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# Metabolism and Biological Activity of Gibberellin $A_4$ in Vegetative Shoots of *Zea mays*, *Oryza sativa*, and *Arabidopsis thaliana*[1]

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**[17-$^{13}$C,$^{3}$H]Gibberellin $A_4$ ($GA_4$) was injected into the shoots of tall (W23/L317), dwarf-1 (*d1*), and dwarf-5 (*d5*) *Zea mays* L. (maize); tall (cv Nipponbare), dwarf-x (*dx*), and dwarf-y (*dy*) *Oryza sativa* L. (rice); and tall (ecotype Landsberg *erecta*), *ga4*, and *ga5* *Arabidopsis thaliana* (L.) Heynh. [$^{13}$C]$GA_4$ and its metabolites were identified from the shoots by full-scan gas chromatography-mass spectrometry and Kovats retention indices. $GA_4$ was metabolized to $GA_1$ in all nine genotypes. $GA_4$ was also metabolized in some of the genotypes to 3-*epi*-$GA_1$, $GA_2$, 2$\beta$-OH-$GA_2$, 3-*epi*-$GA_2$, *endo*-$GA_4$, 16$\alpha$,17-$H_2$-16,17-$(OH)_2$-$GA_4$, $GA_{34}$, *endo*-$GA_{34}$, $GA_{58}$, 15-*epi*-$GA_{63}$, $GA_{71}$, and 16-*epi*-$GA_{82}$. No evidence was found for the metabolism of $GA_4$ to $GA_7$ or of $GA_4$ to $GA_3$. The bioactivities of $GA_4$ and $GA_1$ were determined using the six dwarf mutants for assay. $GA_4$ and $GA_1$ had similar activities for the maize and rice mutants. For the *Arabidopsis* mutants, $GA_4$ was more active than $GA_1$ at low dosages; $GA_4$ was less active than $GA_1$ at higher dosages.**

Information concerning the GA biosynthetic pathways is necessary for an understanding of the origin, identification, and regulation of bioactive GAs. Specific pathways, together with mutants that block specific steps in these pathway(s), have been described for several plant systems (Reid, 1990). In this report we evaluate the role of $GA_4$ and $GA_1$ in the control of shoot elongation for *Zea mays* (maize), *Oryza sativa* (rice), and *Arabidopsis thaliana*. (The structures of the $C_{19}$-GAs relevant to this study are shown in Fig. 1).

The early 13-hydroxylation pathway is the major one present in vegetative shoots of maize. The evidence is based on the isolation of nine members of the pathway from vegetative shoots (Fujioka et al., 1988a, 1988b) and the demonstration of five metabolic steps in the pathway (Spray et al., 1984; Fujioka et al., 1990). The relative roles of $GA_{20}$ and $GA_1$ in the control of growth are based on studies of the two GA mutants, *d1* and *d5*. The *d1* mutant controls the late step, $GA_{20}$ to $GA_1$. In the *d1* mutant, $GA_1$ is bioactive and $GA_{20}$ has less than 1% of the activity of $GA_1$ (Phinney and Spray, 1982). The *d1* mutant accumulates endogenous $GA_{20}$, has trace amounts of $GA_1$ (Fujioka et al., 1988a), and does not metabolize $GA_{20}$ to $GA_1$ (Spray et al., 1984). The *d5* mutant controls the early step, copalylpyrophosphate to *ent*-kaurene (Hedden and Phinney, 1979). In the *d5* mutant, $GA_{20}$ and $GA_1$ have similar bioactivities (Phinney and Spray, 1982), endogenous $GA_{20}$ and $GA_1$ are present in trace amounts (Fujioka et al., 1988a), and the mutant metabolizes $GA_{20}$ to $GA_1$ (Spray et al., 1984). The data suggest that $GA_{20}$ is bioactive in maize shoots only because of its metabolism to bioactive $GA_1$.

$GA_3$ is also endogenous to tall maize shoots but is present in trace amounts only (Fujioka et al., 1988b). $GA_3$ originates from $GA_{20}$ via $GA_5$ (Fujioka et al., 1990). $GA_4$, $GA_7$, $GA_9$, $GA_{15}$, $GA_{24}$, and $GA_{34}$ (GAs that are not members of the early-13-hydroxylation pathway) are also present in trace amounts in tall maize (Fujioka et al., 1988b). Nothing is known about their biosynthetic origin or their biological role in the control of maize shoot growth.

Five endogenous GAs have been identified from vegetative shoots of rice (Kobayashi et al., 1988). All are members of the presumptive early 13-hydroxylation pathway. Studies of the two GA mutants, *dx* and *dy*, indicate that the *dy* mutant controls the step $GA_{20}$ to $GA_1$, and the *dx* mutant controls a step before $GA_{53}$. $GA_1$ has relatively high bioactivity and $GA_{20}$ has low bioactivity when assayed on the *dy* dwarf mutant of rice (Murakami, 1972). The *dy* mutant accumulates $GA_{20}$ and has reduced levels of $GA_1$ (Kobayashi et al., 1989). $GA_{20}$ and $GA_1$ have similar bioactivities when assayed on the *dx* mutant (Murakami, 1972), and levels of endogenous $GA_{20}$ and $GA_1$ are low (Kobayashi et al., 1989). The data support the position that $GA_{20}$ is bioactive because of its metabolism to $GA_1$.

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Abbreviations: AcOH, acetic acid; AE, acidic ethyl acetate-soluble fraction; *endo*, 15,16 double bond isomer; EtOAc, ethyl acetate; *iso*-$GA_3$, 19,2-isomeric lactone of $GA_3$; KRI, Kovats retention index; MeOH, methanol; NB, neutral butanol-soluble fraction; NBE, acidic ethyl acetate-soluble fraction recovered from hydrolyzed NB fraction; Rt, retention time.

