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Phenolic Compounds from the Roots of Jordanian Viper's Grass, *Scorzonera judaica*

Ammar Bader,[†] Nunziatina De Tommasi,*[,‡] Roberta Cotugno,[‡] and Alessandra Braca[§]

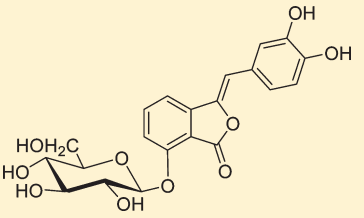
[†]Faculty of Pharmacy, Al-Zaytoonah Private University of Jordan, P.O. Box 130, 11733 Amman, Jordan

[‡]Dipartimento di Scienze Farmaceutiche e Biomediche, Università di Salerno, Via Ponte Don Melillo, 84084 Fisciano (SA), Italy

[§]Dipartimento di Scienze Farmaceutiche, Università di Pisa, Via Bonanno 33, 56126 Pisa, Italy

Supporting Information

ABSTRACT: Nine new phenolic compounds, 3*S*-hydrangenol 4′-*O*-α-L-rhamnopyranoysl-(1→3)-*β*-D-glucopyranoside (**1**), thunberginol F 7-*O*-*β*-D-glucopyranoside (**2**), 2-hydroxy-6-[2-(4-hydroxyphenyl)-2-oxo-ethyl]benzoic acid (**3**), 2-hydroxy-6-[2-(3,4-dihydroxyphenyl)-2-oxo-ethyl]benzoic acid (**4**), 2-hydroxy-6-[2-(3,4-dihydroxyphenyl-5-methoxy)-2-oxo-ethyl]benzoic acid (**5**), hydrangeic acid 4′-*O*-*β*-D-glucopyranoside (**6**), *E*-3-(3,4-dihydroxybenzylidene)-5-(3,4-dihydroxyphenyl)dihydrofuran-2-one (**7**), *Z*-3-(3,4-dihydroxybenzylidene)-5-(3,4-dihydroxyphenyl)-2(3*H*)-furanone (**8**), and 4-[*β*-D-glucopyranosyl)hydroxy]-pinoresinol (**9**), and nine known compounds were isolated from the roots of *Scorzonera judaica*. Structures of **1**−**9** were elucidated by mass spectrometry, extensive 1D and 2D NMR spectroscopy, and CD spectroscopy. All compounds were evaluated for cytotoxic activity.



The genus *Scorzonera* comprises about 170 species distributed worldwide.[1] It belongs to the Cichorieae tribe (synonym: Lactuceae), which consists of approximately 100 genera and 1500 species, many of which are edible and used as vegetables or in salads.[2] *Scorzonera judaica* Eig. (Asteraceae), "Jordanian Viper's Grass", one of nine species that grow in the wild in Jordan, is a perennial herb that grows from 5 to 15 cm tall, with ovoid brown tuberous root, yellow flowers, and lanceolate leaves that are densely wooly with wavy margins. It grows in the desert and dry places,[3] and it is well known as "Ga'fur" by Bedouins, who use its flowers, leaves, and tubers as food.[4] In the past decade the genus *Scorzonera* has attracted the attention of phytochemists due to the many triterpenes,[5] flavonoids,[6] sesquiterpenes,[2] kava lactones,[7] coumarins,[7] dihydrostilbenes,[8] phenolic derivatives, sterols, and dihydroisocoumarins[9] that have been isolated. Several species of *Scorzonera* have been investigated pharmacologically to confirm their use in traditional medicine.[10]

As a part of an ongoing research program to isolate and determine structures of secondary metabolites of Jordanian flora,[11−13] we performed a phytochemical study on the tuberous roots of *S. judaica* collected in Jordan.

RESULTS AND DISCUSSION

The defatted roots of *S. judaica* were extracted successively with $CHCl_3$, $CHCl_3$−MeOH, and MeOH. The extracts were each subjected to column chromatography (CC), followed by RP-HPLC, to afford nine new compounds (**1**−**9**) and nine known phenolic derivatives. The structures of all compounds were determined by detailed NMR and HRESIMS analyses. The known compounds hydrangenol 8-*O*-*β*-D-glucopyranoside (**10**),[14] hydrangenol 4′-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**11**),[15] hydramacrophyllol B (**12**),[16] hydramacrophyllol A (**13**),[16] 4α-hydroxypinoresinol (**14**),[17] hydrangenol 4′-*O*-*β*-D-glucopyranoside (**15**),[18] thunberginol F (**16**),[18,19] hydrangenol (**17**),[14] and scorzotomentosin (**18**)[9] were identified by comparison with published literature data.

