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New Constituents of Sweet *Capsicum annuum* L. Fruits and Evaluation of Their Biological Activity

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Four new acyclic diterpene glycosides named capsianosides (**1**−**4**), together with 12 known compounds, were isolated from the fresh sweet pepper fruits of *Capsicum annuum* L., a plant used as a vegetable food, spice, and external medicine. The chemical structures of new natural compounds, as well as their absolute configurations, were established by means of spectroscopic data including infrared, high-resolution mass spectrometry, and one- and two-dimensional nuclear magnetic resonance and by chemical derivatization. The known capsidiol (**11**) showed bacteriostatic properties in vitro against *Helicobacter pylori* with a minimum inhibitory concentration (MIC) of 200 μg/mL when compared with the commercial drug metronidazole (MIC, 250 μg/mL). Some purified components were also tested for their antioxidant activities.

KEYWORDS: ***Capsicum annuum* L.; *Solanaceae*; *capsianosides*; 2D-NMR; *Helicobacter pylori*; antioxidant activity; liquid chromatography**

INTRODUCTION

The genus *Capsicum* (Solanaceae) includes many species widely cultivated in Asia, Africa, and Mediterranean countries. Peppers are native plants of America, and the fruits (pericarps) are consumed as vegetable foods, spices, and external medicines and are also a source of vitamins A, C, and E (*1*). Hot cultivars are rich in capsaicinoids giving the pungency of some *Capsicum* species. Hot chili pepper has been used for centuries as a condiment to aid digestion. Traditionally, medical doctors advise ulcer patients not to consume spicy foods like pungent capsicum products, while naturopaths and herbalists have tended to use hot seasonings to relieve ulcers. Studies have shown that capsaicin in hot pepper cultivars such as cayenne and jalapeno inhibits the in vitro growth of the gastric pathogen *Helicobacter pylori* (*2*, *3*). Other authors have reported that capsaicin does not possess any in vivo efficacy in the treatment of *H. pylori* (*4*).

Phytochemical investigations have been mainly focused on hot components of pepper species (*5*−*7*) and qualitative and quantitative determinations of phenolic metabolites with antioxidant activities (*8*). In previous works, we reported a detailed chemical analysis of the pericarp and seeds with the identification of new compounds (*9*, *10*). New sesquiterpenes and related compounds have been detected in stems and roots of *Capsicum annuum* (*11*). Several diterpenoid glycosides isolated from the fruits of *C. annuum* exhibited antihypertensive effects (*5*, *6*) and have been found to be related to the improvement and prevention of hypertension (*7*). Among phytochemicals, polyphenols deserve a special mention due to their free radical scavenging properties and in vivo biological activities that are being investigated by many researchers. Epidemiological studies have shown a possible correlation between the dietary intake of polyphenols and the prevention of disease states, including cancer, cardiovascular diseases, and neurovegetative disorders (*12*, *13*).

Several chemical and biological studies have been performed on the hot cultivars, but only a few have been performed on sweet peppers. In recent years, peppers have grown in popularity, and a wide number of varieties are now available in the grocery stores.

Continuing our research on promising active substances of plant origin, we describe in this paper the isolation and the structure elucidation of four new glycosides named capsianoside VIII (**1**), capsianoside IX (**2**), capsianoside I ester (**3**), and capsianoside L (**4**) along with 12 known compounds: capsianosides III (**5**), V (**6**), and I (**7**) (*5*, *6*), inosine, uridine, quercetine 3-*O*-rhamnoside (*14*), 3-*O*-(9,12,15-octadecatrienoyl)glyceryl-β-D-galactopyranoside (**8**) (*15*), oxylipin (**9**) (*16*, *17*), phos-

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phatidylcholine (**10**) (*18*), capsidiol (**11**) (*19*, *20*), loliolide (**12**) (*21*), and blumenol C glucoside (**13**) (*22*) from fresh sweet pepper fruits (*C. annuum* L). Commonly consumed as a vegetable in southern Italy, this pepper variety is known as "Red Bull's Horn Chile". The structural elucidation has been performed by spectral analysis, including various two-dimensional (2D) nuclear magnetic resonance (NMR) techniques, and by chemical means. In addition, we describe the antibacterial in vitro properties of compounds **11** and **12** against *H. pylori* indicated as the undisputed cause of chronic gastritis. *H. pylori* is strongly associated with ulcer disease and has recently been recognized as a probable cofactor in the development of gastric cancer (*23*, *24*).

MATERIALS AND METHODS

General Methods. Fast atom bombardment mass spectrometry (FAB-MS), electron ionization mass spectrometry (EI-MS), and high-resolution (HR) FAB-MS were recorded on a Fisons VG Prospec instrument. Optical rotations were determined on a Perkin-Elmer 141 polarimeter.

^{1}H and ^{13}C NMR spectra were determined on a Varian Unity INOVA spectrometer at 500.13 and 125.77 MHz, respectively, equipped with an indirect detection probe. Chemical shifts were referenced to the solvent signals: deuterated methanol (CD_3OD) and deuterated chloroform ($CDCl_3$). Residual CHD_2OD: δ_H 3.31, δ_C 49.0; residual $CHCl_3$: δ_H 7.26, δ_C 77.0. For **4**, the heteronuclear multiple bond correlation (HMBC) experiment was recorded with a Varian Unity INOVA 700 MHz spectrometer equipped with a triple resonance cryoprobe.

The heteronuclear single-quantum coherence (HSQC) spectra were optimized for an average $^1J_{CH}$ of 140 Hz; the gradient-enhanced HMBC experiment was optimized for a $^3J_{CH}$ of 8 Hz.

Droplet countercurrent chromatography (DCCC) was performed on a DCC-A apparatus (Tokyo Rikakikai Co., Tokyo, Japan) equipped with 250 glass columns. High-performance liquid chromatography (HPLC) was performed using a Waters 510 pump equipped with a Waters U6K injector and a Waters 401 differential refractometer as the detector, using a 150 mm × 4.60 mm i.d., 3 μm, Luna C-18 (Phenomenex, Torrance, CA) and 30 cm × 3.9 mm i.d., C_{18} μ-Bondapak (Waters, Milford, MA) columns; the flow rate was 1 mL min^{-1}.

Plant Material. The fresh sweet pepper fruits of *C. annuum* were collected at the ripening stage at Campobasso (Italy) during the summertime of 2004 and identified at the Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, University of Molise (Campobasso, Italy). Red Bull's Horn Chile is a tapered, 8−12 in. long, nonbell sweet red pepper with the largest of stuffing pericarps, Italian Heirloom pre-1920. A voucher specimen (PEP 5104) is preserved at the Dipartimento di Scienze e Tecnologie per l'Ambiente e il Territorio, University of Molise (Isernia, Italy).

