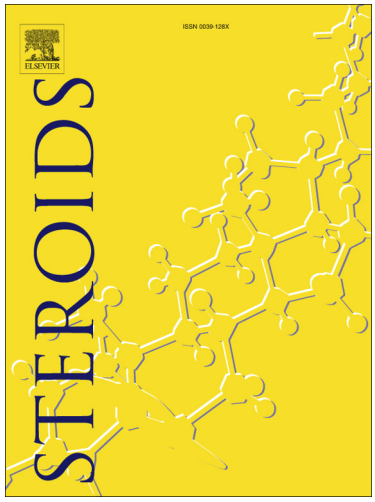
Journal Pre-proofs



Two new 23*S*,26*R*-hydroxylated spirostanoid saponins from the fruits of *Solanum indicum* var. *recurvatum*

Joseph Sakah Kaunda, Ying-Jun Zhang

PII: S0039-128X(19)30196-5
DOI: https://doi.org/10.1016/j.steroids.2019.108506
Reference: STE 108506

To appear in: *Steroids*

Received Date: 20 August 2019
Revised Date: 19 September 2019
Accepted Date: 30 September 2019

Please cite this article as: Kaunda, J.S., Zhang, Y-J., Two new 23*S*,26*R*-hydroxylated spirostanoid saponins from the fruits of *Solanum indicum* var. *recurvatum*, *Steroids* (2019), doi: https://doi.org/10.1016/j.steroids.2019.108506

Two new 23*S*,26*R*-hydroxylated spirostanoid saponins from the fruits of *Solanum indicum* var. *recurvatum*

Joseph Sakah Kaunda[a,b] and Ying-Jun Zhang[a,c,*]

[a]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China*

[b]*Graduate School of the Chinese Academy of Sciences, Beijing 100039, P. R. China*

[c]*Yunnan Key Laboratory of Natural Medicinal Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China*

* Corresponding author:

Ying-Jun Zhang

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China

*Tel/Fax: +86-871-6522-3235. E-mail: zhangyj@mail.kib.ac.cn

ABSTRACT

Solanum indicum var. *recurvatum*, distributed mainly in Hekou, Simao and Menghai areas of Yunnan province, China, is characterized by stellate pubescent leaves with irregular lengths and oblong, short, and downwardly curved anthers. The original species, *S. indicum*, has been used as an anti-inflammatory, wound-healing agent, an analgesic, and for the treatment of rhinitis, cough, and breast cancer in Chinese folk medicine. The variety was chemically studied for the first time, leading to the isolation of two new rare 23*S*,26*R*-hydroxylated spirostanoid saponins, together with five known steroidal components, two of which the rare natural ketosteroids 6-hydroxyandrosta-1,4-diene-3,17-dione and rostadienedione have been isolated from the genus *Solanum* for the first time. Elucidation of the structures was accomplished by extensive 1D and 2D NMR, UV, and HRESIMS spectroscopic methods.

Keywords: *Solanum indicum* var. *recurvatum*; Solanaceae; 23*S*,26*R*-hydroxylated spirostanoid saponins; ketosteroids

1. Introduction

The genus *Solanum* is regarded to be one of the largest among the Angiosperms and the most representative and largest genus of the family Solanaceae [1]. It is comprised of about 1500 species distributed across subtropical and tropical regions of Asia, tropical Africa, non-arid Africa, Americas, and Australia. Many species belonging to this genus possess a rich repertoire of medicinal, economic, and ornamental importance [1-2]. Within the past 30 years, *Solanum* has attracted significant attention in chemical and biological studies [1]. Previous phytochemical investigations on *Solanum* species led to the identification of steroidal saponins, steroidal alkaloids, terpenes, flavonoids, lignans, sterols, phenolics, coumarins, and so on.

Solanum indicum var. *recurvatum*, much the same as its original species, *S. indicum*, is characterized by stellate pubescent leaves with irregular lengths and oblong, short, and downwardly curved anthers [3]. It is distributed mainly in Hekou, Simao and Menghai areas of Yunnan province, China [3]. Whereas *S. indicum* has been used in Chinese folk medicine as an anti-inflammatory, wound-healing, and analgesic agents, as well as for the treatment of rhinitis, cough, and breast cancer [4], the available literature data did not reveal any local uses of the variety. Driven by the aspiration to unearth its chemical constituents and investigate theirbioactivities, our study on methanolic extract of the fruits of *S. indicum* var. *recurvatum* uncovered four steroidal saponins (**1**-**4**), one steroidal sapogenin (**5**) and two ketosteroids (**6**-**7**). Compounds **1**-**2** are new rare 23*S*,26*R*-hydroxylated spirostanoid saponins. All the isolates were obtained from the titled plant for the first time. The new isolates were also evaluated for their cytotoxic, antifungal, and anti-inflammatory activities. Herein, we report the study.

