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Cytotoxic Cardiac Glycosides and Other Compounds from *Asclepias syriaca*

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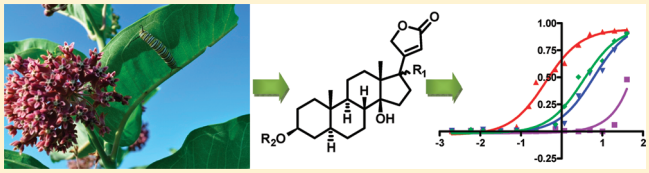
Supporting Information

ABSTRACT: Phytochemical investigation of the dried biomass of *Asclepias syriaca* afforded five new compounds (**1**–**5**), along with 19 known structures. Overall, the secondary metabolites isolated and identified from this plant showed a wide structural diversity including pentacyclic triterpenes, cardiac glycosides, flavonoid glycosides, lignans, a phenylethanoid, and a glycosylated megastigmane. In addition, the isolates were tested against the cancer breast cell line Hs578T, and those showing IC_{50} values lower than 50 μM (**1** and **6**–**9**) were further investigated in three additional breast cancer cell lines (MCF-7, T47D, and Sk-Br-3) and the normal breast cell line Hs578Bst.



Asclepias syriaca L. (Asclepiadaceae), also known as "common milkweed", has received a great deal of attention due to its potential as an alternative crop for different products such as natural rubber from latex, alternative fuels, and fibers for paper fabrication. Although promising, several factors prevented its commercialization.[1,2] Nevertheless, in recent years *A. syriaca* has received industrial crop status in the United States due to the use of its silky seed floss in hypoallergenic pillows, comforters, and insulating fiber manufacture.[3] Conversely, the remaining plant biomass is typically disposed without regard to its potentially valuable products.[4] In fact, the boiled or infused roots, whole plant preparations, and the milky latex of *A. syriaca* have been used medicinally as an expectorant, against asthma, and as an emetic and cathartic.[1,5] Also, the plant or its latex have been used by the Cherokee and other tribes for wort removal, treatment of venereal disease, edema, and kidney stones.[6] Furthermore, the boiled young sprouts, floral buds, and immature fruits have been used historically and currently as food, especially in soup, by the Omaha, Dakota, Pawnee, Ponca, and Winnebago tribes.[7,8] The bitter-tasting compounds have been shown to be removed by four minutes of boiling then changing the water to complete the cooking process, which presumably removes the toxic glycosides.[1] Previous phytochemical investigations of this species, using plant material obtained from Japan, have reported mainly pregnane and cardiac glycosides, as well as glycosylated flavonoids.[9−13] Therefore, as part of our ongoing effort to study the chemical diversity and medicinal potential of the flora from Kansas, we investigated the common milkweed with the aim of identifying bioactive molecules with potential therapeutic applications, and the results are presented herein.

RESULTS AND DISCUSSION

The dried plant material of *A. syriaca* was extracted exhaustively with mixtures of MeOH and DCM, 1:1 (v/v). After organic solvent removal, the resulting extract was subjected to a series of separation steps including liquid−liquid partition, recrystallization, and a number of normal- and reverse-phase chromatographic techniques. Five new compounds were isolated and identified including the cardiac glycoside **1** (4.5 mg), the quercetin triglycoside **2** (24.7 mg), the neolignan **3** (16.1 mg), the phenylethanoid **4** (7.8 mg), and the megastigmane glycoside **5** (6.5 mg), along with 19 known compounds (Scheme 1). The known compounds included the pentacyclic triterpenes α- and β-amyrin, α- and β-amyrin acetate, lupeol acetate, and oleanolic acid;[14,15] the cardiac glycosides 3-*O*-β-D-glucopyranosyl-(1→4)-6-deoxy-β-D-allopyranosyluzarigenin (**6**), uzarin (**7**), and desglucouzarin (**8**);[16] the glycosylated flavonoids quercetin 3-*O*-β-galactopyranosyl-(1→2)-β-xylopyranoside, kaempferol 3-*O*-β-galactopyranosyl-(1→2)-β-xylopyranoside (**9**), 3′-*O*-methylquercetin 3-*O*-β-galactopyranosyl-(1→2)-β-xylopyranoside, and quercetin 3-*O*-β-galactopyranoside;[13,17] the lignans episyringaresinol and prupaside,[18,19] 4-(β-glucopyranosyloxy)benzoic acid;[20] and other low MW phenolics (see Experimental Section). The structures of the new compounds were elucidated using a range of spectroscopic techniques, including 1D and 2D NMR and HRMS. In the case of the known compounds, their structures were identified by comparison of their measured spectroscopic data with literature values.

