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Metabolism of Tritiated Gibberellin A_9 by Shoots of Dark-grown Dwarf Pea, cv. Meteor[1]

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ABSTRACT

Tritium-labeled gibberellin A_9 (^{3}H-GA_9) was metabolized by etiolated shoots of dwarf pea (*Pisum sativum* cv. Meteor) to GA_{20}, GA_{10}, 2,3-dihydro-GA_{31}, and a number of highly polar, acidic GA-like substances. Identifications were made by gas-liquid radiochromatography and combined gas chromatography-mass spectrometry. Kinetic studies showed that GA_{20} and 2,3-dihydro-GA_{31} were produced within 5 hours following ^{3}H-GA_9 application to pea shoots. The polar GA-like substances were produced between 5 and 10 hours after ^{3}H-GA_9 application. Levels of GA_{10} increased with time, and since no GA_{10} was produced during the purification procedures, GA_{10} was, in all probability, produced from ^{3}H-GA_9 within the plant tissue. The radioactive interconversion products produced by pea from ^{3}H-GA_9 have chromatographic properties similar to biologically active GA-like substances present in etiolated shoots of dwarf pea. Large scale applications of ^{3}H-GA_9 with very low specific activity to etiolated pea shoots showed that the radioactivity of the interconversion products was correlated exactly with biological activity as assayed by dwarf rice (*Oryza sativa* cv. Tan-ginbozu).

Dwarf peas contain two main fractions exhibiting gibberellin (GA)-like activity (15, 17) and, in seeds of the dwarf cultivar Progress No. 9, these two GAs have been identified as GA_{20} and GA_{29} (10). Jones (14) detected the presence of two more zones of GA-like activity in buffer extracts from a tall variety of pea, cv. Alaska. One zone appeared more polar than A_{20}, while the second was less polar than GA_{20}. Railton and Reid (unpublished data) have confirmed the findings of Jones (14) and furthermore have detected the presence of at least six zones of GA-like activity in extracts of chloroplasts and leaves of tall peas, cv. Alaska (25, 26). One of these zones has similar chromatographic properties to those of GA_9.

Studies have been carried out previously on the metabolism of radioactive GAs in dwarf pea (16, 21). ^{3}H-GA_1 was metabolized to a more polar compound, but its identity was not established (15). Similarly, ^{3}H-GA_5 was converted to a more polar compound with thin layer chromatographic properties similar to those of GA_1 (21). Re-examination of the above cited work using superior analytical techniques, has shown that radioactive GA_1 was metabolized by dwarf pea to GA_8 (Railton, Durley, and Pharis, unpublished data) and that radioactive GA_5 was converted to GA_3 and another chromatographically similar compound (7). The metabolism of ^{3}H-GA_{14}, a precursor of gibberellins produced by the fungus, *Gibberella fujikuroi*, has been studied in seedlings of dwarf pea, cv. Meteor (8). ^{3}H-GA_{14} was converted to GA_1, GA_8, GA_{18}, GA_{23}, GA_{28}, and GA_{38} and the biosynthetic sequence $GA_{14} \rightarrow GA_{18} \rightarrow GA_{38} \rightarrow GA_{23} \rightarrow GA_1 \rightarrow GA_8$ was strongly indicated.

We now report the metabolism of ^{3}H-GA_9 in shoots of etiolated seedlings of dwarf pea, cv. Meteor. Gibberellin biosynthesis is believed to proceed in the order of increasing hydroxylation (4, 11, 27) and GA_9, a nonhydroxylated GA could therefore serve as an efficient precursor to other GAs. This, in conjunction with the fact that a compound chromatographically similar to GA_9 occurs in shoots of etiolated dwarf pea seedlings (Railton, Durley, and Pharis, unpublished data), prompted such a study.

MATERIALS AND METHODS

Synthesis of ^{3}H-GA_9. Synthesis was carried out in a manner similar to that described by Cross *et al.* (4). GA_9 nor-ketone was converted to 17-^{3}H-GA_9 via a Wittig reaction using triphenylphosphine and ^{3}H-methyl iodide. The product was crystallized from acetone-light petroleum (b.p. 60–80 C) and stored at −20 C in absolute ethanol. 17-^{3}H-GA_9 had a specific activity of 20 mc/mmole.

Gas-Liquid Chromatography and Gas Liquid Radiochromatography. Preparation of methyl esters and trimethylsilyl ether derivatives of the methyl esters and conditions for GLC[4] (3) and GLRC (7, 8, 23) were similar to those described previously.

Combined Gas Chromatography-Mass Spectrometry. This was carried out with a Varian 1200 GLC connected by a double stage Biemann-Watson type molecular separator to a Varian Mat CH5 mass spectrometer. Columns for GLC were 1.8 m × 2 mm i.d. and contained 2% QF-1 on Gaschrom Q (80–100 mesh) at a temperature of 198 C using helium carrier gas at a flow rate of 18 ml/min.

