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Sterol metabolism of insects

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Samenvatting

Er wordt een overzicht gegeven van de huidige kennis omtrent het sterolmetabolisme van insekten. De nadruk wordt er op gelegd, dat een hoge graad van zuiverheid van de sterolen in het voedsel en het uitschakelen van de invloed van symbionten van essentieel belang zijn voor een ondubbelzinnige interpretatie van de resultaten. Ter sprake komt, dat de scherpe scheidslijnen tussen zoösterolen, mycosterolen en fytosterolen niet meer gelden. Opmerkelijk is de aanwezigheid van insektenhormonen en -derivaten in planten. Kennis van het sterolmetabolisme kan leiden tot de ontwikkeling van selectieve bestrijdingsmiddelen van insekten. Tenslotte worden bij de omzetting van C_{28}- en C_{29}-sterolen in C_{27}-sterolen door insekten nog niet eerder gepubliceerde resultaten vermeld.

Summary

This article surveys the present knowledge of the sterol metabolism of insects. It is emphasized that a high degree of purity of the dietary sterols and the elimination of the influence of symbionts are essential to prevent ambiguity in interpreting results. It is pointed out that a sharp distinction between zoosterols, mycosterols and phytosterols can no longer be made. A remarkable fact is the occurrence of insect hormones and their derivatives in plants. Knowledge of the sterol metabolism may help in developing selective insecticides. Finally, this paper gives results on the conversion of C_{28}- and C_{29}-sterols into C_{27}-sterols by insects; these results have not been published previously.

Introduction

Unlike most plants and animals, insects do not possess the complete enzymic system necessary for the synthesis of sterols from more simple building-stones, like acetate and mevalonate [1]. The few exceptions stated in the literature, such as the silkworm [2] and a primitive insect, the so-called silverfish [3], may probably be attributed to the activity of symbionts [4].

Until quite recently, this inability to synthesize sterols was considered characteristic for insects. Actually, forms of life so different as vertebrates, plants and yeasts can indeed synthesize sterols from more simple building-stones [5]. The dogfish is an exception in that it can synthesize squalene from acetate, but not cholesterol [6]. It has also been found, however, that some arthropodes [7], as well as a snail [8], an annelid [9], some micro-organisms [10], an oyster and a sea-urchin [11] cannot synthesize sterols.

Though insects themselves are unable to synthesize sterols, these substances are vitally important for them, because they are structural units of membranes as well as the basic materials for some hormones that control essential life processes. Therefore, sterols are indispensable components in the insects' food.

For some functions they may be utilized as such by the insect, but to satisfy other requirements they must first be converted into other sterols or steroids. These conversions have attracted particular attention in the last few years, since knowledge of their mechanisms may lead to the development of specific methods in insect pest control. Methods, in fact, that are associated with physiological processes, characteristic for insects; other forms of life need then not be affected.

This review focuses on results obtained with cockroaches, because they have been the most thoroughly investigated with regard to the sterol metabolism of insects and, also, because most of our own experience was gained with cockroaches. If useful, reference will frequently be made to points of agreement, or difference, with results obtained in investigations, where other species of insects were used. Among these latter investigations, those of the housefly, *Musca domestica* L., and the hide beetle, *Dermestes vulpinus* Fabr. (= *Dermestes maculatus* Deg.), occupy an important place.

In the Centraal Laboratorium TNO, three species of cockroaches are reared for investigations concerning sterols and insects, viz. *Blattella germanica* L., *Periplaneta americana* L. and *Eurycotis floridana* Walker (Figure 1).

These investigations are carried out within the scope of the "Study group on integrated pest control, TNO".

The influence of the chemical nature of sterols on their capacity to cover the sterol requirement of insects

Until some years ago, research on the nature and the role of sterols in insects had been restricted to

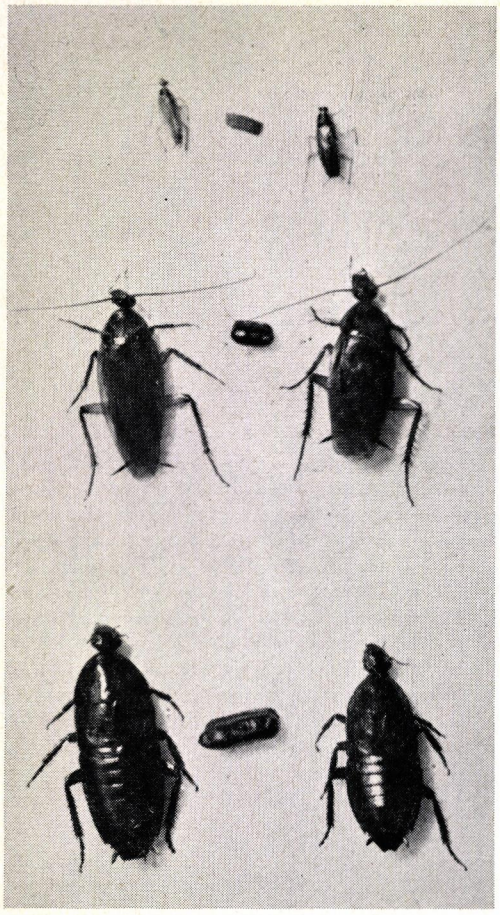

Fig. 1. Three species of cockroaches (of each: one male specimen, ootheca and female specimen), which are reared in the Centraal Laboratorium TNO for investigations concerning sterols and insects. From the top downward Blattella germanica *L.*, Periplaneta americana *L. and* Eurycotis floridana *Walker.*

