DOI:10.1021/jf9024147

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Floral Markers of Strawberry Tree (*Arbutus unedo* L.) Honey

Carlo I. G. Tuberoso,*,† Ersilia Bifulco,† Pierluigi Caboni,†
Filippo Cottiglia,§ Paolo Cabras,† and Ignazio Floris#

†Dipartimento di Tossicologia, and §Dipartimento Farmaco Chimico Tecnologico, University of Cagliari, via Ospedale 72, 09124 Cagliari, Italy, and #Dipartimento Protezione Piante, Sez. Entomologia Agraria, University of Sassari, Via E. De Nicola, 07100 Sassari, Italy

Strawberry tree honey, due to its characteristic bitter taste, is one of the most typical Mediterranean honeys, with Sardinia being one of the largest producers. According to specific chemical studies, homogentisic acid was identified as a possible marker of this honey. This work, based on HPLC-DAD-MS/MS analysis of strawberry tree (*Arbutus unedo* L.) honeys, previously selected by sensory evaluation and melissopalynological analysis, showed that, in addition to the above-mentioned acid, there were other high levels of substances useful for the botanical classification of this unifloral honey. Two of these compounds were isolated and identified as (±)-2-*cis*,4-*trans*-abscisic acid (*c,t*-ABA) and (±)-2-*trans*,4-*trans*-abscisic acid (*t,t*-ABA). A third compound, a new natural product named unedone, was characterized as an epoxidic derivative of the above-mentioned acids. Structures of *c,t*-ABA, *t,t*-ABA, and unedone were elucidated on the basis of extensive 1D and 2D NMR experiments, as well as HPLC-MS/MS and Q-TOF analysis. In selected honeys the average amounts of *c,t*-ABA, *t,t*-ABA, and unedone were 176.2 ± 25.4, 162.3 ± 21.1, and 32.9 ± 7.1 mg/kg, respectively. Analysis of the *A. unedo* nectar confirmed the floral origin of these compounds found in the honey. Abscisic acids were found in other unifloral honeys but not in such high amount and with a constant ratio of about 1:1. For this reason, besides homogentisic acid, these compounds could be used as complementary markers of strawberry tree honey.

KEYWORDS: *Arbutus unedo* L. honey; botanical characterization; abscisic acids; unedone; HPLC-DAD; HPLC-MS/MS; Q-TOF; NMR

INTRODUCTION

Strawberry tree (*Arbutus unedo* L., Ericaceae) unifloral honey is a typical product of some Mediterranean regions, and the island of Sardinia is probably the largest producer in the world. Because of the characteristic taste, this honey is known as "bitter honey". The honey flow period is late autumn, during the flowering of strawberry tree shrubs, when unfavorable weather conditions often remarkably limit production. Due to the low production per hive and the presumed biological properties, the bitter honey is of remarkable economic importance and its market price is from 4 to 8 times higher than that of a sweet honey. This honey has been traditionally employed mainly for curative aims since ancient times, as was attested in the the ancient Greek and Roman world by Dioscoride in his treaty on the medical matter (*1*). The sensory characteristics are easily recognizable: it is an amber-colored when liquid and beige-brown when crystallized; the smell is intense, characteristic, ripe, and similar to that of coffee; the taste is a bit sweet initially and decidedly bitter and astringent later. On the contrary, the melissopalynological approach shows several limits in the botanical classification of this honey. In fact, *Arbutus* pollen is normally underrepresented in the bitter honey and the frequent incidence of overrepresented "contaminating" pollens, such as *Eucalyptus* ones, causes a wide variation in the absolute number of pollen grains in the sediment and also in the percentages of *Arbutus* pollen (*2*). To overcome the limits of melissopalynological methods in the botanical classification of this unifloral honey, chemical investigation of the phenolic fraction highlighted homogentisic acid as a marker of strawberry tree honey (*3*). Moreover, volatile (*4*, *5*) and semivolatile (*6*) fractions showed the presence of several norisoprenoid compounds that are useful to characterize such a honey.

The aim of this work was to investigate strawberry tree honey in order to evaluate the presence of further characteristic compounds.

MATERIALS AND METHODS

Honey and Nectar Samples and Melissopalynological Analyses. Strawberry tree honey samples (n = 25) were collected in triplicate from professional beekeepers in different areas of Sardinia (Italy) during the period 2005−2007 (**Table 1**). Samples were stored at 4 °C in dark glass bottles until analysis. Strawberry tree nectars (n = 4) were collected from flowers growing in three characteristic shrubland areas with strawberry tree as dominant essence, located in northern, central, and southern Sardinia, respectively. The nectars were collected in winter 2007 from 4 to 12 p.m. during the main nectar flow (*1*), from about 2500 flowers for each sample using homemade pipets with two bulbs (*7*). Qualitative and quantitative melissopalynological analyses were carried out following the method of the International Commission of Bee Botany (*8*).

*Author to whom correspondence should be addressed (fax +39 070 6758612; e-mail tuberoso@unica.it).

