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Spirostane Steroidal Saponins from the Undergroud Parts of *Tupistra chinensis*

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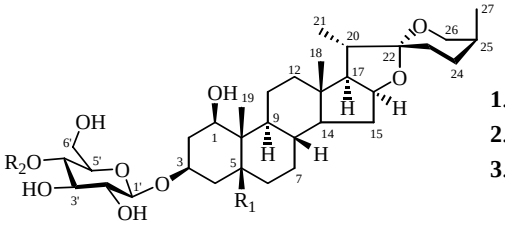
Abstract: Two new spirostanol saponins, (25*S*)-spirostane-1β,3β,5β-triol 3-*O*-β-D-glucopyranoside **1** and rhodeasapogenin 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside **2**, together with a known saponin, rhodeasapogenin 3-*O*-β-D-glucopyranoside **3**, were isolated from the underground parts of *Tupistra chinensis* Bak.. Their structures were determined by spectroscopic analysis.

Key words: *Tupistra chinensis* Bak., Liliaceae, steroidal saponins, (25*S*)-spirostane-1β,3β,5β-triol 3-*O*-β-D-glucopyranoside, rhodeasapogenin 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside.

Tupistra chinensis is a medicinal plant used for treatment of rheumatic diseases and snake-bite in China[1]. Previous phytochemical studies have reported the isolation of several spirostanol sapogenins and flavans from this plant[2–4]. During our ongoing screening for antifungal natural products for post-harvest preservation of litchi fruits, we found that the methanolic extract of the underground parts of this plant showed potent inhibitory activity against *Peronophythora litchii*, a main pathogen of litchi fruits. This prompted us to investigate its chemical constituents. The investigation has led to the isolation of two new steroidal saponins, (25*S*)-spirostane-1β,3β,5β-triol 3-*O*-β-D-glucopyranoside **1** and rhodeasapogenin 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside **2**, together with a known saponin, rhodeasapogenin 3-*O*-β-D-glucopyranoside **3**. We here describe the characterization of these two new compounds.



1. R_1 = OH, R_2 = H
2. R_1 = H, R_2 = Glc
3. R_1 = H, R_2 = H

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The EtOAc fraction of the MeOH extract of the underground parts of *T. chinensis* collected from Shennongjia, Hubei, was separated by a combination of silica gel, ODS, and Sephadex LH-20 column chromatography to afford **1–3**. Compound **3** was identified by comparison of its spectral data with those of previously reported[5,6].

Compound **1** was obtained as colorless needles, mp 276–278 °C. Its molecular formula was assigned as $C_{33}H_{54}O_{10}$ by the ^{13}C NMR data and the negative APCIMS which showed a $[M - H]^-$ ion peak at *m/z* 609 and a $[M - H - \text{hexose}]^-$ fragment peak at *m/z* 447. The ^1H NMR spectrum of **1** showed signals for two tertiary methyl groups at δ 1.53 (s, 3H) and 0.83 (s, 3H), and two secondary methyls at δ 1.14 (d, 3H, *J*=6.8 Hz) and 1.09 (d, 3H, *J* = 6.8 Hz). The structure of **1** based upon a (25*S*)-spirostanol derivative was suggested by the above ^1H NMR data, together with a quaternary carbon signal at δ 109.7 in the ^{13}C NMR spectrum[7] and by the characteristic IR absorptions at 987, 920, 900 and 850 cm^{-1} with the absorption at 920 cm^{-1} being of greater intensity than that at 900 cm^{-1} [8–10]. The presence of a β-D-glucopyranosyl moiety in **1** was readily recognized by the appearance of an anomeric proton signal at δ 5.00 (d, 1H, *J* = 8.0 Hz) in the ^1H NMR spectrum (**Table 1**) and the characteristic six carbon signals at δ 103.0 (CH), 75.5 (CH), 78.9 (CH), 71.5 (CH), 78.4 (CH), 62.7 (CH_2) in the ^{13}C NMR spectrum (**Table 1**), as well as the detection of D-glucose in the acid hydrolysis products. After having excluded signals due to the glucose residue, the remaining carbon and proton signals indicated a spirostanetriol moiety due to the presence of two oxymethine [δ_H 4.15 (br s), δ_C 73.1; δ_H 4.57 (br d, *J* = 3.6 Hz), δ_C 75.5] and an oxygenated quaternary carbon (δ 75.3) resonances. In the ^1H-^1H COSY spectrum, the methylene protons at δ 2.56 (br d, 1H, *J* = 14.8 Hz) and 2.01 (br d, 1H, *J* = 14.8 Hz) were shown to be coupled to both of the two oxymethine protons at δ 4.15 and δ 4.57. The oxymethine proton at δ 4.57 was further coupled to the methylene protons at δ 2.34 (dd, 1H, *J* = 15.6, 3.6 Hz) and 2.18 (br d, 1H, *J* = 15.6 Hz). In the HMBC spectrum, the correlations from the oxymethine proton at δ 4.15 to the carbons at δ 75.5 (CH), 75.3 (C), 44.0 (CH_2), 33.1 (CH_2), and 13.6 (CH_3), from the oxymethine proton at δ 4.57 to the carbons at δ 73.1 (CH) and 75.3, and from the exchangeable proton at δ 6.45 (br s) to the carbons at δ 75.3, 44.0, and 35.5 were observed. These findings allowed the placement of these three hydroxyl groups at C-1, C-3 and C-5 positions. The β-axial orientations of all these hydroxyl groups were indicated by the small $J_{1,2ax}$, $J_{3,2ax}$, and $J_{3,4ax}$ values in the ^1H NMR spectrum, as well as by the NOESY spectrum in which the NOE correlations were observed between H-1/H-19, H-1/H_2-2, H-3/H_2-2, H-3/H_2-4, and between the proton of the hydroxyl group at C-5 and H-4eq and H-19. The aglycone part was thus established as the moiety of (25*S*)-spirostane-1β,3β,5β-triol which was convallagenin A[11]. The linkage position between the β-D-glucose and the aglycone was established at C-3 by the HMBC correlation between H-1′ and C-3 and the NOESY correlation between H-1′ and H-3. In conclusion, **1** was elucidated as (25*S*)-spirostane-1β,3β,5β-triol 3-*O*-β-D-glucopyranoside.

