An Iridoid Glucoside and the Related Aglycones from *Cornus florida*

Yangqing He,† Jiangnan Peng,§ Mark T. Hamann,⊥ and Lyndon M. West*‡

†Department of Applied Chemistry, College of Science, Xi’an University of Technology, Xi’an 710054, People’s Republic of China
‡Department of Chemistry and Biochemistry, Florida Atlantic University, Boca Raton, Florida 33431, United States
§Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78229, United States
⊥Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, Oxford, Mississippi 38677, United States

Supporting Information

ABSTRACT: A new iridoid glucoside, cornusoside A (1), and four new natural product iridoid aglycones, cornolactones A–D (2–5), together with 10 known compounds were isolated from the leaves of *Cornus florida*. The structures of compounds 1–5 were established by interpretation of their spectroscopic data. Cornolactone B (3) is the first natural cis-fused tricyclic dilactone iridoid containing both a five- and a six-membered lactone ring. A biosynthesis pathway is proposed for cornolactones C (4) and D (5), the C-6 epimers of compounds 1–3.

The plant genus *Cornus* (dogwood) belongs to the family Cornaceae and consists of approximately 55 species distributed mainly in the northern hemisphere, eastern Asia, and eastern and northern parts of the United States.1 This genus is a rich source of diverse iridoid glucosides, which have raised interest because of their wide range of promising bioactivities. These include antidiabetic,2,3 antioxidant,4 anti-inflammatory,5 anti-amnesic,6 and immunosuppressive effects.7

*Cornus florida* L., commonly known as flowering dogwood, is a tree native to eastern North America that has been traditionally used for the treatment of malaria.8,9 Previous chemical investigations of *C. florida* have resulted in the isolation of a number of compounds including anthocyanins and other flavonoids, triterpenoids, and sterols.9,10 The anthocyanins impart bright colors to several fruits and vegetables and possess anti-inflammatory,10–12 antioxidative,11,12 antineoplastic,10,13 and antidiabetic activities.14

In the present study the chemical constituents of *C. florida* collected from Oxford, Mississippi, were investigated. A large-scale extraction of the leaves of *C. florida* yielded a new iridoid glucoside, cornusoside A (1), and four new natural product iridoid aglycones, cornolactones A–D (2–5). Cornolactone A (2) was previously reported as a synthetic intermediate in the enantioselective synthesis of semperoside A;15 however, this is the first report of this compound from a natural source. In addition, 10 known compounds were also isolated, which included five iridoids, two megastigmane compounds, and two ellagic acid derivatives, together with a flavonoid. The structures of the new compounds were assigned by detailed spectroscopic analysis and those of the known compounds by comparison with literature data. Cornusoside A (1) is one of a small number of C10 iridoid glucosides with a ring-opening between C-1 and O-2 and a γ-lactone linkage between C-6 and C-11. Cornolactone B (3) is the first natural cis-fused tricyclic dilactone iridoid containing both a five- and a six-membered lactone ring. Herein are reported the isolation and structure elucidation of 1–5 and a possible biosynthetic pathway to cornolactones C (4) and D (5) as C-6 epimers of compounds 1–3.

A 90% aqueous ethanol extract of the dried leaves of *C. florida* (15 kg) was fractionated initially on silica gel (step gradient elution of hexane to EtOAc to MeOH). The 20% MeOH in EtOAc was then subjected to column chromatography on polymeric HP-20 (step gradient elution 10% Me₂CO

Received: March 12, 2014
Published: August 21, 2014
Table 1. NMR Spectroscopic Data for Cornusoside A (1) and Cornolactone A (2)\(^{a,b}\)

