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METABOLISM OF [^{3}H]GIBBERELLIN A_5 AND [^{2}H]GIBBERELLIN A_5 IN CELL SUSPENSION CULTURES OF *PRUNUS PERSICA*

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Key Word Index—*Prunus persica*; Rosaceae; [^{3}H]gibberellin A_5; [^{2}H]gibberellin A_5; cell suspension cultures; metabolism.

Abstract—Metabolism of the native gibberellin A_5 (GA_5), fed as either [1-^{3}H] GA_5 (3.1 Ci/mmol) or [17,17-^{2}H]GA_5, was investigated in cell suspension cultures of peach (*Prunus persica*) after 48 hr of incubation. Extractable radioactivity in free GA metabolites was 57.5%, with 21.6% in the putative GA glucosyl conjugates fraction.

Tentative identifications, based on comparison with authentic standard GAs after sequential chromatography on silica gel column→gradient-eluted C_{18} reversed phase HPLC-radiocounting (RC)→isocratic-eluted C_{18} reversed phase HPLC-RC [→Nucleosil N(Me)$_2$ HPLC-RC, when necessary], showed that only 13.9% of the extractable radioactivity remained as [^{3}H]GA_5, with 2.7% as [^{3}H]GA_5 glucosyl ether (GA_5-G) and 4.3% as [^{3}H]GA_5 glucosyl ester (GA_5-GE). [^{3}H]GA_5 was converted to the free GAs, GA_1, GA_3, GA_6, GA_8 and GA_{22}, and the putative GA glucosyl conjugates, GA_1-0(3)-G, GA_1-0(13)-G, GA_3-0(3)-G, GA_3-0(13)-G, GA_5-G, GA_5-GE, GA_6-GE, GA_8-0(2)-G and GA_8-0(?)-G. From the feeds of [17,17-^{2}H]GA_5 the presence of GA_1, GA_3, GA_5, GA_6, GA_8, GA_{22}, GA_5-G and GA_5-GE was further confirmed by GC-SIM, using five characteristic ions and Kovats Retention Indices.

INTRODUCTION

Among the gibberellins (GAs), GA_5 and GA_{32} are found in immature seeds of peach (*Prunus persica* L.) [1], although GA metabolism in that tissue has not yet been studied. However, in developing seeds of *P. armeniaca* (apricot), which contains structurally similar GAs [2], the metabolism of [^{3}H]GA_5 and [^{3}H]GA_1 has been investigated. Metabolites of GA_5 in apricot include putative GA_1, GA_3, GA_5 methyl ester, GA_6, GA_{22}, GA_{29}, and putative glucosyl conjugates of GA_1, GA_3, GA_5, GA_6, GA_8 and GA_{29} [3]. Metabolites of GA_1 in apricot include putative GA_8 and putative glucosyl conjugates of GA_1 and GA_8 [4]. Gibberellin A_5 is a potential precursor of GA_1 [3–7] and GA_3 [3–8] which as yet have not been identified in peach, although GA_1 has been characterized from developing seeds of apricot [2]. Gibberellin A_5 is also a possible precursor of GA_{32}. Cell suspension cultures of *Pharbitis nil* have been shown to have very similar metabolic pathways (for GA_5) to the developing seeds of *P. nil* [5]. Hence, preparatory to an examination of the GA metabolic sequences in developing seeds of *Prunus persica*, we have analysed the metabolism of [1-^{3}H]GA_5 and [17,17-^{2}H]GA_5 to free GAs and putative glucosyl conjugates in cell suspension cultures of *P. persica*.