Compound **1** was isolated as a colorless, optically active, amorphous powder, $[\alpha]_D$ −46 (*c* 0.1, MeOH). In the ESIMS spectrum, an $[M + Na]^+$ signal was observed at *m*/*z* 587, consistent with the molecular formula $C_{27}H_{32}O_{13}$. A peak at *m*/*z* 441 $[M + Na - 146]^+$ was observed, due to loss of a deoxyhexose unit. The ^1H and ^{13}C NMR spectra of compound **1** (Table 1) showed a pattern typical of hydrangenol plus signals ascribable to sugar moieties.[14] Particularly, the ^1H NMR spectrum of **1** displayed signals ascribable to a 1,4-disubstituted aromatic ring [AA′BB′ spin system at *δ* 7.19 (2H, d, *J* = 8.5 Hz) and 7.48 (2H, d, *J* = 8.5 Hz)], a 1,2,3-trisubstituted benzene ring [*δ* 6.87 (1H, d, *J* = 8.5 Hz), 6.92 (1H, d, *J* = 8.5 Hz), and 7.53 (1H, dd, *J* = 8.5, 8.5 Hz)], a *δ*-lactone [*δ* 3.18 (1H, dd, *J* = 16.0, 3.0 Hz), 3.34 (1H, overlapped signal), and 5.67 (1H, dd, *J* = 12.0, 3.0 Hz)] and two sugar moieties with anomeric protons at *δ* 4.99 (1H, d, *J* = 7.8 Hz) and 5.24 (1H, d, *J* = 1.8 Hz), which correlated respectively with signals at 101.6 and 102.1 ppm in the HSQC spectrum. Assignments of all proton and carbon signals were based on a combination of 1D-TOCSY, DQF-COSY, and HSQC analysis and supported the presence of a hydrangenol aglycone, a glucose, and a rhamnose unit. Hydrolysis of **1** with 1 N HCl yielded D-glucose and L-rhamnose, as determined by

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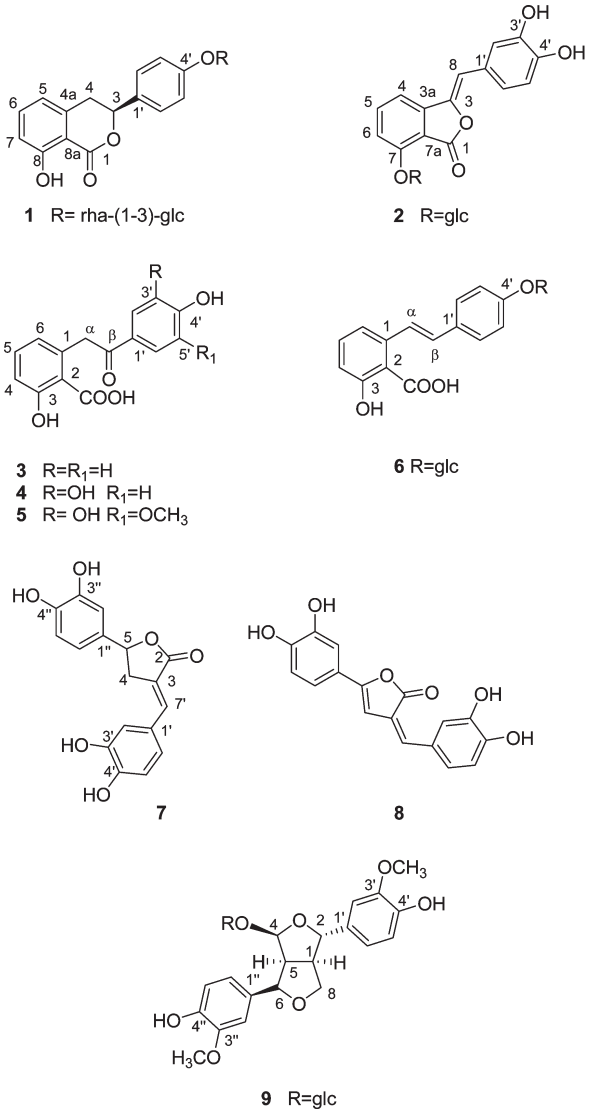

GC of their trimethylsilylated derivatives on a chiral column. The position of the glycoside linkage was established unequivocally by a HMBC experiment that showed correlation peaks between δ 4.99 (H-1_{glc}) and 159.1 (C-4′) and between δ 5.24 (H-1_{rha}) and 83.0 (C-3_{glc}). The absolute configuration at C-3 was ascertained by the circular dichroism (CD) spectrum, which showed a CD curve characteristic for 3*S*-dihydroisocoumarin (negative Cotton effect at 260 nm and positive Cotton effect at 241 nm).[14,15] Thus, compound **1** was determined to be 3*S*-hydrangenol 4′-*O*-α-L-rhamnopyranosyl-(1→3)-*β*-D-glucopyranoside.

