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Steroids and Anthraquinones from *Astragalus hoantchy*

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【ABSTRACT】 AIM: To study the chemical constituents of the roots of *Astragalus hoantchy* Franch.. METHOD: Isolation and elucidation of the chemical constituents were conducted by chromatography and spectral evidences. RESULTS and CONCLUSION: Six steroids and four anthraquinones were isolated from the roots of *A. hoantchy*. Their structures were identified to be stigmastane-3, 6-dione (**1**), 5α, 8α-epidioxy-(22*E*, 24*R*)-ergosta-6, 22-dien-3β-ol (**2**), stigmastane-3β, 6α-diol (**3**), daucosterol (**4**), β-sitosterol (**5**), stigmasterol (**6**), chrysophanol (**7**), emodin (**8**), physion (**9**) and aloe-emodin (**10**) on the basis of spectral data and physical constants. Among them, compounds **1**, **2**, **3**, **7**, **8**, **9**, **10** were isolated from the genus *Astragalus* for the first time.

【KEY WORDS】 *Astragalus hoantchy*; steroids; anthraquinones
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Astragalus hoantchy Franch. belongs to subgenus *Pogonphace*, genus *Astragalus*, family Fabaceae, mainly distributed in the west provinces of China. The roots of *Astragalus hoantchy* are often used as substitute for *Radix Astragali* in Neimeng and Ningxia provinces. Continued our previous research[1], in the recent study, we have isolated six steroids and four anthraquinones from the roots of *A. hoantchy*. By the means of MS, NMR and physical constant, they were identified as stigmastane-3, 6-dione (**1**), 5α, 8α-epidioxy-(22*E*, 24*R*)-ergosta-6, 22-dien-3β-ol (**2**), stigmastane-3β, 6α-diol (**3**), daucosterol (**4**), β-sitosterol (**5**), stigmasterol (**6**), chrysophanol (**7**), emodin (**8**), physion (**9**) and aloe-emodin (**10**), respectively. Among them, **1**, **2**, **3**, **7**, **8**, **9** and **10** were isolated from the genus *Astragalus* for the first time.

1 Results and Discussion

Hoanosterone (**1**) was isolated as an amorphous powder, which gave positive response to Libermann-Burchard reaction. The molecular formula was determined to be $C_{29}H_{48}O_2$ by EI-MS m/z 428 $[M]^+$ and ^1H, ^{13}CNMR analysis. The ^{13}CNMR (Table 1) showed 29 carbon signals that included two carbonyl carbons (δ211.0, 208.9). The absorptions of the IR spectrum (1715 cm^{-1}) and the UV spectrum (λmax 289nm) also suggested the presence of saturated carbonyl carbons. The ^{1}HNMR spectrum showed signals due to two angular methyl groups [δ0.69 (s, 3H, H_3-18), 0.96 (s, 3H, H_3-19)], three other methyl groups [δ0.93 (3H, d, $J = 6.8$ Hz, H_3-21), 0.82 (3H, d, $J = 6.8$ Hz, H_3-26), 0.80 (3H, d, $J = 8.0$ Hz, H_3-27)], [δ0.85 (3H, t, $J = 7.2$ Hz, H_3-29)], which suggested a 24-ethylcholestane structure[2]. The structure of the side-chain was determined by EI-MS and HMBC spectrum. The fragment ion was observed at m/z 287 due to the loss of a $C_{10}H_{21}$ from m/z 428 $[M]^+$ that suggested a saturated C_{10} side-chain. The HMBC spectrum observed from the H_3-21 methyl group to C-17, C-20 and C-22, the H_3-26 to C-24, C-25 and C-27, the H_3-27 to C-26 and C-24, the H_3-29 to C-28 and C-24 confirmed the presence of a saturated 24-ethylated cholestane side-chain. The sterochemistry at C-24 was established as *R* in comparison with the ^{13}CNMR data for stigmastane (24*R*) and poriferastane (24*S*)[2].

