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# CHEMISTRY, BIOLOGY AND POTENTIAL APPLICATIONS OF HONEY BEE PLANT-DERIVED PRODUCTS

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## Valuable Analytical Tools in Analysis of Honeybee Plant-Derived Compounds: Nuclear Magnetic Resonance Spectroscopy

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**Abstract:** Over the past fifty years, Nuclear Magnetic Resonance (NMR) spectroscopy proved to be a powerful tool in the structural characterisation of bioactive compounds from natural sources. In this chapter we cover the basic theory behind each NMR technique used to determine the structure of several families of compounds (*e.g.* carbohydrates, phenolics and sesquiterpenoids) present in honey and propolis. We also provide basic information how 1D and 2D NMR techniques can help in the structure establishment of honeybee constituents. The <sup>1</sup>H and <sup>13</sup>C NMR data of several of these constituents are compiled and described, being some of them used as botanical and geographical markers. In the case of propolis, a list of compounds identified by NMR is presented. A basic overview in quantitative NMR determinations and in NMR coupled to chemometric methodologies highlights their use to detect honey adulteration and assign their authenticity.

**Keywords:** Adulteration, Authenticity, Botanical marker, <sup>13</sup>C NMR, COSY, DEPT, DOSY, Geographical marker, HMBC, HMQC, <sup>1</sup>H NMR, Honeybee, HSQC, Natural products, NMR spectroscopy, NOESY, Propolis, ROESY, Structure elucidation, TOCSY.

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## 1. INTRODUCTION

The most important tools used by chemists for the structural elucidation of natural and synthetic compounds are based on spectroscopic techniques, being NMR spectroscopy a useful resource on a routine basis. The data provided by 1D NMR techniques, such as  $^1\text{H}$  and  $^{13}\text{C}$  NMR experiments, is the first step to achieve this goal. The complexity of molecular structures implies the use of more advanced 1D and 2D NMR techniques for the complete assignment of proton, carbon or other nuclei resonances. It is a non-destructive technique and with the greatest advances over the past years, excellent results can be obtained from samples weighing less than a milligram.

Thus, a brief discussion on the basic concepts of the commonly used NMR experiments from the point of view of structural elucidation of organic compounds is required to highlight the usefulness of these spectroscopic techniques.

The  $^1\text{H}$  nuclei is the most commonly observed nuclei in NMR spectroscopy and is the starting point for most structure determinations. Valuable information can be obtained from a  $^1\text{H}$  NMR spectrum: the chemical shift ( $\delta$ , ppm; using TMS as standard) that is correlated with its chemical environment; the coupling constant ( $J$ , Hz) that are determined by the interactions between individual nuclei and promoted by electrons in a chemical bond and finally, under suitable conditions, the area of a resonance that is related to the number of nuclei giving rise to the  $^1\text{H}$  NMR signal [1, 2].

The  $^{13}\text{C}$  NMR spectrum offers further characterization of a molecule and is directly related to the carbon skeleton. Typically, this spectrum is recorded with broadband decoupling of all protons, appearing each carbon resonance as a single line ( $\delta$ , ppm; using TMS as standard) [1, 2]. The chemical shift of each resonance is once more indicative of their environment, being possible to identify certain functional groups that are not detected in a  $^1\text{H}$  spectrum (*e.g.* carbonyls).

The  $^{15}\text{N}$  NMR spectrum although having great importance for structural NMR analysis, since *N*-containing functional groups and N atoms are present in several molecular skeletons, the low natural abundance of  $^{15}\text{N}$  (about 0.4%) and its

extremely low relative sensitivity make difficult these measurements [1, 2]. The typical range of  $^{15}\text{N}$  chemical shift is about 600 ppm and the recommend reference is nitromethane ( $\delta = 0$  ppm), although these values can also be quoted with respect to saturated aqueous solution of ammonium chloride ( $\delta = -359.5$  ppm) or ammonium nitrate ( $\delta = -3.9$  ppm). Unfortunately, the analysis and comparison of the reported data is many times hampered due to the lack of information of the reference standard used.

The  $^1\text{H}$ - $^1\text{H}$  COSY (CORrelation SpectroscopY) spectrum provides a means of identifying mutually coupled protons (typically geminal and vicinal couplings), allowing the assignment of all proton resonances [1, 3]. In this technique, 1D spectrum is displayed along each axis with a contour projection of this spectrum along the diagonal axis. Off-diagonal peaks represent proton shift correlations (or proton couplings). A related COSY technique, DQF-COSY (Double Quantum Filtered-CORrelated SpectroscopY), is sometimes used to simplify the diagonal of the COSY spectrum where the peaks are greatly reduced in intensity with consequent clarification of this region. A further advantage of DQF-COSY is that in the phase sensitive mode, both diagonal and cross peaks can be adjusted to have a pure absorption line shape. A similar spectrum to  $^1\text{H}$ - $^1\text{H}$  COSY can be obtained by a TOCSY (TOtal CORrelated SpectroscopY) experiment. The correlations observed between all the protons within a given spin system is irrespective of whether they are directly coupled or not. This technique is quite useful in severe resonance overlapping, in which  $^1\text{H}$ - $^1\text{H}$  COSY spectra can leave to ambiguous assignments.

NOE (Nuclear Overhauser Effect) difference experiment provides information about the spatial proximity of two protons within a molecule [1, 3]. It involves the application of a radio-frequency field to a single resonance in which the corresponding protons become saturated (it means that the difference of population between their high and low energy levels are forced to zero). Recording of the proton spectrum after the period of saturation may, therefore, show changes in signal intensities for the protons in the vicinity of the saturated proton. Thus, 1D NOE studies generally presents the differences between the original proton spectrum and the irradiated proton spectra. In the 2D NOESY (Nuclear Overhauser Effect SpectroscopY) experiments, only one spectrum is

recorded and provides all the information from the NOE studies [1, 3].

2D ROESY (Rotating-frame Overhauser Effect Spectroscopy) is used to measure homonuclear ROE effects under spin-locked conditions, these cross-peaks arise from proton resonances that are spatially close even if they are not bonded [3]. It is quite useful for molecules with large molecular sizes (NOEs are too weak to be detectable). Since in NOESY the cross-relaxation rate constant goes from positive (for small molecules) to negative (for large molecules) as the correlation time increases, or even null, in ROESY the cross-relaxation rate constant is positive for all rotational correlation times.

The first attempt to assign the signals of a complicated  $^{13}\text{C}$  NMR spectrum is through DEPT (Distortionless Enhancement by Polarization Transfer) experiments which provide information about the number of protons attached to a carbon atom (C, CH,  $\text{CH}_2$ ,  $\text{CH}_3$ ) [1, 3]. This technique is more sensitive than the normal  $^{13}\text{C}$  and also very useful to assign the type of carbons in molecules bearing aromatic, aliphatic and/or olefinic moieties. In a DEPT experiment, sequences of pulses with various delay times are used to create the DEPT spectra. This requires acquiring and processing three separate spectra termed as DEPT-45, DEPT-90 and DEPT-135 (the number indicates the flip angle of the editing proton pulse in the sequence). A DEPT-45 spectrum presents positive signals for all H-bearing carbon peaks; in a DEPT-90 spectrum only methine carbons are seen as positive signals and for DEPT-135 spectrum methyl and methine carbons peaks appear as positive signals and methylene carbons peaks give negative signals. Quaternary carbons are not detected in DEPT spectra because the technique relies on polarization transfer, which in this case is the transfer of proton magnetization onto the directly bound carbon atoms.

Similar information is obtained from the 2D HSQC (Heteronuclear Single Quantum Correlation) and HMQC (Heteronuclear Multiple Quantum Coherence) experiments [3]. These two techniques provide single bond heteronuclear shift correlation, identifying one-bond H-C or H-N connectivities within a molecule. The resulting 2D spectrum displays in one axis  $^1\text{H}$  chemical shifts and the other for the heteronuclei, most often  $^{13}\text{C}$  or  $^{15}\text{N}$  chemical shifts. The cross-peaks in the contour plot define to which carbon or nitrogen a particular proton (or group of

protons) is directly attached.

HMBC (Heteronuclear Multiple Bond Correlation) is closely related to HMQC, although much less sensitive, that operates in essentially the same manner [1, 3]. In this experiment, however, the sequence timings are optimised for much smaller coupling constants and therefore establish long-range correlations. These  $^1\text{H}$ - $^{13}\text{C}$  couplings typically occur with significant intensity over 2 and 3 bonds ( $^{2,3}J_{\text{C-H}}$  usually  $< 10$  Hz), but may be apparent over 4 bonds in conjugated systems. Correlations can also be observed to quaternary centres (for example in the assignment of carbonyl resonances) and across heteroatoms other than carbon (for example hydroxyl protons).

Taking into consideration the information provided by the different techniques previously described, we will highlight below their application in the identification of several families of compounds present in honeybee plant-derived products. Commonly, the identification of the constituents of honeybee products by NMR can be done through three different approaches: i) isolation and complete structure elucidation of pure compounds by using 1D and 2D NMR techniques; ii) isolation and simple structure elucidation of pure compounds by using 1D NMR techniques, which data are compared with those of authentic samples; or iii) using NMR as a complementary tool of hyphenated chromatographic and/or chemometric techniques, identifying characteristic  $^1\text{H}$  and/or  $^{13}\text{C}$  NMR signals from complex mixtures of compounds. In the first case we will exhaustible describe the structure elucidation of such compounds and depict the corresponding structure. In the second case, the structure will be depicted and some important NMR features will be described. In the last case, we will only list the identified compounds.

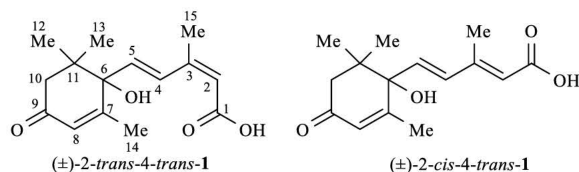
## **2. HONEY**

In the last few decades, alternative or complementary methodologies to mellissopalynology have been developed in order to identify and classify honeys origin focused on the presence of unique molecules or their mixtures. In this context, a series of compounds were isolated and characterized by NMR spectroscopy and used as markers of their botanical origins. In 1996, abscisic acid was

detected in Portuguese heather honeys (*Erica* spp.) and identified for the first time in a monofloral honey [4]. The authors isolated two isomers of abscisic acid, ( $\pm$ )-2-*trans*-4-*trans*-abscisic-**1** and ( $\pm$ )-2-*cis*-4-*trans*-abscisic acid-**1** (Fig. 1), being their NMR spectra very similar (Table 1). The signal of H-4 of the *cis*, *trans*-**1** isomer is deshielded and that of C-4 are shielded compared with those of the *trans*,*trans*-**1** isomer. It is important to highlight that this work has a problem in the assignment of carbons C12-C15 of both isomers. The authors missed the assignment of C-15 and according to a deep analysis of the NMR data of *cis*, *trans*-**1** these assignments must be: i) C-12 and C-13 at 23.1 and 24.3 ppm, ii) C-14 at 19.1 ppm and iii) C-15 at 21.5 ppm [5].

