

CONVERSION OF 6-PROTOILLUDENE INTO ILLUDIN-M AND -S BY OMPHALOTUS OLEARIUS

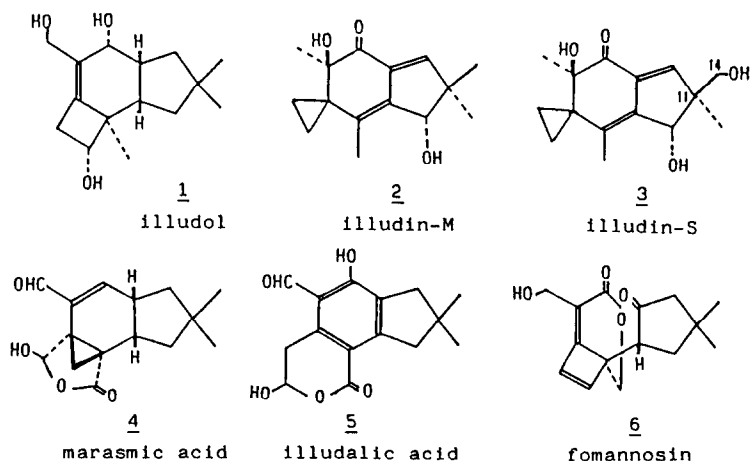
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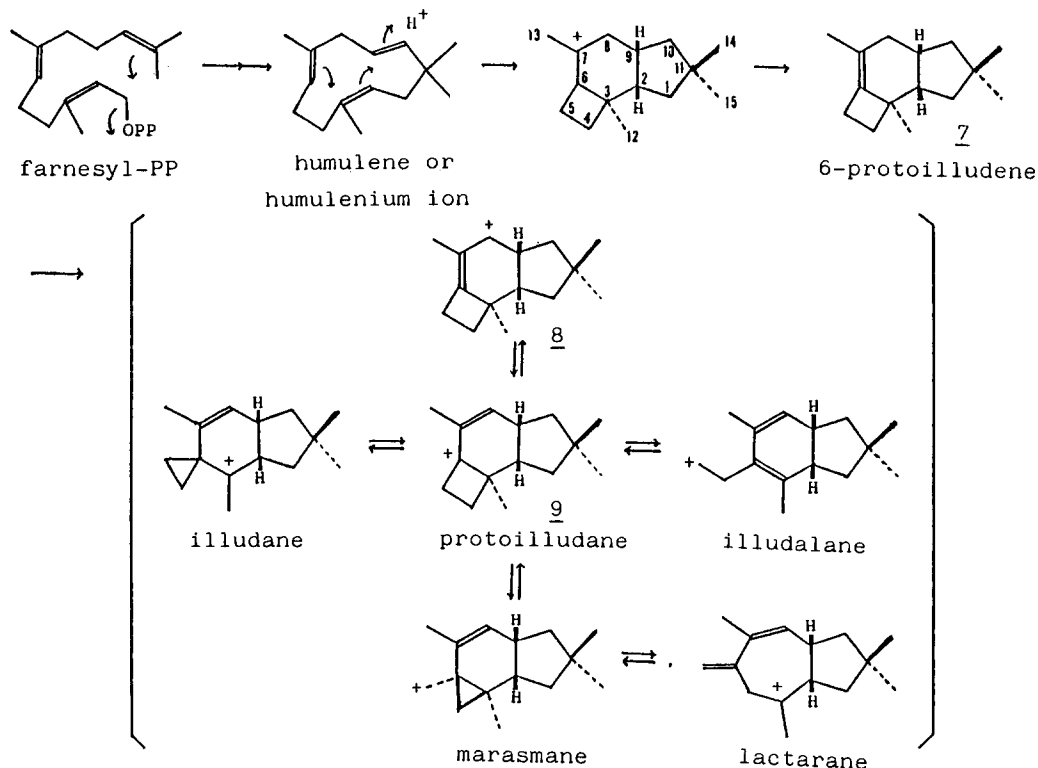
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Summary: Deuterium labeled dl-6-protoilludene (7) was synthesized and fed to the illudin-producing fungus Omphalotus olearius (ATCC 11719). The hydrocarbon (7) was incorporated into illudin-M (2) and -S (3).

