

An Analytical Study on Fatty Acids in Pollen Extract

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Fatty acids in the fat-soluble fraction of pollen extract (Cernitin™ GBX™) were analyzed. Fatty acids were determined on a Dani 3860 PTV GC. Identification was based on the retention times of known mixtures of free fatty acids and their methyl esters in GC/MS. The major part of the fatty acid fraction was in free form. Bound fatty acids were characterized by a high content of α -linolenic acid (70%). The mechanism of antiatherosclerotic action of this pollen extract may be, at least in part, due to polyunsaturated fatty acids.

Keywords: Pollen extract, Fatty acids.

Introduction

Reports on the serum lipid-lowering effect of orally administered pollen extracts to rats (Samochowiec and Wojcicki, 1981; Wojcicki and Samochowiec, 1984) have been confirmed in humans (Wojcicki *et al.*, 1983).

Pollen extracts-Cernitin™ T60™ and Cernitin™ GBX™ (AB Cernelle, Vegeholm, Sweden) were taken from six plant species: rye grass, maize, timothy grass, pine alder flower, and orchard grass. Cernitin™ T60™ contains water-soluble substances (6.0-9.2% of α -amino acids) while those in Cernitin™ GBX™ are mainly fat-soluble (10-16% phytosterols).

The chemical composition of pollen has been investigated (Kvanta, 1968; Nielson *et al.*, 1957; Lelson and Holmstrom, 1957). Numerous chemical substances have been identified and

isolated: 21 amino acids, all known vitamins, enzymes, coenzymes, sterols, minerals and trace elements.

This study was to analyze the fatty acids in the fat-soluble fraction of pollen extract (Cernitin™ GBX™) with regard to its proven antiatherosclerotic activity (Wojcicki *et al.*, 1986).

Materials and Methods

The fatty acid composition of the fat-soluble pollen extract (Cernitin™ GBX™) was analyzed by gas chromatography. Bound fatty acids were transesterified by modifying the method of Hiltunen *et al.*, (1979) as follows:

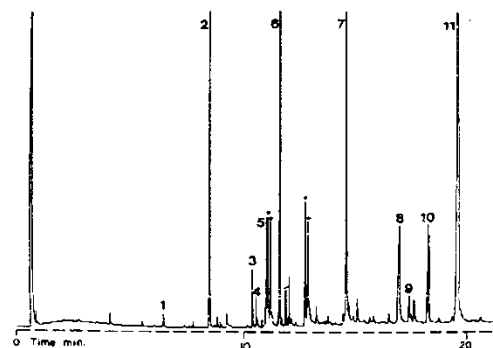
A sample (100 mg) of the fat-soluble pollen extract (batch No 759) was dissolved in 1mL petroleum spirit (b.p. 40-60°C), transmethylated with 0.5mL 0.5 N NaOMe at 40°C for 5 min and

neutralized with 1mL of 15% NaHSO₄. Petroleum spirit was added and 1μL taken from the upper layer for gas chromatography. Fatty acids were determined on a Dani 3860 PTV GC as follows: column OV-351 Nordion fused silica (25 m, 0.32 mm ID) oven programmed from 100°C at 10⁰/min to 225°C, programmed temperature vaporizer (PTV)-injector from 70⁰ to 250°C, carrier gas (H₂) 0.8 bar, detector (FID) 250°C, sampling mode split (40:1). Identification was based on the retention times of known mixtures of free fatty acids and their methyl esters. Analyses after transesterification of triolein confirmed that no free fatty acids were formed under the conditions used. Other constituents such as aliphatic hydrocarbons and alcohols were identified by GC/MS.

Results and Discussion

GLC analyses of the fat-soluble pollen extract revealed that the major part (more than 60%) of the fatty acid was in the free form (Table 1, Fig. 1). Bound fatty acids, which rather reflect the compositional profile of pollen, were characterized by a high content of α-linolenic acid (18: 3n-3, α-LLA) (70%) followed by small amounts of linoleic (18: 2n-6) and oleic acid (18:1n-9) only. Palmitic acid (16:0) was the most abundant saturated

Previous studies have revealed that the pollen extract has beneficial properties, lowering serum lipid levels, reducing atherosclerotic plaque intensity (Wojcicki *et al.*, 1986) and decreasing platelet aggregation both *in vitro* (Kosmider *et al.*, 1983) and *in vivo* (Wojcicki *et al.*, 1983). If fatty acids are involved in these effects, the role of α-linolenic acid as a precursor of eicosapentaenoic acid (20: 5n-3, EPA) is significant, since EPA is considered to be responsible for reduced platelet aggregation (Dyer-berg and Bang, 1979). EPA *in vivo* is incorporated into platelet phospholipids, to some extent replacing arachidonic acid and exerting an antithrombotic effect either by competing with remaining arachidonic acid for cyclo-oxygenase and lipoxygenase or by being converted to less proaggregatory PGH₃ and TXA₃ (Moncada and



Vane, 1984). Studies in humans suggest that a diet supplemented with polyunsaturated Figure 1. GC chromatogram of pollen extract fatty acids on OV-351 column. Peak numbering as in Table 1. *Aliphatic hydrocarbons and/or alcohols.

Fatty acids decreases whole blood viscosity, and reduces triglyceride and cholesterol levels in patients with cardiovascular disease (Saynor *et al.*, 1984). Recent clinical observations are in favour of a linolenic acid supply, leading to higher levels of phospholipid eicosapentaenoic and docosahexaenoic acids (Jacotot *et al.*, 1986). The metabolic conversion from α-LLA into EPA, which is known to occur in humans (Budowski *et al.*, 1984; Sanders and Younger, 1983), would at least in part explain the mechanism of antiatherosclerotic action of pollen extract (Wojcicki *et al.*, 1986).

Figure 1.

Table 1. Fatty acid composition of the fat-soluble pollen extract

Compound No	Relative amount (%)
Methyl esters	
1 14:0	0.21
2 16:0	7.64
3 18:0	0.98
4 18:1n-9	0.68
5 18:2n-6	1.85
6 18:3n-3	26.73
Total	38.09
Free fatty acids	
7 16:0	17.44
8 18:0	1.20
9 18:1n-9	0.83
10 18:2n-6	3.50
11 18:3n-3	38.93
Total	61.90

Peak numbers refer to constituents in Fig. 1.

Figure 2.

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