Natural Occurrence of Abscisic Acid in Heather Honey and Floral Nectar

Federico Ferreres,[†] Paula Andrade,[‡] and Francisco A. Tomás-Barberán*,[†]

Departamento de Ciencia y Tecnología de Alimentos, Laboratorio de Fitoquímica, CEBAS (CSIC), P.O. Box 4195, Murcia 30080, Spain, and Faculdade de Farmácia, Laboratorio de Farmacognosia, Universidade do Porto, P-4000 Porto, Portugal

HPLC analysis of Portuguese heather honey (*Erica* spp.) fractions revealed that two non-flavonoid compounds were the main constituents, while flavonoids and other phenolic metabolites were only present as minor constituents. These two substances were isolated and identified as *cis*,*trans*-abscisic acid and *trans*,*trans*-abscisic acid by EIMS, ^{1}H NMR, and ^{13}C NMR techniques. Their content ranged between 2.5 and 16.6 mg/100 g of honey. When *Erica* flower nectar was analyzed, both isomers were also observed as the main constituents and confirmed the floral origin of the compounds found in honey. These were not detected in any of the different monofloral honey samples analyzed so far and, therefore, could be useful markers of heather honey. More heather samples from other geographical and botanical (*Erica* spp., *Calluna* spp., etc.) origins should be analyzed in order to prove that abscisic acid could be a useful floral marker of heather honey.

Keywords: *Abscisic acid; honey; heather; Erica; nectar; characterization*

INTRODUCTION

In the last few years there has been an increasing interest in finding objective analytical methods that could complement pollen analysis in the determination of the floral origin of honey. In this context, it has been suggested that the next step in this type of research will be an attempt to correlate floral source with the presence of certain compounds, originating either in the nectar or in some biochemical modifications of nectar compounds carried out by the bee (Bonaga and Giumanini, 1986). Volatile compounds (Bonaga et al., 1986), aromatic and degraded carotenoid-like substances (Tan et al., 1988, 1989a,b, 1990; Wilkins et al., 1993), amino acids (Davies, 1975; Bosi and Battaglini, 1978), degradation products of phenylalanine (Speer and Montag, 1987), aromatic aldehydes and heterocycles (Häusler and Montag, 1990), aromatic acids and their esters (Speer and Montag, 1984; Steeg and Montag, 1988) and phenolic compounds (Amiot et al., 1989; Ferreres et al., 1992, 1994a,b; Sabatier et al., 1992) have been found in honey and have been related to the floral origin. In fact, the flavonoid hesperetin proved to be a useful marker for the floral origin of citrus honey (Ferreres et al., 1993), and as a marker showed some advantages over methyl anthranilate, another biochemical marker of the floral origin of citrus honey (Ferreres et al., 1994b), and the flavonol kaempferol was found in both rosemary nectar and honey (Gil et al., 1995).

Heather honey is produced in Portugal from *Erica* spp. (Ericaceae), while in Spain and France heather honey come from either *Calluna* or *Erica* species (Soler et al., 1995). In New Zealand, *Calluna*-derived honeys are considered as heather (Tan et al., 1989a). This honey is characterized by its strong flavor and dark brown color.

Previous studies on Portuguese heather honey phenolic metabolites have shown that flavonoids are minor constituents in the phenolic fraction, although some of them seem to be related to its floral origin (Ferreres et al., 1994a). Ellagic acid is another phenolic constituent which seems to be characteristic of this monofloral honey (Ferreres et al., 1995). In these studies two major unknown compounds were detected in the chromatograms of Portuguese heather honey extracts. In previous studies in our laboratory, different monofloral (rosemary, etc.) honey samples were analyzed as well as multifloral honey samples from different geographical locations, but these compounds were not detected in any of the previously analyzed samples (Ferreres et al., 1992, 1993; Tomás-Barberán et al., 1993b, 1994; Soler et al., 1995; Gil et al., 1995). Therefore, of the possible relevance of these compounds as floral markers of heather honey, the aim of the present work was the isolation and identification of these metabolites, their detection in heather floral nectar, and the determination of their content in different Portuguese heather honey samples.

