Journal of Traditional and Complementary Medicine Vo1. 2, No. 2, pp. 154-157

Journal of Traditional and Complementary Medicine
Journal homepage http://www.jtcm.org

Triterpenes from *Kadsura coccinea*

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Abstract

In vitro anti-allergic screening of medicinal herbal extracts revealed that the 80% acetone extract of the rhizome of *Kadsura coccinea* inhibited nitric oxide (NO) production in a lipopolysaccharide (LPS) and recombinant mouse interferon-γ (IFN-γ) activated murine macrophage like cell line, RAW264.7. Further fractionation of the EtOAc extract led to the isolation of one new 3,4-seco-lanostane type triterpene named kadsuracoccin acid A (1) together with two known triterpenes anwuweizonic acid (2) and neokadsuranic acid B (3). Compounds 1−3 did not exhibit any inhibitory activities for NO production and IFN-γ activation.

Key words: *Kadsura coccinea*; Triterpene; NO production inhibitory activities

Introduction

Schisandraceae family plants are known to be a rich source of lignanoids and triterpenoids with various biological activities (Zhonghuabencao Compilation Committee, 1999). The genus *Kadsura* (Schisandraceae) is closely related to *Schisandra*, and many of its species are extensively used as a substitute for *Schisandra* in Chinese Medicine in Taiwan, Japan and the mainland of China.

In our previous study of anti-inflammatory compounds from Kadsurae Coccineae Caulis et Folium (黑老虎 hēi lǎo hǔ; the stem and leaf of *K. coccinea*), a series of lignans were isolated from the CHCl3 extract (Li et al., 2006a; 2006b). To continue our search for anti-inflammatory compounds from *K. coccinea*, we studied EtOAc extract of this plant. Fractionation of this extract led to isolation of one new 3,4-seco-lanostane type triterpene named kadsuracoccin acid A (1) and two known triterpenes anwuweizonic acid (2) and neokadsuranic acid B (3). Herein, we describe the structure elucidation of compound 1 (Figure 1) and the biological evaluation of three compounds (1−3).

Material and Methods

General

Mp: Fisher-Johns melting point apparatus; uncorrected. IR Spectrum: Mattson Genesis II FT-IR spectrometer, in cm^{-1}. The UV spectrum was obtained in MeOH on a Shimadzu UV-160 spectrophotometer. The NMR spectra were recorded on a Varian JMercury-300BB spectrometer, with TMS as an internal standard. The mass spectra (MS) were obtained on a Jeol ECA-600 spectrometer. Column chromatography was carried out with silica gel (Wako gel C-300, Wako Pure Chemical Industry Ltd.). Thin-layer chromatography (TLC) was performed on Merck TLC plates (0.25 mm thickness), with compounds visualized by spraying with 5% (v/v) H_2SO_4 in ethanol solution and then heating on a hot plate. HPLC was performed on a JASCO PU-2089 apparatus equipped with a JASCO UV-2075. A COSMOSIL (10 × 250 mm i.d.) column, Fluofix 120N (10 × 250 mm i.d.) and a Senshu Pak PEGASIL ODS 2 were used for preparative purposes.

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Plant material

The dried rhizome of *K. coccinea* was collected in Guangxi Province, People's Republic of China, in April 2004 and was identified by Dr. Bao-Lin Guo, Peking Union Medical College, Beijing, People's Republic of China. Voucher specimens were deposited in the Department of Pharmacognosy, College of Pharmacy, Nihon University.

Extraction and isolation

The dried rhizome of *K. coccinea* (1.75 kg) was extracted three times with 80% acetone. Evaporation of the solvent under reduced pressure from the combined extract gave the extract 82.5 g (NO inhibitory effect 100 μg/ml, 78.5%). The extract was dissolved and suspended in water (2 L) and partitioned with chloroform (3 × 2 L), ethyl acetate (3 × 2 L), and n-butanol (3 × 2 L). The amounts extracted were 44.7 g (47.8%), 4.0 g (4.3%), and 14.2 g (15.4%), respectively, and the residual aqueous extract yielded 20.6 g (24.9%). The EtOAc fraction was subjected to CHP-20P column chromatography (40 × 1000 mm, eluted with H_2O and MeOH in reducing polarity, H2O-MeOH, 100:0 → 60:40). The column chromatographic fractions (100 mL each) were combined according to TLC monitoring into seven portions (toluene-EtOAc-acetic Acid, 70:33:3). Portion seven was further purified by HPLC (Fluofix-120N, 10 × 250 mm, H_2O-CH_3CN, 5:95, 3 mL/min, UV detector λ = 210 nm) to give 1 (20.2 mg), 2 (10.1 mg) and 3 (3 mg).