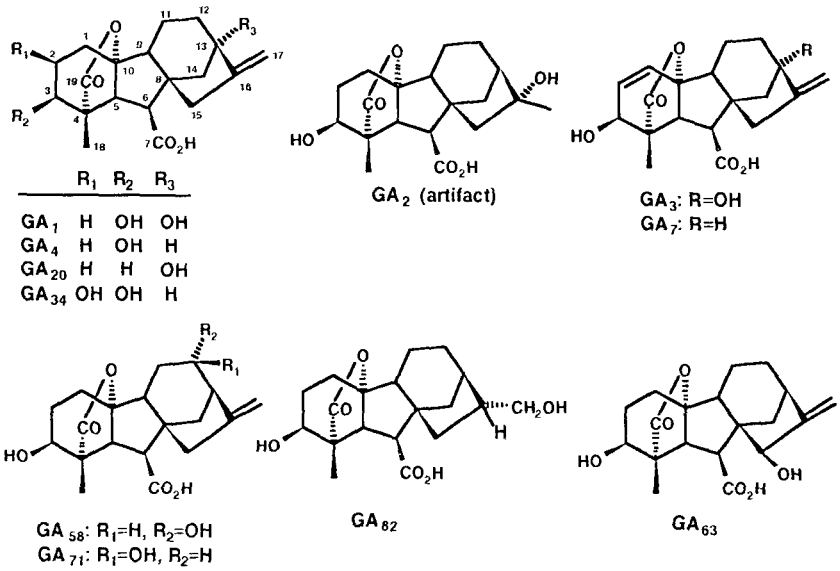


**Figure 1.** The structures of the $C_{19}$-GAs relevant to this study.

In addition to the GAs identified from rice shoots, $GA_4$, $GA_9$, $GA_{12}$, $GA_{17}$, $GA_{24}$, $GA_{34}$, $GA_{44}$, and $GA_{51}$ have been isolated from reproductive organs (Kobayashi et al., 1984, 1988). This is the only identification of $GA_4$ in rice. Metabolic steps have been defined from anthers only. They are $GA_{12}$ to $GA_{15}$, $GA_{53}$ to $GA_{44}$, and $GA_{20}$ to $GA_1$ (Kobayashi et al., 1990).

Twenty GAs have been isolated from the early bolting stage of tall *Arabidopsis* (ecotype Landsberg *erecta*) (Talon et al., 1990b). They are members of three biosynthetic pathways, the early 13-hydroxylation pathway (eight GAs), the early-3-hydroxylation pathway (seven GAs), and the early non 3,13-hydroxylation pathway (five GAs). The three hypothetical pathways originate from $GA_{12}$ and converge to $GA_1$. The two dwarf mutants *ga4* and *ga5* have been analyzed in terms of these three pathways (Talon et al., 1990b; Zeevaart and Talon, 1992). The *ga4* mutant blocks 3$\beta$-hydroxylation at three different steps, $GA_{20}$ to $GA_1$ (late in the early 13-hydroxylation pathway), $GA_9$ to $GA_4$ (late in the early non-3,13-hydroxylation pathway), and probably $GA_{12}$ to $GA_{14}$ (early in the early 3-hydroxylation pathway). GA levels are relatively high before the blocks and relatively low after the blocks. $GA_{20}$ had relatively low bioactivity, and $GA_1$ had high bioactivity on the *ga4* mutant.

The *ga5* mutant blocks the oxidative removal of carbon-20 and also, possibly, the oxidation of the carbon-20 methyl group to the alcohol. The mutant controls the steps $GA_{53}$ to $GA_{44}$ and $GA_{19}$ to $GA_{20}$ (early 13-hydroxylation pathway), $GA_{12}$ to $GA_{15}$ and $GA_{24}$ to $GA_9$ (early non-3,13-hydroxylation pathway), and $GA_{14}$ to $GA_{37}$ and $GA_{36}$ to $GA_4$ (early 3-hydroxylation pathway). GA levels are relatively high for GAs before the blocks and relatively low for GAs after the blocks. It has been concluded (Talon et al., 1990b; Zeevaart and Talon, 1992) that 3$\beta$-hydroxylation is the critical feature for stem elongation, not the per se activity of $GA_1$, and that both $GA_4$ and $GA_1$ are active per se.

Here, we demonstrate the metabolism of $GA_4$ to $GA_1$ in vegetative shoots of maize, rice, and *Arabidopsis*. We also show that $GA_4$ and $GA_1$ have similar bioactivities when assayed on the *d1* mutant of maize and on the *dx* and *dy* mutants of rice. For *d5*, maize $GA_1$ is more active than $GA_4$. For both the *ga5* and *ga4 Arabidopsis* mutants, $GA_4$ is more active than $GA_1$ at low dosages and less active than $GA_1$ at higher dosages.

## MATERIALS AND METHODS

### GAs

[17-$^{13}$C,$^3$H]$GA_4$ (0.979 atoms of $^{13}$C molecule$^{-1}$, 0.52 GBq mmol$^{-1}$) was synthesized from unlabeled $GA_4$ by the method of Fujioka et al. (1988a) for the synthesis of [17-$^{13}$C,$^3$H]$GA_1$. [17-$^{13}$C,$^3$H]$GA_4$ was purified by silica gel column chromatography (*n*-hexane:EtOAc:AcOH, 7:3:0.2), followed by silica gel TLC (EtOAc:$CHCl_3$:AcOH, 15:5:1); [17-$^{13}$C,$^3$H]$GA_4$ was recovered from the zone with $R_F$ 0.6 to 0.7. No radioactive impurities were detected after HPLC analysis of the recovered [17-$^{13}$C,$^3$H]$GA_4$, and GC-MS analysis showed its purity to be greater than 99.9%. The $GA_4$ and $GA_1$ samples used for the bioassays were also shown to be greater than 99.9% pure by GC-MS. [17-$^{13}$C,$^3$H]$GA_4$ was dissolved in ethanol:$H_2O$ (1:1, v/v) for metabolic studies; $GA_1$ and $GA_4$ were dissolved in acetone:$H_2O$ (1:1, v/v) for bioassays.

