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Antimicrobial Furostanol Saponins from the Seeds of *Capsicum annuum* L. Var. *acuminatum*§

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Three new furostanol saponins named capsicoside E (**1**), capsicoside F (**2**), and capsicoside G (**5**) were obtained from the seeds of *Capsicum annuum* L. var. *acuminatum* along with known oligoglycosides (**3**, **4**, and **6**−**10**). On the basis of chemical and spectroscopic analyses, the structures of these new furostanol oligoglycosides were elucidated as 26-*O*-β-D-glucopyranosyl-22-*O*-methyl-5α-furost-25(27)-en-2α,3β,22ξ,26-tetraol-3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl(1→2)-[β-D-glucopyranosyl(1→3)]-β-D-glucopyranosyl(1→4)-β-D-galactopyranoside (**1**), 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furost-20(22)-en-2α,3β,26-triol-3-*O*-β-D-glucopyranosyl (1→3)-β-D-glucopyranosyl(1→2)-[β-D-glucopyranosyl(1→3)]-β-D-glucopyranosyl(1→4)-β-D-galactopyranoside (**2**), and 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furosta-3β,22ξ,26-triol-3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl(1→2)-[β-D-glucopyranosyl(1→3)]-β-D-glucopyranosyl(1→4)-β-D-galactopyranoside (**5**). The isolated saponins showed higher antimicrobial activity against yeasts than against common fungi. Data indicated that the antiyeast activity was related to the combination of the oligosaccharide chain (S1, S2, or S3) with an *O*-methyl group at R_3 and the presence of a hydroxyl group at the C-2 position.

KEYWORDS: ***Capsicum annuum* L.; Solanaceae; red pepper; seeds; furostanol saponins; antiyeast activity**

INTRODUCTION

Peppers, *Capsicum* species (Solanaceae), native plants of America (peppers, *Solanaceae*), are widely cultivated in Asia, Africa, and Mediterranean countries and are very important vegetables used as food, spices, and external medicine. Pepper fruits are a good source of vitamins (*1*), flavonoids, and phenolic components, which play an important role as dietary antioxidants (*2*, *3*). The typical "heat" sensation of peppers is due to a group of compounds named capsaicinoids, and capsaicin is used in medicine to mitigate pain as a topical analgesic. The postulated presence of a vanilloid receptor has been confirmed by the recent cloning of a vanilloid receptor subtype 1 (VR1), which proved the correlation between pungency and this receptor in vivo (*4*−*6*).

The chemical constituents of the polar fractions of ripe fruits of *C. annuum* L. var. *acuminatum* from various countries have been previously investigated (*7*−*9*). As a part of our contribution to the study of this genus, we have established phytochemical screening of the pulverized seeds of *C. annuum* L. var. *acuminatum*, which is a plant material known to include furostanol and spirostanol glycosides (*10*). Steroidal glycosides of the spirostane and furostane series, from various plants, possess a wide range of biological activity, including antitumor, antioxidant, antimicrobial, and fungicidal effects (*11*, *12*). Therefore, we determined the antiyeast activity of major isolated saponins to obtain more detailed information about the relationship between structure and bioactivity. This paper deals with the isolation and structural elucidation, by extensive NMR experiments, of a total of nine furostanol saponins, three of which (**1**, **2**, and **5**; **Figures 1** and **2**), appeared to be new compounds.

MATERIALS AND METHODS

General Methods. Electrospray ionization ESI-MS spectra were recorded in H_2O on an LCQ ThermoQuest instrument. HRFABMS spectra were recorded in a glycerol matrix on a VG Prospec instrument. Optical rotations were determined on a Perkin-Elmer 141 polarimeter; infrared spectra were recorded on a Bruker IFS-48 FT-IR spectrometer. GLC analyses were performed on a Carlo Erba Fractovap 4160 with a 30 m × 0.32 mm i.d. SPB-1, Supelco capillary column operated at 152 °C with He carrier at a flow rate of 10 mL min^{-1}. ^1H and ^{13}C NMR spectra were recorded at 500.13 and 125.76 MHz, respectively, on a Bruker AMX-500 spectrometer equipped with a Bruker X32 computer, using the UXNMR software package. ^1H NMR spectra were measured in C_5D_5N and a few drops of D_2O using TMS as an internal

