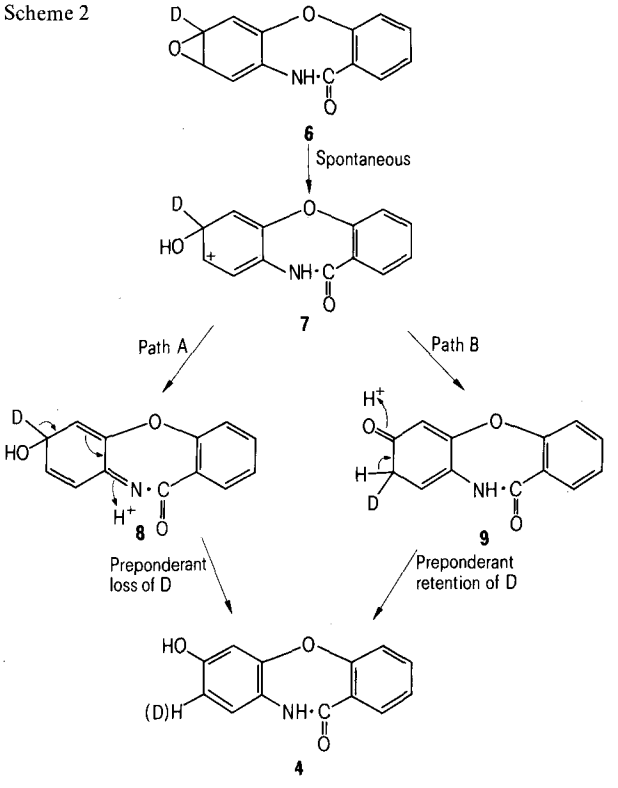
Scheme 2



in an aromatic substrate to an adjacent position is a characteristic of a process involving in whole or in part, an arene oxide intermediate[3]. The results in the table implicate an arene oxide intermediate in the hydroxylation of lactam **2** to 7-hydroxylactam **4**. The quantitative retention of deuterium in both unmetabolised **2** and in the 4-hydroxylactam **3**, where metabolism occurs remote from the site of deuteriation, indicate that low deuterium retention in **4** is a consequence of hydroxylation at C-7 and not of alternative exchange reactions. The absence of metabolites hydroxylated at C-6 or C-8 suggests the formation of only 1 oxide which rearranges selectively to give **4**. The tentative assignment of the deuterium in **4** to C-8 is consistent only with the 7,8-oxide (**6**) (scheme 2).

The low retention (about 13%) of deuterium in **4** is consistent with an oxide in which the substituent para to the deuterium has a readily ionisable proton. It has been suggested[3,11] that after the initial spontaneous ring-opening leading in this instance to **7**, that rearrangement via path A (scheme 2) is favoured where the intervention of intermediates such as **8** result mainly in the loss of deuterium. Rearrangement via path B involving the dienone intermediate **9** result largely in the retention of deuterium. Low retentions of deuterium have been reported with other aromatic amides. Thus, acetanilide-4-d_1 showed 34% retention (in vivo, rat)[12] and the more closely related benzanilide-4-d_1, 21% retention[11].

The absence of metabolites derived from the reaction of nucleophiles with the oxide (**6**), especially mercapturic acid derivatives, suggests low arylating potential. This is consistent with the anticipated facile ring-opening and consequent ready isomerisation to the phenol that is associated with oxides from electron-rich arenes such as **2**. Experiments designed to differentiate between insertion and addition mechanisms for the in vitro conversion of **1** to **2** using a kinetic approach were unsuccessful[15].

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15 Incubation of **1** with the cytosol fraction prepared from rat liver homogenate gave good conversions (> 70%) into the lactam **2**. In an attempt to distinguish between the alternative insertion and addition pathways, i.e. between enol lactam and oxaziridine intermediates for this transformation, a kinetic isotope approach was adopted. Using a 1:1 mixture of **1**-11-d and non-deuteriated **1** (H/D = 1), the H/D ratio was determined in unchanged **1** throughout the course of the incubation (0–45 min). The H/D ratio did not deviate significantly from unity with time. In the absence of a full kinetic analysis of the system and a certain knowledge of the rate-determining step, no conclusions can be drawn from this result. A similar view has been suggested recently[13] and must cast doubt on the validity of a meaningful interpretation based on the absence of a primary kinetic isotope effect[14].

Conversion of β-sitosterol into both fucosterol and isofucosterol in *Tenebrio molitor*[1]

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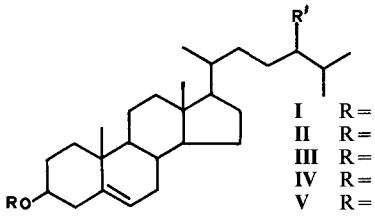
Summary. It has been demonstrated with cold trap experiments that *Tenebrio molitor* transforms β-sitosterol not only into fucosterol, but also into isofucosterol.

Many species of insects are known to convert phytosterols into cholesterol[2], fucosterol (**I**) having been implicated[3-5] as intermediate in this process, since it has been isolated as a metabolite of β-sitosterol (**II**).

On the other hand, as isofucosterol (**III**) is utilized by *Bombix mori*[5] and *Tenebrio molitor*[6], the question arises whether this compound also is a real β-sitosterol metabolite, or whether its utilization is due to enzymes arising from an inductive process.

We wish now to report experimental evidence that, not only fucosterol (**I**), but also isofucosterol (**III**) is formed when β-sitosterol (**II**) is metabolized by *Tenebrio molitor*.

