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Podocarpaside, a Triterpenoid Possessing a New Backbone from *Actaea podocarpa*

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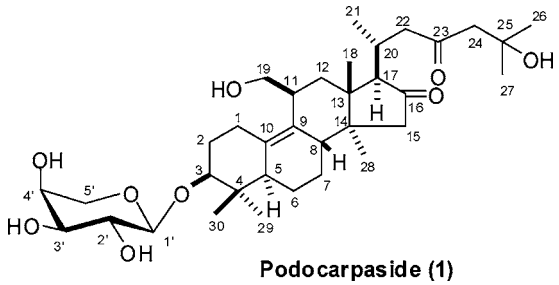
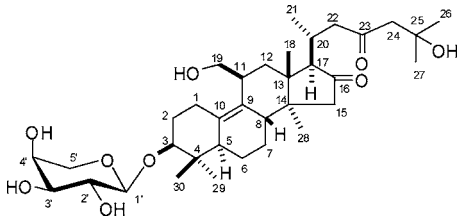
ABSTRACT



Podocarpaside (1)

Podocarpaside (1), a novel arabinoside possessing a unique triterpene skeleton was isolated from *Actaea podocarpa*, a closely related species to black cohosh (dietary supplement used for menopausal disorders). Podocarpaside belongs to a new class of triterpenoids, for which the name "ranunculane" is proposed. Compound 1 possesses anticomplement activity.

Actaea, belonging to the family Ranunculaceae, consists of about 28 species distributed throughout East Asia, Europe, and North America.[1] Black cohosh (*A. racemosa*), a rich source of cyclolanostane-type triterpenes,[2−5] is one of the important *Actaea* species and is used as a dietary supplement for treating menopausal disorders.[2] It is usually collected from the wild where the risk of adulteration is high due to cogrowth of *A. podocarpa* (summer cohosh), *A. pachypoda* (white cohosh), and *A. rubra* (red cohosh).[6] As part of our program aimed at the issues of adulteration, misidentification, and bioequivalence of black cohosh related species in comparison with black cohosh, we reported the isolation and structure elucidation of several *seco*-lanostatane- and cyclolanostane-type triterpenes from *A. podocarpa*, *A. pachypoda*, and *A. rubra*.[7−9] Here, we describe the isolation, the structure determination, and a possible biogenetic origin of the unique structural features of podocarpaside (**1**), from the roots of *A. podocarpa*.



The roots of *A. podocarpa* DC. were collected in North Carolina (August 2004) and identified by Mr. Gregory Gust,

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William L. Brown Center, Missouri Botanical Garden, Missouri. A voucher specimen has been deposited at the Missouri Botanical Garden. Powdered, freeze-dried roots of *A. podocarpa* (543 g) were extracted with methanol (1.5 L × 24 h × 5). The combined extracts were concentrated under reduced pressure to afford colored powder (53.3 g). Part (38.0 g) of this was subjected to vacuum liquid column chromatography over silica gel (600 g) and eluted initially with EtOAc to give three fractions, A (5.0 g), B (6.3 g), and C (0.5 g), and then with methanol to afford fraction D (21.1 g). Fraction B (6.0 g) was divided into 12 fractions (B1−B12) by column chromatography (cc) over reverse-phase silica (RP−18, 300 g, MeOH/H_2O 7:3). Podocarpaside (**1**, 69.3 mg) was obtained by cc from fraction B7 (silica gel, EtOAc/$CHCl_3$/MeOH/H_2O 15:8:4:1).

Podocarpaside (**1**),[10] $[\alpha]_D^{27}$ −14.3 (*c* 0.14, MeOH), was obtained as a white powder, which showed a pseudomolecular ion at *m*/*z* 643 $[M + Na]^+$ in the positive ESIMS. The ^{13}C NMR spectrum showed 35 signals, which in agreement with HR-ESIMS (found 643.3817, calcd 643.3822 for $C_{35}H_{56}NaO_9$) led to a molecular formula, $C_{35}H_{56}O_9$. The HSQC spectrum resolved the 35 carbon signals as 7 methyls, 10 methylenes, 10 methines, and 8 quaternary carbons.

