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A 4,5-Secolanostane Triterpenoid from the Sclerotium of *Poria cocos*

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A 4,5-*seco*-lanostane triterpenoid, daedaleanic acid A, together with six known compounds, was isolated for the first time from the sclerotium of *Poria cocos* (Schw.) Wolf (Polyporaceae). Their structures were identified by comparing their spectral data with the literature values or that of authentic samples.

Key word: *Poria cocos*; *4,5-seco*-lanostane; daedaleanic acid A

Fu-Ling, or Hoelen, the dried sclerotium of the fungus *Poria cocos* (Schw.) Wolf (Polyporaceae) devoid of its surface layer, has been used in combination with other drugs in more than 2000 traditional Chinese prescriptions that are generally used as a diuretic, sedative, diabetic, and tonic medicines in varied dosage forms.[1] In addition, it induces relaxation of the intestine, protects against ulcer formation, reduces the acidity of gastric juice and has antinephritic and anti-emetic effects.[2-3] It is also reported to have an antineoplastic effect and anti-inflammatory activity.[2-4] Note, in China and Japan, the surface of the sclerotia is usually removed and only the inner part is used as "Fu-Ling". It has been reported to contain several triterpenoids of the lanostane type isolated from the inner part and 3,4-*seco*-lanostane type from the surface part of *P. cocos*.[5-11] Being interested in exploring biologically active components from Chinese herbs, we examined the polar fraction of this fungus.

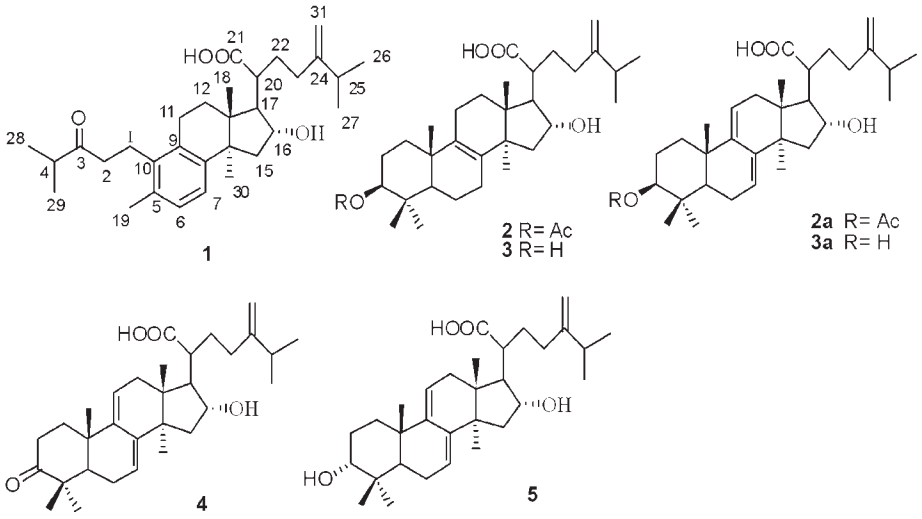

Fig. 1 Structure of triterpenoids isolated from *Poria cocos*.

The MeOH-soluble parts of the 95% ethanol extract in the dried sclerotium of the fungus *P. cocos* were subjected to silica gel column chromatography, yielding seven known triterpenoids. The known compounds (see Figure 1) were identified as daedaleanic acid A (**1**),[13] pachymic acid (**2**),[5,6] tumulosic acid (**3**),[5,6] polyporenic acid C (4),[5] 3-epidehydrotumulosic acid (**5**),[7] a mixture of pachymic acid (**2**) and dehydropachymic acid (**2a**),[5,6] a mixture of tumulosic acid (**3**) and dehydrotumulosic acid (**3a**)[5,6] by comparing their spectral data with the reported values or with that of authentic samples.

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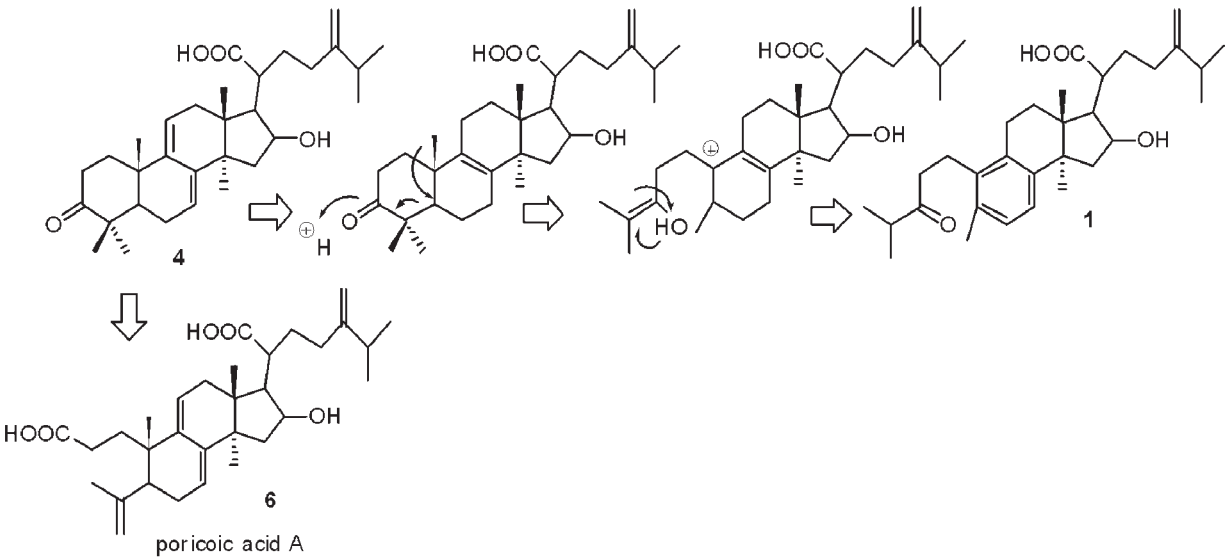

