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Ethylene-Mediated Regulation of Gibberellin Content and Growth in *Helianthus annuus* L.[1]

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ABSTRACT

Elongation of hypocotyls of sunflower can be promoted by gibberellins (GAs) and inhibited by ethylene. The role of these hormones in regulating elongation was investigated by measuring changes in both endogenous GAs and in the metabolism of exogenous [^{3}H]- and [$^2H_2$]GA_{20} in the hypocotyls of sunflower (*Helianthus annuus* L. cv Delgren 131) seedlings exposed to ethylene. The major biologically active GAs identified by gas chromatography-mass spectrometry were GA_1, GA_{19}, GA_{20}, and GA_{44}. In hypocotyls of seedlings exposed to ethylene, the concentration of GA_1, known to be directly active in regulating shoot elongation in a number of species, was reduced. Ethylene treatment reduced the metabolism of [^{3}H]GA_{20} and less [$^2H_2$]GA_1 was found in the hypocotyls of those seedlings exposed to the higher ethylene concentrations. However, it is not known if the effect of ethylene on GA_{20} metabolism was direct or indirect. In seedlings treated with exogenous GA_1 or GA_3, the hypocotyls elongated faster than those of controls, but the GA treatment only partially overcame the inhibitory effect of ethylene on elongation. We conclude that GA content is a factor which may limit elongation in hypocotyls of sunflower, and that while exposure to ethylene results in reduced concentration of GA_1 this is not sufficient *per se* to account for the inhibition of elongation caused by ethylene.

Seedlings of most, if not all, plant species produce ethylene as well as GAs.[2] Gibberellins promote elongation of stem cells directly through the action of GA_1 in several species (*e.g.* 13, 14), while ethylene inhibits elongation of stems but promotes radial growth. It has been shown that GA treatment will reverse the effect of ethylene on elongation (*e.g.* 21) and that ethylene inhibits GA-induced elongation (*e.g.* 3).

As part of ongoing studies on the role of GAs and other hormones in control of growth and development in *Helianthus* (4, 5, 17), we have investigated GA metabolism and its interaction with ethylene in sunflower seedlings. We have used ethylene as a tool to modify elongation in our investigation of GA metabolism.

We describe the effect of GA and ethylene treatment on elongation of hypocotyls of sunflower seedlings, and report the identification of the major endogenous GAs and the effect of ethylene on the concentration of these GAs in the hypocotyls. We also report the results of studies of the metabolism of [^{3}H]- and [^{2}H]GA_{20} in sunflower seedlings.

MATERIALS AND METHODS

Plant Material

In experiment 1, for identification of biologically active GAs, seeds of sunflower (*Helianthus annuus* L. cv Delgren 131) were germinated and grown in vermiculite, watered with tap water, for 6 d at 26°C in continuous light, 90 μE m^{-2} s^{-1}. At harvest, hypocotyls were excised from 255 plants, measured and weighed, and frozen at −70°C. The total fresh weight was 70.7 g. The mean hypocotyl length was 63 mm (SE ± 6 mm). The hypocotyls were elongating rapidly; samples from the population of seedlings indicated a relative elongation rate (change in length per day divided by initial length) of about 0.31 d^{-1} in the day preceding harvest, and a rate of about 0.21 d^{-1} in the day after harvest.

In experiment 2, to test the effect of exogenous GA on elongation, seeds were sown in Terragreen (a baked clay medium) in 10 cm pots and grown in Perspex chambers for fumigation with ethylene from the time of planting (15). The chambers were illuminated with fluorescent Gro-Lux lights (320 μmol m^{-2} s^{-1}) during a 16 h day. The temperature was 26°C during the day, and 14°C at night. The seedlings were watered automatically with half-strength Hoaglands solution for 45 s h^{-1}. The CO_2 concentration in the chambers was maintained at 350 μL L^{-1}. After 7 d, seedlings were treated with GA_3. Three days later, seedlings were harvested and hypocotyl length was measured. This experiment was repeated, and later repeated again with GA_3 and GA_1 applied to plants grown in conditions slightly modified from those described above (*i.e.* manual watering, GAs applied at 6 d and seedlings harvested at 9 d). This latter experiment was also repeated with seedlings which were grown for 7 d in the chambers before being fumigated with ethylene (500 nL L^{-1} only) for the next 2 d. Here, GA_1 and GA_3 were applied in two doses, at 6 h before and 18 h after ethylene treatment started. The seedlings were harvested at 9 d after planting.