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RESULTS AND DISCUSSION

Separation and identification of metabolites of [1-^{3}H]GA_5

Because of the high specific radioactivity of [^{3}H]GA_5, tentative identifications from the [^{3}H]GA_5 feeds were based on a series of sequential chromatography steps, with comparison of R_ts with those of authentic standards at each step (Table 1). However, the reader is referred to Koshioka *et al.* [5] and the results (Table 2) from the [^{2}H]GA_5 feeds for more definitive analyses of certain of the metabolites of GA_5. The C_{18} HPLC profiles of free GA fractions from cells or medium were quite similar to each other. The HPLC profile for the cells is shown in Fig. 1. As shown in Table 1, each of the gradient-eluted C_{18} HPLC column fraction groupings, frs I–V, of the free GA metabolites, contained one or two significant radioactive peaks when chromatographed on isocratic-eluted C_{18} HPLC-RC and/or Nucleosil N(Me)$_2$ HPLC-RC. From C_{18} HPLC fr. I, only [^{3}H]GA_8 was observed. The R_t of the radioactive peak from C_{18} HPLC fr. II, where authentic GA_{29} should have eluted, did not coincide with any of the logical metabolites, including GA_{29}. The R_ts of radioactive peaks from C_{18} HPLC fr. III coincided with those of authentic [^{3}H]GA_1 and [^{3}H]GA_3. The R_ts of radioactive peaks from C_{18} HPLC fr. IV coincided with those of authentic GA_6 and GA_{22}. The R_t of the radioactive peak from C_{18} HPLC fr. V coincided with that of the precursor [^{3}H]GA_5. We therefore conclude that [^{3}H]GA_1, [^{3}H]GA_3, [^{3}H]GA_6, [^{3}H]GA_8 and [^{3}H]GA_{22} are metabolites of [^{3}H]GA_5. The C_{18} HPLC-RC profiles of the methanol-soluble fraction from the silica gel partition column of cells or medium, and of the butanol fraction of the medium, were also quite similar to

Table 1. HPLC separation and identification of the metabolites of [1-^{3}H]GA_5 in cell suspension cultures of *P. persica* by sequential analyses

	R_t (min) of peak			
Compound Free GA metabolites	Gradient-eluted C_{18} HPLC (min)	Isocratic-eluted C_{18} HPLC (min)	Nucleosil $N(Me)_2$ HPLC (min)	Identity
I (Fig. 1)	18–19		19–24	[^{3}H]GA_8
II (Fig. 1)	21–24		24–30	Unknown
III (Fig. 1)	25–27	23–25 (A)*	23–27	[^{3}H]GA_3
		27–30 (A)	20–22	[^{3}H]GA_1
IV (Fig. 1)	28–31	9–12 (B)	33–40	[^{3}H]GA_6
		13–15 (B)		[^{3}H]GA_{22}
V (Fig. 1)	32–34	26–30 (B)	27–33	[^{3}H]GA_5
GA_1	25–27	28–29 (A)	21–22	
GA_3	25–27	23–24 (A)	24–26	
GA_5	33–34	27–29 (B)	29–31	
GA_6	29–30	9–11 (B)	34–36	
GA_8	18–19		20–22	
GA_{22}	29–30			
GA glucosyl metabolites	Gradient-eluted C_{18} HPLC	Isocratic-eluted C_{18} HPLC	Sephadex A-25 column fraction	
I (Fig. 2)	11–13		Glucosyl ether	
Hydrolysate	18–19			[^{3}H]GA_8
II (Fig. 2)	15–17		Glucosyl ether	
Hydrolysate	18–19			[^{3}H]GA_8
III (Fig. 2)	20–21		Glucosyl ether	
Hydrolysate	22–24			Unknown
IV (Fig. 2)	22–24		Glucosyl ether	
Hydrolysate	25–27	23–25 (A)		[^{3}H]GA_3
IV (Fig. 2)	22–24		Glucosyl ether	
Hydrolysate	25–27	27–30 (A)		[^{3}H]GA_1
V (Fig. 2)	25–26		Glucosyl ether	
Hydrolysate	25–27	23–25 (A)		[^{3}H]GA_3
V (Fig. 2)	25–26		Glucosyl ether	
Hydrolysate	25–27	27–30 (A)		[^{3}H]GA_1
VI (Fig. 2)	28–30		Glucosyl ester	
Hydrolysate	29–31	9–12 (B)		[^{3}H]GA_6
VI (Fig. 2)	28–30		Glucosyl ether	
Hydrolysate	32–34	26–30 (B)		[^{3}H]GA_5
VII (Fig. 2)	32–33		Glucosyl ester	
Hydrolysate	32–34	26–30 (B)		[^{3}H]GA_5

