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Gibberellin Biosynthesis in Maize. Metabolic Studies with GA_{15}, GA_{24}, GA_{25}, GA_7, and 2,3-Dehydro-GA_9[1]

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[17-^{14}C]-Labeled GA_{15}, GA_{24}, GA_{25}, GA_7, and 2,3-dehydro-GA_9 were separately injected into normal, *dwarf-1 (d1)*, and *dwarf-5 (d5)* seedlings of maize (*Zea mays* L.). Purified radioactive metabolites from the plant tissues were identified by full-scan gas chromatography-mass spectrometry and Kovats retention index data. The metabolites from GA_{15} were GA_{44}, GA_{19}, GA_{20}, GA_{113}, and GA_{15}-15,16-ene (artifact?). GA_{24} was metabolized to GA_{19}, GA_{20}, and GA_{17}. The metabolites from GA_{25} were GA_{17}, GA_{25} 16α,17-H_2-17-OH, and HO-GA_{25} (hydroxyl position not determined). GA_7 was metabolized to GA_{30}, GA_3, isoGA_3 (artifact?), and trace amounts of GA_7-diene-diacid (artifact?). 2,3-Dehydro-GA_9 was metabolized to GA_5, GA_7 (trace amounts), 2,3-dehydro-GA_{10} (artifact?), GA_{31}, and GA_{62}. Our results provide additional in vivo evidence of a metabolic grid in maize (i.e. pathway convergence). The grid connects members of a putative, non-early 3,13-hydroxylation branch pathway to the corresponding members of the previously documented early 13-hydroxylation branch pathway. The inability to detect the sequence $GA_{12} \rightarrow GA_{15} \rightarrow GA_{24} \rightarrow GA_9$ indicates that the non-early 3,13-hydroxylation pathway probably plays a minor role in the origin of bioactive gibberellins in maize.

The biosynthesis of the gibberellins (GAs) has been recently reviewed (MacMillan, 1997). In all systems studied, the pathway has been shown to proceed from the cyclic diterpene *ent*-kaurene to GA_{12} aldehyde then to GA_{12}. Depending on the sequence of hydroxylation at the 3β- and 13-positions, parallel pathways branch from GA_{12} to the C_{19}-GAs, the number of these branch pathways varying from species to species. For maize (*Zea mays* L.) we previously demonstrated (see Fig. 1) the presence of the early 13-hydroxylation branch pathway, a pathway originating from GA_{12} and leading to the hydroxylated C_{19}-GAs, GA_1, GA_3, and GA_5 (Kobayashi et al., 1996 and refs. therein; Spray et al., 1996). As shown in Figure 1, the steps from GA_{12} to bioactive GA_1, GA_3, and GA_5, the early 13-hydroxylation branch pathway, have been established by feeding studies using labeled substrates; the immediate metabolites were identified by full-scan gas chromatography-mass spectrometry (GC-MS) and Kovats retention index (KRI) data (Fujioka et al., 1990; Kobayashi et al., 1996). All members of this branch pathway are native to maize (Fujioka et al., 1988a, 1988b).

There is indirect evidence for the presence of a second pathway from GA_{12}, the non-early 3,13-hydroxylation branch pathway. The pathway originates from GA_{12} and leads via GA_9 to the 3β-hydroxylated C_{19}-GAs GA_4, and GA_7 (see Fig. 1). While the pathway has been shown to be present in a number of plant species (for review, see MacMillan, 1997), its presence in maize is based solely on the identification from maize of the five members GA_{15}, GA_{24}, GA_9, GA_4, and GA_7. Moreover, in vivo feeding studies have provided no evidence for the metabolism of GA_{12} to GA_{15} (Kobayashi et al., 1996), GA_9 to GA_4 (Davis et al., 1998), and GA_4 to GA_7 (Kobayashi et al., 1993).

In the present study, we describe the metabolism of [17-^{14}C]GA_{15}, [17-^{14}C]GA_{24}, [17-^{14}C]GA_{25}, and [17-^{14}C]GA_7 in seedlings of tall, *dwarf-1 (d1)*, and *dwarf-5 (d5)* maize. Given the previous demonstration of the sequence $GA_{20} \rightarrow GA_5$ (2, 3-dehydro-GA_{20}) $\rightarrow GA_3$ in maize (Fujioka et al., 1990), the possible existence of a parallel sequence of $GA_9 \rightarrow$ 2,3-dehydro-$GA_9 \rightarrow GA_7$ was tested by feeding 2,3-dehydro-[17-^{14}C]GA_9, a GA not reported to be present in maize (Fujioka et al., 1988b). The data obtained, together with the previous results from the metabolism of [17-^{13}C,^{3}H]GA_9 and [17-^{13}C,^{3}H]GA_4, are discussed in terms of the biosynthesis of GAs in maize.