In experiment 3, to determine the effect of ethylene on the content of endogenous GAs, seedlings were grown for 10 d, as described for the first trial in experiment 2 except that no GA treatment was given. The hypocotyls were frozen in liquid N_2 immediately after harvest and later freeze-dried.

In experiment 4, to investigate GA_{20} metabolism, seedlings

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[2] Abbreviations: GA, gibberellin; EtOH, ethanol; MeOH, methanol; EtOAc, ethyl acetate; SIM, selected ion monitoring; M^+, molecular ion; amu, atomic mass unit; MeTMSi, methyl ester trimethylsilyl ether; KRI, Kovats' retention index.

were grown as described for experiment 2, except that they were fumigated with ethylene from 5 d, treated with [^{3}H]/[$^2H_2$]GA_{20} at 7 d and harvested at 9 d after planting. At harvest, the hypocotyls were excised and measured. The cotyledons were briefly rinsed in MeOH to remove any GA_{20} substrate which had not been absorbed. The roots were washed free of adhering Terragreen and blotted dry. All seedling parts were frozen at −70°C and lyophilized.

GA Treatment

In experiment 2, GA_3 (Abbott ProGibb) or GA_1 (synthesized by Prof. L. Mander, Australian National University) (1, 10, or 100 μg in 10 μL of 50% EtOH) or 50% EtOH alone was applied to the cotyledonary petioles of each of 10 seedlings in each of the four ethylene treatments. In experiment 4, a mixture of [17,17-^{2}H]GA_{20} (25.4 ng, synthesized by Prof. L. N. Mander as described in Lombardo [11]) and [2,3-^{3}H]GA_{20} (2945 Bq, stereochemistry of labeling uncertain, with associated GA_{20}, 23.9 ng [12]) in 95% EtOH was applied in 2 μL drops to the cotyledonary petioles of each of 23 seedlings in each of the four ethylene treatments. The [^{3}H]GA_{20} was purified by C_{18} HPLC and analyzed by GC-MS before it was applied; only GA_{20} was detected.

Ethylene Treatment

In experiments 2 and 3 seedlings were fumigated with ethylene for 10 d at nominal concentrations of 0, 5, 50, or 500 nL L^{-1}. The concentration of ethylene was difficult to regulate at low values and so actual concentrations varied somewhat from these nominal conditions. In the 0 nL L^{-1} treatment ambient air was scrubbed of ethylene (<0.01 nL L^{-1}) after passage through glass columns filled with beads of Purafil (Circul-Aire Inc., Montreal) (15). In experiment 4, seedlings were fumigated for 4 d at nominal concentrations of 0, 25, 125, or 500 nL L^{-1}. The actual concentrations measured by GC-FID in samples taken on each day of fumigation were 0, 19 to 27, 135 to 141, and 528 to 553 nL L^{-1}, respectively, in the four treatments.