*Isocratic-eluted conditions.

each other. The C_{18} HPLC-RC profile of this putative conjugate fraction of cell extracts is shown in Fig. 2. From the methanol-soluble fraction of the silica gel partition column, several significant radioactive peaks (frs I ~ VII) were observed on gradient-eluted C_{18} HPLC-RC. The frs were further subjected to Sephadex A-25 column chromatography which will separate GA-G from GA-GE. The results are shown in Table 1. Each of the cellulase hydrolysates of putative GA glucosyl conjugate fractions from Sephadex A-25 columns contained from one to two peaks when run on isocratic-eluted C_{18} HPLC-RC (Table 1). Both of the hydrolysates of frs I and II, which contained putative GA-Gs based on Sephadex A-25 column analyses, coincided with [^{3}H]GA_8. Thus, these fractions probably contained one or more of GA_8-0(2)-G, GA_8-0(3)-G and GA_8-0(13)-G, respectively. The hydrolysate of fr. III, which contained a putative GA-G, yielded a radioactive peak which coincided with an unknown [^{3}H] compound found in the free GA fraction. The hydrolysates of Fr. IV, which contained putative GA-Gs, yielded [^{3}H] peaks which coincided with [^{3}H]GA_1 and [^{3}H]GA_3. Thus, fr. IV probably contained C-13 glucosyl ethers of these two free GAs based on their initial HPLC R_t [5]. The hydrolysates of fr. V which contained putative GA-Gs yielded [^{3}H] peaks on C_{18} HPLC which coincided with [^{3}H]GA_1 and [^{3}H]GA_3. Fraction V thus probably contained C-3 glucosyl ethers of these two free GAs based on their initial HPLC R_ts [5]. From fr. VI, both GA-G- and GA-GE-like compounds were found in Sephadex A-25 column analysis. The hydrolysates of

Table 2. Kovats Retention Indices (KRIs) and relative intensities of characteristic *m/z* ions by GC-SIM for MeTMSi derivatives from bioactive fractions obtained from extracts of cell suspension cultures of *P. persica*, when [17,17-^{2}H]GA_5 was fed

Compound (C_{18} HPLC fraction)	KRI	GC-SIM spectrum with relative abundance and percentage intensity in parenthesis, *m/z* value (abundance: percentage intensity)					Identity
I (Fig. 1)	2821	596 (7:100)	450 (t*:10)	381 (t:10)	377 (t:10)	240 (t:10)	[^{2}H]GA_8
GA_8	2822	594 (7:100)	448 (2:25)	379 (1:20)	375 (1:15)	238 (2:28)	
III (Fig. 1)	2683	508 (18:100)	493 (t:10)	450 (t:10)	379 (t:10)	315 (t:10)	[^{2}H]GA_1
[^{2}H]GA_1	2684	508 (18:100)	493 (2:13)	450 (4:20)	379 (2:12)	315 (3:17)	
III (Fig. 1)	2706	506 (196:100)	491 (17:8)	475 (6:3)	445 (15:8)	370 (29:14)	[^{2}H]GA_3
[^{2}H]GA_3	2707	506 (196:100)	491 (16:8)	475 (4:2)	445 (16:8)	370 (25:13)	
IV (Fig. 1)	2634	434 (540:100)	419 (83:15)	405 (30:6)	375 (135:25)	304 (86:16)	[^{2}H]GA_6
GA_6	2634	432 (540:100)	417 (76:14)	403 (32:6)	373 (124:23)	302 (22:4)	
IV (Fig. 1)	2713	506 (27:100)	491 (6:22)	403 (20:74)	389 (9:33)	372 (5:19)	[^{2}H]GA_{22}
GA_{22}	2713	504 (27:100)	489 (8:28)	401 (16:58)	387 (5:20)	370 (5:20)	
V (Fig. 1)	2534	418 (947:100)	403 (142:15)	387 (28:3)	359 (203:21)	345 (195:21)	[^{2}H]GA_5
[^{2}H]GA_5	2534	418 (947:100)	403 (161:17)	387 (29:3)	359 (198:21)	345 (189:20)	
Hydrolysate of VI (Fig. 2)	2534	418 (584:100)	403 (76:13)	387 (17:3)	359 (128:22)	345 (134:23)	[^{2}H]GA_5
[^{2}H]GA_5	2534	418 (584:100)	403 (99:17)	387 (18:3)	359 (123:21)	345 (117:20)	
Hydrolysate of VII (Fig. 2)	2534	418 (651:100)	403 (78:12)	387 (21:3)	359 (111:17)	345 (113:17)	[^{2}H]GA_5
[^{2}H]GA_5	2534	418 (651:100)	403 (111:17)	387 (20:3)	359 (137:21)	345 (130:20)	

