Fitoterapia 82 (2011) 251–254

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

New anti-HBV caryophyllane-type sesquiterpenoids from *Euphorbia humifusa* Willd

Ying Tian, Li-Min Sun, Bin Li, Xi-Qiao Liu, Jun-Xing Dong *

Department of Pharmaceutical Chemistry, Beijing Institute of Radiation Medicine, Beijing 100850, PR China

ARTICLE INFO

Article history:
Received 4 August 2010
Accepted in revised form 26 September 2010
Available online 16 October 2010

Keyword:
Euphorbia humifusa
Caryophllane
Sesquiterpenoid
Anti-HBV activity

ABSTRACT

Activity-guided fractionation of *Euphorbia humifusa* for anti-HBV activity led to the isolation of two novel sesquiterpenoids, named humifusane A (1) and humifusane B (2). Their structures were elucidated by spectral data to show that they have a caryophyllane-type precursor structure. The two new sesquiterpenoids showed anti-HBV activities through specifically inhibiting the secretion of HBsAg in HepG2.2.15.

1. Introduction

Euphorbia humifusa Willd., mainly distributed in South China and widely used as the Traditional Chinese Medicine for the treatment of bacillary dysentery, enteritis and hepatitis caused by virus [1], was found during the screening studies on anti-HBV natural products. Several phenols [2], flavones [3], alkaloids [4] and triterpenes [5,6] have been isolated from this plant. In the investigation on anti-HBV active substances from the herb, we had discovered a series of interesting compounds mainly including flavones [7] and phenols [8]. In this paper, the isolation, structure elucidation and anti-HBV activities of two novel compounds were described which were caryophyllane-type sesquiterpenoids from *E. humifusa*.

2. Experimental

2.1. General

Macro porous resin AB-8 (NanKai College Chemical Inc.; Tianjin, China), SP825 (Mitsubishi, Japan), polyamide (100–200 mesh, LinJiang Chemical Reagent Co.; Jiangsu, China), Sephadex LH-20 (Pharmacia, USA) and MCI GEL CHP 20P (75–150 μ, Mitsubishi, Japan) were used for column chromatography. UV spectra were carried out on a Shimadzu UV 2501-PC spectrophotometer. IR spectra were recorded on a Bruker Vertex 70 spectrometer with KBr pellets. Optical rotations were measured with a polAAr3005 polarimeter. Melting points were determined on a RY-1 melting point apparatus, uncorrected. NMR spectra were measured on Japanese electronics JUM-ECA-400 superconducting NMR with TMS as internal standard. HRMS were obtained on Bruker Apex-Qe-FTMS and Waters Synapt MS.

2.2. Plant materials

E. humifusa Willd. were bought from BoZhou medicinal trading center, PR China in May 2007 and identified by Professor Bin Li, Department of Pharmaceutical Chemistry, Beijing Institute of Radiation Medicine.

2.3. Extraction and isolation

The dried and powdered material (20 kg) was extracted with 70% ethanol under room temperature. The filtrate was evaporated in vacuo to give a residue (2080 g) which was suspended in H_2O, then partitioned with chloroform. The H_2O

* Corresponding author. Tel.: +86 10 66931314; fax: +86 10 68164257.
E-mail address: hq6106@yahoo.com.cn (J.-X. Dong).

0367-326X/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.fitote.2010.10.005

Table 1
NMR, ^1H–^1H COSY and HMBC data of compound 1 (CD_3OD, δ in ppm).

Position	δ_H	^1H–^1H COSY	δ_C	HMBC
1	2.50(1H, dt, J = 10.8,8.0 Hz)	H-2,9	42.03	H-2α,3,9,10α,12,13
2	2α 1.57(1H, m) 2β 1.76(1H, m)	H-1,3	21.57	H-3
3	3α 2.86(1H, t, J = 11.2 Hz) 3β 1.92(1H, dd, J = 12.8,9.2 Hz)	H-2	28.85	H-5,14
4			156.33	H-2,3,14
5	6.07(1H, s)		130.26	H-3β,7,14
6			194.87	
7	5.99(1H, s)		128.76	H-5,15
8			158.52	H-1,9,10β,15
9	3.90(1H, q, J = 9.2 Hz)	H-1,10	34.14	H-1,2β,7,10,15
10	10α 1.49(1H, dd, J = 11.4,8.4 Hz) 10β 2.27(1H, dd, J = 11.4,10.0 Hz)	H-9	31.26	H-9,12,13
11			38.33	H-1,10,12,13
12	3.76(2H, s)		75.42	H-1,10β,13
13	1.08(3H, s)		18.59	H-1,10,12
14	2.01(3H, s)		25.17	H-3,5
15	2.07(3H, s)		22.47	H-7,9,10β

Table 2
NMR, ^1H–^1H COSY and HMBC data of compound 2 (CD_3OD, δ in ppm).

