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ARTICLE

Lumichrome and Phenyllactic Acid as Chemical Markers of Thistle (*Galactites tomentosa* Moench) Honey

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HPLC-DAD-MS/MS chromatograms of thistle (*Galactites tomentosa* Moench) unifloral honeys, previously selected by sensory evaluation and melissopalynological analysis, showed high levels of two compounds. One was characterized as phenyllactic acid, a common acid found in honeys, but the other compound was very unusual for honeys. This compound was extracted from honey with ethyl acetate and purified by SPE using C_{18}, SiOH, and NH_2 phases. Its structure was elucidated on the basis of extensive 1D and 2D NMR experiments as well as HPLC-MS/MS and Q-TOF analysis, and it was identified as lumichrome (7,8-dimethylalloxazine). Lumichrome is known to be the main product of degradation obtained in acid medium from riboflavin (vitamin B_2), and this is the first report of the presence of lumichrome in honeys. Analysis of the *G. tomentosa* raw honey and flowers extracts confirmed the floral origin of this compound. The average amount of lumichrome in thistle honey was 29.4 ± 14.9 mg/kg, while phenyllactic acid was 418.6 ± 168.9 mg/kg. Lumichrome, along with the unusual high level of phenyllactic acid, could be used as a marker for the botanical classification of unifloral thistle (*G. tomentosa*) honey.

KEYWORDS: Lumichrome; *Galactites tomentosa* Moench; honey; botanical characterization; HPLC-DAD; HPLC-MS/MS; Q-TOF; NMR

INTRODUCTION

Monofloral honeys have a high value in the marketplace because of their distinguishing sensorial characteristics. Thistle honey is a common unifloral honey and, depending on the area of production, several species belonging to the Asteraceae family can be linked to the botanical origin of this honey. Star thistle (*Centaurea solstitialis* L.), nodding thistle (*Carduus nutans* L.), Mediteranean thistle (*Galactites tomentosa* Moench), and other plants belonging to the genus *Carduus* or *Cirsium*, are the main sources of thistle honey. In the scientific literature, only a few papers on the characterization of thistle honey can be found. Volatile compounds from *Carduus nutans* honey have been studied by GC-MS, and linalool derivatives were proposed as possible marker compounds (*1*). Physicochemical, sensory, and melissopalynological parameters of a *Cirsium* spp. honey sample were determined (*2*), but data were not useful to assess the botanical origin of the honey. No data on thistle honey from *G. tomentosa* have been reported, although this unifloral honey is a common product in some Mediterranean regions. The flowering period of *G. tomentosa* is spring, when a lot of other flowering species can interfere with this unifloral honey production. The sensory characteristics are easily recognizable. This honey is amber colored with green reflection when in liquid state and light brown when crystallized. Its aroma is of medium intensity, quite persistent, with floral−fruity notes and faint animal nuances. Its taste is usually sweet and tart, with a light bitter and slightly astringent aftertaste. As for other honeys with underrepresented pollen in their spectra, the botanical classification of thistle honey based on melissopalynology is quite difficult (*3*).

The aim of this work was to investigate by a direct RP-HPLC-DAD technique the nonvolatile fraction of thistle (*G. tomentosa* Moench) unifloral honey in order to find characteristic compounds that can be proposed as suitable marker compounds of this honey.

MATERIALS AND METHODS

Honey and Nectar Samples and Melissopalynological Analyses. Thistle honey samples ($n = 23$) were collected in triplicate from professional beekeepers in different areas of Sardinia (Italy) during the period 2007−2009 (**Table 1**). Samples were stored at 4 °C in dark glass bottles until analysis (within 3 months). A sample of thistle honey produced in Sicily (Italy) in 2008 was also analyzed. Qualitative and quantitative melissopalynological analyses were carried out by following the method of the International Commission of Bee Botany (*4*, *5*). Because of flower morphology, it was not possible to collect its nectar directly from the flowers as done previously (*6*, *7*), so different approaches were used to correlate thistle honey with *G. tomentosa* flowers. In a first trial, three packages of bees with new frames were placed in an area covering more than 90% by *G. tomentosa* in early flowering. During the flowering period (April−May), at intervals of about 15 days, unripe honey samples were collected by pipet from at least 100 uncapping cells from 2−3 combs and