*Trace amount.

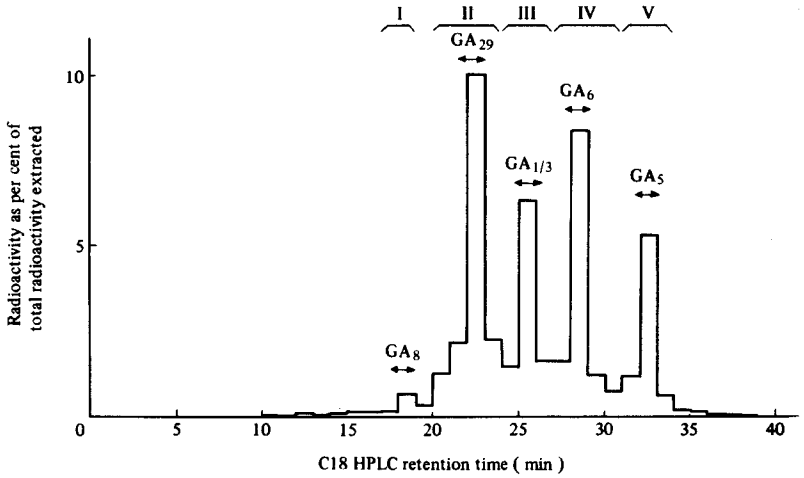

Fig. 1. Elution pattern of radioactivity from a gradient-eluted C_{18} HPLC of the free GA fraction from a silica gel partition column of an extract of cells of *P. persica* fed [1-^{3}H]GA_5.

these GA-G- and GA-GE-like compounds coincided with [^{3}H]GA_5 and [^{3}H]GA_6, respectively. The hydrolysate of fr. VII (which contained a putative GA-GE) coincided with [^{3}H]GA_5. Thus, two [^{3}H]GA_1-Gs, two [^{3}H]GA_3-Gs, [^{3}H]GA_5-G, [^{3}H]GA_5-GE, [^{3}H]GA_6-GE and at least two [^{3}H]GA_8-Gs were tentatively identified from the putative conjugates fraction.

Separation and identification of metabolites of [17,17-^{2}H]GA_5

The sequential analyses for [^{2}H]GA_5 feeds were done in essentially the same way as noted above for the [^{3}H]GA_5 feeds. [^{2}H] Metabolites were collected from HPLC column fractions corresponding to [^{3}H] metabolite HPLC fractions, and then bioassayed prior to GC-SIM analyses. The biological activity profiles of Nucleosil $N(Me)_2$ HPLC fractions after C_{18} HPLC separations are shown in Fig. 3. As shown in Table 2, [^{2}H]GA_8 was found in a gradient-eluted C_{18} HPLC fr. I corresponding to C_{18} HPLC fr. I of the [^{3}H]GA_5 feeds (see Fig. 1), [^{2}H]GA_1 and [^{2}H]GA_3 were found in fr. III, [^{2}H]GA_6 and [^{2}H]GA_{22} were found in fr. IV and [^{2}H]GA_5 was found in fr. V. All identifications were confirmed by GC-SIM, using five characteristic ions and Kovats Retention Indices (KRIs) [9]. From the putative GA glucosyl conjugate fractions of C_{18} HPLC fr. VI, [^{2}H]GA_5 was obtained by cellulase hydrolysis of both of the GA-G and

