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Giffonins A−I, Antioxidant Cyclized Diarylheptanoids from the Leaves of the Hazelnut Tree (*Corylus avellana*), Source of the Italian PGI Product "Nocciola di Giffoni"

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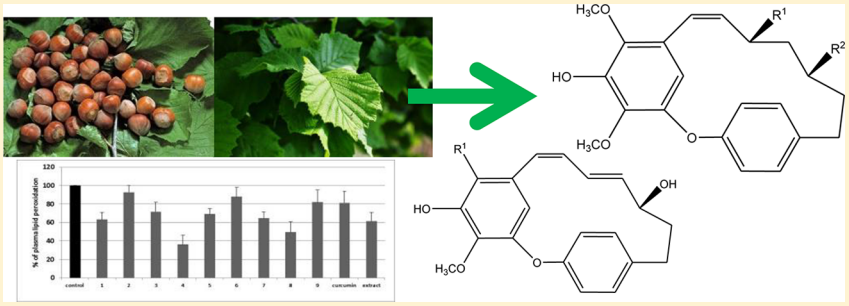
Supporting Information



ABSTRACT: Eight new diaryl ether heptanoids, giffonins A−H (**1**−**8**), and one diaryl heptanoid, giffonin I (**9**), were isolated from the methanol extract of the leaves of *Corylus avellana*. Its hazelnut is the PGI product of the Campania region (Italy) known as "Nocciola di Giffoni". The MeOH extract of *C. avellana* leaves and giffonins A−I (**1**−**9**) were evaluated for their inhibitory effects on human plasma lipid peroxidation induced by H_2O_2 and H_2O_2/Fe^{2+}, by measuring the concentration of TBARS (thiobarbituric acid reactive substances). Compounds **4** and **8** at 10 μM reduced both H_2O_2- and H_2O_2/Fe^{2+}-induced lipid peroxidation by more than 60% and 50%, respectively, indicating higher activity than curcumin used as reference compound.

Hazelnut (*Corylus avellana* L.), belonging to the Betulaceae family, is one of the most popular tree nuts on a worldwide basis and ranks second in tree nut production after almond. Italy is the second largest producer of hazelnut (13%) after Turkey. The main products of *C. avellana* are kernels, nutritious food with a high content of healthy lipids,[1] used by the confectionary industry, consumed raw (with skin) or preferably roasted (without skin). The leaves of *C. avellana* are used in traditional medicine for the treatment of varicose veins and hemorrhoidal symptoms and also for their mild antimicrobial effects.[2] Antioxidant activity was reported for hazelnuts and leaves of *C. avellana*.[1] Previous phytochemical investigations on the leaves resulted in the isolation of phenolic constituents, such as flavonoids, caffeic acid, and diarylheptanoid derivatives.[3]

The Italian "Nocciola di Giffoni", also known as "tonda di Giffoni", is a labeled PGI (protected geographical indication) product of the Campania region, representing an important economic resource. Although the nutritive features of the PGI "Nocciola di Giffoni" hazelnut are well known,[4,5] no studies are reported on the chemical composition of the leaves of *C. avellana*, the source of PGI hazelnut. As part of an ongoing effort to search for new bioactive compounds,[6−8] the MeOH extract of the leaves of *C. avellana* was investigated. Herein, the isolation and the structural elucidation of eight new diaryl ether heptanoids (**1**−**8**) along with a new diaryl heptanoid (**9**) are described as well as their effects on oxidative damage of human plasma lipids, induced by H_2O_2 and H_2O_2/Fe^{2+}.

RESULTS AND DISCUSSION

The MeOH extract of the leaves of *C. avellana* was fractionated on a Sephadex LH-20 column, and the fractions were purified by semipreparative HPLC to afford giffonins A−I (**1**−**9**).

The HR-MALDI-TOF-MS of **1** (*m*/*z* 377.1369 $[M + Na]^+$, calcd for $C_{21}H_{22}O_5Na$, 377.1365) and the ^{13}C NMR data supported a molecular formula of $C_{21}H_{22}O_5$. The IR spectrum showed a band at 1705 cm^{-1} for the presence of a ketocarbonyl group.

The ^1H NMR spectrum displayed signals for five aromatic protons ascribable to two aromatic rings: a signal at δ 4.41 (s), typical of the proton of a pentasubstituted aromatic ring and signals at δ 7.41 (2H, d, J = 8.3 Hz) and 7.01 (2H, d, J = 8.3 Hz), due to the proton of a 1,4-disubstituted aromatic ring. The ^1H NMR data displayed further signals due to a disubstituted *trans*-olefinic group at δ 6.34 (d, J = 15.7 Hz) and 5.20 (dt, J = 8.0, 15.7 Hz) and four methylene groups at δ 2.32 (2H, m),

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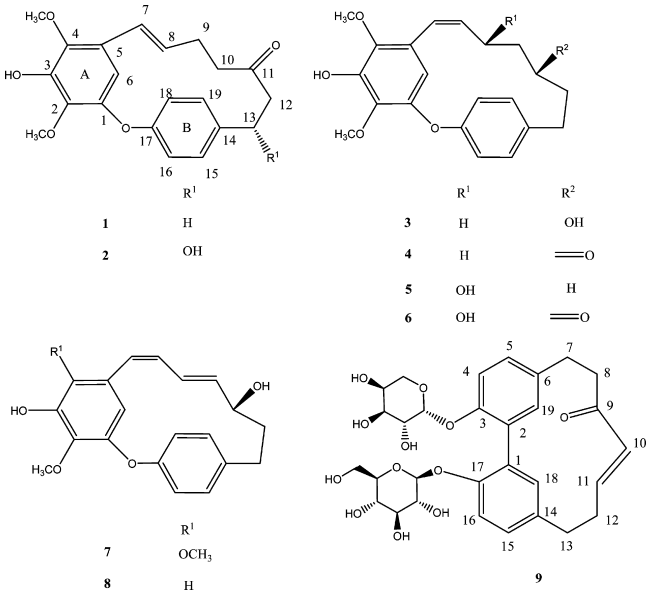

2.44 (2H, t, J = 5.5 Hz), 2.86 (2H, t, J = 5.5 Hz), and 3.03 (2H, t, J = 5.5 Hz) (Table 1). Moreover, two signals for two methoxy groups at δ 4.00 and 3.67 were evident. The ^{13}C NMR spectrum of **1** showed 21 carbon signals (Table 1), typical of diaryl ether heptanoid derivatives,[9−11] comprising a signal at δ 211.1, ascribable to a ketocarbonyl group. The signal at δ 4.41 was attributed to H-6, which generally resonates at an abnormally high field, due to the anisotropic effect of the A ring in diaryl ether heptanoids.[10] This observation together with the ROESY correlations of H-6 at δ 4.41 with H-18 (δ 7.01) and H.16 (δ 5.20) suggested the ether linkage between C-1 and C-17 of the aryl moieties. The HMBC correlations of H-6 (δ 4.41) with the ^{13}C NMR resonances at δ 152.7 (C-1), 136.9 (C-2), 140.4 (C-4), 127.8 (C-5), and 125.5 (C-7) suggested the 1,2,3,4-tetrahydroxylation of the A ring. The disposition of the heptanoid chain and the position of the keto group were determined by HSQC, HMBC, and COSY experiments. In the HMBC spectrum of **1**, the methylene protons at δ 3.03 were assigned to C-13 on the basis of their correlations with the ^{13}C NMR resonances at δ 132.2 (C-15 and C-19), 141.0 (C-14), 46.2 (C-12), and 211.1 (C-11). The COSY correlation between the methylene protons at δ 3.03 and 2.86 allowed the latter methylene group to be located at C-12, and therefore the keto group to be located at C-11. The HMBC correlations of the proton at δ 6.34 (H-7) with the ^{13}C NMR resonances of the B ring at δ 140.4 (C-4), 127.8 (C-5), and 109.0 (C-6) and the linear connectivity observed in the COSY spectrum from H-7 to H-10 were used to assign the heptanoid chain. Finally, the HMBC correlations between the protons at δ 4.00 and 3.67 with the ^{13}C NMR resonances at δ 136.9 (C-2) and 140.4 (C-4), respectively, allowed the methoxy groups to be located at C-2 and C-4 of the A ring. The foregoing spectroscopic data allowed the structure of compound **1**, named giffonin A, to be assigned as shown.