2. Experimental

2.1. General

Optical rotations were measured on Rudolph Autopol VI polarimeter (Rudolph Research Analytical, Hacketstown, NJ, USA). IR spectra were measured on a Bio-Rad FTS-135 series spectrometer with KBr pellets. UV spectra were recorded on a UV 210A Shimadzu spectrometer. CD spectra were obtained on a JASCO 810 spectrometer. One- and two-dimensional (1D and 2D) NMR spectra were recorded in C_5D_5N with Bruker DRX-600 and Avance 800 spectrometer operating at 600 and 800 MHz for 1H and at 150 and 200 MHz for ^{13}C, respectively. Coupling constants are expressed in hertz, and chemical shifts are given on a δ (parts per million, ppm) scale with tetramethylsilane (TMS) as an internal standard. ESI mass spectra were recorded on a VG Auto Spec-300 spectrometer. High-resolution (HR) ESI mass spectra were recorded on an API QSTAR Pular-1 spectrometer. Column chromatography (CC) was performed on MCI gel CHP20P (75−100 μm, Mitsubishi Chemical Co., Ltd.), silica gel (200-300 mesh, Qingdao Marine Chemical Co., Qingdao, China) and RP-18 gel (40−60 μm) (Merck, Darmstadt, Germany). Preparative HPLC and semi-preparative reversed-phase were performed using a Capcell C18 (Agilent, America) column (10 mm × 250 mm) and Cosmosil cholester (Kyoto, Japan) column (4.6 mm I.D. × 150 mm); the flow rate used for Preparative HPLC was 3.0 mL/min, and the separation and detection were achieved using Newstyle NU3000 SERIALS UV/VIS detector (Jiangsu Hanbon Science & Technology Co. Ltd, China). Thin-layer chromatography (TLC) was performed on precoated silica gel H plates, 0.20−0.25 mm thick (Qingdao Haiyang Chemical Co.), with chloroform/methanol/water (7:3:0.5 or 8:2:0.2 v/v/v), and spots were visualized under UV light and by spraying with 10% H_2SO_4 in EtOH followed by heating. Water was purified in a Milli-Q (Millipore, America). Acetonitrile (chromatographic grade) was purchased from Merck (Darmstadt, FR, Germany).

2.2. Plant material

Fruits of *S. indicum* var. *recurvatum* were collected in December 2018 in the vicinity of Xiding township, Menghai county, Xishuangbanna, Yunnan province, China. The plant material was identified by Prof. C.-R. Yang and a voucher specimen (KIB-Z-2018012) was deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China.

2.3. Extraction and isolation

Dried fruits (1.5 kg) of *S. indicum* var. *recurvatum* were crushed into powder and extracted in a Soxhlet apparatus with methanol for 72 h. The methanol extract (4.5 L) was then concentrated under reduced pressure in a rotary evaporator to give a residue (122 g) which was suspended into water (1000 mL) and extracted with *n*-butanol (each 500 mL, 4 times) to yield *n*-butanol (63 g) and water (45 g) fractions, after concentration under reduced pressure. The butanol fraction (63 g) was subjected to MCI gel, eluting with MeOH: H_2O (0:1→1:0), to give 7 fractions, M1-M7. Fraction M5 (5.5 g) was applied to silica gel (120 g) column (4.0 x 35 cm), eluting with chloroform/methanol/water (100:0:0, 98:2:0, 96:4:0, 94:6:0, 92:8:0, 90:10:0, 90:10:1, 85:15:1, 80:20:2 v/v) to generate compounds **1** (6 mg) and **2** (7 mg), and fractions M5c-M5f. Fr. M5g (850 mg) was applied to silica gel (36 g) column (3.5 x 30 cm) chromatography (CC), eluting with chloroform/methanol/ water (100:0:0, 98:2:0, 96:4:0, 94:6:0, 92:8:0, 90:10:0, 90:10:1, 85:15:1, 80:20:2 v/v) to furnish **3** (27 mg) and **4** (43 mg). Fraction M6 (6 g) was applied to silica gel CC (120 g, 4.0 x 30 cm), eluting with chloroform/methanol/water (100:0:0, 98:2:0, 96:4:0, 94:6:0, 92:8:0, 90:10:0, 90:10:1, 85:15:1, 80:20:2 v/v), to afford fractions M6a-M6h. Fr. M6a (900 mg), was subjected to silica gel (36 g) CC (3.5 x 30 cm), eluting as outlined above, to afford two fractions, M6a-1 (300 mg) and M6a-2 (420 mg). Fr. M6a-1 was purified on RP-18 to

bring forth fractions Fr. M6a-1a (29 mg), Fr. M6a-1b (25 mg), and Fr. M6a-1c (46 mg), which were submitted for further purification by preparative HPLC with an isocratic mobile phase system of acetonitrile/water. Fr. M6a-1a furnished **6** (t_R = 26.3 min, 2.0 mg) (35:65, v/v), Fr. M6a-1b gave **7** (t_R = 38.7 min, 2.0 mg) (40:60, v/v), and Fr. M6a-1c afforded **5** (t_R = 18.9 min, 7.0 mg) (25:75, v/v).

