



δ -Tocomonoenol: A new vitamin E from kiwi (*Actinidia chinensis*) fruits

Antonio Fiorentino^{a,*}, Claudio Mastellone^a, Brigida D'Abrosca^a, Severina Pacifico^a, Monica Scognamiglio^a, Giuseppe Cefarelli^a, Romualdo Caputo^b, Pietro Monaco^a

^a Dipartimento di Scienze della Vita, Laboratorio di Fitochimica, Seconda Università degli Studi di Napoli, via Vivaldi 43, I-81100 Caserta, Italy

^b Dipartimento di Chimica Organica e Biochimica, Università Federico II, Complesso Universitario di Monte Sant'Angelo-Via Cinthia 4, I-80126 Napoli, Italy

ARTICLE INFO

Article history:

Received 31 July 2008

Received in revised form 8 October 2008

Accepted 26 November 2008

Keywords:

Actinidia chinensis (kiwi)

δ -Tocomonoenol

Vitamin E

NMR analysis

GC–MS

Antioxidant activity

ABSTRACT

A new vitamin E, δ -tocomonoenol, has been isolated from *Actinidia chinensis* (kiwi) fruits. The new structure, 2,8-dimethyl-2-(4,8,12-trimethyltridec-11-enyl)chroman-6-ol, has been elucidated on the basis of EIMS, 1D, and 2D NMR spectral data. GC–MS analysis of peels and pulps of kiwi showed that the new compound, together with δ -tocopherol, is mainly present in the fruit peel, whilst α -tocopherol is present in a similar amount in both matrices. The compound was tested for its radical-scavenging and antioxidant capabilities, by measuring its ability to scavenge DPPH (2,2'-diphenyl-1-picrylhydrazyl radical) and anion superoxide radical, and inhibit the formation of methyl linoleate conjugated diene hydroperoxides and TBARS (thiobarbituric acid reactive species).

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1. Introduction

Reactive radical species are common by-products of aerobic metabolic processes, whose overproduction causes serious damage to biological membranes and macromolecules. In fact, the innate oxidant defence system is not sufficient to adequately deal with the amount of free radicals produced. In recent years increasing attention has turned to the role of diet in human health. Much research shows that a high plant products dietary intake is related to a reduced risk of chronic diseases. Thus food antioxidants are essential for health and well-being (Zhu, Hackman, Ensunsa, Holt, & Keen, 2002). It has been widely demonstrated that micronutrients such as vitamins C and E, carotenoids and phenolic compounds are able to scavenge free radicals and can delay or inhibit the oxidation of both lipids and other molecules by suppressing the initiation/propagation steps of oxidative chain reactions.

The phytochemical and antioxidant screening of fruits, such as *Malus domestica* cv. Annurca (Cefarelli, D'Abrosca, Fiorentino, Izzo & Monaco, 2005; Cefarelli et al., 2006; D'Abrosca, Fiorentino, Monaco & Pacifico, 2005; D'Abrosca, Fiorentino, Oriano, Monaco, & Pacifico, 2006), *M. domestica* cv. 'Limoncella' (D'Abrosca, Pacifico, Cefarelli, Mastellone & Fiorentino, 2007), *Cydonia vulgaris* (Fiorentino et al., 2006; Fiorentino et al., 2007; Fiorentino et al., 2008) and *Prunus cerasus* (Piccolella et al., 2008) showed that they are a rich source of metabolites with significant antioxidant capa-

bility towards free radicals. In the search for naturally occurring antioxidants, we report the isolation and chemical characterisation of a new vitamin E, from kiwi fruit, the edible berry of woody vine *Actinidia chinensis* (also known as *A. deliciosa*), besides α - and δ -tocopherol. The antioxidant capacity of the new compound was compared with that of accompanying tocopherols using five different methods, in order to evaluate its ability to act as DPPH and anion superoxide radical-scavenger, as well as its activity as anti-lipoperoxidant and Mo(VI) reducing agent.

