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Radical Scavenging Constituents from Leaf of *Humulus scandens*

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Four compounds were obtained from the methanol extract leaves of *Humulus scandens* and identified as *N-trans-p*-coumaroyl octopamine (**1**), dehydrodiconiferyl alcohol (**2**), tribulusamide A (**3**), simiarenol (**4**), all of which were isolated from this plant for the first time. Among them, compounds **1**, **2** and **3** exhibited significant radical scavenging activity against 1,l-diphenyl-2-picrylhydrazyl (DPPH) with IC_{50} values of 16.2, 12.5 and 10.8 μM, respectively.

Keywords: *Humulus scandens*, Free radical scavenging activity, Terpenoids, Phenolics.

INTRODUCTION

Humulus scandens (Lour.) Merr. (Sankoh) is a traditional medicine, widely used for the treatment of inflammatory and diseases of digestive tract, as well as gastrointestinal symptoms, such as nephritis, cystitis, gastric ulcer, diarrhea, dyspepsia, and gastrointestinal spasm [1]. It was reported to have a variety of biological activities such as antimicrobial, swollen, fever, eczema, diuretic, anti-tuberculosis, and antioxidant effects [2-5]. A previous study showed that the effective constituents of *H. scandens* included mainly glycosides, flavones, phenolics, alkaloids, terpenoids and organic acids [2].

Evaluation of the antioxidant ability of the compound by using scavenging activity toward DPPH radical has been referenced frequently [6]. Our laboratory had reported that the methanolic extract of the stem leaf of *H. scandens* showed positive antioxidant activity. The present study was undertaken to investigate the chemical constituents and biological activity of the methanol extract leaf of *H. scandens*, we isolated four known compounds three aromatic compounds (**1**, **2** and **3**) and a terpenoid (**4**) (Fig. 1) from this plant. The structural characterization of the four compounds is described and their free radical scavenging activities are evaluated in this paper. All compounds were isolated from the stem leaf of *H. scandens*.

EXPERIMENTAL

IR spectra were recorded on a Prestige-21 Fourier transform infrared spectrometer (Shimadzu, Japan) with KBr pellets. 1D and 2D NMR spectra were performed on AV-300, spectrometers

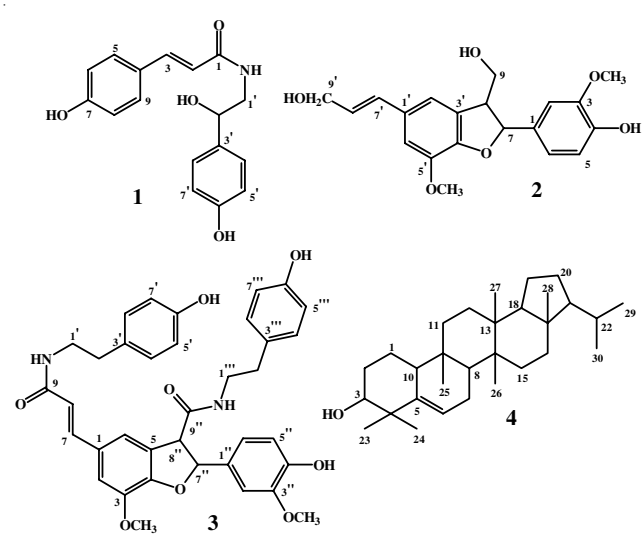

Fig. 1. Chemical structures of the compounds isolated from the *Humulus scandens*

(Bruker, Switzerland) with TMS as the internal standard. ESIMS analyses were carried out on a 1200RRLC-6410B mass spectrometer (Agilent, USA). Semi-preparative HPLC was carried out using a LC-6AD (Shimadzu, Japan) ODS-A (10 × 250 mm, 5 μm, YMC*GEL, Japan). Column chromatography was performed using silica gel (100-200 mesh, Qingdao Marine Chemical Inc., China), Reversed-phase packing ODS-A (150 μm, YMC*GEL, Japan) and TLC plates (GF_{254}, 0.2-0.25 mm, Qingdao Marine Chemical Inc., China) were used for column chromatography. V_C (Sigma-Aldrich) and DPPH

(Sigma-Aldrich) were used for determination of biological activity. HPLC solvents used for chromatographic grade.

The aerial parts of *H. scandens* were collected during July 2013 from Changbai Mountain area, Jilin Province, China. The plant was identified by professor Ji-Xing Nan, College of Pharmacy, Yanbian University, Jilin, China.

