Bambusicolaside I and II, Two New Glucosides from the Aphid *Pseudoregma bambusicola*

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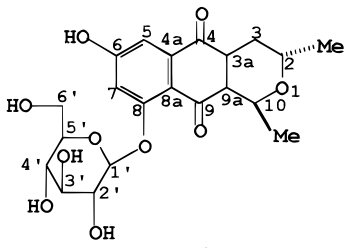
The structures of bambusicolaside I (**1**) and bambusicolaside II (**2**), two new glucosides with novel aglycons from the aphid *Pseudoregma bambusicola* T., were established by spectral data interpretation. The structure of **2** was confirmed by X-ray crystallographic analysis.

A number of aphids have been chemically investigated concerning their pheromone constituents.[1] Past studies have also dealt with triglycerides from different aphids and their different seasonal forms,[2] selective accumulation of carotenoids in 19 species of aphids,[3] and diterpenoid alkaloid production in *Brachycaudus aconiti* Mordv.[4] The aphid *Pseudoregma babusicola* T. (Aphididae),[5] distributed in Sichuan Province, is used in folk medicine directly, or in the form of an alcoholic extract in some areas of China, as a remedy for coughs caused by various pathogens and topically used for hemiplegia.[6] This study of an enthanol extract of *P. bambusicola* led to the isolation of two new glucosides, bambusicolasides I (**1**) and II (**2**), which possess novel aglycons with pyrano[*c*]naphthoquinone and pyrano[*c*]naphthalene skeletons, respectively. Their structures were determined predominantly on the basis of spectral data. The structure and configurations of C-2 and C-10 of **2** were confirmed by X-ray crystallographic analysis. Glucosides of pyrano[*c*]naphthoquinone and pyrano[*c*]naphthalene or of the dimer of pyrano[*c*]naphthoquinone and pyrano[*c*]naphthalene were identified in *Aphis nerii*,[7] *Tuberolachnus salignus*,[8] and in the genera *Dysaphis* and *Dactynotus*.[9] The hosts of *Aphis nerii* and *Tuberolachnus salignus* are oleander and willow, respectively. However, the host of *P. bambusicola* is bamboo. There are no reports about the characterization of pyrano[*c*]-naphthoquinone and pyrano[*c*]naphthalene or their dimers in oleander, willow, and bamboo. Therefore, it could not be concluded that the aglycons of **1** and **2** were from the plants the aphids used as food sources.

Bambusicolaside I (**1**) was isolated as a yellow amorphous powder. The presence of a β-D-glucopyranosyl linkage was indicated by the ^{1}H NMR signals at δ 4.93 (J = 7 Hz) and ^{13}C NMR signals at δ 101.14 (d), 73.06 (d), 77.11 (d), 69.45 (d), 76.10 (d), and 65.54 (t) as well as the detection of D-glucose in the aqueous solution resulting after acidic hydrolysis. The UV spectrum showed typical absorptions for a substituted 1,4-naphthoquinone,[10] with a λ_{max} 213, 270, 290 (sh), 398 nm, which is in agreement with the IR absorptions at 1650, 1631 cm^{-1} and the presence of 10 ^{13}C NMR signals above 102 ppm (Table 1), two of which correspond to carbonyl groups at δ 183.16 and 180.34. The molecular formula $C_{21}H_{24}O_{10}$ was postulated from the ion peak at m/z 439 ([M + 3H]$^+$) in FABMS (positive) and 437.1527 ([M + H]$^-$) in HRFABMS (negative), considering the reduction process normally associated with FABMS of quinones.[11,12] ^{1}H NMR signals at δ1.23 (d, 3H, J = 6 Hz, CH_3-10) and 4.08 (q, 1H, J = 6 Hz, H-10) provided the moiety −CH(CH_3)O− (^{13}C NMR 66.17 for CH and 20.94 for −CH_3). Another moiety −CH_2CH(CH_3)O− was supported by ^{1}H NMR signals at δ 2.06 (dd, 1H, J = 18, 3 Hz, H-3α), 2.58 (dd, 1H, J = 18, 8 Hz, H-3β) and 3.94 (m, 1H, H-2) as well as a signal at δ 1.41 (d, 3H, J = 7 Hz, CH_3-2). In view of the correlations between H-5 at δ 6.95 (d, 1H, J = 2 Hz) and C-4 at δ 180.34, C-4 and H-3α at δ 2.06 in HMBC, the moieties −CH_2CH(CH_3)O− and −CH(CH_3)O− could be attached to C-3a and C-9a, respectively. Therefore, the signal at δ 7.10 (d, 1H, J = 2 Hz) should be assigned to H-7. One proton appearing at δ 10.83 (br, D_2O exchangeable) was assigned to an OH proton at C-6 on the basis of the NOE effects between this proton and H-5 and between this proton and H-7. The fact that UV absorptions are not changed upon addition of $AlCl_3$ and then diluted HCl suggested that there was no intramolecular hydrogen bond between the carbonyl group and aromatic hydroxyl group. Thus, the β-glucopyranosyl moiety is attached to C-8, which was further confirmed by HMBC correlations between H-7 and C-8 at δ 159.52 and between H-1′ at δ 4.93 (d, 1H, J = 7 Hz) and C-8. The ^{13}C NMR assignments for C-3a, C-4a, C-8a, and C-9a were based on C,H-COSY and HMBC experiments. No NOE effect was observed between CH_3-2 and CH_3-10 consistent with the trans relative stereochemistry assigned to C-2 and C-10.