The HR-MALDI-TOF-MS of **2** (m/z 393.1318 [M + Na]$^+$, calcd for $C_{21}H_{22}O_6Na$, 393.1314) and the ^{13}C NMR data supported a molecular formula of $C_{21}H_{22}O_6$. The ^{1}H NMR spectrum displayed five aromatic proton signals, which suggested the occurrence of a pentasubstituted [δ 4.39 (s)] and a 1,4-disubstituted aromatic ring [δ 7.70 (dd, J = 8.3, 1.9 Hz), 7.45 (dd, J = 8.3, 1.9 Hz), 7.16 (dd, J = 8.3, 1.9 Hz), 7.00 (dd, J = 8.3, 1.9 Hz)] (Table 1), as in **1**. The NMR data of **2** revealed that it differed from **1** by the presence of a secondary hydroxy group suggested by a signal at δ 5.23 (dd, J = 5.4, 8.9 Hz) (Table 1). The HMBC correlations of the proton at δ 5.23

Table 1. ^{13}C and ^{1}H NMR Data (J in Hz) of Compounds 1−4 (600 Mz, δ ppm, in Methanol-d_4)

	1		2		3		4	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	152.7		152.7		151.8		151.8	
2	136.9		137.0		137.5		137.6	
3	144.3		144.1		144.7		145.0	
4	140.4		140.6		141.6		141.9	
5	127.8		128.0		124.7		125.7	
6	109.0	4.41, s	109.2	4.39, s	108.3	5.34, s	108.0	5.18, s
7	125.5	6.34, d (15.7)	125.6	6.36, d (15.7)	124.6	6.35, d (11.4)	125.9	6.40, d (11.4)
8	135.5	5.20, dt (8.0, 15.7)	135.5	5.14, ddd (15.7, 10.8, 4.0)	132.5	5.51, ddd (11.4, 7.3, 7.3)	131.0	5.48, dt (11.4, 8.0)
9	26.7	2.32, (2H) m	26.1	2.52, m; 2.00, m	27.0	1.90, m; 1.78, m	23.0	1.94, (2H) m
10	44.3	2.44, (2H) t (5.5)	45.2	2.57, dt (17.0, 3.0); 2.29, ddd (3.0, 11.0, 12.0)	40.1	1.05, m; 0.43, m	46.5	1.94, (2H) m
11	211.1		209.5		72.6	3.31, overlapped	213.9	
12	46.2	2.86, (2H) t (5.5)	54.2	3.04, (2H) t (5.5)	40.9	1.96, m; 1.72, m	45.0	2.66, (2H) t (6.8)
13	29.8	3.03, (2H) t (5.5)	71.2	5.23, dd (5.4, 8.9)	34.2	3.04, dt (12.8, 3.7); 2.74, td (12.8, 5.0)	33.6	3.03, (2H) t (6.8)
14	141.0		143.4		140.0		138.6	
15	132.2	7.41, d (8.3)	131.4	7.45, dd (8.3, 1.9)	130.8	7.37, dd (8.3, 1.9)	131.8	7.26, d (8.3)
16	125.2	7.01, d (8.3)	124.4	7.00, dd (8.3, 1.9)	124.9	7.17, dd (8.3, 1.9)	124.5	7.06, d (8.3)
17	155.8		158.5		156.3		157.0	
18	125.2	7.01, d (8.3)	125.8	7.16, dd (8.3, 1.9)	123.8	6.98, dd (8.3, 1.9)	124.5	7.06, d (8.3)
19	132.2	7.41, d (8.3)	128.4	7.70, dd (8.3, 1.9)	132.8	7.30, dd (8.3, 1.9)	131.8	7.26, d (8.3)
2-OCH_3	61.7	4.00, s	61.6	4.00, s	61.3	4.03, s	61.4	4.02, s
4-OCH_3	61.2	3.67, s	61.0	3.66, s	61.0	3.68, s	61.2	3.69, s

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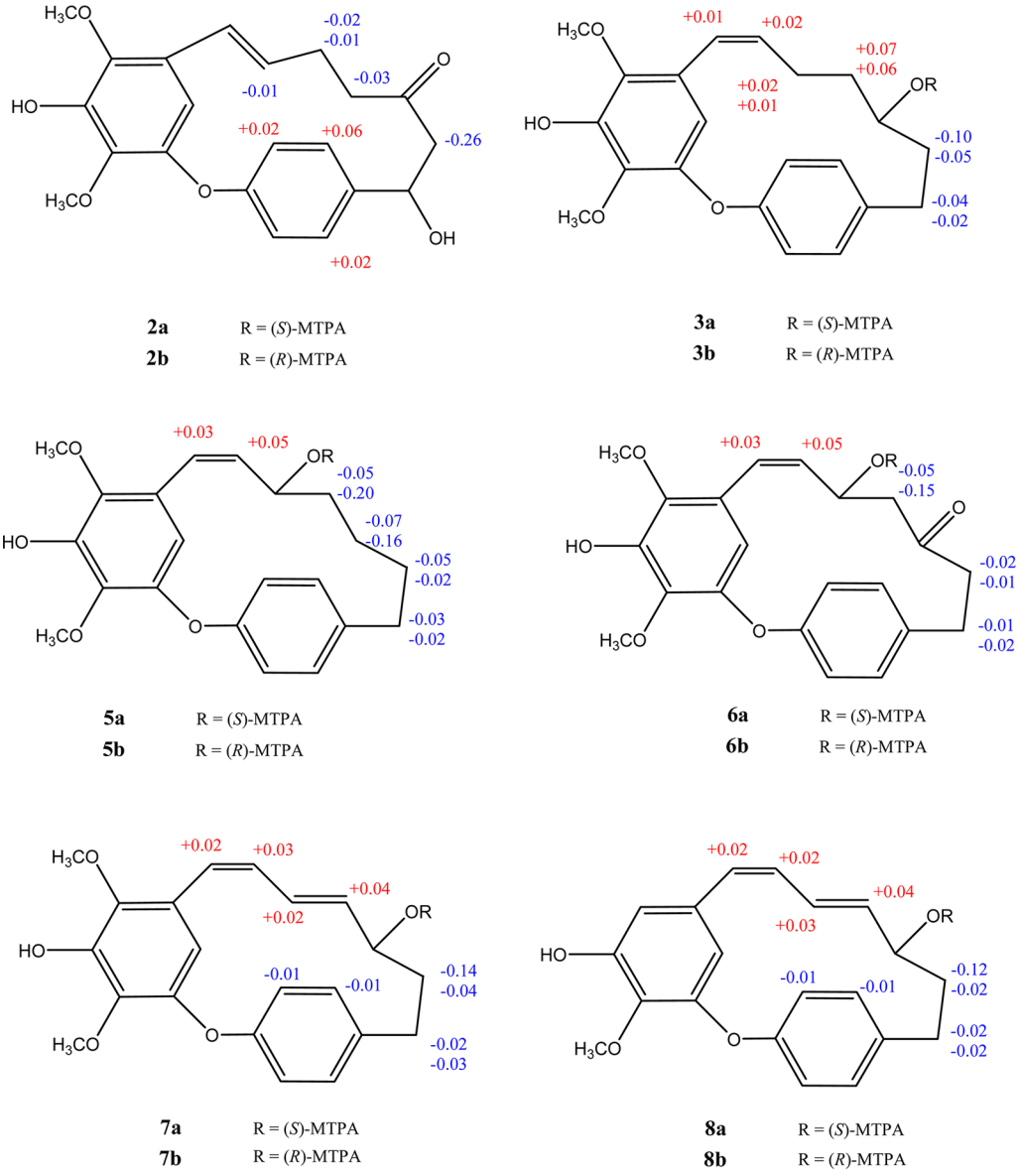

