

BIOSYNTHESIS OF DAPHNIPHYLLUM ALKALOIDS

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(Received in Japan 10 January 1973; received in UK for publication 30 January 1973)

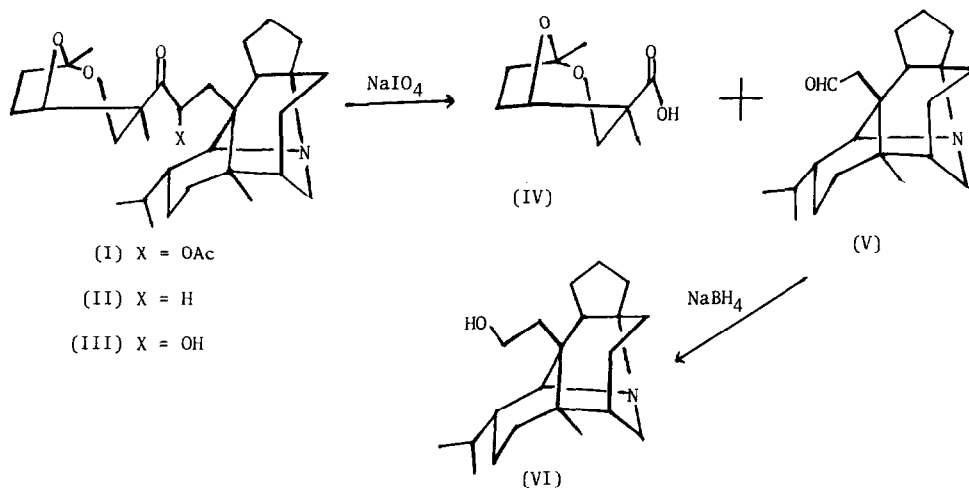
Recently, many alkaloids have been isolated from Daphniphyllum macropodum Miq.,<sup>1</sup> and are mainly divided into four types: daphniphylline<sup>2</sup> and daphmacrine<sup>3</sup>, secodaphniphylline<sup>4</sup>, daphnilactone-B<sup>5</sup> and yuzurimine<sup>6</sup>. In the present paper, the biosynthesis of daphniphylline, a main alkaloid of Daphniphyllum macropodum Miq., is described.

Alkaloidal components as well as the amounts varied with season, and the highest incorporation of DL-mevalonic acid (MVA) into daphniphylline (I) was recorded in June and July. A typical example of the feeding experiment is as follows. A fresh spray of Daphniphyllum macropodum Miq. with 60-70 leaves was immersed in an aqueous solution (15 ml) of DL-[2-<sup>14</sup>C]MVA (50  $\mu$ Ci) at room temperature. After the aqueous solution had been absorbed by the plant, suitable amounts of water to be absorbed were added to it for 12 days (ca. 80 ml/day). Finally, the alkaloidal components were isolated according to the procedure reported,<sup>2</sup> and then by preparative tlc (Kiesel gel GF<sub>254</sub> nach Stahl in Et<sub>2</sub>O-n-hexane-Et<sub>2</sub>NH (20 : 20 : 1)) to give an inseparable mixture of daphniphylline (I) and codaphniphylline (II),<sup>2</sup> which was diluted with small amounts of unlabelled daphniphylline and codaphniphylline (ca. 50 mg). The mixture was then treated with 2N NaOH in methanol and water (1 : 1) followed by preparative tlc to afford the unchanged codaphniphylline (II) and desacetyl daphniphylline (III) in pure states [II, 1.41 x 10<sup>5</sup> dpm (total incorporation,

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0.13%); III,  $1.62 \times 10^5$  dpm (total incorporation, 0.14%)]<sup>2</sup>. For the degradation studies, further dilution with unlabelled desacetyl daphniphylline afforded the pure ketal III with constant specific activity ( $1.95 \times 10^5$  dpm/mM), which was oxidized with  $\text{NaIO}_4$  to the ketal acid IV and the aldehyde V. The latter was subsequently reduced with  $\text{NaBH}_4$  to daphnialcohol (VI), as shown below.<sup>2</sup>