MATERIALS AND METHODS

Plant Material

Normal (tall), *dwarf-1* (*d1*), and *dwarf-5* (*d5*) maize (*Zea mays* L.) seedlings came from seed stocks of known genotype (Spray et al., 1996). The seeds were pre-soaked in water for 12 h and planted in vermiculite:soil (1:1). The

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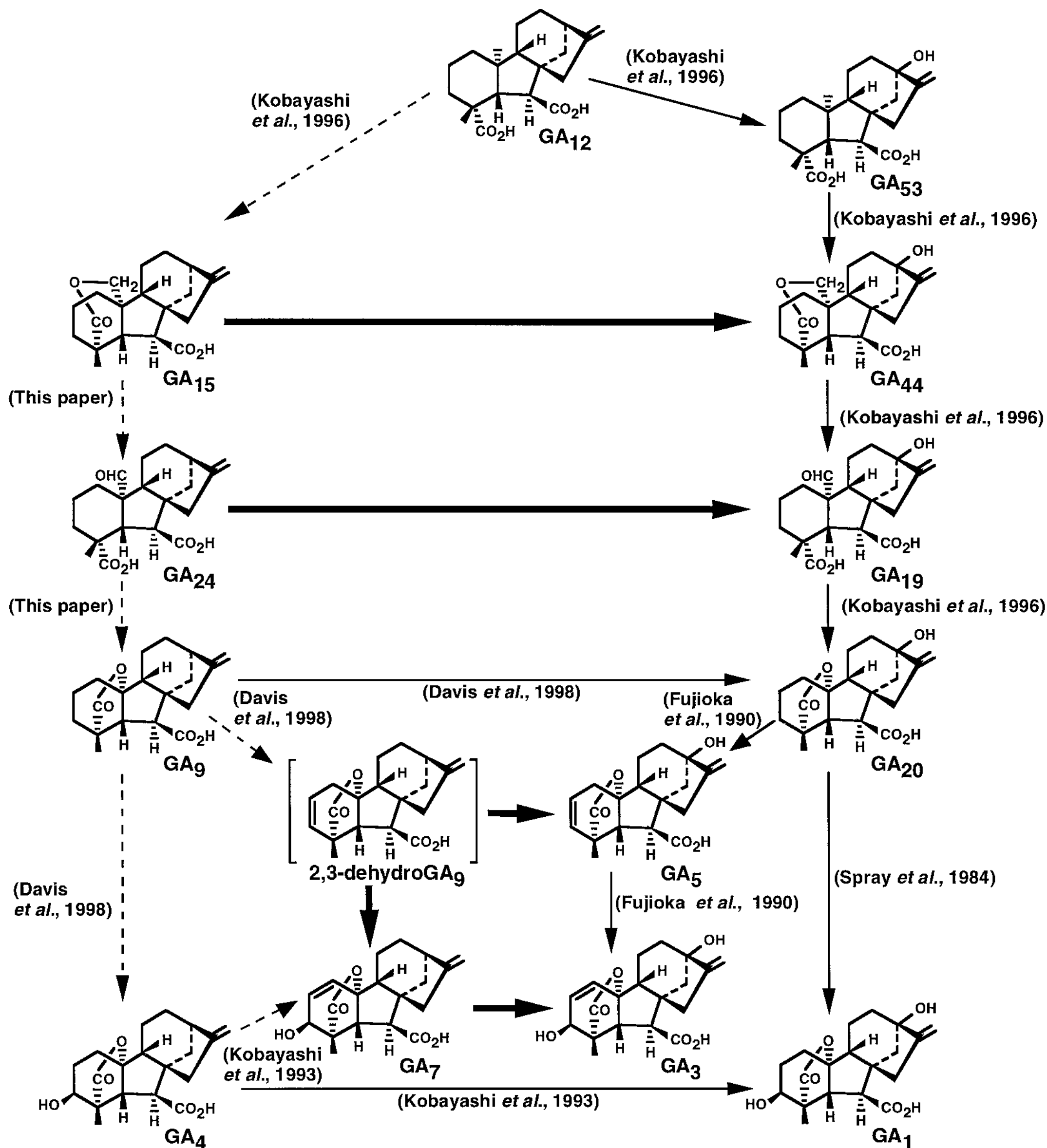

Figure 1. Maize branch pathways from GA_{12}: right vertical row, the early 13-hydroxylation branch pathway; left vertical row, the presumptive non-early 3,13-hydroxylation branch pathway. All of the GAs are endogenous to maize except 2,3-dehydroGA_9, shown in brackets. →, Steps established in this paper; →, steps previously established; - - →, steps tested, not observed.

seedlings were then grown in the greenhouse at the University of California, Los Angeles. Three- to four-week-old seedlings (three- to four-leaf stage) were used for feeds.