Compound **2** was isolated as white amorphous solid. Its negative APCIMS showed a $[M–H]^-$ ion peak at *m/z* 755 and fragment ions at *m/z* 593 $[M–H–\text{hexose}]^-$ and 431 $[M–H–\text{hexose}–\text{hexose}]^-$. Compared with the spectrum of **1**, the aglycone of **2** had 16 less mass units, suggesting the aglycone of **2** had one less hydroxyl group than that of **1**.

Table 1 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data for **1** and **2** (in pyridine-d_6, δ ppm)

Position	**1**		**2**	
	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)	^{13}C
1	4.15 br s	73.1	3.87 br s	72.5
2ax	2.01 br d (14.8)	33.1	1.80 – 1.85 [a]	32.0
2eq	2.56 br d (14.8)		2.27 br d (14.0)	
3	4.57 br d (3.6)	75.5	4.44 – 4.57 [a]	74.9
4ax	2.34 dd (15.6, 3.6)	35.5	1.80 – 1.85 [a]	29.3
4eq	2.18 br d (15.6)		1.89 – 1.97 [a]	
5		75.3	2.36 m	31.0
6ax	1.41 – 1.49 [a]	36.6	1.20 – 1.31 [a]	26.6
6eq	1.94 m		1.20 – 1.31 [a]	
7ax	1.00 m	28.9	0.99 m	26.6
7eq	1.41 – 1.49 [a]		1.62 – 1.72 [a]	
8	1.63 – 1.66 [a]	35.0	1.54 – 1.59 [a]	35.9
9	1.13 m	45.6	1.19 m	42.2
10		44.0		40.4
11	1.38 m	21.6	1.20 – 1.31 [a]	21.1
12ax	0.95 – 1.10 [a]	40.7	1.05 m	40.3
12eq	1.63 – 1.66 [a]		1.62 – 1.72 [a]	
13		40.1		40.7
14	0.95 – 1.10 [a]	56.3	1.07 m	56.4
15α	2.00 m	32.3	2.01 m	32.3
15β	1.41 – 1.49 [a]		1.43 m	
16	4.57 m	81.5	4.59 m	81.2
17	1.79 br d (8.0)	62.9	1.80 – 1.85 [a]	63.2
18	0.83 s	16.7	0.83 s	16.7
19	1.53 s	13.6	1.24 s	19.2
20	1.90 m	42.6	1.94 m	42.6
21	1.14 d (6.8)	14.9	1.13 d (6.8)	15.0
22		109.8		109.8
23ax	1.83 m	26.4	1.62 – 1.72 [a]	26.6
23eq	1.41 – 1.49 [a]		1.17 – 1.31 [a]	
24ax	2.12 m	26.2	1.89 – 1.97 [a]	26.3
24eq	1.30 m		1.20 – 1.31 [a]	
25	1.57 m	27.6	1.54 – 1.59 [a]	27.6
26ax	4.06 br d (11.2)	65.2	4.03 br d (10.8)	65.2
26eq	3.36 br d (11.2)		3.36 br d (10.8)	
27	1.09 d (6.8)	16.4	1.06 d (7.2)	16.4
5-OH	6.45 br s			
1′	5.00 d (8.0)	103.0	4.95 d (8.0)	101.1
2′	3.97 t (8.0)	75.5	3.94 t (8.0)	74.7
3′	4.26 t (8.0)	78.9	4.25 – 4.33 [a]	76.7
4′	4.23 t (8.0)	71.5	4.25 – 4.33 [a]	81.2
5′	3.99 m	78.4	4.25 – 4.33 [a]	76.9
6′a	4.57 dd (12.0, 1.6)	62.7	4.44 – 4.57 [a]	62.1
6′b	4.40 dd (12.0, 5.2)		4.44 – 4.57 [a]	
1″			5.19 d (7.6)	105.0
2″			4.09 t (8.0)	75.0
3″			4.20 t (8.0)	78.5
4″			4.19 t (8.0)	71.6
5″			4.01 m	78.3
6″a			4.44 – 4.57 [a]	62.5
6″b			4.25 – 4.33 [a]	