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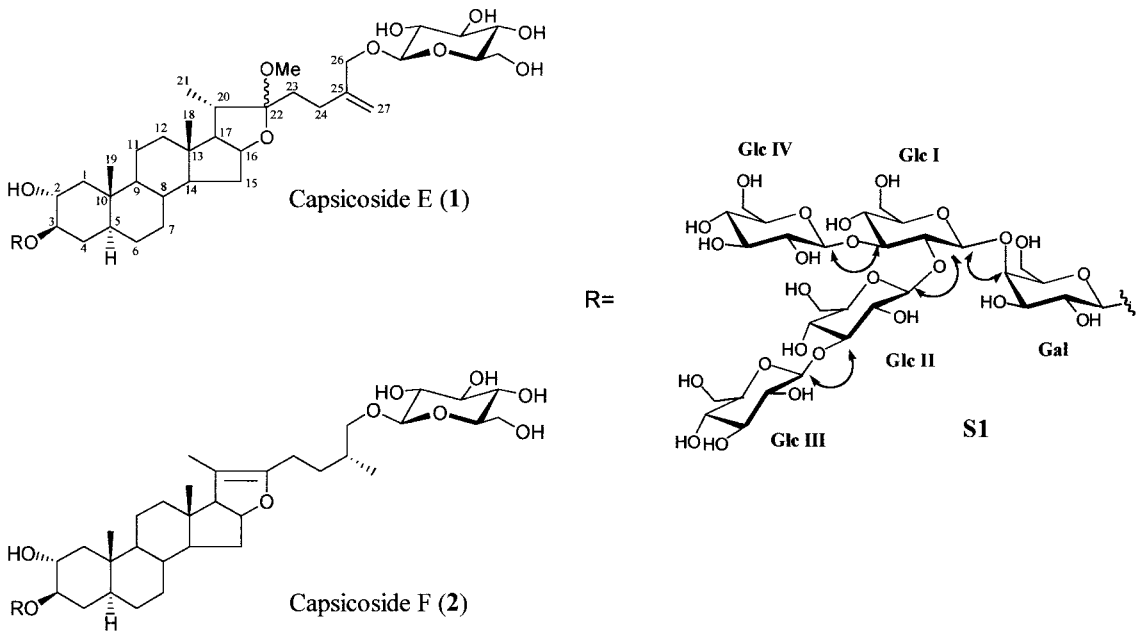
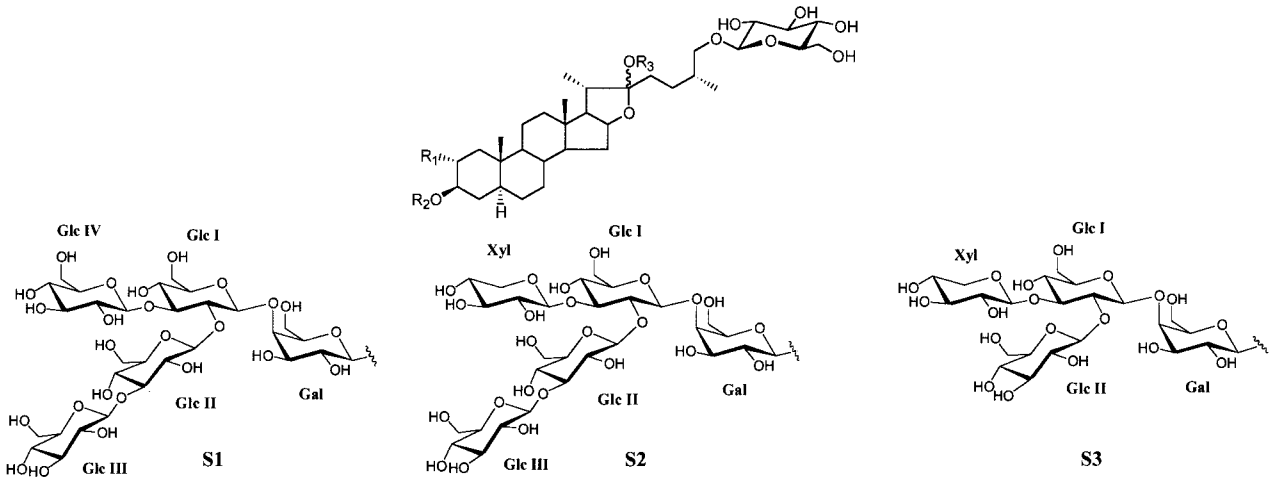


Figure 1. Saponins isolated from seeds of *C. annuum* L. var. *acuminatum*. Significant HMBC correlations are shown by arrows.



Saponins	R_1	R_2	R_3
Capsicoside A (**3**)	OH	S1	H
4	OH	S1	Me
Capsicoside G (**5**)	H	S1	H
6	H	S1	Me
Capsicoside D (**7**)	H	S2	H
8	H	S2	Me
9	H	S3	Me

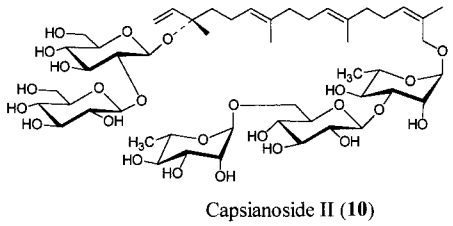


Figure 2. Additional saponins isolated from seeds of *C. annuum* L. var. *acuminatum*.

standard; ^{13}C chemical shifts were referenced to the residual solvent signal ($C_5D_5N + H_2O$, δ_C 135.5). The multiplicities of ^{13}C resonances were determined by DEPT experiments. The ^1H-detected one-bond and multiple-bond ^{13}C multiple-quantum coherence experiments (HMQC and HMBC, respectively) utilized a 5-mm probe with reverse geometry, and the sample was not spun. The magnitude of delay for optimizing one-bond correlations in the HMQC spectrum and suppressing them in the HMBC spectrum was 3.5 ms, and the evolution delay for long-range couplings in the latter was set to 60 ms. HPLC was performed on a Waters model 510 pump equipped with a Waters U6K injector and a differential refractometer, Waters model 401, with a 30 cm × 3.9 mm i.d. reversed phase C_{18} μ-Bondapak column.

Plant Material. Ripe fruits of *Capsicum annuum* L. var *acuminatum* were collected at Campobasso (Italy) in July 1998, separated from the seeds, and identified at the Dipartimento di Scienze Animali, Vegetali e dell'Ambiente (University of Molise). A voucher specimen is kept at the Dipartimento di Scienze e Tecnologie Agro-Alimentari, Ambientali e Microbiologiche dell'Università del Molise, Campobasso, Italy.

Table 1. ^{1}H and ^{13}C NMR Data ($C_5D_5N + D_2O$) of Aglycon Moiety in Saponins **1**, **2**, **4**, and **5**

