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雪茶化学成分研究

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摘要：由管枝衣科地茶属植物雪茶（*Thamnolia vermicularis*）丙酮提取物中分离得到10个成分，通过波谱分析及与已知品对照等方法，最终确定其中的9个化合物分别为：Thamnolin（**1**），鳞片衣酸（**2**），坝巴酸（**3**），羊角衣酸（**4**），3β-羟基-5α，8α-桥二氧麦角甾-6，22-二烯（**5**），3β-羟基-5α，8α-桥二氧麦角甾-6，9，22-三烯（**6**），麦角甾烷-7，22-二烯-3-醇（**7**），麦角甾烷-5，8，22-三烯-3-醇（**8**），亚油酸（**9**）。其中，化合物**1**为新化合物，化合物**3**和**6**～**9**系首次由该种植物中分离得到。研究结果初步明确了云南中甸地区产雪茶为主要含有坝巴酸（**3**）和羊角衣酸（**4**）等酚性成分，而非主含地茶酸等酚性成分的植物品种类型，这为合理开发利用当地植物资源提供了科学依据。

关键词：雪茶；地衣；化学成分；酚性化合物；甾体化合物；Thamnolin
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Constituents from *Thamnolia vermicularis*

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Abstract: From the acetone extract of *Thamnolia vermicularis* (Sw.) Ach., a new phenolic compound, thamnolin (**1**), was isolated, along with eight known constituents, squamatic acid (**2**), barbatinic acid (**3**), baeomycesic acid (**4**), 5, 8-epidioxy-5α, 8α-ergosta-6, 22*E*-dien-3β-ol (**5**), 5, 8-epidioxy-5α, 8α-ergosta-6, 9, 22*E*-tien-3β-ol (**6**), 5α-ergosta-7, 22*E*-dien-3β-ol (**7**), ergosta-5, 8, 22*E*-trien-3β-ol (**8**) and linoleic acid (**9**). Their structures were determined by spectral methods, and compounds **3** and **6**～**9** were obtained from this plant for the first time. According to the phenols mentioned above, *T. vermicularis* distributed in Zhongdian, Yunnan, should be ascribed to the species which mainly contained barbatinic acid and baeomycesic acid instead of thamnolic acid. The conclusion was obviously useful for the utilization of this plant.

Key words: *T. vermicularis*; Lichen; Chemical constituents; phenols; steroids

Thamnolia vermicularis (Sw.) Ach., an algo-fungus symbiont with a commercial name

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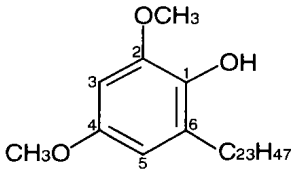
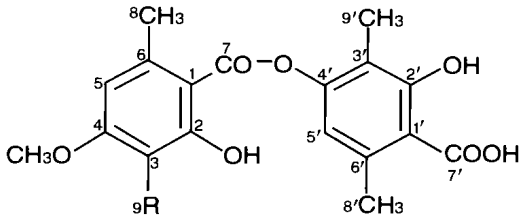
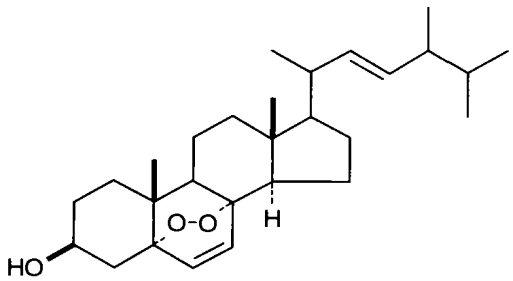
"Xuecha" in China, has long been used for medicinal purposes in common people of southwest China to cure sore throats, hypertension, cough caused by lung-heat, tidal fever due to *yin* deficiency, summer-heat and neurasthenia (Xie *et al*, 1996). It was reported previously that several phenolic compounds, thamnolic acid (Wachtmeister, 1955), vermicularin and baemycesic acid (Sun, 1985), had been isolated from this plant, and some of those isolates had been identified to be the main bioactive constituents of the plant (Xie *et al*, 1996; Sun, 1985). In continuation of our research on bioactive constituents from the lichens, we have reinvestigated on the chemical constituents of *T. vermicularis* collected in Zhongdian recently. As a result, a new compound, thamnolin (**1**), together with eight known ones, squamatic acid (**2**), barbatinic acid (**3**), baeomycesic acid (**4**), 5, 8-epidioxy-5α, 8α-ergosta-6, 22*E*-dien-3β-ol (**5**), 5, 8-epidioxy-5α, 8α-ergosta-6, 9, 22*E*-tien-3β-ol (**6**), 5α-ergosta-7, 22*E*-dien-3β-ol (**7**), ergosta-5, 8, 22*E*-trien-3β-ol (**8**) and linoleic acid (**9**), were obtained from the EtOAc extract, and compounds **3** and **6** ~ **9** were isolated from this plant for the first time.