Gibberellin Extraction and Purification

In experiment 1, the hypocotyls were extracted in MeOH in a Waring Blendor. The extract was filtered and the residue reextracted with MeOH until most of the color was removed. The total volume of filtrate was 530 mL. To this was added 1010 Bq of [1,2-^{3}H]GA_1 (Amersham; 1.2 TBq $mmol^{-1}$) and 1150 Bq of [1,2-^{3}H]GA_4 (Amersham; 1.4 TBq $mmol^{-1}$) as internal standards. The filtrate was dried at reduced pressure at 35°C and the residue was later redissolved with MeOH:water (80:20) and forced through a C_{18} preparative column (9) to remove nonpolar compounds. The eluate (77 mL) was reduced to about 1 mL at reduced pressure at 35°C, MeOH (25 mL) and then EtOAc (25 mL) were added, and the suspension was filtered. The filtrate was dried at reduced pressure at 35°C and later purified by chromatography on a preparative normal phase (SiO_2) partition column (9). The less-polar EtOAc:hexane eluate, expected to contain the majority of free GAs, was dried and further purified by preparative SiO_2 adsorption chromatography. The sample was redissolved in EtOAc and transferred to a 1 cm diameter column of 4 g of activated silica (Woelm, 32–100 μm) which was then eluted with 10 mL volumes of EtOAc. Fractions containing the radioactive internal standards were combined and rerun twice on new columns to further reduce mass before being purified by C_{18} HPLC: 10 μm μBondapak C_{18}, 10 cm × 8 mm i.d. (Radial Pak, Waters Associates), eluted with MeOH:water:acetic acid (from 37:62:1 to 64:35:1 in a linear gradient over 10 min, then isocratic) at 1 mL min^{-1}. Fractions expected to contain the major biologically active GAs (based on the results of preliminary experiments) were combined and further purified by $N(CH_3)_2$ HPLC (24): 5 μm Nucleosil $N(CH_3)_2$, 15 cm × 4.6 mm i.d. (Alltech Associates), eluted with 99.9 MeOH:0.1 acetic acid at 1 mL min^{-1}. Two groups of fractions were selected, based on preliminary bioassay results, for analysis by GC-MS.

In experiment 3, 1.0 g of freeze-dried hypocotyls from seedlings from each ethylene treatment (94, 94, 98, and 78 hypocotyls, respectively, from each of the 0, 5, 50, and 500 nL L^{-1} treatments) was extracted in 80% MeOH. Internal standards of [^{3}H]GA_1 (1440 Bq) and [^{3}H]GA_4 (1370 Bq) were added. After preparative C_{18} and SiO_2 partition chromatography, the fraction expected to contain free GAs was analyzed by C_{18} HPLC and bioassay. The group of fractions which contained the [^{3}H]GA_1 internal standard, and the biologically active fractions expected to contain GA_{19} and GA_{20}, were further purified on $N(CH_3)_2$ HPLC after 100 ng of each of the internal standards [17,17-^{2}H]GA_{19} and [17,17-^{2}H]GA_{20} had been added. After bioassay those fractions expected to contain GA_{20} and [$^2H_2$]GA_{20}, GA_{19} and [$^2H_2$]GA_{19}, and GA_1 were analyzed by GC-MS-SIM, the latter after 10 ng of [17,17-^{2}H]GA_1 was added.

In experiment 4, the freeze-dried hypocotyls, epicotyls, cotyledons, and roots from all plants in each treatment were each extracted as described above. The extracts of the roots were not further analyzed. An internal standard of 10 ng [1,2,2,3,6-^{2}H]GA_1 (a gift from Prof. N. Murofushi, University of Toyko) was added to the extracts of hypocotyls. After preparative C_{18} and SiO_2 partition chromatography, the less-polar EtOAc:hexane eluate (expected to contain most of the free GAs) and the more-polar MeOH eluate (expected to contain GA glucosyl conjugates) were each purified by HPLC, and fractions were then pooled according to their radioactivity. From the hypocotyls, the HPLC fractions expected to contain GA_1 were analyzed by GC-MS-SIM. From the control treatment, the most polar group of fractions 5 to 13 expected to contain conjugated GAs were pooled and hydrolyzed with cellulase. The hydrolysate was extracted with EtOAc, and the acidic EtOAc phase was analyzed for free GAs by GC-MS or GC-MS-SIM. From the epicotyls and cotyledons, those fractions from C_{18} HPLC expected to contain conjugated GAs (from all of the ethylene treatments and the control) were combined in groups of equivalent polarity, and analyzed by chromatography on DEAE-Sephadex (23). The early eluting fractions expected to contain (neutral) GA-glucosyl esters were hydrolyzed and after partitioning the acidic EtOAc phase was analyzed by GC-MS as above.