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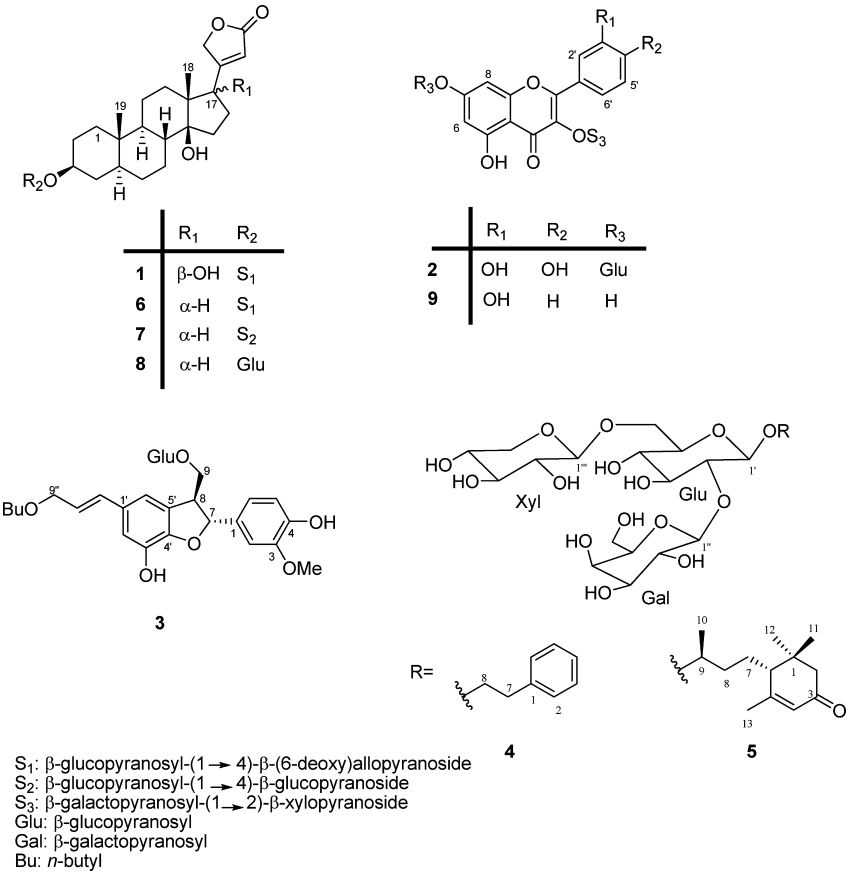
Scheme 1. Structure of Compounds 1−9



Compound **1** was isolated as a white, amorphous powder. The HRMS showed an $[M + Na]^+$ ion at m/z 737.3388, suggesting a molecular formula of $C_{35}H_{54}NaO_{15}$ (calcd 737.3360). The ^{1}H NMR spectrum of **1** showed the characteristic proton signals of the butenolide ring at δ_H 6.26 (dd, J = 1.8, 1.7 Hz, H-22), 5.23 (dd, J = 18.3, 1.7 Hz, H-21a), and 5.09 (dd, J = 18.3, 1.8 Hz, H-21b). The cardenolide steroidal tetracyclic ring system was confirmed using the key HMBC correlations between CH_3-18 (δ_H 1.22, s, H-18) and C-1, C-2, C-5, and C-10 and between CH_3-19 (δ_H 0.67, s, H-19) and C-12, C-14, C-15, and C-17 (Table 1). Moreover, the ^{1}H,^{1}H−COSY, HSQC, and HMBC experiments permitted full assignment of the signals in the aglycone that was identified as 17β-hydroxyuzarigenin. The spectroscopic data of the aglycone were in agreement with previously reported values of similar compounds.[10] Two anomeric proton signals at δ_H 5.42 (d, J = 7.9 Hz, H-1′) and 5.10 (d, J = 7.6 Hz, H-1″) and the corresponding carbon resonances at δ_C 99.7 (CH, C-1′) and 106.8 (CH, C-1″) suggested the presence of an equal number of sugar units, and after assignment of the NMR data using 1D and 2D NMR experiments, these were identified as 6-deoxyallose and glucose, both with a β-linkage based on the coupling constants of the anomeric protons. In addition, HMBC correlations between H-1′ and C-3 and between H-1″ and C-4′ clearly established the connectivity of the sugars. When taken in conjunction with the acid hydrolysis results (Experimental Section), the structure of compound **1** was determined to be 3-*O*-β-D-glucopyranosyl-(1→4)-6-deoxy-β-D-allopyranosyl-17β-hydroxyuzarigenin. Although the 17β-hydroxyuzarigenin has been reported with other C-3 sugar moieties,[10] compound **1** represents a new structure.

Compound **2** was isolated as a yellow, amorphous powder, and the molecular formula of $C_{32}H_{38}NaO_{21}$ was suggested on the basis of the HRMS $[M + Na]^+$ ion at m/z 781.1816 (calcd 781.1803). Two aromatic rings were assigned by inspection of the ^{1}H NMR data: the first showing two signals at δ_H 6.73 (d, J = 2.2 Hz, H-6) and 6.78 (d, J = 2.2 Hz, H-8); the second with three signals at δ_H 8.32 (d, J = 2.3 Hz, H-2′), 7.31 (d, J = 8.6 Hz, H-5′), and 8.36 (dd, J = 8.6, 2.3 Hz, H-6′). The presence of 12 aromatic carbon resonances was confirmed by ^{13}C NMR (Table 1). The observed oxygenation pattern in the aromatic rings, the presence of an additional carbonyl at δ_C 179.3 (C-4), and two olefinic carbons at δ_C 157.5 (C-2) and 135.5 (C-3) suggested a flavonol carbon skeleton, which was confirmed by HMBC correlations and corroborated by comparison with literature data of related structures.[17,21] In addition, three sugar units were identified on the basis of the presence of the same number of anomeric proton signals at δ_H 6.62 (d, J = 7.7 Hz, H-1″), 5.48 (d, J = 7.2, H-1‴), and 5.76 (d, J = 7.8, H-1⁗). Using 1D and 2D NMR data, the signals of the sugar units were fully assigned (Table 1) and their identities were established as β-glucose, β-galactose, and β-xylose. The correlations observed in the HMBC experiment between the glucosyl anomeric proton (H-1⁗) and C-7, between the galactosyl anomeric proton (H-1″) and C-3, and between the xylosyl anomeric proton (H-1‴) and C-2″ established the connectivity of the sugar units. Consequently, the structure of **2** was defined as quercetin-7-*O*-β-glucopyranosyl-3-*O*-β-D-galactopyranosyl-(1→2)-β-D-xylo-