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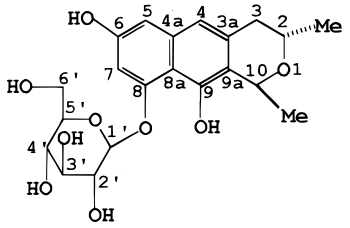

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Table 1. ^{1}H and ^{13}C NMR Shift Values and Long-Range C−H Connectivities in Compounds **1** and **2** Established by HMBC (DMSO-d_6 for **1** and CD_3OD for **2**)

	compound					
	1			**2**		
position	δ_H (mult, J)[a]	δ_C (multi)[b,c]	$^2J_{CH}$, $^3J_{CH}$	δ_H (multi, J)[a]	δ_C (multi)[b,c]	$^2J_{CH}$, $^3J_{CH}$
2	3.94 (m)	61.64 (d)	C-3a	4.08 (m)	64.39 (d)	
3	2.06 (dd, 8, 3) 2.58 (dd, 8, 8)	28.79 (t)	C-4	2.70 (dd, 16, 3) 2.54 (dd, 16, 11)	37.09 (t)	C-3a
3a		134.64 (s)			134.66 (s)	
4		180.34 (s)		6.78 (s)	117.28 (d)	C-8a, C-9a
4a		138.24 (s)			137.41 (s)	
5	6.95 (d, 2)	108.21 (d)	C-4, C-7, C-6, C-8a	6.67 (d, 2)	105.03 (d)	C-4, C-6, C-7, C-8a
6		162.77 (s)			156.57 (s)	
7	7.10 (d, 2)	107.43 (d)	C-5, C-6, C-8, C-8a	6.90 (d, 2)	103.73 (d)	C-5, C-6, C-8, C-8a
8		159.52 (s)			155.81 (s)	
8a		112.80 (s)			110.14 (s)	
9		183.16 (s)			149.81 (s)	
9a		147.15 (s)			119.27 (s)	
10	4.08 (q, 6)	66.17 (d)	C-3a, C-9a, Me-10	5.20 (q, 6)	70.11 (d)	C-2, C-3a, C-9a
Me-2	1.41 (d, 3H, 7)	19.30 (q)	C-2	1.25 (d, 3H, 6)	22.03 (q)	C-3
Me-10	1.23 (d, 3H, 7)	20.94 (q)	C-10	1.53 (d, 3H, 6)	19.64 (q)	C-10, C-9a
1′	4.93 (d, 7)	101.14 (d)	C-8	5.08 (d, 8)	104.05 (d)	C-8
2′	3.20−3.60 (m, 6H), 3.80 (br, 4H, −OH)	73.06 (d)		3.20−3.57 (m, 6H)	73.06 (d)	
3′		77.11 (d)			77.11 (d)	
4′		69.45 (d)			71.10 (d)	
5′		76.10 (d)			78.11 (d)	
6′		60.54 (t)			62.36 (t)	
HO-6	10.83 (br)					

[a] 1H, unless noted. J values in Hz. [b] Multiplicities were determined by DEPT. [c] Assignments are based on C,H-COSY and HMBC.

