Constituents of *Cydonia vulgaris*: Isolation and Structure Elucidation of Four New Flavonol Glycosides and Nine New α-Ionol-Derived Glycosides

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In a previous paper, we described the isolation of four new sesterterpenes from *Cydonia vulgaris* Pers. Here we report the isolation of four new flavonol glycosides (**1**−**4**) and nine new α-ionol-derived glycosides (**5**−**13**) together with the known 3-oxo-α-ionol 9-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**14**), vomifoliol 9-*O*-*β*-D-glucopyranoside (roseoside) (**15**), and vomifoliol 9-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside (**16**) from the MeOH extract of the aerial parts of *C. vulgaris* Pers. (Rosaceae). All structures were elucidated by spectroscopic methods, including the concerted application of one-dimensional NMR and two-dimensional NMR techniques (COSY-90 and HETCOR).

Keywords: *Cydonia vulgaris; Rosaceae; flavonol glycosides; α-ionol glycosides; ^1H and ^{13}C NMR (one- and two-dimensional)*

INTRODUCTION

Interest in edible plants as a source of natural antioxidant prompted us to investigate phenolic constituents of *Cydonia vulgaris*, a small tree collected in Southern Italy whose fruit is traditionally used to prepare jams.

In the course of our study, four new flavonol glycosides (**1**−**4**) and twelve α-ionol-derived glycosides (**5**−**16**), among which nine were never reported (**5**−**13**), were isolated from the MeOH extract of the aerial parts of *C. vulgaris*.

Ionol derivatives are highly potent C_{13} norisoprenoids, aroma compounds widely distributed in fruits and vegetables (Williams et al., 1993), and most notably in the leaf products tea and tobacco (Winterhalter, 1992). The formation of these compounds has been attributed to the degradation of higher molecular weight terpenoids such as carotenoids during the curing and aging process (Wahlberg et al., 1977).

In a previous paper, we described the isolation of four new sesterterpenes from the 9:1 $CHCl_3$−MeOH extract of the leaves of *C. vulgaris*. (De Tommasi et al., 1996). Here we report the isolation and structure elucidation of **1**−**16** by chemical and spectroscopic methods.

EXPERIMENTAL PROCEDURES

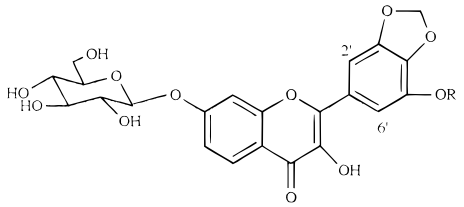
Material. The aerial parts of *C. vulgaris* Pers. were collected in April 1991 in Benevento, Southern Italy, and identified by Dr. V. De Feo, Università degli Studi di Salerno. A voucher sample is deposited at the herbarium of Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli.

Apparatus. The FABMS spectra, in negative ion mode, were obtained by dissolving the samples in a glycerol−thioglycerol matrix and placing them on a copper probe tip prior to bombardment with Ar atoms with an energy of 2−6 kV.

The NMR spectra were obtained in CD_3OD using a Bruker WM-250 Spectroscopin or Bruker AMX-500 spectrometer. The COSY experiments were carried out by employing the conventional pulse sequence. The HETCOR experiments were performed on a 1024 × 512 data matrix, using a CH coupling of 135 Hz and relaxation delay of 1.5 s. The data matrix was processed using a q sine window function.

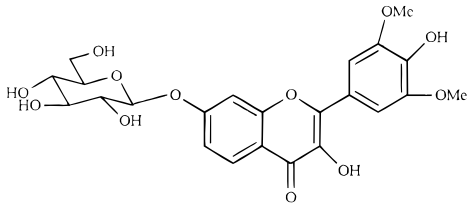
Droplet counter current chromatography (DCCC) was performed on a Buchi apparatus equipped with 300 tubes. GLC

