

UTILIZATION OF STEROID DERIVATIVES BY LARVAE OF *DERMESTES MACULATUS*

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ABSTRACT

The utilization of campesterol, β -sitosterol and stigmasterol derivatives by larvae of *Dermestes maculatus* has been studied. None of the compounds studied was utilized, with two exceptions:

- (a) It was found that the larva does not have an absolute requirement for cholesterol; it develops and pupates normally in the presence of campesterol, but not the other sterols tested.
- (b) Campesteryl fluoride has shown a "sparing effect" on larval growth and pupation.

INTRODUCTION

Since it has been found (e.g., Bergmann and Levinson, 1966, also for references) that cholesterol derivatives and analogs are not utilized by *Musca vicina* larvae which require free cholesterol or such simple derivatives as cholesteryl acetate, similar observations have been reported on other insects. A case in point is *Dermestes maculatus*. We have now studied the utilization, by this insect, of various C₂₈ and C₂₉ sterols which are found in soya beans and other plant sources, as well as that of their derivatives.

Insects require dietary sources of cholesterol or related sterols for larval growth and development. The sterols have probably several functions, such as being building stones for cell membranes or precursors of hormones that regulate moulting and metamorphosis (Clayton, 1964). Phytophagous insects are generally able to transform the common plant sterols into cholesterol, whereas carnivorous insects have lost this metabolic activity. Thus, *Musca domestica* is unable to convert β -sitosterol (C₂₉) to cholesterol, and thus must rely on a dietary source of cholesterol; however, it does utilize campesterol (Kaplanis et al., 1965). *D. maculatus* has even been used in a specific test for the presence of cholesterol in a mixture of sterols.

In the present communication the ability of *D. maculatus* to utilize campesterol (C₂₈) and its derivatives, as well as β -sitosterol and stigmasterol (C₂₉) derivatives is reported.

MATERIALS AND METHODS

The following compounds were prepared and tested. Their purity was assessed by their melting points and by gas chromatography. Most of the substances were known from the literature.

- Campesterol, m.p. 162-163° (Thompson et al., 1963).
 Campestanol, m.p. 149-150° (Fernholz and Ruigh, 1941).
 Campesteryl acetate (synth.), m.p. 138° (Ikan et al. V, 1970).
 24 β -Methylcholesteryl acetate (synth.), m.p. 140° (Fernholz and Ruigh, 1940).
 Campesteryl chloride, m.p. 105°
 Campestan-3-one, m.p. 157-158° (Bergmann, 1934).
 Campesteryl methyl ether, m.p. 102-103°.
 Campesteryl isopropyl ether, m.p. 110°.
 Campesteryl fluoride, m.p. 105° (Ikan and Klein, 1971).
 6 β -Fluoro-5 α -hydroxycampesteryl acetate, m.p. 227-230° (Ikan and Klein, 1971).
 β -Sitosteryl ethyl ether, m.p. 74°.
 β -Sitosteryl isopropyl ether, m.p. 89°.
 Stigmasteryl chloride, m.p. 95° (Windaus and Hauth, 1906).
 Stigmastanol, m.p. 139-140° (Ives and O'Neill, 1958).
 Epistigmastanol, m.p. 197-198° (Dalmer et al. 1935).
 Stigmastanone, m.p. 156-157° (Ives and O'Neill, 1958).
 H Δ ^{4,22} Stigmastadien-3-one, m.p. 125° (Jones et al. 1942).
 H Δ ^{5,22} Stigmastadien-3-one, m.p. 109°-110° (German Patent, 1953).
 Stigmasteryl methyl ether, m.p. 122°.
 Clionasteryl acetate, m.p. 139° (Ikan et al. 1971).

The composition of the semi-synthetic diet was according to Levinson et al. (1967).

The dietary components were thoroughly extracted with ether (4 portions) in order to remove traces of sterols. The residues of the ethereal solutions were subjected to alkaline hydrolysis, and the nonsaponifiable fraction (NSF) was tested for sterols by the Liebermann-Burchard reaction. The last two of the four extracts always proved to be sterol-free. Sterol additions made to the diet were 0.1%, unless otherwise indicated. Each of the compounds investigated was tested on 20 larvae of *Dermestes maculatus* in 2-3 replications.

RESULTS AND DISCUSSION

The results of the experiments with *Dermestes maculatus* are summarized in Tables 1 and 2.

A few interesting conclusions can be drawn from the data in Table 1. Campesterol is as completely utilized as cholesterol, whilst clionasterol, β -sitosterol and even the 24-stereoisomer of campesterol (24 β -methyl-cholesterol) are not. This last compound shows a slight activity in maintaining the larvae; one might venture to think that its optical purity was not absolute but that it contained traces of the epimer, campesterol.