2.3.1 (22*R*,23*S*,25*R*,26*R*)-spirost-5-ene-3*β*,23,26-triol 3-*O*-*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranoside (**1**):

Yellow amorphous powder; $[\alpha]_D^{25}$ -73.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 196.6 (0.54) nm ; IR (KBr) ν_{max} 3424, 2934, 2906, 2850, 1714, 1636, 1509, 1454, 1436, 1383, 1305, 1274, 1259, 1197, 1065, 1038, 914, 894, 874, 856, 837 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 199 (1.00), 237 (-0.17), 250 (-0.07); ^1H and ^{13}C NMR (600/150 MHz, pyridine-d_5) data, see Table 1; HRESIMS *m/z*: 777.4031 $[M+Na]^+$ (calcd for $C_{39}H_{62}O_{14}Na$, 777.4032).

2.3.2 22*R*,23*S*,25*R*,26*R*)-spirost-5-ene-3 *β*,23,26-triol 3-*O*-*β*-D-glucopyranoside (**2**):

White amorphous powder; $[\alpha]_D^{25}$ -19.3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 192.8 (0.61), 196.8 (0.78), 269.2 (0.09), 283.2 (0.10) nm ; IR (KBr) ν_{max} 3431, 2933, 2853, 1712, 1634, 1516, 1454, 1436, 1384, 1318, 1274, 1258, 1159, 1074, 1044, 917, 895, 856, 837 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 202 (0.35), 297 (-0.35); ^1H (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5) data, see Table 1; HRESIMS *m/z*: 631.3458 $[M + Na]^+$ (calcd for $C_{33}H_{52}O_{10}Na$, 631.3453).

2.3.3 Acid hydrolysis of compounds **1**–**2** and GC analysis

Compounds **1-2** (2 mg, each), were separately dissolved in 5% HCl (1 mL) and heated (90 °C) for 2 h. HCl was then removed by evaporation in vacuum. The reaction mixtures were diluted

with H_2O and extracted with EtOAc. The aqueous layer was neutralized with 0.1 M NaOH and essentially dried to give the monosaccharide mixtures. In to the solution of the sugar mixtures in pyridine (2 mL), was added L-cysteine methyl ester hydrochloride (about 2 mg) and the reaction mixture kept at 60 °C for 1 h before addition of trimethylsilylimidazole (2 mL) in to the mixtures. Following this, the reaction mixtures were further kept at 60 °C for 1 h, before stopping the reaction and immediately submitting them for GC analysis [5], run on Hewlett Packard (HP) 5890 series II gas chromatography equipped with flame ionization detector (FID) and thermal conductivity detector (TCD). The column was HP-5: column temperature: 280 °C, increasing at the rate of: 3 °C/min; carrier gas: N_2 (1.5 mL/min); injector and detector temperature: 250 °C; injection volume: 1.0 μL; and split ratio: 1/50. The retention times of the samples were compared with those of the derivatives of authentic sugars, under the same condition. The sugar moieties of **1** were determined to be D-glucose (t_R: 28.232 min) and L-rhamnose (t_R: 24.265 min) by crosschecking with the standards, D-glucose (t_R: 28.418 min) and L-rhamnose (t_R: 24.348 min), respectively. The sugar moiety of **2** was resolved to be D-glucose (t_R: 28.358 min) by matching.

2.4. Cytotoxicity assay

The cytotoxic activities of compounds **1**–**2** were evaluated against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7 and SW480) which were cultured in RPMI-1640 or DMEM medium by means of 4-{5-[3-(carboxymethoxy)phenyl]-2- (4,5-dimethyl-1,3- thiazol- 2-yl)-2H-tetrazol-3-ium-3-yl}benzene-1-sulfonate (MTS) assay [6]. Cells in RPMI-1640 or DMEM medium containing 10% fetal bovine serum and inoculated with 3000-15000 cells per well, seeded into a 96-well cell culture plate with a volume of 100 μL per well, and kept at 37 °C in the incubator for 48 h. The compounds were dissolved in DMSO. After 48 h, 100 μL supernatant culture solutions from each well was discarded and 20 μL MTS solution was added.

The samples were incubated further for 4 h. Cisplatin (DDP) and taxol compounds were used as positive control in each experiment. Cell growth curve was plotted with the concentration as abscissa and cell viability as ordinate. The optical density (OD) was measured at 492 nm using MULTISKAN FC to determine inhibition rates of the 5 strains.