Extraction and isolation: The air-dried, powdered plant material (2.5 kg) was extracted three times (each for 24 h) with methanol at room temperature. After filtration, removal of solvent of combined three times extracting solution afforded a crude residue (230 g). The residue was then suspended in H_2O and partitioned with petroleum either, EtOAc, and *n*-BuOH with equal volume, successively. Obtained by petroleum ether (Pet), EtOAc, and *n*-BuOH layers extract were concentrated. The EtOAc soluble fraction extract (5.8 g) was subjected to silica gel column chromatography (CC) by gradient elution (Pet-EtOAc-MeOH 10:1:0, 5:1:0, 2:1:0, 1:1:0, 1:2:0, 1:5:0, 1:10:0, 0:1:0, 0:10:1).Then detected by TLC, similar fractions were combined to give eleven fractions (Fr. 1-11). The Fr. 7 (501.3 mg) was subjected to ODS-A column chromatography by gradient elution (H_2O-MeOH 5:1, 1:1, 1:5, 1:10) to produce twelve subfractions (Fr. 7.1-7.12). The Fr. 7.3 (35.8 mg) was subjected to ODS-A column chromatography by gradient elution (H_2O-MeOH 10:1, 5:1, 2:1) to produce eight subfractions (Fr. 7.3.1-7.3.7). Subfraction 7.3.7 (17.6 mg) was further purified with semi-preparative HPLC (70 % MeOH) as eluent, yielding **1** (10.6 mg). Fr. 7.4 (22.5 mg) was subjected to ODS-A column chromatography by gradient elution (H_2O-MeOH 10:1, 5:1, 2:1, 1:1) to produce six subfractions (Fr. 7.4.1-7.4.6). Subfraction 7.4.5 (5.2 mg) was further purified with semi-preparative HPLC (55 % MeOH) as eluent, yielding **2** (1.0 mg). Fr. 7.7 (83.9 mg) was subjected to ODS-A column chromatography by gradient elution (H_2O-MeOH 5:1, 1:1, 1:5) to produce nine subfractions (Fr. 7.7.1-7.7.9). Subfraction 7.7.7 (25.6 mg) was further purified with semi-preparative HPLC (55 % MeOH) as eluent, yielding **3** (3.0 mg). Subfraction 7.10.5 (4.1 mg) was further purified with semi-preparative HPLC (35 % MeOH) as eluent, yielding **4** (0.8 mg).

***N-trans-p*-coumaroyloctopamine (1):** White amorphous powder. IR (KBr, ν_{max}, cm^{-1}): 3314, 1698, 1617, 1321, 1259, 1021; ESI-MS *m/z*: 322.2 $[M + Na]^+$; ^1H NMR (300 MHz, CD_3OD) δ 7.44 (1H, d, *J* = 15.7 Hz, H-3), 7.40 (2H, d, *J* = 8.6 Hz, H-5, 9), 7.21 (2H, d, *J* = 8.4 Hz, H-4′, 8′), 6.78 (2H, d, *J* = 8.6 Hz, H-6, 8), 6.74 (2H, d, *J* = 8.4 Hz, H-5′, 7′), 6.43 (1H, d, *J* = 15.7 Hz, H-2), 4.71 (1H, dd, *J* = 7.8, 4.9 Hz, H-2′), 3.52 (1H, dd, *J* = 13.5, 4.9 Hz, H-1′a), 3.41 (1H, dd, *J* = 13.5, 7.8 Hz, H-1′b); ^{13}C NMR (75 MHz, CD_3OD) δ 169.6 (C-1), 160.6 (C-6′), 158.1 (C-7), 142.0 (C-3), 134.8 (C-3′), 130.6 (C-5, 9), 128.5 (C-4′, 8′), 127.7 (C-4), 118.3 (C-2), 116.7 (C-6, 8), 116.1 (C-5′, 7′), 73.5 (C-2′), 47.8 (C-1′).

Dehydrodiconiferyl alcohol (2): Yellow amorphous powder. IR (KBr, ν_{max}, cm^{-1}): 3407, 1597, 1211, 1057, 1008; ESI-MS *m/z*: 381.2 $[M + Na]^+$; ^1H NMR (300 MHz, CD_3OD) δ 6.96 (1H, s, H-6′), 6.95 (1H, s, H-2′), 6.94 (1H, d, *J* = 2.1 Hz H-2), 6.82 (1H, dd, *J* = 2.1, 8.2 Hz, H-6), 6.76 (1H, d, *J* = 8.2 Hz, H-5), 6.54 (1H, d, *J* = 10.8 Hz, H-7′), 6.22 (1H, dt, *J* = 10.8, 6.3 Hz, H-8′), 5.52 (1H, d, *J* = 6.3 Hz, H-7), 4.19 (2H, dd, *J* = 5.8, 1.5 Hz, H-9′), 3.87 (3H, s, 5′-OMe), 3.83 (2H, m, H-9), 3.81 (3H, s, 3-OMe), 3.49 (1H, d, *J* = 6.0 Hz, H-8); ^{13}C NMR (75 MHz, CD_3OD) δ 149.2 (C-4′), 149.1 (C-3), 147.6 (C-4), 145.5 (C-5′), 134.6 (C-1), 132.6 (C-1′), 132.0 (C-7′), 130.3 (C-3′), 127.6 (C-8′), 119.7 (C-6), 116.5 (C-2′), 116.2 (C-5), 112.1 (C-6′), 110.5 (C-2), 89.3 (C-7), 64.9 (C-9), 63.9 (C-9′), 56.7 (3-Me), 56.4 (5′-Me), 55.2 (C-8).

