Bioassay-Guided Isolation of the Antidiabetic Principle from *Sorbus decora* (Rosaceae) Used Traditionally by the Eeyou Istchee Cree First Nations

José A. Guerrero-Analco,[†] Louis Martineau,[‡] Ammar Saleem,[†] Padma Madiraju,[‡] Asim Muhammad,[†] Tony Durst,[†] Pierre Haddad,[‡] and John Thor Arnason*[,†]

Centre for Advanced Research in Environmental Genomics, University of Ottawa, Ottawa, ON, K1N6N5, Canada, and Department of Pharmacology and Montreal Diabetes Research Centre, Université de Montréal, Montréal, QC, H3T 1J4, Canada

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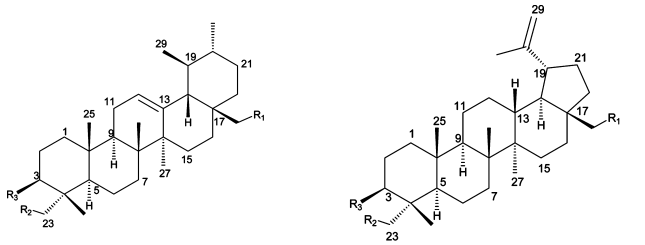
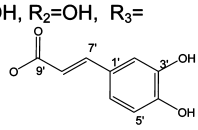
Bioassay-guided fractionation of a crude extract (80% EtOH in H_2O) of stem bark of *Sorbus decora* led the isolation of three new pentacycle triterpenes (compounds **1**−**3**). The structures of **1**−**3** were established on the basis of spectroscopic methods (IR, HREIMS, 1D and 2D NMR) as 23,28-dihydroxyursan-12-ene-3β-caffeate, 23,28-dihydroxylupan-20(29)-ene-3β-caffeate, and 3β,23,28-trihydroxy-12-ursene, respectively. Compound **2** significantly enhanced glucose uptake in C2C12 cells, but compounds **1** and **3** did not. In addition, triterpenoids **4**−**8**, catechin, and epicatechin were also isolated. This is the first comprehensive report of the phytochemical constituents of *S. decora* since the initial study by Narashmachari and von Rudloff (1962) and includes evaluation of their antidiabetic activity.

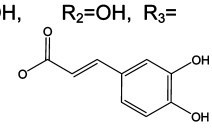
Sorbus decora C.K. Schneid. (Rosaceae), commonly known as "Showy mountain ash", is a widely distributed tree of the boreal forest of North America.[1,2] The plant is known as maskuminan-ahtikw (southern James Bay region) or miskumishi (northern James Bay region). Traditionally the bark is used by the Eeyou Istchee Cree First Nations of the James Bay region of Quebec, Canada, to treat symptoms associated with diabetes mellitus and other diseases.[3−7] In collaboration with healers of the Cree communities, we have validated in vivo antidiabetic properties of the bark extracts.[7] We now describe the first comprehensive phytochemical investigation of these extracts via a bioassay-guided separation based on in vitro glucose uptake by C2C12 cells of various fractions and the isolated pure components. This effort has resulted in the isolation and structure elucidation of three new triterpenoids (**1**−**3**) and seven known compounds (**4**−**10**). One of the triterpenes, compound **2**, identified as 23,28-dihydroxylupan-20(29)-ene-3β-caffeate, showed significant antidiabetic properties as determined by an increase in glucose uptake by C2C12 cells.



