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藏药船型乌头中的生物碱成分

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摘　要:为了研究藏药臭蚤草的活性成分,从其总碱中分离并通过核磁共振和质谱等方法鉴定了 8 个生物碱,包括 6 个二萜类生物碱 13-*O*-acetylhetisine(**1**)、2-acetyl-13-dehydro-11-epihetisine(**2**)、2-acetyl-13-dehydro-11-hetisine(**3**)、hetisinone(**4**)、尼奥林(**5**)和 foresticine(**6**),以及异喹啉(**7**)和 dianthrmide(**8**)。所有化合物均首次从该植物中分离得到。

关键词:藏药;船型乌头;二萜生物碱

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Alkaloids of the Tibetan Medicinal Plant *Aconitum naviculare* Stapf

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Abstract: Six diterpenoid alkaloids 13-O-acetylhetisine (**1**),2-acetyl-13-dehydro-11-epihetisine (**2**),2-acetyl-13-dehydro-11-hetisine (**3**),hetisinone (**4**),neoline (**5**),and foresticine (**6**),together with isoquinoline (**7**) and dianthrmide (**8**),were isolated from the Tibetan folk drug *Aconitum naviculare*. Their structures were elucidated based on ^{1}H and ^{13}C NMR and MS. This is the first report of the presence of compounds **1-8** in this plant.

Key words: Tibetan folk drug; *Aconitum naviculare*; diterpenoid alkaloids

Introduction

The plant genus *Aconitum* (Ranunculaceae) has over 100 species distributed in the northern hemisphere. The whole plants of *Aconitum naviculare* Stapf are used in traditional Tibetan medicine system for the treatment of some inflammations like gastritis, hepatitis, nephritis [1]. Crude preparations from *Aconitum* species were popularly used in Asia,Alaska,and Europe [2] in folklore and traditional medicine for the treatment of traumatic injury [3],as febrifuge and bitter tonic [4],and as ingredients in intoxicating liquor [5]. However, little phytochemistry study has been reported on *A. naviculare*. In this paper,eight alkaloids were isolated from *A. naviculare*.

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Experimental Section

General

Optical rotations determinations were carried on an OA AA-50 digital polarimeter. ^{1}H and ^{13}C NMR Spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with $CDCl_3$ or CD_3OD as solvent and TMS as standard. The MS data were recorded by a VG Autospec-3000 mass spectrometer; in *m/z* (rel. %). Column chromatography were carried out with SiO_2 (200-300 mesh, Qingdao Marine Chemistry Inc. $CHCl_3$/MeOH) and Sephadex LH-20 (25-100 μm,Phamacia Fine Chemical Co. Ltd. $CHCl_3$/MeOH 1:1)

Plant Material

Whole plants of *A. naviculare* were collected from Tibet (China) in June and August 2006. The specimen identified by professor Jiang Si-ping and stored in Plateau Institute of Biology Lhasa.

Extraction and Isolation

The dried material (20 kg) was extracted with methanol, a crude methanol extract (2.1 kg) was obtained. It's solution was acidified with 0.1 M H_2SO_4. The acidic aqueous extract was then basified (pH 10) with 20% aqueous Na_2CO_3 solution and then extracted with $CHCl_3$. The combined chloroform solution was washed with H_2O and dried with Na_2SO_4, and the solvent was evaporated to afford a crude alkaloid extract (40 g), which was subjected to repeated column chromatography (CC) (SiO_2; $CHCl_3$/MeOH, 100:0 to 0:100). The fractions eluted by $CHCl_3$/MeOH 80:20 (3.6 g) was further purified by repeated CC (1. SiO_2, $CHCl_3$/MeOH, 100:1; 2. Sephadex LH-20, $CHCl_3$/MeOH 1:1) to afford compounds **1** (1.5 mg), **2** (26.7 mg), **3** (1.6 mg), **4** (13.7 mg), **5** (40.5 mg), **6** (12.0 mg), **7** (2.1 mg), and **8** (13.0 mg).

Result and Discussion

13-*O*-Acetylhetisine (1) $C_{22}H_{27}NO_4$, white powder, $[\alpha]_D^{26}$ +20.0 (*c* 0.0015, MeOH), EI-MS (70 eV) *m/z* 369 $[M]^+$ (45), 352 (12), 340 (30), 326 (38), 310 (100), 282 (48), 253 (22), 160 (21), 144 (13), ^{1}H NMR (CD_3OD, 400 MHz) δ: 5.02 (2H, d, *J* = 2.2 Hz), 4.78 (1H, s), 4.29 (1H, d, *J* = 9.0 Hz), 3.62 (1H, d, *J* = 1.6 Hz), 3.58 (1H, d, *J* = 1.7 Hz), 3.43 (1H, s), 2.72 (2H, m), 2.57 (1H, d, *J* = 4.9 Hz), 2.53 (1H, s), 2.46 (1H, d, *J* = 2.6 Hz), 2.30 ~ 2.06 (9H, overlap), 1.84-1.76 (3H, overlap), 1.21 ~ 1.17 (4H, overlap); ^{13}C NMR (CD_3OD, 100 MHz) δ: 215.4 (s), 172.4 (s), 146.7 (s), 109.3 (t), 74.9 (d), 74.8 (d), 71.6 (d), 66.7 (d), 63.7 (t), 61.3 (d), 55.6 (d), 50.82 (s), 50.80 (d), 48.6 (d), 45.6 (s), 40.5 (t), 36.7 (s), 36.2 (t), 34.0 (t), 33.7 (t), 29.8 (q), 21.3 (q). Comparing the data to the reported results in literature [6], the structure is defined as 13-*O*-acetylhetisine.

