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Short Communication

Gas chromatographic analysis of underivatized phenolic constituents from propolis using an electron-capture detector

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ABSTRACT

Underivatized phenolic constituents from propolis (flavonoid aglycones, phenolic acids and their esters) were analysed by capillary gas chromatography using an electron-capture detector The analysis was possible because of the good electron-capture response of these compounds, which belong to the so-called "conjugated electrophores"

INTRODUCTION

Phenolic compounds are widespread in plants and they are important as active ingredients of many phytogenic preparations in cosmetics and medicine [1,2] Phenolic compounds (flavonoid aglycones, phenolic acids and their esters) are the main components of propolis (bee glue) [3] and are thought to be responsible for its valuable biological activity [4] Among the various methods used for the separation and analysis of complex mixtures of natural phenolics, such as propolis, capillary gas chromatography (GC) is of major importance due to its sensitivity and resolving power It is common practice to prepare derivatives of phenolic compounds before GC analysis [methyl or trimethylsilyl (TMS) ethers] and to use flame ionization detection [5] The derivatization is thought to be necessary to increase the volatility of the phenolic compounds, but it has some disadvantages, especially when flavonoids are to be analysed [5,6] Some recent reports have shown that under the conditions of pyrolysis GC–mass spectrometry some flavonoid aglycones have been detected [7] This is an indication that even the underivatized compounds of this type are volatile enough and transmit well through suitable GC columns at 300–350°C without thermal degradation The main groups of propolis phenolics (flavonoid aglycones, phenolic acids and their esters) are also known to belong to the so-called "conjugated electrofores" which have a good electron-capture response [8] and in these compounds the electron-capture detector might be more sensitive than the flame ionization detector This was confirmed by experiments to use electron capture for the detection of the TMS derivatives of phenolic

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compounds For this reason we tried to determine if underivatized phenolic compounds could be determined by capillary GC using an electron-capture detector

EXPERIMENTAL

The flavonoids pinocembrin (**2**), tectochrysin (**3**) and galangin (**5**) were isolated from propolis as described by Bankova *et al* [9] The methodology of β-phenylethyl caffeate (**6**) synthesis has been described by Bankova [10] Caffeic acid (**1**) was purchased from Merck and chrysin (**4**) from Roth Propolis was collected in south Bulgaria near Plovdiv

Extraction of propolis

Propolis (1 g) was grated after cooling and refluxed with 15 ml of methanol for 1 h The hot extract was filtered, diluted with water and extracted successively with light petroleum (b p 40–60°C) (3 ×) and diethyl ether (3 ×) The ether extracts were combined and evaporated to dryness This extract (1 mg) was dissolved in 100 μl of acetone, and 1–2 μl of this solution were injected into the gas chromatograph

Trimethylsilylation

A 1 mg mass of the model mixture or of the ether extract of propolis was silylated with 50 μl of N,O-*bis*(trimethylsilyl)trifluoroacetamide at 65°C for 30 min, 1–2 μl of the solution was injected into the gas chromatograph

Gas chromatography

GC analysis was carried out on a Perkin-Elmer 8700 instrument The separation was accomplished on a 9 m × 0 25 mm I D SE-54 fused-silica capillary column with a film thickness of 0 25 μm The linear velocity of the nitrogen carrier gas was 9 cm s^{-1} (split ratio 1 25) The temperature programme was as follows 80–280°C, rate 20°C min^{-1}, 280–300°C, rate 2°C min^{-1}, with a 10-min hold at 300°C The injector temperature was 320°C and the detector temperature was 350°C At the end of the column the gas flow was split in a ratio of 1 1 using two 10 cm × 0 25 mm, 0 25 μm film thickness SE-54 capillaries, the first of them going into the flame ionization detector and the other into the electron-capture detector

