Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

The Chemical constituents of the twigs of Ammopiptanthus nanus

Teng-Fei Ji [b], Jin Li [c] & Chun-Hui Liang [a]

[a] Department of Pharmacy, 3rd Hospital, Hebei Medical University, Shijiazhuang, 050051, China

[b] State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, 100050, China

[c] Department of Life Sciences and Chemistry, Xinjiang Normal University, Urumqi, 830054, China

Version of record first published: 22 Apr 2013.

To cite this article: Teng-Fei Ji , Jin Li & Chun-Hui Liang (2013): The Chemical constituents of the twigs of Ammopiptanthus nanus , Journal of Asian Natural Products Research, DOI:10.1080/10286020.2012.762765

To link to this article: http://dx.doi.org/10.1080/10286020.2012.762765

Journal of Asian Natural Products Research, 2013
http://dx.doi.org/10.1080/10286020.2012.762765

Taylor & Francis
Taylor & Francis Group

The Chemical constituents of the twigs of *Ammopiptanthus nanus*

Teng-Fei Ji[b], Jin Li[c] and Chun-Hui Liang[a]*

[a]*Department of Pharmacy, 3rd Hospital, Hebei Medical University, Shijiazhuang 050051, China;* [b]*State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China;* [c]*Department of Life Sciences and Chemistry, Xinjiang Normal University, Urumqi 830054, China*

(*Received 27 June 2012; final version received 25 December 2012*)

Two new isoflavone glycosides, ammopiptanosides A and B, have been isolated from the 95% EtOH extract of the twigs of *Ammopiptanthus nanus* (M.Pop.) Cheng f., together with six known compounds, and their structures were characterized by spectroscopic methods and compared with the data in the literature.

Keywords: *Ammopiptanthus nanus*; isoflavone glycoside; ammopiptanoside A; ammopiptanoside B

1. Introduction

Ammopiptanthus nanus (M.Pop.) Cheng f., a survivor of the evergreen broadleaf forest in the central Asian desert from the Tertiary period, is a genus of the tribe Thermopsideae of the Leguminosae, which is mainly distributed in Kizilsu Autonomous Prefecture in the southwest of The Xinjiang Uygur Autonomous Region [1]. The aerial parts of *Ammopiptanthus* have been used as a folk medicine for the treatment of cough, congelation, chronic rheumatic arthritis, etc. The genus comprises two species, and the other is *Ammopiptanthus mongolicus* (Maxim.) Cheng f. Alkaloids and flavonoids are the characteristic chemical constituents of *Ammopiptanthus*, according to the literature [2]. As part of our ongoing study on the chemical constituents of Chinese medicinal plants, two new isoflavone glycosides, ammopiptanosides A and B, have been isolated from the 95% EtOH extract of the twigs of *A. nanus* (M.Pop.) Cheng f., together with six known compounds formononetin (**3**), ononin (**4**), isolupalbigenin (**5**), daidzein 4′,7-di-*O*-β-D-glucoside (**6**), genistein 7,4′-di-*O*-β-D-glucoside (**7**), and (+)-maackiain (**8**).

2. Results and discussion

The 95% EtOH extract of the twigs of *A. nanus* was prepared by reflux method. A series of column chromatographies over silica gel, Sephadex LH-20, and reversed-phase ODS led to the isolation of two new compounds glycitein-4′,7-di-*O*-β-D-glucoside named ammopiptanoside A (**1**) and 4′-*O*-β-D-glucopyranosyl-glycitein 7-α-L-rhamnopyranosyl-(1 → 6)-*O*-β-D-glucopyranoside named ammopiptanoside B (**2**), together with six known compounds formononetin (**3**) [3], ononin (**4**) [4], isolupalbigenin (**5**) [5], daidzein 4′, 7-di-*O*-β-D-glucoside (**6**) [6], genistein 7,4′-di-*O*-β-D-glucoside (**7**) [7], and (+)-maackiain (**8**) [8]. The structures of **1** and **2** were elucidated by 1D and 2D NMR and MS techniques, and those of **3**–**8** were identified by comparing their NMR and MS spectral data with reported values.

