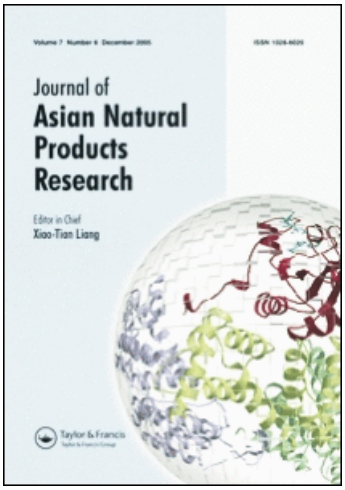

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A novel steroid from Tylophora atrofolliculata Metc

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A NOVEL STEROID FROM *TYLOPHORA ATROFOLLICULATA* METC.

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A novel steroid, tylophoriside A, was isolated from *Tylophora atrofolliculata* Metc. The structure was elucidated on the basis of spectroscopic methods and X-ray diffraction.

Keywords: Steroid; Tylophoriside A; *Tylophora atrofolliculata* Metc; HRFABMS

INTRODUCTION

Tylophora atrofolliculata Metc. is a liana, widely distributed in Guangxi province in China. The roots of this species have been well documented as herbal medicine for the treatment of rheumatism [1]. Pharmacological study showed that it possessed antitumor activity [2], but no chemical work has been done about this species. In the present paper, we report the isolation and structure elucidation of a novel steroid named tylophoriside A (**1**).

RESULTS AND DISCUSSION

Tylophoriside A (**1**) was obtained as a white powder (mp 231–232°C). The molecular formula $C_{28}H_{40}O_9$ of this compound was determined by its HRFABMS (*m/z* 521.2724 calcd. for $C_{28}H_{40}O_9$, 521.2750 $[M + 1]^+$). It gave oleandrose on hydrolysis, which was identified by comparison with authentic sample on TLC. The peak at *m/z* 376 $[M\text{-sugar}]^+$ also revealed the presence of an oleandrose moiety. Its IR spectrum showed the presence of hydroxyl (3521 cm^{-1}), carbonyl (1728 cm^{-1}) and double bond (1653, 1456, 987, 903 cm^{-1}) groups.

The anomeric proton signal at δ 4.88 (1H, dd, $J = 10, 2$) in the ^{1}HNMR was in good agreement with the ^{13}CNMR chemical shifts for the sugar moiety and those reported for methyl β-D-oleandropyranoside [3,4] and established the β mode of linkage for the sugar. A detailed comparison of the ^{1}HNMR and ^{13}CNMR (pyridine) spectral data of this compound

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and glaucoside-A (see Fig. 2) [5] showed that they have similar signals. Therefore, they should have the same skeletal structure (see Fig. 1) except for the stereochemistry and absolute configuration. The sugar was linked with the C-3 of the aglycone moiety, which was constructed on the basis of cross peaks in HMQC and HMBC experiments. (see Table I). The stereochemistry was elucidated on the basis of single crystal X-ray diffraction. The configuration of **1** is shown in Fig. 3. Thus the structure of **1** was (2R, 3R, 8R, 9S,10R, 16S, 17R, 20R)-3-[(2, 6-dideoxy -3-O-methyl-b-D-arabino-hexopyranosyl) oxy]-1, 2, 3, 4, 7, 8, 9, 10, 11, 12, 15, 16, 17, 20-tetradecahydro-2-hydroxy-10, 20-dimethyl-6H-16a, 18a, 20a-trioxapentaleno [1^1, 6^1:5,6,7] cyclonona [1, 2-a] naphthalene-14-one named tylophoriside A.

EXPERIMENTAL SECTION

General Experimental Procedures

NMR spectra were measured on Varian INOVA-500 spectrometer using TMS as internal standard; IR spectra were recorded on Nicolet-Impact 400 spectrometer; HRFABMS was taken by AutoSpec-Ultima mass spectrometer and EIMS was obtained on a ZAB-2F mass spectrometer; Melting point was determined on XT4-100X apparatus and are uncorrected. Silica gel (160–200 mesh) was used for column chromatography(CC), TLC plates were prepared with precoated Si gel GF254, and spots visualized by spraying with 10% H_2SO_4 in water. X-ray analysis was done with a MAC DIP-2030K diffractometer.