Methylene and methine protons absorbed at δ1.03,

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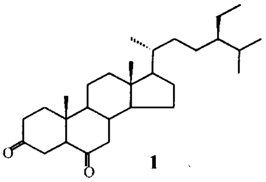
2.60 and their overlap is such that their individual multiplicity is not clear. The chemical shifts of some protons were established from the correlation peaks in ^1H-^1HCOSY and ^{13}C-^1HCOSY spectra. The HMBC spectrum showed the partial structures of the A, B rings. As shown in Figure 1, correlations of H_4 (δ 2.32, 2.09)/carbon (δ 211.0), H_5 (δ 2.60)/carbon (δ 211.0), H_4/carbon (δ 208.9), H_5/carbon (δ 208.9) and H_7 (δ 2.01, 2.39)/carbon (δ 208.9) indicated the presence of 3, 6-dione, C-3 (δ 211.0) and C-6 (δ 208.9). Therefore, the structure of hoanosterone (**1**) could be elucidated as stigmastane-3, 6-dione. ^{13}CNMR assignments were carried out by NMR spectra and with reference to those for stigmastane-3β, 6α-diol[2] and 3-oxoteasteone[3].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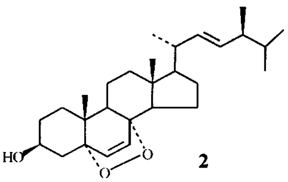
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Tab 1. ^{1}HNMR and ^{13}CNMR data of hoanosterone (**1**) ($CDCl_3$, 400 MHz)

No.	δ_C	δ_H	HMBC
1	37.1		
2	37.5		
3	211.0		
4	38.2	2.32, 2.09	C-3, 6
5	57.5	2.60	C-3, 6, 19
6	208.9		
7	46.7	2.01, 2.39	C-6
8	38.1		
9	53.5		
10	41.3		
11	21.8		
12	39.4		
13	43.1		
14	56.6		
15	24.1		
16	28.1		
17	56.0		
18	12.1	0.69 (3H, S)	C-12, 13, 14, 17
19	12.7	0.96 (3H, S)	C-5, 9, 10
20	36.1		
21	18.8	0.93 (3H, d, J=6.8)	C-17, 20, 22
22	33.9		
23	26.1		
24	45.8		
25	29.2		
26	19.9	0.82 (3H, d, J=6.8Hz)	C-24, 25, 27
27	19.1	0.80 (3H, d, J=8.0Hz)	C-24, 26
28	23.1		
29	12.1	0.85 (3H, t, J=7.2Hz)	C-24, 28

Compound **2**, appeared as colorless crystals and gave positive response to Liebermann-Burchard reaction. The ESI-MS showed quasi-molecular ion peaks at m/z 429 $[M+H]^+$, 451 $[M+Na]^+$ and 467 $[M+K]^+$, combining with the analysis of ^{1}H and ^{13}CNMR spectrum, the molecular formula was obtained to be $C_{28}H_{44}O_3$. Inspection of spectral data that **2** was identical to 5α, 8α-epidioxy (22*E*, 24*R*)-ergosta-23-methylergosta-6, 22-dien-3β-ol expect for the side-chain and the ^{1}H and ^{13}CNMR data of the side-chain were identical with those of 3β, 5α, 9α, 14α-tetrahydroxy-(22*E*, 24*R*)-ergosta-7, 22-dien-6-one[4]. The presence of a peroxy group was further confirmed by the mass fragment ion of m/z 396 $[M+H-O_2]^+$ and the ^{13}C NMR signals [δ 79.4 (C-8), δ 82.5 (C-5)][5]. The mass fragment ion of m/z 271 $[M+H-H_2O-C_9H_{17}]^+$ was due to the loss of a C_9H_{17} side-chain. The *E* configuration of Δ^{22} and the *Z* configuration of Δ^6 were shown by the coupling constants: J=15.2Hz and J=8.4Hz, respectively. Therefore, **2** should be 5α, 8α-epidioxy-(22*E*, 24*R*)-ergosta-6, 22-dien-3β-ol.

