Steroids 131 (2018) 32–36

Contents lists available at ScienceDirect

Steroids

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

Three new withanolides from the calyces of *Nicandra physaloides*

Qin Xiao[a,1], Cui-Fang Wang[b,1], Jun Chen[a], Chen-Lei Lian[a], Ying Xu[a], Lei Xiao[a], Jie-Qing Liu[a,c,*]

[a] School of Biomedical Sciences, Huaqiao University, Quanzhou 362021, China
[b] College of Oceanology and Food Science, Quanzhou Normal University, Quanzhou 362000, China
[c] Engineering Research Center of Molecular Medicine, Xiamen 361021, China

ARTICLE INFO

Keywords:
Nicandra physaloides
Solanaceae
Steroids
Withanolides
Nicphysatones A-C

ABSTRACT

Chemical investigation on ethyl acetate extract of the calyces of *Nicandra physaloides* resulted in the isolation of three new withanolides named as nicphysatone A (**1**), nicphysatone B (**2**), nicphysatone C (**3**), together with five known withanolides, nic 17 (**4**), nic 7 (**5**), nic 2 (**6**), withahisolide G (**7**) and nicaphysalin B (**8**). The structures were determined by comprehensive spectroscopic experiments. The discovery enriched the diversity of natural withanolides and could serve as scaffolds for the synthesis of more potent modified withanolides.

1. Introduction

Withanolides, a class of C_{28} steroidal lactones structurally possessing a δ- or γ-lactone ring between C-26 and C-22 or C-23 in the side chain, are abundant in plants of the family Solanaceae, especially in the genera *Withania*, *Physalis*, *Datura*, *Nicandra*, *Tubocapsicum*, and *Jaborosa* [1]. A number of withanolides have been obtained and characterized since the first withanolide-type isolated in 1965. This type of steroid has attracted significant attention from numerous researchers, not only because of their complex structural features, but also their diverse bioactivities and potential in drug research and development, such as their antitumor [2,3], anti-inflammatory [4,5], immunomodulatory [4–6], insect-antifeedant [7,8], and insecticidal [9] activities.

The genus *Nicandra* comprises three species, *N. john-tyleriana*, and *N. yacheriana*. *N. physaloides*, a well-known and the most widespread species of the genus, is widely distributed in Yunnan, Guangxi, Guizhou and some other places in China [10]. Previous literature reports indicate that *N. physaloides* is also an abundant source of withanolides with structurally diverse and biologically significant withanolides being identified from this species [11,12]. Our continuing interest in the chemical composition of various parts of this plant has yielded a variety of new and known chemical structures belonging to withasteroids [13]. However, previous work mainly focused on its leaves, which have been reported to decrease blood sugar, have anti-tumor activity and serve as insect antifeedant [12,14,15], and its fruits, which have been found to be a good source of antioxidants [16], while few phytochemical investigations had been done for its calyces. The calyces of *Physalis alkekengi* var. *franchetii*, which have traditionally been used as herbal medicine, is a rich source of withanolides that display a variety of biological activities [17]. In this study, it was hypothesized that the calyces of *N. physaloides* could be a source of withanolides with diverse biological activities. Therefore, a phytochemical investigation was developed to reveal and characterize the chemical constituents of the calyces of *N. physaloides*.

Three new withanolides named nicphysatone A (**1**), nicphysatone B (**2**), nicphysatone C (**3**), together with five known withanolides, nic 17 (**4**) [18], nic 7 (**5**) [19], nic 2 (**6**) [20], withahisolide G (**7**) [21] and nicaphysalin B (**8**) [22] were isolated and underwent a cytotoxicity activity test (Fig. 1). Herein, we report the isolation and structure elucidation of the above withanolides.