Fig. 2 Postulated biogenetic pathways of **1** from polyporenic acid C (**4**).

Note, the novel 4,5-*seco*-lanostane triterpenoid, daedaleanic acid A (**1**), previously only 3,4-*seco*-lanostane triterpenes as poricoic acid A (**6**) reported from the surface layer of *P. cocos*,[7] was isolated for the first time from this fungus[12] and independently identified by Yoshikawa's laboratory, isolated from *Daedalea dickisii*.[13] The possible biogenetic pathway of daedaleanic acid A (**1**) may be converted from polyporenic acid C (**4**) in figure 2.

The structure of daedaleanic acid A (**1**) was further supported by mass spectrum, a base peak at *m/z* 449 [M^+-CH_3-H_2O] and prominent peaks at *m/z* 467 [M^+- CH_3], 464 [M^+-H_2O], 403 [M^+-CH_3-H_2O-HCOOH], and 363 [M^+-CH_3-H_2O-$CH_2COCH(CH_3)_2$] also indicated compound **1** has a hydroxy-4,5-*seco*-lanostane skeleton as well as a benzene moiety in ring B, as depicted in figure 3.

Extraction and Isolation: The dried sclerotium of the fungus *P. cocos* (10.0 kg) was extracted with 95% ethanol (50 L×2) at room temperature. The combined extract was concentrated under reduced pressure to yield a brown syrupy mass (80.71 g). This crude extract was dissolved in 95% MeOH/H_2O and then partitioned (1:1) with *n*-hexane to obtain *n*-hexane soluble fraction (2.13 g). The 95% MeOH layer was concentrated to obtain a MeOH-soluble fraction (78.58 g). The MeOH soluble fraction was subjected to column chromatography over a silica gel (70-230 mesh) using CH_2Cl_2-MeOH step gradient mixtures (0-20%) as eluents yielded pachymic acid (**2**, 0.43 g), tumulosic acid (**3**, 1.68 g), a mixture of pachymic acid (**2**) and dehydropachymic acid (**2a**), a mixture of tumulosic acid (**3**) and dehydrotumulosic acid (**3a**), polyporenic acid (**4**, 0.35 g), 3-epidehydrotumulosic acid (**5**, 0.38 g) and daedaleanic acid A (**1**, 7.0 mg).

Daedaleanic acid A (1): white powder; $[\alpha]_D^{26}$ +14.0° (*c* 0.4, pyridine); UV (MeOH) λmax (log ε): 271 (4.20) nm; IR (KBr) νmax: 3407, 2957, 1705, 1690, 1638, 1457, 1380, 1266, 1071, 1014, 890, 814 cm^{-1}; EI-MS *m/z* (rel. int. %) 482 ($[M]^+$, 12), 464 (13), 449 (100), 431 (24), 421 (15), 403 (37), 396 (23), 378 (16), 295 (35), 270 (23), 222 (26), 207 (25), 183 (48), 169 (37), 157 (28), 143 (20), 69 (18), 55 (30); ^{1}H NMR (C_5D_5N, 500 MHz) δ 0.98 (3H, d, *J* = 6.3 Hz, H-26), 0.99 (3H, d, *J* = 6.3 Hz, H-27), 1.02 (6H, d, *J* = 7.2 Hz, H-28, 29), 1.03 (3H, s, H-18), 1.58 (3H, s, H-30), 2.25 (3H, s, H-19), 2.91 (1H, m, H-20), 2.95 (1H, m, H-17), 4.62 (1H, br s, H-16), 4.84, 4.98 (each 1H, brs, H-31), 6.95 (1H, d, *J*= 7.7 Hz, H-7), 7.06 (1H, d, *J* = 7.7 Hz, H-6); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 213.4 (s, C-3), 178.6 (s, C-21), 156.1 (s, C-24), 145.9 (s, C-8), 138.0 (s, C-10), 133.3 (s, C-9), 133.1 (s, C-5), 128.1 (d, C-6), 123.1 (d, C-7), 107.0 (t, C-31), 76.7 (d, C-16), 57.3 (d, C-17), 49.4 (s, C-14), 48.7 (d, C-20), 45.4 (s, C-13), 45.1 (t, C-15), 40.9 (d, C-4), 39.1 (t, C-2), 34.1 (d, C-25), 33.2 (t, C-23), 31.4 (t, C-22), 29.7 (t, C-12), 29.3 (q, C-30), 23.8 (t, C-1), 23.4 (t, C-11), 22.0 (q, C-26), 21.9 (q, C-27), 19.7 (q, C-19), 18.4 (q, C-29), 18.3 (q, C-28), 18.0 (q, C-18); HREIMS *m/z* 482.3386 (calcd for $C_{31}H_{46}O_4$ $[M]^+$ 482.3396).