*Corresponding author. Email: liangchunhui2010@163.com

Compound **1** was isolated as white amorphous powder. The ESI-MS displayed the *quasi*-molecular ion at *m/z* 631 $[M + Na]^+$, and the HR-ESI-MS exhibited *quasi*-molecular ion at *m/z* 609.1825 $[M + H]^+$ and *m/z* 631.1642 $[M + Na]^+$, indicating the molecular formula of $C_{28}H_{32}O_{15}$. In the ^{1}H NMR spectrum, one proton singlet at δ_H 8.42 (1H, s) was the characteristic of an isoflavone and assignable to H-2 and two doublets at δ_H 7.52 (d, 2H, $J = 8.0$ Hz) and 7.08 (d, 2H, $J = 8.0$ Hz) due to 1′,4′-disubstituted B-ring; the signals at δ_H 7.48 (s, 1H), and 7.33 (s, 1H) attributed to 6,7-substituted aromatic protons of the A-ring. The presence of a methoxy group was exhibited by a proton signal at δ_H 3.88 (s, 3H). The aglycone of **1** was identified as glycitein because its NMR spectral data were similar to those of glycitein [2]. Acid hydrolysis of **1** afforded D-glucose on the base of HPLC. The β-anomeric configurations for both glucoses were judged from their coupling constants ($^3J_{H1,H2} > 7.0$). ^{1}H NMR spectrum of **1** contained two anomeric proton signals at δ_H 5.17 (d, 1H, $J = 7.5$ Hz) and 4.90 (d, 1H, $J = 7.0$ Hz) [9]. One and two-dimensional NMR techniques (HMBC) permitted assignments of all ^{1}H and ^{13}C signals of **1** (Table 1). The HMBC correlations between the OCH_3 at δ_H 3.88 and C-6 at δ_C 147.5, H-5 (δ_H 7.48)/H-8 (δ_H 7.33) and C-6 at δ_C 147.5 indicated that the methoxyl group could be attached at C-6 position. In addition, the HMBC correlations of H-1″ at δ_H 5.17 with C-7 at δ_C 151.6 and H-1″ at δ_H 4.90 with C-4′ at δ_C 157.1 were also shown in the HMBC spectrum [10]. Thus, compound **1** was determined to be glycitein-4′,7-di-*O*-β-D-glucoside (Figure 1) and named ammopiptanoside A.

Compound **2** was isolated as white amorphous powder. (+)-ESI-MS displayed the *quasi*-molecular ion at *m/z* 777 $[M + Na]^+$, and the (+)-HR-ESI-MS exhibited a *quasi*-molecular ion at *m/z* 755.2329 $[M + H]^+$, indicating the molecular formula of **2** to be $C_{34}H_{42}O_{19}$. In the ^{1}H NMR spectrum of **2**, one proton singlet at δ_H 8.40 (1H, s) was characteristic signal of an isoflavone and assignable to H-2, two doublets at δ_H 7.48 (d, 2H, $J = 7.5$ Hz) and 7.10 (d, 2H, $J = 7.5$ Hz) due to 1′,4′-disubstituted B-ring and the signals at δ_H 7.50 (s, 1H) and 7.36 (s, 1H) attributable to aromatic protons of 6,7-disubstituted A-ring. The presence of a methoxyl group was exhibited by a proton signal at δ_H 3.87 (s, 3H). The ^{1}H NMR spectrum of **2** also contained three anomeric proton signals at δ_H 5.15 (d, 1H, $J = 7.0$ Hz), 4.91 (d, 1H, $J = 6.5$ Hz) and 5.62 (s, 1H) and a methyl at δ_H 1.55 (3H, d, $J = 6.0$ Hz). Comparison of the spectral data for **2** with those of compound **1** showed their structural similarity, except that compound **2** had additional hexose unit, which was supported by the molecular weight of **2**, more 146 amu than that of **1**. The position of rhamnosyl unit could be determined by an HMBC experiment. In the HMBC spectrum, the correlations between H-1‴′ (δ_H 5.62) of rhamnose and C-6″ (δ 69.4) of glucose, between H-6″ (δ_H 4.63) of glucose and C-5″ (δ 77.6) of glucose, between H-1″ (δ 5.15) and C-5″ (δ 77.6) of glucose, and between H-1″ (δ 5.15) and C-7 (δ 150.9) of aglycone, indicated that the rhamnopyranosyl fragment could be attached at C-6 position of the glucose at C-7 of aglycone. Thus, compound **2** was determined to be 4′-*O*-β-D-glucopyranosyl-glycitein 7-α-L-rhamnopyranosyl-(1 → 6)-*O*-β-D-glucopyranoside (Figure 1) and named as ammopiptanoside B.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an XT_4-100_x micromelting apparatus (Electro-Optical Scientific Instrument Factory, Beijing, China) and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 MC polarimeter (Perkin-Elmer, Waltham, MA, USA). NMR spectra were recorded on a Inova 500