Bambusicolaside II (**2**) was crystallized from MeOH and gave colorless cubic crystals. The molecular formula $C_{21}H_{26}O_9$ was established from the ion peak at m/z 423.1623 ($[M + H]^+$) in HRFABMS (positive). Hydrolysis of **2** in dilute HCl led to the identification of D-glucose. An ^{1}H NMR signal at δ 5.08 (d, J = 7 Hz) and a ^{13}C NMR signal (Table 1) at δ 104.05 (d) revealed the presence of a β-D-glucopyranosyl unit in this molecule. Three ^{1}H NMR signals at δ 6.78 (s, 1H, H-4), 6.67 (d, 1H, J = 2 Hz, H-5), and 6.90 (d, 1H, J = 2 Hz, H-7) indicating aromatic protons were observed. Two moieties, $-CH(CH_3)O-$ (A) and $-CH_2CH(CH_3)O-$ (B), were recognized from two groups of signals in ^{1}H NMR: δ 1.53 (d, 3H, J = 6 Hz, CH_3-10) and 5.20 (q, 1H, J = 6 Hz, H-10); 2.70 (dd, 1H, J = 16, 3 Hz, H-3α), 2.54 (dd, 1H, J = 16, 11 Hz, H-3β), and 4.08 (m, 1H, H-2) as well as 1.25 (d, 3H, J = 6 Hz, CH_3-2). The moieties A and B could be connected at the C-terminus to C-9 and C-3a, respectively, from HMBC correlations, with a doublet signal at δ 6.67 (H-5) correlated with the signal at δ 117.28 (C-4) suggesting that another doublet signal, at δ 6.90, could be assigned to H-7. Cross-peak correlations between H-1′ and C-8 at δ 155.81 and H-7 and C-8, as well as enhancement of the signal for H-7 when H-1′ was irradiated suggested that the β-D-glucopyranosyl moiety was attached to C-8. There was no NOE effect between CH_3-10 and CH_3-2, indicating that the relative configurations of C-2 and C-10 were trans. From the known configurations of D-glucose the stereochemistry of C-2 and C-10 could be determined as that shown.

Experimental Section

General Experimental Procedures. Melting points are uncorrected. NMR spectra were collected using a Brucker AMX 400 system at normal probe temperature (^{1}H NMR, 400 MHz; ^{13}C NMR, 100 MHz; TMS as internal reference). IR spectra were recorded on a NICOLET MX-1 spectrophotometer and UV spectra on a PE260 spectrophotometer. Optical rotations were measured on a PE-241 polarimeter. FABMS and HR-FABMS spectra were recorded on a MAR Z11 mass spectrometer and VG AutoSpec-3000 spectrometer, respectively

Animal Material. The aphid *P. bambusicola*, whose host plant is bamboo, was collected in Sichuan Province, China, in July 1995 and identified in the Biology Department of Sichuan University, where a specimen is deposited (voucher no. 95-06-11).

Extraction and Isolation. Dried *P. bambusicola* (4 kg) was soaked three times (10 L × 3) with 95% EtOH. After EtOH was removed under reduced pressure, 600 g of extract was obtained. The extract was then subjected to column chromatography (silica gel 160−200 mesh), elution with $CHCl_3$−95% EtOH (5:1), to give six fractions. The fraction with R_f 0.40−0.45 on TLC (plated with silica gel G, $CHCl_3$−95% EtOH 5:1) was separated using column chromatography, elution with $CHCl_3$:MeOH:CH_3COCH_3 5:1:1, to yield **1** (300 mg) and **2** (210 mg).

Bambusicolaside I (1): yellow powder; mp > 205 °C dec; $[\alpha]^{20}_D$ −18.0° (c = 0.15, H_2O); IR ν_{max} (KBr) 3248, 1650, 1631, 1598, 1572, 1463, 1361, 1340, 1275, 1175, 1100−1000, 840, 820, 760, 728 cm^{-1}; FABMS (positive) m/z 439 ($[M + 3H]^+$), 276, 261; HRFABMS (negative) m/z 437.1527 ($C_{21}H_{25}O_{10}$, $[M + H]^-$, calcd 437.1448); CD (5.68 mM, EtOH) $[\theta]_{400}$+1907° (max), $[\theta]_{309}$ −136° (min); UV λ_{max} (MeOH) 213 (log ϵ 4.38), 270 (4.24), 290 (sh), 398 (3.90) nm; NMR data see Table 1.