Chart 1



1 R = CH_3

2 R = H



3

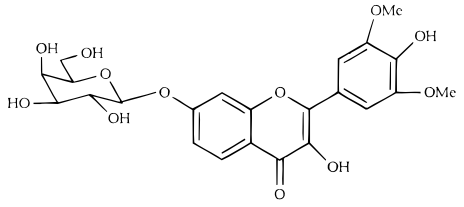


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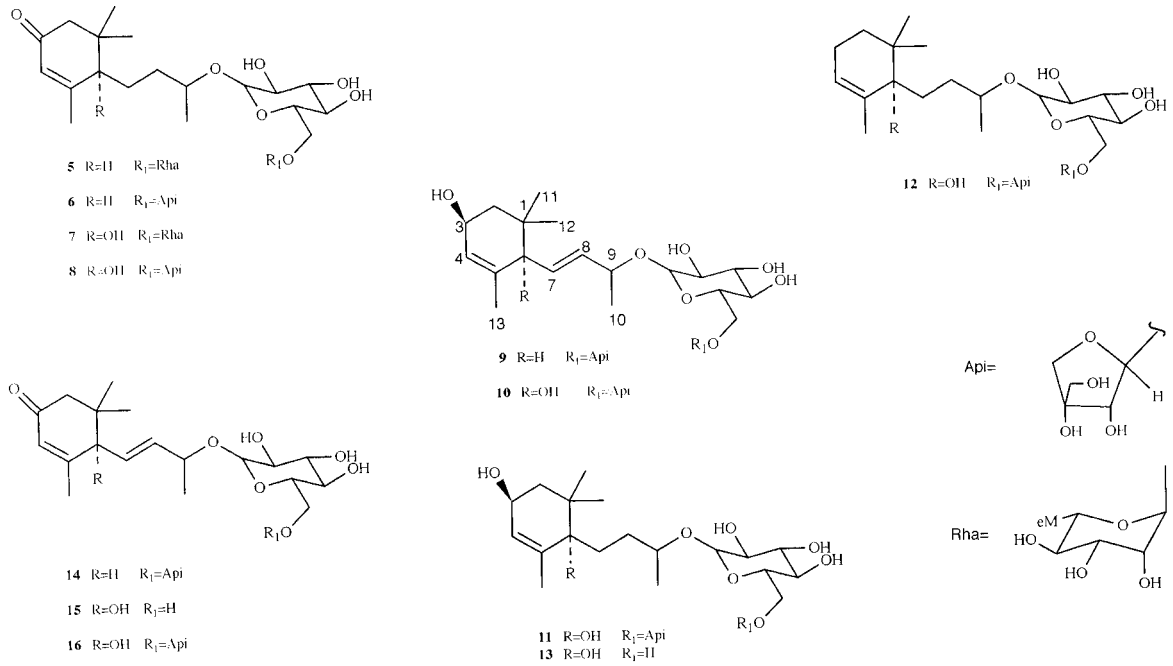
Chart 2



analyses were performed on a Supelco SP200 capillary column (30 m, 0.32 mm i.d., 0.25 mm film thickness, He as carrier gas, 5 mL/min, 156 °C).

HPLC separations were performed on a Waters 590 series pumping system with a Waters R401 refractive index detector equipped with a Waters μ-Bondapak C18 column.

Extraction and Isolation. The air-dried leaves (300 g) of *C. vulgaris* were defatted with petroleum ether and $CHCl_3$ and extracted successively with 9:1 $CHCl_3$−MeOH and MeOH to give 16 g of residue. The MeOH extract was partitioned between *n*-BuOH and H_2O to afford an *n*-BuOH soluble portion (10 g) which was in part (4 g) chromatographed on a Sephadex LH-20 column (100 × 5 cm) with MeOH as eluent. Fractions (9 mL) were collected and checked by TLC [Sigel plates in *n*-BuOH−HOAc−H_2O (60:15:25)]. Fractions 18−27 (800 mg) containing the crude ionol glycoside mixture were purified by DCCC [*n*-BuOH−Me_2CO−H_2O (60:18:22), ascending mode, the lower phase was the stationary phase] to give A (180 mg), B (270 mg), and C (310 mg) which were submitted to HPLC on a C18 μ-Bondapak column using MeOH−H_2O as eluent in a 2:3 ratio for A (flow rate of 2 mL/min) and a 1:1 ratio for B and C (flow rate of 1.5 mL/min). Compounds **14** (13 mg, t_R = 6.5 min), **15** (6 mg, t_R = 10 min), and **16** (24 mg, t_R = 5.5 min) were obtained from A. **5** (4.7 mg, t_R = 7 min), **6** (5.9 mg, t_R = 8 min), **7** (10.9 mg, t_R = 2 min), and **8** (12.6 mg, t_R = 4 min) were from B; **9** (14 mg, t_R = 7.5 min), **10** (11 mg, t_R = 5 min), **11** (13 mg, t_R = 9 min), **12** (15 mg, t_R = 13 min), and **13** (19 mg, t_R = 14.5 min) were from C. Fractions 60−77 (120 mg) from Sephadex were purified by HPLC on a C18 μ-Bondapak column using 2:3 MeOH−H_2O as eluent (flow rate of 2 mL/min) to give **1** (5 mg, t_R = 14 min), **2** (4 mg, t_R = 8 min), **3** (14 mg, t_R = 10 min) and **4** (10.8 mg, t_R = 10.5 min).

Methanolysis of Compounds 5−16: **Carbohydrate Constituents.** A solution of each compound (2 mg) in anhydrous 2 N HCl−MeOH (0.5 mL) was heated at 80 °C in a stoppered reaction vial for 12 h. After cooling, the solution was neutralized with Ag_2CO_3 and centrifuged. The supernatant was evaporated to dryness under N_2. The residue was reacted with TRISIL-Z (Pierce) and analyzed by GLC. Retention times were identical to those of the authentic methyl sugars.