Extraction and Isolation. The fresh sweet pepper fruits of *C. annuum* (259 g), immediately analyzed, were chopped and soaked in methanol (MeOH, 6 L) three times at room temperature for 12 h. The methanol extracts, concentrated under vacuum, afforded 16.6 g of a glassy material and were subjected to a modified Kupchan's partition procedure (*25*) as follows. The methanol extract was dissolved in 10% aqueous methanol and partitioned against *n*-hexane. The water content (% v/v) of the MeOH was adjusted to 20 and 40% and partitioned against tetrachloride (CCl_4) and $CHCl_3$, respectively. The aqueous phase was concentrated to remove MeOH and then extracted with *n*-buthanol (*n*-BuOH). Four extracts were obtained as follows: *n*-hexane (600 mg), CCl_4 (1.4 g), $CHCl_3$ (1.2 g), and *n*-BuOH (11.4 g). The $CHCl_3$ extract (1.2 g) was chromatographed by DCCC using $CHCl_3$/MeOH/H_2O (7:13:8) in the ascending mode (the lower phase was the stationary phase); the flow rate was 18 mL/h; 6 mL fractions were collected and combined on the basis of their similar thin-layer chromatography (TLC) behavior on SiO_2 plates (Alugram SIL G/UV$_{254}$ TLC, Macherey-Nagel, Germany) with $CHCl_3$/MeOH/H_2O (80:18:2) as the eluent. Five fractions were obtained and purified by HPLC as summarized in **Table 1**.

Table 1. DCCC Fractionation and HPLC Purification of $CHCl_3$ and *n*-BuOH Extracts

fractions	amount (mg)	compound	MeOH/H_2O
		$CHCl_3$ extract[a]	
24–32	40.3	**13**	35:65 (+1% HCOOH)
66–76	32.5	**12**	6:4
77–84	22.0	**9**, **11**	6:4 (+1% HCOOH)
85–87	7.9	**10**	9:1 (+1% HCOOH)
161–175	22.5	**8**	9:1
		n-BuOH extract[b]	
A (47–55)	67.2	inosine	1:9
B (56–63)	45.6	uridine	2:98
C (74–83)	36.4	**5**	6:4
D (84–899)	31.7	**1**	6:4
E (90–96)	37.8	**1**, **2**	6:4
F (106–112)	21.9	**1**, **6**	6:4
G (113–117)	12.0	**6**	6:4
H (132–142)	24.0	**3**	75:25
I (225–247)	23.9	**7**	1:1
L (266–274)	34.7	**4**	7:3
M (275–300)	76.0	quercetin 3-*O*-rhamnoside	2:8

[a] All fractions were purified on C_{18} μ-Bondapak, except for fractions 85–87 purified on Luna C-18. [b] All fractions were purified on C_{18} μ-Bondapak column.

The *n*-BuOH extract (3.3 g) was chromatographed by DCCC using *n*-BuOH/Me_2CO/H_2O (3:1:5) in descending mode (the upper phase was the stationary phase); the flow rate was 18 mL/h; 6 mL fractions were collected and combined as described above [TLC on SiO_2 with *n*-BuOH/HOAc/H_2O (12:3:5) as eluent]. Eleven fractions (A−M) were obtained and purified by HPLC as summarized in **Table 1**.

Capsianoside VIII (1). Yield, 40.6 mg $[\alpha]_D^{25}$ −26.8° (c 0.3, MeOH). FAB-MS *m/z* 1083 $[M - H]^-$. HRFAB-MS *m/z* 1083.5218 $[M - H]^-$ (calcd for $C_{50}H_{83}O_{25}$, 1083.5223). ^{1}H and ^{13}C NMR are in **Table 2**.

Capsianoside IX (2). Yield, 0.9 mg; $[\alpha]_D^{25}$ −37.8° (*c* 0.09, MeOH). FAB-MS *m/z* 937 $[M - H]^-$, *m/z* 792 $[(M - H) - 146]^-$, and *m/z* 646 $(792 - 146)^-$. HR-FAB-MS *m/z* 937.4639 $[M - H]^-$ (calcd for $C_{44}H_{73}O_{21}$, 937.4644). ^{1}H and ^{13}C NMR are in **Table 2**.

16-OMe Ester of Capsianoside I (3). Yield, 4.8 mg; $[\alpha]_D^{25}$ −7.5° (*c* 0.08, MeOH). FAB-MS *m/z* 673 $[M - H]^-$, *m/z* 641 $[(M - H) - 32]^-$, and *m/z* 479 $(641 - 162)^-$. ^{1}H and ^{13}C NMR are in **Table 2**.

Enzymatic Hydrolysis of 3 To Give 3a. Compound **3** (3.5 mg) in a citrate buffer (1 mL; pH 4.5) was incubated with a glycosidase mixture (5 mg) of *Charonia lampas* (Shikagaku Kogyo, CO. LTD, Tokyo, Japan) at 37 °C. After 3 days, the TLC analysis showed that the starting material had disappeared and was replaced by one major spot. The mixture was passed through a C-18 Sep-Pak cartridge, washed with H_2O, and eluted with MeOH. The MeOH was evaporated to dryness, and the residue was submitted to HPLC on the C-18 μ-Bondapak column in MeOH:H_2O 85:15 to give compound **3a** (2 mg); FAB-MS *m/z* 349 $[M - H]^-$.

Preparation of (*R*)- and (*S*)-α-Methoxy-α-(trifluoromethyl)-phenylacetyl Chloride (MTPA) Esters (3a′ and 3a″) from 3a. A solution of **3a** (1 mg) in 1 mL of dry CH_2Cl_2 was reacted with (+)-MTPA-Cl (5 μL) in the presence of triethylamine (10 μL) and a catalytic amount of 4-N,N′-(dimethylamino)pyridine (DMAP), and the mixture was stirred at 25 °C for 30 min. After the solvent was removed, the mixture was purified by Si gel column chromatography performed in a Pasteur pipet filled with a slurry of Si gel using $CHCl_3$ as the eluent to afford **3a′**. Compound **3a″** was then prepared through a similar procedure from **3a** (1 mg) using (−)-MTPA-Cl (5 μL), triethylamine (10 μL), and 4-DMAP.