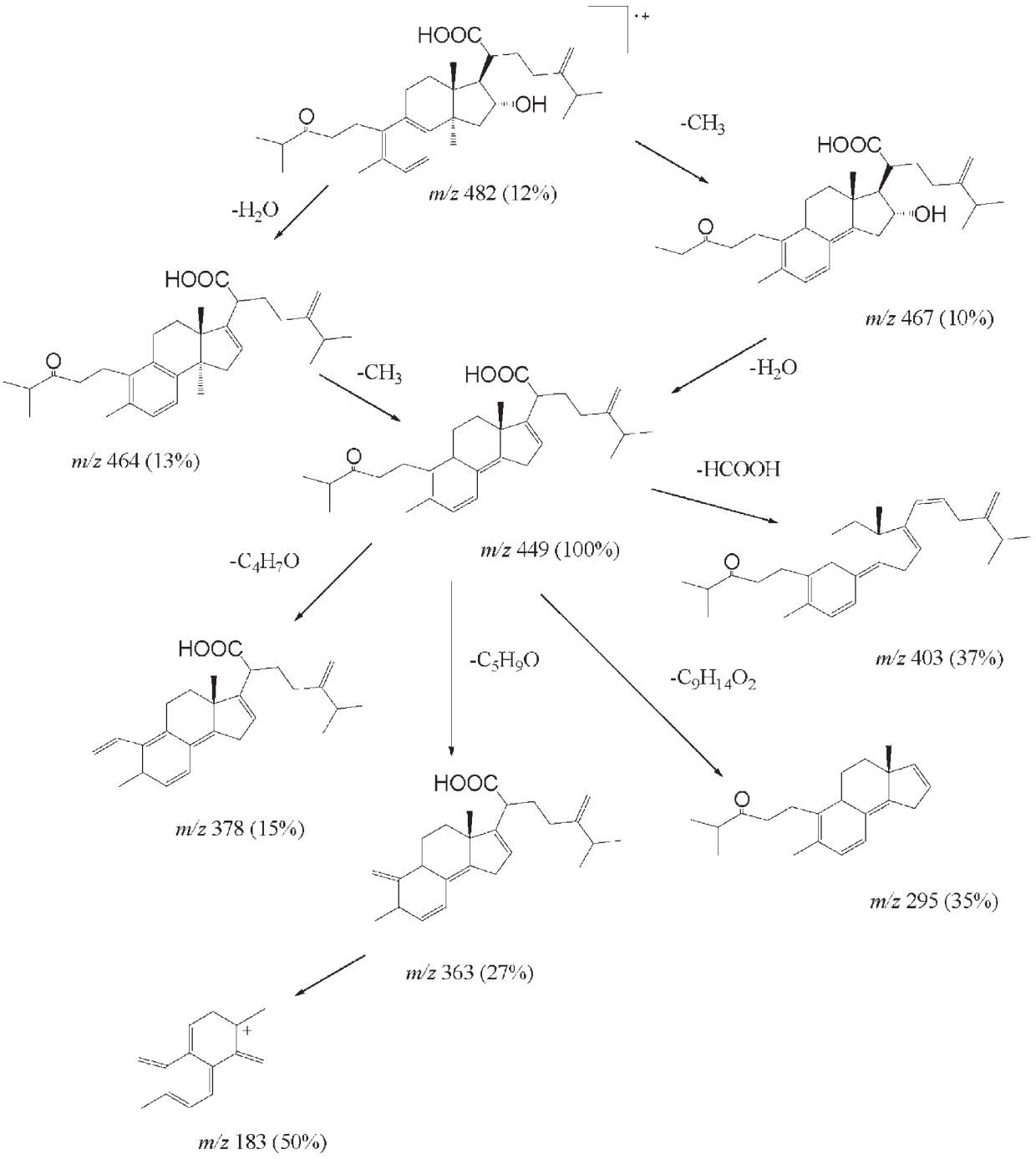

Fig. 3 Major fragmentation process for compound **1**.

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