1 R_1=OH, R_2=OH, R_3=



2 R_1=OH, R_2=OH, R_3=



3 R_1=OH, R_2=OH, R_3=OH

4 R_1=OH, R_2=OH, R_3=OH

5 R_1=OH, R_2=H, R_3=OH

6 R_1=OH, R_2=H, R_3=OH

7 R_1=H, R_2=H, R_3=OH

8 R_1=COOH, R_2=H, R_3=OH

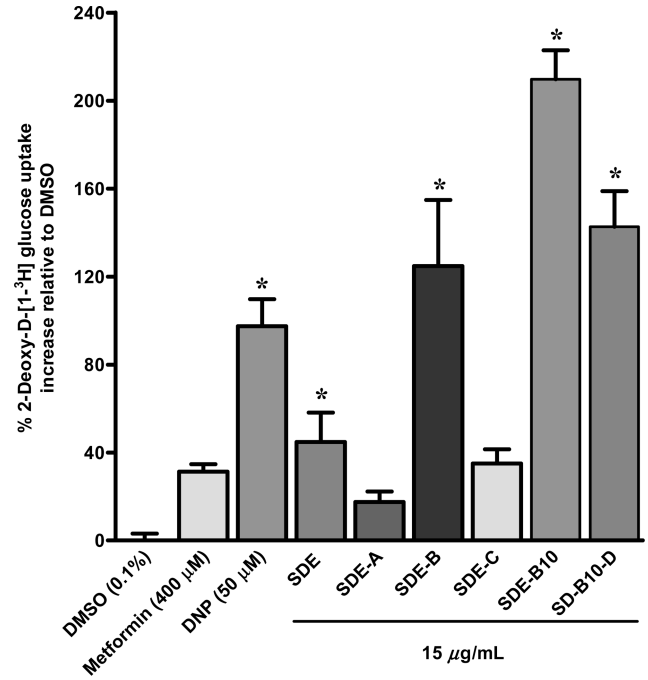


Figure 1. Effect of the crude extract (SDE) and active fractions (SDE-B, SDE-B10, SDE-B10-D) of *S. decora* at the maximum concentration (15 μg/mL) tested on glucose uptake assay in C2C12 cells. Each value is the mean ± SEM (n = 4 wells) in each group. *p < 0.05 significantly different after one-way ANOVA analysis followed by Dunnett's t test vs the negative control group (DMSO 0.1%).

Results and Discussion

Stem bark of *S. decora* was extracted with 80% EtOH in H_2O. The crude extract (SDE) was dissolved in MeOH−H_2O (1:1) and partitioned between hexanes and EtOAc. The EtOAc fraction (SDE-B) was the most active with respect to glucose uptake (Figure 1). This fraction was chromatographed over a silica gel column and separated into 15 fractions (SDE-B1−SDE-B15). The most active subfraction, SDE-B10 (Figure 1), was rechromatographed on a second silica gel column and separated into five fractions (SDE-B10-A−SDE-B10-E), which were tested using the same glucose-uptake bioassay. The active fraction SDE-B10-D (Figure 1) yielded compounds **1** and **2** after preparative reversed-phase HPLC separa-

* Corresponding author. Tel: + 613 562 5262. Fax: + 613 562 5765. E-mail: John.Arnason@uOttawa.ca.
[†] University of Ottawa.
[‡] Université de Montréal.

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Table 1. NMR Data (400 MHz, C_5D_5N) of Compounds **1**−**3**