| Position | \(\delta_{\text{H}}\) (J in Hz) \(\delta_{\text{C}}\) mult. | \(\delta_{\text{H}}\) (J in Hz) \(\delta_{\text{C}}\) mult. |
|----------|---------------------------------|---------------------------------|
| 1a       | 66.7, CH2 3.98, dd (10.4, 3.6) 61.4, CH2 3.87, dd (11.0, 4.0) |
| 1b       | 3.38, m 3.58, dd (11.0, 9.0) |
| 2        | 169.0, qC |
| 4a       | 47.8, CH 3.94, d (5.6) 29.7, CH1 2.66, dd (18.8, 4.7) |
| 4b       | 2.59, dd (18.8, 9.8) |
| 5        | 45.5, CH 3.37, q (7.2) 40.2, CH1 3.14, m |
| 6        | 83.6, CH 5.03, t (6.8) 84.9, CH1 5.00, t (6.0) |
| 7α       | 40.6, CH2 1.99, dd (13.6, 6.0) 41.9, CH1 2.20, dd (14.0, 5.0) |
| 7β       | 1.47, ddd (13.6, 11.6, 6.0) 1.44, ddd (14.0, 11.8, 5.5) |
| 8        | 32.0, CH 1.90, m 32.8, CH1 1.82, m |
| 9        | 48.0, CH 1.83, m 50.6, CH1 1.79, m |
| 10       | 17.3, CH2 0.95, d (5.6) 17.6, CH1 1.02, d (5.6) |
| 11       | 172.8, qC 178.3, qC |
| OMe      | 52.7, CH3 3.68, s |
| 1′       | 103.0, CH 4.05, d (7.8) |
| 2′       | 73.3, CH 2.92, td (8.0, 4.4) |
| 3′       | 76.7, CH 3.10, m |
| 4′       | 70.1, CH 3.03, m |
| 5′       | 76.9, CH 3.07, d (3.9) |
| 6a′      | 61.1, CH 3.64, m |
| 6b′      | 3.43, m |
| OH-2′    | 4.72, d (4.3) |
| OH-3’    | 4.94, d (5.0) |
| OH-4’    | 4.93, d (5.5) |
| OH-6’    | 4.45, t (5.9) |

\(^{a}\)\(^{1}\)H NMR measured at 400 MHz; \(^{13}\)C NMR measured at 100 MHz. \(^{b}\) Measured in DMSO-d_6. \(^{b}\) Measured in CDCl_3.

Figure 1. Selected 2D NMR correlations for cornusoside A (1), and cornolactone A (2).

Figure 2. Selected NOE correlations observed for cornusoside A (1), and cornolactone A (2).
of the cyclopentane ring in a β-orientation. NOE correlations from H-8 to H-7α and H-1 confirmed the α-orientation of H-8 and the glucosylated side chain. A long-range W-coupling in the COSY spectrum between Me-10 and H-7α was consistent with the 1,2-diaxial arrangement of these two groups. Finally, the α-orientation of H-4 was indicated by a NOE correlation observed between H-4 and H-8. This was substantiated by a small coupling (5.6 Hz) observed between H-4 and H-5 that was similar to the coupling constants (ca. 5.0 Hz in both cases) of the previously reported C-1 to O-2 ring-opened iridoids gelsemio14 and borreriagenin.25 The absolute configuration of 1 was determined based on biogenetic grounds in that nearly all iridoids found in Nature have a common core. The reported synthetic compound.15 Since this is similar to the molecular formula, this indicated that cornolactone B (3) is tricyclic. The only possible connection was between the C-1 oxygen and the ester carbonyl C-3 to form a δ-lactone unit. The connection was confirmed with HMBC correlations observed between both H2-1 (δH 4.47, 4.05) and H-4 (δH 3.80) to the ester carbonyl carbon at δC 170.2 (C-3). The relative configuration of 3 at C-5, C-6, C-8, and C-9 was determined to be identical to that of 1 and 2 by NOE correlations observed in a NOESY experiment and scalar coupling (Figure 4). The absence of any NOE correlations observed to or from H-4 made it difficult to assign the configuration at C-4. However, the presence of a large 1H NMR coupling constant (J = 9.6 Hz) between H-4 and H-5 suggested the cis-relationship of these two protons and the β-orientation of H-4. This coupling constant is consistent with that observed for the cis-fused tricyclic iridoids semperoside (J = 10.5 Hz), 9-hydroxysemperoside (J = 11.4 Hz), and dihydrobrasoside (J = 10.5 Hz),24 together with the dilactone compounds, asperuloside trisaccharide lactone (J = 9.8 Hz) and dihydroasperuloside tetracetate lactone (J = 10.0 Hz), produced from the oxidation of the iridoid glucoside asperuloside.26 Thus, the configuration of cornolactone B (3) was defined as 45,5S,6S,8S,9R.