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Table 1. NMR Spectroscopic Data (500 MHz, Pyridine-d_5) for Compounds 1 and 2

	compound 1			compound 2			
position	δ_C, type	δ_H (J in Hz)	HMBC[a]	position	δ_C, type	δ_H (J in Hz)	HMBC[a]
1	37.6, CH_2	1.65, m 0.96, m	2, 10, 19	2	157.5, C		
2	30.4, CH_2	2.10, m 1.66, m	1, 3	3	135.5, C		
3	77.5, CH	3.92, m 1.78, m	2, 1′	4	179.3, C		
4	35.1, CH_2	1.36, ddd (12.6, 12.5, 11.5)	3, 5	4a	107.3, C		
5	44.6, CH	0.90, m	4, 6	5	162.7, C		
6	29.2, CH_2	1.15, m 1.10, m	5, 7	6	100.6, CH	6.73, d (2.2)	4a, 5, 7, 8
7	27.3, CH_2	1.12, m 2.27, m	6, 8	7	164.1, C		
8	41.8, CH	1.75, m	7, 9	8	94.6, CH	6.78, d (2.2)	6, 7, 8a
9	50.1, CH	0.79, ddd (12.1, 11.9, 3.2)	8, 10	8a	157.1, C		
10	36.3, C			1′	123.6, C		
11	21.6, CH_2	1.41, m 1.11, m	9, 12	2′	118.0, CH	8.32, d (2.3)	2, 1′, 3′, 4′, 6′
12	33.6, CH_2	1.06, m 0.95, m	11, 13	3′	151.1, C		
13	52.2, C			4′	147.4, C		
14	88.0, C			5′	116.5, CH	7.31, d (8.6)	1′, 3′, 4′, 6′
15	31.7, CH_2	2.02, m 2.12, m	14, 16	6′	122.9, CH	8.36, dd (8.6, 2.3)	2, 1′, 2′, 4′, 5′
16	37.6, CH_2	2.36, m	15, 17				
17	87.0, C			1″	100.9	6.62, d (7.7)	3, 3″
18	13.4, CH_3	1.22 s	1, 6, 9, 10	2″	82.2	4.94, dd (9.3, 7.7)	1″, 3″, 1‴
19	12.5, CH_3	0.67 s	12, 13, 14, 17	3″	76.0	4.30, m	4″
20	173.6, C			4″	70.2	4.57, m	3″, 5″, 6″
21	73.7, CH_2	5.23, dd (18.3, 1.7) 5.09, dd (18.3, 1.8)	20, 22	5″	79.6	4.15, m	1″, 3″, 6″
22	117.0, CH	6.26, dd (1.8, 1.7)	17, 21	6″	62.3	4.33, m	5″
23	174.4,C						
1′	99.7, CH	5.42, d (7.9)	3, 3′	2‴	76.1	4.24, m	1‴, 4‴
2′	72.5, CH	3.96, m	1′	3‴	78.2	4.14, m	2‴, 4‴
3′	72.9, CH	5.10, brd (7.8)	4′, 5′	4‴	71.4	4.17, m	3‴
4′	84.0, CH	3.87, dd (9.6, 2.5)	5′, 6′, 1″	5‴	67.6	4.41, m 3.68, dd (11.2, 9.2)	1‴, 3‴, 4‴
5′	69.2, CH	4.56, dq (9.4, 6.2, 6.2, 6.2)	1′, 4′, 6′				
6′	18.9, CH_3	1.76, d (6.2)	4′, 5′	1⁗	102.1	5.76, d (7.8)	7
				2⁗	75.2	4.33, m	1⁗, 3⁗
1″	106.8, CH	5.10, d (7.6)	4′, 3″	3⁗	78.9	4.42, m	2⁗, 4⁗
2″	75.6, CH	4.02, m	3″	4⁗	71.5	4.36, m	3⁗, 5⁗
3″	78.7, CH	4.28, m	2″, 4″	5⁗	78.3	4.14, m	4⁗, 6⁗
4″	72.0, CH	4.29, m	3″, 5″, 6″	6‴′	62.8	4.56, m 4.42, m	4⁗, 5⁗
5″	78.6, CH	3.98, m	4″, 3″				
6″	62.9, CH_2	4.48, ddd (11.8, 5.1, 2.6) 4.38, ddd (11.8, 5.9, 5.1)	4″, 5″				

[a]HMBC correlations are from proton(s) to the indicated carbon.

pyranoside. This compound has not been previously reported, and we named it syriacatin.

Compound **3** was isolated as a white, amorphous powder. The HRMS $[M + Na]^+$ ion at m/z 585.2298 suggested a molecular formula of $C_{29}H_{38}NaO_{11}$ (calcd 585.2312). Two independent aromatic spin systems were identified in the ^1H NMR spectrum: the first with only two signals at δ_H 7.36 (d, J = 1.4 Hz, H-2′) and 7.41 (d, J = 1.4 Hz, H-6′), suggesting a 1,2,3,5-tetrasubstitution pattern, and the second displaying three signals at δ_H 7.34 (d, J = 1.6 Hz, H-2), 7.16 (d, J = 8.6 Hz, H-5), and 7.19 (dd, J = 8.6 Hz, 1.6, H-6), indicating a 1,2,4-trisubstituted ring. The previous observation was further confirmed by assignment of the corresponding 12 aromatic carbon resonances aided by HSQC and HMBC spectra (Table 2). Furthermore, two C_3 (propyl) equivalents linked to the aromatic rings were identified by means of $^1H,^1H$−COSY and HMBC spectra, suggesting the presence of a lignan structure. The first propyl fragment showed two vinylic protons at δ_H