	1		**2**		**3**	
position	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)
1	38.3, CH_2	H-1a 1.55, m H-1b 1.55, m	38.2, CH_2	H-1a 1.67, m H-1b 1.67, m	39.3, CH_2	H-1a 1.58, m H-1b 1.58, m
2	26.2, CH_2	H-2a 1.96, m H-2b 1.96, m	27.1, CH_2	H-2a 1.75, m H-2b 1.75, m	27.9, CH_2	H-2a 1.91, m H-2b 1.91, m
3	74.2, CH	5.58, dd (11.6, 4.4)	74.3, CH	5.59, dd (11.6, 4.8)	73.7, CH	4.20, br d (10.4)
4	40.0, qC		42.6, qC		40.6, qC	
5	47.6, CH	1.69, m	50.3, CH	1.55, m	48.7, CH	1.50, m
6	17.7, CH_2	H-6a 1.70, m H-6b 1.35, m	20.7, CH_2	H-6a 1.30, m H-6b 1.15, m	18.8, CH_2	H-6a 1.64, m H-6b 1.40, m
7	32.4, CH_2	H-7a 1.76, m H-7b 1.30, m	33.8, CH_2	H-7a 1.45, m H-7b 1.33, m	33.2, CH_2	H-7a 1.61, m H-7b 1.29, m
8	38.1, qC		40.8, qC		38.9, qC	
9	46.6, CH	1.78, m	48.1, CH	1.67, m	48.4, CH	1.67, m
10	35.8, qC		36.6, qC		37.2, qC	
11	23.2, CH_2	H-11a 1.99, m H-11b 1.85, m	17.7, CH_2	H-11a 1.67, m H-11b 1.33, m	23.9, CH_2	H-11a 2.03, m H-11b 1.86, m
12	124.5, CH	5.22 br, s	23.5, CH_2	H-12a 1.77, m H-12b 1.15, m	125.3, CH	5.20 br s
13	139.2, qC		37.2, CH	1.66, m	140.0, qC	
14	41.9, qC		42.1, qC		42.6, qC	
15	23.5, CH_2	H-15a 1.82, m H-15b 1.46, m	25.3, CH_2	H-15a 1.85, m H-15b 1.08, m	26.8, CH_2	H-15a 2.03, m H-15b 1.24, m
16	23.3, CH_2	H-16a 1.85, m H-16b 1.85, m	29.6, CH_2	H-16a 2.39, m H-16b 1.18, m	24.0, CH_2	H-16a 1.51, m H-16b 1.51, m
17	42.0, qC		48.7, qC		43.1, qC	
18	54.3, CH	1.58, m	46.9, CH	1.53, m	54.9, CH	1.53, m
19	39.4, CH	1.30, m	47.9, CH	2.61, ddd (10.8, 10.8, 6.0)	40.0, CH	1.30, m
20	39.5, CH	1.30, m	150.8, qC		40.1, CH	1.30, m
21	30.8, CH_2	H-21a 1.46, m H-21b 1.46, m	29.9, CH_2	H-21a 2.12, m H-21b 1.53, m	31.5, CH_2	H-21a 1.50, m H-21b 1.50, m
22	36.3, CH_2	H-22a 1.95, m H-22b 1.67, m	34.5, CH_2	H-22a 2.39, m H-22b 1.15, m	36.4, CH_2	H-22a 1.96, m H-22b 1.68, m
23	63.7, CH_2	H-23a 3.90, d (10.8) H-23b 3.47, d (10.8)	63.7, CH_2	H-23a 3.63, d (11.2) H-23b 3.56, d (11.2)	68.2, CH_2	H-23a 4.19, d (10.4) H-23b 3.72, d (10.4)
24	13.6, CH_3	0.91, s	16.4, CH_3	0.89, s	13.4, CH_3	1.07, s
25	15.9, CH_3	0.97, s	15.7, CH_3	1.00, s	16.0, CH_3	1.00, s
26	16.6, CH_3	1.02, s	14.5, CH_3	0.98, s	17.3, CH_3	1.03, s
27	23.1, CH_3	1.17, s	13.4, CH_3	0.89, s	23.8, CH_3	1.16, s
28	68.7, CH_2	H-28a 3.64, d (11.2) H-28b 3.57, d (11.2)	59.0, CH_2	H-28a 4.07, d (10.6) H-28b 3.65, d (10.6)	69.4, CH_2	H-28a 3.91, d (10.4) H-28b 3.47, d (10.4)
29	17.3, CH_3	0.91, d (6.0)	109.6, CH_2	H-29a 4.86, d (2.4) H-29b 4.72, d (2.4)	18.0, CH_3	0.93, d (6.0)
30	21.2, CH_3	0.93, d (6.0)	18.8, CH_3	1.74, s	21.8, CH_3	0.94, d (6.0)
1′	126.6, qC		126.6, qC			
2′	115.5, CH	7.64, d (1.8)	115.5, CH	7.64, d (1.8)		
3′	147.4, qC		147.4, qC			
4′	150.0, qC		150.0, qC			
5′	116.3, CH	7.23, d (8.7)	116.3, CH	7.23, d (8.7)		
6′	121.6, CH	7.20, dd (8.7, 1.8)	121.6, CH	7.20, dd (8.7, 1.8)		
7′	145.2, CH	7.98, d (16.0)	145.1, CH	7.97, d (16.0)		
8′	115.3, CH	6.65, d (16.0)	115.3, CH	6.65, d (16.0)		
9′	167.1, qC		167.1, qC			

tion. A similar approach resulted in the isolation of compounds **3/4**, **5**−**8**, and **9/10** from fractions SDE-B3, SDE-B11, and SDE-B12, respectively. The structures of compounds **1**−**10** were established by 1D and 2D NMR experiments and comparison of their physical and spectroscopic data with those previously reported in the literature.[8−13] Among the 10 pure compounds only the lupane caffeate ester **2** showed significant activity in the glucose uptake assay.

