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Metabolism of ^{3}H-Gibberellin A_1 and ^{3}H-Gibberellin A_4 by *Phaseolus coccineus* Seedlings

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ABSTRACT

[^{3}H]-Gibberellin A_1 (GA_1) and ^{3}H-GA_4 were applied separately to *Phaseolus coccineus* seedlings grown under red light. ^{3}H-GA_1 was converted to a compound with gas-liquid radiochromatography retention times identical to those of GA_8. ^{3}H-GA_4 underwent conversion to at least three metabolites, none of which corresponded to GA_{1-38}. The rate of metabolism of ^{3}H-GA_4 was significantly higher than that of ^{3}H-GA_1.

Seedlings of the 'Scarlet Runner' bean (*Phaseolus coccineus*) contain a number of gibberellin-like compounds, four of which have been characterized by combined gas chromatography-mass spectrometry as GA_1, GA_4, GA_5, and GA_{20} (Fig. 1) (1). On structural grounds GA_4, GA_5, and GA_{20} could all be immediate precursors of GA_1. In order to obtain information on endogenous GA synthesis pathways in *Phaseolus*, a preliminary investigation has been made of the metabolism of ^{3}H-GA_1 and ^{3}H-GA_4 in seedlings grown under red light.

MATERIALS AND METHODS

Seedlings of *Phaseolus coccineus* cv. Prizewinner were grown in water culture (4) under red light (1.1 μw cm^{-1} at 660 nm). 1,2-^{3}H-GA_1 (specific radioactivity 420 mCi mM^{-1}) was prepared by selective hydrogenation of GA_3 (10, 14). 1,2-^{3}H-GA_4 (specific radioactivity 1.87 Ci mM^{-1}) was prepared by selective hydrogenation of GA_7 (5). Fifty μCi of each GA, dissolved in 50% aqueous ethanol, were applied to the apical buds of 40 6-day-old seedlings. After 24 hr the seedlings were macerated in methanol, and the acidic ethyl acetate-soluble fraction was obtained (4). The acidic butanol-soluble fraction was not examined. In addition, in the absence of plant material, ^{3}H-GA_1 (8 μCi) and ^{3}H-GA_4 (10 μCi) blanks were similarly processed. The acidic ethyl acetate-soluble fractions were purified by G-10 Sephadex (3) and charcoal-celite column chromatography (1) prior to high efficiency liquid-liquid column chromatography (Reeve and Crozier, in preparation). The column consisted of a silicic acid support with a 0.5 M formic acid stationary phase and was developed with a gradient of increasing amounts of ethyl acetate in hexane. Approximately 50 successive fractions were collected, and 1/100 aliquots were assayed for radioactivity in a Packard Tricarb liquid scintillation spectrometer. The residual portions of the

