JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Spectroscopic Identification and Antioxidant Activity of Glucosylated Carotenoid Metabolites from *Cydonia vulgaris* Fruits

ANTONIO FIORENTINO,* BRIGIDA D'ABROSCA, SEVERINA PACIFICO, CLAUDIO MASTELLONE, VINCENZO PISCOPO, AND PIETRO MONACO

Laboratorio Integrato per la Qualità e la Sicurezza degli Alimenti, Centro Regionale di Competenza "Produzioni Agroalimentari", Dipartimento di Scienze della Vita, Seconda Università degli Studi di Napoli, via Vivaldi 43, I-81100 Caserta, Italy

Carotenoid metabolites are common plant constituents with significant importance for the flavor and aroma of fruits. Three new carotenoid derivatives, (2*E*,4*E*)-8-hydroxy-2,7-dimethyl-2,4-decadiene-1,10-dioic acid 1-*O*-β-D-glucopyranosyl ester (**1**), (2*Z*,4*E*)-8-β-D-glucopyranosyloxy-2,7-dimethyl-2,4-decadiene-1,10-dioic acid (**3**), and 3,9-dihydroxymegastigmast-5-ene-3-*O*-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (**5**), as well as three known compounds, have been isolated from the ethanolic extract of peels of *Cydonia vulgaris*, the fruit of a shrub belonging to the same family as the apple. All the compounds were identified by spectroscopic techniques, especially 1D and 2D NMR. Antioxidant activities of all the isolated metabolites were assessed by measuring their ability to scavenge DPPH radical and superoxide radical ($O_2^{\bullet -}$) and to induce the reduction of Mo(VI).

KEYWORDS: ***Cydonia vulgaris*; degraded carotenoids; NMR spectroscopy; antioxidant activity**

INTRODUCTION

Antioxidants are compounds that protect against oxidation, or cellular damage caused by free radicals. Plants are an important source of organic antioxidant chemicals, which are widely used as ingredients in dietary supplements. These important additives are utilized for health purposes for the prevention of pathologies such as cancer, heart disease, diabetes, neurodegenerative processes, etc. (*1*−*4*). Fruits and vegetables are the main sources of dietary antioxidants. In fact, high dietary intakes of fruits and vegetables are associated with reduced risks of cancer and cardiovascular disease (*5*). John et al. (*6*) reported that the effects of the intervention of fruit and vegetable consumption, plasma antioxidants, and blood pressure reduce cardiovascular disease in the general population. Paganga et al. (*7*) correlated the major polyphenols of some fruits and vegetables with the antioxidant activities of the extracts.

Recently we reported the complete characterization of the organic extracts of the apple cv Annurca, a variety growing in the southern regions of Italy (*8*). We evaluated the antioxidant activities of a number of compounds belonging to several classes of secondary metabolites (*9*−*11*). In the search for new antioxidant chemicals from food, we have continued the phytochemical study of edible plants growing in southern Italy.

In this study we undertook the isolation, spectroscopic analysis, and antioxidant activity determination of six glucosylated carotenoid metabolites, three of them isolated for the first time, from the peels of fruits of *Cydonia vulgaris* Pers., a small shrub belonging to the same family as the apple and pear (Rosaceae). *C. vulgaris* is the sole member of the genus. It is a small tree with bright golden yellow pome fruits, when mature. The fruit of *C. vulgaris*, known as quince, resembles an apple, but differs in having many seeds in each carpel. Pomes of quince, known in Italy as *cotogna* apple, have a hard flesh of high flavor, but very acidic, and are largely used for marmalade, liqueur, jelly, and preserves. It has been reported that the leaves and fruits of quince have some positive effects in the medical treatment of various conditions including cardiovascular diseases, hemorrhoids, bronchial asthma, and cough (*12*). The antioxidant and radical scavenging activities of all the pure metabolites have been evaluated by measuring their capacity to scavenge the 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) and the superoxide radical ($O_2^{\bullet -}$) and by testing their ability to reduce molybdenum(VI).

MATERIALS AND METHODS

Fruit Collection and Extraction. *C. vulgaris* Pers. (syn. *Cydonia oblonga* Mill.) fruits were collected in Durazzano, near Caserta (Italy), in October 2005 when the fruit had just been harvested. The fruits were sliced, and the peels (3.10 kg) were infused in ethanol (5 L) for 7 days in a refrigerated chamber at 4 °C in the dark. After removal of the solvent under vacuum, we obtained a crude extract (261.6 g).

General Experimental Procedures. NMR spectra were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C on a Varian Mercury 300 Fourier transform NMR spectrometer in CD_3OD, at 25 °C. Proton-detected heteronuclear correlations were measured using HSQC (optimized for $^1J_{HC}$ = 140 Hz) and HMBC (optimized for $^nJ_{HC}$ = 8

* To whom correspondence should be addressed. Phone: +39 0823274576. Fax: +39 0823274571. E-mail: antonio.fiorentino@unina2.it.

