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NATIVE GIBBERELLINS AND THE METABOLISM OF [^{14}C]GIBBERELLIN A_{53} AND OF [17-^{13}C, 17-$^{3}H_2$]GIBBERELLIN A_{20} IN TASSELS OF *ZEA MAYS*

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Key Word Index—*Zea mays*; Gramineae; tassels; normal; *dwarf* mutants; metabolism; gibberellins; GC/RC; GC/MS and GC/SIM identification; Kovats retention index.

Abstract—The native hormones from tassels of maize (*Zea mays*) were re-investigated. The previous identification by GC/SIM of GA_1, GA_8 and GA_{29} in normal tassels was confirmed by full GC/MS scans at the correct Kovats retention indices. In tassels of *dwarf*-1 mutants, GA_{44}, GA_{19}, GA_{17}, GA_{20} and the 16,17-dihydro, 7β,16α,17-trihydroxy derivative of *ent*-kaurenoic acid were identified by GC/MS. Gibberellin A_1 was not found in the mutant tassels. [^{14}C]Gibberellin A_{53} was fed to tassels of the *dwarf*-5 mutant. In the ethyl acetate-soluble acidic fraction from the feeds, [^{14}C]GA_{44} was identified by GC/MS; [^{14}C]GA_{19} and [^{14}C]GA_{29} were identified by GC/SIM. The GA_{29} is probably a metabolite of the feeds because the *dwarf*-5 mutant is known to control the step copalyl pyrophosphate to *ent*-kaurene in the maize GA-biosynthetic pathway and because GA_{29} was not identified in a control experiment. The *n*-butanol fractions obtained from the feeds were shown, by GC/MS, to contain [^{14}C]GA_{53} after hydrolysis, suggesting that conjugated [^{14}C]GA_{53} is a major metabolite from GA_{53} feeds. [17-^{13}C, 17-$^{3}H_2$]Gibberellin A_{20} was fed to normal, *dwarf*-1 and *dwarf*-5 tassels. In each case, analysis of the purified ethyl acetate-soluble acidic extracts by GC/MS led to the identification of [^{13}C]GA_{29} and unmetabolized [^{13}C]GA_{20} in which no ^{13}C-isotope dilution was observed.

INTRODUCTION

The single-gene mutants of *Zea mays* L., *dwarf*-1, -2, -3, and -5, are non-allelic, simple recessives which respond to exogenously applied gibberellins (GAs) by normal phenotypic growth [1–3]. These mutants provide an opportunity to examine in detail both the GA-metabolic pathway and the hormonal action of GAs in relation to elongation growth of stems and tassels. This paper is confined to tassels; recent metabolic studies with seedlings of maize are published elsewhere [4, 5].

This paper extends the data previously reported [6] on the nature of GAs in maize tassels. It also presents results on the metabolism of [^{14}C]GA_{53} of sufficiently high specific activity to detect the ^{14}C-label in metabolites by GC/MS. The *dwarf*-5 mutant was selected for these experiments because the genetic block occurs at the cyclization step of copalyl pyrophosphate (CPP) to *ent*-kaurene [7]. As a result, the levels of GAs are low or zero [8]. This paper also presents the results of feeding [17-^{13}C, 17-$^{3}H_2$]GA_{20} to tassels of normal, *dwarf*-1 and *dwarf*-5 in an attempt to elucidate the later steps in the GA-pathway and to define the position of the block in *dwarf*-1.

RESULTS

Identification of native GAs in tassels

In a previous paper [6], we reported the identification of GAs in extracts from tassels of normal maize by GC/MS. The following GAs were identified by full scan mass spectral comparison with reference spectra: GA_{17}, GA_{19}, GA_{20}, GA_{44} and GA_{53} (Fig. 1). In addition, the presence of small amounts of GA_1, GA_8 and GA_{29} (Fig. 1) was indicated by capillary GC/SIM.

We have re-examined the original extract, prepared in 1979, together with more recent extracts from *dwarf*-1 tassels, by capillary GC/MS using a more sensitive mass spectrometer. The results are shown in Table 1. All identifications were made from full scan comparison of the derivatives at the correct Kovats retention index. These results are discussed later.

In a small-scale control experiment, in connection with the feed of [^{14}C]GA_{53} to *dwarf*-5 tassels, three tassels of *dwarf*-5 were injected with methanol, incubated for 24 hr and extracted. No GAs were detected by GC/SIM.