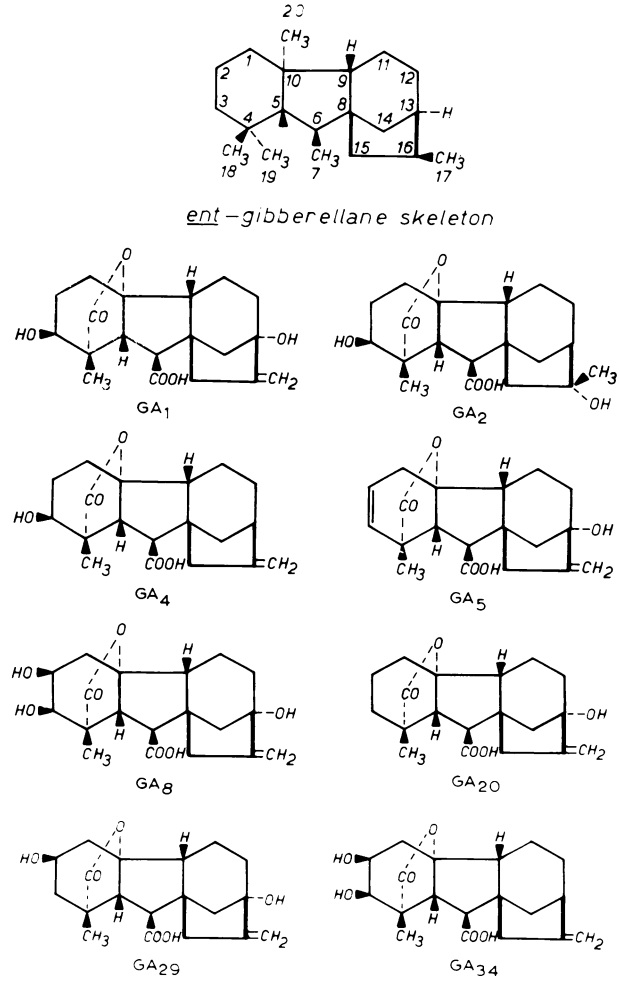

FIG. 1. Structures of the *ent*-gibberellane skeleton, GA_1, GA_2, GA_4, GA_5, GA_8, GA_{20}, GA_{23}, and GA_{34}.

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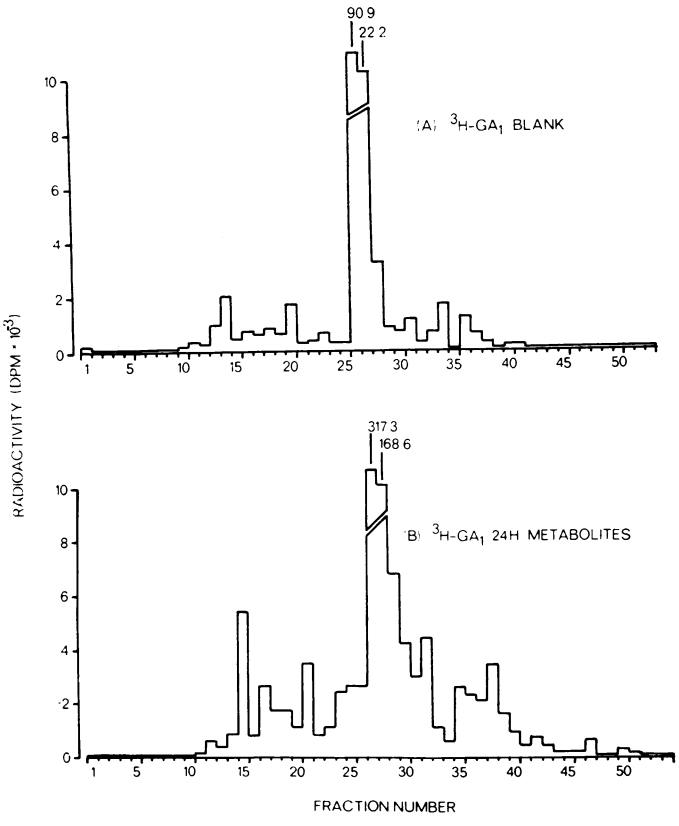

FIG. 2. Distribution of radioactivity in a ^{3}H-GA$_1$ blank and ^{3}H-GA$_1$ 24-hr-metabolites extract following LC. 1/100 aliquots.

LC[4] fractions that contained radioactivity were converted to the trimethylsilyl ether derivatives of the methyl esters (2) and examined by gas-liquid radiochromatography on SE-30, QF-1, and XE-60 columns (5, 6).

RESULTS

Metabolism of ^{3}H-GA$_1$. The distribution of radioactivity in the ^{3}H-GA$_1$ blank indicates the levels of impurities formed by self radiolysis and/or the chromatographic and purification procedures (Fig. 2A). The GA$_1$ located in fractions 26 to 27 represents 63% of the original radioactivity. The GA$_1$ in the 24-hr metabolites extract was eluted in LC fractions 27 to 29 (Fig. 2B). Several other peaks of radioactivity were present but GLRC indicated that the only one that was a genuine metabolite was in fractions 35 to 37. The GLRC retention times of the major compound in these fractions corresponded with those of GA$_8$ (Fig. 1) on all three columns (Table I). Forty-six per cent of the radioactivity applied to the seedlings was recovered as ^{3}H-GA$_1$. The radioactivity in fractions 35 to 37 represented 0.6% of the original dose. However, GLRC demonstrated that only about one-third of this was actually associated with GA$_8$ (Table I).