	capsicoside E (**1**)		capsicoside F (**2**)		compound **4**		capsicoside G (**5**)	
position	δ_H[a]	δ_C	δ_H[a]	δ_C	δ_H[a]	δ_C	δ_H[a]	δ_C
1	2.16, 1.15	45.5	2.18, 1.18	44.3	2.18, 1.18	44.5	1.53, 0.80	35.8
2	3.98 m	69.6	3.98 m	69.7	3.98 m	69.7	2.14	28.9
3	3.92 m	82.8	3.96	83.0	3.96 m	83.0	4.62	79.6
4	1.88, 1.49	33.0	1.87, 1.49	33.2	1.87, 1.49	32.8	1.85, 1.40	33.6
5	0.98 m	43.3	1.00 m	43.5	0.98 m	43.6	0.91 m	43.7
6	1.17, 1.03	27.0	1.17, 1.05	27.3	1.17, 1.05	27.3	1.06	27.0
7	1.49	31.0	1.50	31.1	1.50	31.2	1.51, 0.78	31.2
8	1.33	33.4	1.34	33.6	1.34	33.7	1.95	33.0
9	0.51 br t	53.1	0.57 br t	53.5	0.57 br t	53.4	0.50 br t	53.3
10		35.7		36.0		36.0		34.8
11	1.43, 1.20	20.2	1.45, 1.22	20.7	1.47, 1.19	20.5	1.41, 1.20	20.1
12	1.63, 1.00	38.7	1.67, 1.02	39.2	1.63, 0.98	39.0	1.70, 1.04	38.9
13		40.0		43.2		40.3		40.0
14	0.92 m	55.0	1.00 m	55.0	0.98 m	55.3	1.00 m	55.3
15	1.96, 1.31	31.6	2.41, 1.40	34.2	1.98, 1.36	31.3	1.39	30.9
16	4.46 m	81.8	4.73	84.3	4.47 m	80.6	4.49 m	80.2
17	1.78	63.4	2.45 d (10.0)	64.5	1.76	63.4	1.74	62.1
18	0.76 s	15.7	0.70 s	14.2	0.79 s	15.7	0.83 s	15.1
19	0.66 s	12.2	0.68 s	12.4	0.69 s	12.5	0.68 s	12.2
20	2.22 m	39.2		103.3	2.22 m	39.6	2.06	40.0
21	1.17 d (6.6)	15.5	1.62 s	11.5	1.20 d (6.6)	15.5	1.33 d (6.8)	15.5
22		111.7		152.0		112.0		109.8
23	2.17, 2.05	31.5	2.45, 1.40	33.4	2.03, 1.78	29.9	1.80	34.7
24	2.40 m	28.8	1.73, 1.50	23.7	1.80, 1.32	27.2	1.38	26.5
25		145.9	1.78	31.5	1.92	33.2	1.94	33.1
26	4.65 dd (11.0, 2.1), 4.38	71.6	3.98, 3.60 m	74.8	4.00, 3.63 m	74.5	4.00, 3.64 m	74.3
27a	5.06 br s	109.8	1.01 d (6.8)	17.3	1.02 d (6.6)	16.3	0.99 d (6.8)	16.2
27b	5.36 br s							
OMe	3.32 s	46.5			3.32 s	46.6		

[a] Coupling constants are in parentheses and given in hertz.

Extraction and Isolation. The powdered, dried seeds (18 g) were extracted in MeOH at room temperature (600 mL). Evaporation of MeOH extracts afforded 1.05 g of a glassy material, which was then subjected to Kupchan's partitioning methodology (*13*) to give four extracts: *n*-hexane (320 mg), CCl_4 (52 mg), $CHCl_3$ (47 mg), and *n*-BuOH (212 mg). The water phase was concentrated under reduced pressure, and then the residue was dissolved in MeOH to yield 40 mg of a MeOH soluble fraction containing the crude saponin mixture. Thin-layer chromatography (TLC) on SiO_2 with *n*-BuOH/HOAc/H_2O (12:3:5) for development was used. Spots were visualized with cerium sulfate in 2 N H_2SO_4. The *n*-BuOH extract (212 mg) was purified by HPLC on a 30 cm × 3.9 mm i.d. C_{18} column μ-Bondapak with MeOH/H_2O (6:4) as eluant, flow rate = 2 mL/min, to afford furostanol saponins in this order: **2**, **1**, fraction A, **4**, capsianoside II (**10**), fraction B, and **9**. Fractions A and B were mixtures and were next separated by HPLC under the above conditions to give saponins **7** and **5** (fraction A) and **8** and **6** (fraction B). The crude saponin mixture from MeOH (40 mg), submitted to the HPLC separation under the above conditions, furnished mainly saponins **3**, **7**, and **5** (retention times = 5.2, 8.8, and 9.6 min, respectively).

Capsicoside E (1): yield, 3.5 mg; $[\alpha]_D^{25}$ −57.4° (pyridine, *c* 0.2); ^{1}H and ^{13}C NMR (aglycon moiety), see **Table 1**; ^{1}H and ^{13}C NMR (oligosaccharide moiety), see **Table 2**; HRMSFAB, (*m*/*z*) $[M + Na]^+$ calcd. for $C_{64}H_{106}O_{35}$ 1457.6412, found 1457.6423.

Capsicoside F (2): yield, 2.2 mg; $[\alpha]_D^{25}$ −42.0° (pyridine, *c* 0.07); ^{1}H and ^{13}C NMR (aglycon moiety), see **Table 1**; ^{1}H and ^{13}C NMR (oligosaccharide moiety), see **Table 2**; HRMSFAB, (*m*/*z*) $[M + Na]^+$ calcd. for $C_{63}H_{104}O_{34}$ 1427.6307, found 1427.6326.

Capsicoside A (3): yield, 10.5 mg; $[\alpha]_D^{25}$ −34.4° (pyridine, *c* 1); ^{1}H NMR ($C_5D_5N + D_2O$) (aglycon moiety) δ_H 3.62 (1-H, m, $H_{2'}$-26), 1.33 (3H, d, J = 6.8 Hz, H_3-21), 0.99 (3H, d, J = 6.8 Hz, H_3-27), 0.83 (3H, s, H_3-18), 0.66 (3H, s, H_3-19), 0.52 (1H, m, H-9); ^{13}C NMR ($C_5D_5N + H_2O$) (aglycon moiety) δ_C 44.1 (C-1), 69.5 (C-2), 82.8 (C-3), 32.4 (C-4), 43.3 (C-5), 27.0 (C-6), 31.0 (C-7), 33.3 (C-8), 53.1 (C-9), 35.7 (C-10), 20.3 (C-11), 38.8 (C-12), 40.1 (C-13), 55.0 (C-14), 31.0 (C-15), 80.2 (C-16), 62.2 (C-17), 15.1 (C-18), 12.2 (C-19), 40.0 (C-20), 15.6 (C-21), 110.0 (C-22), 35.6 (C-23), 26.8 (C-24), 33.0 (C-25), 74.5 (C-26), 16.2 (C-27); ^{1}H NMR (anomeric protons) δ_H 5.59, 5.33, 5.16, 5.11, 4.96, 4.82; remaining signals and ^{13}C NMR (oligosaccharide moiety) are virtually identical to those reported for **2**; ESI-MS (positive ion), *m*/*z* 1445 $[M + Na]^+$, *m*/*z* 1283 $[1445 - 162]^+$, and *m*/*z* 1121 $[1283 - 162]^+$.

