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SHORT COMMUNICATION

STUDIES IN THE CRUCIFERAE: STEROLS IN POLLEN OF *BRASSICA NAPUS* L.

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Abstract—Using GLC with mass spectrometry the main sterols of pollen from *Brassica napus L. f. annua* (*cv.* Giant English rape) have been shown to be 24-methylenecholesterol and 24-ethylidenecholesterol.

INTRODUCTION

STUDIES carried out at Gif-Sur-Yvette have shown that pollens may contain unusual sterols, and sterols not found in other parts of the plant. Thus it has been shown that 24-methylenecholesterol is often the principal sterol of pollen,[1,2] occurring in *Zea mays* L., *Pyrus malus* L., *Salix* spp. and *Cistus ladanifera* L. Cholesterol is the principal sterol in pollen of *Hypochoeris radicata* L.[2,3] A new sterol, pollinastanol, was isolated from pollen of *Castanea vulgaris Lam.* and *Corylus avellana* L.[4] *Corylus avellana* pollen also contained mono- and diunsaturated C_{27}, C_{28} and C_{29} sterols. During work involving studies of the effects of *Plasmodiophora brassicae woron* in the Cruciferae, it was found that marked changes in sterol composition occurred in plants at different stages of development. As a part of a study of this effect, pollen of *Brassica napus L. f. annua* (*cv.* Giant English rape) was collected and the sterol composition studied.

RESULTS AND DISCUSSION

Sterols were isolated in the usual way[5] after the pollen grains had been ruptured as far as possible by grinding in light petroleum suspension. Figure 1 shows the gas chromatograms obtained from this sterol fraction as trimethylsilyl ethers using SE-30 and OV-17 stationary phases. The two main peaks (3 and 6) correspond in mobility with the derivatives of 24-methylenecholesterol and Δ^5-avenasterol (24-ethylidenecholesterol) respectively. The identity of these two compounds was confirmed by combined gas chromatography-mass spectrometry (CG-MS) using SE-30 stationary phase. The obtained mass spectra of the trimethylsilyl ethers were identical with previously described spectra for these two compounds.[6] The spectrum from peak 5+6 showed two extra ions indicating the presence of some β-sitosterol, and peak 5 in the OV-17 trace corresponds in retention time to β-sitosterol.

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[2] M. DEVYS and M. BARBIER, *Compt. Rend. Acad. Sci., (Paris)* **261**, 4901 (1965).
[3] M. DEVYS and M. BARBIER, *Phytochem.* **5**, 1031 (1966).
[4] M. F. HUGEL, *Ann. Abeille* **8**, 309 (1965); *Chem. Abs.* **64**, 19718 (1966).
[5] B. A. KNIGHTS, *Memoirs of the Society for Endocrinology No.* 16 (edited by J. K. GRANT), 211 (1967).
[6] B. A. KNIGHTS, *J. Gas Chromatog.* **5**, 273 (1967).

GLC and mass spectral data for peak 1 indicated that this compound was cholesterol and the mass spectrum of peak 2 was consistent with the possibility of this compound being pollinastanol. The identity of compound 4 is uncertain, but mass spectral data suggest it corresponds to a C_{29} diunsaturated sterol with a 4-methyl group.

The relative percentages of each sterol, obtained by triangulation of peaks in the GLC traces, are respectively:

Compound 1, (cholesterol) 2·8 per cent; compound 2, (pollinastanol?) 2·4 per cent; compound 3, (24-methylenecholesterol) 56 per cent; compound 4 (?) 3 per cent; compound 5, (β-sitosterol) 4·2 per cent; compound 6, (24-ethylidenecholesterol $\equiv \Delta^5$-avenasterol) 31·6 per cent.

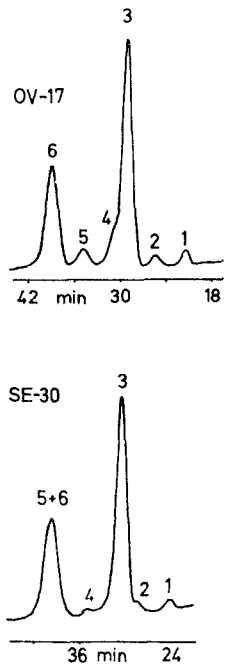

FIG. 1. POLLEN STEROL TRIMETHYLSILYL ETHERS.

Operating conditions 9 ft columns; 3 per cent OV-17 256°, N_2 carrier gas—68 ml/min; 1 per cent SE-30 250°, N_2 carrier gas—60 ml/min.

EXPERIMENTAL

Pollen from flowering plants was collected by detaching the flowers and scraping the anthers with a knife blade. The yield of sterol, isolated in the usual way,[5] was approximately 0·5 per cent. GLC was carried out in a Pye 104 model 14 chromatograph using 9 ft columns packed with either 1 per cent SE-30 coated on Gas Chrom P[7] or 3 per cent OV-17 coated on Gas Chrom Q (Applied Science Laboratories pretested packing). The GC-MS analysis was obtained using an LKB-9000 gas chromatograph-mass spectrometer equipped with a 10 ft 1 per cent SE-30 column.

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