Small-scale feed of [^{14}C]GA_{53} *to* dwarf-5

The distribution and percentage recovery of label in the various fractions are shown in Table 2. The total amount of substrate injected into the three tassels was calculated to be 5.4 μg (3.3×10^4 Bq) of which 21% was found in the acidic fraction and 53% in the *n*-butanol fraction. Nineteen per cent of label was unaccounted for.

The acidic fraction (7.0×10^3 Bq, 7.3 mg) was fractionated by TLC into six zones of radioactivity which

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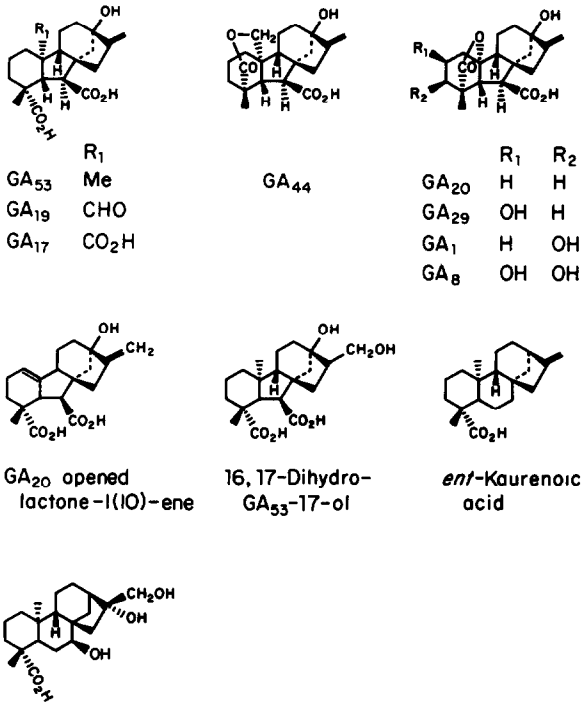

Fig. 1. The structures of the compounds referred to in the text.

Table 1. Compounds identified in normal and *dwarf*-1 tassels and their Kovats retention indices (KRI)

Compound	KRI	Normal	*Dwarf*-1
GA_{20} opened lactone-1(10)-ene	2423	+	+
GA_{20}	2477	+	+
GA_{53}	2492	+	−
GA_{17}	2569	+	tr
GA_{19}	2590	+	tr
GA_1	2664	+	−
GA_{29}	2681	+	−
GA_{53}, 16,17-dihydro-17-ol	2750	+	−
GA_{44}	2778	+	+
GA_8	2815	+	−
ent-Kaurenoic acid	2231	+	−
ent-Kaurenoic acid, 16,17-dihydro, 7α, 16β,17-triol	2860	+	+

tr = trace.

were eluted and assayed for radioactivity (Table 3). Fraction 1 from TLC had too great a mass for GC/MS analysis but the other fractions were analysed by this method after methylation and trimethylsilylation.

From fraction 4, $[^{14}C]GA_{44}$ was identified as the MeTMSi derivative by a full mass spectrum from a peak at R_t 29.6 min. The presence of ^{14}C-label in GA_{44} was shown by the $[M]^+$ cluster which contained an $[M+8]^+$ ion at m/z 440 (9%).

From fraction 5, unmetabolized $[^{14}C]GA_{53}$ was identified from a full mass scan of a peak at R_t 24.7 min; the mass spectrum showed an $[M+8]^+$ ion at m/z 456 (1%). The

Table 2. Partition of label from the 24 hr feeds of $[^{14}C]GA_{53}$ to the *dwarf*-5 mutant; small- and large-scale feeds

Phase/fraction	Small-scale feed	Large-scale feed
Total feed	35051 Bq	50232 Bq
(wt)	(5.7 μg)	(10.2 μg)
Net feed	3.3×10^4 Bq	5.0×10^4 Bq
(wt)	(5.4 μg)	(10 1 μg)
Radioactivity (% net feed)		
Methanolic extract	80.9	80.0
Acidic fraction	21.1	17 7
Basic fraction	1 1	0.6
n-BuOH fraction	53.3	54.0
Hydrolysate acidic fraction	16.2	17.1
Hydrolysate water fraction	36.0	35 4
Water filtrate	1 0	5.4
Acidic water fraction	1.0	0.6
Combined washes	3.4	1.8
Combined distillates	0.0	0 0
Unaccounted	19.2	20.0

reconstructed TIC contained several other unidentified peaks.