MATERIALS AND METHODS

Honey Samples. Heather honey samples (22) were produced in the Serra da Lousa (Coimbra, Portugal) and provided by the Direcçao da Circunscriçao Florestal de Coimbra. Samples were stored at 0 °C until analyzed (for more details about honey origin and monofloral origin, see Ferreres et al. (1994)). Pollen analysis of these samples to guarantee their monofloral origin has been previously published (Ferreres et al., 1994).

Collection of Heather Nectar. Due to the small size of heather flowers, and to the relatively small amount of nectar that they produce, this was obtained by extraction from the honey bee stomach of bees gathering heather nectar in geographical areas where heather was the only flower available for nectar collection. Bees were trapped with liquid nitrogen and stored at −20 °C until analyzed. The bees were thawed and the honey stomach was dissected with a knife and forceps. The contents of the different honey stomachs were placed into Eppendorf tubes and stored at −20 °C until needed.

Extraction of Metabolites from Nectar. Heather nectar (5 mL) was diluted with 100 mL of distilled water and filtered through cotton to remove solid particles. The filtrate was mixed with the Amberlite XAD-2 nonionic polymeric resin

[†] Laboratorio de Fitoquímica.
[‡] Laboratorio de Farmacognosia.

S0021-8561(95)00755-2 CCC: $12.00

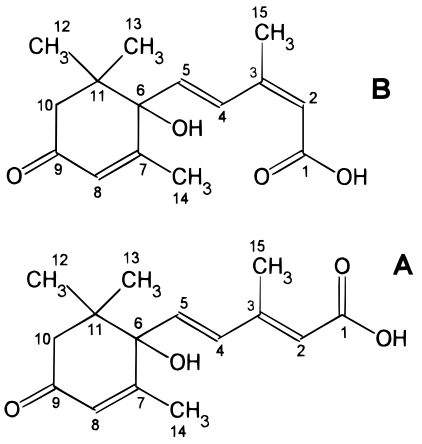
(Fluka Chemie; pore size 9 nm, particle size 0.3−1.2 mm) necessary to fill in a column of 19 × 3 cm and was stirred with a magnetic stirrer for 1 h. The Amberlite XAD-2 with the phenolic compounds adsorbed was packed into the column and washed with 1 L of distilled water. The phenolic metabolites were then eluted with methanol (200 mL) and concentrated with a rotary evaporator (40 °C).

Extraction of Metabolites from Honey. These were extracted as described previously (Ferreres et al., 1991). The available honey samples (~50 g) were thoroughly mixed with 5 parts of water (adjusted to pH 2 with HCl) until completely fluid and filtered through cotton to remove solid particles. The filtrate was then passed through a column (25 × 2 cm) of Amberlite XAD-2 (Fluka Chemie, pore size 9 nm, particle size 0.3−1.2 mm). The phenolic compounds present in honey remained in the column while sugars and other polar compounds eluted with the aqueous solvent. The column was washed with acid water (water adjusted to pH 2 with HCl, 100 mL) and subsequently with distilled water (~300 mL). The whole phenolic fraction was eluted with methanol (~300 mL) and then taken to dryness under reduced pressure (40 °C). The residue was redissolved in 5 mL of distilled water and partitioned with ethylic ether (5 mL × 3). The ether extracts were then combined, and the ether was removed under reduced pressure. The residue was dissolved in 0.5 mL of methanol, filtered through 0.45 μm filters, and analyzed by HPLC.

HPLC Analysis of Nectar Metabolites. The metabolites present in heather nectar were analyzed on a reversed-phase column LiCrochart RP-18 (Merck, Darmstadt, Germany) thermostated at 40 °C (12.5 × 0.4 cm, 5 μm particle size), using water−formic acid (19:1, v:v) and methanol as mobile phases. Elution was performed at a solvent flow rate of 1 mL/min, with a linear gradient elution starting with 15% methanol and 85% water−formic acid, to reach 55% methanol and 45% water−formic acid in 30 min. The different compounds were detected with a diode array detector (Shimadzu SPD-M6A), and the different UV spectra were registered.