13-(*R*)-MTPA Ester (3a′). EI-MS *m/z* 566 $[M]^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H (ppm) 7.31−7.57 (5H, aromatic protons), 6.71 (1H, dd, J = 1.5, 8.6 Hz, H-14), 5.95 (1H, dd, J = 17.0, 11.0 Hz, H-2), 5.84 (1H, m, H-13), 5.12 (1H, t, J = 6.8 Hz, H-10), 5.23 (1H, d, J = 17.5 Hz, H-1), 5.10 (1H, t, J = 6.8 Hz, H-6), 5.09 (1H, d, J = 11.2 Hz, H-1), 3.68 (3H, s, -OCH_3), 3.53 (3H, s, -OCH_3), 2.43 (1H, dd, J =

Table 2. ^{1}H and ^{13}C NMR (CD_3OD, 500 and 125 MHz) of Compounds **1**–**3**

	1		**2**		**3**	
position	δ_H[a]	δ_C	δ_H[a]	δ_C	δ_H[a]	δ_C
1	5.25 d (J = 18.0), 5.21 d (J = 11.1)	115.7	5.25,[b] 5.21[b]	115.8	5.23 d (J = 17.5), 5.21 d (J = 11.2)	115.7
2	6.14 dd (J = 11.1, 18.0)	144.2	5.95 dd (J = 11.0, 17.1)	144.5	6.13 dd (J = 11.2, 17.5)	144.5
3		81.0		81.3		81.8
4	1.61[b]	42.8	2.30,[b] 1.63[b]	42.9	1.60[b]	42.7
5	2.06[b]	23.3	2.17[b]	23.2	2.06[b]	23.3
6	5.13 br t (J = 7.2)	125.6	5.30 t (J = 7.2)	129.3	5.13 t (J = 6.8)	125.6
7		135.9		139.4		135.5
8	1.98[b]	40.6	2.13[b]	36.0	1.98[b]	40.3
9	2.06[b]	27.5	2.13[b]	27.9	2.08[b]	27.4
10	5.13 br t (J = 7.2)	125.6	5.15[b]	126.0	5.18 t (J = 6.8)	129.0
11		135.0		135.7		131.1
12	2.01[b]	40.6	2.02 t (J = 7.7)	40.9	2.30 dd (J = 6.9, 13.5), 2.13 dd (J = 6.9, 13.5)	47.6
13	2.18 m	27.1	2.19[b]	27.3	4.54 m	67.6
14	5.41 t (J = 8.1)	131.1	5.40 t (J = 8.1)	131.3	6.60 dd (J = 1.5, 8.6)	128.6
15		132.1		132.4		144.9
16	1.78 s	21.6	1.78 s	21.9		169.7
17	4.34 d (J = 11.5), 4.13 d (J = 11.5)	67.4	4.33 d (J = 11.2), 4.13 d (J = 11.2)	67.7	1.84 s	12.7
18	1.61 s	16.0	1.62 s	16.3	1.66 s	16.4
19	1.61 s	16.0	4.08 s	59.7	1.60 s	15.8
20	1.39 s	23.2	1.39 s	23.2	1.39 s	23.1
–OCH_3					3.74 s	52.1
			Glc I			
1	4.46 d (J = 7.7)	98.3	4.37 d (J = 7.6)	99.5	4.48 d (J = 7.7)	98.2
2	3.44[b]	83.0	3.19[b]	75.2	3.43[b]	83.0
3	3.50 t (J = 9.0)	77.8	3.31[b]	78.2	3.52[b]	77.8
4	3.32[b]	71.3	3.28[b]	71.7	3.34[b]	71.3
5	3.18[b]	77.3	3.16[b]	77.6	3.19[b]	77.3
6	3.79,[b] 3.63[b]	62.3	3.82,[b] 3.65[b]	62.8	3.81 dd (J = 11.9, 2.3), 3.63 dd (J = 11.9, 5.6)	62.5
			Glc II			
1	4.21 d (J = 7.8)	101.9	4.22 d (J = 7.6)	102.1		
2	3.20[b]	75.0	3.22[b]	75.2		
3	3.44[b]	76.5	3.44[b]	76.7		
4	3.56 t (J = 9.4)	78.9	3.58 t (J = 9.2)	79.3		
5	3.38[b]	75.2	3.39[b]	75.4		
6	3.93,[b] 3.61[b]	66.4	3.93,[b] 3.63[b]	66.9		
			Glc III			
1	4.54 d (J = 7.6)	105.7			4.56 d (J = 7.7)	105.6
2	3.25[b]	76.4			3.25[b]	76.3
3	3.26[b]	78.1			3.27[b]	77.9
4	3.33[b]	71.4			3.37[b]	71.4
5	3.37[b]	77.4			3.38[b]	77.5
6	3.80,[b] 3.70[b]	62.3			3.83 dd (J = 11.9, 2.5), 3.70 dd (J = 11.9, 5.0)	62.5
			Rha I			
1	4.82 s	102.5	4.83 s	102.7		
2	3.83[b]	72.2	3.83[b]	72.2		
3	3.65[b]	72.0	3.65[b]	72.0		
4	3.39[b]	73.7	3.40[b]	73.5		
5	4.00[b]	70.3	4.00[b]	70.3		
6	1.26 d (J = 6.6)	17.7	1.26 d (J = 6.1)	17.8		
			Rha II			
1	4.71 s	101.4	4.72 s	101.6		
2	3.85[b]	72.0	3.85[b]	72.0		
3	3.69[b]	72.1	3.69 dd (J = 9.6, 3.3)	72.1		
4	3.36[b]	73.6	3.38[b]	73.6		
5	3.72[b]	69.6	3.74[b]	69.6		
6	1.28 d (J = 6.6)	17.8	1.28 d (J = 6.1)	18.1		

[a] Coupling costants (in Hz) are given in parentheses. [b] Overlapped signals.

13.5, 6.9 Hz, H-12), 2.26 (1H, dd, J = 13.5, 6.9 Hz, H-12′), 1.96 (3H, s, CH_3-17), 1.60 (3H, s, CH_3-18), 1.57 (3H, s, CH_3-19), 1.30 (3H, s, CH_3-20).

13-(*S*)-MTPA Ester (3a″). EI-MS *m*/*z* 566 $[M]^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ_H (ppm) 7.30−7.58 (5H, aromatic protons), 6.57 (1H, dd, J = 1.5, 8.6 Hz, H-14), 5.93 (1H, dd, J = 17.0, 11.0 Hz, H-2), 5.82 (1H, m, H-13), 5.22 (1H, d, J = 17.5 Hz, H-1), 5.21 (1H, t, J = 6.8 Hz, H-10), 5.09 (1H, t, J = 6.8 Hz, H-6), 5.07 (1H, d, J = 11.2 Hz, H-1), 3.68 (3H, s, -OCH_3), 3.53 (3H, s, -OCH_3), 2.50 (1H, dd, J = 13.5, 6.9 Hz, H-12), 2.31 (1H, dd, J = 13.5, 6.9 Hz, H-12′), 1.94 (3H, s, CH_3-17), 1.67 (3H, s, CH_3-18), 1.58 (3H, s, CH_3-19), 1.30 (3H, s, CH_3-20).

