Antioxidant Phenolic Glycosides from *Moricandia arvensis*

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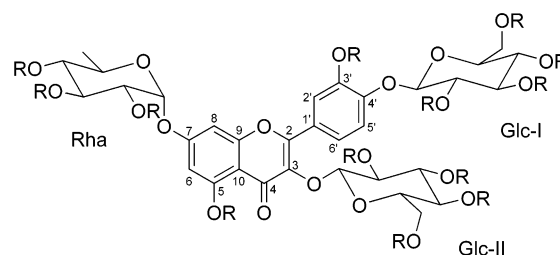
The new phenolic glycosides quercetin 3,4′-di-*O*-*β*-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside (moricandin) (**1**), *β*-D-glucopyranosyl 4-*O*-*β*-D-glucopyranosylcaffeate (**2**), methyl 3-*O*-*β*-D-glucopyranosyl-5-hydroxycinnamate (**3**), and *β*-D-glucopyranosyl 4-*O*-*β*-D-glucopyranosylbenzoate (**4**), together with the previously known *β*-D-glucopyranosyl 4-hydroxybenzoate (**5**), methyl 4-*O*-*β*-D-glucopyranosylcaffeate (**6**), 1-*O*-caffeoyl-*β*-D-glucopyranoside (**7**), and 2-phenylethyl-*β*-D-glucopyranoside (**8**), were isolated from the flowers of *Moricandia arvensis*. Their structures were elucidated by extensive spectroscopic analysis and chemical methods. Compounds **1**−**8** were evaluated for antioxidant activity using DPPH, TEAC, and reducing power assays, where the caffeic acid derivative **7** and the quercetin triglycoside **2** proved to possess the most potent scavenging activity.

The genus *Moricandia* (Cruciferae) includes five species distributed in North Africa, South Europe, and Western Asia.[1] In Tunisia, the leaves of *Moricandia arvensis* (L.) DC. are used in traditional cookery, and a decoction of leaves and stems is employed in the treatment of syphilis.[2] In the limited phytochemical work reported for this species, an indole derivative and several glucosinolates and fatty acids have been characterized.[3−5]

In the course of our search for antioxidants in plants, we detected in the methanolic extract of fresh violet flowers of *M. arvensis* several polar compounds that reduced the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical in a TLC autographic assay.[6] Following a bioassay-guided isolation procedure, four new phenolic glycosides have been identified, namely, quercetin 3,4′-di-*O*-*β*-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside, designated moricandin (**1**), *β*-D-glucopyranosyl 4-*O*-*β*-D-glucopyranosylcaffeate (**2**), methyl 3-*O*-*β*-D-glucopyranosyl-5-hydroxycinnamate (**3**), and *β*-D-glucopyranosyl 4-*O*-*β*-D-glucopyranosylbenzoate (**4**). In addition, the known compounds *β*-D-glucopyranosyl 4-hydroxybenzoate (**5**), methyl 4-*O*-*β*-D-glucopyranosylcaffeate (**6**), 1-*O*-caffeoyl-*β*-D-glucopyranoside (**7**), and 2-phenylethyl-*β*-D-glucopyranoside (**8**) were identified for the first time from this plant. Quantitative evaluation of the antioxidant activity of compounds **1**−**8** was carried out using the TEAC (Trolox equivalent antioxidant capacity),[7] DPPH (2,2-diphenyl-1-picrylhydrazyl),[8] and reducing power assays.[9] Details of the isolation, structure elucidation, and free-radical-scavenging properties of these compounds are discussed in this paper.



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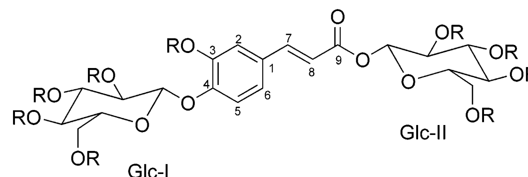
1a R = Ac



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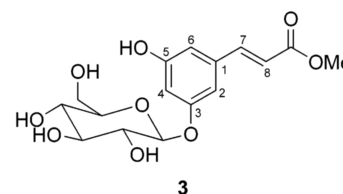

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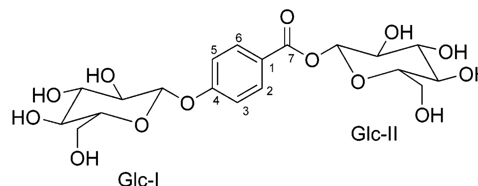

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Results and Discussion

Compound **1** was isolated as a yellow amorphous powder, and its molecular formula determined as $C_{33}H_{40}O_{21}$ by HRESIMS. In the IR spectrum, **1** exhibited carbonyl absorption bands at ν_{max} 1654 and 1600 cm^{-1} and a strong hydroxyl band at ν_{max} 3400 cm^{-1}, whereas its UV spectrum showed maxima at 256, 265, and 344 nm.

