Concinnasteoside A, a New Bisdesmosidic Cholestane Glycoside from the Stems of *Dracaena concinna*

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A new bisdesmosidic cholestane glycoside, designated as concinnasteoside A, was isolated from the fresh stems of *Dracaena concinna*. The structure was determined to be $1\beta,3\beta,16\beta$-trihydroxy-5α-cholesta-7,23-dione 1-*O*-α-L-rhamnopyranosyl 16-*O*-β-D-glucopyranoside on the basis of spectroscopic analysis, including 2D NMR techniques. Concinnasteoside A is believed to be the first example of a cholestane glycoside from a plant of the genus *Dracaena*.

The family Agavaceae, with more than 480 species, has a distribution in the tropic and subtropic dry climate regions throughout the world. The occurrence of steroidal saponins in several Agavaceae, especially those belonging to the representative genera *Agave* and *Yucca,* is well documented.[1,2] Previously, we have analyzed the steroidal constituents of the three Agavaceae plants, *Nolina recurvata*,[3–5] *Sansevieria trifasciata*,[6,7] and *Cordyline stricta*,[8,9] and isolated a variety of steroidal glycosides, such as spirostanol saponins, furostanol saponins, pregnane glycosides, and cholestane glycosides, among which a trisdesmosidic steroidal saponin, recurvoside E,[3] from *N. recurvata* is unique in structure, having a fructose as the carbohydrate component.

As part of our chemical investigation on Agavaceae plants, we have now examined the fresh stems of *Dracaena concinna* Kunth, which is native to Mauritius and cultivated as an excellent foliage plant. This research led to the isolation of a new bisdesmosidic cholestane glycoside (**1**), named concinnasteoside A. This paper reports the structural assignment of **1** on the basis of spectroscopic analysis, including 2D NMR techniques, and acid-catalyzed hydrolysis.

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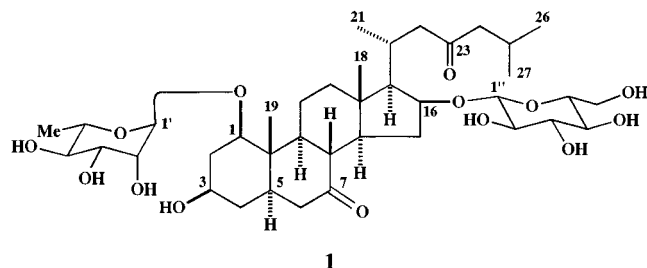
The concentrated *n*-BuOH-soluble phase of the MeOH extract of *D. concinna* stems was subjected to Diaion HP-20, Si gel, and octadecylsilanized (ODS) Si gel column chromatography to yield concinnasteoside A (**1**) (30.8 mg).

Compound **1** ($C_{39}H_{64}O_{14}$, positive-ion FABMS *m/z* 779 [M + Na]$^+$, negative-ion FABMS *m/z* 755 [M − H]$^-$) was obtained as an amorphous solid, $[\alpha]_D$ −15.2° (MeOH). Preliminary inspection of the ^{1}H-NMR data for **1** in pyridine-d_5 showed signals attributable to two tertiary methyl groups at δ 1.17 and 0.88 (each 3H, s); three

