wahrscheinlich gemacht haben, dass Implantationshemmung und Laktationshemmung auf der primären Hemmung der Prolactininkretion beruht. Die Befunde mit α-Ergokryptin sind mit der Annahme einer solchen gemeinsamen Primärwirkung in Einklang.

Für 2-Br-α-Ergokryptin (Figur 2) verlaufen nur die beiden Dosiswirkungsbeziehungen für die beiden Kriterien der Laktationshemmung wie zu erwarten konvergent auf eine maximal wirksame Dosis hin. Die Dosiswirkungsbeziehung für Fertilitätshemmung hat hingegen einen viel steileren Verlauf; es werden 90 (und 100) % Fertilitätshemmung mit Dosen erreicht, die weniger als 50% Gewichtszuwachs- bzw. weniger als 20% Milchfleckhemmung im Laktationstest bewirken. Diese Dosiswirkungsverhältnisse können nicht mit der Vorstellung einer für Fertilitäts- und Laktationshemmung gemeinsamen Primärwirkung in Einklang gebracht werden. Es muss postuliert werden, dass 2-Br-α-Ergokryptin bei Ratten eine spezifisch fertilitätshemmende Wirkung entfaltet.

Die Bromierung von α-Ergokryptin in Stellung 2 des Moleküls bewirkt somit eine Steigerung der antifertilen Aktivität und eine Dissoziation dieser Eigenschaft von der laktationshemmenden Wirkung.

*Summary.* The inhibitory actions of α-ergocryptin on fertility and lactation in the rat are altered differentially in the 2-bromo derivative of this ergot alcaloid. It is therefore unlikely that the fertility inhibiting and the lactation inhibiting effects of 2-bromo-α-ergocryptin are governed by a single mechanism of action.

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## Desmosterol as a Common Intermediate in the Conversion of a Number of $C_{28}$ and $C_{29}$ Plant Sterols to Cholesterol by the Tobacco Hornworm

Although the accumulated data from nutritional studies suggest that a number of $C_{28}$ and $C_{29}$ phytosterols are converted to cholesterol by insects, only the conversion of ergosterol to 22-dehydrocholesterol[1] and the dealkylation of β-sitosterol to cholesterol[2–5] have been conclusively demonstrated. In the tobacco hornworm (*Manduca sexta*) the transformation of β-sitosterol to cholesterol proceeds through the intermediate desmosterol (24-dehydrocholesterol)[5] and certain vertebrate hypocholesterolemic agents (diazasterols and triparanol) block this conversion in the hornworm at the terminal step – the reduction of desmosterol to cholesterol[6]. The present paper reports on the conversion of a number of $C_{28}$ and $C_{29}$ phytosterols to cholesterol by the tobacco hornworm larva and presents evidence that desmosterol is a common intermediate in all these transformations.

The hornworms (6 larvae/test) were reared as previously described[5]. The sterols[7] were added to the artificial diet at a concentration of 0.026% (wet weight) either alone or in combination with an equal concentration of 20,25-diazacholesterol dihydrochloride. Insects reared on diets containing β-sitosterol as the sole added sterol served as controls for both growth and sterol metabolism studies. The total sterols were isolated from either prepupae (normal growth) or 20-day-old larvae (retarded growth) reared on each of the test diets and analyzed by 3 gas-liquid chromatography (GLC) systems as previously reported[5].

The hornworms fed diets containing each of the sterols shown in the Figure underwent normal growth and metamorphosis. However, insects reared on diets containing either brassicasterol or dihydrobrassicasterol developed more slowly than those on campesterol, stigmasterol, 24-methylenecholesterol or fucosterol. The growth rate of insects fed these last 4 sterols was equivalent to that of the controls.

Analyses of the sterol content of the insects indicated that cholesterol was the major sterol ($> 70\%$) in all cases, demonstrating that each of the sterols tested was efficiently dealkylated and converted to cholesterol (Table). These results show that the hornworm larva is capable of dealkylating α- or β-methyl, α-ethyl, methylene and ethylidene groups from the C-24 position of the sterol side chain and can also saturate the $\Delta^{22}$-double bond.

When 20,25-diazacholesterol was fed in the diets in combination with each of the test sterols, severe retardation of growth and development occurred. The effect on growth rate was most severe in insects fed diets containing brassicasterol or dihydrobrassicasterol plus the diazasterol. In addition to growth inhibition, the diazasterol brought about abnormalities in development such as formation of prepupae an instar earlier than normal and the production of prepupal-pupal intermediates. These results suggest that the azasterol may in part be effecting its inhibitory action by interfering with certain endocrine-mediated processes in the hornworm.

In all cases, GLC analyses of the sterols isolated from insects fed a phytosterol plus diazasterol showed an accumulation of desmosterol and a concurrent decrease in cholesterol content (Table). Apparently, desmosterol is an intermediate in the formation of cholesterol from all these sterols, indicating a similarity in the terminal step

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[7] All sterols used in this study except fucosterol were either obtained from commercial sources or prepared by reported methods. A generous gift of fucosterol from Dr. G. W. Patterson, Department of Botany, University of Maryland, is gratefully acknowledged. The compounds were purified by recrystallization and/or adsorption chromatography, except for campesterol, which was purified by preparative gas-liquid chromatography (GLC). The physical constants of the purified sterols agreed well with those reported in the literature, and the purity was determined by melting point, spectroscopic analyses and GLC. All the compounds were shown to be $> 99\%$ pure by these methods except campesterol which was $> 96\%$ pure and contained about 3% β-sitosterol.

of the conversion of these phytosterols to cholesterol by the hornworm.

