and shown to be identical to the natural spiroacetals from *D. cucurbitae* by comparison of their GC retention time and mass spectra. The first derivative (**2**) (0.1 µg/insect per day) showed a molecular ion at m/z 184.1536 corresponding to $C_{11}H_{20}O_2$, m/z 184 (10%), 140 (25), 115 (95), 114 (30), 112 (100), 97 (50), 69 (40), 55 (40), 43 (40), whilst (**3**) (0.01 µg/insect per day) showed a molecular ion at m/z 184.1516 and ions at m/z 184 (5%), 140 (10), 125 (10), 115 (100), 114 (40), 112 (55), 97 (70), 69 (50), 55 (50).

Determination of relative configuration of spiroacetals has been carried out on the basis of solvent-depending shifts in ^{1}H-NMR and γ-effects in ^{13}C-NMR spectra[8]. Derivatives (**2**) and (**3**) have been reported previously as scent marks from the mandibular gland of the bees, *Andrena wilkella*[9], *A. ocreata* and *A. ovatula*[10] and may fulfil a similar role in fruit fly ecology. It would appear that these spiroacetals are not produced in the rectal gland of the female *D. cucurbitae*, because neither (**2**) nor (**3**) were identified by Baker et al.[4] as components of the rectal gland secretions.

From the aeration extract of female *D. dorsalis* three spiroacetals were identified. The two major components were (E,E)-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane (**2**) (0.1 µg/insect per day) identified by comparison with a synthetic sample and the novel compound 8-ethyl-2-methyl-1,7-dioxaspiro[5.5]undecane (0.1 µg/insect per day). The latter showed characteristic fragment ions at m/z 112, 115 in the mass spectrum: m/z 198 (M^+, 10%), 169(20), 154(10), 140(20), 129(60), 115(100), 112(95), 97(45), 55(25). The molecular ion appeared at m/z 198 and the ion at m/z 169 (M − 29) arising from loss of ethyl indicated the alkyl substitution of the spiroacetal[7]. That 8-ethyl-2-methyl-1,7-dioxaspiro[5.5]undecane was the (E,E)-isomer (**4**) was indicated on the basis of the relatively short GC retention time. The minor spiroacetal component was identified as (E,E)-8-methyl-2-propyl-1,7-dioxaspiro[5.5]undecane (**5**) (0.01 µg/insect per day). This showed a mass spectrum and GC properties consistent with literature data[7,10].

Finally, a bishomologous series of ethyl esters of C_{12}, C_{14} and C_{16} saturated alkanoic acids was identified from *D. dorsalis*, together with the ethyl ester of the C_{16} monounsaturated alkanoic acid, all of which have been reported for *D. cucurbitae*[4] and *D. dorsalis*[11] from female rectal gland secretions. The role of the spiroacetals in the female fruit fly biology is as yet unknown, but is under investigation. It is of interest that only the female of the two *Dacus* species examined emits spiroacetals; the male aeration extracts have been examined but no spiroacetals were present.

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Structural characteristics of the carotenoids binding to the blue carotenoprotein from *Procambarus clarkii*

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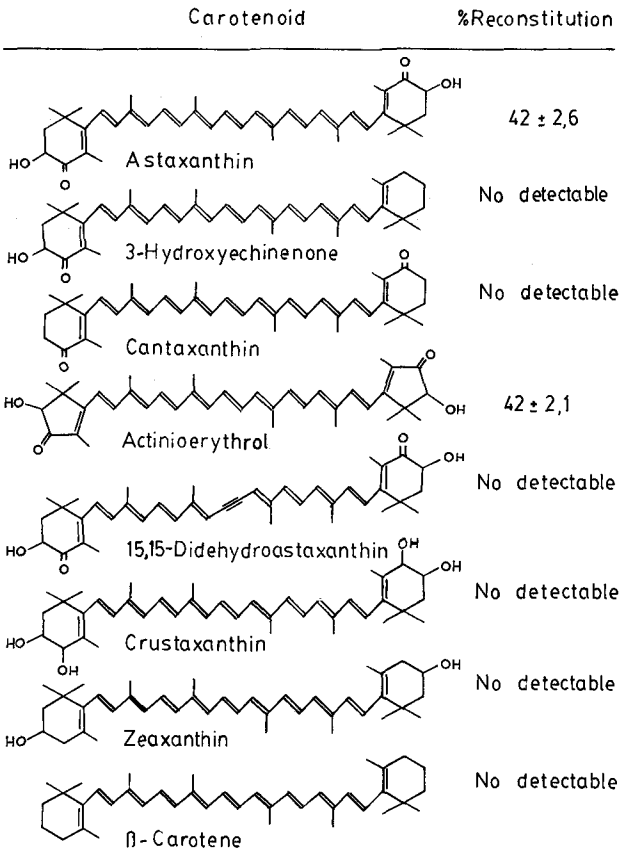
Summary. A blue carotenoprotein from the crayfish *Procambarus clarkii* was extracted and purified. This carotenoprotein contains the carotenoid astaxanthin as a prosthetic group. In the present work we have identified by reconstitution, after removing the native carotenoid, some characteristics of the carotenoids that could bind to the apoprotein. The carotenoid must have two oxo groups at positions 4, 4′ and two hydroxyl groups at positions 3, 3′ the hexagonal or pentagonal end structure being indifferent. It has been proved that changes in the polyene chain structure such as triple bonds destroy this binding capacity.
Key words. Actinioerythrol; astaxanthin; carotenoprotein; *Procambarus clarkii;* reconstitution.