Bambusicolaside II (2): colorless cubic crystals; mp190−19 °C (MeOH); $[\alpha]^{20}_D$ −115.5° (c = 0.6, MeOH); IR ν_{max} (KBr) 3437, 3428, 3417, 3377, 1642, 1621, 1590, 1361, 1300, 1252, 1168, 1100−1000, 863 cm^{-1}; FABMS (positive) m/z 423 ($[M + H]^+$), 261, 245; HRFABMS (positive) m/z 423.1631 ($C_{21}H_{27}O_9$, $[M + H]^+$, calcd 423.1655); CD (4.74 mM, EtOH) $[\theta]_{300}$ +5275° (max),

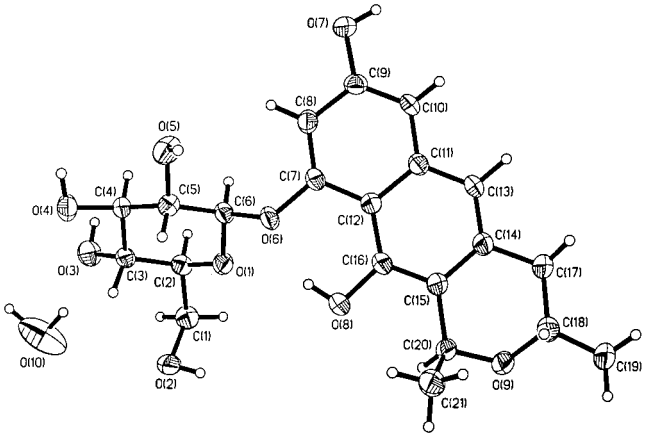

Figure 1. ORTEP diagram (40% probability ellipsoids) showing the crystallographic atom numbering scheme and solid-state conformation of bambusicolaside II (**2**).

$[\theta]_{309}$ +5802° (max), $[\theta]_{348}$ −528° (min); UV λ_{max} (MeOH) 236 (log ϵ 4.69), 280 (sh), 294 (3.74), 307 (3.76), 337 (3.58), 344 (3.60) nm; NMR data see Table 1.

X-ray Crystallographic Analysis of 2·H_2O. Single-crystal X-ray diffraction was used to characterize compound **2**. Crystals suitable for X-ray diffraction were obtained after recystallization from MeOH under a $CHCl_3$-saturated atmosphere at room temperature. Crystal data: $C_{21}H_{26}O_9 \cdot H_2O$, MW 440.43, orthorhombic, space group $P2_12_12_1$ with a = 7.4178(8) Å, b = 9.2380(9) Å, c = 30.983(4) Å, V = 2122.9(4) Å^3, Z = 4, D_{calcd} = 1.378 g cm^{-3}, μ(Mo Kα: radiation, λ = 0.710 73 Å) 0.110 mm^{-1}. Crystal dimensions: 0.66 × 0.46 × 0.46 mm.

Diffraction experiments were carried out at 296(2) K on a Siemens P4 diffractometer using graphite-monochromated Mo Kα radiation. Crystals were orthogonal, and the cell constants were obtained from a least-squares fit to data for 32 well-centered reflections in the range 5.92° ≤ 2θ ≤ 36.46°. From the systematic absences the space group was determined. Intensity data were collected with 0 ≤ h ≤ 10, 0 ≤ k ≤ 12, −1 ≤ l ≤ 42. A total of 3340 unique reflections was measured using an $\omega/2\theta$ scan mode. After data reduction, the unique data set contains 3318 reflections, 2272 are observed with $I > 2\sigma(I)$.

The structure was solved by direct methods using the program Siemens SHELXTL-5.03 and refined by full-matrix least-squares refinement on F^2. The refinements included 391 parameters, a scale factor, and atomic coordinates and anistropic temperature factors for non-hydrogen atoms. All hydroxyl hydrogen atoms were located in difference Fourier maps, and positions of all the other hydrogen atoms were calculated from an idealized geometry with standard bond lengths and were included in structure factor calculations with fixed parameters. The refinement converged to a standard residual of R = 0.038 and wR = 0.084. The final difference Fourier map showed no recognizable residual electron density (−0.34 ≥ $\Delta\rho$ ≥ 0.372 e Å^{-3}). A view of the solid-state conformation is presented in Figure 1.[13]

References and Notes

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(13) Hydrogen coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors have been deposited with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, upon request, from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK. This information can also be obtained from Dr. Guo-lin Zhang, Chengdu Institute of Biology, Academia Sinica, Chengdu 610041, the People's Republic of China.

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