 Published on Web 11/18/2009

Table 1. Characteristics of the *Arbutus unedo* L. Honey and Nectar Samples[a]

sample	year of production	*Arbutus* pollen (%)	water (g/100 g)	homogentisic acid (mg/kg)	unedone (mg/kg)	*t*,*t*-ABA (mg/kg)	*c*,*t*-ABA (mg/kg)
honey 1	2005	36.1	17.3	330.6	28.6	163.9	185.1
honey 2	2005	28.2	18.7	417.5	36.9	191.6	223.0
honey 3	2005	44.5	16.9	331.3	22.8	137.4	148.3
honey 4	2005	51.9	17.7	336.7	19.2	147.7	151.3
honey 5	2005	21.4	16.2	462.4	29.7	207.6	227.4
honey 6	2005	46.3	17.4	379.6	21.9	188.9	198.7
honey 7	2006	34.6	19.4	350.6	26.8	161.8	177.3
honey 8	2006	22.6	18.3	329.6	38.7	168.6	180.8
honey 9	2006	14.5	16.8	383.3	37.4	182.1	202.1
honey 10	2006	40.7	18.7	422.2	31.2	172.9	208.3
honey 11	2006	60.4	16.2	374.2	28.3	166.4	183.3
honey 12	2006	38.0	19.1	401.1	31.3	123.8	127.1
honey 13	2007	42.8	18.1	454.7	36.9	162.2	172.4
honey 14	2007	19.2	18.2	590.3	47.1	189.4	203.8
honey 15	2007	23.6	17.3	490.0	36.0	136.1	141.4
honey 16	2007	55.0	16.1	432.0	35.9	137.8	146.3
honey 17	2007	69.6	16.6	522.1	33.7	147.6	164.9
honey 18	2007	76.3	19.6	481.4	33.8	163.0	172.4
honey 19	2007	20.3	16.2	352.9	36.5	165.4	174.5
honey 20	2007	38.0	18.1	481.7	39.4	192.2	201.2
honey 21	2007	7.3	15.4	358.8	30.7	155.2	162.8
honey 22	2007	51.6	19.8	325.2	19.6	158.4	169.4
honey 23	2007	28.0	17.7	424.6	42.4	143.5	160.1
honey 24	2007	65.4	17.3	482.4	37.2	154.7	159.2
honey 25	2007	18.3	16.6	437.7	40.9	138.3	164.8
total av		38.2 ± 18.3	17.6 ± 1.2	414.1 ± 69.8	32.9 ± 7.1	162.3 ± 21.1	176.2 ± 25.4
2005 av		38.1 ± 11.7**a**	17.4 ± 0.8**a**	376.4 ± 54.4**a**	26.5 ± 6.5**a**	172.8 ± 27.5**a**	189.0 ± 34.1**a**
2006 av		35.1 ± 15.9**a**	18.1 ± 1.3**a**	376.8 ± 33.5**a**	32.3 ± 4.8**ab**	162.6 ± 20.2**a**	179.8 ± 28.7**a**
2007 av		39.6 ± 22.3**a**	17.5 ± 1.3**a**	418.4 ± 64.4**b**	36.2 ± 8.6**b**	157.2 ± 17.9**a**	168.7 ± 17.8**a**
nectar 1	2007		74.6	142.9	18.9	89.3	108.6
nectar 2	2007		86.2	92.6	15.4	82.8	105.4
nectar 3	2007		90.2	135.4	15.6	40.5	50.0
nectar 4	2007		87.5	111.0	12.9	40.1	47.9
total av			84.6 ± 6.9	120.5 ± 23.0	15.7 ± 2.5	63.2 ± 26.6	78.0 ± 33.6

[a] Means ± SD in each column followed by different letters are significantly different at $P < 0.05$ (GLM ANOVA followed LSD test). Values are means of triplicate determinations.

Chemicals. Methanol, acetonitrile, ethyl acetate, acetic acid, 85% phosphoric acid, and silica gel preparative TLC plates (20 × 20 cm, 60 F254) were purchased from Merck (Darmstadt, Germany). Standards of (±)-2-*cis*,4-*trans*-abscisic acid and homogentisic acid were purchased from Sigma-Aldrich (Milan, Italy). Standard of (±)-2-*trans*,4-*trans*-abscisic acid was purchased from A.G. Scientific, Inc. (San Diego, CA). All of the chemicals used in this study were of analytical grade. Ultrapure water (18 mΩ) was distilled and then purified with a Milli-Q Advantage A10 System apparatus (Millipore, Milan, Italy).

Honey Extraction, TLC Separation, and Preparative HPLC-DAD. Honey was extracted with ethyl acetate as reported from Tuberoso et al. (*9*), and the extract was used both for the preparative thin-layer chromatography (TLC) and for the preparative HPLC-DAD.

TLC on silica gel plates were developed in hexane/ethyl acetate/formic acid (3:6:1, v/v/v). Individual bands (detected under UV light at 254 and 366 nm) were scraped and extracted with ethyl acetate, and the organic layer was dried under a gentle nitrogen flow. Residue was dissolved in the eluting mixture, filtered to remove silica gel traces (Acrodisc CR 13 mm, 0.45 μm PTFE membrane, Pall Life Science, Varese, Italy), and injected for HPLC-DAD analysis. Extraction procedure was applied on 500 g of honey using 25 TLC plates, and 9 mg of compound with R_f = 0.84 and HPLC-DAD t_R = 14.8 min was obtained.