Capsianoside L (4). Yield, 0.6 mg; $[\alpha]_D^{25}$ −10.0° (*c* 0.06, MeOH). HR-FAB-MS *m*/*z* 1579.7889 (calcd, 1579.7896). FAB-MS *m*/*z* 1579

Table 3. ^{1}H and ^{13}C NMR (CD_3OD, 500 and 125 MHz) of Compound **4**

position	δ_H[a]	δ_C	position	δ_H[a]	δ_C
1	5.21,[b] 5.25[b]	115.8	1′	5.23[b]	115.8
2	5.95 dd (J = 17.7, 11.0)	144.5	2′	6.14 dd (J = 17.6, 10.9)	144.5
3		81.3	3′		81.4
4	1.64[b]	42.8	4′	1.62[b]	42.8
5	2.16[b]	23.5	5′	2.06	23.3
6	5.31[b]	129.3	6′	5.13 brt	125.7
7		139.3	7′		135.7
8	2.13[b]	35.6	8′	2.00[b]	40.5
9	2.09[b]	28.0	9′	2.11[b]	27.7
10	5.14[b]	128.9	10′	5.13 brt	125.7
11		131.5	11′		135.5
12	2.30,[b] 2.15[b]	47.7	12′	2.02[b]	40.6
13	4.55 m	67.7	13′	2.19[b]	27.3
14	6.72 dd (J = 8.6, 1.5)	129.0	14′	5.41[b]	131.0
15		145.0	15′		132.3
16		169.6	16′	1.78 s	21.8
17	1.89 s	13.2	17′	4.34 d (J = 11.5), 4.13 d (J = 11.5)	67.3
18	1.68 s	16.5	18′	1.62 s	16.3
19	4.09[b]	59.8	19′	1.62 s	16.3
20	1.38 s	23.1	20′	1.39 s	23.4
		Glc I			
1	4.48 d (J = 7.6)	98.3	4	3.31[b]	71.5
2	3.44[b]	83.1	5	3.19[b]	77.5
3	3.50[b]	78.0	6	3.81[b], 3.63[b]	62.5
		Glc IV			
1	4.36 d (J = 7.6)	99.4	4	3.28[b]	71.7
2	3.18[b]	75.2	5	3.17[b]	77.5
3	3.30[b]	78.0	6	3.82,[b] 3.64[b]	62.8
		Glc II			
1	4.21 d (J = 8.0)	102.1	4	3.56[b]	78.0
2	3.21[b]	75.0	5	3.38[b]	75.2
3	3.45[b]	77.0	6	3.95,[b] 3.65[b]	66.8
		Rha I			
1	4.83 s	102.6	4	3.58[b]	71.6
2	4.00 d (J = 3.0)	70.2	5	4.02[b]	70.1
3	4.68 dd (J = 10.0, 3.0)	75.6	6	1.28 d (J = 6.3)	18.1
		Glc III			
1	4.56 d (J = 7.5)	105.7	4	3.35[b]	71.2
2	3.24[b]	76.5	5	3.37 [b]	77.4
3	3.26[b]	78.1	6	3.83,[b] 3.70[b]	62.8
		Rha II			
1	4.72 s	101.6	4	3.37[b]	73.8
2	3.85[b]	72.1	5	3.76[b]	69.7
3	3.70[b]	72.2	6	1.29 d (J = 6.3)	18.0

[a] Coupling costants (in Hz) are given in parentheses. [b] Overlapped signals.

$[M - H]^-$, *m/z* 1083 $[(M - H) - 496]^-$, *m/z* 921 $[1083 -$ hexose$]^-$, *m/z* 775 $[921 -$ deoxyhexose$]^-$, *m/z* 467 $[775 -$ deoxyhexose and hexose$]^-$. ^{1}H and ^{13}C NMR are in **Table 3**.

Capsianoside III (5). Yield, 2.5 mg; $[\alpha]_D^{25}$ −23.3° (*c* 0.12, MeOH). FAB-MS *m/z* 1123 $[M + Na]^+$. ^{1}H, ^{13}C, and 2D NMR data were virtually identical to those reported in the literature (*5*, *6*).

Capsianoside V (6). Yield, 1.0 mg; $[\alpha]_D^{25}$ +1.7° (*c* 0.1, MeOH). FAB-MS *m/z* 537 $[M + Na]^+$. ^{1}H, ^{13}C, and 2D NMR data were virtually identical to those reported in the literature (*5*, *6*).

Capsianoside I (7). Yield, 4.7 mg; $[\alpha]_D^{25}$ −8.5° (*c* 0.1, MeOH). FAB-MS *m/z* 683 $[M + Na]^+$. ^{1}H and ^{13}C NMR were virtually identical to those reported in the literature (*5*, *6*).

3-*O*-(9,12,15-Octadecatrienoyl)glyceryl-β-D-galactopyranoside (8). Yield, 6.9 mg. FAB-MS *m/z* 515 $[M + H]^+$. ^{1}H, ^{13}C, and 2D NMR data were virtually identical to those reported in the literature (*15*).

Oxylipin (9). Yield, 2.6 mg. ESI-MS (*m/z* 327 $[M]^-$). ^{1}H and ^{13}C NMR are in **Table 4**.

Phosphatidylcholine (10). Yield, 2.8 mg. FAB-MS *m/z* 518 $[M + Na]^+$, *m/z* 459 (*18*).

Capsidiol (11). Yield, 2.7 mg; $[\alpha]_D^{25}$ +18.9° (*c* 0.15, MeOH). EI-MS *m/z* 236 $[M]^+$. ^{1}H NMR (500 MHz, CD_3OD): δ_H (ppm) 5.90

Table 4. ^{1}H and ^{13}C NMR (CD_3OD, 500 and 125 MHz) of Oxylipin **9**

position	δ_H[a]	δ_C
1		178.1
2	2.27 t (J = 7.4 Hz)	35.1
3	1.61 m	26.3
4	1.36 m[b]	30.1
5	1.36 m[b]	30.1
6	1.36 m[b]	30.1
7	1.56–1.35[b]	26.4
8	1.74–1.42[b]	33.9
9	3.47 ddd (J = 9.8, 7.5, 2.2)	73.3
10	3.41 dd (J = 7.5, 4.9)	77.8
11	4.59 dd (J = 8.4, 4.9)	69.4
12	5.51 ddt (J = 12.1, 8.4, 1.5)	129.4
13	5.53 m	132.6
14	2.99 dt (J = 15.7, 6.8), 2.85 dt (J = 15.7, 6.6)	27.1
15	5.35 m	127.9
16	5.39 m	132.7
17	2.10 quintet (J = 7.5)	21.1
18	0.97 t (J = 7.5)	14.0

[a] Coupling costants (in Hz) are given in parentheses. [b] Overlapped signals.

(1H, dd, J = 6.8, 2.1 Hz, H-9), 4.70 (1H, t, J = 1.5 Hz, H-12), 4.68 (1H, d, J = 1.1 Hz, H-12), 4.50 (1H, dt, J = 12.7, 4.3 Hz, H-3), 4.27 (1H, dd, J = 3.5, 2.7 Hz, H-1), 2.21 (1H, tt, J = 12.4, 3.8 Hz, H-7), 2.05 (1H, m, H-8), 1.89 (1H, m, H-8′), 1.83 (1H, H-2), 1.75 (1H, dd, J=13.9, 3.0 Hz, H-6), 1.73 (3H, s, H_3-13), 1.69 (1H, m, H-4), 1.63 (1H, ddd, J = 13.4, 12.7, 3.5 Hz, H-2′), 1.35 (3H, s, H_3-15), 1.32 (1H, m, H-6), 0.87 (3H, d, J = 7.0 Hz, H_3-14). ^{13}C NMR (125 MHz, CD_3-OD): δ_C (ppm) 150.8 (C-11), 141.4 (C-10), 129.3 (C-9), 109.2 (C-12), 75.6 (C-1), 66.0 (C-3), 46.6 (C-6), 41.6 (C-7), 40.1 (C-5), 36.9 (C-2), 32.6 (C-15), 31.5 (C-8), 21.1 (C-13), 9.6 (C-14).