Tribulusamide A (3): White amorphous powder. IR (KBr, ν_{max}, cm^{-1}): 3368, 1687, 1593, 1473, 1239, 1047; ESI-MS *m/z*: 623.2 $[M-H]^-$; ^1H NMR (300 MHz, CD_3OD) δ 7.41 (1H , d, *J* = 15.7 Hz, H-7), 7.09 (2H, d, *J* = 8.5 Hz, H-4′ ,8′), 6.73-7.05 (6H, H-5′, 7′, 2″, 5″, 4‴, 8‴), 6.71 (1H, s, H-6), 6.69 (1H, s, H-2), 6.37 (1H, d, *J* = 15.7 Hz, H-8), 5.87 (1H, d, *J* = 8.3 Hz, H-7″), 4.13 (1H, d, *J* = 8.3 Hz, H-8″), 3.86 (2H, s, H-1′), 3.82 (1H, m, H-1‴), 3.81 (3H, s, 3-OMe), 3.47 (3H, s, 3″-OMe), 3.33 (2H, m, H-2′), 2.74 (2H, m, H-2‴); ^{13}C NMR (75 MHz, CD_3OD) δ 172.9 (C-9″), 169.0 (C-9), 156.9 (C-6′), 156.9 (C-6‴), 151.2 (C-4), 149.3 (C-4″), 148.1 (C-3″), 146.0 (C-3), 141.8 (C-7), 132.6 (C-1″), 131.3 (C-3′), 131.1 (C-3‴), 130.9 (C-4′, 8′), 130.8 (C-4‴, 8‴), 130.4 (C-1), 129.4 (C-5), 120.0 (C-8), 119.4 (C-6″), 118.1 (C-6), 116.5 (C-5′, 7′), 116.4 (C-5″), 116.3 (C-5‴, 7‴), 113.1 (C-2), 110.5 (C-2″), 89.9 (C-7″), 58.7 (C-8″), 56.8 (3-OMe), 56.4 (3″-OMe), 42.6 (C-1′), 42.2 (C-1‴), 35.8 (C-2′), 35.3 (C-2‴).

Simiarenol (4): White amorphous powder. IR (KBr, ν_{max}, cm^{-1}): 3411, 1397, 1233, 1124; ESI-MS *m/z*: 449.2 $[M + Na]^+$; ^1H NMR (300 MHz, CD_3OD) δ 5.62 (1H, br.d, *J* = 5.9 Hz, H-6), 3.47 (1H, br.s, H-3a), 1.14 (3H, s, H-24), 1.04 (3H, s, H-23), 1.00 (3H, s, H-26), 0.92 (3H, s, H-27), 0.89 (3H, s, H-25), 0.86 (3H, d, *J* = 6.5 Hz, H-30), 0.83 (3H,d, *J* = 6.5 Hz, H-29), 0.78 (3H, s, H-28). ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.1 (C-5), 122.2 (C-6), 76.5 (C-3), 60.2 (C-21), 51.9 (C-18), 50.4 (C-10), 44.4 (C-8), 42.9 (C-17), 40.9 (C-14), 39.5 (C-4), 38.8 (C-13), 35.6 (C-16), 34.9 (C-9), 34.3 (C-11), 30.9 (C-22), 29.9 (C-15), 29.2 (C-23), 29.1 (C-12), 28.5 (C-20), 27.9 (C-2), 25.6 (C-24), 24.2 (C-7), 23.1 (C-30), 22.1 (C-29), 20.1 (C-19), 18.2 (C-1), 18.0 (C-25), 16.2 (C-28), 15.9 (C-26), 15.1 (C-27).

Biological assays: All compounds were assayed at 1 mg/mL. Compounds were first dissolved in MeOH and then, diluting it 10 and 100 times and pipetting 160 µL simple and 40 µL DPPH from micro-plate. Positive reference and control solution selected 0.1 mg/mL ascorbic acid (V_C) and mixture of 160 µL methanol and 40 µL DPPH. After spotting the completion of the reaction at room temperature for 0.5 h, when measured absorbance with a microplate reader at 520 nm. The extract concentration for a 50 % inhibition (IC_{50}) was calculated from the data and compared with that of the reference compound ascorbic acid.

RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous powder. Its molecular formula was deduced to be $C_{17}H_{17}NO_4$ based on the ^{13}C NMR data and the ESI-MS data (*m/z* 322.2 $[M + Na]^+$), indicating ten indices of hydrogen deficiency. The IR absorption bands at 3314 and 1698 cm^{-1} indicated the presence of OH and α,β-unsaturated carbonyl functionalities, respectively. ^1H NMR spectrum indicated the presence of two *para*-disubstituted aromatic moieties (δ 7.40, 2H, d, *J* = 8.6 Hz, H-5, 9; δ 6.78, 2H, d, *J* = 8.6 Hz, H-6, 8) and (δ 7.21, 2H, d, *J* = 8.4 Hz,

4′, 8′; δ 6.74, 2H, d, *J* = 8.4 Hz, 5′, 7′), two olefinic protons with *trans*-configuration at δ 7.44 (1H, d, *J* = 15.7 Hz, H-3) and 6.43 (1H, d, *J* = 15.7 Hz, H-2), an oxygenated methine proton 4.71 (1H, dd, *J* = 7.8, 4.9 Hz, H-2′), a methylene group. ^{13}C NMR spectrum exhibited the presence of 30 carbon signals and also showed two olefinic carbon signals at δ 142.0 and 118.3 and an oxygenated sp^3 hybridization of carbon signal at δ 73.5. By comparison of its spectral data with those of literature values [7], Compound **1** was determined to be *N-trans-p*-coumaroyloctopamine.

Compound **2** was obtained as a yellow amorphous powder. IR spectrum of **2** indicated the presence of OH (3407 cm^{-1}) group. Its ESI-MS spectrum gave a pseudomolecular ion [M + Na]$^+$ at *m/z* 381.2 consistent with the molecular formula $C_{20}H_{22}O_6$, indicating ten indices of hydrogen deficiency. ^{1}H NMR spectrum indicated the presence of a 1,3,4-trisubstituted and a 1,3,4,5-tetrasubstituted aromatic ring, a *trans*-3-hydroxy-1-prophenyl group, an oxymethine and a hydroxymethyl, a methine proton, and two methoxy groups. ^{13}C NMR spectrum displayed 20 carbon signals including nine methine, two methylene, two methyl and seven quaternary carbons. Thus, the structure of **2** was determined as dehydrodiconiferyl alcohol, by comparison of its physicochemical and spectral data with those of literatures [8].

Compound **3** was obtained as a white amorphous powder, and its molecular formula was determined to be $C_{36}H_{36}N_2O_8$ based on the ^{13}C NMR data and the ESI-MS spectrum. The IR spectrum of **3** indicated the presence of conjugated carbonyl (1687 cm^{-1}) and OH (3368 cm^{-1}) groups. Analysis of the ^{1}H and ^{13}C NMR spectra indicated the presence of one 1,3,4-trisubstituted and 1,3,4,5-tetrasubstituted phenyl group, two *para*-substituted phenyl groups, four sp^3 methylenes, one sp^3 methine, one sp^3 oxymethine, two olefinic protons with *trans*-configuration at δ 7.41 (1H, d, *J* = 15.7 Hz, H-7) and 6.37 (1H, d, *J* = 15.7 Hz, H-8), two amide protons, two methoxy groups and two carbonyl carbons. Thus, the structure of **3** was determined as tribulusamide A. The NMR spectral and physical data of compound **3** were in good agreement with those reported in the literature [9].

Compound **4** was obtained as a white amorphous powder. Its molecular formula was deduced to be $C_{30}H_{50}O$ based on the ^{13}C NMR data and the ESI-MS data (*m/z* 449.2 [M + Na]$^+$), indicating six indices of hydrogen deficiency. IR spectrum showed the presence of hydroxy group (3411 cm^{-1}). ^{1}H NMR spectrum showed an olefinic proton at δ 5.62 (1H, br.d, *J* = 5.9 Hz, H-6), a carbinol proton at ′ 3.47 (1H, br.s, H-3a). ^{13}C NMR spectrum exhibited the presence of 30 carbon signals and also showed two olefinic carbon signals at δ 142.1 and 122.2 and an oxygenated sp^3 hybridization of carbon signal at δ 76.5. The above data were consistent with that for triterpene type compound. The structure of **4** was determined to be simiarenol on the basis of the above evidences, together with a comparison of the above data with those published in the literature [10].

Conclusion

Four compounds (**1**-**4**) were obtained from the methanolic extract of leaves of *H. scandens*. The isolated compounds (**1**-**4**) were screened for radical scavenging activity against DPPH. Among them, compounds **1**, **2** and **3** exhibited significant radical scavenging activity with IC_{50} value of 16.2, 12.5 and 10.8 μM, respectively, which were more potent than the positive control, ascorbic acid (IC_{50} 20.0 μM).

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