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A new steroidal glycoside from the seeds of *Hyoscyamus niger*

Wenna Zhang, Wei Zhang, Jianguang Luo and Lingyi Kong*

Department of Natural Medicinal Chemistry, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, P.R. China

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A new steroidal glycoside hyoscyamoside G (**1**), together with two known analogues hyoscyamoside E (**2**) and hyoscyamoside F_1 (**3**), was isolated from the seeds of *Hyoscyamus niger*. The structure of **1** was established as (22*R*,24*Z*)-1α,3β,7β,22,26-pentakishydroxylergost-22-*O*-β-D-gulcopyranosyl-5,24-diene-26-*O*-β-D-glucopyranoside, by means of chemical and spectroscopic methods including HRESI-MS, 1D and 2D NMR. *In vitro*, compound **2** showed cytotoxicity against human lung cancer cell H460 with IC_{50} value of 66 μg/mL.

Keywords: *Hyoscyamus niger*; steroidal glycoside; Solanaceae; cytotoxicity

1. Introduction

The genus *Hyoscyamus* (Solanaceae) is well known for its anticholinergic properties and alkaloidal constituents (Chinese Pharmacopoeia Commission 2010). However, the presence of some non-alkaloidal constituents, such as anolides, lignanamides (Ma et al. 2002), tyramine derivatives and steroidal glycosides (Lunga et al. 2008a, 2008b) has also been reported in *Hyoscyamus niger* L. (Li et al. 2011). We earlier reported the lignanamides from the seeds of *H. niger* (Zhang et al. 2012). In our continuing search for bioactive constituents from *H. niger*, three steroidal glycosides were obtained. Herein, we report the isolation and structural elucidation of a new steroidal glycoside (**1**).

2. Results and discussion

Hyoscyamoside G (**1**) was obtained as white amorphous powder, $[\alpha]_D^{25}$ −42 (*c* 0.09, CH_3OH), negative-ion HRESI-MS (*m/z*, 785.4329, $[M - H]^-$) revealed the molecular formula of compound **1** to be $C_{40}H_{66}O_{15}$ (calcd for 785.4328). The ^1H NMR spectrum showed signals due to four tertiary methyl groups at δ 0.78, 1.02, 1.84, 1.86; a secondary methyl groups at δ 1.04 (d, $J = 6.5$ Hz); one olefinic proton (δ 5.74, d, $J = 4.5$ Hz), together with two anomeric protons [δ 4.26 (d, $J = 7.5$ Hz), 4.29 (d, $J = 7.5$ Hz)]. Moreover, one set of oxygenated methylene protons at δ 4.13, 4.42 (each 1H, ABq, $J = 10.5$ Hz) indicated the presence of $-CH_2O-$ group. The DEPT spectrum showed 40 carbon signals, of which 28 could be assigned to the signals of the aglycone composed of five methyl groups (δ 12.1, 13.5, 18.2, 18.9, 19.3), one hydroxymethyl (δ 71.3), four oxygenated methine carbons (δ 66.0, 66.7, 73.6, 83.2) and two double bonds (δ 127.4, 128.1, 134.6, 144.3). The full assignments of ^1H NMR and ^{13}C NMR signals were accomplished by a combination of HSQC, HMBC and ROESY experiments (Figure 1). The NMR data of the aglycone were similar with those of cilistol v isolated from

*Corresponding author. Email: cpu_lykong@126.com

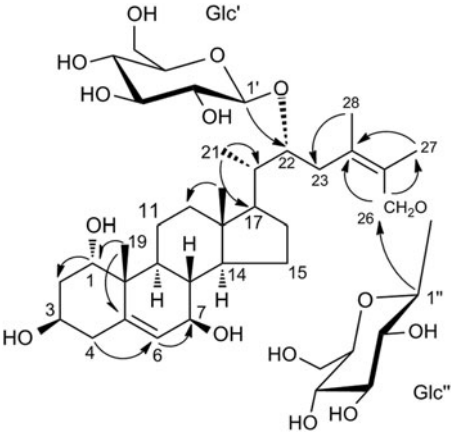

Figure 1. Structure of compound 1 with selected HMBC (H–C) correlations.