HYDROGENATIONS

GA_5 Methyl Ester. Adams catalyst (5 mg) and glacial acetic acid (2 ml) were shaken under hydrogen for 30 min. GA_5

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[4] Abbreviations: GLC: gas liquid chromatography; GLRC: gas liquid radio-chromatography; TMSMe: trimethyl silyl ether derivatives of the methyl esters; GC-MS: combined gas chromatography-mass spectrometry.

methyl ester (3 mg), prepared by treating the free acid with ethereal diazomethane, was added, and the mixture was shaken under hydrogen until uptake of hydrogen ceased. The solution was filtered and evaporated, and the residue was crystallized from ether-light petroleum (b.p. 60–80 C) giving methyl tetrahydro-GA_5 as needles, m.p. 208–210 C, identical (m.p. and infrared spectrum) with authentic material (19). Methyl tetrahydro-GA_5 chromatographed as two distinct peaks (C-16 epimers) on GLC, but in a single peak as its TMSMe derivative on each of the three columns (see Table II). GC-MS of the tetrahydro-GA_5 TMSMe derivative gave a scan, $M^+ = 420$ (52%), with characteristic ions at m/e 405 (11), 399 (2), 377 (100), 361 (6), 333 (6), 331 (5), 318 (6), 303 (10), 209 (24), 145 (5), 143 (8), 105 (6), 91 (7), 75 (15), and 73 (26).

GA_{31} Methyl Ester. Adams catalyst (5 mg) and ethanol (2 ml) were shaken under hydrogen for 30 min. GA_{31} methyl ester, prepared by treating GA_{31} (30 μg) with ethereal diazomethane, was introduced, and the mixture was shaken under hydrogen for 40 min. The solution was filtered and evaporated, leaving tetrahydro-GA_{31} as a white powder. The TMSMe derivative of tetrahydro-GA_{31} chromatographed as two peaks (C-16 epimers) on GLC using the QF-1 and XE-60 columns and as a single peak using the SE-30 column (Table II). The shorter retention time peak (on all columns) was about 10 times the area of the other. GC-MS of the larger peak gave a scan, $M^+ = 420$ (24%) with characteristic ions at m/e 405 (25), 389 (12), 388 (12), 373 (87), 360 (9), 345 (42), 330 (32), 313 (45), 298 (40), 286 (56), 285 (100), 270 (49), 243 (54), 225 (63), 183 (65), 93 (46), 75 (71), and 73 (off scale).

Growth and Treatment of Plants. Seeds of dwarf pea (*Pisum sativum cv.* Meteor) were soaked in running tap water for 20 hr, planted in moist vermiculite, and kept in darkness. Five days after sowing, before elongation of the second internode, ^{3}H-GA_9 was applied in 5-μl droplets of ethanol to the plumular hook. In most experiments, 206 μg (13.5 μc) were applied to a total of 40 seedlings.

In preliminary experiments, seedlings were harvested 20 hr after ^{3}H-GA_9 application. In later experiments where the kinetics of interconversion were followed, harvests were made 5, 10, 20, 30, and 44 hr after application of ^{3}H-GA_9.

Extraction and Purification. After separating the shoots from seeds and roots, the shoots were surface washed by brief agitation in absolute methanol. They were then ground in a precooled mortar with acid-washed sand and ice-cold 80% methanol. After filtering, the methanol was removed *in vacuo,* and an equal volume of 0.5 M phosphate buffer, pH 9.0, was added to the residual aqueous phase. This was partitioned (6X) against equal volumes of diethyl ether and then against ethyl acetate (6X) at pH 3.0. Finally, the aqueous phase was partitioned against 1-butanol (4X) at pH 3.0. The ethyl acetate-soluble fraction was reduced *in vacuo* and subjected to TLC on Silica Gel H (Merck) irrigated with ethyl acetate-chloroform-formic acid (50:50:1, v/v, solvent 1). Radioactive zones were eluted with water-saturated ethyl acetate and analyzed by GLRC. Very polar radioactive zones were further eluted with absolute methanol. Further purification and separation of polar compounds was carried out on TLC with Silica Gel H and chloroform-methanol-acetic acid-water (40:12:2:2, v/v, solvent 2) as developing solvent.

Liquid scintillation spectrometry was carried out using a Packard Tri-Carb scintillation spectrometer and a modified Bray's solution (2). Quenching and counting efficiencies were determined using internal standards.