**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data ( $\delta$  in  $\text{CD}_3\text{OD}$ , ppm;  $J$  in parenthesis, Hz) of ( $\pm$ )-2-*trans*-4-*trans*-abscisic acid **1** and ( $\pm$ )-2-*cis*-4-*trans*-abscisic acid **1**. (Adapted from [4]).

Position	( $\pm$ )-2- <i>trans</i> -4- <i>trans</i> - <b>1</b>		( $\pm$ )-2- <i>cis</i> -4- <i>trans</i> - <b>1</b>	
	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$
1		171.51		170.60
2	5.87, s	119.94	5.78 s	118.10
3		161.95		163.00
4	6.47 d (15.9)	134.18	7.82 d (15.9)	128.50
5	6.18 d (15.9)	135.66	6.18 d (15.9)	136.90
6		79.69		79.90
7		153.04		151.50
8	5.95 s	127.31	5.98 s	127.10
9		197.70		198.30
10	2.49 d (17.1)	49.75	2.50 d (17.1)	49.70
	2.31 d (17.1)		2.30 d (17.1)	
11		41.63		41.70
12	1.11 s	14.38	1.12 s	19.10
13	1.02 s	23.08	1.04 s	23.10
14	1.90 s	24.36	1.93 s	21.40
15	2.29 s		2.05 s	



**Fig. (1).** Structures of  $(\pm)$ -2-*trans*-4-*trans*-abscisic-1 and  $(\pm)$ -2-*cis*-4-*trans*-abscisic acid-1.

In a strawberry-tree (*Arbutus unedo*) honey the phenolic acid homogentisic acid (2,5-dihydroxyphenylacetic acid), which structure was assigned based in the MS and NMR ( $^1\text{H}$ ,  $^{13}\text{C}$  and DEPT-135 spectra) data and confirmed by spectral comparison with an authentic sample, was used as marker of this monofloral honey [6]. Complementary markers of strawberry-tree honeys were  $(\pm)$ -2-*cis*-4-*trans*-abscisic acid,  $(\pm)$ -2-*trans*-4-*trans*-abscisic acid and unedone **2** (Fig. 2) [7]. The structure of unedone **2** was identified by MS, HPLC-MS and NMR ( $^1\text{H}$ ,  $^{13}\text{C}$ , DEPT-90 and 135, COSY, HSQC and HMBC) (Table 2).

**Table 2.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data ( $\delta$  in  $\text{CDCl}_3$ , ppm;  $J$  in parenthesis, Hz) of unedone **2**. (Adapted from [7]).

Position	$\delta_{\text{H}}$	$\delta_{\text{C}}^{\text{a}}$	$^{23}\text{J}_{\text{H-C}}$ connectivities
1		37.1 s	C-1, C-3, C-4, C-6, C-11, C-12
2	2.38 dd (17.1, 1.2) (H-2 $\alpha$ )	51.2 t	C-1, C-3, C-6, C-11, C-12
	2.50 br d (17.1) (H-2 $\beta$ )		
3		197.0 s	
4	6.03 br s	129.6 d	C-2, C-6, C-13
5		161.2 s	
6		68.7 s	
7	3.10 d (8.7)	66.0 d	C-5, C-6
7	4.01 dd (8.7, 3.9)	72.1 d	C-7, C-9, C-10
8	3.84 m	69.2 d	C-7, C-10
10	1.25 d (6.6)	19.7 q	C-8, C-9
11	1.16 s	25.7 q	C-1, C-2, C-6, C-12
12	1.01 s	26.9 q	C-1, C-2, C-3, C-6, C-11
13	1.78 d (1.2)	18.0 q	C-4, C-5, C-6

<sup>a</sup> The multiplicity was determined by DEPT experiments.

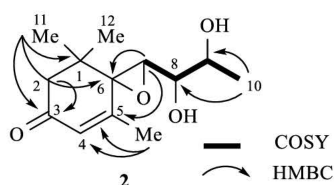


Fig. (2). Main COSY and HMBC correlations of unedone 2.

Later on, the phenolic ester methyl syringate (methyl 4-hydroxy-3-5-dimethoxybenzoate) was identified as a chemical marker of the asphodel (*Asphodelus microcarpus* Salm. Et Viv.) monofloral honey [8]. The  $^1\text{H}$  NMR spectrum of this compound was particularly important in the structure elucidation because of their four singlets (3.87 and 3.91 ppm of the methoxyl groups, 5.88 ppm of the hydroxyl group and 7.30 ppm corresponding to the symmetrical aromatic methines). 4-Hydroxyquinaldic acid (kynurenic acid) **3** and their tautomer 4-quinolone-2-carboxylic acid **4** (Fig. 3) were reported as markers of the floral origin of chestnut honey [9]. Their structures were assigned based on the HPLC-DAD-MS-MS and NMR data (Table 3). From the same type of honey, Beretta *et al.* isolated and elucidated the structure of two kynurenic acid related compounds 3-(2'-pyrrolidinyl)kynurenic acid **5** and its  $\gamma$ -lactam derivative **6** (Fig. 3) [10]. A detailed NMR study was necessary to establish these structures 1D ( $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{15}\text{N}$ ) and 2D (COSY, HMQC and HMBC) NMR techniques (Table 3). In the  $^{15}\text{N}$  NMR of compound **5**, the authors assigned the protonated aliphatic secondary amine N-1'' resonance at 435 ppm (through direct acquisition) and that of the quinolone heterocycle ring N-1 at 130 ppm (through a HMBC detection) [10]. However, they did not indicate the reference used in the  $^{15}\text{N}$  NMR spectrum as well as the coupling constants used in the HMBC spectrum acquisition.

Table 3.  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{15}\text{N}$  NMR spectroscopic data ( $\delta$  in DMSO- $d_6$ , ppm) of compounds 3-6.

Position	3		4		5			6		
	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$	$^{15}\text{N}$	$^{13}\text{C}$	$^1\text{H}$	$^{15}\text{N}$	$^{13}\text{C}$
1						130			n.d.	

(Table 3) contd....

Position	3		4		5			6		
	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>15</sup> N	<sup>13</sup> C	<sup>1</sup> H	<sup>15</sup> N	<sup>13</sup> C
2		140.0		145.3			139.2			140.9
3	6.68	109.0	8.88	107.5			113.4			114.9
4		178.0		178.1			177.8			174.0
5	8.09	123.5	8.28	124.4	8.16		125.2	8.17		125.5
6	7.32	119.6	7.85	119.9	7.48		119.9	7.41		119.8
7	7.66	132.0	7.59	133.8	7.79		132.8	7.72		133.7
8	7.99	124.6	7.86	125.0	7.93		125.0	7.92		124.6
9		125.6		126.0			125.9			126.8
10		142.8		139.8			141.9			141.1
2''		163.8		166.5			164.1			166.7
1''					8.88, 9.95	435			n.d.	
2''					5.11		57.6	4.66		62.3
3''					2.10, 2.20		24.9	2.30-2.40		29.2
4''					2.20		29.6	1.14		29.4
5''					3.32		46.1	3.35, 3.60		42.0

n.d. = not detected

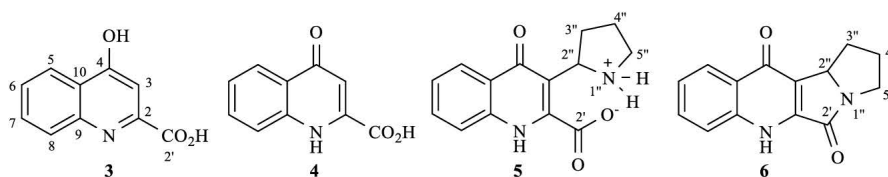


Fig. (3). Structures of compounds 3-6.

Later, the same group of researchers used DOSY NMR analysis to demonstrate that among various honeys of different botanical origins chestnut honey showed the highest content of quinolone alkaloids (compounds 4-6 and 4-quinolone) [11]. This technique uses the molecular diffusion rate in solution to separate resonances generated by different compounds in a mixture, with no need of separation.

A rapid and easy differentiation of oak honeydew honey from other honey types can be based on the methylene <sup>1</sup>H (two multiplets at 1.98 and 1.80 ppm) and <sup>13</sup>C (37.87 ppm) NMR resonance of quercitol (a glucosidase inhibitor that blocks the

absorption and metabolism of carbohydrates) [12]. These resonances only appear in the NMR spectra of the oak honeydew honey being quercitol a good taxonomic marker of this honey and of the genus *Quercus*. The semi-quantitative evaluation of quercitol, using the unambiguously distinguished signal at 1.80 ppm (H-6 axial), can be determined by the ratio intensity to the anomeric proton of the  $\alpha$ - and  $\beta$ -glucopyranose, always present in high amounts in honey [12].

Low Field  $^1\text{H}$  Nuclear Magnetic Resonance (LF  $^1\text{H}$  NMR) was used to differentiate the botanical origin of eight (eucalyptus, “assa-lipto”, oranges, Barbados cherry, cashew tree, “assa-peixe”, “cipó-uva”, and polyfloral) Brazilian honeys [13]. For instance, comparison of the continuous distributed NMR ( $T_2$ ) relaxation curves revealed differences in the water mobility among the different botanical sources. Cashew honey that has higher water activity exhibited broader  $T_2$  distribution than did the other with lower water activity. The bi-exponential fitting of the transversal relaxation ( $T_2$ ) data revealed two water populations  $T_{21}$  and  $T_{22}$  in all the samples, corresponding to relaxation times of 0.6-1.8 ms and 2.3-5.4 ms, respectively (Table 4). Good linear correlations were observed between the  $T_2$  and  $T_{21}$  parameters and the physical and chemical data, including water contents, water activity, pH and colour [13].

**Table 4.** Range of the LF  $^1\text{H}$  NMR parameters obtained in honeys according to the different botanical origins. (Adapted from [13]).

