NOTE

Steroid Compounds from *Sida szechuensis*

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A new steroid compound, 2 β ,3 β ,14 α ,20,21,22R,24-heptahydroxycholest-7-en-6-one (**1**), was isolated from *Sida szechuensis*. Its structure was determined by spectroscopic analysis, including extensive 1D and 2D-NMR data. Ten previously known compounds were also identified *i.e.*, 3-O- β -D-glucopyranoside- α -ecdysone, loliolide, 3-O- β -D-galactopyranoside- β -ecdysone, 3, 4,5,6-tetrahydro-3-methyl- β -carboline-5-carboxylic acid, β -ecdysone, polypodine B, α -ecdysone, β -sitosterol, astragalin 6"-O-(4-hydroxycinnamoyl) and pterosterone. The antibacterial assay of the new compound showed that it has inhibitory effect on *Escherichia coli* with a concentration of 20 mg/mL.

Keywords: *Sida szechuensis*, 2 β ,3 β ,14 α ,20,21,22R,24-Heptahydroxycholest-7-en-6-one, Steroid, Antimicrobial activity.

Sida szechuensis has been used as a folk medicine in China to treat problems like traumatic injury, enteritis and dysentery¹. In previous papers, four sterols were reported *e.g.*, α -ecdysone, β -ecdysone, polypodine A, polypodine B^{2,3}. In order to find more bioactive compounds, the chemical components of *Sida szechuensis* were investigated. As a result, one new compound together with ten known compounds, 3-O- β -D-glucopyranoside- α -ecdysone (**2**), loliolide (**3**), 3-O- β -D-galactopyranoside- β -ecdysone (**4**), 3,4,5,6-tetrahydro-3-methyl- β -carboline-5-carboxylic acid (**5**), β -ecdysone (**6**), polypodine B (**7**), α -ecdysone (**8**), β -sitosterol (**9**), astragalin 6"-O-(4-hydroxycinnamoyl) (**10**), pterosterone (**11**), were isolated from the ethyl acetate extract of this plant. Presence of compounds **1-4** in this plant is reported for the first time herein. In addition, compounds **1-11** were tested for their antibacterial activity using disk diffusion method⁴.

The above ground part of *Sida szechuensis* were collected from Xishuangbanna, Yunnan, P.R. China. And a voucher specimen is kept at the laboratory of Research Group on Ethnomedicine, Xishuangbanna Tropical Botanical Garden Chinese Academy of Sciences.

Optical rotation was measured on a SEPA-3000 high sensitive polarimeter. IR (KBr) spectra were measured on a Bio-Rad FTS-135 Spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 instrument (400 or 500 MHz for ¹H NMR and 100 or 125 MHz for ¹³C NMR), using TMS as an internal standard. FAB-MS spectra were recorded on VG

Auto Spec-3000 spectrometer. UV spectra were on Shimadzu Double-beam 210A Spectrophotometer. Precoated silica gel plates were used for TLC. Detection was one by spraying the plates with 10 % sulfuric acid-ethanol, followed by heating.

Extraction and isolation: The air-dried above ground tissues of *Sida szechuensis* (18.5 kg) were extracted three times with 95 % ethanol under room temperature, 24 h each time. The combined extract was concentrated and dissolved in H₂O and then extracted with petroleum ether, ethyl acetate and *n*-butyl alcohol. The ethyl acetate extract was concentrated and applied to a silica gel column, eluting with CHCl₃-MeOH (1:9 $\rightarrow$ 9:1), repeatedly to give 2 β , 3 β , 14 α , 20, 21, 22R, 24-hepta-hydroxyl-cholest-7-en-6-one (1.074 g), together with other ten known compounds.

Characterization of compound (1): Colorless crystal; m.p. 244-246 °C; [α]_D²⁰ + 65.79° (c0.76, CH₃Cl₃); UV (MeOH) λ_{max} (log ϵ): 242 nm; IR (KBr, ν_{max} , cm⁻¹): 3375.26, 3020.72, 2964.24, 2948.17, 2876.96, 1644.51, 1463.02, 1379.03; HR-TOFMS m/z : 496.3035 (Calcd. for C₂₇H₄₄O₈); FAB-MS m/z (rel. int.): 496 [M + 1]⁺ (100), 480 (30), 109 (6), 81 (13), 69 (16); ¹H NMR (Pyridine-*d*₅, 400 MHz, δ ppm): 1.93 (1H, H-1 α), 2.24 (1H, H-1 β), 4.13 (1H, d, J = 12.86, H-2), 4.22 (1H, s, H-3), 1.82 (1H, H-4 α), 2.01 (1H, H-4 β), 2.98 (1H, d, J = 12.86, H-5), 6.27 (1H, s, H-7), 3.58 (1H, m, H-9), 1.75 (1H, H-11 α), 1.88 (1H, H-11 β), 1.91 (1H, H-12 α), 2.49 (1H, H-12 β), 1.89 (1H, H-15 α), 2.14 (1H, H-15 β), 2.18 (1H, H-16 α), 2.62 (1H, H-16 β), 3.14 (1H, H-17), 1.22 (3H, s, H-18

Me), 0.99 (3H, s, H-19 Me), 1.02 (3H, s, H-21 Me), 3.94 (1H, m, H-22), 1.85 (1H, H-23 α), 2.16 (1H, H-23 β), 3.94 (1H, d, J = 3.99, H-24), 1.75 (1H, H-25), 1.01 (3H, s, H-27). ^{13}C NMR spectral data Table-1.