2. Experimental

2.1. General methods

Optical rotations were obtained with a JASCO P-1020 polarimeter. UV spectra were run on a UV 210A spectrophotometer. 1D- and 2D-NMR experiments were measured on DRX-600 instruments (Bruker, Zurich, Switzerland) using *pyridine-d_5* as solvent, with Me_4Si (TMS) as internal standard. ESIMS and HRESIMS data were recorded on an API QSTAR Pulsar spectrometer and infrared spectra were recorded on a Bruker Tensor-27 instrument by using KBr pellets. Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with a YMC ODS-C18 (5 μm, 9.4 × 250 mm) column. Silical gel (200–300) mesh, (Qingdao Marine Chemical, Inc.), Lichroprep RP-18 (40–63 μm, Merck) and Sephadex LH-20 (GE Healthcare, Piscataway, NJ, USA)

* Corresponding author at: School of Biomedical Sciences, Huaqiao University, Quanzhou 362021, China.
E-mail address: liujieqing@hqu.edu.cn (J.-Q. Liu).
[1] These authors contributed equally to this work and should be considered co-first authors.

https://doi.org/10.1016/j.steroids.2018.01.003
Received 27 September 2017; Received in revised form 17 December 2017; Accepted 11 January 2018
0039-128X/

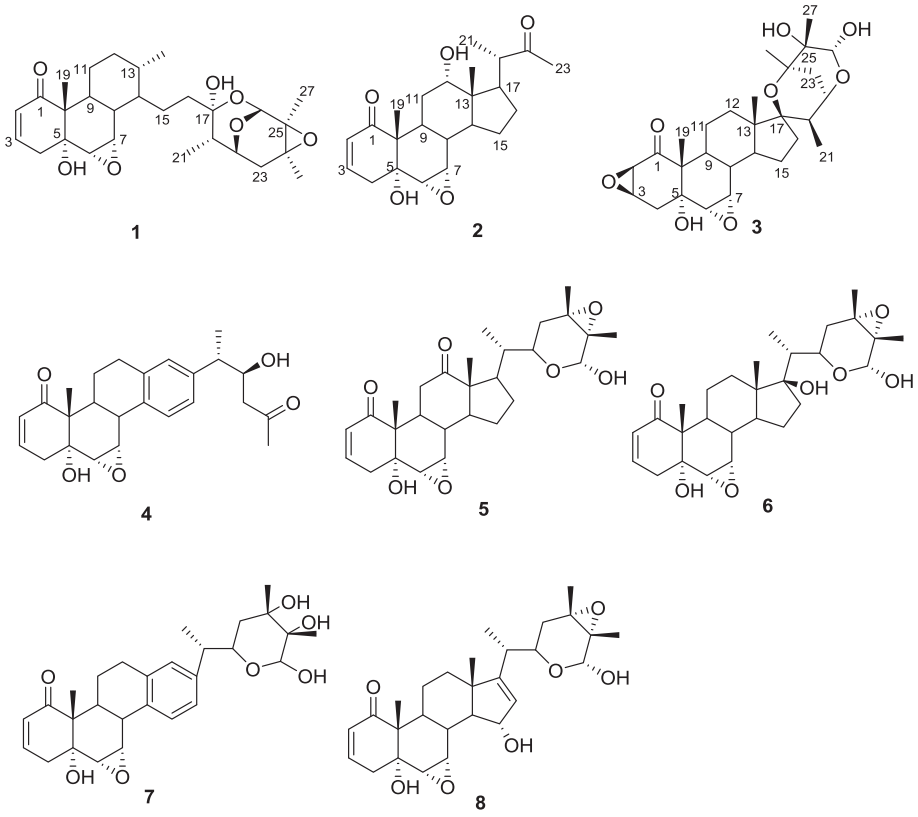

Fig. 1. Structures of isolates from the calyces of *Nicandra physaloides*.

were used for column chromatography. Analytical TLC was performed on precoated TLC plates (200–250 μM thickness, F254 Silical gel 60, Qingdao Marine Chemical, Inc.). Spots were visualized by spraying with 10% aqueous H_2SO_4 following then heating by heat gun. Isolation and purification procedures were done by using analytical grade solvents (Fischer chemicals).

2.2. Plant material

The calyces of *N. physaloides* were collected from Lijiang of Yunnan province, People's Republic of China, in June 2015. The plant material was identified by Prof. Liu Jie-Qing. A voucher specimen (HQ20150601) is deposited in the school of biomedical sciences, Huaqiao University, China.