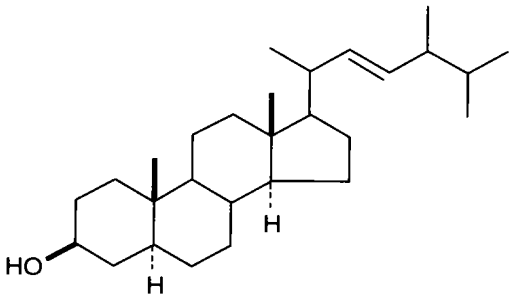
1



2:R=COOH
3:R=CH3
4:R=CHO



5
6:9(11)-ene



7:7-ene
8:5,8(9)-diene

Results and discussion

Thamnolin (**1**), amorphous substance, has a molecular formula of $C_{31}H_{56}O_3$ established by HRE-IMS (obsd 476.4237, calcd 476.4230). From the signals of IR spectrum at 1600, 1580, 1495 cm^{-1}, ^1H NMR at δ 6.33 ~ 6.26 and ^{13}C NMR at δ 96.77 ~ 152.79, combining with the analysis of

its MS spectrum, compound **1** obviously possessed a phenolic skeleton with the substitutes of two methoxy and one alkyl groups. Also observed from the spectra of EIMS and DEPT were that the alkyl was tricosyl, a linear alkyl group, because of the observation of twenty-two methylenes and one methyl in the spectra. The positions of all substitutes were determined by the experiment of HMBC, and the key correlations of HMBC spectrum were shown in Fig. 1. Thus, compound **1** was elucidated as 6-tricosyl-2, 4-dimethoxy-phenol (Jiang *et al*, 2001).

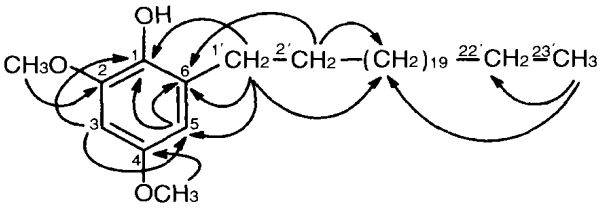


Fig. 1 Key HMBC correlations of compound **1**

Compounds **2** ~ **9** were identified as squamatic acid (**2**), barbatinic acid (**3**), baeomycesic acid (**4**), 5, 8-epidioxy-5α, 8α-ergosta-6, 22*E*-dien-3β-ol (**5**), 5, 8-epidioxy-5α, 8α-ergosta-6, 9, 22*E*-tien-3β-ol (**6**), 5α-ergosta-7, 22*E*-dien-3β-ol (**7**), ergosta-5, 8, 22*E*-trien-3β-ol (**8**) and linoleic acid (**9**), respectively, by comparing their physical and spectral data with those reported in the literature.

According to the type of depside, Asahina Y divided *T. vermicularis* into two species in 1937. One of them contained only thamnolic acid, and the other mainly contained barbatinic acid and baeomycesic acid (Asahina, 1954). Therefore, the plant used in this research should be the latter based on the depsides isolated, which was important to the utilization of *T. vermicularis* distributed in Zhongdian area.

Experimental

General IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. UV spectra were obtained on a UV 210A spectrometer. MS were recorded on a VG Auto Spec-3000 spectrometer. 1D-and 2D-NMR spectra were run on a Bruker AM-400 and DRX-500 instrument with TMS as internal standard, respectively. HPLC were performed on a HEWLETT PACKARD 1100 and SHIMADZU SCL-10A apparatus, respectively.

Plant Material Purchased at Dali city in May, 1997, the plant used in this research was originally collected from Zhongdian prefecture of Yunnan Province, in 1996, and air-dried. The identity of plant material was verified by Prof. Zhong-Wen Lin, and a voucher specimen was deposited in Kunming Institute of Botany, Academia Sinica.

