Biosynthesis of Enmein and Oridonin from 15-Oxygenated Kaurenoids and 14-Deoxyoridonin

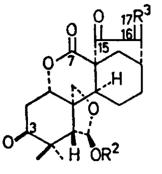
By Tetsuro Fujita
(*Faculty of Pharmaceutical Sciences, Tokushima University, Tokushima* 770, *Japan*)
and Sachiko Takao, Yoshimitsu Nagao, and Eiichi Fujita*
(*Institute for Chemical Research, Kyoto University, Uji, Kyoto-Fu* 611, *Japan*)

Summary Incorporation of *ent*-16-kauren-15-one (**3f**) into enmein (**1a**) and of 14-deoxyoridonin (**2b**) into oridonin (**2a**) have been demonstrated by tracer experiments.

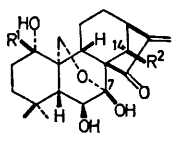
Recently, we reported the incorporation of *ent*-16-kaurene (**3a**) into enmein (**1a**) and oridonin (**2a**) in *Isodon japonicus* Hara.[1] Functionalisation at C-15 may occur by (i) direct oxygenation at the allylic position of *ent*-16-kaurene (**3a**), (ii) oxygenation of *ent*-15-kaurene (**4a**) accompanied by allylic rearrangement, or (iii) epoxidation of (**4a**) followed by rearrangement. We now present evidence supporting pathway (i) in the biosynthesis of enmein, and also report the incorporation of 14-deoxyoridonin (**2b**) into oridonin (**2a**).

The [17-^{14}C]-*ent*-16-kaurene (**3b**)[1] was isomerized by treatment with iodine to give [17-^{14}C]-*ent*-15-kaurene (**4b**), which on epoxidation afforded [17-^{14}C]-*ent*-15,16β-epoxykaurane (**5**). *ent*-15-Kaurene (**4a**) was converted into *ent*-16-kauren-15β-ol (**3c**)[2] by photosensitized oxygenation, and (**3c**) was transformed to [17-^{14}C]-*ent*-16-kauren-15β-ol (**3d**) *via* acetylation, ozonolysis, and Wittig reaction with $Ph_3{}^{14}CH_3PI$. The radioactive alcohol (**3d**) on oxidation gave [17-^{14}C]-*ent*-16-kauren-15-one (**3f**), which on reduction with sodium borohydride yielded [17-^{14}C]-*ent*-16-kauren-15α-ol (**3e**), the C-15 epimer of (**3d**). [1β-^{3}H]-14-Deoxyoridonin (**2b**)[3] was derived from trichokaurin (**6**),[4] a diterpene from *Isodon trichocarpus* Kudo, *via* Jones oxidation, sodium borotritiide reduction, treatment with $LiAlH_4$, and oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

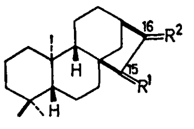
The labelled compounds (**3b**), (**4b**), (**5**), (**3d**), (**3e**), (**3f**), and (**2b**), respectively, dissolved in acetone, were applied[5] to the reverse side of leaves of growing *I. japonicus* plants, and the leaves were harvested after a week. The diterpenes, (**1a**) and (**2a**), isolated from each feeding plant were purified to constant radioactivity as their acetates (**1b**) and (**7**). The results are summarized as follows: (i) incorporation of *ent*-16-kaurene (**3b**) into both enmein (**1a**) and oridonin (**2a**) again occurred although the incorporations (0·001% for enmein and 0·0004% for oridonin) were lower than those in the previous experiments.[1] (ii) *ent*-15-Kaurene (**4b**) was not incorporated into enmein, while no definite conclusion was obtained in the oridonin case.† (iii) The 15,16-epoxide (**5**) was not incorporated into either diterpenoid. (iv) The alcohol (**3d**) was not incorporated into oridonin, while no definite conclusion was obtained in the enmein case.† (v) The alcohol (**3e**) and the ketone (**3f**) were incorporated into enmein (0·01% and 0·02%, respectively), but no conclusions could be drawn about oridonin biosynthesis in either case.† (vi) 14-Deoxyoridonin (**2b**) was shown to be incorporated into oridonin in 0·003%, in spite of the trace amount of oridonin,† but it was not incorporated at all into enmein.