Compound **3** was identified by direct comparison with the NMR data and EI-MS with those of stigmastane-3β, 6α-diol[2]. Compounds **4**, **5** and **6** were identified as β-daucosterol, β-sitosterol and stigmasterol by comparison of EI-MS spectra, the mixed melting points and R_fs with authentic samples[6].

Compounds **7**, **8**, **9**, **10** were identified as chrysophanol, emodin, physion and aloe-emodin on the basis of ^{1}H, ^{13}CNMR data[7] and physical constants. Compounds **7**, **9** were also confirmed by EI-MS spectra.

2 Experiment

2.1 *General experimental procedures*

UV spectra were measured and IR (KBr) spectra were recorded on a Pekin-Elmer FT-IR instrument. NMR spectra were run on a JEOL JNM-EX-400 spectrometer with TMS as internal standard. MS spectra were carried out on JEOL DX-300 and FISONS VG QUATTRO mass spetrometer. Silica gel and Sephadex LH-20 was used for TLC and column chromatography.

2.2 *Plant material*

The roots of *Astragalus hoantchy* were collected from Luoshan, Yinchuan city of Ningxia province, PR China, in 1998 and authenticated by one of the authors DUAN Jin-Ao. A voucher specimen was kept in the department of Natural Medicinal Resources, China Pharmaceutical University.

2.3 *Extraction and isolation*

Dry roots of *A. hoantchy* (6 Kg) were powdered and extracted with EtOH (80%) for four times under reflux (each process lasting for 1 h). After removal of solvent by evaporation, the combined extract was suspended in H_2O, and extracted with Et_2O. The Et_2O portion (60 g) was chromatographed repeatedly on silica gel column and purified on sephadex LH-20 column to yield **1** (10 mg), **2** (15 mg), **3** (5 mg), **4** (50 mg), **5** (20mg), **6** (15 mg), **7** (40 mg), **8** (20 mg), **9** (20 mg) and **10** (mg).

2.4 *Identification*

stigmastane-3, 6-*dione* (**1**) (*hoanosterone*) mp 201 ~ 203 ℃, a amorphous powder. EI-MS *m/z* 428 [M]$^+$, 287 [M-$C_{10}H_{21}$]$^+$; IR (KBr) cm^{-1}: 1714 (C=O); UVλmax (nm): 289; ^{1}H and ^{13}CNMR data were shown in Table 1.

5α, 8α-*epidioxy*-(22*E*, 24*R*)-*ergosta*-6, 22-*dien*-3β-*ol* (**2**) mp 182 ~ 184 ℃, was obtained as colorless crystals. ESI-MS *m/z* 467 [M+K]$^+$, 451 [M+Na]$^+$, 429 [M+H]$^+$, 411 [M+H-H_2O]$^+$, 396 [M+H-O_2]$^+$. IR (KBr) cm^{-1}: 3411 (OH), 1631, 976, 724 (C=C); ^{1}HNMR (C_5D_5N, 400 MHz) δ0.77 (3H, s, H_3-18), 0.85 (3H, d, *J*=6.8Hz, CH_3-26), 0.86 (3H, d, *J* = 6.8Hz, CH_3-27), 0.89 (3H, s, CH_3-19), 0.95 (3H, d, *J*=6.8Hz, CH_3-28), 1.02 (3H, d, *J*=6.4Hz, CH_3-21), 4.38 (1H, m, H-3), 5.18 (1H, dd, *J*=15.2, 8.2Hz, H-23), 5.26 (1H, dd, *J*=15.2, 7.6Hz, H-22), 6.31 (1H, d, *J* = 8.4Hz, H-6), 6.52 (1H, d, *J* = 8.4Hz, H-7); ^{13}CNMR (C_5D_5N, 100 MHz) δ35.7 (C-1), 31.5 (C-2), 66.0 (C-3), 38.5 (C-4), 82.5 (C-5), 136.3 (C-6), 131.0 (C-7), 79.4 (C-8), 52.1 (C-9), 37.7 (C-10), 21.4 (C-11), 39.8 (C-12), 44.9 (C-13), 52.3 (C-14), 23.9 (C-15), 29.3 (C-16), 56.5 (C-17), 13.3 (C-18), 18.6 (C-19), 40.3 (C-20), 21.4 (C-21), 136.0 (C-22), 132.3 (C-23), 43.3 (C-24), 33.6 (C-25), 20.1 (C-26), 20.4 (C-27), 18.1 (C-28).

