DOI 10.1007/s10600-019-02826-0
Chemistry of Natural Compounds, Vol. 55, No. 5, September, 2019

PHENOLIC ACIDS FROM THE SEEDS OF *Sophora alopecuroides*

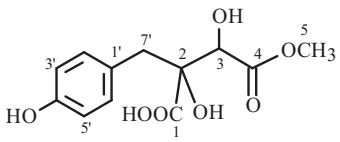
Ping Song[1], Hao Chen[2], Yun Huang[2], Yanzhang Wen[2], Ji Hao[2], Yibing Lv[2], Shihao Deng[2*], and Xinzhou Yang[2*]

Chemical profiling of the EtOAc extract from a traditional Chinese medicine Sophora alopecuroides *yielded a new phenolic acid, (3*S*,2*R*)-methyl 2-(4-hydroxybenzyl)tartrate (**1**), along with four known compounds, eucomic acid (**2**), methyl eucomate (**3**), piscidic acid (**4**), and ethyl 2-(4-hydroxybenzyl)tartrate (**5**). The structure of **1** was determined by extensive spectral analyses including 1D and 2D NMR and MS data. All compounds were evaluated for their cytotoxic activities against HepG2 and Hep3B hepatocellular carcinoma (HCC) cell lines. Compounds **1–5** showed weak anti-HCC activities.*

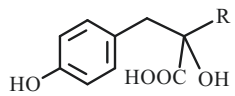
Keywords: *Sophora alopecuroides*, phenolic acid, cytotoxity.

Sophora alopecuroides L. is widely distributed in the desert of northwest and north of China. The seeds of this herb have been used as the traditional Chinese ethnic medicine "Buya" in northwest of China to treat acute diarrhea and enteritis [1]. Pharmacological studies show that the plant exhibits a variety of biological activities including anticancer, antivirus, antibacterial, and anti-neuroinflammatory effects [1–3]. Modern chemical research is mainly focused on its alkaloids and flavonoids [4, 5]. However, studies of phenolic compounds are rare. This prompted us to further study its chemical compositions. Five phenolic natural products were isolated from *S. alopecuroides*. On the basis of the spectral data, they were determined as (3*S*,2*R*)-methyl 2-(4-hydroxybenzyl)tartrate (**1**), eucomic acid (**2**) [6], methyl eucomate (**3**) [7], piscidic acid (**4**) [8], and ethyl 2-(4-hydroxybenzyl)tartrate (**5**) [9]. Among them, compound **1** is a new compound, and compounds **1–3**, and **5** were isolated from the seeds of *S. alopecuroides* for the first time. The cytotoxity of all isolated compounds was tested against two human hepatocellular carcinoma cell lines, HepG2 and Hep3B. All compounds **1–5** exhibited weak anticancer activity against HepG2 and Hep3B cell lines.

Compound **1**, (3*S*,2*R*)-methyl 2-(4-hydroxybenzyl)tartrate, was isolated as a white powder. The molecular formula $C_{12}H_{14}O_7$ was indicated by EI-MS at *m/z* 269 $[M - H]^-$, combined with the NMR spectral data. The characteristic maximum absorptions at 205, 225, and 272 nm in the UV spectrum suggested that **1** was an aromatic derivative. Its IR spectrum showed strong absorption bands for a hydroxyl group (3390 cm^{-1}) and benzene ring (1612, 1608 cm^{-1}).



1



2 - 5

2: R = CH_2-COOH; **3:** R = CH_2-$COOCH_3$
4: R = CH(OH)-COOH; **5:** R = CH(OH)-$COOCH_2CH_3$

1) Division of Science & Technology, Qinghai University for Nationalities, 810007, Xiling, P. R. China; 2) School of Pharmaceutical Sciences, South-Central University for Nationalities, 430074, Wuhan, P. R. China, fax: +86 27 67841196, e-mail: s791476858@163.com; xzyang@mail.scuec.edu.cn. Published in *Khimiya Prirodnykh Soedinenii*, No. 5, September–October, 2019, pp. 720–722. Original article submitted August 6, 2018.

TABLE 1. Cytotoxic Activity of Compounds **1–5** (IC_{50}, μM; mean ± SD, n = 3)

Compound	HepG2	Hep3B	Compound	HepG2	Hep3B
1	72.5 ± 2.3	86.1 ± 3.2	**4**	92.5 ± 2.3	> 100
2	73.2 ± 1.9	78.6 ± 2.7	**5**	88.6 ± 2.5	> 100
3	84.7 ± 2.5	75.6 ± 3.6	Cisplatin*	6.7 ± 1.6	8.6 ± 1.8

*Positive control.