The n-*butanol fraction* (1.7×10^4 Bq, 14.1 mg) was hydrolysed to give an ethyl acetate-soluble acidic fraction (5.3×10^3 Bq, 1.0 mg) which was separated into six zones of radioactivity by TLC. Zones 1–4 contained low levels of radioactivity and were discarded. Fractions 5 and 6 contained high levels of radioactivity and low mass and were derivatized for GC/MS. No GAs were identified in fraction 5 either by full mass scans or from SIM profiles. Fraction 6 contained several peaks, one of which, at R_t 23.1 min, gave a full mass scan, corresponding to that of GA_{53} MeTMSi. The mass spectrum contained an ion of $[M+8]^+$ at m/z 456 showing the presence of four ^{14}C-atoms.

Control for $[^{14}C]GA_{53}$ *feeds to* dwarf-5 *tassels*

To check the absence of non-enzymatic conversion of GA_{53}, $[17\text{-}^3H_2]GA_{53}$ was injected into three tassels of lengths 3.0, 4.0 and 4.0 cm. The tassels were immediately immersed in liquid nitrogen, then extracted and partitioned as for the feeds. The net feed was 1.6×10^4 Bq (1.9 μg) and the distribution of label was: 69% acidic fraction; 6% basic fraction; 3% *n*-butanol fraction; 0% water filtrate; 1% acidic water and 1% recombined water washes. The remaining 21% was unaccounted for. Only GA_{53} was identified as its MeTMSi derivative in the acidic fraction. No GAs were detected in any other fraction.

Large-scale feed of $[^{14}C]GA_{53}$ *to* dwarf-5

The net feed to 12 tassels was 5.0×10^6 Bq (10.1 μg). The distribution of label in the isolated fractions is shown in Table 2. Twenty per cent of the label was unaccounted for.

The acidic fraction (8.8×10^3 Bq, 21.5 mg) was separated into five radioactive fractions by TLC (Table 3). Fractions 1 and 2 were not examined further because of their high mass. Fractions 3–5 were derivatized and analysed by GC/MS.

Fraction 3 contained many peaks from which recognizable full mass scans were not obtained. The reconstructed TIC trace was therefore examined by mass

Table 3. Distribution of radioactivity from the acidic and hydrolysate acidic fractions from TLC of the feeds of $[^{14}C]GA_{53}$ to the *dwarf*-5 mutant

Fraction No.	Acidic fraction R_f range	Wt (mg)	Radioactivity (Bq)	% Net feed	Hydrolysate acidic fraction R_f range	Wt (mg)	Radioactivity (Bq)	% Net feed
Small-scale feed								
1	0.00–0.11	2.4	6.7×10^2	2.0	0.00–0.13	—	—	—
2	0.36–0.48	<1.6	7.2×10^2	2.2	0.23–0.33	—	—	—
3	0.48–0.59	<2.6	6.3×10^2	1.9	0.33–0.46	—	—	—
4	0.59–0.66	<4.6	1.2×10^3	3.6	0.46–0.60	—	—	—
5	0.66–0.72	<4.6	1.4×10^3	4.2	0.60–0.67	<0.5	6.7×10^2	2.0
6	0.72–0.79	<1.3	3.5×10^2	1.1	0.67–0.78	<0.5	3.0×10^3	9.1
Large-scale feed								
1	0.00–0.06	3.0	1.2×10^3	2.4	0.00–0.09	<0.5	9.2×10^2	1.8
2	0.22–0.29	2.5	8.2×10^2	1.6	0.09–0.17	<0.5	6.0×10^2	1.2
3	0.36–0.46	<0.5	1.3×10^3	2.6	0.17–0.28	<0.5	6.8×10^2	1.4
4	0.51–0.62	<0.5	2.3×10^3	4.6	0.43–0.53	<0.5	5.2×10^2	1.4
5	0.69–0.80	<0.5	6.3×10^2	1.3	0.68–0.78	<0.5	5.8×10^3	11.6

fragmentography. Three ions, characteristic of GA_{19}, at m/z 434, 238 and 208, were observed at R_t 23.7 min. Authentic GA_{19} MeTMSi showed the same ions at the same R_t. The ion at m/z 434 corresponds to $[M-28]^+$ (CO) and was accompanied by an ion at m/z 436 indicating the presence of ^{14}C-label.

Also in fraction 3, three ions characteristic of GA_{29} MeTMSi, at $[M]^+$ 506 and m/z 303 and 208, were detected at R_t 25.5 min, the R_t of authentic GA_{29} MeTMSi. The intensities of these ions were too low to detect isotopic ^{14}C-ions. However, it might be expected that the detected GA_{29} is a metabolite of the fed $[^{14}C]GA_{53}$ since GA_{29} was not detected in extracts of *dwarf*-5 tassels.