Types of honeys	$T_{21}$ [ms]	$T_{22}$ [ms]
Cashew tree	1.88±0.10 <sup>a</sup> (1.65-2.06)	5.49±0.12 <sup>a</sup> (5.15-5.68)
Polyfloral	1.58±0.07 <sup>b</sup> (1.44-1.71)	4.91±0.08 <sup>b</sup> (4.71-5.05)
Barbados cherry	1.36±0.12 <sup>c</sup> (1.11-1.63)	3.53±0.10 <sup>c</sup> (3.33-3.72)
“Assa-Peixe”	1.31±0.07 <sup>c</sup> (1.16-1.42)	4.07±0.10 <sup>c</sup> (3.89-4.24)
“Assa-Lipto”	1.07±0.07 <sup>d</sup> (0.96-1.22)	3.43±0.09 <sup>c</sup> (3.32-3.59)
Eucalyptus	1.09±0.12 <sup>d</sup> (0.89-1.32)	2.95±0.09 <sup>d</sup> (2.75-3.10)
Oranges	0.84±0.05 <sup>e</sup> (0.73-0.94)	3.30±0.12 <sup>c</sup> (3.07-3.50)
“Cipó-Uva”	0.64±0.04 <sup>f</sup> (0.57±0.74)	2.43±0.06 <sup>e</sup> (2.29-2.54)

<sup>a, b, c,...</sup> Different letters in a column indicate significant differences ( $p < 0.01$ ) within each treatment (ANOVA).

The LF  $^1\text{H}$  NMR was also used to detect honey adulteration of high fructose corn syrup [14].

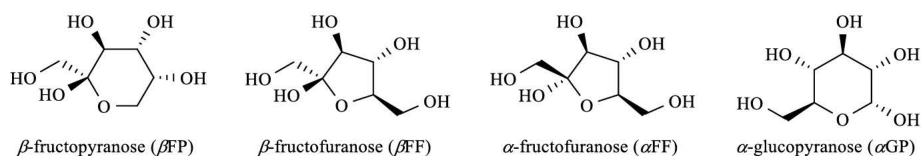
Markers of geographical origin could also be characterized by a combination of NMR data and chemometric analysis. Unsupervised principal component analysis (PCA) is a powerful tool to distinguish  $^1\text{H}$  NMR spectra of polyfloral and of acacia honey samples and of geographical differentiation for the later ones [15].

$^{13}\text{C}$  NMR spectra of the acacia honey allow to identify sugar isoforms. The  $\beta$ -fructopyranose ( $\beta\text{FP}$ ) and  $\beta$ -fructofuranose ( $\beta\text{FF}$ ) ratio and the  $\alpha\text{FF}$ ,  $\beta\text{FP}$ ,  $\beta\text{FF}$  and  $\alpha$ -glucopyranose ( $\alpha\text{GP}$ ) (Fig. 4) chemical shifts suggested possible geographical markers of Argentinian and Hungarian honey samples [15].  $^1\text{H}$  NMR spectroscopy and multivariate analysis were used to classify the geographical differentiation of Corsican and non-Corsican honeys [16]. In this case, 2D TOCSY spectra were used to determine the molecular structural characteristics of the main components of the honey samples. Comparative analysis of  $^{13}\text{C}$  NMR spectra of basic and depolymerized galactomannan polymers (Table 5), obtained from a hot water extract of the seeds of Chinese honey locust (*Gleditsia sinensis* Lam.), with those of the corresponding monosaccharides revealed that the galactomannan macromolecule possesses residues of 1,4- $\beta$ -D-mannopyranose, substituted with residues of  $\alpha$ -D-galactopyranose at C-6 [17].

In the carbohydrate region of the  $^1\text{H}$  NMR spectra of several Brazilian honeys, the resonances of the main monosaccharides were identified [18]. Those signals are almost equal to all analyzed honeys with only small intensity variations.

**Table 5.**  $^{13}\text{C}$  NMR spectroscopic data ( $\delta$  in  $\text{DMSO}-d_6$ , ppm) of depolymerized galactomannan from the seeds of *Gleditsia sinensis*. (Adapted from [17]).

	C-1	C-2	C-3	C-4	C-5	C-6
$\alpha$ -D-galactopyranosyl	99.7	69.5	70.0	70.6	72.2	62.1
$\alpha$ -D-galactopyranose	93.5	69.6	70.4	70.6	71.7	62.4
4-O- $\beta$ -D-mannopyranosyl	101.2	71.0	72.3	77.3	76.1	61.4
				77.6		
4,6-D-di-O- $\beta$ -D-mannopyranosyl	101.0	71.0	72.4	77.6	74.4	67.4
				78.0		
$\alpha$ -mannopyranose	94.9	72.5	74.3	67.9	77.4	62.3



**Fig. (4).** Structures of  $\beta$ -fructopyranose ( $\beta$ FP),  $\beta$ -fructofuranose ( $\beta$ FF),  $\alpha$ -fructo-furanose ( $\alpha$ FF) and  $\alpha$ -glucopyranose ( $\alpha$ GP).

The assignments of the major  $^1\text{H}$  and  $^{13}\text{C}$  NMR signals of the major constituents were done by 2D NMR experiments (gCOSY, TOCSY, gHSQC, gHMBC) (Table 6). The presence of typical resonances (chemical shift and coupling constant) allowed the assignment of some minor constituents (Table 7). Chemometric analysis PCA and Hierarchical Cluster Analysis (HCA) of these  $^1\text{H}$  NMR spectra discriminate its botanic origin, namely eucalyptus (higher amount of lactic acid), citrus (higher amount of sucrose) and wildflower (higher amount of phenylalanine and tyrosine) [18]. A similar study conducted by Beretta *et al.* of solid-phase extracted samples allowed the identification of the botanical origin of Italian honeys [19]. Honeydew honey showed typical resonances of an aliphatic component (sets of signals at 0.85-1.60, 2.15, 4.60-5.20 ppm, one doublet at 5.70 ppm and a double of triplets at 6.75 ppm). Chestnut honey presented typical resonances of kynurenic acid **3** and a structurally related metabolite while linden honey showed signals assigned to cyclohexane-1,3-diene-1-carboxylic acid and its 1-*O*- $\beta$ -gentiobiosyl ester [19].

**Table 6.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data ( $\delta$  in  $\text{D}_2\text{O}$ , ppm;  $J$  in parenthesis, Hz) of the main saccharides present in Brazilian honeys:  $\alpha$ -glucopyranose ( $\alpha$ GP),  $\beta$ -glucopyranose ( $\beta$ GP),  $\beta$ -fructopyranose ( $\beta$ FP),  $\beta$ -fructofuranose ( $\beta$ FF) and  $\alpha$ -fructo-furanose ( $\alpha$ FF). (Adapted from [18]).

Position	$\alpha$ GP		$\beta$ GP		$\beta$ FP	
	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$
1	5.22 d (3.70)	94.6	4.63 d (8.0)	98.4	3.52-3.57 m	66.5
1'					3.66-3.72 m	66.5
2	3.49-3.54 m	74.0	3.23 dd (9.2, 8.0)	76.7		110.6
3	3.66-3.73 m	75.4	3.30-3.50 m	78.3	3.74-3.79 m	70.2
4	3.35-3.42 m	72.1 or 72.2	3.35-3.46 m	72.1 or 72.2	3.85-3.90 m	72.3

(Table 6) contd....

Position	$\alpha$ GP		$\beta$ GP		$\beta$ FP	
	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$
5	3.78-3.84 m	73.9	3.40-3.50 m	78.4	3.97-3.99 m	71.8
6	3.67-3.73 m	63.3	3.72-3.77 m	63.4	3.65-3.72 m	65.9
6'	3.79-3.85 m	63.3	3.85-3.90 m	63.4	3.97-4.03 m	65.9
Position	$\beta$ FF		$\alpha$ FF			
	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$		
1	3.53-3.58 m	65.4	3.62-3.65 m	65.6		
1'						
2		104.1		107.0		
3	4.07-4.10 m	78.0	4.07-4.10 m	84.5		
4	4.07-4.10 m	77.0	3.95-4.00 m	78.6		
5	3.77-3.85 m	83.2	4.02-4.07 m	83.8		
6	3.75-3.82 m	65.0	3.64-3.68 m	63.7		
6'	3.62-3.68 m	65.0	3.77-3.80 m	63.7		

**Table 7.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data ( $\delta$  in  $\text{D}_2\text{O}$ , ppm;  $J$  in parenthesis, Hz) of the minor compounds present in Brazilian honeys. (Adapted from [18]).

Position	$^1\text{H}$	$^{13}\text{C}$	Position	$^1\text{H}$	$^{13}\text{C}$
<b>Acetic acid</b>			<b>Alanine</b>		
$\text{CO}_2\text{H}$		179.8	$\text{CO}_2\text{H}$		178.3
$\text{CH}_3$	2.00 s	23.6	$\text{CH}$	3.75-3.85 m	53.1
<b>Citric acid</b>			$\text{CH}_3$	1.46 d (7.3)	18.9
1,5- $\text{CO}_2\text{H}$		176.5	<b>5-Hydroxymethylfurfural</b>		
2- $\text{CH}$	2.79 d (15.5)	46.1	2-C		154.3
2'- $\text{CH}$	2.94 d (15.5)	46.1	3- $\text{CH}$	7.54 d (3.7)	129.7
3-C		76.1	4- $\text{CH}$	6.68 d (3.7)	113.7
4- $\text{CH}$	2.79 d (15.5)	46.1	5-C		164.1
4'- $\text{CH}$	2.94 d (15.5)	46.1	$\text{CH}_2$	4.69 s	58.8
6- $\text{CO}_2\text{H}$		180.2	$\text{CHO}$	9.45 s	183.2
<b>Formic acid</b>			<b>Phenylalanine</b>		
$\text{HCO}_2\text{H}$	8.45 s	173.5	Aromatic 2,6- $\text{CH}$	7.31 d (7.2)	132.1
<b>Lactic acid</b>			Aromatic 3,5- $\text{CH}$	7.41 d (7.2)	131.8

(Table 7) contd....

Position	<sup>1</sup> H	<sup>13</sup> C	Position	<sup>1</sup> H	<sup>13</sup> C
CO <sub>2</sub> H		183.7	Aromatic 4-CH	7.35-7.39 m	130.4
CH	4.30-4.40 m	70.6	<b>Tyrosine</b>		
CH <sub>3</sub>	1.35 d (6.9)	22.5	Aromatic 1-C		129.4
<b>Ethanol</b>			Aromatic 2,6-CH	7.18 d (8.4)	133.5
CH <sub>2</sub>	3.56-3.66 m	60.1	Aromatic 3,5-CH	6.88 d (8.4)	118.6
CH <sub>3</sub>	1.15 t (7.1)	19.6	Aromatic 4-COH		157.5

Honey adulteration increased exponentially both in terms of geographic and botanical origin, leading to a huge demand for novel analytical methods to effectively control the quality of honey. Israeli citrus honeys authenticity were determined by the D/H(CH<sub>3</sub>) isotope ratio of the ethanols, produced by alcoholic fermentation (dry yeast *Saccharomyces bayanos*), measured by deuterium NMR (Table 8) [20]. Ethanols obtained from fermentation of citrus honeys have D/H(CH<sub>3</sub>) values similar to those obtained from citrus juice and 5 ppm higher than the values obtained for other honeys.