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Table 2. NMR Spectroscopic Data (500 MHz, Pyridine-d_5) for Compound 3

position	δ_C, type	δ_H (J in Hz)	HMBC[a]
1	133.3, C		
2	111.2, CH	7.34, d (1.6)	1, 3, 4, 7
3	149.1, C		
4	148.5, C		
5	116.8, CH	7.16, d (8.6)	1, 3, 4, 6
6	120.1, CH	7.19, dd (8.6, 1.6)	1, 2, 4, 5, 7
7	88.8, CH	5.99, d (6.5)	1, 2, 6, 8, 9, 4′, 5′
8	52.5, CH	4.08, m	1, 7, 9, 4′, 5′, 6′
9	71.9, CH_2	4.66, m	7, 8, 5′, 1‴
		4.44, m	
1′	132.1, C		
2′	114.9, CH	7.36, d (1.4)	1′, 3′, 4′, 6′
3′	143.3, C		
4′	148.5, C		
5′	133.0, C		
6′	116.4, CH	7.41, d (1.4)	8, 1′, 2′, 4′, 5′
7′	130.2, CH	6.78, d (16.0)	1′, 2′, 6, 8, 9
8′	124.9, CH	6.47, ddd (16.0, 6.0, 6.0)	1′, 9′
9′	72.1, CH_2	4.12, m	8′, 7′, 1⁗
1″	105.2, CH	5.03, d (7.6)	9, 3″
2″	75.5, CH	4.12, m	1″, 4″
3″	79.0, CH	4.30, m	2″, 4″, 5″
4″	72.0, CH	4.29, m	3″, 5″
5″	79.1, CH	4.02, m	4″, 6″
6″	63.0, CH_2	4.65, m	4″, 5″
		4.45, m	
9′-OBu			
1⁗	70.3, CH_2	3.43, dd (6.5, 6.5)	9′, 2⁗, 3⁗
2⁗	32.6, CH_2	1.57, dddd (7.5, 7.5, 6.5, 6.5)	1⁗, 3⁗, 4⁗
3⁗	20.0, CH_2	1.37, ddq (7.5, 7.5, 7.3, 7.3, 7.3)	1⁗′, 2⁗, 4⁗
4⁗	14.4, CH_3	0.85, dd (7.3, 7.3)	2⁗, 3⁗

[a]HMBC correlations are from proton(s) to the indicated carbon.

6.78 (d, J = 16.0 Hz, H-7′) and 6.47 (ddd, J = 16.0, 6.0, 6.0 Hz, H-8′) and an oxygenated methylene at δ_H 4.12 (2H, m, H-9′). The olefin signals for H-7′ and H-8′ clearly showed HMBC correlations with C-1′, thus indicating a linkage to the first aromatic ring. The second propyl equivalent showed two methine protons at δ_H 5.99 (d, J = 6.5 Hz, H-7) and 4.08 (m, H-8) and an oxygenated methylene at δ_H 4.66, 4.44 (each 1H, m, H-9). The HMBC correlations observed from H-2, H-5, H-6 to C-7 and from H-6′ to C-8, as well as the chemical shift of C-7 (δ_C 88.8, CH), typical of a benzylic ether linkage, suggested the presence of a 4′,7-epoxy-8,3′-neolignan, which was further confirmed by NMR data comparison with reported structures containing the same benzofuran neolignan skeleton.[22,23] The 7,8-*trans* relative configuration of the dihydropyran ring was proposed on the basis of the coupling constant of H-7 and the NOE signal observed between H-7 and H-9. Also, an anomeric proton at δ_H 5.03 (d, J = 7.6 Hz, H-1″) indicated the presence of a sugar moiety further identified as glucose using 2D NMR experiments. Furthermore, the spin system of an *n*-butoxy group was readily identified from a ^{1}H ^{1}H−COSY experiment. Finally, the HMBC correlations observed between the glucose anomeric proton (H-1″) and C-9 and between the butoxymethylene (H-1‴) and C-9′ established the linkage of the sugar and butyl units to the neolignan skeleton. Consequently, the structure of **3** was determined to be 9′-*O*-butyl-3-*O*-demethyl-9-*O*-*β*-D-glucopyranosyl dehydrodiconiferyl alcohol, differing from previously described neolignans by the presence of an unusual *O*-butyl substituent.[23] Although other plant-derived metabolites containing *n*-butyl substituents are known,[24,25] an artificial origin of compound **3** cannot be ruled out, as *n*-butanol was used during the initial liquid−liquid partition of the crude extract.

Compound **4** was obtained as an amorphous, white powder and displayed an $[M + Na]^+$ ion in HRMS at m/z 601.2087, suggesting a molecular formula of $C_{25}H_{38}NaO_{15}$ (calcd 601.2108). The ^{1}H NMR showed three aromatic signals from a monosubstituted benzene ring and two methylene signals corresponding to a phenylethanoid group and confirmed by ^{1}H,^{1}H-COSY and HMBC correlations. In addition, the ^{1}H NMR spectrum also revealed three anomeric protons at δ_H 4.83 (d, J = 7.8 Hz, H-1′), 5.33 (d, J = 7.9 Hz, H-1″), and 4.88 (d, J = 7.0 Hz, H-1‴), which were used as starting points to fully characterize each structure using ^{1}H,^{1}H-COSY, HSQC-TOCSY, HSQC, and HMBC experiments. Hence, the sugar moieties were identified as *β*-galactose, *β*-glucose, and *β*-xylose. The connectivity of the phenylethanoid and sugar moieties was elucidated using the HMBC correlations between the galactosyl anomeric proton (H-1′) and C-8, the glucosyl anomeric proton H-1″ and C-2′, and the xylosyl anomeric proton H-1‴ and C-6′. Thus, compound **4** was determined to be phenylethyl-*β*-D-glucopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→6)]-*β*-D-galactopyranoside, and we named it kansanoside A. The occurrence of phenylethanoids is not common in the Asclepiadaceae family,[26,27] and this is the first report of this compound class in the *Asclepias* genus.