The reconstructed TIC trace from fraction 4 contained several peaks, two of which were identified as $[^{14}C]GA_{53}$ and $[^{14}C]GA_{44}$ by full mass scans at R_t 21.4 and 26.9 min, respectively (Table 4). The mass spectra showed $[M+2]^+$ ions at m/z 450 (2%) for $[^{14}C]GA_{53}$ MeTMSi and at m/z 436 (3%) for $[^{14}C]GA_{44}$ MeTMSi.

In fraction 5, no GAs were identified either by full mass scans or SIM.

The n-butanol fraction (2.7×10^4 Bq, 61.3 mg), on hydrolysis, gave an ethyl acetate-soluble fraction (8.3×10^3 Bq, 61.3 mg), which was separated into five radioactive fractions (Table 3). Fractions 1–4 contained high mass and were not examined further. Fraction 5 was derivatized and analysed by GC/MS. A full mass spec-

Table 4. GC/MS data obtained from the feeds of $[^{14}C]GA_{53}$ to the *dwarf*-5 mutant

Fraction	TLC (fraction)	Time of scan (min)	Peaks in mass spectrum m/z values (relative intensity)	Assignment
Small-scale feed				
Acidic	4	29.6	440 $[M+8]^+$ (9), 432 $[M]^+$ (61), 417 (12), 373 (22), 259 (5) 251 (9), 238 (47), 209 (42), 208 (47), 207 (100), 180 (14), 75 (34), 73 (100)	$[^{14}C]GA_{44}$
Acidic	5	24.7	456 $[M+8]^+$ (1), 448 $[M]^+$ (17), 419 (7), 416 (9), 389 (26), 251 (36), 241 (21), 235 (26), 209 (55), 208 (92), 207 (100), 193 (28), 181 (67), 75 (33), 73 (100)	$[^{14}C]GA_{53}$
Hydrolysate acidic	6	23.1	456 $[M+8]^+$ (30), 448 $[M]^+$ (100), 419 (1), 416 (3), 389 (25), 251 (9), 241 (9), 235 (13), 209 (22), 208 (18), 207 (47), 193 (12), 181 (11), 167 (1), 75 (26), 73 (100)	$[^{14}C]GA_{53}$
Large-scale feed				
Acidic	4	26.9	436 $[M+2]^+$ (3), 432 $[M]^+$ (8), 373 (3), 259 (100), 251 (1), 238 (15), 209 (15), 208 (17), 207 (38), 180 (5), 75 (100), 73 (100)	$[^{14}C]GA_{44}$
Acidic	4	21.3	450 $[M+2]^+$ (2), 448 $[M]^+$ (16), 419 (5), 416 (5), 389 (13), 251 (29), 241 (17), 209 (64), 208 (91), 207 (100), 193 (34), 181 (59), 73 (100)	$[^{14}C]GA_{53}$
Hydrolysate acidic	5	18.1	450 $[M+2]^+$ (15), 448 $[M]^+$ (53), 419 (1), 416 (1), 389 (15), 251 (3), 241 (1), 235 (13), 209 (28), 208 (24), 207 (59), 193 (13), 181 (16), 167 (8), 75 (30), 73 (100)	$[^{14}C]GA_{53}$

trum at R_t 18.1 min fitted that of the GA_{53} MeTMSi and showed the presence of ^{14}C-label by an $[M+2]^+$ ion at m/z 450 (15%).

Feeds of [17-^{13}C, 17-$^{3}H_2$]GA_{20} *to tassels of normal*, dwarf-1 *and* dwarf-5

In each case, ten tassels were injected with labelled GA_{20}. The amount fed and the distribution of radioactivity following extraction and fractionation are shown in Table 5. Only the acidic ethyl acetate fractions were investigated further.

The acidic ethyl acetate fractions obtained from each of the three feeds were purified further by reversed-phase HPLC [5]. After the adjacent radioactive fractions had been combined, the resulting pooled fractions were derivatized and analysed by GC/MS [5].

(a) *Normal tassels.* The total feed of [17-^{13}C, 17-$^{3}H_2$]GA_{20} was 9.7×10^4 Bq (7.6 μg) of which 5.0×10^4 Bq (51%) was recovered in the acidic ethyl acetate fraction. After reversed-phase HPLC, two fractions were analysed further by GC/MS. The combined fractions eluting from the column from 12–19 min contained 5.1×10^3 Bq. This fraction, after derivatization, was shown to contain [^{13}C]GA_{29} MeTMSi by full scan GC/MS at the correct KRI; the combined fractions eluting from the column over 19–28 min contained 2.2×10^4 Bq in which unmetabolized [^{13}C]GA_{20} MeTMSi was identified by full scan GC/MS at the correct KRI. In neither case was any dilution of the ^{13}C-label observed (Table 6).