HPLC Analysis of Honey Metabolites. This was achieved as recently reported (Ferreres et al., 1994) on the same column and with the same mobile phases used in nectar analysis. In this case, elution was performed at a solvent flow rate of 1 mL/min, starting with 30% methanol in water−formic acid, which remained isocratic until 15 min, and then installing a linear gradient to obtain 40% methanol at 20 min, 45% methanol at 30 min, 60% methanol at 50 min, and 80% methanol at 52 min, and which then became isocratic until 60 min. Detection was accomplished with a diode array detector, and chromatograms were recorded at 340 and 290 nm.

Extraction and Isolation of Compounds A and B. Heather honey (1.4 kg) was dissolved in distilled water (4 L) and mixed with 200 g of the nonionic polymeric resin Amberlite



XAD-2 (Fluka Chemie; pore size 9 nm, particle size 0.3−1.2 mm) and stirred with a magnetic stirrer overnight at room temperature to adsorb phenolic compounds on the Amberlite XAD-2 particles. Amberlite particles were packed into a glass column (50 × 4 cm) and washed with distilled water (4 L) to remove sugars and other polar compounds, and the phenolic fraction was then eluted with methanol (500 mL). This extract was mixed with 100 mL of water and concentrated under reduced pressure (40 °C) until only water remained (50 mL). The water layer was extracted with diethyl ether (100 mL × 3), and the ether extract was then taken to dryness at room temperature and redissolved in 5 mL of methanol. This was then chromatographed on a Sephadex LH-20 (Pharmacia, Upsala) column (25 × 2.5 cm) using methanol as mobile phase, and fractions were followed under UV light (360 and 254 nm). The nature and composition of the different fractions was tested by analytical HPLC on reversed-phase columns with the conditions described for analysis of honey phenolics. Compounds **A** and **B** were then purified by semipreparative HPLC on an ODS-2 column (Teknochroma, Barcelona) (25 × 0.7 cm, 5 μm particle size), with a flow rate of 2 mL/min using as mobile phase an isocratic mixture of methanol−water (3:7). The purity of the compounds was tested by analytical HPLC with diode array detection. The pure isolated compounds were freeze-dried and stored dried until NMR and electron impact mass spectometry (EIMS) analyses were run.

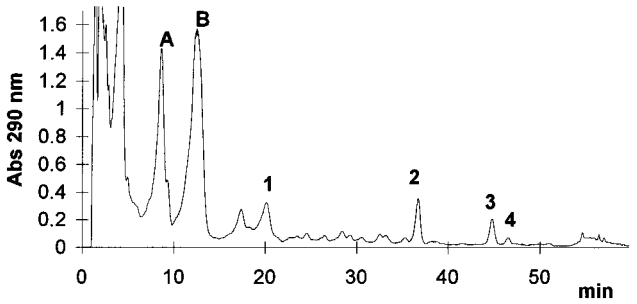


Figure 1. HPLC analysis of Amberlite XAD-2 extracts from heather honey: (**A**) *trans,trans*-abscisic acid; (**B**) *cis,trans*-abscisic acid; (**1**) pinobanksin; (**2**) pinocembrin; (**3**) chrysin; (**4**) galangin. For HPLC conditions, see Materials and Methods (HPLC Analysis of Honey Metabolites).

NMR Analyses. These were achieved in a Brücker ARX500 instrument, ^{1}H NMR at 500 MHz and ^{13}C NMR at 60 MHz. Samples were dissolved in CD_3OD (Aldrich), and analyzed in a 3 mm tube at 20 °C.