2. Materials and methods

2.1. General experimental procedures

NMR spectra were recorded at 300 MHz for ¹H and 75 MHz for ¹³C on a Varian (Palo Alto, CA) 300 spectrometer Fourier transform NMR in CDCl₃ at 25 °C. The spectrum width was 2300 Hz. The initial matrix of 2 k × 2 k data points was zero-filled to give a final matrix of 4 k × 4 k points. The TOCSY experiments were performed in the phase-sensitive mode with a mixing time of 90 ms. The spectral width was 3000 Hz. The initial matrix of 512 × 512 data points was zero-filled to give a final matrix of 1 k × 1 k points. The NOESY experiments were performed in the phase-sensitive mode. The mixing time was 500 ms and the spectral width was 3000 Hz. Proton-detected heteronuclear correlations were measured using a gradient heteronuclear single-quantum coherence (HSQC), optimised for ¹J_{HC} = 140 Hz, and a gradient heteronuclear multiple bond coherence (HMBC), optimised for ²J_{HC} = 8 Hz. The

* Corresponding author. Tel.: +390823274576; fax: +39 0823274571.

E-mail address: antonio.fiorentino@unina2.it (A. Fiorentino).

HSQC experiment was performed in the phase-sensitive mode with field gradient. The spectral width was 22,000 Hz in f_1 (^{13}C) and 3200 Hz in f_2 (^1H) and the matrix of $1\text{ k} \times 1\text{ k}$ data points was zero-filled to give a final matrix of $2\text{ k} \times 2\text{ k}$ points. The HMBC experiment was performed in the absolute value mode with field gradient. The spectral width was 20,000 Hz in f_1 (^{13}C) and 1100 Hz in f_2 (^1H) and the matrix of $1\text{ k} \times 1\text{ k}$ data points was zero-filled to give a final matrix of $4\text{ k} \times 4\text{ k}$ points. UV spectra were measured on a UV-1700 Shimadzu (Kyoto, Japan) spectrophotometer in EtOH solutions. Optical rotations were measured on a Perkin-Elmer 141 (Perkin-Elmer Co., Norwalk, CT) in EtOH solutions. Electron impact mass spectra (EIMS) were obtained with a HP 6890 GC instrument (Agilent Technologies, Santa Clara, CA) equipped with a 5973N mass spectrometer. The GC was equipped with a fused silica capillary column (Zebtron ZB-5MS, $30\text{ m} \times 0.25\text{ mm}$ i.d., film thickness $0.25\text{ }\mu\text{m}$, Phenomenex, Torrance, CA), with He as carrier gas (flow 1.2 ml/min). The column head pressure was set at 10.27 psi . Temperature conditions were as follows: injector port at $250\text{ }^\circ\text{C}$; initial oven temperature $60\text{ }^\circ\text{C}$ for 1 min, then increased at $20\text{ }^\circ\text{C/min}$ to $180\text{ }^\circ\text{C}$, then increased at $2.5\text{ }^\circ\text{C/min}$ to $275\text{ }^\circ\text{C}$ and held for 23 min. A $5\text{ }\mu\text{l}$ volume sample was injected in splitless mode. Full-scan mass spectra were collected between 15 and 600 amu at 2 scan/s . The MS was operated in the electron impact (EI) ionisation mode with electron energy of 70 eV . The ion source and quadrupole temperatures were maintained at 230 and $150\text{ }^\circ\text{C}$, respectively.

The preparative HPLC apparatus consisted of a pump (Shimadzu LC-10AD), a refractive index detector (Shimadzu RID-10A) and a Shimadzu Chromatopac C-R6A recorder. Preparative HPLC was performed using an RP-8 (Luna $10\text{ }\mu\text{m}$, $250 \times 10\text{ mm}$ i.d., Phenomenex) column. The flow was 1.5 ml/min . Analytical TLC was performed on Merck Kieselgel 60 F_{254} or RP-8 F_{254} plates with 0.2 mm layer thickness. Spots were visualised by UV light or by spraying with $\text{H}_2\text{SO}_4/\text{AcOH}/\text{H}_2\text{O}$ (1:20:4). The plates were then heated for 5 min at $110\text{ }^\circ\text{C}$. Preparative TLC was performed on Merck Kieselgel 60 F_{254} plates, with 0.5 or 1 mm film thickness. Column chromatography (CC) was performed on Merck Kieselgel 60 (70–240 mesh) or NH_2 -silica (LiChroprep NH_2 , $40\text{--}63\text{ }\mu\text{m}$; Merck, Darmstadt, Germany).