### Plant Material

The origin of the *Zea mays* L. (maize) seed of the tall phenotypes segregating for the *d1* and *d5* mutants is given by Fujioka et al. (1988a). The seed of the tall phenotype of *Oryza sativa* L. (rice) (cv Nipponbare, previously called Nihonbare) and the dwarf mutants *dx* (cv *Tan-ginbozu*) and *dy* (cv *Waito-C*) originated from Dr. Hitoshi Saka (National Institute of Agrobiological Resources, Tsukuba-shi, Ibaraki

305, Japan). The *Arabidopsis thaliana* (L.) Heynh. seed of the tall phenotype (ecotype Landsberg *erecta*) and dwarf mutants *ga4* and *ga5* originated from Dr. Maarten Koornneef (Agricultural University, Wageningen, the Netherlands) via Dr. Judy Brusslan (Department of Biology, University of California, Los Angeles, CA).

For bioassay and metabolic studies, maize and *Arabidopsis* plants were grown in the University of California, Los Angeles, greenhouse under natural sunlight supplemented with Sylvania incandescent 300-W PS35 Excel bulbs to give a daylength of at least 12 h. The rice plants used for bioassay and metabolic studies were grown in an incubator chamber maintained at 28°C under continuous light supplied by General Electric F96PG17CW fluorescent bulbs.

### Bioassays

The maize seedling bioassay was described by Phinney and Spray (1982), and the rice microdrop bioassay was discussed by Murakami (1968). For the *Arabidopsis* assay, seeds were soaked in 0.1% agarose at 4°C for 48 h and sown in 15- × 10-cm plastic trays filled with soil:vermiculite:perlite (1:1:1), 24 seeds per tray, and covered with a 3-mm layer of fine vermiculite. Before treatment, the plants were thinned to 10 per tray. The GA solution of 2 μL was added to the base of the fully developed vegetative rosette just before bolting (plants were about 3 weeks old). Five dosage levels were used with 10 plants per dosage level. After shoot elongation (10–14 d after treatment), plant height was measured from the soil level to the top of the inflorescence. Response data were calculated from the averages of the 10 treatments at each dosage level.

### Metabolic Studies: Treatment, Extraction, and Purification

[17-$^{13}$C,$^{3}$H]$GA_4$ was injected into the coleoptilar node of maize and rice seedlings at the three- to four-leaf stage; for *Arabidopsis*, the labeled $GA_4$ was injected into the basal petiole of the uppermost leaf of the vegetative rosette just before bolting. For maize, five seedlings of each genotype were each injected with 1510 Bq of [17-$^{13}$C,$^{3}$H]$GA_4$ (2 μL); for rice, 97 tall and 100 *dx* and *dy* seedlings were each injected with 152 Bq of [17-$^{13}$C,$^{3}$H]$GA_4$ (1 μL); for *Arabidopsis*, 30 plants of each genotype were each injected with 215 Bq of [17-$^{13}$C,$^{3}$H]$GA_4$ (0.5 μL). Incubation times were 8 (rice), 12 (maize), and 24 h (*Arabidopsis*). After incubation, the shoots were harvested and immediately frozen in dry ice. The frozen plant material was homogenized and extracted twice at 4°C with MeOH:$H_2O$ (4:1, v/v). After filtration and removal of the MeOH, the aqueous residue was solvent fractionated to give the AEs as described by Fujioka et al. (1988a) and the NBs, as described by Yokota et al. (1980; see fig. 2.8, p. 126). The AEs and NBs were evaporated to dryness in vacuo and purified further (see below).

#### *AE*

The AE in $H_2O$ (1 mL) was loaded onto a Bond Elut $C_{18}$ column (1 g; Analytichem International, Harbor City, CA). The column was washed with $H_2O$ (2 mL) and then eluted with MeOH (2 × 2 mL). The MeOH eluates were combined, evaporated to dryness redissolved in MeOH (1 mL), and loaded onto a Bond Elut DEA column (500 mg) that had a head of DEAE-Sephadex A-25 (1 mL of gel suspended in MeOH). The column was washed with MeOH (1 mL) and then eluted with 1 N AcOH in MeOH (5 × 1 mL). The fractions containing radioactivity (usually fractions 2–4) were combined and dried under a stream of $N_2$. This combined fraction was dissolved in 40% MeOH (aqueous) (200–250 μL) and injected onto a HPLC column of Nucleosil 5 $C_{18}$ (10 cm × 6 mm, i.d.). The column was eluted as described by Kobayashi et al. (1990). Fractions were collected at 1-min intervals, and an aliquot of each fraction was analyzed by liquid scintillation spectrometry. Radioactive fractions were combined according to their Rt, and each of the combined fractions was purified further by HPLC on a column of Nucleosil 5 $N(CH_3)_2$ (10 cm × 6 mm, i.d.). The column was eluted with 0.05% AcOH in MeOH at a flow rate of 1.5 mL $min^{-1}$, and fractions were collected at 1-min intervals. The radioactivity in each fraction was determined by liquid scintillation spectrometry, and radioactive fractions were combined according to their Rt, concentrated, and derivatized for GC-MS analysis (Gaskin and MacMillan, 1991).

#### *NB*

The NB was passed through the two Bond Elut columns as described for the AE, and the recovered radioactive fraction was hydrolyzed with cellulase (30 mg, type I; Sigma) in acetate buffer (1 N, pH 4.5, 1 mL) at 30°C. After 24 h, the solution was acidified with 6 N HCl and extracted with EtOAc. The EtOAc extract (NBE) was dried, redissolved in MeOH (1 mL), and purified using a Bond Elut DEA column, followed by HPLC as described for the AE. The fractions containing radioactivity were combined according to their Rt values and dried. Each dried fraction was redissolved in MeOH and methylated with excess diazomethane, dried, and trimethylsilylated with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide, to give methyl ester, trimethylsilyl ether derivatives for GC-MS analysis (Gaskin and MacMillan, 1991).