The aromatic region of the ^{1}H NMR spectrum of **1** displayed a three-spin system at δ 7.20 (1H, d, $J = 8.7$ Hz), 7.65 (1H, d, $J = 8.6$ Hz), and 7.68 (1H, brs) and two singlets at δ 6.44 and 6.85 (Table 1). The appearance of a broad singlet at δ 12.6 and a carbon resonance at δ_C 177.7, in addition to the characteristic IR bands for α,*β*-unsaturated and chelated ketones (1654 and 1600 cm^{-1}, respectively), suggested the presence of quercetin as the basic skeleton, which was confirmed by the observed HMBC correlations

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Table 1. NMR Data of Compounds **1** and **1a**

	1[a]		**1a**[b]	
position	δ_H (J = Hz)	δ_C	δ_H (J = Hz)	δ_C
aglycon				
2		156.1		154.0
3		134.2		136.2
4		177.7		171.8
5		160.7		150.2[d]
6	6.44 (s)	99.4	6.76 (d, 2.3)	109.3
7		161.7		159.2
8	6.85 (s)	94.6	7.09 (d, 2.3)	101.7
9		156.1		157.3
10		105.8		112.7
1′		124.4		125.3
2′	7.68 (brs)	116.6	7.70 (d. 2.1)	123.9
3′		146.3		139.8
4′		147.7		150.7[d]
5′	7.20 (d, 8.7)	115.5	7.12 (d. 8.9)	114.6
6′	7.65 (d, 8.6)	121.1	7.96 (dd. 8.9, 2.1)	128.2
rhamnose				
1	5.55 (brs)	98.4	5.56 (brs)	95.8
2	3.08−3.83[c]	70.2[d]	5.10−5.46[c]	69.2[e]
3	3.08−3.83[c]	70.1[d]	5.10−5.46[c]	68.5[e]
4	3.31 (m)	71.6	5.02 (t, 9.8, 9.6)	71.5[f]
5	3.08−3.83[c]	70.0[d]	3.89−4.00[c] (m)	68.4[e]
6	1.11 (d, 6.0)	17.9	1.23 (d, 6)	17.5
glucose-I				
1	4.87 (d, 6.9)	100.7	5.10−5.46[c]	98.5[g]
2	3.41 (m)	73.2[e]	5.10−5.46[c]	70.5
3	3.41 (m)	75.8[f]	5.10−5.46[c]	72.3[h]
4	3.08−3.83[c]	69.7[d]	5.10−5.46[c]	68.0[i]
5	3.41 (m)	77.2[g]	3.89−4.00[c]	72.6[h]
6	3.31−3.71[c]	60.7[h]	4.33 (dd, 12.0, 5.6); 4.19−4.23[c]	61.4[j]
glucose-II				
1	5.51 (d, 7.5)	101.5	5.63 (d, 8.0)	98.7[g]
2	3.20 (m)	74.1[e]	5.10−5.46[c]	70.5
3	3.20 (m)	76.4[f]	5.10−5.46[c]	71.9[f]
4	3.08−3.83[c]	69.8[d]	5.10−5.46[c]	68.1[i]
5	3.15 (m)	77.7[g]	3.89−4.00[c]	72.6[h]
6	3.31−3.71[c]	60.9[h]	4.19−4.23[c]; 4.01 (dd,12.4, 4.4)	61.8[j]
CO-*Me*			1.98−2−44[c]	20.6−21.1[c]
CO-Me				168.7−170.4[c]

[a] Measured in DMSO-d_6. [b] Measured in $CDCl_3$. [c] Overlapped signals. [d−j] These values may be interchanged in the same column.

(Figure 1). The presence of three sugar units and two phenol groups in the molecule was corroborated by the anomeric proton resonances at δ 5.55 (1H, brs), 5.51 (1H, d, J = 7.0 Hz), and 4.87 (1H, d, J = 6.9 Hz), linked to carbons at δ_C 98.4, 101.5, and 100.7, respectively, and the formation of a peracetylated derivative (**1a**) whose pseudomolecular ion peak at *m/z* 1319 $[M + H]^+$ accounted for 13 acetyl groups. Moreover, the ^1H and ^{13}C NMR spectra showed a methyl doublet at δ 1.11 (J = 6.0 Hz) and δ_C 17.9, indicative of a rhamnosyl moiety, which was confirmed from the acid hydrolysis of **1**, which yielded quercetin, L-rhamnose, and D-glucose, and by its FDMS, whose base peak at *m/z* 302 resulted from the loss of the three sugar groups.[10] The identity of the two sugar units was confirmed by GLC analysis of their acetylated dithioacetal derivatives, using a described procedure.[11] The location of the sugar units was deduced from the NOESY spectrum and glycosylation effects on the chemical shifts of the aglycon (Figure 1). The attachment of rhamnopyranose at C-7 was evidenced from the NOE cross-peaks of H-6 and H-8 with the anomeric proton of rhamnose (δ 5.55), which was also in agreement with the upfield shift of −2.2 ppm observed for the resonance of C-7 (δ_C 161.7), relative to that of quercetin.[12] In comparison to the ^{13}C NMR data of quercetin, the downfield shifts of +9.2, +3.7, +2.0, and +1.4 ppm, observed for carbons C-2, C-1′, C-3′, and C-5′ of compound **1**, supported the glucosylation sites at C-3 and C-4′,[13] which