2.2. Fruit samples collection

Kiwi (*A. chinensis*) fruits were collected in Rotondi, near Caserta (Italy), in October 2007, just after their harvest. Fruits were stored in a climatic cell at $4\text{ }^\circ\text{C}$ and 98% humidity.

2.3. Extraction procedure

The kiwi fruits (17.0 kg) were sliced and peels (4.2 kg) dried in a ventilated incubator at $45\text{ }^\circ\text{C}$ overnight. The dried material (1.1 kg) was infused in hexane for 5 days in a refrigerated chamber at $4\text{ }^\circ\text{C}$ in the dark. After removal of the solvent, a crude organic extract (8.1 g) was obtained.

2.4. Organic extract fractionation

The whole hexane extract was chromatographed on silica gel and eluted with petroleum ether (PE)/ Me_2CO solutions. The fraction eluted with PE/ Me_2CO (9:1) was rechromatographed by NH_2 -silica CC using PE/EtOAc solutions and collecting 5 ml fractions. Fractions 7–11, eluted with PE/EtOAc (16:1), contained α -tocopherol ($2, 45\text{ mg}$); fractions 31–38 eluted with PE/EtOAc (47:3) gave a fraction which, when purified by RP8 HPLC eluting with $\text{MeOH}/\text{MeCN}/\text{H}_2\text{O}$ (15:4:1), contained pure compound **1** (22 mg , $\text{RT} = 20.1\text{ min}$) and **3** (30 mg , $\text{RT} = 24.0\text{ min}$). Compound **1** was 2,8-dimethyl-2-(4,8,12-trimethyltridec-11-enyl)chroman-6-ol δ -tocomonoenol):

Table 1

NMR data of δ -tocomonoenol (**1**) registered in CDCl_3 at 300 MHz (for ^1H) and 75 MHz (for ^{13}C). J values (Hz) are reported in brackets. *brs* = broad singlet, *d* = doublet, *s* = singlet, *ov* = overlapped; *m* = multiplet, and *t* = triplet.

Position	$\delta\text{ }^1\text{H}$	COSY H \rightarrow H	DEPT	$\delta\text{ }^{13}\text{C}$	HMBC H \rightarrow C
2	–	–	C	75.6	–
3	1.72 <i>m</i> 1.83 <i>m</i>	4	CH_2	31.3	2, 4 2, 4
4	2.69 <i>t</i> (6.9)	3	CH_2	22.5	2, 3, 5, 9, 10
5	6.38 <i>d</i> (2.4)	6	CH	112.5	6, 7, 9
6	–	–	C	147.6	–
7	6.47 <i>d</i> (2.4)	5	CH	115.6	5, 6, 9
8	–	–	C	127.3	–
9	–	–	C	146.0	–
10	–	–	C	121.3	–
1'	1.52 <i>m</i>	2'	CH_2	39.9	17'
2'	1.29 <i>ov</i>	1'	CH_2	20.9	–
3'	1.25 <i>ov</i>	–	CH_2	37.1	–
4'	1.40 <i>ov</i>	–	CH	32.4	–
5'	1.25 <i>ov</i>	–	CH_2	37.3	–
6'	1.29 <i>ov</i>	–	CH_2	24.4	–
7'	1.25 <i>ov</i>	–	CH_2	37.4	–
8'	1.40 <i>ov</i>	–	CH	32.7	–
9'	1.25 <i>ov</i>	–	CH_2	37.4	–
10'	1.92 <i>m</i>	11'	CH_2	25.6	–
11'	5.12 <i>t</i> (7.2)	10'	CH	125.1	13', 14'
12'	–	–	C	130.9	–
13'	1.61 <i>s</i>	–	CH_3	25.7	11', 12', 14'
14'	1.69 <i>s</i>	–	CH_3	17.6	11', 12', 13'
15'	0.85 <i>d</i> (6.6)	8'	CH_3	19.9	7', 8', 9'
16'	0.86 <i>d</i> (6.3)	4'	CH_3	19.6	3', 4', 5'
17'	1.24 <i>s</i>	–	CH_3	24.1	1', 2, 3
Me	2.12 <i>s</i>	–	CH_3	16.0	7, 8
OH	4.22 <i>brs</i>	–	–	–	–

UV (EtOH) λ_{max} ($\log \epsilon$) 297.0 (3.53), $[\alpha]_{\text{D}}^{20} - 1.18$ ($c = 0.25$, EtOH); EIMS: m/z 400 $[\text{M}]^+$, 177, 137, 69; Anal. Calcd for $\text{C}_{27}\text{H}_{44}\text{O}_2$: C, 80.94; H, 11.07. Found: C, 80.91; H, 11.10. ^1H NMR (300 MHz , CDCl_3) and ^{13}C NMR (75 MHz , CDCl_3): see Table 1.

