study gibberellins (GA_{17}, GA_{25}, GA_{45}), abscisic acid (ABA), phaseic acid and dihydrophaseic acid were identified in pear seeds [1] and in addition chromatographic evidence for the presence of zeatin (Z) in pear fruit was published [2]. Recent advances in hormone methodology have made possible the absolute identification of many plant hormones in minute quantities [3]. These procedures are deemed important in a continuing effort toward elucidating the participation of hormones in the fruit set phenomena. The purpose of the current investigation was to identify ABA-GE,† IAA, Z and its riboside (ZR) in pear receptacles.

RESULTS AND DISCUSSION

In the process of comparing extraction–purification procedures for IAA it was evident that information on partition coefficients for this compound was limited to two publications. One contained work accomplished prior to the availability of sensitive assay procedures and involved use of a single organic solvent [4]. The other publication though more recent still lacked useful detail [5].

The information on the partitioning of IAA between water and several organic solvents is noteworthy (Table 1). Clearly one must be careful when selecting a partitioning solvent for the purification procedure. A much used method includes an ether partition at pH 8, yet this step results in some loss of IAA (Table 1). Even more serious would be the ethyl acetate partition at pH 8 where little or no IAA remains in the water phase after three partitions. This may easily explain why some investigations have been unable to find IAA in plant extracts in which ethyl acetate has been used at pH 8. One is well advised to check partition coefficients particularly within the system employed using the plant material under investigation. The coefficients listed in Table 1 were achieved without added plant extract. Doubtless some change in partition coefficients would occur when plant material is added and could vary with the type of plant.

A commercial standard of IAA and standards of ABA-GE, Z and ZR prepared by ourselves and presumed ABA-GE, IAA, Z and ZR from pear receptacles displayed similar HPLC and GC retention times (R_t) (Table 2). Following base hydrolysis at pH 11, pear ABA-GE displayed a single EC-GC peak at the R_t of ABA; subsequent UV treatment of the released ABA resulted in a 1:1 ratio of *c*, *t*-ABA and *t*, *t*-ABA as judged by EC-GC analysis. Pear receptacle samples were subjected to base hydrolysis at pH 11 or β-glucosidase or α-glucosidase at a pH appropriate for each enzyme and subsequent assay by EC-GC. Similar ABA peaks were found for pH 11 hydrolysis and enzymatic release by β-glucosidase with no peak for α-glucosidase. Hydrolysis of both putative ABA-GE and authentic ABA-GE was achieved with β-glucosidase, while neither compound was hydrolysed by α-glucosidase [6].

The UV maximum for pear ABA-GE tetra-acetate coincided with the standard at 271 nm and agrees closely with the previously reported maximum at 270 nm [7]. The mass spectrum obtained by direct probe analysis was similar to that of authentic acetylated ABA-GE. No $[M]^+$ at 594 was observed but the ion at *m/z* 534 (0.5% ion intensity) probably represents $[M-60]^+$ of acetic acid. Following the base peak at *m/z* 43 (100) other notable ions included: 331 (17.3), 271 (1.5), 247 (4.4), 242 (3.0), 229 (3.0), 190 (13.8), 169 (54.8), 157 (16.8), 145 (11.2), 127 (10.6), 115 (27.2), 109 (35.9). The mass spectrum for Me-TMS-

†Abbreviations: (+) - 1 - abscisyl - β - D - glucopyranoside (ABA-GE); indole - 3 - acetic acid (IAA); zeatin (Z); zeatin riboside (ZR); high performance liquid chromatography (HPLC); rotary film evaporator (RFE); methyl (Me); trimethylsilyl (TMS); electron capture GC (EC-GC); triethylamine bicarbonate (TEAB).