**Table 8.** Isotope ratio parameters in various samples of honey. (Adapted from [20]).

Source	D/H(CH <sub>3</sub> ) <sup>a</sup>	δ <sup>13</sup> C‰ <sup>b</sup>
citrus	105.4	-23.7
citrus	105.2	-24.1
citrus	105.2	
citrus	104.9	-24.0
citrus	104.8	-24.4
citrus	104.7	-23.3
citrus	104.6	-23.9
citrus	104.1	-24.3
field flowers <sup>c</sup>	100.0	-25.7
field flowers <sup>c</sup>	100.0	-24.1
field flowers <sup>c</sup>	99.6	-23.6
field flowers <sup>c</sup>	99.2	-24.4
field flowers <sup>c</sup>	98.9	-25.0
field flowers <sup>c</sup>	97.8	
onion	100.3	-24.1

(Table 8) contd....

Source	D/H(CH <sub>3</sub> ) <sup>a</sup>	δ <sup>13</sup> C‰ <sup>b</sup>
cotton	99.1	-25.1
eucalyptus	98.9	-24.1
zaatar <sup>d</sup>	98.2	-24.1
thorns <sup>e</sup>	97.8	-24.1
beet sugar <sup>f</sup>	95.3	

<sup>a</sup> ppm D/H of methyl group of ethanols obtained by alcoholic fermentation.

<sup>b</sup> δ<sup>13</sup>C ) [ [(<sup>13</sup>C/<sup>12</sup>C)<sub>sample</sub> / (<sup>13</sup>C/<sup>12</sup>C)<sub>standard</sub> ] - 1 ] x 1000.

<sup>c</sup> Mainly Cruciferae, Compositae, Labiatae, and Leguminosae. <sup>d</sup> *Majorana syriaca*. <sup>e</sup> *Centaurea verutum* L. <sup>f</sup> The bees were fed with beet sugar solutions.

The application of multivariate analysis *e.g.* PCA, Cluster Analysis (CLA), Correspondence Analysis (CA), Factor Analysis (FA), and General Discriminant Analysis (GDA) to the chromatographic and spectroscopic (*e.g.* 1D and 2D NMR) data proved to be extremely useful to group and detect honeys of different origins [21 - 23].

PCA and Partial Least Squares Discriminant Analysis (PLS-DA) of <sup>1</sup>H and <sup>13</sup>C NMR based sugar profiles of herbhoneys and Polish honeys did not identify any distinct cluster between them but some tendency appeared regarding the β GP and β FP content [24]. The traditional honeys seem to be a little bit rich in fructose than the herbhoneys. In both PCA and PLS-DA methods there is a cluster related to sucrose content allowing the fast detection of adulteration. The authors collected 1D and 2D NMR spectra of mono and disaccharides from artificial honey to allow a reliable assignment of these compounds in real honey samples (Table 9 only shows the NMR data of disaccharides which were not yet described in this chapter) [24].

**Table 9.** <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data (δ in DMSO-*d*<sub>6</sub>, ppm) of disaccharides present in an artificial honey sample. (Adapted from [27]).

Position	Sucrose			α-Maltose			β-Maltose		
	<sup>1</sup> H	<sup>1</sup> H (OH)	<sup>13</sup> C	<sup>1</sup> H	<sup>1</sup> H (OH)	<sup>13</sup> C	<sup>1</sup> H	<sup>1</sup> H (OH)	<sup>13</sup> C
1	4.86		92.27	4.88	6.35	92.52	4.29	6.71	97.11
2	3.17	5.11	71.95	3.16	4.72	72.23	2.92	5.06	74.76
3	3.50	n.d.	73.20	3.44	5.34	73.39	3.36	5.47	76.85
4	3.10	4.88	70.18	3.27		80.59	3.24		80.06

(Table 9) *contd....*

Position	Sucrose			$\alpha$ -Maltose			$\beta$ -Maltose		
	$^1\text{H}$	$^1\text{H (OH)}$	$^{13}\text{C}$	$^1\text{H}$	$^1\text{H (OH)}$	$^{13}\text{C}$	$^1\text{H}$	$^1\text{H (OH)}$	$^{13}\text{C}$
5	3.21		72.95	3.17		75.40	3.77		75.82
6	3.41, 3.46	n.d.	60.92	3.55	4.52	61.05	3.48, 3.62	4.59	61.19
1'	3.36, 3.51	n.d.	62.51	4.94		101.20	4.98		101.13
2'			104.36	3.44	5.43	71.03	3.21	5.45	72.78
3'	3.84	4.65	77.44	3.21	4.68	72.82	3.33	4.66	73.82
4'	3.74	5.32	74.66	3.52	4.87	66.98	3.03	4.88	70.26
5'	3.54		82.78	3.63		73.33	3.43		73.79
6'	3.47, 3.70	4.49	62.56	3.26, 3.36	4.50	63.69	3.41, 3.57	4.60	61.31

n.d. = not detected

$^1\text{H}$  and 2D TOCSY NMR spectroscopy allowed structure elucidation (by comparing with chemical shifts and  $J$  coupling constants of authentic samples) of several biomarkers of Corsican honeys, namely, kynurenic acid **3** (chestnut honey),  $\alpha$ -isophorone and 2,5-dihydroxyphenylacetic acid (strawberry-tree honey) [25]. A complete NMR study ( $^1\text{H}$ ,  $^{13}\text{C}$ , TOCSY, COSY, NOESY, HMQC and HMBC spectra) of semi-purified compounds allowed to identify some known and new biomarkers of different types of honeys [26]. The same authors have characterized a complete set of biomarkers and other compounds **7-27** (Fig. 5) extracted from monofloral honeys [27]. The complete assignment of proton and carbon resonances were carried out with the aid of 2D NMR techniques (DQF-COSY, TOCSY, NOESY, HMQC and HMBC spectra) (Tables **10-12**).

**Table 10.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data ( $\delta$  in  $\text{CDCl}_3$ , ppm;  $J$  in parenthesis, Hz) of compounds **7-12**.

Position	7		8 <sup>a</sup>		9 <sup>a</sup>	
	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$
1		154.6		154.3		142.1
2	7.18 dt (5.8, 1.7)	136.8	7.18 d (5.8)	116.0	7.24 d (5.8)	120.0
3	6.18 dt (5.8, 1.7)	115.8	6.18 d (5.8)	136.2	6.19 d (5.8)	136.5
4		125.3		125.3		126.2
5	2.47 t (9.6)	21.6	2.48 m	23.8	2.52 m	23.9
6	2.32 t (9.6)	23.6	2.32 m	21.7	2.52 m	21.5
7		170.9		172.0		172.3

(Table 10) contd....

Position	7		8 <sup>a</sup>		9 <sup>a</sup>	
	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C
8		73.2		73.0		144.3
9	1.42 s	28.1	1.40 s	28.6	5.31, 5.16 s	115.5
10	1.42 s	28.1	1.40 s	28.6	1.97 s	20.3
11	3.76 s	63.4				
Position	10		11		12	
	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C
1		125.0	1.33	29.3		
2	8.08 d (8.1)	130.1		71.2		159.8
3	7.62 d (8.1)	124.4	5.73 dt (15.7, 1.1)	143.4		
			5.64 ddd (15.7, 7.9, 6.6)	121.9		
4		155.3	2.25 dd (7.9, 1.1)	44.9		n.d.
5	7.62 d (8.6)	130.1	2.30 dd (6.6, 1.1)	44.9	8.3 dd (8.0, 1.1)	126.2
6	8.08 d (8.6)	124.4		73.0	7.66 d (8.1)	127.0
7	n.d.	170.1	5.93 dd (17.3, 10.8)	144.5	7.46 t (7.5)	125.9
8		73.0	5.07 dd (10.8, 1.1)	112.2	7.74 td (7.0, 1.4)	134.2
			5.22 dd (17.3, 1.1)	112.2		
9	1.61 s	31.0				149.2
10	1.61 s	31.0				120.9
11						
1 <sup>'</sup>					3.2 t (7.9)	32.9
2 <sup>'</sup>			1.33	29.3	2.3 t (7.6)	19.8
3 <sup>'</sup>					4.2 t (7.2)	46.4
6 <sup>'</sup>			1.29	27.4		

<sup>a</sup> These data were taken from reference [28].**Table 11.** <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data ( $\delta$  in CDCl<sub>3</sub>, ppm; *J* in parenthesis, Hz) of compounds 13-19.

Position	13		14		15 <sup>a</sup>		16 <sup>a</sup>	
	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C
1	n.d.		n.d.					
2		162.5	7.95 d (6.6)	139.5	5.44	78.8		163.1
3	6.68 d (9.5)	121.8	6.3 d (6.6)	109	3.10, 2.84	43.8, 43.3	6.68	103.0
4	7.78 d (9.5)	141.0		180.1		197.3		181.8

(Table 11) contd....

Position	13		14		15 <sup>a</sup>		16 <sup>a</sup>	
	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C
5	7.58 dd (8.1, 1.2)	127.9	8.07 dd (7.8, 1.3)	130.7	12.05	165.4	12.81	161.4
6	7.24 td (8.1, 1.2)	122.4	7.59 td	121.6	6.01	95.6	6.31	98.1
7	7.52 td (8.4, 1.2)	130.5	7.59 td	132.4	12.05	168.3	12.81	164.3
8	7.17 d (8.4)	115.1	7.89 dd	115.8	6.01	97.2	6.48	94.1
9		137.8		144.8		164.6		157.0
10		120.2		129.9		103.3		105.1
1'								130.6
2'					7.44	127.3	7.89	126.3
3',5'					7.44	129.6	7.54	129.1
4'					7.44	129.7	7.54	132.0
6'					7.44	127.3	7.89	126.3
Position	17		18 <sup>a</sup>		19 <sup>a</sup>			
	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C		
1					4.00	67.9		
2	5.04 d (11.9)	83.1		151.8		136.8		
3	4.57 d (11.9)	72.4			5.43	125.7		
4		198.0		148.0	2.08	23.2		
5	11.25 s	162.8		107.8	1.61	42.4		
6	6.08 d (2.2)	94.4		155.1		73.1		
7		163.7			5.93	145.8		
8	6.11 d (2.2)	95.4	7.55	141.2	5.09, 5.24	111.2		
9		169.0						
10		101.0	3.42	27.7				
11	3.81 s	55.6	3.61	29.8				
12			4.01	33.2				
1'		138.9						
2'	7.44 m	128.7			1.68	13.3		
3',5'	7.46 m	125.8						
4'	7.42 m	128.2						
6'	7.44 m	128.7			1.30	13.3		

<sup>a</sup> These data were taken from reference [29].

**Table 12.** <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data ( $\delta$  in CDCl<sub>3</sub>, ppm; *J* in parenthesis, Hz) of compounds 20-27.