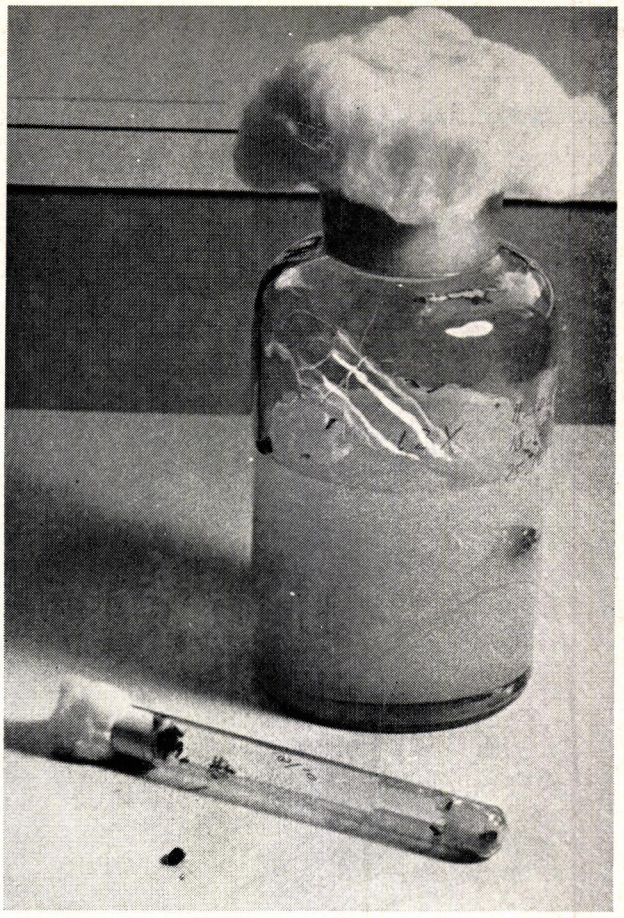

Fig. 2. Culture of cockroaches under aseptic conditions according to the method of Clayton [13]. Immediately after sterilization by immersion in alcohol, mercuric chloride, alcohol and water respectively, the ootheca is placed in a hatching and sterility testing tube containing agarthioglycollate medium. Failure of fungal or bacterial growth to appear on the test medium during 4 days after hatching of the ootheca is taken as an index of asepsis of the colony. The cockroaches are then placed in a sterile glass bottle containing an agar medium, as a source of water, and a tube with the diet.

analysis of the sterol contents of the insects and to nutritional studies, in which the function of the sterols, as a growth factor, was examined. In this work "utilizable" and "non-utilizable" sterols are distinguished. Some more recent investigations with synthetic sterol derivatives are also restricted to this aspect [12].

The results of investigations concerning sterols and insects should be regarded critically on account of the important role which symbionts may play, and on account of the possibility of selective absorption of sterols by insects.

Especially Clayton [4] has paid much attention to the influence of symbionts, which can sometimes synthesize considerable quantities of sterols; a point which is often not taken into consideration. In our laboratory we work with cockroaches which, from their eggs, are reared under aseptic conditions according to the Clayton method [13] (Figures 2 and 3).

That small amounts of sterols can be selectively absorbed in certain tissues, in quantities that may occur as impurities in other sterols, is shown by the following two examples.

Clayton et al [14] reared *E. floridana* on a diet, the sterols of which consisted of 5% cholesterol (I) labelled with ^{14}C and 95% tritiated cholestanol (II). Some tissues showed a sterol composition corresponding to that of the food. In the ventral nerve chord, however, about 50% cholesterol and 50% cholestanol were found. Since the cholesterol was labelled exclusively with ^{14}C, and not with ^{3}H, this cholesterol cannot have originated from a conversion of cholestanol. Therefore, we may conclude that cholesterol has been selectively absorbed in the ventral nerve chord.

If the sterols had not been labelled differently, and the presence of cholesterol besides the principal sterol in the diet had not been known, the large quantity of cholesterol in the ventral nerve chord

might easily have led to the wrong conclusion that cholestanol had been converted by the cockroach into cholesterol.

An even more striking example of selective absorption was found with the housefly, *Musca domestica,* by Thompson et al [15]. This insect is often reared on a standard medium (CSMA) and is reported to contain a special sterol, "muscasterol", according to previous investigations [16]. This sterol, however, now appeared to be a mixture, containing 74% campesterol (III) and 21% β-sitosterol (IV). The sterol content of the standard medium contained only 18% campesterol besides 79% β-sitosterol.

When cholesterol was also investigated, the more general rule could be established that the housefly, reared on a medium with more than one Δ^5-3-hydroxysterol, selectively concentrates in its tissues that sterol of which the side-chain most resembles that of cholesterol (see Table).

TABLE

Sterol contents of pupae of houseflies reared aseptically on media with varying sterol combinations (calculated from data of Thompson et al [15b])

relative composition of the sterols in the diet (%)			relative composition of the sterols in the housefly (%)		
cholesterol	campesterol	β-sitosterol	cholesterol	campesterol	β-sitosterol
50	50	—	77	19	—
50	—	50	89	—	9
—	50	50	—	77	21
—	30	70	—	76	21

As, according to Kaplanis et al [17], *M. domestica* is unable to convert β-sitosterol into cholesterol or campesterol, the explanation is to be found, actually, in a selective absorption of cholesterol and campesterol with regard to β-sitosterol, and not in a dealkylation in the 24-position. Strictly speaking, it also remains to be proved that in *M. domestica* no conversion takes place of campesterol into cholesterol.

The problem of selective absorption arises especially when the composition of the sterols in the diet is uncertain. In investigating the sterol metabolism of insects, the dietary sterols should therefore be as pure as possible, and preferably one should work with radioactively labelled sterols.

The fact mentioned above that *M. domestica* is unable to convert β-sitosterol into cholesterol, is one of the exceptions in the insect kingdom. For many other insects, including the oriental housefly [18], such a dealkylation has been made plausible. The conversion of β-sitosterol into cholesterol has been proved for the case of the cockroach, by Robbins et al [19], and this example could be extended with the conversion of many other sterols with 28 or 29 C-atoms into C_{27}-sterols, in an investigation of Ritter, Clayton and Bloch [20]. In a subsequent chapter of this paper, the conversion of phytosterols into zoosterols will be treated in detail.