Table 1. IAA partition coefficients and percent IAA remaining in the aqueous phase after one partition of organic solvent against buffers at pH 3.0 or 8.0*

Solvent	pH 3.0		pH 8.0	
	K†	% Remaining in aqueous phase	K†	% Remaining in aqueous phase
n-Butanol (water satd)	53.3	2	17.7	5
Ether	13.9	7	0.03	97
Ethyl acetate water (satd)	28.2	3	19.4	5
Methylene chloride	3.3	23	0.09	92
Petrol (bp 60–80°)	—	—	0.02	98
Toluene	—	—	0.05	95

*Water at pH 3.0 with hydrochloric acid; pH 8 with potassium hydroxide.
†$K = C_{organic}/C_{aqueous}$.

Table 2. Average times R_t of ABA-GE, IAA, Z and ZR on HPLC and GC

Compound	HPLC-R_t*					GC R_t†
	Partisil‡ 10 PAC	ODS§ semi-prep.	ODS§ anal.	Hypersil‖	Partisil¶ 10	3% OV 1
IAA (standard)	—	11:40	—	—	5:10	6:30
Pear IAA	—	11:40	—	—	5:10	6:30
ZR (standard)	3:30	21:30	15:40	—	—	5:25
Pear ZR	3:30	21:30	15:40	—	—	5:25
Z (standard)	3:10	13:30	9:00	—	—	6:30
Pear Z	3:10	13:30	9:00	—	—	6:30
ABA-GE (standard)	—	10:30	—	—	5:40	—
Pear ABA-GE	—	10:30	—	—	5:40	—
ABA-GE tetra-acetate (standard)	—	—	—	2:30	—	—
Pear ABA-GE tetra-acetate	—	—	—	2:30	—	—

*Average retention time ($R_t \pm 5$ sec); polar phase Partisil 10 PAC (250 × 4.5 mm i.d.); non-polar phase ODS semi-prep. (150 × 10 mm i.d.); ODS anal. (150 × 4.5 mm i.d.); absorption phase, Hypersil (150 × 4.5 mm i.d.); Partisil 10 (250 × 10 mm i.d.). Flow rates: 2 ml/min anal., 5 ml/min semi-prep.
†Average retention time ($R_t \pm 5$ sec).
‡Isocratic acetonitrile: H_2O (90:10).
§Acetonitrile: pH 7H_2O (TEAB) 7–11% in 30 min; TEAB.
‖Isocratic chloroform.
¶Isocratic chloroform: methanol (9:1).

IAA was similar to that of the standard compound. The $[M]^+$ at 261 (32.4) and base peak at m/z 202 (100) were followed by other notable ions at m/z values: 170 (10.2), 145 (11.2), 137 (10.5). The strong peak at m/z 73 representing TMS was removed from the spectrum. Other interfering m/z values which were not part of the Me-TMS-IAA GC peak included 204 (33.5), 157 (25.6), 157 (21.1). The latter were judged to be contaminants in the extract. The basic aspects of the IAA spectra agree with those published for Me-TMS-IAA [8]. The mass spectrum of pear Z revealed $[M]^+$ at 261 (8.5) and base peak at 230 (100). Strong ions also occurred at the following m/z values: 231 (20.8), 188 (10.0), 162 (13.1), 134 (10.9), 133 (12.0), 119 (8.9). Contaminants in the peak for this Z occurred at m/z 125 (15.0), 111 (11.2), 103 (11.4). The essentials of this spectrum agree closely to that of standard Z and those previously published [9]. The mass spectrum of

pear ZR displayed a $[M]^+$ at 421 (8.9) and a base peak at 216 (100). Other prominent ions included those at *m/z* values: 391 (20.6), 390 (70.2), 348 (7.0), 246 (4.7), 202 (6.8), 174 (19.6), 101 (9.3) and 45 (31.9). This spectrum agrees closely with that of standard ZR and that previously published [9].

On the basis of the chromatographic and mass spectral data presented above the identity of ABA-GE, IAA, Z and ZR is established in extracts from pear receptacles.