Sample Preparation for GC-MS. ^{3}H-GA_9 (4.1 μc) was added to GA_9 (2 mg) in ethanol (2 ml), and the solution was applied to a total of 400 etiolated dwarf pea seedlings in the same manner as described above. Extraction procedures were also identical to those described above. The acidic, ethyl acetate-soluble fraction, in the form of a brown gum, was dissolved in a minimal volume of 0.1 M phosphate buffer, pH 8.0, and purified further on a column of poly-N-vinylpyrrolidone (12). Extracts were then separated on TLC using ethyl acetate-chloroform-formic acid (50:50:1, v/v), and radioactive zones were eluted as described above. Estimates of amounts of GAs present in these zones were determined by bioassay. Prior to GC-MS, individual zones of activity were purified further on a silica gel partition column (6, 22), using a modified gradient, chambers 1 to 4 containing respectively, ethyl acetate-hexane, 35:65 (137.5 ml), 50:50 (131.3 ml), 65:35 (125.2 ml), and 100:0 (111 ml).

Bioassays. Dwarf rice (*Oryza sativa* cv. Tan-ginbozu) was the assay material throughout (5, 20).

RESULTS

When ^{3}H-GA_9 was applied to shoots of etiolated seedlings of dwarf pea and the acidic, ethyl acetate-soluble fraction chromatographed on TLC (ethyl acetate-chloroform formic acid [50:50:1, v/v] solvent 1) four main zones of radioactivity were found. Approximately equal numbers of counts were observed in zone 1 (R_F 0.0–0.2) and zone 3 (R_F 0.5–0.7). Significantly less radioactivity was associated with zone 2 (R_F 0.2–0.4). Zone 4 (R_F 0.8–0.9) contained high levels of radioactivity and co-chromatographed with ^{3}H-GA_9.

The butanol fraction contained relatively high levels of radioactivity, suggesting interconversion of ^{3}H-GA_9 to very polar compounds such as GA-glycosides. The distribution of radioactivity between the extraction solvents was: diethyl ether, 1.2×10^5 dpm; ethyl acetate, 57.6×10^5 dpm; butanol, 13.6×10^5 dpm; and residual buffer, 18.4×10^5 dpm.

Each zone of radioactivity associated with the acidic, ethyl acetate fraction was analyzed further by GLRC. Following elution from TLC with water-saturated ethyl acetate, the radioactive zones were reduced to dryness, converted to the TMSMe derivatives, and analyzed by GLRC on three separate liquid stationary phases, 2% QF-1, 2% SE-30, and 1% XE-60 on Gaschrom Q. The retention times of the compounds in the metabolite zones are depicted in Table I. Zone 1 was associated with high levels of impurities which made preliminary analysis by GLRC impossible. Zone 2 contained two compounds, one with identical retention times to those of the TMSMe derivative of GA_{10}, and the second, with similar retention times to those of the TMSMe derivative of GA_{29}, although the low levels of radioactivity associated with the latter compound precluded definite identification. Zone 3 separated into two radioactive compounds on GLRC. One compound had identical retention times to those of the TMSMe derivative of GA_{20} and the other similar, though not identical, retention times to those of the TMSMe derivative of GA_{31}. The close chromatographic similarity of the radioactive metabolite to derivatized GA_{31} on 2% SE-30 and its shorter retention time on 1% XE-60 suggested a compound structurally similar to GA_{31} but lacking one double bond. Zone 4 contained only GA_9.

In order to test the possibility that the GA_{31}-like metabolite present in zone 3 might be a dihydro derivative of GA_{31}, a portion of the residue from zone 3 was hydrogenated over Adams catalyst as described under "Materials and Methods." Samples of authentic GA_{31} and GA_5 were also hydrogenated in an identical manner. The retention times of the TMSMe derivatives of the hydrogenation products were compared on GLRC (Table II). Tetrahydro-GA_5 had identical retention times to those of the hydrogenated GA_{20} metabolite, further

Table I. *GLRC Retention Times of TMSMe Derivatives of Standard GAs and of Eluates from TLC Zones 2, 3, and 4*

Extracts were separated on Silica Gel H using ethyl acetate-chloroform-formic acid (50:50:1, v/v) as developing solvent.

	R_F	Retention Time			Identity (incorporation)[1]
		2% QF-1 (206 C)	2% SE-30 (203 C)	1% XE-60 (209 C)	
		min			
TLC Zone					
2	0.2–0.4	14.1	11.4	16.7	GA_{10} (0.4%)
			15.9	15.9	GA_{29}?
3	0.5–0.7	9.6	8.6	12.0	GA_{20} (3.6%)
		12.9	10.4	16.6	2,3-dihydro-GA_{31} (5.6%)
4	0.7–0.9	6.9	4.9	9.6	
GA standards					
A_1		14.0	15.3	15.3	
A_4		10.0	9.1	11.7	
A_9		6.9	5.0	9.6	
A_{10}		14.2	11.4	16.8	
A_{20}		9.6	8.5	11.9	
A_{29}		14.2	15.9	15.9	
A_{31}		14.5	10.8	18.7	

[1] Incorporation measured by comparison of estimation of dpm in peak and dpm of [^{3}H]-GA_9 applied to seedlings.