Compound **2** had the molecular formula $C_{21}H_{20}O_{10}$ as deduced from MS and NMR data. The ESIMS spectrum (positive ions) indicated the loss of a hexose moiety from the quasi molecular ion $[M + Na]^+$ at *m*/*z* 455. The ^{13}C NMR spectrum (Table 1) showed 21 carbon signals, of which 15 were assigned to the aglycone and six to a sugar unit. Twelve aromatic C atoms (three quaternary, three oxygenated quaternary, and six methine), one sp^2 methyne, one quaternary sp^2, and one lactone group were observed in the ^{13}C NMR spectrum of the aglycone portion. The ^1H and ^{13}C NMR data of **2** showed the presence of two trisubstituted benzene rings, a conjugated olefin, and a hexose residue. The NMR signals were all assigned using DQF-COSY and HSQC spectra. HMBC correlations between δ 6.61 (H-8) and 117.9 (C-2′), 124.7 (C-6′), and 143.5 (C-3); δ 7.50 (H-2′) and 109.7 (C-8) and 147.3 (C-4′); δ 7.17 (H-6′) and 109.7 (C-8), 117.9 (C-2′), and 147.3 (C-4′); and δ 7.59 (H-4) and 112.5 (C-7a), 115.7 (C-6), 143.5 (C-8), 144.6 (C-3a), and 157.5 (C-7) indicated that the aglycone of **2** was thunberginol F.[19] Structural elucidation of the glucopyranose moiety was performed on the basis of 1D TOCSY data, and the configuration of the sugar unit was assigned as described for compound **1**. The HMBC experiment showed a correlation between the resonance at δ 5.18 (H-1_{glc}) and 157.5 (C-7) of thunberginol F. Therefore, compound **2** was defined as thunberginol F 7-*O*-*β*-D-glucopyranoside.

The ESIMS of compound **3** in the negative mode showed peaks at *m*/*z* 271 $[M - H]^-$ and 227 $[M - H - 44]^-$, which, together with ^{13}C NMR data (Table 2), indicated a molecular formula of $C_{15}H_{12}O_5$. The ^1H NMR spectrum of **3** (Table 2) indicated the presence of two signals with double intensity of an AA′BB′ spin system characteristic of a *para*-disubstituted aromatic ring, three further proton aromatic signals in the 1-, 2-,

Table 1. ^1H and ^{13}C NMR Data of Compounds 1 and 2 (CD_3OD, 600 MHz)[a]

	1		2	
position	δ_H	δ_C	δ_H	δ_C
1		169.5		167.3
3	5.67 dd (12.0, 3.0)	81.6		143.5
3a				144.6
4	3.18 dd (16.0, 3.0), 3.34[b]	35.4	7.59 d (8.0)	114.6
4a		141.7		
5	6.87 d (8.5)	118.3	7.72 t (8.5)	138.3
6	7.53 dd (8.5, 8.5)	136.5	7.24 d (8.0)	115.7
7	6.92 d (8.5)	116.9		157.5
7a				112.5
8		163.5	6.61 s	109.7
8a		109.7		
1′				127.9
2′	7.48 d (8.5)	128.5	7.50 d (2.0)	117.9
3′	7.19 d (8.5)	129.0		146.9
4′		117.5		147.3
5′	7.19 d (8.5)	159.1	6.82 d (8.0)	116.2
6′	7.48 d (8.5)	117.5	7.17 dd (8.0, 2.0)	124.7
Glc 1	4.99 d (7.8)	129.0	5.18 d (7.8)	101.6
2	3.60 dd (9.0, 7.8)	101.6	3.67 dd (9.5, 7.8)	74.4
3	3.64 t (9.0)	75.0	3.56 t (9.5)	78.3
4	3.50 t (9.0)	83.0	3.47 t (9.5)	71.2
5	3.50 m	70.0	3.57 m	78.3
6a	3.93 dd (12.0, 3.0)	77.0	3.94 dd (12.0, 3.5)	62.6
6b	3.74 dd (12.0, 5.0)	62.0	3.72 dd (12.0, 5.0)	
Rha 1	5.24 d (1.8)	102.1		
2	4.01 dd (3.0, 1.8)	71.8		
3	3.76 dd (9.5, 3.0)	71.7		
4	3.43 t (9.5)	73.8		
5	4.05 m	70.0		
6	1.30 d (6.0)	17.4		

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in ppm. [b] Under the solvent.

Table 2. ^{1}H and ^{13}C NMR Data of Compounds 3−6 (CD_3OD, 600 MHz)[a]