10.1021/jf062125e CCC: $33.50
Published on Web 11/14/2006

Table 1. NMR Data of Carotenoid Glucosides **1**, **3**, and **5** in CD_3OD[a]

	1		3		5	
position	$\delta(^{13}C)$	$\delta(^{1}H)$	$\delta(^{13}C)$	$\delta(^{1}H)$	$\delta(^{13}C)$	$\delta(^{1}H)$
1	168.7		176.9		38.8	
2	125.1		123.0		47.4	1.82, ddd (J = 12.1, 3.7 and 2.1 Hz)
						1.50, ov
3	141.6	7.32, d (J = 11.4 Hz)	133.7	6.98, d (J = 11.1 Hz)	73.3	3.46, m
4	128.5	6.48, dd (J = 15.0 and 11.4 Hz)	129.3	6.4, dd (J = 15.0 and 11.1 Hz)	39.6	2.35, dd (J = 15.6 and 4.8)
						2.10, ov
5	144.4	6.18, dt (J = 15.0, 6.9 and 7.5 Hz)	136.0	5.95, dt (J = 15.0, 6.9 and 7.5 Hz)	125.1	
6	37.2	2.09, m	32.6	2.05, m	138.5	
		2.50, m		2.65, m		
7	39.9	1.70, m	36.9	1.89, m	25.5	2.05, m
8	73.5	3.70, m	83.0	4.03, m	40.6	1.49, m
9	41.2	2.26, dd (J = 15.3 and 9.0 Hz)	38.8	2.44, m	69.2	3.66, m
		2.42, dd (J = 15.3 and 3.3 Hz)		2.38, m		
10	179.9		180.2		23.2	1.17, d (J = 6.0 Hz)
11	15.8	0.90, d (J = 6.9 Hz)	16.0	0.95, d (J = 6.6 Hz)	20.0	1.64, s
12	12.5	1.93, s	12.9	1.89, s	29.0	1.06, s
13	96.0	5.53, d (J = 7.8 Hz)	104.2	4.36, d (J = 7.8 Hz)	30.3	1.07, s
14	73.9	3.15, ob	75.2	3.17, dd (J = 8.6 and 7.8 Hz)		
15	78.8	3.32, ov	78.0	3.32, ob		
1′	71.1	3.30, ov	71.8	3.30, ob	102.2	4.43, d (J = 8.1 Hz)
2′	78.0	3.32, ov	78.0	3.30, ob	75.1	3.15, dd (J = 8.1 and 7.9 Hz)
3′	62.3	3.67, dd (J = 11.7 and 2.1 Hz)	63.0	3.62, dd (J = 12.1 and 1.8 Hz)	78.0	3.32, ob
		3.80, dd (J = 11.7 and 5.6 Hz)		3.80, dd (J = 12.1 and 5.7 Hz)		
4′					71.4	3.32, ob
5′					77.9	3.32, ob
6′					69.7	4.12, dd (J = 11.7 and 2.1 Hz)
						3.81, dd (J = 11.7 and 5.4 Hz)
1″					104.8	4.36, d (J = 7.8 Hz)
2″					75.1	3.21, dd (J = 7.8 Hz)
3″					78.0	3.32, ob
4″					71.6	3.32, ob
5″					77.9	3.32, ob
6″					62.7	3.87, dd (J =12.3 and 1.5 Hz)
						3.66, ov

[a] Key: d = doublet; dd = doublet of doublets; dt = doublet of triplets; m = multiplet; ob = obscured by the solvent; ov = overlapped; s = singlet; t = triplet.

Hz). UV spectra were obtained in MeOH solutions on a Perkin-Elmer Lambda 7 spectrophotometer. IR spectra were determined in $CHCl_3$ solutions on an FT-IR Perkin-Elmer 1740 spectrophotometer.

Optical rotations were measured on a Perkin-Elmer 343 polarimeter in MeOH solutions. Electrospray mass spectra were recorded using a Waters ZQ mass spectrometer (Waters Co., Milford, MA) equipped with an electrospray ionization (ESI) probe operating in positive or negative ion mode. The scan range was m/z 80−2000. The Shimadzu preparative HPLC apparatus consisted of an LC-10AD pump, an RID-10A refractive index detectorn and a C-R6A Chromatopac recorder. Preparative HPLC was performed using a 250 × 10 mm i.d., 10 μm, Luna RP-18 (Phenomenex, Torrance, CA) column. Analytical HPLC was performed using 250 × 4.6 mm i.d., 5 μm, RP-18 and RP-8 Luna columns. Analytical TLC was performed on Merck Kieselgel 60 F_{254} or RP-18 F_{254} plates with a 0.2 mm layer thickness. Spots were visualized by UV light or by spraying with H_2SO_4/AcOH/H_2O (1:20:4). The plates were then heated for 5 min at 110 °C. Preparative TLC was performed on Merck Kieselgel 60 F_{254} plates, with a 0.5 or 1 mm film thickness. Flash column chromatography (FCC) was performed on Merck Kieselgel 60 (230−400 mesh) at medium pressure. Column chromatography (CC) was performed on Merck Kieselgel 60 (70−240 mesh), Amberlite XAD-4 (Fluka, Buchs, Switzerland), on Sephadex LH-20 (Pharmacia, Piscataway, NJ).