Extraction and Isolation The dried and powdered materials (4.0 kg) were extracted with 75% Me_2CO (3 × 16 L) and filtered. The filtrate was concentrated to yield a pale yellow substance (about 360 g) which was further dissolved with acetone for many times (1.2 L at a time) and then filtered. A portion (1.0 g) of the filter residue (286 g) was subjected to column chromatography on a silica gel column (eluting with the solution of $CHCl_3$-MeOH-H_2O at a proportion of 5:4:0.1) and a RP-18 gel column (eluting with a solution of pyridine/water from 3:7 to 8:2) successively, finally yielding compounds **3** (497 mg) and **4** (261 mg). The acetone filtrate was evaporated *in vacuo* to give a light yellow residue (68 g), which was chromatographed over Si gel (200 – 300 mesh, 1.5 kg) eluting with $CHCl_3$ containing increasing amounts of Me_2CO to yield fractions I-III. Fraction I (250 mg) ws subjected to column chromatograph on Si gel eluting with petroleum ether-chloroform (15:1) to produce **1** (91 mg). After laying up for a short time, compound **3** (some about 17 g) was crystallized from the fraction II, and the mother liquid was separated successively by passage over Si gel

(cyclohexane-isopropanol, 7:1) and DYNAMAX - 300 ODS (SHIMADZU SCL - 10A preparative HPLC, MeOH-CH_3CN-H_2O, 91:4:5) to afford compounds **5** (59 mg), **6** (28 mg), **7** (16 mg), **8** (13 mg) and **9** (3.27 g). Fraction III was purified using Si gel CC ($CHCl_3$-MeOH-H_2O, 10:2:0.1), finally yielding **2** (2.61 g) in appropriate fraction.

Thamnolin (**1**), $C_{31}H_{56}O_3$, amorphous powder, UV $\lambda_{max}^{CHCl_3}$ nm: 289 (log ε 3.61); IR ν_{max}^{KBr} cm^{-1}: 3379, 2919, 2850, 1616, 1503, 1467, 1430, 1388, 1302, 1221, 1200, 1151, 1114, 1077, 1052, 926, 823, 811; EIMS (70 eV) m/z (%): 476 [M^+] (3), 462 (5), 448 [$M^+ - CH_2 = CH_2$] (58), 434 (19), 420 [$M^+ - 2 \times CH_2 = CH_2$] (52), 406 (2), 392 [$M^+ - 3 \times CH_2 = CH_2$] (100), 378 (4), 334 (1), 306 (3), 292 (2), 278 (2), 264 (3), 250 (3), 236 (1), 222 (1), 208 (2), 194 [$M^+ - C_{20}H_{42}$] (2), 180 [$M^+ - C_{21}H_{44}$] (5), 168 [$M^+ - C_{22}H_{44}$]$^+$ (98), 167 [$M^+ - C_{22}H_{45}$]$^+$ (52), 153 (14), 139 (38), 137 (13), 125 (5), 109 (3), 95 (6), 83 (4), 77 (6), 69 (8), 57 (12); HREIMS m/z: 476.4237, calcd for $C_{31}H_{56}O_3$ 476.4230; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.33 (1H, d, J = 2.80 Hz, H-3), 6.26 (1H, d, J = 2.80 Hz, H-5), 2.58 (2H, t, J = 7.76 Hz, H_2-1'), 1.57 (2H, m, H_2-2'), 1.30 (2H, m, H_2-3'), 1.23 (38H, overlap, H-4'-22'), 0.86 (3H, t, J = 6.8 Hz, H-23'), 3.82 (3H, s, OMe-2), 3.73 (3H, s, OMe-4); ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.66 (s, C-1), 146.76 (s, C-2), 96.77 (d, C-3), 152.79 (s, C-4), 106.01 (d, C-5), 128.80 (s, C-6), 31.91 (t, C-1'), 30.03 (t, C-2'), 29.67 (t, C-3'-20'), 29.33 (t, C-21'), 22.65 (t, C-22'), 14.03 (q, C-23'), 55.97 (q, OMe-2), 55.75 (q, OMe-4).

Squamatic acid (**2**), $C_{19}H_{18}O_9$, colorless crystals, FAB (-) MS m/z (%): 389 [M-1] (100), 375 (7), 208 (28); HRFAB (-) MS m/z: 389.0926, calcd for $C_{19}H_{18}O_9$ 389.0873; ^{1}H NMR (400 MHz, C_5D_5N): δ 7.02 (1H, s, H-5'), 6.55 (1H, s, H-5), 3.90 (3H, s, OMe-4), 2.81 (3H, s, Me-8'), 2.62 (3H, s, Me-8), 2.57 (3H, s Me-9'); ^{13}C NMR (100 MHz, C_5D_5N): (114.6 (s, C-1), 166.9 (s, C-2), 105.1 (s, C-3), 162.5 (s, C-4), 105.4 (d, C-5), 144.3 (s, C-6), 163.0 (s, C-7), 21.4 (q, C-8), 176.0 (s, C-9), 112.6 (s, C-1'), 164.1 (s, C-2'), 117.1 (s, C-3'), 153.5 (s, C-4'), 116.5 (d, C-5'), 140.8 (s, C-6'), 173.5 (s, C-7'), 24.0 (q, C-8'), 9.9 (q, C-9'), 56.5 (q, OMe).