2.5. Antifungal assay

The test was performed in Potato Dextrose medium (Scientific Research Special) according to the procedure of Y. Liu et al. [7]. A volume of 100 μL aliquot from the stock solutions of the samples which had been initially prepared was added into the wells of the 96-well plates. 100 μL of the inoculum was added to achieve a final inoculum concentration of 1×10^5 CFU/mL in each well. The volume in each well was finally adjusted to 200 μL. Amphotericin B (Sigma-Aldrich) was used as reference substance. After incubation at 37 °C for 24 hours, plates were read at 625 nm to calculate the inhibitory rate of *C. albicans* - ATCC 10231 (Microbiologics).

2.6. Anti-inflammatory assay

As previously described by N. Soonthornsit et al. [8], RAW264.7 cells (Cell Bank of Chinese Academy of Sciences, Shanghai, China) were inoculated into 96-well plates, and induced stimulation with 1 μg/mL LPS (Sigma-Aldrich), and a test compound (final concentration 50 μM) was added to prepare a drug-free group and an L-NMMA-positive drug group (Sigma-Aldrich) as control. After the cells were cultured overnight, the medium was taken to detect NO production, and the absorbance was measured at 570 nm. MTS was added to the remaining medium for cell viability assay to eliminate the toxic effects of the compounds on the cells. NO production inhibition rate (%) = (non-drug treatment group OD_{570} nm - sample group OD_{570} nm) / non-drug treatment group OD_{570} nm × 100%.

3. Results and discussion

The MeOH extract of the fruits of *S. indicum* var. *recurvatum* was subjected to repeated column chromatography over MCI-gel CHP20P, silica gel, and semi-preparative HPLC, to yield seven steroidal compounds (**1**-**7**). Among them, compounds **1**-**2** are new 23*S*,26*R*-hydroxylated spirostanoid saponins. The known constituents were identified, on the basis of comparison of their NMR spectroscopic and MS data with those reported in the literature, as two steroidal glycosides, indioside A (**3**) [2,4] and 26-*O*-*β*-D-glucopyranosyl-22-methoxy-3,26-dihydroxy-(25*R*)-furost-5-ene 3-*O*-L-rhamnopyranosyl-(1→2)-[-*α*-L-arabinopyranosyl-(1→3)]-*β*-D-glucopyranoside (**4**) [9], one steroidal sapogenin indioside L (**5**) [10], and two ketosteroids, 6*β*-hydroxyandrosta-1,4-diene-3,17-dione (**6**) and rostadienedione (**7**) [11,12] (Fig. 1). All of them were reported from the titled plant for the first time. The only reports on the isolation of 23*S*,26*R*-hydroxylated spirostanoid glycoside were featured by Yohara et al. [2] and Mona et al. [4] who obtained compound **3** from the fruits of *S. indicum*. Compounds **6**-**7** were identified as rare ketosteroids in plants, only isolated from *Aglaia rubiginosa* (Meliaceae) [11]. They were previously obtained by chemical synthesis and microbiological biotransformations [12].

Compound **1** was obtained as a yellow, amorphous powder. Its molecular formula was assigned as $C_{39}H_{62}O_{14}Na$ based on a quasi-molecular ion peak at *m/z* 777.4031 $[M+Na]^+$ in the HR-ESI-MS (calcd for $C_{39}H_{62}O_{14}Na$, 777.4032). The ^1H NMR spectrum of **1** indicated two tertiary methyls at δ_H 1.01 and 0.87 (each 3H, s, CH_3-18, CH_3-19) and two secondary methyls at δ_H 1.30 (3H, d, J = 7.0 Hz, CH_3-21) and 1.18 (3H, d, J = 6.2 Hz, CH_3-27), which showed characteristics of a typical steroidal glycoside [4]. The two anomeric proton signals at δ_H 4.98 (1H, d, J = 7.7 Hz) and 5.94 (1H, s) suggested the existence of two sugar moieties in **1**. The ^{13}C NMR spectrum showed 27 signals that emerged from the aglycone of the glycoside; these signals