Compound 1: $C_{23}H_{22}O_{12}$; FABMS *m/z* [M − H]$^-$ 489, [(M − H) − 178]$^-$ 311, [(M − H) − (178 + 15)]$^-$ 296; see Table 1 for ^{13}C NMR and ^{1}H NMR.

Compound 2: $C_{22}H_{20}O_{12}$; FABMS *m/z* [M − H]$^-$ 475, [(M − H) − 178]$^-$ 297; ^{13}C NMR aglycon signals δ 158.2 (C-2) 134.7 (C-3), 178.1 (C-4), 124.0 (C-5), 114.3 (C-6), 159.2 (C-7), 98.0 (C-8), 159.2 (C-9), 116.2 (C-10), 121.9 (C-1′), 100.1 (C-2′), 149.2 (C-3′), 133.1 (C-4′), 143.2 (C-5′), 106.2 (C-6′), 102.5 (OCH_2O); ^{1}H NMR aglycon signals δ 7.14 (1H, dd, *J* = 2 and 8 Hz, H-6), 7.27 (1H, d, *J* = 8 Hz, H-8), 7.50 (1H, d, *J* = 2 Hz, H-2′), 7.53 (1H, d, *J* = 2 Hz, H-6′), 7.86 (1H, d, *J* = 2 Hz, H-5), 5.80 (2H, s, OCH_2O).

Compound 3: $C_{23}H_{24}O_{12}$; FABMS *m/z* [M − H]$^-$ 491, [(M − H) − 178]$^-$ 313, [(M − H) − (178 + 15 × 2)]$^-$ 283; see Table 1 for ^{13}C NMR and ^{1}H NMR.

Compound 4: $C_{23}H_{24}O_{12}$; FABMS *m/z* [M − H]$^-$ 491, [(M − H) − 178]$^-$ 313, [(M − H) − (178 + 15 × 2)]$^-$ 283; ^{13}C NMR sugar signals δ 101.6 (C-1″), 72.8 (C-2″), 75.1 (C-3″), 70.2 (C-4″), 77.0 (C-5″), 62.3 (C-6″); ^{1}H NMR sugar signals δ 5.00 (1H, d, *J* = 7.5 Hz, H-1″), 4.00 (1H, dd, *J* = 3.0 and 12.0 Hz, H-6″a), 3.80 (1H, dd, *J* = 5.0 and 12.0 Hz, H-6″b), 3.54 (1H, dd, *J* = 4 and 7.5 Hz, H-3″).

Compound 5: $C_{25}H_{42}O_{11}$; FABMS *m/z* [M − H]$^-$ 517, [(M − H) − 146]$^-$ 371, [(M − H) − 162]$^-$ 355, [(M - H) − (146 + 162]$^-$ 209; see Table 3 for ^{13}C NMR aglycon signals; see Table 2 for ^{1}H NMR aglycon signals; see Table 4 for ^{13}C NMR and ^{1}H NMR sugar signals.

Compound 6: $C_{24}H_{40}0_{11}$; FABMS *m/z* [M − H]$^-$ 503, [(M − H)-132]$^-$ 371, [(M − H) − (132 + 162)]$^-$ 209; see Table 4 for ^{13}C NMR and ^{1}H NMR sugar signals.

Compound 7: $C_{25}H_{42}O_{12}$; FABMS *m/z* [M − H]$^-$ 533, [(M − H) − 146]$^-$ 387, [(M − H) − (146 + 162)]$^-$ 225; see Table 3 for ^{13}C NMR aglycon signals; see Table 2 for ^{1}H NMR aglycon signals.

Compound 8: $C_{24}H_{40}O_{12}$; FABMS *m/z* [M − H]$^-$ 519, [(M − H) − 132]$^-$ 387, [(M − H) − (132 + 162)]$^-$ 225.

Compound 9: $C_{24}H_{40}O_{11}$; FABMS *m/z* [M − H]$^-$ 503; see Table 3 for ^{13}C NMR aglycon signals; see Table 2 for ^{1}H NMR aglycon signals; see Table 4 for ^{13}C NMR and ^{1}H NMR sugar signals

Compound 10: $C_{24}H_{40}O_{12}$; FABMS *m/z* [M − H]$^-$ 519; see Table 3 for ^{13}C NMR aglycon signals; see Table 2 for ^{1}H NMR aglycon signals.

Compound 11: $C_{24}H_{42}O_{12}$; FABMS *m/z* [M − H]$^-$ 521, [(M − H) − 132]$^-$ 389, [(M − H) − (132 + 162)]$^-$ 227; see Table 3 for ^{13}C NMR aglycon signals; see Table 2 for ^{1}H NMR aglycon signals.

