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THE EFFECT OF EXOGENOUS GIBBERELLIC ACID ON GIBBERELLIN BIOSYNTHESIS BY *GIBBERELLA FUJIKUROI*

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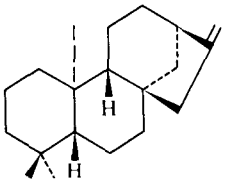
Key Word Index—*Gibberella fujikuroi*; Ascomycetes; biosynthesis; *ent*-kaurene; gibberellic acid.

Abstract—The addition of gibberellic acid and some other gibberellins to cultures of *Gibberella fujikuroi* suppresses the incorporation of [2-^{14}C]MVA and ^{14}C-labelled *ent*-kaurene into the gibberellin metabolites.

INTRODUCTION

Recently there has been interest in compounds that regulate the biosynthesis of the gibberellin plant growth hormones [1, 2]. Several stages such as the cyclization of geranylgeranyl pyrophosphate to *ent*-kaurene (**1**) [3–5] and the ring contraction of *ent*-7α-hydroxykaur-16-en-19-oic acid to gibberellin A_{12} 7-aldehyde [6] are points at which potential plant growth regulators act. Since many hormonal substances regulate their own biosynthesis by a feed-back control, we have examined the role of exogenous gibberellic acid (**2**) and some other metabolites in the context of gibberellic acid biosynthesis in *Gibberella fujikuroi*.



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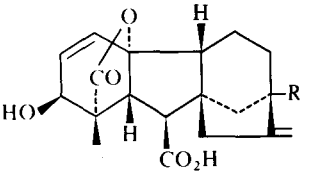
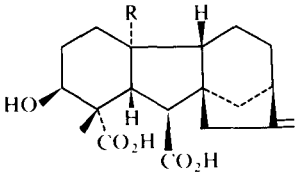


2 R = OH
3 R = H, A_4 lacks 1,2-double bond



4 R = CO_2H
5 R = Me

Sephadex LH 20 and the elution patterns compared to controls. The effect of adding gibberellic acid (gibberellin A_3) (**2**), gibberellins A_4/A_7 (**3**), gibberellin A_{13} (**4**) and gibberellin A_{14} (**5**) on metabolite production was examined by comparing the incorporation of [2-^{14}C]mevalonate and ^{14}C-labelled *ent*-kaurene (**1**) with control fermentations. The incorporation from [2-^{14}C]mevalonate should reveal effects on general terpenoid biosynthesis whilst the pattern from ^{14}C-labelled *ent*-kaurene might reveal effects on the later stages of gibberellin biosynthesis. Gibberellin A_3 (**2**) and gibberellin A_{13} (**4**) were isolated from the first group of fermentations and crystallized to constant activity whilst gibberellins A_3, A_4/A_7, A_{13} and A_{14} were separated by further TLC and crystallized from the second group of experiments. The results are given in Tables 1 and 2.

RESULTS AND DISCUSSION

Previous studies have shown that in our hands, the wild-type strain of *Gibberella fujikuroi* ACC 917 affords 50–70 mg/l of gibberellic acid in shake culture. Consequently, the exogenous substrates were added at a concentration of 200 μM. The gibberellin metabolites of the fungus were separated by chromatography on

Gibberellin A_3 (**2**) reduced the incorporation of radioactivity from both [2-^{14}C]MVA and ^{14}C-labelled *ent*-kaurene into the gibberellins including those that precede it in the biosynthetic sequence [9, 10]. Consequently, it is exerting a regulatory role on its own biosynthesis. The gibberellin A_4/A_7 mixture also reduced

Table 1. The effect of added gibberellins on the incorporation of [2-^{14}C]MVA into gibberellic acid and gibberellin A_{13}

Compound added	Gibberellin A_3		Gibberellin A_{13}	
	Total activity (dpm × 10^{-6})	Incorporation (%)	Total activity (dpm × 10^{-6})	Incorporation (%)
None (Control 1)	4.00	7.20	2.72	4.91
None (Control 2)	3.94	7.11	2.78	5.01
Gibberellin A_3	0.79	1.42	1.35	2.43
Gibberellin A_4/A_7	0.36	0.65	2.74	4.93
Gibberellin A_{13}	3.38	6.10	1.28	2.31
Gibberellin A_{14}	0.06	0.12	0.63	1.14

