Three New Alkaloids from *Hippophae rhamnoides* LINN. subsp. *sinensis* ROUSI

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Two novel organic amide alkaloids, 4-[(*E*)-*p*-coumaroylamino]butan-1-ol (**1**) and 4-[(*Z*)-*p*-coumaroylamino]butan-1-ol (**2**), together with a rare pyridoindole alkaloid, hippophamide (**3**), were isolated from the seed residue of *Hippophae rhamnoides* LINN. subsp. *sinensis* ROUSI. Their structures were determined by spectroscopic means. The results show that compounds **1** and **2** are (*E*/*Z*)-isomers, compound **3**, a pyridoindole alkaloid concerted with γ-lactam ring.

Introduction. – *Hippophae rhamnoides* LINN. subsp. *sinensis* ROUSI belongs to the family of Elaeagnaceae, which occurs mainly in Asia and Europe [1–3]. The fruits with beautiful orange color and pleasantly acid flavor are edible. It has high resistibility, adaptability, and drought tolerance. These characteristics determine that it is very suitable for the land reclamation and farmstead protection [4]. This plant is reported to be widely used in the traditional Chinese medicine, Mongolian, and Tibetan pharmacopoeia. It is considered to have significant medicinal value for the treatment of skin disorders resulting from bed confinement, and shows antimicrobial, antioxidant, cardiovascular protective, and antitumor activities [5–10]. Previous phytochemical investigations on the genus *Hippophae* were mainly focused on the isolation and identification of various flavonoids and triterpenoids, while there are only few reports about the systematic study of the alkaloid chemical composition of the seed residue of *Hippophae rhamnoides* LINN. subsp. *sinensis* ROUSI [6][10–17]. Our work was focused on the isolation and structural elucidation of three new alkaliods: 4-[(*E*)-*p*-coumaroylamino]butan-1-ol (**1**), 4-[(*Z*)-*p*-coumaroylamino]butan-1-ol (**2**), and hippophamide (**3**), a pyridoindole alkaloid concerted with γ-lactam ring.

Results and Discussion. – The AcOEt- and BuOH-soluble fractions of the seed residue of *Hippophae rhamnoides* LINN. subsp. *sinensis* ROUSI were purified by repeated column chromatography to afford compounds **1**, **2**, and **3**. Their structures were elucidated on the basis of the physical and spectroscopic data, including those by 1D- and 2D-NMR techniques.

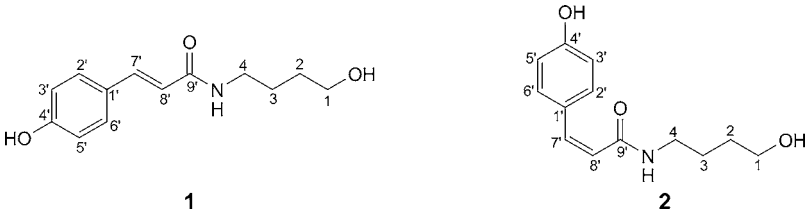
Compound **1**, which is named as 4-[(*E*)-*p*-coumaroylamino]butan-1-ol (=(2*E*)-*N*-(4-hydroxybutyl)-3-(4-hydroxyphenyl)prop-2-enamide), was obtained as red-brown

powder and was analyzed to have the molecular formula $C_{13}H_{17}NO_3$ through HR-ESI-MS (positive-ion mode; *m/z* 236.1275 ($[M+H]^+$, $C_{13}H_{18}NO_3^+$; calc. 236.1281)). The NMR data (*Table 1*), combined with HMBC analysis, revealed 13 C-atom signals, including four CH_2, two CH, and three quaternary C-atoms. The planar structure of **1** (*Fig. 1*) was revealed through the HMBC spectrum. HMBCs (*Fig. 2*) of H–C(1) with C(2) and C(3), H–C(2) with C(3), H–C(3) with C(2), H–C(4) with C(2), C(3), and C(9′), 4-NH with C(9′), H–C(2′) with C(3′), C(4′), C(5′), C(6′), and C(7′), H–C(6′) with C(2′), C(4′), C(5′), C(6′), and C(7′), H–C(3′) with C(1′), C(4′), and C(5′), H–C(5′) with C(1′), C(3′), and C(4′), H–C(7′) with C(8′), C(1′), C(9′), C(2′), and C(6′), H–C(8′) with C(1′) and C(9′) were observed. Thus, compound **1** was unambiguously characterized as 4-[(*E*)-*p*-coumaroylamino]butan-1-ol.