	3		4		5		6	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		139.8		140.0		139.4		130.0
2		117.2		117.5		117.0		115.0
3		164.4		164.2		163.6		157.0
4	6.86 d (8.0)	117.0	6.86 d (8.0)	117.4	6.85 d (8.0)	117.1	6.77 d (8.5)	116.2
5	7.33 t (8.5)	134.2	7.35 t (8.5)	134.6	7.32 t (8.5)	134.3	7.40 dd (8.5, 8.0)	128.7
6	6.73 d (8.0)	124.5	6.73 d (8.0)	124.8	6.71 d (8.0)	124.5	7.20 d (8.0)	124.4
α	4.48 s	47.0	4.60 s	47.2	4.64 s	47.0	7.41 d (16.0)	119.6
β		200.0		200.0		199.5	7.10 d (16.0)	130.6
1′		132.2		132.0		131.0		135.0
2′	7.96 d (8.0)	131.5	7.47 d (2.0)	116.0	7.24 d (1.5)	110.8	7.23 d (8.0)	128.6
3′	6.86 d (8.0)	115.8		147.0		145.3	7.09 d (8.0)	115.3
4′		163.8		147.5		139.4		154.0
5′	6.86 d (8.0)	115.8	8.84 d (8.5)	115.9		148.6	7.09 d (8.0)	115.3
6′	7.96 d (8.0)	131.5	7.52 dd (8.5, 2.0)	122.9	7.22 d (1.5)	104.6	7.23 d (8.0)	128.6
OMe					3.89 s	56.7		
COOH		177.2		177.0		177.5		177.0
Glc 1							4.93 d (7.8)	104.0
2							3.53 dd (9.5, 7.8)	75.0
3							3.43 t (9.5)	77.9
4							3.40 t (9.5)	71.1
5							3.49 m	77.9
6a							3.92 dd (12.0, 3.0)	62.3
6b							3.71 dd (12.0, 5.0)	

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in ppm.

and 3-positions, and a methylene group. The ^{13}C NMR spectrum indicated the presence of carboxylic acid and ketone functionalities. HSQC and HMBC experiments in combination with the ^{1}H NMR coupling pattern established the substitution pattern of the bibenzyl moiety; key correlation peaks were observed between H-6 (*δ* 6.73) and C-α (*δ* 47.0) and COOH (*δ* 177.2, weak), between H-4 (*δ* 6.86) and C-2 (*δ* 117.2), C-6 (*δ* 124.5), and C-3 (*δ* 164.4), and between H-2′ (*δ* 7.96) and C-*β* (*δ* 200.0), C-1′ (*δ* 132.2), and C-4′ (*δ* 163.8). Compound **3** was thus established as 2-hydroxy-6-[2-(4-hydroxyphenyl)-2-oxo-ethyl]benzoic acid.

Compound **4** showed an $[M - H]^-$ peak at *m/z* 287 and fragments at *m/z* 243 and 177, supporting a molecular formula of $C_{15}H_{12}O_6$. Comparison of NMR data of compound **4** (Table 2) with those of **3** showed it to differ only in the B ring. The ^{1}H NMR spectrum of **4** contained an ABX system for ring B. Hence, the structure of compound **4** was established as 2-hydroxy-6-[2-(3,4-dihydroxyphenyl)-2-oxo-ethyl]benzoic acid.

The ESIMS spectrum of compound **5** ($C_{16}H_{14}O_7$) showed a main signal at *m/z* 317 $[M - H]^-$ and two ion fragments at *m/z* 273 $[M - H - 44]^-$ and 177 $[M - H - 140]^-$. Comparison of the MS and NMR data of **5** with those of **4** showed an additional OCH_3 group in the B ring of **5**, in a *meta* position. Thus, compound **5** was established to be 2-hydroxy-6-[2-(3,4-dihydroxyphenyl-5-methoxy)-2-oxo-ethyl]benzoic acid.

Compound **6** was isolated as a pale yellow, amorphous powder. Its UV spectrum was characteristic for a stilbene derivative, with absorption maxima at 226, 300, and 320 nm. The ESIMS spectrum of **6** showed a $[M - H]^-$ peak at *m/z* 417, indicating the molecular formula $C_{21}H_{22}O_9$. The spectral data of compound **6** showed close similarities to those of scorzoerzincanin.[9] The ^{1}H NMR spectrum (Table 2) indicated that the molecule consisted of a *para*-disubstituted aromatic ring [AA′BB′ spin system at *δ* 7.09 (2H, d, *J* = 8.0 Hz), 7.23 (2H, d, *J* = 8.0 Hz)] and a trisubstitued benzene ring [*δ* 6.77 (1H, d, *J* = 8.5 Hz), 7.20 (1H, d, *J* = 8.0 Hz), 7.40 (1H, dd, *J* = 8.5, 8.0 Hz)]. In addition, the ^{1}H NMR signals indicated the presence of one glucopyranose moiety. The ESIMS/MS further pointed to a hexose and a carboxyl group. The sugar unit was D-glucose. The positions of substituents were assigned by correlations in the HMBC spectrum between signals at *δ* 4.93 ($H\text{-}1_{glc}$) and 154.0 (C-4′), between *δ* 7.10 (H-*β*) and 135.0 (C-1′), 124.4 (C-6), 128.6 (C-2′), and between *δ* 7.20 (H-6) and 130.0 (C-1) and 119.6 (C-α). Thus, **6** was identified as hydrangeic acid 4′-*O*-*β*-D-glucopyranoside.

