Taccasterosides A—C, Novel C_{28}-Sterol Oligoglucosides from the Rhizomes of *Tacca chantrieri*

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Three novel C_{28}-sterol oligoglucosides, named taccasterosides A—C (1—3), were isolated from the rhizomes of *Tacca chantrieri* (Taccaceae). Their structures were determined by detailed spectroscopic analysis, including 2D NMR data, and a few chemical transformations.

Key words *Tacca chantrieri*; Taccaceae; C_{28}-sterol oligoglucoside; taccasteroside

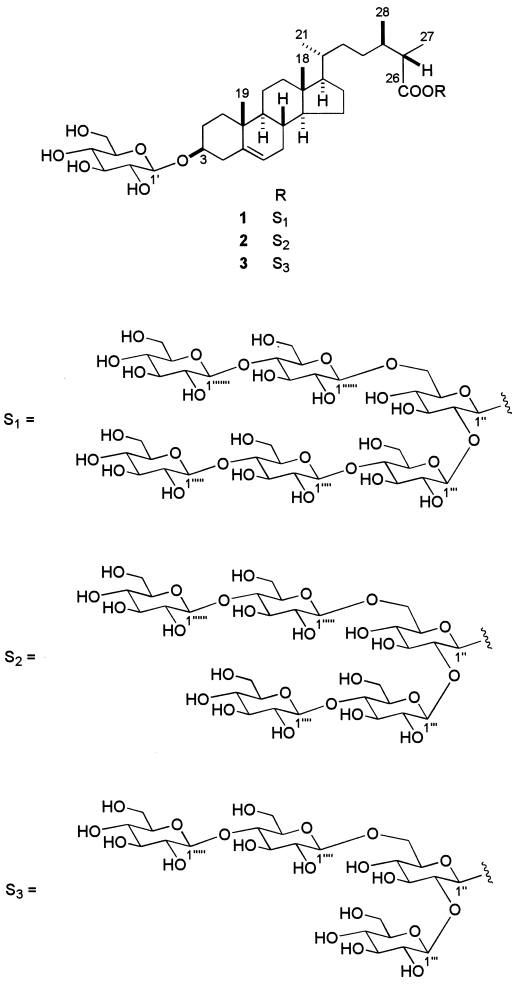
Tacca chantrieri ANDRÉ (Taccaceae) is a perennial plant that grows in southeastern China. Its rhizomes have been used in Chinese folk medicine for the treatment of gastric ulcer, enteritis, and hepatitis.[1)] Previously, we reported the isolation and structural characterization of diarylheptanoids, diarylheptanoid glucosides, and steroidal glycosides such as spirostan, furostan, pseudo-furostan, pregnane, and withanolide glycosides from the rhizomes of *T. chantrieri*, as well as their cytotoxic activities against cultured tumor and normal cells.[2—6)] Further phytochemical analysis of the MeOH extract of *T. chantrieri* rhizomes resulted in the isolation of three novel C_{28}-sterol oligoglucosides, named taccasterosides A—C (**1**—**3**). This communication deals with the structural determination of the new compounds in extensive spectroscopic studies, including 2D NMR data, and acid hydrolysis followed by chromatographic and spectroscopic analyses and further chemical transformations.

The MeOH extract (630 g) of *T. chantrieri* rhizomes (7.3 kg, dry weight) was passed through a Diaion HP-20 column, and the MeOH eluate portion (115 g) was subjected to column chromatography using silica gel and ODS silica gel, as well as to preparative HPLC, to yield taccasterosides A (**1**) (17 mg), B (**2**) (25 mg), and C (**3**) (13 mg).