### Control Experiment for Metabolic Studies

[$^{13}$C,$^{3}$H]$GA_4$ was injected into *d1* maize seedlings, and the seedlings were frozen immediately after injection, extracted, purified, and analyzed as described in "Metabolic Studies: Treatment, Extraction, and Purification."

### GC-MS

Mass spectra were obtained using a computerized VG 7050 mass spectrometer (VG Analytical, Manchester, UK) fitted with a DANI 3800 GC (Kontron Instruments, St. Albans, UK) equipped with a WCOT vitreous silica column (25 m × 0.2 mm, i.d.; GC$^2$ Chromatography, Altrincham, UK) coated with a 0.25-μm layer of bonded OV-1. The initial GC column temperature was 50°C. After injection, the temperature was held at 50°C for 2 min, followed by temperature programming at 10°C $min^{-1}$ to 150°C and then 3°C $min^{-1}$ to 300°C. The use of the GC-MS instrument and GC column was described by Gaskin et al. (1984).

**Table I.** *$^{13}C$-Labeled metabolites identified by GC-MS after injection of [$^{13}C$,$^{3}H$]$GA_4$ into tall, d1, and d5 maize (4.85 μg, 7.55 × $10^3$ Bq each), tall (9.44 μg, 14.7 × $10^3$ Bq), dx, and dy rice (9.76 μg, 15.2 × $10^3$ Bq each), and tall, ga4, and ga5 Arabidopsis (4.14 μg, 6.45 × $10^3$ Bq each)*

| Plant Material (fresh weight, g) | Fraction | Recovered Radioactivity | ODS-HPLC Fraction | $N(CH_3)_2$-HPLC Fraction | Radioactivity | Metabolites[a] |
|---|---|---|---|---|---|---|
| | | % | | | Bq | |
| Maize | | | | | | |
| Tall (8.9) | AE | 41.1 | 7–8 | 6–9 | 151 | 16α,17-$H_2$-16,17-$(OH)_2$-$GA_4$ |
| | | | 9–10 | 16 | 13 | $GA_1$ |
| | | | 11–15 | 6–9 | 170 | $GA_2$; 15-*epi*-$GA_{63}$; 16-*epi*-$GA_{82}$ |
| | | | 19–22 | 17–18 | 67 | $GA_{34}$ |
| | | | 23–26 | 7–11 | 3575 | $GA_4$ |
| | NBE | 28.2 | 8–10 | 11–12 | 80 | 2β-OH-$GA_2$; 16α,17-$H_2$-16,17-$(OH)_2$-$GA_4$ |
| | | | 13–15 | 9–10 | 158 | $GA_2$; 3-epi-$GA_2$ |
| | | | 21–22 | 16 | 7.7 | $GA_{34}$ |
| | | | 24–25 | 10–12 | 123 | $GA_4$ |
| *d5* (4.9) | AE | 38.8 | 10 | 28–30[b] | 211 | $GA_1$; 3-*epi*-$GA_1$ |
| | | | 19–20 | 12–13 | 17 | $GA_4$ |
| | | | 23–25 | 8–10 | 1658 | $GA_4$ |
| | NBE | 18.8 | 10 | 11 | 57 | $GA_1$; 3-*epi*-$GA_1$ |
| | | | 13–14 | 6–7 | 57 | $GA_2$ |
| | | | 21–22 | 13–14 | 30 | $GA_{34}$ |
| | | | 23–25 | 7–9 | 276 | $GA_4$ |
| *d1* (5.2) | AE | 17.5 | 10 | 28–30[b] | 63 | $GA_1$; 3-*epi*-$GA_1$; $GA_{58}$ |
| | | | 23–25 | 9–10 | 753 | $GA_4$ |
| | NBE | 31.2 | 9–10 | 15–16 | 30 | $GA_1$; 3-*epi*-$GA_1$ |
| | | | 21–22 | 13–14 | 37 | $GA_{34}$ |
| | | | 23–25 | 9–10 | 504 | $GA_4$ |
| Rice | | | | | | |
| Tall (21.3) | AE | 46.6 | 8–11 | 9–10 | 20 | $GA_2$; 16α,17-$H_2$-16,17-$(OH)_2$-$GA_4$ |
| | | | 8–11 | 13 | 11 | $GA_1$ |
| | | | 12–15 | 8–10 | 51 | $GA_2$ |
| | | | 19–23 | 10–13 | 1226 | $GA_4$; *endo*-$GA_4$ |
| | | | 19–23 | 14–15 | 19 | $GA_{34}$ |
| | | | 24–25 | 11–12 | 1322 | $GA_4$ |
| | NBE | 12.9 | 8–10 | 9–10 | 109 | 2β-OH-$GA_2$; 16α,17-$H_2$-16,17-$(OH)_2$-$GA_4$ |
| | | | 8–10 | 13 | 1.9 | $GA_1$ |
| | | | 12–15 | 8–10 | 45 | $GA_2$ |
| | | | 21–22 | 14 | 6.3 | $GA_{34}$ |
| *dx* (16.5) | AE | 43.8 | 8–11 | 9–10 | 47 | 16α,17-$H_2$-16,17-$(OH)_2$-$GA_4$ |
| | | | 8–11 | 13 | 25 | $GA_1$; $GA_{58}$ |
| | | | 12–16 | 8–10 | 274 | $GA_2$; 15-epi-$GA_{63}$; 16-*epi*-$GA_{82}$ |
| | | | 19–23 | 14–16 | 90 | $GA_{34}$ |
| | | | 24–26 | 10–14 | 4604 | $GA_4$ |
| | NBE | 8.1 | 8–10 | 9–10 | 60 | 16α,17-$H_2$-16,17-$(OH)_2$-$GA_4$ |
| | | | 14–15 | 8–10 | 40 | $GA_2$; 15-*epi*-$GA_{63}$; 16-*epi*-$GA_{82}$ |
| | | | 21–22 | 13–14 | 44 | $GA_{34}$ |
| | | | 25 | 11 | 24 | $GA_4$ |
| *dy* (15.1) | AE | 42.0 | 7–11 | 9–10 | 32 | 16α,17-$H_2$-16,17-$(OH)_2$-$GA_4$ |
| | | | 7–11 | 13 | 23 | $GA_1$ |
| | | | 12–15 | 8–10 | 95 | $GA_2$; 15-*epi*-$GA_{63}$; 16-*epi*-$GA_{82}$ |
| | | | 18–22 | 13–15 | 57 | $GA_{34}$ |
| | | | 23–26 | 9–14 | 4291 | $GA_4$ |
| | NBE | 9.4 | 8–10 | 9–10 | 44 | 16α,17-$H_2$-16,17-$(OH)_2$-$GA_4$ |
| | | | 14–15 | 8 | 18 | $GA_2$; 16-*epi*-$GA_{82}$ |
| | | | 21–22 | 14 | 13 | $GA_{34}$ |
| | | | 24–25 | 11 | 59 | $GA_4$ |

