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INVESTIGATIONS ON CAROTENOIDS IN INSECTS. IV.

THE OCCURENCE OF PARTICULAR CAROTENOIDS IN *APIS MELIFERA L.* (APIDAE)

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SUMMARY

By means of columnar and thin-layer chromatography, the presence of carotenoids in *Apis mellifera* L. was studied. The investigations covered larvae of workers, drones, queens and adult workers, drones and queens.

The investigations revealed the presence of the following carotenoids : α-, β-carotene, echinenone, canthaxanthin, β-cryptoxanthin, β-carotene epoxide, isocryptoxanthin, lutein, lutein epoxide, flavoxanthin, zeaxanthin, isozeaxanthin, aurochrome, astaxanthin, astaxantin ester, mutatochrome and neoxanthin.

The analysis of the presence of carotenoids in *Apis mellifera* showed the most common carotenoids were canthaxanthin, β-cryptoxanthin, lutein epoxide ans astaxanthin (the pure and ester forms together).

INTRODUCTION

Examining carotenoid content of various insect species both water and land we managed to prove the dominance of particular carotenoids in definite insect species. Besides, particular carotenoids belonging to separate orders are rather frequent as for example in Ephemeroptera β-carotene, β-carotene epoxide, β-cryptoxanthin or mutatochrome belong to such carotenoids. Then, as in larvae of odonates, to such carotenoids belong the following ones : canthaxanthin and astaxanthin – particularly its ester form (CZECZUGA and MIRONIUK, 1979). In this we are interested in the problem of which carotenoids are peculiar to particular evolutionary form of *Apis mellifera* as a typical phytophagous species feeding with nectar and pollen of particular plants. As it is known, insects as well as animals are not able to produce carotenoids *de novo* but they take them only with the food as plants are the source of all

carotenoids for animals. The animals are only able to transform some of them by oxidation.

MATERIALS AND METHODS

The investigations covered larvae and adults of drone, queen and worker honey bee. The whole material was taken from the same source in June.

The material was prepared immediately on collection by placing it into dark glass containers and covering it with 96 % acetone. It was kept in a refrigerator until the spectrophotometric determinations were made.

The carotenoid pigments were extracted by means of 96 % acetone in a dark room. Saponification was carried out by means of 10 % KOH in ethanol at a temperature of about 20 °C for 24 hours in the dark in a nitrogen atmosphere.

Columnar and thin-layer chromatography, described in detail in our previous papers (CZECZUGA, 1971) were used for the separation of the various carotenoids. A glass column (Quicklift – England) approximately 1 cm diameter and 15-20 cm in length, filled with Al_2O_3, was used in column chromatography. The extract was passed through the column after which the different fractions were eluted with the solvent. Silica gel was used for the thin-layer chromatography, with the appropriate solvent systems, the R_f values being determined for each spot.

The pigments were identified by the following methods : *a*) behaviour on column chromatography; *b*) absorption spectra of the pigments in various solvents were recorded by a Beckman spectrophotometer model 2 400 DU; *c*) the partition characteristics of the carotenoid between hexane and 95 % methanol; *d*) comparison of R_f on thin-layer chromatography; for identification of α-, β-carotene, echinenone, canthaxanthin, lutein, zeaxanthin and astaxanthin co-chromatography was applied identical carotenoids (firms Hoffmann – La Roche and Co. Ltd., Basel, Switzerland and Sigma Chemical Company, U.S.A.); *e*) the presence of allylic hydroxyl groups was determined by acid chloroform; and *f*) the epoxide test.