Table I. *Principle Ions and KRI of GAs Identified from Sunflower Seedlings*

Gibberellin	KRI	Principle Ions (Relative Intensities)
GA_1[a]	2664	506(100), 491(10), 448(21), 447(9), 377(19), 375(17), 313(14)
GA_8[b]	2811	594(100), 579(4), 535(8), 448(22), 379(38), 238(33)
GA_{19}[a]	2599	462(7), 434(100), 402(29), 375(42), 374(53), 345(18)
GA_{20}[a]	2492	418(100), 403(12), 387(6), 375(42), 359(21), 301(13)
GA_{29}[b]	2679	506(100), 491(11), 447(10), 375(19), 313(10), 303(30)
GA_{44}[a]	2795	432(38), 417(6), 373(12), 238(35), 207(100)
GA_{67}[a,c]	2618	506(53), 491(26), 462(13), 447(7), 416(100), 389(15), 372(25), 357(22)

[a] Identified as the MeTMSi derivative after GC-MS by comparison of mass spectrum and KRI with standards; relative intensities of ions given here are from GC-MS-SIM. [b] Indicated as the MeTMSi derivative by GC-MS and identity confirmed from GC-MS-SIM. [c] After cellulase hydrolysis of the neutral fraction from DEAE-Sephadex chromatography (of fractions 4–10 from C_{18} HPLC of the polar fraction from SiO_2 chromatography) of an extract of cotyledons. All the other GAs were found in extracts of hypocotyls.

Table II. *Effect of Ethylene on Length and GA Content of Hypocotyls of Sunflowers*[a]

Ethylene concn.	Gibberellin Content[b]			Hypocotyl length
	GA_{19}	GA_{20}	GA_1	
nL L⁻¹				*mm ± SE*
0	204(2.2)	8.4(0.09)	9.9(0.11)	74.5 ± 3.4
5	221(2.4)	7.6(0.08)	12.8(0.14)	68.5 ± 2.1
50	374(3.8)	8.2(0.08)	—[c]	51.8 ± 2.2
500	224(2.9)	10.8(0.14)	2.3(0.03)	50.8 ± 1.6

[a] Results of experiment 3. Seedlings were exposed to ethylene from sowing until harvested at 10 d. [b] Values expressed as ng g^{-1} dry weight and, in parentheses, ng per hypocotyl. [c] No data available.

GC-MS

Samples were methylated with ethereal diazomethane, and centrifuged to remove insoluble material before being silylated with 1:1 pyridine:*N,O*-bis(trimethylsilyl)trifluoroacetamide plus 1% TMCS (Pierce). The sample was dried then dissolved in hexane (about 5–20 μL depending on the sample), and a 1 μL aliquot was introduced by cool on-column injection into a DB1–15N capillary column (J & W Scientific) installed in a Hewlett Packard 5790 GC, with a capillary direct interface to a HP 5970B Mass Selective Detector. The GC temperature program was 60°C to 195°C at 15°C min^{-1}, with a second ramp to 275°C at 5°C min^{-1}. Mass spectra were acquired at 70 eV (nominal voltage) in either the scan or SIM modes under control of a HP 300 computer.

RESULTS

Identification and Quantification of Gibberellins

The major biologically active GAs in the hypocotyls were GA_{19}, GA_{20}, GA_{44}, and GA_1, identified initially in experiment 1 by comparison of KRI and mass spectra with those of standards after analysis by GC-MS (Table I). The inactive GA_8 and GA_{29} were identified in experiment 4 by GC-MS and GC-SIM (Table I) after cellulase hydrolysis of the relatively polar fractions (5–13) from C_{18} HPLC of the MeOH eluate from SiO_2 partition chromatography. From an equivalent fraction derived from an extract of cotyledons, GA_{67} (15β-OH GA_{20}) was identified after hydrolysis of the neutral fraction from DEAE-Sephadex chromatography (Table I).

The concentration of GA_{19}, the most abundant biologically active GA in the hypocotyls, was little affected by ethylene treatment (experiment 3, Table II). The concentration of GA_{20}, lower than that of GA_{19}, was also similar in all treatments. The concentration of GA_1 in the 500 nL L^{-1} treatment was about one-fifth of that in the 0 or 5 nL L^{-1} treatments. This effect on the GA_1 content of the hypocotyls of seedlings exposed to ethylene was confirmed by the analysis of endogenous GA_1 from the seedlings fed with [^{2}H]- and [^{3}H]GA_{20} (experiment 4, Table III). There, the endogenous GA_1 concentration decreased in hypocotyls of seedlings exposed to progressively higher ethylene concentrations.