Figure 1. $\Delta\delta$ ($\delta_S - \delta_R$) values (in ppm) for the MTPA esters of **2**, **3**, and **5**–**8**.

with the ^{13}C NMR resonances at δ 131.4 (C-15), 128.4 (C-19), 143.4 (C-14), 54.2 (C-12), and 209.5 (C-11) suggested the secondary hydroxy group to be located at C-13. The presence of hydroxy groups on the heptane moiety causes the different chemical shifts for the corresponding protons on the aromatic ring (H-15 and H-19; H-16 and H-18).[11] The ROESY spectrum showed correlations between H-6 at δ 4.39 and H-8 (δ 5.14). The aromatic proton H-19 at δ 7.70 showed a correlation with the methylene protons H-9 (δ 2.00), and H-13 at δ 5.23 showed a strong correlation with H-15 (δ 7.45). The absolute configuration at C-13 of compound **2** was determined through the application of the modified Mosher's method.[12] Mosher derivatization was performed on compound **2**, which was treated with (*R*)- and (*S*)-MTPA chloride to form (*S*)-MTPA and (*R*)-MTPA esters, respectively. To determine the absolute configuration of C-13 in **2**, the $\Delta\delta$ ($\delta_S - \delta_R$) values were observed for signals of the protons close to C-13, revealing a 13*S* configuration for **2**. Thus, on the basis of the above data the structure of **2**, named giffonin B, was determined as reported.

The positive-ion HR-MALDI-TOF-MS data of **3** showed a pseudomolecular ion at *m*/*z* 379.1524 $[M + Na]^+$ (calcd for $C_{21}H_{24}O_5Na$, 379.1521), which in combination with the ^{13}C NMR data supported a molecular formula of $C_{21}H_{24}O_5$. Comparison of the ^{1}H NMR spectrum of **3** in the aromatic region with that of giffonin B (**2**) suggested that they share the same aromatic substitution patterns (Table 1). Moreover, the ^{1}H NMR spectrum displayed signals due to a disubstituted *cis*-olefinic function at δ 6.35 (d, J = 11.4 Hz) and 5.51 (ddd, J = 11.4, 7.3, 7.3 Hz), a proton linked to an oxymethine carbon at δ 3.31, and two methoxy groups at δ 4.03 (s) and 3.68 (s) (Table 1). On the basis of the COSY experiment the connectivity from H-7 to H-13 was established and the hydroxy group was located at C-11 (δ 72.6). The methoxy groups were placed at C-2 and C-4 via the HMBC correlations between the protons at δ 4.03 and 3.68 with the ^{13}C NMR resonances at δ 137.5 and 141.6, respectively. The hydroxy group at C-11, bulkier than a hydrogen atom, should be directed away from the inside of the macrocyclic ring.[11] The ROE correlations between H-15 (δ 7.37) and H-13β (δ 3.04) and between H-19 (δ 7.30) and H-

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Table 2. ^{13}C and ^{1}H NMR Data (J in Hz) of Compounds 5−8 (600 Mz, δ ppm, in Methanol-d_4)

	5		6		7		8	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	151.9		152.1		150.4		155.6	
2	137.6		138.3		136.7		136.9	
3	144.5		145.2		144.2		151.7	
4	141.6		142.4		140.4		110.8	6.30, d (1.7)
5	125.7		124.7		125.1		133.5	
6	108.5	5.11, s	108.3	4.89, s	110.0	5.46, s	109.6	5.51, d (1.7)
7	123.0	6.33, d (11.6)	124.9	6.46, d (12.0)	124.5	6.36, d (11.4)	129.9	6.09, d (11.2)
8	136.8	5.32, dd (11.6, 8.6)	132.2	5.31, dd (12.0, 8.8)	129.0	6.01, dd (11.4, 9.2)	128.8	5.94, t (11.2)
9	69.5	3.86 ddd (12.1, 8.6, 3.6)	65.3	4.33, t (9.8)	126.6	5.67, dd (15.4, 9.2)	126.8	5.68, dd (15.4, 11.2)
10	39.0	1.32, m; 0.31, br t (12.1)	51.3	2.49, m; 1.74, br s	139.9	5.64, dd (15.4, 8.6)	140.1	5.63, dd (15.4, 8.6)
11	22.9	1.78, q (10.8); 1.08, m	215.4		73.2	4.07, dt (8.6, 3.3)	73.2	4.09, dt (8.6, 3.4)
12	30.5	2.11, m; 1.30, m	44.7	2.84, td (12.5, 5.6); 2.51, m	42.3	2.05, dq (14.2, 3.3); 1.66, tt (14.2, 3.3)	42.5	2.05, dq (14.2, 3.4); 1.67, tt (14.2, 3.4)
13	36.3	3.05, m; 2.47, td (12.7, 5.4)	32.9	3.08, m; 3.03, td (12.1, 5.6)	34.3	3.04, dt (12.8, 3.0); 2.71, td (12.8, 3.0)	34.6	3.04, dt (13.0, 3.3); 2.72, td (13.0, 3.3)
14	140.1		138.6		139.3		140.9	
15	130.9	7.40, dd (8.3, 1.9)	130.5	7.52, dd (8.3, 1.9)	132.8	7.33, dd (8.3, 1.9)	133.2	7.36, dd (8.3, 1.9)
16	124.9	7.15, dd (8.3, 1.9)	125.0	7.20, dd (8.3, 1.9)	122.8	7.20, dd (8.3, 1.9)	123.0	7.19, dd (8.3, 1.9)
17	155.8		156.9		155.2		156.7	
18	123.2	7.00, dd (8.3, 1.9)	123.8	6.98, dd (8.3, 1.9)	125.6	7.01, dd (8.3, 1.9)	126.3	7.03, dd (8.3, 1.9)
19	134.0	7.39, dd (8.3, 1.9)	134.0	7.12, dd (8.3, 1.9)	130.5	7.42, dd (8.3, 1.9)	130.7	7.44, dd (8.3, 1.9)
2-OCH_3	61.4	4.03, s	61.2	4.03, s	61.2	4.05, s	60.0	4.03, s
4-OCH_3	61.2	3.71, s	61.2	3.71, s	61.0	3.69, s		

13α (δ 2.74) and H-11 (δ 3.31) were observed. Mosher derivatization was performed on compound **3**, which was treated with (*R*)- and (*S*)-MTPA chloride to form (*S*)-MTPA and (*R*)-MTPA esters, respectively. Analysis of the $\Delta\delta(S-R)$ values of the protons close to the oxygenated methine according to the Mosher model[12] (Figure 1) allowed the assignment of the 11*R* absolute configuration of **3**. Thus, the structure of compound **3**, named giffonin C, was elucidated as depicted.

The ^{13}C NMR and HR-MALDI-TOF-MS data of **4** (m/z 377.1367 $[M + Na]^+$, calcd for $C_{21}H_{22}O_5Na$, 377.1365) supported a molecular formula of $C_{21}H_{22}O_5$. The IR spectrum showed a band at 1713 cm^{-1} for the presence of a ketocarbonyl group. The NMR data of compound **4** were comparable to those of giffonin C (**3**) except for the presence of a carbonyl group replacing the secondary hydroxy group in **3**. Analyses of HMBC and COSY experiments confirmed the presence of a carbonyl group at C-11 (δ 213.9). Notably, the absence of the hydroxy group on the heptanoid chain induced similar chemical shifts of H-15 and H-19 (each, δ 7.26, d, J = 8.3 Hz) and H-16 and H-18 (each, δ 7.06, d, J = 8.3 Hz) (Table 1). On the basis of the reported data the structure of **4**, named giffonin D, was deduced as depicted.