RESULTS AND DISCUSSION

Experiments were carried out to see if the TMS ethers of propolis phenolics have a significant electron-capture response In these experiments a model mixture of representatives of the three main groups of propolis phenolics (caffeic acid, **1**, flavonoid aglycones pinocembrin, **2**, galangin, **5**, and the ester β-phenylethyl caffeate **6**) and propolis extract were used At the end of the column both detectors were run simultaneously It is shown that the electron-capture response was about one order of magnitude higher than the flame ionization response (Fig 1) When the injector temperature was increased (280–320°C), higher responses were observed for both detectors because of the increasing vapour pressure of the compounds analysed An increase in the detector temperature (320–350°C) resulted in a lower electron-capture response (15–40% for the different compounds) This is an indication that the electron-capture process in this instance occurs in a way which represents an undissociative attachment producing a stable negative molecular ion [8] The high electron-capture response of the conjugated electrophores (silylated flavonoids and cinnamic acid derivatives) encouraged the analysis of the low-volatile underivatized compounds by capillary GC with electron-capture detection The same column was used for the separation of derivatized and underivatized propolis phenolic components (caffeic acid, **1**, pinocembrin, **2**, tectochrysin, **3**, chrysin, **4**, galangin, **5**, and β-phenylethyl caffeate, **6**) A satisfactory resolution (not the optimum solution) of the underivatized compounds was achieved under the conditions used for the analysis of the TMS ethers, so these conditions were used for the comparison study (Fig 2)

The injector temperature was 320°C, when it was increased to 350°C, only a slight increase in the relative areas of the peaks with the longest retention times (chrysin **4** and galangin **5**) was observed The percentage of caffeic acid **1** (retention time 4 5 min) in these samples was low (less than 1%) [11] and was below the limit of detection It is interesting to note that when underivatized propolis phenolic components were analysed using the two detection methods simultaneously, only the largest peaks pinocembrin and galangin, were satisfactorily detected by flame ionization detection at an acceptable signal-to-noise ratio

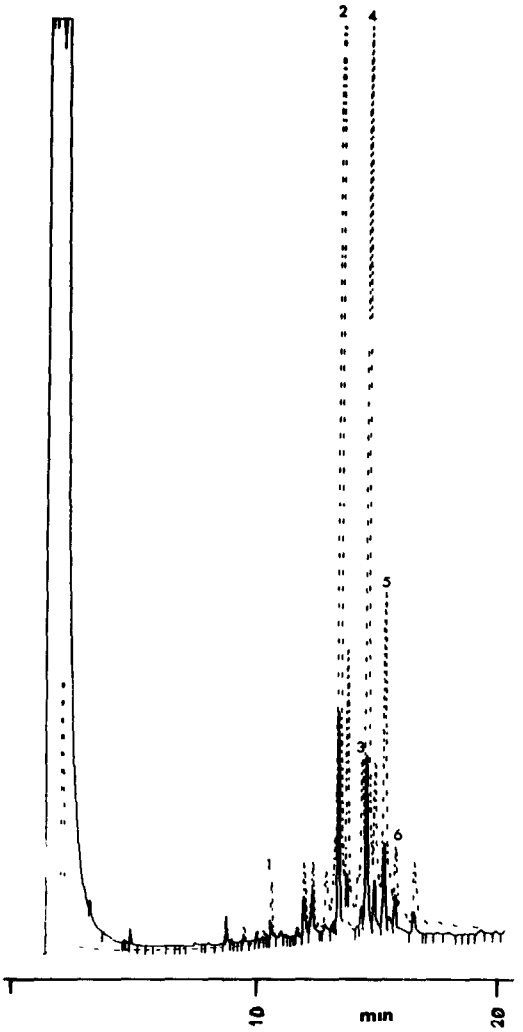

Fig 1 Capillary gas chromatogram of TMS ethers of propolis phenolic constituents For GC conditions, see under Experimental Peaks **1** = caffeic acid, **2** = pinocembrin, **3** = tectochrysin, **4** = chrysin, **5** = galangin, **6** = β-phenylethyl caffeate (———) Flame ionization response, (----) electron-capture