Labeled Substrates

[17-^{14}C]GA_{15} (2.07 TBq mol^{-1}), [17-^{14}C]GA_{24} (2.07 TBq mol^{-1}), and [17-^{14}C]GA_7 (2.07 TBq mol^{-1}) were purchased from Prof. L.N. Mander (Australian National University, Canberra). [17-^{14}C]GA_{25} (2.07 TBq mol^{-1}) was prepared from [17-^{14}C]GA_{24} (300 kBq; a gift from Prof. L.N. Mander) with cell lysates (3.5 mL) from *Escherichia coli* NM522 containing clone E5 by methods detailed by Lange (1997) and purified as described by Lange and Graebe (1993). 2,3-Dehydro-[17-^{14}C]GA_9 (1.75 TBq mol^{-1}) was prepared as described in MacMillan et al. (1997).

Treatment, Purification, and Analysis

Each of the five labeled GAs, [17-^{14}C]GA_{15}, [17-^{14}C]GA_{24}, [17-^{14}C]GA_{25}, [17-^{14}C]GA_7, and 2,3-dehydro-[17-^{14}C]GA_9, was dissolved in 90 μL of ethanol:water (1:1). Two microliters of the [17-^{14}C]GA_{15} solution (1,570 Bq; 250 ng) were individually injected into the coleoptile nodes of three sets of 10 plants (normal, *d1*, and *d5*). Similar injections were made with [17-^{14}C]GA_{24} (1,490 Bq; 250 ng), [17-^{14}C]GA_{25} (1,420 Bq; 250 ng), and [17-^{14}C]GA_7 (1,550 Bq; 250 ng). One

Table I. *Analysis of metabolites from feeds of [17-^{14}C]GA$_{15}$ (250 ng, 1.57 × 10^{3} Bq each) to normal, d1, and d5 seedlings of maize*

Plant Material	ODS-HPLC Fraction	$N(CH_3)_2$-HPLC Fraction	Radioactivity	[^{14}C]Product[a]
			Bq	
Normal (12.0 g)	18–21	31–34	128	GA_{113}
	22–25	31–34	49	GA_{44}
	30–34	26–29	34	GA_{15}-15,16-ene
	30–34	30–33	628	GA_{15} (feed)
d1 (7.2 g)	22–25	31–32	109	GA_{44}
	30–34	28–31	452	GA_{15} (feed)
d5 (7.0 g)	18–21	31–34	228	GA_{20}, GA_{113}
	22–25	28–30	102	GA_{44}
	22–25	45–47	94	GA_{19}
	30–34	28–31	726	GA_{15} (feed)

[a] Identified by data shown in Table II.

set of 10 *d5* seedlings was used for the 2,3-dehydro-[17-^{14}C]GA$_9$ injections (485 Bq; 88 ng).

The seedlings were incubated in the greenhouse for 24 h, harvested as sets of 10, frozen with dry ice, and stored at −80°C. Each set of frozen seedlings was homogenized, extracted, and solvent-fractionated to give an acidic ethyl acetate-soluble (AE) fraction. Each fraction was concentrated and purified using Bond Elut (Varian, Harbor City, CA) columns and two steps of HPLC (Davis et al., 1998). All samples were methylated and the GAs in each sample were identifed by full-scan GC-MS and KRI (Gaskin and MacMillan, 1991; Spray et al., 1996).

Isotopic Dilution

To determine whether 2,3-dehydro-GA$_9$ is endogenous to maize, [17-^{14}C]2,3-dehydro-GA$_9$ (1.75 TBq mol^{-1}) was used in an isotopic dilution experiment. Fifteen nanograms (3 Bq) was dissolved in 100 μL of 50% (v/v) aqueous ethanol and added to a homogenate from 50 normal maize seedlings (200 g fresh weight). The homogenate was extracted immediately and solvent fractionated to give an AE fraction. The fraction was processed for the determination of isotopic dilution using the isotope dilution fit program described by Croker et al. (1994).

Table II. *Representative GC-MS and KRI data used for the identification of GA metabolites (listed in Table I) from the feeds of [17-^{14}C]GA$_{15}$ to maize*