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Table 1. Phenyllactic Acid and Lumichrome Amounts (mg/kg) in Thistle Honey Samples[a]

sample	year of production	water (g/100 g)	phenyllactic acid (mg/kg)	lumichrome (mg/kg)
CA07	2007	15.7 ± 0.8	271.5 ± 16.3	20.4 ± 1.4
CA05	2007	15.7 ± 0.7	308.7 ± 17.9	22.6 ± 1.5
CA11	2007	16.4 ± 0.9	760.7 ± 40.3	72.1 ± 5.1
CA126B	2007	15.8 ± 0.8	329.4 ± 19.5	18.5 ± 1.4
CA21A	2007	16.3 ± 0.7	578.0 ± 32.4	43.3 ± 3.5
CA9	2008	15.7 ± 0.9	241.3 ± 14.5	14.2 ± 1.2
CA-CRA1	2008	15.8 ± 0.6	692.2 ± 42.5	56.2 ± 4.3
CA-CRA2	2008	15.9 ± 0.7	601.5 ± 35.9	43.4 ± 2.9
CA-CRA3	2008	16.9 ± 0.9	390.3 ± 22.4	23.0 ± 1.6
CA-CRA4	2008	16.1 ± 0.9	607.1 ± 35.6	37.7 ± 2.8
CA03	2008	16.8 ± 0.8	292.6 ± 17.6	14.6 ± 1.0
CA10	2008	15.2 ± 0.8	368.3 ± 22.3	23.5 ± 1.9
CA15	2008	15.5 ± 0.5	121.7 ± 6.2	12.3 ± 0.9
CA93	2009	14.8 ± 0.6	582.4 ± 35.1	20.0 ± 1.5
CA19	2009	16.7 ± 0.9	601.5 ± 33.8	43.5 ± 2.9
CA02	2009	14.7 ± 0.8	392.1 ± 23.5	26.2 ± 1.8
CA04	2009	15.4 ± 0.8	552.3 ± 33.2	45.5 ± 3.2
CA08	2009	15.3 ± 0.8	398.4 ± 21.9	25.8 ± 1.7
CA06	2009	16.0 ± 0.9	319.0 ± 18.1	23.2 ± 1.5
CA14	2009	16.2 ± 0.8	376.9 ± 22.0	26.7 ± 1.9
CA16	2009	15.1 ± 0.6	340.3 ± 20.4	20.5 ± 1.5
CA17	2009	16.0 ± 0.8	311.7 ± 17.1	23.4 ± 1.7
CA18	2009	14.6 ± 0.5	189.2 ± 11.4	19.8 ± 1.2
total average (mean ± SD)		15.8 ± 0.6a	418.6 ± 168.9a	29.4 ± 14.9a
2007 average (mean ± SD)		16.0 ± 0.3a	449.7 ± 211.7a	35.4 ± 22.8a
2008 average (mean ± SD)		16.0 ± 0.6a	414.4 ± 200.9a	28.1 ± 16.0a
2009 average (mean ± SD)		15.5 ± 0.7a	406.4 ± 133.2a	27.5 ± 9.3a
Sicilian thistle honey	2008	17.1 ± 0.2	528.6 ± 22.1	20.8 ± 1.1
raw honey I (09/05/2010)	2010	23.2 ± 0.3	191.3 ± 27.3	11.1 ± 1.6
raw honey II (25/05/2010)	2010	22.3 ± 0.3	269.6 ± 17.8	22.8 ± 1.7

[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.

stored at −20 °C until analysis was performed (raw honeys sample I and II). The unripe honey sampled in this way was representative of the nectar collected by bees from the dominant flowers of *G. tomentosa* during all the flowering period. A second trial was carried out directly on *G. tomentosa* flowers. Because nectar is water soluble, flowers of *G. tomentosa* were gently soaked in water and the water solution was extracted with ethyl acetate and purified on SPE as described for honey samples (see section 2.3, Honey Extraction and SPE Purification).