A group of sesquiterpenes with unique carbon skeletons such as protoilludanes (i.e., illudol 1), illudanes (i.e., illudin-M 2 and -S 3), marasmanes (i.e., marasmic acid 4) and illudalanes (i.e., illudalic acid 5) are produced by Basidiomycetes.<sup>1)</sup> Metabolites formed by further rearrangement (i.e., lactaranes) or C-C bond cleavage (i.e., fomannosin 6) are also known. We isolated 6-protoilludene 7 as a probable precursor of these compounds from the mycelia of Fomitopsis insularis<sup>2)</sup> and Omphalotus olearius. In addition to 7, F. insularis produces fomannosin and various metabolites belonging to the group, i.e., protoilludanes, marasmanes, illudalanes and lactaranes<sup>3)</sup>, and O. olearius produces illudin-M (2) and -S (3).

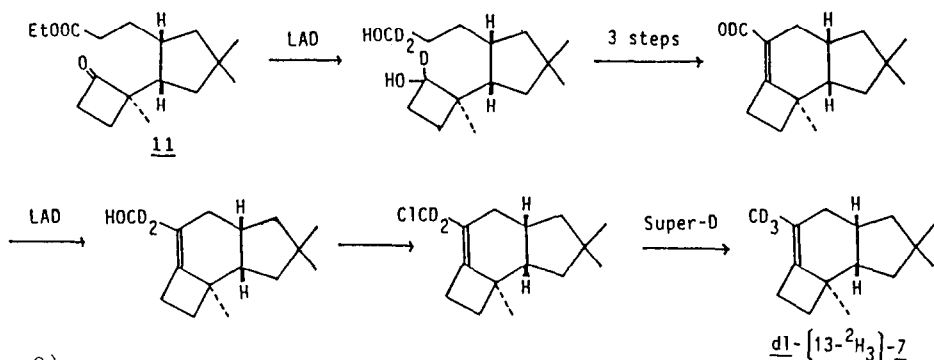
It has been postulated that these sesquiterpenes are formed biogenetically from farnesylpyrophosphate via an 11-membered intermediate, humulene or





humulenium ion, and that cyclization of this intermediate followed by deprotonation would afford the hydrocarbon 7.<sup>2)</sup> Allylic carbonium ions, such as 8 and/or 9 which presumably are generated from the hydrocarbon by the introduction of a functional group such as hydroxy group at C-8 followed by solvolytic removal of the group, would undergo the cyclobutyl-cyclopropylcarbinyl-homoallyl cation rearrangement to form illudane, marasmane and illudalane carbon skeletons.<sup>4)</sup>

In order to prove the intermediary role of hydrocarbon 7 in the biosynthesis of these sesquiterpenes, we synthesized  $[13-^2\text{H}_3]$ -6-protoilludene (7) and fed it to the growing mycelium of *O. olearius* (ATCC 11719). This fungus, when cultured in medium containing sodium glutamate,<sup>5)</sup> produced illudin-M (2) and -S (3) in better yield and in less time than that previously reported.<sup>6)</sup> The feeding substrate  $d_1$ - $[13-^2\text{H}_3]$ -7 was synthesized by a route<sup>7)</sup> in which  $\text{LiAlD}_4$  and super-deuteride were used for deuterium labeling. Starting from 490 mg of ketoester 11, 48 mg of labeled 7 was obtained ( $m/z$  207:206=100:4). The feeding experiment was carried out as follows. Mycelium of *O. olearius* (ATCC 11719), precultured for 16 days at 27°C on a mixture of rice bran and beech shavings (1:2) wetted with a solution containing glucose (2%) and yeast extract (0.5%), was inoculated in 100 ml of sodium glutamate-containing medium<sup>8)</sup> in a 1 L Roux bottle at 27°C. Half of a mixture of labeled 7 (46 mg), Tween 80 (15 mg) and water (1 ml) was administered to a 12-day-old surface



culture<sup>9)</sup> and the rest was fed two days later. After another 4 days, the culture filtrate was extracted with EtOAc (EtOAc-extract: 260 mg). Both illudin-M (2) and -S (3) were separated by silica gel column chromatography. Illudin-M (2) (crude, 110 mg) was further purified by recrystallization from EtOH-H<sub>2</sub>O (mp 180-181°C). Illudin-S (crude, 50 mg) was isomerized to isoilludin-S with Al<sub>2</sub>O<sub>3</sub><sup>10)</sup> and then recrystallized from AcOEt (mp 130-131°C). The methyl group on C-8 of isoilludin-S (10) is known to migrate from C-7 of illudin-S.<sup>6,10)</sup>