[^{14}C]GA Metabolite/Ref. Compound	KRI[a]	Diagnostic Ions										
[^{14}C]GA_{15}	2,587	*m/z*	346	314	300	286	241	213	195			
		intensity	19	19	13	54	100	9	30			
GA_{15} ref.	2,605	*m/z*	344	312	298	284	239	211	193			
		intensity	25	27	18	70	100	13	33			
[^{14}C]GA_{15}-15,16-ene	2,542	*m/z*	346	314	288	286	243	229	217	199	185	159
		intensity	30	59	53	62	100	42	66	24	28	54
GA_{15}-15,16-ene ref.	2,551	*m/z*	344	312	286	284	243	227	217	197	183	159
		intensity	18	60	39	69	100	30	36	19	23	41
[^{14}C]GA_{19}	2,584	*m/z*	464	436	404	376	347	317	287	258	241	210
		intensity	17	100	24	52	18	18	23	28	46	40
GA_{19} ref.	2,596	*m/z*	462	434	402	374	345	315	285	258	239	208
		intensity	4	100	7	4	24	5	21	30	33	32
[^{14}C]GA_{20}	2,473	*m/z*	420	405	377	303	237	209	207			
		intensity	100	3	47	19	15	52	58			
GA_{20} ref.	2,482	*m/z*	418	403	375	301	235	207				
		intensity	100	16	46	12	8	30				
[^{14}C]GA_{44}	2,768	*m/z*	434	419	375	240	209	182				
		intensity	60	7	11	32	100	12				
GA_{44} ref.	2,786	*m/z*	432	417	373	238	207	180				
		intensity	46	6	14	33	100	11				
[^{14}C]GA_{113}	2,801	*m/z*	434	402	374	312	298	284	239	227		
		intensity	75	26	18	49	35	86	100	30		
GA_{113} ref.	2,801	*m/z*	432	400	372	310	296	282	237	225		
		intensity	100	31	27	56	36	83	82	38		

[a] The discrepancies between the KRI values for the metabolites and for the standards (ref.) are due to batch-to-batch variations in the GC columns used.

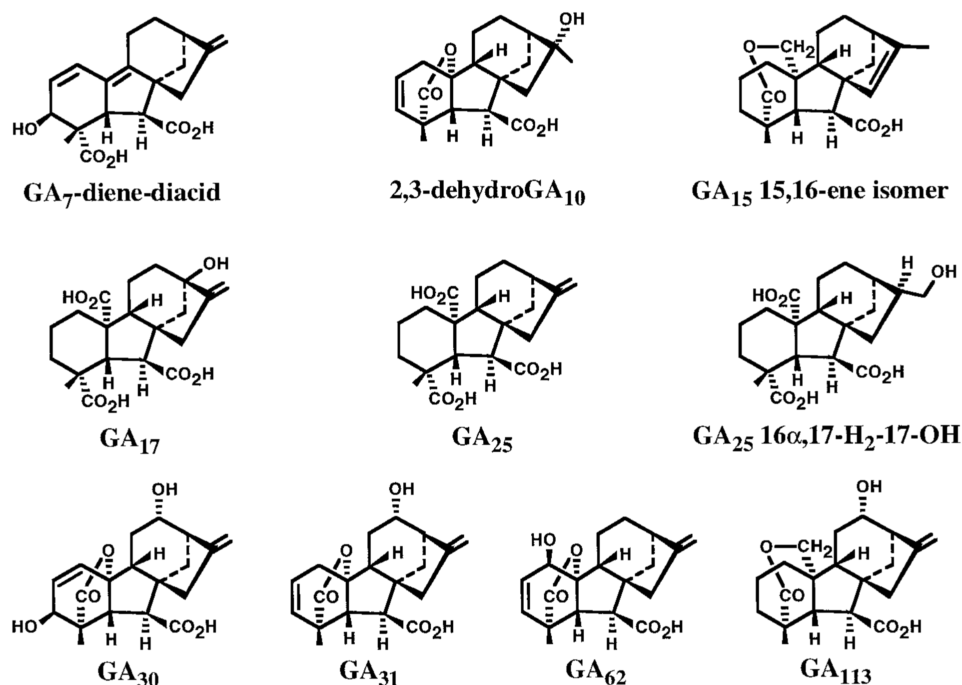

Figure 2. Structures of metabolites not shown in Figure 1.

RESULTS AND DISCUSSION

Metabolism

[17-^{14}C]GA$_{15}$

The recovered [^{14}C]labeled metabolites GA_{44}, GA_{19}, GA_{20}, GA_{113}, and GA_{15}-15,16-ene (artifact?) are shown in Table I, and are based on identification by the full-scan GC-MS and KRI data presented in Table II. The step from GA_{15} to GA_{44} (Fig. 1) is a direct 13-hydroxylation that is new for maize. The observed 13-hydroxylation of GA_{15} to GA_{44} in maize (Fig. 1) has also been reported in a cell-free preparation from germinating barley (Grosslindemann et al., 1992). In addition, the opened lactone of GA_{15} is metabolized to GA_{44} from in vitro studies using seeds of pea (Kamiya and Graebe, 1983) and bean (Takahashi et al., 1986). The steps $GA_{44} \rightarrow GA_{19}$ and $GA_{19} \rightarrow GA_{20}$ have been previously demonstrated in maize seedlings (Kobayashi et al., 1996). The step from GA_{15} to GA_{113} (Fig. 2) is a direct 12α-hydroxylation, which is new for maize and for higher plants. GA_{113} has not been found to occur naturally in maize but has been recently isolated from the seeds and shoots of the Japanese radish (Nakayama et al., 1998). The relatively high levels of endogenous GA_{44} and GA_{19} present in the normal and *d1* seedlings compared with the *d5* seedlings (Fujioka et al., 1988a) may create feedback inhibition and thus account for the absence of the labeled metabolite GA_{19}, in the normal and *d1* seedlings, in contrast to the recovery of [^{14}C]GA_{19} from *d5* seedlings.