The molecular formula of **5** was established as $C_{21}H_{24}O_5$ by HR-MALDI-TOF-MS (m/z 379.1526 $[M + Na]^+$, calcd for $C_{21}H_{24}O_5Na$, 379.1521) and ^{13}C NMR data. The ^{1}H NMR spectrum of **5** displayed signals at δ 7.40 (dd, J = 8.3, 1.9 Hz), 7.39 (dd, J = 8.3, 1.9 Hz), 7.15 (dd, J = 8.3, 1.9 Hz), 7.00 (dd, J = 8.3, 1.9 Hz), 5.11 (s), 4.03 (s), and 3.71 (s), ascribable to the two aryl moieties with the same substitution pattern as in **3** (Table 2). Further signals of a disubstituted *cis*-olefinic function at δ 6.33 (d, J = 11.6 Hz) and 5.32 (dd, J = 11.6, 8.6 Hz) and of a proton linked to an oxymethine carbon at δ 3.86 (ddd, J = 12.1, 8.6, 3.6 Hz) (Table 2) were observed. The position of the hydroxy group was determined by COSY and HMBC correlations. In the HMBC spectrum, correlations between the proton signals at δ 6.33 (H-7) and the ^{13}C NMR resonances at δ 141.6 (C-4), 125.7 (C-5), 108.5 (C-6), 136.8 (C-8), and 69.5 (C-9) were observed. In the COSY spectrum the correlation between the proton at δ 6.33 (H-7) and the proton at δ 5.32 (H-8), which in turn correlated with the proton at δ 3.86 (H-9), allowed the hydroxy group to be located at C-9. The ^{1}H and ^{13}C NMR chemical shifts of the heptene moiety and the coupling constant of the proton of the secondary hydroxy function (δ 3.86, ddd, J = 12.1, 8.6, 3.6 Hz) of compound **5** were almost superimposable to those of 3,5′-dihydroxy-4′-methoxy-3′,4″-oxy-1,7-diphenyl-1-heptene, isolated from *Betula platyphylla* var. *japonica*.[13] The absolute configuration of compound **5** was determined through the application of the Mosher methodology.[12] The $\Delta\delta$ ($\delta_S - \delta_R$) values observed for signals of the protons close to C-9 in compounds **5a** and **5b** revealed a 9*S* configuration for **5.** On the basis of these observation, the structure of **5**, named giffonin E, was determined as depicted.

The ^{13}C NMR and HR-MALDI-TOF-MS data of **6** (m/z 393.1319 $[M + Na]^+$, calcd for $C_{21}H_{22}O_6Na$, 393.1314) supported a molecular formula of $C_{21}H_{22}O_6$. The IR spectrum showed a band at 1710 cm^{-1} for the presence of a carbonyl group. The NMR data of compound **6** were similar to those of giffonin E (**5**) except for the presence of the resonance of a carbonyl group (δ 215.4). The location of the carbonyl group at C-11 was confirmed by the HMBC correlations of the proton signals at δ 3.08 and 3.03 (H-13) and δ 4.33 (H-9) with the carbon resonance at δ 215.4. Mosher derivatization was performed on compound **6**, which was treated with (*R*)- and (*S*)-MTPA chloride to form (*S*)-MTPA and (*R*)-MTPA esters,

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respectively. Analysis of the $\Delta\delta(S-R)$ values of the protons neighboring the oxygenated methine according to the Mosher model[12] (Figure 1) allowed the assignment of the 9*S* configuration of **6**. Accordingly, the structure of compound **6**, named giffonin F, was determined as shown.

The molecular formula of **7** was established as $C_{21}H_{22}O_5$ by HR-MALDI-TOF-MS (*m*/*z* 377.1367 $[M + Na]^+$, calcd for $C_{21}H_{22}O_5Na$, 377.1365) and the ^{13}C NMR data. Comparison of the ^1H NMR spectrum of **7** with those of **1**−**6** suggested that they share the same aromatic substitution patterns. Furthermore, the ^1H NMR spectrum displayed four signals at δ 6.36 (d, J = 11.4 Hz), 6.01 (dd, J = 11.4, 9.2 Hz), 5.67 (dd, J = 15.4, 9.2 Hz), and 5.64 (dd, J = 15.4, 8.6 Hz), ascribable to *Z*- and *E*-olefinic protons, respectively, and a signal at δ 4.07 (dt, J = 8.6, 3.3 Hz), corresponding to a proton linked to an oxymethine carbon (Table 2). The COSY experiment showed the connectivity from H-7 to H-10, allowing a conjugated diene system (C-7/C-10) flanked by a hydroxy group at C-11 (δ 4.07) to be identified. ROESY experiment showed correlations of H-15 (δ 7.33) with H-13β (δ 3.04) and of H-19 (δ 7.42) with H-13α (δ 2.71) and H-11 (δ 4.07). To determine the absolute configuration of C-11 in **7**, (*S*)- and (*R*)-MTPA esters of **7** were synthesized. The $\Delta\delta$ ($\delta_S - \delta_R$) values observed for signals of the protons close to C-11 revealed an 11*S* configuration for **7**. On the basis of the reported data, the structure of compound **7**, named giffonin G, was determined as depicted.

The ^{13}C NMR and HR-MALDI-TOF-MS data of **8** (*m*/*z* 347.1261 $[M + Na]^+$, calcd for $C_{20}H_{20}O_4Na$, 347.1259) supported a molecular formula of $C_{20}H_{20}O_4$. The ^1H NMR data for the heptadiene moiety were comparable to those of **7**, while differences were observed for signals due to an aryl moiety. In particular, the ^1H NMR spectrum showed aromatic signals at δ 7.44 (dd, J = 8.3, 1.9 Hz, H-19), 7.36 (dd, J = 8.3, 1.9 Hz, H-15), 7.19 (dd, J = 8.3, 1.9 Hz, H-16), and 7.03 (dd, J = 8.3, 1.9 Hz, H-18), corresponding to the 1,4-disubstituted aromatic ring, and at δ 6.30 (d, J = 1.7 Hz, H-4) and 5.51 (d, J = 1.7 Hz, H-6), corresponding to a 1,2,3,5-tetrasubstituted aromatic ring (Table 2). Moreover, a signal at δ 4.03 (s), typical of a methoxy group, was observed. The HMBC correlation between the proton signal at δ 4.03 and the carbon resonance at δ 136.9 allowed the methoxy group to be placed at C-2. Mosher derivatization was performed on compound **8**, which was treated with (*R*)- and (*S*)-MTPA chloride to form (*S*)-MTPA and (*R*)-MTPA esters, respectively. Analysis of the $\Delta\delta(S-R)$ values of the protons neighboring the oxygenated methine according to the Mosher model[12] (Figure 1) allowed the assignment of an 11*S* configuration of **8**. Therefore, the structure of compound **8**, named giffonin H, was defined as shown.