Compound 12: $C_{24}H_{42}O_{11}$; FABMS *m/z* [M − H]$^-$ 505, [(M − H) − 132]$^-$ 373, [(M − H) − (132 + 162)]$^-$ 211; see Table 3 for ^{13}C NMR aglycon signals; see Table 2 for ^{1}H NMR aglycon signals.

Table 1. ^{13}C and ^{1}H NMR Data of Compounds 1 and 3 in CD_3OD

	1		3	
position	δ_C	δ_H	δ_C	δ_H
2	158.2	−	158.2	−
3	134.6	−	134.6	−
4	178.0	−	179.0	−
5	124.0	7.87, d, J = 8.0 Hz	121.0	7.84 d, J = 8 Hz
6	114.0	7.14, dd, J = 2.0 and 8.0 Hz	114.0	7.14, dd, J = 2.0 and 8.0 Hz
7	159.0	−	159.0	−
8	98.0	7.26, d, J = 2.0 Hz	98.8	7.24, d, J = 2.0 Hz
9	159.0	−	158.8	−
10	116.0	−	116.2	−
1′	121.0	−	121.1	−
2′	100.1	7.60, d, J = 2.0 Hz	103.9	7.26 d, J = 2.0 Hz
3′	148.0	−	144.9	−
4′	131.0	−	136.1	−
5′	145.5	−	147.5	−
6′	105.1	7.62, d, J = 2.0 Hz	103.9	7.26, d, J = 2.0 Hz
1″	101.3	5.03,d, J = 7.2 Hz	101.3	5.05, d, J = 7.2 Hz
2″	74.8	*a*	74.7	*a*
3″	78.1	*a*	77.9	*a*
4″	70.9	*a*	70.6	*a*
5″	78.2	*a*	78.0	*a*
6″	62.8	3.70, dd, J = 5.0 and 12 Hz 3.90, dd, J = 3.5 and 12 Hz	62.6	3.72, dd, J = 5.0 and 12.0 Hz 3.91, dd, J = 3.5 and 12.0 Hz
OCH_2O	102.5	5.80, s	−	−
OMe	57.4	3.87, s	57.1	3.9, s
OMe	−	−	57.1	3.9, s

[a] Submerged by the solvent signal.

Compound 13: $C_{19}H_{34}O_8$; FABMS m/z $[M - H]^-$ 389, $[(M - H) - 162]^-$ 227; ^{13}C NMR sugar signals δ 104.5 (C-1′), 75.4 (C-2′), 78.6 (C-3′), 71.9 (C-4′), 78.2 (C-5′), 62.8 (C-6′); ^{1}H NMR sugar signals δ 4.52 (1H, d, J = 7.5 Hz, H-1′), 3.60 (1H, dd, J = 5.0 and 12.0 Hz, H-6′a), 3.80 (1H, dd, J = 3.5 and 12.0 Hz, H-6′b).

Compound 14: $C_{24}H_{38}O_{11}$; FABMS m/z $[M - H]^-$ 501.

Compound 15: $C_{19}H_{30}O_8$; FABMS m/z $[M - H]^-$ 385.

Compound 16: $C_{24}H_{38}O_{12}$; FABMS m/z $[M - H]^-$ 517.

Further NMR data have been given as supporting information.

RESULTS AND DISCUSSION

Compound **1** has a molecular formula of $C_{23}H_{22}O_{12}$, as suggested by ^{13}C, ^{13}C DEPT NMR data, and FABMS analysis in negative ion mode which showed a quasi-molecular anion $[(M - H)^-]$ at m/z 489 and a prominent fragment at m/z 311 indicating the loss of a hexose unit. The ^{13}C NMR data (Table 1) indicated for the aglycon moiety of **1** a flavonol structure with an unusual substitution pattern. The ^{1}H NMR spectrum (Table 1) exhibited a signal at δ_H 5.03 (d, J = 7.2 Hz), adapting well to an anomeric proton of a β-D-glucopyranose unit linked to a phenolic function. Further features were signals at δ_H 7.14 (dd, J = 8 and 2 Hz), 7.26 (d, J = 2 Hz), and 7.87 (d, J = 8 Hz), suggesting the occurrence of only one hydroxyl group at C-7 (Shirataki et al., 1986) and a pair of meta-coupled ^{1}H doublets at δ_H 7.60 (J = 2 Hz) and 7.62 (J = 2 Hz), in agreement with a 3′,4′,5′ trisubstitution pattern. Two singlets at δ_H 3.87 (3H, s) and 5.80 (2H, s) indicated, respectively, the presence of a methoxy group and a methylenedioxy function whose locations were established at C-5′ and C-3′−C-4′ on the basis of the ^{13}C NMR values for ring B (Ma et al., 1991). The attachment of the β-D-glucopyranosyl unit at C-7 of the aglycon moiety was deduced from the resonances of H-6 and H-8, shifted downfield (about +0.25 ppm)

