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CHEMICAL AND BIOLOGICALLY ACTIVE CONSTITUENTS OF *Salsola collina*

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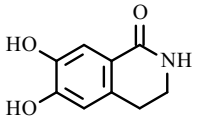
UDC 547.945+547.972.913

*A new natural product (*N*-acetyltryptophan), together with 22 known constituents, including seven alkaloids, six flavonoids, six organic acids, and other compounds, were isolated from* Salsola collina *Pall. Their structures were elucidated on the basis of chemical reaction and spectral evidence. Among the isolated compounds,* N*-acetyltryptophan (**8**) showed moderate inhibition of α-amylase activity, and terrestric acid (**9**) showed positive antifungal activity.*

Keywords: *Salsola collina* Pall., alkaloids, constituents, antifungal activity, inhibition of α-amylase activity.

Salsola collina Pall., which is widely distributed in northeastern and southwestern China, has been used as food or folk medicine for the treatment of hypertension, headache, and vertigo in China. Few phytochemical investigations of the plant have been previously reported, and they mainly refer to the isolation of sterols [1], sugars, sugar esters [2], flavonoids [3–6], and alkaloids [7, 8]. Salsoline A, an alkaloid isolated from *Salsola collina* Pall., showed appreciable antibacterial activity and moderate antiviral activity against influenza virus A and B [9]. To isolate new compounds and to search for biologically active compounds from *Salsola collina* Pall., we have carried out a phytochemical investigation of it, obtaining 23 compounds, which were pericampylinone-A (**1**) [10], salsoline A (**2**) [7], moupinamide (**3**) [11], 7′-hydroxymoupinamide (**4**) [12], 7′-hydroxy-3′-methylmoupinamide (**5**) [12], uracil (**6**) [13], uridine (**7**) [14], *N*-acetyltryptophan (**8**) [15, 16], terrestric acid (**9**) [17], anisic acid (**10**), protocatechuic aldehyde (**11**), vanillin (**12**) [18], corchoionoside C (**13**) [19], acetyl ferulic acid (**14**) [20], *p*-hydroxycinnamic acid (**15**) [8], salicylic acid (**16**), isorhamnetin (**17**) [21], tricin (**18**) [22], tricin-7-*O*-β-D-glucopyranoside (**19**) [8], 5,2′-dihydroxy-6,7-methylenedioxyisoflavone (**20**) [23], quercetin (**21**) [24], rutin (**22**) [25], and *p*-hydroxybenzoic acid (**23**). Among these compounds, *N*-acetyltryptophan (**8**) was a new natural product, and compounds **1**–**9** and **11**–**14** were isolated from this plant for the first time. The biologically active results showed that *N*-acetyltryptophan (**8**) had moderate α-amylase inhibitory activity (Table 1), and terrestric acid (**9**) had positive antifungal activity (Table 2).



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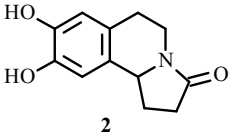

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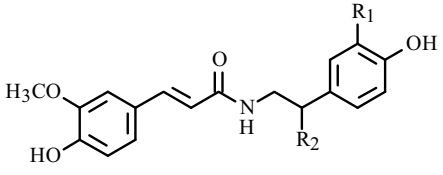

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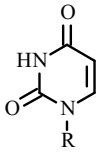
3: $R_1 = R_2 = H$; **4:** $R_1 = H$, $R_2 = OH$
5: $R_1 = OCH_3$, $R_2 = OH$



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6: R = H; **7:** R = ribose

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TABLE 1. Inhibition of α-Amylase Activities by Compounds **2**, **8**, and **18**

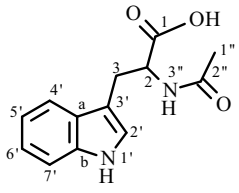
Entry*	Activity test, ($\bar{x} \pm s$)	Activity control, ($\bar{x} \pm s$)	Inhibition, %
2	0.205 ± 0.012	0.502 ± 0.005	17
8	0.314 ± 0.005	0.513 ± 0.005	44
18	0.289 ± 0.026	0.679 ± 0.055	7
Acarbose	0.466 ± 0.010	0.484 ± 0.010	95
Control group	0.132 ± 0.004	0.489 ± 0.010	0

*Concentration: 0.556 mg/mL.