The ^1H NMR spectrum displayed signals for six tertiary methyls [δ_H 1.09 (s, Me-18), 1.21 (s, Me-26, Me-27), 0.93 (s, Me-28), 1.06 (s, Me-29), 0.70 (s, Me-30)], a secondary methyl [δ_H 1.01 (d, J = 6.0 Hz, Me-21)], an anomeric methine [δ_H 4.36 (d, J = 6.2 Hz, H-1′)], and two isolated methylenes [δ_H 1.88/2.17 (each d, J = 18.0 Hz, 2H-15); 2.60 (s, 2H-24)]. The ^{13}C NMR spectrum of **1** showed two carbonyls [δ_C 219.7 (s, C-16) and 212.2 (s, C-23)], two olefinic carbons [δ_C 133.5 (s, C-9) and 135.1 (s, C-10)], and three oxygenated carbons [δ_C 88.1 (d, C-3), 69.1 (t, C-19), and 70.3 (s, C-25)], in addition to five more oxygenated carbons assignable to α-L-arabinopyranose [δ_C 106.4 (C-1′), 72.8 (C-2′), 74.1 (C-3′), 68.8 (C-4′), and 65.7 (C-5′)].[2,11]

To resolve the overlapping of signals, ^1H NMR spectra of **1** were recorded in acetone-d_6, acetone-d_6−D_2O, pyridine-d_5, pyridine-d_5−D_2O,[12] $CDCl_3$,[13] CD_3OD, and DMSO-d_6 at 400 or 600 MHz (see Supporting Information). Acetone-d_6 showed the best resolution, and NMR data recorded in this solvent were utilized for structure elucidation.

The following unambiguous HMBC correlations (in acetone-d_6 or pyridine-d_5) located the sugar at C-3 [$\delta_{H/C}$ 3.31 (H-3)/106.4, 25.2, 15.0, 28.9, 31.7 (C-1′, 29, 30, 1, 2); $\delta_{H/C}$ 4.36 (H-1′)/88.1 (C-3)] and two carbonyls at C-16 and C-23 [$\delta_{H/C}$ 1.88, 2.17 (2H-15)/219.7 (C-16); 2.32 (H-17)/219.7 (C-16) and $\delta_{H/C}$ 2.45, 3.25 (2H-22)/212.2 (C-23); 2.60 (2H-24)/212.2 (C-23)]. Furthermore, the position of the C_9−C_{10} double bond and the unique hydroxymethyl at C-11 were revealed by HMBC correlations [H-19a/C-12, 11, 9; H-11/C-8, 9, 10, 13, 19; H-5/C-10; H-12β/C-9; H-1β/C-9, 10; H-2α/C-10; and H-7β/C-9]. Detailed HMBC associations are shown in Figure 2. Analysis of the ^1H−^1H COSY spectrum led to partial structures (Figure 1), which were

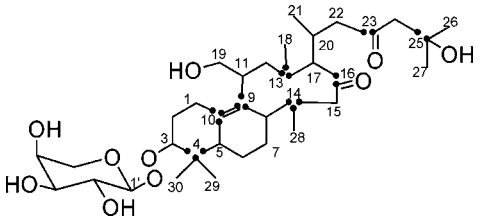

Figure 1. Partial structures of **1**.

connected by using HMBC correlations (Figure 2). The NMR assignments (Table 1) were done by analyzing ^1H−^1H COSY

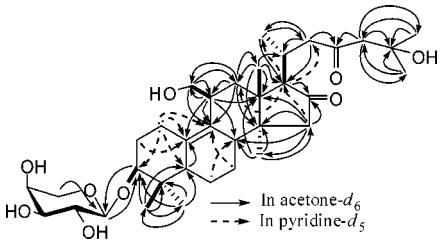

Figure 2. Unambiguous HMBC correlations of **1**.

(Figure 1), HSQC, HMQC, 2D *J*-resolved, HMBC (Figure 2), and ROESY (Figure 3) spectra.

The coupling constants of H-3 (J = 4.8, 11.6 Hz) revealed its axial α-orientation and ultimately equatorial β-oriented 3-hydroxylation. Assuming a β-oriented 3-hydroxylation and hence 3*S*-absolute confirmation, we defined the configuration of the remaining stereogenic carbon atoms from the observed

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(10) Podocarpaside (**1**). White powder; $[\alpha]_D^{27}$ −14.3 (*c* 0.14, MeOH); IR (KBr) ν_{max} 3380, 2939, 1731, 1652 cm^{-1}; NMR, see Table 1; positive ESIMS *m*/*z* 643 $[M + Na]^+$; HR-ESIMS found 643.3817, calcd for $C_{35}H_{56}NaO_9$ 643.3822.

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(12) ^1H NMR (C_5D_5N−D_2O, 600 MHz) δ_H 4.76 (d, J = 7.2 Hz, H-1′), 4.37 (dd, J = 7.2, 8.8 Hz, H-2′), 4.18 (dd, J = 3.6, 8.8 Hz, H-3′), 4.32 (br s, H-4′), 4.21 (dd, J = 3.2, 11.2 Hz, H-5′β), 3.71 (overlap, H-5′α).