Chemicals. Methanol, acetonitrile, ethyl acetate, 85% phosphoric acid, formic acid, and trichloromethane were purchased from Merck (Darmstadt, Germany). Standards of 5-hydroxymethylfurfural (HMF), lumichrome, and phenyllactic acid were purchased from Sigma-Aldrich (Milan, Italy). All 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 and SPE Purification. The best procedure to obtain pure compound **2** (**Figure 1**) was an initial extraction of honey using ethyl acetate and then a purification with three types of SPE (solid-phase extraction): C_{18}, silica, and amino columns. Pure honey (1 kg) was extracted, for 5 times, with 300 mL of ethyl acetate, and the extracts collected together were dried under vacuum. Residue was suspended in ultra pure water and purified by SPE C_{18} column (Varian Bond Elut 500 mg, 3 mL) previously activated with methanol and washed with ultra pure water. The elution was carried out with 50% CH_3OH, and a bright-yellow band was collected. Methanol was evaporated under vacuum, and the aqueous part was extracted with ethyl acetate (5 mL × 3 times). Ethyl acetate was dried under vacuum, and the residue was dissolved in trichloromethane and then purified using a SPE silica gel (SiOH) column (J. T. Baker Bakerbond 40 μm APD, 60A, 3 mL) previously activated with methanol and washed with trichloromethane. Then 10 mL of trichloromethane were used to wash the column from interfering substances, and compound **2** was eluted with a mixture of trichloromethane/methanol (9:1, v/v). The collected trichloromethane/methanol mixture was dried under vacuum, dissolved in CH_3CN, and purified using a SPE NH_2 column (Varian Bond Elut 500 mg, 3 mL) previously activated with CH_3CN/methanol (9:1, v/v) and washed with CH_3CN. The elution solvent was CH_3CN and the yellow band was collected and then dried under a nitrogen gentle flow. Purity of fractions was monitored using the HPLC-DAD apparatus described in next paragraph. At the end of the SPE purifications, from 1 kg of thistle honey about 10 mg of pure compound **2** was collected and used for the LC-MS/MS, LC-Q TOF, IR, and NMR analysis.

HPLC-DAD Analysis. The analysis was performed as reported by Tuberoso et al. (*7*) using an HPLC-DAD Varian system ProStar fitted with a pump module 230, an autosampler module 410 with a 10 μL loop, and a ThermoSeparation diode array detector SpectroSystem UV 6000lp (ThermoSeparation, San Jose, CA) set at 210, 280, and 360 nm.

Separation was obtained with a Phenomenex Gemini C18 110A column (150 mm × 4.60 mm, 3 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, keeping 90% of solvent A for 5 min, then decreasing to 65% in 15 min, 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. 4.0 data system (ThermoQuest, Rodano, Milan, Italy).

Honey and raw honey samples were diluted with ultrapure water (1:10, w/v) and then filtered through Econofilter RC membrane (0.45 μm, ∅ 25 mm, Agilent Technologies, Milan, Italy).

Calibration curves were plotted with the external standard method, correlating the peak area with the concentration. Phenyllactic acid and lumichrome standard solutions were prepared in methanol and working standard solutions in ultrapure water. The correlation coefficients were > 0.998 in the range of 2−100 and 1−20 mg/kg for phenyllactic acid and lumichrome, respectively. Validation of the method was performed in agreement with EMEA note guidance on validation of analytical methods (*8*). To evaluate precision, the repeatability was established by intra- and interday assays, performing six injections of the same standard at five concentration levels for each compound for six consecutive days.

