Phytochemistry, 1968, Vol. 7, pp. 1361 to 1365. Pergamon Press. Printed in England

POLLEN STEROLS—A MASS SPECTROGRAPHIC SURVEY

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(*Received* 31 *January* 1968)

Abstract—The fractions of sterol in pollens from fifteen species of plants in eleven families were analyzed by mass spectrometry. 24-Methylene-cholesterol was the principal sterol of red clover (*Trifolium pratense* L.), saguaro cactus (*Carnegiea gigantea* (Engelm.) Britt. & Rose), mustard (*Brassica nigra* (L.) Koch), London-rocket (*Sisymbrium irio* L.), rye (*Secale cereale* L.), timothy (*Phleum pratense* L.), and sweet corn (*Zea mays* var. *saccharata* (Sturtev.) Bailey). β-Sitosterol was the principal sterol of mule fat (*Baccharis viminea* DC.), juniper (*Juniperus utahensis* (Engelm.) Lemm.), heartsease (*Polygonum* sp.), waterleaf (*Hydrophyllum capitatum* Dougl.), Scotch pine (*Pinus sylvestris* L.), European alder (*Alnus glutinosa* (L.) Gaertner), and Lombardy poplar (*Populus nigra* var. *italica* Muenchh.). Cholesterol was the principal sterol of cottonwood (*Populus fremontii* S. Wats.). The presence of cholesterol in the pollen from cottonwood has been confirmed by gas–liquid chromatography. 24-Methylene-cholesterol in saguaro cactus was further verified by the preparation of 24-ketocholesterol by ozonolysis, saponification of the acetate, and then a direct comparison of the thin-layer chromatograph of the free sterol with an authentic sample of 24-ketocholesterol.

INTRODUCTION

24-METHYLENE-cholesterol is often but not always the principal sterol in pollens from some species of plants.[1–5] Mass spectrographic analyses of ten species showed a series of C_{27}, C_{28}, and C_{29} sterols: 24-methylene-cholesterol was the major sterol in three, β-sitosterol in five, and stigmasterol and cholesterol each in one.[2, 3] Also, sterols of the C_{27}, C_{28}, and C_{29} series have been found in many plant extracts,[6] and, since they correspond to successive methylations by S-adenosylmethionine,[4, 7, 8] 24-methylene-cholesterol could be an intermediate in such a biosynthesis.

Although the results of previous studies[1–5, 9] indicated that no taxonomic relationships existed between the sterols in the pollen and the plant families, the number of such analyses was enlarged to confirm this conclusion. The present paper reports the mass spectrographic analyses of sterol fractions of pollen from fifteen species of plants.

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TABLE 1. PERCENTAGE OF MONO- AND DI-UNSATURATED C_{27}, C_{28}, AND C_{29} STEROLS OF BEE-GATHERED AND HAND-COLLECTED POLLENS

Pollens	Sterols* (in % of total sterols)						Plant family	Main sterol
	C_{27}		C_{28}		C_{29}			
	Di-unsat.	Mono-unsat.	Di-unsat.	Mono-unsat.	Di-unsat.	Mono-unsat.		
BEE-GATHERED								
Red clover, *Trifolium pratense* L.	Traces	3	82	6	6	3	Leguminosae	24-Methylene-cholesterol
Cactus, saguaro, *Carnegiea gigantea* (Engelm.) Britt. & Rose	Traces	Traces	94	6	Traces	Traces	Cactaceae	24-Methylene-cholesterol
Mule fat, *Baccharis viminea* DC.	Traces	Traces	4	11	11	74	Compositae	β-Sitosterol
Mustard, *Brassica nigra* (L.) Koch	Traces	9	37	15	32	7	Cruciferae	24-Methylene-cholesterol
Juniper, *Juniperus utahensis* (Engelm.) Lemm.	Traces	15·5	6 5	17	11	50	Cupressaceae	β-Sitosterol
Heartsease, *Polygonum* sp.	4	21	21	8	17	29	Polygonaceae	β-Sitosterol
London-rocket, *Sisymbrium irio* L.	6	15	37	12	12	18	Cruciferae	24-Methylene-cholesterol
Cottonwood, *Populus fremontii* S. Wats.	8	59	6	7	5	15	Salicaceae	Cholesterol
Waterleaf,† *Hydrophyllum capitatum* Dougl.	Traces	4	15	16	26	39	Hydrophyllaceae	β-Sitosterol
HAND-COLLECTED								
Rye, *Secale cereale* L.	Traces	6·5	49	17	15	12·5	Gramineae	24-Methylene-cholesterol
Scotch pine, *Pinus sylvestris* L.	Traces	7	9	14	16	54	Pinaceae	β-Sitosterol
European alder, *Alnus glutinosa* (L.) Gaertner	Traces	2 5	5·5	11	17	64	Betulaceae	β-Sitosterol
Timothy, *Phleum pratense* L.	Traces	2·5	61·5	13	10	13	Gramineae	24-Methylene-cholesterol
Sweet corn, *Zea mays* var. *saccharata* (Sturtev.) Bailey	Traces	4·5	64·5	13	9	9	Gramineae	24-Methylene-cholesterol
Lombardy poplar, *Populus nigra* var. *italica* Muenchh.	Traces	15	3	15	6	61	Salicaceae	β-Sitosterol