Plant Material

Roots of *T. atrofolliculata* were collected in November 1999 at Nanning, Guangxi province, China. It was authenticated by Prof. Liu Shouyang, Department of Pharmacognosy, Guangxi College of Chinese Traditional Medicine and a voucher specimen was deposited in the herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences.

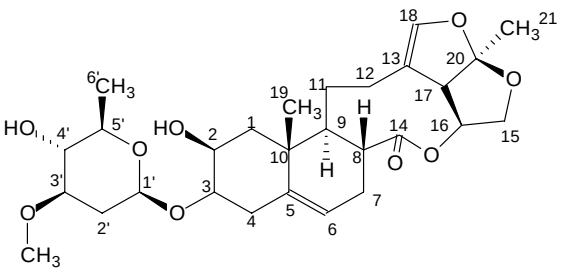

FIGURE 1 Structure of tylophoriside A(1).

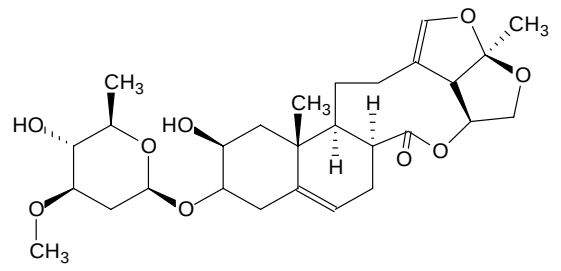

FIGURE 2 Structure of glaucoside-A.

Extraction and Isolation

The dried roots (7 kg) of *T. atrofolliculata* were extracted with hot 95% EtOH. The ethanolic extract was concentrated under reduced pressure and 0.5% HCl added. The deposit was filtered off. The filtrate was then made alkaline (pH = 9) with NH_4OH and extracted repeatedly with $CHCl_3$ to give 20 g extract. Eighteen gram of which was subjected to successive silica gel column chromatography eluted with P.E.-$CHCl_3$; $CHCl_3$–MeOH gradients, yielding seven fractions (A–G). 1 (61 mg) was isolated from fraction D after silica gel column chromatography ($CHCl_3$–MeOH, 8:1), and purified by crystallization in MeOH.

Tylophoriside A(**1**) was obtained as white needles (MeOH) with mp 231–232°C and $[\alpha]_D^{18} + 58$ (c 1.0, CH_3OH). IR (KBr) ν_{max} (cm^{-1}): 3521, 2935, 1728, 1653, 1456, 1383, 1309, 1265, 1163, 1082, 1034, 987, 903, 845, 796, 642, 600. HRFABMS (*m/z* 521.2724 calcd. for $C_{28}H_{40}O_9$, 521.2750 $[M + 1]^+$); EIMS (*m/z*): 69, 87, 95, 113, 145, 159, 169, 221, 258, 294, 312, 330, 358, 376, 520. The ^{1}HNMR and ^{13}CNMR data, see Table I.

X-ray crystallographic analysis: compound **1** crystallized in the monoclinic space group $P2_1$ with molecules of composition $C_{28}H_{40}O_9$ ($Z = 2$), accurate cell constants of $a = 7.559(1)$, $b = 9.801(1)$, $c = 18.759(1)$ Å, $\beta = 78.805(3)°$, $v = 1363.3(2)$ Å^3. All reflections were collected on the Nonius CAD-4 diffractometer, with graphite-monochromator, Mo K_α radiation ($\lambda = 0.71073$ Å), maximum 2θ value of 50.0°, independent reflections: 2307, observed number of reflections: 2303 [$|F|^2 \geq 8\delta|F|^2$]. The structure was resolved by direct method SHELXS-86 and expanded using difference Fourier techniques, refined by fullmatrix least-squares calculation. Hydrogen atoms were fixed at calculated positions. The

TABLE I ^{1}H (500 MHz), ^{13}C (125 MHz)NMR and 2D NMR data for tylophoriside A (C_5D_5N)