2.5. Methylation of compound 1

To a solution of **1** (2.5 mg in 3 M ethanolic KOH), dimethyl sulphate ($25\text{ }\mu\text{l}$) was added. The mixture was kept under magnetic stirring for 12 h at room temperature. After acidification of the solution with 2 M HCl, the reaction product was extracted with CHCl_3 in a separating funnel.

2.6. GC–MS analyses

Aliquots (about 100 mg) of both lyophilised peels and pulps of kiwi fruits were extracted by ultrasound-assisted extraction using a ultrasound bath (Elma, TP680DH) for 1 h with 1 ml of hexane/EtOAc (17:3) solution containing $50\text{ }\mu\text{g/ml}$ of BHT (butylated hydroxytoluene) as antioxidant. The extraction was repeated three times for each sample. After removal of the solvent the residue was dissolved in heptane ($500\text{ }\mu\text{l}$) and analysed by GC–MS. The compounds concentration was determined by calibration curves of the three pure standards. The concentrations were calculated in $\text{mg}/100\text{ g}$ of fresh weight using the average peak area compared between standard and sample after triplicate injections.

2.7. DPPH radical-scavenging capacity

The DPPH radical-scavenging capacity of metabolites was measured according to the method of Brand-Williams, Cuvelier, and Berset (1995). Pure isolated metabolites ($100\text{ }\mu\text{l}$, 0.4 mM) were added to a DPPH radical (Fluka, Buchs, Switzerland) methanolic solution (1.40 ml , 10^{-4} M). The absorbance at 517 nm was

measured at 30 min vs. blank (100 μ l MeOH in 1.40 ml of DPPH radical solution) using a UV-1700 Shimadzu spectrophotometer. The analysis was carried out in triplicate and the results were expressed in terms of percentages of radical-scavenging capacity (RCS).

2.8. Superoxide radical-scavenging capacity

The assay of superoxide radical-scavenging capacity was based on the capacity of each isolated metabolite (0.4 mM) to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) (Fluka, Buchs, Switzerland) in the riboflavin-light-NBT system (Dasgupta & De, 2004). The reaction mixture was prepared from 13 mM methionine (Fluka), 2 μ M riboflavin (Riedel-de Haën, Seelze, Germany), 100 μ M EDTA (Carlo Erba Reagents, Rodano, Milano, Italy), 75 μ M NBT and 100 μ l sample solution in 50 mM sodium phosphate buffer (pH 7.8). The reaction was monitored through the increase in absorbance at 560 nm after 10 min illumination by a fluorescent lamp. The tests were carried out in triplicate and the results were expressed in terms of percentages of radical-scavenging capacity (RCS).

2.9. Evaluation of total antioxidant capacity

The spectrophotometric evaluation of antioxidant capacity through formation of a phosphomolybdenum complex was carried out according to Prieto, Pineda, and Aguilar (1999). Sample solutions (100 μ l) containing the reducing metabolites (0.4 mM) were combined in eppendorf tubes with 1.0 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated in a thermal block at 95 °C for 90 min and then cooled to room temperature. The absorbances of samples were measured at 820 nm against a blank. The tests were carried out in triplicate and the antioxidant capacities were expressed as caffeic acid equivalents (CAE).

2.10. Inhibition of auto-oxidation of methyl linoleate

The auto-oxidation of methyl linoleate results in conjugated diene (CD) hydroperoxides (Kähkönen et al., 1999). Methyl linoleate (0.1 mmol) containing the single metabolites under investigation (100 μ l, 0.4 mM) was placed in a test tube and incubated at 60 °C in the dark. After 48 h, each sample was dissolved in 1.0 ml of ethanol. The formation of hydroperoxides was spectrophotometrically monitored by measuring the formation of conjugated diene hydroperoxides at 232 nm. The antioxidant capacities were expressed as percentages of methyl linoleate conjugated diene hydroperoxides vs. an authentic sample (100%).