Position	20		21		22		23		24		25		26	
	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C
1		42.5		n.d.		170.4		170.4		178.0		178.0		121.0
2	2.51 d (17.2)	49.8	2.51 d (17.2)	n.d.	5.85 dt (15.6)	120.1	5.85 dt (15.6)	120.1	2.37 m	33.2	2.37 m	33.2	7.34 s	106.5
	2.34 d (17.2)	49.8	2.34 d (17.2)											
3		196.8		n.d.	7.07 dt (15.6)	151.7	7.07 dt (15.6)	151.7	1.66 m	24.4	1.66 m	24.4		147.5
4	5.97 s	128.2	6.01 s	126.1	2.26 m	31.8	2.26 m	31.8	1.38 m	29.1	1.38 m	29.1		139.9
5		160.8		n.d.	1.51 m	27.4	1.51 m	27.4	1.51 m	27.4	1.51 m	27.4		147.5
6		79.3	2.74 d (9.6)	55.0	1.38 m	29.1	1.38 m	29.1	1.51 m	27.4	1.38 m	29.1	7.34 s	106.5
7	6.47 d (15.9)	130.3	6.68 dd (15.7, 9.6)	143.5	1.37 m	29.3	1.66 m	24.4	1.38 m	29.1	1.66 m	24.4	3.96 s	56.3
8	6.84 d (15.9)	145.0	6.2 d (15.7)	133.2	1.66 m	24.4	2.37 m	33.2	1.66 m	24.4	2.37 m	33.2	3.96 s	56.3
9		198.0		n.d.	2.37 m	33.2		178.0	2.37 m	33.2		178.0	3.91 s	51.8
10	2.31 s	28.4	2.31 s	34.0		178.0				178.0				167.3
1'	1.03 s	23.8	1.03 s	n.d.										
1''	1.11 s	24.7	1.11 s	n.d.										
5'	1.89 s	18.8	1.92 s	23.0										

Position	27		Position	27	
	<sup>1</sup> H	<sup>13</sup> C		<sup>1</sup> H	<sup>13</sup> C
1	3.56 dd (5.4, 2.8)	68.2	2a (R3, R2)	2.33 dt (15.1, 7.4)	34.5
2	5.21 m	69.5	2b (R3, R2)	2.30 dt (15.1, 7.4)	34.5
3	4.18 dd (11.9, 6.4)	61.8	3 (R2, R3)	1.64 m	25.2
3'	4.35 dd (11.9, 6.4)	61.8	4-7, 12-17 (R2, R3)	1.31 m	29.1
1 (R1)	3.45 m	71.4	9, 10 (R2, R3)	5.36 m	129.9

(Table 12) contd....

Position	27		Position	27	
	<sup>1</sup> H	<sup>13</sup> C		<sup>1</sup> H	<sup>13</sup> C
2(R2)	1.56 m	29.8	8, 11 (R2, R3)	2.04 b	27.8
3 (R3)	1.3 m	22.5	18 (R1, R2, R3)	0.91 t (7.2)	14.0

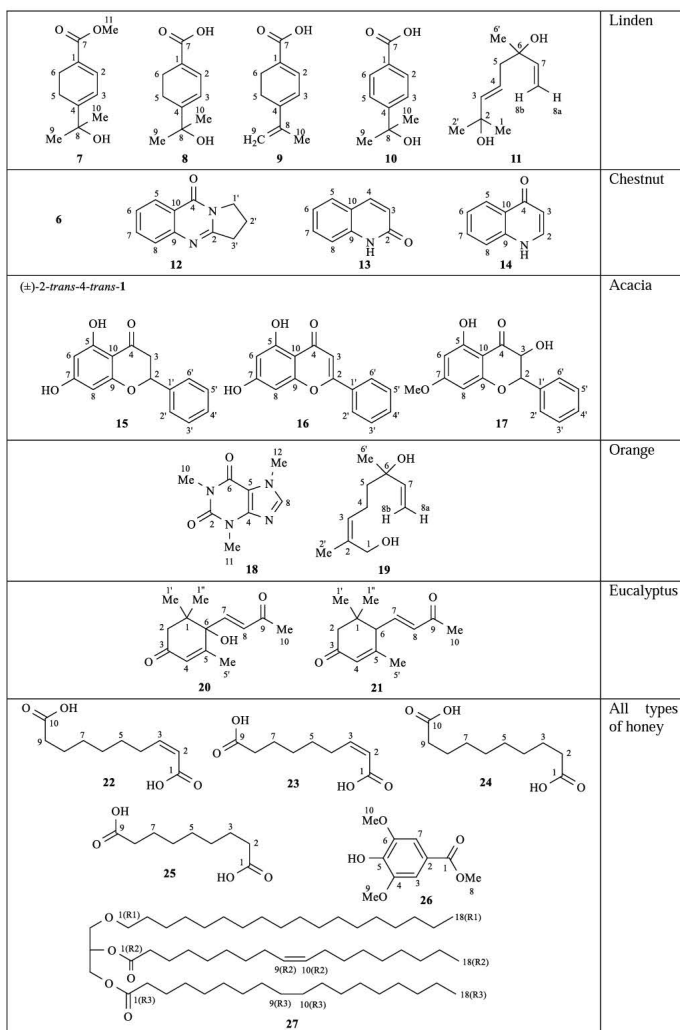


Fig. (5). Structures of compounds 7-27.

Lianda and Castro, isolated for the first time from a Brazilian *Citrus* honey, the flavonol morin (2',3,4',5,7-pentahydroxyflavone) [30]. The characterization of this compound was based on its  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra and further comparison with the literature.

The combination of NMR spectroscopy ( $^1\text{H}$  saccharide content) and chemometrics allowed a very good differentiation for multifloral honey samples from different countries and also for rhododendron and “high mountain multifloral” honeys from very closely related regions. NMR appears to be one of the elective techniques to identify the authenticity of honey, offering the possibility to assign the metabolite contents of honeys ( $^1\text{H}$  and  $^{13}\text{C}$  anomeric resonances of several identified saccharides) with a single experiment and avoiding derivatization (Table 13) [31]. The saccharide contents can be used to characterize honey samples and to construct an identity card of saccharides for each floral source, being the adulteration by carbohydrate addition identified by analysing the different ratios of the present saccharides [32].

**Table 13.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data ( $\delta$  in  $\text{D}_2\text{O}$ , ppm) of all the identified saccharides anomeric signals. (Adapted from [31]).

Saccharide	Abbreviation	$^1\text{H}$	$^{13}\text{C}$
Erllose	Erl	5.3505	102.53 94.71
Fructose	Frc	4.0524	77.64
Gentiobiose	Gnt	5.1732 4.5860 4.4455	94.71 98.63 105.46
Glucose	$\alpha/\beta\text{Gl}$	5.1732 4.5860	94.71 98.63
Isomaltose	Imt	5.1874 4.9055 4.6194	94.71 101.07 98.63
Isomaltotriose	Im3	5.1874 4.8988 4.6194	94.71 101.12 98.63
Kojibiose	Kjb	5.3841 5.3304 5.0404 4.7506	92.27 100.57 99.11 99.11

(Table 13) contd....

Saccharide	Abbreviation	<sup>1</sup> H	<sup>13</sup> C
Kestose	Kst	5.3729	95.19
		4.2284	79.45
Maltose	Mlt	5.3505	102.53
		5.1732	94.71
		4.5860	98.63
Maltotetraose	Ml4	5.3567	102.53
		5.1732	94.71
		4.5860	98.63
Maltotriose	Ml3	5.3383	102.53
		5.1732	94.71
		4.5860	98.63
Maltulose	Mtl	5.1874	103.23
		5.1536	101.06
		5.1034	100.59
Melezitose	Mlz	5.3924	94.71
		5.1316	103.01
Melibiose	Mlb	5.1732	101.28
		4.9251	94.71
		4.6150	98.63
Nigerose	Ngr	5.3159	102.04
		5.3003	102.04
		5.1732	94.71
		4.6115	98.63
Palatinose	Plt	4.9183	101.06
Raffinose	Rff	5.3729	95.19
		4.9397	101.06
Sucrose	Scr	5.3505	94.71
Turanose	Trn	5.2444	101.07
		5.2444	103.49
		5.1536	99.59
		5.1034	100.57

The origin of Polish monofloral and multifloral honeys has been done with the application of chemometric studies using NMR spectroscopy [33]. The markers of dark monofloral honeys were: i) heather honey presents two dominant chemical markers, dehydrovomifoliol **28** (formed from abscisic acid **1**) and phenyl acetic acid **29** (formed from phenylpyruvic acid); and ii) in the buckwheat honey formic acid **30** and tyrosine **31** were the identified new chemical markers (Fig. 6) since the expected ones were not observed. For light monofloral honeys we can

conclude: i) in the case of rape honey it was not possible to identify any markers although there are two typical  $^1\text{H}$  (8.704 and 8.328 ppm) and  $^{13}\text{C}$  (178.2 and 113.1 ppm) NMR resonances; and ii) in the case of lime honey there are high levels of acetic acid and 4-(1-hydroxy-1-methylethyl)cyclohexane-1,3-diene-1-carboxylic acid **32** (Fig. 6). In this case there are also some sets of  $^1\text{H}$  and  $^{13}\text{C}$  characteristic chemical shifts (1.369/29.98 and 6.16/118.56 ppm). The chemical composition of multifloral honeys is similar to that of light honeys. Even so, the authors have constructed a heatmap from the characteristic NMR peaks (Table 14) to differentiate the different types of multifloral honeys [33].

**Table 14.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopic data ( $\delta$  in  $\text{D}_2\text{O}$ , ppm) of compounds **28-32**.

Position	28		29		30		31		32	
	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$	$^1\text{H}$	$^{13}\text{C}$
1									2.3	24
2			7.30	129.1	8.06	166.8	6.74	118.9	2.3	24
3	2.53	50.4	7.29	131			7.04	133.8		
4			7.37	131					6.1	123
5	5.94	128.8	7.29	131			7.04	133.8	6.9	123
6			7.30	129.1			6.74	118.9		
7			3.37	50.7						
8	6.27	130.8					2.62	46.1		
9	6.91	145								
12	1.03	24.2							1.2	28
13	1.11	22.8							1.2	28
15	1.89	18.6								
16	2.32	28.3								

The identification of individual carbohydrates of multicomponent artificial mixtures using a computer-aided analysis of their  $^{13}\text{C}$  NMR spectra, without previous purification, is based in the assignment of each component by comparison the signals of the mixture spectrum with those of the pure reference spectra compiled in a library. Each compound is identified by taking into consideration three parameters: i) the number of observed carbons with respect with the number of expected signals; ii) the number of overlapped signals of

carbons which possess the same chemical shift; and iii) the difference in the chemical shift of each signal in the mixture and in the reference [34, 35]. The quantitation of mono-, di- and trisaccharides from the referred artificial mixtures was made using 1,4-dioxane as internal standard, without the need to use inverse gated decoupling technique (technique used to acquire quantitative  $^{13}\text{C}$  NMR spectra) or a relaxation reagent (a paramagnetic species allowing the shortening of the relaxation time) [36].