secondary methyl groups at δ 1.00 (3H, d, $J = 6.5$ Hz), 0.91 (3H, d, $J = 6.6$ Hz), and 0.90 (3H, d, $J = 6.7$ Hz), indicating a sterol structure for the aglycon; and two anomeric protons at δ 5.59 (1H, d, $J = 1.9$ Hz) and 4.69 (1H, d, $J = 7.7$ Hz). A signal at δ 1.68 (3H, d, $J = 6.1$ Hz) was ascribable to the methyl group of 6-deoxyhexopyranose. The presence of two carbonyl groups in **1** was shown by the ^{13}C-NMR signals at δ 211.5 and 210.6 (quaternaries by DEPT 135° spectrum), and the IR absorption at ν_{max} 1695 cm^{-1}. Acid hydrolysis of **1** with 1 M HCl in dioxane−H$_2$O (1:1) gave D-glucose and L-rhamnose in a ratio of 1:1. The ^{13}C-NMR data for **1** showed a total of 39 resonance lines, 12 of which could be assigned to a glucose and a rhamnose unit, which anomeric carbons were observed at δ 106.8 and 98.5. This implied a $C_{27}H_{44}O_5$ composition for the aglycon portion, possessing six degrees of unsaturation, two of which were due to two carbonyl groups. Consequently, the aglycon of **1** was assumed to have the usual C_{27} steroid skeleton with a four-ring system. The ^{13}C-NMR spectrum showed three CHOH functions together with two carbonyl groups which accounted for all the oxygen atoms of the molecule. The presence of the carbinol groups at C-1, C-3, and C-16 of the steroid nucleus was suggested by the resonances at δ 80.5 (C-1), 67.0 (C-3), and 82.4 (C-16) in comparison with literature data.[10] Strongly deshielded signals for C-6 (δ 45.5) and C-8 (δ 50.0), as well as C-22 (δ 50.1) and C-24 (δ 52.4), suggested the location of two carbonyl groups at C-7 and C-23. These data were confirmed by detailed interpretation of the ^{1}H−^{1}H COSY spectrum combined with the HOHAHA data, together with one-bond ^{1}H−^{13}C connectivities through the HMQC spectrum, which were measured in a mixed solvent of pyridine-d_5 and MeOH-d_4 (11:1) to remove signals due to exchangeable protons and to minimize signal overlap. These allowed assignment of all the ^{1}H- and ^{13}C-NMR signals arising from the aglycon moiety, as shown in Table 1. Analysis of the HMBC spectrum optimized for an $^nJ_{C,H}$ parameter of 8 Hz led to a complete structure assignment. The quaternary carbon signal observed at δ 42.4 showing $^2J_{C,H}$ and $^3J_{C,H}$ correlation peaks with the proton signals at δ 0.89 (3H, s, Me-18), 3.29 (H-15α), and 1.21 (dd, $J = 11.0, 7.9$ Hz, H-17) was assigned to C-13. Another quaternary carbon signal at δ 42.2 was assignable to C-10, because it was correlated to δ 1.18 (3H, s, Me-19), 2.09 (dd, $J = 12.2, 3.0$ Hz, H-6eq), and 1.47 (ddd, $J = 12.5, 11.1, 4.7$ Hz, H-9). The correlation peaks between signal at δ 211.0 (C=O) and each of the proton

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Table 1. ^{1}H- and ^{13}C-NMR Chemical Shift Assignment of **1**[a]

position	^{1}H		J (Hz)	^{13}C
1	3.72	dd	11.4, 4.1	80.8
2 eq	2.80	ddd	12.2, 4.5, 4.1	37.3
2 ax	1.75	ddd	12.2, 12.2, 11.4	
3	3.83	m	20.4[b]	67.2
4	1.71 (2H)			39.3
5	1.55			45.2
6 eq	2.09	dd	12.2, 3.0	45.7
6 ax	2.49	dd	12.2, 12.2	
7				211.0
8	2.37	dd	11.1, 11.1	50.3
9	1.47	ddd	12.5, 11.1, 4.7	56.7
10				42.2
11 eq	2.74			24.9
11 ax	1.69			
12 eq	2.04	br d	12.7	39.8
12 ax	1.29			
13				42.4
14	1.44			47.9
15 α	3.29			37.4
15 β	1.77			
16	4.30	ddd	7.9, 7.9, 4.9	82.6
17	1.21	dd	11.0, 7.9	60.7
18	0.89	s		13.9
19	1.18	s		7.8
20	2.86			27.5
21	1.01	d	6.6	19.5
22a	3.18	br d	14.9	50.2
22b	2.33	dd	14.9, 10.3	
23				211.9
24a	2.50	dd	16.5, 6.7	52.6
24b	2.46	dd	16.5, 6.8	
25	2.27	m		24.7
26	0.93	d	6.3	22.9
27	0.91	d	6.3	22.9
1′	5.56	d	1.4	98.7
2′	4.47	dd	3.1, 1.4	73.2
3′	4.41	dd	9.3, 3.1	72.8
4′	4.25	dd	9.3, 9.3	73.7
5′	4.13	dq	9.3, 6.1	71.4
6′	1.68	d	6.1	18.8
1″	4.68	d	7.8	107.0
2″	3.93	dd	9.2, 7.8	75.6
3″	4.12	dd	9.2, 9.2	78.8
4″	4.23	dd	9.2, 9.2	71.6
5″	3.85	ddd	9.2, 4.8, 2.0	78.3
6″a	4.41	dd	11.8, 2.0	62.8
6″b	4.32	dd	11.8, 4.8	