Metabolism of [^{2}H]- and [^{3}H]GA_{20}.

In experiment 4 the seedlings were harvested 48 h after applying the labeled substrates to the cotyledonary petioles. Most of the radioactivity recovered was in the epicotyls (48–61% of that applied, Table IV). The cotyledons, including their petioles, contained 12 to 20% of the applied radioactivity. The hypocotyls and roots each contained less than 1% of the applied radioactivity. The radioactivity recovered decreased in hypocotyls of seedlings exposed to progressively higher concentrations of ethylene.

In the hypocotyls the major radioactive metabolites eluted from C_{18} HPLC in fractions expected to contain [^{3}H]GA_{20}, [^{3}H]GA_1, [^{3}H]GA_8, and glucosyl conjugates of [^{3}H]GA_{20}, [^{3}H]GA_8, and [^{3}H]GA_{29} and/or other polar compounds (Table V). Subsequently, from these fractions from the control (no

Table III. *Effect of Ethylene on Length and GA_1 Content in Hypocotyls of Sunflower Seedlings Fed with [$^2H_2$]- and [^{3}H]GA_{20}*[a]

Ethylene concn.	GA_1[b,c]	[$^2H_2$]GA_1[b]	Hypocotyl length
nL L⁻¹			*mm ± SE*
0	6.1(0.12)	0.39(0.007)	49.1 ± 2.3
25	4.3(0.06)	0.62(0.009)	40.3 ± 1.5
125	3.6(0.05)	0.29(0.004)	38.1 ± 1.3
500	3.0(0.04)	ND[d]	34.8 ± 1.2

[a] Results of experiment 4. Seedlings were exposed to ethylene from 5 d, fed GA_{20} at 7 d and harvested at 9 d. [b] Values expressed as ng g^{-1} dry weight and, in parentheses, ng per hypocotyl. [c] This is endogenous GA_1, calculated as the total GA_1 found minus the amount of GA_1 formed from the exogenous GA_{20} (the latter calculated from the amount of [$^2H_2$]GA_1 found and the GA_{20}:[$^2H_2$]GA_{20} ratio in the substrate, assuming equal metabolism of GA_{20} and [$^2H_2$]GA_{20}). [d] Not detected (*i.e.* less than 0.1 ng g^{-1} dry weight).

Table IV. *Recovery of Radioactivity from Sunflower Seedlings Fed with [$^2H_2$]- and [^3H]GA_{20}*[a,b]

Ethylene Concn.	Radioactivity Recovered from					
	Epicotyls	Hypocotyls	Roots	Cotyledons	MeOH rinse of cotyledons	Total
nL L^{-1}	*% of amount applied*					
0	48.1	0.77	0.12	12.3	7.5	68.8
25	48.2	0.48	0.16	20.4	9.4	78.6
125	60.7[c]	0.43	0.18	13.0	2.7	77.1
500	60.2[c]	0.24	0.06	12.7	4.9	78.1

[a] Loss of an unknown amount of radioactivity as [^3H]H_2O from hydroxylation of the substrate or metabolites at C-2 or C-3 is expected. [b] Result of experiment 4. [c] It was not possible to prevent some of the applied solution from coming into contact with the epicotyl in these plants because the cotyledons were small. Therefore this value may be higher than that found for the epicotyls from the zero and 25 nL L^{-1} treatments as a result of direct absorption into the epicotyls rather than through movement from the cotyledons.

ethylene) treatment, GA_{20} (in fractions 31–35), and GA_1 and [$^2H_2$]GA_1 (in fractions 18–20) were indicated after analysis by GC-SIM for the M^+ of these GAs and the [$^2H_5$]GA_1 internal standard (Table III). The presence of [$^2H_2$]GA_{20} could not be confirmed because of contamination of the ions monitored. [$^2H_2$]GA_8 and [$^2H_2$]GA_{29} were indicated by GC-MS-SIM through enrichment of M^+ plus 2 amu in the spectra of their endogenous analogs (data not shown), in the acidic-EtOAc phase from partition of the enzymic hydrolysate of fractions 5 to 13 from C_{18} HPLC of the more-polar fraction from SiO_2 chromatography (Table V).