EXPERIMENTAL

Plant material. Large samples of 'Winter Nelis' pear receptacles were collected in liquid N_2 and freeze-dried to constant dry wt. Prior to extraction receptacles were ground with a mortar and pestle to a fine powder. 10.0 g samples for identification of ABA-GE and IAA were processed as outlined in Fig. 1. 100 g samples were processed for Z and ZR as outlined in Fig. 2. Conditions for HPLC, GC and GC/MS are listed (Tables 2, 3).

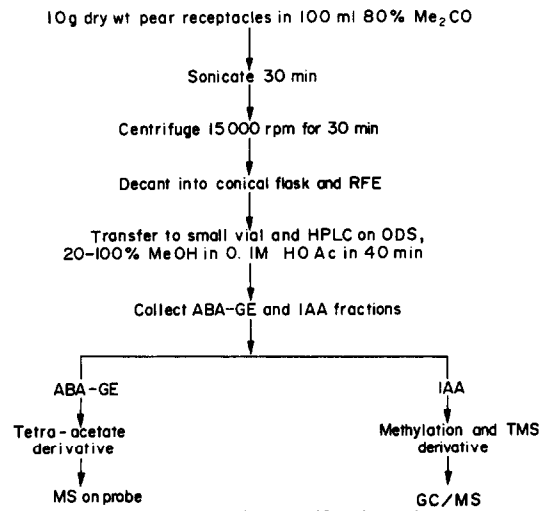

Fig. 1. Scheme for extraction–purification of ABA-GE and IAA in pear receptacles.

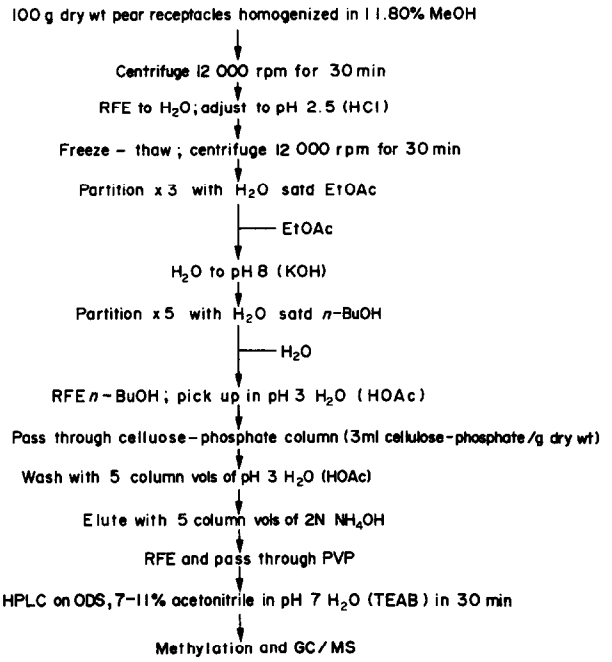

Fig. 2. Scheme for extraction–purification of Z and ZR in pear receptacles.

IAA partition coefficients. [^{14}C]IAA following HPLC purification was added at 10000 dpm to pH 3 H_2O (HCl) or pH 8 H_2O (KOH) plus an equal vol. of organic solvent, shaken and allowed to stand for 1 hr. Equal vols were taken from each layer for counting by standard scintillation techniques.

Analysis procedures. Methylation with CH_2N_2 or TMS derivatives were used as previously described [1, 10]. Z and ZR were permethylated by the procedure of Hakomori [11] with modifications [12]. Zones of cytokinin activity were located at each purification step by the *Amaranthus* bioassay [13].