Table 1. The ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) spectral data of **1** and **2** (DMSO-d_6, δ ppm).

	1		**2**	
Position	δ_C	δ_H	δ_C	δ_H
2	153.4	8.42 (s, 1H)	153.0	8.40 (s, 1H)
3	122.7		122.8	
4	174.2		173.9	
5	103.4	7.48 (s, 1H)	103.1	7.50 (s, 1H)
6	147.5		147.8	
7	151.6		150.9	
8	104.7	7.33 (s, 1H)	104.5	7.36 (s, 1H)
9	151.2		150.8	
10	117.8		118.1	
1′	125.5		125.6	
2′	129.9	7.52 (d, 2H, 8.0 Hz)	130.1	7.48 (d, 2H, 7.5 Hz)
3′	116.0	7.08 (d, 2H,8.0 Hz)	116.2	7.10 (d, 2H, 7.5 Hz)
4′	157.1		157.1	
5′	116.0	7.08 (d, 2H, 8.0 Hz)	116.2	7.10 (d, 2H, 7.5 Hz)
6′	129.9	7.52 (d, 2H, 8.0 Hz)	130.1	7.48 (d, 2H, 7.5 Hz)
1″	99.6	5.17 (d, 1H, 7.5 Hz)	99.8	5.15 (d, 1H, 7.0 Hz)
2″	73.0	5.40 (s,1H)	73.2	5.45 (s,1H)
3″	76.7	5.08 (s, 1H)	76.8	5.04 (s, 2H)
4″	69.6		69.6	
5″	77.0		77.6	
6″	60.6	4.57 (m, 2H)	69.4	4.63 (m, 2H)
1‴	100.4	4.90 (d, 1H, 7.0 Hz)	100.5	4.91 (d, 1H, 6.50 Hz)
2‴	73.2	5.31 (s, 1H)	73.0	5.31 (s, 1H)
3‴	76.6	5.08 (s, 1H)	76.5	5.08 (s, 2H)
4‴	69.7	5.01 (d, 1H, 3.5 Hz)	69.1	5.01 (d, 1H, 3.5 Hz)
5‴	77.2		77.1	
6‴	60.7	4.57 (m, 2H)	60.7	4.57 (m, 2H)
1‴			102.5	5.62 (s, 1H)
2⁗			72.1	4.69 (s, 1H)
3⁗			72.4	4.54 (s, 1H)
4⁗			72.2	4.28 (m, 1H)
5⁗			70.1	4.72 (1H, d, $J = 6.0$ Hz)
6⁗			18.2	1.55 (3H, d, $J = 6.0$ Hz)
OCH_3	55.8	3.88 (s, 3H)	55.9	3.87 (s, 3H)

(^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrometer (Varin, Inc., Palo Alto, CA, USA). ESI-MS was performed with Angilent 1100 LC/MSD (Santa Clara, CA, USA). For column chromatography, silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China), ODS (40–60 μm, Alltech) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) were used. The analytical HPLC was performed on an Angilent 1200 LC with DAD and the preparative HPLC was performed on a Shimadzu LC-20A (Shimadzu LC-20A, Kyoto, Japan) with YMC-Pack ODS column (20 × 250 mm, 10 μm, YMC Co. Ltd, Kyoto, Japan).