A mixture of [22, 23(n)-$^2H_2$]-β-sitosterol (Amersham, 15 µCi, sp. act. 47 Ci/mmole), 2 mg of fucosterol and 2 mg of isofucosterol, were deposited onto 140 mg of finely grounded oatmeal and fed to 50 (1.4 g) young larvae of *Tenebrio molitor,* which had been starved for 48 h.



I R=–H; R'=–CH–CH_3 (E)
II R=–H; R'=–C_2H_5
III R=–H; R'=–CH–CH_3 (Z)
IV R=–Ac; R'=–CH–CH_3 (E)
V R=–Ac; R'=–CH–CH_3 (Z)

Specific activity of diastereoisomeric ethylidene sterols (and their acetates) formed during the metabolism of β-sitosterol in *Tenebrio molitor*

Compound		Activity (dpm of ^3H/mM × 10^{-6}
Fucosteryl acetate (**IV**)	3rd crystallization	2.07
	4th crystallization	2.12
	5th crystallization	2.08
Fucosterol (**I**)	3rd crystallization	2.11
	4th crystallization	2.08
	5th crystallization	2.09
Isofucosteryl acetate (**V**)	3rd crystallization	1.35
	4th crystallization	1.38
	5th crystallization	1.36
Isofucosterol (**III**)	3rd crystallization	1.33
	4th crystallization	1.36
	5th crystallization	1.34

After the food has been consumed (~4 days), the larvae were freezed, macerated in ethanol and submitted to alkaline hydrolysis. The unsaponifiable material was fractionated on silica gel-celite and from the free sterol fraction fucosteryl acetate (**IV**) (2.3×10^5 dpm of ^3H) and isofucosteryl acetate (**V**) (1.5×10^5 dpm of ^3H) were obtained by acetylation and $SiO_2/AgNO_3$ 3:1 preparative TLC (hexane – benzene 1:1). Each compound was shown to be pure by $SiO_2/AgNO_3$ TLC and GLC (LAC 796 1%, T_c=200 °C), and then diluted with carrier material and crystallized to constant specific activity (table).

Hydrolysis of the acetates (**IV**) and (**V**) produced the free sterols (**I**) and (**III**), the activity of which also is shown in the table. Our experiment indicates that, besides fucosterol, isofucosterol too is a real metabolite of β-sitosterol in *Tenebrio molitor.* This result is in agreement with the formation of the 2 geometrical isomers, fucosterol and isofucosterol, through 2 different and parallel pathways with opposite stereochemistry or, alternatively, with the enzymic isomerization of one of the isomers into the other.

1 Acknowledgment. We thank Dr A. Longoni, Centro Ricerche antiparassitarie Montedison S.p.A. for *Tenebrio molitor* larvae.
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Disaccharidase rhythm in rat small intestine; no relationship with mitosis rhythm

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Summary. Same circadian difference in the specific activities of sucrase and maltase was observed in the purified brush border fraction as in the crude homogenate of the mucosa of rat small intestine, suggesting that the disaccharidase rhythm is not due to the mitosis rhythm of epithelial cells.

The brush border of the intestinal epithelium contains many hydrolytic enzymes, such as sucrase, lactase, maltase, alkaline phosphatase and leucine aminopeptidase, and some of these enzymes are believed to play important roles in the final stages of digestion. As reported previously[1-4], the activities of these digestive enzymes of the homogenate of the intestinal mucosa show a clear circadian rhythm with the highest activity at midnight in rats kept under normal light-dark cycle and ad libitum feeding condition. When the rats are put on a daytime feeding schedule, the phase of the rhythm shifts about half a day, suggesting that time of feeding is a synchronizer of the enzyme rhythm.

The circadian rhythmicity in the intestinal epithelium has also been described in mitotic index[5], in DNA synthesis[6] and in mitotic rate[7,8], and these rhythms seem to be related to the feeding pattern[8,9]. One explanation about the rhythm in crypt cell proliferation is that more digestive-absorptive cells are required during periods of feeding[9]. According to this view, the enzyme rhythm itself might be secondary to the rhythm in the population of epithelial cells in the intestinal mucosa. To test this, in the present study, we examined the disaccharidase rhythm in the brush border fraction purified from the intestinal mucosa of rats.

Methods. Male Wistar strain rats (200–250 g) were kept at 22±2 °C under 12 h light-dark cycle (light on from 07.00 to 19.00 h) and allowed free access to water and laboratory chow. Individuals were decapitated every 4 h and the upper half of the small intestine was rapidly removed, washed out with cold 0.9% NaCl and everted. The mucosa was scrapped off with a glass slide and homogenized with about 50 vol. of 5 mM ethylenediamine tetraacetate buffer (pH 7.4) in a Warling Blendor for 40 sec. An aliquot of the homogenate was assayed for sucrase and maltase by Dahlqvist's method[10] and for protein by the method of Lowry et al.[11]. The homogenates obtained at 00.00 h and at 12.00 h were centrifuged at 450 × g for 10 min and brush borders were purified according to the method of Forstner et al.[12].

Results and discussion. The figure shows a typical pattern of the circadian rhythm in the activities of sucrase and maltase of the homogenates. The highest activity was observed at 04.00 h and the lowest at 12.00 h, confirming earlier results[1-4]. This pattern of the disaccharidase rhythm seems to be similar to that of the mitosis rhythm reported[7,8].

In the figure, the enzyme activities were expressed as the specific activity based on the protein content of the homogenate of the scrapped mucosa. The changes in the enzyme