2.11. Determination of TBARS

Determination of thiobarbituric acid reactive substances (TBARS) was performed according to the method reported by Sroka and Cisowski (2003) with some modifications. Vegetable fat (Riedel-de Haën, 10.0 μ l) was emulsified with 30.0 mg of Tween-40 (Fluka Chemie) initially dissolved in 1.5 ml of 0.2 M Tris-HCl buffer, pH 7.4. The emulsion was stirred for 24 h and then irradiated with UV light at 254 nm at room temperature for 60 min. Then, the single metabolites under investigation (100 μ l, 0.4 mM) were added and the reaction mixtures were exposed to UV light for 60 min more. TBA reagent (2.0 ml), prepared dissolving 375 mg of thiobarbituric acid, 30 mg of tannic acid and 15 mg of trichloroacetic acid in aqueous HCl (100 ml, 0.2 M), was then added. The test tubes were placed into a boiling water bath for 60 min and then centrifuged using a Beckman GS-15R centrifuge for 3 min at 3500 g. The supernatant was analysed by reading the percentage

of lipid peroxidation against a blank, at 532 nm. The analyses were carried out in triplicate.

3. Results and discussion

Peels of kiwi fruits were dried in a ventilated incubator at 45 °C overnight. From the hexane extract of the dried material, besides α -tocopherol (**2**) and δ -tocopherol (**3**), a new vitamin E, named δ -tocomonoenol (**1**), was isolated and identified on the basis of its spectroscopic features (Fig. 1).

The compound showed a molecular formula $C_{27}H_{44}O_2$ in agreement with elemental analysis and spectral data, suggesting the presence of six unsaturations in the molecule. The EIMS spectrum showed the molecular peak at m/z 400 and fragment peaks at m/z 177, 137 and 69. The 1H NMR spectrum showed, in the downfield region, two protons at δ 6.47 and 6.38, besides a triplet at δ 5.12. The presence of a peak at δ 4.22 exchangeable with D_2O , indicated the presence in the molecule of an OH group. In the upfield of the spectrum six methyl signals were evident as four singlets at δ 2.12, 1.69, 1.61 and 1.24 and two doublets at δ 0.86 and 0.85, besides two methylenes at δ 2.69 and 1.92, and other overlapped protons.

The ^{13}C NMR spectrum showed 27 carbons, identified, on the basis of a DEPT experiment, as six methyls, 10 methylenes, five methines and six tetrasubstituted carbons. The band at 297 nm in the UV-Vis spectrum suggested the presence of an aromatic ring, confirmed by the presence of aromatic carbons in the ^{13}C NMR. The HMBC experiment showed correlation between carbon at δ 147.6 with the protons at δ 6.38 and 6.47, which, in their turn, are correlated (HSQC) with the aromatic carbons at δ 112.5 and 115.6; the signal at δ 6.38 correlated with the carbons at δ 121.3, 146.0, whilst the proton at δ 6.47 showed cross peaks with the carbons at δ 146.0 and 127.3, which was in turn correlated with the methyl at δ 2.12. The homocorrelations between these protons in a COSY experiment indicated their *meta* position in a 1,2,3,5-tetrasubstituted aromatic ring. The carbons at δ 112.5 and 121.3 were both correlated with the methylene protons at δ 2.69 (bonded to the carbon at δ 22.5), which showed cross peaks with the carbons at δ 31.3 and 75.6. The latter carbon was also correlated to the methyl at δ 1.24 and the methylene at δ 1.52. These data were in good agreement with the presence of a 6-hydroxy-2,7-dimethylchromane moiety in the molecule. In order to confirm the structure, an aliquot of **1** was treated with dimethyl sulphate in ethanolic KOH. The NOESY experiment on the *O*-methyl derivative showed NOE between the methoxyl group and both the aromatic protons as well as between the aromatic proton at δ 6.47 and the methyl singlet at δ 2.12.