Taccasteroside A (**1**)[7)] was obtained as an amorphous solid, $[\alpha]_D^{25}$ −10.0° (c=0.10, MeOH), with a molecular formula of $C_{70}H_{116}O_{38}$, which was deduced from ESI-TOF-MS, ^{13}C-NMR with DEPT spectra, and elemental analysis data. The ^{1}H-NMR spectrum showed three-proton singlet signals at δ 0.93 and 0.62, and three three-proton doublet signals at δ 1.23 (J=6.9 Hz), 0.97 (J=7.1 Hz), and 0.95 (J=6.7 Hz), which were recognized as typical steroid methyls. Signals for seven anomeric protons at δ 6.30 (d, J=8.4 Hz), 5.23 (d, J=7.7 Hz), 5.17 (d, J=7.7 Hz), 5.16 (d, J=8.1 Hz), 5.13 (d, J=8.0 Hz), 5.07 (d, J=7.7 Hz), and 4.93 (d, J=7.7 Hz) were also observed. The ^{13}C-NMR (δ 175.2) and IR (1739 cm^{-1}) spectra suggested the presence of an ester carbonyl group. Acid hydrolysis of **1** with 1 M HCl in dioxane–H_2O (1 : 1) gave a C_{28}-sterol as the aglycon (**1a**, $C_{28}H_{46}O_3$) and D-glucose.[8)] The above data, along with seven anomeric carbon signals at δ 106.1, 105.0, 105.0, 104.9, 104.6, 102.6, and 93.5, suggested that **1** is a C_{28}-sterol heptaglucoside.

The structure of **1a**, except for the absolute configurations of C-24 and C-25, was identified as 3β-hydroxyergost-5-en-26-oic acid by analysis of its ^{1}H-, ^{13}C-, and 2D NMR spectra.[9)] To determine the absolute configurations of the C-24 and C-25 chiral centers, as well as the other steroidal skeleton, the following chemical transformations and spectroscopic analysis were carried out with **1a**. Compound **1a** was reduced with $LiAlH_4$ to give ergost-5-ene-3β,26-diol (**1b**), which was then converted to the diastereomeric pairs of the C-26-(*R*)-MTPA (**1b-R**) and C-26-(*S*)-MTPA (**1b-S**) esters. In the ^{1}H-NMR spectra of **1b-R** and **1b-S**, the H_2-26 methylene protons of **1b-R** was observed as a d-like signal at δ 4.20 (J=6.3 Hz) whereas those of **1b-S** had a dd pattern at δ 4.30 (J=10.8, 6.6 Hz) and 4.09 (J=10.8, 7.2 Hz). Application of these spectral data to the empirical rule reported by Yasuhara *et al.*[10)] allowed us to assign the C-25 configuration as *S*. On the other hand, **1b** was treated with *p*-TsCl to give 26-*O*-tosylate (**1b-T**) of **1b**, which was then reduced with $LiAlH_4$, affording (24*R*)-ergost-5-en-3β-ol, that is, campesterol. Thus the structure of **1a** was determined to be (24*R*,25*S*)-3β-hydroxyergost-5-en-26-oic acid (Chart 1).

The exact structures of the sugar moieties and their linkage positions to the aglycon were resolved by detailed analysis of the 1D TOCSY and 2D NMR spectra. The ^{1}H-NMR subspectra of individual monosaccharide units were obtained



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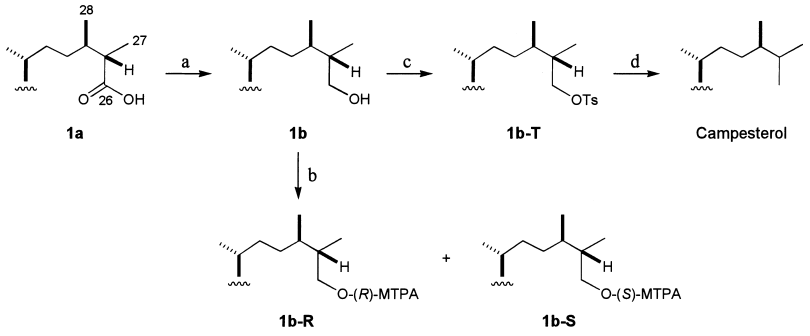

Reagents and conditions: a, $LiAlH_4$, THF, 0°C, 5 h; b, (*R*)-MTPA or (*S*)-MTPA, EDC·HCl, 4-DMAP, CH_2Cl_2, r.t., 12 h; c, *p*-TsCl, pyridine, r.t., 6 h; d, $LiAlH_4$, THF, 0°C, 5 h

Chart 1

Table 1. ^{1}H- and ^{13}C-NMR Data for the Glycoside Moieties of **1** in C_5D_5N at 303 K