Metabolism of ^{3}H-GA$_4$. The distribution of radioactivity in the ^{3}H-GA$_4$ blank indicates a recovery of 64% of the original radioactivity in the form of GA$_4$ (Fig. 3A). The activity in LC fractions 8 to 9 of the 24-hr metabolites extract represents an 18% recovery of GA$_4$ (Fig. 3B). Two major peaks of radioactivity were eluted in LC fractions 14 to 17 and 19 to 22.

[4] Abbreviations: LC: liquid-liquid column chromatography; GLRC: gas-liquid radiochromatography; TMSMe: trimethylsilyl ether derivative of methyl esters.

When the fractions 14 to 17 were combined and examined by GLRC they were found to contain two metabolites representing 0.6 and 1.9% of the applied label. Neither compound had GLRC retention times corresponding with those of GA$_{1-38}$ (Table II). The radioactivity in LC fractions 19 to 22 was equivalent to 0.9% of the original application. GLRC showed a single compound with identical retention times to those of GA$_2$ (Table II, Fig. 1). However, this metabolite is not GA$_2$, since GA$_2$ has a different retention volume on the LC column and is eluted in fractions 26 to 29. A small quantity of a compound with GA$_2$-like GLRC retention indices was, in fact, detected in these fractions in both the blank and metabolite extracts. Presumably, this compound is GA$_2$ and is an artifact generated

Table I. *GLRC Retention Times of TMSMe Derivatives of LC Fractions from ^{3}H-GA$_1$ Extract, with Comparison Standards*[1]

Comparison was made between the radioactive peaks of derivatized metabolites and flame ionization detected peaks of appropriate coinjected derivatized standards.

	Retention Time on 3 columns			Radioactivity of Peak	Accumulation of Applied Radioactivity
	2% QF1	2% SE30	1% XE60		
	min			*dpm* × 10^{-6}	%
LC fractions					
27–29	14.0	15.4	15.2	47.0	
35–37	17.5	25.4	17.6	0.27	0.24
Standard GAs					
A$_1$	14.0	15.3	15.3		
A$_5$	16.4	16.8	18.5		
A$_8$	17.4	25.4	17.5		