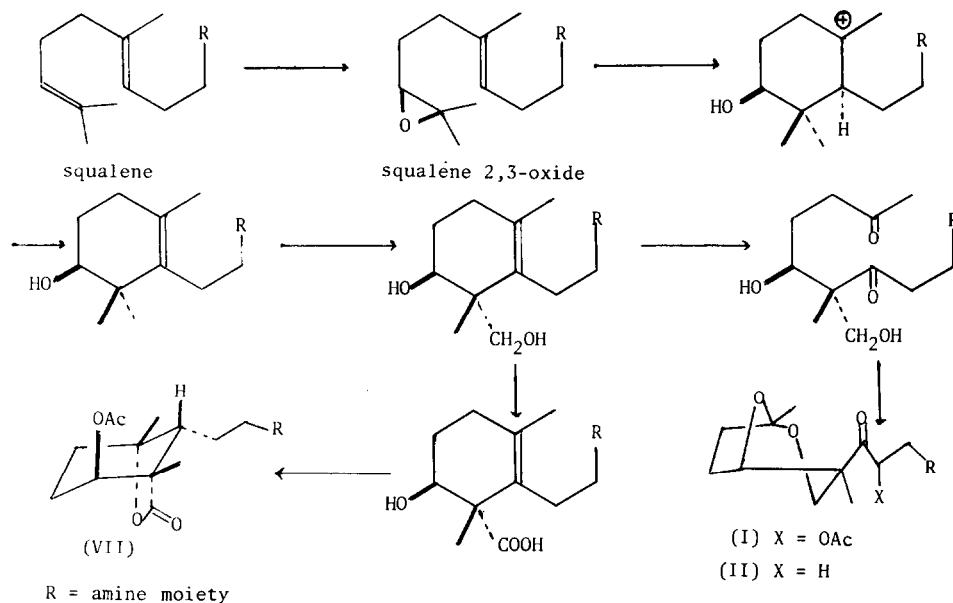


Source	(III)	(IV)	(VI)	(VI)/(IV)
DL-[2- <sup>14</sup> C]MVA	$1.95 \times 10^5$ dpm/mM	$0.65 \times 10^5$ dpm/mM	$1.30 \times 10^5$ dpm/mM	2.0
DL-[5- <sup>14</sup> C]MVA	$4.37 \times 10^4$ dpm/mM	$0.69 \times 10^4$ dpm/mM	$3.52 \times 10^4$ dpm/mM	5.1

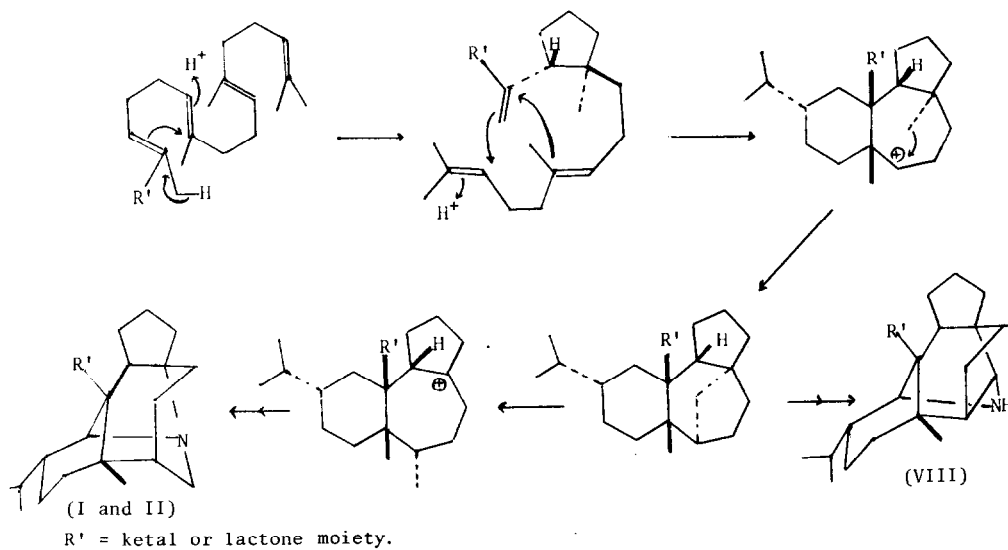
Furthermore, when (3R, 4R and 3S, 4S)-[4-<sup>3</sup>H]MVA (100  $\mu\text{Ci}$ ) and DL-[2-<sup>14</sup>C]MVA (50  $\mu\text{Ci}$ ) were fed to the plant, doubly labelled desacetyl daphniphylline was obtained, in which the value of  $2 \times {}^{14}\text{C}/{}^3\text{H}$  was ca. 1.27 (<sup>3</sup>H,  $8.40 \times 10^4$  dpm/mM; <sup>14</sup>C,  $5.30 \times 10^4$  dpm/mM).

Accordingly, five tritium atoms must be incorporated into daphniphylline as expected. Finally, when an aqueous solution of <sup>14</sup>C-labelled squalene<sup>7</sup> (2.5  $\mu\text{Ci}$ ) emulsified with Tween 80 was fed to the plant, the total incorporation of it into daphniphylline and co-daphniphylline was 0.008%.

From the above feeding experiments, daphniphylline (I) and codaphniphylline (II) must be biosynthesized from six MVA molecules through a squalene-like intermediate. Particularly, in the light of the co-occurrence of daphmacrine (VII)<sup>3</sup>, the ketal moiety of I must be formed as follows.



On the other hand, the amine moiety of I (or II) is complicated. However, in connection with the co-occurrence of secodaphniphylline (VIII)<sup>1,4</sup> the carbon skeleton of the amine moiety seems to be constructed as described below. Further degradation studies



are now in progress.

The authors wish to thank Professor Reiji Okazaki and Mr. Akio Sugino (Institute of Molecular Biology, Nagoya University) for scintillation measurements. We also wish to express our appreciation to Professor Shigeo Nozoe (Institute of Applied Microbiology, University of Tokyo) for his enlightening discussions and to Mr. Hisayoshi Kobayashi (same Institute) for his assistance in preparing labelled squalene.

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7.  $^{14}\text{C}$ -Labelled squalene was prepared enzymatically from DL-[2- $^{14}\text{C}$ ]MVA; A. Kawaguchi, J. Biochemistry (Tokyo), 67, 219 (1970).