[17-^{14}C]GA$_{24}$

The recovered [^{14}C]labeled metabolites, GA_{19}, GA_{20}, and GA_{17} are shown in Table III, and are based on identification by the full-scan GC-MS and KRI data presented in Table IV.

Table III. *Analysis of metabolites from feeds of [17-^{14}C]GA$_{24}$ (250 ng, 1.49 × 10^{3} Bq each) to normal, d1, and d5 seedlings of maize*

Plant Material	ODS-HPLC Fraction	$N(CH_3)_2$-HPLC Fraction	Radioactivity	[^{14}C]Products[a]
			Bq	
Normal (20.8 g)	19–21	33–36	117	GA_{20}
	22–23	42–45	4,290	GA_{19}
	29–30	40–43	663	GA_{24} (feed)
d1 (9.5 g)	19–21	33–36	452	GA_{20}
	29–30	40–43	852	GA_{24} (feed)
d5 (10.8 g)	19–21	33–36	710	GA_{20}
	24–25	33–35	218	GA_{17}
	29–30	40–43	347	GA_{24} (feed)

[a] Identified by data shown in Table IV.

Table IV. *Representative GC-MS and KRI data used for the identification of GA metabolites (listed in Table III) from the feeds of [17-^{14}C]GA_{24} to maize*

[^{14}C]GA Metabolite/Ref. Compound	KRI[a]	Diagnostic Ions												
[^{14}C]GA_{17}	2,563	*m/z*	494	462	435	434	403	375	374	253	210	195		
		intensity	64	37	28	37	19	26	23	27	100	21		
GA_{17} ref.	2,575	*m/z*	492	460	433	432	401	373	372	251	208	193		
		intensity	43	23	26	15	11	23	14	24	100	22		
[^{14}C]GA_{19}	2,584	*m/z*	464	436	404	376	347	317	287	258	241	210		
		intensity	17	100	24	52	18	18	23	28	46	40		
GA_{19} ref.	2,596	*m/z*	462	434	402	374	345	315	285	258	239	208		
		intensity	4	100	7	4	24	5	21	30	33	32		
[^{14}C]GA_{20}	2,473	*m/z*	420	405	377	361	303	237	209	194	182	169		
		intensity	100	12	50	14	14	6	32	8	8	8		
GA_{20} ref.	2,482	*m/z*	418	403	375	359	301	235	207	192	180	167		
		intensity	100	16	6	2	2	8	30	8	6	7		
[^{14}C]GA_{24}	2,426	*m/z*	376	348	344	316	312	288	287	284	256	229	228	227
		intensity	3	8	33	91	42	72	50	34	58	58	83	100
GA_{24} ref.	2,442	*m/z*	374	346	342	314	310	286	285	282	254	227	226	225
		intensity	4	8	30	80	26	79	72	42	29	70	100	78

[a] The discrepancies between the KRI values for the metabolites and for the standards (ref.) are due to batch-to-batch variations in the GC columns used.

The step from GA_{24} to GA_{19} (Fig. 1) is a direct 13-hydroxylation and is new for maize seedlings. The step from GA_{19} to GA_{20} has been previously established for maize (Kobayashi et al., 1996) with no evidence for the conversion of GA_{19} to GA_{17}. However, the conversion of GA_{19} to GA_{17} has been demonstrated using GA 20-oxidases from spinach (Wu et al., 1996) and pumpkin (Lange et al., 1994), which have been cloned and expressed in *E. coli*.

[17-^{14}C]GA_{25}

The recovered [^{14}C]labeled metabolites GA_{17}, GA_{25} 16α,17-H_2-17-OH, and HO-GA_{25} (hydroxyl position not determined) are shown in Table V, based on identification by the full-scan GC-MS and KRI data presented in Table VI. The metabolism of GA_{25} to GA_{17} (Fig. 2) is a result of direct 13-hydroxylation. This step is new for plants.

[17-^{14}C]GA_7

The [^{14}C]labeled metabolites GA_{30}, GA_3, isoGA_3, and GA_7-diene-diacid (trace amounts) are shown in Table VII, and are based on identification by the full-scan GC-MS and KRI data shown in Table VIII. However, in each case, most of the radioactivity was recovered in fractions (Table VII) that contained products not analyzable by GC-MS. The products are presumed to be conjugates.