Compound 1: Colorless prisms; mp 142-144 ℃; UV (MeOH) λmax nm (log ε): 202 (3.69); IR (KBr) cm^{-1}: 3433, 3076, 1739, 1690, 1272; EI-MS *m/z* (rel. int. %): 482 $[M]^+$ (80), 383 (100), 275 (40), 95 (65); HR-EI-MS *m/z*: 482.3391 ($[M]^+$, Calcd 482.3396 for $C_{31}H_{46}O_4$); ^{1}H- and ^{13}C-NMR: see Table 1.

Inhibitory Activity on NO Production from Activated Macrophages-Like Cell Line, RAW 264.7

The cells were seeded at 1.2 × 10^6 cells/ml onto 96-wells flat bottom plate (Sumitomo Bakelite, #8096R, Tokyo) and then incubated at 37 ℃ for 2 h. Next, the test extract was added to the culture simultaneously with both *Escherichia coil* LPS (100 ng/mL) and recombinant mouse IFN- γ (0.33 ng/mL). Then cells were incubated at 37 ℃ for approximately 16 h and subsequently chilled on ice. One hundred micro liters of the culture supernatant was placed in duplicate in the wells of 96-well flat bottomed plates. A standard solution of $NaNO_2$ was placed in alternate wells on the same plate. To quantify nitrite, 50 μL of Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% *N*-1-naphthyletylenediamide dihydrochloride) was added to each well. After 10 min the reaction products were colorimetrically quantified at 550 nm using a Model 3550 Microplate Reader (BIO-RAD) and the background absorbance (630 nm) was subtracted. Cytotoxicity was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method.

Results and Discussion

Kadsuracoccin acid A (1) was crystallized as colorless prisms and has the molecular formula of $C_{31}H_{46}O_4$ by its HREIMS (found 482.3391, calcd 482.3396). Inspection of the ^{13}C-NMR spectrum revealed the presence of two carboxyl signals at δc 175.1 and 172.6 (C-3 and C-26, respectively). The 1H-NMR spectrum indicated the three olefinic methyls at δH 1.56, 1.74 and 1.90, one isopropenyl (*exo*-methylene protons at δ_H 4.85 and 4.69, H-28a and H-28b; δ_H 1.74, CH_3-29), and three tertiary methyls at δ_H 0.70, 0.80 and 1.04, respectively.

Table 1. ^{1}H- and ^{13}C-NMR Data for Compound 1 (600 MHz, 150 MHz in CDCl3, δ in ppm) [a)]

	δ_C	δ_H (mult; *J*, Hz)		δ_C	δ_H (mult; *J*, Hz)
1	28.9	2.21, m; 2.47, m	17	142.9	
2	32.3	1.78, m; 2.04, m	18	21.2	0.80, s
3	175.1		19	26.7	1.04, s
4	147.6		20	124.8	
5	49.3	2.04, m	21	19.6	1.56, s
6	27.8	1.53, m; 1.78, m	22	33.0	2.05, m; 2.27 , m
7	26.7	1.23, m; 1.63, m	23	29.3	2.47, m; 2.69, m
8	41.5	2.09, m	24	146.2	6.08, (t, 7.3)
9	142.1		25	126.0	
10	42.4		26	172.6	
11	118.4	5.35 (d, 6)	27	20.5	1.90, s
12	36.5	2.24, m; 2.41, m	28	113.7	4.69, (d, 1.7); 4.85, (d, 1.7)
13	47.2		29	23.2	1.74, s
14	46.1		30	19.1	0.70 s
15	33.0	1.42, m; 2.06, m	$-OCH_3$	51.5	3.65, s
16	28.8	2.20, m; 2.48, m			

[a)] Data were recorded on Jeol ECA-600MHz spectrometer (^{1}H, ^{13}C), assignments were confirmed by ^{1}H-^{1}H COSY, HMQC and HMBC.

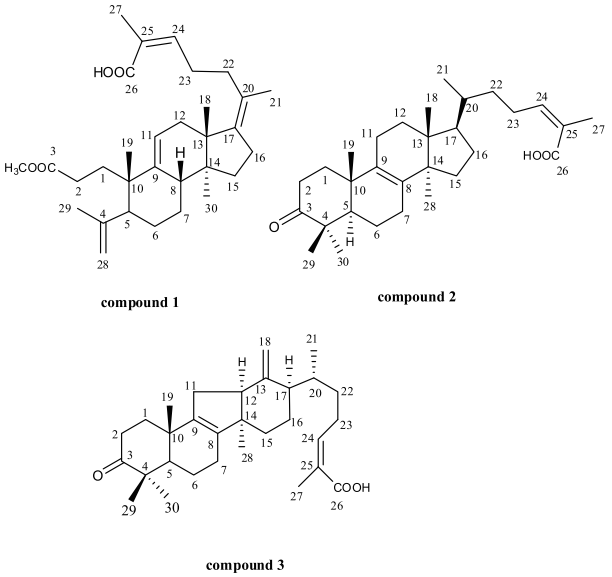

Figure 1. Structures of compounds 1−3.