(b) Dwarf-1 *tassels.* The total feed of [17-^{13}C, 17-$^{3}H_2$]GA_{20} was 6.35×10^4 Bq (5.0 μg) of which 2.84×10^4 Bq (45%) was recovered in the acidic ethyl acetate fraction. Reversed-phase HPLC again gave two combined fractions, which were derivatized and analysed by GC/MS. The fraction eluting from the column over 14–21 min contained 3.32×10^3 Bq, in which [^{13}C]GA_{29}, together with a trace of [^{13}C]GA_{20}, was identified by full scan GC/MS at the correct KRI. The fraction eluting from 21–28 min contained 1.14×10^4 Bq and unmetabolized [^{13}C]GA_{20}. Again ^{13}C-isotope dilution was not observed (Table 6).

(c) Dwarf-5 *tassels.* The total feed of [17-^{13}C, 17-$^{3}H_2$]GA_{20} was 5.93×10^4 Bq (6.7 μg) of which 4.5×10^4 Bq (75.8%) was recovered in the acidic ethyl acetate fraction. Following reversed-phase HPLC, the fraction eluting from the column over 9–14 min contained 2.24×10^3 Bq. This fraction, after derivatization, was shown to contain [^{13}C]GA_{29} MeTMSi by full scan GC/MS at the correct KRI. The fraction eluting over 14–19 min contained 1.86×10^3 Bq; however, no GAs were detected in this fraction by GC/MS. The fraction eluting over 19–24 min contained 1.3×10^4 Bq. This fraction, after derivatization, was shown to contain unmetabolized [^{13}C]GA_{20} MeTMSi, by GC/MS (see Table 7).

The reason for the inconsistency in the HPLC retention times (Table 7) of [^{13}C]GA_{20} and [^{13}C]GA_{29} in the three feeds is not known.

DISCUSSION

Gibberellins A_1, A_8 and A_{29} have now been identified in an extract from tassels of normal maize by full scan mass spectra of the MeTMSi derivatives with the same Kovats retention indices as those of authentic standards. By the same criteria, GA_{53}, GA_{44}, GA_{19}, GA_{17} and GA_{20} have also been identified from tassels of the normal phenotype [6]. Thus all eight GAs, from and including GA_{53}, shown in Fig. 2, for the proposed GA pathway in maize [3, 8] have been finally identified in tassels of normal maize (Table 1).

However, the levels of GA_1, GA_8, and GA_{29} in normal tassels are very low. Thus the presence of GA_{20} in extracts of *dwarf*-1 tassels, and the absence of GA_1, GA_8 and GA_{29} (Table 1), does not, *per se*, provide conclusive evidence that the *d1* block is between GA_{20} and GA_1 [3, 8].

Table 5. Partition of label from feeds of [17-^{13}C, 17-$^{3}H_2$]GA_{20} to tassels of normal, *dwarf*-1 and *dwarf*-5

Plant material	Radioactivity (10^{-4} × Bq)					
	Total fed	MeOH extract	Acidic EtOAc	Acidic *n*-BuOH	Residual aqueous	Neutral basic
Normal	9.7 (7.6 μg)	9.03 (93%)*	5.0 (51%)	4.05 (42%)	negligible (—)	0.04 (0.6%)
Dwarf-1	6.35 (5.0 μg)	5.76 (86%)	2.84 (45%)	2.41 (38%)	negligible (—)	lost
Dwarf-5	5.93 (6.7 μg)	5.82 (98%)	4.5 (75.8%)	0.99 (16.6%)	0.32 (5.5%)	lost

* % total fed is given in parentheses.

Table 6 Percentage ^{13}C in reoovered GA_{20} and metabolite GA_{29} from feeds to normal, *dwarf*-1 and *dwarf*-5, and fit factors

[^{13}C, ^{3}H]GA	Normal	Fit factor	Dwarf-1	Fit factor	Dwarf-5	Fit factor
GA_{20}	^{12}C 9.0 ^{13}C 91.0	0.95	^{12}C 10.8 ^{13}C 89.2	0.96	^{12}C 10.9 ^{13}C 89.1	0.95
GA_{29}	^{12}C 10.5 ^{13}C 89.5	0.93	^{12}C 4.7 ^{13}C 95.3	0.72	^{12}C 10.4 ^{13}C 89.6	0.95

Table 7. HPLC of acidic ethyl acetate fractions from feeds of [17-^{13}C, 17-$^{3}H_2$]GA_{20} to tassels of normal, *dwarf*-1 and *dwarf*-5