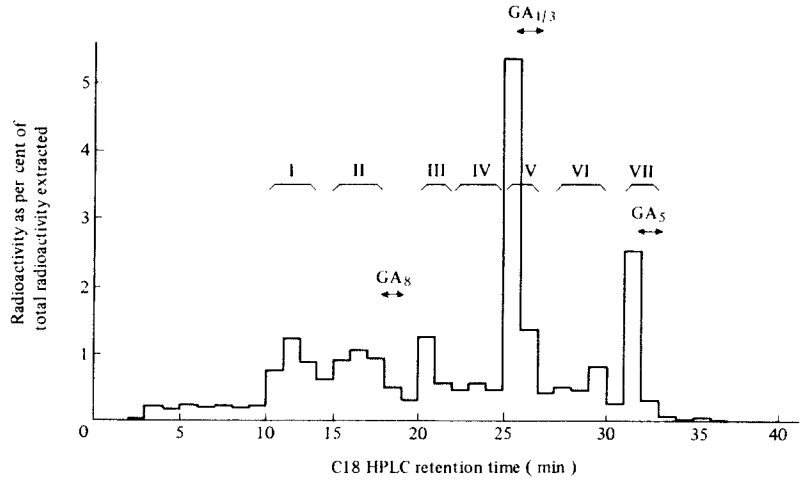

Fig. 2. Elution pattern of radioactivity from a gradient-eluted C_{18} HPLC of the putative GA glucosyl conjugate fraction from a silica gel partition column of an extract of cells of *P. persica* fed [1-^{3}H]GA_5.

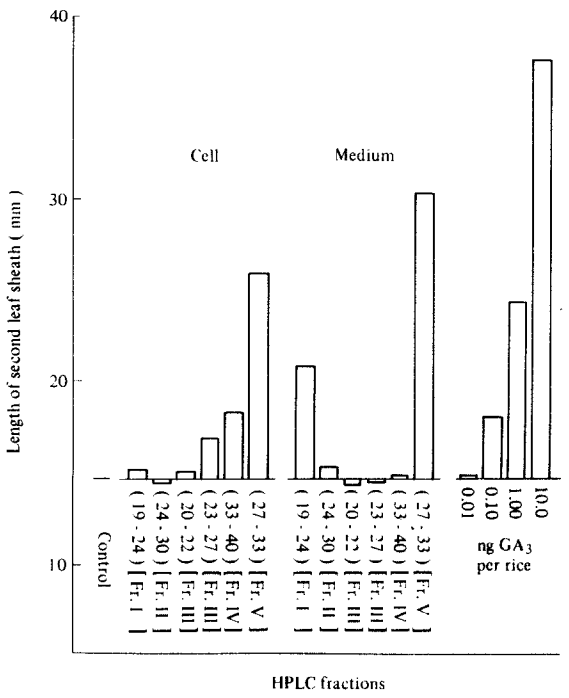

Fig. 3. Biological activity after a sequential C_{18} HPLC →Nucleosil N(Me)$_2$ HPLC of the free GA fractions from an extract of cell suspension cultures of *P. persica* fed [17,17-^{2}H]GA_5. The biological activity of GA_3 standards on the micro-drop assay, expressed as 0.1–10.0 μg/rice plant, is shown on the right. Each Nucleosil HPLC fraction was assayed at 1/300 aliquot. R_ts on the Nucleosil HPLC are shown in parentheses, and original fractions from C_{18} HPLC are shown in brackets (see text for R_ts in min).

GA-GE fractions from the DEAE Sephadex A-25 columns based on GC-SIM (Table 2 and Fig. 2). The permethylated derivatives of frs VI and VII also showed characteristic peaks (G. Schneider and O. Juntilla, unpublished work) of [^{2}H]GA_5-G (*m/z* 389, 329 and 88) and [^{2}H]GA_5-GE (*m/z* 664, 633, 389, 329, and 101) on GC-SIM.

Metabolic pathways of GA_5 in cell suspension cultures of peach

Possible metabolic pathways of GA_5 in cell suspension cultures of *P. persica* are shown in Fig. 4. The pattern of GA_5 metabolism in these cells is thus similar to that found in the immature seeds of *P. armeniaca*, although major quantitative differences occur between these two systems in their metabolism of GA_5.

