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Presence of some cyclitols in honey

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Abstract

Quercitol (1,3,4/2,5-cyclohexanepentol), pinitol (3-*O*-methyl-*chiro*-inositol), 1-*O*-methyl-*muco*-inositol and *muco*-inositol have been identified for the first time in edible honeys by GC–MS of their *O*-trimethylsilyl derivatives, using their Kovats indices and mass spectral data. Individual cyclitol content (including *myo*-inositol for comparison) was estimated by using phenyl-β-glucoside as internal standard. *Myo*-inositol and pinitol were present in the 28 examined honey samples; quercitol, methyl-*muco*-inositol and m*uco*-inositol were only detected in some samples. The presence, in honey, of these compounds could be used to distinguish among honey types, since they probably are collected by bees from wild plants.

Keywords: Cyclitols; Honey; Gas chromatography–mass spectrometry

1. Introduction

Honey is mainly constituted of a complex mixture of carbohydrates (monosaccharides and oligosaccharides) and many studies have been carried out in order to separate, identify and quantify them (Swallow & Low, 1990; Goodall, Dennis, Parker & Sharman, 1995; Low & South, 1995; Mateo & Bosc-Reig, 1997; Horváth & Molnárl-Perl, 1998; Gómez Bárez et al., 2000). Nevertheless, data about cyclitols or polyalcohols in honey are very scarce; only *myo*-inositol and mannitol have been reported in edible honeys (Horvath & Molnar-Perl, 1998).

Cyclitols are present in most animal and plant tissues (Anderson, 1972) and they are implicated in a range of physiological activities (Angyal & Anderson, 1959). *Myo*-inositol is a well-known nutrient for most animal species, whereas other cyclitols and methyl inositols have been mainly found in vegetable sources. Pinitol and quercitol have been described in wasp honeys by Hunt, Rossi, Holmberg, Smith, and Sherman (1998).

A study of carbohydrates in honeys carried out in our laboratory showed several peaks eluting before hexoses. Although their mass spectra indicated a cyclitol-type structure, no references were found on their presence in honeys and they could not be identified. In the present work, we have identified some of these minor peaks, which are reported in honey for the first time, by comparison of their chromatographic retention and mass spectral data with those of natural compounds.

2. Experimental

2.1. Standard substances

Cyclitols commercially available were myo-inositol (Sigma), *muco*-inositol and pinitol (Aldrich Chem. Co.).

2.2. Honey samples

Eight honey samples were commercial products, from different Spanish regions, while another 20 were directly obtained from beekeepers of the Madrid province.

Samples of fresh leaves, bark and acorns of *Quercus pyrenaica* were collected in El Espinar (Segovia) and needles from *Sequoia sempervirens* were collected in the Botanic Garden of Madrid (Real Jardín Botánico, CSIC).

2.3. Analysis

Although the oximation step is not necessary when only hydroxyl groups are present, GC analysis was carried out using a two-step derivatization procedure

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(oximation and trimethylsilylation) which was applied both to standards and to real samples, since the latter always contain reducing sugars. Sample preparation was carried out by diluting 0.5 g of honey to 25 ml with 80% ethanol; 0.5 ml of the extract was mixed with 1 ml ethanol containing 1 mg phenyl-β-glucoside as internal standard. This mixture was evaporated under vacuum and derivatives were formed as previously described (Brobst & Lott, 1966). After reaction, samples were centrifuged at 7000 *g* for 5 min at 5 °C (Li & Schumman, 1981).

GC–MS analysis was carried out with an HP 5890 series instrument with a MD 5971 quadrupole mass detector (both from Hewlett-Packard, Palo Alto, CA, USA) working in EI mode at 70 eV and using helium as carrier gas. The column was a methylsilicone (SPB-1), 25 m×0.25 mm i.d., 0.25 μm film thickness from Supelco (Bellefonte, PA). The temperatures of injector and detector were 300 °C and oven temperature was held at 200 °C for 20 min, then programmed to 270 °C at a heating rate of 15 °C min^{-1} and to 290 °C at 1 °C min^{-1} and finally programmed to 300 °C at 15 °C min^{-1} and held for 40 min in order to elute the remaining oligosaccharides. Injections were made in the split mode, with a split flow of 40 ml/min. Chromatographic peaks were measured using HPChem Station software (Hewlett-Packard, Palo Alto, CA, USA). Kovats retention indices (*I*) were obtained at 200 °C. Hold-up time was measured by injecting *n*-pentane. The amount of each cyclitol was directly estimated from the areas of peaks and internal standard.

3. Results and discussion

A preliminary study was carried out in order to obtain retention and mass spectral data from cyclitols not commercially available.