Compound **7** was assigned the molecular formula $C_{17}H_{14}O_6$. The ESIMS exhibited peaks at *m/z* 313 $[M - H]^-$ and *m/z* 269 (loss of a carboxylic group). The 600 MHz ^{1}H NMR spectrum (Table 3) indicated two 1,3,4-trisubstituted benzene rings, and these were supported by the ESIMS peaks at *m/z* 159 and 109 due to bond cleavage at C-1′/C-5 and C-7′/C-1′. The 1D and 2D NMR spectra suggested that **7** was a nor-lignan. The NMR data revealed an α,*β*-unsaturated lactone (δ_C 175.0), which was coupled with an sp^2 proton (*δ* 7.43, s) and a methine proton (*δ* 5.54, dd, *J* = 8.5, 5.5 Hz) in the HMBC spectrum. Correlations in the 1D TOCSY, DQF-COSY, HSQC, and HMBC spectra revealed a −CH(C-5)−CH_2(C-4)−C(C-3)−CH(C-7)− sequence. HMBC correlations from H-7 to C-2, C-2′, and C-6′,

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Table 3. ^{1}H and ^{13}C NMR Data of Compounds 7−9 (CD_3OD, 600 MHz)[a]

	7		8		9	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1					3.11 m	55.2
2		175.0		172.6	4.99 d (7.5)	85.7
3		133.3		131.0		
4a	3.66 ddd (17.0, 8.5, 2.0)	37.4	7.69 s	124.8	5.71 d (6.0)	106.1
4b	3.10 ddd (17.0, 5.5, 2.0)					
5	5.54 dd (8.5, 5.5)	80.5		139.6	3.45 m	59.4
6					5.54 d (3.0)	81.0
8a					4.08 dd (9.0, 5.5)	71.1
8b					3.59 dd (9.0, 2.0)	
1′		127.5		128.6		133.2
2′	7.06 d (2.0)	117.8	7.26 d (2.0)	111.7	6.98 d (2.0)	111.0
3′		146.2		147.0		148.0
4′		149.7		149.0		146.4
5′	6.87 d (8.0)	116.9	6.91 d (8.0)	116.3	6.78 d (8.0)	115.8
6′	6.99 dd (8.0. 2.0)	124.8	6.81 dd (8.0. 2.0)	121.9	6.86 dd (8.0, 2.0)	120.5
7′	7.43 s	138.3	8.26 s	128.0		
1″		133.7		134.2		135.1
2″	6.80 d (2.0)	114.2	7.30 d (2.0)	109.0	6.96 d (2.0)	110.7
3″		146.8		148.0		148.0
4″		149.8		146.4		146.4
5″	6.80 d (8.0)	116.3	6.91 d (8.0)	116.8	6.76 d (8.0)	115.4
6″	6.73 dd (8.0, 2.0)	118.8	6.81 dd (8.0, 2.0)	121.9	6.85 dd (8.0, 2.0)	119.7
OMe					3.85 s	56.4
Glc 1					4.56 d (8.0)	104.2
2					3.38 dd (9.0, 8.0)	75.2
3					3.28 t (9.0)	78.2
4					3.36 t (9.0)	71.1
5					3.39 m	78.0
6a					3.83 dd (12.0, 2.5)	62.4
6b					3.67 dd (12.0, 4.5)	

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in ppm.

from H_2-4 to C-2, C-3, C-5, C-7′, and C-1″, and from H-5 to C-2, C-3, C-2″, and C-6″ suggested that C-7′ was connected to C-1′ and that C-5 was linked to C-1″. The configuration of the double bond $\Delta^{3(7)}$ was obtained by a NOESY experiment and comparison with known compounds.[20] Thus, the structure of **7** was elucidated as *E*-3-(3,4-dihydroxybenzylidene)-5-(3,4-dihydroxyphenyl)dihydrofuran-2-one.

Compound **8** had the molecular formula $C_{17}H_{12}O_6$ and displayed a peak at *m/z* 311 $[M - H]^-$ and a fragment ion at *m/z* 267 $[M - H - 44]^-$. Its UV spectrum showed maxima at 266 and 425 nm, absorptions characteristic of an extended π-system conjugation. The ^{1}H and ^{13}C NMR spectra (Table 3) indicated the presence of an α,β,γ-unsatured five-membered lactone ring. Additionally, comparing the ^{1}H NMR spectrum of **8** with that of **7**, the signals attributable to H-5 and H_2-4 were absent. On the basis of NMR data, **8** displayed a C=CH at C-4/C-5 instead of a CH−CH_2 group. Since a NOE was observed between H-4 and H-7, the *Z* configuration at $\Delta^{3(7)}$ was established. Therefore, compound **8** was deduced to be *Z*-3-(3,4-dihydroxybenzylidene)-5-(3,4-dihydroxyphenyl)-2(3*H*)-furanone. The *E* derivative of **8** was isolated previously from the mycelia of *Rhizoctonia* strain F23372.[21]

