PERGAMON

Phytochemistry 55 (2000) 887–890

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Identification of endogenous gibberellins in strawberry, including the novel gibberellins GA_{123}, GA_{124} and GA_{125}

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Received 15 March 2000; received in revised form 20 May 2000

Abstract

Extracts of carboxylic acids from immature fruits of strawberry (*Fragaria x ananassa* Duch. cv. Elsanta) were analysed for gibberellins by combined gas chromatography-mass spectrometry. The following previously characterised gibberellins were identified by comparison of their mass spectra and Kovats retention indices (KRIs) with those of standards or published data: GA_1, GA_3, GA_5, GA_8, GA_{12}, GA_{17}, GA_{19}, GA_{20}, GA_{29}, GA_{44}, GA_{48}, GA_{49}, GA_{53}, GA_{77}, GA_{97}, GA_{111} and GA_{112}. Evidence for endogenous 1-*epi* GA_{61} (GA_{119}) and 11α-OH-GA_{12} was also obtained. In addition, a number of putative GAs were detected. Of these, three were shown to be 12α-hydroxy-GA_{53}, 12α-hydroxy-GA_{44}, and 12α-hydroxy-GA_{19} by comparison with authentic compounds prepared by rational synthesis, and have been allocated the descriptors GA_{123}, GA_{124} and GA_{125}, respectively.

Keywords: *Fragaria x ananassa*; Rosaceae; Strawberry; Identification; Gibberellins; GA_{123}; GA_{124}; GA_{125}; Immature fruit

1. Introduction

There is considerable evidence that the gibberellins (GAs) play a major role in the processes of fruit-set and development (García-Martínez and Hedden, 1997). However, studies on the hormone physiology of fruit development in the cultivated strawberry (*Fragaria x ananassa* Duch.) have mostly concentrated on auxins, since Nitsch (1950) demonstrated that removal of achenes (seeds) stopped berry enlargement, and that treatment with synthetic auxins caused growth to continue. It has been suggested that GAs may also have a role in the control of strawberry fruit development (Mudge et al., 1981) and this is supported by the positive effects of exogenous GAs on parthenocarpic berry development (Thompson, 1964, 1967). To date, only one bioassay-based study on the endogenous GA content of strawberry fruit has been reported (Lis et al., 1978). Two studies on the effect of photoperiod on GA levels in strawberry led to the identification of GA_1, GA_5, GA_8, GA_{17}, GA_{19}, GA_{20}, GA_{29}, and GA_{44}, by full-scan gas chromatography — mass spectrometry (GC–MS) in mature leaves of the cv. Elsanta (Taylor et al., 1994), and of GA_1, GA_4, GA_8, GA_{17} (tentative), GA_{19}, GA_{20}, GA_{29}, and GA_{34} in petioles of the cv. Earlisweet (Wiseman and Turnbull, 1999). In this paper, we now report the identification of further endogenous GAs in developing fruit of the cv. Elsanta.

2. Results and discussion

A total of 17 previously characterised GAs were identified by comparison of their full-scan mass spectra and KRIs with those of authentic standards or by comparison with published mass spectra and KRIs (Gaskin and MacMillan, 1991) (data not presented). Thus, GA_1, GA_3, GA_5, GA_{19}, GA_{20}, GA_{44}, GA_{77}, GA_{111} and GA_{112} were identified by comparison with protio-GA standards, while GA_8, GA_{12}, GA_{17}, GA_{29}, GA_{48}, GA_{49}, GA_{53} and GA_{97} were identified by comparison with published information (Gaskin and MacMillan, 1991).

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0031-9422/00/$ - see front matter
PII: S0031-9422(00)00237-5

However, we also used [17,17-$^2H_2$]-GA_8, [17,17-$^2H_2$]-GA_{29} and [17,17-$^2H_2$]-GA_{53} to obtain KRI values (2819, 2690 and 2518 respectively); KRIs of [17-$^2H_2$] GAs differ from those of the corresponding protio-GAs by ca. 2 under our conditions. Tentative identification of endogenous 11α-OH-GA_{12} and 1-*epi* GA_{61} was also made by comparison with published mass spectra and KRI values (Gaskin and MacMillan, 1991). In addition, three novel GAs, 12α-OH-GA_{53}, 12α-OH-GA_{44}, and 12α-OH-GA_{19} were identified by comparisons of their KRIs and mass spectra with those of authentic samples obtained using the same GC–MS conditions (Table 1). These three GAs all show the loss of fragments m/z = 103 and 116 (attributed to the C–12 and C–11–C–12 moieties, CH_2=OTMS and CH_2=CHOTMS, respectively) that is characteristic of 12,13-dihydroxy GAs (Gaskin and MacMillan, 1991). For the GA_{44} and GA_{53} analogues, the fragments are lost from the parent ion, whereas for GA_{19}, the loss follows that of m/z = 28 (C–20). The authentic standards were obtained by rational synthesis from gibberellic acid (Mander and Owen, 1997). According to convention (MacMillan and Takahashi, 1968) 12α-OH-GA_{53} is now identified as GA_{123}, 12α-OH-GA_{44} as GA_{124} and 12α-OH-GA_{19} as GA_{125}. Their structures are shown in Fig. 1.

