Chemistry of Natural Compounds, Vol. 43, No. 5, 2007

CHEMICAL CONSTITUENTS FROM THE ROOTS OF *Polygonum bistorta*

Xiao-Bai Sun, Pei-Hua Zhao, Yang-Jun Xu, Li-Mei Sun, Mei-Ai Cao, and Cheng-Shan Yuan*

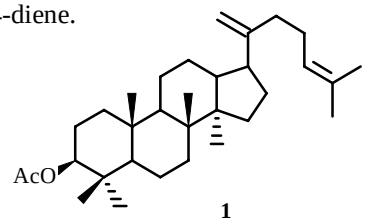
UDC 547.918

Investigation of the roots of Polygonum bistorta *L. afforded seven compounds including five triterpenoids, a coumarin, and a steroid, the structures of which were identified by EIMS, ^{1}H NMR, ^{13}C NMR, DEPT, and HMBC experiments. All the compounds have been isolated from* Polygonum genus *for the first time.*

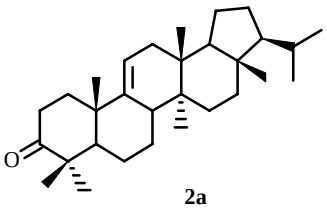
Key words: *Polygonum bistorta*, triterpenoid, coumarin, steroid.

The dried roots of *Polygonum bistorta* L. have been widely used as Chinese folk medicine for the treatment of suppurative dermatitics, hemorrhoids, and hematemesis [1]. Recently, some studies indicate that it has anti-inflammatory, anti-bacterial, and even anti-cancer properties [2], but the chemical constituents of this plant have not been studied systematically. It is necessary to start the study on the chemical components of this oriental medicine. This study attempted to investigate the constituents of the roots of *P. bistorta* L., and seven compounds, including five triterpenoids, a coumarin, and a steroid, were isolated and identified. To the best of our knowledge, those compounds have been reported from *Polygonum* genus for the first time.

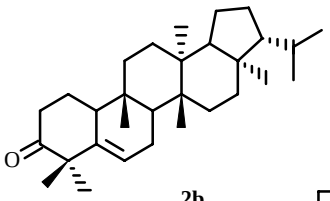
Compound **1** was obtained as white powder. The molecular formula $C_{32}H_{52}O_2$ could be deduced from its EIMS and ^{13}C NMR. The ^{13}C NMR and DEPT spectra of **1** exhibited the presence of 32 carbons, including an ester carbonyl carbon (171.00, C), four double-bond carbons (152.74, C; 124.48, CH; 131.33, CH; 107.49, CH_2), and an oxygen-bearing carbon (80.97, CH). The ^{1}H NMR spectrum revealed the presence of a singlet (δ 5.12, 1H), a methylene (δ 4.73 and 4.88, each 1H, each s), an acetyl methyl (δ 2.04, s), two vinyl methyls (δ 1.61, 1.68, each s), and five shielded methyls (δ 0.81, 0.81, 0.82, 0.84, 0.96, each s). In addition, the multiplet centered at δ 4.47 indicated the presence of a proton on an oxygen-bearing carbon. From the molecular formula and the information above, **1** was considered to be a tetracyclic triterpenoid with an acetyl group. By referencing the published spectral data in the literature [3], the structure of **1** was finally determined as 3β-acetoxy-dammara-20,24-diene.



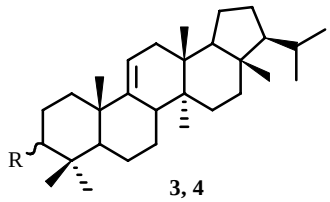
1



2a

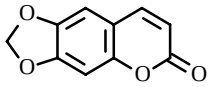


2b

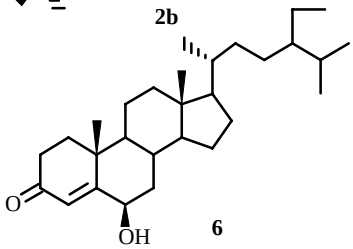


3, 4

3: R = α-OH
4: R = β-OH



5



6

State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, P. R. China, Fax +86 931 912582, e-mail: yuancs@lzu.edu.cn. Published in Khimiya Prirodnykh Soedinenii, No. 5, pp. 463-465, September-October, 2007. Original article submitted May 11, 2006.