The ^{13}C NMR and HR-MALDI-TOF-MS data of **9** (*m*/*z* 611.2107 $[M + Na]^+$, calcd for $C_{30}H_{36}O_{12}Na$, 611.2104) supported a molecular formula of $C_{30}H_{36}O_{12}$. The ^{13}C NMR spectrum of **9** showed 30 carbon signals, of which 19 were assigned to a diaryl heptanoid moiety[13] and 11 to two sugar units (Table 3). The ^1H NMR spectrum showed signals ascribable to two 1,2,4-trisubstituted aromatic rings at δ 7.04 (dd, J = 8.0, 2.2 Hz), 7.03 (dd, J = 8.0, 2.2 Hz), 7.02 (d, J = 2.2 Hz), 6.92 (d, J = 2.2 Hz), 6.83 (d, J = 8.0 Hz), and 6.76 (d, J = 8.0 Hz) and signals due to a disubstituted *trans*-olefinic function at δ 6.62 (d, J = 15.6 Hz) and 7.10 (dt, J = 7.6, 15.6 Hz). The structure of the heptanoid moiety was readily deduced from HSQC, HMBC, and COSY correlations. Thus, the aglycone of **9** was established as alnusone, a natural compound first isolated from *Alnus japonica* and also available via total synthesis.[14,15] The ^1H NMR spectrum displayed in the sugar region signals corresponding to two anomeric protons at δ 5.14 (d, J = 3.7 Hz) and 4.51 (d, J = 7.8 Hz). The NMR data (HSQC, HMBC, COSY, and 1D-TOCSY) indicated the presence of an α-arabinopyranosyl unit (δ 5.14) and a β-glucopyranosyl unit (δ 4.51). The configurations of the arabinose and glucose units were established as L and D, respectively, after hydrolysis of **9** with 1 N HCl, trimethylsilation, and GC analysis.[16] The linkage sites of the sugar units on the diaryl heptanoid moiety were obtained from the HMBC spectrum, which showed correlations between H-1_{ara} (δ 5.14) and the ^{13}C NMR resonance of C-3 (δ 152.4) and between H-1_{glc} (δ 4.51) and C-17 (δ 153.2). On the basis of the reported data, the structure of compound **9**, named giffonin I, was established as shown.

Diarylheptanoid derivatives occur frequently in plants belonging to the Betulaceae family, but so far only one report deals with their presence in the leaves of *C. avellana*.[2] Noteworthy, giffonins A−I differ from the compounds reported by Riethmüller et al.[2] in that they represent cyclized diarylheptanoids, and, in particular, giffonins A−H are diaryl ether heptanoid derivatives. Diarylheptanoids isolated from plants belonging to the Betulaceae family have been reported to possess various biological activities including antioxidant, anti-inflammatory, anticancer, and antiadipogenic.[2,17−19] Therefore,

Table 3. ^{13}C and ^1H NMR Data (J in Hz) of Compound 9 (600 Mz, δ ppm, in Methanol-d_4)

	δ_C	δ_H (J in Hz)
1	127.4	
2	127.5	
3	152.4	
4	117.6	6.83, d (8.0)
5	128.9	7.04, dd (8.0, 2.2)
6	137.6	
7	30.4	3.17, (2H) m
8	40.6	2.83, (2H) m
9	203.0	
10	134.6	6.62, d (15.6)
11	150.4	7.10, dt (7.6, 15.6)
12	36.2	2.62, (2H) m
13	33.8	2.93, (2H) br t (5.5)
14	133.4	
15	129.3	7.03, dd (8.0, 2.2)
16	116.4	6.76, d (8.0)
17	153.2	
18	136.0	6.92, d (2.2)
19	134.2	7.02, d (2.2)
	α-Ara (at C-3)	
1	93.5	5.14, d (3.7)
2	73.7	3.38, dd (8.0, 3.7)
3	74.5	3.69, dd (8.0, 3.0)
4	72.7	3.81, m
5	62.7	3.87, dd (12.5, 3.0); 3.67, dd (12.5, 2.6)
	β-Glc (at C-17)	
1	97.9	4.51, d (7.8)
2	76.0	3.15, dd (9.0, 7.8)
3	77.7	3.37, dd (9.0, 9.0)
4	71.6	3.31, dd (9.0, 9.0)
5	77.7	3.31, m
6	62.5	3.80, dd (12.0, 2.5); 3.70, dd (12.0, 4.5)

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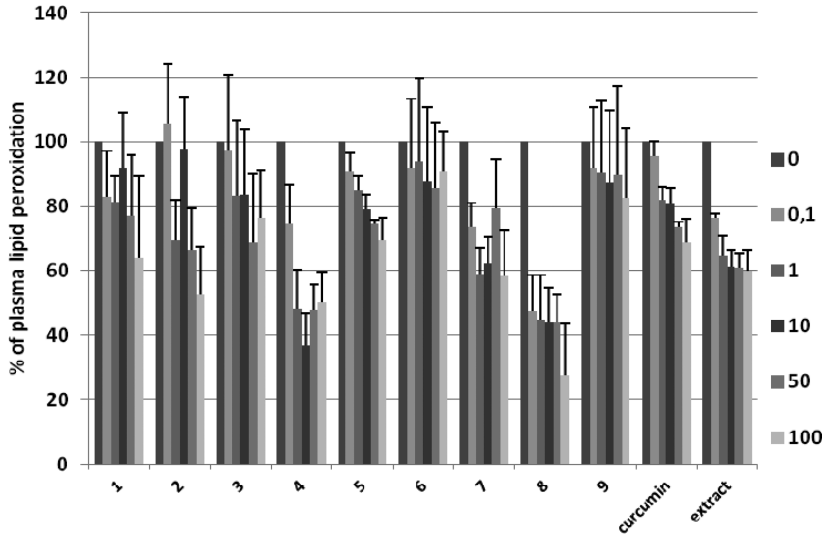

Figure 2. Effects of MeOH extract (0.1−100 μg/mL; 30 min), giffonins A−I (**1**−**9**) (0.1−100 μM; 30 min), and curcumin (0.1−100 μM; 30 min) on plasma lipid peroxidation induced by H_2O_2. The results are representative of three independent experiments and are expressed as means ± SD. The effect of five different concentrations of tested compounds (0.1, 1, 10, 50, and 100 μM) and tested extract (0.1, 1, 10, 50, and 100 μg/mL) was statistically significant according to the ANOVA I test; $p < 0.05$ (for compounds **1**, **5**, **7** and curcumin); $p < 0.02$ (for compounds **4**, **8** and extract); $p > 0.05$ (for compounds **2**, **3**, **6**, and **9**).

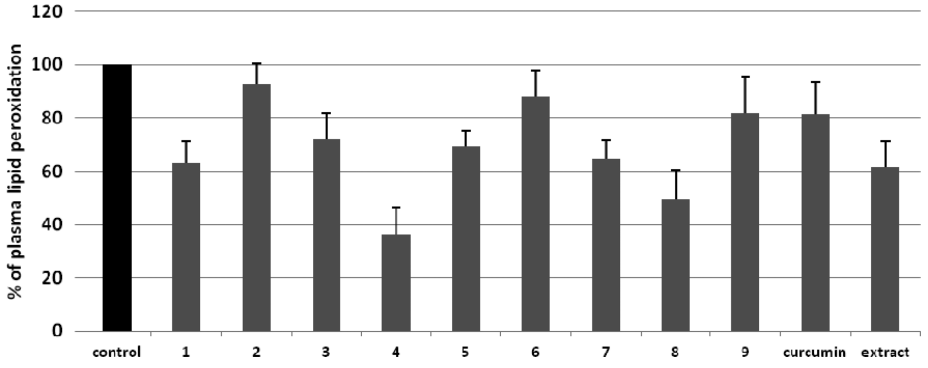

Figure 3. Effects of MeOH extract (10 μg/mL; 30 min), giffonins A−I (**1**−**9**) (10 μM; 30 min), and curcumin (10 μM; 30 min) on plasma lipid peroxidation induced by H_2O_2/Fe^{2+}. The results are representative of three independent experiments and are expressed as means ± SD. The statistical significances were confirmed with the paired Student's *t* test.

the MeOH extract of *C. avellana* leaves and giffonins A−I (**1**−**9**) were evaluated for their inhibitory effects on human plasma lipid peroxidation induced by H_2O_2 and H_2O_2/Fe^{2+}, by measuring the concentration of thiobarbituric acid reactive substances (TBARS).