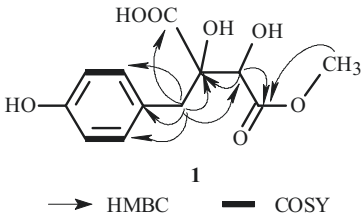


Fig. 1. Key HMBC and ^1H–^1H COSY correlations for compound **1**.

The ^{13}C NMR spectrum showed the presence of two carbonyl carbons at δ 175.7 and 173.4, six aromatic carbons including one oxygenated aromatic carbon at δ 157.2 and five aromatic carbons at δ 132.5, 115.7, and 127.7, one methoxyl carbon at δ 52.4, one methylene carbon at δ 41.7, one oxygenated methine carbon at δ 76.6 and one quaternary carbon at δ 81.8. The ^1H NMR spectrum showed a 1,4-disubstituted phenyl group [δ 7.07 (2H, d, J = 8.5 Hz, H-2′, 6′), 6.65 (2H, d, J = 8.5 Hz, H-3′, 5′)], one isolated methylene [δ 3.13 (1H, d, J = 13.8 Hz, H-7′b), 2.97 (1H, d, J = 13.8 Hz, H-7′a)], and an oxygenated methine at δ 4.57 (1H, s, H-3), similar to the data of piscidic acid (**4**) [8]. An additional signal in **1** is a methoxy at δ 3.70 (3H, s, H-5). The HSQC and ^1H–^1H COSY spectrum of **1** provided unambiguous assignments of proton and carbon signals. In the HMBC spectrum, the key HMBC long-range correlations combined with the chemical shifts of these proton and carbon resonances revealed the presence of a 2-substituted tartaric acid moiety in compound **1**. In addition, HMBC correlations (Fig. 1) from a methylene at δ 2.97 and 3.13 (H-7′) to the carbonyl carbon at δ 175.7 (C-1), a quaternary carbon at δ 81.6 (C-2), and the aromatic carbons at δ 127.9 (C-1′) and 132.5 (C-2′, 6′) determined that the 4′-hydroxybenzyl moiety was located at C-2. The HMBC correlation from methoxyl at δ 3.70 (4-OMe) to δ 173.4 (C-4) demonstrated that the tartaric acid was esterified at the 4-carboxyl moiety. Thus, **1** was elucidated as methyl 2-(4-hydroxybenzyl)tartrate. The similarities of the positive optical rotations for **1** ($[\alpha]_D^{25}$+46.96°, *c* 0.12, MeOH) and piscidic acid [8] suggest that compound **1** possesses the same absolute configuration. Therefore, the structure of compound **1** was determined as (3*S*,2*R*)-methyl 2-(4-hydroxybenzyl)tartrate.

Compounds **1–5** were evaluated against the hepatocellular carcinoma cell lines HepG2 and Hep3B. Preliminary toxicity towards human cells was investigated using a human embryonic kidney cell line, HEK293 (IC_{50} > 100 μM). All compounds **1–5** exhibited weak anticancer activity against HepG2 and Hep3B cell lines (Table 1).

EXPERIMENTAL

General. All melting points were determined on a Buchi 510 melting point apparatus and are uncorrected. Optical rotations were measured on a PerkinElmer polarimeter 341. IR spectra were determined on a Nicolet Magna FT-IR 750 spectrometer (ν_{max} in cm^{-1}). The NMR spectra were recorded on the Bruker DRX-600 NMR spectrometer for ^1H and ^{13}C NMR. The chemical shifts were given on the δ (ppm) scale with tetramethylsilane as internal standard, and the coupling constants (J) were in Hz. The EI-MS data were obtained on a Finnigan-MAT-95 mass spectrometer. Commercial silica gel (Qing Dao Hai Yang Chemical Group Co., 200–300 and 300–400 mesh) was used for column chromatography. Precoated Silical gel plates (Yan Tai Zi Fu Chemical Group Co., G60F-254) were used for analytical TLC.

Plant Material. The seeds of *Sophora alopecuroides* L. were purchased from the herbal market during May of 2015 at Xining, Qinghai Province, China and identified by one of our authors, Prof. Xinzhou Yang. A voucher specimen (No. SC0217) has been deposited in the Herbarium of South-Central University for Nationalities, Wuhan, China.

