Metabolism of Steviol and its Derivatives by *Gibberella fujikuroi*, Mutant B1-41a

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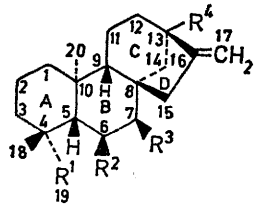
Summary The low substrate specificity of enzymes operating beyond the genetic defect in mutant B1-41a of the fungus, *Gibberella fujikuroi*, is shown by the metabolism of the non-fungal diterpene steviol (*ent*-13-hydroxykaur-16-en-19-oic acid) and some of its derivatives to higher plant gibberellins and their derivatives.

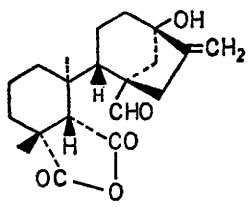
THE preceding communication[1] showed that the mutant B1-41a of *Gibberella fujikuroi* is blocked for gibberellin (GA) biosynthesis at the step between *ent*-kaurenal (**1**) and *ent*-kaurenoic acid (**2**) and briefly described the metabolism by B1-41a of substrates normally produced by the wild-type parent strain GF-1a. The low substrate specificity of enzymes operating beyond the block has been established by the metabolism of substrates which are not produced by the parent strain and is illustrated below for steviol (**3**) and some of its derivatives. Methods for the incubation of substrates with B1-41a and the characterisation of the resultant metabolites are described in the preceding communication.[1]

(a) Steviol (**3**) is rapidly metabolised to *ent*-7α,13-dihydroxykaurenoic acid (**4**) and further metabolised to *ent*-

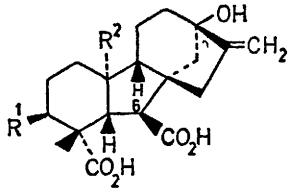


	R¹	R²	R³	R⁴
(**1**)	CHO	H	H	H
(**2**)	CO_2H	H	H	H
(**3**)	CO_2H	H	H	OH
(**4**)	CO_2H	H	OH	OH
(**5**)	CO_2H	OH	OH	OH
(**6**)	CO_2H	H	H	OAc
(**7**)	CO_2Me	H	H	OH
(**8**)	CO_2Me	H	OH	OH
(**9**)	CO_2H	H	OAc	H

6α,7α,13-trihydroxykaurenoic acid (**5**), *ent*-6β,7α,13-trihydroxykaurenoic acid 19,6-lactone, 13-hydroxyfujenal (**10**) and the corresponding di-acid, GA_1 (**17**), 13-hydroxy-GA_{12} (**11**), GA_{18} (**12**), GA_{19} (**13**), and traces of GA_{20} (**15**). GA_1 is the major product. 13-Hydroxy-GA_{12} has not been found naturally; GA_{18}, GA_{19}, and GA_{20} are typical GAs of higher plants.[2] Ruddat *et al.*[3] observed the conversion of [^{14}C]-



(**10**)



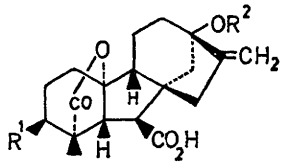
(**11**) R^1=H, R^2=Me
(**12**) R^1=OH, R^2=Me
(**13**) R^1=H, R^2=CHO
(**14**) R^1=H, R^2=CO_2H

steviol by the wild-type strain 45-339 into an unidentified [^{14}C]-metabolite with GA-like biological properties. The microbiological conversion of steviol (**3**) into *ent*-6β,7α,13-trihydroxykaurenoic acid 19,6-lactone by the wild-type strain ACC 917 has been observed by Hanson and White.[4]

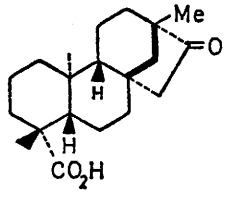
(b) Steviol acetate (**6**) is converted, *inter alia*, into the acetate (**16**) hydrolysis of which gives the common higher plant gibberellin, GA_{20} (**15**), in *ca.* 30% overall yield.

(c) Steviol methyl ester (**7**) is metabolised to several mono- and dihydroxy-steviol methyl esters not hydroxylated in ring A and so far unidentified except for methyl *ent*-7α,13-dihydroxykaurenoic acid (**8**). No GAs were formed indicating that methylation of the carboxy-group in steviol prevents ring B contraction.

(d) Acetylation of *ent*-7α-hydroxykaurenoic acid likewise prevents ring B contraction to GAs and the acetate (**9**) is metabolised to a monohydroxy derivative.



(**15**) R^1=H, R^2=H
(**16**) R^1=H, R^2=Ac
(**17**) R^1=OH, R^2=H



(**18**)

(e) iso-Steviol (**18**) is metabolised to the ring C/D rearranged compounds corresponding to GA_{17} (**14**), GA_{20} (**15**), and 13-hydroxy-GA_{12} (**11**). The ring C/D rearrangement products of (**4**), (**5**), and *ent*-6β,7α,13-trihydroxykaurenoic acid 19,6-lactone were also produced. This result indicates a remarkable lack of enzyme-substrate specificity in the GA-biosynthetic pathway.

The conversion of steviol into GAs of higher plants by the mutant B1-41a suggests that many of the fungal enzymes controlling GA-biosynthesis are similar, or identical, to those present in higher plants. The mutant, therefore, provides both a convenient model for the study of GA-biosynthesis in higher plants and a convenient system for the production of plant GAs and GA-analogues of biological interest.

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[1] J. R. Bearder, P. Hedden, J. MacMillan, C. M. Wels, and B. O. Phinney, preceding communication.
[2] J. MacMillan and R. J. Pryce in 'Phytochemistry,' ed. L. P. Miller, van Nostrand Reinhold Co., New York, 1973, vol. 3, ch. 11.
[3] M. Ruddat, E. Heftmann, and A. Lang, *Arch. Biochem. Biophys.*, 1965, **110**, 496.
[4] J. R. Hanson and A. F. White, *Tetrahedron*, 1968, **24**, 6291.