Barbatinic acid (**3**), $C_{19}H_{20}O_7$, colorless crystals, FAB (-) MS m/z (%): 359 [M-1] (100), 195 (21), 181 (60), 163 (25), 136 (13), 119 (5); ^{1}H NMR (400 MHz, C_5D_5N): δ 6.77 (1H, s, H-5'), 6.56 (1H, s, H-5), 3.83 (3H, s, OMe-4), 2.83 (3H, s, Me-8'), 2.76 (3H, s, Me-8), 2.32 (3H, s Me-9'), 2.31 (3H, s, Me-9); ^{13}C NMR (100 MHz, C_5D_5N): δ 112.9 (s, C-1), 164.0 (s, C-2), 106.1 (s, C-3), 162.7 (s, C-4), 107.2 (d, C-5), 141.2 (s, C-6), 170.7 (s, C-7), 24.0 (q, C-8), 9.5 (q, C-9), 111.5 (s, C-1'), 162.9 (s, C-2'), 116.7 (s, C-3'), 152.6 (s, C-4'), 116.3 (d, C-5'), 140.7 (s, C-6'), 176.0 (s, C-7'), 24.8 (q, C-8'), 9.8 (q, C-9'), 55.9 (q, OMe).

Baeomycesic acid (**4**), $C_{19}H_{18}O_8$, colorless crystals, FAB (-) MS m/z (%): 373 [M-1] (64), 209 (20), 181 (100), 163 (47), 137 (13), 119 (10); HRFAB (-) MS m/z: 373.0986, calcd for $C_{19}H_{17}O_8$ 373.0923; ^{1}H NMR (400 MHz, C_5D_5N): δ 10.27 (1H, s, CHO-9), 6.85 (1H, s, H-5'), 6.40 (1H, s, H-5), 3.79 (3H, s, OMe-4), 2.85 (3H, s, Me-8'), 2.54 (3H, s, Me-8), 2.51 (3H, s, Me-9'); ^{13}C NMR (100 MHz, C_5D_5N): δ 115.6 (s, C-1), 164.9 (s, C-2), 104.2 (s, C-3), 163.7 (s, C-4), 104.2 (d, C-5), 141.0 (s, C-6), 163.9 (s, C-7), 21.5 (q, C-8), 193.8 (d, C-9), 109.5 (s, C-1'), 163.9 (s, C-2'), 116.2 (s, C-3'), 152.3 (s, C-4'), 115.3 (d, C-5'), 140.9 (s, C-6'), 177.4 (s, C-7'), 23.8 (q, C-8'), 10.0 (q, C-9'), 56.4 (q, OMe).

5, 8-Epidioxy-5α, 8α-ergosta-6, 22E-dien-3β-ol (**5**), $C_{28}H_{44}O_3$, colorless crystals, EIMS (70 eV) m/z (%): 428 [M]$^+$ (18), 410 (21), 396 (100), 377 (12), 363 (25), 337 (11), 303 (9), 285 (13), 267 (14), 251

Table 1 ^{13}C NMR data for compounds 5 ~ 8 (125.8 MHz, $CDCl_3$)

C	5*	6*	7	8
1	34.73 (t)	32.62 (t)	37.17 (t)	35.67 (t)
2	30.09 (t)	30.58 (t)	31.48 (t)	31.97 (t)
3	66.42 (d)	66.28 (d)	71.10 (d)	71.48 (d)
4	36.94 (t)	36.08 (t)	37.99 (t)	42.24 (t)
5	82.16 (s)	83.06 (s)	40.29 (d)	138.90 (s)
6	135.43 (d)	135.44 (d)	29.66 (t)	119.57 (d)
7	130.73 (d)	130.73 (d)	117.47 (d)	29.03 (t)
8	79.43 (s)	78.91 (s)	139.58 (s)	126.50 (s)
9	51.20 (d)	143.13 (s)	49.48 (d)	132.02 (s)
10	36.94 (s)	38.32 (s)	34.24 (s)	37.41 (s)
11	20.63 (t)	119.63 (d)	21.57 (t)	22.87 (t)
12	39.39 (t)	41.22 (t)	39.48 (t)	36.76 (t)
13	44.59 (s)	43.38 (s)	43.32 (s)	41.93 (s)
14	51.71 (d)	48.20 (d)	55.99 (d)	51.91 (d)
15	23.40 (t)	21.48 (t)	22.95 (t)	23.00 (t)
16	28.55 (t)	28.55 (t)	28.12 (t)	29.09 (t)
17	56.29 (d)	55.93 (d)	55.13 (d)	54.60 (d)
18	12.86 (q)	12.86 (q)	12.11 (q)	11.52 (q)
19	18.13 (q)	25.52 (q)	13.05 (q)	19.65 (q)
20	39.61 (d)	39.78 (d)	40.49 (d)	40.57 (d)
21	20.86 (q)	20.95 (q)	21.13 (q)	22.30 (q)
22	135.19 (d)	135.17 (d)	135.69 (d)	135.73 (d)
23	132.36 (d)	132.48 (d)	131.91 (d)	131.91 (d)
24	42.78 (d)	42.78 (d)	42.83 (d)	42.82 (d)
25	33.07 (d)	33.04 (d)	33.11 (d)	33.10 (d)
26	19.60 (q)	19.60 (q)	19.96 (q)	20.94 (q)
27	19.89 (q)	19.83 (q)	19.66 (q)	19.95 (q)
28	17.52 (q)	17.52 (q)	17.61 (q)	17.62 (q)