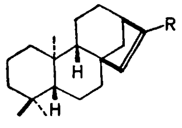
(1a) $R^1=R^2=H, R^3=CH_2$
(1b) $R^1=R^2=Ac, R^3={}^{14}CH_2$
(1c) $R^1=Ac, R^2=H, R^3={}^{14}CH_2$
(1d) $R^1=R^2=Ac, R^3=\alpha\text{-}{}^{14}CH_3, \beta\text{-}H$



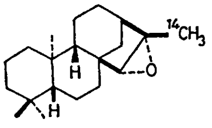
(2a) $R^1=H, R^2=OH$
(2b) $R^1={}^{3}H, R^2=H$



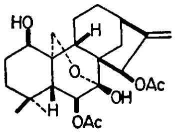
(3a) $R^1=H_2, R^2=CH_2$
(3b) $R^1=H_2, R^2={}^{14}CH_2$
(3c) $R^1=\alpha\text{-}OH, \beta\text{-}H, R^2=CH_2$
(3d) $R^1=\alpha\text{-}OH, \beta\text{-}H, R^2={}^{14}CH_2$
(3e) $R^1=\alpha\text{-}H, \beta\text{-}OH, R^2={}^{14}CH_2$
(3f) $R^1=O, R^2={}^{14}CH_2$



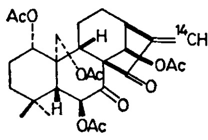
(4a) $R=CH_3$
(4b) $R={}^{14}CH_3$



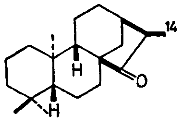
(5)



(6)



(7)



(8)

Specific activity

	Enmein diacetate (**1b**) dpm/mmol	Oridonin tetra-acetate (**7**) dpm/mmol
(**3b**)	$1{\cdot}29 \times 10^4$	$1{\cdot}04 \times 10^4$
(**3e**)	$3{\cdot}87 \times 10^4$	
(**3f**)	$4{\cdot}12 \times 10^4$	
(**2b**)		$3{\cdot}60 \times 10^4$

These results exclude pathways (ii) and (iii) for functionalisation at C-15 of the kaurene nucleus, and support direct oxygenation at the allylic position of *ent*-16-kaurene (**3a**) [path (i)]. The higher incorporation of the ketone (**3f**) suggested it was an important precursor in the earlier stages of the biosynthesis of enmein from (**3a**). The ketone (**3f**) may be formed *via* hydroperoxidation from the less hindered α-side of (**3b**) followed by dehydration. The observed incorporation of the 15β-ol (**3e**) into enmein probably occurs *via ent*-15-oxokaurene.

Ozonolysis of the radioactive enmein diacetate (**1b**), in which (**3b**) or (**3f**) had been incorporated, led to nearly quantitative recovery (93·8 and 98·5% for enmein, respectively) of the radioactivity from C-17 as formaldehyde, demonstrating specific incorporation in both cases. In contrast, the recovery of radioactivity from (**1b**), in which (**3e**) had been incorporated, was 58·1%. Reinvestigation of the 3-monoacetate (**1c**) gave the same result, showing a recovery of 57·4%. Generally, separation of pure dihydroenmein and enmein from the mixture is very difficult. *Isodon japonicus* plants have been shown to contain enmein uncontaminated by dihydroenmein.[6] N.m.r. investigations of radioactive enmein diacetate gave no evidence for contamination by dihydroenmein diacetate, but low localization of activity at C-17 was observed in this case. We suggest the following explanation for this. (i) The 15β-ol (**3e**) was converted into dihydroenmein *via* [17-^{14}C]-*ent*-15-oxokaurane (**8**) derived enzymatically or non-enzymatically by a garryfoline–cuauchichicine type rearrangement.[2] (ii) The content of dihydroenmein was so low that it was not detected by n.m.r. investigations. (iii) Dihydroenmein has a higher specific activity than enmein.

(*Received*, 11*th June* 1974; *Com.* 678.)

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† In these cases, dilution with the corresponding non-labelled compounds was used, because of the small amounts of the corresponding diterpene present.

[1] T. Fujita, S. Takao, and E. Fujita, *J.C.S. Chem. Comm.*, 1973, 434.
[2] M. F. Barnes and J. MacMillan, *J. Chem. Soc.* (*C*), 1967, 361.
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