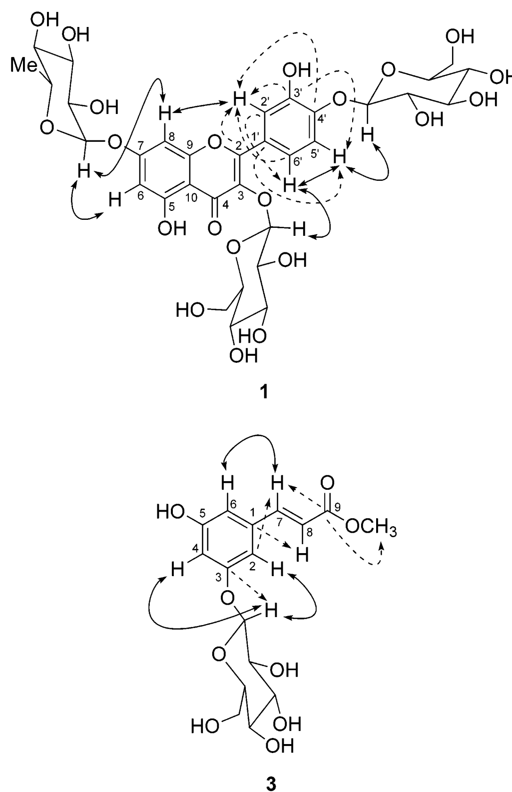

Figure 1. Relevant HMBC (- - ->) and NOE (→) correlations of **1** and **3**.

was confirmed by the NOE effects of H-5′and H-6′ with the anomeric protons at δ 4.87 (Glc I) and 5.51 (Glc II), respectively. The singlet displayed by the anomeric proton of rhamnose was indicative of an α-configuration, whereas the coupling constants of the anomeric protons of both glucose units (6.9 and 7.5 Hz) indicated their β-configuration. Detailed analysis of COSY, DEPT, HMQC, and HMBC spectra, and comparison with literature data,[13−18] confirmed the structure of the new flavonol glycoside **1** as quercetin 3,4′-di-*O*-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside, which has been named moricandin.

Compound **2** was obtained as a colorless oil, whose molecular formula, $C_{21}H_{28}O_4$, was inferred from the HRESIMS. Its IR spectrum displayed a strong hydroxyl band (3370 cm^{-1}) and carbonyl absorptions at ν_{max} 1710 and 1632 cm^{-1}. Upon acetylation, compound **2** formed a nona-acetate (**2a**), as indicated by its pseudomolecular ion peak at *m/z* 905 $[M + Na]^+$. The ^1H and ^{13}C NMR data of **2** (Table 2) exhibited the characteristic pattern of a caffeate moiety: three aromatic protons at δ 7.07 (1H, d, J = 8.1 Hz), 7.13 (1H, brs), and 7.21 (1H, d, J = 8.2 Hz) and two *trans*-olefinic protons at δ 6.41 (1H, d, J = 16.0 Hz) and 7.69 (1H, d, J = 15.6 Hz), both displaying HMBC correlations with a carbonyl ester at δ_C 167.4. In addition, the NMR spectra of **2** showed the presence of two pyranoses, for which the anomeric proton and carbon resonances appeared at δ 4.85 (1H, d, J = 7.2 Hz), δ_C 103.5, and δ 5.58 (1H, d, J = 7.4 Hz), δ_C 95.9. This assumption was also corroborated by the FDMS, which showed mass fragments at *m/z* 341 and 179, resulting from the successive loss of two sugar units. HMBC cross-peaks of C-4 and C-9, with the anomeric protons at δ 4.85 and 5.58, respectively, confirmed the glycosylation sites of caffeic acid. The acid hydrolysis of **2** afforded glucose as the only sugar, identified by GLC.[11] The new compound was thus identified as β-D-glucopyranosyl 4-*O*-β-D-glucopyranosylcaffeate (**2**), whose