The MeOH extract, compounds **1**−**9**, and curcumin, used as reference compound, were tested at doses ranging from 0.1 to 100 μg/mL and from 0.1 to 100 μM, respectively. The tested compounds, curcumin, and the plant extract did not exert any effect on autoperoxidation of human plasma (data not shown). The MeOH extract of leaves at 10 μg/mL reduced by about 38% plasma lipid peroxidation stimulated by H_2O_2 or H_2O_2/Fe^{2+}. Compounds **4** and **8** at 10 μM reduced both H_2O_2- and H_2O_2/Fe^{2+}-induced lipid peroxidation by more than 60% and 50%, respectively, being more active than curcumin (Figure 2). Among the nine compounds, the weakest activity was displayed by compounds **2**, **6**, and **9** (Figures 2 and 3 and Table 4), which exerted a protective action against oxidative stress induced by H_2O_2 or H_2O_2/Fe^{2+}, similar to that shown by curcumin.

This study demonstrates the potential benefits of *C. avellana* leaves as a rich source of phenolic compounds. Moreover, it extends and reinforces the notion of the antioxidant capacity of

Table 4. Inhibitory Effects of the Extract (10 μg/mL; 30 min), Compounds 1−9 (10 μM; 30 min), and Curcumin (10 μM; 30 min) on Plasma Lipid Peroxidation Induced by H_2O_2 or H_2O_2/Fe^{2+}[a]

compound	inhibition of lipid peroxidation induced by H_2O_2 (%)	inhibition of lipid peroxidation induced by H_2O_2/Fe (%)
1	8.1 ± 3.6 ($p < 0.05$)	36.8 ± 8.8 ($p < 0.02$)
2	2.2 ± 2.4 ($p > 0.05$)	7.3 ± 3.8 ($p > 0.05$)
3	16.4 ± 3.9 ($p < 0.05$)	28.2 ± 4.9 ($p < 0.05$)
4	64.3 ± 14.5 ($p < 0.002$)	63.2 ± 10.7 ($p < 0.002$)
5	20.8 ± 5.1 ($p < 0.05$)	30.8 ± 6.8 ($p < 0.02$)
6	12.1 ± 4.9 ($p < 0.05$)	12.0 ± 5.2 ($p < 0.05$)
7	37.6 ± 7.7 ($p < 0.02$)	35.5 ± 7.6 ($p < 0.02$)
8	55.8 ± 13.9 ($p < 0.01$)	50.6 ± 14.5 ($p < 0.01$)
9	12.6 ± 4.5 ($p < 0.05$)	18.1 ± 4.8 ($p < 0.05$)
curcumin	19.2 ± 4.4 ($p < 0.05$)	18.6 ± 5.1 ($p < 0.05$)
extract	38.8 ± 6.1 ($p < 0.02$)	38.3 ± 7.2 ($p < 0.02$)

[a]The results are representative of three independent experiments and are expressed as means ± SD. The statistical significances were confirmed with the paired Student's *t* test.

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C. avellana leaf extracts, confirming the beneficial value of the diarylheptanoid derivatives. Furthermore, the discovery of phytochemicals with health benefits in the PGI byproducts could be useful to give an interesting and economically feasible opportunity to use waste materials, such as leaves, as a source of functional ingredients for nutraceutical, herbal, and cosmetic formulations.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO DIP 1000 polarimeter. IR measurements were obtained on a Bruker IFS-48 spectrometer. NMR experiments were performed on a Bruker DRX-600 spectrometer (Bruker BioSpin GmBH, Rheinstetten, Germany) equipped with a Bruker 5 mm TCI CryoProbeat 300 K. All 2D NMR spectra were acquired in methanol-d_4 (99.95%, Sigma-Aldrich), and standard pulse sequences and phase cycling were used for DQF-COSY, HSQC, and HMBC spectra. The NMR data were processed using UXNMR software. The ROESY spectra were acquired with t_{mix} = 400 ms. Exact masses were measured using a MALDI-TOF MicroMX (Micromass) mass spectrometer. A mixture of analyte solution and α-cyano-4-hydroxycinnamic acid (Sigma) was applied to the metallic sample plate and dried. Mass calibration was performed with the ions from the ACTH (fragment 18−39) at 2465.1989 Da, with α-cyano-4-hydroxycinnamic acid at 190.0504 Da as an internal standard. GC analysis was performed on a Thermo Finnigan Trace GC apparatus with an FID detector and using a l-Chirasil-Val column (0.32 mm × 25 m). Column chromatography was performed over Sephadex LH-20 (Pharmacia). HPLC separations were carried out on a Waters 590 system equipped with a Waters R401 refractive index detector, a Waters XTerra Prep MSC18 column (300 × 7.8 mm i.d.), and a Rheodyne injector.

Plant Material. The leaves of the "Nocciola di Giffoni" (*Corylus avellana*) were collected at Giffoni, Salerno, Italy, in November 2012 and identified by V. De Feo (Department of Pharmacy, University of Salerno, Italy). A voucher specimen (No. 133) has been deposited in this Department.

Extraction and Isolation. The leaves of "Nocciola di Giffoni" (*C. avellana* L.) (910 g) were dried and extracted at room temperature using solvents of increasing polarity such as *n*-hexane (2.5 L for 3 days, three times), $CHCl_3$ (2.5 L for 3 days, three times), and MeOH (2.5 L for 3 days, three times). After filtration and evaporation of the solvent to dryness in vacuo, 30 g of crude MeOH extract was obtained. The dried MeOH extract (3.0 g) was fractionated on a Sephadex LH-20 (Pharmacia) column (100 × 5 cm), using MeOH as mobile phase, affording 88 fractions (8 mL), monitored by TLC. Fractions 34 and 35 (56.5 mg) were chromatographed by semipreparative HPLC using MeOH−H_2O (3:2) as mobile phase (flow rate 2.5 mL/min) to yield compounds **2** (1.8 mg, t_R = 11.1 min), **6** (1.5 mg, t_R = 19.5 min), **3** (2.9 mg, t_R = 30.2 min), and **5** (1.6 mg, t_R = 52.2 min). Fractions 36−40 (90.0 mg) were chromatographed by semipreparative HPLC using MeOH−H_2O (13:7) as mobile phase (flow rate 2.5 mL/min) to yield compounds **8** (2.4 mg, t_R = 18.0 min), **1** (2.0 mg, t_R = 19.2 min), **7** (3.5 mg, t_R = 20.5 min), and **4** (4.1 mg, t_R = 21.2 min). Fraction 41 (13.5 mg) was chromatographed by semipreparative HPLC using MeOH−H_2O (9:11) as mobile phase (flow rate 2.5 mL/min) to yield compound **9** (2.5 mg, t_R = 20.0 min). This procedure was repeated three times to accumulate sufficient amounts of compounds **2**, **3**, and **5**−**8** to subject to the modified Mosher's method.

*Giffonin A (**1**):* amorphous, white solid; IR (KBr) ν_{max} 3440, 2935, 1705, 1660 cm^{-1}; ^1H and ^{13}C NMR (methanol-d_4, 600 MHz) data, see Table 1; HR-MALDI-TOF-MS $[M + Na]^+$ *m/z* 377.1369 (calcd for $C_{21}H_{22}O_5Na$, 377.1365).

*Giffonin B (**2**):* amorphous, white solid; $[\alpha]^{25}_D$ −14 (*c* 0.1 MeOH); IR (KBr) ν_{max} 3430, 2940, 1705, 1660 cm^{-1}; ^1H and ^{13}C NMR (methanol-d_4, 600 MHz) data, see Table 1; HR-MALDI-TOF-MS $[M + Na]^+$ *m/z* 393.1318 (calcd for $C_{21}H_{22}O_6Na$, 393.1314).

*Giffonin C (**3**):* amorphous, white solid; $[\alpha]^{25}_D$ −28 (*c* 0.03 MeOH); IR (KBr) ν_{max} 3450, 2940, 1655 cm^{-1}; ^1H and ^{13}C NMR (methanol-d_4, 600 MHz) data, see Table 1; HR-MALDI-TOF-MS $[M + Na]^+$ *m/z* 379.1524 (calcd for $C_{21}H_{24}O_5Na$, 379.1521).