Compound **9** was an amorphous, optically active powder, $[\alpha]_D$ +26 (*c* 0.1, MeOH). The $[M + Na]^+$ peak at *m/z* 559 (ESIMS) indicated the molecular formula $C_{26}H_{32}O_{12}$. Inspection of the ^{13}C NMR spectrum of **9** (Table 3) showed two OCH_3 functions, a sugar residue, and 18 additional C atoms. The ^{1}H NMR spectrum (Table 3) indicated two trisubstituted aromatic rings and two OCH_3 groups, suggesting a pinoresinol-type lignan framework. The 1D TOCSY, DQF-COSY, and HSQC experiments allowed the sequential assignments of all proton and carbon resonances and confirmed the proposed aglycone skeleton. 1D TOCSY and 2D NMR experiments indicated the presence of a β-glucopyranose moiety. Hydrolysis of **9** yielded D-glucose, as was described for **1**. Locations of the OH groups and sugar moiety were confirmed by the HMBC spectrum, and the relative configuration of **9** was determined on the basis of a 2D NOESY experiment and by comparison of chemical shifts, multiplicities of the signals, and values of the coupling constants with literature data.[22,23] Compound **9** was thus identified as 4β-[β-D-glucopyranosyl)hydroxy]pinoresinol. A stereoisomer of **9** was previously isolated from *Ligularia virgaurea* ssp. *oligocephala*, a plant belonging to the Asteraceae family, subfamily Senecioineae.[23]

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Compounds **1**−**9** were each assayed against human uterine cervical adenocarcinoma (HeLa), human lymphocyte T cell (Jurkat), and human breast adenocarcinoma (MCF7) cell lines. None of the compounds were cytotoxic, as they all had IC_{50} values in excess of 100 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and a 1 dm microcell. UV spectra were recorded on a Perkin-Elmer-Lambda spectrophotometer. CD spectra were measured on a JASCO J-810 spectropolarimeter with a 0.1 cm cell in MeOH at room temperature with the following conditions: speed 50 nm/min, time constant 1 s, bandwidth 2.0 nm. NMR experiments were performed on a Bruker DRX-600 spectrometer at 300 K. 2D NMR spectra were acquired in CD_3OD or DMSO-d_6 in the phase-sensitive mode with the transmitter set at the solvent resonance and TPPI (time proportional phase increment) used to achieve frequency discrimination in the ω_1 dimension. The standard pulse sequence and phase cycling were used for DQF-COSY, TOCSY, HSQC, HMBC, and NOESY experiments. The NMR data were processed on a Silicon Graphic Indigo2 workstation using UXNMR software. HRESIMS were acquired in positive ion mode on a Q-TOF premier spectrometer equipped with a nanoelectrospray ion source (Waters-Milford, MA, USA). Column chromatography was performed over silica gel or Sephadex LH-20. HPLC separations were conducted on a Shimadzu LC-8A series pumping system equipped with a Waters R401 refractive index detector and Shimadzu injector on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, 10 μm Waters, flow rate 2.0 mL min^{-1}). GC analyses were performed using a Dani GC 1000 instrument on a L-CP-Chirasil-Val column (0.32 mm × 25 m).

Plant Material. The roots of *S. judaica* were collected during the flowering stage in the Dab'a desert reserve (50 km South of Amman), Jordan, during April 2009 and were identified by one of the authors (A. Bader). A voucher specimen (N. Jo-It 2009/1) is deposited at Al-Zaytoonah University of Jordan, Amman, Jordan.

Extraction and Isolation. The dried powdered roots of *S. judaica* (700 g) were successively extracted for 48 h with *n*-hexane, $CHCl_3$, $CHCl_3$−MeOH (9:1), and MeOH (each 3 × 2 L) to give 34.4, 15.8, 14.4, and 63.7 g of the respective residues. Part of the $CHCl_3$ extract (5.0 g) was subjected to silica gel CC eluting with $CHCl_3$ followed by increasing concentrations of MeOH in $CHCl_3$ (between 1% and 50%). Fractions of 25 mL were collected, analyzed by TLC (silica gel plates, in $CHCl_3$ or mixtures of $CHCl_3$−MeOH, 99:1, 98:2, 97:3, 9:1, 4:1), and grouped into seven fractions (A−G). Fraction D (700 mg) was subjected to RP-HPLC with MeOH−H_2O (6.5:3.5) as eluent to give compound **17** (20.3 mg). Fraction E (182 mg) was separated by RP-HPLC [MeOH−H_2O (5.5:4.5)] to yield compound **18** (6.1 mg). Fraction F (326 mg) was subjected to RP-HPLC [MeOH−H_2O (1:1)] to give compound **14** (17 mg). The $CHCl_3$−MeOH extract (7.0 g) was chromatographed over Sephadex LH-20 to give eight major fractions (A−H) grouped by TLC. Fractions D (340 mg) and G (155 mg) were separately purified by RP-HPLC [MeOH−H_2O (2:3)] to give compounds **9** (8.1 mg), **10** (4.2), **11** (9.6 mg), and **1** (16.2 mg) from fraction D and compounds **3** (7.3 mg) and **17** (15.5 mg) from fraction G. Fraction F (124 mg) was chromatographed by RP-HPLC [MeOH−H_2O (3.5:6.5)] to give compounds **12** (4.5 mg), **13** (2.5 mg), **14** (1.5 mg), and **15** (5.5 mg). Fraction H (72 mg) was also separated by RP-HPLC [MeOH−H_2O (1:1)] to yield compounds **8** (3.2 mg), **7** (6.8 mg), and **16** (5.2 mg). The MeOH extract was partitioned between *n*-BuOH and H_2O, to afford a *n*-BuOH residue (4.5 g), which was submitted to Sephadex LH-20 CC using MeOH as eluent to obtain nine major fractions (A−K) grouped by TLC. Fraction B (124 mg) was purified by RP-HPLC [MeOH−H_2O (3.5:6.5)] to afford compound **9** (3.0 mg). Fractions C (99.2 mg), D (136.6 mg), and G (70.8 mg) were separately subjected to RP-HPLC [MeOH−H_2O (2:3)] to give compounds **11** (10.5 mg), **15** (1.5 mg), and **1** (17.8 mg) from fraction C, compounds **6** (2.7 mg), **10** (5.7 mg), **11** (5.9 mg), and **15** (4.5 mg) from fraction D, and compound **2** (6.0 mg) from fraction G. Finally, fraction F (70.0 mg) was subjected to RP-HPLC [MeOH−H_2O (4.5:5.5)] to give compounds **5** (2.6 mg), **4** (3.5 mg), and **3** (4.7 mg).