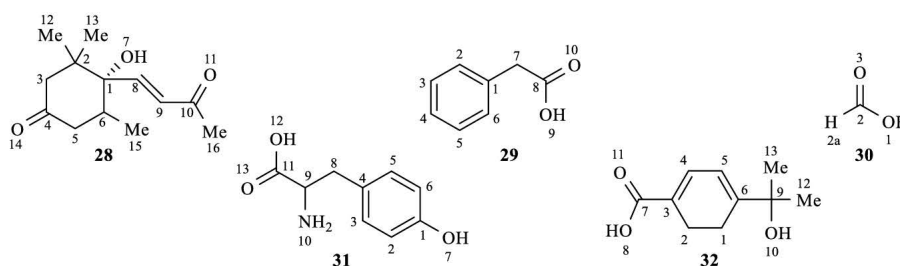


Fig. (6). Structures of compounds 28-32.

The referred two procedures were used to the direct identification and quantitation of carbohydrates present in six Corsican authentic honeys of different floral types (mixed floral, *Castanea*, *Robinia*, *Asphodelus*, *Anthyllus* and *Clementina*) (Table 15) [36]. Together with the mono- and disaccharides, several oligosaccharides (identification of some of these is not easy by chromatographic methods) were observed in levels ranging from 0.4 to 3.3% [36].

Table 15.  $^{13}\text{C}$  NMR quantitative analysis of carbohydrates (%) in six honeys<sup>a</sup>. (Adapted from [36]).

Components	Honey floral type					
	Mixed floral	<i>Castanea</i>	<i>Robinia</i>	<i>Asphodelus</i> <sup>b</sup>	<i>Anthyllis</i>	<i>Clementina</i>
Fructose	35.6	37.1	32.4	32.7	31.5	33.4
Glucose	26.8	23.0	25.9	22.7	28.7	28.4
Turanose	2.2 <sup>c</sup>	1.7 <sup>c</sup>	0.8 <sup>c</sup>	1.8 <sup>c</sup>	1.7 <sup>c</sup>	1.8 <sup>c</sup>
Maltulose	0.8 <sup>c</sup>	1.0 <sup>c</sup>	0.6 <sup>c</sup>	0.7 <sup>c</sup>	0.6 <sup>c</sup>	1.8 <sup>c</sup>
Maltose			0.7	0.9		
Isomaltose	1.4				1.7	

(Table 15) contd....

Components	Honey floral type					
	Mixed floral	Castanea	Robinia	Asphodelus <sup>b</sup>	Anthyllis	Clementina
Nigerose					2.6	2.3
Isomaltotriose	2.5	1.7		1.7		3.3
Melezitose				1.1		
Erllose		0.4		1.7		
Carbohydrates	69.3	64.9	60.4	63.3	66.8	71.0
Oligosaccharides	6.9	4.8	2.1	7.9	6.6	9.2
F+G	62.4	60.1	58.3	55.4	60.2	61.8
F/G	1.33	1.6	1.25	1.44	1.1	1.17

<sup>a</sup> All percentages are expressed with respect to fresh matter (raw honey).

<sup>b</sup> Repeatability of the measurement given at 95% for four analyses of *Asphodelus* honey: fructose 32.2±1.7%; glucose, 24.6±1.8%; turanose, 1.9±0.2%; maltulose, 0.9±0.2%; maltose, 0.7±0.2%; isomaltotriose, 1.8±0.2%; melezitose, 1.0±0.2%; erlose, 1.4±0.3%. <sup>c</sup> Correction factors applied since the minor anomeric forms ( $\alpha$ -FF) of maltulose and of turanose are not detected and estimated at 8.9% and 17.0%, respectively, in the solution of maltulose and turanose.

Independent Component Analysis (ICA) has been used to solve the overlap of <sup>1</sup>H NMR spectra of foodstuffs, including honey. The ICA strategy has been proven to effectively extract spectra of pure components and concentrations from the NMR of mixtures [37].

Selective 1D TOCSY experiments were used to isolate the features of a single spin system from a crowded forest of peaks (*e.g.* amino acids from honey), thus allowing for more certain identification of a species, and cleaner and more accurate quantification (Table 16) [38]. PCA of data sets (integrals of minor honey components, namely amino acids) derived from selective TOCSY spectra of commercial honey samples constitute a more sensitive way to distinguish the origin of honey than the classical metabonomics approach [38].

**Table 16.** Amino acids observed in honey by 1D TOCSY. (Adapted from [38]).

Amino acid	Excitation (ppm)		TOCSY peaks (ppm)	
	$\beta$ 1	(2.33)	$\alpha$	(4.12)
Pro	$\beta$ 1	(2.33)	$\delta$ 1	(3.40)
			$\delta$ 2	(3.32)
			$\beta$ 2	(2.06)
			$\gamma$	(1.98)

(Table 16) contd....

Amino acid	Excitation (ppm)		TOCSY peaks (ppm)	
Pro	$\gamma$	(1.98)	$\alpha$ $\delta 1$ $\delta 2$ $\beta 1$	(4.12) <sup>a</sup> (3.40) (3.32) (2.33)
Ala	$\beta$	(1.46)	$\alpha$	(3.80) <sup>a</sup>
Thr	CH <sub>3</sub> $\gamma$	(1.31)	$\alpha$ $\beta$	(3.60) <sup>a</sup> (4.25)
Tyr	CH	(6.90)	CH	(7.19) <sup>d</sup>
Tyr	CH	(7.19)	CH	(6.90)
ethanol	CH <sub>3</sub>	(1.17)	CH <sub>2</sub>	(3.65) <sup>a</sup>
Ile	CH <sub>2</sub> $\gamma 1$	(1.46)	$\beta$ CH <sub>2</sub> $\gamma 2$ CH <sub>3</sub> $\gamma$ CH <sub>3</sub> $\delta$	(1.98) (1.25) (0.99) (0.91)
Ile	CH <sub>2</sub> $\gamma 2$	(1.25)	$\beta$ CH <sub>2</sub> $\gamma 1$ CH <sub>3</sub> $\gamma$ CH <sub>3</sub> $\delta$	(1.98) (1.46) (0.99) (0.91)
Phe <sup>ab</sup>	na <sup>c</sup>		na <sup>c</sup>	

<sup>a</sup> Used in PCA analysis. <sup>b</sup> Phe quantitation based on peak height at 7.42 ppm. <sup>c</sup> Not applicable.

DOSY (Diffusion Ordered SpectroscopY) was directly applied to the monofloral manuka honey for simultaneously identify and in part quantify their components and also to correlate the antibacterial activity with the components identified. It was shown that the use of an internal standard is essential for the calibration and quantisation of diffusion, and the relationship between diffusion and molecular mass needs to be established for each component [39].

The concentration of methylglyoxal of manuka honey has been determined for the first time by a quantitative NMR method (qNMR), without the need of chromatographic separation or a derivatisation procedure [40]. Methylglyoxal **33** is a naturally occurring dicarbonyl compound that undergoes a “spontaneous” reaction with water, leading to the two major compounds methylglyoxal monohydrate **34** and methylglyoxal dihydrate **35**, remaining less than 1% unreacted (Fig. 7). To determine the concentration of methylglyoxal **33** in a sample, the methyl peaks of methylglyoxal monohydrate **34** (2.297-2.314 ppm, 3H, s), methylglyoxal dihydrate **35** (1.369-1.386 ppm, 3H, s) and TSP (3-

trimethylsilyl[2,2,3,3-D<sub>4</sub>]propionic acid) normally used as reference in aqueous solution) (-0.01 to 0.01 ppm, 9H, s) was integrated [40]. The concentration of methylglyoxal **33** in the NMR sample was calculated using equation 1.

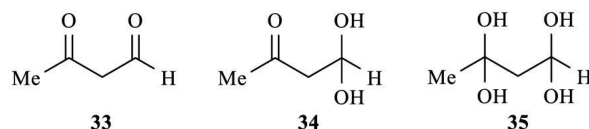


Fig. (7). Structures of methylglyoxal **33**, methylglyoxal monohydrate **34** and methylglyoxal dihydrate **35**.

$$3 \left( \frac{A_m + A_d}{A_{TSP}} \right) \times [TSP] = [MGO_{NMR}] \quad (1)$$

where  $A_m$  corresponds to the area of the methyl resonance of methylglyoxal monohydrate **34**;  $A_d$  to the area of methylglyoxal dihydrate **35**;  $A_{TSP}$  to the area of the TSP internal standard; TSP to the concentration of TSP (M); and  $MGO_{NMR}$  the concentration of methylglyoxal **33** (M). This value was converted to a concentration of methylglyoxal **33** in undiluted honey using equation 2. This approach has shown that previously applied methodology may overestimated the methylglyoxal concentration in manuka honey [40].

$$72.06 \times \left( \frac{[MGO_{NMR}]}{Honey} \right) \times 1000 = [MGO_{Honey}] \quad (2)$$

### 3. PROPOLIS

A wide variety of compounds can be found in propolis samples and their chemical composition varies according to its variety and environmental factors. Generally, propolis from Europe and China contains flavonoid aglycones (flavones and flavanones), phenolic acids and their esters. In Japanese propolis many prenylflavonoids has been identified. Propolis from Solomon Islands seems to contain prenylflavonoids similar to those found in Japanese samples. In a study conducted in thirty three propolis samples, the application of PCA to <sup>1</sup>H NMR data allowed to identify different geographical origins and divided the samples into three groups, African, Asian and European propolis [41].

Papotti *et al.* [42] stated that the harvesting method used for gathering propolis can also lead to variations on their chemical composition. For that, they analyzed sixty propolis samples and used NMR and multivariate statistical methods to develop an efficient and appropriate model for classifying them. NMR spectroscopy coupled to an appropriate spectral analytical tool (for spectra pre-treatment and analysis) was used for the recognition and detection of several phenolic compounds in propolis extracts [43]. This work demonstrated that ten (caffeic acid, *p*-coumaric acid, naringenin, quercetin, apigenin, pinostrobin, pinocembrin, kaempferol, chrysin and galangin) out of twelve typical phenolic propolis compounds were identified as statistically significant in most of the sixty five samples tested.

Since it is possible to identify in propolis samples several families of compounds and would be a hard work the description of their structural characterization according to their origin, we will summarize below the compounds identified in American (Table 17), Asian (Table 18), African and Oceania (Table 19) propolis, in which NMR techniques were used to elucidate their structures.