Cornolactone C (4) was isolated as a colorless gum. The molecular formula of this compound, C16H14O6, as determined from the HRESIMS of the [M + Na]+ ion at m/z 251.0884, required four degrees of unsaturation. The NMR data of 4 were similar to those of cornolactone B (3), except that the addition of a methoxy group at δH 3.80, 3.34 (OMe-11) to δC 169.2 (C-11) confirmed the presence of a methyl ester at C-11. Additional 1H-1H COSY and HMBC correlations (Figure 3) further supported this assignment. The relative configuration of 4 was determined by NOE correlations observed in a NOESY experiment and scalar coupling (Figure 4). NOE correlations from H-5 to H-4, H-7β, and H-9, together with correlations from H-9 to H-7β and H-9, established the cis-fusion of the cyclopenta[c]pyran skeleton with H-4, H-5, H-7β, and H-9 in the β-orientation. Finally, the α-orientation of H-6 was indicated by an NOE correlation observed from H-6 to H-7α and H-8 on the underside of the cyclopentane ring. Thus, the configuration of cornolactone C (4) was defined as 4R,5S,6R,8S,9R.

Cornolactone D (5) was isolated as a colorless gum. The molecular formula, C16H14O6, was determined from the HRESIMS of the [M + H]+ ion at m/z 193.0834 (calc 193.0835) and required three degrees of unsaturation. An initial inspection of the NMR data revealed that 5 is similar to 4, except for the absence of the signals for the methane at C-4 and the methyl ester at C-3 and the appearance of a methylene group at δH 2.57 (2H, d, J = 5.2 Hz, H-4). This suggested that 5 did not have a C-11 methyl ester unit. The observation of 1H-1H COSY correlations from H2-4 to H-5 and HMBC correlations from H2-4 to C-3, C-5, and C-9, together with a correlation from H-6 to C-4, confirmed this assignment (Figure 3). The similarity of proton–proton coupling constants and 1H and 13C NMR chemical shifts together with a NOESY correlation from H2-4 and H-5 to C-3, C-5, and C-9, together with a correlation from H-6 to C-4, confirmed this assignment (Figure 3).

Figure 3. Selected 2D NMR correlations for cornolactones B–D (3–5).
spectrum of 5 showed the same relative configuration as 4 at the four chiral centers (Figure 4). Thus, the configuration of cornolactone D (5) was defined as 5R, 6R, 8S, 9R.

Compounds 1–5 and cornin were evaluated for cell growth inhibitory activities against human embryonic stem cells (BG02) and human breast cancer cell lines (MCF-7 and MDA-MB-231). No cytotoxicity was observed for any of the compounds at 100 μM except for slight cytotoxicity being observed for compound 5 against the MDA-MB-231 cell line. The compounds were also examined for agonistic activity against peroxisome proliferator-activated receptor γ (PPARγ), but no activity was observed.

Cornusoside A (1) is one of a small number of C10 iridoid glucosides where the δ-lactone is ring-opened between the C-1 and O-2 positions and contains a γ-lactone linkage between C-6 and C-11. Other examples reported include gelsemiol 3-O-β-D-glucoside,24 gelsemiol 6′-trans-caffeyl-1-glucoside,27 and verbenabrasides A and B.28 Cornolactone B (3) is the first natural cis-fused tricyclic dilactone iridoid containing both a five- and a six-membered lactone ring. Interestingly, cornolactones C (4) and D (5) have an opposite configuration at C-6 compared to that of compounds 1–3. This suggests that rather than cleavage of the γ-lactone in 3 by methanolysis at C-11, to give the C-6 epimer of 4 (6-epi-4) with retention of configuration, an alternative biosynthetic pathway is necessary (Figure 5).