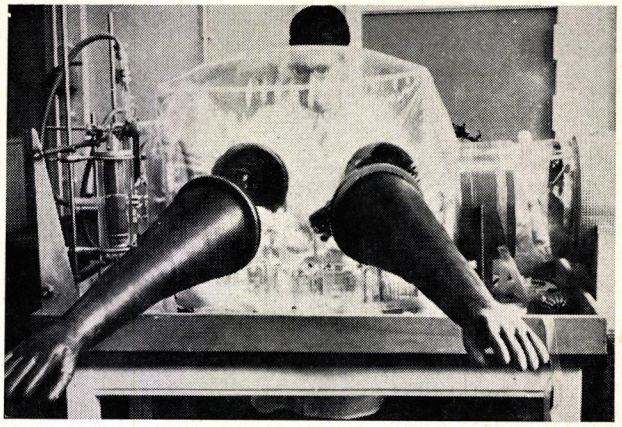

Fig. 3. Sometimes the cultures are kept in "germ-free" plastic bags in which sterile air is blown. This gives additional protection against microbial contamination and facilitates experimental work under sterile conditions. The equipment was kindly constructed by the Radiobiological Institute TNO, Rijswijk-Z.H.

Apart from one exception, a Mexican cactus fly [21], all the insects investigated so far, can do with the C_{27}-sterol cholesterol as the sole sterol in their food. This applies to both plant-eating (herbivorous and omnivorous) insects and non-plant-eating, strictly carnivorous insects.

With the plant-eaters, including the omnivorous cockroaches, the cholesterol can be fully replaced by a plant sterol, such as the C_{29}-sterol β-sitosterol. With the carnivorous insects, such as the hide beetle and the carpet beetle, on the contrary, the cholesterol in the diet can be largely replaced by sterols of the ergostane - or stigmastane series (resp. with 28 or 29 C-atoms); a slight quantity of cholesterol, however, is indispensable [4]. According to Budowski et al [22] phytosterols even have an inhibitory effect on the growth of the hide beetle.

Clark and Bloch [23] discovered that a large part of the total sterol requirement of the hide beetle can be covered by any one of a large number of sterols which, by themselves, are inadequate to support a normal growth. For example, β-sitosterol could cover 97% of the normal sterol requirement, but the remaining 3% had to be cholesterol.

A similar cholesterol-sparing effect was also found with *B. germanica* and *E. floridana.* These insects normally grow on a diet containing 0.1% cholesterol, but do not grow if, instead, the diet contains 0.1% of the saturated compound cholestanol. If besides cholestanol, however, at least 0.005% cholesterol (i.e. about 5% of the total sterol requirement) is added, growth and development are normal [14].

Originally this was interpreted as follows. The minimum quantity of cholesterol that could not be replaced, would have a strictly metabolic function, e.g. for the synthesis of hormones. The less specific sterol requirement, on the other hand, could be met by a so-called sparing sterol, which could perform the structural and metabolically inert func-

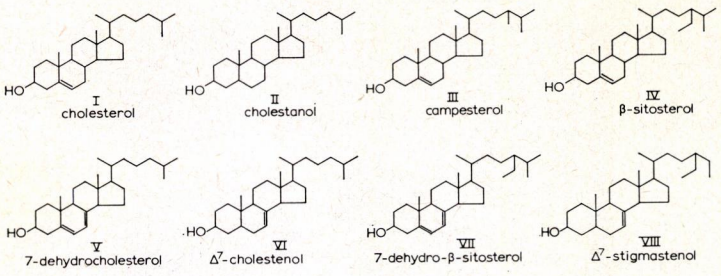

Fig. 4. Formulae of some sterols.

tion of cholesterol [23]. This difference can no longer be considered to be very distinct.

From investigations by Clayton et al [14, 24], it appeared that the minimum quantity of cholesterol mentioned above was selectively absorbed in most tissues of *E. floridana* as a component of subcellular membranes, and that this cholesterol was strongly bound in these membranes, showed a low rate of turnover and could not be replaced entirely by a sparing sterol.

With the adult *P. americana,* Vroman et al [25], found also only a slow movement of labelled cholesterol into and out of the roach tissues.

That this low rate of turnover is not characteristic for all insects, appears from the fact that the adult boll weevil, shows a high rate of turnover [26]. Consequently, this insect needs a constant supply of dietary cholesterol to stay alive.

Clark, Clayton and Bloch tried to correlate certain structural characteristics of sterols with their cholesterol-sparing ability [23, 27]. A complete interpretation of these data is still difficult, but it does appear from them that both the position of the hydroxyl group and the position of the double bonds, as well as the nature of the side-chain, are of basic importance.

Other investigators also studied the influence of the structure of sterols on their biological activity in insects.

For cholesterol derivatives with modified side-chains, and other synthetic steroid derivatives, the activities as cholesterol-replacing dietary components were investigated by Bergmann et al [12], using the oriental housefly and the hide beetle. None of the cholesterol derivatives with modified side-chains was able to replace cholesterol completely. Only 27-norcholesterol was satisfactory as a growth-factor for the larvae of the housefly, but normal pupation could not be obtained with it.

In accordance with Fraenkel et al [28], and Clark and Bloch [23], they found that only 7-dehydrocholesterol (V) was as effective as cholesterol for the hide beetle. According to Clayton [4], however, this would be no more the case after a thorough purification of 7-dehydrocholesterol.

Several investigators tried to synthesize cholesterol derivatives that could act as growth-inhibiting antimetabolites in insect control. A critical consideration by Clayton [4] of all these investigations, leads to the conclusion, however, that it is very doubtful whether one of these cholesterol derivatives really has any inhibiting effect on the growth of insects. It is true that a competitive inhibition is sometimes found, but this effect is usually completely reversed by cholesterol in normal concentrations.

Earle et al [29], however, recently reported a partial inhibition of growth in the boll weevil by two different azasterols even in the presence of cholesterol.

Svoboda et al [30] found in the tobacco hornworm also an inhibition of growth by another diazacholesterol. (This investigation will be mentioned again in connection with the dealkylation of phytosterols.)

Introduction of a Δ^7-bond in the nucleus of sterols by insects

So far the introduction of a double bond in sterols by insects has only been found at the 7-position, but this is very interesting from a biochemical point of view.