3S-Hydrangenol 4′-O-α-L-rhamnopyranoysl-(1→3)-β-D-glucopyranoside (***1***): amorphous powder; $[\alpha]_D^{25}$ −46 (*c* 0.1, MeOH); CD $[\theta]_{25}$ (*c* 0.05, MeOH, nm) +4390 (241), −8000 (260); ^1H and ^{13}C NMR (CD_3OD, 600 MHz), see Table 1; ESIMS *m/z* 587 $[M + Na]^+$, 569 $[M + Na - 18]^+$, 441 $[M + Na - 146]^+$, HRESIMS *m/z* 565.1933 $[M + H]^+$ (calcd for $C_{27}H_{32}O_{13}$, 564.1843).

Thunberginol F 7-O-β-D-glucopyranoside (***2***): amorphous powder; $[\alpha]_D^{25}$ −83 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 223 (4.03), 331 (sh) (3,54), 380 (3.88) nm; ^1H and ^{13}C NMR (CD_3OD, 600 MHz), see Table 1; HRESIMS *m/z* 433.1127 $[M + H]^+$ (calcd for $C_{21}H_{20}O_{10}$, 432.1056); ESIMS *m/z* 455 $[M + Na]^+$, 293 $[M + Na - 162]^+$.

2-Hydroxy-6-[2-(4-hydroxyphenyl)-2-oxo-ethyl]benzoic acid (***3***): amorphous powder; UV (MeOH) λ_{max} (log ε) 280 (3.95) nm; ^1H and ^{13}C NMR (CD_3OD, 600 MHz), see Table 2; ESIMS *m/z* 271 $[M - H]^-$, 227 $[M - H - 44]^-$, 177 $[M - H - 94]^-$; HRESIMS *m/z* 273.0764 $[M + H]^+$ (calcd for $C_{15}H_{12}O_5$, 272.0685).

2-Hydroxy-6-[2-(3,4-dihydroxyphenyl)-2-oxo-ethyl]benzoic acid (***4***): amorphous powder; UV (MeOH) λ_{max} (log ε) 280 (sh) (3.86), 308 (4.02) nm; ^1H and ^{13}C NMR (CD_3OD, 600 MHz), see Table 2; ESIMS *m/z* 287 $[M - H]^-$, 243 $[M - H - 44]^-$, 177 $[M - H - 110]^-$; HRESIMS *m/z* 289.0728 $[M + H]^+$ (calcd for $C_{15}H_{12}O_6$, 288.0634).

2-Hydroxy-6-[2-(3,4-dihydroxyphenyl-5-methoxy)-2-oxo-ethyl]benzoic acid (***5***): amorphous powder; UV (MeOH) λ_{max} (log ε) 304 (3.92) nm; ^1H and ^{13}C NMR (CD_3OD, 600 MHz), see Table 2; ^1H NMR (DMSO-d_6, 600 MHz) δ 3.80 (3H, s, OCH_3), 4.64 (1H, s, H_2-α), 6.83 (1H, d, *J* = 8.0 Hz, H-4), 7.14 (2H, m, H-5 and H-6), 7.16 (1H, s, H-2′), 7.24 (1H, s, H-6′); ^{13}C NMR (DMSO-d_6, 600 MHz) δ 43.7 (C-α), 55.1 (OCH_3), 103.0 (C-6′), 108.5 (C-2′), 114.9 (C-4), 116.3 (C-2), 119.0 (C-6), 125.2 (C-5), 127.0 (C-1′), 138.4 (C-1 and C-4′), 144.7 (C-3′), 147.9 (C-5′), 159.0 (C-3), 176.5 (COOH), 195.8 (C-β); ESIMS *m/z* 317 $[M - H]^-$, 273 $[M - H - 44]^-$, 177 $[M - H - 140]^-$; HRESIMS *m/z* 319.0830 $[M + H]^+$ (calcd for $C_{16}H_{14}O_7$, 318.0740).