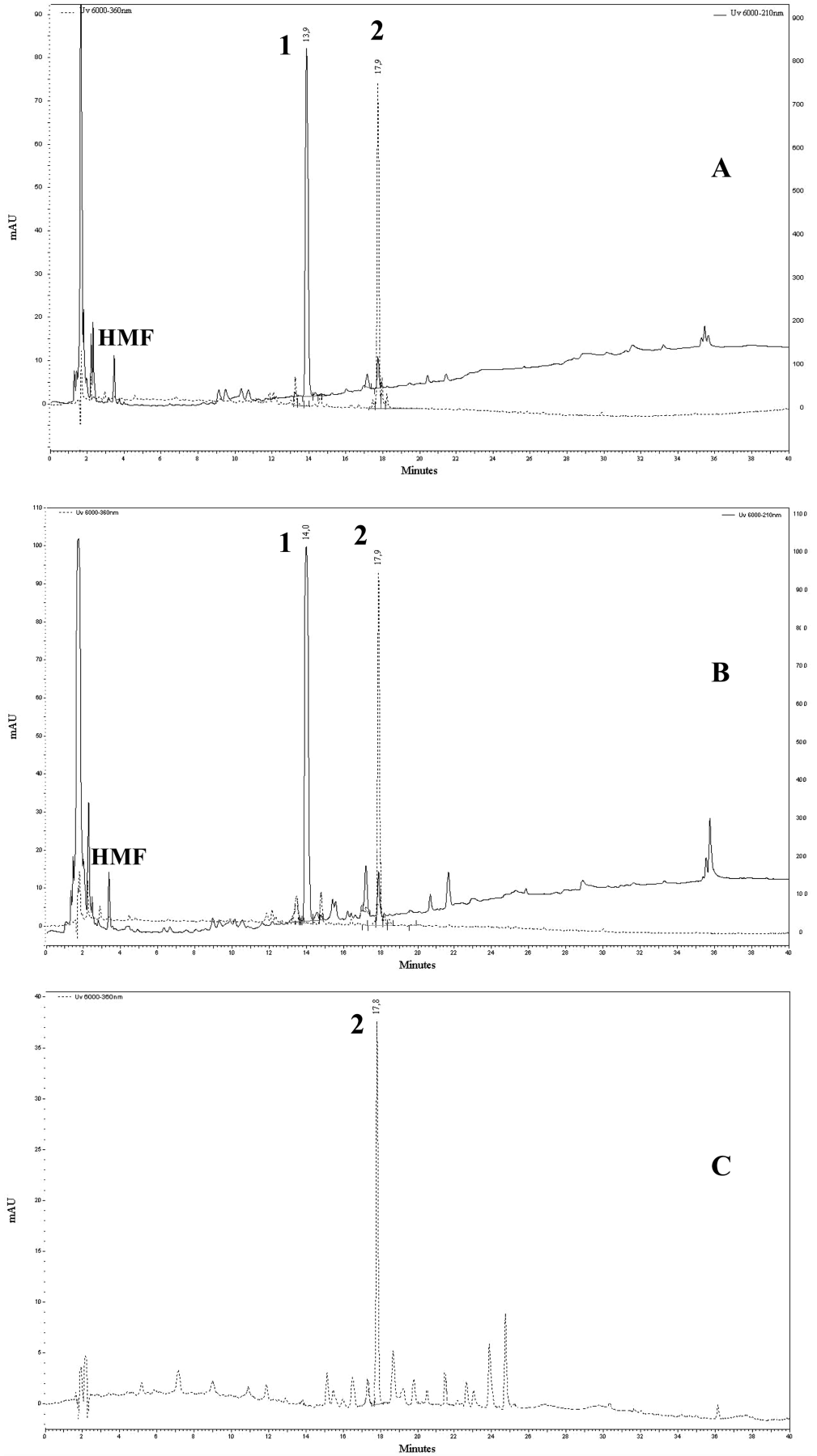

Figure 1. HPLC chromatograms at λ = 210 nm (—) and λ = 360 nm (---) of thistle (**A**) honey, (**B**) raw honey, and (**C**) flower extract: (**1**) phenyllactic acid, (**2**) lumichrome, (HMF) 5-(hydroxymethyl)furfural.