0009-3130/07/4305-0563

TABLE 1. ^{13}C NMR Chemical Shifts of Compounds **1-4** and **6** ($CDCl_3$ solution, TMS as int. standard)

No.	1*	2a	2b	3	4	6
1	35.62	36.66	38.13	30.74	36.07	38.56
2	34.12	34.86	33.63	26.04	27.83	34.76
3	80.97	217.06	215.34	76.60	78.97	200.36
4	40.51	47.63	49.95	38.17	39.64	126.35
5	55.98	53.33	142.72	46.90	52.34	168.41
6	18.18	26.30	121.37	21.69	21.44	73.31
7	28.92	22.62	24.08	26.95	26.69	37.11
8	37.92	41.12	43.89	41.39	40.99	29.73
9	50.88	147.47	35.03	149.17	148.88	53.63
10	37.16	39.42	51.24	39.93	39.08	37.98
11	21.39	115.67	21.35	114.42	114.33	20.97
12	24.94	36.14	28.96	36.40	36.02	39.60
13	45.30	36.76	38.68	37.11	36.79	42.51
14	49.44	38.23	39.32	38.62	38.20	56.06
15	31.37	29.66	29.03	29.96	29.66	24.15
16	27.07	35.92	35.41	36.29	35.94	28.17
17	47.82	42.81	42.85	43.21	42.96	59.81
18	15.96	52.04	51.75	52.43	52.09	11.97
19	15.65	20.17	19.93	20.59	20.18	19.81
20	131.33	28.18	28.28	28.53	28.21	36.11
21	107.49	59.65	60.05	60.00	59.65	18.72
22	38.79	30.75	30.75	31.09	30.77	33.90
23	23.72	25.59	28.39	28.6	28.21	26.10
24	124.48	22.02	24.66	22.84	15.63	45.86
25	152.74	21.65	17.40	22.25	22.12	23.08
26	27.98	16.94	15.46	17.36	17.03	12.00
27	17.69	15.31	15.07	15.63	15.29	29.16
28	16.27	13.99	16.04	14.32	13.99	19.50
29	16.49	22.96	22.88	23.29	22.98	19.03
30	25.69	22.11	21.95	22.44	22.12	

*The ^{13}C-NMR chemical shifts of the acetyl group are 21.21 and 171.00 ppm.

Compound **2** was obtained as a white powder. It was a mixture of **2a** and **2b**. Compounds **2a** and **2b** had the same formula $C_{30}H_{48}O$, which was identified by ^{13}C NMR and DEPT. The ^{13}C NMR and DEPT spectra showed that each of them had a ketone carbonyl carbon (217.06 and 215.34), a trisubstituted double bond (147.47, C; 115.67, CH, and 142.72, C; 121.37, CH), and an isopropyl group (30.75, CH; 22.96, CH_3; 22.11, CH_3, and 30.75, CH; 22.88, CH_3; 21.95, CH_3). Based on the evidences above, **2a** and **2b** could be deduced to be pentacyclic triterpenoids, and **2a** was identified as arborinone, whose data agreed with the literature [4], while **2b** was adianenone, whose data agreed with literature [5].

Compound **3** was obtained as colorless needles. The molecular formula $C_{30}H_{50}O$ could be determined by analysis of its EIMS and ^{13}C NMR spectra. In the ^{13}C NMR spectrum, the resonances at δ 149.17 (C) and 114.42 (CH) could be assigned to olefinic carbons, and a downfield resonance at δ 76.60 (CH) could be assigned to an oxygen-bearing carbon.

Furthermore, the existence of an isopropyl group was indicated by signals at δ 31.09 (CH), 23.29 (CH_3) and 22.44 (CH_3). The ^1H NMR spectrum revealed two doublets [δ 0.88 (3H, d, J = 6.8 Hz), δ 0.82 (3H, d, J = 6.8 Hz)] and other six C-methyls (δ 0.74, 0.76, 0.81, 0.87, 0.95, 1.04, each s). In addition, the 1H-singlet at δ 3.42 indicated the presence of a proton on an oxygen-bearing carbon, which also showed the existence of an α-OH. By comparison of the data with the literature [6], compound **3** was identified as arborinol, the structure of which was also supported by its HMBC experiments.

Compound **4** was obtained as a white powder. The ^1H NMR, ^{13}C NMR, and DEPT spectra showed it to possess the molecular formula $C_{30}H_{50}O$ and showed the same characteristics as compound **3**. With the exception of signals of H-3, which appeared as a doublet at δ 3.20 (1H, dd, J = 7.6, 3.2 Hz) in **4** and as a broad singlet at δ 3.42 in **3**, the ^1H NMR spectra of **4** and **3** were very similar. Thus, the difference was that **4** had a 3β-OH while **3** had an 3α-OH. Based on the former evidence,

compound **4** could be determined as isoarborinol, the spectral data of which have been previously described in the literature [6]. Compound **5**, which had the formula $C_{10}H_6O_4$, was crystallized from Me_2CO as colorless platelets. The information from ^1H NMR, ^{13}C NMR, and EIMS spectra indicated that **5** was a coumarin. A strong singlet at δ 6.07 (2H, s) in ^1H NMR spectrum was typical of a dioxymethylene group. From the spectral data, compound **5** was assigned as 6,7-methylene-dioxycoumarin, and its spectral data agreed with the literature [7].