22-O-Methylcapsicoside A (4): yield, 15.0 mg; $[\alpha]_D^{25}$ −49.7°-(pyridine, *c* 1); ^{1}H and ^{13}C NMR (aglycon moiety), see **Table 1**; ^{1}H and ^{13}C NMR (oligosaccharide moiety), virtually identical to those reported for **2**.

Capsicoside G (5): yield, 5.3 mg; $[\alpha]_D^{25}$ −55.2° (pyridine, *c* 0.5); ^{1}H and ^{13}C NMR (aglycon moiety), see **Table 1**; ^{1}H and ^{13}C NMR (oligosaccharide moiety), virtually identical to those reported for **2**; HRMSFAB, (*m*/*z*) $[M + Na]^+$ calcd. for $C_{63}H_{106}O_{34}$ 1429.6463, found 1429.6485.

22-O-Methylcapsicoside G (6): yield, 6.6 mg; $[\alpha]_D^{25}$ −59.3° (pyridine, *c* 0.4); ^{1}H ($C_5D_5N + D_2O$) (aglycon moiety) δ_H 3.64 (1-H, m, $H_{2'}$-26), 3.32 (−OMe), 1.21 (3H, d, J = 6.8 Hz, H_3-21), 1.03 (3H, d, J = 6.8 Hz, H_3-27), 0.81 (3H, s, H_3-18), 0.66 (3H, s, H_3-19), 0.51 (1H, m, H-9); ^{13}C NMR ($C_5D_5N + H_2O$) (aglycon moiety) δ_C 36.0 (C-1), 28.7 (C-2), 79.6 (C-3), 33.6 (C-4), 43.6 (C-5), 27.1 (C-6), 31.2 (C-7), 33.1 (C-8), 53.3 (C-9), 42.9 (C-10), 20.2 (C-11), 38.9 (C-12), 40.0 (C-13), 55.2 (C-14), 31.0 (C-15), 80.5 (C-16), 63.1 (C-17), 15.2 (C-18), 11.2 (C-19), 39.3 (C-20), 15.5 (C-21), 112.0 (C-22), 29.6 (C-23), 27.7 (C-24), 34.1 (C-25), 74.2 (C-26), 16.0 (C-27), 46.5 (OMe); ^{1}H and ^{13}C NMR (oligosaccharide moiety), virtually identical to those reported for **2** and **4** (**Table 2**).

Capsicoside D (7): yield, 8.3 mg; $[\alpha]_D^{25}$ −44.0° (pyridine, *c* 0.5); ^{1}H ($C_5D_5N + D_2O$) (aglycon moiety) δ_H 3.63 (1-H, m, $H_{2'}$-26), 1.32 (3H, d, J = 6.8 Hz, H_3-21), 0.99 (3H, d, J = 6.8 Hz, H_3-27), 0.81 (3H, s, H_3-18), 0.66 (3H, s, H_3-19), 0.54 (1H, m, H-9); ^{13}C NMR ($C_5D_5N + H_2O$) virtually identical to those reported for **5**; ^{1}H and ^{13}C NMR (oligosaccharide moiety), see **Table 2** (**S2**); ESI-MS (positive ion), *m*/*z* 1399 $[M + Na]^+$, *m*/*z* 1237 $[M + Na - 162]^+$, and *m*/*z* 1075 $[1237 - 162]^+$.

22-O-Methyl-capsicoside D (8) (sativoside R1): yield, 4.4 mg; $[\alpha]_D^{25}$ −57.7° (pyridine, *c* 0.3); ^{1}H and ^{13}C NMR (aglycon moiety), virtually identical to those reported for **6**; ^{1}H and ^{13}C NMR (oligosaccharide

Table 2. ^{1}H and ^{13}C NMR Shifts ($C_5D_5N + H_2O$) of Oligosaccharide Moiety for Saponins **1** and **2** and Sugar Chains **S2** and **S3**