*Giffonin D (**4**):* amorphous, white solid; IR (KBr) ν_{max} 3425, 2930, 1713, 1665 cm^{-1}; ^1H and ^{13}C NMR (methanol-d_4, 600 MHz) data, see Table 1; HR-MALDI-TOF-MS $[M + Na]^+$ *m/z* 377.1367 (calcd for $C_{21}H_{22}O_5Na$, 377.1365).

*Giffonin E (**5**):* amorphous, white solid; $[\alpha]^{25}_D$ −28 (*c* 0.03 MeOH); IR (KBr) ν_{max} 3430, 2935, 1665 cm^{-1}; ^1H and ^{13}C NMR (methanol-d_4, 600 MHz) data, see Table 2; HR-MALDI-TOF-MS $[M + Na]^+$ *m/z* 379.1526 (calcd for $C_{21}H_{24}O_5Na$, 379.1521).

*Giffonin F (**6**):* amorphous, white solid; $[\alpha]^{25}_D$ −24 (*c* 0.03 MeOH); IR (KBr) ν_{max} 3432, 2940, 1710, 1665 cm^{-1}; ^1H and ^{13}C NMR (methanol-d_4, 600 MHz) data, see Table 2; HR-MALDI-TOF-MS $[M + Na]^+$ *m/z* 393.1319 (calcd for $C_{21}H_{22}O_6Na$, 393.1314).

*Giffonin G (**7**):* amorphous, white solid; $[\alpha]^{25}_D$ −71 (*c* 0.1 MeOH); IR (KBr) ν_{max} 3430, 2930, 1620 cm^{-1}; ^1H and ^{13}C NMR (methanol-d_4, 600 MHz) data, see Table 2; HR-MALDI-TOF-MS $[M + Na]^+$ *m/z* 377.1365 (calcd for $C_{21}H_{22}O_5Na$, 377.1365).

*Giffonin H (**8**):* amorphous, white solid; $[\alpha]^{25}_D$ −22 (*c* 0.03 MeOH); IR (KBr) ν_{max} 3430, 2930, 1620 cm^{-1}; ^1H and ^{13}C NMR (methanol-d_4, 600 MHz) data, see Table 2; HR-MALDI-TOF-MS $[M + Na]^+$ *m/z* 347.1261 (calcd for $C_{20}H_{20}O_4Na$, 347.1259).

*Giffonin I (**9**):* amorphous, white solid; $[\alpha]^{25}_D$ −4 (*c* 0.1 MeOH); IR (KBr) ν_{max} 3425, 2920, 1710 cm^{-1}; ^1H and ^{13}C NMR (methanol-d_4, 600 MHz) data, see Table 3; HR-MALDI-TOF-MS $[M + Na]^+$ *m/z* 611.2107 (calcd for $C_{30}H_{36}O_{12}Na$, 611.2104).

Preparation of (*S*)- and (*R*)-MTPA Esters of 2, 3, and 5−8. (*R*)-(−) and (*S*)-(+)-MTPA-Cl (15 μL) and a catalytic amount of DMAP were separately added to two different aliquots of **2** (each 1.5 mg) in anhydrous pyridine. The resulting mixtures were maintained at room temperature under vigorous stirring overnight, and then ^1H NMR spectra were recorded. The related proton signals were assigned by analyzing COSY spectra. By the same procedure, the (*S*)- and (*R*)-MTPA esters of **3** and **5**−**8** were prepared, and the related proton signals were also assigned by analyzing COSY spectra.

*(S)-MTPA ester of 2 (**2a**):* selected ^1H NMR values (600 MHz, methanol-d_4) δ_H 7.80 (dd, *J* = 8.5, 1.9 Hz, H-19), 7.74 (dd, *J* = 8.5, 1.9 Hz, H-15), 7.29 (dd, *J* = 8.5, 1.9 Hz, H-18), 5.79 (dd, *J* = 5.4, 8.9 Hz, H-13), 5.25 (ddd, *J* = 15.5, 10.5, 4.0, H-8), 3.17 (t, *J* = 5.5 Hz, H-12), 2.65 (dt, *J* = 16.0, 3.0 Hz, H-10), 2.60 (m, H-9), 2.15 (m, H-9).

*(R)-MTPA ester of 2 (**2b**):* selected ^1H NMR values (600 MHz, methanol-d_4) δ_H 7.74 (dd, *J* = 8.5, 1.9 Hz, H-19), 7.72 (dd, *J* = 8.5, 1.9 Hz, H-15), 7.28 (dd, *J* = 8.5, 1.9 Hz, H-18), 5.79 (dd, *J* = 5.4, 8.9 Hz, H-13), 5.26 (ddd, *J* = 15.5, 10.5, 4.0, H-8), 3.43 (t, *J* = 5.5 Hz, H-12), 2.68 (dt, *J* = 16.0, 3.0 Hz, H-10), 2.61 (m, H-9), 2.17 (m, H-9).

*(S)-MTPA ester of 3 (**3a**):* selected ^1H NMR values (600 MHz, methanol-d_4) δ_H 6.49 (d, *J* = 11.5 Hz, H-7), 5.65 (ddd, *J* = 11.5, 7.5, 7.5 Hz, H-8), 4.23 (m, H-11), 3.15 (dt, *J* = 12.5, 3.7 Hz, H-13), 2.85 (td, *J* = 12.5, 5.2 Hz, H-13), 2.21 (m, H-12), 2.01 (m, H-9), 1.98 (m, H-12), 1.97 (m, H-9), 1.30 (m, H-10), 0.75 (m, H-10).

*(R)-MTPA ester of 3 (**3b**):* selected ^1H NMR values (600 MHz, methanol-d_4) δ_H 6.48 (d, *J* = 11.4 Hz, H-7), 5.63 (ddd, *J* = 11.5, 7.5,7.5 Hz, H-8), 4.23 (m, H-11), 3.19 (dt, *J* = 12.5, 3.7 Hz, H-13), 2.87 (td, *J* = 12.5, 5.2 Hz, H-13), 2.31 (m, H-12), 2.00 (m, H-9), 2.03 (m, H-12), 1.95 (m, H-9), 1.24 (m, H-10), 0.68 (m, H-10).

*(S)-MTPA ester of 5 (**5a**):* selected ^1H NMR values (600 MHz, methanol-d_4) δ_H 6.43 (d, *J* = 11.2 Hz, H-7), 5.20 (dd, *J* = 11.2, 8.6 Hz, H-8), 5.05 (ddd, *J* = 12.0, 8.5, 3.4 Hz, H-9), 3.07 (m, H-13), 2.45 (td, *J* = 12.5, 5.4 Hz, H-13), 2.16 (m, H-12), 1.98 (q, *J* = 10.8 Hz, H-11), 1.45 (m, H-10), 1.38 (m, H-12), 1.12 (m, H-11), 0.35 (br t, *J* = 11.8 Hz, H-10).

*(R)-MTPA ester of 5 (**5b**):* selected ^1H NMR values (600 MHz, methanol-d_4) δ_H 6.40 (d, *J* = 11.2 Hz, H-7), 5.15 (dd, *J* = 11.2, 8.6 Hz, H-8), 5.05 (ddd, *J* = 12.0, 8.5, 3.4 Hz, H-9), 3.09 (m, H-13), 2.48 (td, *J* = 12.5, 5.4 Hz, H-13), 2.21 (m, H-12), 2.05 (q, *J* = 10.8 Hz, H-11), 1.65 (m, H-10), 1.40 (m, H-12), 1.28 (m, H-11), 0.40 (br t, *J* = 11.8 Hz, H-10).