Organic Extract Fractionation. The ethanolic extract (261.6 g) was dissolved in water (1.0 L) and shaken with EtOAc (1.5 L) to obtain an organic and an aqueous fraction. The aqueous fraction (8.05 g) was chromatographed on Amberlite XAD-4 eluting with water first (1.5 L) and then with pure MeOH (2.0 L). The methanolic fraction was chromatographed on Sephadex LH-20 eluting with water and a water−methanol solution (1:4) to obtain three fractions, A−C.

Fraction A (137.5 mg), eluted with water, was rechromatographed on RP-8 by column chromatography eluting with MeOH/MeCN/H_2O (8:1:1) to obtain pure **6** (9.4 mg) and two other fractions. One of these fractions was purified by TLC (0.5 mm) eluting with the lower organic phase of a $CHCl_3$/MeOH/H_2O (13:7:3) biphasic solution to give pure (2*E*,4*E*)-8-hydroxy-2,7-dimethyl-2,4-decadiene-1,10-dioic acid 1-*O*-β-D-glucopyranosyl ester (**1**) (19.6 mg): colorless oil; UV (MeOH) λ_{max} (nm) (log ϵ) 271.0 (4.29); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3685, 2925, 2854, 1724, 1604; ^1H NMR and ^{13}C NMR (CD_3OD) see **Table 1**; ESI-MS m/z 427 $[M + Na]^+$, 264 $[M - C_6H_{11}O_5 + Na]^+$, 247 $[M - C_6H_{12}O_6 + Na]^+$; $[\alpha]_D = -3.3$ (c = 0.43, MeOH). Anal. Calcd for $C_{18}H_{28}O_{10}$: C, 53.46; H, 6.98. Found: C, 53.71; H, 6.77.

The other fraction, purified on RP-8 HPLC with MeOH/MeCN/H_2O (8:2:1), gave pure 3-hydroxymegastigmasta-5,7-dien-9-one-3-*O*-β-D-glucopyranoside (**4**) (2.3 mg).

Fraction B (237.6 mg), eluted with water, was rechromatographed on RP-8 by column chromatography eluting with MeOH/MeCN/H_2O (8:1:1) to obtain a fraction which, purified by TLC (0.5 mm) using as eluent the lower organic phase of a $CHCl_3$/MeOH/H_2O (13:7:3) biphasic solution, furnished 3,9-dihydroxymegastigmast-5-ene-3-*O*-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (**5**) (4.6 mg): UV (MeOH) λ_{max} (nm) (log ϵ) 205.5 (3.5); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3682, 2925, 1728, 1609; ^1H NMR and ^{13}C NMR (CD_3OD) see **Table 1**; ESI-MS m/z 559 $[M + Na]^+$, 396 $[M - C_6H_{11}O_5 + Na]^+$, 234 $[M - C_{12}H_{22}O_{10} + Na]^+$; $[\alpha]_D = -2.2$ (c = 0.23, MeOH). Anal. Calcd for $C_{25}H_{44}O_{12}$: C, 55.96; H, 8.26. Found: C, 56.11; H, 8.19.

Fraction C, eluted with MeOH/H_2O (1:4), was rechromatographed on RP-8 by column chromatography eluting with MeOH/MeCN/H_2O (9:1:1) to give compounds **2** (15.3 mg) and **3** (3.0 mg). Data for (2*Z*,4*E*)-8-β-D-glucopyranosyloxy-2,7-dimethyl-2,4-decadiene-1,10-dioic acid (**3**): colorless oil; UV (MeOH) λ_{max} (nm) (log ϵ) 264.5 (5.7); IR ($CHCl_3$) ν_{max} (cm^{-1}) 3682, 3595, 2920, 1734, 1604; ^1H NMR and ^{13}C NMR (CD_3OD) see **Table 1**; ESI-MS m/z 427 $[M + Na]^+$, 264 $[M - C_6H_{11}O_5$

