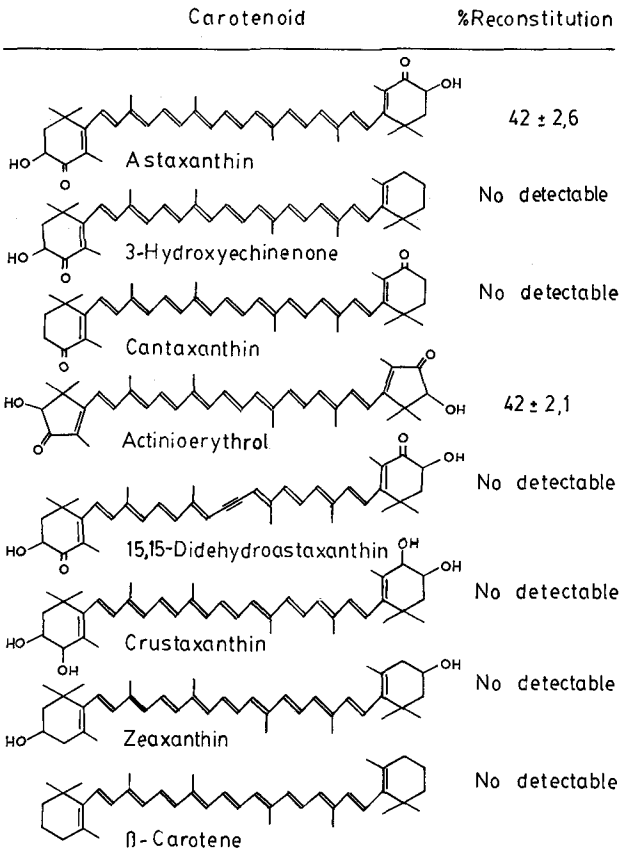

Formulae and percentage of reconstitution obtained for eight different carotenoid structures. Values are the mean ± SEM of five experiments.

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)

Results. At an air temperature of 2 °C, the bees produced heat at a rate dependent upon the number of bees in the group. For a group size of 4252 bees (608 g), the heat production was 3.78 W (0.90 cal/sec) for a constantly maintained cluster core temperature of 34 °C. The average total heat conductance through the bees and comb amounted to 0.028 cal/sec °C, a value which compares with the insulating values of birds and mammals[2]. The entire cluster volume was about 1.686 cm^3 for this group. The theoretical minimum volume of 617 cm^3 for the 4252 bees packed as tightly as possible in a sphere was calculated from volume measurements (acetone displacement of individual bees). The minimum spherical diameter was 10.5 cm, and minimum surface area 353 cm^2. The actual volume was about 2¾ times greater than the theoretical minimum. This is due to the intrusion of wax combs and the resulting nonspherical shape assumed by the bees. The bees occupied the spaces inbetween 3 wax combs, and packed themselves into oval shaped masses of varying thickness depending on the distance between the combs. They formed tight layers on the combs trapping air in each cell. The combs interrupt the cluster, however they also can provide insulation. The temperature on one side of an empty comb (i.e., without honey, pollen or brood) adjacent to the bees was maintained at 33.0 °C, while on the other side of the comb where there were no bees (2 cm separation), the temperature was only 8.3 °C (at an air temperature of 2 °C). This yields an average thermal conductivity of only 0.065×10^{-3} cal/sec cm °C for the comb when covered with the tightly packed layer of living bees[3]. Thermal conductivity figures from the direct measurements are higher ($0.36–0.44 \times 10^{-3}$ cal/sec cm °C). However these values represent only the comb alone without the bees actively forming a tight layer, trapping the air.

Of the total cluster surface area of 934 cm^2, 353 cm^2 were exposed to the cold air inbetween the frames, and an additional 581 cm^2 were adjacent to the insulating combs on both sides of the cluster. Although the total surface area was some 2½ times greater than the theoretical minimum, only 32% of the surface (identical to the theoretical minimum) is actually exposed to the cold air temperatures while the remaining 68% of the surface is adjacent to the insulating combs.

The effectiveness of the comb is clearly seen when bees without comb are examined. With no comb, the same mass and volume of bees would require 39% more heat to maintain the same temperature differential. Insulation by the wax combs is provided by the combined effect of combs and behavioral response to low temperatures by the bees.