EIMS Analyses. These were achieved in a Hewlett-Packard electron impact mass spectrometer. Samples were introduced by direct inlet, and analyses were achieved at 70 eV.

RESULTS AND DISCUSSION

HPLC analysis of the Amberlite XAD extracts from heather honey showed that the major compounds were not flavonoids. In fact, the phenolic metabolites were minor constituents of these extracts. This is clearly shown in the chromatogram in Figure 1, in which the main substances are those marked as **A** and **B**. These had identical UV spectra when recorded with a diode array detector (identical shape and maximum at 262 nm) and suggested that they could be isomers. Four flavonoids were identified, by their UV spectra and chromatographic comparisons with authentic markers previously isolated and identified from other honey samples (Ferreres et al., 1991), as pinobanksin (**1**), pinocembrin (**2**), chrysin (**3**), and galangin (**4**) (Figure 1). However, other minor flavonoids, up to 18, that had already been found in heather honey were not detected under these analytical conditions since a further step in purification, using Sephadex LH-20 chromatography, was necessary in order to detect all these minor flavonoids and ellagic acid (Ferreres et al., 1995). The total amount of flavonoids detected in those heather honey samples ranged between 0.06 and 0.5 mg/100 g of honey, and most of the flavonoids were in amounts smaller than 0.005 mg/100 g of honey (Ferreres et al., 1994a).

Table 1. ^1H Chemical Shifts (ppm) and $^1H-^1H$ Coupling Constants (Hz) in CD_3OD

	A	B
H_2	5.87 s	5.78 s
H_4	6.47 d (J_{4-5} 15.9)	7.82 d (J_{4-5} 15.9)
H_5	6.18 d (J_{4-5} 15.9)	6.18 d (J_{4-5} 15.9)
H_8	5.95 s	5.98 s
H_{10A}	2.49 d ($J_{10A-10B}$ 17.1)	2.50 d ($J_{10A-10B}$ 17.1)
H_{10B}	2.31 d ($J_{10A-10B}$ 17.1)	2.30 d ($J_{10A-10B}$ 17.1)
H_{12} (3)	1.11 s	1.12 s
H_{13} (3)	1.02 s	1.04 s
H_{14} (3)	1.90 s	1.93 s
H_{15} (3)	2.29 s	2.05 s

Table 2. ^{13}C Chemical Shifts (ppm) in CD_3OD

carbon no.	A	B	abscisic acid[a]
1	171.51	170.58	170.60
2	119.94	118.13	118.10
3	161.95	162.80	163.00
4	134.18	128.49	128.50
5	135.66	136.97	136.90
6	79.69	79.97	79.90
7	153.04	151.51	151.50
8	127.31	127.19	127.10
9	197.70	198.16	198.30
10	49.75	49.82	49.70
11	41.63	41.74	41.70
12	14.38	19.05	19.10
13	23.08	23.20	23.10
14	24.36	21.44	21.40

[a] Commercial.

Table 3. Quantitation of Abscisic Acid Isomers in Portuguese Heather Honey and Floral Nectar

samples	A*	% A	B*	% B	A + B*
16	4.3	32	9.2	68	13.5
15	4.7	36.4	8.2	63.6	12.9
13	4.1	33	8.3	67	12.4
1	5.2	36	9.2	64	14.4
8	4.1	37	7.0	63	11.1
20A	2.7	34.6	5.1	65.4	7.8
19	4.4	33.3	8.8	66.7	13.2
6	2.9	32.6	6.0	67.4	8.9
28	4.6	38.7	7.3	61.3	11.9
20	3.3	42	4.6	58	7.9
50	7.0	42	9.6	58	16.6
40	4.4	38	7.2	62	11.6
23	2.2	32	4.7	68	6.9
9	4.7	38.2	7.6	61.8	12.3
24	2.8	30.4	6.4	69.6	9.2
12	3.2	41.6	4.5	58.4	7.7
14	3.1	46.3	3.6	53.7	6.7
10	2.4	35.8	4.3	64.2	6.7
7	6.5	41.4	9.2	58.6	15.7
11	1.2	36.4	2.1	63.6	3.3
3	0.9	36	1.6	64	2.5
2	0.9	36	1.6	64	2.5
nectar		25		75	