The specificity, evaluated as the noninterference with other substances detected in the region of interest, was assessed by the ChromQuest purity calculation software index (total peak purity ≥0.99) and resulted to be specific with no any other peak interfering at the retention times of the two marker compounds in the HPLC-DAD detection mode. Sensitivity of the method was evaluated establishing limit of quantification and detection (LOQ, LOD). LOQ and LOD were determined using the signal-to-noise ratio by preparing standard additions of lumichrome and phenyllactic acid into asphodel (*Asphodelus microcarpus* Salzm. et Viv.) honey because this unifloral honey lacks in these compounds. LOD, calculated at the signal/noise ratio of 3, were 0.1 and 0.2 mg/kg for lumichrome and phenyllactic acid, respectively. LOQ, calculated at the signal/noise ratio of 10, were 0.2 and 0.5 mg/kg for lumichrome and phenyllactic acid, respectively.

HPLC-MS/MS Analysis. HPLC−MS/MS analysis was performed as reported by Tuberoso et al. (*7*) using an HPLC-MS/MS Varian (Varian Palo Alto, CA, USA) system fitted with a 1200 L triple quadrupole mass spectrometer with an electrospray ionization source (ESI). A Varian MS workstation version 6.7 software was used for data acquisition and processing. Rapid identification was achieved with direct infusion of the purified molecule, dissolved in methanol, on the mass spectrometer source. The system was optimized to work in positive mode for lumichrome. The electrospray capillary potential was set to 75 V. Nitrogen was used as desolvatation solvent gas at 200 °C, while the housing API temperature was kept at 55 °C. Protonated analyte molecules of the parent compounds were subjected to collision-induced dissociation using argon at 2.40 mTorr in the multiple reaction monitoring (MRM) mode. The scanning time was 0.2 s, and the voltage detector was set to 1350 V.

HPLC-MS Q-TOF Analysis. The extract was 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; gradient, solvent B 20−100% over 10 min. Then 1 μL of the extract, dissolved in CH_3CN-H_2O (80:20, v/v), was 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 and IR Experiments. NMR spectra were recorded at 25 °C on Varian Unity INOVA 400 MHz spectrometer, operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C, respectively. The compounds were measured in DMSO-d_6 and the spectra referenced against residual nondeuterated solvents. IR spectra were performed with a Perkin-Elmer system 2000 FT-IR spectrophotometer using KBr mulls.

Statistical Analyses. Data were expressed as mean ± SD. For the statistical analysis, data were analyzed by the general linear models (GLM) of ANOVA, and means were separated by least-squares means using the Statigraphics Plus version 4 software (Manugistics Inc. Rockville, MD).

RESULTS

Sensory and Melissopalynological Analyses. The sensory characteristics and the melissopalynological analyses confirmed the floral origin of the sampled honeys. In particular, from the melissopalynological analyses, 32 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 literature (*3*). The average percentages of *Galactites* pollen, estimated based on a total counts of at least 1000 pollen grains (*4*), varied between 5 and 25%. Apart from *Galactites*, the most frequent pollens were *Acacia*, *Borago*, *Brassica*, *Capsella*, *Cistus*, *Citrus*, *Echium*, *Eucalyptus*, *Hedysarum, Lotus*, *Matricaria*, *Olea*/*Phyllirea*, *Ononis*, *Papaver*, *Smyrnium*, *Trifolium*, and *Vicia*, all commonly present in the spectra of Sardinian honeys (*3*). Because of the incidence of contaminant pollens in the spectra of thistle honey samples, it was not possible to record a correlation between the percentages of *Galactites* or *Carduus* f. pollen and the content of the two marked compounds.