Compound **1** was obtained as a white powder with IR absorption bands at 3445, 1705, 1684, 1269, and 1176 cm^{-1}, suggesting the presence of hydroxyl, α,β-unsaturated ester, and an isolated C═C bond group in the molecule. The HREIMS gave an accurate ion peak at *m/z* 620.0769 corresponding to the molecular formula $C_{39}H_{56}O_6$ (calcd 620.5834), which was also in agreement with ^{13}C NMR and DEPT data. The ^{13}C NMR spectrum in C_5D_5N (Table 1) indicated the presence of 39 unique carbon atoms assigned to six methyl groups, 11 methylenes including two hydroxymethyl, 12 methines including three olefinic, three aromatic, and one hydroxymethine, and 10 quaternary carbons including one olefinic, one carbonyl, and three aromatics. The ^1H NMR spectrum of **1** (Table 2) exhibited three downfield signals typical of a 1,2,4-trisubstituted aromatic ring at δ 7.20 (dd, J = 8.7, 1.8 Hz, H-6′), 7.23 (d, J = 8.7 Hz, H-5′), and 7.64 (d, J = 1.8 Hz, H-2′). The chemical shifts of the three aromatic protons combined with those due to a *trans*-conjugated double bond [δ 7.98 (d, J = 16.0 Hz, H-7′) and 6.65 (d, J = 16.0 Hz, H-8′)] were strongly suggestive of

Table 2. Effective Concentration for 50% Increase in Glucose Uptake in C2C12 Cells

fraction or compound tested	EC_{50}
SDE	25.5[a]
SDE-B	1.31[a]
SDE-B10	1.28[a]
SDE-B10-D	1.7[a]
compound **2**	1.47[b]

[a] μg/mL. [b] μM.

a caffeic acid ester moiety. Indeed these signals were essentially identical to signals for known caffeic acid esters.[6−9] Two doublets [δ 0.93 (d, J = 6.0 Hz, H-30) and 0.91 (d, J = 6.0 Hz, H-29)] and four singlets for six methyl groups in the ^{1}H NMR and olefinic signals at δ 5.22 (bs) combined with carbon resonances at δ 124.5 and 139.2 in the ^{13}C NMR spectrum indicated the presence of a Δ^{12}-ursene skeleton in **1**.[9,10]

The oxidation pattern in **1** was deduced from the presence of signals for two isolated hydroxymethyl groups [δ 3.90 (d, J = 10.8 Hz, H-23a), 3.47 (d, J = 10.8 Hz, H-23b), 3.64 (d, J = 11.2 Hz, H-28a), 3.57 (d, J = 11.2 Hz, H-28b)] and a hydroxymethine at δ 5.58 (dd, 11.6, 4.4 Hz, H-3). These data combined with the six remaining methyl groups in the molecule were consistent with the structure of **1** as 24,28-dihydroxyursene caffeic acid ester. Structural confirmation and specific assignments of the protons in **1**, in particular those of the two hydroxymethyl groups, were provided by a combination of COSY, HMQC, and HMBC data. Specifically, the HMBC spectrum displayed long-range correlations from the methylene protons at δ 3.90 (H-23a) and 3.47 (H-23b) to the carbon signals at δ 74.2 (C-3) and 13.6 (C-24), from the methylene protons at δ 3.64 (H-28a) and 3.57 (H-28b) to the carbon signal at δ 54.3 (C-18), and from the methine proton at δ 5.58 (H-3) to the carbonyl carbon of caffeic acid at δ 167.1 (C-9′). On the basis of these data, compound **1** was assigned as 23,28-dihydroxyursan-12-ene-3β-caffeate. The complete ^{1}H and ^{13}C chemical shift assignments based on the COSY, HMQC, and HMBC data are shown in Table 1.

