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 (Sanochowiec and Wójcicki, 1981; Wójcicki and Samochowiec, 1984) have been confirmed in humans (Wójcicki *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;* Nelson and Homström, 1957). Numerous chemical substances have been identified and isolated: 21 aminoacids, 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 (Wójcicki *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% $NaHSO_4$. 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 10°/min to 225°C, programmed temperature vaporizer (PTV)-injector from 70° to 2500°C, carrier gas (H_2) 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.

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.

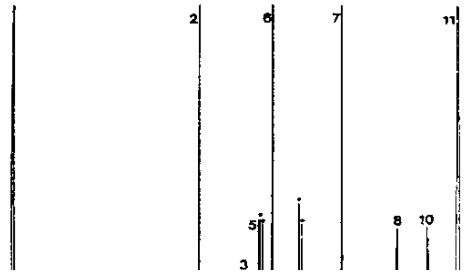

Figure 1. GC chromatogram of pollen extract fatty acids on OV-351 column. Peak numbering as in Table 1. *Aliphatic hydrocarbons and/or alcohols.

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 (181n-9) only. Palmitic acid (16:0) was the most abundant saturated fatty acid.

Previous studies have revealed that the pollen extract has beneficial properties, lowering serum lipid levels, reducing atherosclerotic plaque intensity (Wójcicki *et al.,* 1986) and decreasing platelet aggregation both *in vitro* (Kośmider *et a1.,1983)* and *in vivo* (Wójcicki *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 (Dyerberg 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 cylco-oxygenase and lipoxygenase or by being converted to less proagreggatory PGH_3 and TXA_3 (Moncada and Vane, 1984). Studies in humans suggest that a diet supplemented with polyunsaturated 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 eicosapaentoic 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 (Wójcicki *et al*, 1986).

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