The Stereochemistry of Some Stages in Gibberellin Biosynthesis

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Summary The stereospecific labelling of the gibberellins and the kaurenolides with 2(*R*) and 5(*R*)-[^{3}H]mevalonate leads to the conclusion that the dehydrogenation of ring A is a *cis*-process and that hydroxylation of ring B takes place with retention of configuration.

It has been shown[1] that the biosynthesis of the gibberellins and kaurenolides lies through the tetracyclic diterpene (−)-kaurene (I). This biosynthesis is followed by stepwise hydroxylation leading to the formation of 7β-hydroxy-(−)-kaur-16-en-19-oic acid which acts as a precursor of both the kaurenolides and the gibbane aldehyde (II).[2] The absolute stereochemistry of the mevalonodi hydrogen atoms of the open-chain terpenoid precursors has been defined[3] and we now extend this information to gibberellin and kaurenolide biosynthesis.

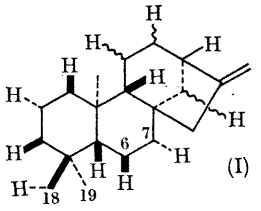
The formation of the antipodal (−)-kaurene is a function of the relative orientation of the double bonds of a geranylgeraniol precursor but does not disturb the pro "*R*" or pro

The incorporation of mevalonic acid into the metabolites of Gibberella fujikuroi

	2(*R*)-^{3}H		5(*R*)-^{3}H	
	^{3}H : ^{14}C	No. of ^{3}H	^{3}H : ^{14}C	No. of ^{3}H
Mevalonic acid	9·0 : 1	–	9·5 : 1	–
(−)-Kaurene (I)	9·0 : 1	4	9·5 : 1	4
7-Hydroxykaurenolide (III)	9·0 : 1	4	9·4 : 1	4
7,18-Dihydroxykaurenolide (IV)	8·0 : 1	3·6	9·15 : 1	4
Gibberellic acid (V)†	6·6 : 1	3	4·96 : 1	2
Gibberellin A_4 (VI)†	7·0 : 1	3	–	–
Gibberellin A_{13} (VII)†	6·8 : 1	3	7·43 : 1	3

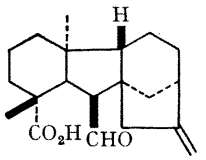
† Purified as their methyl esters.

"*S*" character of the individual hydrogen atoms. On the basis of analogy with the steroids, those that would be expected to originate from [2(*R*), 4(*R*), and 5(*R*)-^{3}H]mevalonic acid are shown in (I). We have already described[4]



(I)

H= label from 2(*R*)-, 4(*R*)-, or 5(*R*)-mevalonate



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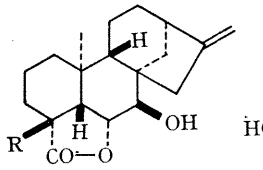
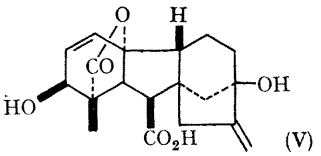


(III) R=Me (IV) R=CH_2OH



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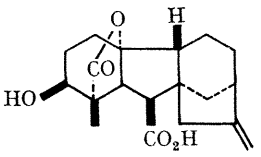

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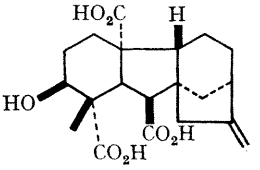

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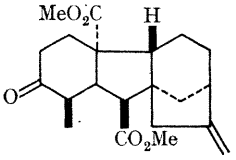

(VIII)

our results with doubly-labelled [4(*R*)-^{3}H;2-^{14}C]mevalonic acid and we now detail our results with [2(*R*)-^{3}H;2-^{14}C]- and [5(*R*)-^{3}H;2-^{14}C]mevalonic acid, the latter fed as its DBED salt.

7-Hydroxykaurenolide from the (2(*R*)-^{3}H] experiment was oxidized to 7-oxokaurenolide [^{3}H:^{14}C, 7·2:1] whilst the 7,18-dihydroxykaurenolide was oxidized to 7-oxo-18-norkaurenolide [^{3}H:^{14}C, 5·0:0·75] thus locating a mevalonoid label at positions 7α and 18. Methyl gibberellate was converted into methyl gibberate [^{3}H:^{14}C, 6·68:1] with no loss of label.

The metabolites from the [5(*R*)-^{3}H] experiment were degraded as follows. 7-Hydroxykaurenolide was oxidized to 7-oxokaurenolide [^{3}H:^{14}C, 9·77:1] and the latter treated with base. The gummy product showed [^{3}H:^{14}C, 7·25:1]. Alternatively, the kaurenolide was converted[5] into 6-oxo-(−)-kaur-16-en-19-oic acid [^{3}H:^{14}C, 7·35:1] thus locating a label at the 6α-position. Gibberellin A_{13} trimethyl ester was oxidized to the corresponding ketone [^{3}H:^{14}C, 7·43:1] and the latter treated with alkali and then remethylated with diazomethane to form the nor-gibbane (VIII) [^{3}H:^{14}C, 4·85:1].[6] Methyl gibberellate was converted into methyl allogibberate [^{3}H:^{14}C, 4·99:1] and thence to methyl gibberate [^{3}H:^{14}C, 4·96:1], which was oxidized to methyl gibberdionate [^{3}H:^{14}C, 2·66:1],[7] thus locating a label at (the gibbane) position 11 in gibberellic acid. Hydrolysis of methyl gibberate with alkali gave an acid [^{3}H:^{14}C, 2·48:1] with the loss of only one tritium from the enolizable position of the cyclopentanone. Thus there was no evidence for the presence of a label at the 10-position.

A number of conclusions may be drawn from these results. Hydroxylation of ring B to form the kaurenolides must take place with retention of configuration at C-6 and C-7. However, ring-contraction to form the gibberellins which takes place at the aldehyde oxidation level, results in the loss of 5(*R*)-mevalonoid hydrogen from C-6 (C-10 of the gibbane skeleton). This suggests that the leaving group which initiates ring contraction possesses the 6β-stereochemistry. Secondly there is evidence for the stereochemistry of processes on ring A of gibberellic acid. The dehydrogenation step to form the Δ^3-double bond involves a "*cis*" elimination of hydrogen from the "α" face of a saturated gibberellin. This must exclude processes involving hydroperoxidation of Δ^2-gibberellins as this would require the elimination of a β-hydrogen atom (*cis* to the hydroperoxide) at C-4.

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