Both *E. floridana* [31] and *B. germanic*a [31, 32] are able to convert cholestanol into Δ^7-cholestenol (VI). This dehydrogenation is so special, because there is no activation of hydrogen atoms by an adjacent unsaturated group. Moreover, Clayton and Edwards [31b] could prove by means of tracer experiments that the 7 β- and 8 β-hydrogen atoms were removed without intermediate formation of a 7-hydroxyl group. In their opinion, the enzymes bringing about this conversion are probably located in the gastric caeca.

Also cholesterol can be dehydrogenated at the 7-position [33] by cockroaches *(B. germanica* and *P. americana).*

With aseptically reared *E. floridana,* Clayton and Edwards [31] did not find this conversion. As Robbins et al [33a] and Ishii et al [33b] had performed their experiments with non-aseptically reared *B. germanica* and *P. americana,* in Clayton's opinion [4] the small quantities of 7-dehydrocholesterol (V) found, could possibly be attributed to these non-aseptic conditions.

Robbins et al [34] managed to remove this doubt, however, by investigations with aseptically reared *B. germanica.* In the sterol fraction of extracts of complete cockroaches that had received radioactively labelled cholesterol in their diet, 3% 7-dehydrocholesterol was found, besides 96% cholesterol. Gas-chromatographic analysis of organs of *B. germanica,* which we reared under aseptic conditions on media with radioactively labelled cholesterol, also indicates that this cockroach might, to a slight degree, convert cholesterol into 7-dehydrocholesterol.

The finding reported by Robbins et al [34] that,

during the last moulting of the nymphs of *P. americana*, 25% of the sterols of the prothoracic gland consists of 7-dehydrocholesterol is very interesting in regard of the fact that this gland is considered to be the source of the moulting hormone ecdysone, which is also a Δ^7-sterol.

The conversion of cholesterol into 7-dehydrocholesterol has also been observed in the housefly, *M. domestica* [35]. Moreover, this insect is reported to be able to carry out the analogous conversion of β-sitosterol into 7-dehydro-β-sitosterol (VII) [17a].

In the confused flour beetle [36] and the Virginia pine sawfly [1m], indications were found for the presence of 7-dehydrocholesterol. With the latter insect, this sterol is supposed to originate from the phytosterols in the pine foliage.

It is important to point out the fact here that the sterol requirement of the Mexican cactusfly, *Drosophila pachea* Patterson and Wheeler, cannot be covered by cholesterol or β-sitosterol, but only by a cactus sterol, Δ^7-stigmastenol (VIII) or another Δ^7- or $\Delta^{5,7}$-sterol [21]. Apparently this insect cannot introduce a double bond at the 7-position, and this Δ^7-bond is vitally important, in view of the structures of the moulting hormones.

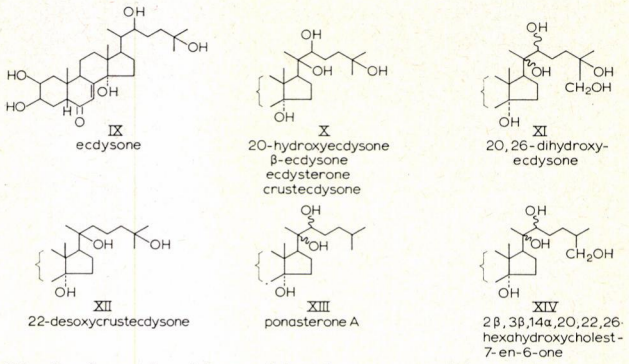

Fig. 5. Steroids with moulting hormone activity.

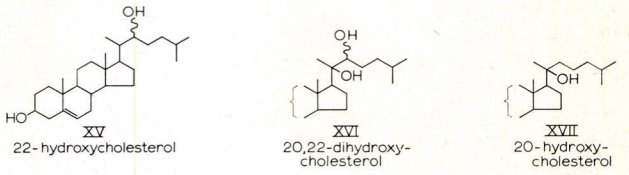

Fig. 6. Precursors of steroid hormones in vertebrates.

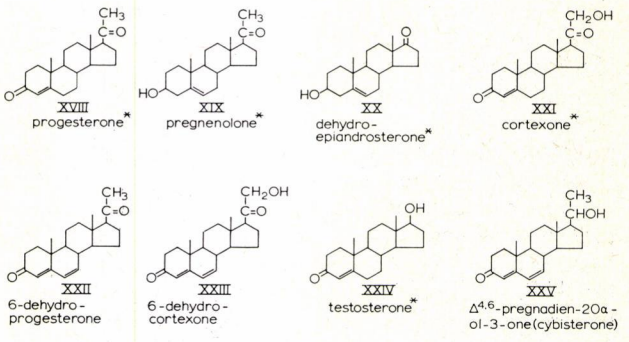

Fig. 7. C_{19}- and C_{21}-steroids, which are demonstrated in insects. The compounds marked with an asterisk, are common in vertebrates.

The presence of ecdysone and other steroid hormones in insects

In 1963 Karlson et al [37] found that the moulting hormone ecdysone (IX) is a sterol, synthesized from cholesterol by the blowfly, *Calliphora erythrocephala*.

After the complete elucidation of the structure of this hormone in 1965 by means of X-ray analysis [38] ecdysone was almost simultaneously synthesized by German-Swiss and American research teams [39].

These teams of investigators synthesized also steroids related to ecdysone, which possess more or less the same properties as ecdysone [39g, 40]. The synthesis of ecdysone is now also under study by other investigators [41]. In addition to ecdysone, 20-hydroxyecdysone (X) has meanwhile been isolated from various insects and other arthropodes. It also possesses a moulting hormone activity and has been given various names: β-ecdysone [42], ecdysterone [43], crustecdysone [44] and 20-hydroxyecdysone [40, 45]. In the tobacco hornworm, besides 20-hydroxyecdysone [46a] also 20,26-dihydroxyecdysone (XI) [46b] has been found. The latter compound has a somewhat lower activity.