pointed out the presence of a hemiacetal [δ_C 96.6 (C-26)], a ketal [δ_C 114.2 (C-22)], three oxygenated methines [δ_C 78.7 (C-3), 82.2 (C-16), 67.9 (C-23)], a tri-substituted double bond [δ_C 141.3 (C-5), 122.2 (C-6)], eight methylenes [δ_C 37.9 (C-1), 30.7 (C-2), 39.7 (C-4), 32.7 (C-7), 21.6 (C-11), 40.7 (C-12), 32.6 (C-15), 38.2 (C-24)], six methines [δ_C 32.0 (C-8), 50.7 (C-9), 57.2 (C-14), 63.0 (C-17), 36.0 (C-20), 38.9 (C-25)], four methyls (δ_C 17.1 (C-18), 19.8 (C-19), 15.3 (C-21), 17.9 (C-27), and two quaternary carbons [δ 37.5 (C-10), 41.5 (C-13)]. Furthermore, the spectrum unveiled the sapogenol moiety as a spirostanol derivative containing one hemiacetal, a ketal, and three oxygen-bearing carbons. Also displayed on the ^{13}C NMR spectrum were 12 carbon signals that designated the existence of a glucosyl and one terminal rhamnosyl moieties, which were determined to be D-glucose (t_R: 28.232 min) and L-rhamnose (t_R: 24.265 min) on the basis of acidic hydrolysis, followed by GC analysis of the corresponding trimethylsilylated L-cysteine adduct. The HMBC experiment showed correlations from δ_H 1.18 (H_3-27) to δ_C 67.9 (C-23), 38.9 (C-25) and 96.6 (C-26), from δ_H 1.30 (H_3-21) to δ_C 63.0 (C-17), 36.7 (C-20) and 114.2 (C-22) and from δ_H 4.04 (1H, dd, J = 4.9, 7.8 Hz, H-23) to C-22 (Fig. 2), suggesting that the aglycone of **1** was a 3,23,26-trihydroxyspirost-5-ene derivative [4], even as confirmed by the ^{1}H–^{1}H COSY, HMBC and ROESY correlations (Fig. 2). The H-26 signal appeared as a doublet at δ_H 5.22 (J = 8.0 Hz), expressing *trans*-diaxial coupling between H-26 and H-25 [3]. Moreover, in the ROESY spectrum, correlations were observed between H-20 (δ 3.04) and H-23 (δ 4.04) as well as between H-23 and H-25 (δ 2.02) (Fig. 2), conveying that C-22 and C-25 were both in the *R* configuration [2, 4]. In the sugar moiety, HMBC correlations were observed from the rhamnosyl H-1 (δ 5.94) to glucosyl C-4 (δ 77.8) and from the glucosyl H-1 (δ_H 4.98) to the aglycone C-3 (δ 78.7). Except for the sugar moieties, the above NMR features of **1** were quite similar to those of compound **3**, the first 23*S*,26*R*-hydroxylated spirostanoid saponin with three

sugar moieties at aglycone C-3 obtained previously from the fruits of *S. indicum* [2,4]. Instead of one rhamnosyl and one arabinosyl moieties linked to the inner glucosyl C-2 and C-3, resp, in **3**, compound **1** has only one rhamnosyl group linked to the inner glucosyl C-2. Accordingly, the structure of **1** was characterized as (22*R*,23*S*,25*R*,26*R*)-spirost-5-ene-3*β*,23,26-triol 3-*O*-*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranoside .

Compound **2** was obtained as a white amorphous powder. Its molecular formula was established as $C_{33}H_{52}O_{10}$, in reference to the quasi-molecular ion peak at *m/z* 631.3458 $[M+Na]^+$ in the HR-ESI-MS (calcd for $C_{33}H_{52}O_{10}Na$, 631.3453). There was absolute similarity of signals in ^1H and ^{13}C NMR spectra of **1** and **2**, however, ^{13}C NMR spectrum of **2** exhibited 33 signals implying a loss of one hexosyl unit from the sugar chain in relation to compound **1**. The absent sugar moiety was determined to be *α*-L-rhamnopyranosyl, which existed in compound **1** at C-4" (δ 77.8), whereas in compound **2**, the ^{13}C NMR chemical shift signal assignable to C-4" of glucosyl was upfield shifted to δ 72.2. The single anomeric proton displayed at δ_H 5.06 (1H, d, J = 7.7 Hz) indicated only one glycosidic moiety in **2**[4], which was resolved to be D-glucose (t_R: 28.358 min) by acidic hydrolysis, followed by GC analysis of the corresponding trimethylsilylated L-cysteine adduct. The HMBC correlation was observed from the glucosyl H-1 (δ_H 5.06) to C-3 (δ 79.0) of the aglycone moiety. Compound **2** was thus determined to be (22*R*,23*S*,25*R*,26*R*)-spirost-5-ene-3 *β*,23,26-triol 3-*O*-*β*-D-glucopyranoside.

Compounds **1-2** were evaluated for their cytotoxicities on five human cancer cell lines (HL-60, SMMC- 7721, A-549, MCF-7 and SW480), antifungal activity on *Candida albicans* (ATCC 10231), and inhibition activity on nitric oxide (NO) production; none of them was active at concentrations of 40 μM, 200 μM, and 50 μM, respectively.