A preparative HPLC-DAD was performed to separate the *c*,*t*-ABA from its isomer *t*,*t*-ABA. A Varian system ProStar HPLC was employed, fitted with a pump module 230, an autosampler module 410 with a 100 μL loop, and a ThermoSeparation diode array detector SpectroSystem UV 6000lp (ThermoSeparation, San Jose, CA) set at 280 nm. Separation was obtained with a Polaris C8-A column (250 × 100 mm, 5 μm, Varian, Milan, Italy) using 0.2 M phosphoric acid and acetonitrile 70:30 (v/v) as mobile phase at a constant flow rate of 3.0 mL/min. The injected samples were obtained from the honey extract after ethyl acetate evaporation and dissolution in water/acetonitrile 70:30 (v/v). Eluent mixtures corresponding to retention times of 21.9 min (*t*,*t*-ABA) and 23.1 min (*c*,*t*-ABA) were collected in two screw-capped 40 mL tubes. After 50 HPLC injections, 5 mL of ethyl acetate and 0.5 g of NaCl were added to both of the two collected eluent mixtures, and then the organic phase was recovered. Pure compounds (10 mg each) were obtained after the ethyl acetate was dried under a gentle nitrogen flow.

HPLC-DAD Analysis. The analysis was performed using the same HPLC-DAD previously described, but fitted with a 10 μL loop. Separation was obtained with a Phenomenex Synergi Hydro-RP 80A C18 column (150 × 4.60 mm, 4 μm, Chemtek Analitica, Anzola Emilia, Bologna, Italy) using 0.2 M phosphoric acid (solvent A) and acetonitrile (solvent B) as mobile phase at a constant flow rate of 1.0 mL/min. The gradient (v/v) was generated by keeping 90% of solvent A for 5 min, then decreasing to 65% in 15 min and to 10% in 20 min, and remaining at this concentration for 10 min. Before each injection, the system was stabilized for 10 min with the initial A/B ratio (90:10, v/v). Chromatograms and spectra were elaborated with a ChromQuest V. 2.51 data system (ThermoQuest, Rodano, Milan, Italy).

Honey and nectar samples were diluted with ultrapure water (1:10 w/v) and then filtered through the Acrodisc CR PTFE membrane.

Calibration curves were built with the external standard method, correlating the area of the peaks with the concentration. Homogentisic acid, *c*,*t*-ABA, and *t*,*t*-ABA standard solutions were prepared in methanol and working standard solutions in ultrapure water. The correlation coefficients were 0.9998 in the range of 1−100 mg/kg.

HPLC-MS/MS Analysis. HPLC-MS/MS analysis was performed as reported by Tuberoso et al. (*9*) using an HPLC-MS/MS Varian (Varian, Palo Alto, CA) system fitted with a 1200 L triple-quadrupole mass

