Biosynthesis of the Diterpenes Enmein and Oridonin from *ent*-16-Kaurene

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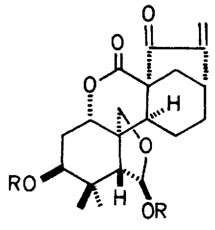
Summary *ent*-16-Kaurene (**3**) has been shown by tracer experiments to be incorporated into enmein (**1**) and oridonin (**2**) in *Isodon japonicus* Hara.

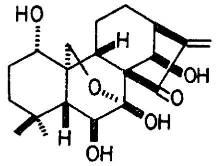
THE diterpenes, enmein (**1**)[1] and oridonin (**2**)[2] have been found as major components in the leaves of *Isodon japonicus* Hara, a home remedy for gastrointestinal diseases in Japan. *ent*-16-Kaurene (**3**) has been considered to be a precursor for them. Oxygenations at the C-1, -6, -7, -14, -15, and -20 positions would lead to formation of the oridonin molecule, and enmein is presumably formed by the oxidative cleavage[3] of the 6,7 C–C bonding of an oridonin-like precursor. We now present experimental evidence supporting this.

[17-^{14}C]-*ent*-16-Kaurene (**4**), prepared from 17-nor-*ent*-kauran-16-one (**5**) and ^{14}C-methyl iodide *via* Wittig reaction,[4] was dissolved in acetone, and applied to the reverse side of leaves of the growing plants. The leaves were harvested after a week. The diterpenes, (**1**) and (**2**),

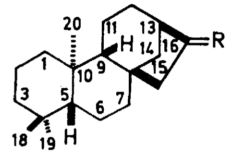


(1) R = H

(6) R = Ac



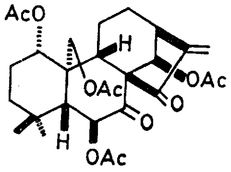
(2)



(3) $R = CH_2$

(4) $R = {}^{14}CH_2$

(5) R = O



(7)

isolated were purified to constant radioactivity as their acetates (**6**) and (**7**). The incorporation was 0·004% for enmein (**1**) and 0·003% for oridonin (**2**).

Ozonolyses of the acetates led to nearly quantitative recoveries (115% for enmein, and 89% for oridonin) of the radioactivity from C-17 as formaldehyde, demonstrating specific incorporations.

In this experiment, the recovery of the radioactivity was low (total activity fed: $1{\cdot}38 \times 10^7$ d.p.m.; total activity recovered from the crude methanolic extract: $1{\cdot}46 \times 10^6$ d.p.m.; recovery 10·6%), just as experienced by Bennett *et al.*[5,6] in feeding experiments with [17-^{14}C]-*ent*-16-kaurene (**4**). They assumed a rapid oxidative decomposition of 17-exocyclic methylene into carbon dioxide. Then, carbon dioxide was trapped by 2-aminoethanol during 3 h† after the administration of [17-^{14}C]-*ent*-16-kaurene (**4**), but it scarcely showed radioactivity (recovery: 0·004%), contrary to their assumption. From a sublimate collected as a solution in hexane, however, 11·6% of the radioactivity was recovered. Its thin layer radio-chromatogram suggested the formation of a polar product, presumably an oxidized kaurene derivative.

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† Preliminary experiments showed that the methanolic extract of the leaves harvested after 3 h recovered 27·4% of the radioactivity which was initially fed.

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