Material	R_t (min)	Radioactivity (10^{-4} × Bq)	Identified GA
Normal	12–19	0.51	[^{13}C]GA_{29}
	19–28	2.2	[^{13}C]GA_{20}
Dwarf-1	14–21	0.33	[^{13}C]GA_{29} + [^{13}C]GA_{20} (trace)
	21–28	1.14	[^{13}C]GA_{20}
Dwarf-5	9–14	0.22	[^{13}C]GA_{29}
	14–19	0.19	—
	19–24	1.3	[^{13}C]GA_{20}

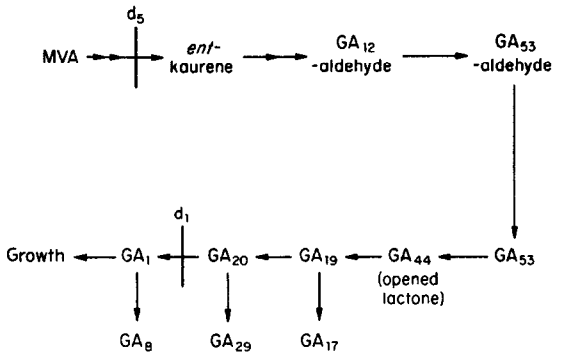

Fig. 2. Presumptive early 13-hydroxylation pathway for maize [8, 15].

The failure to detect GAs in extracts of *dwarf*-5 tassels is expected from previous evidence that the *d5* block is between CPP and *ent*-kaurene. However, low levels of GAs might not have been detected since only three tassels were extracted.

The identification of [^{14}C]GA_{44} from feeds of [^{14}C]GA_{53} to tassels of *dwarf*-5 by capillary GC/MS with a full scan spectrum of the MeTMSi derivative at the correct R_t, and of [^{14}C]GA_{19} by capillary GC/SIM, provides evidence for the two steps after GA_{53} in the proposed pathway shown in Fig. 2. The identification of GA_{29} as a metabolite of [^{14}C]GA_{53} is less secure since the intensity of the $[M]^+$ was too low to allow the detection of ^{14}C-isotope peaks. It seems likely that the detected GA_{29} was a metabolite of [^{14}C]GA_{53} since no GA_{29} was detected in a control experiment. However, the failure to detect [^{14}C]GA_{20} from [^{14}C]GA_{53} is surprising and leaves the issue unresolved.

In the feeds of [^{14}C]GA_{53} to *dwarf*-5 tassels, an appreciable proportion of the substrate was identified in the ethyl acetate-soluble acidic fraction of the hydrolysed *n*-butanol fraction. Conjugation of the fed [^{14}C]GA_{53} appeared, therefore, to be a major process in *dwarf*-5 tassels.

From the feeds of [17-^{13}C, 17-$^{3}H_2$]GA_{20} to tassels of *dwarf*-1, *dwarf*-5 and normal, the only metabolite identified in each case was [^{13}C]GA_{29}. The ^{13}C-content in the unmetabolized GA_{20}, and in the metabolite, GA_{29}, was the same as that in the fed [^{13}C]GA_{20} within experimental error, except for the [^{13}C]GA_{29} identified in tassels of *dwarf*-1. In this case, the ^{13}C-content was considerably higher than that of the fed [^{13}C]GA_{20} but the spectrum was weak and the fit factor was very low. These ^{13}C-contents in unmetabolized [^{13}C]GA_{20} and in [^{13}C]GA_{29} indicate that there was no detectable dilution of the ^{13}C-label by endogenous GA_{20} and GA_{29}. The levels of endogeneous GA_{20} and GA_{29} in the tassels must therefore be very much lower than the amount of [^{13}C]GA_{20} which was fed.

In the [^{13}C]GA_{20} feeds, the failure to detect metabolism to [^{13}C]GA_1 and/or [^{13}C]GA_8 was unexpected, particularly in the case of normal and *dwarf*-5 tassels. However, the endogenous levels of GA_1 and GA_8 are very low in tassels of normal plants. Thus, the conversion of exogenous [^{13}C]GA_{20} to [^{13}C]GA_1 may be too low for detection.

In summary, the results presented in this paper provide evidence for the conversion of GA_{53} to GA_{44} (open lactone) and GA_{19}; and for GA_{20} to GA_{29}, in tassels of maize. Direct evidence for the remaining steps in the proposed pathway, shown in Fig. 2, for tassels of maize must await further experimentation. In the meantime [5], from feeds of [17-^{13}C, 17-$^{3}H_2$]GA_{20} to seedlings of maize, it has been shown that GA_{20} is metabolized to GA_1 in normal and *dwarf*-5 seedlings, but not in *dwarf*-1 seedlings.