Position	^{13}CNMR	^{1}HNMR	HMBC
1	43.0	2.35(1H, dd, J = 11.5, 2.5) 1.20(1H, dd, J = 11.5, 2.5)	C10,C19,C9,C5,C2,C3
2	69.1	4.52(1H, m)	C1,C10,C3,C4
3	80.4	3.87(1H, m)	C2,C1,C4,C5
4	33.4	3.06(1H, dd, J = 11.5, 2) 2.40(1H, dd, J = 11.5, 3)	C2,C3,C5,C10,C6
5	141.6		
6	120.1	5.50(1H, d, J = 5.0)	C4,C5,C7,C8,C10
7	30.1	2.16(1H, m) 1.44(1H, m)	C5,C6,C8,C9,C14
8	53.8	1.24(1H,m)	C6,C7,C9,C10,C11,C14
9	40.3	2.55(1H, m)	C5,C7,C8,C10,C11,C12,C14
10	38.7		
11	24.0	2.60(1H, m) 1.38(1H, m)	C8,C9,C10,C12,C13
12	28.4	2.70(1H, m) 2.20(1H, m)	C9,C11,C13,C17,C18
13	118.5		
14	175.6		
15	67.8	3.90(1H, dd, J = 10, 9) 4.20(1H, dd, J = 9, 7)	C16,C17
16	75.6	5.45(1H, ddd, J = 10,8, 7)	C13,C15,C17,C20
17	56.2	3.56 (1H, d, J = 8)	C12,C13,C15,C16,C18,C20,C21
18	143.8	6.45(1H, s)	C12,C13,C17
19	21.3	1.35(3H, s)	C1,C5,C9,C10
20	114.4		
21	24.8	1.53(3H, s)	
1′	98.9	4.88(1H, dd, J = 10, 2)	C2′,C3′,C5′
2′	37.2	2.46(1H, m) 1.67(1H, m)	C1′,C3′,C4′
3′	81.5	3.50(1H, m)	
4′	76.3	3.50(1H, m)	
5′	73.5	3.60(1H, m)	
6′	18.9	1.54(3H, d, J = 6.5)	C4′,C5′
OMe	56.9	3.44(3H, s)	

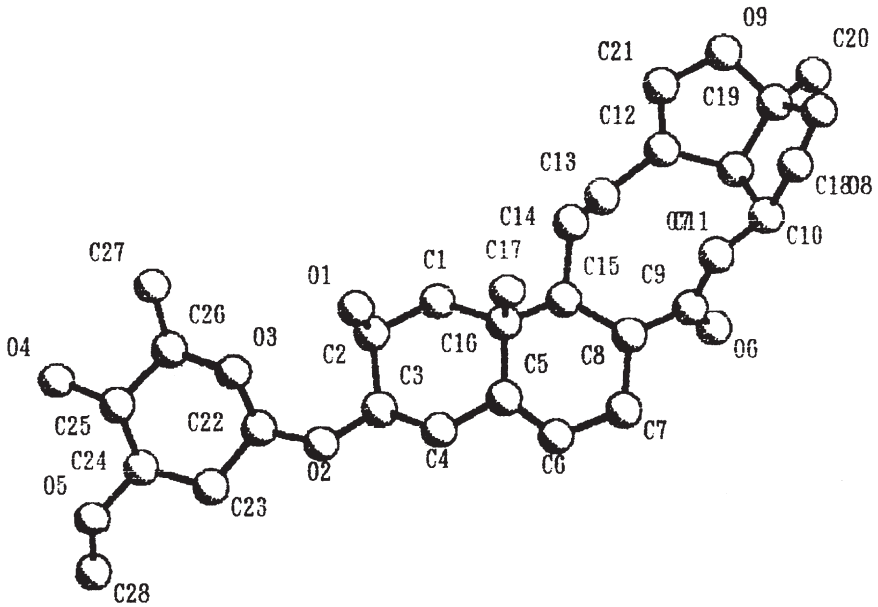

FIGURE 3 A perspective view of the structure (**1**).

final indices were $R_f = 0.0051$, $R_w = 0.051$. The fractional atomic coordinates of **1** are deposited in the editorial office of JANPR.

Acknowledgements

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References

[1] Xie, Z.W., Fang, S.C. and Zhu, Z.Y. (1996), *Quan Guo Zhong Cao Yao Hui Bian (B)*, 818.
[2] Institute of Medical and Pharmaceutical Science of Kwangsi Chuang Autonomous(1977), *J. Biochem. Biophys.* **9**(2), 131–138, See also, pp. 139–145.
[3] Monmoret, C. and Conreur, C. (1978), *Carbohydr. Res.* **65**, 35–39.
[4] Abe, F. and Yamauch, T. (1978), *Chem. Pharm. Bull.* **26**, 3023–3027.
[5] Nakaguwa, T. and Hayashi, K. (1983), *Tetrahedron* **39**, 607–612.