(13) ^{13}C NMR ($CDCl_3$, 600 MHz) δ_C 28.2 (C-1), 30.6 (C-2), 88.1 (C-3), 41.1 (C-4), 48.1 (C-5), 23.2 (C-6), 25.2 (C-7), 41.2 (C-8), 132.2 (C-9), 134.5 (C-10), 37.9 (C-11), 31.7 (C-12), 45.2 (C-13), 42.3 (C-14), 48.9 (C-15), 220.0 (C-16), 60.9 (C-17), 17.8 (C-18), 68.4 (C-19), 27.2 (C-20), 21.0 (C-21), 50.8 (C-22), 213.0 (C-23), 53.6 (C-24), 70.1 (C-25), 29.5 (C-26), 29.6 (C-27), 18.9 (C-28), 24.7 (C-29), 14.7 (C-30), 105.3 (C-1′), 73.0 (C-2′), 71.7 (C-3′), 68.2 (C-4′), 65.6 (C-5′); ^1H NMR ($CDCl_3$, 600 MHz, most of the signals appeared broad) δ_H 4.36 (br d, J = 5.4 Hz, H-1′), 3.91 (br, H-4′, H-5′e), 3.72 (br, H-2′), 3.67 (br, H-3′), 3.53 (overlap, H-5′a, H-19a), 3.47 (br, H-19b), 3.30 (br, H-3), 3.21 (br d, J = 13.8 Hz, H-22a), 2.71 (br d, J = 13.2 Hz, H-1β), 2.66 (d, J = 18.0 Hz, H-24a), 2.56 (d, J = 18.0 Hz, H-24b), 2.34 (br overlap, H-8, H-21, H-22b), 2.19 (d, J = 8.4 Hz, H-17), 2.17 (br d, J = 14.4 Hz, H-12β), 2.11 (d, J = 17.7 Hz, H-15β), 2.04 (br, H-2β), 1.92 (d, J = 17.7 Hz, H-15α), 1.88 (br, H-6β), 1.84 (br, H-12α), 1.78 (br, H-5), 1.74 (br, H-1α), 1.52 (m, H-2α), 1.43 (br, H-7β), 1.25 (s, 3H-27), 1.23 (s, 3H-26), 1.22 (overlap, H-6α, H-7α), 1.07 (s, 3H-18), 1.01 (br overlap, 3H-21, 3H-29), 0.88 (s, 3H-28), 0.68 (s, 3H-30).

Table 1. NMR Data of Podocarpaside (**1**)

no.	$\delta_C{}^a$ ($\delta_C{}^b$) (mult)	$\delta_H{}^a$ (mult., *J*, Hz)	$\delta_H{}^b$ (mult., *J*, Hz)
1α	28.9 (29.0) t	1.72 (m)	1.83 (overlap)
1*β*		2.77 (br d, 13.6)	2.89 (br d, 8.4)
2α	31.7 (31.8) t	2.02 (m)	2.33 (m)
2*β*		1.52 (m)	1.83 (overlap)
3α	88.1 (88.0) d	3.31 (dd, 4.8, 11.6)	3.56 (dd, 3.8, 11.0)[c]
4	42.0 (41.9) s		
5α	49.1 (48.9) d	1.93 (m)	1.91 (overlap)
6α	24.1 (23.9) t	1.75 (m)	1.72 (m)
6*β*		1.32 (m)	1.21 (overlap)
7α	26.2 (26.0) t	1.29 (m)	1.21 (overlap)
7*β*		1.47 (m)	1.34 (m)
8*β*	42.2 (41.9) d	2.42 (overlap)	2.38 (overlap)
9	133.5 (133.3) s		
10	135.1 (134.9) s		
11α	39.3 (39.5) d	3.10 (br m)	3.33 (br m)
12α	32.1 (32.2) t	1.82 (dd, 8.8, 14.4)	1.93 (overlap)
12*β*		2.37 (br d, 14.4)	2.71 (d, 13.2)[c]
13	46.1 (45.9) s		
14	43.1 (42.9) s		
15α	49.5 (49.5) t	1.88 (d, 18.0)	1.99 (d, 18.0)
15*β*		2.17 (d, 18.0)	2.22 (d, 18.0)
16	219.7 (219.9) s		
17α	61.6 (61.6) d	2.32 (d, 9.0)[c]	2.41 (d, 8.4)
18	18.2 (18.3) q	1.09 (s)	1.16 (s)
19a	69.1 (68.9) t	3.45 (dd, 6.0, 11.0)	3.82 (overlap)
19b		3.49 (dd, 6.0, 11.0)[c]	
20	28.1 (28.1) d	2.32 (overlap)	2.64 (m)
21	21.2 (21.4) q	1.01 (d, 6.0)	1.15 (d, 6.6)
22a	51.6 (51.8) t	2.45 (dd, 8.4, 17.0)	2.69 (dd, 8.4, 17.0)[c]
22b		3.25 (dd, 1.6, 17.0)	3.56 (dd, 3.0, 17.0)[c]
23	212.2 (211.4) s		
24a	55.4 (56.4) t	2.60 (s)	2.80 (d, 15.0)
24b		2.60 (s)	2.83 (d, 15.0)
25	70.3 (70.1) s		
26	30.1 (30.6) q	1.21 (s)	1.48 (s)
27	30.3 (30.9) q	1.21 (s)	1.47 (s)
28	19.1 (19.2) q	0.93 (s)	0.90 (s)
29	25.2 (25.4) q	1.06 (s)	1.31 (s)
30	15.0 (15.3) q	0.70 (s)	0.88 (s)
1′a			
x	106.4 (107.5) d	4.36 (d, 6.2)	4.78 (d, 6.9)
2′eq	72.8 (73.4) d	3.64 (dd, 6.2, 8.2)	4.41 (dd, 6.9, 8.4)
3′a			
x	74.1 (75.1) d	3.57 (dd, 3.2, 8.2)	4.16 (dd, 2.4, 8.4)
4′eq	68.8 (69.9) d	3.81 (br s)	4.32 (br s)
5′a			
x	65.7 (67.1) t	3.52 (dd, 3.2, 12.0)	3.78 (dd, 2.4, 12.0)[c]
5′eq		3.82 (dd, 3.6, 12.0)	4.27 (br d, 12.0)