Compound **2**, which is named as 4-[(*Z*)-*p*-coumaroylamino]butan-1-ol (=(2*Z*)-*N*-(4-hydroxybutyl)-3-(4-hydroxyphenyl)prop-2-enamide), was obtained as yellow powder and was analyzed to have the molecular formula $C_{13}H_{17}NO_3$ through HR-ESI-MS (positive-ion mode; *m/z* 258.1104 ($[M+Na]^+$, $C_{13}H_{17}NNaO_3^+$; calc. 258.1101)). The NMR data (*Table 1*), combined with the HMBC spectrum, revealed 13 C-atom signals, including four CH_2 groups, two CH groups, and three quaternary C-atoms. The formula of **2** (*Fig. 1*) was revealed through the HMBC spectrum. HMBCs (*Fig. 2*) of H–C(1)

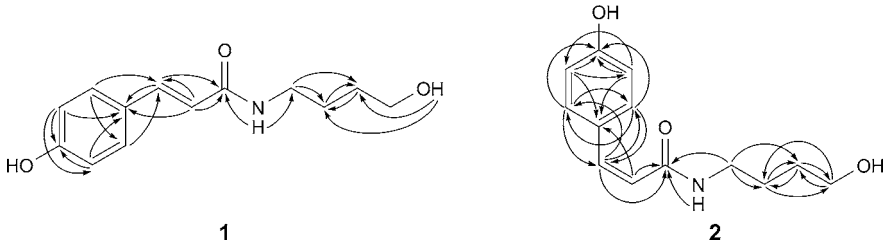
Table 1. *^{1}H- and ^{13}C-NMR Data* (600 MHz; (D_6)DMSO) *of Compounds* **1** *and* **2**. δ in ppm, *J* in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	3.39 (*t*, *J* = 6.0)	60.5 (*t*)	3.38 (*td*, *J* = 6.0, 4.5)	60.4 (*t*)
2	1.39 – 1.49 (*m*)	25.9 (*t*)	1.40 – 1.46 (*m*)	25.6 (*t*)
3	1.39 – 1.49 (*m*)	30.0 (*t*)	1.37 – 1.43 (*m*)	30.0 (*t*)
4	3.14 (*td*, *J* = 7.0, 5.0)	38.5 (*t*)	3.08 (*td*, *J* = 7.2, 5.3)	38.4 (*t*)
1′		126.0 (*s*)		126.3 (*s*)
2′,6′	7.37 (*d*, *J* = 8.4)	129.1 (*d*)	7.59 (*d*, *J* = 8.6)	131.8 (*d*)
3′,5′	6.77 (*d*, *J* = 8.4)	115.7 (*d*)	6.68 (*d*, *J* = 8.6)	114.7 (*d*)
4′		158.8 (*s*)		157.8 (*s*)
7′	7.29 (*d*, *J* = 15.8)	138.5 (*d*)	6.46 (*d*, *J* = 12.9)	136.1 (*d*)
8′	6.39 (*d*, *J* = 15.8)	118.8 (*d*)	5.75 (*d*, *J* = 12.9)	121.0 (*d*)
9′		165.3 (*s*)		166.1 (*s*)
1-OH	4.40 (br. *s*)		4.37 (*t*, *J* = 4.5)	
NH	7.95 (*t*, *J* = 5.0)		7.99 (*t*, *J* = 5.3)	
4′-OH	9.84 (br. *s*)		9.68 (br. *s*)	



1 **2**

Fig. 1. *Structures of compounds* **1** *and* **2**

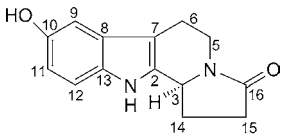


1 **2**

Fig. 2. *Key HMBCs* (H → C) *of compounds* **1** *and* **2**

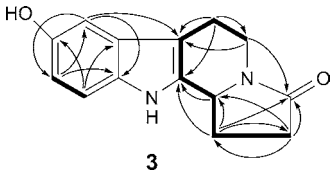
with C(2) and C(3), H–C(2) with C(3), H–C(3) with C(2), H–C(4) with C(2), C(3), and C(9′), 4-NH with C(9′), H–C(2′) with C(4′), C(6′), and C(7′), H–C(6′) with C(2′), C(4′), and C(7′), H–C(3′) with C(1′), C(4′), and C(5′), H–C(5′) with C(1′), C(3′), and C(4′), H–C(7′) with C(9′), C(2′), and C(6′), H–C(8′) with C(1′) and C(9′) were observed. Thus, compound **2** was unambiguously characterized as 4-[(*Z*)-*p*-coumaroylamino]butan-1-ol.