Moreover, a small amount of 22-desoxycrustecdysone (XII) was isolated from crustaceans. This compound also has a moulting hormone activity [47]. The structures of these moulting hormones have led to the speculation that they actually could be precursors of another hormone with a shorter side-chain [44b]. The well-known conversion of cholesterol via pregnenolone (XIX) into progesterone (XVIII) and other hormones of vertebrates easily leads to this supposition, because 22-hydroxycholesterol (XV), 20,22-dihydroxycholesterol (XVI) and 20-hydroxycholesterol (XVII) were found as intermediary products [48]. However, the 20-keto-compound, which would be formed if a similar side-chain degradation would occur with ecdysone, has recently been synthesized and does not show any appreciable moulting hormone activity [82].

Hora et al [49] examined the ecdysone-antagonistic action of a number of derivatives of cholestane and 24β_F-methylcholestane containing 3β-hydroxy- and 6-ketogroups. These derivatives prevent the hardening of the cuticle of *Pyrrhocoris*.

As far as we know, the Δ^7-6-ketosteroids mentioned above, are typical hormones of arthropodes. Since 1964 some publications have appeared, however, which show that in insects also steroid hormones with a Δ^4-3-ketogroup occur, which are common in vertebrates.

In the confused flour beetle, *Tribolium confusum*, progesterone (XVIII), pregnenolone (XIX) and dehydroepiandrosterone (XX) were reported to be found [50]. Schildknecht et al reported that cortexone (XXI) [51a], progesterone (XVIII), 6-dehydroprogesterone (XXII), 6-dehydrocortexone

(XXIII) [51b] and testosterone (XXIV) [51c] occur in exceptionally high concentrations in the excrements which certain waterbeetles secrete as a deterrent when attacked by fishes. Also a new steroid $\Delta^{4,6}$-pregnadien-20α-ol-3-one (XXV) has been shown to be present in such a beetle [51d].
Rothschild and Ford [52] described equally spectacular experiments with the rabbit flea, whose sexual development is strongly influenced by Δ^4-3-ketosteroids. It was found that the life-cycle of the flea depends on the hormone cycle of the rabbit. The authors think that in all probability the hormones of the host have an indirect influence, and thus affect the secretion of the real insect hormones.

It is plausible that besides ecdysone other hormones are produced from cholesterol by insects. After administration of radio-active cholesterol to insects, several investigators [35, 33, 26, 53, 14b] found always a certain percentage of the radioactivity in the so-called polar fractions of some tissues. So far, the nature of these radio-active components is completely unknown.
In the cockroach *E. floridana* the highest concentration of polar steroids has been found in the midintestine and the gastric caeca. It is noteworthy that the female reproductive organs seem to contain more polar steroids than the male ones [14b]. Until recently it was assumed that in the polar metabolites the cholesterol side-chain would be still intact [14b]. During an investigation now in progress at our laboratory, however, indications have been obtained that in certain organs of *B. germanica* the side-chain is degraded. In this investigation cholesterol is administered, which has been labelled on the 26-position in the side-chain by ^{14}C, and which moreover has been tritiated generally or at a certain position in the nucleus. It is too early, however, to make definite statements with regard to this investigation.

The discovery of ecdysone derivatives in plants

Remarkably, substances with moulting hormone activity can also be isolated from plants and, moreover, in much bigger quantities than from insects or crustaceans.
Nakanishi et al [54] isolated four active substances from the conifer *Podocarpus nakaii*. One of these four components, ponasterone A (XIII), has the structure of 25-desoxyecdysterone, but the exact configuration of the hydroxyl groups at C_{20} and C_{22} is unknown. Of the other three components we only know that they contain more hydroxyl groups than does ponasterone A.
Takemoto et al [55a, b] isolated two components from *Achyrantes fauriei*, 2 β, 3 β, 14 α, 20, 22, 26-hexahydroxycholest-7-ene-6-one (XIV) and 20-hydroxyecdysone (X). Also extracts of other Amaranthaceae show moulting hormone activity [55a]. *Bosea yervamora* is exceptionally rich in 20-hydroxyecdysone.
Galbraith and Horn [56] succeeded in isolating from *Podocarpus elatus* an active component whose physical properties correspond to those of 20-hydroxyecdysone (X). They suggest a possible relationship between the presence of this hormone and the longknown resistance of this conifer to insects. Staal et al [57] could also prove the presence of considerable amounts of that hormone in *Taxus baccata*.
From quite different plants, viz. *Polypodium vulgare* [58] and *Vitex megapotamica* [59], 20-hydroxyecdysone has been isolated. Jizba et al [58] assume that insects receive moulting hormones with their food.
All the sterols with moulting hormone activity mentioned so far are C_{27}-sterols; rather surprising is the recent isolation of a C_{29}-sterol with moulting hormone activity from *Cyathula capitata* by Takemoto et al [55c].
The above findings open up prospects for a practical application of insect hormones. The possibility of using these hormones in selective insect control has been considered for some time. Recently, Williams [60] pointed out that they promise to provide specific insecticides. He classified them as "third-generation pesticides", the first generation being exemplified by lead arsenate, the second by DDT. He especially referred to the juvenile hormone and its analogues, which show promising prospects. Until recently the application of moulting hormones was only a theoretical possibility. The earlier described synthesis of ecdysone and its derivatives, was already an important step into the right direction. The presence of ecdysone derivatives in abundantly available plant material might render this application realizable.