EXPERIMENTAL

Plant material. Seeds that would segregate 3 normal: 1 *dwarf* were soaked in H_2O for 24 hr before being planted in moist vermiculite in aluminium trays. After 7 days, the plants were transferred to pots (22 cm × 21 cm) containing a vermiculite–soil mixture (1:1) and grown in a greenhouse for 48 days. The tassels were removed from the plants by dissection under green light. Tassels ranging in length from 3.0 to 5.5 cm were selected for feeding studies. (The internodes of the tassels were non-elongated and meiosis had not yet occurred in the young anthers.)

Radiolabelled substrates. [17-^{13}C, 17-$^{3}H_2$]Gibberellin A_{20} (88% ^{13}C, 1.27 × 10^9 Bq/mmol) was synthesized as described by Ingram *et al.* [9]. [^{14}C]Gibberellin A_{53} was prepared by two separate procedures. In the first, the incubation of [2-^{14}C]MVA (78.2 mg; 2.1 × 10^9 Bq/mmol) with a cell-free system obtained from the endosperm of *Cucurbita maxima* L. (S_8, 15 ml), as described by Graebe *et al.* [10], gave [^{14}C]GA_{12}-aldehyde (1.03 × 10^6 Bq) and [^{14}C]GA_{12} (1.17 × 10^5 Bq). A mixture of the latter was incubated with a cell-free system derived from immature seed of *Pisum sativum* L. (S_2, 12 ml), as described by Ropers *et al.* [11], to yield [^{14}C]GA_{53} (1.08 mg; 4.44 × 10^9 Bq/mmol). Dilution with unlabelled GA_{53} gave a product with sp. act. of 2.5 × 10^9 Bq/mmol. The identity of the sample was confirmed by

GC/MS comparison of its MeTMSi derivative with that of authentic GA_{53}. The mass spectrum showed a prominent $[M+8]^+$ ion (*m/z* 456).

In the second preparation, [^{14}C]GA_{12}-aldehyde (23 μg; 1.48 × 10^9 Bq/mmol + 20 μg; 4.44 × 10^9 Bq/mmol) and [^{14}C]GA_{12} (3.96 μg; 4.44 × 10^9 Bq/mmol) were incubated with a cell-free system from immature seed of *P. sativum* [11]. The recovered [^{14}C]GA_{53} (13.7 μg; 1.67 × 10^9 Bq/mmol) was identified by comparison of the MeTMSi derivative with that of authentic GA_{53}. The mass spectrum of this product exhibited a strong $[M+2]^+$ ion (*m/z* 450).

[17-$^3H_2$]Gibberellin A_{53} was isolated from a feed of [17-$^3H_2$]steviol (7.5 mg; 2.1 × 10^9 Bq/mmol) to a resuspension culture (100 ml) of *Gibberella fujikuroi*, mutant B1-41a, as described by Bearder *et al.* [12, 13].

Feeds of radiolabelled substrates to young tassels. Feeds were performed by injecting into the base of each tassel with a soln of the radiolabelled substrate in MeOH. After injection, each tassel was transferred to a glass vial (2.5 × 10 cm) and incubated in the dark at 30° for 24 hr.

[^{14}C]Gibberellin A_{53} was fed to *dwarf*-5 tassels in two separate experiments. In a small-scale feed, three tassels (lengths 3.0, 4.0 and 4.0 cm) were each injected with [^{14}C]GA_{53} (see Table 2 for details). In a large-scale feed, 12 tassels were each injected with [^{14}C]GA_{53} (see Table 2 for details). Each tassel was injected with the radiolabelled substrate dissolved in MeOH (5 μl).

[17-^{13}C, 17-$^3H_2$]Gibberellin A_{20} was fed to normal, *dwarf*-1 and *dwarf*-5 tassels. In each case, a total of 10 tassels were injected with a soln of the labelled GA_{20} in MeOH (3 μl/tassel) (see Table 5 for details).

Extraction and fractionation of metabolites. After incubation, the tassels were removed from the vials, frozen in dry ice, and ground to a fine powder with a pestle and mortar. The resultant powder was extracted for *ca* 15 hr at 5° with MeOH–H_2O (4:1; 20 ml/tassel). The slurry was filtered and the residue was washed with MeOH–H_2O (4:1; 3 × 10 ml). The combined filtrate and washes were evapd under red. pres. at 12° to remove the MeOH. The aq. residue was partitioned using a modification of the procedure described by Rademacher [14].