The blue carotenoproteins from crustaceans contain as prosthetic group the red carotenoid astaxanthin[1]. The interaction between the carotenoid and the colorless apoprotein changes the red color of the carotenoid into the blue color of the carotenoid-protein complex[2]. The carotenoid is easily removed by addition of organic solvents like acetone or DMF (dimethylformamide)[3,4], recovering its original red color. This change in color also occurs by denaturation of the protein by coccion, as is well known. Zagalsky has demonstrated[1] that after elimination of the astaxanthin from crustacyanin (a blue carotenoprotein from *Homarus gammarus*) the reconstitution of the carotenoprotein is only achieved if the carotenoids contain oxo groups at C-4. According to Remstrom et al.[5] the reconstitution of α-crustacyanin requires the presence of hydroxyl groups at C-3 in at least one of the rings of the carotenoid. In this work the reconstitution of the blue carotenoprotein from *Procambarus clarkii* with different kinds of carotenoids has been studied.

The blue carotenoprotein from the carapace of the crayfish *Procambarus clarkii* was extracted using the method of Quarmby et al.[6]. The crude extract was fractionated with ammonium sulfate, and the blue precipitate resuspended and dialyzed against phosphate buffer 20 mM pH 7.5, then adsorbed on to a DE-52 Whatman ionic exchange column. The blue fraction so obtained was chromatographed through a Sephacryl S-300 column, and eventually concentrated. This carotenoprotein solution showed only one band on electrophoresis by the method of Davis and Ornstein[7,8], indicating its purity. Reconstitution of the native carotenoprotein was achieved by the method of Britton et al.[4] using the following carotenoids: astaxanthin, 15,15′-didehydroastaxanthin, actinioerythrol, 3-hydroxyechinenone,



Formulae and percentage of reconstitution obtained for eight different carotenoid structures. Values are the mean ± SEM of five experiments.

cantaxanthin, zeaxanthin, crustaxanthin and β-carotene whose formulae and percentage of reconstitution are represented in table. Only astaxanthin and actinioerythrol are able to bind to the apoprotein forming a blue reconstituted carotenoprotein with a maximum absorption of 625 nm against the 635 nm of the native carotenoprotein which contains astaxanthin as prosthetic group. The rest of the carotenoids tested were unable to bind to the apoprotein. Hence, the keto groups at positions 4 and 4' and hydroxyl groups at positions 3 and 3' are necessary for binding to the apoprotein. On the other hand, the high yield of reconstitution obtained with actinioerythrol is evidence that the complex can be reconstituted by compounds with either hexagonal or pentagonal end ring structures.

However, 15,15'-Didehydroastaxanthin, which has these groups, cannot bind to the apoprotein. This fact suggests that minimal variations in the polyenic chain prevent the interaction between protein and carotenoid, showing the high specificity of the *Procambarus clarkii* blue caroteno-protein binding site.

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Thermal conductivity of wax comb and its effect on heat balance in colonial honey bees (*Apis mellifera* L.)

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Summary. Wax comb was found to have a thermal conductivity of 0.36×10^{-3} cal/cm sec °C. At low air temperatures, honey bees, *Apis mellifera* L., form clusters inbetween the combs in their nests. The combs provide insulation and the bee behavior actually increases the insulating effectiveness of the combs. When they form a compact living layer over the wax comb, the conductivity can be reduced to 0.065×10^{-3} cal/cm sec °C. Some aspects of the role of the wax comb in heat balance are examined in this paper.
Key words. Apis mellifera; thermoregulation; heat conductance; energy balance; wax comb; colony; cold tolerance.

Material and methods. Groups of bees (*Apis mellifera* L.) were tested overnight in a dark temperature cabinet at 2 ± 1.0 °C. An array of iron-constantan thermocouples was used to record temperatures within and outside the colony. Heat production was measured indirectly by oxidative metabolism, VO_2, and conversion to watts or calories[1]. The overall conductance of heat through the bees (for any specific mass) was calculated from VO_2 and the temperature differential maintained between the core and air outside the cluster surface, following the relation:

$$C = \frac{MR}{T_c - T_e} \quad (1)$$

where C = thermal conductance (cal/sec °C)
MR = metabolic rate (cal/sec)
T_c = maintained core temperature (°C)
T_e = environmental temperature (°C)

The thermal conductivity of isolated wax comb without bees, was determined using a closed cell styrofoam chamber with a 28.3 cm^2 hole in the top. Heat was produced by a tungsten resistance element with power (wattage, voltage and milliamperage) controlled by a transformer (Desega Desatronic 2000/300). Power was held constant at 996 ± 23 mW. Using thermocouples on each side of the test comb, the temperature differential maintained (at 2 °C air temperature) was determined and used to calculate conductivity with the equation:

$$C' = \frac{H \times d}{T_1 - T_2} \quad (2)$$

where C' = thermal conductivity (cal/sec cm °C)
H = power/unit area (cal/sec cm^2)
d = thickness (cm)
T_1 = temperature inside (°C)
T_2 = temperature outside (°C)