The conversion of "phytosterols" (C_{28} and C_{29}) into "zoosterols" (C_{27}) by insects

The conversion of the phytosterol β-sitosterol into the zoosterol cholesterol has been discussed before. The ability to perform such a dealkylation at the 24-position appears to be characteristic for plant-eating insects, including the omnivorous cockroaches. As most well-known plant sterols belong to the ergostane- or stigmastane series (resp. C_{28}- and C_{29}-sterols), this ability to dealkylate can be considered of vital importance for those phytophagous insects whose natural source of nutrition does not contain cholesterol.
In view of these facts, substances inhibiting the dealkylation of C_{28}- and C_{29}-sterols open up new perspectives for the development of specific pesticides. These would, biologically, be more acceptable than the insecticides of general toxicity, because the former would affect the phytophagous insects only and not their natural enemies; whether these are mammals, birds or insectivorous insects. On the other hand, the specificity of these former insecticides would not be so great as that of e.g. sex-attractants, whose limited applicability is less attractive from a practical-commercial point of view.
It has become clear, however, in the last few years that the "zoosterol" cholesterol may also occur in plants [61].
Johnson et al [61d] demonstrated the presence of cholesterol in the potato plant. On the other hand,

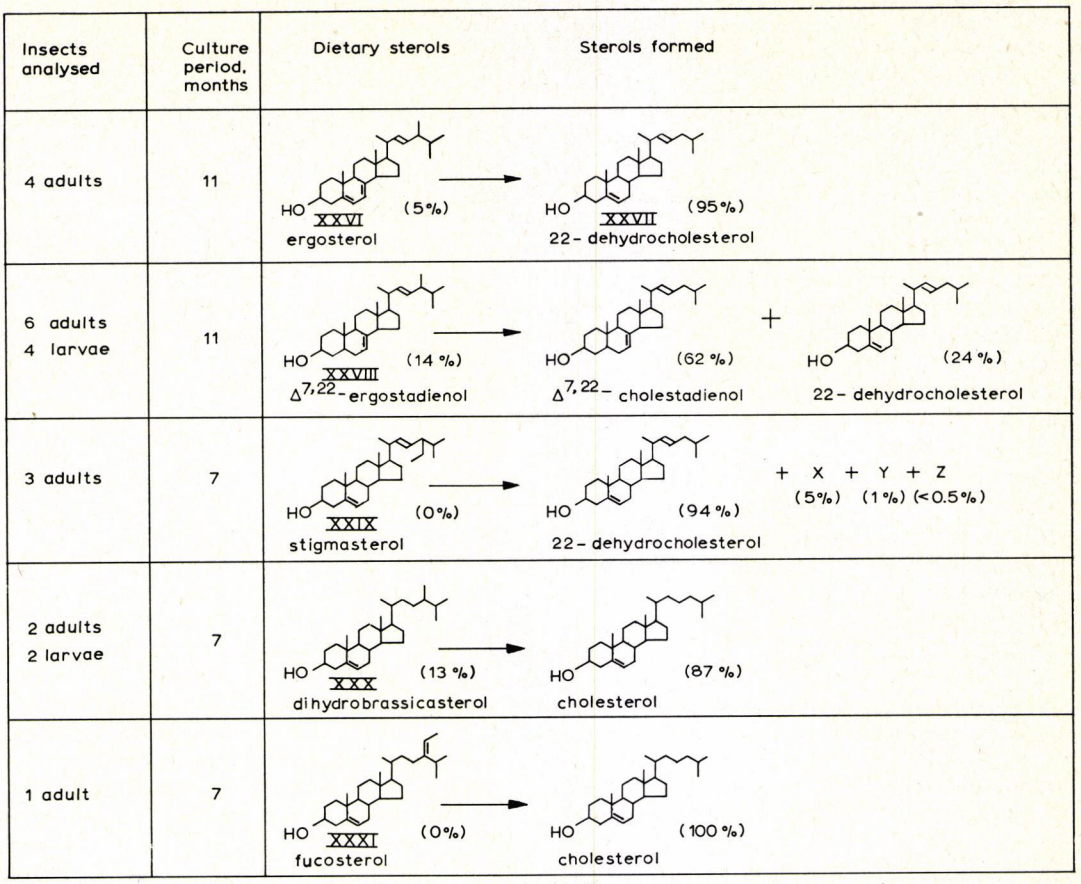

Fig. 8. Conversion of C_{28}- and C_{29}-sterols into C_{27}-sterols by the cockroach B. germanica. *The figures in brackets indicate the percentages of the sterols found in the insects.*

Schreiber et al [62] initially found in the Colorado beetle, which feeds on potato plants, only the "phytosterol" β-sitosterol, which also occurs in the potato plant, but not the "zoosterol" cholesterol.

Also in view of these remarkable facts we examined the sterol contents of both the potato plant and the Colorado beetle [63]. Like Johnson et al we found cholesterol in the potato plant; in certain leaves it even represented about 35% of the sterol fraction. In the Colorado beetle, however, cholesterol could also be shown to be present. Schreiber et al [64] reached the same conclusion in a second investigation.

As cholesterol is present in the potato plant, the question whether or not the Colorado beetle can dealkylate plant sterols would require further investigation.

The same is true for publications in which the conclusion that plant sterols are converted into cholesterol was exclusively based on the fact that in these phytophagous insects cholesterol was demonstrated by a biological [65, 18] or chemical [36, 66] assay of their respective sterol contents.

Clark and Bloch [67] were the first to demonstrate the dealkylation of a sterol by an insect directly. They proved that *B. germanica* converted ergosterol (XXVI) into 22-dehydrocholesterol (XXVII); a fact confirmed by Clayton [68] and Ritter et al [20], who used aseptic cockroaches.

Robbins et al [19] proved, for the same insect, the conversion of β-sitosterol into cholesterol, Ritter et al [20] did it for *E. floridana,* Ikekawa et al [69] for the silkworm and Svoboda et al [70] for the tobacco hornworm.

Schaefer et al [1m] demonstrated the dealkylation of β-sitosterol and campesterol from pine foliage by a pine sawfly. In this process, besides cholesterol, 7-dehydrocholesterol is probably formed too. Earle et al [29] found a conversion into cholesterol after administering a mixture of β-sitosterol and campesterol to the boll weevil.

Two communications about the conversion of ergosterol into cholesterol, respectively by the silk worm [71] and the rice moth [1k], require further analytical verification.

In collaboration with Clayton and Bloch, Ritter [20] studied the conversion by *B. germanica* of a number of different sterols of the ergostane- and stigmastane series.

