NATURE OF HOUSE FLY STEROLS

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Insects generally require dietary sterols for growth and development. They vary considerably in the nature of the sterols that will support their growth and that are consumed in their diet. A variety of sterols might therefore be expected to occur in insects and complex mixtures might be present in any individual species. Cholesterol is considered to be the typical sterol of insects (Bergmann, 1949). Honey bees, Apis mellifera L., contain 24-methylene cholesterol (Barbier et al., 1959a and b), as does the pollen food source for these insects (Barbier et al., 1960). Clark and Bloch (1959) have isolated C^{14}- labelled 22-dehydrocholesterol from Blattella germanica (L.) nymphs fed on uniformly labelled ergosterol. The chrysalis oil of the silk worm, Bombyx mori L., contains 'bombicysterol' (Menozzi and Moreschi, 1910), which appears to be a mixture of cholesterol and sitosterol (Bergmann, 1934). Preliminary studies by Louloudes (unpublished data) have shown that the major sterol from unfed adult house flies, Musca domestica L., reared by the CSMA procedure (Anonymous, 1959) has different physical properties and infra-red spectra than those of cholesterol. These flies also contain 'fast acting' sterols and a sterol which gives the typical ultra-violet spectra of a conjugated 5-7 diene and a positive color test with activated glycerol dichlorohydrin as does ergosterol but not 7-dehydrocholesterol (Kaplanis et al., 1960).

In the present study sterols were extracted from house flies reared by the CSMA procedure and then purified by digitonin precipitation and chromatography on a silicic acid-celite column using a gradient of Skelly-

solve C (b.p. 90-100°C) and benzene for elution. Three sterols were recovered from the adults and eggs, and four from the larvae and pupal exuviae. The major fraction in each case appeared to be the same sterol. Melting points (uncorrected, °C) of this major sterol and its derivatives (free sterol 145-146.5, acetate 135-137, acetate dibromide 125-126 and benzoate 141-142) differed from those of cholesterol (free sterol 148-149, acetate 115-116, acetate dibromide 116 and benzoate 146); the mixed melting points of the free sterols and acetates were 145 and 114-129 respectively. Elemental analyses on the free fly sterol, and its acetate, benzoate and bromide acetate derivatives were similar to those calculated for a $C_{27}H_{46}O$ sterol, but failed to differentiate it with certainty from either a $C_{26}H_{44}O$ or a $C_{28}H_{48}O$ sterol. One double bond was present based on perbenzoic acid titration and formation of a dibromide, the unsaturation occupying the 5-6 position as ascertained by the optical rotations (Bergmann, 1952), and response to Liebermann-Burchard reagent (Idler and Baumann, 1953). Infrared spectra of all these derivatives were almost identical with the corresponding derivative of cholesterol. X-ray powder diffraction patterns were greatly different for the acetates of the fly sterol and cholesterol but the differences were much less in the case of benzoates and free sterols. Chromatography of the fly sterol and cholesterol or their acetates on the above-mentioned column yielded a slight separation, the fly sterol being a little less polar than cholesterol. The optical rotatory dispersion curves (260-700 mμ) for the two sterol acetates showed no Cotton effect and were similar in shape; however, the rotation intensity was uniformly less for the fly sterol acetate than for cholesterol acetate. Nuclear magnetic resonance spectra of these two acetates indicated no difference in the immediate environment of the C_{18} and C_{19} angular methyl groups, but the signals assigned to the isopropyl hydrogens, C_{26} and C_{27}, of cholesterol were absent in the fly sterol.

It would appear that the major fly sterol varies from cholesterol only in the side chain, where the difference resides, at least in part, in that

the isopropyl structure is missing. Sterols identical to cholesterol in the carbon structure to C_{24} but with the side chain extended unbranched to C_{26}-C_{28} have been prepared (De Vries and Backer, 1950). The fly sterol differs in properties from these unbranched sterols. Branching in all the known natural sterols occurs at the C_{24} position and not at C_{22} or C_{23}. A C_{26} sterol with branching at C_{24} would result in a terminal isopropyl structure which must be presumed absent based on the nuclear magnetic resonance spectra. It would therefore appear that the side chain of the major fly sterol starting at C_{24} is either $-CH(C_2H_5)_2$ or the α or β form of $-CH(CH_3)C_2H_5$.

The origin of this unusual major fly sterol (designated as 'muscasterol') is not known. It appears to be present as the major sterol throughout the life cycle of this insect, but is a minor component or absent in the fermenting larval media. Incorporation of cholesterol -4-C^{14} into the fly eggs by prefeeding the adult flies with cholesterol -4-C^{14} acetate and then rearing these eggs in CSMA media containing cholesterol -4-C^{14} acetate yielded little, if any, conversion of cholesterol to the major fly sterol, or to other sterols recovered in lesser amounts. These minor fly sterols behaved on chromatography, color development time with Liebermann-Burchard reagent, and in ultra-violet spectra like methostenol (4-α-methyl-Δ^7-cholestenol), Δ^7-cholestenol and 7-dehydrocholesterol, with the methostenol being present only in the larvae and pupal exuviae. But as with the major sterol these sterols might have had side chain or other modifications from the cholesterol type compound. The precursor of the fly sterol would appear to be a phytosterol which was dealkylated in the fermenting media and concentrated by the fly or dealkylated directly in the fly. It is interesting in this respect that Bergmann and Levinson (1958) have noted that pupae of Musca vicina Macq. reared on wheat bran with a culture of Eschericia coli contain, in addition to a considerable quantity of beta-sitosterol, sterols that will support the growth of Dermestes vulpinus Fabr., an insect which cannot grow on phytosterols, but has been found to

develop only when cholesterol or 7-dehydrocholesterol is added to the diet (Fraenkel et al., 1941). The sterol requirement of house flies (Bergmann and Levinson, 1954, and Bergmann et al., 1959) is such that the structural difference between cholesterol and 'muscasterol' might not greatly impede the utilization of this sterol for growth and development. Studies with roach nymphs, Perplaneta americana (L.), indicate that their major sterol is not cholesterol, but that in contrast to the fly they can convert C^{14}-cholesterol to their major natural sterol.

A more detailed account of these investigations will be presented elsewhere.

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