Compound **6** was obtained as a white powder. The molecular formula $C_{29}H_{48}O_2$ could be deduced from its ^{13}C NMR and DEPT. From the molecular formula and characteristics in the ^1H NMR spectrum, **6** was considered to be a C_{29}-steroid. The ^{13}C NMR spectrum showed the presence of 29 carbons, including a ketone carbonyl carbon (200.36), two olefinic carbons (168.41, C; 126.35, CH), and an oxygen-bearing carbon (73.31, CH). Thus compound **6** could be 6-hydroxystigmast-4-en-3-one, which was established by comparison of their spectral data with those reported in the literature [8].

EXPERIMENTAL

General Experimental Procedures. ^1H NMR (400 MHz), ^{13}C NMR (100 MHz), DEPT, and HMBC spectra were recorded on a Varian Mercury-400BB NMR spectrometer; EIMS data were obtained on a HP 5988A-GC/MS instrument. Sephadex LH-20 (Pharmacia); silica gel (200–300 mesh) for CC. and silica GF254 for TLC was supplied by the Qingdao Marine Chemical factory.

Plant Material. The root of *Polygonum bistorta* L. was purchased from Focitang Medicine Material Corporation in Lanzhou and was identified by Prof. Pei-Jun Yu, the Second Hospital of Lanzhou University. A voucher specimen (No. 200503PB) is deposited in the Institute of Organic Chemistry, Lanzhou University.

Extraction and Isolation. The dried milled roots of *P. bistorta* (10.0 kg) were percolated with 90% aq. MeOH three times (one week each time) at room temperature. After vaporation of MeOH under reduced pressure, the aq. solution was extracted with petroleum ether (30–60°), EtOAc, and *n*-BuOH successively.

The petroleum ether extract (32.5 g) was chromatographed over Si-gel using petroleum ether–Me_2CO (100:1 to 0:1) gradient and divided into 6 crude fractions (Fr.A–Fr.F). Fr.B was subjected to Si-gel CC eluted with petroleum ether–Me_2CO (100:1 to 20:1) to give 6 fractions (Fr.B-1–Fr.B-6). Fr. B-3 gave **1** (20 mg) after recrystallization from Me_2CO. Fr.B-4 was submitted to preparative TLC (petroleum ether–EtOAc–Me_2CO, 10:10:1) to yield **2a**+**2b** (16 mg). Fr.B-5 was purified on Sephadex LH-20 column (petroleum ether: $CHCl_3$–MeOH, 5:5:1) to give **3** (8 mg). Fr.C was subjected to Si-gel CC eluted with petroleum ether–Me_2CO (80:1 to 1:1) to give 5 fractions (Fr.C-1–Fr.C-5). Fr.C-3 gave **4** (8 mg) after reseparation by Si-gel (petroleum ether–$CHCl_3$, 5:1 to 0:1). Fr.E was subjected to Si-gel CC eduted with petroleum ether–Me_2CO (50:1 to 1:1) to give 6 fractions (Fr.E-1–Fr.E-6). Fr.E-2 gave **5** (9 mg) after recrystallization from Me_2CO. Fr.E-4 was futher separated by Si-gel CC using petroleum ether–Me_2CO (20:1) to afford **6** (15 mg).

Structure and Identification. **3*β*-Acetoxy-dammara-20, 24-diene** (**1**): white powder, $C_{32}H_{52}O_2$, ^1H NMR ($CDCl_3$, δ, ppm): 5.12 (1H, s, H-24), 4.88, 4.73 (each H, each s, H-21), 4.47 (1H, m, H-3), 2.04 (3H, s, CO-CH_3), 1.68 (3H, s, H-27), 1.61 (3H, s, H-26), 0.96 (3H, s), 0.84 (3H, s), 0.82 (3H, s), 0.81 (6H, s). EIMS (I_{rel}, %): 468 (3) $[M]^+$, 299(3), 249 (8), 229 (5), 218 (10), 204 (13), 189 (61), 175 (14), 161 (18), 147 (17), 135 (33), 121 (28), 109 (54), 105 (23), 93 (48), 81 (32), 69 (82), 55 (24), 43 (100). For ^{13}C NMR data, see Table 1.

Arborinone (2a): white powder, $C_{30}H_{48}O$, ^1H NMR ($CDCl_3$, δ, ppm): 5.28 (1H, d, J = 5.6Hz, H-11). For ^{13}C NMR data, see Table 1.