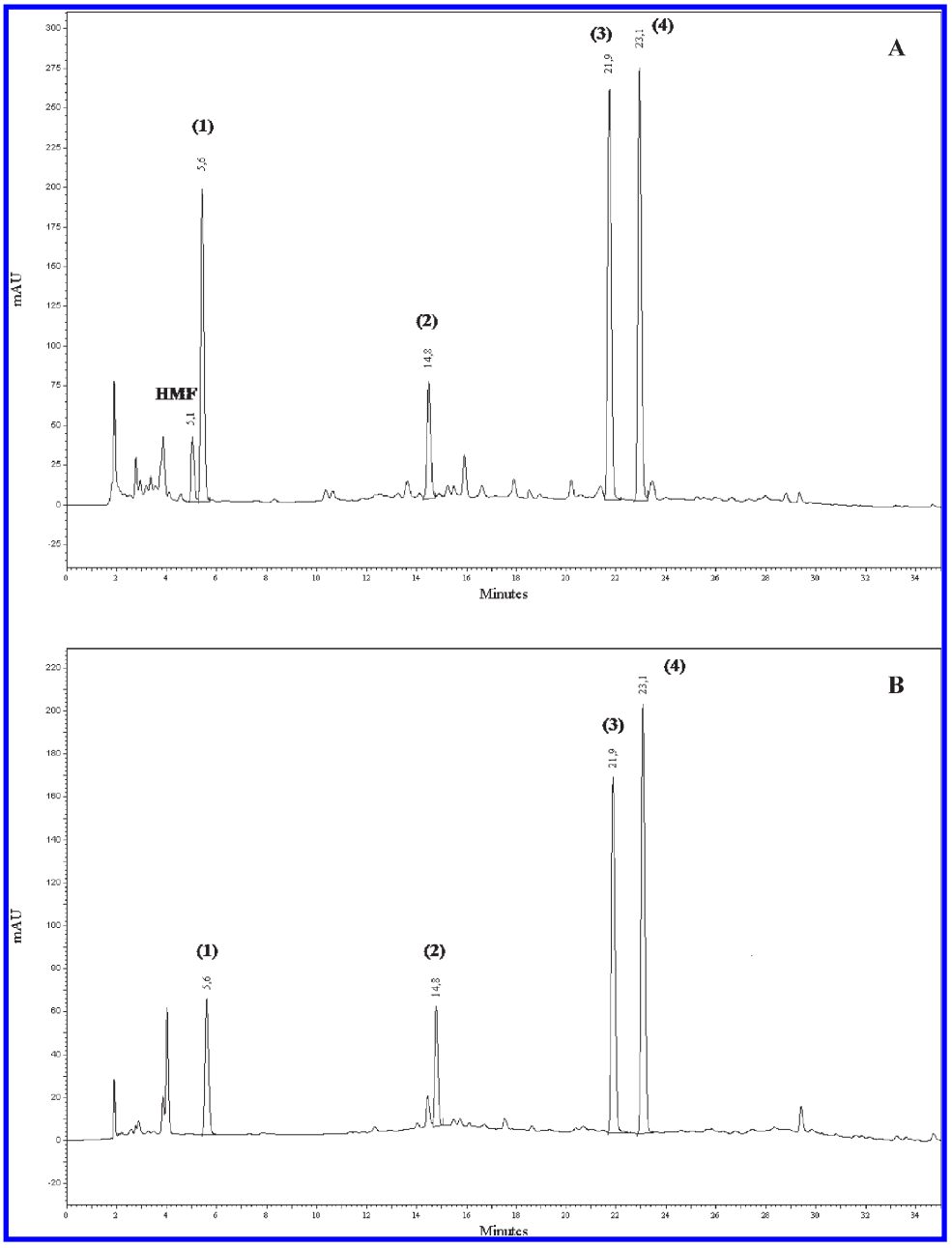


Figure 1. HPLC chromatograms at λ = 280 nm of *Arbutus unedo* L. (**A**) honey and (**B**) nectar: (**1**) homogentisic acid; (**2**) unedone; (**3**) *t,t*-ABA, (**4**) *c,t*-ABA; (**HMF**) 5-(hydroxymethyl)furfural.

spectrometer with an electrospray ionization source (ESI). Separation was obtained with a Licrocart Purosher Star RP-18e column (Merck KGaA, Darmstadt, Germany) using water and acetonitrile 60:40 (v/v) as mobile phase. The flow was maintained at 0.4 mL/min, and the injection volume was 10 μL. A Varian MS workstation version 6.7 software was used for data acquisition and processing. The system was optimized to work in negative mode for the ABAs and in positive mode for unedone. In negative mode the electrospray capillary potential was set to −55 V. Air was used as desolvatation solvent gas at 300 °C, and the housing API temperature was kept at 43 °C. Deprotonated analyte molecules of the parent compounds were subjected to collision-induced dissociation using argon at 2.18 mTorr in the multiple reaction monitoring (MRM) mode. The scanning time was 0.2 s, and the detector voltage was set to 1050 V. For unedone, the compound with t_R = 14.8 min, the system was optimized for working in positive mode. The electrospray capillary potential was set to −40 V. Nitrogen was used as desolvatation solvent gas at 300 °C, whereas the housing API temperature was kept at 40 °C.

Protonated analyte molecules of the parent compounds were subjected to collision-induced dissociation using argon at 2.18 mTorr in the MRM mode. The scanning time was 0.2 s, and the voltage detector was set to 1500 V.

HPLC-MS Q-TOF Analysis. The honey extracts were analyzed by reverse-phase HPLC on an Agilent 1200 series HPLC system fitted with a microchip technology using an Agilent Zorbax 300 SB-C18 5 μm, 43 mm × 75 μm (Agilent, Santa Clara, CA). The HPLC conditions were as follows: flow rate, 0.4 μL/min; solvent A, 0.1% formic acid in water; solvent B, acetonitrile; and gradient, solvent B, 5−100% over 10 min. The samples (1 μL) were then analyzed by ESI in positive mode using an Agilent 6520 time of flight (TOF) MS. Mass spectral data were acquired in the range m/z 100−1000 with an acquisition rate of 1.35 spectra/s, averaging 10000 transients. The source parameters were adjusted as follows: drying gas temperature, 250 °C; drying gas flow rate, 5 L/min; nebulizer pressure, 45 psi; and fragmentor voltage, 150 V. Data acquisition and processing were done using Agilent MassHunter Workstation Acquisition v. B.02.00 software.

NMR Analyses. NMR spectra were recorded at 25 °C on a Varian Unity INOVA 400 MHz spectrometer, operating at 400 MHz for ^{1}H and at 100 MHz for ^{13}C, respectively. The compounds were measured

in $CDCl_3$ and the spectra referenced against residual nondeuterated solvents.

Statistical Analyses. Statistical analysis was applied to the comparison between the abscisic acids and unedone contents and to the percentage of *Arbutus* pollen in the honeys sampled, considering the years of honey production as independent variable. Data were analyzed by the general linear models (GLM) of ANOVA, and means were separated by least-squares means using Statigraphics Plus ver. 4 software (Manugistics Inc., Rockville, MD).