Antibacterial activity: It was reported by Islam and his co-workers⁵ and others⁷ that the extract from *Sida rhombifolia* has antibacterial effects, so we tested antibacterial activity of those compounds on *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* with a concentration of 20 mg/mL, using disk diffusion method. It is shown that compound **1** has inhibitory effect on *Escherichia coli*. The results were shown in Table-2.

TABLE-1
 ^{13}C NMR SPECTRA OF COMPOUND **1**

Carbon	δ_{c}	Carbon	δ_{c}	Carbon	δ_{c}
C-1	36.23 (t)	C-10	38.73 (s)	C-19	24.51 (q)
C-2	68.20 (d)	C-11	21.12 (t)	C-20	78.18 (s)
C-3	68.11 (d)	C-12	31.76 (t)	C-21	66.00 (q)
C-4	32.53 (t)	C-13	47.97 (s)	C-22	78.18 (d)
C-5	51.44 (d)	C-14	84.27 (s)	C-23	37.98 (t)
C-6	203.73 (s)	C-15	31.37 (t)	C-24	77.17 (d)
C-7	121.82 (d)	C-16	21.71 (t)	C-25	33.89 (d)
C-8	166.27 (s)	C-17	47.56 (d)	C-26	17.11 (t)
C-9	34.44 (d)	C-18	18.05 (q)	C-27	19.68 (q)

TABLE-2
ANTIBACTERIAL ACTIVITY OF THOSE COMPOUNDS
FROM *Sida szechuensis* (ALL THE COMPOUNDS
WERE TESTED AT 20 mg/mL CONCENTRATION)

No. of compound	Test organisms and zone of inhibition (mm)		
	<i>E. coli</i>	<i>S. aureus</i>	<i>B. subtilis</i>
1	11.4 $\pm$ 1.0	-	-
2	8.7 $\pm$ 0.5	-	-
3	-	-	-
4	7.0 $\pm$ 0.6	-	-
5	-	-	-
6	-	7.0 $\pm$ 0.7	-
7	-	-	-
8	6.0 $\pm$ 1.0	-	6.7 $\pm$ 0.4
9	-	-	-
10	-	-	-
11	-	-	-
Penicillin	9.3 $\pm$ 1.4	9.5 $\pm$ 0.5	6.5 $\pm$ 0.5

Results are the means of diameter values $\pm$ standard deviation, - no activity

Compound **1** (Fig. 1) was obtained as colorless crystal. Its molecular formula was assigned as $\text{C}_{27}\text{H}_{44}\text{O}_8$ on the basis of positive ion HRFABMS ($[\text{M}^+]$, m/z 496.3035) and ^{13}C DEPT spectrum. The sterol-like structure of compound **1** was clearly indicated by the NMR spectrum. The ^1H NMR showed two tertiary methyl groups resonating in the high field region, reminiscent of the angular methyl of a sterane and the ^{13}C NMR spectrum confirmed presence of one oxygen-linked tetrasubstituted carbon atoms which indicated the presence in the molecule of one ketone (δ 203.73), as well as a trisubstituted

Fig. 1. Structure of compound **1**

double bond [δ 121.82 (C-7) and δ 166.27 (C-8)], conjugated with one of the carbonyl groups as shown by the very deshielded ^{13}C chemical shift of the β -olefine quaternary carbons and by UV absorption at λ_{max} = 243 nm, together with two oxy-methine groups [δ 68.20 (C-2) and δ 68.10 (C-3)]. All of these above data were in good agreement with the structural features of an ecdysteroid-type nucleus. And a 2H singlet at 4.36 were reasonably attributable to an oxymethylene of a saturated side chain hydroxylated at C-20. Two methyl doublets at δ 0.99 and δ 1.01, characteristic of methyl of 26 and 27, respectively. In addition, the ^{13}C NMR spectrum also confirmed presence of a oxymethylene [δ 66 (C-21)] and another two oxymethine groups [δ 78.18 (C-22) and δ 77.17 (C-24)]. And compare with known compounds⁸⁻¹⁰, this compound was shown to be 2 β ,3 β ,14 α ,20,22R,24-heptahyd-roxycholest-7-en-6-one.

In conclusion, present results have shown that compound **1** from *Sida szechuensis* possess antibacterial activity. Further comprehensive pharmacological investigations are needed to elucidate the exact mechanism of the antimicrobial effect of *Sida szechuensis*. All the compounds were tested at 20 mg/mL concentration.

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