Lumichrome Identification. HPLC-DAD analysis of thistle honeys showed two main compounds at retention times of ca. 14.0 and 18.0 min, respectively (**Figure 1A**). The first peak at t_R = 14.0 min was phenyllactic acid (compound **1**), a common compound present in many types of honeys. The peak at 18.0 min (compound **2**) was investigated by NMR, IR, HPLC-MS/MS, and high resolution mass spectrometry (HR-MS Q-TOF) techniques to determine its structure. Compound **2**, isolated by SPE (see Honey Extraction and SPE purification paragraph), was obtained as a bright-yellow product with reduced solubility in most solvents. UV−vis spectrum (MeOH) was characterized by a maximum at λ_{max} = 217, 260, 353, and 387 nm (**Figure 2**). HPLC-MS/MS for compound **2** was optimized in the positive mode and showed the prevalence of *m*/*z* 242.9 pseudomolecular ion $[M + H]^+$ and two adducts ($[M + Na]^+ = 264.8$ and $[2M + Na]^+ = 506.8$), which suggested a MW = 242.0. The MS/MS transitions observed, using argon as collision gas at 2.40 mTorr, for the precursor ion *m*/*z* 243.0 were 243.0 → 197.9 (CE-34V), 243.0 → 171.8 (CE-44V), 243.0 → 144.9 (CE-26V), 243.0 → 241.7 (CE-44V), and 243.0 → 199.6 (CE-30V). The molecular formula of **2** was established as $C_{12}H_{10}N_4O_2$ by HR-MS (Q-TOF) (*m*/*z* 243.0874 $[M + H]^+$, calcd 243.0882). The ^{1}H NMR spectrum of compound **2** showed two methyl singlets at 2.50 and 2.52 ppm, two aromatic protons at 7.74 and 7.95 ppm, and two downfield proton signals at 11.69 and 11.87 ppm. The signals at δ 11.69 and 11.87 have been easily assigned to imide protons because an absorption band at 3582 cm^{-1} could be detectable in the IR spectrum (**Figure 2**). The IR spectrum also showed a carbonyl group at 1727 cm^{-1}. The ^{13}C NMR spectrum revealed the presence of two methine (125.9, 128.8 ppm), two methyl (19.7, 20.3 ppm), and eight quaternary (160.7, 150.1, 146.6, 144.7, 141.7, 138.9, 138.4, 130.7 ppm) carbons. A detailed analysis of 1D and 2D (DQF-COSY, HSQC, HMBC) NMR experiments allowed to identify compound **2** as 7,8-dimethylalloxazine, known also as lumichrome. The NMR spectral data of compound **2** matched up with those of lumichrome reported in the literature (*9*, *10*). Direct comparison with analytical and spectral data obtained from commercial lumichrome confirmed the structure reported in **Figure 2** for compound **2**. The main transition *m*/*z* 198.0 observed in the HPLC-MS/MS analysis corresponds to fragment $C_{11}H_8N_3O$ due to the loss of HNCO, as suggested by Phillips et al. (*11*). The formation of ion *m*/*z* 198 occurs in both ESI^+ and ESI^- analysis (*12*), with differences in the elimination of $HCONH_2$ from the $[M + H]^+$ ion in positive ion mode and of HNCO from the $[M - H]^-$ ion in negative ion mode.

Quantitative Analysis of Phenyllactic Acid and Lumichrome. Thistle honey samples were analyzed to quantify compounds **1** and **2** (**Table 1**). Average levels of phenyllactic acid (**1**) were 418.6 ± 168.9 mg/kg and lumichrome (**2**) 29.4 ± 14.9 mg/kg. The statistically significant correlation between the two molecules (R^2 = 0.7845) suggests a common origin for both compounds. The amount of the two compounds is constant over years, and there is a highly significant (R^2 = 0.9900) correlation between the average concentration of these compounds and the three years of collection. Moreover, the ANOVA proved that there are not statistically significant differences from one year to another between the mean contents of water (F = 1.2535, $P > 0.05$), phenyllactic acid (F = 0.0645, $P > 0.05$), and lumichrome (F = 0.4104, $P > 0.05$), respectively. This result evidence that the content of each compound is not affected by the environmental and seasonal condition. Even the thistle honey produced in Sicily showed similar composition (with levels of phenyllactic acid and lumichrome of 528.6 ± 22.1 and 20.8 ± 1.1 mg/kg, respectively),