RESULTS AND DISCUSSION

Melissopalynological Analysis. The sensory characteristics and the melissopalynological analyses confirmed the floral origin of the analyzed honeys. In particular, from the melissopalynological analyses 65 different elements (pollen types and spores) were identified in the sediment of the honey samples, with the presence of the main plant species already marked for this type of honey in the literature (*1*). The average percentages of *Arbutus* pollen were 38.1 ± 11.7, 35.1 ± 15.9, and 39.6 ± 22.3 for the years 2005, 2006, and 2007, respectively (**Table 1**). Apart from *Arbutus*, the most frequent pollen was *Eucalyptus*, a typical contaminant pollen, which was found in all samples with percentages varying from 0.32 to 62.41%. Among the other pollens, the most frequent were *Quercus*, *Hedera*, *Rubus*, *Citrus*, *Ononis*, *Pistacia*, *Rosmarinus*, *Cistus*, *Trifolium*, *Hedysarum*, and *Echium*, *Lotus*, all commonly present in the spectra of Sardinian honeys (*1*).

Unedone Identification. HPLC-DAD analysis of strawberry tree honey showed four main compounds at retention times of 5.6 (**1**), 14.8 (**2**), 21.9 (**3**), and 23.1 min (**4**), respectively (**Figure 1A**). To assess if such compounds are derived from *A. unedo* L. nectar, several nectars from strawberry tree were analyzed. HPLC-DAD analysis of strawberry tree nectar showed the same compounds (**Figure 1B**), confirming the botanical origin of such compounds. The first peak at t_R = 5.6 min was found to be homogentisic acid (**1**), the typical marker of strawberry-tree honey (*3*). The peak at 14.8 min (compound **2**) was investigated by NMR, HPLC-MS/MS, and high-resolution mass spectrometry (HR-MS Q-TOF) techniques to determine its structure. HPLC-MS/MS for compound **2** was optimized in the positive mode and showed the prevalence of m/z 241.1 pseudomolecular ion $[M + H]^+$. The MS/MS transitions observed, using argon as collision gas, for the precursor ion m/z 241.1 were 241.1 → 109.0 (CE − 14 eV), 241.1 → 137.0 (CE − 10 eV), 241.1 → 153.0 (CE − 10 eV), and 241.1 → 111.0 (CE − 16 eV). Compound **2** was further isolated by preparative TLC (see Honey Extraction and TLC Separation) and obtained as a colorless semisolid product. Optical rotation measured in MeOH at 25 °C using a Perkin-Elmer 241 polarimeter showed a value $[\alpha]^{25}_D$ +20.0, and the UV−vis spectrum (MeOH) was characterized by a maximum at λ_{max} = 245 nm. The ^{13}C NMR spectrum of compound **2** showed 13 carbon signals (**Table 2**), which were sorted by DEPT 90 and 135 experiments into four CH_3, one CH_2, four CH, and four quaternary carbons. This corresponds to a molecular formula of $C_{13}H_{20}O_4$, in agreement with the ion peak $[M + H]^+$ at m/z 241.1437 in the HR-MS (Q-TOF) with an accuracy of 1.2 ppm on the calculated exact mass 241.1434. In the ^1H NMR spectrum (**Table 2**) the signals at δ_H 1.01 (s) and 1.16 (s) indicated the presence of geminal dimethyl groups (CH_3-11 and CH_3-12), whereas the signals at δ_H 2.38 (dd, J = 17.1, 1.2) and 2.50 (br, d, J = 17.1) could be attributed to two methylene protons (CH_2-2). In the same spectrum, the resonances at δ_H 1.78 (d, J = 1.2) and δ_H 6.03 (br s) were attributed to the vinyl methyl (CH_3-13) and to the olefinic methine (C-4), respectively. A HSQC experiment was utilized to assign the protons to their attached carbons. All of the above-mentioned resonances, together with a ketonic function (δ_C 197.0, C-3) and an oxygenated quaternary carbon (δ_C 68.7, C-6) observed in the ^{13}C NMR spectrum (**Table 2**), suggested the presence of an α-ionone derivative. In addition to these signals, the ^{13}C NMR spectrum exhibited resonances for three oxymethine (δ_C 66.0 (C-7); 69.2 (C-9); 72.1 (C-9)) protons and one further methyl group at δ_C 19.7 (C-10). The use of $^1H-^1H$ DQF-COSY and $^1H-^{13}C$ HMBC experiments allowed us to deduce the remaining carbon−carbon connectivities of the unknown compound. HMBC correlations between the oxygenated methine at δ_H 3.10 (d, J = 8.7) and C-5 (δ_C 161.2), C-6 (δ_C 68.7), suggested this proton to be located at position C-7 (**Figure 2** and **Table 2**). The locations of the methine protons at δ 4.01 (dd, J = 8.7, 3.9) and 3.84 (m) were established by the $^1H-^1H$ DQF-COSY technique, indicating cross-peaks between H-8 (δ 4.01) and H-7 (δ 3.10) and H-9 (δ 3.84), whereas H-9 correlated with H-8 and H_3-10 (δ 1.25 d, J = 6.6) but not with H-7 (**Figure 2**). Because the C-6, C-7, C-8, and C-9 were oxygenated, according to the molecular ion at m/z 241.1437 in the HR-MS (Q-TOF), the side chain must contain an epoxy group. The placement of the C-6(7)-epoxide group was inferred by the C-6 chemical shift value, which resonated in the molecule at higher field than that of similar compounds bearing a free hydroxyl group at this position. This observation was corroborated by a direct comparison of the ^{13}C values, obtained for the molecule, to those of 7α,8α-epoxyblumenol B (*10*). Complete assignment of all proton and carbon resonances was achieved after careful analysis of $^1H-^1H$ DQF-COSY, HSQC, HMBC, and ROESY experiments. The absolute or relative configurations of compound **2** could not be determined, due to the fact that a crystalline derivative suitable for an X-ray structure determination could not be obtained from the compound isolated. Thus, the substance was identified as 2-(1,2-dihydroxypropyl)-4,8,8-trimethyl-1-oxaspiro[2.5]oct-4-en-6-one and was given the trivial name unedone (**Table 3**). This compound is an abscisic acid epoxidic derivative, and it is a new natural product.