Metabolism of [1-^{3}H]GA_5 (Table 3)

In *P. persica* cell suspension cultures most (98.1%) of the applied radioactivity was recovered. The distribution of extracted radioactivity was: 71.8% in cells and 28.2% in medium (of which 22.4% was in the acidic ethyl acetate extract, 3.0% in the acidic butanol extract and 0.7% in the aqueous residue). Of the extracted radioactivity from the cells, 57.5% existed as free GA metabolites, with 21.6% putative GA glucosyl conjugates. Within the cells 79.1% of the radioactivity represented GAs other than GA_5, and their putative glucosyl conjugates, with only 13.9% remaining as free [^{3}H]GA_5, and 2.7% as putative [^{3}H]GA_5-G and 4.3% as putative [^{3}H]GA_5-GE. The major free GA metabolites were [^{3}H]GA_3 (17.2%), [^{3}H]GA_6 (11.6%) and the unknown compound (14.1%), while the major putative conjugates were [^{3}H]GA_3-Gs (8.2%), [^{3}H]GA_8-Gs (7.1%) and [^{3}H]GA_5-G/GE (7.0%) in GA glucosyl conjugate metabolites. As expected [3–7], [^{3}H]GA_6 was observed in relatively large amounts (11.6%) but amounts of its putative glucosyl conjugate ([^{3}H]GA_6-GE) were low (only 0.1%). This means the conversion of [^{3}H]GA_6 to [^{3}H]GA_3 may proceed much faster than that of [^{3}H]GA_6 to [^{3}H]GA_6-GE. [^{3}H]GA_8 and putative [^{3}H]GA_8-Gs were also present in relatively high amounts (8.6%) in the cell extracts, but there was no evidence for [^{3}H]GA_{32} and/or its glucosyl conjugates. The presence of [^{3}H]GA_{29} and its glucosyl conjugates was not indicated in these [^{3}H]GA_5 feeds to cell suspension cultures of *P. persica*, although immature seeds of *P.*