	capsicoside E (**1**)		capsicoside F (**2**)			**S2**[a]		**S3**[b]	
	δ_H	δ_C	δ_H	δ_C		δ_H	δ_C	δ_H	δ_C
Gal					Gal				
1	4.96 (7.8)	101.6	4.96 (7.8)	102.0	1	4.94 (7.8)	101.8	4.97 (7.9)	101.4
2	4.56	71.6	4.56	71.8	2	4.42	71.9	4.42	72.1
3	4.17	74.1	4.17	74.2	3	4.15	74.0	4.16	74.1
4	4.55	78.8	4.55	78.9	4	4.59	79.0	4.57	78.9
5	3.80	76.4	3.80	76.3	5	3.78	76.4	3.79	76.2
6	4.54, 4.32	60.2	4.54, 4.32	60.3	6	4.68, 4.26	59.8	4.60, 4.27	60.0
Glc I					Glc I				
1	5.10 (8.0)	102.9	5.12 (8.0)	103.3	1	5.11 (7.6)	103.6	5.14 (7.9)	103.6
2	4.26	86.7	4.26	87.0	2	4.26	86.6	4.25	86.5
3	4.32	80.4	4.32	79.9	3	4.31	80.0	4.32	79.9
4	3.95	69.1	3.95	69.5	4	3.94	69.5	3.91	69.7
5	3.84	76.5	3.84	76.9	5	3.83	76.9	3.84	76.3
6	4.44, 4.36	61.6	4.44, 4.36	61.8	6	4.43, 4.24	62.9	4.44, 4.23	61.3
Glc II					Glc II				
1	5.32 (7.8)	102.3	5.35 (7.8)	102.3	1	5.33 (7.8)	103.4	5.56 (7.8)	103.7
2	4.05	73.3	4.05	73.3	2	4.06	73.8	4.05	74.0
3	4.23	86.8	4.23	86.9	3	4.22	86.5	4.23	77.3
4	3.98	68.9	3.98	69.0	4	3.97	68.0	4.21	70.7
5	3.98	76.8	3.95	76.9	5	3.95	76.8	3.96	76.7
6	4.20, 3.98	61.2	4.20, 3.98	61.2	6	4.18, 3.98	61.5	4.34, 4.00	61.7
Glc III					Xyl				
1	5.14 (7.8)	103.7	5.16 (7.8)	104.1	1	5.21 (8.0)	103.7	5.18 (7.8)	103.5
2	4.02	74.7	4.02	74.9	2	3.95	74.2	3.93	74.3
3	4.28	77.3	4.28	77.5	3	3.98	76.5	4.00	77.0
4	4.21	70.3	4.21	70.3	4	3.69	70.0	3.65	70.2
5	3.97	77.5	3.97	77.5	5	4.22, 3.67	65.8	4.21, 3.67	65.9
6	4.44, 4.20	61.5	4.44, 4.20	61.6					
Glc IV					Glc III				
1	5.59 (7.8)	103.7	5.59 (7.8)	103.5	1	5.16 (8.0)	104.0		
2	4.06	74.8	4.06	74.8	2	4.03	74.2		
3	4.30	77.5	4.30	77.5	3	4.25	76.9		
4	4.23	70.8	4.23	70.8	4	4.19	70.3		
5	3.93	77.6	3.93	77.5	5	3.97	76.8		
6	4.40, 4.18	61.6	4.42, 4.20	61.6	6	4.39, 4.20	61.5		
C-26 Glc					C-26 Glc				
1	4.90 (7.8)	103.6	4.86 (7.8)	103.8	1	4.84 (7.8)	104.1	4.84 (7.8)	103.7
2	4.06	74.6	4.07	74.5	2	4.04	74.4	4.03	74.4
3	4.28	77.0	4.31	77.5	3	4.30	77.1	4.31	77.0
4	4.23	70.5	4.21	70.7	4	4.20	70.5	4.22	70.7
5	3.98	77.0	3.99	77.3	5	3.98	77.1	3.99	77.4
6	4.36, 4.00	61.4	4.36, 3.99	61.7	6	4.36, 3.98	61.5	4.36, 3.99	61.7

[a] Data extracted from the spectrum of capsicoside D (**7**). [b] Data extracted from the spectrum of timosaponin I2 (**9**).

moiety), virtually identical to those reported for **7** (**Table 2**, **S2**); ESI-MS (positive ion), *m/z* 1413 $[M + Na]^+$, *m/z* 1251 $[M + Na - 162]^+$, *m/z* 1089 $[1251 - 162]^+$.

*Timosaponin I2 (**9**):* yield, 3.2 mg; $[\alpha]_D^{25}$ −52.5° (pyridine, *c* 0.1); ^{1}H ($C_5D_5N + D_2O$) (aglycon moiety) δ_H 3.62 (1-H, m, $H_{2'}$-26), 3.32 (−OMe), 1.20 (3H, d, $J = 6.8$ Hz, H_3-21), 1.01 (3H, d, $J = 6.8$ Hz, H_3-27), 0.79 (3H, s, H_3-18), 0.64 (3H, s, H_3-19), 0.50 (1H, m, H-9); ^{13}C NMR ($C_5D_5N + D_2O$, aglycon moiety), identical to those reported for **8**; ^{1}H and ^{13}C NMR (sugars), see **Table 2** (**S3**); ESI-MS (positive ion), *m/z* 1251 $[M + Na]^+$, *m/z* 1089 $[1251 - 162]^+$.

*Capsianoside II (**10**):* yield, 2.3 mg. Compound **10** (*9*) was identified on the basis of spectroscopic data compared with data of an authentic sample.

Methanolysis and Sugar Analysis. A solution of **1**−**9** (each 0.5 mg) in anhydrous 2 M HCl/MeOH (0.5 mL) was heated at 80 °C in a stoppered reaction vial for 8 h. Once cool, the reaction mixture was neutralized with Ag_2CO_3 and centrifuged, and the supernatant was evaporated to dryness under N_2. The residue was trimethylsilylated with 1-(trimethylsilyl)imidazole and pyridine (1:1) for 15 min at 70 °C. GLC analysis gave peaks that coeluted with those of silylated methyl glucoside, methyl galactoside, and methyl xyloside.

*Enzymatic Hydrolysis of Capsicoside G (**5**) To Give **5a**.* Compound **5** (3.5 mg) in a citrate buffer (1 mL; pH 4.5) was incubated with a glycosidase mixture (8.0 mg) of *C. lampas* (Shikagaku Kogyo) at 37 °C. After 3 h, TLC analysis showed that the starting material had disappeared and displayed 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 to give compound **5a**: ESI-MS (positive ion), *m/z* 1249 $[M + Na]^+$, *m/z* 1087 $[1249 - 162]^+$, *m/z* 925 $[1087 - 162]^+$; ^{1}H ($C_5D_5N + D_2O$) (aglycon) δ_H 0.64 (3H, s, H_3-18), 0.70 (3H, d, $J = 6.8$ Hz, H_3-27), 0.86 (3H, s, H_3-19), 1.13 (3H, d, $J = 6.8$ Hz, H_3-21); ^{1}H NMR (anomeric protons) δ_H 5.57, 5.27, 5.15, 5.05, 4.94; ^{13}C NMR ($C_5D_5N + H_2O$) (aglycon) δ_C 36.0 (C-1), 30.0 (C-2), 79.5 (C-3), 34.0 (C-4), 43.7 (C-5), 27.1 (C-6), 31.3 (C-7), 33.2 (C-8), 53.5 (C-9), 42.9 (C-10), 20.4 (C-11), 39.1 (C-12), 40.2 (C-13), 55.4 (C-14), 31.2 (C-15), 80.4 (C-16), 62.5 (C-17), 15.3 (C-18), 12.0 (C-19), 42.0 (C-20), 14.7 (C-21), 109.3 (C-22), 32.1 (C-23), 29.2 (C-24), 30.7 (C-25), 67.1 (C-26), 17.2 (C-27); ^{13}C NMR (anomeric carbons) δ_C 104.1, 103.8, 103.3, 103.4, 104.0.