Table 2. ^1H and ^{13}C NMR Spectral Data of Unedone (**2**) in $CDCl_3$ (δ; J, Hz)

	unedone		
position	δ_C	δ_H	$^2J/^3J$ $^1H-^{13}C$ correlations
1	37.1 s		
2	51.2 t	2.38, dd (17.1, 1.2) H-2α	C-1, C-3, C-4, C-6, C-11, C-12
		2.50, br d (17.1) H-2β	C-1, C-3, C-6, C-11, C-12
3	197.0 s		
4	129.6 d	6.03, br s	C-2, C-6, C-13
5	161.2 s		
6	68.7 s		
7	66.0 d	3.10, d (8.7)	C-5, C-6
8	72.1 d	4.01 dd (8.7, 3.9)	C-7, C-9, C-10
9	69.2 d	3.84, m	C-7, C-10
10	19.7 q	1.25, d (6.6)	C-8, C-9
11	25.7 q	1.16, s	C-1, C-2, C-6,C-12
12	26.9 q	1.01, s	C-1, C-2, C-3, C-6, C-11
13	18.0 q	1.78, d (1.2)	C-4, C-5, C-6

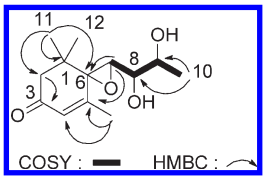
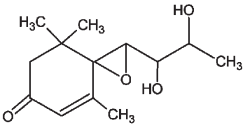
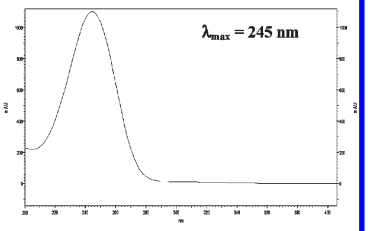
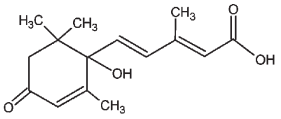
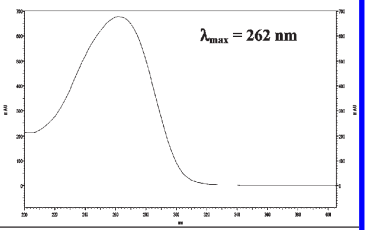
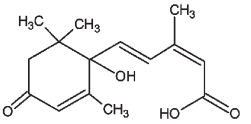
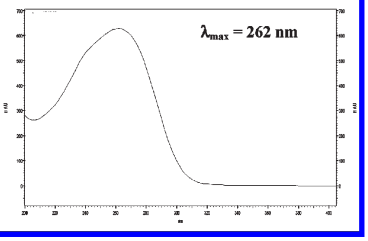


Figure 2. Important $^1H-^1H$ COSY and $^1H-^{13}C$ HMBC of unedone (**2**).

Abscisic Acid Isomer Identification. Compounds **3** and **4**, at retention times of 21.9 and 23.1 min, respectively, showed the

Table 3. Chemical Characteristics of the Norisoprenoid Compounds Detected in *A. unedo* L. Nectar and Honey Samples

Name	MW	Formula	UV spectra
Unedone (**2**)	240.1434		
(±)2-*trans*, 4-*trans* abscisic acid (**3**)	264.3162		
(±)2-*cis*, 4-*trans* abscisic acid (**4**)	264.3162		

same UV−vis spectrum and, to determine their structure, they were investigated by HPLC-MS/MS. This analysis gave the same mass m/z 264 for each peak. Taking into account this information, it was supposed that the two compounds were isomers. NMR analysis of the purified compounds by preparative HPLC demonstrated that such compounds were isomers of abscisic acid: *t*,*t*-ABA (**3**) and *c*,*t*-ABA (**4**) (**Table 3**). Confirmation was obtained with the injection of pure standards of *t*,*t*-ABA (**3**) and *c*,*t*-ABA (**4**) in HPLC-MS/MS by cochromatography. These compounds ionized better in negative mode, and both of them showed the prevalence of the mass m/z 263.3 corresponding to the pseudomolecular ion $[M - H]^-$. The transitions observed for the precursor ion 263.3 (m/z) were 263.3 → 152.8 (CE + 16 eV), 263.3 → 203.0 (CE + 28 eV), and 263.3 → 218.8 (CE + 18 eV) for *c*,*t*-ABA; and 263.3 → 203.0 (CE + 28 eV) and 263.3 → 218.8 (CE + 18 eV) for *t*,*t*-ABA. Thus, the two ABA isomers could be discriminated also for their mass fragmentation because only *c*,*t*-ABA formed the fragment at m/z 152.8.