Table V. *Analysis of metabolites from feeds of [17-^{14}C]GA_{25} (250 ng, 1.42 × 10^3 Bq each) to normal, d1, and d5 seedlings of maize*

Plant Material	ODS-HPLC Fraction	$N(CH_3)_2$-HPLC Fraction	Radioactivity	[^{14}C]Product[a]
			Bq	
Normal (17.1 g)	19–21	23–25	88	GA_{25} 16α, 17-H_2 17-OH
	19–21	26–28	101	HO-GA_{25}, unknown position of hydroxyl
	23–25	27–29	412	GA_{17}
	29–31	26–28	498	GA_{25} (feed)
d1 (6.0 g)	19–21	26–28	35	HO-GA_{25}, unknown position of hydroxyl
	23–25	27–29	560	GA_{17}
	29–31	26–28	365	GA_{25} (feed)
d5 (5.2 g)	19–21	23–25	41	GA_{25} 16α, 17-H_2 17-OH
	19–21	26–28	78	HO-GA_{25}, unknown position of hydroxyl
	23–25	27–29	563	GA_{17}
	29–31	26–28	595	GA_{25} (feed)

[a] Identified by data shown in Table VI.

Table VI. *Representative GC-MS and KRI data used for the identification of GA metabolites (listed in Table V) from the feeds of [17-^{14}C]GA$_{25}$ to maize*

[^{14}C]GA Metabolite/Ref. Compound	KRI[a]		Diagnostic Ions									
[^{14}C]GA$_{17}$	2,539	*m/z*	494	462	435	434	403	375	374	253	210	195
		intensity	77	94	67	94	33	41	41	11	100	11
GA$_{17}$ ref.	2,575	*m/z*	492	460	433	432	401	373	372	251	208	193
		intensity	43	23	26	15	11	23	14	24	100	22
[^{14}C]GA$_{25}$	2,455	*m/z*	406	374	314	255	286	227	199			
		intensity	0	19	63	9	100	37	6			
GA$_{25}$ ref.	2,440	*m/z*	404	372	312	253	284	225	197			
		intensity	0	13	82	8	100	41	4			
[^{14}C]HO-GA$_{25}$, unknown position of hydroxyl[b]	2,667	*m/z*	494	462	460	434	432	402	400	374	372	
		intensity	3	77	19	36	14	41	17	100	31	
[^{14}C]GA$_{25}$ 16α,17-H$_2$ 17-OH	2,738	*m/z*	496	464	436	404	376	342	314	286	227	
		intensity	0	69	22	100	66	28	36	57	37	
[^{14}C]GA$_{25}$ 16α,17-H$_2$ 17-OH ref.	2,760	*m/z*	494	462	434	402	374	340	312	284	225	
		intensity	0	86	26	100	63	30	78	97	93	

[a] The discrepancies between the KRI and ion abundance values for the metabolites and for the standards (ref.) are due to the change in the GC-MS instrument from a DANI-3800 GC-VG Analytical 70–250 (Micromass, Beverly, MA) mass spectrometer to a Thermoquest GCQ (Thermoquest, San Jose, CA) gas chromatograph with a WCOT BPX5 capillary column (25-m × 0.22-mm × 0.25-μm film thickness; Scientific Glass Engineering). [b] No reference data available; identification by analogy with known HO-GA$_{25}$ examples.

2,3-Dehydro-[17-^{14}C]GA$_9$

The recovered [^{14}C]labeled metabolites, GA$_5$, GA$_7$ (trace amounts), 2,3 dehydro-GA$_{10}$ (artifact), GA$_{31}$, and GA$_{62}$ are shown in Table IX, based on identification by the full-scan GC-MS and KRI data shown in Table X. Four of the metabolites are formed by hydroxylation at C-1β (GA$_{62}$, Fig. 2), at C-3β (GA$_7$, Fig. 1), at C-12α (GA$_{31}$, Fig. 2), and at C-13 (GA$_5$, Fig. 1). 2,3-Dehydro-GA$_{10}$ (Fig. 2) is the product of hydration of the 16,17-double bond and this step may be non-enzymatic. The metabolism of 2,3-dehydro-[17-^{2}H$_2$]GA$_9$ to [^{2}H$_2$]GA$_7$ has been previously reported from cell-free systems from seeds of wild cucumber and apple (Albone et al., 1990). The metabolism of 2,3-dehydro-GA$_9$ to GA$_{62}$, to GA$_{31}$, and to GA$_5$ are the first examples of these conversions in plants.