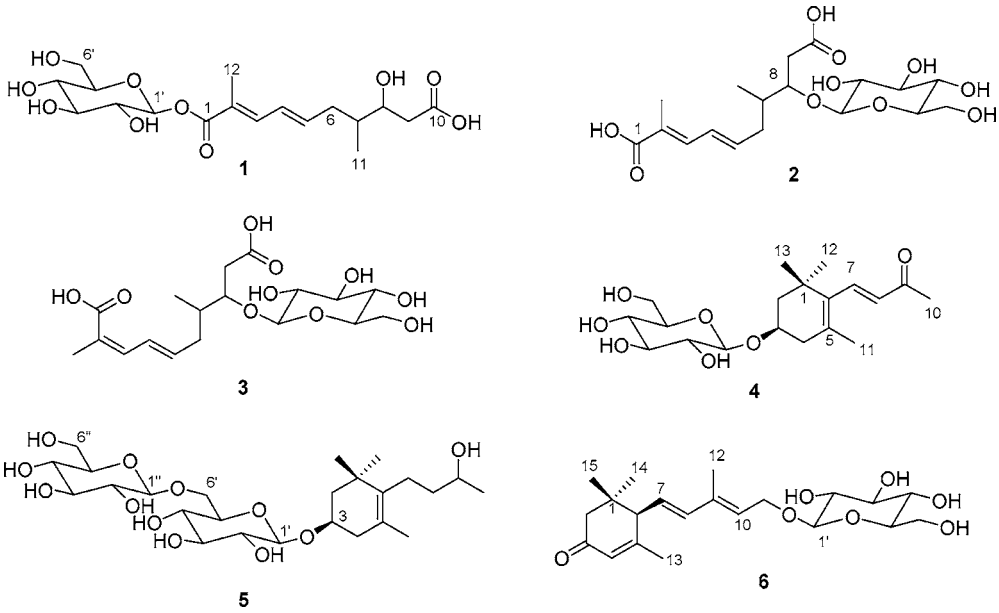

Figure 1. Chemical structures of C_{12} carotenoid glucosides **1**–**3**, C_{13} carotenoid glucosides **4** and **5**, and C_{15} carotenoid glucoside **6**.

+ Na]$^+$; $[\alpha]_D = -14.8$ ($c = 0.15$, MeOH). Anal. Calcd for $C_{18}H_{28}O_{10}$: C, 53.46; H, 6.98. Found: C, 53.60; H, 6.91.

DPPH Radical Scavenging Activity. The scavenging activity of the metabolites was measured according to the method of Brand-Williams (*13*). The method was based on the reduction of methanolic DPPH• in the presence of a hydrogen-donating antioxidant.

DPPH• (Fluka) solution showed an absorption band at 517 nm and was intensely violet colored. The adsorption and color decreased when DPPH• was reduced by an antioxidant compound. The remaining DPPH• corresponded inversely to the radical scavenging activity of the antioxidant (*14*). DPPH• (2 mg) was dissolved in 54 mL of MeOH. The investigated metabolites were prepared by dissolving 0.1 mg of each compound in 1 mL of MeOH. Then 38 μL of each solution containing compound was added to 1.462 mL of DPPH• solution at room temperature (*15*). The absorbance at 517 nm was measured in a cuvette at 30 min vs a blank (38 μL of MeOH in 1.462 mL of DPPH• solution) using a UV-1601 Shimadzu spectrophotometer. The analysis was carried out in triplicate, and the results are expressed in terms of the percentage reduction of the initial DPPH• adsorption by the test compounds.

Superoxide Radical Scavenging Activity. The assay of superoxide radical scavenging activity was based on the capacity of each isolated metabolite (0.1 mg/mL) to inhibit the photochemical reduction of nitroblue tetrazolium (NBT; Fluka) in the riboflavin−light−NBT system (*16*). Each 3 mL reaction mixture contained 50 mM sodium phosphate buffer (pH 7.8), 13 mM methionine (Fluka), 2 μM riboflavin (Riedel-de Haën, Seelze, Germany), 100 μM EDTA (Carlo Erba Reagents, Rodano, Milano, Italy), 75 μM NBT, and 100 μL of sample solution. The production was followed by monitoring the increase in absorbance at 560 nm after 10 min of illumination from a fluorescent lamp. The analysis was carried out in triplicate.

Evaluation of the Total Antioxidant Activity. Spectrophotometric evaluation of the antioxidant activity through the formation of a phosphomolybdenum complex was carried out according to Prieto et al. (*17*). Sample solutions (100 μL) containing reducing metabolites (0.2 mg in 1 mL of dimethyl sulfoxide) were combined in an Eppendorf tube with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95 °C for 90 min. After the samples were cooled to room temperature, the absorbance of an aqueous solution of each was measured at 820 nm against a blank. The analysis was carried out in triplicate, and the antioxidant activity is expressed relative to that of caffeic acid, an antioxidant substance significantly better than α-tocopherol (*18*).