Furthermore, the olefin proton at δ 5.12, bonded to the carbon at δ 125.1, showed long range heterocorrelations with the quaternary

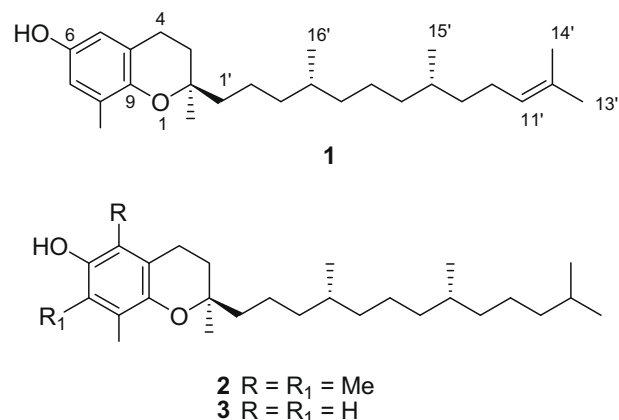


Fig. 1. δ -Tocomonoenol (**1**) and tocopherols isolated from *Actinidia chinensis*.

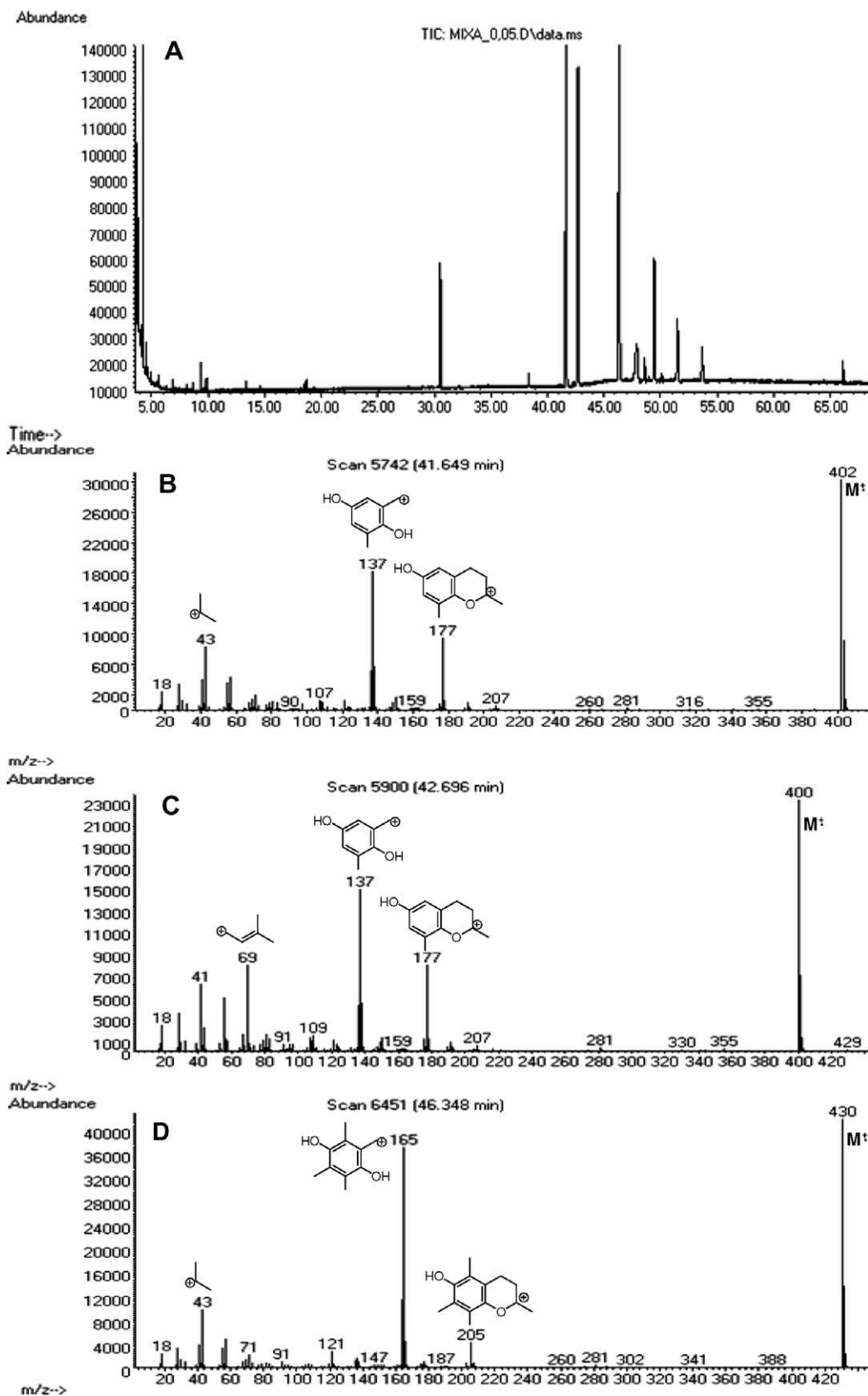


Fig. 2. A: Total ion current (TIC) chromatogram; B: EIMS spectrum of δ -tocopherol (3); C: EIMS spectrum of δ -tocomonoenol (1); D: EIMS spectrum of α -tocopherol (2).

carbon at δ 130.9, and with the methyls at δ 17.6 and 25.7 as well. The corresponding protons at δ 1.69 and 1.61 showed cross peaks with the carbons at δ 125.1, 130.9, respectively, and with the

mutual methyl carbons. These data suggested the presence of a terpenoidic chain with a double bond between the last two carbons. The presence of the peak at m/z 69 in the EIMS spectrum,

due to the 3-methylbut-2-en-1-ylm cation confirmed this hypothesis.

The spectral data were in good accordance with the presence of 2,8-dimethyl-2-(4,8,12-trimethyltridec-11-enyl)chroman-6-ol. The configurations of the C-2, C-4' and C-8' carbons were tentatively assigned on the basis of biogenetic considerations.

In order to investigate the presence of δ -tocomonoenol (**1**), and the other tocopherols in the kiwi fruits, a GC–MS analysis was performed. Aliquots of lyophilised pulps and peels were extracted in hexane/EtOAc (17:3) solution, in the presence of BHT. The extracts were dried, resuspended in heptane, and analysed by GC–MS. All the analyses were carried out in triplicate. The identification of the analytes was obtained by comparison of the retention times with those of pure standards (Fig. 2A) and by the fragmentation patterns in the EIMS spectra. In fact, in the EIMS spectra of δ -tocopherol (Fig. 2B), besides the molecular peak at m/z 402, were identified peaks at m/z 177, due to the fragmentation of C2–C1' bond with formation of a 6-hydroxy-2,8-dimethylchroman-2-ylm cation, and at m/z 137, corresponding to a (2,5-dihydroxy-3-methylphenyl)methylm cation. The peak at m/z 43 was attributed to an isopropylm cation coming from the breaking of the C11'–C12' bond. Analogously, in the EIMS spectrum of δ -tocomonoenol (Fig. 2C), were evident the same peaks at m/z 177 and 137, due to the chromane moiety of the molecule, and a diagnostic peak at m/z 69 due to allylic 3-methylbut-2-en-1-ylm cation generated by the fragmentation of the C9'–C10' bond. Finally, in the EIMS spectrum of α -tocopherol (Fig. 2D) peaks at m/z 205 and 165 were evident, due to 6-hydroxy-2,5,7,8-tetramethylchroman-2-ylm and (2,5-dihydroxy-3,4,6-trimethylphenyl)methylm cations. The results of the qualitative GC–MS analysis are reported in Table 2. α -Tocopherol was present in the same amounts in both peels and pulps, but δ -tocopherol and δ -tocomonoenol were more abundant in the peels than in the flesh.

The antioxidant capacities of compounds **1–3** were assessed using five different methods. Radical-scavenging capacity of the investigated substances was evaluated against both DPPH radical and anion superoxide radical. Antioxidant capacity was evaluated by measuring the formation of methyl linoleate conjugated diene hydroperoxides and of TBARS substances. Both methods are commonly used to evaluate the molecule/extract antioxidant efficacy in biological and food systems (Kulisic, Radonic, Katalinic, & Milos, 2004). The capacity of compounds **1–3** to induce formation of phosphomolybdenum (V) complex was also investigated. The results are reported in Table 3.