Hydrangeic acid 4′-O-β-D-glucopyranoside (***6***): amorphous powder; $[\alpha]_D^{25}$ −60 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 226 (4.10), 300 (4.05), 320 (3.88) nm; ^1H and ^{13}C NMR (CD_3OD, 600 MHz), see Table 2; ESIMS *m/z* 417 $[M - H]^-$, 255 $[M - H - 162]^-$, 211 $[M - H - 162 - 44]^-$; HRESIMS *m/z* 419.1359 $[M + H]^+$ (calcd for $C_{21}H_{22}O_9$, 418.1264).

E-3-(3,4-Dihydroxybenzylidene)-5-(3,4-dihydroxyphenyl)dihydrofuran-2-one (***7***): amorphous powder; $[\alpha]_D^{25}$ +18 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 301 (sh) (3.54), 330 (3.87) nm; ^1H and ^{13}C NMR (CD_3OD, 600 MHz), see Table 3; ESIMS *m/z* 313 $[M - H]^-$, 269 $[M - H - 44]^-$, 159 $[M - H - C_6H_5O_2]^-$, 109 $[C_6H_5O_2]^-$; HRESIMS *m/z* 315.0870 $[M + H]^+$ (calcd for $C_{17}H_{14}O_6$, 314.0790).

Z-3-(3,4-Dihydroxybenzylidene)-5-(3,4-dihydroxyphenyl)-2(3H)-furanone (***8***): amorphous powder; UV (MeOH) λ_{max} (log ε) 266 (3.94), 425 (4.30) nm; ^1H and ^{13}C NMR (CD_3OD, 600 MHz), see Table 3; ESIMS *m/z* 311 $[M - H]^-$, 267 $[M - H - 44]^-$; HRESIMS *m/z* 313.0700 $[M + H]^+$ (calcd for $C_{17}H_{12}O_6$, 312.0634).

4-[β-D-Glucopyranosyl)hydroxy]pinoresinol (***9***): amorphous powder; $[\alpha]_D^{25}$ +26 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 231 (3.80), 281 (4.05) nm; ^1H and ^{13}C NMR (CD_3OD, 600 MHz), see Table 3; ESIMS *m/z* 559 $[M + Na]^+$, 397 $[M + Na - 162]^+$; HRESIMS *m/z* 537.1965 $[M + H]^+$ (calcd for $C_{26}H_{32}O_{12}$, 536.1894).

Acid Hydrolysis of Compounds 1, 2, 6, and 9. A solution of each compound (2.0 mg) in 1 N HCl (1 mL) was stirred at 80 °C in a

stoppered reaction vial for 4 h. After cooling, the solution was evaporated under a stream of N_2. The residue was dissolved in 1-(trimethylsilyl)-imidazole and pyridine (0.2 mL), and the solution was stirred at 60 °C for 5 min. After drying the solution, the residue was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ layer was analyzed by GC using a L-CP-Chirasil-Val column (0.32 mm × 25 m). The temperature of both the injector and detector was 200 °C. A temperature gradient system was used for the oven, starting at 100 °C for 1 min and increasing up to 180 °C at a rate of 5 °C/min. Peaks of the hydrolysate were identified by comparison of retention times of authentic samples of L-rhamnose (10.2 min) and D-glucose (14.7 min) (Sigma Aldrich) after similar treatment with 1-(trimethylsilyl)imidazole in pyridine.

Cell Culture and Cytotoxic Assay. HeLa (human cervical carcinoma), Jurkat (human lymphocyte T cells), and human breast adenocarcinoma (MCF7) cell lines were obtained from the American Type Cell Culture (ATCC) (Rockville, MD, USA). Cells were maintained in DMEM and/or RPMI 1640, supplemented with 10% FBS, 100 mg/L streptomycin and 100 IU/mL penicillin (Gibco) at 37 °C in a humidified atmosphere of 5% CO_2. To ensure logarithmic growth, cells were subcultured every two days. Stock solutions (1 mg/mL) of all compounds were prepared in DMSO and stored at 4 °C. In all experiments, the final concentration of DMSO did not exceed 0.3% (v/v). Cells were seeded in 96-well plates (1×10^4/well). One day after seeding, cells were exposed to increasing concentrations of each compound or vehicle only and incubated for the established times. The number of HeLa, Jurkat, and MCF7 viable cells was quantified by MTT assay[24] and CellTiter-Blue1 cell viability assay (Promega). Appropriate controls were included in preliminary experiments to exclude any interferences of each compound with the assays. The IC_{50} values were obtained from dose−response curves.

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra of compounds **1**−**9** are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +39-089-969754. Fax: +39-089-969602. E-mail: detommasi@unisa.it.

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