Compound **3**, which was given the trivial name hippophamide (=(11b*S*)-1,2,5,6,11,11b-hexahydro-8-hydroxy-3*H*-indolizino[8,7-*b*]indol-3-one), with $[\alpha]_D^{23.9} = -5.396$ ($c = 0.210$, MeOH) and UV (MeOH): 204.50 (0.6731), 219.00 (0.6252), 277.00 (0.2131), 297.00 (0.1430), was obtained as yellow powder and analyzed to have the molecular formula $C_{14}H_{14}N_2O_2$ through HR-ESI-MS (positive-ion mode; *m*/*z* 265.0950 ($[M+Na]^+$, $C_{14}H_{14}N_2NaO_2^+$; calc. 265.0947)). The NMR data (*Table 2*), combined with ^{1}H,^{1}H-COSY, HMQC, and HMBC spectrum analyses, revealed one lactam C=O group, eight aromatic C-atoms, one phenolic OH group, one CH, and four CH_2 groups. The formula of **3** was revealed through the ^{1}H,^{1}H-COSY, HMQC, and HMBC spectrum (*Fig. 3*). ^{1}H,^{1}H-COSY correlations (*Fig. 4*) of C(3) to C(6), C(3) to C(14), C(3) to C(15), C(5) to C(6), C(11) to C(12), C(14) to C(15) were observed, as well as HMBCs (*Fig. 4*) of H–C(3) with C(2), H–C(5) (δ(H) 4.39–4.41 (*m*)) with C(7), H–C(5) (δ(H) 3.04–3.06 (*m*)) with C(16), H–C(6) with C(5), C(7), and C(2), H–C(9) with C(7), C(11), C(13), and C(10), H–C(11) with C(9) and C(13), H–C(12) with C(8) and C(10), H–C(14) (δ(H) 2.60–2.61 (*m*)) with C(15) and C(16), H–C(14) (δ(H) 1.87–1.90 (*m*)) with C(15), C(2), C(3) and C(16), H–C(15) (δ(H) 2.61–2.64



3

Fig. 3. *Structure of compound* **3**



3

Fig. 4. *Key* ^{1}*H,*^{1}*H-COSY correlations* (—) *and HMBCs* (H → C) *of compound* **3**

Table 2. *^{1}H- and ^{13}C-NMR Data* (600 MHz, CD_3OD) *of Compound* **3**. δ in ppm, J in Hz.

Position	**3**	
	δ(H)	δ(C)
2		135.6 (*s*)
3	4.96 (*t*-like, $J = 6.9$)	56.4 (*d*)
5	3.04 – 3.06 (*m*), 4.39 – 4.41 (*m*)	38.9 (*t*)
6	2.66 – 2.78 (*m*)	22.1 (*t*)
7		107.0 (*s*)
8		128.8 (*s*)
9	6.78 (br. *s*)	103.4 (*d*)
10		151.5 (*s*)
11	6.64 (br. *d*, $J = 8.6$)	112.3 (*d*)
12	7.12 (*d*, $J = 8.6$)	112.5 (*d*)
13		133.0 (*s*)
14	1.87 – 1.90 (*m*), 2.60 – 2.61 (*m*)	26.8 (*t*)
15	2.41 – 2.43 (*m*), 2.61 – 2.64 (*m*)	32.6 (*t*)
16		176.0 (*s*)

(*m*)) with C(14) and C(16), H–C(15) (δ(H) 2.41 – 2.43 (*m*)) with C(14), C(3) and C(16). Thus, compound **3** was unambiguously characterized as hippophamide.