Identification of the 13-hydroxylated GAs (GA_1, GA_3, GA_5, GA_8, GA_{17}, GA_{19}, GA_{20}, GA_{29}, GA_{44} and GA_{53}) in berries confirms that the early 13-hydroxylation GA biosynthetic pathway exists in fruit tissues of strawberry, as well as in vegetative tissues (Taylor et al., 1994; Wiseman and Turnbull, 1999). Given the presence of the potential intermediary, GA_5, it seems likely that GA_3 is produced from GA_{20} via GA_5 as was shown in maize (Fujioka et al., 1990). The occurrence of the new gibberellins, GA_{123}, GA_{124} and GA_{125}, together with GA_{77} and GA_{111}, raises the possibility that an early 12,13-dihydroxylation pathway is also operative in developing fruit of strawberry (Fig. 2). In this context, it is interesting to note the presence of two further 12-hydroxylated C_{19} GAs, GA_{48} and GA_{49}, although their biosynthetic origin in strawberry fruit remains unknown. The identification of GA_{97} (2β-OH-GA_{53}) in this study, lends support to the suggestion that this GA may be widespread in higher plants, having been found previously in spinach, pea, tomato and barley (Mander et al., 1996).

3. Experimental

3.1. Plant material

Immature fruit (< 10 mm diameter) were collected from the 'Junebearing' strawberry cv. Elsanta on 27 July 1999, from plants grown in peat grow bags (35 l, Bulrush Peat Company Ltd., N. Ireland) and cropping under polythene tunnels at a commercial growers holding at Mereworth, Kent. Berries were plunged immediately into liquid N_2 and stored at −20°C until analysis.

3.2. Extraction and purification

The method described for extraction and purification of GAs was based on that used previously for GA analyses of vegetative tissues of the same cultivar (Taylor et al., 1994). Frozen samples (100 g fresh weight) of whole (achenes + receptacle tissue) berries were homogenised in cold (4°C) 80% MeOH (vol/vol) containing 20 mg l^{-1} BHT and stirred overnight at 4°C. After filtration, the residue was re-extracted with MeOH overnight and refiltered. The filtrates were pooled, 0.67 KBq of [1,2-^{3}H]-GA_1 (1406 GBq $mmol^{-1}$; Du Pont de Nemours GmbH, NEN Division, Dreiech, Germany) was added, and the MeOH removed at 35°C under reduced pressure on a rotary film evaporator (RFE). An equal volume of 0.5 M K–Pi buffer pH 8.2 was added to the aqueous residue, which was then frozen, thawed and filtered, using further K–Pi buffer. The filtrate was loaded onto a column of insoluble PVPP (25 g) pre-equilibrated with

Table 1
Comparison of Kovats retention indices (KRI) and relative intensities of characteristic ions for MeTMSi derivatives of GA-like compounds in immature fruits of strawberry with those of authentic compounds

Compound	Kovats retention index	Diagnostic ions (m/z) with % abundance in reference and sample									HPLC fraction
12α-OH-GA_{44}	2950	Ion Standard	520 (M^+)	505	430	417	404	295	238	147	29
(GA_{124})	2950	Sample	33	10	15	73	18	27	76	100	
			28	8	13	68	15	24	71	100	
12α=OH-GA_{19}	2732	Ion Standard	550 (M^+)	522	432	419	406	239	193	147	30
(GA_{125})	2732	Sample	2	14	21	19	16	100	67	80	
			2	12	19	18	14	100	59	78	
12α-OH-GA_{55}	2642	Ion Standard	536 (M^+)	521	504	477	446	433	420	147	30
(GA_{123})	2642	Sample	68	12	18	27	19	72	48	100	
			59	10	18	23	17	68	43	100	

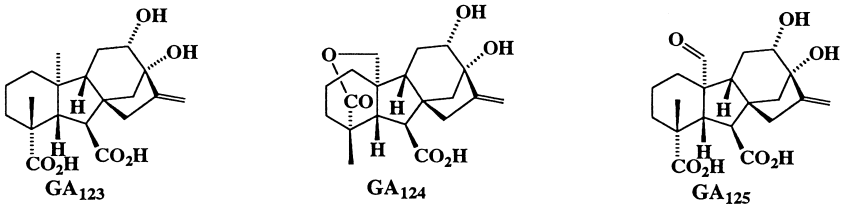

Fig. 1. Structures of GAs in strawberry.