Antimicrobial Assay. Inhibitory activity against each microorganism adopted (ATCC, American Type Culture Collection, Rockville, MD; CBS, Centraalbureau voor Schimmelcultures, Baarn, Delft, The Netherland; DMS, Deutsche Sammlung von Mikroorganismen und Zellkuturen GmbH, Braunschweig, Germany; DIPROVAL, Dip. di Protezione e Valorizzazione Agroalimentare, Università di Bologna, Italy) was determined using an agar dilution method. Briefly, 0.5 mL of a serial 2-fold dilution of test samples was mixed with 9.5 mL of culture medium. The culture media used were standard plate count agar (PCA) medium (Oxoid) for bacteria, yeast peptone dextrose medium (YPDA),

and malt agar medium (Oxoid) for yeasts and fungi, respectively. Each culture medium containing a test sample was transferred into a Petri dish, onto which each microorganism was streaked. After an adequate incubation period (36 and 48 h, 28 °C), the inhibitory activity of the sample was assessed as the minimum inhibitory concentration (MIC), the lowest concentration tested at which no growth was observed. A microbiology computerized reader−analyzer able to perform the growth (turbidity development) of up to 200 cultures simultaneously by recording of growth curves parameters and MIC responses (Bioscreen C, Labsystems, Helsinki, Finland) was used, and the results were confirmed.

RESULTS AND DISCUSSION

The *n*-butanol extracts and the soluble MeOH fraction of *C. annuum* L. var *acuminatum* were repeatedly chromatographed by HPLC to furnish nine furostanol saponins (**1**−**9**) and the known capsianoside II (**10**), also found in the ripe fruits (*7*, *9*).

Saponins named capsicoside E (**1**), capsicoside F (**2**), and capsicoside G (**5**) are new compounds and are the minor components obtained from the polar mixture. By extensive 1D- and 2D-NMR experiments, capsicoside A (**3**) and capsicoside D (**7**), previously isolated from seeds of *C. annuum* var. *conides* and var. *fasciculatum* (*10*), and the corresponding 22-*O*-methyl capsicoside D (**8**), present in bulbs, roots, and leaves of *Allium sativum* L. (*14*), were also identified. Compound **9** was identified as timosaponin I2 (*15*), spectral data being consistent with the indicated literature values. Saponins **4** and **6** are 22-*O*-methyl derivatives of capsicosides A (**3**) and G (**5**), respectively, and could be considered as secondary products formed from the corresponding 22-hydroxyfurostanosides during the extraction of the plant material with methanol (*16*, *17*).

Capsicoside E (**1**) was isolated as a white amorphous solid and showed in the positive ESI-MS spectrum a pseudomolecular ion peak at *m*/*z* 1457 $[M + Na]^+$ and fragment ion peaks at *m*/*z* 1295 $[1457 - 162]^+$ and *m*/*z* 1133 $[1295 - 162]^+$. The molecular formula $C_{64}H_{106}O_{35}$, established by HRMSFAB, was supported by ^1H and ^{13}C NMR spectra (**Tables 1** and **2**). Compound **1** was deduced to possess a furostanol structure, assigned by combination of 1D- and 2D-NMR experiments and comparison with literature data (*10*, *18*).

The ^1H NMR of **1** in pyridine-d_5 + D_2O displayed signals due to three steroidal methyl groups at δ 0.66 (s, 19-CH_3), δ 0.76 (s, 18-CH_3), and δ 1.17 (d, J = 6.6 Hz, 21-CH_3). The fourth methyl signal was replaced by unsaturated downfield methylene protons at δ 5.06 and 5.36 (each broad singlet), suggesting the presence of a double bond between C-25 and C-27. A typical methoxy signal at δ 3.32 was also detectable in the ^1H NMR spectrum and was indicative of a 22-methoxy functionality. Detailed analysis of the ^{13}C NMR, with the aid of $^1H-^1H$ COSY, HMQC, and HMBC experiments, indicated the presence of a furost-25(27)-en-22-*O*-methyl-2,3,22,26-tetrahydroxy skeleton.

On acid methanolysis with 2 N HCl/MeOH, compound **1** afforded glucoside and galactoside in the molar ratio of 5:1 as estimated by GLC analysis. Among 64 carbon signals in the ^{13}C NMR spectrum, 28 signals were assigned to the aglycon; the remaining 36 signals were indicative of the presence of six hexoses, in good agreement with six anomeric signals appearing at δ 5.59 (J = 7.8 Hz), 5.32 (J = 7.8 Hz), 5.14 (J = 7.8 Hz), 5.10 (J = 8.0 Hz), 4.96 (J = 7.8 Hz), and 4.90 (J = 7.8 Hz). These correlated with the corresponding carbon signals at δ_C 103.7, 102.3, 103.7, 102.9, 101.6, and 103.6, respectively, in the HMQC experiment.

The 3,26-bisdesmoside structure of **1** and the interglycosidic linkages were characterized by an HMBC experiment. Specifically, long-range correlations were observed between the anomeric proton at δ 4.96 (d, J = 7.8 Hz, Gal H-1) with the carbon at 82.8 ppm (aglycon C-3) and the anomeric proton at δ 4.90 (d, J = 7.8 Hz, 26-Glc H-1) with the carbon at 71.6 ppm (aglycon C-26). The assignments of the anomeric proton of each sugar unit and the spin sequence were confirmed by $^1H-^1H$ COSY and TOCSY experiments. The sequence in the oligoglycoside chain was pursued by HMBC, which showed cross-peaks between H-1 of Glc I (δ 5.10) and C-4 Gal (78.8 ppm), between H-1 of Glc II (δ 5.32) and C-2 Glc I (86.7 ppm), between H-1 of Glc IV (δ 5.59) and C-3 Glc I (80.4 ppm), and between H-1 of Glc III (δ 5.14) and C-3 Glc II (86.8 ppm), thus clarifying the interglycosidic linkages, which were identical to those reported for the known capsicoside A (*10*). The 2α-hydroxy stereochemistry in the furostanol structure was characterized by a rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiment, which showed ROE correlations between H-2 (δ 3.98 m) and CH_3-19 (δ 0.66 s) and between H-9 (δ 0.51 br t) and H-5 (δ 0.98 m), confirming the A/B trans junction of steroidal nucleus. Finally, by comparison of the NMR data for **1** (**Tables 1** and **2**) with those of related furostanol saponins (*10*, *19*), the structure of capsicoside E was elucidated as 26-*O*-β-D-glucopyranosyl-22-*O*-methyl-5α-furost-25(27)-en-2α,3β,22ξ,26-tetraol-3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl(1→2)-[β-D-glucopyranosyl(1→3)]-β-D-glucopyranosyl(1→4)-β-D-galactopyranoside.