Table 2. NMR Spectroscopic Data for Cornolactones B–D (3–5) in CDCl₃<sup>a</sup>

| position | δ<sub>C</sub>, mult.  | δ<sub>H</sub> (J in Hz)  | δ<sub>C</sub>, mult.  | δ<sub>H</sub> (J in Hz)  | δ<sub>C</sub>, mult.  | δ<sub>H</sub> (J in Hz) |
|----------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1a       | 68.8, CH₂       | 4.47, dd (12.2, 4.8) | 69.3, CH₂       | 4.27, dd (12.0, 5.6) | 68.6, CH₂       | 4.18, dd (11.6, 4.0) |
| 1b       | 4.05, dd (12.2, 8.0) |                | 4.00, dd (11.6, 6.0) |                | 4.08, dd (12.0, 3.2) |                |
| 3        | 164.7, qC       | 3.80, d (9.6)    | 169.0, qC       | 50.4, CH       | 3.58, d (6.6)    | 174.0, qC       |
| 4        | 45.7, CH       | 3.35, dt (9.5, 6.4) | 46.1, CH       | 2.76, dt (11.6, 7.2) | 42.6, CH       | 2.39, m         |
| 5        | 85.1, CH       | 5.04, t (5.5)    | 77.4, CH       | 3.82, ddd (10.2, 7.4, 6.0) | 77.8, CH       | 3.71, ddd (10.2, 7.8, 5.5) |
| 6        | 41.2, CH₂       | 2.36, dd (14.8, 6.8) | 43.2, CH₂       | 2.12, td (11.7, 6.0) | 42.6, CH₂       | 2.01, m         |
| 7α       | 1.68, ddd (14.8, 9.4, 5.6) |                | 1.38, dt (12.1, 10.2) |                | 1.27, dt (12.1, 11.2) |                |
| 7β       | 34.2, CH       | 2.04, m         | 33.9, CH       | 1.78, m        | 33.1, CH       | 1.79, m         |
| 9        | 43.1, CH       | 2.10, m         | 42.8, CH       | 2.20, ddd (11.7, 9.4, 6.3) | 43.2, CH       | 1.97, m         |
| 10       | 19.4, CH₂       | 1.12, d (6.7)    | 18.9, CH₂       | 1.07, d (6.3)   | 18.7, CH₂       | 1.03, d (6.4)   |
| 11       | 170.2, qC       |                | 169.2, qC       |                | 53.4, CH₂       | 3.80, s         |

<sup>a</sup>H NMR measured at 400 MHz, 13C NMR measured at 100 MHz.

Figure 5. Plausible biosynthetic route to 3 and potential pathways to inversion of configuration at C-6 in 4 and 5.
plausible pathway leading to inversion of configuration at C-6 could occur through the S\textsubscript{2} hydrolysis of 3 at C-6, followed by esterification to give cornolactone C (4) or decarboxylation to give cornolactone D (5). Alternatively, cornolactones C (4) and D (5) could also have been formed from the reduction followed by oxidation of the co-isolated iridoid cornin (6). Previously it has been shown that reduction of 6 with NaBH\textsubscript{4} gives approximately a 1:1 mixture of both epimers at C-6.\textsuperscript{18} It is also conceivable that 6-epi-4 could undergo a lactonization reaction to give cornolactone B (3) and could provide an explanation of why no 6-epi-4 was isolated.

**EXPERIMENTAL SECTION**

**General Experimental Procedures.** Optical rotations were measured on a JASCO P-2000 polarimeter (c g/100 mL) equipped with a halogen lamp (589 nm) and a 1 mL microcell. IR spectra were recorded on a Thermo Electronic Corporation Nicolet IR-100 spectrophotometer. All NMR spectra were acquired with a Varian MercuryPlus 400 spectrometer using solvent signals (DMSO-d\textsubscript{6}; δ\textsuperscript{1}H, δ\textsuperscript{13}C, δ\textsuperscript{31}P 7.24 ppm; 1\textsuperscript{1}H, δ\textsuperscript{13}C, δ\textsuperscript{31}P 77.23 ppm) as references. Short- and long-range H–H COSY correlations were determined with gradient-enhanced inverse-detected HSQC and HMBC experiments, respectively. NOE correlations were detected with NOESY experiments with a 0.5 s mixing time. The HRESIMS were obtained using an Agilent 6220 series TOF mass spectrometer. HPLC was performed on a Shimadzu LC-20AT instrument with a Shimadzu SPD-M20A UV/vis photodiode detector and a Shimadzu ELSD-LTII detector.

**Plant Material.** The leaves of *Cornus florida* L. were collected from a one-mile radius around Timber Lake, Oxford, Mississippi, during the spring and summer of 2011 by M.T.H. Voucher specimens are kept in the Hamann Laboratory at the University of Mississippi, School of Pharmacy, Oxford, MS (CZ2011).