Position	^{1}H	*J* (Hz)	^{13}C	Position	^{1}H	*J* (Hz)	^{13}C
1′	5.07 d	7.7	102.6	1‴′	5.13 d	8.0	105.0
2′	4.07 dd	8.4, 7.7	75.4	2‴′	4.06 dd	8.4, 8.0	74.7
3′	4.32 dd	8.4, 8.2	78.6	3‴′	4.20 dd	9.1, 8.4	78.1
4′	4.29 dd	8.2, 8.0	71.7	4‴′	4.17 dd	9.1, 8.2	71.5
5′	4.00 m		78.5	5‴′	4.00 m		78.5
6′ a	4.58 br d	11.6	62.9	6″ a	4.54 br d	11.7	62.4
b	4.43 dd	11.6, 4.0		b	4.29 dd	11.7, 4.7	
1″	6.30 d	8.4	93.5	1⁗	4.93 d	7.7	105.0
2″	4.12 dd	9.2, 8.4	82.9	2⁗	4.01 dd	9.0, 7.7	74.7
3″	4.31 dd	9.4, 9.2	77.9	3⁗	4.21 dd	9.4, 9.0	76.5
4″	4.28 dd	9.4, 9.4	70.3	4⁗	4.31 dd	9.4, 8.7	80.9
5″	4.03 m		77.7	5⁗	3.82 m		76.6
6″ a	4.63 br d	10.4	69.2	6⁗ a	4.53 br d	11.3	62.0
b	4.26 br d	10.4		b	4.41 br d	11.3	
1‴	5.23 d	7.7	106.1	1′′′′′	5.17 d	7.7	104.9
2‴	4.01 dd	8.9, 7.7	75.7	2′′′′′	4.10 dd	9.0, 7.7	74.7
3‴	4.21 dd	8.9, 8.8	76.3	3′′′′′	4.22 dd	9.0, 8.9	78.1
4‴	4.28 dd	8.8, 8.8	81.5	4′′′′′	4.20 dd	9.0, 8.9	71.4
5‴	3.92 m		76.7	5′′′′′	3.99 m		78.5
6‴	4.57 br d	11.3	62.5	6′′′′′ a	4.52 br d	11.4	62.4
	4.48 br d	11.3		b	4.31 dd	11.4, 3.7	
1⁗	5.16 d	8.1	104.6				
2⁗	4.07 dd	8.3, 8.1	74.7				
3⁗	4.23 dd	9.1, 8.3	76.5				
4⁗	4.27 dd	9.1, 8.4	80.9				
5⁗	3.98 m		76.5				
6⁗ a	4.48 br d	11.8	61.8				
b	4.31 dd	11.8, 3.7					

by using selective irradiation of easily identifiable anomeric proton signals, as well as irradiation of other nonoverlapping proton signals in a series of 1D TOCSY experiments.[11—13] Subsequent analysis of the ^{1}H–^{1}H COSY spectrum resulted in the sequential assignments of all the proton resonances due to the seven glucosyl units, including identification of their multiplet patterns and coupling constants, as shown in Table 1. The HSQC and HSQC-TCSY spectra correlated the proton resonances with those of the corresponding one-bond coupled carbons, leading to unambiguous assignments of the carbon shifts (Table 1). Comparison of the carbon chemical shifts thus assigned with those of reference methyl α-D- and β-D-glucosides,[14] taking into account the known effects of *O*-glycosylation, indicated that **1** contained three terminal β-D-glucopyranosyl moieties (Glc′, Glc⁗, Glc′′′′′), three C-4 substituted β-D-glucopyranosyl moieties (Glc‴, Glc⁗, Glc′′′′′), and a C-2 and C-6 disubstituted β-D-glucopyranosyl moiety (Glc″). The β-orientations of anomeric centers of all the glucosyl moieties were supported by the relatively large *J* values of their anomeric protons (7.7—8.4 Hz). In the HMBC spectrum, the anomeric proton of the terminal glucosyl unit (Glc′) at δ 5.07 exhibited a long-range correlation with C-3 of the aglycon at δ 78.2, indicating that one glucosyl unit was attached at the C-3 hydroxyl group of the aglycon. Consequently, an oligoglucoside composed of six glucosyl units was presumed to form an ester linkage with the C-26 carboxyl group. Further HMBC correlations from δ 6.30 (H-1 of Glc″) to C-26 of aglycon at δ 175.2, δ 5.23 (H-1 of Glc‴) to C-2 of Glc″ at δ 82.9, δ 5.17 (H-1 of Glc′′′′′) to C-4 of Glc⁗ at δ 80.9, δ 5.16 (H-1 of Glc⁗) to C-4 of Glc‴ at δ 81.5, δ 5.13 (H-1 of Glc⁗) to C-4 of Glc⁗ at δ 80.9, and δ 4.93 (H-1 of Glc′′′′′) to C-6 of Glc″ at δ 69.2