Enzymatic Hydrolysis of Compound 2. To a solution of pure compound **2** (4 mg) in acetate buffer (0.5 M, pH 5.0, 5 mL) was added 16 mg of β-glucosidase (Sigma, St. Louis, MO). After 24 h at 37 °C with stirring, the mixture was extracted with EtOAc (5 mL × 2), dried over Na_2SO_4, and evaporated in vacuo. The crude extract consisted of pure aglycon **2a**: ^{1}H NMR (300 MHz, CD_3OD): δ 7.17 (1H, d, $J = 11.7$, H-3), 6.45 (1H, dd, $J = 15.0$ and 11.7 Hz, H-4), 6.10 (1H, dt, $J = 15.0$, 7.5, and 7.8 Hz, H-5), 2.07 (1H, m, H-6a), 2.44 (1H, m, H-6b), 1.70 (1H, m, H-7), 3.83 (1H, m, H-8), 2.32 (1H, dd, $J = 15.3$ and 9.0 Hz, H-9a), 2.51 (1H, dd, $J = 15.3$ and 3.6 Hz, H-9b), 0.91 (3H, d, $J = 6.9$ Hz, H-11), 1.98 (3H, s, H-12); ^{13}C NMR (75 MHz, CD_3OD) δ 176.2 (C, C-1), 126.4 (C, C-2), 140.0 (CH, C-3), 128.8 (CH, C-4), 142.6 (CH, C-5), 37.1 (CH_2, C-6), 40.1 (CH, C-7), 73.0 (CH, C-8), 40.3 (CH_2, C-9), 176.9 (C, C-10), 15.8 (CH_3, C-11), 12.7 (CH_3, C-12).

RESULTS AND DISCUSSION

The phytochemical study of the ethanol extract of the peels of *C. vulgaris* led to the isolation of six carotenoid metabolites (**Figure 1**), three of which (**1**, **3**, and **5**) were isolated for the first time. All the compounds have been identified on the basis of their spectroscopic features.

The compounds from *C. vulgaris* should be derived from the oxidation of carotenoid precursors. Biodegradation of carotenoids, due to photooxygenation and autoxidation processes or enzymatic cleavage, has been suggested as a pathway giving rise to the formation of a number of volatile degradation products (*19*). Carotenoid metabolites so obtained include fragments containing 12, 13, and 15 carbons. It has been demonstrated that C_{13} norisoprenoids are important carotenoid-related aroma compounds in fruits. For quince fruits a considerable number of C_{13} norterpenes have been reported (*20*).

Compound **1**, isolated as a colorless oil, showed a molecular formula of $C_{18}H_{28}O_{10}$, as inferred from ESI-MS analysis, which revealed the pseudomolecular ion at *m*/*z* 427 [M + Na]$^+$, and by the ^{13}C NMR spectrum, showing signals of 18 carbons. The ESI-MS spectrum also showed fragments due to the loss of a sugar moiety at *m*/*z* 264 [M − $C_6H_{11}O_5$ + Na]$^+$ and 247 [M − $C_6H_{12}O_6$ + Na]$^+$.

The ^{1}H NMR spectrum showed, in the olefinic region, three signals at δ 7.32, 6.48, and 6.18, whose values suggested the

presence of two conjugated double bonds; in the aliphatic region, two diasterotopic methylenes at δ 2.09/2.50 and 2.26/2.42, a methine as a multiplet at δ 3.70, and two methyls as a singlet at δ 1.93 and a doublet at δ 0.90 were evident. The sugar moiety was confirmed by the presence in the same spectrum of a doublet at δ 5.53 (J = 7.8 Hz), two doublets of doublets at δ 3.80 and 3.67, and other overlapped protons ranging from δ 3.15 to δ 3.32. The homonuclear two-dimensional experiments (g-COSY, DQ-COSY, and TOCSY) revealed correlations between the olefinic doublet at δ 7.32 with the doublet of doublets at δ 6.48; this proton was correlated with the third olefinic proton at δ 6.18, which showed cross-peaks with the methylenes at δ 2.09 and 2.50. Both these protons were correlated with the methine at δ 3.70, which was, finally, correlated with the methylene protons at δ 2.26 and 2.42.

The ^{13}C NMR spectrum showed 18 signals identified, on the basis of a DEPT experiment, as two methyls, three methylenes, ten methines, two carboxylic carbons at δ 168.7 and 179.9, and a quaternary olefinic carbon at δ 125.1. The signals of the sugar moiety were in good accordance with those reported for glucose. The value of the coupling constant suggested a β configuration for the anomeric carbon. Furthermore, the downfield value of the anomeric proton and the upfield shift of the corresponding carbon, in the respective spectra, suggested an ester bond between the glucose and the aglycon. In fact, the HMBC experiment showed heterocorrelations between the anomeric proton and the carboxyl at δ 168.7, which was also correlated with the methine proton at δ 7.32, bonded to the carbon at δ 141.6. The proton at δ 7.32 was also heterocorrelated with the tetrasubstituted olefinic carbon, the methines at δ 128.5 and 144.4. The carboxyl at δ 168.7, together with the quaternary olefinic carbon, showed correlations with the methyl protons at δ 1.93. The proton at δ 6.18 (H-5), correlated in the HSQC experiment to the carbon at δ 144.4, showed heterocorrelations with the methylene carbon at δ 37.2 and the methine at δ 39.9. This latter signal showed correlations with the doublet methyl at δ 0.90 and with the methylenes at δ 2.26 and 2.42, which were, finally, correlated to the further carboxyl group at δ 179.9. These data were in accordance with a C_{12} diene α,ω-diacid esterificated with a glucose moiety.