2.3. Extraction and isolation

The air-dried calyces of *N. physaloides* (5 kg) were smashed and then extracted for 3 h each with methanol (30 L × 3) under conditions of reflux. The methanol solubles were combined and concentrated *in vacuo* to obtain the crude extract (0.5 kg), which was then suspended in water and partitioned with petroleum ether and EtOAc, respectively. The EtOAc extract (48 g) was chromatographed on silica gel ($CHCl_3$/MeOH, 150:1, 80:1, 50:1, 20:1, 10:1, 5:1) to afford six main fractions (Fr.1–Fr.-6). Fr.2 (4.8 g) was subjected to silica gel CC and eluted with a gradient system of petroleum ether/acetone (10:1, 8:1, 5:1, 3:1, 1:1) to yield seven subfractions (Fr.2-1–Fr.2-7) by TLC analysis. Fr.2-2 (50 mg) was successively purified by Sephadex LH-20 ($CHCl_3$/MeOH 1:1), RP-18 column (40% MeOH/H_2O → 80% MeOH/H_2O) and by P-TLC (CH_2Cl_2/MeOH) to yield compound **1** (2.0 mg). Fr.3 (2.6 g) was subjected to silica gel RP-18 and eluted with a gradient system of MeOH/ H_2O (50% → 100%) to yield seven subfractions (Fr.3-1–Fr.3-7) by TLC. Fr.3-1 (29 mg) was successively purfied by Sephadex LH-20 (MeOH), silica gel CC (petroleum ether/acetone), and by P-TLC ($CHCl_3$/IPA) to yield compound **2** (3.0 mg). Fr.3-5 (88 mg) was successively purified by Sephadex LH-20 (MeOH) and recrystallization to yield compound **3** (2.5 mg). Fr.4 (48 mg) was subjected to silica gel CC and eluted with MeOH to yield five subfractions (Fr.4-1–Fr.4-5) by TLC. Fr.4-1 (42 mg) was successively purified by silica gel CC (CH_2Cl_2/MeOH 100:1), vacuum liquid chromatography (CH_2Cl_2/MeOH 150:1 → 130:1 → 50:1) and P-TLC (CH_2Cl_2/MeOH 60:1) to yield compound **4** (2.5 mg, nic 17). Fr.4-2 (361 mg) was successively purified by silica gel CC (petroleum ether/ethyl acetate 4:1), recrystallization to yield compound **5** (10.5 mg, nic 3) and P-TLC (CH_2Cl_2/MeOH 25:1) to yield compound **6** (4.5 mg, nic 2). Fr.4-3 (15 mg) was successively purified by vacuum liquid chromatography (CH_2Cl_2/MeOH 50:1) and P-TLC (CH_2Cl_2/MeOH 15:1) to yield compound **7** (4.0 mg, withahisolide G). Fr.4-4 (630 mg) was successively purified by silica gel CC (CH_2Cl_2/MeOH 50:1, petroleum ether/ethyl acetate 1:3), P-TLC (CH_2Cl_2/MeOH 20:1, ethyl acetate) and recrystallization to yield compound **8** (10.0 mg, nicaphysalin B).

2.3.1. Nicphysatone A (1)

White powder; [α]25 D −19.2° (*c* 0.15, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (3.48) nm; IR (KBr) ν_{max} 3440 (OH), 2926 (CH), 2848 (CH), 1686 (α,β-unsaturated carbonyl group), 1633 (C═C), 1451 (CH), 1108 (C—O) cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 511 $[M+Na]^+$; HRESIMS, *m/z* at 511.2668 (calcd for $C_{28}H_{40}NaO_7$ $[M + Na]^+$, 511.2672).

2.3.2. Nicphysatone B (2)

Yellow powder; [α]25 D −28.0° (*c* 0.10, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 242 (3.52) nm; IR (KBr) ν_{max} 3517 (OH), 3444 (OH), 2945 (CH), 1693 (α,β-unsaturated carbonyl group), 1631 (C═C), 1561 (C═C),