Isotopic Dilution of 2,3-Dehydro-GA$_9$

In view of the observed conversion of 2,3-dehydro-GA$_9$ to GA$_7$, we investigated the possible natural occurrence of 2,3-dehydro-GA$_9$ in maize. Thus, we determined the level of isotopic dilution of 2,3-dehydro-[17-^{14}C]GA$_9$ added to a homogenate of normal maize seedlings. No dilution of label was observed based on a full-scan GC-MS analysis of

Table VII. *Analysis of metabolites from feeds of [17-^{14}C]GA$_7$ (250 ng, 1.55 × 10^3 Bq each) to seedlings of normal, d1, and d5 seedlings of maize*

Plant Material	ODS-HPLC Fraction	N(CH$_3$)$_2$-HPLC Fraction	Radioactivity	[^{14}C]Product[b]
			Bq	
Normal (14.2 g)	8–9	34–35	17[a]	GA$_{30}$
	10–11	33–34	23[a]	GA$_3$, isoGA$_3$
	19–22	33–35	18[a]	GA$_7$-diene-diacid
	24–26	31–34	216[a]	[c]
	24–26	44–47	265[a]	[c]
d1 (8.3 g)	8–9	34–35	33	GA$_{30}$
	10–11	33–34	54	GA$_3$, isoGA$_3$
	19–22	33–35	24	GA$_7$-diene-diacid
	24–26	31–34	432	[c]
	24–26	44–47	723	[c]
d5 (8.1 g)	8–9	34–35	53	GA$_{30}$
	10–11	33–34	98	GA$_3$, isoGA$_3$
	19–22	33–35	15	GA$_7$-diene-diacid
	24–26	31–34	470	[c]
	24–26	44–47	393	[c]

[a] One-half of the original feed. [b] Identified by data shown in Table VIII. [c] No ^{14}C-labeled compounds were identified by GC-MS.

Table VIII. *Representative GC-MS and KRI data used for the identification of GA metabolites (listed in Table VII) from the feeds of [17-^{14}C]GA$_7$ to maize*

[^{14}C]GA Metabolite/Ref. Compound	KRI[a]	Diagnostic Ions										
[^{14}C]GA$_3$	2,685	*m/z*	506	491	447	433	372	349	313	240	210	
		intensity	100	6	8	5	12	6	8	12	19	
GA$_3$ ref.	2,692	*m/z*	504	489	445	431	370	347	311	238	208	
		intensity	100	7	12	9	24	9	14	21	37	
iso[^{14}C]GA$_3$	2,625	*m/z*	506	501	477	447	372	240	223			
		intensity	100	9	20	17	16	24	22			
isoGA$_3$ ref.	2,633	*m/z*	504	499	475	445	370	238	221			
		intensity	100	10	12	9	12	28	12			
[^{14}C]GA$_7$[b] (feed)	2,520	*m/z*	418	386	358	300	284	225	224	195	181	155
		intensity	11	12	17	21	25	60	100	28	28	31
GA$_7$ ref.[b]	2,525	*m/z*	416	384	356	298	282	223	222	193	179	155
		intensity	9	22	22	19	35	73	100	43	42	48
[^{14}C]GA$_7$ di-acid 9,10-ene	2,399	*m/z*	432	372	313	283	223	195				
		intensity	14	45	59	100	100	60				
GA$_7$ di-acid 9,10-ene ref.	2,405	*m/z*	430	370	311	281	221	193				
		intensity	17	23	80	100	77	24				
[^{14}C]GA$_{30}$	2,754	*m/z*	506	446	416	384	371	315	282	223	195	
		intensity	11	6	13	18	38	44	34	100	42	
GA$_{30}$ ref.	2,759	*m/z*	504	444	414	382	369	315	280	221	193	
		intensity	30	10	26	21	50	17	37	100	47	

[a] The discrepancies between the KRI values for the metabolites and for the standards (ref.) are due to batch-to-batch variations in the GC columns used. [b] Data for [^{14}C]GA$_7$ is reported, although not recovered from feed.

the recovered 2,3-dehydro-[17-^{14}C]GA$_9$ (data not shown), thus indicating that 2,3-dehydro-GA$_9$ is not endogenous to maize.

General

The structures of the substrates and metabolites presented in this report are shown in Figures 1 and 2, with the exception of the HO-GA$_{25}$ metabolite for which the hydroxylation site was not determined. In maize, the 13-hydroxylation of GA$_{15}$ to GA$_{44}$, GA$_{24}$ to GA$_{19}$, GA$_9$ to GA$_{20}$, and GA$_4$ to GA$_1$ results in the formation of a grid connecting members of the (presumptive) non-early 3,13-hydroxylation pathway to members of the early 13-hydroxylation pathway (Fig. 1). The two steps, GA$_{15}$ $\rightarrow$ GA$_{44}$ and GA$_{24}$ $\rightarrow$ GA$_{19}$, represent the first demonstration of in vivo crossovers between C$_{20}$-GAs. A similar grid connecting the two branch pathways has been demonstrated from in vitro studies from a number of plant species (Kamiya and Graebe, 1983; Takahashi et al., 1986; Grosslindemann et al., 1992). The 13-hydroxylation of GA$_{15}$, GA$_{24}$, GA$_9$, and GA$_4$ in maize may reside in a single 13-hydroxylase with low substrate specificity or with the presence of separate substrate-specific enzymes. The failure to detect the sequence GA$_{12}$ $\rightarrow$ GA$_{15}$ $\rightarrow$ GA$_{24}$ $\rightarrow$ GA$_9$ $\rightarrow$ GA$_4$ $\rightarrow$ GA$_7$ could be because the K_m values for these substrates are much lower for the 13-hydroxylase(s) than for the 20-oxidase(s).