[a] Spectra were measured in a mixed solvent of pyridine-d_5 and MeOH-d_4 (11:1). Assignments were established by interpretation of the ^{1}H$-^1$H COSY, HOHAHA, and HMQC spectra. [b] $W_{1/2}$.

signals at δ 2.09 (H-6eq) and 2.37 (dd, J = 11.1, 11.1 Hz, H-8) and between signal at δ 211.9 (C=O) and signals at δ 3.18 (br d, J = 14.9 Hz, H-22a) and 2.50 (dd, J = 16.5, 6.7 Hz, H-24a) allowed the locations of the carbonyl groups to C-7 and C-23. Thus, the aglycon of **1** was shown to be a 1,3,16-trihydroxy-7,23-dioxo-cholestane derivative (Figure 1).

The usual steroidal ring junctions, A/B trans, B/C trans, and C/D trans were ascertained by the ^{13}C-NMR resonance of C-19 (δ 7.8),[11] and NOE correlations observed between H-8 and H-15β (δ 1.77), H-8 and Me-18, and H-8 and Me-19. The C-1β and C-3β orientations of the oxygen atoms were confirmed by the ^{1}H-NMR coupling constant of H-1 and H-3 protons ($^3J_{H-1\ (δ\ 3.72),\ H-2ax\ (δ\ 1.75)}$ = 11.4 Hz and $^3J_{H-1,\ H-2eq\ (δ\ 2.80)}$ = 4.1 Hz, and $W_{1/2\ (H-3,\ δ\ 3.83)}$ = 20.4 Hz), and supported by NOEs observed between H-1 and H-9, and H-3 and H-5 (δ 1.55). The β-configurations of C-16 carbinol group and of C-17, which attached the side chain, were verified by NOEs observed between H-14 (δ 1.44) and H-16 (δ

4.30, ddd, J = 7.9, 7.9, 4.9 Hz), H-15α and H-16, and H-16 and H-17 (Figure 2). The stereochemistry at C-20 was examined by using molecular modeling, $^3J_{H,H}$ value, and NOESY data. A combination of molecular mechanics (MM) and molecular dynamics (MD) calculations in the MM2 force field as implemented in Macro-model 5.5 was performed on two possible compounds with C-20*S* and C-20*R* configurations. The $H_{17}-C_{17}-C_{20}-H_{20}$ torsion angle was almost identical between the minimum energy conformers of C-20*S* and C-20*R* models; −175.6° for C-20*S* and −177.8° for C-20*R*. The experimental J value (11.0 Hz) of H-17/H-20 almost corresponded to that (12.3 Hz) calculated through the application of the given dihedral angle to the advanced Karplus-type equation proposed by Altona et al.[12] Because the above data indicated that H-20 lay toward Me-18 and that the C-17/C-20 bond was frozen, NOE correlations observed between Me-18 and H-20 (δ 2.86) and H-16 and H-22a made it possible to assign the C-20 configuration as *S*.

Assignment of the ^{1}H$-$ and ^{13}C-NMR signals arising from the saccharide part of **1**, which was composed of a D-glucose and an L-rhamnose, was performed by the ^{1}H$-^1$H COSY and HOHAHA spectra combined with the HMQC data, indicating that each monosaccharide was directly attached to the aglycon and was not substituted. The respective linkage positions of β-D-glucopyranose ($^4C_1$; δ 4.68, d, J = 7.8 Hz) and α-L-rhamnopyranose ($^1C_4$; δ 5.56, d, J = 1.4 Hz) were revealed to be, respectively, at C-16 and C-1 of the aglycon by observing three-bond C,H correlations of each anomeric proton signal with the respective carbon signal of the aglycon [δ$_H$ 4.68 to δ$_C$ 82.6 (C-16) and δ$_H$ 5.56 to 80.8 (C-1)]. All of these data were consistent with the structure 1β,3β,16β-trihydroxy-5α-cholesta-7,23-dione 1-*O*-α-L-rhamnopyranosyl 16-*O*-β-D-glucopyranoside for compound **1**.

Concinnasteoside A (**1**) is a new naturally occurring cholestane bisdesmoside and, to the best of our knowledge, is the first example of a cholestane glycoside from a plant of the genus *Dracaena*.