stigmastane-3β, 6α-*diol* (**3**) mp 207 ~ 209 ℃, White crystals and gave positive response to Liebermann-Burchard reaction. EI-MS *m/z* 432 (M)$^+$, 414 [M-H_2O]$^+$, 399, 396 [M-2H_2O]$^+$, 381, 283; IR (KBr) cm^{-1}: 3433 (OH); ^{1}H NMR (CD_3OD, 400MHz) δ0.69 (3H, s, H_3-18), 0.83 (3H, s, H_3-19), 0.93 (3H, d, *J*=6.4 Hz, H_3-21), 0.85 (3H, d, *J*=6.8 Hz, H_3-26), 0.83 (3H, d, *J* = 6.8 Hz, H_3-27), 0.86 (3H, t, *J* = 7.6 Hz, H_3-29); ^{13}CNMR (CD_3OD, 100MHz) δ38.7 (C-1), 31.9 (C-2), 72.0 (C-3), 33.0 (C-4), 52.9 (C-5), 70.0 (C-6), 42.7 (C-7), 35.7 (C-8), 55.3 (C-9), 37.5 (C-10), 22.3 (C-11), 41.3 (C-12), 43.8 (C-13), 57.7 (C-14), 25.3 (C-15), 29.4 (C-16), 57.5 (C-17), 12.4 (C-18), 13.9 (C-19), 37.4 (C-20), 19.3 (C-21), 35.1 (C-22), 27.2 (C-23), 47.3 (C-24), 30.4 (C-25), 20.3 (C-26), 19.5 (C-27), 24.2 (C-28), 12.5 (C-29).

daucosterol (**4**) mp 289 ~ 290 ℃, white powder. It was identified in comparison with the mixed melting point and Rf with an authentic sample.

β-*sitosterol* (**5**) mp 142 ~ 143 ℃, and stigmasterol (**6**), mp 170 ℃, were all isolated as colorless needles. They were identified as β-sitosterol and stigmasterol in comparison with the mixed melting points and R_{fs} with authentic samples.

chrysophanol (**7**) mp 200 ~ 201 ℃, orange-red crystals. EI-MS *m/z* 254 [M]$^+$, 226 [M-CO]$^+$, 198 [M-2CO]$^+$; IR (KBr) cm^{-1}: 3437 (OH), 1628 (C=O); UVλmax (nm): 300, 259, 290, 435; ^{1}HNMR ($CDCl_3$, 400MHz) δ7.82 (1H, br dd, *J* = 7.6, 1.2Hz), 7.68 (1H, d, *J*= 7.6Hz), 7.66 (1H, m), 7.29 (1H, br dd, *J*=7.6, 1.2Hz), 7.10 (1H, m), 2.47 (3H, CH_3); ^{13}CNMR ($CDCl_3$, 100MHz) δ162.5 (C-1), 124.3 (C-2), 149.2 (C-3), 121.3 (C-4), 133.2 (C-4a), 119.8 (C-5), 136.8 (C-6), 124.5 (C-7), 162.2 (C-8), 115.8 (C-8a), 192.3 (C-9), 113.7 (C-9a), 181.8 (C-10), 133.5 (C-10a), 22.4 (CH_3).