Solanum cilistum (Zhu et al. 2001) with the exception of the carbon signal δ 66.0 (C-7) instead of δ 32.3, indicating an additional OH group at C-7, which was further confirmed by the HMBC correlation between H-6 (δ 5.74) and C-7 (δ 66.0). From the anomeric protons of each monosaccharide moiety, all the protons within each spin system were assigned by HMBC and ROESY analyses (Figure 1). The β-configuration of the anomeric proton of the glucopyranosyl residue was assigned based on its J_{H1-H2} value ($J = 7.5$ Hz). Acid hydrolysis of **1** liberated D-glucose, which was identified by comparison Thin-layer chromatography (TLC) analysis and optical rotation dispersion with authentic glucose. The aglycone *was deduced* as a new compound on the basis of 1D and 2D NMR data. The linkages of sugars at C-22 and C-26 were determined by HMBC correlations between H-1′ (δ 4.26) and C-22 (δ 83.2) and H-1″ (δ 4.29) and C-26 (δ 71.3), respectively. Thus, the plain structure for **1** is presented as shown. The geometry at the double bond at C-24 was deduced as *Z* on the basis of ROESY between methyl groups at C-27 and C-28 (Zhu et al. 2001). Since the proton signals at C-1, C-3 displayed as a broad singlet ($W_{1/2} = 2.0$ Hz), multiplet ($W_{1/2} = 23$ Hz), the stereo configurations of the hydroxyl groups at C-1, C-3 were concluded to be α, β, respectively (Zhu et al. 2001). Furthermore, the ROESY correlations of H-9 (δ 1.94) with H-14 (δ 1.55) and H-7 (δ 3.74), and of H-8 (δ 1.48) with H-18 (δ 0.78) and H-19 (δ 1.02) suggested that B/C and C/D trans ring junctions as usual steroids (Lu et al. 2011) and the 7-OH in β-orientation. The multiplicity of H-22 as a broad double triple with couplings of about 9.0 and 3.5 Hz was consistent with a proton adjacent to one methylene and one methane. This is very similar to the appearance of H-22 in steroids with straight chain reported from Solanaceae family (Waiss et al. 1993). The configuration at C-22 was thus deduced as R based on identical biogenesis. Therefore, compound **1** was formulated as (22*R*,24*Z*)-1α,3β,7β,22,26-pentakishydroxylergost-22-*O*-β-D-glucopyranosyl-5,24-diene-26-*O*-β-D-glucopyranoside (Figure 1), and named hyoscyamoside G.

The structures of the known compounds were identified as hyoscyamoside E (**2**) (Lunga et al. 2008b) and hyoscyamoside F_1 (**3**) (Lunga et al. 2008b) by comparison of their spectroscopic data to previously reported values.

Compounds **1**–**3** were tested for *in vitro* cytotoxic activity against the HCT116 (human colon cancer), H460 (human lung cancer) and SMMC-7721 (human hepatoma cancer) cells. The result showed that compound **2** exhibited moderate cytotoxic activities against human lung cancer cell H460, with IC_{50} of 66 μg/mL. Fluorouracil was used as a positive control with IC_{50} of 12 μg/mL.

3. Experimental

3.1. *General*

Optical rotations were measured with a JASCO P-1020 polarimeter (Jasco, Tokyo, Japan); IR spectra (KBr disks) were recorded on a Bruker Tensor 27 spectrometer (Bruker, Karlsruhe, Germany). NMR spectra were measured in CD_3OD at 303 K on Bruker ACF-500 NMR instruments (^{1}H NMR: 500 MHz and ^{13}C NMR: 125 MHz), with tetramethylsilane as internal standard. Electrospray ionisation (ESI) and high-resolution ESI mass spectral data were acquired on an Agilent 1100 series LC/MSD ion trap mass spectrometer. All solvents used were of analytical grade (Jiangsu Hanbang Science and Technology Co., Ltd, Nanjing, China). Column chromatography was performed on silica gel H (200–300 mesh, Qingdao Marine Chemical Industry, Qingdao, China), D-101 macroporous resin (Bonchem, Cangzhou, China), MCI gel CHP-20 (Mitsubishi, Tokyo, Japan) and ODS (40–63 μm, FuJi, Aichi, Japan). TLC was performed on precoated silica gel GF254 plates (Qingdao Marine Chemical Co. Ltd) and detected by spraying with 10% H_2SO_4 in EtOH (v/v).

3.2. *Plant material*

The seeds of *H. niger* were purchased from local market in Bozhou, Anhui Province, China, in September 2010. The botanical identification was made by Prof. Min-jian Qin, Department of Medicinal Plants, China Pharmaceutical University. A voucher specimen (No. 100705) is deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

3.3. *Extraction and isolation*

Air-dried seeds of *H. niger* (15 kg) were ground and extracted with 95% EtOH under reflux. After concentration of the solution, the obtained crude extract (1 kg) was suspended in water, and then partitioned with petroleum ether and $CHCl_3$. Subsequently, the H_2O layer (22 g) was subjected to macroporous resin D101 column chromatography using a gradient of EtOH in H_2O (0:100, 30:70, 70:30, 100:0, v/v) to afford four fractions (AA–AD). The fraction AC (EtOH–H_2O, 70:30) was subjected to an MCI gel, eluting with CH_3OH–H_2O (30:70–100:0, gradient system) to obtain fractions of AC1–AC4. The subfraction AC2 (1 g) was further separated to ODS column chromatography and medium-pressure preparation liquid chromatography CH_3OH–H_2O (40:60–100:0, gradient system) to give rise to compound **1** (7.0 mg). The subfraction AC3 (2.5 g) was isolated to column chromatography on silica gel eluting with mixtures of $CHCl_3$–CH_3OH–H_2O (70:25:5) to afford compounds **2** (28.0 mg) and **3** (54.5 mg).