**Table I.** *Continued.*

| Plant Material (fresh weight, g) | Fraction | Recovered Radioactivity | ODS-HPLC Fraction | $N(CH_3)_2$-HPLC Fraction | Radioactivity | Metabolites[a] |
|---|---|---|---|---|---|---|
| | | % | | | *Bq* | |
| *Arabidopsis* | | | | | | |
| Tall (42.4) | AE | 61.7 | 8–10 | 10–12 | 47 | $GA_1$ |
| | | | 19–22 | 13–16 | 248 | $GA_{34}$ |
| | | | 23–26 | 8–10 | 2196 | $GA_4$ |
| | | | 23–26 | 11–12 | 40 | $GA_{34}$ |
| | NBE | 5.9 | 24 | 10 | 29 | $GA_4$ |
| *ga5* (18.8) | AE | 72.5 | 8–12 | 8–10 | 68 | $GA_1$; 2$\beta$-OH-$GA_2$ |
| | | | 13–16 | 6–9 | 415 | $GA_4$; *endo*-$GA_4$ |
| | | | 18–22 | 7–11 | 179 | *endo*-$GA_{34}$ |
| | | | 23–26 | 7–8 | 178 | $GA_4$; *endo*-$GA_4$ |
| | NBE | 7.7 | 25 | 8–9 | 28 | $GA_4$ |
| *ga4* (10.0) | AE | 45.9 | 8–12 | 9–11 | 60 | $GA_1$; $GA_{58}$; $GA_{71}$ |
| | | | 13–15 | 6–8 | 89 | $GA_2$; 15-*epi*-$GA_{63}$; 16-*epi*-$GA_{82}$ |
| | | | 20–23 | 11–14 | 89 | $GA_{34}$ |
| | | | 24–27 | 7–10 | 1409 | $GA_4$; *endo*-$GA_4$ |
| | NBE | 12.0 | 13–15 | 7 | 11 | $GA_2$ |
| | | | 23–25 | 8–9 | 54 | $GA_4$ |

[a] Metabolites identified by comparison of GC-MS data and KRIs with published values (Gaskin and MacMillan, 1991). Representative data are presented in Table II. [b] Unusual Rt due to a lower concentration of AcOH in the solvent system.

## RESULTS

The fractions from each genotype that were analyzed for $^{13}C$-metabolites are shown in Table I. The $^{13}C$-metabolites were identified by comparison of the full-scan GC-MS and KRI data with those of reference compounds (Kovats, 1958; Gaskin and MacMillan, 1991); typical characterizing data are given in Table II.

In the free GA fraction, [$^{13}C$]$GA_1$ was identified as a metabolite of [$^{13}C$,$^{3}H$]$GA_4$ from all nine kinds of plant material. Eleven additional $^{13}C$-labeled products were identified from other radioactive fractions. They were $GA_2$, $GA_{34}$, $GA_{58}$, 15-*epi*-$GA_{63}$, and 16-*epi*-$GA_{82}$ from maize, rice, and *Arabidopsis*; 16$\alpha$,17-$H_2$-16,17-$(OH)_2$-$GA_4$ from maize and rice; *endo*-$GA_4$ from rice and *Arabidopsis*; 3-*epi*-$GA_1$ from maize; and 2$\beta$-OH-$GA_2$, *endo*-$GA_{34}$, and $GA_{71}$ from *Arabidopsis*.

In the hydrolyzed fraction (NBE) of the presumptive bound GAs, [$^{13}C$]$GA_1$ was identified from maize and rice. Other $^{13}C$-labeled products also identified were $GA_2$ and $GA_4$ from maize, rice, and *Arabidopsis*; 2$\beta$-OH-$GA_2$, 16$\alpha$,17-$H_2$-16,17-$(OH)_2$-$GA_4$, and $GA_{34}$ from maize and rice; 3-*epi*-$GA_1$ and 3-*epi*-$GA_2$ from maize; and 15-*epi*-$GA_{63}$ and 16-*epi*-$GA_{82}$ from rice.

A control experiment was designed to determine whether any of the identified GAs were artifacts of extraction and purification rather than metabolites of the added [$^{13}C$,$^{3}H$]$GA_4$ (see "Materials and Methods"). Six HPLC fractions contained radioactivity; one had the Rt corresponding to $GA_4$; it was not analyzed further. The second fraction was shown by GC-MS to contain [$^{13}C$]$GA_2$. The other four fractions had only traces of radioactivity, and they were not analyzed chemically.