Furthermore, two pair of non-equivalent methylene protons at δ_H 2.21, 2.47 (H-1) and δ_H 2.04, 1.78 (H-2) showed correlations in the COSY spectrum (Figure 2) with each other, and both of them showed long-range correlations with C-3 in the HMBC spectrum. An ester methyl signals at δ_H 3.65 and δ_C 51.5 were found in ^{1}H and ^{13}C-NMR spectrum. Additionally, the HMBC cross peak of ester methyl signal (δ_H 3.65) with C-3 (δ_C 175.1) confirmed the presence of a methyl ester.

The COSY spectrum of 1 also showed cross-peaks between δ_H 1.90 (CH_3-27) and 6.08 (t, J = 7.3 Hz, H-24); H-24 with two non-equivalent methylene protons at δ_H 2.47, 2.69 (H-23). The HMBC spectrum of 1 showed the long-rang correlations between CH_3-27 and carbons at δ_C 172.6 (C-26), 126.0 (C-25) and 146.2 (C-24); H-24 with C-26, C-25, C-27 (δ_C 20.5), C-23 (δ_C 29.3) and C-22 (δ_C 33.0). The facts above indicated the presence of a side-chain with correlated protons: HOOC-C(CH3)=CH-CH_2-CH_2-. Irradiation of CH_3-27 resulted in NOE of H-24 showed the double-bond in C-24 has a Z-configuration.

These data above were closely related to those previously reported kadsuric acid (Yamada et al., 1976), but clearly differed in the signals corresponding to the CH_3-21, C-17 and C-22. Both proton signals of methine on CH-17 and CH-20 disappeared. And the most apparently, the three-proton doublet (CH_3-21) disappeared and downshifted to δ_H 1.56 as a single signal. The facts above indicated the presence of a double bond between C-17 and C-20. The orientation of H-8 was determined on the basis of NOSEY correlations of CH_3-19/H-8 and CH_3-18/H-8 (Figure 2). In addition, the correlations of CH_3-30/H-5 suggested

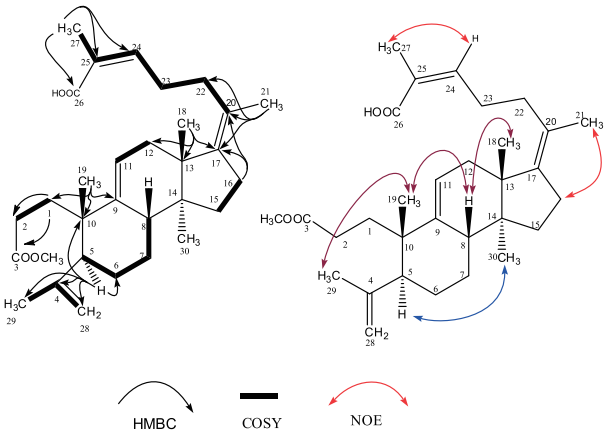

Figure 2. Key COSY, HMBC correlations and NOEs of compound 1.

that H-5 has an α-orientation; H-16 at δ_H 2.20 showed cross peaks with CH_3-21 and CH_3-18 at δ_H 0.80 showed cross peaks with non-equivalent methylene protons at δ_H 2.05, 2.27 (CH_2-22) indicating the double bond in C-17 has a Z-configuration. Thus, the structure of 1 was assigned as shown in Figure 1. Thus 1 was suggested to be kadsuracoccinic acid A ester (Li et al., 2008). Therefore, the structure of 1 was suggested to be 3,4-*seco*-lanosta-4(28),9(11),17(20),17(Z),24(Z)-tetraene-26-oic acid-3-oic acid methyl ester.

Compounds 2 and 3 (Ito et al., 2001; Li et al., 1989) were known compounds, whose structures were elucidated by comparison with those in the literature.

Compounds 1−3 were tested for their ability to inhibit nitric oxide (NO) production in a LPS and IFN-γ activated murine macrophage like cell line RAW 264.7.Compounds 1– 3 did not exhibit any inhibitory activity.

Acknowledgements

Project 30901853 supported by NSFC; The Project-sponsored by SRF for ROCS, SEM; the project 09KJB350002 supported by University Science Research Project of Jiangsu Province; the research work was supported by the PAPD (A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions).

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