* Relative composition determined by the comparison of the intensities of the M-60 peaks in the mass spectra of the acetate (values are given at ± 10 per cent). Mono-unsaturated sterols in C_{27}, C_{28}, C_{29} respectively furnish the peaks at m/e 368, 382, 396. Di-unsaturated sterols of the same series give peaks at m/e 366, 380, and 394.

† *Osmia lignaria*.

RESULTS AND DISCUSSION

The percentages of mono- and di-unsaturated C_{27}, C_{28}, and C_{29} sterols in the nine species of bee-gathered and in the six species of hand-collected pollens examined are summarized in Table 1. The results[1–5] obtained earlier at the Institut de Chimie des Substances Naturelles for an additional six species of bee-gathered and four hand-collected pollens are shown in Table 2. The data in both tables support our previous observations concerning the lack of relationship between the kind and amount of C_{27}, C_{28}, and C_{29} sterols in pollens from different plants and the taxonomic family to which they belong. Furthermore, quantitative variations in the relative quantities of sterols in both bee-gathered and hand-collected pollens were as great in species belonging to the same plant family as in species belonging to different families. For example, though willow (*Salix* sp.), cottonwood (*Populus fremontii* S. Wats.), and Lombardy poplar (*P. nigra* var. *italica* Muenchh.) all belong to Salicaceae, the pollens vary widely in the content of C_{27}, C_{28}, and C_{29} sterols.

TABLE 2. PRINCIPAL STEROL OF SIX SPECIES OF BEE-GATHERED AND FOUR SPECIES OF HAND-COLLECTED POLLENS

Pollen	Plant family	Principal sterol
BEE-GATHERED		
Apple, *Malus sylvestris* Mill.	Rosaceae	24-Methylene-cholesterol (60%)
Willow, *Salix* sp.	Salicaceae	24-Methylene-cholesterol (50%)
Heather, *Calluna vulgaris* (L.) Salisb.	Ericaceae	Stigmasterol (80%)
Cat's-ear, *Hypochaeris radicata* L.*	Compositae	Cholesterol (90%)
Dandelion, *Taraxacum officinale* Web. ex Wigg.	Compositae	β-Sitosterol (38%)
Sweet chestnut, *Aesculus hippocastanum* L.	Hippocastanaceae	β-Sitosterol (74%)
HAND-COLLECTED		
Hazel, *Corylus avellana* L.	Betulaceae	β-Sitosterol (75%)
Corn, *Zea mays* L.	Gramineae	24-Methylene-cholesterol (59%)
Pine, *Pinus mugo* Turra	Pinaceae	β-Sitosterol (65%)
Sunflower, *Helianthus annus* L.	Compositae	β-Sitosterol (42%)

* Pollen pellets sorted and separated by hand.

24-Methylene-cholesterol was the principal sterol of the pollens from species belonging to Gramineae. This sterol is also the major constituent of the sterol fraction of pollens from plants belonging to Leguminosae, Cactaceae, Cruciferae, Rosaceae, and Salicaceae. In fact, the sterol mixture isolated from the pollen of saguaro cactus (*Carnegiea gigantea* (Engelm.) Britt. & Rose, Cactaceae) was found to contain about 94 per cent 24-methylene-cholesterol, but as far as we know this pollen is the only vegetable material containing such large amounts. Although 24-methylene-cholesterol has been previously isolated from two marine invertebrates,[10] researchers have generally assumed that it came from the phyto-plankton associated with these organisms because animals have never been found to biosynthesize C_{28} sterols.

One of the most interesting aspects of this and an earlier study[11] was the discovery of cholesterol in pollens from the cottonwood and cat's-ear, *Hypochaeris radicata* L. (Table 2).

[10] D. R. IDLER and U. H. M. FAGERLUND, *J. Am. Chem. Soc.* 77, 4142 (1955).
[11] M. DEVYS and M. BARBIER, *Phytochem.* 5, 1031–1033 (1966).