Compound **2** showed the same molecular formula as **1**, as inferred on the basis of the ^{13}C NMR spectrum, the ESI-MS spectrum, which had the pseudomolecular peak at m/z 427 $[M + Na]^+$, and elemental analysis. The ^{1}H NMR spectra showed differences relative to the upfield shift of the anomeric proton at δ 4.34 and the downfield shift of the H-7 and H-8 protons at δ 1.90 and 4.05, respectively. ^{13}C NMR showed the C-8 carbon at δ 82.5, suggesting a different location for the sugar moiety. In fact, the HMBC experiment showed correlations between the C-8 carbon and the anomeric proton and between the anomeric carbon and the H-8 proton at δ 4.05. The nature of the sugar was confirmed by an enzymatic hydrolysis of compound **2** with β-glucosidase. This compound has been isolated from the plant *Oryctanthus* sp. and reported as a VEGF receptor binding inhibitor (*21*).

The new compound **3** has been identified as the *Z* isomer of **2**. Elemental analysis, the ESI-MS spectrum, and the ^{13}C NMR spectrum indicated the same molecular formula, $C_{18}H_{28}O_{10}$, as the previous compounds. The differences were shown by the ^{1}H NMR and ^{13}C NMR chemical shifts. In the ^{1}H NMR spectrum, in fact, the doublet H-3 and the H-5 protons were shifted to δ 6.98 and 5.95, respectively. Major differences were shown in the ^{13}C NMR: the C-2−C-5 carbons of the conjugated

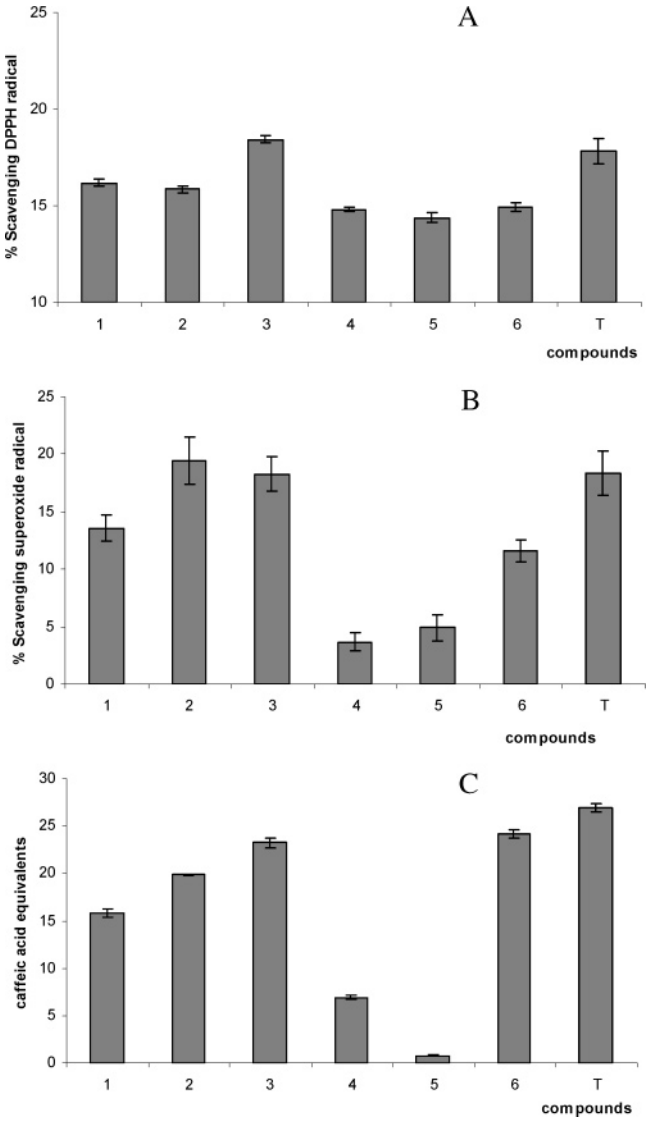

Figure 2. (**A**) DPPH radical scavenging activity of compounds **1**–**6**. Values are reported as percentages vs a blank. (**B**) Superoxide radical scavenging activity of compounds **1**–**6**. Values are reported as percentages vs a blank. (**C**) Evaluation of the antioxidant activity of compounds **1**–**6** by reduction of Mo(VI). Values are expressed as caffeic acid equivalents. In all parts, T = α-tocopherol.

dienic moiety resonated at δ 123.0, 133.7, 129.3, and 136.0. Furthermore, the C-6 carbon was upfield shifted at δ 32.6. These data were in accordance with a *Z* geometry for the C-2/C-3 double bond. Two-dimensional NMR data confirmed the scalar connections of the molecule. In particular, the NOESY experiment confirmed the hypothesized structure by showing an NOE effect between the H-12 methyl and the H-3 proton.