Experimental Part

1. *General.* Reagents and solvents used were of anal. grade and purchased from *Tianjin Baishi Chemical Co., Ltd.* (Tianjin, P. R. China). Column chromatography (CC): *D101* and *AB-8* macroporous resins (*Qingdao Marine Chemical Factory*, Qingdao, P. R. China), *Sephadex LH-20* (20 – 100 μm; *GE Healthcare*, Stockholm, Sweden). *MCI Gel CHP20P* (75 – 150 mm; *Mitsubishi Kasei Chemical Industries*, Tokyo, Japan). HPLC (anal. and prep.): *Agilent 1260 Infinity system*, including a *G1311A QuatPump*, a *G1329B* autosampler with 20 μl injection loop, a *G1316A Thermostatted* column compartment, and a *G1315B* diode array detector, *Agilent Eclipse XDB-C18* column (150 × 4.6 mm i.d. 5 μm; *Agilent Technologies Co., Ltd.*, Santa Clara, CA, USA). *Hanbon-NP7010 system*, including two *NP7000C* series liquid phase pumps, a *UV2000D* detector, and a *Rheodyne 7725 sampler* with 20 ml injection loop. *Hanbon Dubhe C18* column (250 × 20 mm i.d.10 μm, *Jiangsu Hanbon Science & Technology CO., Ltd.*, Huaian, P. R. China). UV/VIS: *UV-2401PC* (*Shimadzu*, Japan); λ_{max} (log ε) in nm. Optical rotation: *P-1020* (*JASCO*, Japan). NMR: *Varian INOVA 600 NMR* spectrometer (*Varian*, *Palo Alto*, CA, USA); δ in ppm rel. to Me_4Si as internal standard, J in Hz. HR-ESI-MS: *Thermo Velos Pro* with *Orbitrap elite* detector (*Thermo Fisher Scientific Inc.*, MA, USA); in *m*/*z*.

2. *Plant Material. Hippophae rhamnoides* LINN. subsp. *sinensis* ROUSI seeds were collected from Datong Country, Qinghai, P. R. China, in August 2013, and dried under shade at r.t.

3. *Extraction and Isolation.* The dried and powdered seed residue (13 kg, firstly extracted by supercritical CO_2) of *Hippophae rhamnoides* LINN. subsp. *sinensis* ROUSI were extracted with 70% aq. EtOH to obtian the EtOH extracts (2262.5 g). Then, the EtOH extracts were dissolved in H_2O and partitioned with AcOEt and BuOH, resp. to give the AcOEt-, BuOH-, and H_2O-soluble fractions (54.6 g, 462.3 g, 1482.5 g, resp.). The AcOEt-soluble fraction (50 g) was fractioned with *AB-8* macroporous resin column chromatography (CC) and eluted with a EtOH/H_2O gradient (0 : 100 → 10 : 90 → 30 : 70 → 50 : 50 → 70 : 30) to give four fractions (*Fr. E1* (4.763 g), *Fr. E2* (11.759 g), *Fr. E3* (8.753 g), and *Fr. E4* (2.668 g)). *Fr. E2* (2.0 g) was subjected to *Sephadex LH-20* gel CC and eluted with MeOH to yield nine fractions *Fr. E2-1* (503 mg), *Fr. E2-2* (226 mg), *Fr. E2-3* (68 mg), *Fr. E2-4* (108 mg), *Fr. E2-5* (219 mg), *Fr. E2-6* (142 mg), *Fr. E2-7* (128 mg), *Fr. E2-8* (237 mg), and *Fr. E2-9* (183 mg)). *Fr. E2-2* contained pure

compound **1** (226 mg). *Fr. E1* (1.5 g) was separated by *Sephadex LH-20* gel CC and eluted by MeOH/H_2O (0:100 → 30:70 → 50:50 → 70:30 → 100:0) to yield six fractions (*Fr. E1-1* (127 mg), *Fr. E1-2* (173 mg), *Fr. E1-3* (241 mg), *Fr. E1-4* (386 mg), *Fr. E1-5* (239 mg), *Fr. E1-6* (205 mg)). *Fr. E1-2* (173 mg) was further purified by prep. HPLC (MeOH/H_2O 10:90) to give compound **2** (13 mg). The BuOH-soluble fraction (200 g) was subjected to CC (*D101* macroporous resin; EtOH/H_2O 0:100 → 10:90 → 30:70 → 50:50 → 70:30) to give seven fractions (*Fr. N1* (10.01 g), *Fr. N2* (10.481 g), *Fr. N3* (20.457 g), *Fr. N4* (15.375 g), *Fr. N5* (3.513 g), *Fr. N6* (2.481 g), and *Fr. N7* (1.923 g)). *Fr. N1* (4.0 g) was subjected to CC (*MCI* gel) and eluted by H_2O to yield ten fractions (*Fr. N1-1* (926 mg), *Fr. N1-2* (674 mg), *Fr. N1-3* (284 mg), *Fr. N1-4* (214 mg), *Fr. N1-5* (370 mg), *Fr. N1-6* (426 mg), *Fr. N1-7* (302 mg), *Fr. N1-8* (237 mg), *Fr. N1-9* (109 mg), and *Fr. N1-10* (178 mg)). *Fr. N1-9* (109 mg) was further purified by prep. HPLC (MeOH/H_2O 10:90 → 30:70) to obtain compound **3** (8 mg).

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