2-Acetyl-13-dehydro-11-epihetisine (2) $C_{22}H_{27}NO_4$, white powder, $[\alpha]_D^{26}$ + 48.7 (*c* 0.01335, MeOH), ESI-MS (pos.) *m/z* 370 $[M+H]^+$, ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.03 (1H, d, *J* = 9.0 Hz), 4.83 (1H, s), 4.68 (1H, s), 4.05 (1H, d, *J* = 9.2 Hz), 3.77 (2H, s), 3.39 (1H, d, *J* = 13.9 Hz), 3.34 (1H, s), 2.99 (1H, d, *J* = 12.6 Hz), 2.50 ~ 2.03 (14H, overlap), 1.74 (1H, d, *J* = 12.6 Hz), 1.16 (3H, s), ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 210.1 (s), 171.1 (s), 142.9 (s), 109.6 (t), 75.1 (d), 69.6 (d), 69.1 (d), 65.6 (d), 61.4 (t), 57.4 (d), 54.9 (s), 52.1 (d), 49.8 (d), 49.1 (t), 47.2 (d), 44.2 (s), 42.7 (t), 41.3 (s), 34.0 (t), 32.5 (t), 28.0 (q), 21.1 (q). Comparing the data to the reported results in literature [7], the structure is defined as 2-acetyl-13-dehydro-11-epihetisine.

2-Acetyl-13-dehydro-11-hetisine (3) $C_{22}H_{27}NO_4$, white powder, $[\alpha]_D^{26}$ + 100.0 (*c* 0.0003, MeOH), ESI-MS (pos.) *m/z* 370 $[M+H]^+$ (60); ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.11 (1H, d, *J* = 9.5 Hz), 4.94 (1H, s), 4.77 (1H, s), 4.30 (1H, d, *J* = 9.0 Hz), 3.90 (2H, s), 3.48 (2H, d, *J* = 13.3 Hz), 3.10 (1H, *J* = 12.8 Hz), 2.65-2.14 (14H, overlap), 1.89 (1H, d, *J* = 12.4 Hz), 1.18 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 211.6 (s), 170.0 (s), 143.4 (s), 109.7 (t), 74.1 (d), 73.1 (d), 70.3 (d), 65.3 (d), 63.5 (t), 59.7 (d), 55.4 (s), 54.2 (d), 49.8 (t), 48.9 (d), 47.8 (d), 44.9 (t), 44.4 (s), 42.3 (s), 35.1 (t), 33.1 (t), 28.5 (q), 21.0 (q). Comparing the data to the reported results in literature [7], the structure is defined as 2-acetyl-13-dehydro-11-hetisine.

Hetisinone (4) $C_{20}H_{25}NO_3$, white powder. $[\alpha]_D^{26}$ + 16.0 (*c* 0.00685, MeOH), ESI-MS (pos.) *m/z* 328 $[M+1]^+$ (83), ^{1}H NMR (CD_3OD, 400 MHz) δ: 4.71 (1H, s, br), 6.63 (1H, s, br), 4.28 (1H, m), 4.20 (s, br), 4.16 (1H, m), 3.69 (1H, m), 3.62 (1H, m), 3.20 (1H, m), 2.78 (1H, d, *J* = 6.7 Hz), 2.74 (1H, d, *J* = 4.0 Hz), 2.62 (2H, m), 2.30 ~ 2.39 (5H, overlap), 1.79 ~ 2.11 (4H, overlap), 1.32 (3H, s); ^{13}C NMR (CD_3OD, 100 MHz) δ: 211.3 (s), 144.7 (s), 109.2 (t), 74.6 (d), 70.8 (d), 70.7 (d), 67.2 (d), 61.4 (t), 57.2 (d), 54.7 (d), 50.8 (d), 50.6 (d), 49.8 (d), 49.3 (t), 44.6 (t), 44.4 (s), 41.7 (s), 34.3 (t), 32.9 (t), 28.3 (q). Comparing the data to the reported results in literature [8-10], the structure is defined as helisinone.