Compound **4** was identified as 3β-glucopyranosyloxy-β-ionone; the aglycon was previously isolated from the weed *Chenopodium album* (*22*).

Compound **5** showed the molecular formula $C_{25}H_{44}O_{12}$ on the basis of elemental analysis and the ^{13}C NMR spectrum, which showed signals for 25 carbons. The ESI-MS spectrum showed the pseudomolecular peak at m/z 559. The ^{1}H NMR spectrum showed two anomeric doublets at δ 4.43 and 4.36 besides other overlapped protons in the region of the proton geminal to oxygen, ranging from δ 3.15 to δ 4.12. In the aliphatic part of the spectrum three singlet methyls were

distinguishable at δ 1.64, 1.07, and 1.06, and a doublet methyl was shown at δ 1.17. In the DQ-COSY experiment the doublet methyl was correlated to the carbinol methine at δ 3.66, which showed cross-peaks with the methylene protons at δ 1.49. The latter correlated with the methylene protons at δ 2.05, in the DQ-COSY experiment, and with the carbons at δ 38.8 (C-1), 125.1 (C-5), and 138.5 (C-6), in the HMBC experiment. In the same experiment, the anomeric proton at δ 4.43 was correlated with the C-3 carbon at δ 73.3, and vice versa, the H-3 proton at δ 3.46 was correlated with the anomeric carbon at δ 102.2. The anomeric proton at δ 4.36 was correlated with the diasterotopic doublet of doublets at δ 4.12 and 3.81, attributed to the H-6′ protons on the basis of a TOCSY experiment. These data suggested the presence of a C_{13} norterpene bonded, across the C-3 carbon, with a gentiobiose moiety, and the HMBC correlations confirmed this hypothesis.

Compound **6** was identified as a C_{15} carotenoid metabolite that had previously been isolated from quince fruits (*23*).

The metabolites isolated from the ethanolic extract were tested for their antioxidant activity. Evaluation of antioxidant activity was performed using three different methods. Two of these methods estimate the radical scavenging activities of the investigated substances against the DPPH radical and the superoxide radical; the remaining test evaluates the capacity of the substances to induce the formation of a phosphomolybdenum complex. The standard used in all the methods was α-tocopherol, a known natural antioxidant, and the results are reported in **Figure 2**. The evaluation of the results allows us to state that the activity was influenced by chemical and stereochemical functions: the presence of the free carboxylic functions and the stereochemistry of the double bonds of the aglycon moiety. All of the metabolites showed weak DPPH-inhibiting radical activity (**Figure 2A**). The strongest activity was observed for compounds **1**−**3** (16.2%, 15.9%, and 18.4%, respectively). Compound **3**, characterized by *Z* geometry for the C-2/C-3 double bond, was more active than α-tocopherol. Among compounds **4**−**6**, metabolite **6** was more active. The activity seemed to be correlated to the presence of two unsaturations on the side chain and a free oxygenated function on the cyclohexenyl ring.

Moderate activity was observed when the superoxide radical ($O_2^{\bullet -}$) scavenging activity was tested (**Figure 2B**). Isomers **2** and **3** were more active compounds, inhibiting by 19.4% and 18.2% the production of superoxide radical in assay media. Although these activities could be considered insignificant, they assumed more meaning when we compared them with the activity shown by α-tocopherol (18.3%). Compounds **4** and **5** were inactive, while metabolite **6** showed an inhibition activity of only 12%.

Spectrophotometric quantitation of the antioxidant capacity of the tested metabolites through the formation of a phosphomolybdenum complex showed that the substances were able to induce the reduction of Mo(VI) to Mo(V). The reducing capacity of the compounds may serve as a significant indicator of their potential antioxidant activity. The results (**Figure 2C**), reported as equivalents of caffeic acid, demonstrated again that compound **2** and particularly metabolite **3** are responsible for the antioxidant activity.

The presence of new carotenoid-related compounds in *Cydonia* fruits with potential antioxidant activity and the bioactivity of C_{12} and C_{15} isoprenoids suggest a significant role of these compounds as health-protecting factors.

LITERATURE CITED

(1) Radisky, D. C.; Levy, D. D.; Littlepage, L. E.; Liu, H.; Nelson, C. M.; Fata, J. E.; Leake, D.; Godden, E. L.; Albertson, D. G.; Nieto, M. A.; Werb, Z.; Bissel, M. J. Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. *Nature* **2005**, *436*, 123−127.

(2) Houstis, N.; Rosen, E. D.; Lander, E. S. Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature* **2006**, *440*, 944−948.