TABLE 2. *In vitro* Antifungal Activities of Compounds **2**, **3**, **8**, and **9**

Entry	MIC80, g/mL*			
	C. albicans (Y0109)	*C. albicans* (SC5314)	*C. neoformans* (BLS108)	*T. rubrum* (CMCCFTa)
2	32	> 64	32	64
3	> 64	64	64	64
8	> 64	> 64	64	64
9	8	32	16	64
ICZ	0.125	0.25	0.125	0.0156
TBR	4	1	0.125	0.0625
KCZ	< 0.125	0.25	0.125	0.25
AMB	8	0.25	4	16
VCZ	< 0.125	0.25	0.125	0.0156
FCZ	0.5	16	0.125	0.0156

*The tested concentration ranged from 0.00024 to 64 μg/mL. The given data are mean values of three parallel experiments.



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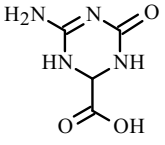

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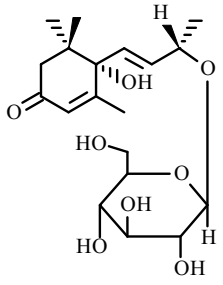

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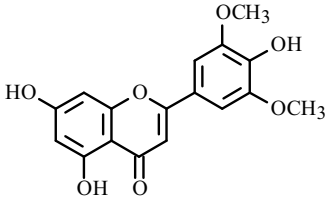

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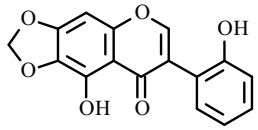

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EXPERIMENTAL

General Procedures. ESI-MS was performed on a Waters Q-Tof micromass spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE II-300 spectrometer, and chemical shifts are reported in parts per million relative to DMSO-d_6 (2.50 ppm for ^{1}H and 39.51 ppm for ^{13}C). All solvents used were of analytical grade (Shanghai Chemical Co., Ltd.). Silica gel (100–200 and 200–300 mesh, Qingdao Haiyang Chemical Co., Ltd.) and Sephadex LH-20 (Pharmacia Co., Ltd.) were used for column chromatography. Acarbose (Bayer, Wuppertal, German) and α-amylase (Wako Pure Chemicals Ind., Ltd., Osaka, Japan) were used to study the inhibition of α-amylase assay.

Plant Material. The whole plants of *Salsola collina* Pall. were collected in Shandong province, People′s Republic of China, in September, 2001, and were authenticated as *Salsola collina* Pall. by Prof. Hanmin Zhang, Department of Pharmacognosy, School of Pharmacy, Second Military Medical University.

Extraction and Isolation. The air-dried and powdered aerial parts of *Salsola collina* Pall. (10 kg) were extracted by percolation with 80% EtOH. The extract was evaporated under vacuum to obtain about 250 g of a residue, and the water-ethanol solution was then extracted with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH successively. Combining the