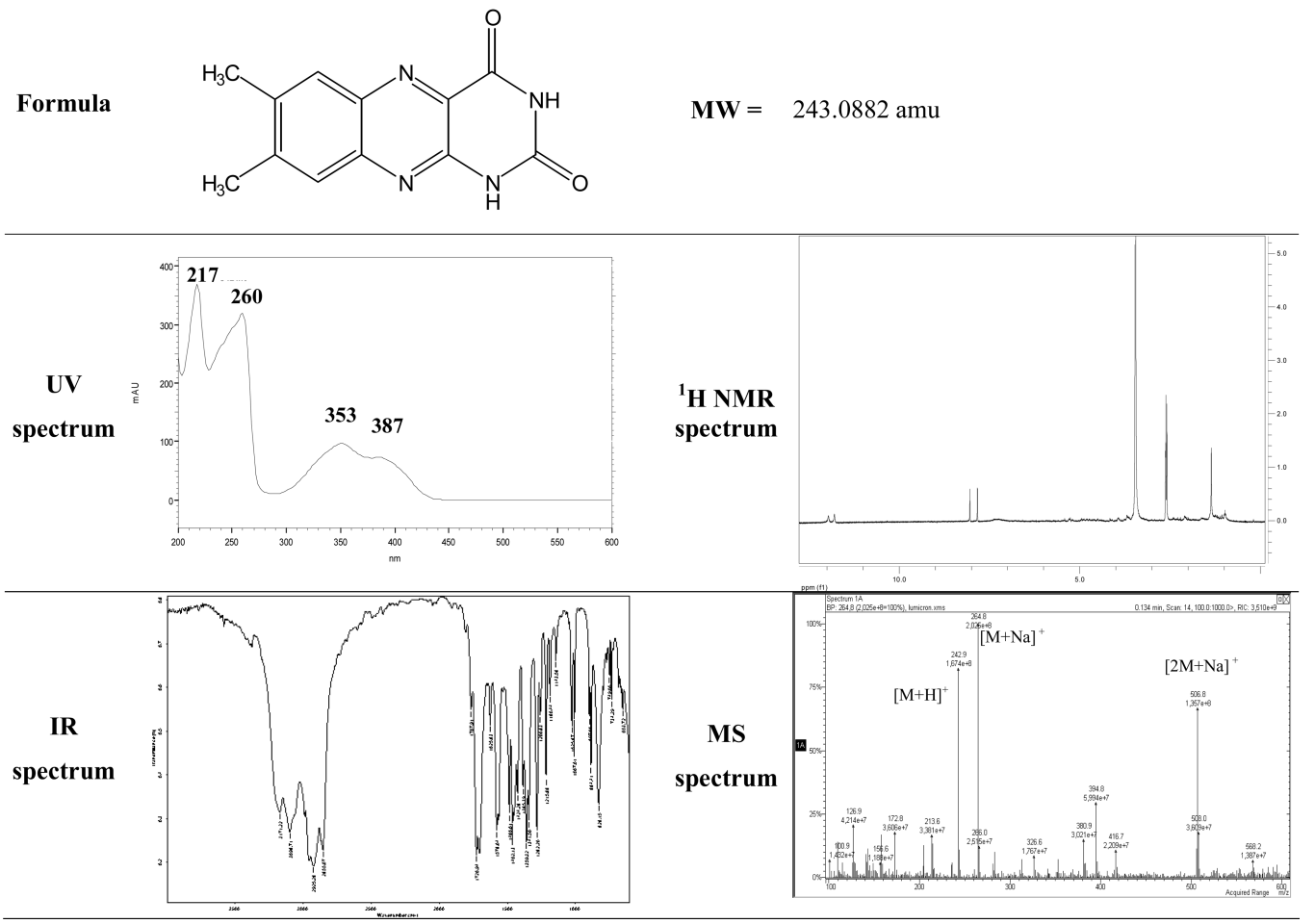

Figure 2. Chemical characteristics of lumichrome detected in honey samples and thistle flower extracts.

indicating that these compounds are not influenced by geographical origin.