The <sup>2</sup>H-NMR spectra of these samples, i.e. illudin-M (2) and isoilludin-S (10), showed that deuterium was enriched only at the methyl group on C-7 in illudin-M (2) ( $\delta$  1.33, CHCl<sub>3</sub> solution) and at the methyl on C-8 in isoilludin-S (10) ( $\delta$  1.50, CHCl<sub>3</sub> solution). This clearly shows that the hydrocarbon 6-protoilludene was converted into illudin-M (2) and -S (3). The deuterium content of partially deuterated illudin-M (2) and -S (3) was calculated by

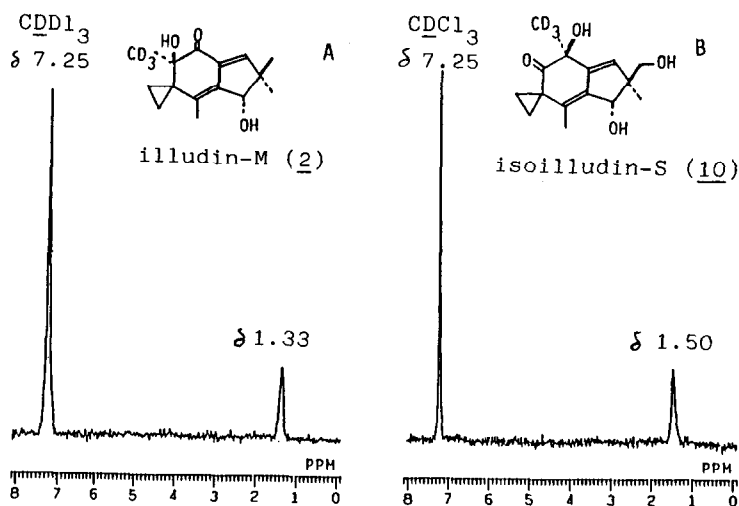


Figure 1. 60 MHz <sup>2</sup>H-NMR spectra of illudin-M and isoilludin-S derived from deuterated 7

A. illudin-M (12 mg) in CHCl<sub>3</sub> (620 mg), 28000 scans

B. isoilludin-S (4.5 mg) in CHCl<sub>3</sub> (545 mg), 95000 scans

comparing the integration of the  $^2\text{H}$ -NMR signal of the methyl group ( $\delta$ 1.33 or  $\delta$ 1.50) with that of deuterium which exists in natural abundance, 0.015%, in chloroform used as the solvent for the  $^2\text{H}$ -NMR measurements. The ratio,  $\text{CD}_3/\text{CDCl}_3$ , was 0.31 for illudin-M (2) and 0.57 for illudin-S. The conversion ratio of 7 to illudin-M (2) and -S (3) was calculated to 0.59% and 1.2%, respectively.

Thus, 6-protoilludene (7) was shown to be the biosynthetic precursor of illudin-M (2) and -S (3). The hydrocarbon 7 may also be the precursor of marasmanes and illudalanes via the cation 9.

The study on the incorporation of [ $1,2\text{-}^{13}\text{C}$ ] acetate into (-)-illudin-S (3) showed that  $\text{CH}_2\text{OH}$  on C-11 of 3 was derived from  $\text{CH}_3$  of R-MVA.<sup>6e</sup> Therefore, pro-R- $\text{CH}_3$  (C-14) of 6-protoilludene (7) should also be derived from  $\text{CH}_3$  of R-MVA. This would be satisfied when 2S,3R,9S-enantiomer (7) is the precursor of illudin-M (2) and -S (3) on considering the cyclization mechanism of FPP to 6-protoilludene (7). Biosynthesis of fomannosin (6)<sup>11</sup> also supports this conclusion.

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#### REFERENCES AND NOTES

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8. Medium containing (per litre) glucose (40 g), cornsteep liquor (5 g),  $\text{KH}_2\text{PO}_4$  (1 g), KCl (0.5 g),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.5 g), dry yeast (5 g), Na glutamate (10 g).
9. The production of illudin-M (2) and -S (3) began to increase about this time and continued for about ten days.
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