It is interesting to note that the synthetic campesterol (Ikan *et al.*, 1970) is as active as the purified natural product; one can thus exclude the possibility that the biological activity of the latter is due to the presence of a highly active impurity. Thus *D. maculatus* resembles *Musca domestica* in that it does not have an absolute requirement for cholesterol, as has been generally assumed. In this connection, attention should be drawn to the fact that in the 24-ethyl series *both* epimers (β -sitosterol and clionasterol) are inactive; considering the similarity in structure of a 24-methylated and a 24-ethylated side-chain, this emphasizes even more the special position of campesterol.

In order to clarify this point further, we have fed a *second* generation of *D. maculatus* larvae solely on campesterol; these larvae were the progeny of a generation brought up on this same sterol. The larvae developed and pupated normally; examination of the adults obtained from them for sterols, using gas chromatography (3% XE 60 and Gas Chrom Q, at 240°) and mass spectroscopy, showed unequivocally that the adults contained only one sterol, campesterol. Thus campesterol is utilized as such and not, after de-alkylation, as cholesterol. We believe, taking into consideration the observations on the other sterols tested, that the absorption of campesterol is specifically easier than that of the other C₂₈- or C₂₉-sterols.*

Practically all the derivatives of campesterol, sitosterol and stigmasterol tested were unable to sustain the development of the *D. maculatus* larvae and their pupation.

Unexpectedly, campesteryl fluoride (and the same is true for the other fluorine derivative of this sterol, 6 β -fluoro-5 α -hydroxycampesteryl acetate) does not inhibit the action of campesterol, even if the former is present in 10 times the amount of the latter. Table 2 shows that campesteryl fluoride, but not the chloride, has a sparing effect on cholesterol if the latter is used in suboptimal concentrations (0.02% instead of 0.1%). It is interesting that in these suboptimal concentrations campesterol is not able to sustain larval growth and pupation. *Quantitatively*, it is thus less active than cholesterol.

* One of the Referees has expressed the view that the difference is not due to easier absorption, but to easier utilization — while the absorption of all sterols may be equally easy. Our experiments until the present do not permit a decision between these two hypotheses.

Table 1
Effect of various sterols and their derivatives on
Dermestes maculatus

Sterol derivative tested	Average weight of larvae (mg)			% Larvae pupating	Mortality of larvae
	10 days	20 days	25 days		
Sterol-free control	1.0	—	—	none	complete
Cholesteryl acetate	2.1	20.4	38.0*	100	—
Campesterol (natural)	1.6	15.3	38.0	100	—
Campestanol	2.0	3.0	5.0	none	complete
Campesteryl acetate (synth.)			33.0	95	1 in 20
24 β -Methylcholesteryl acetate (synth.)	2.0	4.3	6.7*	none	10 in 20
Campesteryl chloride	—	—	—	none	complete
Campesteryl fluoride	—	2.0	1.0	none	17 in 20
Campestan-3-one	1.0	1.0	—	none	complete
Campesteryl methyl ether	—	—	—	none	complete
Campesteryl isopropyl ether	—	—	—	none	complete
Campesteryl fluoride (1%) + campesterol (0.1%)	3.0	15.6	42.0*	90	1 in 10
6 β -Fluoro-5 α -hydroxy- campesteryl acetate (1%) + campesterol (0.1%)	2.0	13.2	37.3*	100	—
Clionasteryl acetate	—	—	3.0	none	complete
β -Sitosteryl acetate (natural)	—	—	2.6	none	complete
β -Sitosteryl acetate (synth.)	—	—	2.0	none	complete
β -Sitosteryl ethyl ether	—	—	—	none	complete
β -Sitosteryl isopropyl ether	—	—	—	none	complete
Stigmasteryl chloride	—	—	—	none	complete
Stigmastanone	—	—	—	none	complete
H $\Delta^{4,22}$ -Stigmastadien-3-one	—	—	—	none	complete
N $\Delta^{5,22}$ -Stigmastadien-3-one	—	—	—	none	complete
Stigmasteryl methyl ether	0.5	—	—	none	complete

* after 30 days.

Table 2

Effect of adding campesteryl fluoride to cholesterol
and campesterol on *Dermestes maculatus*

Sterol derivative tested	Average weight of larvae (mg)			% Larvae pupating	Mortality of larvae
	10 days	20 days	30 days		
Cholesterol (0.02%)	2	11	22	55	8 in 20
Campesterol (0.02%)	—	—	—	—	complete
Cholesterol (0.02%) + campesteryl chloride (0.1%)	2	4	7	25	14 in 20
Cholesterol (0.02%) + campesteryl fluoride (0.1%)	2	18	35	80	4 in 20

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