Table 2. ^{1}H NMR Data of the Aglycon Moieties of Compounds 5, 7, and 9−12[a]

position	5	7	9	10	11	12
2	2.15, d, J=16.0	2.20, d, J = 17.0	1.55, dd, J = 16.0, 2.0	1.55, dd, J = 17.0, 2.0	1.65, dd J = 17.0, 4.5	1.45, m
	2.52, d, J = 16.0	2.90, d, J = 17.0	1.80, dd, J = 16.0, 4.5	1.90, dd, J = 17.0, 4.5	1.88, dd, J = 17.0, 3.0	1.47, m
3	−	−	4.39, br dd, J = 4.5, 3.0	4.39, br dd, J = 4.5, 3.0	4.42, br dd, J = 4.5, 3.0	2.20, m
						2.39, m
4	5.90, br s	5.87, br s	5.35, br s	5.30, br s	5.23, br s	5.34 d J = 2.5, 4.5
6	2.40, dd, J = 8.0, 1.5	−	2.70, d, J = 8.7	−	−	−
7	1.48, br m	1.50, ddd, J = 12.0, 3.0, 4.5	5.60, dd, J = 15.0, 8.7	5.90, br m	1.58, ddd, J = 12.0 3.0, 4.5	1.58, ddd, J = 12.0, 3.0, 4.5
	1.70, br m	1.95, ddd, J = 12.0, 6.0, 5.0			1.92, ddd, J = 12.0, 6.0, 5.0	1.92, ddd, J = 12.0, 6.0, 5.0
8	1.65, m	1.60, m	5.85, dd, J = 15.0, 6.5	5.95, br m	1.60 m	1.60, m
	1.95, m	1.85, m			1.83, m	1.83, m
9	4.10, m	4.15, m	4.45, q, J = 6.5	4.30, q, J = 7.0	4.25, m	4.25, m
10	1.35, d, J = 6.5	1.35, d, J = 7.0	1.35, d, J = 6.5	1.35, d, J = 7.0	1.35, d, J = 6.5	1.35, d, J = 6.5
11	1.05, s	1.08, s	1.08, s	1.10, s	1.07, s	1.07, s
12	1.08, s	1.10, s	1.10, s	1.10, s	1.07, s	1.07, s
13	1.98, d, J = 1.2	1.90, d, J = 1.5	1.90, br s	1.92, d, J = 1.5	1.95, d, J = 1.5	1.95, d, J = 1.5

[a] Assignments confirmed by COSY-90 and HETCOR experiments. J values are in hertz.

Table 3. ^{13}C NMR Data of the Aglycon Moieties of Compounds 5, 7, and 9−12 in CD_3OD[a].

position	5	7	9	10	11	12
C-1	36.2	43.1	39.1	40.1	40.0	42.2
C-2	49.5	50.4	39.4	38.1	37.1	28.4
C-3	203.6	201.1	69.0	70.1	70.1	31.0
C-4	124.0	126.6	125.1	124.0	124.3	131.4
C-5	166.1	168.1	148.7	150.0	148.9	140.6
C-6	49.0	79.2	52.1	80.2	78.4	79.1
C-7	28.0	38.1	129.0	131.3	38.7	38.1
C-8	32.1	26.5	138.0	138.1	26.4	25.1
C-9	79.7	78.3	76.9	77.4	77.9	79.0
C-10	21.2	21.2	20.5	22.4	21.1	21.1
C-11	27.4	19.3	27.6	19.0	19.2	19.3
C-12	27.9	24.5	28.1	25.4	25.0	24.3
C-13	23.7	23.2	24.0	24.0	23.4	23.2

[a] Assignments confirmed by HETCOR experiments.

when compared with analogous values in unglycosylated models (Shirataki et al., 1986). Consequently, compound **1** was established to be 7-hydroxy-5′-methoxy-3′,4′-methylenedioxyflavonol 7-*O*-*β*-D-glucopyranoside.

Compound **2** ($C_{22}H_{20}O_{12}$) differed from **1** only in the substitution of the methoxy group at C-3′ with a phenolic function, as suggested by the absence of the signals at δ_H 3.87 in the ^1H NMR spectrum and at δ_C 57.1 in the ^{13}C NMR spectrum and small differences in the carbon resonances of ring B.

NMR data of compound **3** ($C_{23}H_{24}O_{12}$) were very similar to those of **2**, the main differences being the absence of the signals ascribable to the methylenedioxy function (δ_H 5.80 in the ^1H NMR spectrum and δ_C 102.5 in the ^{13}C NMR spectrum) and the occurrence of signals for a further methoxy group (δ_H 3.90, s, 6H, in the ^1H NMR spectrum and δ_C 57.1 in the ^{13}C NMR spectrum) (Table 1). This evidence allowed us to establish a 3′,5′-dimethoxy-4′-hydroxy substitution pattern and led to the identification of **3** as 4′,7-dihydroxy-3′,5′-dimethoxyflavonol 7-*O*-*β*-D-glucopyranoside.