3.4. *Determination of cytotoxic activities*

Compounds **1**–**3** were evaluated for cytotoxic activity against HCT116 (human colon cancer), SMMC-7721 (human hepatoma cancer) and H460 (human lung cancer) cells by an 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay as described in the literature (Lu et al. 2009). The concentration of test compounds that gave 50% inhibition of cell growth was expressed as the IC_{50} value. Fluorouracil was used as a positive control, and the experiments were conducted for three independent replicates.

3.5. *Hyoscyamoside G (1)*

White amorphous powder (CH_3OH); $[\alpha]_D^{25}$ −42 (*c* 0.09, CH_3OH); IR (KBr) ν_{max} (cm^{-1}): 3439, 2932, 1076, 1046; ESI-MS: *m/z* 785 $[M - H]^-$, 605 $[M - H–Glu–H_2O]^-$; HRESI-MS: *m/z* $[M - H]^-$ calcd for $C_{40}H_{65}O_{15}$, 785.4328; found: 785.4329. ^{1}H NMR (500 MHz, CD_3OD): δ 3.84

(H, brs, H-1), 1.75 (H, m, H-2), 2.04 (H, m, H-2), 3.93 (H, m, H-3), 2.34 (H, m, H-4), 2.37 (H, m, H-4), 5.74 (H, d, $J = 4.5$ Hz, H-6), 3.74 (H, t, $J = 4.5$ Hz, 7.5 Hz, H-7), 1.94 (H, m, H-9), 1.52 (H, m, H-11), 1.58 (H, m, H-11), 1.25 (H, m, H-12), 2.06 (H, m, H-12), 1.84 (H, m, H-16), 1.22 (H, m, H-16), 1.24 (H, m, H-17), 0.78 (3H, s, H-18), 1.02 (3H, s, H-19), 2.18 (H, m, H-20), 1.04 (3H, d, $J = 6.5$ Hz, H-21), 3.91 (H, br dt, $J = 9.0$ Hz, 3.5 Hz, H-22), 2.02 (H, m, H-23), 2.48 (H, dd, $J = 11.0$ Hz, 18.0 Hz, H-23), 4.13(H, d, $J = 10.5$ Hz, H-26), 4.42 (H, d, $J = 10.5$ Hz, H-26), 1.86 (3H, s, H-27), 1.84 (3H, s, H-28), 4.26 (H, d, $J = 7.5$ Hz, H-1′-Glc), 4.29 (H, d, $J = 7.5$ Hz, H-1″’-Glc). ^{13}C NMR (500 MHz, CD_3OD): δ 73.6 (C-1), 39.1 (C-2), 66.7 (C-3), 42.4 (C-4), 144.3 (C-5), 127.4 (C-6), 66.0 (C-7), 39.0 (C-8), 35.1 (C-9), 43.3 (C-10), 21.0 (C-11), 40.6 (C-12), 43.7 (C-13), 50.7 (C-14), 28.7 (C-15), 25.3 (C-16), 54.4 (C-17), 12.1 (C-18), 18.9 (C-19), 42.1 (C-20), 13.5 (C-21), 83.2 (C-22), 34.5 (C-23), 128.1 (C-24), 134.6 (C-25), 71.3 (C-26), 19.3 (C-27), 18.2 (C-28), 105.7 (C-1′, 22-*O*-Glc), 75.9 (C-2′), 77.9 (C-3′), 71.8 (C-4′), 78.0 (C-5′), 62.9 (C-6′), 103.3 (C-1″, 26-*O*-Glc), 75.1 (C-2″), 78.0 (C-3″), 71.8 (C-4″), 78.0 (C-5″), 62.9 (C-6″).

3.5.1 *Acid hydrolysis of* ***1***

Compound **1** (2.0 mg) was treated in 1 M H_2SO_4 (dioxane: H_2O, 1:1, 2 mL) at 100°C for 2 h. The acid aqueous layer was neutralised with $BaCl_2$ to give a $BaSO_4$ precipitate. After filtering, the aqueous was concentrated under reduce pressure to dryness. TLC analysis compared with the authentic glucose (*n*-BuOH–HOAc–H_2O, 4:1:5, v/v/v, upper layer) and optical rotation dispersion $[\alpha]_D^{25}$ + 52.6 (*c* 0.03, H_2O) indicated the presence of D-glucose.

Supplementary material

Supplementary material relating to this article is available online, alongside Figures S1–S8.

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