The Lipids of the Common House Cricket, *Acheta domesticus* L. III. Sterols

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ABSTRACT

Sterols constitute 1.95% of the total extractable lipids of *Acheta domesticus* L., of which 18% are esterified. The free sterols consist of cholestane-3β-ol (0.5%), Δ^5-cholestene-3β-ol (83.5%), Δ^7-cholestene-3β-ol (2.3%) $\Delta^{5,7}$-cholestadiene-3β-ol (3%), $\Delta^{5,22}$-cholestadiene-3β-ol (4%), $\Delta^{5,7,22}$-cholestatriene-3β-ol (0.2%), campestane-3β-ol (0.03%), Δ^5-campestene-3β-ol (1.0%), Δ^7-campestene-3β-ol (trace), $\Delta^{5,7}$-campestadiene-3β-ol (0.2%), stigmastane-3β-ol (0.09%), Δ^5-stigmastene-3β-ol (2.1%), Δ^7-stigmastene-3β-ol (0.04%), $\Delta^{5,7}$-stigmastadiene-3β-ol (0.4%), $\Delta^{5,22}$-stigmastadiene-3β-ol (0.1%). The same sterols are present in the esterified sterol fraction. Δ^7-Sterols and $\Delta^{5,7}$-sterols are present in significantly larger amounts in the esterified fraction than in the free sterol fraction. By a comparison with the sterols of the cricket food, it is clear that *A. domesticus* is capable of removing methyl and ethyl groups from C-24 of sterols of the campestane and stigmastane type. The ability to introduce a Δ^7 double bond into saturated and Δ^5-sterols is indicated, and it is suggested that Δ^7-sterols of the C_{27}, C_{28}, and C_{29} sterol series may be intermediates in the conversion of Δ^5-sterols to $\Delta^{5,7}$-sterols.

INTRODUCTION

UNLIKE VERTEBRATES, insects are unable to synthesize sterols from such precursors as acetate, mevalonate, squalene or lanosterol (1-7). Consequently, insects provide an excellent system for studying such fundamental problems as sterol function in biological membranes (8,9) and metabolic transformations which involve skeletal modifications of the preformed steroid system, uncomplicated by the multitude of possible reactions which do or can occur in plants and animals during sterol biogenesis.

Cholesterol is the major sterol of insects. Other sterols which have been encountered in smaller amounts or in only certain species include 7-dehydrocholesterol, cholestanol, 22-dehydrocholesterol, Δ^7-cholestenol and β-sitosterol (10,11). In addition, in a number of the more careful studies, a small fraction of polar sterols has been reported but never characterized (3, 12-14). Since insects are unable to synthesize sterols, they require a dietary source. Phytophagous insects are generally able to transform the common plant sterols, such as β-sitosterol and stigmasterol, into cholesterol by removal of the ethyl group from C-24, whereas carnivorous insects have lost this metabolic capability and require cholesterol or 7-dehydrocholesterol in their diets (10,15). The ability to utilize ergosterol is less widespread (5, 16-18).

In this study the simple sterols of the common house cricket, *Acheta domesticus* L., are characterized and analyzed quantitatively. A much less complete study of the sterols of *A. domesticus* has appeared (19). This study is preliminary to a more ambitious effort to characterize the minor sterols of the polar sterol fraction. It is in this fraction that important sterol metabolites, either derived from dietary sterols or from cholesterol, are likely to be found. *Acheta domesticus* has been chosen for this study as a consequence of its commercial availability in quantities necessary to permit a successful isolation of minor components.

MATERIALS AND METHODS

Lipid Extraction

Lipids were extracted from 8 to 8½ week old crickets by the procedure described in the first paper of this series (20).

Separation of Sterols

In a typical run, 25.25 g of lipid extract was chromatographed over 1275 g of Florisil (Fisher) containing 7% water. Hydrocarbons were eluted with 4 liters of petroleum ether (bp 40-60, redistilled). A simple ester fraction weighing 2.356 g, which contained the sterol esters, was eluted with 7 liters of ether-petroleum

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ether (5:95). There was some contamination by triglycerides. Triglycerides were eluted in 7 liters of ether-petroleum ether (15:85), and 1.086 g of sterols were eluted with 8 liters of ether-petroleum ether (25:75).