Position	δ_H	^1H–^1H COSY	δ_C	HMBC
1	2.13(1H, m)	H-2,9	40.85	H-3α,9,10α,12
2	2α 1.64(1H, m) 2β 1.79(1H, m)	H-1,3	27.35	H-1,3β,9
3	3α 2.51(1H, m) 3β 2.68(1H, m)	H-2	31.11	H-5,14
4			157.94	H-14
5	5.90(1H, s)		130.61	H-14
6			201.59	H-7
7	2.99(1H, d, J = 13.0 Hz) 3.06(1H, d, J = 13.0 Hz)		56.74	H-5,15
8			73.82	H-7,9,15
9	2.39(1H, q, J = 8.8 Hz)	H-1,10	44.99	H-7,10,15
10	10α 1.53(1H, dd, J = 12.0,9.6 Hz) 10β 1.95(1H, m)	H-9	29.72	H-9,12,13
11			35.55	H-10,13
12	3.73(1H, d, J = 9.6 Hz) 3.77(1H, d, J = 9.6 Hz)		76.28	H-10β,13
13	1.09(3H, s)		19.53	H-10,12
14	1.95(3H, s)		26.67	H-5
15	1.26(3H, s)		22.97	H-7,9

extract (435 g) was subsequently separated into five fractions by macro porous resin AB-8. Among them, 20% ethanol fraction (57 g) exhibited anti-HBV activity in vitro, therefore further subjected to SP825. The 20% ethanol fraction (16 g) through SP825 was submitted to polyamide with acetone–H_2O (3:7 → 10:0) to give five subfractions (Fr.). Fr.1 (4.85 g) was further submitted to MCI GEL CHP 20P with MeOH-H_2O (4:6 → 10:0) and Sephadex LH-20 with EtOH–acetone–H_2O (7:1:2), EtOH and acetone–MeOH-H_2O (6:3:1) repeatedly to afford compounds 1 (60 mg) and 2 (80 mg).

2.4. Cells and cell culture

The HepG2.2.15, human hepatoblastoma cell line stably transfected with an HBV clone, was used as the model in vitro. They were plated at a density of 5×10^4 cells mL^{-1} on 96-well cell culture plates and routinely cultured with Dulbecco's modified Eagle's medium (DMEM, Gibico, USA) supplemented with 10% (v/v) fetal calf serum (Gibico, USA), penicillin G 100 units mL^{-1}, streptomycin 80 units mL^{-1}, and G418 380 μg mL^{-1}, at 37 °C under 5% CO_2. Different concentrations of the studied compounds were supplemented to the medium after cells were plated. Control cultures received the carrier solvent (DMEM with 0.2% DMSO). Cells were grown in the presence of the studied compounds for 8 days changing the medium at the 4th day. The suspension and the cells were separated and collected for antigen assay and cytotoxicity test immediately.

2.5. HBeAg/HBsAg assay of the suspension

The suspension was collected as described above. The concentration of HBeAg/HBsAg was detected by a diagnostic kit for hepatitis B antigen (ELISA) (Shanghai SIIC Kehua Biotech CO., LTD) according to the manufacturer's protocol.

2.6. Cell proliferation assay

HepG2.2.15 cells were cultured with test compounds for 8 days. After cultivation, cell proliferation was determined by MTT [3-(4,5-dimethylthiazol)-2,5-diphenyl tetrazolium bro-

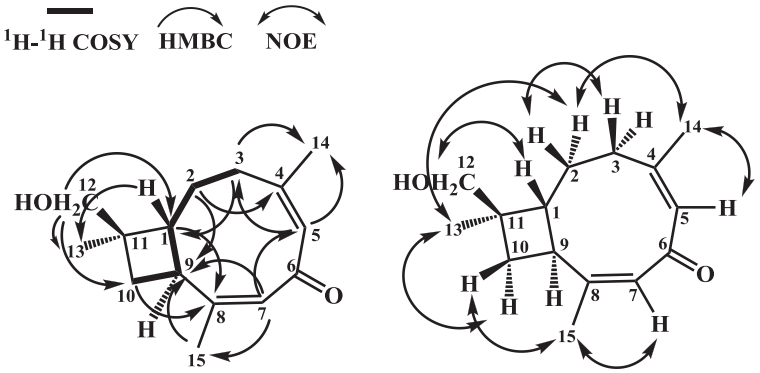

Fig. 1. Key ^1H–^1H COSY, HMBC and NOESY correlations observed for 1.