Further experiments on thermal relations at cold temperature by bees and a more detailed discussion will be published elsewhere.

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Marine indoles of novel substitution pattern from the acorn worm *Glossobalanus* sp.[1]

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Summary. 4,6-Dibromoindole and 4,6-dibromo-2-methylindole have been isolated from the acorn worm *Glossobalanus* sp. The biosynthetic implications of this finding are discussed.

Key words. Marine brominated indoles; biogenesis; antibiotics.

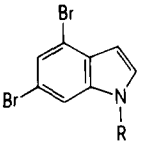
In previous work[4] on the acorn worm *Glossobalanus* sp. (class Enteropneusta, phylum Hemichordata) one of us detected by mass spectrometry the presence of a dibromoindole and a methyldibromoindole in the acetone extract. The amount of material available was limited, which precluded the isolation of these metabolites at that time. When a larger quantity of the organism had been obtained we were able to isolate and characterize the compounds as 4,6-dibromoindole (**1**) and 4,6-dibromo-2-methylindole (**2**), which belong to a novel substitution class of halogenated marine indoles.

Specimens of the animal (5 kg including ingested sand) accumulated in several collections at Miibaru Beach, Okinawa, in March and April, 1983 were thoroughly extracted with acetone in a blender. The extract was concentrated and successively extracted with hexane and ethyl acetate (EtOAc) to yield 10.5 and 3.3 g of oily residues, respectively. The latter oil was chromatographed on silica gel by eluting with hexane and increasing amounts of EtOAc. Of the many fractions exhibiting antibacterial activity[5], fractions eluted with 5:1 hexane-EtOAc were subjected to two more separations on a LiChroprep Si 60 column (3:2 hexane-$CHCl_3$) to give 21 mg of 4,6-dibromoindole (**1**) as a colorless oil and 35 mg of 4,6-dibromo-2-methylindole (**2**) as colorless crystals, m.p. 96–96.5 °C.

Compound **1**, $C_8H_5Br_2N$, showed EIMS at m/z 277, 275, 273 (M^+) and ^1H NMR ($CDCl_3$) δ6.56 (1H, t, 3-H), 7.21 (1H, t, 2-H), 7.42 (1H, d, 5-H), 7.47 (1H, d, 7-H), and 8.28 (1H, br s, N-H).

Compound **1** was unstable. Treatment of **1** with hot acetic anhydride and pyridine gave the acetyl derivative **3**[6]. ^1H NMR ($CDCl_3$) of **3** contained signals at δ2.59 (3H, s, $COCH_3$), 6.65 (1H, dd, J = 3.6, 0.7 Hz, 3-H), 7.42 (1H, d, J = 3.6 Hz, 2-H), 7.56 (1H, d, J = 1.5 Hz, 5-H), and 8.60 (1H, dd, J = 1.5, 0.7 Hz, 7-H). The low-field shifts of the 2-H and 7-H comparing to the chemical shifts of the parent compound **1**, the observed coupling constant between 2-H and 3-H, and the long range coupling[7] between 3-H and 7-H enabled us to locate unambiguously two bromine atoms at the 4- and 6-positions.

Compound **2**, $C_9H_7Br_2N$, exhibited EIMS at m/z 291, 289, 287 (M^+) and ^1H NMR ($CDCl_3$) δ2.40 (3H, s, 2-CH_3), 6.22 (1H, br s, 3-H), 7.31 (1H, br s, 7-H), 7.35 (1H, d, J = 1.5 Hz, 5-H), and 7.95 (1H, br s, N-H). Compound **2** was similarly acetylated to give a 1-acetyl derivative **4**[8] which showed ^1H NMR ($CDCl_3$) δ2.60 (3H, s, 2-CH_3), 2.66 (3H, s, $COCH_3$), 6.40 (1H, br s, 3-H), 7.52 (1H, d, J = 1.5 Hz, 5-H), and 8.23 (1H, dd, J = 1.5, 0.7 Hz, 7-H). These resonances clearly showed that the 2-, 4-, and 6-positions



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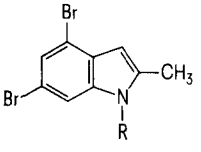
3, R = Ac



2, R = H
4, R = Ac