Quantitative determinations of the concentrations of carotenoid solutions were made from the quantitative absorption spectra. These determinations were based on the extinction coefficient $E_{cm}^{1\%}$ at the wavelengths of maximal absorbance in petroleum ether or hexane. If x g of the carotenoid dissolvent in y ml solution gives an extinction of E at its wavelength of maximal absorption, then is :

$$x = \frac{E\,y}{E\,\dfrac{1\,\% \times 100}{1\ \text{cm}}}$$

RESULTS

The results of chromatography analysis of workers are presented in Table 1. Then carotenoids among which lutein epoxide (23.9 %) and zeaxanthin (20.6 %) made up the majority occurred in vault larvae. Beside lutein epoxide which also occurred in large quantity (20.6 %), β-cryptoxanthin (31.6 %) was found to be the majority in the 21 days old worker. Flying worker similarly as its larvae had zeaxanthin in largest quantity (20.1 %). Besides two carotenoids (α- and β-carotene) and neoxanthin were being not observed in larvae or in 21 days old workers. The total content varied from 2.825 μg/g to 3.203 μg/g fresh weight.

TABL. I. — *Content of carotenoids in honey bee workers* (Apis mellifera *L.*) *in % of the total carotenoid content.*

Carotenoid	gummed up worker larvae	21 days old worker	flying worker
α-carotene	–	–	3.1
β-carotene	–	–	7.0
β-cryptoxanthin	7.0	31.6	3.1
β-carotene epoxide	2.5	5.7	–
canthaxanthin	trace	7.8	13.7
isocryptoxanthin	–	5.0	–
lutein	12.2	7.1	–
lutein epoxide	23.9	20.6	19.5
zeaxanthin	20.6	7.4	20.1
isozeaxanthin	8.0	–	–
aurochrome	–	4.9	–
astaxanthin	8.1	–	–
astaxanthin ester	11.8	trace	12.9
flavochrome	5.9	–	5.5
mutatochrome	–	9.9	4.7
neoxanthin	–	–	10.4
Total content in μg/g fresh weight	2.825	2.911	3.203

TABL. 2. — *Content of carotenoids in honey bee drones of* (Apis mellifera *L.*) *in % of the total carotenoid content*

Carotenoid	non-gummed up drone larvae	gummed up drone larvae	24 days old drone
β-carotene	11.5	–	–
β-cryptoxanthin	5.0	6.3	2.1
β-carotene epoxide	–	–	2.8
canthaxanthin	12.2	2.8	28.8
lutein epoxide	30.3	25.2	29.5
zeaxanthin	7.8	–	–
astaxanthin	32.0	47.2	28.2
astaxanthin ester	–	14.1	8.6
flavoxanthin	1.2	1.8	–
mutatochrome	–	2.6	–
Total content in μg/g fresh weight	2.905	2.998	3.100

Table 2 presents chromatography analysis data of drone larvae and adults. Astaxanthin (28.2-47.2 %) and lutein epoxide (25.2-30.3 %) were found to be in the largest quantity in all forms. Only in non-gummed up drone larvae the presence of β-carotene was confined and canthaxanthin made up 28.8 % of all carotenoids in adult drones. The total carotenoid content ranged from 2.905 to 3.100 μg/g fresh weight.

As regards the analysis of queens they are given in Table 3. In the examined material 13 carotenoids were identified among which astaxanthin (28.2 %) and β-

TABL. 3. — *Content of carotenoids in honey bee queens* (Apis mellifera *L.*) *in % of the total carotenoid content*

Carotenoid	queen larvae	queen
β-carotene	3.3	11.0
echinenone	1.5	
β-cryptoxanthin	31.2	7.0
β-carotene epoxide	2.7	20.4
canthaxanthin	1.5	2.8
isocryptoxanthin	2.0	5.4
lutein	8.5	
lutein epoxide	3.8	
zeaxanthin	10.3	4.5
isozeaxanthin	7.0	10.0
astaxanthin	28.2	14.6
astaxanthin ester		22.4
flavoxanthin		1.9
Total content in μg/g fresh weight	2.108	3.415

cryptoxanthin (31.2 %) occurred in largest quantities, while adult queens had β-carotene epoxide (20.4 %) and astaxanthin ester (22.4 %) in largest quantities. Both in larvae as well as in adult queens the following carotenoids occurred: β-carotene, canthaxanthin, β-cryptoxanthin, β-carotene epoxide, isocryptoxanthin, astaxanthin (free and ester form) zeaxanthin, isozeaxanthin and flavoxanthin. Attention should be drawn to the fact that lutein epoxide (3.8 %) was observed in small quantities and in all stages except in adult queens. This carotenoid occured in workers as well as in drones in proportionally large quantities. The total carotenoid content varied from 2.108 μg/g queen larvae to 3.415 μg/g fresh weight (adult queen).