Table II. *GLRC Retention Times of TMSMe Derivatives of Standard GAs and of Zone 3 (R_F 0.5–0.7) and Hydrogenated TLC Zone 3*

	Retention time		
	2% QF-1 (194 C)	2% SE-30 (194 C)	1% XE-60 (209 C)
	min		
Fraction			
Hydrogenated	14.6	12.1	12.4
	19.0	13.4	15.7
	21.9		18.5
Nonhydrogenated	13.6	11.7	12.2
	18.1	14.6	16.6
GA standards			
GA_{20}	13.6	11.6	12.2
GA_{31}	20.1	14.5	18.5
Tetrahydro-GA_{31}	18.9	13.4	15.7
	21.8		18.5
Tetrahydro-GA_5	14.5	12.1	12.3

confirming GA_{20} as a metabolite of ^{3}H-GA_9. Tetrahydro-GA_{31} had identical retention times to those of the hydrogenated GA_{31}-like metabolite from zone 3. The metabolite was thus identified as dihydro-GA_{31}. The indicated metabolism of ^{3}H-GA_9 applied to etiolated shoots of dwarf pea is summarized in Figure 1.

In order to obtain further evidence of the identity of the metabolites of ^{3}H-GA_9 from TLC zones 2 and 3 by GC-MS, and to determine the biological activity of these metabolites, the above experiment was carried out on a larger scale (see "Materials and Methods"). The correlation of radioactivity from TLC and biological activity are shown in Figure 2. Four zones of radioactivity were present, corresponding to zone 1, zone 2 containing GA_{10} and the GA_{20}-like metabolite,

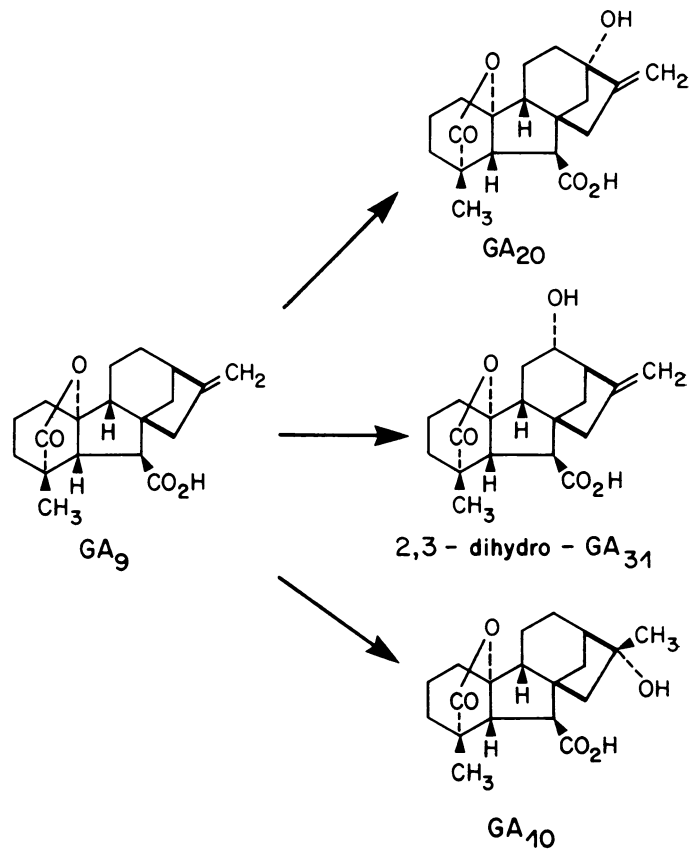

FIG. 1. Metabolism of ^{3}H-GA_9 applied to etiolated shoots of dwarf pea.

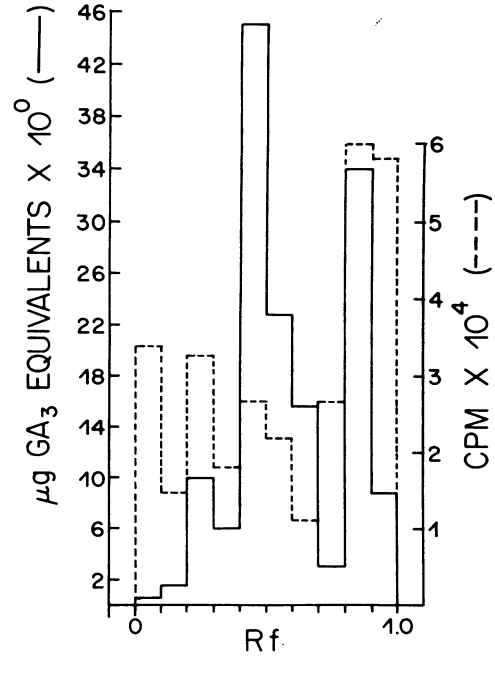

FIG. 2. Distribution of radioactivity (cpm) and biological activity (dwarf rice micro-drop assay) on TLC (ethyl acetate-chloroform-formic acid, 50:50:1, v/v) from extracts of etiolated shoot of dwarf pea fed with 2 mg of cold GA_9 and 4.1 μc ^{3}H-GA_9. Tissue was extracted 24 hours following GA_9 application.

zone 3 containing GA_{20} and dihydro GA_{31}, and zone 4 containing precursor GA_9. Significant biological activity was associated with all zones except zone 1. The lack of biological activity in zone 1 may be the result of impurities which were markedly inhibitory to GA-induced rice leaf sheath growth. Further purification of zone 1 on columns of charcoal-celite has shown that it does indeed exhibit a low level of biological activity (Railton, Durley, and Pharis, unpublished data).