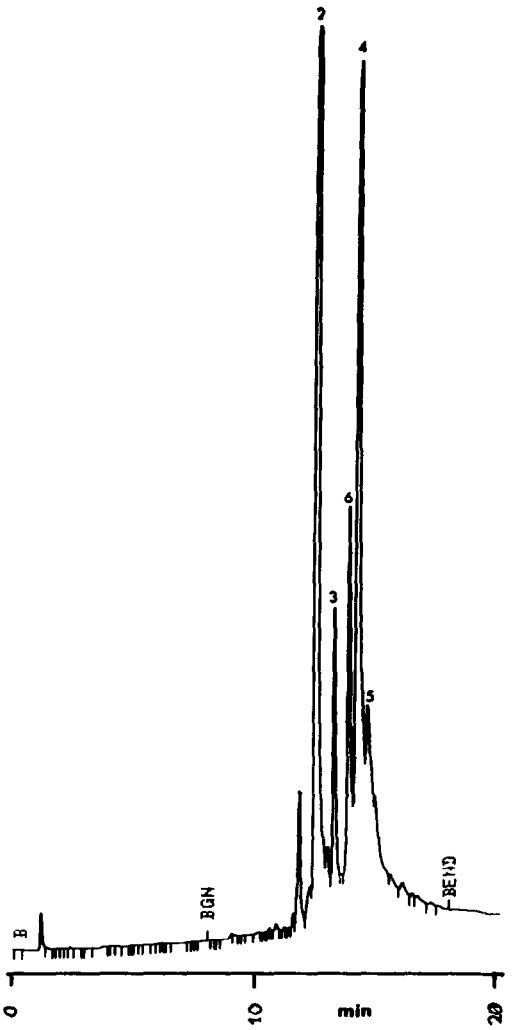

Fig 2 Capillary gas chromatogram of underivatized propolis phenolic constituents using electron-capture detection For GC conditions, see under Experimental For peak identification, see Fig 1

To the best of our knowledge this is the first analysis of underivatized flavonoid aglycones by capillary GC It was possible because of the good electron-capture response of these compounds The method proposed allows the rapid qualitative analysis of the main biologically active components of propolis [12] The good reproducibility of the peak areas is an indication that the method could also be used for the quantitative analysis of this valuable natural product

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REFERENCES

1 E E Conn, *The Biochemistry of Plants*, Vol 7, Academic Press, New York, 1981, p 425

2 J W McClure, in J Harborne, T J Mabry and H Mabry (Editors), *The Flavonoids*, Chapmann & Hall, London, 1975, Ch 18, p 970
3 P Walker and E Crane, *Apidologie*, 18 (1987) 327
4 E Ghisalberty, *Bee World*, 60 (1979) 59
5 C S Creaser, M R Koupai-Abyazani and G R Stephenson, *J Chromatogr*, 478 (1989) 415
6 W Greenaway, T Scaysbrook and F R Whatley, *Proc R Soc London B* 232 (1987) 249
7 H -R Schulten, N Simmleit and R Mueller, *Anal Chem*, 61 (1989) 221
8 K Peltonen, *LC GC Int*, 3 (1990) 52
9 V Bankova, S Popov and N Marekov, *J Nat Prod*, 46 (1983) 471
10 V Bankova, *J Nat Prod*, 52 (1990) 821
11 V Bankova, R Christov, G Stoev, S Popov, *J Chromatogr*, in press
12 V Bankova, A Kuyumgiev, A Ignatova, Al Dyulgerov, O Pyreb, Z Zamyansan and S Popov, in R Vlahov (Editor), *Proc Int Conf Chem Biotechnol Biol Active Nat Prod, September 18–23, 1989, Varna*, Vol 2, Bulgarian Academy of Sciences, Sofia, 1989, p 239