The dosage-response curves for $GA_4$ and $GA_1$ are shown in Figure 2. For the *d1* maize and *dx* and *dy* rice mutants, $GA_4$ and $GA_1$ have similar bioactivities. For *d5* maize, $GA_1$ is more active than $GA_4$. For *ga5* and *ga4 Arabidopsis* mutants, $GA_4$ is more active than $GA_1$ at low dosages and less active at high dosages.

## DISCUSSION

The metabolism of [$^{3}H$]$GA_4$ to presumptive $GA_1$ has been reported for seedlings of rice (Durley and Pharis, 1973) and *Phaseolus coccineus* (Turnbull et al., 1986), maturing seeds of maize (Rood et al., 1983), germinating seeds of bean (*Phaseolus vulgaris*) (Yamane et al., 1975), germinating pollen of knobcone pine (*Pinus attenuata*) (Kamienska et al., 1976), and cell cultures of anise (*Pimpinella anisum*) (Koshioka et al., 1983a) and carrot (*Daucus carota*) (Koshioka et al., 1983b). However, in all of these examples, the presence of $GA_1$ was based on HPLC and/or GC Rt values. GC-MS-single ion-current monitoring has been used to identify [$^{2}H$]$GA_1$ as a metabolite of [$^{2}H$]$GA_4$ from cell cultures of rice (Koshioka et al., 1991). For *Arabidopsis* there have been two preliminary reports of the metabolism of $GA_4$ to $GA_1$ with GC-MS identification (Kobayashi et al., 1991; Zeevaart and Talon, 1992). The results of the study reported here establish the metabolism of [$^{13}C$,$^{3}H$]$GA_4$ to [$^{13}C$]$GA_1$ in shoots of maize, rice, and *Arabidopsis* using full-scan GC-MS and KRIs.

The amounts of [$^{13}C$]$GA_1$ formed from [$^{13}C$,$^{3}H$]$GA_4$ can be estimated from the radioactivities of the fractions from which [$^{13}C$]$GA_1$ was identified (Table I). For example, for tall plants of maize, rice, and *Arabidopsis*, the amounts of [$^{13}C$]$GA_1$ formed are 0.93, 0.39, and 0.71 ng $g^{-1}$ fresh weight of tissue, respectively. These values are comparable to the reported endogenous levels of 0.12 ng $g^{-1}$ fresh weight for maize