(3) Fresquet, F.; Pourageaud, F.; Leblais, V.; Brandes, R. P.; Savineau, J.-P.; Marthan, R.; Muller, B. Role of reactive oxygen species and gp91phox in endothelial dysfunction of pulmonary arteries induced by chronic hypoxia. *Br. J. Pharmacol.* **2006**, *148*, 714−723.

(4) Manton, K. G.; Volovik, S.; Kulminski, A. ROS effects on neurodegeneration in Alzheimer's disease and related disorders: On environmental stresses of ionizing radiation. *Curr. Alzheimer Res.* **2004**, *1*, 277−293.

(5) Hu, F. B.; Willtee, W. C. Optimal diet for prevention of coronary heart disease. *JAMA, J. Am. Med. Assoc.* **2002**, *288*, 2569−2578.

(6) John, J. H.; Ziebland, S.; Yudkin, P.; Roe, L. S.; Neil, H. A. Oxford Fruit and Vegetable Study Group., Effect of fruit and vegetable consumption on plasma antioxidant concentrations and blood pressure: a randomised controlled trial. *Lancet* **2002**, *359*, 1969−1974.

(7) Paganga, G.; Miller, N.; Rice Evens, C. A. The polyphenolic content of fruit and vegetables and their antioxidant sctivities. What does a serving constitute? *Free Radical Res.* **1999**, *30*, 153−62.

(8) Cefarelli, G.; D'Abrosca, B.; Fiorentino, A.; Izzo, A.; Mastellone, C.; Pacifico, S.; Piscopo, V. Free radical scavenging and antioxidant activities of secondary metabolites from reddened cv. Annurca apple fruits. *J. Agric. Food Chem.* **2006**, *54*, 803−809.

(9) Cefarelli, G.; D'Abrosca, B.; Fiorentino, A.; Izzo, A.; Monaco, P. Isolation, characterization, and antioxidant activity of *E*- and *Z*-*p*-coumaryl fatty acid esters from cv. Annurca apple fruits. *J. Agric. Food Chem.* **2005**, *53*, 3525−3529.

(10) D'Abrosca, B.; Fiorentino, A.; Monaco, P.; Pacifico, S. Radical scavenging activity of new hydroxylated ursane triterpenes from cv. Annurca apples. *Chem. Biodiversity* **2005**, *2*, 953−958.

(11) D'Abrosca, B.; Fiorentino, A.; Oriano, P.; Monaco, P.; Pacifico, S. Annurcoic acid: a new antioxidant ursane triterpene from fruits of cv. Annurca apple. *Food Chem.* **2006**, *98*, 285−290.

(12) Yildirim, A.; Oktay, M.; Bilalo_lu, V. The antioxidant activity of the leaves of Cydonia vulgaris. *Turk. J. Med. Sci.* **2001**, *31*, 23−27.

(13) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. Technol.* **1995**, *28*, 25−30.

(14) Kulisic, T.; Radonic, A.; Katalinic, V.; Milos, M. Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chem.* **2004**, *85*, 633−640.

(15) Sroka, Z.; Cisowski, W. Hydrogen peroxide scavenging, antioxidant and antiradical activity of some phenolics acids. *Food Chem. Toxicol.* **2003**, *41*, 753−758.

(16) Dasgupta, N.; De, B. Antioxidant activity of *Piper betle* L. leaf extract in vitro. *Food Chem.* **2004**, *88*, 219−224.

(17) Prieto, P.; Pineda, M.; Aguilar, M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem.* **1999**, *269*, 337−341.

(18) Lu, Y.; Foo, L. Y. Antioxidant activities of polyphenols from sage (*Salvia officinalis*). *Food Chem.* **2001**, *75*, 197−202.

(19) Baldermann, S.; Naim, M.; Fleischmann, P. Enzymatic carotenoid degradation and aroma formation in nectarines (*Prunus persica*). *Food Res. Int.* **2005**, *38*, 833−836.

(20) Winterhalter, P.; Harmsen, S.; Trani, F. A C_{13}-norisoprenoid gentiobiose from quince fruit. *Phytochemistry* **1991**, *30*, 3021−3025.

(21) Hegde, V. R.; Pu, H.; Patel, M.; Jachens, A.; Gullo, V. P.; Chan, T.-M. A new compound from the plant *Oryctanthus* sp. as a VEGF receptor binding inhibitor. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4907−4909.

(22) DellaGreca, M.; Di Marino, C.; Zarrelli, A.; D'Abrosca, B. Isolation and phytotoxicity of apocarotenoids from *Chenopodium album. J. Nat. Prod.* **2004**, *67*, 1492−1495.

(23) Lutz, A.; Winterhalter, P. Isolation of additional carotenoid metabolites from quince fruit (*Cydonia oblonga* Mill.*). J. Agric. Food Chem.* **1992**, *40*, 1116−1120.

Received for review July 26, 2006. Revised manuscript received September 14, 2006. Accepted September 29, 2006.

JF062125E