Adianenone (2b): white powder, $C_{30}H_{48}O$, ^1H NMR ($CDCl_3$, δ, ppm): 5.66 (1H, d, J = 6.4 Hz, H-6). For ^{13}C NMR data, see Table 1.

Arborinol (3): colorless needles, $C_{30}H_{50}O$, ^1H NMR ($CDCl_3$, δ, ppm, J/Hz): 5.26 (1H, d, J = 5.6, H-11), 3.42 (1H, s, H-3), 1.04 (3H, s, H-25), 0.95 (3H, s, H-23), 0.88 (3H, d, J = 6.8, H-29), 0.87 (3H, s, H-24), 0.82 (3H, d, J = 6.8, H-30), 0.81 (3H, s, H-26), 0.76 (3H, s, H-27), 0.74 (3H, s, H-28). EIMS (I_{rel}, %): 426 (9) $[M]^+$, 411 (12) $[M\text{-}CH_3]^+$, 393 (8) $[M\text{-}CH_3\text{-}H_2O]^+$, 259 (24), 241 (16), 137 (26), 133 (23), 123 (23), 119 (37), 109 (46), 105 (41), 95 (65), 91 (31), 81 (54), 69 (88), 55 (82), 43 (100). For ^{13}C NMR data, see Table 1.

Isoarborinol (4): white powder, $C_{30}H_{50}O$, ^1H NMR ($CDCl_3$, δ, ppm, J/Hz): 5.22 (1H, d, J = 4.8, H-11), 3.20 (1H, dd, J = 7.6, 3.2, H-3), 0.95 (3H, s, H-25), 0.89 (3H, s, H-23), 0.86 (3H, d, J = 7.6, H-29), 0.83 (3H, d, J = 7.6, H-30), 0.81 (3H, s, H-24), 0.80 (3H, s, H-26), 0.76 (3H, s, H- 27), 0.75 (3H, s, H-28). For ^{13}C NMR data, see Table 1.

6,7-Methylenedioxycoumarin (5): colorless platelets, $C_{10}H_6O_4$, ^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 7.57 (1H, d, J = 9.6, H-4), 6.82 (2H, s, H-5, H-6), 6.27 (1H, d, J = 9.6, H-3), 6.07 (2H, s, 6,7-OCH_2O-). ^{13}C NMR ($CDCl_3$, δ, ppm): 161.16 (C-2), 151.28 (C-8), 144.91 (C-8a), 143.41 (C-4), 143.41 (C-6), 113.41 (C-3), 112.69 (C-4a), 105.02 (C-5), 102.32 (6, 7-OCH_2O-), 98.41 (C-8). EIMS (I_{rel}, %): 190 (100) $[M]^+$, 162 (95) $[M\text{-}CO]^+$, 161 (98) $[M\text{-}CO\text{-}H]^+$, 76 (38), 69 (22), 62 (11), 55 (5), 53 (38), 51 (34).

6-Hydroxystigmast-4-en-3-one (6): white powder, $C_{29}H_{48}O_2$, ^{1}H NMR ($CDCl_3$, δ, ppm, J/Hz): 5.81 (1H, s, H-4), 4.34 (1H, s, H-6), 1.37 (3H, s, H-18), 0.91 (3H, d, J = 6.4, H-21), 0.84 (3H, t, J = 7.2, H-26), 0.83 (3H, d, J = 9.6, H-28), 0.80 (3H, d, J = 9.6, H-29). For ^{13}C NMR data, see Table 1.

ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (No. 20021001 QT Program).

REFERENCES

1. Committee of pharmacopoeia, *Pharmacopoeia of People's Republic of China*, Chemical industry press, 2000, p. 239.
2. M. Duwiejua, I. J. Zeitlin, A. I. Gray, and P. G. Waterman, *Planta Med.*, **65**, 371 (1991).
3. Q. F. Zhang, S. D. Luo, and H. Y. Wang, *Acta Botanica Yunnanica*, **20** (3), 362 (1998).
4. A. Toshihiro, Y. Kazuhiro, T. toshitake, K. Yumiko, I. Takashi, N. Toshio, and C. C. Frederic, *Chem. Pharm. Bull.*, **40** (3), 789 (1992).
5. H. A. Loycl, M. J. Taysir, S. L. Evans, and J. F. Morton, *Phytochemistry*, **16** (6), 1301 (1977).
6. K. C. Ajit, M. Kazuo, S. Hideki, and A. Hiroyuki, *Tetrahedron*, **50** (9), 2865 (1994).
7. L. D. Silvia, L. N. Elena, D. C. Jorge, D. K. Norbert, and B. Marc, *Phytochemistry*, **48** (4), 707 (1998).
8. M. S. George, C. M. Yu, and S. Annalisa, *Phytochemistry*, **28** (10), 2861(1989).