Loliolide (12). Yield, 0.7 mg; $[\alpha]_D^{25}$ −61.7° (*c* 0.07, MeOH). EI-MS *m*/*z* 196 $[M]^+$. ^{1}H NMR (500 MHz, CD_3OD): δ_H (ppm) 5.75 (1H, s, H-7), 4.22 (1H, quintet, J = 3.4 Hz, H-3), 2.42 (1H, dt, J = 13.7, 2.4 Hz, H-4), 2.01 (1H, dt, J = 14.3, 2.5 Hz, H-2), 1.76 (3H, s, H_3-11), 1.75 (1H, dd, J = 13.7, 4.2 Hz, H-4), 1.53 (1H, dd, J = 14.3, 3.8 Hz, H-2), 1.47 (3H, s, H_3-9 ax), 1.27 (3H, s, H_3-10). ^{13}C NMR (125 MHz, CD_3OD): δ_C (ppm) 185.4 (C-6), 174.3 (C-8), 113.3 (C-7), 88.7 (C-5), 67.2 (C-3), 48.1 (C-2), 46.5 (C-4), 37.1 (C-1), 31.0 (C-10), 27.5 (C-11), 27.0 (C-9).

Blumenol C Glucoside (13). Yield, 1.0 mg; $[\alpha]_D^{25}$ +48° (*c* 0.1, MeOH). FAB-MS *m*/*z* 395 $[M + Na]^+$ (*22*).

Bacterial Strain. The isolate of *H. pylori* (strain DSMZ 4867, originating from human gastric samples) was obtained from the DSMZ Culture Collection. The strain was cultured on Columbia agar (Difco, Italy) with 4% horse blood (Difco).

Minimum Inhibitory Concentration (MIC) Determination. The MIC was performed by the NCCLS (*26*) agar microdilution procedure (*27*). The bacterium was recultured on Columbia agar, as described above, at 37 °C for 5−7 days under microaerobic conditions. In a tube of brain heart infusion broth (Difco), a bacterial suspension was made to a density of a 0.5 McFarland standard (10^8 CFU/mL). The inoculum was diluted in sterile saline such that the final organism suspensions contained a viable count of 6×10^{-5} CFU/mL. Compounds **11** and **12** were dissolved in dimethyl sulfoxide (DMSO) (*27*). Metronidazole (dissolved in DMSO) was used as a positive control.

Three different concentrations of **11** and **12** (50−200 μg/mL) were prepared and added to 5 cm diameter Petri plates where the bacterial suspension (0.1 mL) and 5 mL of Mueller−Hinton agar with 5% aged (>2 weeks old) sheep blood (Difco) were previously inoculated at 45 °C. The Petri plates were incubated at 37 °C for 72 h under microaerobic conditions. After incubation, the bacterial colonies were counted. The lowest drug concentration showing no growth was read as the MIC.

RESULTS AND DISCUSSION

The methanol extracts of Red Bull's Horn Chile pericarps were very rich in secondary metabolites. The *n*-buthanol soluble portion was subjected to DCCC and HPLC purification to furnish four new glycosides named capsianosides and classified in two groups, monomeric diterpene glycosides (**1**−**3**) and dimeric esters (**4**). Capsianosides **1**, **2**, and **4** contain a trisaccharide chain with an unusual glucose unit, a branching point never found before in other related compounds. By HPLC purification were also isolated the known compounds **5**, **6**, and **7**, inosine, uridine, and quercetine 3-*O*-rhamnoside.

Analysis of the chloroform soluble fraction led to the isolation and structural elucidation of **8**, **9**, **10**; two sesquiterpenes, **11** and **12**; and the megastigmane blumenol C glucoside (**13**) previously identified from several plants.

In the sweet variety examined, acyclic diterpene glycosides constituted the main components when compared with other products obtained in minor amounts. Compound **8** is a very rare metabolite, and its biological functions have not been fully clarified (*28*). It is generally accepted that plants respond to pathogen attack with a multicomponent defense response. Formation of oxygenated fatty acids, collectively named oxylipins, is one of the early responses of plant cells to environmental stress (*17*), and in this plant material, it is associated with the presence of **11**, a natural phytoalexin with antifungal activity. Capsidiol synthesis occurs in many solanaceous species and is synthesized in response to several environmental stimuli (*20*).

The FAB mass spectrum of **1** exhibited a quasi-molecular ion peak at *m*/*z* 1083 $[M - H]^-$, and high-resolution measurements indicated the molecular formula $C_{50}H_{84}O_{25}$.

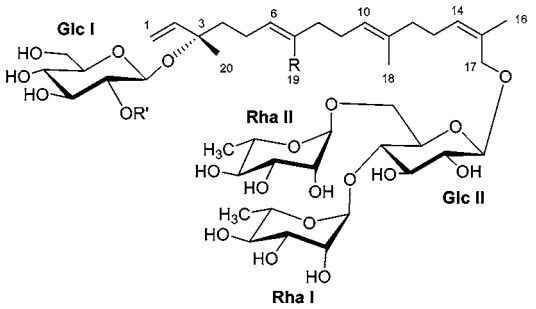
A comparative analysis of ^{1}H and ^{13}C NMR and ^{1}H−^{1}H correlation spectroscopy (COSY) spectra of **1** with those of **5** (*5*, *9*) suggested the presence of a 6*E*,10*E*,14*Z*,17-hydroxygeranyllinalool as the basic skeleton, which was confirmed by the observed HMBC correlations. Significant differences were attributable to NMR data of oligosaccharide chains with respect to the reference compounds, and the new compound **1** shows a branching glucose unit never found before in other related metabolites.

The presence of five sugar units was corroborated by the anomeric proton resonances at δ_H 4.54, 4.82, 4.21, 4.71, and 4.46, which were correlated with the corresponding anomeric carbon signals at 105.7, 102.5, 101.9, 101.4, and 98.3 ppm in the HSQC experiment. The identity of the five sugar units was determined by acid methanolysis and GLC analysis, as glucose and rhamnose in the ratio 3:2. The ^{13}C NMR confirmed the presence of 20 carbon signals including one oxygenated methylene carbon (C-17, 67.4 ppm), one quaternary carbon bearing one oxygen atom (C-3, 81.0 ppm), along with signals of β-glucose (×3) and α-rhamnose (×2). HMBC cross-peaks of C-3 and C-17 with the anomeric protons at δ_H 4.46 (Glc I) and δ_H 4.21 (Glc II), respectively, confirmed the glycosylation sites of geranyllinalool aglycone. Two different oligosaccharide chains were present in **1**, and a comparative analysis of NMR data with those of known capsianoside III suggested the presence of the same disaccharide chain linked at C-3 of aglycone. By HMBC experiment, the linking of Glc III at Glc I from a cross-peak between H-1 (δ_H 4.54) of Glc III with C-2 (δ_C 83.0) of Glc I was established. The remaining sugar units, glucose and rhamnose (×2), are components of the second oligosaccharide chain in which the glucose unit (Glc II) was linked to C-17 of aglycone.

