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Bioactive steroidal alkaloids from the fruits of *Solanum nigrum*

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ABSTRACT

The investigation of the fruits of *Solanum nigrum* led to the isolation of four previously undescribed steroidal alkaloids, named solanine A, 7α-OH khasianine, 7α-OH solamargine and 7α-OH solasonine, together with six known ones. The structures of the isolated compounds were elucidated unambiguously by spectroscopic data analyses and chemical methods. Solanine A represents an unusual steroidal alkaloid with an unprecedented 6/5/6/5/5/6 hexacyclic ring system, and its structure was confirmed by X-ray single crystal diffraction analysis. Compounds **2**–**4** were rare naturally occurring steroidal alkaloid glycosides bearing a hydroxyl group at C-7 position. Solanine A showed the most potent inhibitory activity against the LPS-induced NO production in murine RAW264.7 macrophages with an IC_{50} value of 3.85 ± 0.71 μM and significant cytotoxicity against MGC803, HepG2 and SW480 cancer cell lines with IC_{50} values of 6.00 ± 0.52 μM, 9.25 ± 0.49 μM and 6.23 ± 0.26 μM, respectively.

1. Introduction

Solanum nigrum Linn. (Solanaceae) is now a very widespread species, having naturalised in many areas throughout the world. The whole herb of *S. nigrum*, locally known as "Longkui", has long been used in traditional folk medicine for centuries because of its detumescent, diuretic and antipyretic effects. More specifically, it has been used to cure cancer, hepatic damage, trachitis, asthma, inflammation, cough, and dropsy in oriental medicine (Chinese Pharmacopoeia Commission, 1978; Sultana et al., 1995). Previous phytochemical studies revealed the presence of steroidal alkaloids, steroidal glycosides, steroidal genin, tannin, polyphenolic compounds (Saijo et al., 1982; Eltayeb et al., 1997; Hu et al., 1999; Kuo et al., 2000). In recent years, this plant has attracted much attention because of its proved remarkable antitumor activity (Sepide, 2016). Pharmacological studies indicated the plant extract has potential application for treating many types of cancer, including liver cancer, cervical cancer, breast cancer, lung cancer, stomach cancer, colon cancer, skin cancer and bladder cancer (Zhao and Zeng, 2002; Raju et al., 2003; Son et al., 2003; Li et al., 2008; Ding et al., 2012). As a part of a program to search for novel bioactive constituents from traditional Chinese medicines, a methanol extract of the fruits of *S. nigrum* was investigated, and four previously undescribed steroidal alkaloids (**1**–**4**), together with six known ones (**5**–**10**) were isolated (Fig. 1). In this paper, we describe the isolation, structural elucidation, *in vitro* anti-inflammatory and cytotoxic activity evaluation of these steroidal alkaloids.

2. Results and discussion

2.1. Structural elucidation

The H_2O-soluble fraction prepared from a MeOH extract from the fruits of *S. nigrum* were subjected to macroporous resin (D101), silica gel, ODS, and preparative HPLC to yield four previously undescribed steroidal alkaloids (**1**–**4**) and six known steroidal glycosides, as shown in Fig. 1.

Compound **1** was obtained as a colorless crystal with a molecular formula of $C_{27}H_{39}NO_2$, which was determined by HRESIMS at

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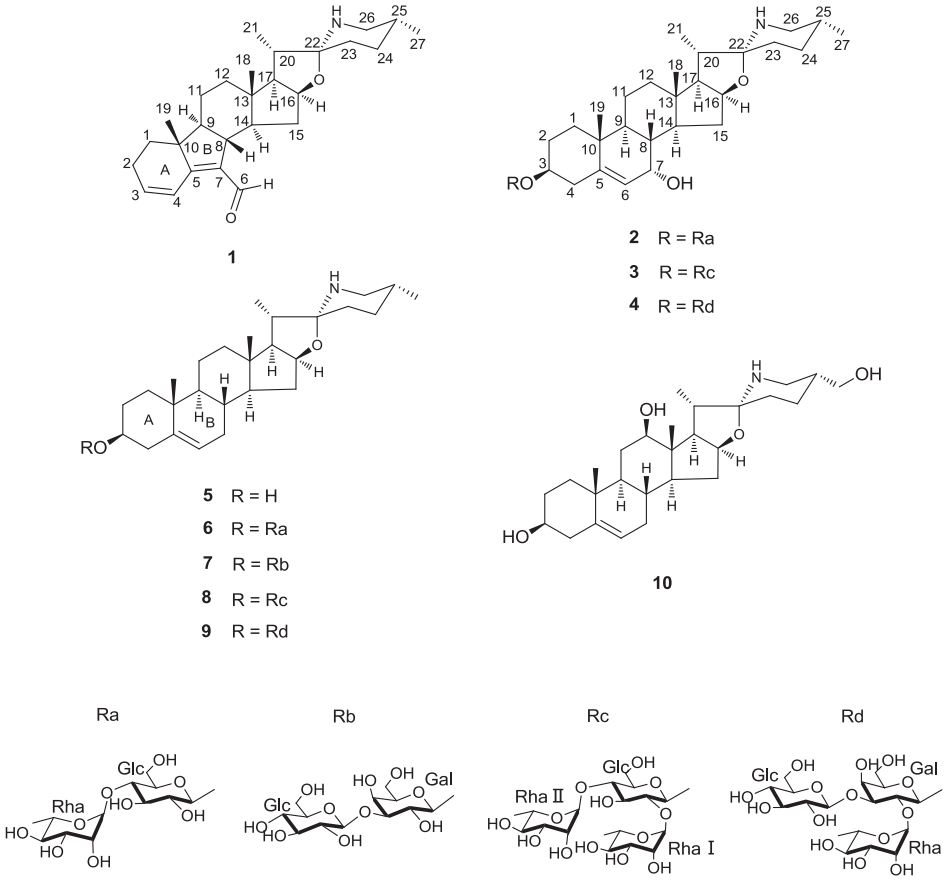