confirmed the hexaglucoside sequence to be Glc-(1→4)-Glc-(1→4)-Glc-(1→2)-[Glc-(1→4)-Glc-(1→6)]-Glc, which was attached at C-26 of the aglycon. All of these data were consistent with the structure (24*R*,25*S*)-3*β*-[(*β*-D-glucopyranosyl)oxy]ergost-5-en-26-oic acid *O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranosyl ester, which was assigned to **1**.

Taccasteroside B (**2**)[15] was analyzed for $C_{64}H_{106}O_{33}$ on the basis of HR-ESI-TOF-MS. The molecular formula of **2** was less than that of **1** by $C_6H_{10}O_5$, corresponding to the lack of one hexose unit. The ^{1}H-NMR spectrum of **2** showed signals for six anomeric protons at δ 6.29 (d, J=8.1 Hz), 5.24 (d, J=7.8 Hz), 5.18 (d, J=7.1 Hz), 5.16 (d, J=7.1 Hz), 5.06 (d, J=7.7 Hz), and 4.92 (d, J=7.8 Hz), along with the signals for five steroid methyl groups at δ 1.23 (d, J=7.0 Hz), 0.98 (d, J=7.0 Hz), 0.95 (d, J=6.5 Hz), 0.93 (s), and 0.62 (s). Acid hydrolysis of **2** with 1 M HCl in dioxane–H_2O (1 : 1) resulted in the production of **1a** and D-glucose. In the ^{13}C-NMR spectrum of **2**, the signals due to C-3 and C-26 of the aglycon residue were observed at δ 78.2 and 175.2, respectively, indicating that the sugar linkages were both at C-3 and C-26, as in **1**. Using the same procedures as described for **1**, all the ^{13}C-NMR signals for the sugar moieties were assigned to three terminal *β*-D-glucopyranosyl units (Glc′, Glc‴, Glcp⁗), two C-4 substituted *β*-D-glucopyranosyl units (Glc‴, Glc⁗), and a C-2 and C-6 disubstituted *β*-D-glucopyranosyl unit (Glc″). In the HMBC spectrum of **2**, long-range correlations were observed from δ 6.29 (H-1 of Glc″) to C-26 of aglycon at δ 175.2, δ 5.24 (H-1 of Glc‴) to C-2 of Glc″ at δ 82.8, δ 5.18 (H-1 of Glc⁗) to C-4 of Glc‴ at δ 81.8, δ 5.16 (H-1 of Glc⁗′) to C-4 of Glc⁗ at δ 81.0, δ 5.06 (H-1 of Glc′) to C-3 of the aglycon at δ 78.2, and δ 4.92 (H-1 of Glc⁗) to C-6 of Glc″ at δ 69.2. Thus the structure of **2** was established to be (24*R*,25*S*)-3*β*-[(*β*-D-glucopyranosyl)oxy]ergost-5-en-26-oic acid *O*-*β*-D-glucopyranosyl-(1→4)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranosyl ester.

The ^{1}H-NMR spectrum of taccasteroside C (**3**) ($C_{58}H_{96}O_{28}$)[16] showed five anomeric proton signals at δ 6.32 (d, J=8.0 Hz), 5.30 (d, J=7.8 Hz), 5.18 (d, J=7.8 Hz), 5.07 (d, J=7.7 Hz), and 4.93 (d, J=7.8 Hz), along with five steroid methyl signals at δ 1.26 (d, J=7.0 Hz), 0.98 (d, J=6.7 Hz), 0.95 (d, J=6.4 Hz), 0.92 (s), and 0.61 (s). Acid hydrolysis of **3** with 1 M HCl afforded **1a** and D-glucose. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **3** with those of **1** and **2** indicated that one *β*-D-glucopyranosyl unit (Glc′) was linked to C-3 of the aglycon and the sugar chain attached at C-26 was made up of two terminal *β*-D-glucopyranosyl moieties (Glc‴, Glc⁗), a C-4 substituted *β*-D-glucopyranosyl unit (Glc⁗), and a C-2 and C-6 disubstituted *β*-D-glucopyranosyl unit (Glc″). In the HMBC spectrum of **3**, long-range correlations were observed from δ 6.32 (H-1 of Glc″) to C-26 of aglycon at δ 175.3, δ 5.30 (H-1 of Glc‴) to C-2 of Glc″ at δ 82.5, δ 5.18 (H-1 of Glc⁗) to C-4 of Glc⁗ at δ 80.9, and δ 4.93 (H-1 of Glc⁗) to C-6 of Glc″ at δ 69.2. The structure of **3** was characterized as (24*R*,25*S*)-3*β*-[(*β*-D-glucopyranosyl)-oxy]ergost-5-en-26-oic acid *O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranosyl ester.