Abscisic acids are norisoprenoid compounds, cyclohexene products formed by carotenoid degradation. Abscisic acids act as a plant hormone, mainly in higher plants, to inhibit growth, to promote seed dormancy and germination, and to help the plant in tolerating stressful conditions and controlling stomal closure (*11*).

Quantitative Composition of Strawberry Tree Nectar and Honeys. Strawberry tree nectar and honey samples were analyzed to quantify compounds **1**−**4**, and data are reported in **Table 1**. Nectars showed the presence of homogentisic acid (**1**) (120.5 ± 23.0 mg/kg), unedone (**2**) (15.7 ± 2.5 mg/kg), *t*,*t*-ABA (**3**) (63.2 ± 26.6 mg/kg), and *c*,*t*-ABA (**4**) (78.0 ± 33.6 mg/kg). The ratio between the two abscisic isomers varied from 1.19 to 1.27. Honeys showed levels of homogentisic acid (**1**) (414.1 ± 69.8 mg/kg), unedone (**2**) (32.9 ± 7.1 mg/kg), *t*,*t*-ABA (**3**) (162.3 ± 21.1 mg/kg), and *c*,*t*-ABA (**4**) (176.2 ± 25.4 mg/kg). The amounts of homogentisic acid confirmed data previously published (*3*, *12*). The amounts of this acid showed significant differences between 2007 and 2005−2006 samples. Moreover, unedone showed significant differences between 2007 and 2005 samples, whereas no statistically significant differences were observed for the two ABA isomers (**Table 1**). The *c*,*t*-ABA/*t*,*t*-ABA ratio ranges from 1.0 to 1.20, with an average value of 1.08 ± 0.05, and the amounts of the two ABA isomers are statistically different ($F = 4.84$, $P = 0.325$). HPLC fingerprinting of other Sardinian unifloral honeys evidenced the absence of abscisic acids and unedone in asphodel (*Asphodelus microcarpus* Salzm. and Viv.), sulla (*Hedysarum coronarium* L.), thistle (*Galactites tomentosa* Moench.), and eucalyptus (*Eucalyptus* spp.) honeys.

ABA isomers were found in many honeys, with remarkable differences both in quantitative amount and in relative percentage of each isomer (*13*−*22*). Usually, ABA isomer amount is < 100 mg/kg, and *t*,*t*-ABA was never found in buckwheat (*Fagopyrum esculentum*), clover (*Melilotus* spp.), fireweed (*Epilobium agustifolium*), lime tree (*Tilia europaea*), soybean (*Glycine max*), or tupelo (*Nyssa aquatica*) honeys (*13*, *15*, *21*). From literature data, it is evident that no other unifloral honeys showed a similar ABA isomer composition, in either amount or isomer ratio.

In conclusion, ABA isomer levels and the presence of unedone could represent further useful markers for fingerprinting the botanical origin of strawberry tree honey.

ABBREVIATIONS USED

c,*t*-ABA, (±)-2-*cis*,4-*trans*-abscisic acid; *t*,*t*-ABA, (±)-2-*trans*,4-*trans*-abscisic acid; DQF-COSY, double-quantum filtered correlation spectroscopy; HSQC, heteronuclear single quantum coherence; HMBC, heteronuclear multiple-bond correlation; ROESY, rotating-frame Overhauser enhancement spectroscopy.

Supporting Information Available: NMR spectra of unedone (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Floris, I.; Prota, R.; Marras, P. M.; Ricciarelli, G. *Caratterizzazione Botanica dei Mieli della Sardegna* (*I. Aspetti generali*). *Atti convegno "Stato Attuale e Sviluppo della Ricerca in Apicoltura" Sassari*, Italy, 25-26/10/1991; pp 169−188.

(2) Floris, I.; Satta, A.; Ruiu, L. Honeys of Sardinia (Italy). *J. Apic. Res.* **2007**, *46*, 198–209.

(3) Cabras, P.; Angioni, A.; Tuberoso, C.; Floris, I.; Reniero, F.; Guillou, C.; Ghelli, S. Homogentisic acid: a phenolic acid as a marker of strawberry-tree (*Arbutus unedo*) honey. *J. Agric. Food Chem.* **1999**, *47*, 4064–4067.

(4) Bianchi, F.; Cereri, M.; Musci, M. Volatile norisoprenoids as markers of botanical origin of Sardinian strawberry-tree (*Arbutus unedo* L,) honey: characterisation of aroma compounds by dynamic headspace extraction and gas chromatography−mass spectrometry. *Food Chem.* **2005**, *89*, 527–532.