**Table II.** *GC-MS data used for the identification of GAs listed in Table I*

| GA from Purified Fractions/ Reference Compound[a] | KRI | | Ions (mass/intensity [Int.]) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| [$^{13}$C]$GA_1$ | 2674 | Mass | 131 | 168 | 181 | 194 | 208 | 236 | 239 | 314 | 377 | 417 | 449 | 492 | 507 |
| | | Int. | 21 | 18 | 16 | 18 | 38 | 14 | 11 | 14 | 19 | 5 | 21 | 10 | 100 |
| $GA_1$ reference | 2673 | Mass | 131 | 167 | 180 | 193 | 207 | 235 | 238 | 313 | 376 | 416 | 448 | 491 | 506 |
| | | Int. | 9 | 6 | 7 | 6 | 23 | 6 | 5 | 9 | 14 | 5 | 18 | 9 | 100 |
| [$^{13}$C]3-*epi*-$GA_1$ | 2793 | Mass | 181 | 208 | 236 | 314 | 377 | 417 | 432 | 449 | 460 | 479 | 492 | 507 | |
| | | Int. | 14 | 28 | 10 | 10 | 28 | 8 | 9 | 31 | 9 | 3 | 8 | 100 | |
| 3-*epi*-$GA_1$ reference | 2793 | Mass | 180 | 207 | 235 | 313 | 376 | 416 | 431 | 448 | 459 | 478 | 491 | 506 | |
| | | Int. | 11 | 55 | 6 | 7 | 29 | 6 | 8 | 31 | 8 | 3 | 7 | 100 | |
| [$^{13}$C]$GA_2$ | 2761 | Mass | 131 | 226 | 262 | 285 | 290 | 329 | 387 | 419 | 450 | 451 | 493 | 494 | 509 |
| | | Int. | 100 | 31 | 11 | 15 | 24 | 14 | 11 | 42 | 10 | 13 | 10 | 9 | 22 |
| $GA_2$ reference | 2762 | Mass | 130 | 225 | 261 | 284 | 289 | 329 | 386 | 419 | 449 | 451 | 493 | | 508 |
| | | Int. | 100 | 12 | 8 | 10 | 26 | 6 | 7 | 15 | 6 | 6 | 13 | | 29 |
| [$^{13}$C]2$\beta$-OH-$GA_2$ | 2906 | Mass | 131 | 147 | 217 | 262 | 284 | 290 | 373 | 417 | 449 | 507 | 539 | 582 | 597 |
| | | Int. | 100 | 37 | 24 | 14 | 21 | 22 | 11 | 10 | 14 | 22 | 6 | 5 | 61 |
| 2$\beta$-OH-$GA_2$ reference | 2905 | Mass | 130 | 147 | 217 | 261 | 283 | 289 | 372 | 416 | 449 | 506 | 539 | 581 | 596 |
| | | Int. | 94 | 10 | 16 | 13 | 15 | 25 | 13 | 7 | 19 | 18 | 8 | 12 | 100 |
| [$^{13}$C]3-*epi*-$GA_2$ (putative) | | Mass | 131 | 226 | 262 | 290 | 329 | 344 | 372 | 387 | 419 | 451 | 493 | 494 | 509 |
| | | Int. | 100 | 24 | 18 | 41 | 25 | 16 | 25 | 13 | 30 | 12 | 9 | 8 | 21 |
| [$^{13}$C]$GA_4$ | 2511 | Mass | 129 | 202 | 225 | 226 | 234 | 262 | 285 | 290 | 329 | 359 | 387 | 391 | 419 |
| | | Int. | 64 | 37 | 92 | 92 | 47 | 31 | 100 | 57 | 23 | 11 | 16 | 12 | 16 |
| $GA_4$ reference | 2510 | Mass | 129 | 201 | 224 | 225 | 233 | 261 | 284 | 289 | 328 | 358 | 386 | 390 | 418 |
| | | Int. | 54 | 26 | 77 | 78 | 41 | 28 | 100 | 58 | 25 | 10 | 17 | 11 | 22 |
| [$^{13}$C]*endo*-$GA_4$ | 2481 | Mass | 129 | 202 | 226 | 230 | 234 | 262 | 285 | 290 | 301 | 359 | 387 | 391 | 419 |
| | | Int. | 45 | 34 | 47 | 34 | 26 | 49 | 29 | 100 | 19 | 13 | 10 | 6 | 48 |
| *endo*-$GA_4$ reference | 2478 | Mass | 129 | 201 | 225 | 229 | 233 | 261 | 284 | 289 | 300 | 358 | 386 | 390 | 418 |
| | | Int. | 25 | 25 | 41 | 30 | 19 | 46 | 39 | 100 | 22 | 12 | 10 | 10 | 75 |
| [$^{13}$C]16$\alpha$,17-$H_2$-16,17-$(OH)_2$-$GA_4$ | 2932 | Mass | 147 | 239 | 269 | 299 | 359 | 433 | 493 | 507 | 582 | 597 | | | |
| | | Int. | 14 | 11 | 11 | 14 | 6 | 3 | 100 | 1 | 2 | 1 | | | |
| 16$\alpha$,17-$H_2$-16,17-$(OH)_2$-$GA_4$ reference | 2932 | Mass | 147 | 239 | 269 | 299 | 359 | 433 | 493 | 506 | 581 | 596 | | | |
| | | Int. | 6 | 2 | 9 | 13 | 5 | 2 | 100 | 3 | 2 | 1 | | | |
| [$^{13}$C]$GA_{34}$ | 2670 | Mass | 147 | 202 | 217 | 224 | 230 | 262 | 289 | 314 | 388 | 417 | 432 | 460 | 507 |
| | | Int. | 55 | 23 | 36 | 26 | 23 | 16 | 17 | 12 | 7 | 8 | 5 | 4 | 100 |
| $GA_{34}$ reference | 2669 | Mass | 147 | 201 | 217 | 223 | 229 | 261 | 288 | 313 | 387 | 416 | 431 | 459 | 506 |
| | | Int. | 13 | 22 | 28 | 30 | 23 | 14 | 24 | 12 | 7 | 9 | 5 | 4 | 100 |
| [$^{13}$C]*endo*-$GA_{34}$ | 2644 | Mass | 147 | 211 | 230 | 262 | 284 | 314 | 373 | 417 | 460 | 507 | | | |
| | | Int. | 15 | 33 | 10 | 11 | 100 | 11 | 6 | 3 | 5 | 49 | | | |
| *endo*-$GA_{34}$ reference | 2642 | Mass | 147 | 211 | 229 | 261 | 284 | 314 | 372 | 416 | 459 | 506 | | | |
| | | Int. | 18 | 44 | 8 | 6 | 100 | 9 | 3 | 2 | 9 | 57 | | | |
| [$^{13}$C]$GA_{58}$ | 2738 | Mass | 224 | 228 | 267 | 283 | 327 | 357 | 373 | 385 | 400 | 417 | 447 | 452 | 507 |
| | | Int. | 100 | 44 | 61 | 67 | 27 | 55 | 29 | 55 | 10 | 60 | 7 | 16 | 17 |
| $GA_{58}$ reference | 2740 | Mass | 223 | 227 | 266 | 282 | 326 | 356 | 372 | 384 | 399 | 416 | 446 | 451 | 506 |
| | | Int. | 92 | 48 | 67 | 90 | 42 | 100 | 40 | 94 | 20 | 93 | 10 | 8 | 35 |
| [$^{13}$C]15-*epi*-$GA_{63}$ | 2786 | Mass | 157 | 224 | 283 | 288 | 358 | 373 | 385 | 417 | 447 | 475 | 492 | 507 | |
| | | Int. | 33 | 25 | 22 | 6 | 12 | 10 | 6 | 7 | 18 | 3 | 9 | 100 | |
| 15-*epi*-$GA_{63}$ reference | | Mass | 156 | 223 | 282 | 287 | 357 | 372 | 384 | 416 | 446 | 474 | 491 | 506 | |
| | | Int. | 100 | 28 | 30 | 17 | 8 | 5 | 7 | 10 | 11 | 1 | 24 | 76 | |
| [$^{13}$C]$GA_{71}$ | 2695 | Mass | 129 | 222 | 228 | 267 | 283 | 314 | 327 | 357 | 385 | 417 | 447 | 492 | 507 |
| | | Int. | 95 | 100 | 55 | 35 | 70 | 41 | 28 | 70 | 60 | 57 | 9 | 11 | 18 |
| $GA_{71}$ reference | 2693 | Mass | 129 | 221 | 227 | 266 | 282 | 313 | 326 | 356 | 384 | 416 | 446 | 491 | 506 |
| | | Int. | 27 | 53 | 42 | 58 | 77 | 33 | 39 | 98 | 94 | 100 | 13 | 24 | 46 |
| [$^{13}$C]16-*epi*-$GA_{82}$ | 2821 | Mass | 129 | 226 | 234 | 262 | 285 | 290 | 380 | 449 | 477 | 481 | 491 | 494 | 509 |
| | | Int. | 79 | 100 | 39 | 37 | 31 | 68 | 30 | 16 | 15 | 17 | 13 | 33 | 15 |
| 16-*epi*-$GA_{82}$ reference | 2822 | Mass | 129 | 225 | 233 | 261 | 284 | 289 | 379 | 448 | 476 | 480 | 490 | 493 | 508 |
| | | Int. | 47 | 76 | 56 | 49 | 29 | 100 | 26 | 15 | 20 | 12 | 11 | 5 | 10 |

[a] As methyl ester, trimethylsilyl ether derivatives.