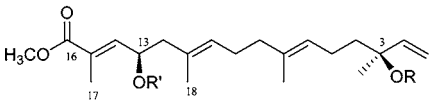
2D-TOCSY and COSY experiments clearly showed correlation signals from the H-1 to H_2-6 spin system of Glc II and for Rha I and Rha II. Taking into account the known effects of glycosylation on the ^{13}C chemical shift (*29*), the position of interglycosidic linkages was determined. On the basis of these considerations, the Rha I and Rha II units were located as terminal sugars by the absence of any ^{13}C glycosylation shifts. The attachment of Rha I at C-4 of Glc II was established by a significant cross-peak H-1 (Rha I) at δ_H 4.82 with C-4 (Glc II) at δ_C 78.9 in the HMBC spectrum. In the same experiment, H-1 of Rha II (δ_H 4.71) showed a correlation with C-6 of Glc II (δ_C 66.4) indicating a 4,6-diglycosylated β-D-glucopyranosyl unit. These data were also in agreement with the downfield shift observed in the ^{13}C NMR for C-4 (δ_C 78.9) and C-6 (δ_C 66.4) of Glc II as expected for a glycosylation shift. Moreover, C-3 (δ_C 76.5) and C-5 (δ_C 75.2) were upfield shifted (γ-effect) with respect to the reference data of a unsubstituted glucose (Glc III) (*29*). The presence of a disubstituted glucose unit in **1** represents a singular exception to the general pattern encountered in the previously isolated capsianosides. The absolute configuration at C-3 was suggested to be 3*S* as in other capsianosides isolated, on the basis of optical rotation. Therefore, the structure of **1** was defined as 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-17-hydroxy-6*E*,10*E*,14*Z*-(3*S*)-geranyllionalool-17-*O*-[α-L-rhamnopyranosyl-(1→4)-{α-L-rhamnopyranosyl-(1→6)}]-β-D-glucopyranoside.

Comparison of ^{1}H and ^{13}C NMR data of **2** with those of **1** clearly indicated structural similarity between the two compounds (**Table 2**). In a negative ion mode spectrum, the quasi-molecular ion peak for **2** was found at *m/z* 937 $[M - H]^-$ followed by two predominant peaks at *m/z* 792 $[(M - H) - 146]^-$ and *m/z* 646 $(792 - 146)^-$ indicating the loss of two deoxyhexose units. The main difference in the compound **2** with respect to **1** was the lack of signals of a glucose unit in the NMR spectrum. ^{1}H NMR and ^{1}H−^{1}H COSY showed four anomeric proton signals at δ_H 4.22 (d, $J = 7.6$ Hz), δ_H 4.37 (d, $J = 7.6$ Hz) typical of two β-glucosyl moieties, and δ_H 4.72 (s) and δ_H 4.83 (s), indicative of two α-rhamnosyl units. Moreover, in **2**, a singlet at δ_H 4.08 (2H) due to a hydroxymethyl group and three methyl groups [δ_H 1.39 (CH_3-20), 1.62 (CH_3-18), and 1.78 (CH_3-16)] was observed, one less than in **1**, when compared with the ^{1}H NMR of **1**. The location of the hydroxymethyl group at C-19 was derived from the HMBC spectrum, which showed correlations between H_2-19 (δ_H 4.08) and C-7 (δ_C 139.4), C-6 (δ_C 129.3), and C-8 (δ_C 36.0) and was also supported by hydroxylation shifts observed in the ^{13}C NMR spectrum for C-6, C-7, and C-8 with respect to the same carbon signals in **1**. A good coincidence in the chemical shift of HSQC data and HMBC correlations was observed for the trisaccharide moiety linked at C-17 with the unusual 4,6-disubstituted glucose as in **1** (**Table 2**). The remaining β-D-glucopyranosyl residue was linked at C-3 of the geranyllinalool moiety as deduced from HMBC correlation for anomeric proton signal (Glc I) at δ_H 4.37 with C-3 (81.3 ppm). Thus, the structure reported in **Figure 1** was assigned to compound **2**, which was given the trivial name of capsianoside IX.

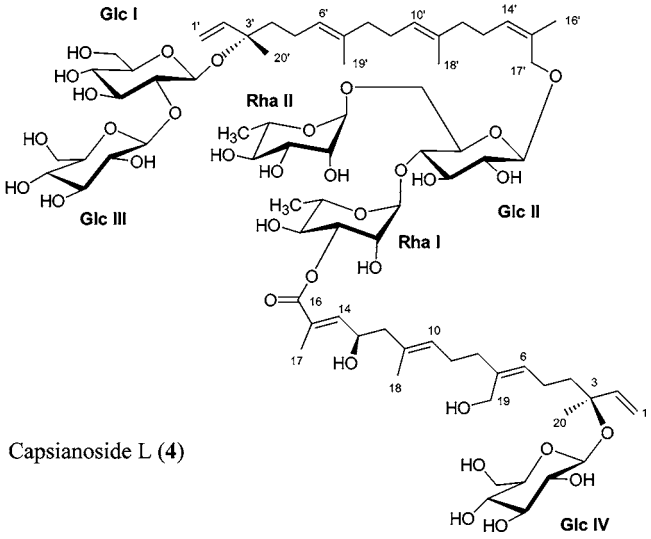
Analysis of NMR data of compound **3**, in comparison with those of **1**, indicated the same disaccharide moiety linked at C-3 of the aglycone and the loss of the trisaccharide chain at C-17. The IR spectrum showed absorption due to hydroxyl (3458 cm^{-1}) functions and a α,β-unsaturated ester carbonyl group (1724, 1652 cm^{-1}). In the FAB-MS spectrum, **3** exhibited a pseudomolecular ion peak at *m/z* 673 $[M - H]^-$ together with fragment ions at *m/z* 641 $[(M - H) - 32]^-$ and *m/z* 479 $(641 - 162)^-$. The ^{13}C NMR spectrum displayed signals assigned to two β-D-glucose units, one hydroxymethine group (δ_C 67.6), one carbonyl ester function (δ_C 169.7), and one −OMe group (δ_C 52.1). The complete elucidation of **3** was achieved by HSQC, HMBC, and ^{1}H−^{1}H COSY experiments (**Table 2**) and by the correlations observed; it was possible to deduce that **3** was the 16-methyl ester of **7** (*5*, *6*), also isolated as component of the same extract. The absolute configuration at C-13 was determined to be *R* by a modified Mosher's method after esterification with MTPA [α-methoxy-α-(trifluoromethyl)phenyl acetate] (*30*) of the aglycone **3a** obtained by enzymatic hydrolysis of **3**. $\Delta\delta S - \Delta\delta R$ values in Hz are shown in **Figure 3**. Compound **4** showed the absorption bands due to hydroxyl (3438 cm^{-1}) and a α,β-unsaturated ester groups (1715, 1647 cm^{-1}) in the IR spectrum. Its molecular formula was determined to be $C_{76}H_{124}O_{34}$ by an HR-FAB-MS spectrum. The negative FAB-MS gave a pseudomolecular ion peak at *m/z* 1579 $[M - H]^-$ and significant mass fragment ions at *m/z* 1083 $[(M - H) - 496]^-$, *m/z* 921 [1083 − hexose]$^-$, *m/z* 775 [921 − deoxyhexose]$^-$, and *m/z* 467 [775 − deoxyhexose and hexose]$^-$. The ^{13}C NMR spectrum of **4** showed 76 signals including six anomeric carbons (**Table 3**) at δ_C 98.3, 99.4, 101.6, 102.1, 102.6, and 105.7, which were correlated with the corresponding anomeric protons in the HSQC experiment. Moreover, the ^{13}C NMR also suggested the presence of the ester carbonyl group at δ_C 169.6; therefore, **4** was estimated to be related to dimeric



