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ISOLATION OF Δ^6-PROTOILLUDENE AND THE RELATED ALCOHOLS

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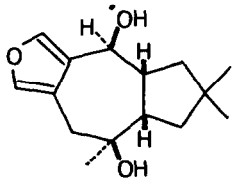
From the cultured mycelium of *Fomitopsis insularis*, a fungus which produces fomannosin (3) at a latter stage of fermentation period, we isolated furanoid sesquiterpenes having the structures 1 and 2[1,2]. Subsequently, several minor metabolites possessing protoilludane and marasmane skeletons have also been isolated from the same fungus[3]. We have been interested in their biogenetical relationship and also isolating a common precursor of these metabolites. In the present paper, we describe the isolation and characterization of Δ^6-protoilludene (4), a possible precursor of these metabolites, and also of other substances which are assumed to be biosynthetically significant.



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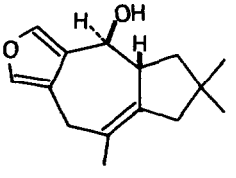

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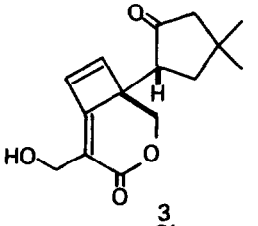

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Cultures of *Fomitopsis insularis* were grown under the shaking conditions in 500 ml. Erlenmyer flasks containing glucose-yeast extract medium (100 ml./flask), which was seeded with a precultured mycelium. After six days growth the mycelium was collected by filtration (ca. 70 g. of wet mycelium/ 1. of culture liquid) and extracted with acetone and then with n-hexane repeatedly. The crude extract (ca. 320 mg./1. of culture liquid) was chromatographed over silica gel eluting with n-hexane to afford a hydrocarbon mixture which was found to

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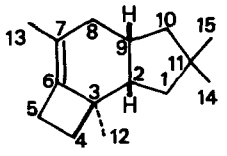
contain sesquiterpene hydrocarbon, $C_{15}H_{24}$ (r.t. 3.8 min, m/e 204)[4a], as a main component and squalene (r.t. 18.3 min)[4a]. The sesquiterpene hydrocarbon was then collected by preparative glc to give a pure sample of 4 (ca. 4 mg./l. of culture liquid); r.t. 12.73 min[4b], R_f= 0.57 (n-pentane/Merck silica gel plate); α_D -52° (MeOH, c=0.5); ir(CCl_4), 1370,1385,1462,1440, 1310 cm^{-1}; uv(MeOH), end absorption; nmr ($CDCl_3$,δ) 0.93 (3H, s), 1.05(6H, s) and 1.56 (3H, s). Its mass spectrum showed peaks at 204 (M^+, 23.6 %), 185 (M-15, 19.3 %), 176 (M-28, 12.9 %),175 (M-29, 91.4 %) and 119 (base peak). The proton-noise decoupled spectrum of ^{13}C nmr of 4 ($CDCl_3$, 25.05 MHz) exhibited 15 lines which contained the signals due to two sp^2 carbons at 123.1 and 141.8. Other signals appeared at 17.3, 20.5, 25.5, 27.5, 29,9, 34.2, 36.9, 39.2, 40.7, 41.1, 45.8, 47.2, and 48.7, of which assignments are under study. Treatment of 4 with Pd-C catalyst in MeOH under an atomosphere of hydrogen (usual hydrogenation condition) afforded an isomerized product 5 ; r.t. 10.92 min[4b], R_f=0.63 (n-pentane/silica gel) instead of a hydrogenated product. This compound showed nmr signals ($CDCl_3$,δ) at 0.98 (6H, s), 1.17 (3H, s), 1.60 (3H, s), 5.13 (1H, bs) and mass fragmentation peaks at m/e 204 (M^+, 23 %), 189 (M-15, 18 %), 176 (M-28, 43 %), 161 (59 %), 148 (23 %), and 108 (base peak). This compound was identified as 5 by a direct comparison with an authentic sample which has been synthesized by Matsumoto et. al.[5] This conversion and the physico-chemical properties mentioned above confirmed that the hydrocarbon possesses the structure of Δ^6-protoilludene 4.



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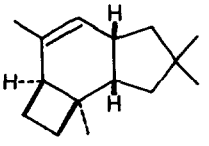

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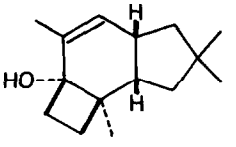

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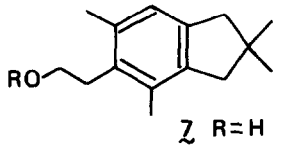
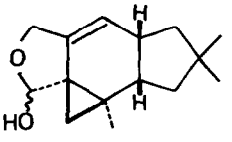


7 R=H

8 R=CHO



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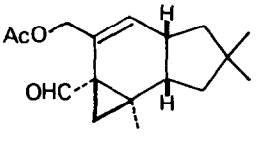