The remainder of the residues from TLC zones 2, 3, and 4 from the large scale experiment were purified further on a silica gel partition column (6, 22) using a modified gradient (see "Materials and Methods") prior to GC-MS. The residue from TLC zone 2 was eluted as two peaks of radioactivity from the silica gel column. The first was derivatized and examined by GC-MS. A scan of the major peak, which corresponded to the TMSMe derivative of GA_{10} (by GLRC), gave a mass spectrum identical with that of the published spectrum of the TMSMe derivative of GA_{10} (1). The second peak contained insufficient GA to obtain an identifiable mass spectrum. TLC zone 3 eluted from the silica gel column in fractions 10 to 13 and these were combined and reduced to dryness *in vacuo*. Half of the sample was converted to the TMSMe derivative, and the other half was esterified with ethereal diazomethane, hydrogenated over Adams catalyst (see "Materials and Methods"), and subsequently converted to the TMSMe derivatives. The nonhydrogenated sample was examined by GC-MS and scans were obtained of the two peaks corresponding to dihydro-GA_{31} and GA_{20} observed during the earlier GLRC. The first scan gave a mass spectrum identical with that published for the TMSMe derivative of GA_{20} (1). The second scan gave the mass spectrum shown in Figure 3. The hydrogenated sample was examined by GC-MS in an identical fashion. Scans were obtained corresponding to the two major radioactive peaks observed during earlier GLRC of the hydrogenated radioactive sample. The first scan gave a mass spectrum identical with that of the TMSMe derivative of tetrahydro-GA_5. The second scan gave a mass spectrum identical to that of the TMSMe derivative of the major epimer of the tetrahydro-GA_{31}. TLC zone 4 contained a single compound which when methylated and examined by GC-MS gave a mass spectrum identical to that of the methyl ester of GA_9 (1).

The new metabolite in TLC zone 3 was therefore identified as dihydro-GA_{31}. From the data obtained here, we could not fully ascertain whether the metabolite was 2,3-dihydro-GA_{31} or 16,17-dihydro-GA_{31} (see Figs. 1 and 4 for structures). However, circumstantial evidence favors 2,3-dihydro-GA_{31}, since the metabolite was produced from GA_9 in a similar yield to that of GA_{20}, both conversions requiring a one step hydroxylation. Synthesis of 16,17-dihydro-GA_{31} from GA_9 would require several steps, and if produced, would be expected to be in lower yield. The mass spectral fragmentation pattern of the TMSMe derivative of the metabolite (Fig. 3) was similar to that of the TMSMe derivative of GA_{31}, many of the major ions of the former being 2 atomic mass units larger than those of the latter.

Attempts were made to further purify the polar metabolite in zone 1, and these attempts have only been partially successful. The marked polarity of the metabolite suggested that it could be the glucoside of a weakly polar gibberellin which would partition into ethyl acetate in small amounts. The glucoside of GA_4, for example, partially migrates into ethyl acetate at pH 3.0 (G. Sembdner, personal communication). However, partition studies suggested that the polar metabolite was a free acid, since it was almost wholly removed from 0.5 M phosphate buffer at pH 3.0 by ethyl acetate and could be reextracted again with 0.5 M phosphate buffer at pH 9.0. The