3.2 *Plant material*

The twigs of *A. nanus* were collected in Tulufan Desert Botanical Garden (Chinese Academy of Science), in Xinjiang Uygur Autonomous Region of China, and are identified by Prof. Jin Li (Xinjiang Normal University). A voucher specimen (ID-S-2321) is deposited at Institute of

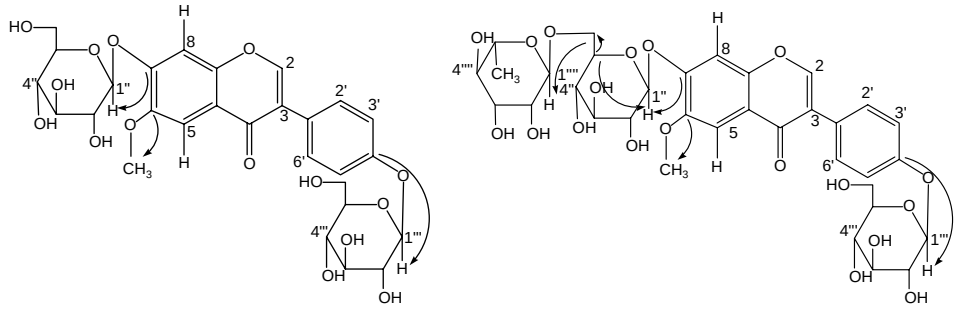

Figure 1. Key HMBC correlations of compounds **1** and **2**.

Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College.

3.3 Extraction and isolation

The air-dried twigs (0.365 kg) were crushed and extracted with 95% EtOH (4 liters) under reflux for three times (2 h each time). The ethanol extract was concentrated *in vacuo* to give a residue (102 g), and the 95% extract was subjected to silica gel column chromatography eluted with petroleum ether, or $CHCl_3$–MeOH gradient system (v/v 100:0, 20:1, 10:1, 4:1, 2:1, 1:1, 0:100), to obtain eight fractions. The separation of fraction 3 (19.2 g) was carried out on silica gel column chromatography eluted with $CHCl_3$–MeOH (v/v 9:1, 4:1) and yielded 20 subfractions 3A ~ 3T. Subfractions 3F ~ 3J were further purified by preparative thin-layer chromatography ($CHCl_3$–MeOH = 4:1) and Sephadex LH-20 column eluted with MeOH to afford compounds **3** (17 mg), **4** (23 mg), **5** (8 mg) and **8** (12 mg). From the fraction 5, compounds **1** (11 mg), **6** (42 mg), and **7** (181 mg) were obtained by silica gel column chromatography eluted with $CHCl_3$–MeOH–H_2O (v/v/v, 4:1:0.1) and Sephadex LH-20 column eluted with MeOH. The separation of fraction 7 was carried out on Sephadex LH-20 column eluted with MeOH to afford 14 subfractions 7A ~ 7N, and subfractions 7D ~ 7E (8.6 g) were further purified by medium pressure liquid chromatography eluted with MeOH–H_2O gradient system (v/v 10:90, 20:80, 30:70, 50:50, 70:30, 80:20, 100:0) to obtain seven mixtures of 7DE1 ~ 7DE7. Compound **2** (6 mg) was obtained by preparative HPLC eluted with MeOH–H_2O (18:82 (0.05% CF_3COOH), flow rate 7 ml/min, 280 nm, $t_R = 21$ min) from 7DE3.

3.4 Acid hydrolysis of compounds 1 and 2

Eighty microliters of D-glucose, L-rhamnose, D-xylose, and L-arabinose aqueous solutions (each 2 mg/ml) were mixed with 80 μl 0.5 mol/l 1-phenyl-3-methyl-5-pyrazolone in CH_3OH and 80 μl 0.3 mol/l NaOH aqueous solution. The mixtures were heated at 70°C for 30 min and then cooled at room temperature, to which 80 μl 0.3 mol/l HCl aqueous solution was added. The resulted mixture was extracted with $CHCl_3$ (0.5 ml, three times), and the water fractions were identified by HPLC analysis (Phenomenex C18, 250 mm × 4.6 mm, 5 μm; flow phase: A: CH_3CN–20 mmol/l NH_4OAc aqueous solution (15:85), B: CH_3CN–20 mmol/l NH_4OAc aqueous solution (40:60); flow rate about 1.2 ml/min by gradient elution, 0 → 20 min, volume fraction of B from 0% to 60%; detection wavelength: 245 nm; sample volume: 20 μl).