Finally, compound **5**, an amorphous, white powder, showed a HRMS $[M + Na]^+$ ion at m/z 689.2974, suggesting a molecular formula of $C_{30}H_{50}NaO_{16}$ (calcd 689.2974). Three anomeric protons at δ_H 4.87 (d, J = 7.7 Hz, H-1′), 5.23 (d, J = 7.8 Hz, H-1″), and 4.89 (d, J = 7.0 Hz, H-1‴) indicated the presence of three sugar units that, after comparison of their NMR data (Table 3), were determined to be the same as those present in compound **4**. In addition, a total of 13 carbons were left to be assigned: four methyls, three methylenes, a trisubstituted olefin, two methines, a quaternary carbon, and a conjugated carbonyl. Several HMBC correlations suggested a megastigmane carbon skeleton, namely, of H-12 with C-1, C-2, C-6, and C-13; H-13 with C-1, C-2, C-6, and C-12; H-2 with C-1, C-3, and C-4; H-4 with C-3, C-13, and C-6; H-6 with C-1, C-4, C-7, and C-8; and H-10 with C-9 and C-8. The proposed skeleton was confirmed by the ^{1}H,^{1}H-COSY cross-peaks showing the spin coupling sequence H-6, H-7, H-8, H-9, and H-10, as well as allylic coupling (J = 1.2 Hz) between H-13 and H-4. In addition, the NMR data were in agreement with reported data for structurally related compounds, including the proposed relative configuration.[28,29] Hence, compound **5** was established as 9-hydroxymegastigma-4-en-3-one *β*-D-glucopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→6)]-*β*-D-galactopyranoside and named oreadoside A. Although a couple of reports have identified megastigmane glycosides in *Asclepias* species,[30,31] this is the first account of this type of compound in *A. syriaca*.

The isolates were screened against the human cancer breast cell line Hs578T; however, only compounds showing IC_{50} < 50 *μ*M (data not shown) were chosen for further testing. Although most of the isolated compounds did not show cytotoxicity in our preliminary screening, many of them have been reported as biologically active and could have potential value against other disease targets. Particularly, the highly abundant pentacyclic

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Table 3. NMR Spectroscopic Data (500 MHz, Pyridine-d_5) for Compounds 4 and 5

	compound 4			compound 5		
position	δ_C, type	δ_H (J in Hz)	HMBC[a]	δ_C, type	δ_H (J in Hz)	HMBC[a]
1	139.8, C			36.7, C		
2	130.3, CH	7.32, d (7.2)	1, 3, 4, 6, 7	48.0, CH_2	2.56, d (17.1)	1, 3, 11, 12
					2.10, d (17.1)	
3	128.0, CH	7.27, dd (7.4, 7.2)	1, 2, 4, 5	198.9, C		
4	126.8, CH	7.17, dd (7.4, 7.4)	2, 3, 5, 6	125.5, CH	5.95, br s	2, 6, 13
5	130.3, CH	7.32, d (7.2)	2, 3, 5, 6	166.0, C		
6	128.0, CH	7.27, dd (7.4, 7.2)	1, 2, 4, 5, 7	51.4, CH	1.82, m	1, 4, 5, 7, 11, 12, 13
7	36.9, CH_2	3.09, m	1, 2, 6, 8	26.1, CH_2	2.02, m	1, 5, 6, 8, 9
					1.59, m	
8	71.2, CH_2	4.26, m	1, 7, 1′	37.6, CH_2	1.70, m	1, 7, 9, 10, 1′
		3.75, m			1.80, m	
9				75.4, CH	4.01, m	7, 8, 10
10				20.2, CH_3	1.34 d (6.2)	8, 9
11				26.6, CH_3	1.01, s	1, 2, 6, 12
12				29.0, CH_3	0.93, s	1, 2, 6, 11
13				24.8, CH	1.95, d (1.2)	4, 5, 6
1′	103.2, CH	4.83, d (7.8)	8, 2′, 3′	101.3, CH	4.87, d (7.7)	9, 3′
2′	84.2, CH	4.08, dd (8.8, 7.8)	1′, 3′, 1″	84.2, CH	4.08, m	1′, 3′, 1″
3′	78.2, CH	4.29, m	2′, 4′	78.1, CH	4.26, m	2′, 4′
4′	69.7, CH	4.38, m	3′, 5, 6′	69.5, CH	4.34, m	3′, 5, 6′
5′	77.2, CH	4.01, m	1′, 3′, 4′, 6′	77.0, CH	4.02, m	1′, 3′, 4′, 6′
6′	69.8, CH_2	4.83, dd (11.5, 2.3)	4′, 5′, 1‴	69.9, CH_2	4.82, m	4′, 5′, 1‴
		4.24, m			4.21, m	
1″	106.9, CH	5.33, d (7.9)	2′, 3″	106.9, CH	5.23, d (7.8)	2′, 3″
2″	77.2, CH	4.13, m	1″, 3″	77.2, CH	4.12, dd (8.9, 7.8)	1″, 3″
3″	78.5, CH	4.28, m	2″, 4″	78.4, CH	4.26, m	2″, 4″
4″	71.9, CH	4.30, m	3″, 5″	71.9, CH	4.30, m	3″, 5″
5″	79.2, CH	3.98, m	3″, 4″, 6″	79.2, CH	3.97, ddd (9.4, 4.6, 2.6)	3″, 4″, 6″
6″	63.1, CH	4.56, dd (11.8, 2.5)	5″, 4″	63.1, CH_2	4.54, dd (11.5, 2.4)	5″, 4″
		4.43, dd (11.8, 4.9)			4.43, dd (11.5, 4.8)	
1‴	105.8, CH	4.88, d (7.0)	6′, 1‴, 3‴	105.8, CH	4.89, d (7.0)	6′, 1‴, 3‴
2‴	72.7, CH	4.48, dd (8.5, 7.0)	1‴, 3‴	72.7, CH	4.46, dd (8.4, 7.0)	1‴, 3‴
3‴	77.2, CH	4.13, m	2‴, 4‴	77.4, CH	4.12, dd (8.4, 3.3)	2‴, 4‴
4‴	74.8, CH	4.14, m	3‴, 5‴	69.5, CH	4.34, m	3‴, 5‴
5‴	67.12, CH_2	4.30, m	1‴, 3″, 4‴	66.9, CH_2	4.33, m	1‴, 3″, 4‴
		3.73, m			3.77, dd (11.8, 1.4)	

[a]HMBC correlations are from proton(s) to the indicated carbon.