The molecular formula of compound **4** ($C_{23}H_{24}O_{12}$) was identical to that of **3**, and NMR data of the aglycon moiety were superimposable on those observed for **3**, suggesting that the difference between the two compounds should be confined to the nature of the sugar unit. The ^1H and ^{13}C NMR sugar signals of **4** could be reasonably assigned to a *β*-D-galactopyranosyl unit; particularly characteristic of H-3 of a *β*-D-galactopyranose were the coupling costants (J = 4.0 and 7.5 Hz) of the signal at δ_H 3.54 (D'Auria et al., 1992). Thus, compound **4** was defined as 4′,7-dihydroxy-3′,5′-dimethoxyflavonol 7-*O*-*β*-D-galactopyranoside.

The molecular formula ($C_{25}H_{42}O_{11}$) of compound **5** was determined by ^{13}C NMR data and FABMS analysis in the negative ion mode. The FABMS spectrum of **5** showed the $[M - H]^-$ ion at m/z 517 and peaks at m/z 371 $[(M - H) - 146]^-$ and m/z 355 $[(M - H) - 162]$ (cleavage of a deoxyhexose unit with or without the glycosidic oxygen) and m/z 209 $[(M - H) - (146 + 162)]$ due to the subsequent loss of a hexose unit. The ^{13}C and DEPT ^{13}C NMR spectra of **5** showed 24 signals, 11 of which were assigned to the saccharide portion and 13 to the aglycon moiety. Examination of the NMR data (Tables 2 and 3) and comparison with literature (Miyase et al., 1988) allowed identification of the aglycon as the ionol-derived blumenol C. The downfield shift (*β*-effect) observed for the C-9 resonance (δ_C 79.7) and the upfield shift (*γ*-effect) experienced by the C-10 resonance (δ_C 21.2), if compared with those reported for blumenol C, suggested the attachment of the disaccharidic chain at C-9 of the aglycon moiety. ^1H and ^{13}C NMR data of the sugar portion of **5** (Table 4) together with the results from GLC led us to establish the disaccharidic chain to be formed by one D-glucose unit and one L-rhamnose unit. The interglycosidic linkage was located at C-6′of the glucose unit on the basis of the downfield shift exhibited by this carbon resonance (δ_C 68.7) if compared with the respective shift in the unglycosylated model (Miyase et al., 1988). A two-dimensional COSY experiment allowed the complete sequential assignment of all sugar proton resonances, starting from the well-isolated anomeric proton signals at δ_H 4.52 (H-1′ Glu) and 5.25 (H-1′ Rha). Chemical shifts, multiplicity of the signals, absolute values of the coupling constants, and their magnitude from the shape of cross-peaks in the COSY spectrum as well as ^{13}C NMR data indicated the *β* configuration at the anomeric position for the glucopyranosyl unit ($J_{H-1-H-2}$ = 7.5 Hz) and the α configuration for the rhamnopyranosyl unit ($J_{H-1-H-2}$ = 1.5 Hz). Thus, compound **5** is the new blumenol C 9-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside.

In the FABMS spectrum of **6** ($C_{24}H_{40}O_{11}$), we observed the $[M - H]^-$ ion at m/z 503 and prominent fragments at m/z 371 $[(M - H) - 132]^-$ and m/z 209 $[(M - H) - (132 + 162)]^-$ due to the subsequent loss of one pentose and one hexose unit. Comparison of NMR data of **6** with those of **5** indicated that the two compounds have the same aglycon, differing only in the sugar portion. The^1H NMR spectrum of **6** showed an anomeric hydrogen signal at δ_H 4.52 (H-1′, d, J = 7.5 Hz) ascribable to a *β*-D-glucopyranose and a second one at δ_H 5.01 (H-1′, d, J=2 Hz) correlated by a COSY experiment (Table 4) to a signal at δ_H 3.92 (H-1″, d, J = 2 Hz), both characteristic of a *β*-D-apiofuranosyl unit (Aquino et al., 1988). The location of the interglycosidic linkage at C-6′ of the glucose unit was established on the basis of the same arguments used for **5**. Thus, compound **6** could be defined as the new blumenol C 9-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside.