It is opined that introducing two hydroxyl groups at C-23 and C-27 of progenin II or dioscin considerably decreased their antimicrobial activities against *Propionibacterium acnes* by comparison of the MIC values of chonglouoside SL-2 and progenin II or chonglouoside SL-3 and dioscin [13]. Regarding biological activities, these compounds mentioned above (progenin II or dioscin) might not be the perfect examples to compare with compounds **1** and **2** owing to different sugar moieties and positions of the hydroxy group. Bizarrely, however, they tend to underscore why such compounds, like **1** and **2**, with hydroxy groups attached directly to the F ring display poor biological activities e.g. antimicrobial, cytotoxic and anti-inflammatory. In order to shed more light on this, it should therefore be of great interest to investigate and compare the biological activities of compound **1** and yamogenin 3-*O*-*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranoside [14], as well as compound **2** and (22*S*,25*S*)-spirost-5-en-3*β*-yl *β*-D-glucopyranoside [15]. This is because the compounds are absolutely similar except for the introduction of hydroxy groups at C-23 and C-26 in **1** and **2.**

4. Conclusion

Two new rare 23*S*,26*R*-hydroxylated spirostanoid saponins (**1**-**2**), together with five known steroidal constituents (**3**-**7**) were isolated from the methanolic extract of the fruits of *Solanum indicum* var. *recurvatum*. The rare natural ketosteroids, **6**-**7**, were isolated from the genus *Solanum* for the first time. Compounds **1** and **2** exhibited no cytotoxicity, antifungal, and anti-inflammatory activities; this should invite further studies to explore other biological effects in future.

Acknowledgements

We are delighted to express our sincere gratitude to members of the analytical center of State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, for spectroscopic data measurements. This work was partially supported by Yunnan Key Laboratory of Natural Medicinal Chemistry (S2017-ZZ14).

Declaration of Competing Interest

The authors declare no conflict of interest.

Supplementary data

Supplementary data to this article can be found online at

References

[1] J.S. Kaunda, Y.J. Zhang, The genus Solanum: An ethnopharmacological, phytochemical and biological properties review, Nat. Prod. Bioprosp. 9 (2019), 77-137.

[2] S. Yahara, T. Nakamura, Y. Someya, T. Matsumoto, T. Yamashita, T. Nohara, Steroidal glycosides, indiosides A-E, from *Solanum indicum*, Phytochemistry 43 (1996), 1319-1323.

[3] Delectis Florae Reipublicae Popularis SInicae, Agendae Academiae Sinicae Edita. Flora Reipublicae Popularis Sinicae. Science Press, Beijing, 1978, 67 (1), p101.

[4] E.A. Mona, H. Miyashita, T. Ikeda, J.H. Lee, H. Yoshimitsu, T. Nohara, K. Murakami, A New spirostanol glycoside from fruits of *Solanum indicum* L., Chem. Pharm. Bull. 57 (2009) 747-748.

[5] C.Z. Gu, J.J. Lv, X.X. Zhang, Y.J. Qiao, H.Yan, Y. Li, D. Wang, H.T. Zhu, H.R. Luo, C.R. Yang, Triterpenoids with promoting effects on the differentiation of PC12 cells from the steamed roots of *Panax notoginseng*, J. Nat. Prod. 78 (2015) 1829-1840.

[6] A.H. Cory, T.C. Owen, J.A. Barltrop, J.G. Cory, Use of an aqueous soluble tetrazolium/ formazan assay for cell growth assays in culture, Cancer Commun. 3 (1991) 207-212.

[7] Y. Liu, G. Tortora, M.E. Ryan, H.M. Lee, L.M. Golub, Potato dextrose agar antifungal susceptibility testing for yeasts and molds: evaluation of phosphate effect on antifungal activity of CMT-3, Ant. Agents and Chemo. 46 (2002) 1455-1461.

[8] N. Soonthornsit, T. Pitaksuteepong, C. Pitaksutheepong, W. Hemstapat, P. Utaisincharoen, In vitro anti-Inflammatory activity of *Morus alba* L. stem extract in LPS-stimulated RAW 264.7 cells, Evidence-based comp. and alternative med. : 2017 (2017) 3928956.

[9] Y. Mimaki, O. Nakamura, Y. Sashida, Y. Satomi, A. Nishino, H. Nishino, Steroidal saponins from the bulbs of *Lilium longiflorum* and their antitumor-promoter activity, Phytochemistry 37 (1994) 227-232.

[10] F.R. Chang, C.T. Yen, M. El-Shazly, C.Y. Yu, M.H. Yen, Y.B. Cheng, et al., Spirostanoids with 1,4-dien-3-one or 3β,7α-diol-5,6-ene moieties from *Solanum violaceum*, Bioorg. & Med. Chem. Lett. 23 (2013) 2738-2742.

[11] S. Weber, J. Puripattanavong, V. Brecht, A.W. Frahm, Phytochemical investigation of *Aglaia rubiginosa*, J. Nat. Prod. 63 (2000), 636-642.

[12] V.V. Kollerov, A.A. Shutov, V.V. Fokina, G.V. Sukhodol'skaya, M.V. Donova, Biotransformation of 3-keto-androstanes by *Gongronella butleri* VKM F-1033, J. Mol. Cat. Enzym. 55 (2008) 61-68.