Of the radioactivity recovered from the hypocotyls the proportion associated with metabolites of [^3H]GA_{20} was lower in seedlings exposed to 125 or 500 nL L^{-1} of ethylene than in those exposed to zero or 25 nL L^{-1} (Table V), indicating slower metabolism of [^3H]GA_{20} in seedlings exposed to higher ethylene concentrations. Consistent with this, the amount of [$^2H_2$]GA_1 was lowest in the hypocotyls of seedlings exposed to the highest concentrations of ethylene (Table III).

Table V. *Distribution of Radioactivity*[a] *after C_{18} HPLC of the Less-Polar (EtOAc:Hexane) and More-Polar (MeOH) Eluates from SiO_2 Chromatography of Extracts of Hypocotyls from [^3H]GA_{20}-Fed Sunflower Seedlings*

Ethylene Concn.	C_{18} HPLC Fractions[b]				
	SiO_2 Less-Polar			SiO_2 More-Polar	
	31–35	18–20	10–12	30–34	5–13
nL L^{-1}					
0	11	4	2	10	66
25	13	8	5	6	57
125	27	2	2	9	54
500	22	5	2	9	46

[a] Values expressed as percent of total radioactivity recovered after HPLC adjusted for known losses. [b] GAs expected in these fractions include: less-polar 31–35 GA_{20}, 18–20 GA_1, and 10–12 GA_8; more-polar, 30–34 GA_{20}-glucosyl conjugates, 5–13 GA_8- and GA_{29}-glucosyl conjugates.

Table VI. *Distribution of Radioactivity*[a] *after C_{18} HPLC of the Less-Polar (EtOAc:Hexane) and More-Polar (MeOH) Eluates from SiO_2 Chromatography of Extracts from Epicotyls and Cotyledons of [^3H] GA_{20}-Fed Sunflower Seedlings*

Ethylene Concn.	C_{18} HPLC Fractions[b]		
	SiO_2 Less-Polar	SiO_2 More-Polar	
	30–35	28–33	4–11
nL L^{-1}			
Epicotyls			
0	74	7	13
25	83	4	6
125	80	4	10
500	87	3	7
Cotyledons			
0	28	27	31
25	53	14	20
125	36	25	22
500	43	22	21

[a] Values expressed as percent of total radioactivity recovered after HPLC adjusted for known losses. [b] GAs expected in these fractions include: less polar, 30–35 GA_{20}; more polar, 28–33 GA_{20}-glucosyl conjugates, 4–11 GA_8- and GA_{29}-glucosyl conjugates.

In the epicotyls and cotyledons, the majority of the radioactivity recovered (74–87% in the epicotyls, and 28 to 53% in the cotyledons) was unmetabolized [^3H]GA_{20}, with most of the remainder in fractions expected to contain [^3H]GA-conjugates or other compounds with similar properties (Table VI). The latter were combined in groups of fractions of equivalent polarity from all ethylene treatments and the control, with fractions from epicotyls and cotyledons kept separate, and then analyzed by DEAE-Sephadex chromatography. Most of the radioactivity (85% for epicotyls, and 73% for cotyledons) recovered from chromatography of the ^3H-metabolites in the most polar fractions from C_{18} HPLC (fractions 4–11, Table VI) eluted from DEAE-Sephadex in fractions expected to contain (neutral) GA glucosyl esters. From GC-MS analysis of the acidic EtOAc-soluble products of subsequent enzymic hydrolysis, [$^2H_2$]GA_8 and [$^2H_2$]GA_{29} were identified from epicotyls through enrichment of ions of two amu higher than those monitored (Table I) for their endogenous analogs; based on the M^+, GA_8:[$^2H_2$]GA_8 was 2:1 and GA_{29}:[$^2H_2$]GA_{29} was 1.14:1. These [$^2H_2$]GAs were also indicated from cotyledons only by enrichment of M+ plus 2 amu.