*(S)-MTPA ester of 6 (**6a**):* selected ^1H NMR values (600 MHz, methanol-d_4) δ_H 6.53 (d, *J* = 11.8 Hz, H-7), 5.25 (dd, *J* = 11.8, 8.6 Hz, H-8), 5.15 (t, *J* = 10.0 Hz, H-9), 3.13 (m, H-13), 3.09 (td, *J* = 12.5, 5.5

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Hz, H-13), 2.90 (td, J = 12.5, 5.5 Hz, H-12), 2.59 (m, H-12), 2.75 (m, H-10), 1.90 (br s, H-10).

(R)-MTPA ester of ***6*** *(****6b****):* selected ^{1}H NMR values (600 MHz, methanol-d_4) δ_H 6.50 (d, J = 11.8 Hz, H-7), 5.20 (dd, J = 11.8, 8.6 Hz, H-8), 5.15 (t, J = 10.0 Hz, H-9), 3.15 (m, H-13), 3.10 (td, J = 12.5, 5.5 Hz, H-13), 2.92 (td, J = 12.5, 5.5 Hz, H-12), 2.60 (m, H-12), 2.80 (m, H-10), 2.05 (br s, H-10).

(S)-MTPA ester of ***7*** *(****7a****):* selected ^{1}H NMR values (600 MHz, methanol-d_4) δ_H 7.52 (dd, J = 8.2, 1.9 Hz, H-19), 7.08 (dd, J = 8.2, 1.9 Hz, H-18), 6.47 (d, J = 11.4 Hz, H-7), 6.05 (dd, J = 11.4, 9.0 Hz, H-8), 5.87 (dd, J = 15.4, 8.4 Hz, H-10), 5.69 (dd, J = 15.4, 9.0 Hz, H-9), 5.57 (dt, J = 8.4, 3.3 Hz, H-11), 3.05 (dt, J = 12.5, 3.0 Hz, H-13), 2.83 (td, J = 12.5, 3.0 Hz, H-13), 2.27 (dq, J = 14.0, 3.3 Hz, H-12), 1.75 (tt, J = 14.0, 3.3 Hz, H-12).

(R)-MTPA ester of ***7*** *(****7b****):* selected ^{1}H NMR values (600 MHz, methanol-d_4) δ_H 7.53 (dd, J = 8.2, 1.9 Hz, H-19), 7.09 (dd, J = 8.2, 1.9 Hz, H-18), 6.45 (d, J = 11.4 Hz, H-7), 6.02 (dd, J = 11.4, 9.0 Hz, H-8), 5.83 (dd, J = 15.4, 8.4 Hz, H-10), 5.67 (dd, J = 15.4, 9.0 Hz, H-9), 5.57 (dt, J = 8.4, 3.3 Hz, H-11), 3.08 (dt, J = 12.5, 3.0 Hz, H-13), 2.85 (td, J = 12.5, 3.0 Hz, H-13), 2.41 (dq, J = 14.0, 3.3 Hz, H-12), 1.79 (tt, J = 14.0, 3.3 Hz, H-12).

(S)-MTPA ester of ***8*** *(****8a****):* selected ^{1}H NMR values (600 MHz, methanol-d_4) δ_H 7.41 (dd, J = 8.3, 1.9 Hz, H-19), 7.30 (dd, J = 8.3, 1.9 Hz, H-18), 6.22 (d, J = 11.4 Hz, H-7), 5.98 (t, J = 11.4 Hz, H-8), 5.88 (dd, J = 15.4, 8.6 Hz, H-10), 5.79 (dd, J = 15.4, 11.4 Hz, H-9), 5.57 (dt, J = 8.6, 3.5 Hz, H-11), 3.06 (dt, J = 13.0, 3.3 Hz, H-13), 2.80 (td, J = 13.0, 3.3 Hz, H-13), 2.24 (dq, J = 14.2, 3.4 Hz, H-12), 1.73 (tt, J = 14.2, 3.4 Hz, H-12).

(R)-MTPA ester of ***8*** *(****8b****):* selected ^{1}H NMR values (600 MHz, methanol-d_4) δ_H 7.42 (dd, J = 8.3, 2.9 Hz, H-19), 7.31 (dd, J = 8.3, 2.9 Hz, H-18), 6.20 (d, J = 11.2 Hz, H-7), 5.96 (t, J = 11.2 Hz, H-8), 5.84 (dd, J = 15.4, 8.6 Hz, H-10), 5.76 (dd, J = 15.4, 11.2 Hz, H-9), 5.57 (dt, J = 8.6, 3.8 Hz, H-11), 3.08 (dt, J = 12.8, 3.3 Hz, H-13), 2.82 (td, J = 12.8, 3.3 Hz, H-13), 2.36 (dq, J = 14.2, 3.8 Hz, H-12), 1.75 (tt, J = 14.2, 3.8 Hz, H-12).

Determination of the Sugar Configuration. The configurations of sugar units of compound **9** were established after hydrolysis of **9** with 1 N HCl, trimethylsilation, and determination of the retention times by GC operating in the reported experimental conditions.[20] The peaks of the hydrolysate of **9** were detected at 14.74 min (D-glucose) and at 8.91 and 9.83 min (L-arabinose). Retention times for authentic samples after being treated in the same manner with 1-(trimethylsilyl)-imidazole in pyridine were detected at 14.71 min (D-glucose) and 8.92 and 9.80 (L-arabinose).

Biological Assay. DMSO, thiobarbituric acid (TBA), and H_2O_2 were purchased from Sigma (St. Louis, MO, USA). All other reagents were of analytical grade and were provided by commercial suppliers. Stock solutions of tested compounds and tested plant extract were made in 50% DMSO. The final concentration of DMSO in samples was lower than 0.05%, and in all experiments its effects were determined.

Fresh human plasma was obtained from medication-free, regular donors at a blood bank (Lodz, Poland). Samples of human plasma were incubated with (1) plant extract at final concentrations of 0.1−100 μg/mL (30 min, at 37 °C); (2) plant extract at final concentrations of 0.1−100 μg/mL plus 2 mM H_2O_2 (30 min, at 37 °C); (3) plant extract at a final concentration of 10 μg/mL plus 4.7 mM H_2O_2/3.8 mM Fe_2SO_4/2.5 mM EDTA (30 min, at 37 °C); (4) test compounds (**1**−**9**) and curcumin at final concentrations of 0.1−100 μM (30 min, at 37 °C); (5) test compounds (**1**−**9**) and curcumin at final concentrations of 0.1−100 μM plus 2 mM H_2O_2 (30 min, at 37 °C); (6) test compounds (**1**−**9**) and curcumin at a final concentration of 10 μM plus 4.7 mM H_2O_2/3.8 mM Fe_2SO_4/2.5 mM EDTA (30 min, at 37 °C).

Lipid Peroxidation Measurement. Lipid peroxidation was quantified by measuring the concentration of TBARS. Incubation of plasma (control, test compounds, treated plasma) was stopped by cooling the samples in an ice-bath. Samples of plasma were transferred to an equal volume of 20% (v/v) cold trichloroacetic acid in 0.6 M HCl and centrifuged at 1200g for 15 min. One volume of clear supernatant was mixed with 0.2 volume of 0.12 M thiobarbituric acid in 0.26 M Tris at pH 7.0 and immersed in a boiling water bath for 15 min; then absorbance at 532 nm (UV/vis Helios alpha Unicam spectrophotometer) was measured.[21] The TBARS concentration was calculated using the molar extinction coefficient (ε = 156 000 M^{-1} cm^{-1}).

Statistical Analysis. The statistical analysis was done by several tests. In order to eliminate uncertain data, the Q-Dixon test was performed. All the values in this study were expressed as mean ± SD. The statistical analysis was performed with one-way ANOVA for repeated measurements. The statistically significant differences were also assessed by applying the paired Student's t test.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR, HSQC, HMBC, COSY, and ROESY spectra of compounds **1**−**9**. 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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