Capsicoside F (**2**) had a molecular formula of $C_{63}H_{104}O_{34}$ established by HRMSFAB as well as from analysis of its ^{13}C NMR and DEPT data. The ESI-MS showed a pseudomolecular ion peak at *m*/*z* 1427 $[M + Na]^+$ and fragment ion peaks at *m*/*z* 1265 $[M + Na - 162]^+$ and *m*/*z* 1103 $[1265 - 162]^+$. Methanolysis of **2** afforded methyl glucoside and methyl galactoside in the ratio of 5:1 by GLC analysis. A comparative analysis of ^1H and ^{13}C NMR and $^1H-^1H$ COSY spectra of **2** with those of capsicoside E (**1**) suggested the presence of the same oligosaccharide chain linked to C-3 and also revealed a β-glucose unit at C-26. We observed a good coincidence in the chemical shifts from C-1 to C-12 of the furostanol part, whereas significant differences were detectable for the data from C-13 to C-27. The ^1H NMR spectrum displayed signals due to four steroidal methyl groups at δ 0.68 (s, 19-CH_3), 0.70 (s, 18-CH_3), 1.01 (d, J = 6.8 Hz, 27-CH_3), and 1.62 (s, 21-CH_3). This last signal, downfield shifted, correlated in the HMQC experiment with the corresponding carbon signal at 11.5 ppm. In the ^{13}C NMR the trisubstituted double-bond carbon signals appeared at 103.3 and 152.0 ppm, suggesting the presence of an unsaturation between C-20 and C-22. These data were supported by an HMBC experiment that showed correlations for 21-CH_3 (δ 1.62)/C-20 (103.3 ppm) and for 17-H (δ 2.45)/C-21 (11.5 ppm). The HMBC also confirmed the 3,26-bisdesmoside structure of **2** and the interglycosidic linkages identical to those observed in capsicoside E (**1**) (**Tables 1** and **2**). The 25*R* stereochemistry in **2** was deduced from the resonances of protons and carbons in positions 25−27, by comparison with literature data (*20*−*22*), and by analogy with capsicoside G (**5**), which was submitted to enzymatic hydrolysis. Thus, capsicoside F (**2**) has been established as 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furost-20(22)-en-2α,3β,26-triol-3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl(1→2)-[β-D-glucopyranosyl(1→3)]-β-D-glucopyranosyl(1→4)-β-D-galactopyranoside.

Saponin (**4**) showed in the ESI-MS spectrum a pseudomolecular ion peak at *m*/*z* 1459 $[M + Na]^+$ and fragment ion peaks at *m*/*z* 1297 $[M + Na - 162]^+$, *m*/*z* 1135 $[1297-162]^+$, and *m*/*z* 973 $[1135 - 162]^+$. The ^1H NMR spectrum displayed

Table 3. Antiyeast Activity of Saponins **1**, **4**, and **6**

yeast, MIC (μg/mL)	**1**	**4**	**6**
Saccharomyces			
cerevisiae (Diproval 404)	50	50	25
cerevisiae (Diproval 6073)	50	50	25
cerevisiae (Diproval 2439)	50	50	25
cerevisiae (Diproval 8167)	50	50	25
cerevisiae (Diproval 7070)	50	50	25
cerevisiae (Diproval 9109)	50	50	25
Saccharomycoides			
ludwigii (Diproval 120)	50	50	12.5
Schizosaccharomyces			
pombe (Diproval 106)	100	50	25
Candida albicans (CBS 562)	25	50	25
Kloeckera apiculata (Distaam AP23)	25	50	12.5
Hanseniaspora			
osmophila (CBS 313)	100	50	25
vinae (CBS 2171)	50	25	12.5
uvarum (CBS 314)	25	25	12.5
guilliermondii (CBS 465)	50	25	12.5

typical signals of a 5α-furosta-2α,3β,26-trihydroxy moiety almost identical to those observed in capsicoside A (**3**). In addition, the proton spectrum revealed a methoxy signal at δ 3.32 associated with the characteristic carbon signals [δ_C 46.6 (−OMe) and δ_C 112.0 (C-22)] of a 22-*O*-methylfurostanol saponin. On acid methanolysis and GLC analysis, **4** afforded methyl galactoside and methyl glucoside in ratio of 5:1. ^{1}H−^{1}H COSY, HMQC, and HMBC experiments further confirmed the presence of the same oligosaccharide chain observed in capsicosides E (**1**), F (**2**), and A (**3**). Thus, the structure of **4** was assigned as 26-*O*-β-D-glucopyranosyl-(25*R*)-22-*O*-methyl-5α-furosta-2α,3β,22ξ,26-tetrarol-3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl(1→2)-[β-D-glucopyranosyl(1→3)]-β-D-glucopyranosyl(1→4)-β-D-galactopyranoside.