Table 2. NMR Data of Compounds **2**, **2a**, and **3**

	2[a]		**2a**[b]		**3**[a]	
position	δ_H (J = Hz)	δ_C	δ_H (J = Hz)	δ_C	δ_H (J = Hz)	δ_C
aglycon						
1		131.0		129.4		128.8
2	7.13 (brs)	116.1	7.26[c]	115.3	7.11 (brs)	121.1
3		148.6		140.4		147.7
4		149.1		150.3	7.11 (brs)	116.3
5	7.21 (d, 8.2)	118.2	7.03 (d, 8.6)	122.9		147.1
6	7.07 (d, 8.1)	122.5	7.35 (dd, 8.6 2.0)	127.6	7.17 (brs)	115.1
7	7.69 (d, 15.6)	147.5	7.64 (d, 16.0)	145.6	7.53 (d, 16.0)	144.7
8	6.41 (d, 16.0)	116.7	6.28 (d, 16.0)	116.0	6.43 (d, 16.0)	115.9
9		167.4		168.4		167.0
glucose-I						
1′	4.85 (d, 7.2)	103.5	5.11−5.31[c]	98.3	4.77 (d, 6.7)	101.8
2′	3.47−3.51[c]	74.8[d]	5.11−5.31[c]	70.5[d]	3.29[c]	73.4
3′	3.47−3.51[c]	77.6[e]	5.11−5.31[c]	72.3[e]	3.29[c]	76.0
4′	3.40−3.44[c]	71.3[f]	5.11−5.31[c]	67.9[f]	3.16 (m)	70.0
5′	3.40−3.44[c]	78.4[g]	3.89 (m)	72.8[e]	3.31[c]	77.4
6′	3.83−3.91[c]	62.4	4.10−4.33[c]	61.5[g]	3.44 (m), 3.69 (m)	60.9
glucose-II						
1″	5.58 (d, 7.4)	95.9	5.82 (d, 8.0)	91.9		
2″	3.47−3.51[c]	74.1[d]	5.11−5.31[c]	70.3[d]		
3″	3.47−3.51[c]	78.2[e]	5.11−5.31[c]	72.5[e]		
4″	3.40−3.44[c]	71.2[f]	5.11−5.31[c]	68.2[f]		
5″	3.40−3.44[c]	78.9[g]	3.89 (m)	72.8[e]		
6″	3.83−3.91[c]	62.4	4.10−4.33[c]	61.9[g]		
CO-*Me*			1.91−2.07[c]	20.3−20.5[c]		
CO-Me				164.4−170.6[c]		
OMe					3.69 (s)	51.5

[a] Measured in DMSO-d_6. [b] Measured in $CDCl_3$. [c] Overlapped signals. [d−g] These values may be interchanged in the same column.

NMR assignments were confirmed by comparison with those reported for related caffeic acid glucosides.[19−21]

Compound **3**, obtained as a white amorphous powder, was assigned a molecular formula of $C_{16}H_{20}O_9$, as determined from the $[M + Na]^+$ ion at *m/z* 379.1005 in HRESIMS. As for compound **2**, its IR spectrum showed hydroxyl and α,β-unsaturated ester absorption bands at ν_{max} 3412, 1681, and 1630 cm^{-1}. The ^1H NMR spectrum of **3** exhibited three *meta*-related proton singlets at δ 7.11 (2H) and 7.17 (1H), two *trans*-olefinic proton doublets at δ 6.43 (J = 16.0 Hz) and 7.53 (J = 16.0 Hz), a methyl ester singlet (δ 3.69), and the characteristic signals of a pyranose ring. These data, supported by the ^{13}C NMR, DEPT, COSY, HMQC, HMBC, and NOESY spectra (Figure 1), were consistent with a methyl cinnamate moiety bearing one hydroxyl and one glycoside substituent (Table 2). The sugar, which was identified as glucose by GLC after acid hydrolysis of **3**, should be linked to C-3, as indicated by the HMBC cross-peak between this carbon (δ_C 147.7) and the anomeric proton (δ 4.77). Accordingly, compound **3** was formulated as methyl 3-*O*-β-D-glucopyranosyl-5-hydroxycinnamate.

Compound **4** was isolated as a colorless oil, whose molecular formula $C_{19}H_{26}O_{13}$ was determined by HRESIMS via a Na^+ adduct at *m/z* 485.1272 $[M + Na]^+$. The IR spectrum displayed absorption bands for hydroxyl (3368 cm^{-1}) and carbonyl groups (1715 cm^{-1}), as well as aromatic carbon to carbon stretching at ν_{max} 1510 and 1606 cm^{-1}. The ^1H and ^{13}C NMR data of **4** showed two pairs of doublets at δ 7.19 (8.9 Hz) and 8.07 (8.8 Hz), an α,β-unsaturated carbonyl ester at δ_C 166.5, and characteristic proton and carbon resonances for two pyranose units, both identified as β-D-glucose after acid hydrolysis of **4** (Table 3). The above data indicated a glucopyranosylbenzoate with a *para* glucosyl substituent, whose site of attachment was confirmed by the NOE correlation of the anomeric proton at δ 5.05 (Glc I) with the aromatic protons H-3 and H-5. Compound **4**, thus identified as β-D-glucopyranosyl 4-*O*-β-D-glucopyranosylbenzoate, is an analogue of the known benzoic acid glucosides 4-(β-D-glucopyranosyloxy)benzoic acid[22] and β-D-glucopyranosyl 4-hydroxybenzoate.[23] The latter corresponds to compound **5**, also found in the extract of *M. arvensis*, whose structure was confirmed by comparison of its spectral data with those reported in the literature.[23]