**Table 17.** Compounds identified in American propolis, which structures were determined using NMR spectroscopy.

Propolis type	Compounds	Ref.
Cuban (methanol extract)	isoprenylated benzophenone	[44]
Red Cuban (methanol extract)	gallic acid isoliquiritigenin (-)-liquiritigenin formononetin biochanin A (3S)-vestitol (3S)-7-O-methylvestitol (3S)-7,4'-dihydroxy-2'-methoxyisoflavan (6aS,11aS)-medicarpin (6aS,11aS)-homopterocarpan (6aR,11aR)-vesticarpan (6aR,11aR)-3,8-dihydroxy-9-methoxypterocarpan (6aR,11aR)-3-hydroxy-8,9-dimethoxypterocarpan (6aR,11aR)-3,4-dihydroxy-9-methoxypterocarpan	[45]

(Table 17) contd....

Propolis type	Compounds	Ref.
Cuban (methanol extract)	Brown-type – isoprenylated benzophenones (nemorosone, scrobiculatones A and B) Red-type – isoflavonoids (isoflavans, isoflavanones and pterocarpanes) Yellow-type – aliphatic compounds (terpenoids and sterols)	[46]
Yellow Cuban (methanol extract)	Type A – 3-hydroxylated triterpenoids Type B – 3-acetylated triterpenoids	[47]
Red Cuban (ethanol extract)	retusapurpurin A and retusapurpurin B	[48]
Red Mexican (methanol extract)	1-(3',4'-dihydroxy-2'-methoxyphenyl)-3-phenylpropane (Z)-1-(2'-methoxy-4',5'-dihydroxyphenyl)-3-phenylprop-2-ene 3-hydroxy-5,6-dimethoxyflavan (-)-7-hydroxyflavanone (+)-pinocembrin (-)-mucronulatol (-)-arizonicanol (+)-vestitol (-)-melilotocarpan A (-)-melilotocarpan D	[49]
Hondoriun(ethanol extract)	(E)-cinnamyl (E)-cinnamate (E)-cinnamyl (Z)-cinnamate hydrocinnamyl (E)-cinnamate benzyl (E)-cinnamate (E)-cinnamyl (E)-p-coumarate (E)-cinnamyl (Z)-p-coumarate (E)-cinnamyl benzoate 6 $\beta$ -hydroxy-3-oxolup-20(29)-en-28-oic acid 3-oxo-oleanoic acid sakuranetin liquiritigenin kukulkanin B (E)-p-coumaric acid (E)-cinnamic acid p-hydroxybenzoic acid	[50]
Brazilian (methanol extract)	3-hydroxy-2,2-dimethyl-8-prenylchroman-6-propenoic acid	[51]
Brazilian (methanol extract)	(Z)-2,2-dimethyl-8-(3-methyl-2-butenyl)benzopyran-6-propenoic acid (E)-2,2-dimethyl-8-(3-methyl-2-butenyl)benzopyran-6-propenoic acid	[52]
Brazilian (methanol extract)	3-prenyl-4-hydroxycinnamic acid 2,2-dimethyl-6-carboxyethenyl-2H-1-benzopyran 3,5-diprenyl-4-hydroxycinnamic acid 2,2-dimethyl-6-carboxyethenyl-8-prenyl-2H-1-benzopyran	[53]

(Table 17) contd....

Propolis type	Compounds	Ref.
Brazilian (São Paulo) (methanol extract)	isomangiferolic acid 24-methylenecycloartane-3 $\beta$ ,26-diol mangiferolic acid mangiferonic acid ambonic acid ambolic acid	[54]
Brazilian (Minas Gerais) (water extract)	chlorogenic acid 4-caffeoylquinic acid 5-caffeoylquinic acid 3,4-di- <i>O</i> -caffeoylquinic acid 3,5-di- <i>O</i> -caffeoylquinic acid 4,5-di- <i>O</i> -caffeoylquinic acid	[55]
Brazilian (Minas Gerais) (water extract)	3,4-di- <i>O</i> -caffeoylquinic acid 3,5-di- <i>O</i> -caffeoylquinic acid 4,5-di- <i>O</i> -caffeoylquinic acid	[56]
Brazilian (Minas Gerais) (methanol and chloroform extract)	bauer-7-en-3 $\beta$ -yl acetate	[57]
Brazilian (Ceará) (ethanol extract)	canaric acid lupeol lupenone germanicone quercetin kaempferol acacetin	[58]
Brazilian type 6 (Bahia) (ethanol 80% extract)	hyperibone A	[59]
Green Brazilian (Minas Gerais) (ethanol extract)	dihydrokaempferide kaempferide isosakuranetin betuletol	[60]
Green Brazilian (Minas Gerais) (ethanol extract)	mixture of $\alpha$ - and $\beta$ -amyrin lupeol ramnocitrin eupalitin acacetin 3-prenyl-4-hydroxycinnamic acid 3,5-diprenyl-4-hydroxycinnamic acid ( <i>E</i> )-3-[4-(3-phenylpropanoyloxy)]-3,5-diprenylcinnamic acid	[61]
Red Brazilian (Alagoas) (ethanol 70% extract)	2,3-epoxy-2-(3-methyl-2-butenyl)naphthalene-1,4-dione	[62]
Red Brazilian (Alagoas) (ethanol 80% extract)	vestitol neovestitol isoliquiritigenin	[63]

(Table 17) contd....

Propolis type	Compounds	Ref.
Brazilian (Manaus-Amazon) (ethanol 80% extract)	xanthochymol gambogenone 7- <i>epi</i> -nemorosone 7- <i>epi</i> -clusianone	[64]
Red Brazilian (ethanol extract)	(6a <i>S</i> ,11a <i>S</i> )-6a-ethoxymedicarpan 2-(2',4'-dihydroxyphenyl)-3-methyl-6-methoxybenzofuran 2,6-dihydroxy-2-[(4-hydroxyphenyl)methyl]-3-benzofuranone	[65]
Red Brazilian (ethanol extract)	guttiferone E/xanthochymol, oblongifolin A, 7,5'-dihydroxy-3'-methoxyisoflavone, retusapurpurin A and retusapurpurin B	[37]
Geopropolis Brazilian (ethanol extract)	6- <i>O-p</i> -coumaroyl- <i>D</i> -galactopyranose 6- <i>O</i> -cinnamoyl-1- <i>O-p</i> -coumaroyl- $\beta$ - <i>D</i> -glucopyranose 7- <i>O</i> -methylnaringenin 7- <i>O</i> -methylaromadendrin 7,4'-di- <i>O</i> -methylaromadendrin 4'- <i>O</i> -methylkaempferol 3- <i>O</i> -methylquercetin 5- <i>O</i> -methylaromadendrin 5- <i>O</i> -methylkaempferol	[66]
Geopropolis Brazilian (methanol extract)	5,7,4'-trihydroxyflavanone 3,5,6,7,4'-pentahydroxyflavonol naringenin-4'- <i>O</i> - $\beta$ - <i>D</i> -glucopyranoside myricetin-3- <i>O</i> - $\beta$ - <i>D</i> -glucopyranoside	[67]

Trying to relate the secondary metabolites of propolis with the vegetation surrounded of the bee hive, Edrada-Ebel *et al.* isolated two new prenylated stilbene derivatives (Table 20) from the Ghanian propolis and a new phloroglucinone from a Cameroon propolis [88]. The *trans* olefinic configuration of protons H- $\alpha$  and H- $\beta$  of (*E*)-5-{2-[8-hydroxy-2-methyl-2-(4-methylpent-3-en-1-yl)-2*H*-chromen-6-yl]vinyl}-2-(3-methylbut-2-en-1-yl)benzene-1,3-diol **36** and 5-[(*E*)-3,5-dihydroxystyryl]-3-[(*E*)-3,7-dimethylocta-2,6-dien-1-yl]-benzene-1,2-diol **37** was established based on their  $^1\text{H}$ - $^1\text{H}$  coupling constant of  $\sim 16$  Hz.

These structures were unequivocally assigned by using 2D NMR, mainly based on the HMBC correlations. The key HMBC correlations are those of H- $\beta$  with C-1', C-2',6' of the resorcinol ring and of the H- $\alpha$  with C-1 and C-5 of chromenol moiety (Fig. 8).

**Table 18.** Compounds identified in Asian propolis, which structures were determined using NMR spectroscopy.

Propolis type	Compounds	Ref.
Taiwanese (ethanol extract)	propolins A, B	[68]
Taiwanese (ethanol extract)	propolin C	[69]
Taiwanese (ethanol extract)	propolins A, B, C, D, E, F	[70]
Taiwanese (ethanol extract)	propolin G	[71]
Japanese (ethanol extract)	propolin C (or nymphaeol-A) propolin D (or nymphaeol-B) propolin F (or isonymphaeol-B) propolin G (or nymphaeol-C)	[72]
Japanese (ethanol extract)	propolins A, B, C, D, E, F, H (or 3'-geranylaringenin) prokinawan	[73]
Thai (methanol extract)	(7''S)-8-[1-(4'-hydroxy-3'-methoxyphenyl)prop-2-en-1-yl]-(2S)- pinoembrin (E)-cinnamyl (E)-cinnamylidenate (E)-cinnamyl (E)-p-methoxycinnamate (E)-cinnamyl (E)-cinnamate (E)-cinnamyl (E)-ferulate (E)-cinnamyl (E)-p-coumarate	[74]
Thai (methanol extract) (cont.)	phenethyl caffeate phenethyl ferulate benzyl (E)-ferulate benzyl (E)-p-coumarate benzyl (E)-isoferulate (2S)-pinostrobin (2S)-pinoembrin (2R,3R)-alpinone-3-acetate (2R,3R)-pinobanksin 3-acetate (2R,3R)-pinobanksin 3-isobutyrate (2R,3R)-pinobanksin 3-(2-methyl)butyrate (2R,3R)-pinobanksin 3-propanoate tectochrysin chrysin izalpinin	
Thai (methanol 80% extract)	cardanol	[75]
Jeju Island, Korean (ethanol extract)	4'-methoxybavachromanol laserpitin isolaserpitin	[76]

(Table 18) contd....

Propolis type	Compounds	Ref.
Korean (ethanol extract)	phenethyl caffeate benzyl caffeate ethyl caffeate benzyl ferulate 3',3'-dimethylallyl ferulate cinnamyl 3,4-dimethoxycaffeate cinnamyl coumarate benzyl coumarate phenethyl cinnamate cinnamyl cinnamate	[77]
Wuhan, Chinese (methanol extract)	2-acetyl-1-coumaroyl-3-cinnamoylglycerol (+)-2-acetyl-1-feruloyl-3-cinnamoylglycerol (-)-2-acetyl-1-feruloyl-3-cinnamoylglycerol 2-acetyl-1,3-dicinnamoylglycerol (-)-2-acetyl-1-( <i>E</i> )-feruloyl-3-[3''(ζ),16'']-dihydroxypalmitoylglycerol	[78]
Al-Baha, Saudi Arabian (ethylacetate extract)	(12 <i>E</i> )-communic acid (12 <i>Z</i> )-communic acid sandaracopimaric acid (+)-ferruginol (+)-totarol 3β-acetoxy-19(29)-taraxasten-20 <i>α</i> -ol cycloartenol 24-methylene-cycloartenol β-amyrin-3β- <i>O</i> -acetate α-amyrin-3β- <i>O</i> -acetate taraxasterol-3β- <i>O</i> -acetate pseudotaraxasterol-3β- <i>O</i> -acetate lupeol-3β- <i>O</i> -acetate	[79]

**Table 19.** Compounds identified in African and Oceania propolis, which structures were determined using NMR spectroscopy.