* Recorded in C_5D_5N, 100.13 MHz.

(23), 233 (13), 152 (26); ^1H NMR (400 MHz, $CDCl_3$): δ 6.48 (1H, d, J = 8.48 Hz, H-7), 6.22 (1H, d, J = 8.48 Hz, H-6), 5.17 (1H, dd, J = 15.28, 8.16 Hz, H-23), 5.13 (1H, dd, J = 15.28, 7.40 Hz, H-22), 3.95 (1H, m, H-3α), 0.97 (3H, d, J = 6.56 Hz, Me-21), 0.89 (3H, s, Me-19), 0.88 (3H, d, J = 6.84 Hz, Me-28), 0.81 (3H, overlap, Me-26), 0.80 (3H, s, Me-18), 0.79 (3H, overlap, Me-27); ^{13}C NMR data see Table 1.

5, 8-Epidioxy-5α, 8α-ergosta-6, 9, 22E-tien-3β-ol (6), $C_{28}H_{42}O_3$, colorless prism, EIMS (70 eV) m/z (%): 426 $[M]^+$ (41), 408 (30), 394 (100), 376 (54), 361 (18), 335 (13), 301 (55), 299 (40), 283 (23), 265 (27), 249 (48), 231 (22), 69 (97); ^1H NMR (400 MHz, $CDCl_3$): δ 6.58 (1H, d, J = 8.52 Hz, H-7), 6.27 (1H, d, J = 8.52 Hz, H-6), 5.47 (1H, m, H-11), 5.17 (1H, dd, J = 15.28, 7.52 Hz, H-23), 5.09 (1H, dd, J = 15.26, 8.04 Hz, H-22), 3.95 (1H, m, H-3α), 1.06 (3H, s, Me-19), 0.96 (3H, d, J = 6.58 Hz, Me-21), 0.88 (3H, d, J = 6.84 Hz, Me-28), 0.81 (3H, overlap, Me-26), 0.80 (3H, overlap, Me-27), 0.71 (3H, s, Me-18); ^{13}C NMR data see Tabl 1.

5α-Ergosta-7, 22E-dien-3β-ol (7), $C_{28}H_{46}O$, colorless needles, FAB (+) MS (70 eV) m/z (%): 399 $[M+1]^+$ (100); ^1H NMR (500 MHz, $CDCl_3$): δ 5.21 ~ 5.13 (3H, overlap, H-22, H-23 and H-7), 3.57 (1H,

m, H－3α), 1.00 (3H, d, J = 6.56 Hz, Me-21), 0.89 (3H, d, J = 6.78 Hz, Me-28), 0.82 (3H, overlap, Me-26), 0.81 (3H, overlap, Me-27), 0.78 (3H, s, Me-19), 0.52 (3H, s, Me-18); ^{13}C NMR data see Table 1.

Ergosta-5, 8, 22E-trien-3β-ol (8), $C_{28}H_{44}O$, colorless needles, FAB (+) MS (70 eV) m/z (%): 397 [M+1]$^+$ (100); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.41 (1H, br s, H－6), 5.22～5.12 (2H, overlap, H－22 and H－23), 3.54 (1H, m, H－3α), 1.17 (3H, s, Me-19), 1.02 (3H, d, J = 6.62 Hz, Me-21), 0.90 (3H, d, J = 6.86 Hz, Me-28), 0.82 (3H, overlap, Me-26), 0.81 (3H, overlap, Me-27), 0.64 (3H, s, Me-18); ^{13}C NMR data see Table 1.

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