Capsianoside VIII (**1**): R = CH_3, R′ = Glc III
Capsianoside IX (**2**): R = CH_2OH, R′ = H



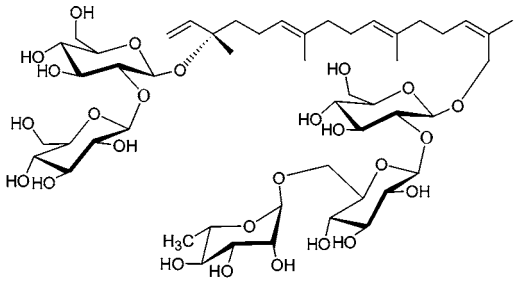
Compound **3** R = GlcI $\xrightarrow{2\rightarrow1}$ Glc III
Compound **3a** R = H, R′ = H
Compound **3a′** R = H, R′ = (R)-MTPA
Compound **3a″** R = H, R′ = (S)-MTPA



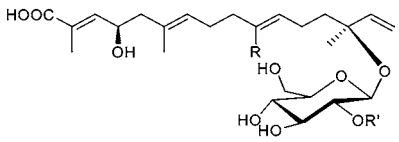
Capsianoside L (**4**)

Figure 1. New compounds isolated from fresh sweet pepper fruits of *C. annuum* L.

(capsianosides A−H) esters of acyclic diterpene glycosides, isolated from different species of *Capsicum* (*5*−*7*). Acid methanolysis of **4** afforded D-glucose and L-rhamnose in the ratio 4:2. The identity of the single sugar chain and the sequence of oligosaccharide chains was determined by ^{1}H−^{1}H COSY, TOCSY, HSQC, and HMBC data. Detailed inspection of 2D NMR experiments indicated a cluster of signals for the H-1′ to H-20′ chain and for Glc I, Glc III, Glc II, and Rha II units, virtually identical to those detected for **1**. In addition, NMR data showed signals relative to a second 6*E*,10*E*,14*E*-geranyllinalool chain with a terminal glucosyl unit (Glc IV) linked at C-3 as indicated by cross-peak H-1 (Glc IV) δ_H 4.36 with C-3 (δ_C 81.3) observed in the HMBC spectrum. The analysis of ^{1}H NMR and ^{1}H−^{1}H COSY from H_2-1 to H_3-20 displayed signals for three tertiary methyl groups (H_3-17, H_3-18, and H_3-20), one oxymethine (δ_H 4.56), one oxymethylene (δ_H 4.09), and a series of olefinic protons. On the basis of HSQC and HMBC experiments, hydroxyl groups were located at C-13 and C-19. This was supported by correlations observed between H-13 (δ_H 4.56)/C-14 (δ_C 129.0) and C-12 (δ_C 47.7) and between H_2-19

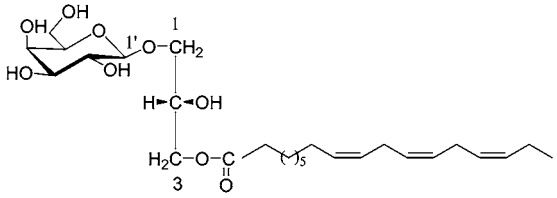


Capsianoside III (**5**)

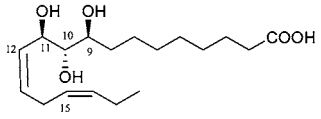


Capsianoside V (**6**) R=CH_2OH, R′=OH

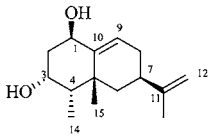
Capsianoside I (**7**) R=CH_3, R′=Glc



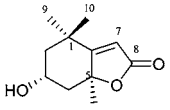
(**8**)



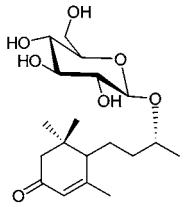
Oxylipin (**9**)



Capsidiol (**11**)



Loliolide (**12**)



Blumenol C glucoside (**13**)

Figure 2. Known compounds isolated from fresh sweet pepper fruits of *C. annuum* L.

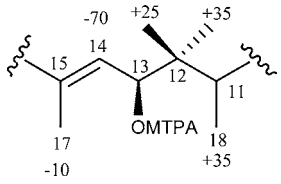


Figure 3. Results of the modified Mosher's method for **3a**. The $\Delta\delta$ values are in Hz (δS–δR, 500 MHz).

(δ_H 4.09) and C-6 (δ_C 129.3), C-7 (δ_C 139.3), and C-8 (δ_C 35.6), respectively. From HMBC data, the carbonyl ester was located at C-16 on the basis of the cross-peaks H_3-17/C-16, H_3-17/C-15, and H-14/C-17. Interpretation of $^1H-^1H$ COSY and TOCSY experiments clearly indicated a different chemical shift values in the spin system sequence for the Rha I unit as compared with those of terminal rhamnosyl residue (Rha II). Thus, a hydroxyl group of Rha I that participated in the ester bonding was suggested. The low-field chemical shift of H-3 (Rha I) at δ_H 4.68 (δ_C 75.6) indicated that the hydroxyl group at C-3 was involved in the ester linkage. Moreover, we also observed the acylation shifts in the 2 (δ_H 4.00; δ_C 70.2) and 4 (δ_H 3.58; δ_C 71.6) positions. These data were confirmed by HMBC correlations observed between C-16 (δ_C 169.9) and H-3 (δ_H 4.68) of Rha I. The absolute configurations at C-3 and C-3′ were suggested to be 3*S* as indicated for previously isolated compounds. The chirality at C-13 was assigned as *R*, as for compound **3**. Thus, structure **4** was indicated for capsianoside L.