DISCUSSION

Comparing all gathered results of particular carotenoid content for various *Apis mellifera* forms it should be stated that in all examined individuals β-cryptoxanthin, canthaxanthin and astaxanthin (pure or ester form) were identified. Besides, in the majority of examined *Apis mellifera* forms zeaxanthin and lutein epoxide (not found in adult queens) were observed. In flowers keto-carotenoids, especially astaxanthin, are comparatively rare (GOODWIN, 1976). Therefore we should assume that other carotenoids taken with the food are transformed by the honey bee individuals into keto-carotenoids of canthaxanthin and astaxanthin type (Fig. 1), as in the case of other insect representatives (CZECZUGA, 1979). Canthaxanthin and astaxanthin have been identified in a number of insects so far (LEUENBERGER and THOMMEN, 1970; CZYGAN, 1972; CZECZUGA, 1976). As we know nectar and flower pollen are the honey bee food, thus the majority of carotenoids of the investigated material was identified so far except canthaxanthin and astaxanthin in pollens or in flowers themselves (GOODWIN,

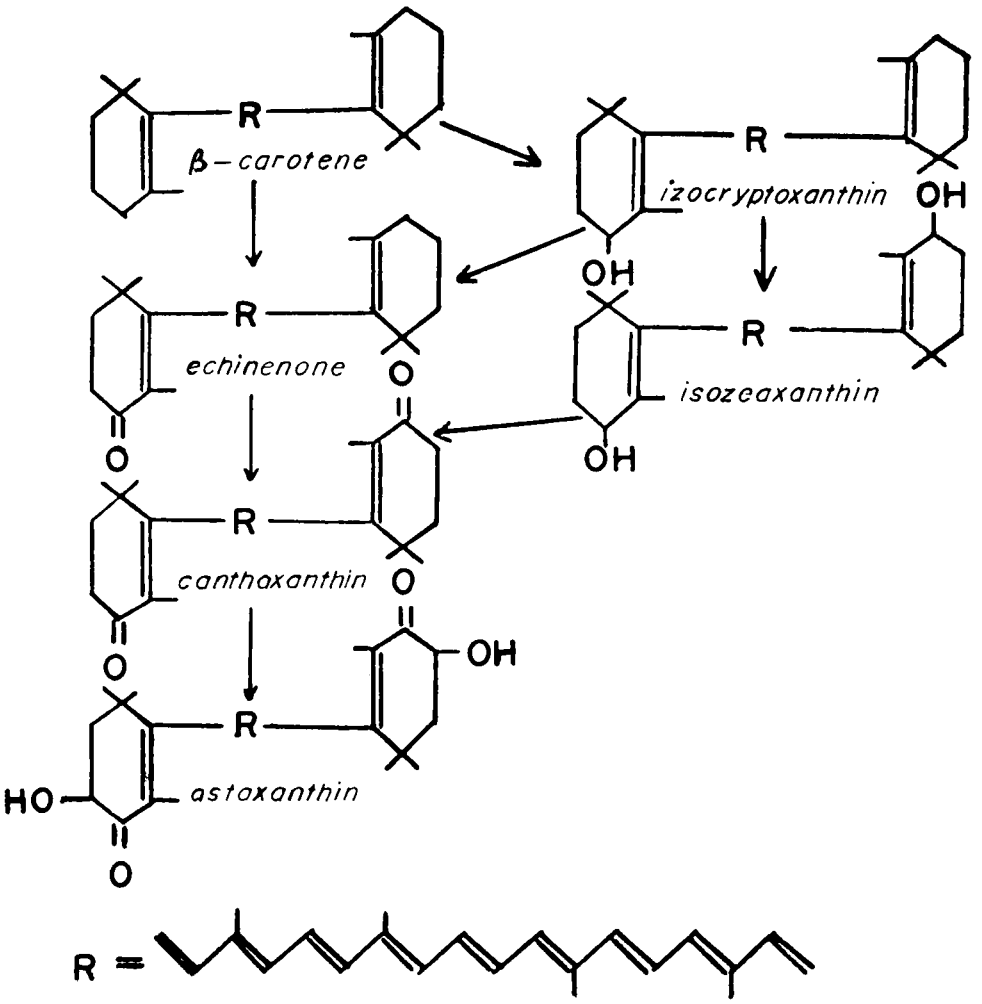

FIG. 1. — *Possible pathway of β-carotene oxidation to astaxanthin in* Apis mellifera *L.*

1976). Besides, these carotenoids observed in small quantities in the investigated material (flavoxanthin and mutatochrome) belong to the common carotenoids in pollens and flowers. This carotenoid was observed in other insects (FELTWELL and ROTHSCHILD, 1974). Evidently α- and β-carotene and various lutein forms, among others epoxide form, occure in pollen first of all.

The presence of α- and β-carotene in the workers should be related to the close contact of the workers with nectar and pollen. It also should be stressed that β-carotene occurs both in queen larvae and in queens themselves. Identified carotenoids in *Apis mellifera* honey bee were also pointed out in other *Hymenoptera* representatives particularly in bumblebees (CZECZUGA, 1979).

