IDENTITY OF THE "HOUSE FLY STEROL"

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Preliminary studies by Louloudes (unpublished data cited by Kaplanis et al., 1960) have shown that the major sterol from unfed adult house flies (Musca domestica L.), reared by the CSMA procedure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 infrared spectra than those of cholesterol. Shortly thereafter, Agarwal and Casida (1960) reported that three sterols were obtained from adults and eggs of house flies reared by the CSMA procedure. These workers designated the major sterol as "Muscasterol" and believed it to differ from cholesterol beyond C-22. Its side-chain starting at C-24 was thought to be either $-CH(C_2H_5)_2$ or α- or β-form of $-CH(CH_3)C_2H_5$. However, Agarwal et al. (1961) in a more detailed account of their studies stated that if an isopropyl group were present in the side-chain the substituent at C-24 would be restricted to a methyl group. The authors speculated that the precursor of the fly sterol could be a phytosterol which was dealkylated in the fermenting media and concentrated by the fly or dealkylated directly in the fly.

A preliminary examination of the unsaponifiable material from the CSMA media showed β-sitosterol to be the major sterol component. Kaplanis et al. (in press; unpublished data) have recently shown that house flies maintained

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on a semidefined diet containing H^3-β-sitosterol do not alter its side-chain. Nevertheless, house flies reared by CSMA procedure contained a major sterol that differed from cholesterol and β-sitosterol. Thus, it appeared desirable to isolate, identify, and determine the origin of the house fly sterol.

The house flies (Musca domestica L.) (2.1 kilograms) were homogenized and saponified according to previously reported methods (Louloudes et al., 1961). The unsaponifiable moiety was chromatographically separated into a hydrocarbon and an alcohol fraction. The alcohol fraction (2.0 g) yielded 1.2 grams of digitonin precipitable material which showed four distinct zones when analyzed by gas-liquid chromatography (VandenHeuvel et al., 1961). The components in order of increasing retention time were present in relative quantities of 2.8, 1.5, 74.3, and 21.4%, respectively. Neither the major sterol nor its derivatives could be separated from the fourth component (21.4% β-sitosterol) by various adsorption chromatographic systems. The sterol mixture recrystallized three times from methanol gave similar physical properties as reported by Agarwal and Casida (1960), yet the relative composition remained unchanged.

Gas-liquid chromatographic analyses of commercial samples of β-sitosterol in this laboratory demonstrated the presence of a major impurity varying in amounts from 5 to 31%. This contaminant exhibited an identical relative retention time as that of the major house fly sterol and also could not be separated from β-sitosterol by adsorption chromatography. However, this sterol could be concentrated from a commercial source of β-sitosterol to 91% purity (GLC analysis) by repeated fractional recrystallization from acetone (10 times) at room temperature. The infrared spectrum of this sterol (m.p. 159-160° (α_D^{20} -33°) was nearly identical to that of an authentic sample of β-sitosterol. Several ester derivatives of the sterol were prepared and their physical properties are given in Table I.

The major house fly sterol was purified under similar conditions and analyzed to be 94% pure. The impurity was shown to be β-sitosterol by GLC analysis. Several ester derivatives of the house fly sterol were prepared

Table I

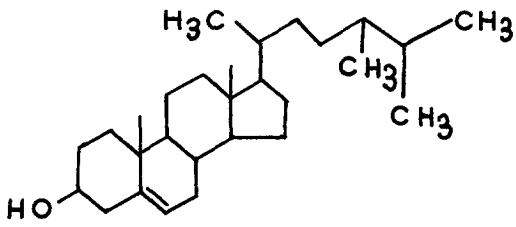
Physical properties of campesterol, house fly sterol, and their derivatives

	M.P. °C	$[\alpha]_D^{20}$ [a]		M.P. °C	$[\alpha]_D^{20}$	Reported M.P. °C	Reported $[\alpha]_D^{20}$	Reference
House fly sterol	159-160	-34°	Campesterol (obtained from commercial source of β-sitosterol)	159-160	-33°	157-158	-33.8°	5
				163-164 [b]	-32°	157-158	-33.8°	17
						157-158	-35.5°	19
Acetate	144-145	-40°	Acetate	141-142	-36°	138-139	-43°	5
						139-140	-37°	17
						137-138	-41°	19
Benzoate	162-163	-12°	Benzoate	164-165	-13°	156-157	-10.8°	5
						162-163	-10.4°	6
						158-160	-14.0°	17
3,5-Dinitrobenzoate	201-203	-10°	3,5-Dinitrobenzoate	209-210°	-6.4°	202-203	-6°	8
						202-203	-7°	8
						201-203	-8.6°	8

a/ Rotations were determined in approximately 1% solutions in chloroform.