Oxylipins are important signaling and defense compounds in plants whose synthesis may occur either enzimatically via the lipoxygenase pathway or by autoxidation (*17*). Among them, several kinds of oxygenated unsaturated C18 fatty acids have been isolated. A 9(*S*),10(*S*),11(*R*)-trihydroxy-12(*Z*),15(*Z*)-octadienoic acid was previously identified with several related compounds, as novel elicitor-inducible oxylipins in potato (*16*) with strong antifungal activity. We report herein the characterization of compound **9** because no clear references about the stereochemical determination were found.

The molecular formula of **9** was determined as $C_{18}H_{32}O_5$ by elemental analysis, ESI-MS (*m*/*z* 327 $[M]^-$), ^{13}C, and 2D NMR. The ^1H NMR **(Table 4**) displayed one methyl group (δ_H 0.97), four olefinic protons (δ_H 5.53, δ_H 5.51, δ_H 5.39, and δ_H 5.35), three oxygen-bearing methine signals [(δ_H 4.59, dd, 1H), (δ_H 3.47, ddd, 1H), and (δ_H 3.41, dd, 1H)], and several methylene groups resonating from δ_H 1.35 to δ_H 2.99. The ^{13}C NMR (**Table 4**) indicated the presence of a carbonyl group (δ_C 178.2), four olefinic carbons, and partially overlapped methylene carbon signals. Inspection of $^1H-^1H$ COSY and TOCSY experiments allowed us to detect the C18 chain, starting from H_3-18 to H_2-6, with 12(*Z*),15(*Z*) unsaturations. The location of the carboxylic function was determined by inspection of the HMBC spectrum, which showed a long-range coupling between the carboxyl group at δ_C 178.2 with the methylene protons at δ_H 2.27 (H_2-2), while the remaining hydroxyl functions at C-9, C-10, and C-11 were supported by cross-peaks between H-10 (δ_H 3.41)/ C-11, C-12, C-9, and C-8 and between H-9 (δ_H 3.47)/ C-10, C-11, C-8, and C-7. The double bond positions were supported by correlations observed for H_2-14 with H-15 and H-13. The *Z* geometry of double bonds was derived from the coupling constants ($J = 12.1$ Hz) between H-12 and H-13, while a very complex signal was detected for H-15 and for H-16. The *Z* stereochemistry was assigned on the basis of high-field chemical shift of the allylic methylene carbons in the ^{13}C NMR spectrum (*31*).

We suggest the 9(*S*),10(*S*),11(*R*) configuration in our molecule, as for the previously isolated oxylipin, by application of the Murata method, a *J*-based configuration analysis for the elucidation of relative stereochemistry in acyclic structures with 1,2-chiral centers using $^3J_{H-H}$ and $^{2,3}J_{C-H}$ values, with a phase-sensitive HMBC (*32*) in combination with ROE data (*33*, *34*). This approach allowed the determination of the predominant staggered rotamers along the C9−C10 axis and the C10−C11 axis. Observation of a large (*anti*) $^3J_{H-H}$ value between H-9 and H-10 led a proton−proton anti arrangement. The *erythro* rotamer with C/C-*anti* configuration was proposed because no ROE was observed between H-9 and H-10. In our case, ROE experiments revealed spatial proximity for H-8 and H-10, H-10 and H-15, and H-11 and H-14. These data were supported by a large $^2J_{C10-H9}$ (−4.6 Hz) and $^2J_{C9-H10}$ (−5.4 Hz) coupling constants. For the segment C10−C11, the observed values of $^3J_{H10-H11}$ small (gauche), $^2J_{C10-H11}$ large, and $^2J_{C11-H10}$ small (−2.1) were consistent with the proposed stereochemistry. On the basis of above evidence, **9** was determined to be 9(*S*),10(*S*),11(*R*)-trihydroxy-12(*Z*),15(*Z*)-octadecadienoic acid.

Other compounds isolated from this plant material were fully characterized by comparison of the NMR data with spectroscopic data reported in the literature as **5**, **6**, **7** (*5*, *6*), **8** (*15*), **10** (*18*), **11** (*19*, *20*), **12** (*21*), and **13** (*22*).

Antimicrobial Activity. *H. pylori* is strongly associated with gastritis and ulcer disease. Environmental sources indicate that *H. pylori* can survive in water, chilled foods, milk, and fresh vegetables for several days. *H. pylori* can be found in human feces and can be transmitted directly from person to person by the fecal−oral or oral−oral route. *H. pylori* can be found in several animal reservoirs; however, the possibility of animal-to-animal or zoonotic transmission is unknown (*35*). Patients with *Helicobacter*-associated peptic ulceration can be cured by antimicrobial treatment, and agents used in the therapy are metronidazole, bismuth salts, furazolidone, ciprofloxacin, etc. (*36*). Widespread antimicrobial use has resulted in a worldwide increase in the prevalence of antibiotic resistance in *H. pylori*, and this problem has led to the evaluation of a number of newer compounds showing high antimicrobial activity without the resistance drawback (*37*, *38*). In recent years, a growing interest in alternative therapies and therapeutic use of natural products, especially those derived from plants, has been observed.

In the present study, we have examined the antibacterial properties of capsidiol (*11*) and loliolide (*12*) using *H. pylori* cultures and NCCLS microdilution method. Comparative efficacies of **11**, **12**, and metronidazole were examined in vitro.

Compound **11** showed bacteriostatic properties in vitro at the concentrations of 200 μg/mL (MIC). The commercial drug metronidazole possessed a similar MIC (250 μg/mL) as compared to **11**. Compound **12** showed no inhibitory activity against *H. pylori*. The results clearly demonstrate that **11** can act as an antibacterial agent against *H. pylori*. In the guidelines established by the NCCLS (*26*), the MIC interpretive standard of metronidazole for *H. pylori* is defined as follows: susceptible, <8 μg/mL; intermediate, $=8$ μg/mL; and resistant, >8. *H. pylori* DSMZ 4867 showed metronidazole resistance.

The capsidiol showed a clear in vitro anti-*H. pylori* activity against DSMZ 4867 isolate; thus, treatment with capsidiol may be a useful treatment for antibiotic-resistant strains and for patients who do not wish to take synthetic antibiotics and would also be a cheaper alternative in developing countries. However, absolutely further animal models and clinical studies should be performed.

Antioxidant Activity. We have also investigated the antioxidant activity of compounds **1**, **5**, **6**, and **8** with different methods: DPPH scavenging test (*39*), assays based upon the reduction of Cu^{++}/Cu^{+} (*40*), and lipid peroxidation (*41*) using the reported procedure. All of the tests gave negative results, which are not reported in this paper.

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