The slower rate of growth that occurred with brassicasterol and dihydrobrassicasterol and the more severe growth retardation that was brought about when these sterols were fed in combination with diazasterol both suggest that the 24-β-alkyls of these sterols may be less efficiently dealkylated than the 24-α-alkyls of campesterol, β-sitosterol and stigmasterol. If such a selectivity exists, it could be related to an adaptation to the normal sterol complex available to this oligophagous species from its major host plant, tobacco, in which we find stigmasterol and β-sitosterol to be the predominant sterols. Saturation of the $\Delta^{22}$-double bond in the sterol side chain by the tobacco hornworm is a biochemical conversion unavailable to the omnivorous German cockroach[1]. This metabolic transformation is important in that it permits the hornworm to convert stigmasterol, which is the major sterol of tobacco leaves, to cholesterol.

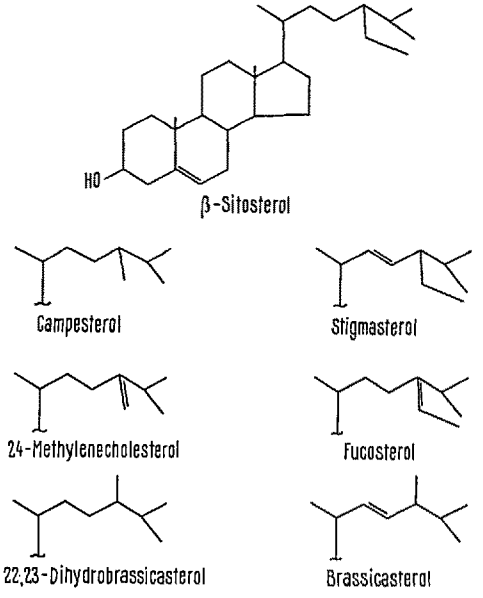
Both fucosterol and 24-methylenecholesterol were most effectively converted to cholesterol in the absence of diazasterol and feeding these compounds in combination with the diazasterol resulted in the greatest accumulations of desmosterol and the least unchanged dietary sterol. The latter effect suggests that the conversion of these 2 sterols with alkene substituents at C-24 is less affected by a feedback mechanism which appears to bring about a decrease in the rate of dealkylation of sterols with alkane substituents at C-24[6]. Both the efficient conversion and the apparent decrease in feedback effect suggest that fucosterol and 24-methylenecholesterol may be normal intermediates in the dealkylation scheme. We previously noted a minor sterol metabolite ($^3$H-labeled)[5] from $^3$H-β-sitosterol in the tobacco hornworm which could correspond to fucosterol. Recently, RITTER and WIENTJENS[8] reported the tentative identification of 24-methylenecholesterol and fucosterol as metabolites of dihydrobrassicasterol and 3-$^3$H-β-sitosterol respectively, in the German cockroach and suggested that these may be intermediates in the dealkylation scheme.

In a previous publication[5], we called attention to an interesting similarity: the terminal step of the dealkylation process in the hornworm resembles the primary step of alkylation in plants[9] in that both involve a $\Delta^{24}$-sterol intermediate. Since both 24-methylene and 24-ethylidene sterols are reported to be intermediates in the alkylation process[9], C-24-sterol dealkylation in insects may, in fact, be the reverse of C-24-sterol alkylation in plants. A final decision on whether or not 24-methylenecholesterol and fucosterol are intermediates in the primary dealkylation scheme or are derived from or converted through minor or alternate pathways must await a complete and detailed mapping of the metabolic steps of sterol dealkylation in insects. This information is a requisite to a more thorough understanding of the comparative sterol biochemistry of phytophagous insects and their host plants. It is also necessary to a rational approach to the development of chemicals that may be used to selectively disrupt the essential dealkylation processes in plant-feeding insects[10].

Sterol content of tobacco hornworms fed various $C_{28}$ and $C_{29}$ phytosterols alone and in combination with 20,25-diazacholesterol

| Dietary sterol | Sterol content of hornworms (% of total sterols) | |
|---|---|---|
| | Cholesterol | Desmosterol |
| Phytosterols alone[a] | | |
| β-Sitosterol | 84.5 | – |
| Campesterol | 74.5 | – |
| Stigmasterol | 75.3 | – |
| Fucosterol | 94.1 | – |
| 24-Methylenecholesterol | 87.5 | – |
| Brassicasterol | 70.5 | – |
| Dihydrobrassicasterol | 74.4 | – |
| 20,25-Diazacholesterol plus[b] | | |
| β-Sitosterol | 5.6 | 21.8 |
| Campesterol | 7.7 | 14.4 |
| Stigmasterol | 5.1 | 12.2 |
| Fucosterol | 6.4 | 79.4 |
| 24-Methylenecholesterol | 8.0 | 39.8 |
| Brassicasterol | 3.5 | 10.5 |
| Dihydrobrassicasterol | 2.8 | 11.3 |

[a] Remainder of sterol was unchanged dietary sterol; desmosterol accounted for < 2% of total. [b] Remainder of sterol was unchanged dietary sterol plus minor unidentified metabolites[4].




$C_{28}$ and $C_{29}$ phytosterols tested as dietary sterols.

*Résumé. Manduca sexta* (Johannson) est capable de transformer 7 phytostérols en cholestérol. Ainsi, cet insecte peut déalcoyler le α- et le β-méthyl de même que les groupes méthylène et éthylidènes du carbone 24 et peut hydrogéner la double liaison 22,23. Quand ces stérols sont combinés au 20,25-diazacholestérol, on a constaté dans tous les cas que la transformation en cholestérol s'effectue par l'intermédiaire commun, le desmostérol.

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[10] The authors gratefully acknowledge the technical assistance of D. J. BOND. We thank G. D. Searle and Company for a sample of 20,25-diazacholesterol dihydrochloride (SC-12937).