Purification of the Sterols

The simple ester fraction, 2.356 g, was saponified by refluxing for 3 hr under nitrogen in 25 ml of methanol containing 1.5 g of 85% potassium hydroxide and 1.5 ml of water. A neutral fraction weighing 225 mg was isolated by conventional techniques. This material consisted of a mixture of sterols, simple alcohols and fatty acid contaminants which persisted through the purification procedure applied to the saponification mixture. Fatty acids were removed by passing the mixture through an ion exchange column. The column was prepared by stirring 50 g of Amberlite IRA-400 ion exchange resin with 100 ml of 0.5*N* sodium hydroxide. This suspension was packed into the column, and washed with distilled water until neutral. The residue from the saponification, 225 mg, dissolved in ether saturated with water, was applied to the column. Slow elution with 1 liter of wet ether removed neutral material. The eluant was dried over sodium sulfate and evaporated, leaving 167 mg of residue. Final purification of the 3β-hydroxysterols was achieved by a digitonide precipitation using the method of Schoenheimer and Dam (21), which was found to work better than the more recent modification of Bergman (22). This procedure gave 88 g of purified sterols (100% of the theoretical amount which should be liberated from the 367 mg of digitonide obtained). Sterol esters, therefore, constitute 0.35% of the total lipids of *A. domesticus*.

Purification of the Free Sterol Fraction

The same three steps, saponification, ion exchange chromatography and digitonide precipitation were utilized to purify the free sterols. From 1.086 g of free sterol fraction, saponification gave 997 mg of a mixture which was reduced to 452 mg of neutral material by ion exchange chromatography. From these 452 mg of material, 1.687 g of digitonide was obtained, from which 402 mg of pure sterols was liberated. Free sterols, therefore, constitute 1.6% of the total lipids of *A. domesticus*.

GLC of Sterol Mixtures

The sterols were converted into their trimethylsilyl ethers for analysis by gas-liquid chromatography (GLC). For this transformation 0.1 ml of TRI-SIL (Pierce Chemical Company) Solvent-Reagent-Catalyst formulation was used per milligram of sterol mixture, according to the procedure suggested on the bottle. Reaction was complete within 5 min.

GLC Conditions

GLC analyses were conducted on a dual column F&M Model 810 chromatograph equipped with dual flame ionization detectors. Two columns were found to be satisfactory for these analyses. An 8 ft × ⅛ in O.D. SE-30 Hi-Pak column (F and M Scientific Corporation), conditioned for 3 hr at 300C and deactivated by repeated injections of tetramethyldisilazane separated the sterols very well, and gave symmetrical peaks. The operating conditions were as follows: column temperature, 250C; injection port temperature, 330C; detector temperature, 310C; helium flow rate, 35 ml/min; sample size, 0.8-1.0 μl. A 6 ft × ⅛ in O.D., 3% QF-1 on 100-120 mesh Gas-Chrom Q (Applied Science Laboratories) column, conditioned for 3 hr at 260C also served well. Operating conditions were as follows: column temperature, 200C; injection port temperature, 265C; detector temperature, 310C; helium flow rate, 25-30 ml/min; sample size, 0.3-1.0 μl.

Sterols were identified by comparing retention times of the components with authentic samples of cholesterol, cholestanol, Δ^7-cholestenol, β-sitosterol, β-sitostanol, stigmasterol, campestanol and campesterol (generously provided by Dr. Richard Rapala of Eli Lilly Company) on both columns. Mixtures were analyzed quantitatively by determining peak area, using triangulation, of duplicate chromatograms on the SE-30 Hi-Pak column.