Experimental Section

General Experimental Procedures. Optical rotation was measured using a JASCO DIP-360 automatic digital polarimeter. IR spectrum was recorded on a Hitachi 260-30 spectrophotometer and MS on a VG AutoSpec E instrument. 1D NMR spectra were recorded on a Bruker AM-400 spectrometer (400 MHz for ^{1}H-NMR), and 2D NMR, on a Bruker AM-500 (500 MHz for ^{1}H-NMR) using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as internal standard. HPLC was performed using a Tosoh HPLC system comprised of a CCPM pump, a CCP controller PX-8010, a UV-8000 detector, and Rheodyne injection port with a 20-μL sample loop. A TSK-gel ODS-Prep column (Tosoh, 4.6 mm i.d. × 250 mm, ODS, 5 μm) was used for HPLC analysis. Computer calculations were performed using the molecular-modeling software Macro-model 5.5 on a Silicon Graphics work station, COMTEC 4D/O2 10000SC.

Plant Material. *D. concinna* was purchased from Exotic Plants Co., Ltd., Japan, and the plant specimen is on file in our laboratory.

Extraction and isolation. The plant material (stems, fresh wt 11.5 kg) was extracted with hot MeOH
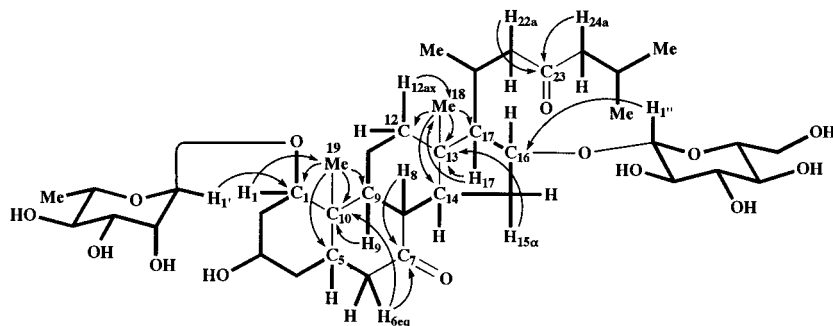
Figure 1. HMBC correlations of **1**.

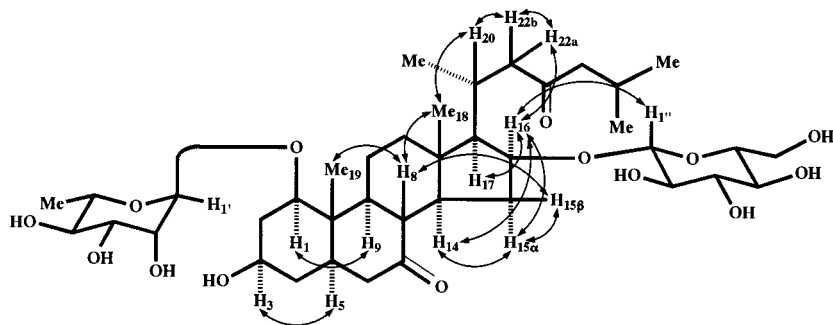**Figure 2.** NOE correlations of **1**.

(20 L × 2). The MeOH extract was concentrated under reduced pressure, and the viscous concentrate (900 g) was partitioned between H_2O and *n*-BuOH. The *n*-BuOH-soluble phase (420 g) was passed through a Diaion HP-20 column with increasing amounts of MeOH in H_2O. The 80% MeOH and MeOH eluate fractions were combined and chromatographed on Si gel eluting with a stepwise gradient mixture of $CHCl_3$–MeOH system (9:1; 4:1; 2:1) and, finally, with MeOH. Fractions with the same TLC profile were combined. Five fractions (I–V) were recovered. Fraction IV was subjected to column chromatography on Si gel eluting with $CHCl_3$–MeOH (6:1; 3:1) and ODS Si gel with MeOH–H_2O (3:2) to give **1** (30.8 mg).