Table 3. NMR Data of Compound **4**[a]

position	δ_H (J = Hz)	δ_C
1		124.4
2	8.07 (d, 8.8)	133.0
3	7.19 (d, 8.9)	117.3
4		163.3
5	7.19 (d, 8.9)	117.3
6	8.07 (d, 8.8)	133.0
7		166.5
glucose-I		
1′	5.05 (d, 7.4)	101.6
2′	3.0.49 (m)	74.8[c]
3′	3.44−3.49[b]	77.9[d]
4′	3.45 (m)	71.3[e]
5′	3.44−3.49[b]	78.3[d]
6′	3.74 (m), 3.90 (m)	62.5
glucose-II		
1″	5.71 (d, 7.7)	96.2
2″	3.49 (m)	74.1[c]
3″	3.44−3.49[b]	78.1[d]
4″	3.45 (m)	71.1[e]
5″	3.44−3.49[b]	78.9[d]
6″	3.74 (m), 3.90 (m)	62.5

[a] Measured in CD_3OD. [b] Overlapped signals. [c−e] These values may be interchanged in the same column.

Compounds **6** and **7** were identified as methyl 4-*O*-β-D-glucopyranosylcaffeate and 1-*O*-caffeoyl-β-D-glucopyranoside, respectively, by comparison of their spectral data with those described in the literature.[19,20] To our knowledge, this is the first reported isolation of methyl 4-*O*-β-D-glucopyranosylcaffeate from a natural source, although it has been previously prepared from the corresponding acid precursor.[19] Compound **8** was characterized as 2-phenylethyl-β-D-glucopyranoside, by comparison of its spectral data with literature values.[24]

Table 4. Scavenging Effects on $ABTS^{\bullet+}$ and Reducing Power of Compounds **1**−**8**

compound	ABTS TEAC (mM)[a]	reducing power IC_{50} (μg/mL)
1	1.23 ± 0.02	48.1 ± 0.7
2	1.06 ± 0.02	95.9 ± 0.5
3	0.35 ± 0.01	630.8 ± 5.1
4	0.28 ± 0.01	735.4 ± 7.5
5	*b*	*b*
6	0.89 ± 0.01	486.2 ± 12.1
7	1.91 ± 0.03	33.5 ± 0.2
8	*b*	*b*
MeOH extract	1.62 ± 0.09	969.2 ± 7.1
quercetin	4.26 ± 0.12	24.4 ± 0.3
tocopherol	*c*	106.9 ± 0.2

[a] TEAC values were calculated at 15 min. [b] No activity observed. [c] Not determined.

The antioxidant activity of the MeOH extract of *M. arvensis* and compounds **1**−**8** was studied in the TEAC, DPPH, and reducing power assays. The TEAC reflects the ability of hydrogen-donating antioxidants to scavenge the $ABTS^{\bullet+}$ radical cation in aqueous phase, absorbing in the near-IR region at 734, 645, and 815 nm, compared with that of Trolox, a water-soluble vitamin E analogue. Antioxidants suppress the absorbance at 734 nm to an extent and on a time scale dependent on the antioxidant activity. The TEAC is defined as the concentration of Trolox solution with equivalent antioxidant potential to a 1.0 mM or 1 mg/mL concentration of the tested sample. The results exhibited a decreasing antioxidant activity in the order **7** > **1** > **2** > **6** > **3** > **4** (Table 4). Compounds **5** and **8** were not active. In the series of dihydroxy-cinnamic acids, the caffeic acid glucoside **7** showed a TEAC of 1.91 mM, a value that is higher than those reported by Re et al. (0.98 mM),[7] Zhang et al. (1.01 mM),[25] and Kuhnau (1.26 mM)[26] for caffeic acid. This result did not corroborate reported conclusions suggesting that the esterification of caffeic acid by a sugar moiety decreases or has no influence on the TEAC value.[27,28] On the other hand, the glucosylation of a phenol group in compounds **2** and **6**, and the absence of an *o*-dihydroxy structure in **3**, clearly contributed to the decrease of their activity. The benzoic acid glucoside **4** displayed a very weak activity, which is in agreement with TEAC values found for *para*-hydroxybenzoic acids and their esters.[28]

The TEAC of moricandin (**1**) was 3.4 times lower than that of quercetin, which is in accordance with structure−antioxidant activity relationships of flavonoids.[28] Although moricandin possesses a 2,3-double bond in conjugation with a 4-oxo function, and a free OH-5 group, which together constitute structural requirements for antioxidant activity, glycosylation at C-7 and C-4′ reduces the radical-scavenging capacity. The attachment of glucose to the OH-3 position does not seem to have any negative effect on the antioxidative capacity.[29]

In the reducing power assay, the formation of Fe^{2+} in the reduction of F^{3+}/ferricyanide complex by antioxidants is monitored at 700 nm. As expected, compounds **1** and **7** proved to be the most active, with IC_{50} values of 48.1 and 33.5 μg/mL, respectively (Table 4).