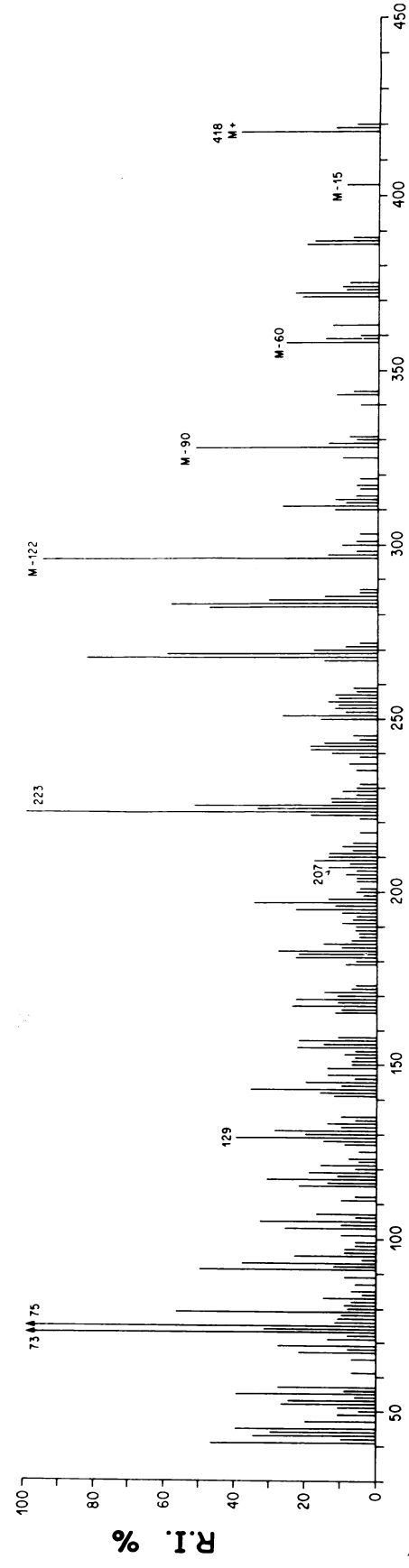

FIG. 3. Mass spectrum of the TMSMe derivative of 2,3-dihydro-GA_{31} isolated from etiolated shoots of dwarf pea seedlings fed with GA_9.

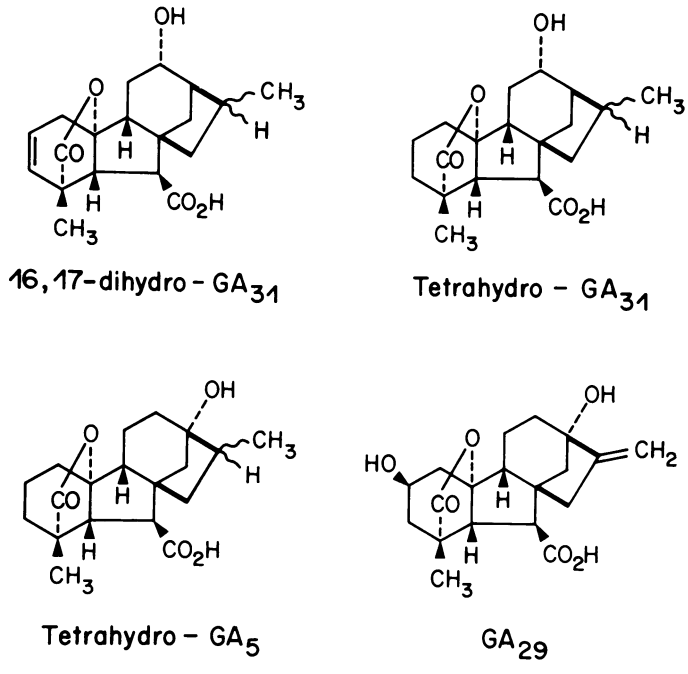

FIG. 4. Structures of gibberellins.

Table III. *Kinetics of the Metabolism of ^{3}H-GA$_9$ by Etiolated Shoots of Dwarf Pea Seedlings*

Radioactivity associated with the various fractions and acidic metabolites of ^{3}H-GA$_9$ at various times (5, 10, 20, 30, 44 hr) following ^{3}H-GA$_9$ application. The 153 × 10^6 dpm originally were applied to a total of 200 etiolated dwarf pea seedlings. Forty seedlings were extracted at each harvest time.

Time	Diethyl Ether Fraction	Ethyl Acetate Fraction	Radioactivity in Main Acidic Metabolites		1-Butyl alcohol Fraction	Residual Buffer Fraction
			GA$_{20}$	Dihydro-GA$_{31}$		
hr	*dpm* × 10^{-5}					
5	7.2	46.2	0.5	5	0.4	20.4
10	1.6	46.9	3.5	7.8	3.2	0.8
20	1.2	57.6	11.0	17.5	13.6	18.4
30	1.6	45.6	9.1	11.0	9.5	49.7
44	2.8	41.9	9.8	8.0	10.4	13.6

compound migrated as a single zone of radioactivity when chromatographed on TLC using solvent 2 and was more polar than GA$_8$ but less polar than GA$_8$-glucoside (Fig. 5A). When the metabolite was treated with ethereal diazomethane and rechromatographed in the same solvent system it separated into three main zones of radioactivity (Fig. 5B) suggesting the presence of free carboxyl groups and indicating that the zone was composed of at least three separate radioactive compounds.