Determination of Sterol Distribution

A complete analysis of the sterol mixtures by GLC is complicated by the fact that the separation of saturated, Δ^5- and $\Delta^{5,7}$-sterols for each series is inadequate to permit acceptable quantitation. On the SE-30 column the saturated and Δ^5-enol of a given series have virtually identical retention times, and on the QF-1 column the stanol is a shoulder on the trailing side of the stenol peak. The $\Delta^{5,7}$-dienols have only slightly greater retention times on the SE-30 column.

In order to obtain a complete quantitative analysis of a sterol mixture, the following series of operations was conducted. First the entire sterol mixture was analyzed by GLC. This gave an accurate value for the Δ^7-enol and a composite value for the saturated, Δ^5- and $\Delta^{5,7}$-sterols. Then a portion of the sterol mixture was oxidized with performic acid, a proc-

TABLE I
Free and Esterified Sterols of *A. domesticus* and Its Food

Sterol	Percentage Composition			
	Cricket Sterols		Cricket Food Sterols	
	Free	Esterified	Free	Esterified
1. $\Delta^{5,22}$-Cholestadiene-3β-ol	4.0	5.5	0.0	0.0
2. Cholestane-3β-ol	0.5	1.5	0.4	0.5
3. Δ^{5}-Cholestene-3β-ol	83.5	69	6.0	4.0
4. $\Delta^{5,7}$-Cholestadiene-3β-ol	3.0	6.5	<0.7[a]	<0.6[a]
5. Δ^{7}-Cholestene-3β-ol	2.3	11	0.0	0.0
6. $\Delta^{5,7,22}$-Cholestatriene-3β-ol	0.2	0.4	<0.7[a]	<0.6[a]
7. Campestane-3β-ol	0.03	0.08	1.0	1.0
8. Δ^{5}-Campestene-3β-ol	1.0	0.9	19	17
9. $\Delta^{5,7}$-Campestadiene-3β-ol	0.2	0.4	<0.7[a]	<0.6[a]
10. Δ^{7}-Campestene-3β-ol	trace	~0.1	0.0	0.0
11. $\Delta^{5,22}$-Stigmastadiene-3β-ol	0.1	~0.05	6.0	4.0
12. Stigmastane-3β-ol	0.09	0.4	2.5	3.5
13. Δ^{5}-Stigmastene-3β-ol	2.1	1.5	64	64
14. $\Delta^{5,7}$-Stigmastadiene-3β-ol	0.4	0.8	<0.7[a]	<0.6[a]
15. Δ^{7}-Stigmastene-3β-ol	0.04	1.0	0.0	0.0
16. Unknown A (sat'd) R_S 3.9 on SE-30	0.03	0.02	0.0	0.2
17. Unknown B (unsat'd) R_S 3.9	0.04	1.0	0.7	3.8
18. Unknown C	0.0	~1.5	0.0	<0.4[a]
19. Other Unknowns	0.0	0.0	0.0	1.5[b]

[a] By ultraviolet analysis.

[b] Three components: R_S 4.1, 4.4, 4.9 on SE-30.

ess which completely removed all of the unsaturated sterols (23). The saturated sterol mixture which remained was then analyzed by GLC. The total $\Delta^{5,7}$-sterol content was then established by spectral examination of the entire sterol mixture at 272 mμ, 282 mμ, and 294 mμ (4% of the free sterols, 8% of the sterol esters). The $\Delta^{5,7}$-dienols were then separated from the rest of the sterols by conversion to their peroxides (24). The peroxides were purified by chromatography on Florisil (7% water), eluting with ether-petroleum ether (40:60). The peroxide mixture was then reduced with sodium and ethanol (25) to a 9:1 mixture of the Δ^{7}-enol and Δ^{5}-enol. The product mixture of the reduction was analyzed by GLC. Thus, the amount of Δ^{5}-enol present could be obtained by subtracting the values for $\Delta^{5,7}$-dienol and saturated sterol from the composite value obtained from the GLC analysis of the entire mixture.

Identification of 22-Dehydrocholesterol

One component of the sterol mixture had a smaller retention time (1.98 relative to cholestane) than cholesterol (2.23 relative to cholestane). 22-Dehydrocholesterol is the only sterol which has been reported to have a shorter retention time than cholesterol under GLC conditions comparable to those used in this work (26,27). The presence of 22-dehydrocholesterol in the sterol mixture was confirmed by subjecting the mixture to oxidative cleavage (28), and detecting isovaleric acid in the product.