To asses if these compounds derived from *G. tomentosa* flowers, raw honeys obtained from thistle in controlled conditions and extract of *G. tomentosa* flowers were analyzed. The raw honeys contained phenyllactic acid (**1**) and lumichrome (**2**) at levels that were comparable to the analyzed thistle honeys (**Table 1**, **Figure 1B**). Thistle flower extracts, obtained according to the procedure described in section Honey Extraction and SPE Purification, contained lumichrome (**Figure 1C**), confirming definitively the botanical origin of such compound.

DISCUSSION

Phenyllactic acid is a compound commonly found in honey. It was found in high concentrations in heather, ling heather, and manuka honeys (828.3, 875.4, and 243.0 mg/kg) (*13*, *14*). Lower concentrations were found in chestnut, *Echium plantagineum*, lime, and clover honeys (47.9, 46.7, 26.4, and 19.0 mg/kg, respectively) (*13*, *14*). Levels of phenyllactic acid lower than 11 mg/kg were found in honey from acacia, bell heather, lavender, rosemary, orange, lemon, repressed, sunflower, eucalyptus, and *fior di sulla* (*14*). Also, in nodding thistle honey (*1*), a small amount of phenyllactic acid was reported (0.7−0.2 mg/kg).

During thistle honey markers quantification, panel tests observed that smell and taste of pure phenyllactic acid standard correlated strongly with the typical sensorial notes of this honey. In particular, the animal nuances were attributed to this phenolic compound.

No data were found in scientific literature concerning the presence of lumichrome in honey. It is well-known that lumichrome (7,8-dimethylalloxazin) is a common breakdown product of riboflavin, formed in the presence of light through a photochemical induced cleavage of the ribityl group under neutral and acidic conditions (*15*). Studies have shown the production of lumichrome from riboflavin catabolism (*16*), but other studies suggest that lumichrome can be produced via an active mechanism and exert biochemical activities. Bacteria, plants, and algae commonly secrete riboflavin or lumichrome. Lumichrome was purified and identified from culture of *S. meliloti* as a compound responsible for stimulating root respiration and plant growth in its symbiotic partner alfalfa (*Medicago sativa*) (*11*). Lumichrome significantly stimulates seedling development in both legume and cereal crops (*17*, *18*), reduces stomatal conductance in bean (*19*), and enhances photosynthesis in corn and soybean (*20*, *21*). Lumichrome obtained from culture of the alga *Chlamydomonas* is as a quorum sensing (QS) signal-mimic compound capable of stimulating the bacteria *Pseudomonas aeruginosa* LasR QS receptor, suggesting that different bacteria could benefit from secreting this compound during host interactions (*22*). These data suggest that lumichrome has important regulatory roles in plants. Lumichrome is a potentially important compound that can enhance the photosynthetic rate and growth of plants.

None of the described papers helps to understand why lumichrome has been found only in thistle honey. Besides a lack in scientific literature, HPLC fingerprinting of other unifloral honeys evidenced the absence of lumichrome in asphodel (*Asphodelus microcarpus* Salzm.

and Viv.) (*6*), strawberry-tree (*Arbutus unedo* L.) (*7*), sulla flower (*Hedysarum coronarium* L.), eucalyptus (*Eucalyptus* spp.), and chestnut (*Castanea sativa* Mill.) honeys (data not shown). Occasionally, lumichrome and phenyllactic acid have been found in these honeys at low levels (phenyllactic acid < 10 mg/L). However, this could be due to contamination from thistle because of the contemporary flowering period of these plants.

In conclusion, the presence of lumichrome and of high levels of phenyllactic acid in thistle (*G. tomentosa*) honey could be a useful marker to fingerprint the botanical origin of this honey.

ABBREVIATIONS USED

DQF-COSY, double-quantum filtered correlation spectroscopy; HSQC, heteronuclear single quantum coherence; HMBC, heteronuclear multiple-bond correlation; Q-TOF, quadrupole time-of-flight.

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