Table 4. Cytotoxicity[a] (IC_{50}, μM, ± SD) Values of Compounds 1 and 6–9 against Breast Cancer Cell Lines MCF-7, T47D, SK-BR-3, and Hs578T and Normal Breast Cell Line Hs578Bst

	cell line				
compound	MCF-7	T47D	SK-BR-3	Hs578T	Hs578Bst
1	>40	>40	>40	>40	14.2 ± 3.3
6	5.3 ± 1.2	1.76 ± 0.21	2.52 ± 0.35	0.593 ± 0.051	0.043 ± 0.010
7	11.6 ± 2.5	9.5 ± 1.0	19.4 ± 1.6	4.58 ± 0.64	0.76 ± 0.51
8	17.0 ± 4.9	11.7 ± 1.3	18.1 ± 2.2	6.28 ± 0.27	1.05 ± 0.11
9	>40	>40	>40	>40	>40
digoxin	4.7 ± 3.0	0.66 ± 0.12	1.08 ± 0.21	0.251 ± 0.026	0.040 ± 0.017
digitoxigenin	9.1 ± 2.6	2.53 ± 0.34	4.42 ± 0.51	1.022 ± 0.058	0.15 ± 0.12
ouabain	10.6 ± 0.5	0.385 ± 0.026	0.403 ± 0.023	0.1100 ± 0.0059	0.006 ± 0.005
doxorubicin	1.54 ± 0.15	1.9 ± 1.1	0.210 ± 0.033	0.546 ± 0.055	0.18 ± 0.17

[a]Cytotoxicity is the average (n = 3) of calculated IC_{50}'s.

triterpenes (see Experimental Section) have been previously reported as chemopreventive,[32] anti-inflammatory,[33] and analgesic agents.[34,35]

The isolates that showed activity in the preliminary screening (**1**, **6**–**9**) were submitted for testing in a panel of three additional breast cancer cell lines (MCF-7, T47D, and Sk-Br-3) and a normal breast cell line (Hs578Bst), and the results are shown in Table 4. In addition, the classic cardiac glycosides digoxin, digitoxigenin, and ouabain were included for comparison purposes. The tested compounds displayed

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cytoxicity in a range of 0.59 to 40 μM, compound **6** being the most active across the panel of cell lines tested. Actually, the cytotoxicity observed for compound **6** was comparable to the positive controls doxorubicin and digoxin. Cardenolides **1** and **6**−**8** showed reduced cytotoxicity when compared to compound **6**, revealing the important role of the C-17 configuration as well as the nature and number of sugars attached to C-3. In addition, the relative potency of the classic cardiac glycosides tested here (ouabain > digoxin > digitoxigenin) is in agreement with previously reported data.[36,37] Even though the kaempferol glycoside **9** showed cytotoxicity during the initial screening (IC_{50} < 50 μM), further testing reveled low activity (IC_{50} > 40 μM) in all cancer cell lines and a low percentage of inhibition (32.7 ± 2.2%) at maximum concentration for the cancer cell line Hs578T.

Furthermore, cytotoxicity data from the paired breast human cell lines Hs578T and Hs578Bst revealed information about selectivity of tested compounds against malignant cells.[38] In our assay the tested compounds showed lower IC_{50} values against the normal cells than cancer cells (Table 4); however the toxicity (expressed as a percentage of the control at maximum concentration) was significantly higher in the cancer cells when compared with normal cells (Table 5). Further investigation is needed to explain the reason behind this behavior, but it can possibly be due to the significant growing rate differences between the two cell lines.

Table 5. Percentage of Toxicity[a] (%, ± SD) for Compounds 1 and 6−9 for the Paired Breast Cell Lines Hs578T (Cancer) and Hs578Bst (Normal)

	cell line	
compound	Hs578T	Hs578Bst
1	56 ± 10	38.2 ± 2.7
6	91.5 ± 3.7	51.7 ± 1.6
7	84 ± 11	54.7 ± 1.0
8	85.5 ± 8.2	51.6 ± 1.6
9	32.7 ± 2.2	13.9 ± 3.9
digoxin	93.5 ± 2.7	47.7 ± 6.8
digitoxigenin	92.3 ± 4.3	49.8 ± 6.9
ouabain	93.0 ± 3.8	55.8 ± 4.1
doxorubicin	98.32 ± 0.43	64.3 ± 6.1

[a]At maximum concentration (40 μM) expressed as percentage of control (n = 3, ± SD).

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were recorded with an OptiMelt automatic apparatus. IR spectra were obtained with a Thermo Nicolet Avatar 380 FT-IR. UV−vis spectra were recorded on a Varian Cary 50 scan; ^{1}H NMR, ^{13}C NMR, and 2D spectra were recorded with a Bruker Avance AV-III 500 with a dual carbon/proton cryoprobe. HRMS were conducted with an LCT Premier (Waters Corp., Milford, MA, USA). Semipreparative HPLC was conducted using an Agilent 1200 HPLC system with a Phenomenex Luna C_{18} column (5 μm, 250 × 10 mm), flow rate of 4.5 mL/min (ca. 160 bar), injection volume of 50 μL (ca. 10 mg of sample), and UV detection using a diode array. Preparative HPLC separations were done using an Agilent 1100 HPLC system with a Phenomenex Luna C_{18} column (5 μm, 250 × 21 mm), flow rate of 35 mL/min (ca. 35 bar), injection volume of 800 μL (ca. 100 mg of sample), and UV detection using a multiwavelength detector.