Fig. 1. Structures of compounds **1**–**10**.

m/z 410.3051 ([M+H]$^+$, calcd for $C_{27}H_{40}NO_2$, 410.3059), implying nine degrees of unsaturation. The IR absorption bands at 1667 and 1609 cm^{-1} indicated the presence of *α*,*β*-unsaturated aldehyde and olefinic groups. In the ^{1}H-NMR spectrum of **1**, two characteristic singlet methyl signals at δ_H 0.91 (3H, s) and δ_H 0.92 (3H, s), two doublet methyl signals at δ_H 0.83 (3H, d, J = 6.1 Hz) and 0.96 (3H, d, J = 7.1 Hz), two olefinic proton signals at δ_H 6.22 (1H, m) and 6.90 (1H, dd, J = 10.0, 1.6 Hz), as well as one aldehyde proton signal at δ_H 10.0 (1H, s) were observed. The ^{13}C NMR, DEPT and HSQC spectra exhibited 27 carbon signals, comprising four methyls, eight methylenes, nine methines, six quaternary carbons (including one *α*,*β*-unsaturated aldehyde) (Table 1). Overall consideration of the NMR data suggested that compound **1** was a steroidal alkaloid similar to the known co-occurring compound solasodine (**5**) (Mahato et al., 1980). The marked difference occurred in the rings A and B of these two compounds. In the ^{13}C NMR spectrum, the signal of C-3 in **1** appeared downfield at *δ* 138.4 as an olefinic carbon but exhibited in **5** at *δ* 71.8 as an oxymethine resonance, demonstrating the presence of a double bond rather than a hydroxyl group at C-3 in **1**. The HMBC correlations (Fig. 2) between H-3 (δ_H 6.22) and C-1 (δ_C 34.2), C-5 (δ_C 163.6), H-4 (δ_H 6.90) and C-2 (δ_C 23.9), C-10 (δ_C 44.6) defined the double bond at $\Delta^{3,4}$. Compared to **5**, an additional aldehyde proton at δ_H 10.0, which corresponded to an aldehydic carbon at δ_C 188.9 was observed in **1**. In HMBC spectrum, the aldehydic proton H-6 (δ_H 10.0) showed a two-bond correlation to C-7 (δ_C 135.4) and three-bond correlations to C-5 (δ_C 163.6) and C-8 (δ_C 44.5), suggesting the attachment of the aldehyde functionality at C-7, and defining the double bond at $\Delta^{5,7}$. Hence, a five-membered ring B with an *α*,*β*-unsaturated aldehyde was present in **1**. Parguesterol A (Wei et al., 2007), a related B ring 5(6 → 7) abeo-sterol with rarely reported 6/5/6/5 fused rings, was previously reported from a Caribbean Sea sponge, *Svenzea zeai*. The chemical shift values for ring B for **1** were comparable to those for parguesterol A. Accordingly, the planar structure of **1** was elucidated. The assignments of all proton and carbon signals could be assigned by 2D NMR experiments. The stereochemistry of **1** was determined by a NOESY experiment and NMR data analyses (Fig. 2). The NOESY correlations H-19*β*/H-8*β*, H-8*β*/H-18*β*, H-9*α*/H-14*α*, H-14*α*/H-16*α*, H-14*α*/H-17*α*, H-16*α*/H-17*α*, H-16*α*/H-21*α* and H-17*α*/H-21*α* indicated the same relative configurations at C-8, C-9, C-10, C-13, C-14, C-16, C-17, C-20 as those of **5**. The stereochemistry of the C-22 and C-25 of **1** were established as 22*α* and 25*R* based on comparison of the NMR data of **1** with those of **5** and tomatidenol (Wanyonyi et al., 2002). The ^{13}C NMR signals of C-22 and C-25 and their neighboring carbons of **1** were similar to those of **5**. The structure of **1** was further confirmed by a single-crystal X-ray diffraction analysis (Fig. 3) and named solanine A.

Compound **2** was obtained as white powder. The molecular formula was determined as $C_{39}H_{63}NO_{12}$ by HRESIMS (*m/z* 738.4443 [M+H]$^+$, calcd for $C_{39}H_{64}NO_{12}$, 738.4429) on the basis of HRESIMS data. The IR spectrum showed the absorption bands for hydroxy (3428 cm^{-1}) and olefinic (1647 cm^{-1}) groups. The ^{1}H-NMR spectrum revealed two singlet methyl signals at δ_H 0.92 (3H, s) and δ_H 0.95 (3H, s), three doublet methyl signals at δ_H 0.77 (3H, d, J = 5.6 Hz), 1.11 (3H, d, J = 7.0 Hz) and 1.72 (3H, d, J = 6.1 Hz), one olefinic proton signal at δ_H 5.81 (1H, d, J = 4.0 Hz), as well as two anomeric proton signals at δ_H 4.89 (1H, d, J = 7.6 Hz) and 5.89 (1H, br s). The ^{13}C NMR spectrum displayed 39 carbons including 27