Extraction and Isolation. Dried seeds of *S. alopecuroides* (1.0 kg) were triturated and then extracted sequentially by maceration with 95% EtOH three times (3 L each) at room temperature. The solvent was evaporated under reduced pressure to obtain a crude extract (170 g). The extract was suspended in water and then partitioned successively with petroleum ether (PE), EtOAc, and *n*-BuOH to afford the PE fraction (12 g), EtOAc fraction (29 g), and *n*-BuOH fraction (55 g), respectively. The EtOAc fraction (20 g) was subjected to the column chromatography over silica gel eluting with CH_2Cl_2–MeOH (1:0→50:1→30:1→20:1→10:1→5:1→1:1→0:1, containing 0.1% formic acid) to give five fractions (1–5). Fraction 2 (4.5 g) was chromatographed over silica gel with EtOAc–acetone (100:1→30:1→20:1→10:1→5:1→0:1, containing 0.1% formic acid) to afford four fractions (2.1–2.4). Fractions 2.2 (200 mg) and 2.3 (315 mg) were further purified on a Sephadex LH-20 column, eluting with MeOH (containing 0.1% formic acid) to afford compounds **3** (22 mg) and **4** (17 mg), respectively. Fraction 3 (4.3 g) was subjected to column chromatography over silica gel with EtOAc–acetone (20:1→10:1→8:1→5:1→0:1, containing 0.1% formic acid) to afford three fractions (3.1–3.3). Fraction 3.3 (370 mg) was further purified on a Sephadex LH-20 column eluted with MeOH (containing 0.1% formic acid) and then purified by semipreparative HPLC (H_2O–CH_3CN, 80%:20% → 30%:70%, 40 min, containing 0.1% formic acid in both mobile phases) to afford **1** (18 mg) and **5** (21 mg), respectively. Fraction 5 (2.2 g) was separated by Sephadex-LH20 eluted with MeOH (containing 0.1% formic acid) to afford five fractions (5.1–5.5). Fraction 5.3 (300 mg) was further purified by semipreparative HPLC (H_2O–CH_3CN, 70%:30% → 30%:70%, 40 min, containing 0.1% formic acid in both phases) to afford compound **2** (31.3 mg).

(3*S*,2*R*)-Methyl 2-(4-hydroxybenzyl)tartrate (1), white powder; $[\alpha]_D^{25}$ +46.96° (*c* 0.12, MeOH). UV (MeOH, λ_{max}, nm) (log ε): 205 (1.04), 225 (1.16), 277 (0.38). IR (film, ν_{max}, cm^{-1}): 3390, 2950, 2810, 1731, 1612, 1608, 1515, 1442, 1226, 1118, 840, 782. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 7.07 (2H, d, J = 8.5, H-2′, 6′), 6.65 (2H, d, J = 8.5, H-3′, 5′), 4.57 (1H, s, H-3), 3.70 (3H, s, 4-OMe), 3.13 (1H, d, J = 13.8, H-7′b), 2.97 (1H, d, J = 13.8, H-7′a). ^{13}C NMR (150 MHz, CD_3OD, δ, ppm): 175.7 (C-1), 173.4 (C-4), 157.2 (C-4′), 132.5 (C-2′, 6′), 127.9 (C-1′), 115.7 (C-3′, 5′), 81.6 (C-2), 76.6 (C-3), 52.4 (C-5), 41.7 (C-7′). ESI-MS *m/z* 269 $[M - H]^-$. HR-ESI-MS *m/z* 269.0665 $[M - H]^-$.

Eucomic acid (2), white powder. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 7.08 (2H, d, J = 8.4, H-2′, 6′), 6.69 (2H, d, J = 8.4, H-3′, 5′), 2.97 (1H, d, J = 16.3, H-3b), 2.96 (1H, d, J = 13.3, H-7′a), 2.88 (1H, d, J = 13.3, H-7′b), 2.58 (1H, d, J = 16.3, H-3a). ^{13}C NMR (150 MHz, CD_3OD, δ, ppm): 175.3 (C-1), 173.0 (C-4), 156.9 (C-4′), 132.0 (C-2′, 6′), 127.4 (C-1′), 115.2 (C-3′, 5′), 76.4 (C-2), 44.7 (C-3), 43.1 (C-7′). ESI-MS *m/z* 239 $[M - H]^-$.

Methyl eucomate (3), white powder. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 7.08 (2H, d, J = 8.4, H-2′, 6′), 6.69 (2H, d, J = 8.4, H-3′, 5′), 3.66 (3H, s, H-5), 2.99 (1H, d, J = 15.8, H-3b), 2.97 (1H, d, J = 13.7, H-7′a), 2.88 (1H, d, J = 13.3, H-7′b), 2.61 (1H, d, J = 15.8, H-3a). ^{13}C NMR (150 MHz, CD_3OD, δ, ppm): 177.3 (C-1), 172.5 (C-4), 157.4 (C-4′), 132.6 (C-2′, 6′), 127.5 (C-1′), 115.8 (C-3′, 5′), 76.9 (C-2), 52.4 (C-5), 45.5 (C-3), 43.8 (C-7′). ESI-MS *m/z* 253 $[M - H]^-$.