Compound **2** was obtained as a white powder from the same fraction as compound **1**. The spectroscopic properties of **2** were similar to those of **1**. IR absorption bands at 3440, 1701, 1684, 1271, and 1180 cm^{-1} indicated the presence of the same functional groups as in **1**. The molecular formula was established by HREIMS to be $C_{39}H_{56}O_6$ (ion peak at 620.4076), isomeric with **1**. The ^{1}H and ^{13}C NMR spectra showed signals assignable to a caffeic acid residue (Table 1), and the remaining 30 carbon resonances indicated that **2** was also a triterpene caffeic acid ester.

The ^{1}H NMR spectrum showed signals at δ 4.86 (d, J = 2.4 Hz) and 4.72 (d, J = 2.4 Hz) that were characteristic of the terminal methylene group (H-29a and H-29b) found in the lupane family of compounds; the ^{13}C peak at δ 109.6 confirmed this assignment. The remaining carbon signals were consistent with the lupane skeleton (Table 1). The combination of five remaining methyl singlets, including one at δ 1.74 due to the C30 olefinic methyl group, and the requisite number of methylenes, methine, and quaternary carbons confirmed the lupane structure for **2**. The appearance of two isolated hydroxymethyl groups [δ 3.63 (d, J = 11.2 Hz, H-23a), 3.56 (d, J = 11.2 Hz, H-23b), 4.07 (d, J = 10.6 Hz, H-28a), 3.65 (d, J = 10.6 Hz, H-28b)] and one hydroxymethine at δ 5.59 (dd, 11.6, 4.8 Hz, H-3) in the ^{1}H NMR spectrum was consistent with the structure assignment for **2** as 23,28-dihydroxy-lupan-12-ene-3β-caffeate. Further support for this structure was obtained from the HMBC data, which displayed long-range correlations from the methylene protons H-23a and H-23b to the carbon signals of C-3 and C-24, from the methylene protons of H-28a and 3.57 H-28b to the carbon signal of C-18, from the methine proton of H-3 to the carbonyl carbon of caffeic acid of C-9′, and from the methylene protons of H-29a and H-29b to the methyl carbon of C-30 and carbon signal of C-19. Complete ^{1}H and ^{13}C chemical shift assignments for **2**, based on COSY, HMQC, and HMBC data, are shown in Table 1.

Compound **3**, also obtained as a white powder, showed IR absorption bands at 3460 and 1709 cm^{-1}, suggesting the presence of OH and alkene groups in the molecule. The molecular formula, determined to be $C_{30}H_{50}O_3$ by HREIMS (m/z 458.3760, calcd 458.7162), was typical of an oxygenated triterpene. The NMR data of **3** were very similar to those of compound **1**, except for the absence of signals for the caffeoyl unit (Table 1). The data in **3**, for its triterpene moiety, were almost identical with those of compound **1**, with the exception of the expected upfield (1.4 ppm) shift of the signals for H-3 [δ 4.20 (br d, J = 10.4 Hz)] in **3** versus δ 5.58 in **1** and further indicated that the caffeoyl unit in **1** was connected to O-3. These data showed that compound **3** was 3β,23,28-trihydroxy-12-ursene.[8−11]

In addition, the well-known compounds 23-hydroxybetulin (**4**), uvaol (**5**), betulin (**6**), α-amyrin (**7**), betulinic acid (**8**), (+)-catechin, and (−)-epicatechin were also isolated (see Experimental Section). The structures of these compounds were established by comparison of their spectroscopic data with those reported in the literature.[8−13]

The information generated in this study indicates that the bark of *S. decora* and one of its major components, compound **2**, have significant activity in antidiabetic studies in vitro. The results also lend scientific support to the use of this species for diabetes in traditional medicine of the Cree First Nation. This work is also in agreement with a previous investigation carried out by our research group where we described caffeic acid ester derivatives as potential leads in diabetic therapy.[14]

Glucose uptake results in the C2C12 cell line were concentration dependent when treated with active extracts or the purified active principle (**2**). The EC_{50} values estimated using linear regression of the uptake versus log C (concentration) varied from 1.28 to 25.5 μg/mL (Table 2). These results show that the fractions have potent concentration-dependent glucose uptake activity and suggest that compound **2** is the most likely component responsible for the antidiabetic effects observed in vivo. Further work is needed to confirm the mode of action of **2**.[7]