The above results correlate well with those obtained in the DPPH model system. At the lowest tested concentration (15 μg/mL), the inhibition percentage of radical-scavenging activity for compounds **7** and **1** was 72% and 60%, respectively, whereas the reference compounds quercetin and α-tocopherol showed a scavenging effect of 87%.

The results presented here indicate a high content of phenol glycosides in the flowers of *M. arvensis*, which constitute its active antioxidant principles.

Experimental Section

General Experimental Procedures. Melting points were determined on a Reichert microscope. Optical rotations were obtained using a Perkin-Elmer 241-MC polarimeter. UV spectra were recorded on a Milton Roy Spectronic 1201 spectrophotometer, and FTIR spectra were measured on a Perkin-Elmer 157G infrared spectrophotometer. The NMR spectra were recorded on a Bruker ARX 400 NMR spectrometer (^1H at 400 MHz; ^{13}C at 100.61 MHz), using CD_3OD, DMSO, or $CDCl_3$ as solvent. EIMS (70 eV) and FDMS were carried out on a Micromass GCTOF spectrometer, ESIMS and HRESIMS experiments were performed on an Agilent MSD1100 single quadropole spectrometer and on an Agilent ESI-TOF instrument, respectively, and FABMS were performed on a Micromass Autospec spectrometer. Gas−liquid chromatography was performed on a Carlo Erba GC 6000 Vega Series 2 chromatograph equipped with a flame-ionization detector. Normal- and reversed-phase silica gel were used for flash chromatography (Merck 13905 and 13900, respectively). TLC were performed on normal- and reversed-phase precoated silica gel F_{254} plates (MN 818133 and Merck 5559, respectively) and visualized under UV light and by spraying with sulfuric or $CeSO_4$ spray reagents, followed by heating. NH_2 HPTLC plates (Merck 15647) were used for sugar analysis. Silica gel plates of 0.50 mm thickness (Merck 5744) were used for preparative TLC.

Plant Material. The flowers of *Moricandia arvensis* were collected in Monastir, in March 2003, and identified by Dr. F. Harzallah-Skhiri, from Ecole Supérieure d'Horticulture et d'Elevage de Chott Mériem, Université du Centre, Sousse, Tunisia, where a voucher specimen (HCM-120) has been deposited.

Extraction and Isolation. The fresh violet flowers (1 kg) were macerated at room temperature in MeOH (3 × 5 L) for 48 h. The combined methanolic extracts were concentrated to dryness, yielding a residue of 37 g, which was dissolved in methanol (1 L) and defatted with petroleum ether (40−60 °C). The resulting methanolic extract (30 g) was chromatographed on silica gel (1 kg) using an eluting mixture of CH_2Cl_2−MeOH (100:0 to 80:20; 2% gradient; 0.6 L each eluent; fractions of 200 mL; 78:22 to 76:24; 2% gradient; 2 L each eluent; fractions of 200 mL; 74:26 to 50:50; 2% gradient; 4 L each eluent; fractions of 200 mL), yielding 12 crude fractions, A_1−A_{12}. The residue (8.4 g) of fraction A_{12} (58:42 to 50:50; 20 L) was subjected to RP-18 flash column chromatography using mixtures of H_2O−MeOH (1:0, 0.5 L; 90:10, 0.5 L; 80:20, 0.5 L; 70:30, 0.5 L; 60:40, 0.5 L; 1:1, 0.5 L; fractions of 50 mL) to yield fractions B_1−B_9. A 100 mg sample of fraction B_5 (145 mg; 70:30 to 60:40; 650 mL) was submitted to RP-18 flash column chromatography using gradient elution by H_2O−MeOH (1:0, 50 mL; 90:10, 50 mL; 80:20, 50 mL; 70:30, 50 mL; fractions of 5 mL) to give 20 mg of **1** (80:20 to 70 30; 55 mL). RP-18 flash column chromatography of 200 mg of fraction B_4 (270 mg; 80:20 to 70:30; 150 mL) using mixtures of H_2O−MeOH (90:10, 0.2 L; 80:20, 0.2 L; 70:30, 0.2 L; fractions of 10 mL) yielded fractions C_1−C_4. Preparative TLC (1 × $CHCl_3$−AcOH−MeOH−H_2O, 60:32:12:8) of fraction C_2 (28 mg; 90:10; 50 mL) yielded 13 mg of **2**, whereas evaporation of C_4 (70:30; 30 mL) gave 10 mg of **3**. Preparative TLC (2 × $CHCl_3$−AcOH−MeOH−H_2O, 60:32:12:8) of 100 mg of fraction B_3 (120 mg; 80:20 to 70:30; 250 mL) afforded 7 mg of **4**. Fraction A_8 (500 mg; 84:16 to 78:22; 2.8 L) was subjected to RP-18 flash column chromatography using mixtures of H_2O−MeOH (95:5, 0.2 L; 90:10, 0.2 L; 80:20, 0.2 L; 70:30, 0.2 L; 60:40, 0.2 L; fractions of 20 mL) to yield 6 mg of **5** (90:10; 200 mL). Fraction A_7 (122 mg; 92:8 to 86:14; 2.4 L) was subjected to RP-18 flash column chromatography (H_2O−MeOH, 95:5, 0.2 L; 90:10, 0.2 L; 85:15, 0.2 L; 80:20, 0.2 L; 75:25, 0.2 L; 70:30, 0.2 L; fractions of 20 mL) to yield three fractions (D_1−D_3) of 45 mg (80:20; 140 mL), 25 mg (75:25; 180 mL), and 15 mg (75:25 to 70:30; 260 mL), respectively. Fractions D_1 (45 mg; 80:20; 140 mL), D_2 (25 mg; 75:25; 180 mL), and D_3 (15 mg; 85:15; 30 mL) were rechromatographed by RP-18 flash column chromatography (H_2O−MeOH, 85:15,