In order to obtain information about the sequence of interconversions of ^{3}H-GA$_9$ into the various metabolites, kinetic studies were carried out. After applying ^{3}H-GA$_9$ to etiolated pea shoots, harvests were made 5, 10, 20, 30, and 44 hr later. The distribution of radioactivity in the various fractions extracted from the shoot is given in Table III. The acidic, ethyl acetate-soluble fractions were chromatographed on TLC as before using solvent 1, and each R_F zone was eluted, derivatized, and examined by GLRC. The distribution of radioactivity on TLC plates at each harvest is shown in Figure 6, and the

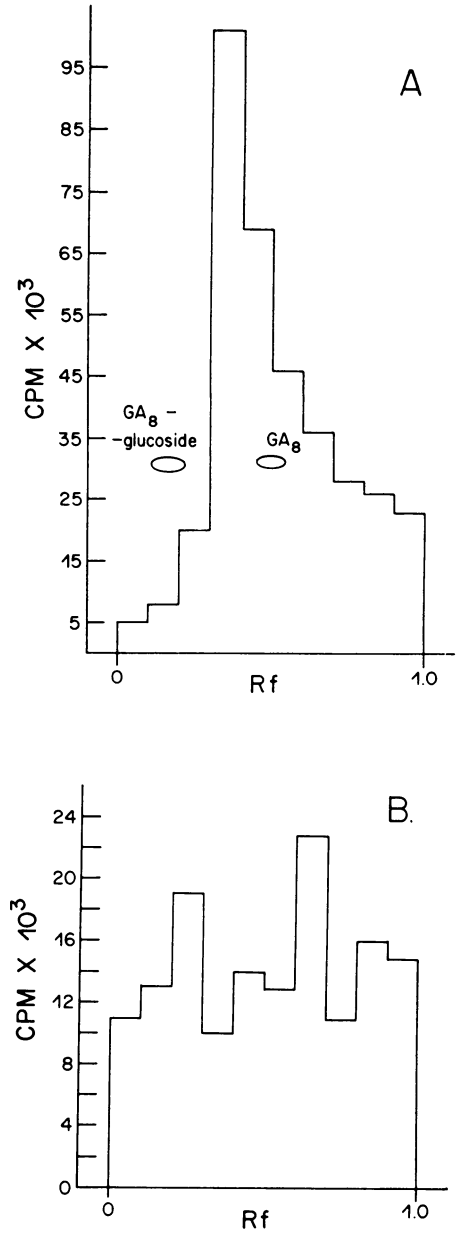

FIG. 5. Distribution of radioactivity on TLC (chloroform-methanol-acetic acid-water; 40:12:2:2, v/v) of zone 1 from extracts of etiolated dwarf pea seedlings treated with ^{3}H-GA$_9$. A: Free acid; B: methyl ester.

radioactivity associated with each metabolite is shown in Table III.

Five hours after ^{3}H-GA$_9$ application, both GA$_{20}$ and 2,3-dihydro-GA$_{31}$ had been produced in significant amounts but the levels of radioactivity associated with GA$_{10}$ and the polar metabolite zone were of a lower order (Fig. 6a). Approximately 10 times more dihydro-GA$_{31}$ than GA$_{20}$ had been produced from ^{3}H-GA$_9$ within 5 hr (Table III). Within the next 5 hr, the levels of radioactivity associated with the polar metabolite zone had increased dramatically as had the levels of both dihydro GA$_{31}$ and GA$_{20}$. Smaller increases were observed in the levels of GA$_{10}$ (Fig. 6b). The low levels of radioactivity associated with the butanol fraction at the 10-hr harvest (Table III) relative to those of the polar metabolite zone further suggested that this zone was not glycosidic in nature.