Experimental Section

General Experimental Procedures. The final preparative scale isolation of the phytochemicals was undertaken using a reversed-phase Gemini Axia 250 × 21.2 mm column, particle size 10 μm (Phenomenex Inc., Torrance, CA), on an Agilent 1200 Series preparative HPLC system comprising a binary pump (flow rate range 5−100 mL/min), an autosampler with a 2 mL loop, a diode array detector with a flow cell (path length 3 mm and maximum pressure limit 120 bar). and a fraction collector (40 mL collection tubes). IR spectra were recorded on a Shimadzu 8400-S FT/IR spectrometer. Optical rotations were registered on a Perkin-Elmer 241 digital polarimeter. NMR spectra were recorded on a Bruker Avance 400 spectrometer in C_5D_5N, at either 400 MHz (^{1}H) or 100 (^{13}C) MHz, using tetramethylsilane (TMS) as an internal standard. EIMS and HREIMS were obtained on a Kratos Concept IIH mass spectrometer. Open column chromatography was carried out on silica gel 60 (70−230 mesh, Merck). TLC analyses were performed on silica gel 60 F_{254} plates (Merck), and visualization of the plates was carried out using a ceric sulfate (10%) solution in H_2SO_4.

Plant Material. Stem bark (1.5 kg) of *S. decora* was collected in Mistissini, Quebec, in August 2007 and identified by Alain Cuerrier from the Institut de Recherche en Biologie Vegetal, Universite de Montreal; a voucher specimen (MIS 03-9) was deposited at the Marie Victorin Herbarium of the Jardin Botanique de Montreal.

Extraction and Isolation. Dried and shredded stem bark (1.6 kg) was extracted twice with EtOH (80% in H_2O) during 24 h each at room temperature. The first extraction was made using 16.0 L of solvent and the second extraction using 8.0 L. The combined extracts were evaporated in vacuo to yield 360 g of a brown residue (crude extract). Dried extract (200 g) (SDE) was partitioned between hexanes, EtOAc, and MeOH−H_2O (1:1) using a continuous liquid−liquid extractor. Evaporation of solvent in vacuo at 30 °C afforded 16.0 g of hexanes fraction (SD-A), 65.0 g of EtOAc fraction (SD-B), and 115.0 g of aqueous fraction (SD-C). Fraction SD-B (60 g) resulting from the partition process was chromatographed in a glass column packed with silica gel (1.0 kg) eluting with hexanes−EtOAc (1:0 → 0:1) and EtOAc−MeOH (1:0 → 0:1) to yield 13 secondary fractions (SD-B1−SD-B13). Fraction SD-B3 (500 mg), eluted with hexanes−EtOAc (8:2), was separated by preparative scale HPLC using a reversed-phase Gemini Axia 250 × 21.2 mm column, particle size 10 μm (Phenomenex Inc., Torrance, CA), using an isocratic mobile phase composition of 45% THF in 55% water at 37.5 mL/min to afford uvaol (**5**, 12.0 mg, 0.0008%), betulin, (**6**, 100 mg, 0.007%), α-amyrin (**7**, 6.5 mg, 0.0004%), and betulinic acid (**8**, 5.0 mg, 0.0003%) at the monitoring