The FABMS spectra of **7** ($C_{25}H_{42}O_{12}$) and **8** ($C_{24}H_{40}O_{12}$) showed quasi-molecular anions at m/z 533 and 519, respectively, and their fragmentation patterns were superimposable, respectively, on those of **5** and **6**. ^1H and ^{13}C NMR spectra of **7** and **8** (Tables 2 and 3) revealed signals due to the aglycon moiety which matched very closely those reported for the ionol derivative blumenol B (Miyase et al., 1988) and sugar signals identical to ±0.02 ppm respectively to those observed in the spectra of **5** and **6**. Thus, **7** was determined as the new blumenol B 9-*O*-α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside and **8** as the new blumenol *β* 9-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside.

In the FABMS spectrum of **9** ($C_{24}H_{40}O_{11}$), we observed the $[M - H]^-$ ion at m/z 503 and a fragmentation pattern identical with those of **6** and **8**. ^{13}C NMR data of **9** (Table 3) for the aglycon moiety showed 13 signals which were sorted by DEPT ^{13}C NMR into four Me groups (δ_C 20.5, 24.0, 27.6, and 28.1), one CH_2 group (δ_C 39.4), an aliphatic CH group (δ_C 52.1), three olefinic CH groups (δ_C 125.1, 129.0, and 138.0), two quaternary carbons, one of which was sp^2 (δ_C 39.4 and 148.7), and two oxygenated CH groups (δ_C 69.0 and 76.9). The ^1H NMR spectrum (Table 2) confirmed the presence of four methyl groups, one of which (δ_H 1.35, d, J = 6.5 Hz) was attached to a CH bearing an oxygen atom and one of which (δ_H 1.90, br s) was linked to a quaternary sp^2 carbon. Further features were three signals (δ_H 2.70, d, J = 8.7 Hz; δ_H 1.80, dd, J = 16.0 and 4.5 Hz; δ_H 1.55, dd, J = 16.0 and 2.0 Hz,) the last two of which were

Table 4. NMR Data of the Sugar Moieties of Compounds 5, 6, and 9[a]

	5			6			9		
position		δ_C	δ_H (J_{HH}, Hz)		δ_C	δ_H (J_{HH}, Hz)		δ_C	δ_H (J_{HH}, Hz)
1′	Glu	104.5	4.52, d (7.5)	Glu	104.5	4.52, d (7.5)	Glu	102.7	4.53, d (7.5)
2′		75.1	3.30, d (7.5, 9.5)		75.1	3.48, d (7.5, 9.5)		75.4	3.31, d (7.5, 9.5)
3′		78.3	3.42, d (9.5, 9.5)		78.3	3.40, d (9.5, 9.5)		78.2	3.42, d (9.5, 9.5)
4′		71.9	3.34, d (9.5, 9.5)		71.9	3.34, d (9.5, 9.5)		71.9	3.34, d (9.5, 9.5)
5′		78.2	3.28, m		78.3	3.28, m		78.3	3.28, m
6′		68.7	3.60, d (12, 3.0)		68.6	3.63, d (12.0, 3.0)		68.7	3.60, d (12, 3.0)
			4.01, d (12.0, 5.0)			4.01, d (12.0, 5.0)			4.00, d (12.0, 5.0)
1″	Rha	102.3	5.25, d (1.5)	Api	110.5	5.01, d (2.0)	Api	110.5	5.01, d (2.0)
2″		72.7	3.94, d (1.5, 3.0)		77.0	3.92, d (2.0)		77.1	3.91, d (2.0)
3″		72.7	3.82, dd (3.0, 9.0)		80.5	–		80.6	–
4″		74.5	3.40, dd (9.0, 9.0)		75.2	3.74, d (10.0)		75.2	3.74, d (10.0)
						4.02, d (10.0)			4.02, d (10.0)
5″		70.0	4.15, dd (3.0, 9.0)		65.9	3.58, br s		65.9	3.58, br s
6″		18.0	1.30, d (6.5)		–	–		–	–

[a] Assignments confirmed by COSY-90 and HETCOR experiments.

assignable to a methylene group. Also evident were signals ascribable to olefinic protons (δ_H 5.35, br s; δ_H 5.60, dd, J = 15.0 and 8.7 Hz; δ_H 5.85, dd, J = 15.0 and 6.5 Hz) and two signals diagnostic for CHOH groups (δ_H 4.39, br dd, J = 3.0 and 4.5 Hz; δ_H 4.45, q, J = 6.5 Hz). A COSY-90 spectrum of **9** identified, for the aglycon moiety, two different spin systems corresponding to the C=CH−CHOH−CH_2 and CH_3−CHOH−CH=CH−CH sequences. A HETCOR experiment, which established the association of protons with corresponding carbons, led to the unambiguous assignment reported in Table 3. The α configuration of H-6 was deduced by examination of NMR data and comparison with those reported for 3-oxo-α-ionol (De Tommasi et al., 1992), while the stereochemistry at the chiral center C-3 was assigned in accordance with the magnitude of the H-3 coupling constants (J = 3.0 and 4.5 Hz) which showed this proton to be equatorial, and therefore, the OH group should be axial and β. NMR data for the saccharidic portion (Table 4) suggested the same sugar chain as in **6** and **8**; the upfield shift exhibited by the anomeric carbon of the glucose unit in **9** when compared to the shift of the same carbon in **6** and **8** (δ_C 102.7 in **9** versus δ_C 104.5 in **6** and **8**) was attributed to the linkage of the sugar unit to an allylic alcoholic function (De Tommasi et al., 1988). Thus, the aglycon of **9** was identified as 3β-hydroxy-α-ionol, previously reported as a product of microbial biotransformation of α-ionone with undetermined stereochemistry at C-3 (Hartman et al., 1988) and **9** as the new 3β-hydroxy-α-ionol 9-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside.