Concinnasteoside A (1): amorphous solid; $[\alpha]^{29}_D$ −15.2° (*c* 0.11, MeOH); IR (KBr) ν_{max} 3400 (OH), 2920 (CH), 1695 (C=O), 1450, 1365, 1255, 1195, 1155, 1060, 1020, 980, 900, 830, 800 cm^{-1}; ^{1}H-NMR (C_5D_5N) δ 5.59 (1H, d, *J* = 1.9 Hz, H-1′), 4.69 (1H, d, *J* = 7.7 Hz, H-1″), 3.72 (1H, dd, *J* = 11.4, 4.1 Hz, H-1), 1.68 (3H, d, *J* = 6.1 Hz, Me-6′), 1.17 (3H, s, Me-19), 1.00 (3H, d, *J* = 6.5 Hz, Me-21), 0.91 (3H, d, *J* = 6.6 Hz, Me-26 or Me-27), 0.90 (3H, d, *J* = 6.7 Hz, Me-26 or Me-27), 0.88 (3H, s, Me-18); ^{13}C-NMR (C_5D_5N) δ 80.5, 37.2, 67.0, 39.3, 45.0, 45.5, 210.6, 50.0, 56.5, 42.1, 24.7, 39.6, 42.2, 47.7, 37.2, 82.4, 60.5, 13.7, 7.6, 27.3, 19.4, 50.1, 211.5, 52.4, 24.5, and 22.7 × 2 (C-1–C-27), 98.5, 73.1, 72.8, 73.6, 71.2, and 18.7 (C-1′–C-6′), and 106.8, 75.6, 78.7, 71.5, 78.1, and 62.7 (C-1″–C-6″); positive-ion FABMS *m/z* 779 [M + Na]$^+$, negative-ion FABMS *m/z* 755 [M − H]$^-$.

Acid Hydrolysis of 1. A solution of **1** (10 mg) in 1 M HCl (dioxane–H_2O, 1:1, 3 mL) was heated at 100 °C for 1 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo) column and fractionated using a Sep-Pak C_{18} cartridge (Waters), eluting with H_2O (5 mL) followed by MeOH (5 mL), to give a sugar fraction (3.2 mg) and an aglycon fraction (6.4 mg).

TLC analysis of the aglycon fraction showed that it contained several unidentified artifactual sapogenols. The monosaccharide mixture was suggested to be composed of glucose and rhamnose by direct TLC comparison with authentic samples; R_f (*n*-BuOH–Me$_2$-CO–H_2O, 4:5:1): 0.63 (rhamnose); 0.38 (glucose). The mixture (2 mg) was dissolved in H_2O (1 mL), to which (−)-α-methylbenzylamine (5 mg) and Na[BH$_3$CN] (8 mg) in EtOH (1 mL) were added. After being set aside at 40 °C for 4 h followed by addition of AcOH (0.2 mL) and evaporation to dryness, the reaction mixture was acetylated with Ac$_2$O (0.3 mL) in pyridine (0.3 mL) at room temperature for 12 h. The crude mixture was passed through a Sep-Pak C_{18} cartridge with H_2O–MeCN (4:1; 1:1; 1:9, each 5 mL) mixtures as solvents. The H_2O–MeCN (1:9) eluate was further passed through a Toyopak IC–SP M cartridge (Tosoh) with EtOH (10 mL) to give a mixture of the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides,[13,14] which was then analyzed by HPLC under the following conditions: solvent, MeCN–H_2O (2:3); flow rate, 0.8 mL min^{-1}; detection, UV 230 nm. The derivatives of D-glucose and L-rhamnose were detected; t_R (min), 24.15 (derivative of D-glucose); 27.17 (derivative of L-rhamnose).

Conformational Analysis. 5800-Step systematic Monte Carlo conformation searches were carried out with the MM2 force field as implemented in Macromodel 5.5 to predict the fully optimized lowest energy structure.[15] Energies were minimized with the PR conjugate gradient minimizer, and convergence was obtained when the gradient root-mean-square was less than 0.001 kJÅ^{-1}M. The MD simulations were carried out with Macro-model beginning with the lowest energy structures obtained by the Monte Carlo conformation search. The following options were used in the MD calculations: time step, 0.001 ps; equilibration time period, 100 ps; and production run time period, 1000

ps. Initial kinetic energy was added to all atoms as random velocities. Translational and rotational momentum was reset to zero every 0.1 ps. To maintain a constant temperature, the system was coupled to an external temperature bath set at 300 K. Coupling between bath and molecule was updated every 0.2 ps. In the production run time at 300 K the conformers were sampled every 5 ps, followed by energy minimizations using the MM2 force field.

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