wavelength of 210 nm, bandwidth 4, reference off. Fraction SDE-B10 (1.0 g), eluted with hexanes−EtOAc (3:7), was further chromatographed over a silica gel column (100 g) using hexanes−EtOAc (7:3 → 0:1) and EtOAc−MeOH (1:0 → 9:1) as the mobile phase to give five fractions (SDE-B10-A−SDE-B10-E). From fraction SDE-B10-D (300 mg), eluted with hexanes−EtOAc (4:6), 23,28-dihydroxyursen-12-ene-3β-caffeate (**1**, 15 mg, 0.001%) and 23,28-dihydroxylupan-12-ene-3β-caffeate (**2**, 20 mg, 0.0013%) were purified by preparative scale HPLC using a reversed-phase Gemini Axia 250 × 21.2 mm column, particle size 10 μm (Phenomenex Inc., Torrance, CA), using an isocratic mobile phase of 60% CH_3CN in 40% of 0.1% trifluoroacetic acid in water at a flow rate of 31.5 mL/min at the monitoring wavelength of 210 nm, bandwidth 4, reference off. Further column chromatography over silica gel (200 g) of SDE-B11 (2.5 g), using a gradient of hexanes−EtOAc (1:1 → 0:1) and EtOAc−MeOH (1:0 → 1:1) as mobile phase, yielded seven fractions (SDE-B11-1−SD-B11-7). From fraction SDE-B11-3 (400 mg), eluted with hexanes−EtOAc (2:8), 3β,23,28-trihydroxy-12-ursene (**3**, 12 mg, 0.0008%) and 23-hydroxybetulin (**4**, 200 mg, 0.013%) were purified by preparative scale HPLC using a reversed-phase Gemini Axia 250 × 21.2 mm column, particle size 10 μm (Phenomenex Inc., Torrance, CA), using a linear gradient of 5−100% CH_3CN in water at a flow rate of 20 mL/min at the monitoring wavelength of 210 nm, bandwidth 4, reference 360, 100. Finally, fraction SDE-B12 (1.2 g), eluted with EtOAc−MeOH (9.5:0.5), was also subjected to column chromatography on silica gel (200 g) using a gradient of hexanes−EtOAc (2:8 → 0:1) and EtOAc−MeOH (1:0 → 1:1) to give 10 fractions (SDE-B12-1−SDE-B12-10). From fraction SDE-B12-5 (100 mg), eluted with EtOAc (100%), (+)-catechin (5.0 mg, 0.0003%) and (−)-epicatechin (25.0 mg, 0.001%) were purified by preparative scale HPLC using a reversed-phase Gemini Axia 250 × 21.2 mm column, particle size 10 μm (Phenomenex Inc., Torrance, CA), using a linear gradient of 5−100% CH_3CN in water at a flow rate of 35 mL/min and the monitoring wavelength of 280 nm, bandwidth 4, reference off.

23,28-Dihydroxyursen-12-ene-3β-caffeate (1): white powder (MeOH); $[\alpha]^{25}_D$ +64.7 (*c* 0.00139, MeOH); UV (MeOH) λ_{max} (log ε) 220 (2.81), 245 (2.64), 295 (2.77), 330 (2.87) nm; IR (KBr) ν_{max} 3445, 2926, 1705, 1684, 1363, 1269, 1176 cm^{-1}; ^{13}C and ^1H NMR, see Table 1; EIMS *m/z* 620 $[M]^+$ (10), 602 $[M - H_2O]^+$ (18), 440 $[M - C_9O_4H_8]^+$ (35); HREIMS *m/z* 621.0769 $[M + H]^+$ (calcd for $C_{39}H_{56}O_6$: 620.5834).

23,28-Dihydroxylupan-12-ene-3β-caffeate (2): white powder (MeOH); $[\alpha]^{25}_D$ +52.5 (*c* 0.00162, MeOH); UV (MeOH) λ_{max} (log ε) 220 (2.90), 245 (2.77), 295 (2.90), 330 (2.95) nm; IR (KBr) ν_{max} 3440, 1701, 1684, 1363, 1271, 1180 cm^{-1}; ^{13}C and ^1H NMR, see Tables 1 and 2; EIMS *m/z* 620 $[M]^+$ (8), 602 $[M - H_2O]^+$ (15), 440 $[M - C_9O_4H_8]^+$ (25); HREIMS *m/z* 621.4076 $[M + H]^+$ (calcd for $C_{39}H_{56}O_6$: 620.7482).

3β,23,28-Trihydroxy-12-ursene (3): white powder (MeOH); $[\alpha]^{25}_D$ +57.4 (*c* 0.00122, MeOH); UV (MeOH) λ_{max} (log ε) 210 (2.66) nm; IR (KBr) ν_{max} 3460, 2925, 1709, 1361, 1218, 1040 cm^{-1}; ^{13}C and ^1H NMR, see Tables 1 and 2; EIMS *m/z* 458 $[M]^+$ (12), 440 $[M - H_2O]^+$ (23), 422 $[M - 2H_2O]^+$ (32); HREIMS *m/z* 459.3760 $[M + H]^+$ (calcd for $C_{30}H_{50}O_3$: 458.7162).