Neoline (5) $C_{24}H_{39}NO_6$, yellow powder. $[\alpha]_D^{26}$ + 5.68 (*c* 0.01935, MeOH), ESI-MS (pos.) *m/z* 438 $[M+1]^+$ (65). ^{1}H NMR (CD_3OD, 400 MHz) δ:

4.22 (1H, d, J = 6.5 Hz), 4.21 (1H, m), 3.82 (s, br), 3.58 (1H, d, J = 7.9 Hz), 3.39 (1H, m), 3.36 (3H, s), 3.33 (3H, s), 3.30 (3H, s), 3.29 (2H, s), 3.27 (1H, m), 2.97 (1H, d, J = 11.1 Hz), 2.89 (1H, s), 2.85 (1H, m), 2.75 (1H, m), 2.57 (1H, d, J = 11.1 Hz), 2.25 (1H, d, J = 6.5 Hz), 2.20 (2H, d, J = 7.1 Hz), 2.20 (1H, m), 2.08 (1H, s), 1.95 (1H, m), 1.65 (1H, m), 1.63 (1H, m), 1.59 (2H, m), 1.27 (1H, s), 1.14 (3H, d, J = 7.1 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ: 84.3 (d), 80.8 (t), 79.5 (d), 76.3 (d), 75.2 (s), 73.2 (d), 64.2 (d), 59.4 (q), 58.6 (t), 58.3 (q), 56.5 (q), 54.4 (d), 50.8 (s), 49.8 (d), 48.5 (d), 45.3 (d), 45.2 (d), 42.5 (t), 41.8 (d), 39.2 (s), 31.4 (t), 30.0 (t), 29.8 (t), 12.6 (q). Comparing the data to the reported results in literature [11], the structure is defined as neonile.

Foresticine (6) $C_{24}H_{39}NO_6$, yellow powder. $[\alpha]_D^{26}$ + 18.5 (c 0.00755, MeOH), ESI-MS (pos.) m/z $[M+1]^+$ (67), ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 4.19 (1H, m), 4.15 (1H, d, J = 6.5 Hz), 3.65 (1H, s), 3.62 (1H, d, J = 8.0 Hz), 3.34 (1H, m), 3.32 (3H, s), 3.31 (3H, s), 3.30 (3H, s), 3.24 (1H, d, J = 8.0 Hz), 2.67 (2H, m), 2.51 (1H, m), 2.45 (1H, m), 2.26 ~ 2.35 (3H, overlap), 2.16 (2H, m), 2.03 (3H, m), 1.83 ~ 1.87 (2H, overlap), 1.56 (1H, m), 1.43-1.55 (3H, m), 1.11 (3H, t, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 83.1 (d), 81.8 (d), 80.2 (t), 75.9 (d), 74.2 (s), 72.2 (d), 63.8 (d), 59.2 (q), 57.9 (q), 57.0 (t), 56.3 (q), 52.1 (d), 49.4 (d), 48.2 (d), 48.2 (t), 44.8 (d), 44.0 (d), 42.8 (t), 40.3 (d), 38.1 (s), 29.8 (t), 29.4 (t), 29.3 (t), 13.0 (q). Comparing the data to the reported results in literature [12], the structure is defined as foresticine.

Isoquinoline (7) C_9H_7N, light yellow oil. ESI + MS m/z 130 $[M+1]^+$ (78). ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 9.12 (1H, s), 8.41 (1H, d, J = 8.2 Hz), 7.86 (1H, m), 7.71 (1H, m), 7.56 (1H, m), 7.50 (2H, m). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 152.2 (d), 142.4 (d), 135.8 (d), 130.3 (d), 128.4 (d), 127.3 (d), 126.9 (d), 126.1 (d), 120.1 (d). Comparing the data to the reported results in literature [13], the structure is defined as isoquinoline.

Dianthramide B (8) *N*-salicyl-4-hydroxyanthranilic acid methyl ester, $C_{15}H_{13}NO_5$, white powder. EI-MS (70 eV) m/z 287 $[M]^+$ (16), 255 ((9), M-MeOH), 167 ((100), M 34ArCOO), 135 ((50), M-ArCOO-MeOH), 121 ((44), ArCOOH); FAB-MS: 286 $[M-H]^-$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 12.3 (1H, s, -OH), 11.9 (1H, s, -OH), 8.68 (1H, d, J = 9.5 Hz), 7.79 (1H, d, J = 8.5 Hz), 7.56 (1H, d, J = 2.9 Hz), 7.44 (1H, s), 7.12 (1H, dd, J = 9.0, 2.9 Hz), 6.99 (2H, m), 3.98 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 170.9 (d), 171.2 (s), 166 (s), 163.4 (s), 144.8 (s), 136.5 (d), 135.2 (d), 129.2 (d), 121.3 (d), 120.2 (d), 118.2 (d), 113.1 (d), 109.4 (d), 109.3 (s), 53.6 (q). Comparing the data to the reported results in literature [14,15], the structure is defined as dianthramide B (*N*-salicyl-4-hydroxyanthranilic acid methyl ester).

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