[13] X.J. Qin, D.J. Sun, N. Wei, C.X. Chen, H. Yan, H. Li, H.Y. Liu. Steroidal saponins with antimicrobial activity from stems and leaves of *Paris polyphylla* var. *yunnanensis*, Steroids 77 (2012) 1242–1248.

[14] M. Yoshikawa, F. Xu, T. Morikawa, Y. Pongpiriyadacha, S. Nakamura, Y. Asao, A. Kumahara, H. Matsuda, Medicinal flowers. XII. New spirostane-type steroid saponins with antidiabetogenic activity from *Borassus flabellifer*, Chem. & Pharm. Bull. 55 (2007) 308-316.

[15] S.J. Schneider, J. John, Metabolism of dehydroisoandrosterone and other steroids by surviving slices of the potato tuber and additional plant tissues, J. Bio. Chem. 245 (1970) 5505-5510.

Table 1
1D NMR data of compounds **1**-**2** in pyridine-d_5 (600 MHz/150 MHz)

	1		**2**	
Position	$^{\delta}$H (*J* in Hz)	$^{\delta}$C	$^{\delta}$H (*J* in Hz)	$^{\delta}$C
1	1.71 m, 0.98 m	37.9	1.71 m, 0.98 m	37.9
2	2.09 m, 1.71 m	30.7	2.14 m, 1.74 m	30.7
3	3.89 m	78.7	3.98 overlapped	79.0
4	2.72 m, 2.46 m	39.7	2.73 m, 2.46 m	39.8
5		141.3		141.4
6	5.31 d (5.0)	122.2	5.29 d (5.1)	122.2
7	2.00 m, 1.50 m	32.7	1.99 m, 1.48 m	32.7
8	1.49 m	32.0	1.50 m	32.0
9	0.89 m	50.7	0.89 m	50.7
10		37.5		37.5
11	1.43 m	21.6	1.43 m	21.6
12	1.76 m, 1.17 m	40.7	1.77 m, 1.14 m	40.7
13		41.5		41.5
14	1.06 m	57.2	1.07 m	57.2
15	2.01 m, 1.81 m	32.6	2.01 m, 1.81 m	32.6
16	4.71 m	82.2	4.71 m	82.2
17	1.95 m	63.0	1.96 m	63.1
18	1.01 s	17.1	1.03 s	17.1
19	0.87 s	19.8	0.86 s	19.9
20	3.04 m	36.7	3.03 m	36.7
21	1.30 d (7.0)	15.3	1.32 d (6.8)	15.4
22		114.2		114.2
23	4.04 m	67.9	4.02 m	67.9
24	2.19 m, 2.03 m	38.2	2.18 m, 2.02 m	38.2
25	2.02 m	38.9	1.98 m	38.9
26	5.22 d (8.0)	96.6	5.21 d (8.0)	96.6
27	1.18 d (6.2)	17.9	1.17 d (6.1)	17.9
	3-*O*-Glc			
1'	4.98 d (7.7)	102.9	5.06 d (7.7)	103.1
2'	4.02 m	76.0	4.07 m	75.8
3'	4.27 m	77.2	4.32 m	79.1
4'	3.89 m	78.7	4.30 m	72.2
5'	3.75 m	77.6	3.98 overlapped	78.6
6'	4.29 m, 4.17 m	62.0	4.57 m, 4.43 m	63.3
	4'-*O*-Rha			
1"	5.94 s	103.2		
2"	4.74 m	73.1		
3"	4.62 m	73.3		
4"	4.39 m	74.5		
5"	5.07 m	70.9		
6"	1.76 d (6.2)	19.0		