A and **B** were extracted and purified from Portuguese heather honey by a combination of Sephadex LH-20 chromatography and semipreparative HPLC. The purity of the isolated compounds was tested by analytical HPLC with a diode array detector. These HPLC analyses revealed that after isolation each compound was partly transformed to the other after a few hours in methanol solution, suggesting that both compounds were isomers. The EIMS analysis of both compounds confirmed that they were isomers and showed that they had a molecular weight of 264 and that relevant fragments at 208, 190, 162, and 134 mu were produced. The ^1H NMR (Table 1) and ^{13}C NMR (Table 2) analyses clearly showed that both compounds were isomers of abscisic acid, with **A** being *trans,trans*-abscisic acid and **B** being *cis,trans*-abscisic acid. The NMR spectra of both compounds were very similar with the only differences at the level of the proton H-4, which appeared at lower fields in the spectrum of **B**, and the signal of carbon C-4 which appeared at 134 ppm in **A**, and only 128 ppm in **B**. The identity of both compounds was then confirmed by HPLC analysis with an authentic sample of *cis,trans*-abscisic acid (Sigma, St. Louis, MO), which coincided with **B**, and by comparison with the ^{13}C NMR spectrum of the authentic sample of *cis,trans*-abscisic acid (Table 2).

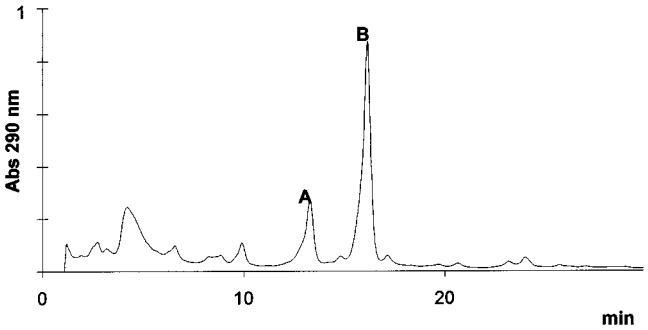


Figure 2. HPLC analysis of *Erica* floral nectar extract: (**A**) *trans,trans*-abscisic acid; (**B**) *cis,trans*-abscisic acid. For HPLC conditions see Materials and Methods (HPLC Analysis of Nectar Metabolites).

Quantitative Study of Abscisic Acid in Portuguese Honey Samples. Twenty-two heather honey samples were analyzed to evaluate their content of abscisic acid isomers **A** and **B** (Table 3). Isomer **A** was always present in smaller amounts than **B**. The total amount of abscisic acid derivatives ranged between 2.5 and 16.66 mg/100 g of honey. This content is much greater than that reported previously for flavonoids in heather honey samples of the same origin (see above) (Ferreres et al., 1994a). In a previous study on New Zealand unifloral heather honey (*Calluna vulgaris*), methyl abscisate was detected in much smaller amounts (between 0.16 and 0.57 mg/100 g) (Tan et al., 1989a). However, this compound was not detected when unmethylated honey extracts were analyzed by GC−MS; the authors suggested that abscisic acid might be present in New Zealand heather honey as free acid isomers. In this previous study, the main compounds detected in the extracts were a whole range of degraded carotenoid compounds (3,5,5-trimethylcyclohex-2-ene skeletons) with 13 carbon atoms, and their contents were in the 3−18 mg/100 g range. The present results indicate that Portuguese heather honey (produced from *Erica* spp.) accumulate abscisic acid, while New Zealand heather honey (*Calluna* spp.) accumulates other chemically related derivatives, but abscisic acid isomers seem to be minor constituents.