**Extraction and Purification Procedures.** The leaves of *C. florida* (15.0 kg, dry weight) were extracted with 90\% aqueous ethanol and dried under reduced pressure to give a crude extract (900 g). A portion of this crude extract (400 g) was separated on a silica gel column (20 × 70 cm) using a stepwise gradient of hexanes/EtOAc (100:0, 80:20, 50:50, and 100:0, v/v, each 3 L) and EtOAc/MEOH mixtures (80:20, 60:40, 50:50, and 0:100, v/v, each 3 L) to afford eight fractions. Fraction E (220 g) was then chromatographed on HP-20 (80:20, 7 mL/min) to yield six subfractions (Fr. E1−E6). Fraction E\textsubscript{1} (40 g) was chromatographed on a preparative C\textsubscript{18} reversed-phase MPLC column (20 × 250 mm; 20−35% CH\textsubscript{3}OH/H\textsubscript{2}O over 40 min, 35−65% CH\textsubscript{3}OH/H\textsubscript{2}O over 20 min; flow rate: 12 mL/min) to afford eight subfractions (Fr. E\textsubscript{1d}−E\textsubscript{1k}). Fraction E\textsubscript{1d} (50 mg) was purified by C\textsubscript{18} reversed-phase HPLC (Polar-C\textsubscript{8}; 5 μm, 10 × 250 mm; 15−45% CH\textsubscript{3}CN/H\textsubscript{2}O over 90 min, 5 mL/min) to yield cornoside A (1, 5.0 mg, t\textsubscript{R} 76.2 min). Fraction E\textsubscript{1d} (832 mg) was subjected to silica gel column chromatography using CH\textsubscript{2}Cl\textsubscript{2}/MeOH (90:10 to 0:100, v/v) to afford nine fractions (Fr. E\textsubscript{1d,1}−E\textsubscript{1d,9}). Fraction E\textsubscript{1d,6} (320 mg) was further purified by C\textsubscript{8} reversed-phase HPLC (Polar-C\textsubscript{8}; 5 μm, 10 × 250 mm; 5−25% CH\textsubscript{3}CN/H\textsubscript{2}O over 70 min, 5 mL/min) to yield two fractions (Fr. E\textsubscript{1d,6a}−E\textsubscript{1d,6b}). Fraction E\textsubscript{1d,6b} (50 mg) was purified by C\textsubscript{18} reversed-phase HPLC (Polar-C\textsubscript{8}; 5 μm, 10 × 250 mm; 5−25% CH\textsubscript{3}CN/H\textsubscript{2}O over 70 min, 5 mL/min) to yield cornolactone C (4, 8.0 mg, t\textsubscript{R} 41.5 min). The second fraction (40 mg) was chromatographed on a polymeric HPLC column (Hamilton PRP-1; 5 μm; 20 × 250 mm; 15−45% CH\textsubscript{3}CN/H\textsubscript{2}O over 70 min, 7 mL/min) to yield cornolactone B (3, 4.0 mg, t\textsubscript{R} 37.9 min). Cornolactones A (2, 15.0 mg) and D (5, 12 mg) were purified from the remaining crude extract (500 g) using a similar procedure (see Supporting Information).

**ASSOCIATED CONTENT**

**Supporting Information**

Full isolation procedures and 1D and 2D NMR spectroscopic data of 1−5 are available including \( ^{1} \)H, \(^{13} \)C, COSY, HSQC, HMBC, and NOESY. This material is available free of charge via the Internet at http://pubs.acs.org.

**AUTHOR INFORMATION**

**Corresponding Author**

†Tel: 661-297-0939. Fax: 661-297-2759. E-mail: lwest@fau.edu.

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

The authors thank Mr. S. Abbas (Ole Miss) for his assistance with plant collections, together with Kraft Foods and the National Institutes of Health (1R01AT007318) for some financial support. We also thank Dr. P. Griffin at the Scripps Research Institute Florida for performing the PPAR\textgamma agonistic activity evaluation, and Drs. A. Robbins and T. Schulz at Viacyte Inc. for cytotoxicity testing. This research was supported by National Institutes of Health grants (P41GM079597 and P01GM085354) and by the Scientific Research Program Funded by Shaanxi Provincial Education Department in China (2010JK749). This research was also supported in part by Xi’an University of Technology Starting
grant (108-211409), awarded to Dr. Y. He, and the Youth Scientists Innovation Team Program.