The two labeled metabolites GA$_{15}$-15,16-ene and GA$_7$-diene-diacid were probably formed by the non-enzymatic rearrangement of a double bond. Additionally, 2,3-dehydro-GA$_{10}$ was probably formed as a result of a non-enzymatic hydration of the 16,17-double bond in the substrate 2,3-dehydro-GA$_9$.

Based on the previous demonstration that GA$_5$ is an intermediate between GA$_{20}$ and GA$_3$ in maize shoots (Fujioka et al., 1990; Spray et al., 1996), we examined the possibility that 2,3-dehydro-GA$_9$ is an intermediate be-

Table IX. *Analysis of metabolites from feeds of 2,3-dehydro-[17-^{14}C]GA$_9$ (88 ng, 485 Bq each) to d5 maize (10.0 g of plant material)*

ODS-HPLC Fraction	N(CH$_3$)$_2$-HPLC Fraction	Radioactivity	[^{14}C]Product[a]	Specific Radioactivity
		Bq		*TBq mol^{-1}*
14–15	13	77	GA$_{31}$	1.81
16–18	9–10	136	2,3-Dehydro-GA$_{10}$	1.74
16–18	14–15	162	GA$_5$	1.76
19–21	10–13	124	GA$_{62}$	Not determined
22–24	9–10	14	GA$_7$ (trace)	Not determined
26–27	12	381	2,3-Dehydro-GA$_9$ (feed)	1.76

[a] Identified by data shown in Table X.

Table X. *Representative GC-MS and KRI data used for the identification of GA metabolites (listed in Table IX) from the feeds of 2,3-dehydro-[17-^{14}C]GA_9 to d5 maize*

[^{14}C]GA Metabolite/Ref. Compound	KRI[a]	Diagnostic Ions												
[^{14}C]GA_5	2,475	*m/z*	418	403	359	345	315	301	209					
		intensity	100	17	24	9	31	402	32					
GA_5 ref.	2,479	*m/z*	416	401	357	343	313	299	207					
		intensity	100	21	24	22	9	58	55					
[^{14}C]GA_7	2,522	*m/z*	418	386	358	300	284	225	224					
		intensity	8	46	8	18	20	60	100					
GA_7 ref.	2,525	*m/z*	416	384	356	298	282	223	222					
		intensity	9	14	22	19	35	30	100					
2,3-Dehydro-[^{14}C]GA_9	2,298	*m/z*	299	286	284	227	226	156						
		intensity	7	41	12	51	100	45						
2,3-Dehydro-GA_9 ref.	2,301	*m/z*	297	284	282	225	224	156						
		intensity	10	46	2	62	100	36						
2,3-Dehydro-[^{14}C]GA_{10}[b]	2,563	*m/z*	420	361	329	286	227	226	143	132				
		intensity	24	24	46	52	58	79	52	100				
[^{14}C]GA_{31}	2,546	*m/z*	418	386	371	296	284	268	251	241	225	224	223	195
		intensity	7	7	10	14	47	33	15	10	58	100	77	44
GA_{31} ref.	2,550	*m/z*	416	384	369	294	282	266	269	239	223	222	221	193
		intensity	6	8	10	19	49	33	23	7	71	100	72	36
[^{14}C]GA_{62}	2,424	*m/z*	418	403	374	315	284	225	224	209				
		intensity	2	7	13	18	36	100	95	26				
GA_{62} ref.	2,424	*m/z*	416	401	372	313	282	223	222	207				
		intensity	0	4	8	10	24	100	93	10				

[a] The discrepancies between the KRI values for the metabolites and for the standards (ref.) are due to batch-to-batch variations in the GC columns used. [b] No reference data available; identification by analogy with known GA-15,16-enes.

tween GA_9 and GA_7. Our results show that 2,3-dehydro-GA_9 is predominantly 13-hydroxylated to GA_5, 12α-hydroxylated to GA_{31}, and 1β-hydroxylated to GA_{62}, and converted into GA_7 in trace amounts. However, isotope dilution studies gave no evidence for the natural occurrence of 2,3-dehydro-GA_9 in maize shoots (data not shown). The metabolic origin of GA_{15}, GA_{24}, GA_9, GA_4, and GA_7 in maize remains unresolved.

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