The identification of putative ABA-GE provided some

Table 3. Conditions for GC/MS of ABA-GE, IAA, Z and ZR

	ABA-GE*	IAA	Z	ZR
GLC Pye 104				
Column (152.4 × 4 mm)	—	3%	OV-1 on Gas Chrom 100–120 mesh	Q
Carrier gas (35 ml/min)	—	He	He	He
Column temperature	—	196°	220–280° (8°/min)	305
MS Kratis MS30				
eV	70	70	70	70
μA	300	300	300	300
Scan speed (sec/decade)	3	3	3	3
Source temperature	150°	190°	190°	200°
Jet temperature	—	230°	280°	280°

*Analysis by probe—therefore GC not used.

special problems because of the putative single glucose moiety. Thus, additional procedures were used to substantiate the probable structure. Base hydrolysis to ABA was carried out as previously described [14] with subsequent measurement of ABA by EC-GC. In addition, the pear ABA following hydrolysis was subjected to 20 min UV light and measured by EC-GC for production of a 1:1 ratio of *c, t*-ABA and *t, t*-ABA.

ABA-GE samples from pear receptacles were subjected to base hydrolysis at pH 11, and two additional samples were dissolved in 0.1 M acetate buffer to which were added commercial preparations of either β-glucosidase or α-glucosidase; incubated for 15 hr at 37°, and subsequently methylated and assayed for ABA by EC-GC. This was done to clarify the nature of the sugar moiety. Finally both the UV spectrum and the direct probe MS of pear ABA-GE tetra-acetate were obtained. Because molecular ions were easily discerned by MS analysis for IAA, Z and ZR additional identification procedures beyond chromatographic data and MS was deemed unnecessary.

Pear ABA-GE was acetylated by the following procedure: the sample was dissolved in 20 drops dry pyridine to which 8 drops re-distilled Ac_2O were added; following 15 hr at ambient temp. 2 ml Et_2O were added and partitioned against 3 ml 2% aq. $NaHCO_3$; the Et_2O washed with 2 × 2 ml H_2O; dried and purified by HPLC on Hypersil (Table 2).

REFERENCES

1. Martin, G. C., Dennis, Jr., F. G., Gaskin, P. and MacMillan, J. (1977) *Phytochemistry* **16**, 605.
2. Ohkawa, M. (1973) *J. Jpn. Soc. Hortic. Sci.* **42**, 183.
3. Horgan, R. (1981) in *Progress in Phytochemistry* (Reinhold, L., Harborne, J. B. and Swain, T., eds.), Vol. 7, p. 137. Pergamon Press, Oxford.
4. Larsen, P. (1955) *Physiol. Plant.* **8**, 343.
5. Atsumi, S., Kuraishi, S. and Hayashi, T. (1976) *Planta* **129**, 245.
6. Lehman, H., Miersch, O. and Schutte, H. R. (1975) *Z. Chem.* **15**, 443.
7. Koshimizu, K., Inui, M., Fukui H. and Mitsui, T. (1968) *Agric. Biol. Chem.* **32**, 789.
8. Savidge, R. A. (1981) Ph.D. thesis, University College of Wales, Aberystwyth.
9. Morris, R. O. (1977) *Plant Physiol.* **59**, 1029.
10. Shaybany, B. and Martin, G. C. (1977) *J. Am. Soc. Hortic. Sci.* **102**, 300.
11. Hakomori, S. (1964) *J. Biochem. (Tokyo)* **55**, 205.
12. Martin, G. C., Scott, I. M. and Horgan, R. (1981) *J. Chromatogr.* **219**, 167.
13. Biddington, N. L. and Thomas, T. H. (1973) *Planta* **111**, 183.
14. Martin, G. C. and Nishijima, C. (1979) *J. Am. Soc. Hortic. Sci.* **104**, 185.

NOTE ADDED IN PROOF

Putative ABA-GE from pear was analysed by insertion via the desorption chemical ionization probe. Using ammonia gas for the chemical ionization resulted in $[M]^+$ at 612 with other notable ions at *m/z* 534, 518, 427, 366, 331, 306, 266, 264, 246 (base peak), 240, 222, 203, 188, 186, 184, 170, 168 and 167.