Consistent with the results from analysis of the hypocotyls, the proportion of radioactivity associated with metabolites of [^3H]GA_{20} was lower in the epicotyls and cotyledons from seedlings exposed to ethylene (Table VI). The results from the plant as a whole (Tables IV, V, and VI) then suggest slower metabolism of the [^3H]GA_{20} in ethylene-treated seedlings, and this is confirmed by the absolute (rather than %) recovery of putative [^3H]GA_{20} from the different treatments (data not shown).

Effect of Exogenous GA_1 and GA_3 on Growth

In seedlings treated with GA_3 or GA_1 (applied to the cotyledonary petioles) the hypocotyls elongated faster than did those of controls (experiment 2, Table VII; greater responses to GA_3—*e.g.* increases in length of 35% and 49% over untreated seedlings—were found in the other trials). In seedlings exposed to ethylene, the hypocotyls elongated more slowly than did controls (in experiments 2 [Table VII], 3 [Table II], and 4 [Table III]). In seedlings exposed to ethylene for 6 or 7 d and subsequently treated with GA_3 or GA_1 for 2 d, the hypocotyls were longer at harvest than those of controls which were not treated with GAs (Table VII). However, over the 2d after GA treatment, the inhibition of elongation which ethylene exerted was not completely reversed. In subsequent experiments to test the effect of GA applied concomitantly with ethylene, seedlings were exposed to ethylene (500 nL L^{-1}) and treated with GA_1 or GA_3 only after 6 d growth. In the 2 d which elapsed before harvest the GA treatment did not overcome the inhibitory effect of ethylene on elongation (results not shown).

DISCUSSION

The GAs identified from vegetative tissue of sunflower seedlings in this study are among those described from sunflower seeds (6). With the exception of GA_{67} they are members of the 13-hydroxylation pathway ($GA_{53} \rightarrow GA_{44} \rightarrow GA_{19} \rightarrow GA_{20} \rightarrow GA_1 \rightarrow GA_8$), which is common in vegetative tissues of many species (*e.g.* 13, 14). In plants fed with [^{3}H]- and [^{2}H]GA_{20} the metabolites included [$^2H_2$]GA_1, [$^2H_2$]GA_{29}, and [$^2H_2$]GA_8, the latter two identified from the hydrolysates of fractions expected to contain GA-glucosyl ester conjugates. The formation of these metabolites is consistent with biosynthesis of GAs through the 13-hydroxylation pathway. The relative concentrations of GA_{19}, GA_{20}, and GA_1 in sunflower hypocotyls are similar to those found in vegetative tissue of other species; *e.g. Zea* (2) and *Oryza* (8); and it has been suggested that GA_{19} serves as a pool of inactive substrate for the synthesis through GA_{20} of the active GA_1 (10). GA_{67} was identified from cotyledons, consistent with its presence in seeds (6), and thus is likely to have been synthesized during seed maturation.

Our measurements of endogenous GAs show that while hypocotyls of ethylene-treated sunflower seedlings contained less GA_1 than those of seedlings grown in ethylene-free air, the content of GA_{19} and GA_{20} was not much altered. One explanation of this observation is that GA biosynthesis was modified by a specific effect (not necessarily direct) of ethylene on the formation or fate of GA_1, rather than through an effect on an earlier biosynthetic step.

This suggestion was supported by the results of feeds of [^{3}H/$^2H_2$]GA_{20} to sunflower seedlings, where there was slower metabolism of [^{3}H]GA_{20} in those seedlings exposed to ethylene (Tables IV, V, and VI). This suggests that the reduced amount of [$^2H_2$]GA_1 found in the hypocotyls of seedlings exposed to higher concentrations of ethylene was at least partly a result of slower synthesis from [$^2H_2$]GA_{20}. Alternatively, there might have been enhanced metabolism of [$^2H_2$]GA_1 to other products, notably the inactive [$^2H_2$]GA_8 and conjugated forms. However, while [^{3}H/$^2H_2$]]GA_8 conjugates were not separately distinguished in this study there was no evidence of increased formation of ^{3}H-metabolites with the chromatographic properties expected of GA conjugates (Tables V and VI); in fact the trend is toward reduced formation of such compounds in ethylene-treated seedlings. Another contributing factor to the reduced content of [$^2H_2$]GA_1 in the hypocotyls was possibly reduced amount of [$^2H_2$]GA_{20} substrate available in the hypocotyl, as indicated by the reduced amounts of radioactivity recovered from the hypocotyls of seedlings treated with ethylene (Table IV).

