Gibberellin Biosynthesis in the Mutant B1-41a of *Gibberella fujikuroi*

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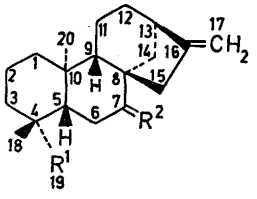
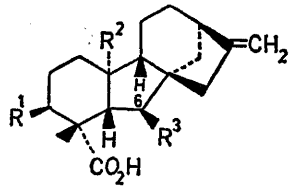
Summary The mutant B1-41a of *Gibberella fujikuroi* is shown to be blocked for gibberellin synthesis at the step between *ent*-kaurenal and *ent*-kaurenoic acid; steps beyond the block have been examined by feeding, to the mutant, substrates which occur beyond this point in the parent strain GF-1a.

CURRENT studies with a u.v.-induced mutant (B1-41a) of *Gibberella fujikuroi* are providing detailed information on the gibberellin (GA) biosynthetic pathway. Some results on the metabolism by the mutant of substrates which normally occur in the parent wild-type strain (GF-1a) are



	R^1	R^2
(**1**)	Me	H_2
(**2**)	CH_2OH	H_2
(**3**)	CHO	H_2
(**4**)	CO_2H	H_2
(**5**)	CO_2H	H,β-OH
(**6**)	CO_2H	O

reported below. Incubations were made at 25° for 20 h with pigmented mycelium re-suspended in a nitrogen-free medium buffered at pH 3·5; metabolites were characterised by g.c.–m.s. and, where appropriate, by g.c.–r.c.



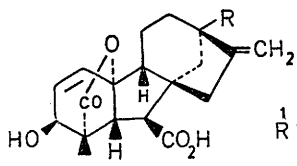
	R^1	R^2	R^3		R^1	R^2	R^3
(**7**)	H	Me	CHO	(**13**)	OH	Me	CHO
(**8**)	OH	Me	CO_2H	(**14**)	OH	CO_2H	CO_2H
(**9**)	H	Me	CO_2H	(**15**)	OH	CHO	CO_2H
(**10**)	H	CH_2OH	CO_2H	(**16**)	OH	CH_2OH	CO_2H
(**11**)	H	CHO	CO_2H	(**17**)	H	Me	CH_2OH
(**12**)	H	CO_2H	CO_2H	(**18**)	OH	Me	CH_2OH

(a) *ent*-Kaurene (**1**), *ent*-kaurenol (**2**), and *ent*-kaurenal (**3**) were not significantly metabolised to GAs. *ent*-Kaurenoic acid (**4**) was, however, completely metabolised to give the same GAs as produced by the parent strain. This block between *ent*-kaurenal (**3**) and *ent*-kaurenoic acid (**4**) is 97·5% effective as determined by comparison of the incorporation of [2-^{14}C]mevalonic acid into GA_3 (**19**) by the mutant and by the parent strain.

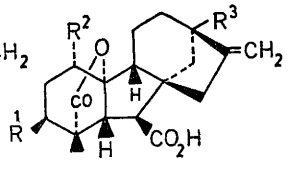
(b) *ent*-7α-Hydroxykaurenoic acid (**5**), assumed to be the next intermediate after *ent*-kaurenoic acid (**4**) was formed from the latter after 1 h. However, it was incompletely metabolised to give the same metabolites as *ent*-kaurenoic

acid but in 10% of the concentration *ent*-7-Oxokaurenoic acid (**6**) was not metabolised.

(c) 6-[^{3}H]-GA_{12}-Aldehyde (**7**) was metabolised mainly to GA_{14} (**8**) after 20 h; the ratio of GA_{14} (**8**) to GA_3 (**19**) was 3 : 1 at pH 3·5 and 22 : 1 at pH 7. After 5 days at pH 3·5 GA_3 was the main metabolite and the concentration of GA_{14} was low; all other GAs, produced by the parent strain, were also present. Since GA_{14} does not accumulate to such a large extent in short term cultures of the parent strain, the rate-limiting step in the parent must occur before *ent*-kaurenoic acid (**4**).



(**19**) R=OH
(**20**) R=H



	R^1	R^2	R^3
(**21**)	H	H	H
(**22**)	OH	H	OH
(**23**)	OH	H	H
(**24**)	OH	OH	H

GA_{14}-Aldehyde (**13**) is an efficient precursor of GA_3 (**19**) and its formation from GA_{12}-aldehyde (**7**) was established by isotope dilution.

GA_{12}-Alcohol (**17**) and GA_{14}-alcohol (**18**) were as efficient precursors of GAs as the corresponding aldehydes (**7**) and (**13**). However their occurrence as intermediates has not been established.[3]

(d) The metabolism of 6-[^{3}H]-GA_{12} (**9**) was quite different from that of [6-^{3}H]-GA_{12}-aldehyde (**7**); the radioactivity was incorporated mainly into the non-3-hydroxylated gibberellins, GA_9 (**21**), GA_{15}[19,20-lactone of (**10**)], GA_{24} (**11**), GA_{25} (**12**), and to a lesser extent into the 3-hydroxylated GAs. At pH 7 this trend was even more pronounced.

(e) GA_{14} (**8**) is metabolised, partially after 20 h, and completely after 48 h, to give the 3-hydroxylated GAs: GA_1 (**22**), GA_3 (**19**), GA_4 (**23**), GA_7 (**20**), GA_{13} (**14**), and GA_{36} (**15**).

(f) GA_{13} (**14**), GA_{36} (**15**), and GA_{37}[19,20-lactone of (**16**)], potential intermediates between GA_{14} (**8**) and GA_4 (**23**) were not metabolised. The 19,20-anhydride of GA_{13} was completely converted into GA_{13} (our methods would not, however, detect the 0·14% conversion of the anhydride into GA_3 reported by Hanson and Hawker[2]).

(g) GA_4 (**23**) is converted mainly into GA_3 (**19**), and to a lesser extent into GA_1 (**22**), GA_7 (**20**), and GA_{16} (**24**). GA_7 (**20**) is metabolised exclusively to GA_3 (**19**) which was not further metabolised after 5 days' incubation. GA_1 and GA_{16} are also terminal GAs. GA_{16} is therefore neither derived from GA_7 by hydration[1] nor converted into GA_7 by dehydration. The metabolism of GA_4 and GA_7 is completely inhibited at pH 7·0.

(h) GA_{15}[19,20-lactone of (**10**)], and GA_{25} (**12**) were not metabolised. GA_{24} (**11**) was converted into GA_{25} (**12**). Thus none of these appear to be precursors of GA_9 (**21**).

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[1] M. Katsumi and B. O. Phinney, 'The Gibberellins,' ed. S. Tamura, Tokyo University Press, 1968, ch. 4.
[2] J. R. Hanson and J. Hawker, *Tetrahedron Letters*, 1972, 4299.
[3] J. R. Hanson and J. Hawker, *Phytochemistry*, 1973, **12**, 1073.