Compounds **1** (5 mg) and **2** (4 mg) were heated in an ampule with 2 ml of aqueous 2M HCl–1,4-dioxane (1:1),

respectively, at 80°C for 6 h. The aglycone was extracted with chloroform, and the aqueous residue was evaporated under reduced pressure. The residue was taken as preparations of the normal sugar derivatives. Then, D-glucose in compound **1**, as well as L-rhamnose and D-glucose in the ratio of 1:2 in compound **2**, was identified by HPLC analysis of the derivatives [11].

3.4.1 Ammopiptanoside A (*1*)

White amorphous powder. m.p. 182–184°C; $[\alpha]_D^{20}$ −39 (*c* 0.1, MeOH); UV (MeOH): $\lambda_{max}(\log \varepsilon)$ 256 (4.21), 315 (1.58) nm; ^{1}H NMR and ^{13}C NMR spectral data see Table 1; ESI-MS *m/z*: 631 $[M + Na]^+$. HR-ESI-MS *m/z*: 609.1825 $[M + H]^+$ (calcd for $C_{28}H_{33}O_{15}$, 609.1814).

3.4.2 Ammopiptanoside B (*2*)

White amorphous powder. m.p. 176–178°C; $[\alpha]_D^{20}$ −26 (*c* 0.05, MeOH); UV (MeOH): $\lambda_{max}(\log \varepsilon)$ 258 (4.30), 320 (1.08) nm; ^{1}H NMR and ^{13}C NMR spectral data see Table 1; ESI-MS *m/z*: 777 $[M + Na]^+$; HR-ESI-MS *m/z*: 755.2329 $[M + H]^+$ (calcd for $C_{34}H_{43}O_{19}$, 755.2322).

Acknowledgement

This work was financially supported by the National Science and Technology Project of China (No. 2009ZX09301-003-4-1).

References

[1] C.L. Li, Q.S. Yu, and A.D. Li, *Chin. Wild Plant Resour.* **5**, 21 (2004).
[2] X.M. Tian, S.Z. Cheng, P.F. Tu, and L.D. Lei, *China J. Chin. Mater. Med.* **19**, 2204 (2008).
[3] N.L. Zhang, J.Z. Cai, R.M. Huang, Y.J. Hu, Y. Yue, and S.X. Qiu, *Chin. Tradit. Herb. Drugs.* **42**, 1909 (2011).
[4] Q. Ma, H.M. Lei, and Y.X. Zhou, *China Pharm J.* **40**, 1058 (2005).
[5] S. Tahara, Y. Katagiri, J. Ingham, and J. Mizutani, *Phytochemistry* **36**, 1261 (1994).
[6] Jun-Ei Kinjo, Jun-Ichi Furuswa, and Junko Baba, *Chem. Pharm. Bull.* **35**, 4849 (1987).
[7] K. Watanabe, J.E. Kinjo, and T. Nohata, *Chem. Pharm. Bull.* **41**, 396 (1993).
[8] S.K. Chaudhuri, L. Huang, F. Fullas, D.M. Brown, M.C. Wani, M.E. Wall, J.C. Tucker, C.W.W. Beecher, and A.D. Kinghorn, *J. Nat. Prod.* **58**, 1966 (1995).
[9] C. Xiang, J. Cheng, H. Liang, Y.Y. Zhao, and J. Feng, *Acta Pharm. Sin.* **2**, 160 (2009).
[10] M. Leda, V. Ivo, J. C, and B. Raimundo, *J. Nat. Prod.* **61**, 1158 (1998).
[11] R. Oshima, Y. Yamauchi, and J. Kumanotani, *Carbohydr. Res.* **107**, 169 (1982).