The observed reduction in [$^2H_2$]GA_1 and endogenous GA_1 in the hypocotyls treated with ethylene is correlated with reduction in the length of the hypocotyls of seedlings in these treatments. However, it seems unlikely that the effect of ethylene on GA_1 content was the complete cause of reduced hypocotyl length, because exogenous GA_1 or GA_3 only partly reversed the inhibitory effect of ethylene even at an apparently saturating dose.

The observation that exogenous GA_1 and GA_3 promoted elongation of the hypocotyls of sunflower seedlings is consistent with the view that elongation of the hypocotyl was limited by GAs in both control seedlings and those seedlings exposed to ethylene, at least in the conditions of these experiments. However, the question of the relationship between elongation rate and GA_1 content was not critically addressed, as the GA_1 content was measured at only one point in time and the elongation rate of the hypocotyls was not measured at that time. While there are many other studies indicating that GA_1 content is related to growth rate, there is no well-defined overall relationship. Examples include work on *Pisum* (16), *Triticum* (22), *Phaseolus* (1), *Brassica napus* (19), and *Zea* (13, 18).

There is other evidence of links between ethylene and GA synthesis and action. Most studies indicate an antagonism of ethylene to the action of GAs (*e.g.* 3, 20, 21). The latter study showed that the *lk* dwarf mutant of pea, which is insensitive to exogenous GA and produces more ethylene than the normal *Lk*, becomes more responsive to applied GA_1 when ethylene synthesis is inhibited. In similar vein, epicotyl seg-

Table VII. *Effect of GA_1 or GA_3 Treatment on Hypocotyl Length of Sunflower Seedlings Exposed to Ethylene*[a]

Gibberellin, Amount Applied		Hypocotyl Length at Each Ethylene Concentration[b]			
		0	5	50	500
μg/seedling		*mm ± SE*			
Control	0	108.3 ± 4.0	80.4 ± 3.3	77.4 ± 3.8	56.7 ± 4.8
GA_1	1	114.5 ± 2.5	95.0 ± 3.5	95.7 ± 5.7	69.5 ± 3.3
	10	114.3 ± 3.2	99.5 ± 4.4	92.4 ± 4.7	66.8 ± 4.2
	100	117.0 ± 5.0	103.5 ± 4.1	96.3 ± 6.6	78.5 ± 5.3
GA_3	1	117.8 ± 4.9	100.5 ± 5.0	92.6 ± 4.5	71.8 ± 6.4
	10	115.0 ± 6.6	112.5 ± 2.6	89.8 ± 3.9	79.9 ± 4.9
	100	126.7 ± 2.5	103.2 ± 4.7	102.9 ± 4.6	77.2 ± 5.0

[a] Results of one trial in experiment 4, where ethylene treatment started at sowing, GA_1 or GA_3 was applied after 6 d and plants were harvested at 9 d. The other two trials yielded similar results in that GA_3 treatment did not reverse the inhibitory effect of ethylene. [b] Ethylene concentrations are given as nL L^{-1}.

ments of cowpea elongated more in response to exogenous GA_1 when ethylene (present in part from GA-induced synthesis) was removed from the system (3). One well-documented case of an apparently opposite response is that of rapid extension in submerged internodes of deepwater rice, apparently linked with an increase in responsiveness to GAs that accompanies increased ethylene synthesis (7).

In summary, the results of this study extend the evidence of interaction between ethylene and GAs, suggesting that inhibition of elongation by ethylene is accompanied by an inhibition of GA_1 biosynthesis, although it is not known whether this is a direct or an indirect effect. In addition we have identified the major GAs in vegetative tissue of sunflowers, indicated the likely pathway of their biosynthesis, and indicated that GAs are involved in regulating elongation in hypocotyls of sunflower seedlings.

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