Piscidic acid (4), white solid. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 6.97 (2H, d, J = 8.5, H-2′, 6′), 6.59 (2H, d, J = 8.5, H-3′, 5′), 4.29 (1H, s, H-3), 2.96 (1H, d, J = 13.8, H-7′b), 2.80 (1H, d, J = 13.8, H-7′a). ^{13}C NMR (150 MHz, CD_3OD, δ, ppm): 174.3 (C-1), 173.3 (C-4), 156.2 (C-4′), 131.6 (C-2′, 6′), 126.9 (C-1′), 115.0 (C-3′, 5′), 80.2 (C-2), 75.3 (C-3), 41.3 (C-7′). ESI-MS *m/z* 255 $[M - H]^-$.

Ethyl 2-(4-hydroxybenzyl)tartrate (5), colorless solid. ^{1}H NMR (600 MHz, CD_3OD, δ, ppm, J/Hz): 7.07 (2H, d, J = 8.5, H-2′, 6′), 6.69 (2H, d, J = 8.5, H-3′, 5′), 4.55 (1H, s, H-3), 4.19 (2H, q, J = 7.1, H-5), 3.15 (1H, d, J = 13.8, H-7′b), 2.99 (1H, d, J = 13.8, H-7′a), 1.26 (3H, t, J = 7.1, H-6). ^{13}C NMR (150 MHz, CD_3OD, δ, ppm): 175.6 (C-1), 173.3 (C-4), 157.1 (C-4′), 132.3 (C-2′, 6′), 127.7 (C-1′), 115.6 (C-3′, 5′), 81.5 (C-2), 76.3 (C-3), 62.3 (C-5), 41.3 (C-7′), 14.2 (C-6). ESI-MS *m/z* 283 $[M - H]^-$.

Cytotoxity Assay. Human hepatocellular carcinoma cell lines HepG2 and Hep3B and human embryonic kidney cell line HEK293 were purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA). The cytotoxic assay was performed as previously described [10–12]. Briefly, three cell lines suspended in RPMI 1640 containing 10% fetal bovine serum (FBS) were seeded at 1×10^4 cells (100 μL) per well in a flat 96-well plate and incubated in a humidified atmosphere of 5% CO_2 in air at 37°C. After 24 h, the medium containing different concentrations of compounds **1–5** was added to incubate cells for 24 h, and 0.1% DMSO was used as solvent control. After that, the cells were fixed with EtOH–H_2O (95:5), stained with crystal violet solution, and lysed with a solution of 0.1 N HCl in MeOH. The absorbance in control and drug-treated wells was measured in an automated microplate reader (Bio-Rad 550) at 550 nm.

ACKNOWLEDGMENT

This work was financially supported by the grant of Natural Science Foundation of Qinghai Province (No. 2016-ZJ-908) and Wuhan Applied Basic Research Program of Science and Technology (2017060201010217).

REFERENCES

1. H. T. Bian, J. Zhao, and H. Huang, *J. Chin. Med. Mater.*, **37**, 72 (2014).
2. C. Guo, L. Yang, and C. X. Wan, *Phytomedicine*, **23**, 1629 (2016).
3. M. Iinuma, J. Yokoyama, and M. Ohyama, *Phytochemistry*, **33**, 203 (1999).
4. X. Zhou, F. Jia, and X. Liu, *Chin. J. Integr. Med.*, **19**, 945 (2013).
5. A. Mun′im, O. Negishi, and T. Ozawa, *Biosci. Biotechnol. Biochem.*, **67**, 410 (2003).
6. J. Z. Song, H. X. Xu, and S. J. Tian, *J. Chromatogr. A.*, **857**, 303 (1999).
7. J. Jiang, Y. Li, and Z. Chen, *Steroids*, **71**, 1073 (2006).
8. H. Toshima, M. Saito, and T. Yoshihara, *Biosci. Biotechnol. Biochem.*, **63**, 964 (1999).
9. C. Luo, W. Zhang, and C. Sheng, *Chem. Biodiv.*, **7**, 2869 (2010).
10. J. Yang, C. Xu, H. Chen, M. Huang, X. H. Ma, S. H. Deng, Y. Huang, Y. Z. Wen, X. Z. Yang, and P. Song, *Biomed. Pharmacother.*, **96**, 1199 (2017).
11. E. M. Suleimen, G. G. Sisengalieva, R. I. Dzhalmakhanbetova, Zh. B. Iskakova, and M. Yu. Ishmuratova, *Chem. Nat. Compd.*, **54**, 1177 (2018).
12. S. A. Pukhov, S. V. Afanas'eva, L. V. Anikina, V. I. Kozlovskii, M. E. Neganova, and S. G. Klochkov, *Chem. Nat. Compd*., **55**, 41 (2019).