Capsicoside G (**5**) displayed in the HRMSFAB a quasimolecular ion peak at *m*/*z* 1429.6485 in accordance with the empirical formula $C_{63}H_{106}O_{34}$. Comparison of its ^{1}H and ^{13}C NMR spectral data with those of capsicoside A (**3**) showed that the structure of **5** was analogous to that of **3**, except for the loss of a hydroxyl group at C-2, which caused the large upfield shift of C-2 to δ 28.9 (69.5 in **3**) along with downfield shifts of C-1 (−8.3 ppm) and C-3 (−3.2 ppm). The lack of the hydroxyl group at C-2 was also apparent from the positive ESI-MS, which showed a pseudomolecular ion peak at *m*/*z* 1429 [M + Na]$^+$ and fragment ion peaks at *m*/*z* 1267 [M + Na − 162]$^+$, *m*/*z* 1105 [1297 − 162]$^+$, and *m*/*z* 943 [1135 − 162]$^+$. The stereochemistry at C-25 was pursued on the basis of NMR data obtained after enzymatic hydrolysis of capsicoside G. After incubation with a glycosidase mixture of *Charonia lampas*, capsicoside G liberated the corresponding monodesmoside **5a** with the loss of the terminal β-D-glucopyranose from the C-26 position. The ^{13}C chemical shifts of F ring part gave evidence for the 25*R* configuration as reported for tigogenin (*18*). The 2D-NMR experiments, including ^{1}H−^{1}H COSY, HMQC, and HMBC, further confirmed the structure of **5** as 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furosta-3β,22ξ,26-triol-3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl(1→4)-β-D-galactopyranoside.

Saponin **6** showed ^{1}H and ^{13}C spectral data similar to those of **5**, except for the presence of an additional methoxyl group at C-22 (−OMe δ_H 3.32, δ_C 46.5; C-22 δ_C 112.0). This evidence was confirmed by the positive ESI-MS, which displayed a pseudomolecular ion peak at *m*/*z* 1443 [M + Na]$^+$ and fragment ion peaks at *m*/*z* 1281 [M + Na − 162]$^+$ and *m*/*z* 1119 [1297 − 162]$^+$. The furostanol structure and the interglycosidic linkages in the oligosaccharide chain were mainly elucidated by 1D- and 2D-NMR experiments; therefore, **6** was identified as 26-*O*-β-D-glucopyranosyl-(25*R*)-22-*O*-methyl-5α-furosta-3β,22ξ,26-triol-3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl(1→3)]-β-D-glucopyranosyl(1→4)-β-D-galactopyranoside.

Antimicrobial Activity of the Saponin Fractions. The antiyeast and antifungal activities are reported in **Tables 3** and **4**, respectively. The pure saponins showed high antiyeast activity, whereas they exhibited less activity against common fungi. Yeast strains related to *Saccharomycoides*, *Kloeckera*, and *Hanseniaspora*, with the exclusion of *H. osmophila*, appeared to be more sensitive, probably due to their typical bipolar sporulation in comparison to other strains (*23*).

From the data reported, it was apparent that for the growth inhibition, the presence of the **S1** oligosaccharide chain, the methyl group at R_3, is essential. Saponin **6**, having a branched-chain pentasaccharide moiety (**S1**) without any oxygen functionality at C-2, exhibited the highest antiyeast activity (**Table 3**), which is also related to the presence of a methyl group at the R_3 position. In saponins **1** and **4**, which differ from **6** by the presence of a hydroxyl group at C-2, we observed a good antiyeast activity but less than that of **6**. The lack of the methyl group at R_3, as in capsicosides A (**3**) and G (**5**), produced a great reduction in activity at MIC > 100 μg/mL. When a different oligosaccharide moiety (**S2** or **S3**) is present, as in **7** and **9**, no inhibition was observed, probably due to the presence of a xylose, which replaced a glucose unit. Saponins **1**, **4**, and **6** also exhibited activity against *Candida albicans*, a pathogenic yeast that causes cutaneous candidiasis. The activity of pure saponins against common fungi (**Table 4**) was not so strong and was detectable only for compounds **1**, **4**, and **6** in the fungi *Phoma terrestris* and *Rhizopus orizea*. No inhibition was observed on *Aspergillus niger* (ATCC 1040), *Aspergillus flavus* (ATCC 11655), and *Aspergillus fumigatus* (ATCC 1028) (MIC > 1000 μg/mL). In our experimental conditions, capsianoside II (**10**) showed no activity in either assay.

The isolated saponins showed no or weak growth inhibition against both Gram-positive and Gram-negative bacteria. MIC determinations against Gram-positive bacteria: >1000 μg/mL (*Bacillus subtilis* DSM 345, *Bacillus licheniformis* ATCC 14580, *Staphylococcus epidermidis* DSM 1798, *Staphylococcus aureus* sub. *aureus* DSM 346, *Ent. faecalis* DSM 2570, *Mi. luteus* DSM 348) and 1000 μg/mL (*Bacillus cereus* DSM 345, *Lact. rhamnosus* DSM 20021, *Lact. plantarum* DSM 20174, *Lact. casei* DSM 20011, *Lact. fermentum* DSM 20052). MIC against Gram-negative bacteria: >1000 μg/mL (*Ps. aeruginosa* DSM 1117; *Ps. stutzeri* ATCC 962) and 1000 μg/mL (*Escherichia coli* ATCC 35346, *E. coli* DSM 682).

Table 4. Antifungal Activity of Saponins **1**, **3**–**7**, **9**, and **10**

fungus, MIC (μg/mL)	**1**	**3**	**4**	**5**	**6**	**7**	**9**	**10**
Penicillium expansum (ATCC 7861)	1000	1000	1000	1000	500	>1000	>1000	1000
Phoma terrestris (ATCC 11321)	125	1000	125	1000	250	1000	1000	1000
Rhizopus orizea (ATCC 56536)	125	1000	125	1000	125	1000	1000	1000
Trichoderma viride (ATCC 28020)	500	>1000	250	1000	250	>1000	>1000	1000

The extract and pure saponins are testeless, colorless, and odorless, exerting no influence on the quality of foods, but it is difficult to use saponin fractions as a food ingredient without long-term toxicity tests because we have no history of these compounds as foodstuffs. On the basis of the results obtained, we believe that the new saponins fraction could be used as an ingredient in foods, beverages, cosmetics, and pharmaceuticals only after adequate safety and long-term toxicity tests.

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