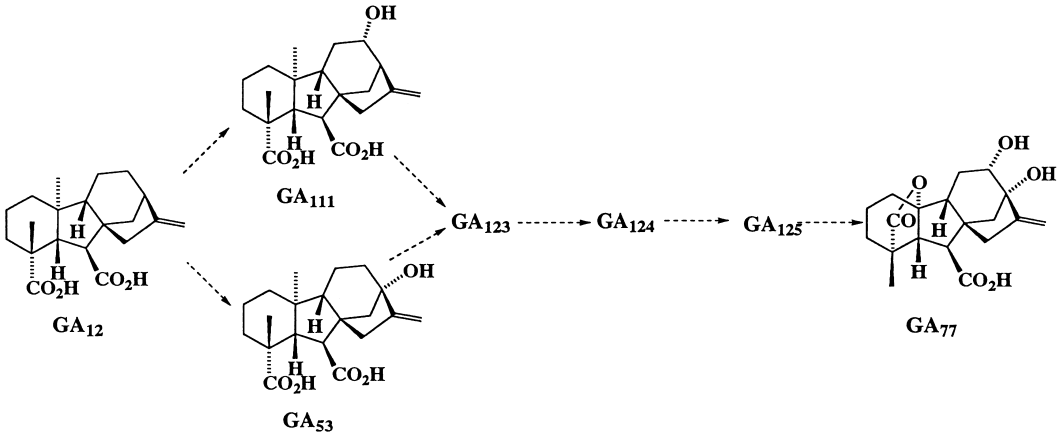

Fig. 2. Possible biosynthetic pathways of endogenous gibberellins in developing fruit of strawberry (*Fragaria x ananassa* Duch. cv. Elsanta).

0.5 M K–Pi buffer (pH 8.2). After washing the column with further K–Pi buffer, the pooled eluates were adjusted to pH 3.0 with phosphoric acid and partitioned against ethyl acetate (5×1/2 volume). The combined ethyl acetate phase was then partitioned against 5% sodium bicarbonate (5×1/5 volume). The combined aqueous phases were adjusted to pH 3.0 with phosphoric acid and extracted with ethyl acetate (5×1/5 volume), which following the addition of water (100 ml) was reduced to the aqueous phase (RFE). The extract was adjusted to pH 8.0 and loaded onto a column (2×10 cm) of QAE-Sephadex A25, pre-equilibrated with sodium formate (0.5 M) and washed with formic acid (0.2 M) and pH 8.0 water. After loading, the column was washed with water (90 ml, pH 8.0) and the GAs were eluted with 0.2 M formic acid (120 ml). The eluate was fed directly through four pre-equilibrated NH_2 Sep-Pak cartridges in series, and after washing with water (20 ml), pH 3.0, the GAs were eluted with 80% (vol/vol) MeOH (20 ml), which was then evaporated to dryness (RFE) after adding 2 ml toluene.

The GAs were purified further by reverse phase HPLC, using a Hypersil 5 ODS column (4.6 mm i.d.×250 mm). The column was eluted at a flow rate of 1 ml min^{-1} with 10% (vol/vol) MeOH for 5 min, followed by a linear gradient to 100% (vol/vol) MeOH over 45 min (solvents contained 50 μl l^{-1} acetic acid). The extract was dissolved in 10% MeOH (200 μl) and injected into the column using a 500 μl loop. Fifty 1 ml fractions were collected and aliquots (1/20) removed for scintillation counting to locate GA_1. Fractions were taken to dryness on a centrifugal vacuum concentrator (CVC), redissolved in 50 μl MeOH and methylated with excess ethereal diazomethane. The extracts were taken to dryness (CVC), redissolved in 25 μl Tri-Sil/BSA (Pierce and Warriner, Chester, UK), heated to 90°C for 15 min and evaporated to dryness (CVC), prior to being redissolved in 10 μl BSTFA (Pierce and Warriner, Chester, UK) to produce the trimethylsilyl ethers of the GA-methyl esters (MeTMSi) for GC–MS.

3.3. Capillary column GC–MS

Derivatised samples were analysed using a VG-TRIO 1 MS coupled to an HP 5890 GC equipped with a split/splitless injector. The CP-SIL 5 CB-MS capillary column (Chrompack (UK) Limited, London; 30 m long×0.25 mm i.d.) was coupled directly to the ion source with an interface temp. of 275°C and the He carrier gas was supplied under electronic pressure control to maintain a linear velocity of 35 cm s^{-1}. Samples (1 μl) were injected (injector temperature of 270°C) at an oven temperature of 90°C with the injection splitter (50:1) closed, and after 1.0 min the splitter opened and 1.0 min later the oven temperature increased at 15°C min^{-1} to 200°C. This temperature was held for 2.0 min and then increased at 2°C min^{-1} to 270°C which was held for 10 min. Mass spectra were acquired 20 min after injection by the VG Lab-Base data system, by scanning every 0.9 s from 50 to 650 amu. The electron

energy was 70 eV and source temp. 200°C. For calculation of KRIs, samples were coinjected with 0.1 μl of a Parafilm extract (Gaskin et al., 1971). A sample of GA_3 was obtained from Zeneca Crop Protection.

Acknowledgements

The authors are grateful to Mrs. M. Regan of Hugh Lowe Farms Ltd. for kindly providing samples of berries used in this study and to Paul Gaskin, IACR, Long Ashton Research Station for advice on GC–MS analysis. The UK Ministry of Agriculture, Fisheries and Food funded the contributions of P.S.B., D.R.T. and C.M.C. to this work.

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