[a] Data were recorded in C_3D_6O at 400 MHz and in C_5D_5N at 600 MHz. [b] Chemical shifts (δ) are in ppm. [c] Coupling constants were recorded on a 2D *J*-resolved spectrum.

ROESY associations (Figure 3). Thus, H-3α shows association with H-5α, H-5α with H-7α, H-7α with Me-28α, and Me-28α with H-17α. The latter association then indicates a *β*-orientation of the C-17 side chain. H-20 associates strongly with Me-18 and hence confirms the *β*-orientation of this methyl group. Finally, Me-18 shows association with both H-8 and the C-19 oxymethylene protons to confirm the *β*-orientation of these groups. These associations then permit

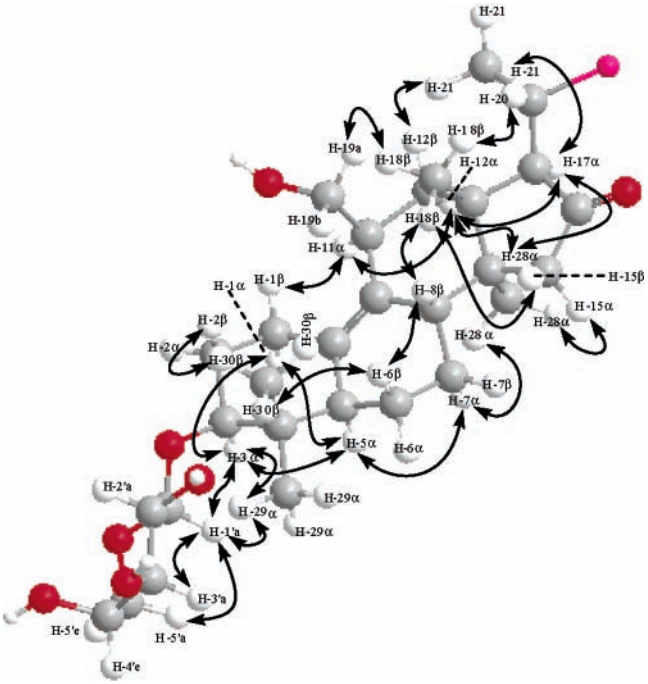

Figure 3. ROESY correlations of **1**.

definition of the absolute configuration of podocarpaside as 3*S*, 5*R*, 8*S*, 11*S*, 13*R*, 14*S*, and 17*R*. The 20*R* absolute configuration is assumed on the basis of the predominance of 20*S*-configured naturally occurring lanostane-type triterpenes.[14] Note that the spatial arrangement of the substituents at C-20 in podocarpaside is the same as that for 20*S*-configured lanostane-type triterpenes.[14] The configuration is inverted because of a reversal of Cahn−Ingold−Prelog sequencing. The *β*-oriented C-11 hydroxymethyl group, based on the *β*-position of the cyclopropyl moiety in the cycloartane skeleton,[2−5] is also supported by the proposed biogenetic pathway (Figure 4). The sugar obtained after