These insects had been reared aseptically. They received a sterol-free diet to which the sterol to be examined had been added. The insects grew badly on the media with ergosterol (XXVI), $\Delta^{7,22}$-ergostadienol (XXVIII) or stigmasterol (XXIX). These sterols have the double bond at the 22-position in common. In the media with dihydrobrassicasterol (XXX), fucosterol (XXXI) or β-sitosterol (IV) which do not have a Δ^{22}-bond, normal growth took place. After 7 to 11 months, the adult insects, any occurring oothecae and larvae were collected and their

sterol contents were subjected to a quantitative and qualitative analysis by gas-chromatography according to the method of Clayton [72]. These analyses resulted in the conclusions shown in Figure 8. In agreement with reports by Clark and Bloch [67], and Clayton [68], ergosterol was found to be converted into 22-dehydrocholesterol. During this conversion, besides a demethylation a reduction of the Δ^7-bond takes place as well. So the cockroach is not only able to convert a Δ^5-compound into a $\Delta^{5,7}$-diene (cf the conversion of cholesterol into 7-dehydrocholesterol), but it can also do the reverse. In this case, the conversion was practically quantitative.

The $\Delta^{7,22}$-ergostadienol was demethylated for the greater part into the corresponding cholestane derivative. Moreover, 24% of the sterols was found, however, in a peak the retention time of which corresponded to that of 22-dehydrocholesterol. This would mean a rearrangement of a Δ^7-bond to a Δ^5-bond.

This rearrangement would be very surprising, since the synthesis of Δ^7-sterols by insects has been shown frequently, as was described before, but a rearrangement of these sterols into Δ^5-sterols has never been found. The possibility must not be ruled out that in the dietary sterol ergosterol was present as an impurity and that this was converted into 22-dehydrocholesterol, which was absorbed selectively.

The stigmasterol appeared to be converted quantitatively for no less than 94% into its dealkylation product, 22-dehydrocholesterol. Six per cent of the sterols, distributed over three compounds, could not be identified.

The C_{28}-sterol dihydrobrassicasterol and the C_{29}-sterol fucosterol were dealkylated for respectively 87 and 100%.

From these results it appears that the dealkylation of C_{28}- and C_{29}-sterols into C_{27}-sterols is a general phenomenon with this cockroach.

Apparently the insect is able to reduce the Δ^7-bond, but cannot reduce the Δ^{22}-bond. This explains why that insect develops better with sterols yielding cholesterol after dealkylation, than with sterols having a double bond at the 22-position.

In connection with the cholesterol-sparing effect of some sterols, which was mentioned before, it seemed interesting to investigate, whether such a dealkylation also occurs, if besides a C_{28}- or C_{29}-sterol, cholesterol is present in the diet.

This aspect may also be of importance with a view to the possible development of specific pesticides for phytophagous insects.

Therefore, some more experiments were done [20], in which two sterols were added to the diet : a sparing sterol and cholesterol in a ratio of 2 : 1. From these experiments it appeared that ergostane derivatives, notably Δ^7-ergostenol and $\Delta^{7,22}$-ergostadienol, could be demethylated in the presence of cholesterol, too.

Moreover, in the experiment with $\Delta^{7,22}$-ergostadienol as a sparing sterol, for the first time indications were obtained about the occurrence of an intermediate during the demethylation. The results of this experiment are shown in Figure 9.

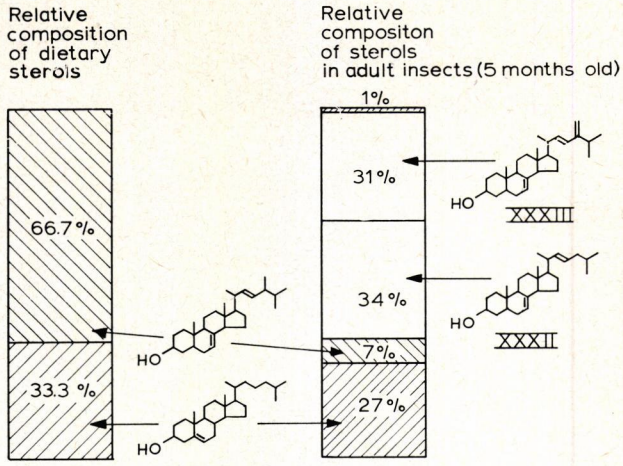

Fig. 9. Conversion of $\Delta^{7,22}$-ergostadienol in the presence of cholesterol by B. germanica.

Whereas cholesterol constituted a third part of the sterols in the diet, it represented 27% of the sterols in the insects. The remaining part of the sterols of the cockroach consisted of 7% unchanged ergostadienol, 34% of the fully demethylated product, $\Delta^{7,22}$-cholestadienol (XXXII), 1% of an impurity probably already present in the diet, and moreover a strikingly large quantity of a product whose properties were in agreement with those to be expected for a hypothetical intermediate of the demethylation, viz. $\Delta^{7,22,24(28)}$-ergostatrienol (XXXIII). The following arguments are in favour of this structure.

(1) It is a 3β-hydroxysterol, since it forms a precipitate with digitonine.

(2) It is a C_{28}-sterol with a Δ^7-bond, because after catalytic hydrogenation, Δ^7-ergostenol is produced.

(3) Since the compound is a C_{28}-sterol, and the insect is unable to synthesize sterols, the compound must be a metabolite of the ergostadienol.

(4) The higher retention time of the compound, compared with that of $\Delta^{7,22}$-ergostadienol, in the gas-chromatographic analysis according to Clayton, suggests a higher degree of unsaturation.

(5) The compound has a λ_{max} in the UV at 230 nm, which according to data in the literature [73a] corresponds to a 22,24(28)-diene system.

(6) The expected retention time of the postulated trienol relative to cholestane, could roughly be calculated from data in the literature [72]. The value found is, within the limits of experimental error, in agreement with the value so calculated.

