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*NOTE*

# Steroid Compounds from *Sida szechuensis*

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A new steroid compound, 2β,3β,14α,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*, 2β,3β,14a,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 dysentery[1]. 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-ecdy-sone (**6**), poly- podine B (**7**), α-ecdysone (**8**), β-sitosterol (**9**), astragalin 6"-*O*-(4-hydroxy-cinnamoyl) (**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 method[4].

The above ground part of *Sida szechuenisis* were collected from Xishuangbanna, Yunnan, P.R. China. And a voucher specimen is kept at the laboratory of Research Group on Ethno-medicine, 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 $H^1$ NMR and 100 or 125 MHz for $^{13}C$ 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 $H_2O$ 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 $CHCl_3$-MeOH (1:9 → 9:1), repeatedly to give 2β, 3β, 14α, 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; $[\alpha]_D^{20}$ + 65.79° (c0.76, $CH_3Cl_3$); UV (MeOH) $\lambda_{max}$ ($\log_\varepsilon$): 242 nm; IR (KBr, $\nu_{max}$, $cm^{-1}$): 3375.26, 3020.72, 2964.24, 2948.17, 2876.96, 1644.51, 1463.02, 1379.03; HR-TOFMS *m/z*: 496.3035 (Calcd. for $C_{27}H_{44}O_8$); 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)δ: 1.93 (1H, H-1α), 2.24 (1H, H-1β), 4.13 (1H, d, *J* = 12.86, H-2), 4.22 (1H, s, H-3), 1.82 (1H, H-4α), 2.01 (1H, H-4β), 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-11α), 1.88 (1H, H-11β), 1.91 (1H, H-12α), 2.49 (1H, H-12β), 1.89 (1H, H-15α), 2.14 (1H, H-15β), 2.18 (1H, H-16α), 2.62 (1H, H-16β), 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-23α), 2.16 (1H, H-23β), 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-workers[5] and others[7] that the extract from *Sida rhombifolia* has antibacterial effects, so we tested antibacterial activity of those compounds on *Bacillus subtlis*, *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 | $\delta_c$ | Carbon | $\delta_c$ | Carbon | $\delta_c$ |
|---|---|---|---|---|---|
| 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) | | |
|---|---|---|---|
| | *E. coli* | *S. aureus* | *B. subtlis* |
| **1** | 11.4 ± 1.0 | - | - |
| **2** | 8.7 ± 0.5 | - | - |
| **3** | - | - | - |
| **4** | 7.0 ± 0.6 | - | - |
| **5** | - | - | - |
| **6** | - | 7.0 ± 0.7 | - |
| **7** | - | - | - |
| **8** | 6.0 ± 1.0 | - | 6.7 ± 0.4 |
| **9** | - | - | - |
| **10** | - | - | - |
| **11** | - | - | - |
| Penicillin | 9.3 ± 1.4 | 9.5 ± 0.5 | 6.5 ± 0.5 |

Results are the means of diameter values ± standard deviation, – no activity

Compound **1** (Fig. 1) was obtained as colorless crystal. Its molecular formula was assigned as $C_{27}H_{44}O_8$ on the basis of positive ion HRFABMS ([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 $^1$H 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

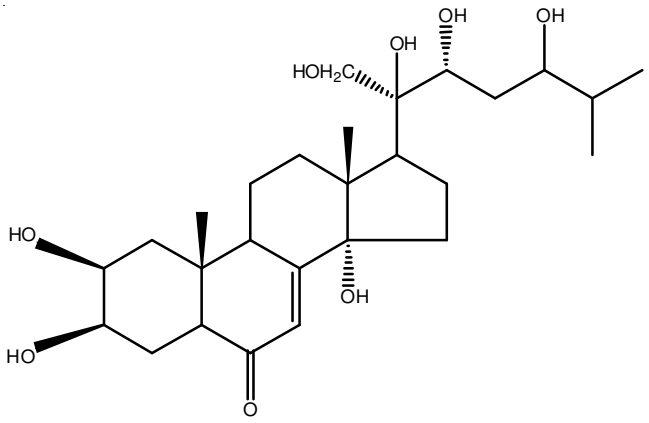


Fig. 1. Structure of compound **1**

double bond [δ 121.82 (C-7) and δ166.27 (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 $\lambda_{max}$ = 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 compounds[8-10], this compound was shown to be 2β,3β,14α,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.

## REFERENCES

1. D.J. Du, X.F. Shi, C.Q. Ran, Z.J. Wen, L.L. Peng and D.C. Xie, *Chin. Tradit. Herbal Drugs*, **26**, 594 (1995).
2. X. Chen, D.J. Du, N.N. Fang *et al*., *Chin. Tradit. Herbal Drugs*, **27**, 63 (1996).
3. C.S. Yao and Y.L. Xu, *Acta Botanica Yunnanica*, **22**, 503 (2000).
4. N. Kawabata and M. Nishiguchi, *Appl. Environ. Microbiol*., **54**, 2532 (1988).
5. M. Ekramul Islam, M. Ekramul Haque and M.A. Mosaddik, *Phytother. Res*., **17**, 973 (2003).
6. S. Venkatesh, Y.S. Rami Reddy, B. Suresh, B.M. Reddy and M. Ramesh, *J. Ethnopharmacol*., **67**, 229 (1999).
7. V. Ravi Kanth and P.V. Diwan, *Phytother. Res*., **13**, 75 (1999).
8. V. Costantino, C. Dell'Aversano, E. Fattorusso and A. Mangoni, *Steroids*, **65**, 138 (2000).
9. K. Vokác, M. Budesnský, J. Harmatha and J. Kohoutova, *Phyotochemistry*, **49**, 2109 (1998).
10. B.M.R. Bandara, L. Jayasinghe, V. Karunaratne, G.P. Wannigama, M. Bokel, W. Kraus and S. Sotheeswaran, *Phytochemistry*, **28**, 1073 (1989).