100 mL; fractions of 5 mL) to afford 15 mg of **6** (85:15; 30 mL), 17 mg of **7** (85:15; 25 mL), and 13 mg of **8** (85:15; 30 mL).

Moricandin (quercetin 3,4′-di-*O*-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside, 1): yellow powder; mp 198−200 °C; $[\alpha]_D^{25}$ −113.5° (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 256 (4.00), 265 (2.10), 344 (3.75) nm; IR ν_{max} 3400, 2925, 1654, 1600 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 773.2141 $[M + H]^+$ (calcd for $C_{33}H_{41}O_{21}$, 773.2140); FD^+MS *m/z* 464 [M − glucose − rhamnose]$^+$ (12), 448 [M − 2 × glucose]$^+$ (22), 302 [M − 2 × glucose − rhamnose]$^+$ (100).

***β*-D-Glucopyranosyl 4-*O*-*β*-D-glucopyranosylcaffeate (2):** colorless oil; $[\alpha]_D^{25}$ −38.5° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (4.10), 233 (3.80), 285 (4.4.15) nm; IR ν_{max} 3368, 2925, 1710, 1632 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRESIMS *m/z* 527.1376 $[M + Na]^+$ (calcd for $C_{21}H_{28}Na\ O_{14}$, 527.1377); FD^+MS *m/z* 341 [M − H − glucose]$^+$ (9), 179 [M − H − 2 × glucose]$^+$ (100).

Methyl 3-*O*-*β*-D-glucopyranosyl-5-hydroxycinnamate (3): white powder; mp 195−197 °C; $[\alpha]_D^{25}$ +100° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (3.90), 230 (3.10), 290 (4.00) nm; IR ν_{max} 3412, 3276, 2925, 1681, 1630, 1597 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRESIMS *m/z* 379.1005 $[M + Na]^+$ (calcd for $C_{16}H_{20}NaO_9$, 379.1005).

***β*-D-Glucopyranosyl 4-*O*-*β*-D-glucopyranosylbenzoate (4):** colorless oil; $[\alpha]_D^{25}$ −18.4° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 211 (4.30), 238 (3.50), 300 (3.40) nm; IR ν_{max} 3368, 2925, 1715, 1606, 1510 cm^{-1}; ^1H and ^{13}C NMR, see Table 3; HRESIMS *m/z* 485.1272 $[M + Na]^+$ (calcd for $C_{19}H_{26}NaO_{13}$, 485.1271).

Acetylation of Compounds 1 and 2. Each compound (5 mg) was dissolved in pyridine−acetic anhydride (0.5:0.5 v/v), and the solution was allowed to stand overnight at room temperature. The reaction mixture was diluted with water and extracted three times with ethyl acetate, and the organic phase was evaporated in vacuo. The acetylated compounds were further purified by RP-18 flash column chromatography (H_2O−MeOH, 30:70, 0.2 L, fractions of 5 mL) to yield **1a** (4 mg) and **2a** (5 mg).

Moricandin peracetate (1a): yellow powder; mp 122−124 °C; $[\alpha]_D^{25}$ −82.4° (*c* 0.17, $CHCl_3$); ^1H and ^{13}C NMR, see Table 1; ESIMS *m/z* 1319 $[M + H]^+$ ($C_{59}H_{67}O_{34}$), 1341 $[M + Na]^+$ ($C_{59}H_{66}NaO_{34}$).