In order to test whether abscisic acid was already present in *Erica* floral nectar or whether it had been produced by the action of bee enzymes on other constituents (i.e., degradation of carotenoids from pollen), *Erica* nectar was collected with the help of bees, and the soluble constituents were analyzed by HPLC. The HPLC chromatogram of nectar extract (Figure 2) shows that the main constituents were **A** and **B** and proves that the compounds detected in heather honey are natural constituents of heather nectar.

Even though abscisic acid is present in all species of Angiosperms, many Gymnosperms, and a few Pteridophytes, and it has been found in phloem and xylem sap and in nectar (Goodwin and Mercer, 1983), this me-

tabolite has not been detected in any of the previously analyzed monofloral honeys (rosemary, sunflower, lavender, chesnut, orange, lemon, grapefruit, rapeseed, *Acacia*, *Eucalyptus*, *Abies*, *Tillium*, etc.) (Ferreres et al., 1992, 1993; Tomás-Barberán et al., 1993, 1994; Soler et al., 1995; Gil et al., 1995), and this suggests that abscisic acid could be a useful marker of the floral origin of heather honey.

However, heather honey samples from other geographical and botanical (different species of *Erica* and *Calluna*) origins should be analyzed to evaluate their content in abscisic acid, in order to prove whether these metabolites are useful floral markers of these monofloral honeys.

ACKNOWLEDGMENT

We are grateful to M. P. de Luis Villota for technical assistance.

LITERATURE CITED

Amiot, M. J.; Aubert, S.; Gonnet, M.; Tacchini, M. Honey phenolic compounds: a preliminary study on their identification and quantitation by families. *Apidologie* **1989**, *20*, 115−125.

Bonaga, G.; Giumanini, A. G. The volatile fraction of chesnut honey. *J. Apic. Res.* **1986**, *25*, 113−120.

Bonaga, G.; Giumanini, A. G.; Gliozzi, G. Chemical composition of chesnut honey: Analysis of the hydrocarbon fraction. *J. Agric. Food Chem.* **1986**, *34*, 319−326.

Bosi, G.; Battaglini, A. G. Gas chromatographic analysis of free and protein amino acids in some unifloral honeys. *J. Apic. Res.* **1978**, *17*, 152−166.

Davies, A. M. C. Amino acid analysis of honey from eleven countries. *J. Apic. Res.* **1975**, *14*, 29−39.

Ferreres, F.; Tomás-Barberán, F. A.; Gil, M. I.; Tomás-Lorente, F. An HPLC technique for flavonoid analysis in honey. *J. Sci. Food Agric.* **1991**, *56*, 49−56.

Ferreres, F.; Ortiz, A.; Silva, C.; García-Viguera, C.; Tomás-Barberán, F. A.; Tomás-Lorente, F. Flavonoids of "La Alcarria" honey. A study of their botanical origin. *Z. Lebensm. Unters. Forsch.* **1992**, *194*, 139−143.

Ferreres, F.; García-Viguera, C.; Tomás-Lorente, F.; Tomás-Barberán, F. A. Hesperetin, a marker of the floral origin of citrus honey. *J. Sci. Food Agric.* **1993**, *61*, 121−123.

Ferreres, F.; Andrade, P.; Tomás-Barberán, F. A. Flavonoids from Portuguese heather honey. *Z. Lebensm. Unters. Forsch.* **1994a**, *199*, 32−37.

Ferreres, F.; Giner, J. M.; Tomás-Barberán, F. A. A comparative study of hesperetin and methyl anthranilate as markers of the floral origin of citrus honey. *J. Sci. Food Agric.* **1994b,** *65*, 371−372.

Ferreres, F.; Tomás-Barberán, F. A.; Soler, C.; García-Viguera, C.; Ortiz, A.; Tomás-Lorente, F. A simple extractive technique for honey flavonoid HPLC analysis. *Apidologie* **1994c**, *25*, 21−30.

Ferreres, F.; Andrade, P.; Gil, M. I.; Tomás-Barberán, F. A. Floral nectar phenolics as biochemical markers of the botanical origin of heather honey. *Z. Lebensm. Unters. Forsch.* **1996**, *202*, 40−44.