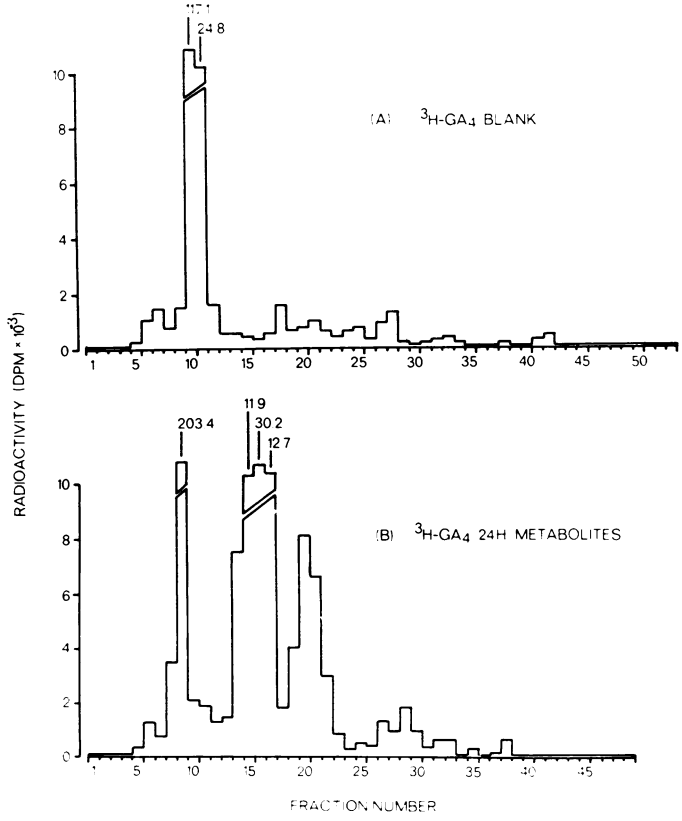

FIG. 3. Distribution of radioactivity in a ^{3}H-GA$_4$ blank and ^{3}H-GA$_4$ 24-hr metabolites extract following LC. 1/100 aliquots.

Table II. *GLRC Retention Times of TMSMe Derivatives of LC Fractions from ^{3}H-GA$_4$ Extract, with Comparison Standards*

Comparison was made between the radioactive peaks of the derivatized metabolites and flame ionization detected peaks of appropriate coinjected derivatized standards.

	Retention Time on 3 Columns			Radioactivity of Peak	Accumulation of Applied Radioactivity
	2% QF1	2% SE30	1% XE60		
	min			*dpm* × 10^{-6}	%
LC fractions					
8–9	10.0	9.2	11.7	19.4	
14–17	14.2	10.5	18.8	0.7	0.6
	15.9	15.3	15.5	2.1	1.9
19–22	19.6	19.5	20.7	1.7	1.6
Standard GAs					
A_1	14.0	15.3	15.3		
A_2	19.7	19.5	20.6		
A_3	16.4	16.8	18.5		
A_4	10.0	9.1	11.7		
A_5	10.1	8.6	13.4		
A_6	16.4	11.3	18.5		
A_7	11.2	9.6	14.8		
A_8	17.4	25.4	17.5		
A_{16}	14.7	13.8	15.6		
A_{20}	9.6	8.4	12.2		
A_{51}	14.4	10.8	18.0		
A_{34}	12.6	15.2	13.4		
A_{35}	11.2	14.0	12.8		

by the purification and separation procedures. Thus ^{3}H-GA$_4$ is converted into three compounds none of which is a characterized GA. All other peaks of radioactivity in Figure 3 are below the sensitivity limits of the GLRC and retention times could not be obtained.

DISCUSSION

The conversion of ^{3}H-GA$_1$ to a compound with GA$_8$-like LC and GLRC retention indices by *Phaseolus* seedlings is in agreement with the general tendency of labeled C_{19}-GAs to undergo 2β-hydroxylation (5, 7–9, 11, 12, 15). Almost without exception, 2β-hydroxylated GAs exhibit much lower biological activity than their deoxy analogues (13). This could be an effective means whereby plant tissues deactivate comparatively high doses of exogenous GA. It is possible that the physiological state of the tissue could have some bearing on the fate of applied GAs. If, for instance, the endogenous GA supply is saturating the major part of the applied GA may be deactivated by 2β-hydroxylation. However, in circumstances where endogenous GAs are limiting and the applied GA induces a growth response, more of the hormone may proceed by the main metabolic pathway with a considerably smaller portion undergoing 2β-hydroxylation.

^{3}H-GA$_4$ was metabolized more rapidly than ^{3}H-GA$_1$. Three metabolites were detected and all had LC and GLRC properties different from those of GA$_{1-38}$. It is perhaps unexpected that the ^{3}H-GA$_4$ did not give rise to detectable quantities of its 2β- and 13α-hydroxy analogues, GA$_{34}$ and GA$_1$, as it does in dwarf rice (5), *Pinus* pollen (7), and vegetative shoots of Douglas fir (15). However, any speculation as to how the metabolism of ^{3}H-GA$_4$ by *Phaseolus* seedlings differs from that of other plants is premature while the major metabolites remain unidentified. It is of importance not only to characterize these metabolites but also to obtain information on their rates of turnover. They could represent end points of side branches in the main GA metabolism pathway and thereby accumulate in sufficient quantities to permit detection while perhaps biologically important main pathway intermediates may have low pool sizes and go undetected.

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