Quercitol is a deoxiinositol (1,3,4/2,5-cyclohexanepentol) whose trivial name is "acorn sugar", present in different species of oaks (Anderson, 1972). As this compound is not commercially available, three aqueous extracts were prepared from fresh leaves, bark and acorns of *Quercus pyrenaica* collected in El Espinar (Segovia). The extracts were evaporated at low temperature under vacuum, silylated and injected as described above. Besides quercitol, the main compounds found in them were fructose and glucose.

1-*O*-methyl-*muco*-inositol has previously been found in *Sequoia sempervirens,* and its Kovats index and mass spectrum have been published (Binder & Haddon, 1984). We identified this compound in the aqueous extract of needles from *Sequoia sempervirens*, collected in the Botanic Garden of Madrid (Real Jardín Botánico, CSIC).

The cyclitols identified in honey samples are listed in Table 1. Compound A was identified as quercitol by comparison of its GC and MS data with those of quercitol found in oak samples, while the compound B analytical data were similar to those obtained for the methyl-*muco*-inositol present in *S. sempervirens*. Compounds C, D and E were identified as pinitol, *muco*-inositol and *myo*-inositol by comparison of their GC and MS data with those of standard compounds. Table 1 presents Kovats indices and most significant MS fragments for these compounds.

Table 2 shows the concentration values obtained for the identified cyclitols. *Myo*-inositol and pinitol were present in all studied honeys, whereas quercitol was found in twenty samples and methyl-*muco*-inositol appeared in 24. Their concentrations were in many cases higher than 1 mg/g. *Muco*-inositol, present at a lower level, could be detected in seventeen samples. No clear correlations were found among the concentrations of the five compounds.

These cyclitols are present in variable amounts in plants. Quercitol appears as an important carbohydrate in oaks (Anderson, 1972), but it has also been found in a variety of plant material (Fletcher, 1948). Pinitol is present in Cupressaceae and Pinaceae whereas 1*D*-1-*O*-methyl-*muco*-inositol has been reported in Araucariaceae, Cistaceae and some species of Cupressaceae, but not in Pinaceae (Dittrich, Gietl, & Kandler, 1971). Cyclitols found in honey are probably directly collected by bees from wild plants, possibly from nonfloral sources, and their presence in honeys could be related to honey origin, but more studies will be necessary.

Table 1
Minor components found in honeys and identified by GC-MS

Compound	Kovats index	Main MS ions (higher than 150)[a]	Assignation
A	1834	191 (5.8%) / 204 (6.3%) / 217 (12.6%) / 265 (3.4%) / 305 (2.4%) / 318 (3.2%)	Quercitol
B	1866	133 (4.1%) / 191 (3.7%) / 204 (1.6) / 217 (8.4%) / 260 (5.5%) / 305 (2.4%) / 318 (3.1%)	1-Methyl-*muco*-inositol
C	1880	133 (3.3%) / 191 (3.2%) / 204 (1.13%) / 217 (5.6%) /260 (4.9%) / 305 (1.8%) / 318 (2.6%)	Pinitol
D	1974	191 (1.1%) / 217 (6.1%) / 305 (3.6%) / 318 (4.6%)	*Muco*-inositol
E	2152	191 (4.7%) / 217 (8.3%) / 305 (5.3%) / 318 (2.9%)	*Myo*-inositol

[a] *m*/*z* Values (relative abundance in brackets).

Table 2
Cyclitol content (mg/g product) in different honey samples

Sample	Quercitol	Methyl-*muco*-inositol	Pinitol	*Muco*-inositol	*Myo*-inositol
1	0.00	0.07	1.86	0.07	0.14
2	3.59	0.14	2.33	0.16	0.80
3	3.35	0.08	0.22	0.08	1.86
4	0.22	0.54	1.29	0.07	1.13
5	0.31	2.59	0.39	0.03	1.30
6	6.57	1.80	7.85	0.21	1.93
7	3.55	1.76	0.61	0.10	1.10
8	1.63	0.43	5.83	0.11	0.73
9	2.34	2.36	3.81	0.18	2.53
10	0.17	0.58	1.33	0.00	1.07
11	0.26	1.85	7.82	0.05	1.37
12	0.00	3.36	3.73	0.05	1.50
13	0.06	0.08	0.35	0.00	2.61
14	0.00	0.05	0.69	0.02	1.58
15	0.00	0.46	2.78	0.00	1.64
16	0.31	0.11	2.04	0.02	0.64
17	0.06	0.65	1.53	0.00	0.55
18	0.05	0.27	1.88	0.07	0.63
19	1.95	0.57	6.04	0.08	0.96
20	0.00	0.00	0.43	0.00	0.27
21	1.37	0.02	0.02	0.03	0.10
22	0.46	3.63	8.15	0.00	2.78
23	0.00	1.03	0.91	0.00	0.87
24	3.12	0.39	0.52	0.00	1.51
25	5.78	0.36	7.01	0.00	0.76
26	5.96	0.00	0.41	0.40	1.07
27	0.00	0.00	0.39	0.00	3.01
28	0.00	0.00	0.09	0.00	0.45

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