$CHCl_3$ partition (12 g) with the EtOAc (26 g) partition, we subjected the new partition (38 g) to silica gel CC eluting with a gradient of $CHCl_3$–MeOH (50:1 to 10:1 gradually) to give four fractions (Fr. A1 to Fr. A4). Fraction A1 was rechromatographed on silica gel eluting with $CHCl_3$–CH_3OH (30:1) and then purified on Sephadex LH-20 to yield compounds **1** (90 mg), **2** (200 mg), **11** (46 mg), and **12** (53 mg). Fraction A2 was rechromatographed on silica gel eluting with $CHCl_3$–CH_3OH (20:1) and then purified on Sephadex LH-20 to yield compounds **4** (63 mg), **5** (45 mg), and **17** (70 mg). Fraction A3 was rechromatographed on silica gel eluting with $CHCl_3$–CH_3OH (15:1) and then purified on Sephadex LH-20 to yield compounds **6** (76 mg), **8** (45 mg), and **18** (230 mg). The *n*-BuOH partition (34 g) was subjected to silica gel CC eluting with a gradient of $CHCl_3$–MeOH (20:1 to 5:1) to give three fractions (Fr. B1 to Fr. B3). Fraction B1 was rechromatographed on silica gel eluting with $CHCl_3$–CH_3OH (15:1 to 10:1) and then purified on Sephadex LH-20 to yield compounds **3** (35 mg), **7** (52 mg), **9** (110 mg), **10** (32 mg), **13** (30 mg), **14** (46 mg), **15** (32 mg), **16** (23 mg), **19** (50 mg), **20** (60 mg), and **23** (45 mg). Their structures were identified on the basis of chemical reaction, spectral analysis, and comparison of their spectroscopic data with those previously described in the literature.

***N*-Acetyltryptophan (8).** $C_{13}H_{14}N_2O_3$, white solid, mp 188–189°C. ESI-MS *m/z:* 247 $[M + H]^+$. ^1H NMR (300 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.79 (3H, s, H-1″), 2.94–3.01 (1H, dd, J = 14.7, 8.7, H-3α), 3.11–3.18 (1H, dd, J = 14.7, 5.7, H-3β), 4.42–4.49 (1H, m, H-2), 6.95–7.00 (1H, dd, J = 8.1, 7.5, H-5′), 7.03–7.08 (1H, dd, J = 8.1, 7.8, H-6′), 7.13 (1H, d, J = 2.4, H-2′), 7.31–7.34 (1H, d, J = 7.8, H-7′), 7.50–7.53 (1H, d, J = 7.5, H-4′), 8.16–8.19 (1H, d, J = 7.8, H-3″), 10.84 (1H, s, H-1′), 12.62 (1H, s, 1-OH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 22.4 (C-1″), 27.2 (C-2), 53.0 (C-3), 110.0 (C-3′), 111.4 (C-4′), 118.2 (C-7′), 118.4 (C-5′), 121.9 (C-6′), 123.2 (C-2′), 127.2 (C-b), 136.1 (C-a), 169.3 (C-2″), 173.6 (C-1).

Inhibition of α-Amylase Assay. The Caraway iodine/potassium iodide (IKI) method [26] was applied to determine the amount of starch hydrolyzed (absorbance at 620 nm). The widely described drug acarbose was used as positive control. The inhibition of α-amylase activity is calculated as follows:

$$\text{Inhibition (\%)} = [1 - (\text{activity test/activity control})] \times 100.$$

***In vitro* Antifungal Activities.** The *in vitro* antifungal activities of the isolated compounds were evaluated by the standard broth microdilution method of the NCCLS [27]. The tested fungi species included *Candida albicans* (Y0109), *Candida albicans* (SC5314), *Cryptococcus neoformans* (BLS108), and *Trichophyton rubrum* (CMCCFTa). The positive controls included four different classes of antifungal drugs currently used in the clinic, including triazoles [flucanazole (FCZ), voriconazole (VCZ), and itraconazole (ICZ)], allylamine (terbinafine, TBR), imidazole (ketoconazole, KCZ), and polyene (amphotericin B, AMB). The minimum 80% inhibitory concentration (MIC80) values are summarized in Table 2.

Results and Disccusion. The results of inhibition of α-amylase assay of *N*-acetyltryptophan (**8**) and salsoline A (**2**) are presented in Table 1. *N*-Acetyltryptophan (**8**) at the concentration of 0.556 mg/mL showed moderate α-amylase inhibition activity (44%). *N*-Acetyltryptophan (**8**) is a derivative of amino acid, which implies that some tyrosine derivatives can have potent α-amylase inhibition activity.

The results of antifungal activities of pericampylinone-A (**1**), salsoline A (**2**), *N*-acetyltryptophan (**8**), and terrestric acid (**9**) are presented in Table 2. Terrestric acid (**9**) showed positive antifungal activity.

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