Plant Material. Above-ground biomass (stems, leaves, and flowers) of *A. syriaca* was collected on June 17, 2009, by Q. Long and R. Loring. The plant material was collected 100 miles east of NE Ohio and 1400 Road, Anderson, Kansas. The GPS location of the collection site was latitude 38.22676°, longitude 95.20080°. Botanical identification was performed by Kelly Kindscher, and a botanic specimen was deposited in the McGregor Herbarium of the University of Kansas (Hillary Loring 3547).

Plant Extraction and Isolation. *A. syriaca* fresh biomass (12 kg) was left to dry at room temperature; then the dry material (2.5 kg) was ground to a fine powder and extracted four times with a mixture of MeOH and DCM (1:1, v/v) at room temperature. The organic solvents (ca. 20 L) were removed under vacuum to afford 196.1 g (7.8% w/w based on dry biomass) of the crude organic extract. The extract was suspended in MeOH and H_2O, 9:1 (v/v, 1 L), and extracted with hexanes (3 × 500 mL). After removal of the MeOH, the volume of aqueous residue was adjusted to 500 mL with distilled H_2O and extracted successively with DCM (3 × 500 mL) and *n*-butanol (3 × 500 mL) to give DCM and *n*-BuOH fractions, respectively. After organic solvent removal, the DCM fraction (32.7 g) was partitioned using silica gel (350 g, 24−40 μm) flash chromatography using mixtures of hexanes and EtOAc as mobile phase. The resulting fractions were purified using an automated flash chromatography apparatus with prepacked silica gel columns (CombiFlash Teledyne Isco, San Diego, CA, USA) using different solvent systems. A mixture of α- and β-amyrin was obtained (1.1 g, 0.04% w/w based on dry biomass), and it was resolved by means of semipreparative HPLC for identification (isocratic 90% MeCN and 10% acetone mixture as mobile phase during 60 min). Also, a mixture of α- and β-amyrin acetates and lupeol acetate (2.1 g, 0.08% w/w based on dry biomass) was separated for identification using semipreparative HPLC (MeCN and acetone mixture as mobile phase). Finally, oleanolic acid (3.5 g, 0.12% w/w based on dry biomass) was obtained by recrystallization (DCM/MeOH, 1:1, v/v). The structures of these pentacyclic triterpenes were elucidated using spectroscopic methods, and NMR data were in agreement with those reported.[14,15] The *n*-BuOH (24.5 g) and H_2O (78.0 g) fractions were combined, suspended in H_2O, adsorbed on an MCI gel column (500 g), and eluted using mixtures of H_2O and MeOH starting with 10% MeOH (v/v) to 100% MeOH in 10% increments (2 L each fraction) to afford a total of 10 fractions (BuOH 1−10). Subsequently, fractions BuOH 4−10 were purified following the next separation steps: first Sephadex LH-20 (500 g) column chromatography (MeOH as eluent), then automated flash chromatography with prepacked silica gel columns using $CHCl_3$/MeOH/H_2O, 10:1:0.1 (v/v/v) as mobile phase, and finally semi-preparative or preparative HPLC chromatography using mixtures of MeCN (solvent A), H_2O (solvent B), or 0.1% HCO_2H acidified H_2O (solvent C) as follows: fractions obtained from BuOH-7 were separated using a linear gradient of solvents A:B from 20:80 to 60:40 (v/v) in 40 min, and fractions obtained from BuOH-4 and BuOH-5 were separated using a linear gradient of solvents A:C from 5:95 to 25:75 (v/v) in 50 min. Furthermore, fractions BuOH 1−3 were subjected to an automated flash chromatography using a reversed-phase C_{18} column (80 g, linear gradient 5% MeOH to 50% MeOH in 60 min), then to an automated normal-phase flash chromatography (EtOAc/MeOH/H_2O, 88:11:8, v/v/v, with 0.5% HCO_2H as mobile phase), and finally purified using semipreparative or preparative HPLC with a linear gradient of solvents A:C from 1:99 to 15:85 in 60 min. From fraction BuOH-7 were isolated the known cardiac glycosides 3-*O*-β-D-glucopyranosyl-(1→4)-6-deoxy-β-D-allo-pyranosyluzarigenin (**6**), uzarin (**7**), and deglucouzarin (**8**); their structures were elucidated using spectroscopic methods and were in agreement with literature data. The glycosylated flavonoids quercetin 3-*O*-β-galactopyranosyl-(1→2)-β-xylopyranoside, kaempferol 3-*O*-β-galactopyranosyl-(1→2)-β-xylopyranoside (**9**), quercetin-7-*O*-β-gluco-pyranosyl-3-*O*-β-D-galactopyranosyl-(1→2)-β-D-xylopyranoside (**2**), 3′-*O*-methylquercetin 3-*O*-β-galactopyranosyl-(1→2)-β-xylopyrano-side, and quercetin 3-*O*-β-galactopyranoside were isolated from fractions BuOH-4 and BuOH-5. In addition, from fraction BuOH-7 the known lignans episyringaresinol and prupaside were obtained. The phenylethanoid **4** and the megastigmane **5** were isolated from fractions BuOH-4 and BuOH-5, respectively. Finally, from the highly polar

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fractions BuOH 1−3 the following compounds were purified: 4-(β-glucopyranosyloxy)benzoic acid, *cis*- and *trans*-cinnamic acids, and isovanillinic acid. The structures of the new compounds **1**−**5** were elucidated using UV, IR, HRMS, and NMR experiments.

