The Oxidative Modification of the Kauranoid Ring B during Gibberellin Biosynthesis

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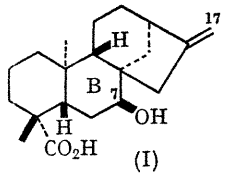
Summary 7β-Hydroxy-(−)-kaur-16-en-19-oic acid has been shown to act as a precursor of the gibberellins and the kaurenolides.

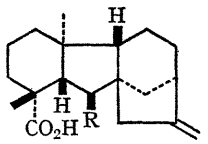
THE gibberellin plant-growth hormones differ from the other tetracyclic diterpenes by possessing a five-membered ring B. However, the tetracyclic diterpenes, (−)-kaurene and (−)-kaur-16-en-19-oic acid, which both possess a six-membered ring B, have been shown[1,2] to act as precursors of gibberellic acid and of the kaurenolides. The subsequent stages in the biosynthesis therefore include the oxidation of ring B and its ring-contraction. The mevalonate labelling-pattern indicated[3] that C-7 is extruded from the six-membered ring whilst various suggestions[4] (including *in vitro* experiments[5]) have been made to account for the ring contraction. We present experimental evidence for the biosynthetic order of oxidation of ring B.

In defining the late stages of this biosynthetic sequence, it is imperative to establish that compounds such as 7β-hydroxy-(−)-kaur-16-en-19-oic acid (I)[6] and the gibbane

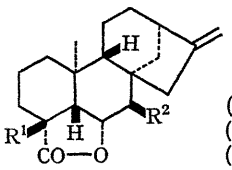
aldehyde (II),[5] which are at present unknown as metabolites of *Gibberella fujikuroi*, are in fact produced by the mould. Therefore [17-^{14}C]-(−)-kaur-16-ene was fed to *Gibberella fujikuroi*. The fermentation was subjected to dilution



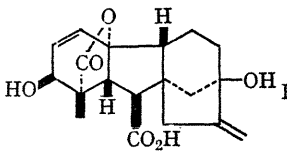
(I)



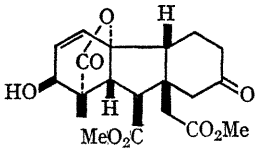
(II) R=CHO
(III) R=CO_2H



(IV) R^1=Me, R^2=H
(V) R^1=Me, R^2=OH
(VI) R^1=CH_2OH, R^2=OH



(VII)



(VIII)

analysis for kaurenolide (IV)[7] and 7β-hydroxy-(−)-kaur-16-en-19-oic acid (I). Only the latter was active, showing a low incorporation of 0·006% compared to 7-hydroxykaurenolide (V) (0·8%) and 7,18-dihydroxykaurenolide (VI) (5·5%). However, this was shown to be specific by ozonolysis to the nor-ketone (inactive) and recovery of the formaldehyde (from C-17) as its dimedone derivative. The latter contained 99% of the activity. [17-^{14}C]-7β-Hydroxy-(−)-kaur-16-en-19-oic acid (as its potassium salt) was fed to *Gibberella fujkuroi*. After 4 hr. the gibbane aldehyde (II) (the expected product of ring contraction at the glycol level) was isolated by dilution analysis and showed a specific incorporation of 0·21% and gibberellin A_{12} (III) 0·29%. After 24 hr. the incorporations were 0·17 and 0·37%, respectively. Specificity was demonstrated in each case. Thus the gibbane aldehyde from the 24 hr. fermentation was oxidized to gibberellin A_{12} (which retained the radioactivity) and the latter, as its dimethyl ester, was ozonized to give the nor-ketone and formaldehyde. The formaldehyde contained 96·5% of the radioactivity whilst the nor-ketone was inactive. In the gibberellin A_{12} case, the formaldehyde contained 98% of the radioactivity and again the nor-ketone was inactive.

A different fermentation was grown for 5 days after feeding the same labelled hydroxy-acid as its potassium salt. The major metabolites were isolated. Gibberellic acid (VII), which was purified as its methyl ester, showed an incorporation of 32·3% whilst 7-hydroxykaurenolide (V) and 7,18-dihydroxykaurenolide (VI) showed incorporations of 0·03 and 0·44%, respectively. Ozonolysis of methyl gibberellate gave a keto-acid which was purified as its dimethyl ester (VIII), and formaldehyde which was isolated as its dimedone derivative. The formaldehyde contained 97·6% of the activity whilst 2·7% remained in the keto-ester. The kaurenolides were also degraded to their (inactive) nor-ketones. The formaldehyde from 7,18-dihydroxykaurenolide accounted for 98·5% of the radioactivity in that metabolite.

Thus the biosynthesis of gibberellic acid involves the conversion of (−)-kaur-16-ene through (−)-kaur-16-en-19-oic acid to 7β-hydroxy-(−)-kaur-16-en-19-oic acid[8] followed by ring-contraction to the gibbane aldehyde (II).

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[4] For summaries see B. E. Cross, *Progr. Phytochem.*, 1968, **1**, 195; and J. R. Hanson, "The Tetracyclic Diterpenes," Pergamon, Oxford, 1968.
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[8] Professor C. A. West has informed us of comparable results involving the isolation of this acid using an enzyme system from *Echinocystis macrocarpa*. C. A. West, M. O. Oster, D. Robinson, F. Lew, and P. J. Murphy in 'Biochemistry and Physiology of Plant Growth Substances' ed. F. Wightman and G. Setterfield, Runge Press, Ottawa, Canada (in the press), being the text of a lecture given at Carletor University in July 1967.