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RÉSUMÉ

RECHERCHES SUR LES CAROTÉNOIDES DES INSECTES
IV. PRÉSENCE DE CAROTÉNOIDES PARTICULIERS CHEZ *APIS MELLIFERA* L.

On a étudié la présence de caroténoïdes chez l'abeille *Apis mellifera* L. au moyen de la chromatographie sur colonne et en couches minces. Les recherches ont porté sur les larves et les adultes des trois castes.

Elles ont montré la présence des caroténoïdes suivants : carotène α et β, échinénone, canthaxanthine, cryptoxanthine β, époxyde de carotène β, isocryptoxanthine, lutéine, époxyde de lutéine, flavoxanthine, zéaxanthine, isozeaxanthine, aurochrome, astaxanthine, ester d'astaxanthine, mutatochrome et néoxanthine.

L'analyse des caroténoïdes présents chez *Apis mellifera* a montré que les plus courants étaient la canthaxanthine, la cryptoxanthine β, l'époxyde de lutéine et l'astaxanthine, sous sa forme pure et estérifiée.

ZUSAMMENFASSUNG

UNTERSUCHUNGEN ÜBER CAROTINOIDE BEI INSEKTEN
IV. DAS VORKOMMEN VON BESONDEREN CAROTINOIDEN BEI *APIS MELLIFERA* L.

Mittels Säulen- und Dünnschichtchromatographie wurde das Vorkommen von Carotinoiden bei *Apis mellifera* L. studiert. Die Untersuchung umfasste Larven von Arbeiterinnen, Drohnen und Königinnen.

Die Untersuchung ergab das Vorhandensein der folgenden Carotinoide : α-, β-Carotin, Echinenon, Canthaxanthin, β-Cryptoxanthin, β-Carotin-epoxid, Isocruptoxanthin, Lutein, Lutein-Epoxid, Flavoxanthin, Zeaxanthin, Isozeaxanthin, Aurochrom, Astaxanthin, Astaxanthinester, Mutatochrom und Neoxanthin.

Die Analyse des Vorkommens von Carotinoiden bei *Apis mellifera* ergab, dass die häufigsten Carotinoide, Canthaxanthin, β-Cryptoxanthin, Lutein-epoxid und Astaxanthin waren, und zwar in reiner wie in Esterform.

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