But the recent discovery that pollinastanol is present in the pollen from cat's-ear[12] is perhaps of equal interest; the presence of this stanol along with large quantities of cholesterol in pollen from one plant species is remarkable. However, during the past several years, cholesterol has been reported from such different plant extracts as leaves of the potato (*Solanum tuberosum* L.), marine red algae (*Rhodophyceae*),[13] and slime mold (*Labyrinthula minuta* var. *atlantica*).[14]

Scientific data are lacking to either refute or substantiate the statement frequently made by beekeepers that pollens from the same species of plants in different geographical areas sometimes vary widely in their nutritive value for honey bees. However, the kind and percentage of C_{27}, C_{28}, and C_{29} sterols are almost identical in the pollen from corn (*Zea mays* var. *saccharata* (Sturtev.) Bailey) of North American (Table 1) and European origin (Table 2).

METHODS AND MATERIALS

Pollens analyzed in this study include fifteen species representing eleven plant families (Table 1). Six were hand-collected, eight were gathered by honey bees (*Apis mellifera* L.), and one was gathered by the insect *Osmia lignaria* Say. The methods of collecting, handling, sorting, and storing pollens previous to analysis have been reported.[15] Also, the methods and materials used to isolate the crude lipid matter from pollens have been previously described.[15] The sterols were separated from the lipid matter by column chromatography,[16] then further purified by digitonin precipitation[17] and by thin-layer chromatography (TLC) on silicic acid, and then developed with pentane and ethyl acetate (7:3).[5] Subsequently, the sterol mixtures were acetylated with acetic anhydride in pyridine (72 hr at 20°), and, after the usual treatment,[1] the sterol acetates were recrystallized once from methanol.

Mass spectra of the sterol acetates of pollen were obtained on an Atlas CH_4 instrument.[18] The temperature when the sample was introduced was 180°. The relative compositions of the sterol mixtures analyzed were calculated on the basis of the M-60 peaks (values ± 10 per cent). Mono-unsaturated sterols of C_{27}, C_{28}, and C_{29}, respectively, have peaks at m/e 368, 382, and 396. Di-unsaturated sterols of the same series have peaks at m/e 366 (C_{27}), 380 (C_{28}), and 394 (C_{29}). The Δ-5 sterol acetates give M-60 ions quantitatively. The Δ-7 sterols, when present, are eliminated by preparative TLC, and their absence is noticeable in the mass spectra (lack of M ions).

Mass spectrometric data do not show the exact positions of substituent groups and their stereochemistry, but in this report we have used the names of the C_{27}, C_{28}, and C_{29} mono- and di-unsaturated (Δ-5) sterols most commonly found in nature.

The presence of cholesterol in the sterol fraction of pollen from cottonwood was verified by preparing trimethylsilyl ether derivatives[19] and analyzing them by gas–liquid chromatography (GLC) at 200° and a flow rate of 120 ml/min on a column 2m × 4mm i.d. packed with

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[13] K. TSUDA, S. AKAGI, Y. KISHIDA, R. HAYATSU and K. SAKAI, *Chem. Pharm. Bull. (Japan)* **6**, 724 (1958).
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[17] W. M. SPERRY and M. WEBB, *J. Biol. Chem.* **187**, 97 (1950).
[18] Mention of a proprietary product or company names does not necessarily imply their endorsement by the U.S. Department of Agriculture.
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Celite-HMDS impregnated with 10 per cent QF-1 flurosilicone. The method of internal standards was used for this GLC analysis.

The presence of 24-methylene-cholesterol in the sterol fraction from six pollens (Table 1) was verified by mass spectrometry (intense peak at m/e 296 in the spectra),[20] i.r. spectrometry [bands at 1639 cm^{-1} (6·1 μ) and at 885 cm^{-1} (11·3 μ)], and by the preparation of 24-keto-cholesterol by ozonolysis[1] for pollen from saguaro cactus. Ozonization was done on 15 mg of the sterol acetate of the pollen in 5 ml of acetic acid.[2] The acetate of 24-ketocholesterol was extracted and then saponified with 2 N KOH for direct TLC comparison of the free sterol with an authentic sample of 24-ketocholesterol. The observed R_f values on TLC were 0·45 when the pentane–ethyl acetate (3:2) system was used with a HCl solution of 2,4-dinitrophenylhydrazine as an indicator.

Acknowledgement—The authors are grateful to Professor E. Lederer of the Institut de Chimie des Substances Naturelles, 91-Gif-sur-Yvette, France, for his interest.

[20] H. E. AUDIER, R. BEUGELMANS and B. C. DAS, *Tetrahedron Letters* **36**, 4341 (1966).