FABMS and NMR data (Tables 2 and 3) of compounds **10**−**12** indicated that they have the same sugar chain as in **6**, **8**, and **9** but different aglycons.

A detailed analysis of ^{1}H and ^{13}C NMR spectra of **10** ($C_{24}H_{40}O_{12}$) and comparison with those of **9** allowed us to establish the occurrence of an additional hydroxyl group at C-6 of the aglycon in **10**. Thus, the aglycon of **10** is the 3β,6α-dihydroxy-α-ionol previously reported as megastigma-4,7-diene-3,7,9-triol, a synthetic product whose hydrolitic chemistry has been studied to account for the origins of many of the volatile norisoprenoids in grapes and wine (Strauss et al., 1986).

^{13}C and DEPT ^{13}C NMR data together with the FABMS spectrum, which showed a $[(M - H)]^-$ ion at m/z 521, suggested for **11** the molecular formula $C_{24}H_{42}O_{12}$. From the inspection of ^{1}H and ^{13}C NMR signals of the aglycon moiety, it was possible to deduce that the aglycon of **11** was closely related to that of **10**, being its 7,8-dihydro derivative, previously reported as the synthetic megastigm-4-ene-3,6,9-triol (Strauss et al., 1986).

A quasi-molecular anion $[(M - H)^-]$ at m/z 505, 16 mass units lower than that of **11**, indicated for **12** the molecular formula $C_{24}H_{42}O_{11}$. The ^{1}H NMR spectrum, if compared to that of **11**, showed the absence of the signal at δ_H 4.42 (br dd, J = 3.0 and 4.5 Hz, H-3) and the occurrence of two signals at δ_H 2.20 (1H, m, H-3a) and 2.39 (1H, m, H-3b) ascribable to a methylene group; a further feature was the upfield shift exhibited by H_2-2 (δ_H 1.45 and 1.47 in **12** versus δ_H 1.65 and 1.88 in **11**). From the examination of ^{13}C NMR data for the aglycon moiety and comparison with those of **11**, it resulted that the resonances at δ_C 37.1 and 70.1 gave rise to the signals at δ_C 28.4 and 31.0 which were correlated by a HETCOR experiment respectively to H_2-2 and H_2-3. Thus, the aglycon of **12** was identified as the never reported 7,8-dihydro-6α-hydroxy-α-ionol.

The FABMS of compound **13** ($C_{19}H_{34}O_8$) gave a quasi-molecular anion peak $[(M - H)^-]$ at m/z 389 and a fragment at m/z 211 corresponding to the loss of a glucose unit. Examination of NMR data and comparison with those of **11** showed **13** to differ from **11** only in the absence of the terminal apiofuranosyl unit.

On the basis of the foregoing data, compounds **10**−**13** were determined as the new 3β,6α-dihydroxy-α-ionol 9-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**10**), 7,8-dihydro-3β,6α-dihydroxy-α-ionol 9-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**11**), 7,8-dihydro-6α-hydroxy-α-ionol 9-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**12**), and 7,8-dihydro-3β,6α-dihydroxy-α-ionol 9-*O*-β-D-glucopyranoside (**13**), respectively. Compounds **14**−**16** were identified, respectively, as 3-oxo-α-ionol 9-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside, vomifoliol 9-*O*-β-D-glucopyranoside (roseoside), and vomifoliol 9-O-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside by comparison of NMR data with reported values (Okamura et al., 1981; De Tommasi et al., 1992).

It is to be noted that the aglycons of **10** and **11** were previously reported as synthetic products that in aqueous acid at pH 3.35 gave a number of volatile C_{13} isoprenoids which occur naturally in grapes and wine (Strauss et al., 1986). Therefore, although never observed as natural products, they were thought to be substances generating the C_{13} norisoprenoid volatiles. The isolation of these two aglycons from a natural source could be useful to confirm their role as precursors of highly fragrant flavor compounds in fruits and vegetables.

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Supporting Information Available: ^{13}C and ^{1}H NMR data for compounds **2**, **4**, **6**−**8**, and **10**−**16** (2 pages). Ordering information is given on any current masthead page.

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