b/ Obtained from hydrolysis of the 3,5-dinitrobenzoate derivative (m.p. 209-210°); GLC analyses indicated a purity of 99%.

and their physical properties are given in Table I. The infrared spectra of the house fly sterol and its derivatives were identical with that of the sterol of 91% purity (derived from a commercial sample of β-sitosterol) and its respective derivatives. Their physical properties were also in complete agreement. The physical properties and infrared spectra of both sterols and their derivatives characterized these sterols to be campesterol (α-methyl at C-24). This sterol has the opposite configuration at C-24 from ergosterol (Fernholz et al., 1941a and 1941b).



campesterol (Fieser and Fieser, 1959)

A comparison of physical properties of campesterol and its derivatives with those cited in the literature is shown in Table I. Table II summarizes the relative retention time of the house fly sterol and the campesterol obtained from a commercial sample of β-sitosterol and their acetates.

Campesterol has been isolated from a wide variety of plant sources including soyabeans (Fernholz et al., 1941; Matagrin, 1950; Humphlett and Wilson, 1959). The commercial sample of β-sitosterol from which we obtained campesterol of 91% purity had been isolated from soya oil.

The secured identity of the house fly sterol as campesterol suggested that it was derived from the CSMA media. Yet, gas-liquid chromatography of the unsaponifiable fraction of the CSMA media showed β-sitosterol to be the major sterol, with campesterol present in a lesser amount. The possibility that the smaller size particles being consumed by the house fly were richer in campesterol was readily eliminated. Experimental results showed that the greater percentage of campesterol was present in the larger size particles of the CSMA media. An experiment was designed to determine whether there

Table II
Comparison of gas chromatographic relative retention times of house fly sterol, campesterol, β-sitosterol and their respective acetates

Compound	Relative retention time[a]	
	QF-1[b]	SE-30[c]
House fly sterol	4.16	2.37
Campesterol (obtained from commercial source of β-sitosterol)	4.16	2.38
β-Sitosterol	5.15	2.96
House fly sterol acetate	7.16	3.40
Campesterol acetate	7.17	3.42
β-Sitosterol acetate	8.79	4.19

a/ Relative to cholestane.

b/ Column 6 ft. x 4 mm. ID, 1% QF-1 (10,000 CS) on 100-140 mesh, Gas-Chrom P, 25 psi, 204° C, cholestane time 6.95 min.

c/ Column 6 ft. x 4 mm. ID, 0.75% SE-30 on 100-140 mesh. Gas-Chrom P, 11 psi, 236° C, cholestane time 7.22 min.

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was a selective uptake or a selective retention of campesterol in preference to β-sitosterol by the house fly. The experiment was extended to include cholesterol. House fly pupae were reared on a semi-defined diet (Monroe, 1962) containing 0.2% each of the following sterols in combination: (A) campesterol - β-sitosterol, (B) campesterol - cholesterol, (C) cholesterol - β-sitosterol. The pupae were homogenized and saponified, and the sterol fraction from the unsaponifiable lipids analyzed by gas-liquid chromatography. These analyses showed the sterol content of pupae reared on sterol combinations A, B, and C was 77% campesterol - 21% β-sitosterol, 77% cholesterol - 19% campesterol, and 89% cholesterol - 9% β-sitosterol, respectively. These results establish that the house fly sterol (campesterol) originates from the CSMA media and that there is a selective uptake and/or retention of campesterol by the house fly.

Insects generally require dietary sterols for growth and development, and cholesterol has been found to serve as a growth-promoting sterol for nearly all insect species studied, regardless of their food habits (Albritton, 1953). The data presented herewith indicate that when the house fly is reared on a diet containing more than one Δ^5-3β-hydroxy sterol, as in the case of the CSMA media, this insect will show selective uptake or retention of that sterol in which the side-chain more nearly approximates that of cholesterol.

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