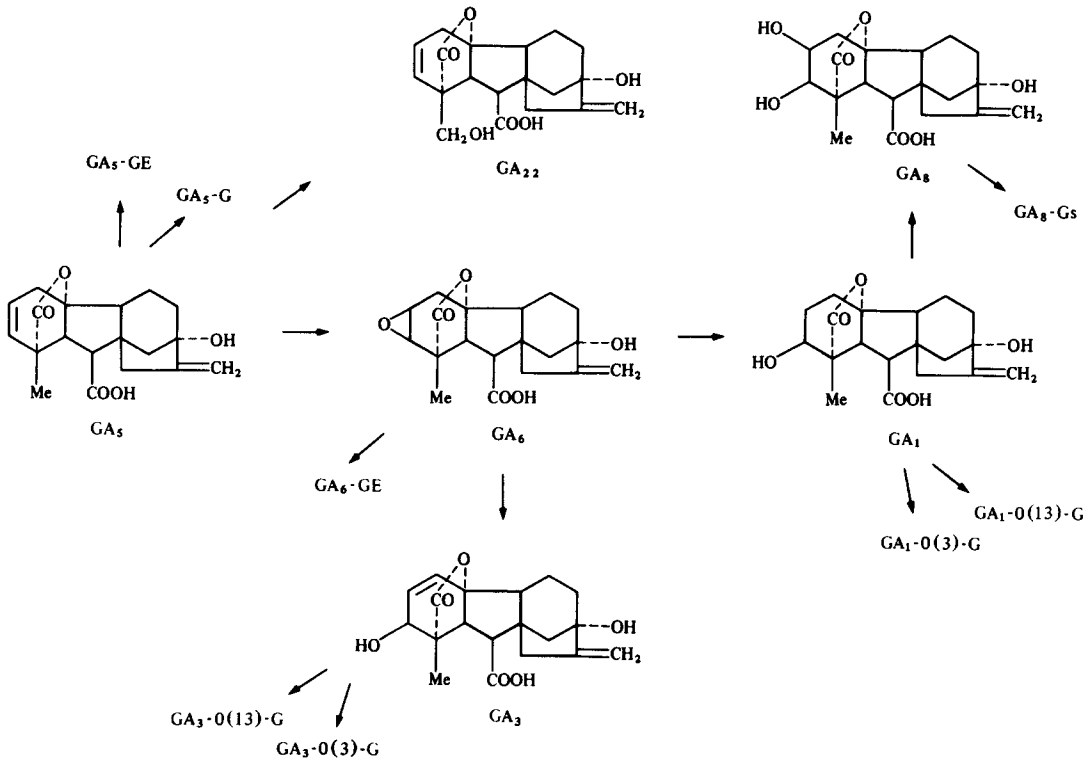

Fig. 4. Possible metabolic pathways of GA_5 in cell suspension cultures of *P. persica*.

Table 3. Radioactivity (as a percentage of extracted radioactivity) from extracts of cells or medium from cell suspension cultures of *P. persica*. incubated with [1-^{3}H]GA_5 for 48 hr

Tentative identity of free GA or GA conjugates	C_{18} HPLC fraction (Figs 1 and 2)	Percentage of radioactivity			
			Medium		
		Cell extract	Free GAs	Putative GA conjugates	Total
Free GAs					
GA_8	I (Fig. 1)	1.3	0.2		1.5
Unknown	II (Fig. 1)	10.2	3.9		14.1
GA_1	III (Fig. 1)	0.8	1.7		2.5
GA_3	III (Fig. 1)	8.6	8.6		17.2
GA_6	IV (Fig. 1)	10.6	1.0		11.6
GA_{22}	IV (Fig. 1)	0.5	—		0.5
GA_5	V (Fig. 1)	7.9	6.0		13.9
Others	(Fig. 1)	7.3	2.8		10.1
Sub-total		47.2	24.2		71.4
Putative GA glucosyl conjugates					
GA_8-Gs	I, II (Fig. 2)	6.9		0.2	7.2
Unknown-G	III (Fig. 2)	2.1		0.3	2.4
GA_1-Gs	IV, V (Fig. 2)	0.9		0.2	1.1
GA_3-Gs	IV, V (Fig. 2)	7.3		0.9	8.2
GA_6-GE	VI (Fig. 2)	0.1		—	0.1
GA_5-G	VI (Fig. 2)	2.1		0.6	2.7
GA_5-GE	VII (Fig. 2)	3.2		1.1	4.3
Others	(Fig. 2)	2.0		0.7	2.7
Sub-total		24.6		4.0	28.6
Total		71.8	24.2	4.0	100.0

armeniaca [3] and *Pharbitis nil* [5], and cell suspension cultures of *Daucus carota* [7] did yield [^{3}H]GA_{29} and its putative glucosyl conjugates as metabolites. Instead of [^{3}H]GA_{29}, an unknown [^{3}H] compound and its putative glucosyl ether were observed in relatively large amounts (16.5%) at the same R_t as GA_{29} on gradient-eluted C_{18} HPLC. The GC-SIM data and KRI data of the MeTMSi derivative of the fraction containing the unknown compound from the [^{2}H]GA_5 feeds did not coincide with those of known GAs. In cells of suspension cultures of *P. nil* [5], *D. carota* [7, 10] and *Pimpinella anisum* [11], most (over 80%) of the extracted GAs were GA glucosyl conjugates for incubations in excess of 48 hr. However, in the present study, cells of *P. persica* contained only 34% of the radioactivity in the putative GA conjugate fraction, with 66% in the free GA fraction. The pattern of GA_5 metabolism in cell suspension cultures of *P. persica* is thus very similar to those found in developing seeds of *P. armeniaca* [3], *P. nil* [5, 6], and *D. carota* [7], although minor qualitative differences do exist (such as absence of GA_{29} and its glucosyl conjugates and presence of the unknown metabolite). Metabolism of GA_5 to free GA metabolites (especially, GA_3, GA_6 and the unknown metabolite) is enhanced in *P. persica* cell suspension cultures relative to cell suspension cultures of the other species [6, 7].