emodin (**8**) mp 266 ~ 268 ℃, orange-red crystals. IR (KBr) cm^{-1}: 3464 (OH), 1627 (C＝O); UVλmax (nm): 300, 269, 289, 441; ^{1}HNMR (DMSO-d_6, 400MHz) δ11.39, 11.98, 12.06 (OH × 3), 7.46 (1H, d, *J* = 0.8Hz), 7.14 (1H, d, *J* = 0.8Hz), 7.09 (1H, dd, *J* = 2.4, 1.2Hz), 6.57 (1H, dd, *J* = 2.4, 1.2Hz), 2.39 (3H, s, CH_3); ^{13}CNMR (DMSO-d_6, 100MHz) δ164.2 (C-1), 107.8 (C-2), 165.3 (C-3), 108.6 (C-4), 134.9 (C-4a), 120.3 (C-5), 148.1 (C-6), 124.0 (C-7), 161.2 (C-8), 113.2 (C-8a), 189.4 (C-9), 108.8 (C-9a), 181.1 (C-10), 132.6 (C-10a), 21.6 (CH_3).

physcion (**9**) mp 209 ~ 210 ℃, orange-yellow needle crystals. EI-MS *m/z* 284 [M]$^{+}$, 256 [M-CO]$^{+}$, 228 [M-2CO]$^{+}$; IR (KBr) cm^{-1}: 3436 (OH), 1630 (C＝O); UVλmax (nm): 300, 269, 289, 440; ^{1}HNMR ($CDCl_3$, 400MHz) δ12.30, 12.10 (OH × 2), 7.61 (1H, d, *J* = 1.2 Hz), 7.35 (1H, d, *J* = 2.4Hz), 7.07 (1H, d), 6.67 (1H, d, *J* = 2.8Hz), 3.94 (3H, s, OCH_3), 2.45 (3H, CH_3); ^{13}CNMR ($CDCl_3$, 100MHz) δ165.0 (C-1), 106.7 (C-2), 166.3 (C-3), 108.1 (C-4), 135.1 (C-4a), 121.2 (C-5), 148.3 (C-6), 124.4 (C-7), 162.3 (C-8), 113.6 (C-8a), 190.5 (C-9), 110.2 (C-9a), 181.8 (C-10), 133.1 (C-10a), 22.3 (CH_3), 56.1 (OCH_3).

aloe-emodin (**10**) mp 221 ~ 223 ℃, orange-red needle crystals. IR (KBr) cm^{-1}: 3430 (OH), 1628 (C＝O); UVλmax (nm): 300, 259, 289, 433; ^{1}HNMR (DMSO-d_6, 400MHz) δ12.5 ~ 11.5 (OH), 7.80 (1H, t, *J* = 7.6, 8.4Hz), 7.71 (1H, dd, *J* = 7.6, 1.2Hz), 7.69 (1H, d, *J* = 0.8Hz), 7.38 (1H, dd, *J* = 8.4, 1.2Hz), 7.29 (1H, d, *J* = 0.8Hz), 5.62 (1H, t, OH), 4.62 (2H, d, *J* = 4.8Hz); ^{13}CNMR (DMSO-d_6, 100MHz) δ161.4 (C-1), 120.6 (C-2), 153.5 (C-3), 117.0 (C-4), 133.0 (C-4a), 119.2 (C-5), 137.2 (C-6), 124.3 (C-7), 161.1 (C-8), 115.8 (C-8a), 191.4 (C-9), 114.4 (C-9a), 181.3 (C-10), 133.2 (C-10a), 62.0 (CH_2).

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贺兰山黄芪中的甾类和蒽醌类成分

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【摘　要】 目的: 对贺兰山黄芪的根进行化学成分研究。方法: 运用层析手段和波谱方法及相关物理常数对照进行化学成分的分离和结构鉴定。结果: 得到六个甾类和四个蒽醌类化合物: 豆甾烷-3, 6-2 酮(**1**), 5α, 8α-二氧-(22*E*, 24*R*)-麦角甾-6, 22-大黄素甲醚-3β-醇(**2**), 豆甾烷-3β, 6α-二醇(**3**), 胡萝卜苷(**4**), β-谷甾醇(**5**), 豆甾醇(**6**), 大黄酚(**7**), 大黄素(**8**), 大黄素甲醚(**9**)和芦荟大黄素(**10**)。结论: 化合物 **1** ~ **3**, **7** ~ **10** 为黄芪属中首次发现。

【关键词】 贺兰山黄芪; 甾类; 蒽醌类