The relative retention time of $\Delta^{7,22,24(28)}$-ergostatrienol with respect to cholestane is not known from the literature, and it cannot be calculated exactly either. If we start, however, from the relative retention time of $\Delta^{7,22}$-ergostadienol and the separation factor for the $\Delta^{24(28)}$-group, which are both known [72], we arrive at a value of 5.55 x 1.18 = 6.55. On account of the conjugation of the Δ^{22}- with the $\Delta^{24(28)}$

double bond, this figure must still be multiplied with an unknown factor. To reach the observed value of 7.43 for the r.r.t. of the trienol, this factor would have to be 1.13. This value is not unreasonable. For the effect of the conjugation when a Δ^7-bond is introduced into Δ^5-ergostenol, a factor of 1.13 is found, too (see Clayton [72]: $\Delta^{5,7}/\Delta^7 : \Delta^5$/stanol = 1.21 : 1.07 = 1.13). This complete agreement is purely accidental. There are other cases, in which for the conjugation factor somewhat different values are found.

Katsuki and Bloch [73b] found for a component to which they had assigned the tentative structure of $\Delta^{5,7,22,24(28)}$-ergostatetraenol, an r.r.t. of 7.6 to 7.8. In view of the data discussed above, we would expect a higher r.r.t. for this ergostatetraenol, but accurate calculations cannot be made.

Also ten fairly big nymphs from the same culture were shown to contain this intermediate, even in a somewhat higher percentage than the adult insects, viz. 39%.

In experiments with β-sitosterol and fucosterol which had been tritiated at the 3-position, and with non-labelled dihydrobrassicasterol, gas-chromatographic analysis also indicated the occurrence of intermediates during the dealkylation. They were tentatively characterized as fucosterol, desmosterol (XXXIV) and 24-methylenecholesterol (XXXV) (see Figure 10).

Besides *B. germanica*, also the bigger *E. floridana* was included in the experiments. With the latter insect, the sterols could be examined in individual organs.

The common aspect of these conversions is the synthesis of an intermediate with a $\Delta^{24(28)}$ double bond. The results were not always reproducible, and the metabolites concerned could only tentatively be indentified on the strength of their relative retention times found in the gas-chromatographic analysis.

Among other things, the impurities in β-sitosterol were a problem in that they are very difficult to remove. At the Centraal Laboratorium TNO, work is now in progress on the synthesis of pure β-sitosterol labelled in its nucleus by ^{14}C. At the same time β-sitosterol is being synthesized which is labelled with ^{14}C at C_{28} and C_{29}. By means of these compounds we will try to find further data about the mechanism of the dealkylation. Recently Svoboda et al [70] successfully demonstrated desmosterol to be an intermediate in the dealkylation of β-sitosterol into cholesterol by the tobacco hornworm. They [30] stated that the conversion of β-sitosterol to cholesterol could be blocked by 22,25-diazacholesterol and triparanol. The site of inhibition, however, appeared to be the conversion of desmosterol to cholesterol. These inhibitors, therefore, would not fulfil the requirements set for specific insecticides for phytophagous insects, since the conversion of desmosterol to cholesterol is also important in vertebrates.

The dealkylation by insects can in principle occur via an oxidative degradation of C_{28} and C_{29}, or via a mechanism that is the reverse of the alkylation, which takes place during the biosynthesis of C_{28}- and C_{29}-sterols in micro-organisms and plants.

As regards the first possibility, it may be remembered that Allais and Barbier [74] added to the diet of locusts a mixture of β-sitosterol, stigmasterol and campesterol, labelled at the 28- and 29-

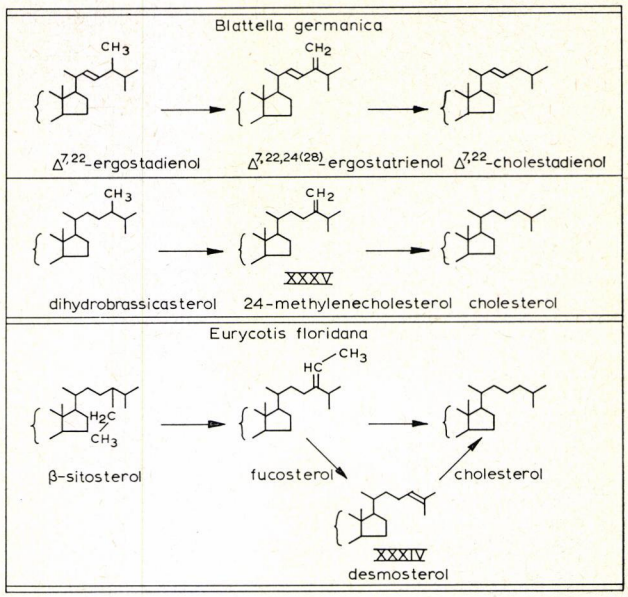

Fig. 10. Tentative schedule of metabolic pathways for the dealkylation of C_{28}- and C_{29}-sterols.

position with ^{14}C. During these preliminary experiments only very little radio-activity was found in oxidative degradation products.

Concerning the second possibility, the following facts are of importance. During the biosynthesis, the C-atoms 28 and 29 are introduced at a late stage and they originate from methionine [5d, 75, 76], whereas C_1 up to C_{27} originate from acetate and mevalonate [5d, 75, 77].

Breivik et al [73a] and Katsuki and Bloch [73b] isolated from yeast $\Delta^{5,7,22,24(28)}$-ergostatetraenol, which appears to occur as an intermediate in the ergosterol biosynthesis. Its resemblance with the intermediate $\Delta^{7,22,24(28)}$-ergostatrienol, found during dealkylation of $\Delta^{7,22}$-ergostadienol by the cockroaches, is obvious. A 24-methylene sterol, as a precursor of ergosterol, was also suggested by Akhtar et al [78a] and Barton et al [78b].

The other compounds, tentatively determined as intermediates in the dealkylation, viz. fucosterol, desmosterol and 24-methylenecholesterol, were also isolated from plants [79, 61f, i, p, q] and are supposed to act as intermediates in the biosynthesis of plant sterols [61f, 75, 5d, 80] or, anyhow, to have similar side-chains [80c, 81].

Though the intermediates found in the dealkylation of C_{28}- and C_{29}-sterols by insects strongly suggest the possibility of a mechanism that is the reverse of the synthesis of these sterols by micro-organisms and plants, they do not exclude the possibility of an oxidative degradation.

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