Glucose Uptake Assay. The deoxy-D-glucose uptake assay was performed as described previously (Spoor et al., 2006), with a few modifications.[4] Confluent and differentiated C2C12 myotubes (in 12-well plates) were incubated for 18 h in differentiation medium (DMEM with 2% HS) containing different concentrations of crude extract or fractions (15, 10, 5, 2, and 1 μg/mL) or pure compounds (15, 10, 5, 2, and 1 μM) of *Sorbus decora.* DMSO (0.1%) and metformin (400 μM) were taken as negative and positive controls, respectively. After 18 h, cells were washed twice with prewarmed (37 °C) Krebs phosphate buffer, pH 7.4 (136 mM NaCl, 20 mM HEPES, 4.7 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 4.05 mM Na_2HPO_4, 0.95 mM NaH_2PO_4), containing glucose (5 mM) and then incubated in the same buffer for 30 min at 37 °C. After 30 min, cells were rinsed three times with Krebs phosphate buffer, pH 7.4 (37 °C), with no glucose, and glucose uptake was initiated by the addition of 500 μL (in each well) of Krebs phosphate buffer (37 °C) containing 10 μM 2-deoxy-D-glucose and 1 μCi/mL [^{3}H-]-2-deoxy-D-glucose and incubated for 10 min at 37 °C. After 10 min incubation, cells were rinsed three times with cold Krebs phosphate buffer (4 °C) containing glucose (5 mM) and were lysed with 500 μL of 0.1 M NaOH for 30 min at RT. Lysates were added to 4 mL of liquid scintillation cocktail (Ready-gel, Beckman Coulter Inc.), and radioactivity from [^{3}H-]-2-deoxy-D-glucose incorporated into cells was measured in a scintillation counter. Repeated measurement analysis of variance (ANOVA) was used to analyze the changes in the glucose uptake. Dunnett range posthoc comparisons were used to determine the source of significant differences where appropriate; $p < 0.05$ was considered statistically significant. Prisma Graph-Pad (version 5.0) software was used for statistics and plotting.

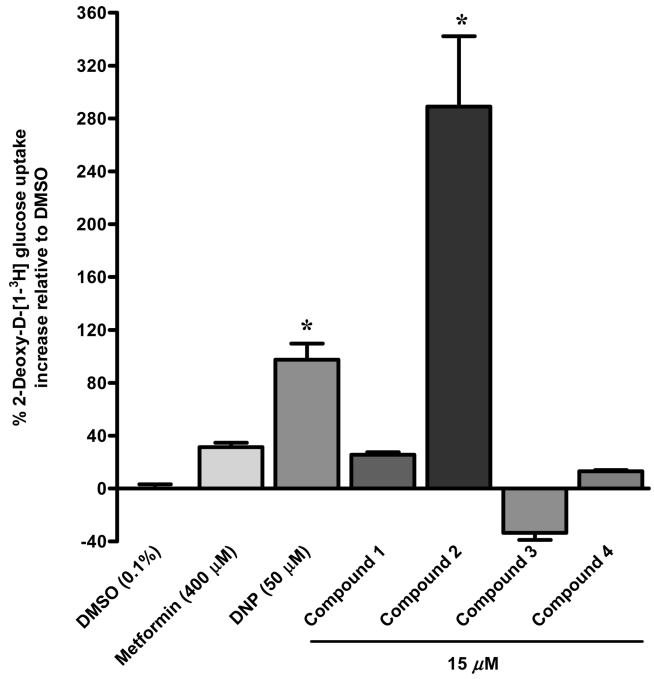

Figure 2. Effect of compounds **1**−**4** of *S. decora* at the maximum concentration (15 μM) tested on glucose uptake assay in C2C12 cells. Each value is the mean ± SEM ($n = 4$ wells) in each group. *$p < 0.05$ significantly different after one-way ANOVA analysis followed by Dunnett's *t* test vs the negative control group (DMSO 0.1%).

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Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds **1**−**3** are available free of charge via the Internet at http://pubs.acs.org.

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