Propolis type	Compounds	Ref.
Tunisian (methanol extract)	5,3'-dihydroxy-3,7,4',5'-tetramethoxyflavone 5,4'-dihydroxy-3,7,3'-trimethoxyflavone (or pachypodol)	[80]

(Table 19) contd....

Propolis type	Compounds	Ref.
Jordanian (methanol extract)	cinnamyl cinnamate tetracosanoic acid tectochrysin pinocembrin pinobanksin-3- <i>O</i> -acetate 3-methylethergalangin chrysin galangin genkwanin alpinone naringenin apigenin cryptomeridiol agathadiol 24-( <i>Z</i> )-3-oxolanosta-1,7,24-trien-26-oic acid 24-( <i>Z</i> )-1 $\beta$ ,3 $\beta$ -dihydroxyeupha-7,24-dien-26-oic acid	[81]
Algerian (methanol extract)	3-methyl-3-butenyl ( <i>E</i> )-caffeate 2-methyl-2-butenyl ( <i>E</i> )-caffeate phenethyl ( <i>E</i> )-caffeate chrysin apigenin kaempferol galangin pinobanksin pinobanksin 3-acetate pinobanksin 3-( <i>E</i> )-caffeate cupressic acid isocupressic acid imbricatoloic acid torulosal isoagathotal torulosol agathadiol cistadiol 18-hydroxy- <i>cis</i> -clerodan-3-ene-15-oic acid myricetin 3,7,4',5'-tetramethyl ether	[82]
Cameroonian (70% ethanol extract)	13 alk(en)ylphenols $\alpha$ -amyrin $\beta$ -amyrin lupeol cycloartenol mangiferonic acid mangiferolic acid ambonic acid ambolic acid isomagiferolic acid 9 alk(en)ylresorcinols	[83]

(Table 19) contd.....

Propolis type	Compounds	Ref.
Solomon Islands (95% ethanol extract)	propolins C, D, G, H	[84]
Solomon Islands (ethanol extract)	solophenol A bonannione A sophoraflavanone A (2 <i>S</i> )-5,7-dihydroxy-4'-methoxy-8-prenylflavanone	[85]
Solomon Islands (ethanol extract)	solophenol B, C, D solomonin	[86]
Australian [hexane:methanol(1:1)]	(2 <i>S</i> )-cryptostrobin (2 <i>S</i> )-stroboponin (2 <i>S</i> )-cryptostrobin 7-methyl ether (2 <i>S</i> )-desmethoxymatteucinol (2 <i>S</i> )-pinostrobin (2 <i>S</i> )-pinocembrin	[87]

**Table 20.** <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data (δ, ppm; *J* in parenthesis, Hz) of compounds **36-38**.

Position	36 (in CDCl <sub>3</sub> )		37 [in (CD <sub>3</sub> ) <sub>2</sub> CO]		Position	38 (in CDCl <sub>3</sub> )	
	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C		<sup>1</sup> H	<sup>13</sup> C
1	6.68 d (1.7)	116.9	6.82 d (1.8)	120.0	1		66.5
2		121.3		119.6	2A	3.52 dd (15.0, 11.7)	31.0
3		139.9		143.5	2B	1.58 m	31.0
3-OH			8.62 s		3	4.94 dd (11.7, 2.9)	85.7
4		145.0		140.0	6		88.6
4-OH	5.42 s		7.39 s		7	2.78 dd (10.7, 8.2)	42.6
5	6.94 d (1.7)	113.1	6.95 d (1.8)	110.5	8A	2.39 m	31.5
6		130.5		128.6	8B	1.88 m	31.5
α	6.86 d (16.2)	128.7	6.91 d (16.3)	129.3	9	2.10 m	44.5
β	6.76 d (16.2)	126.5	6.76 d (16.3)	126.3	10		50.4
1'		137.9		125.6	11		81.9
2'	6.53 br s	106.7	6.49 br d (2.0)	104.9	12		208.4
3'		155.7		159.3	13		205.2
3'-OH	5.35 s		8.37 s		14		68.5
4'		113.2	6.24 br t (2.0)	101.1	15A	2.57 dd (16.3, 6.6)	41.7
5'		155.7		159.3	15B	1.85 d (16.3)	41.7
5'-OH	5.35 s		8.37 s		16		204.4
6'	6.53 br s	106.7	6.49 br d (2.0)	104.9	17		84.3

(Table 20) contd....

Position	36 (in CDCl <sub>3</sub> )		37 [in (CD <sub>3</sub> ) <sub>2</sub> CO]		Position	38 (in CDCl <sub>3</sub> )	
	<sup>1</sup> H	<sup>13</sup> C	<sup>1</sup> H	<sup>13</sup> C		<sup>1</sup> H	<sup>13</sup> C
1''	6.34 d (10.0)	123.1	3.34 d (7.2)	28.7	18	1.20 s	21.5
2''	5.59 d (10.0)	130.3	5.36 t (7.2)	122.5	19	1.16 s	20.6
3''		80.8		135.4	20	1.29 s	28.2
4''	1.74 m	41.6	2.02 m	40.1	21	1.09 s	17.8
5''	2.06 m	31.0	2.05 m	29.7	22	1.48 s	24.9
6''	5.07 br t (6.3)	124.2	5.08 m	124.7	23	1.35 s	22.7
7''		132.6		130.6	24		192.2
8''	1.65 s	26.1	1.74 s	15.9	25/33		135.3
9''	1.57 s	18.1	1.57 s	17.5	26/30	7.16 d (8.0)	128.9
10''	1.41 s	27.0	1.63 s	25.6	27/29	7.28 t (8.0)	128.2
1'''	3.41 br d (7.0)	22.9			28	7.40 t (7.2)	132.4
2'''	5.26 br t (7.0)	122.0			30		128.9
3'''		135.2			31A	2.59 d (6.5)	29.8
4'''	1.75 s	26.2			31B	1.25 m	29.8
5'''	1.81 s	18.3			32	5.09 t (6.9)	118.9
					33		135.3
					34	1.69 s	26.1
					35	1.68 s	18.2

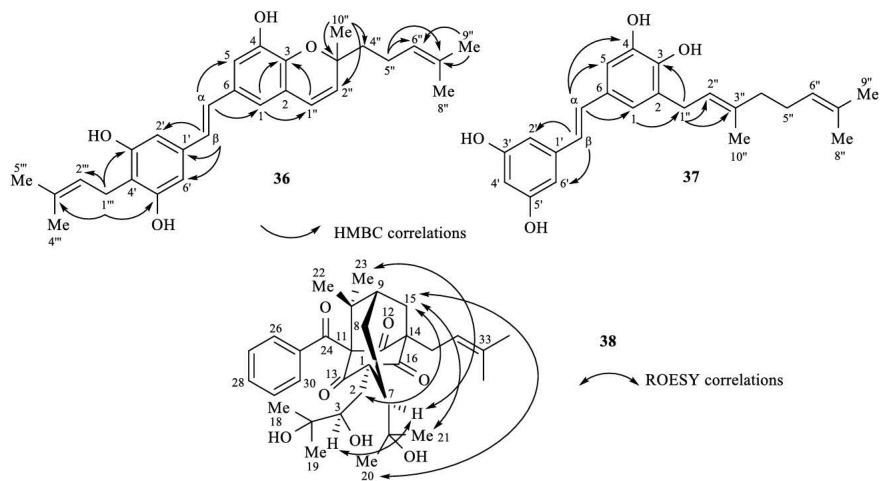


Fig. (8). HMBC correlations of compounds 36 and 37 and ROESY correlations of compound 38.

The NMR data of the prenylated phloroglucinone **38** (Table 20) was assigned based on those of plukenetione C isolated before from *Clusia plukenetii* [89]. The relative stereochemistry of compound **38** was determined by its ROESY correlations (Fig. 8).

In conclusion, we have shown that NMR spectroscopy is a powerful analytical tool to: identify and quantify the constituents of honey and propolis and to elucidate their structures; detect their adulteration and differentiate their botanical and geographical origin.

#### CONFLICT OF INTEREST

The authors confirm that there is no conflict of interest to declare for this publication.

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#### ABBREVIATIONS

$\delta$	= Chemical shift
CA	= Correspondence analysis
CLA	= Cluster analysis
$^{13}\text{C}$ NMR	= Carbon-13 nuclear magnetic resonance spectroscopy
cont.	= Continuation
COSY	= Correlation spectroscopy
d	= Doublet
dd	= Double doublet
ddd	= Double doublet of doublets
dt	= Double triplet
DEPT	= Distortionless enhancement by polarization transfer
DQF	= Double quantum filtered
DOSY	= Diffusion ordered spectroscopy

<b>FA</b>	= Factor analysis
<b>GDA</b>	= General discriminant analysis
<b>HCA</b>	= Hierarchical cluster analysis
<b>HMBC</b>	= Heteronuclear multiple bond correlation
<b>HMQC</b>	= Heteronuclear multiple quantum coherence
<b><sup>1</sup>H NMR</b>	= Proton nuclear magnetic resonance spectroscopy
<b>HPLC-DAD-MS</b>	= High pressure liquid chromatography-diode array detector-mass spectrometry
<b>HPLC-MS</b>	= High pressure liquid chromatography-mass spectrometry
<b>HSQC</b>	= Heteronuclear single quantum correlation
<b>Hz</b>	= Hertz
<b>ICA</b>	= Independent component analysis
<b><i>J</i></b>	= Coupling constant
<b>m</b>	= Multiplet
<b>n.d.</b>	= Not detected
<b>NOESY</b>	= Nuclear Overhauser effect spectroscopy
<b>PCA</b>	= Principal component analysis
<b>PLS-DA</b>	= Partial least squares discriminant analysis
<b>ppm</b>	= Parts-per-million
<b>ROESY</b>	= Rotating-frame Overhauser effect spectroscopy
<b>s</b>	= Singlet
<b>t</b>	= Triplet
<b>td</b>	= Triplet of doublets
<b>TMS</b>	= Tetramethylsilane
<b>TOCSY</b>	= Total correlated spectroscopy

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