Analysis of Dietary Sterols

Isolation, separation, purification and analysis of the dietary sterols were conducted by the same series of operations that were applied to the cricket sterols.

RESULTS AND DISCUSSION

The results of the characterization of the sterols of *A. domesticus* and its food are presented in Table I. Cholesterol (entry 3) is the major sterol of *A. domesticus*. The cricket, a phytophagous insect, has a dietary sterol supply which consists of over 90% sterols with methyl or ethyl groups attached to C-24 of the sterol side chain (entries 7-15), whereas over 90% of the sterols of the adult cricket are unsubstituted at C-24 (entries 1-6). Only small amounts of the major dietary plant sterols (entries 8, 11, 12, 13 and 17) persist in the cricket sterols. The ability on the part of the cricket to dealkylate C-24 is clearly indicated.

Several other metabolic capabilities are indicated by the results summarized in Table I. The Δ^{7}-enols (entries 5, 10 and 15) are completely absent in the food, but are present in the significant amounts, especially Δ^{7}-cholestene-3β-ol, in the cricket. Irreversible conversion of cholestane-3β-ol to Δ^{7}-cholostene-3β-ol has been demonstrated in *Eurycotis floridana*

(29, 30) and *Blatella germanica* (29-31), and the dealkylation of saturated sterols of the stigmastane-3β-ol type has been demonstrated in *Blatella* (32). Thus the saturated sterols (entries 2, 7 and 12) are likely precursors to the Δ^7-cholestene-3β-ol, by way of dealkylation and oxidation.

$\Delta^{5,7}$-Dienes, particularly $\Delta^{5,7}$-cholestadiene-3β-ol (entry 4), are also present in larger amounts in the cricket than in its food. Desaturation of Δ^5-cholestene-3β-ol to $\Delta^{5,7}$-cholestadiene-3β-ol by *Musca domesticus* has been demonstrated (12), and such a process may represent the origin of the $\Delta^{5,7}$-dienes in the cricket. Desaturation of a Δ^7-enol to a $\Delta^{5,7}$-dienol has never been suggested as a possible route to the $\Delta^{5,7}$-dienols, but is a definite possibility.

Δ^7-Cholestene-3β-ol and $\Delta^{5,7}$-cholestadiene-3β-ol are seen to be concentrated in the esterified sterol fraction. $\Delta^{5,7}$-Cholestadiene-3β-ol is believed to be a precursor of physiologically active growth and fertility hormones (26, 33). This may suggest that subsequent transformations of the $\Delta^{5,7}$-dienol occur in the esterified compound. Similarly, the concentration of the Δ^7-enol in the esterified fraction and Δ^5-enols in the free sterol fraction may suggest that Δ^7-enols are closer to $\Delta^{5,7}$-dienols than the Δ^5-enols in a biosynthetic pathway, as would be the case if the sequence Δ^5-enol $\rightarrow$ saturated sterol $\rightarrow$ Δ^7-enol $\rightarrow$ $\Delta^{5,7}$-dienol were operative. These are suggestions which will require testing by suitable labeling experiments.

Finally, $\Delta^{5,22}$-cholestadiene-3β-ol (entry 1) is present in the crickets and not in the food. It probably arises through the dealkylation of $\Delta^{5,22}$-stigmastadiene-3β-ol (entry 11). A similar conversion of ergosterol to $\Delta^{5,22}$-cholestadiene-3β-ol has been demonstrated in *B. germanica* (2, 34). Subsequent reduction of the Δ^{22}-unsaturation by *germanica* was not observed.

$\Delta^{5,24}$-Cholestadiene-3β-ol, or desmosterol, recently shown to be an intermediate in the dealkylation process by which sitosterol is converted to cholesterol (27) was not detected in the cricket sterols. Its presence in small amounts would have gone undetected, however.

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