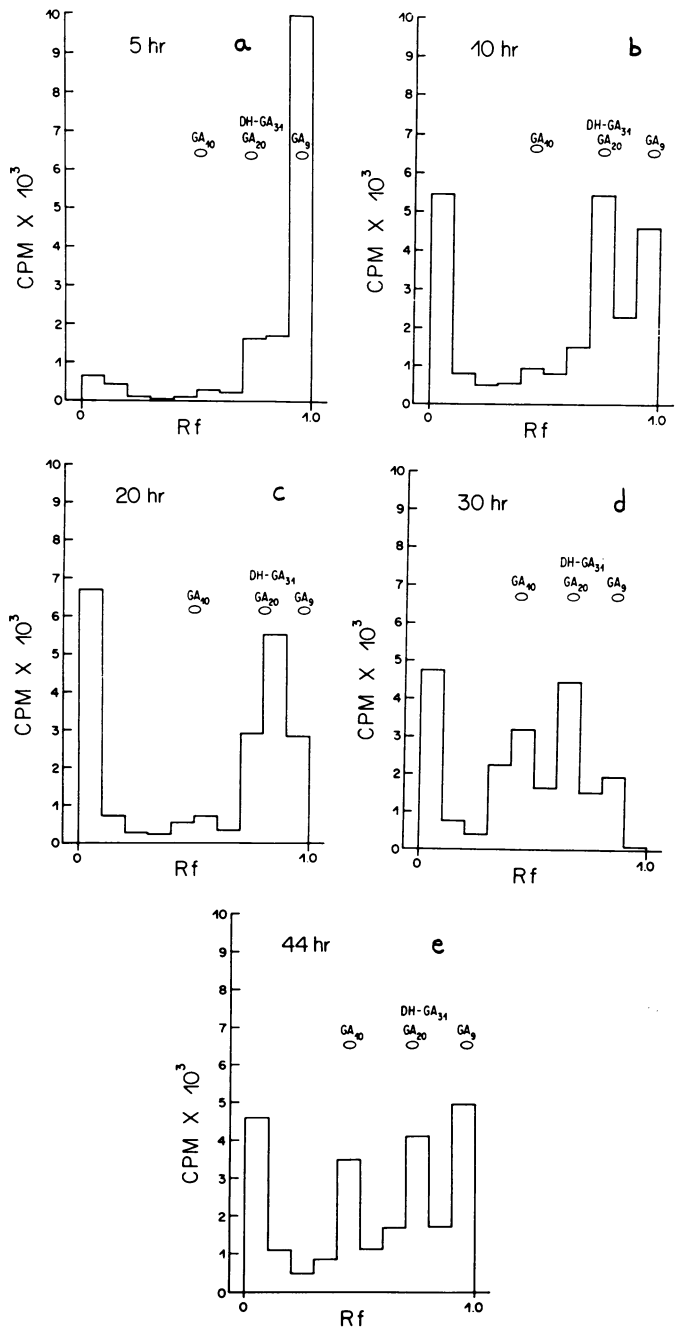

FIG. 6. Distribution of radioactivity (cpm) on TLC (ethyl acetate-chloroform-formic acid; 50:50:1, v/v) in extracts of etiolated shoots of dwarf pea at various times (5, 10, 20, 30, 44 hr.) following ^{3}H-GA_9 application. The 30.6 × 10^6 dpm (206 μg) were applied to five batches of 40 seedlings. One-fiftieth of each extract was applied per TLC run.

Twenty hours after ^{3}H-GA_9 application, the levels of the polar metabolite zone, 2,3-dihydro-GA_{31} and GA_{20} were maximal (Table III). Radioactivity associated with the butanol fraction had also reached a maximum by 20 hr and thereafter decreased to a steady level at the 30- and 44-hr harvests. Levels of radioactivity in the residual aqueous phases inexplicably varied widely from harvest to harvest.

The data suggested that ^{3}H-GA_9 was simultaneously hydroxylated at C-12 and C-13 to give 2,3-dihydro GA_{31} and GA_{20}, respectively. The rapid rise in levels of the polar metabolite zone between 5 and 10 hr following ^{3}H-GA_9 application suggests that this zone was produced from both 2,3-dihydro-GA_{31} and GA_{20}, or directly from GA_9. Levels of GA_{10} increased slowly with time. GA_9 is known to be converted into GA_{10} under conditions of mild acidity (13) and whether GA_{10} was produced enzymatically from GA_9 is not known. It is possible that any GA_9 migrating into the plant cell vacuole could be nonenzymatically converted to GA_{10} under conditions of mild acidity.

In order to investigate further the sequence of interconversions, ^{3}H-GA_{20} was synthesized by a novel method (N. Murofushi, Durley, and Pharis, unpublished data) and applied to etiolated seedlings of dwarf peas. ^{3}H-GA_{20} was converted into a single zone of radioactivity on TLC with identical chromatographic properties to those of metabolite zone 2 produced from ^{3}H-GA_9. GLRC analysis indicated a single radioactive compound with identical retention times to those of GA_{29} and the unequivocal identity of this compound has been achieved by GC-MS (24). The presence of GA_{20} was indicated in preliminary analyses of the metabolites derived from ^{3}H-GA_9 but was present in very small amounts. GA_9 is therefore converted by etiolated shoots of dwarf pea into 2,3-dihydro-GA_{31} and GA_{20}. GA_{20} is further metabolized to GA_{29}. Both 2,3-dihydro-GA_{31} or GA_9 or both could therefore be directly converted to the polar metabolites of TLC zone 1.

GA_{20} and GA_{29} are endogenous to seeds of a dwarf cultivar of pea (10), and GA_{20} is endogenous to pods and seeds of tall cultivars of pea (9, 18). The fact that GA_{20} was produced from ^{3}H-GA_9 in shoots of dwarf Meteor pea seedlings strongly suggests that GA_9 or a closely related GA is the precursor of GA_{20}. GA_{20} was further converted into GA_{29} in etiolated shoots of pea (24) and hence GA_{29} may also occur naturally in shoots of dwarf peas. Extracts of etiolated shoots of dwarf pea have been shown to contain at least four biologically active GA-like substances with chromatographic properties identical to those of the three metabolite zones produced from ^{3}H-GA_9 and a compound similar to GA_9 (Railton, Durley, and Pharis, unpublished data). The present results suggest that these endogenous GA-like substances are part of metabolic sequence with GA_9 or a closely related GA serving as a precursor.

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