[a]Signal pattern unclear due to overlapping.

A preliminary inspection of the ^{1}H and ^{13}C NMR spectra of **2** readily indicated the presence of two monosaccharide units through easily identifiable signals for anomeric protons at δ 5.19 (d, 1H, *J* =7.6 Hz) and δ 4.95 (d, 1H, *J* = 8.0 Hz) in the ^{1}H NMR spectrum and carbons at δ 105.0 and 101.1 in the ^{13}C NMR spectrum. Acid hydrolysis afforded only D-glucose besides the aglycone. Detailed examination of the ^{13}C NMR spectrum indicated that the sugar part was β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside moiety [7], and this was supported by the HMBC correlation from H-1″ to C-4′. The ^{1}H and ^{13}C NMR signals for the aglycone part were closely similar to those of rhodeasapogenin [7,12] except that the carbon signal of C-3 shifted downfield by 6.7 ppm while the carbon signals of C-1, C-2, and C-4 shifted upfield by 0.9, 0.9, and 3.4 ppm, respectively, in **2**. This together with the presence of a HMBC correlation between H-1′ and C-3 indicated that the sugar moiety linked at C-3 [7]. By combination of ^{1}H-^{1}H COSY, HMQC and HMBC spectra, the ^{1}H and ^{13}C NMR signals were assigned (see **Tables 1**), and the assignments supported the structure. Consequently, compound **2** was determined as rhodeasapogenin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside.

It is noted that the occurrence of **3** in this plant is reported for the first time.

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References

1. Jiangsu New Medical College, *Dictionary of Traditional Chinese Medicines* (in Chinese), Shanghai Science and Technology Press, Shanghai, 1979, p. 907.
2. W. B. Pan, F. R. Chang, Y. C.Wu, *Chem. Pharm. Bull.*, **2000**, *48*, 1350.
3. W. B. Pan, F. R. Chang, Y. C.Wu, *J. Nat. Prod.*, **2000**, *63*, 861.
4. W. B. Pan, F. R. Chang, L. M. Wei, *et al.*, *J. Nat. Prod.*, **2003**, *66*, 161.
5. K. Kudo, K. Miyahara, N. Marubayashi, *et al*., *Chem. Pharm. Bull.*, **1984**, *32*, 4229.
6. K. Miyahara, K. Kudo, T. Kawasaki, *Chem. Pharm. Bull.*, **1983**, *31*, 348.
7. P. K. Agrawal, D. C. Jain, R. K. Gupta, *et al*., Thakur, R.S., *Phytochemistry*, **1985**, *24*, 2479.
8. C. R. Eddy, M. E.Wall, M. K. Scott, *Anal. Chem.*, **1953**, *25*, 266.
9. M. E. Wall, C. R. Eddy, M. L. McClennan, *et al*., *Anal. Chem.*, **1952**, *24*, 1337.
10. R. N. Jones, K. Katzenellenbogen, K. Dobriner, *J. Am. Chem. Soc.*, **1953**, *75*, 158.
11. M. Kimura, M. Tohma, I. Yoshizawa, *Chem. Pharm. Bull.*, **1966**, *14*, 50.
12. P. K. Agrawal, P. Bunsawansong, G. A. Morris, *Phytochemistry*, **1998**, *47*, 255.

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