Cell suspension cultures of *P. persica* may thus be a useful initial tool in examining the metabolic fates of other native peach GAs.

EXPERIMENTAL

Culture origin and maintenance. To obtain the cell suspension cultures of *Prunus persica*, we obtained leaf discs from a 3-year-old tree of cultivar 'Hakuho' (*P. persica* L. Batsch, Rosaceae). The cell suspension cultures derived from these discs were maintained by bi-weekly passages according to Matsuta *et al.* [12].

Application of [^{2}H]GA_5 *and* [^{3}H]GA_5. Filter-sterilized [17,17-^{2}H]GA_5 (99.2% enrichment, prepared by a modification of the Nozaki procedure [13], 220 μg dissolved in 100 μl of 95% EtOH) and [1-^{3}H]GA_5 (6.4 μCi, 3.1 Ci/mmol [14], dissolved in 100 μl of 95% EtOH) were each applied to a separate 40 ml volume of cell suspension cultures of *P. persica* (5-day-old innoculum). After 2 days of incubation the cells and the media were separated, frozen with liquid N_2 and freeze-dried.

Extraction and separation. Freeze-dried cells (0.34 g for [^{2}H]GA_5 feeds and 0.35 g for [^{3}H]GA_5 feeds) were extracted with 40 ml of aq. 80% MeOH and the extract was purified by the method of Koshioka *et al.* [15]. Freeze-dried medium was then dissolved in 20 ml of pH 3.0 H_2O and shaken 3 times with equal vols of EtOAc, followed by *n*-BuOH ($\times$ 2). The cell extracts and the EtOAc extracts of the medium were subjected to chromatography on separate small silica gel partition columns in order to separate most free GAs from putativae GA glucosyl conjugates [16]. The free GA fractions from the silica gel partition columns, and the subsequent MeOH-soluble fractions which will contain glucosyl conjugates and much of the polyhydroxylated GA group, and the BuOH extracts of the media were further purified by a gradient-eluted C_{18} reversed phase HPLC column [16] with the following gradient: 10% MeOH in 1% aq. HOAc, isocratic (10 min), linear gradient from 10% MeOH in 1% aq. HOAc to 73% MeOH in 1% aq. HOAc (10–40 min), 100% MeOH, isocratic (40–50 min); flow rate, 2 ml/min. Radioactive metabolite peaks were detected by an in-line Berthold radiocounter (RC) and bioactive GA-like substances were detected by use of the dwarf rice micro-drop assay [17], or dwarf rice immersion assay [18]. The C_{18} HPLC fractions of putative GA glucosyl conjugates were further chromatographed on DEAE Sephadex A-25 columns designed to separate GA glucosyl esters (GA-GEs) from GA glucosyl ethers (GA-Gs) [19]. Parts of the putative glucosyl conjugates were then hydrolysed with cellulase [20] prior to additional HPLC analysis. Furthermore, isocratic-eluted C_{18} HPLC-RC was used further to separate discrete radioactive free GA fractions, or hydrolysed fractions of the putative conjugates derived from the earlier gradient-eluted C_{18} HPLC-RC. The isocratic conditions were A: 15% MeOH in 1% aq. HOAc and B: 30% MeOH in 1% aq. HOAc. The radioactive or biologically active peaks from isocratic-eluted C_{18} HPLC columns were further separated on Nucleosil $N(Me)_2$ HPLC columns when necessary, using the following conditions: 99.9% MeOH, 0.1% HOAc, flow rate 1 ml/min. The biologically active fractions from C_{18} HPLC and/or Nucleosil $N(Me)_2$ HPLC columns were further analysed by GC-SIM, with cool on-column injection into a DBI-15N column (film thickness 0.25 μm, internal diameter 0.25 mm, length 15m; J & W Scientific), temperature programmed from 60° (1 min) to 250° at 25°/min, installed in a Hewlwtt Packard 5790A GC with Capillary direct interface to a HP 5970A Mass Selective Detector, as the methyl ester trimethylsilyl ether (MeTMSi) derivatives for free GAs, or the permethylated derivatives [21] for GA glucosyl conjugates according to methods described in a previous report [7].

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