Nona-*O*-acetyl-*β*-D-glucopyranosyl 4-*O*-*β*-D-glucopyranosylcaffeate (2a): colorless oil; $[\alpha]_D^{25}$ −15.8° (*c* 0.2, $CHCl_3$); ^1H and ^{13}C NMR, see Table 2; ESIMS *m/z* 905 $[M + Na]^+$.

Identification of Sugar Units of Compounds 1−4. Each compound (1 mg) was refluxed for 2 h in MeOH (0.5 mL) acidified with 2 M HCl (1 mL). The reaction mixture was neutralized with NaOH and concentrated in vacuo, and the aglycon extracted with EtOAc (2 × 5 mL). The aqueous layer was analyzed for its sugar composition on NH_2 TLC plates (CH_3CN−H_2O, 7:3), using commercial standards of D-glucose and L-rhamnose, and α-naphthol as spray reagent. These aqueous fractions were evaporated in vials under nitrogen, derivatized with (+)-1-phenylethanethiol, and then acetylated with acetic anhydride containing DMAP to form the corresponding acetylated dithiocetals,[11] which were applied to GLC. GLC conditions: J&W DB-5 column, 28 m × 0.25 mm, 0.1 μm; column temperature, 280 °C; He flow rate, 30 cm/s; t_R of derivatives, L-rhamnose 5.90 mn, D-glucose 9.50 mn. Standards of D-glucose and L-rhamnose were derivatized and analyzed as described above.

Radical Cation ABTS$^{\bullet+}$ Scavenging Activity. The radical cation ABTS$^{\bullet+}$ scavenging activity was evaluated according to the modified TEAC method of Re et al.[7] ABTS$^{\bullet+}$ (7 mM) was dissolved in milli Q water and added to potassium persulfate to reach a 2.4 mM final concentration. The reaction mixture was left 16 h in the dark at room temperature, and the radical cation solution was further diluted with water (1 mL solution in ca 60 mL of water) to give an absorbance value of 0.700 ± 0.03 at 734 nm. Samples were diluted with H_2O−EtOH so that after the addition of 15 μL to 1.485 mL of ABTS$^{\bullet+}$ a 20%−80% decrease in the initial absorbance at 734 nm was observed. The decrease of absorbance was recorded at time intervals of 1, 2, 5, 10, and 15 min, for a range of 5−8 concentrations (0 to 20 μM final concentration after addition of ABTS$^{\bullet+}$) for each sample. Assays were performed in triplicate, and solvent blanks were run in each assay. The percentage inhibition of absorbance at 734 nm was calculated and plotted as a function of sample concentration and that of the antioxidant standard Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The scavenging activity is estimated within the range of the dose−response curve of Trolox and expressed as the Trolox equivalent antioxidant capacity (TEAC), which is defined as the concentration (mM) of Trolox having the antioxidant capacity equivalent to a 1.0 mM or 1 mg/mL of the tested sample solution.

Reducing Power Assay. Reducing power was determined according to the method of Oyaizu.[9] Samples (0.02−1.0 mg) were dissolved in 1.0 mL of milli Q water to which was added 2.5 mL of a 0.2 mM phosphate buffer (pH 6.6) and 2.5 mL of a 1% (w/v) solution of potassium ferricyanide. The mixture was incubated in a water bath for 20 min at 50 °C, followed by the addition of 2.5 mL of a 10% (w/v) trichloroacetic acid solution. The mixture was then centrifuged at 5000 rpm for 10 min, and a 2.5 mL aliquot of the resulting upper layer was combined with 2.5 mL of Milli Q water and 0.5 mL of a 0.1% (w/v) $FeCl_3$ solution. The absorbance of the reaction mixture was read spectrophotometrically at 700 nm against a blank sample. The mean absorbances from three independent samples were plotted against concentration, and a linear regression analysis was carried out to calculate the IC_{50} value, defined as the effective concentration at which the decrease in absorbance was 0.50 at 700 nm. α-Tocopherol and quercetin were used as standards.

DPPH Assay. DPPH$^\bullet$-scavenging activity was measured according to the procedure described by Blois.[8] Briefly, each test sample (50 μL) of various concentrations (0.015−2.0 mg/mL) was added to 950 μL of freshly prepared DPPH solution (0.004% in MeOH), and the mixture vortexed for 15 s. The decrease in absorbance at room temperature was determined at 515 nm after 30−45 min of incubation, until the reaction reached a steady state. All experiments were performed in triplicate. The inhibition percentage (%) of radical-scavenging activity was calculated as $(1 - A_s/A_0) \times 100$, where A_0 and A_s are the absorbance of the control and sample, respectively, at 515 nm. α-Tocopherol and quercetin were used as standards.

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Supporting Information Available: Structures of compounds **5**−**8**, and figures of the scavenging effect of compounds **1**−**4**, **6**, and **7** on DPPH radicals, and reducing power of compounds **1**−**4**, **6**, and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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