(5) de la Fuente, E.; Sanz, M. L.; Martínez-Castro, I.; Sanz, J.; Ruiz-Matute, A. I. Volatile and carbohydrate composition of rare unifloral honeys from Spain. *Food Chem.* **2007**, *105*, 84–93.

(6) Dalla Serra, A.; Franco, M. A.; Mattivi, F.; Ramponi, M.; Vacca, V.; Versini, G. Aroma characterization of Sardinian strawberry tree (*Arbutus unedo* L.) honey. *Ital. J. Food Sci.* **1999**, *11*, 47–56.

(7) Demianowicz, S. *La Secretion du Nectar et le Rendement en Miel des Plus Important Plantes Mellifères en Pologne*; XIX Congrès d'Apimondia: Prague, Czech Republic, 1963; pp 180−183.

(8) Louveaux, J.; Maurizio, A.; Vorwohl, G. Methods of melissopalynology. *Bee World* **1978**, *59*, 139–157.

(9) Tuberoso, C. I. G.; Bifulco, E.; Jerković, I.; Caboni, P.; Cabras, P.; Floris, I. Methyl syringate: a chemical marker of asphodel (*Asphodelus microcarpus* Salzm. et Viv.) monofloral honey. *J. Agric. Food Chem.* **2009**, *57*, 3895–3900.

(10) Su, B. N.; Park, E. J.; Nikolic, D.; Schunke Vigo, J.; Graham, J. G.; Cabieses, F.; Van Breemen, R. B.; Fong, H. H. S.; Farnsworth, N. R.; Pezzuto, J. M.; Kinghorn, A. D. Isolation and characterization of miscellaneous metabolites of *Deprea subtriflora*. *J. Nat. Prod.* **2003**, *66*, 1089–1093.

(11) Xiong, L.; Zhu, J.-K. Regulation of abscisic acid biosynthesis. *Plant Physiol.* **2003**, *133*, 29–36.

(12) Scanu, R.; Spano, N.; Panzanelli, A.; Pilo, M. I.; Piu, P. C.; Sanna, G.; Tapparo, A. Direct chromatographic methods for the rapid determination of homogentisic acid in strawberry tree (*Arbustus unedo* L.) honey. *J. Chromatogr., A* **2005**, *1090*, 76–80.

(13) Tomás-Barberán, F. A.; Martos, I.; Ferreres, F.; Radovic, B. S.; Anklam, E. HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *J. Sci. Food Agric.* **2001**, *81*, 458–496.

(14) Yao, L.; Jiang, Y.; Singanusong, R.; Datta, N.; Raymont, K. Phenolic acids in Australian *Melaleuca*, *Guioa*, *Lophostemon*, *Banksia* and *Helianthus* honeys and their potential for floral authentication. *Food Res. Int.* **2005**, *38*, 651–658.

(15) Gheldof, N.; Wang, X.-H.; Engeseth, N. J. Identification and quantification of antioxidant components of honeys from various floral sources. *J. Agric. Food Chem.* **2002**, *50*, 5870–5877.

(16) Tan, S. T.; Wilkins, A. L.; Holland, P. T.; McGhie, T. K. Extractives from New Zealand honeys. 3. Unifloral thyme and willow honey constituents. *J. Agric. Food Chem.* **1990**, *38*, 1833–1838.

(17) Tan, S. T.; Wilkins, A. L.; Holland, P. T.; McGhie, T. K. Extractives from New Zealand unifloral honeys. 2. Degraded carotenoids and other substances from heather honey. *J. Agric. Food Chem.* **1989**, *37*, 1217–1221.

(18) Truchado, P; Ferreres, F; Bortolotti, L; Sabatini, A. G.; Tomás-Barberán, F. A. Nectar flavonol rhamnosides are floral markers of acacia (*Robinia pseudacacia*) honey. *J. Agric. Food Chem.* **2008**, *56*, 8815–8824.

(19) Yao, L.; Jiang, Y.; Singanusong, R.; Datta, N.; Raymont, K. Phenolic acids and abscisic acid in Australian *Eucalyptus* honeys and their potential for floral authentication. *Food Chem.* **2004**, *86*, 169–177.

(20) Yao, L.; Datta, N.; Tomás-Barberán, F. A.; Ferreres, F.; Martos, I.; Singanusong, R. Flavonoids, phenolic acids and abscisic acid in Australian and New Zealand *Leptospermun* honeys. *Food Chem.* **2003**, *81*, 159–168.

(21) Wang, X. H.; Gheldof, N.; Engeseth, N. J. Effect of processing and storage on antioxidant capacity of honey. *J. Food Sci.* **2004**, *69*, C96–C101.

(22) Ferreres, F.; Andrade, P.; Tomás-Barberán, F. A. Natural occurrence of abscisic acid in heather honey and floral nectar. *J. Agric. Food Chem.* **1996**, *44*, 2053–2056.

Received for review July 13, 2009. Revised manuscript received October 27, 2009. Accepted November 03, 2009. This paper was supported by a grant of Fondazione Banco di Sardegna (436/2007.0065).