The Formation of an *ent*-6α,7α-Epoxykaurene of Possible Biosynthetic Significance by *Gibberella fujikuroi*

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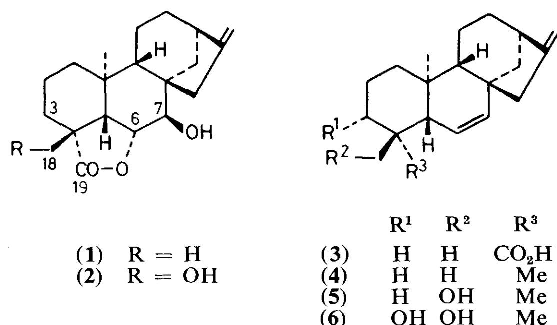
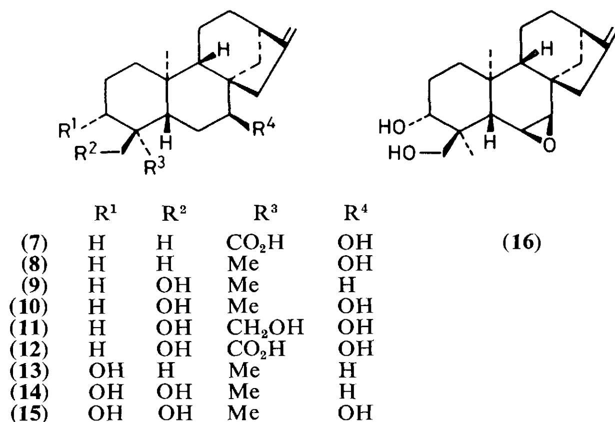
Whereas *ent*-kaur-6,16-diene affords 7-hydroxykaurenolide and *ent*-18-hydroxykaur-6,16-diene affords 7,18-dihydroxykaurenolide on incubation with *Gibberella fujikuroi, ent*-6α,7α-epoxy-3β,18-dihydroxykaur-16-ene, the structure of which was determined by *X*-ray crystallography was obtained from *ent*-3β 18-dihydroxykaur-6,16-diene.

The fungus *Gibberella fujikuroi* produces both the gibberellins and a second group of diterpenoid lactones, the kaurenolides (*e.g.* **1**).[1] Recently *ent*-kaur-6,16-dien-19-oic acid (**3**)[2,3] rather than *ent*-7α-hydroxykaur-16-en-19-oic acid (**7**) has been shown[4] to be an efficient precursor of 7-hydroxykaurenolide (**1**) in *Cucurbita maxima* and in a mutant of *G. fujikuroi*. It was suggested that a 6,7-epoxide was formed first and that this then underwent a *trans* diaxial ring-opening to afford the kaurenolides. However although both the 6α,7α- and 6β,7β-epoxides can be prepared chemically in the presence



	R^1	R^2	R^3
(3)	H	H	CO_2H
(4)	H	H	Me
(5)	H	OH	Me
(6)	OH	OH	Me



	R^1	R^2	R^3	R^4
(7)	H	H	CO_2H	OH
(8)	H	H	Me	OH
(9)	H	OH	Me	H
(10)	H	OH	Me	OH
(11)	H	OH	CH_2OH	OH
(12)	H	OH	CO_2H	OH
(13)	OH	H	Me	H
(14)	OH	OH	Me	H
(15)	OH	OH	Me	OH

(16)

of a 19-methyl ester,[5] it was not possible[2,3] to detect a 6,7-epoxide *in vivo* presumably owing to rapid hydrolysis in the presence of a 19-carboxylic acid. We now present evidence for the formation of an *ent*-6α,7α-epoxykaurene by *G. fujikuroi* using an 'artificial' substrate in which the microbiological oxidation of C-19 is inhibited.