## REFERENCES

(1) Fan, C.; Xiong, Q. Y. *Am. J. Bot.* 2001, 88, 1131−1138.

(2) Yamabe, N.; Kang, K. S.; Matsuo, Y.; Tanaka, T.; Yokozawa, T. *Biol. Pharm. Bull.* 2007, 30, 1289−1296.

(3) Yokozawa, T.; Yamabe, N.; Kim, H. Y.; Kang, K. S.; Hur, J. M.; Park, C. H.; Tanaka, T. *Biol. Pharm. Bull.* 2008, 31, 1422−1428.

(4) Wang, W.; Sun, F. L.; An, Y.; Ai, H. X.; Zhang, L.; Huang, W. T.; Li, L. *Eur. J. Pharmacol.* 2009, 613, 19−23.

(5) Díaz, A. M.; Abad, M. J.; Fernandez, L.; Recuero, C.; Villaescusa, L.; Silva, A. M.; Bermejo, P. *Biol. Pharm. Bull.* 2000, 23, 1307−1313.

(6) Lee, K. Y.; Sung, S. H.; Kim, S. H.; Jang, Y. P.; Oh, T. W.; Kim, Y. C. *Arch. Pharm. Res.* 2009, 32, 677−683.

(7) Meng, J.; Chen, L.; Tang, S. B.; Chen, J. S.; Lin, S. F.; Zhao, S. B. *Chin. J. Pathophysiol.* 2005, 21, 927−930.

(8) Hasegawa, G. R. *Mil. Medicine* 2007, 172, 650−655.

(9) Graziose, R.; Rojas-Silva, P.; Rathinasabapathy, T.; Dekock, C.; Grace, M. H.; Poulev, A.; Lila, M. A.; Smith, P.; Raskin, I. *J. Ethnopharmacol.* 2012, 142, 456−461.

(10) Vareed, S. K.; Reddy, M. K.; Schutzki, R. E.; Nair, M. G. *Life Sci.* 2006, 78, 777−784.

(11) Seeram, N. P.; Schutzki, R.; Chandra, A.; Nair, M. G. *J. Agric. Food Chem.* 2002, 50, 2519−2523.

(12) Wang, H.; Nair, M. G.; Strasburg, B. M.; Chang, Y. C.; Booren, A. M. J. *Nat. Prod.* 1999, 62, 294−296.

(13) Sefton, M. A.; Francis, L.; Williams, P. J. *J. Agric. Food Chem.* 1990, 38, 2045−2049.

(14) He, Y. Q.; Ma, G. Y.; Peng, J. N.; Ma, Z. Y.; Hamann, M. T. *Biochem. Biophys. Acta* 2012, 1820, 1021−1026.

(15) Jensen, S. R.; Kjær, A.; Nielsen, B. *Acta Chem. Scand.* 1973, 27, 2581−2585.

(16) Marini, S. D.; Borbone, N.; Zollo, F.; Iannaro, A.; Meglio, P. D.; Iorizzo, M. J. *Agric. Food Chem.* 2004, 52, 7525−7531.

(17) Setton, M. A.; Francis, L.; Williams, P. J. *J. Agric. Food Chem.* 1990, 38, 2045−2049.

(18) Sadly, W. E.; Yazaki, Y. *Phytochemistry* 1973, 12, 2969−2977.

(19) Xie, X. Y.; Wang, R.; Shi, Y. P. *Biochem. Syst. Ecol.* 2012, 45, 120−123.

(20) Jensen, S. J.; Kirk, O.; Nielsen, B. J.; Norrestam, R. *Phytochemistry* 1987, 26, 1725−1731.

(21) Vieira, I. J.; Mathias, L.; Braz-Filho, R.; Schripsema, J. *Org. Lett.* 1999, 1, 1169−1171.

(22) Berkowitz, W. F.; Choudhry, S. C.; Hrabie, J. A. J. *Org. Chem.* 1982, 47, 824−829.