3-*O*-β-D-Glucopyranosyl-(1→4)-6-deoxy-β-D-allopyranosyl-17β-hydroxyuzarigenin (1): amorphous, white powder; mp 263.2−264.8 °C; $[\alpha]^{25}_{D}$ −6.7 (*c* 0.1, MeOH); IR ν_{max} (film) cm^{-1}: 3343.6 (OH), 1706.0 (C═O), 1735.8 (C═O), 1158.4, 1059.8; UV_{max} 217.1 nm; HRMS *m*/*z* 737.3388 $[M + Na]^+$ (737.3360 calcd for $C_{35}H_{54}NaO_{15}$); ^1H and ^{13}C NMR data, see Table 1.

Syriacatin (2): amorphous, yellow powder; mp 195.6−197.0 °C; $[\alpha]^{25}_{D}$ −39 (*c* 0.4, MeOH); IR ν_{max} (film) cm^{-1} 3282.4 (OH), 1651.6 (C═O), 1044.8, 989.1; UV_{max} 358.0, 256.9 nm; HRMS *m*/*z* 781.1816 $[M + Na]^+$ (781.1803 calcd for $C_{32}H_{38}NaO_{21}$); ^1H and ^{13}C NMR data, see Table 1.

9′-*O*-Butyl-3-*O*-demethyl-9-*O*-β-D-glucopyranosyldehydrodiconiferyl alcohol (3): amorphous, white powder; mp 234.2−235.9 °C; $[\alpha]^{25}_{D}$ +25 (*c* 0.7, MeOH); IR ν_{max} (film) cm^{-1} 3326.9 (OH), 3025.1 (Ar−H), 1336.3, 1073.6, 1058.5; UV_{max} 278.9, 220.5 nm; HRMS *m*/*z* 585.2298 $[M + Na]^+$ (585.2312 calcd for $C_{29}H_{38}NaO_{11}$); ^1H and ^{13}C NMR data, see Table 2.

Kansanoside A (4): amorphous, white powder; mp 267.2−269.0 °C; $[\alpha]^{25}_{D}$ +12 (*c* 0.3, MeOH); IR ν_{max} (film) cm^{-1} 3310.28 (OH), 1161.4, 1055.5; UV_{max} 237.9 nm; HRMS *m*/*z* 601.2087 $[M + Na]^+$ (601.2108 calcd for $C_{25}H_{38}NaO_{15}$); ^1H and ^{13}C NMR data, see Table 3.

Oreadoside A (5): amorphous, white powder; mp 254.3−255.9 °C; $[\alpha]^{25}_{D}$ −29 (*c* 0.7, MeOH); IR ν_{max} (film) cm^{-1} 3350.2 (OH), 1642.6 (C═O), 1377.6, 1058.9; UV_{max} 278.9, 262.0, 256.0 nm; HRMS *m*/*z* 689.2974 $[M + Na]^+$ (689.2997 calcd for $C_{30}H_{50}NaO_{16}$); ^1H and ^{13}C NMR data, see Table 3.

Acid Hydrolysis of 1−5. Aliquots of pure compounds (approximately 1−2 mg) were hydrolyzed using 3 mL of 1 M HCl (dioxane/H_2O, 1:1, v/v) for 4 h at 70 °C. The resulting mixtures were neutralized with 3 M NaOH and extracted three times with EtOAc. The aqueous layer was concentrated, and the residue was fractionated in a small silica gel column using mixtures of $CHCl_3$, MeOH, and H_2O. The purified sugars were compared by TLC with authentic samples, and the optical rotation values recorded in H_2O after equilibration during 24 h. Compound **1** afforded D-glucose, $[\alpha]^{25}_{D}$ +50.1 (*c* 0.1, H_2O), lit. +56;[39] 6-deoxy-D-allose, $[\alpha]^{25}_{D}$ +2.1 (*c* 0.1, H_2O), lit. +2.[40] Compound **2** afforded D-glucose, $[\alpha]^{25}_{D}$ 48.7 (*c* 0.1, H_2O); D-galactose, $[\alpha]^{25}_{D}$ +62.6 (*c* 0.1, H_2O), lit. +84.2;[41] and D-xylose, $[\alpha]^{25}_{D}$ +18.2 (*c* 0.1, H_2O), lit. +19.4.[42] Compound **3** afforded D-glucose, $[\alpha]^{25}_{D}$ +51.3 (*c* 0.1, H_2O). Compounds **4** and **5** afforded D-glucose, $[\alpha]^{25}_{D}$ +50.4 (*c* 0.1, H_2O); D-galactose $[\alpha]^{25}_{D}$ +68.6 (*c* 0.1, H_2O); and D-xylose, $[\alpha]^{25}_{D}$ +17.3 (*c* 0.1, H_2O).

Cytotoxicity Assay. Four cancer breast (Hs578T, T47D, Sk-Br-3, and MCF-7) and one normal breast (Hs578Bst) cell line were seeded in separate 384-well plates (seeding density of 3000 cells per well, in a volume of 30 μL per well) and allowed to attach and grow overnight in a cell incubator. The compounds were added using a Lybcyte ECHO acoustic liquid handling instrument (14 concentrations in the range 0.002−40 μM), and plates were incubated for 72 h. Cell viability was determined adding 10 μL of CellTiter-Glo reagent, shaking the plates for 2 min followed by reading of luminescence after a 15 min stabilizing period. Each dose−response curve was determined in triplicate. The data were normalized dividing by the median, and IC_{50} calculation was done using GraphPad Prism software.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H NMR and ^{13}C NMR spectra of compounds **1**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

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