Taccasterosides A—C (**1**—**3**) are novel bisdesmosideic oligoglucosides of (24*R*,25*S*)-3*β*-hydroxyergost-5-en-26-oic acid. Phytosterols and their monoglucosides such as campesterol, stigmasterol, and *β*-sitosterol, and their 3-*O*-glucoside, widely occur in the plant kingdom. However, compounds **1**—**3** are believed to be the first representatives of oligoglucosides of a phytosterol derivative, which have sugar moieties with a total of five to seven glucose units.

References and Notes

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7) Data for **1**. ESI-TOF-MS (positive mode) *m/z*: 1587 $[M+Na]^+$. *Anal.* Calcd for $C_{70}H_{116}O_{38}\cdot 4H_2O$: C, 51.34; H, 7.63. Found: C, 51.20; H, 7.93. ^{13}C-NMR for the aglycon moiety (C_5D_5N) δ: 37.5, 30.3, 78.2, 39.3, 140.9, 122.0, 32.2, 32.0, 50.3, 36.9, 21.3, 39.9, 42.4, 56.8, 24.5, 28.5, 56.1, 12.0, 19.4, 36.0, 19.0, 33.8, 31.4, 35.4, 44.1, 175.2, 11.5, 15.5 (C-1—C-28).
8) D-Glucose was identified by direct HPLC analysis of the hydrolysate, which was performed on an aminopropyl-bonded silica gel column, with detection being carried out using a combination of refractive index (RI) and optical rotation (OR) detectors.
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15) Data for **2**. $[\alpha]_D^{25}$ −10.0° (c=0.10, MeOH). HR-ESI-TOF-MS (positive mode) *m/z*: 1425.6469 $[M+Na]^+$ (Calcd for $C_{64}H_{106}O_{33}Na$, 1425.6514). IR (film) cm^{-1}: 3270 (OH), 2933 (CH), 1740 (C=O), 1072. ^{13}C-NMR for the sugar moieties (C_5D_5N) δ: 102.6, 75.3, 78.6, 71.7, 78.4, 62.9 (C-1′—C-6′), 93.5, 82.8, 77.8, 70.3, 77.7, 69.2 (C-1″—C-6″), 106.0, 75.7, 76.3, 81.8, 76.7, 62.4 (C-1‴—C-6‴), 105.1, 74.8, 78.3, 71.5, 78.5, 62.4 (C-1⁗—C-6⁗), 105.6, 74.6, 76.6, 81.0, 76.5, 61.9 (C-1⁗′—C-6⁗′), 104.9, 74.7, 78.1, 71.4, 78.5, 62.4 (C-1⁗″—C-6⁗″).
16) Data for **3**. $[\alpha]_D^{25}$ −8.0° (c=0.10, MeOH). HR-ESI-TOF-MS (positive mode) *m/z*: 1241.6163 $[M+H]^+$ (Calcd for $C_{58}H_{97}O_{28}$, 1241.6166). IR (film) cm^{-1}: 3129 (OH), 2869 (CH), 1745 (C=O), 1075. ^{13}C-NMR for the sugar moieties (C_5D_5N) δ: 102.5, 75.3, 78.6, 71.7, 78.4, 62.9 (C-1′—C-6′), 93.7, 82.5, 77.8, 70.3, 77.7, 69.2 (C-1″—C-6″), 106.4, 76.3, 78.2, 71.6, 78.8, 62.4 (C-1‴—C-6‴), 105.0, 74.6, 76.6, 80.9, 76.5, 61.9 (C-1⁗—C-6⁗), 104.9, 74.7, 78.1, 71.4, 78.5, 62.8 (C-1⁗′—C-6⁗′).