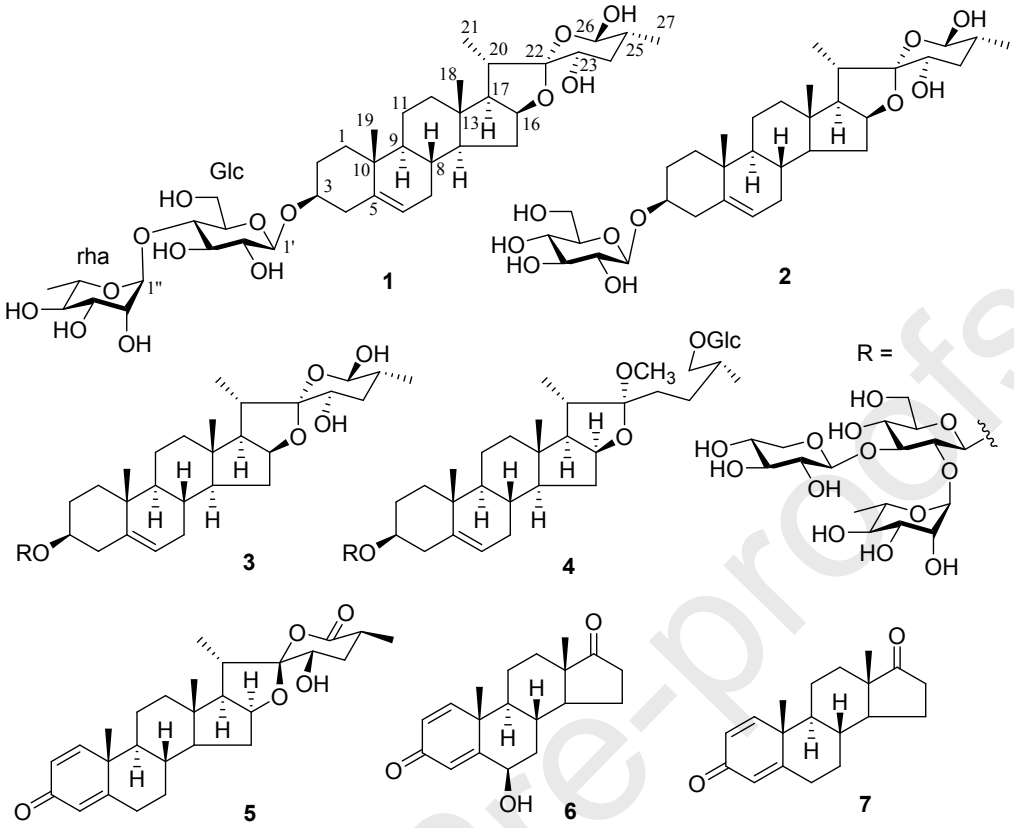


Fig. 1. Compounds **1-7** isolated from the fruits of *Solanum indicum* var. *recurvatum*

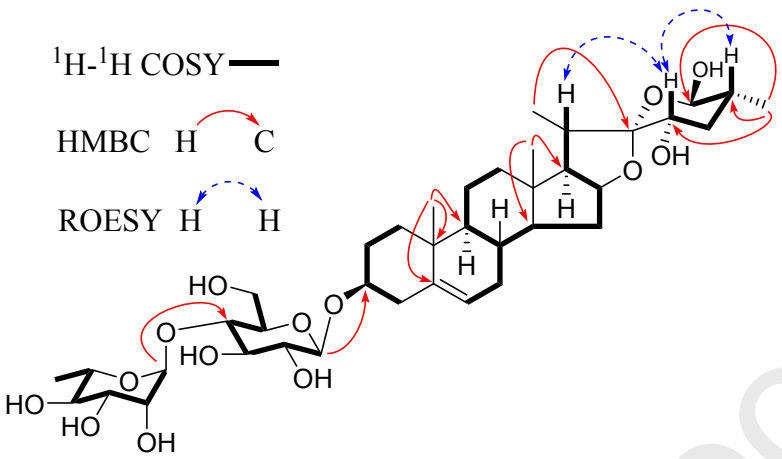
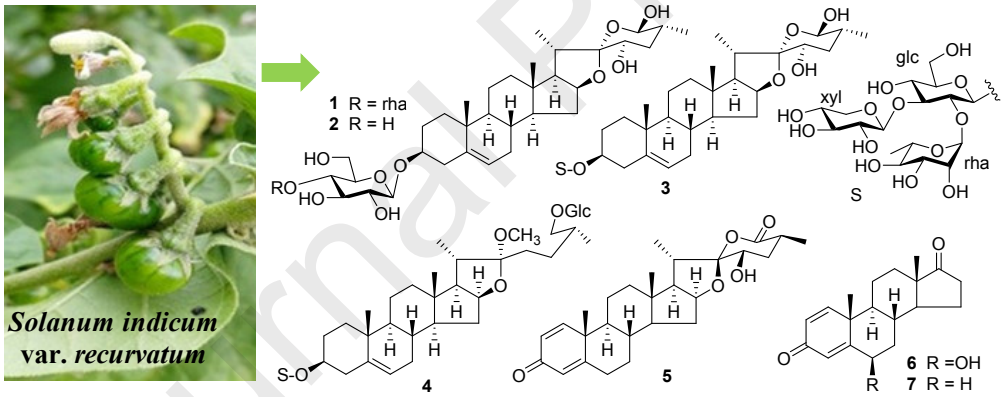


Fig. 2. Key ^{1}H-^{1}H COSY, HMBC, and ROESY correlations of **1**

Graphical Abstract

Two new 23*S*,26*R*-hydroxylated spirostanoid saponins from the fruits of *Solanum indicum* var. *recurvatum*

Joseph Sakah Kaunda and Ying-Jun Zhang



Highlights

1. The fruits of *S. indicum* var. *recurvatum* were studied chemically.

2. Two new rare 23*S*,26*R*-hydroxylated spirostanoid saponins were obtained.
3. Two rare ketosteroids were isolated from the genus *Solanum* for the first time.

The first author, Joseph Sakah Kaunda: Investigation; Writing - original draft and revision.

Corresponding author, Ying-Jun Zhang: Funding acquisition; Supervision; Writing - review & editing.