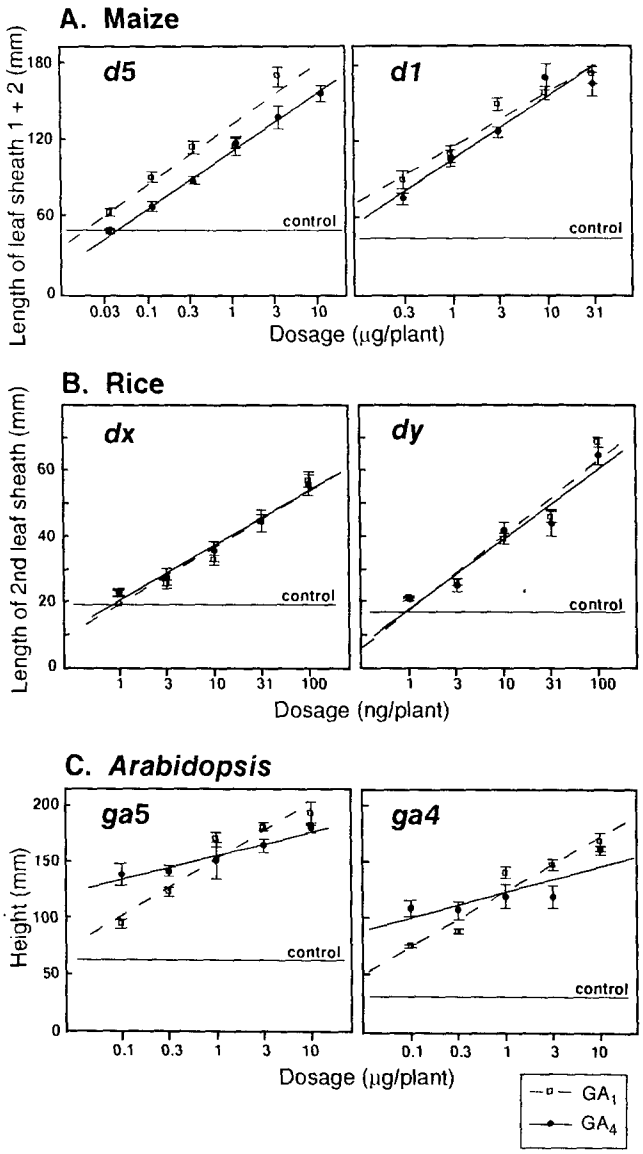


**Figure 2.** Dosage-response curves showing relative bioactivities of $GA_4$ and $GA_1$ assayed on *d5* and *d1* mutants of maize (A), *dx* and *dy* mutants of rice (B), and *ga5* and *ga4* mutants of *Arabidopsis* (C). Each point represents the mean of 10 to 20 measurements. Vertical bars represent SE.

(Fujioka et al., 1988a), 0.16 to 0.25 ng $g^{-1}$ fresh weight for rice (Kobayashi et al., 1989), and 0.34 ng $g^{-1}$ dry weight for *Arabidopsis* (Talon et al., 1990b).

In addition to [$^{13}$C]$GA_1$, 12 other $^{13}$C-labeled metabolites were identified. Of these, $GA_{34}$ (2$\beta$-OH-$GA_4$) is a bioinactive metabolite from $GA_4$ (Yamane et al., 1973). 16$\alpha$,17-$H_2$-16,17-$(OH)_2$-$GA_4$, $GA_{58}$, 15-*epi*-$GA_{63}$, $GA_{71}$, and 16-*epi*-$GA_{82}$ are metabolites from $GA_4$, unrelated to the biosynthesis of $GA_1$. $GA_{58}$ has been identified as a natural product from seeds of *Cucurbita maxima* (Beale et al., 1984), $GA_{71}$ from sporophytes of *Cyathea australis* (Yamane et al., 1985) and from the *gai* mutant of *Arabidopsis* (Talon et al., 1990a), and 16$\alpha$,17-$H_2$-16,17-$(OH)_2$-$GA_4$, and 16-*epi*-$GA_{82}$ from seeds of *Lupinus albus* (Gaskin et al., 1992). 15-*epi*-$GA_{63}$ has not been identified as a natural product. [$^{13}$C]$GA_2$ is an artifact because of its identification from a control experiment using [$^{13}$C,$^{3}$H]$GA_4$ and *d1* seedlings. [$^{13}$C]3-*epi*-$GA_1$ has been shown to be an artifact from [$^{13}$C]$GA_1$ in maize (our unpublished data).

There was no evidence in any of the experiments for the metabolism of [$^{13}$C,$^{3}$H]$GA_4$ to [$^{13}$C]$GA_7$ or to [$^{13}$C]$GA_3$ (Table I). By contrast, $GA_4$ is metabolized to $GA_1$ and to $GA_3$ via $GA_7$ in the fungus *Gibberella fujikuroi* (Bearder et al., 1975).

The data concerning the bioassays from all six mutants can be interpreted to mean that both $GA_4$ and $GA_1$ are active per se. Our metabolic studies show that at least some of the $GA_4$ bioactivity could be due to its metabolism to $GA_1$. Although the question of per se activity for $GA_4$ and $GA_1$ can be discussed at length from these kinds of data, the question will probably not be resolved from bioassay and metabolic studies alone. The answer could come from bioassay and metabolic studies conducted in conjunction with the use of mutants and/or chemicals that block 13-hydroxylation (Zeevaart and Talon, 1992). The answer could also come from the isolation of a GA receptor followed by receptor-hormone interaction studies.

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