Gil, M. I.; Ferreres, F.; Ortiz, A.; Subra, E.; Tomás-Barberán, F. A. Phenolic compounds in rosemary honey and floral nectar. *J. Agric. Food Chem.* **1995**, *43*, 2833−2838.

Goodwin, T. W.; Mercer, E. I. *Introduction to Plant Biochemistry*; Pergamon Press: Oxford, U.K., 1983; pp 606−617.

Greenaway, W.; Scaysbrook, T.; Whatley, F. R. The composition and plant origin of propolis: a report of work at Oxford. *Bee World* **1990**, *71*, 107−118.

Häusler, H.; Montag, A. Minor flavour/aroma compounds in honey. III. Distribution of aromatic aldehydes in floral types, and occurrence of heterocyclic N- and S-compounds. *Dtsch. Lebensm. Rundsch.* **1990**, *86*, 171−174.

Sabatier, S.; Amiot, M. J.; Tacchini, M.; Aubert, S. Identification of flavonoids in sunflower honey. *J. Food Sci.* **1992**, *57*, 773−777.

Soler, C.; Gil, M. I.; García-Viguera, C.; Tomás-Barberán, F. A. Flavonoid patterns of French honey with different floral origin. *Apidologie* **1995**, *26*, 53−60.

Speer, K.; Montag, A. Occurrence of benzoic acid and phenylacetic acid in honey. *Dtsch. Lebensm. Rundsch.* **1984**, *80*, 103−105.

Speer, K.; Montag, A. Phenylalanine decomposition products as flavour compounds in honey. *Dtsch. Lebensm. Rundsch.* **1987**, *83*, 103−107.

Steeg, E.; Montag, A. Minor components of honey with organoleptic significance. Part I. Aromatic carboxylic acids and their esters. *Dtsch. Lebensm. Rundsch.* **1988**, *84*, 103−108.

Tan, S. T.; Holland, P. T.; Wilkins, A. L.; Molan, P. C. Extractives from New Zealand honeys. 1. White clover, Manuka and Kanuka unifloral honeys. *J. Agric. Food Chem.* **1988**, *36*, 453−460.

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.* **1989a**, *37*, 1217−1222.

Tan, S. T.; Wilkins, A. L.; Molan, P. C.; Holland, P. T.; Reid, M. A chemical approach to the determination of floral sources of New Zealand honeys. *J. Apic. Res.* **1989b**, *28*, 212−222.

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.

Tomás-Barberán, F. A.; Blázquez, M. A,; García-Viguera, C.; Ferreres, F.; Tomás-Lorente, F. A comparative study of different Amberlite XAD resins in flavonoid analysis. *Phytochem. Anal.* **1992**, *3*, 178−181.

Tomás-Barberán, F. A.; Ferreres, F.; García-Viguera, C.; Tomás-Lorente, F. Flavonoids in honey of different geographical origin. *Z. Lebensm. Unters. Forsch.* **1993**, *196*, 38−44.

Tomás-Barberán, F. A.; Ortiz-Valbuena, A.; Ferreres, F.; Fernández-Maeso, M. C. *Study on the flavonoid content of La Alcarria honey: Its application to the geographical/botanical characterization*; CSIC-JJCC Castilla-La Mancha: Madrid. 1994.

Wilkins, A. L.; Lu, Y.; Tan, S. T. Extractives from New Zealand honeys. 4. Linalool derivatives and other components from nodding thistle (*Carduus nutans*) honey. *J. Agric. Food Chem.* **1993,** *41*, 873−878.

Received for review November 14, 1995. Accepted May 13, 1996.[⊗] We are grateful to the Spanish CICYT (Grants ALI91-0486 and ALI92-0151 for financial support of this work.

JF9507553

[⊗] Abstract published in *Advance ACS Abstracts*, July 15, 1996.