Table 2. The effect of added gibberellins on the incorporation of ^{14}C-labelled *ent*-kaurene

Compound added	Gibberellin A_3		Gibberellin A_4/A_7		Gibberellin A_{13}		Gibberellin A_{14}	
	Total activity (dpm × 10^{-6})	Incorporation (%)	Total activity (dpm × 10^{-6})	Incorporation (%)	Total activity (dpm × 10^{-6})	Incorporation (%)	Total activity (dpm × 10^{-6})	Incorporation (%)
None (Control 1)	2.65	5.09	0.47	0.90	0.75	1.45	1.51	2.91
None (Control 2)	2.80	5.39	0.47	0.90	0.81	1.55	1.50	2.89
Gibberellin A_3	0.96	1.84	0.24	0.46	0.34	0.65	0.56	1.07
Gibberellin A_4/A_7	1.47	2.82	1.06	2.03	0.73	1.40	1.43	2.74
Gibberellin A_{13}	2.65	5.10	0.44	0.84	0.53	1.01	1.37	2.63
Gibberellin A_{14}	1.44	2.78	0.32	0.61	0.59	1.14	0.79	1.51

the incorporation of [2-^{14}C]MVA and ^{14}C-labelled *ent*-kaurene into gibberellin A_3. However, the incorporation of *ent*-kaurene into gibberellin A_4/A_7 was enhanced, suggesting that the effect was largely one of dilution of a pool by the added substrate and consequent trapping of the label. Gibberellin A_{14} inhibited the incorporation of radioactivity from ^{14}C-labelled *ent*-kaurene both into itself and into the later metabolites. It also inhibited the incorporation of [2-^{14}C]MVA into gibberellins A_3 and A_{13}, suggesting that it was exerting a control over the biosynthesis perhaps at the 3-hydroxylation stage. On the other hand, gibberellin A_{13}, whilst inhibiting the incorporation of radioactivity into itself, has little effect on the incorporation into the other metabolites. This is not surprising in view of the fact that gibberellin A_{13} appears to be a terminal metabolite on a branch of the main gibberellin pathway in *G. fujikuroi* [9–11]. It is significant that exogenous gibberellic acid can inhibit the biosynthesis not only of itself but also of other gibberellins, an effect that could in the extreme be counter-productive in those situations in which gibberellic acid does not elicit the major response. Since there are commercial mutants that accumulate relatively large quantities of gibberellic acid, it is possible that in these cases, the regulatory mechanism has been blocked.

EXPERIMENTAL

G. fujikuroi ACC 917 was grown as described previously [12].

Administration of [2-^{14}C]*MVA*. Six groups of 3-day-old shake cultures (200 ml each group) were fed [2-^{14}C]MVA (25 μCi per group) in H_2O (0.2 ml). 200-μM quantities of the gibberellins (Table 1) were added in EtOH (1 ml) to four groups of flasks and two were retained as controls. After a further 8 days' growth, the cultures were harvested and the metabolites recovered in EtOAc. They were separated on Sephadex LH-20 using the biphasic system petrol–EtOAc–AcOH–MeOH–H_2O (100:80:5:40:7). The fractions containing gibberellins A_3 and A_{13} were further purified by TLC on Si gel in $CHCl_3$–EtOAc–AcOH (5:5:1) and crystallized to constant sp. activity (Table 1).

Administration of ^{14}C-*labelled* ent-*kaurene*. Six groups of 3-day-old cultures (200 ml in each group) were fed [1,7,12,18-^{14}C]*ent*-kaurene (prepared biosynthetically from [2-^{14}C]MVA) (23.45 μCi) in EtOH (0.1 ml). 200-μM quantities of the gibberellins A_3, A_4/A_7, A_{13} and A_{14} in EtOH (1 ml) were added to four groups whilst the remaining two received EtOH (1 ml) only as controls. After a further 9 days growth, the cultures were harvested and the metabolites isolated as before and purified by TLC on Si gel. The solvent systems used were: C_6H_6–AcOH (9:1) for gibberellin A_{14}, di-isopropyl ether–AcOH (19:1) for gibberellins A_4/A_7 and A_{13} and $CHCl_3$–EtOAc–AcOH (5:5:1) for gibberellin A_3. The metabolites were crystallized to constant sp. activity (Table 2).

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