Table 2

Amount of δ -tocomonoenol (**1**), δ -tocopherol (**3**), and α -tocopherol (**2**) found in pulp and peel of kiwi fruits. Values are reported as mg/100 g of fresh weight.

	α -Tocopherol	δ -Tocopherol	δ -Tocomonoenol
Pulp extract	1.02 \pm 0.03	0.64 \pm 0.01	0.85 \pm 0.02
Peel extract	1.05 \pm 0.06	2.49 \pm 0.12	1.45 \pm 0.08

Table 3

Radical-scavenging capacities (RSC, \pm SD) and antioxidant capacities (\pm SD) of δ -tocomonoenol (**1**), α -tocopherol (**2**) and δ -tocopherol (**3**) from kiwi fruit.

	DPPH-RSC (%) ^b	O ₂ -RSC (%) ^b	Hydroperoxide conjugate dienes formation (%) ^b	TBARS determination (%) ^b	Total antioxidant capacity (CAE ^a)
1	23.96 \pm 1.60	29.20 \pm 2.28	26.88 \pm 0.87	46.60 \pm 1.12	0.13 \pm 0.01
2	25.21 \pm 0.50	27.07 \pm 0.99	33.08 \pm 0.46	53.01 \pm 1.24	0.16 \pm 0.01
3	23.40 \pm 0.75	29.73 \pm 1.57	25.48 \pm 0.77	43.17 \pm 1.14	0.15 \pm 0.01

^a CAE = Caffeic acid equivalent.

^b Values are presented as percentage differences vs. blank.

The radical-scavenging capacities of δ -tocomonoenol (**1**) and δ -tocopherol (**3**) appear quite similar. In particular, δ -tocomonoenol (**1**) was able to reduce the DPPH radical by 24% and the anion superoxide radical by 29.2%. The auto-oxidation of lipid substrates, such as methyl linoleate, was measured by the formation of conjugated diene hydroperoxides. All the three compounds inhibited the lipoperoxidative process; α -tocopherol was the most effective molecule showing an antioxidant power of 33%. δ -Tocopherol was slightly less active than the corresponding tocomonoenol form. The effect of the metabolites on lipid peroxidation, estimated by measuring thiobarbituric acid reactive substances (TBARS), confirmed the strong antioxidant efficacy of α -tocopherol.

Spectrophotometric quantitation of the antioxidant capacity of the tested metabolites, through the formation of a phosphomolybdenum complex, whose results are reported as equivalents of caffeic acid, showed for all of them a significant propensity to induce reduction of Mo(VI) to Mo(V).

The regular dietary uptake of antioxidant vitamins is believed to be effective in the prevention or delaying of the oxidative stress-related diseases (cardiovascular disease, atherosclerosis, Alzheimer's disease and cancer) (Frei, 2004). Recently, vitamin E has become a topic of increasing interest as a health promoter. The term vitamin E is used for a family of eight fat-soluble and structurally related molecules, tocopherols and tocotrienols (Schneider, 2005), characterised by a chromanol ring possessing a different pattern of methyl groups at the positions 5, 7 and 8 in the diverse naturally occurring forms. The four tocopherol forms (α -, β -, γ -, and δ -) display in addition a 16-carbon saturated phytol side chain; where the tocotrienols display an unsaturated C16 isoprenoid chain. It is widely demonstrated that all vitamin E molecules have antioxidant activity, although α -tocopherol is chemically and biologically the most active. The interception of the lipid autooxidation radical chain process seems to be the rationale of the antioxidant power of α -tocopherol. In fact, it reacts quickly with the primary products of lipoperoxidation and takes away the radical character from the oxidising fatty acid, preventing further radical reactions.

Clinical trials have focused on assessing the effect of vitamin E as a natural cancer fighter. Several studies have shown that vitamin E consumption decreases the risk of prostate, colon and lung cancers (Giovannucci, 2000; Stone & Pappas, 1997; Woodson et al., 1999). Plant foods are the main source for dietary uptake of vitamin E whose daily value (DV), suggested by US Food and Drug Administration, is 12–15 International Units (1 IU = 1 mg of α -tocopherol).

In conclusion, our work reported the identification and characterisation of a new tocopherol analogue in the kiwi fruit. Data on its radical-scavenging and antioxidant capacities showed its involvement in the total antioxidant activity generally attributed to this fruit.

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