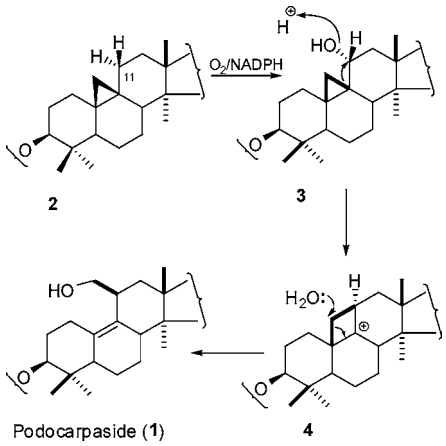

Figure 4. Proposed biogenetic pathway for podocarpaside.

standard acid hydrolysis was identified as L-arabinose by comparing its TLC (EtOAc−$CHCl_3$−MeOH−H_2O 12:8:8:4) and specific rotation $[\alpha]_D^{28}$ +102.8 (*c* 0.51, H_2O) with a reference sample of L-arabinose purchased from Sigma-Aldrich. On the basis of these spectroscopic data, compound **1** was defined as 3β,19,25-trihydroxy-16,23-dioneranuncul-9(10)-ene 3-*O*-α-L-arabinopyranoside.

Podocarpaside belongs to a new class of triterpenoids, for which the name "ranunculane" is proposed. It probably originates from a cycloartane precursor (**2**) by initial oxygenation of the α-equatorial C-11 hydrogen (Figure 4). The α-alcohol (**3**) is then susceptible to acid-catalyzed rearrangement of the strained cyclopropane into cyclobutane analogue **4**. Hydrolytic ring cleavage then affects the equivalent of the methyl migration and generation of the C_9−C_{10} double bond. The lanostane and its rearranged classes of triterpenoids are shown in Figure 5.

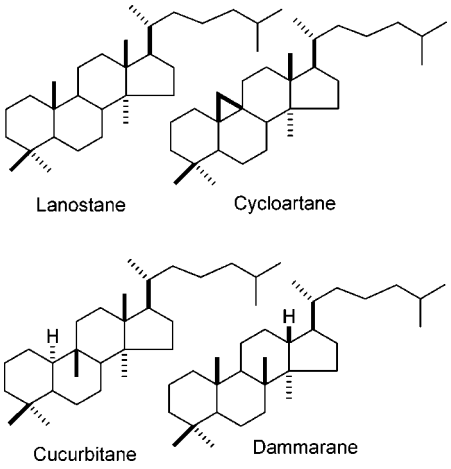

Figure 5. Lanostane and its rearranged classes.

Podocarpaside was tested for cytotoxicity toward mammalian kidney fibroblasts (Vero) and epithelial (LLC-PK_1) cells and for anticancer activity in a panel of human solid tumor cells (SK-MEL, KB, BT-549, and SK−OV-3) in an assay described earlier.[15] The compound was not cytotoxic to kidney cells up to 40 μM. It did not exhibit any estrogenic effect up to 500 μM, in a cell-based assay.[16] No antioxidant activity was observed up to 30 μM in HL-60 cells by a DCFH-DA assay.[17] However, it inhibited Human Complement activity with an IC_{50} value of 190 μM in an in vitro assay using sensitized RBCs and human serum.[7] Ursolic acid and oleanolic acid were used as positive controls showing IC_{50} values of 54 and 80 μM, respectively. The anticomplement activity of some triterpenes has been reported earlier with IC_{50} values in the range of 101−180 μM.[18] The Human Complement System (HCS) is an integral part of the body's immune response against infections, but it may cause adverse effects when activated inappropriately. Activation of HCS is known to be initiated by a number of agents such as pathogens, foreign molecules, and immune complexes. Therefore, the modulation of complement activity becomes important under pathological conditions of inflammation and during organ transplantation.[18,19]

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Supporting Information Available: ^{1}H NMR spectra of podocarpaside (**1**) in acetone-d_6, acetone-d_6−D_2O, pyridine-d_5, pyridine-d_5−D_2O, $CDCl_3$, CD_3OD, and DMSO-d_6; and ^{13}C and 2D NMR spectra in acetone-d_6 and pyridine-d_5. This material is available free of charge via the Internet at http://pubs.acs.org.

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