The aq. residue was adjusted to pH 2.5 (1 M HCl) and extracted with EtOAc (4 × 1/4 vols.). The aq. residue was then extracted with *n*-BuOH (5 × 1/5 vols.) and the combined n-BuOH extracts were evapd at red. pres. to give the acidic *n*-BuOH fraction.

The combined EtOAc extracts were re-extracted with 0.1 M Pi buffer, pH 8.5 (3 × 1/2 vols.) and the buffer extracts combined. The EtOAc residue was washed with H_2O (5 × 1/20 vols.), the washes were discarded and the EtOAc was evapd at red. pres. to give the neutral/basic EtOAc fraction. The combined buffer extracts were adjusted to pH 2.5 (HCl) and extracted with EtOAc (4 × 1/4 vols.). The combined EtOAc extracts were washed with H_2O (pH 2.5; 5 × 1/20 vols.) and evapd at red. pres. and 19° to give the acidic EtOAc fraction. The residual H_2O phases were discarded.

Enzymatic hydrolysis. The *n*-BuOH fractions derived from the [^{14}C]GA_{53} feeds to *dwarf*-5 tassels were dissolved in 0.1 M Pi buffer, pH 4.5 (3.0 ml). Cellulase (type 1, practical grade, Sigma) was added (5.2 mg/mg sample). The mixture was readjusted to pH 4.5 and incubated at 35° for 24 hr. A hydrolysate acidic EtOAc fraction was obtained as described above.

TLC and GC/RC. The acidic EtOAc and the hydrolysate acidic EtOAc fractions obtained from the feeds of [^{14}C]GA_{53} to *dwarf*-5 tassels were purified on silica gel 60G in the solvent system $CHCl_3$–EtOAc–HOAc (40:60:1). Radioactive regions were detected by a TLC radioscanner and eluted. Prior to GC/RC, the eluted products were methylated with ethereal CH_2N_2. Samples were injected (300°) onto a column packed with Anakrom (100–110 mesh) coated with 3% OV-210. The column was maintained at 170° for 1 min, then programmed at 5°/min to 265°. The Ar flow rate was 67 ml/min and the column effluent was split 10:1 (10 parts to radiocounter, 1 part to FID at 270°). The radioactivity monitoring conditions (Analytical Biochemistry Labs. Series 7357 radiocounter) were: detector tube plateau voltage, 1850 V; Ar flow rate, 60 ml/min; H_2 flow rate, 2 ml/min; quench gas flow rate, 6.2 ml/min. The sensitivity was 300 cpm full scale deflection.

Reversed-phase HPLC. The acidic EtOAc fractions obtained from feeds of [17-^{13}C, 17-$^3H_2$]GA_{20} to normal, *dwarf*-5 and *dwarf*-1 tassels were dissolved in MeOH–H_2O (1:1; 2 ml) and passed through a Sep-Pak C_{18} cartridge. The cartridge was eluted with MeOH–H_2O (1:1; 5 ml). The combined eluates for each feed were dried, redissolved in MeOH–H_2O (1:9; 2 ml) and purified by reversed-phase HPLC on a μBondapak C_{18} column (7.8 × 300 mm) as described by Spray *et al.* [5].

Radioactive monitoring. At all stages of the extraction, fractionation and purification procedures, liquid scintillation counting was used to determine the radioactivity of each fraction.

GC/MS. (1) [^{14}C]Gibberellin A_{53} feeds to *dwarf*-5 tassels. Samples were methylated with ethereal CH_2N_2 and trimethylsilylated with BSTFA (90°, 30 min) and analysed using a Finnigan 4015 GC/MS data system. Samples from the small-scale feed were injected (260°) into a fused SiO_2 capillary column (3% OV-1) by the Grob splitless method. The column was maintained at 50° for 1 min, then programmed at 15°/min to 200° and at 4°/min to 260°. The He flow rate was 2 ml/min and the column effluent was led directly into the source (250°). The electron energy was 70 eV, the emission current 0.21 mA. Scanning was performed from 200°.

For the large-scale feeds, a 3% OV-101 packed column was used as described above, except that the temp. programme was from 190° to 260° at 4°/min. The source temp. was 290° and scanning was begun at 190°.

(2) [17-^{13}C, 17-$^3H_2$]Gibberellin A_{20} feeds to normal, *dwarf*-5 and *dwarf*-1 tassels. The experimental details were as described by Spray *et al.* [5].

(3) Re-examination of the hormones native to young normal tassels. The GC/MS details were as described by Spray *et al.* [5].

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