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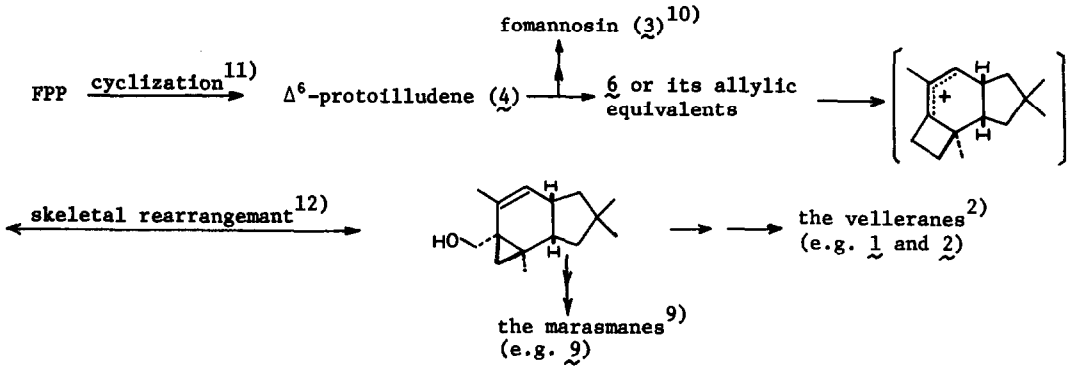
We have observed that Δ^7- and $\Delta^{7(13)}$-isomers of protoilludene were not present in the mycelial extracts of this fungus.

Gc-ms analysis[6] of the metabolites which was extracted from 14-days-old mycelium of same fungus (still-culture) revealed the presence of two new alcohols having the molecular formula of $C_{15}H_{22}O$ and $C_{15}H_{20}O$ among the variety of metabolites in higher oxidation levels. These alcohols (6 and 7) were isolated as a pure form by repeated column chromatography. The compound 6; r.t. 3.2 min[4c], R_f=0.40 (benzene:ether/9:1, silica gel); ir ($CHCl_3$), 3600, 2880, 1451, 1052, exhibited the nmr signals ($CDCl_3$, δ) at 0.95 (3H, s), 0.99 (3H, s), 1.20 (3H, s), and 1.73 (3H, bs) due to four methyl groups and a methine proton signal at 2.61 (1H, m) and an olefinic proton signal at 5.24 (1H, m). The mass spectrum of 6 showed the peaks at m/e 220 (M^+, 7.3 %), 205 (M-15, 2.2 %), 202 (M-18, 2.9 %), 192 (M-28, 64 %), 177 (M-43, 20%), 135 (base peak), and 124 (95 %). The more polar compound 7; r.t. 15.8 min[4c], R_f=0.19 (benzene;ether/9:1), uv (MeOH); 218 nm (ε, 7000), 272.5 (860), 277 (740), 282 (890); ir($CHCl_3$); 3620, 2945, 2860, 1460, 1382, 862 cm^{-1} , showed the nmr signals at 1.13 (6H, s), 1.55 (1H, exchangeable with D_2O), 2.20 (3H, s), 2.30 (3H, s), 2.65 (4H, m), 2.93 (2H, t, J=7.5 Hz), 3.74 (2H, t, J=7.5 Hz), and 6.83 (1H, s). The mass spectrum of 7 exhibited the peaks at m/e 218 (M^+, 15.5 %), 188 (M-30, 16.7 %), 187 (M-31, base peak), 173 (M-45, 3.8 %), and 157 (8.3 %). Formolysis of the alcohol 6 with anhydrous formic acid in tetrahydrofuran afforded the formate 8; m/e 246 (M^+, $C_{16}H_{22}O_2$) which was hydrolyzed to yield the compound 7 whose structure was confirmed by a direct comparison with a synthetic sample.[7] The above data indicated that the former is Δ^7-protoilluden-6-ol (6). This compound contains same allylic alcohol system with that of neoilludol which has recently been isolated from *Clitocybe illudens*[8]. Beside this, the compound 9 with a marasmane skeleton[9] was also obtained from same fraction as a minor component; m/e 234 (M^+, $C_{15}H_{22}O_2$), ir, 3045, 3020, 1175, 1015 cm^{-1}; acetate 10, m/e 276 (M^+, $C_{17}H_{24}O_3$), uv (MeOH); 220 nm (ε, 6300), ir, 2700, 1710, 3020 cm^{-1}; nmr ($CDCl_3$,δ), 1.01 (6H, s), 1.16 (3H, s), 1.77 (1H, d, J=4.0 Hz), 1.97 (3H, s), 4.57 and 4.95 (2H, ABq, J_{AB}=12.0 Hz), 5.27 (1H, d, J=3.0 Hz), and 9.57 (1H, s).

Our present views of the role of the hydrocarbon 4 and the alcohol 6 in the biosynthesis of the compounds 1, 2, 3, and 9 is summarized as follows. Biosynthetic investigations along this line are in progress.



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3. Presented at 92th Annual Meeting of the Pharmaceutical Society of Japan, April 1972. The details will be given in a full report.
4. Gc analyses were performed on a Shimadzu GC4C fitted with; a)1.5 m × 4 mm, OV-17;100°→280°C, 10°/min; b) 30 m × 0.3 mm glass capillary column, 1% OV-17, 90°C, 0.5 kg/cm^2; c) 1.5 m × 4 mm, 1.5% OV-17, 140°C.
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6. Gc-ms analysis was performed on an LKB-9000 fitted with 1.5 m, 1.5 % OV-17 column.
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11. For cyclization, see J.R.Hanson, T.Marten and R.Nyfeler, J. Chem. Soc. Perkin-1, 876(1976).
12. Cf. K.B.Wiberg, J.E.Hiatt and K.Hseih,J. Am. Chem. Soc., 92, 544 (1970); K.B.Wiberg, J.G. Pfeiffer, ibid., 92, 553 (1970).