The plant growth regulator, AMO-1618, whilst blocking the formation of *ent*-kaur-16-ene by *G. fujikuroi*, does not appear to modify the post-kaurenoid metabolic steps.[6,7] Hence by blocking the formation of endogenous kaurenoid metabolites, it is possible to isolate the metabolites of post-kaurenoid 'artificial' substrates and thereby to draw some conclusions concerning the structural requirements of post-kaurenoid metabolism by the fungus. Previously we have shown[8] that incubation of *ent*-7α-hydroxykaurene (**8**) with *G. fujikuroi* afforded products which were characteristic of the normal metabolism of *ent*-7α-hydroxykaurenoic acid (**7**) but we were unable to detect any kaurenolides. On the other hand *ent*-18-hydroxykaur-16-ene (**9**) gave 7,18-dihydroxy-kaurenolide (**2**). Nevertheless this kaurenolide (**2**) was not formed from *ent*-7α,18-dihydroxykaur-16-ene (**10**),[9] *i.e.* although minor pathways may exist, an *ent*-7α-hydroxy-group was incompatible with efficient microbiological transformation into the kaurenolide branch of the diterpenoid pathways.

Incubation of *ent*-kaur-6,16-diene (**4**)[10] gave 7-hydroxy-kaurenolide (**1**) and 7,18-dihydroxykaurenolide (**2**) but no detectable gibberellin metabolites. Similarly *ent*-18-hydroxy-kaur-6,16-diene (**5**)[10] gave 7,18-dihydroxykaurenolide (**2**). Thus in contrast with the effect of an *ent*-7α-hydroxy-group, the presence of a 6-ene appears to direct microbiological transformation into the kaurenolide pathway paralleling the biosynthetic results.[2,3]

The major metabolites of *ent*-7α,18-dihydroxykaur-16-ene (**10**) were the 19-alcohol (**11**) and the 19-acid (**12**). On the other hand *ent*-3β-hydroxykaur-16-ene (**13**)[11] was not metabolized whilst *ent*-3β,18-dihydroxykaur-16-ene (**14**) gave

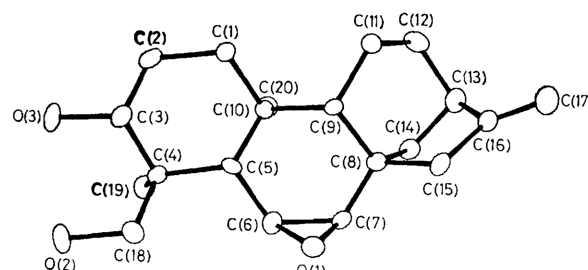


Figure 1. Molecular structure of *ent*-6α,7α-epoxy-3β,18-dihydroxykaur-16-ene (**16**).

ent-3β,7α,18-trihydroxykaur-16-ene (**15**), *i.e.* an *ent*-3β-hydroxy-group inhibited hydroxylation at C-19 by *G. fujikuroi.* This afforded a means of detecting the formation of a 6,7-epoxide from a 6,7-ene. Incubation of *ent*-3β,18-dihydroxy-kaur-6,16-diene (**6**) with *G. fujikuroi* ACC 917 gave a good yield of *ent*-6α,7α-epoxy-3β,18-dihydroxykaur-16-ene (**16**). The structure (Figure 1), in particular the stereochemistry of the epoxide ring, was established by *X*-ray analysis.

It has been suggested that the microbiological epoxidation of an alkene is sometimes equivalent to the hydroxylation of the corresponding alkane. The divergence in the oxidative modification of ring B in kaurenolide–gibberellin biosynthesis may depend on the order in which dehydrogenation and hydroxylation (epoxidation) occurs.

Crystal data: compound (**16**), $C_{20}H_{30}O_3$, M 318.5, monoclinic, $a = 10.640(2)$, $b = 7.614(6)$, $c = 11.158(3)$ Å, $\beta = 107.91(2)°$, $U = 860.1$ Å^3, $Z = 2$, $D_c = 1.23$ g cm^{-3}, $F(000) = 348$; Mo-K_α radiation, $\lambda = 0.71069$ Å, $\mu = 0.9$ cm^{-1}. Space group $P2_1$ from systematic absences of $0k0$ for k odd and successful structure refinement. Data were measured on an Enraf-Nonius CAD 4 diffractometer. 911 reflections were used in the structure refinement. The structure was solved using the MULTAN programme, and refined to an R-factor of 0.104.†

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† The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.