6 Duka, T., Höllt, V., Przewtocki, R., and Wasche, D., Biochem. biophys. Res. Commun. *85* (1978) 1119.
7 Lemaire, J., Tseng, R., and Lemaire, S., Proc. natn. Acad. Sci. *75* (1978) 6240.
8 Suda, T., Liotta, A.S., and Krieger, D.T., Science *202* (1978) 221.
9 Haddox, M.K., and Russell, D.H., Life Sci. *25* (1979) 615.
10 Huidobro, J.P., Huidobro, F., and Croxatto, R., Life Sci. *24* (1979) 679.
11 Larsson, L.J., Childers, S., and Snyder, S.H., Nature *282* (1979) 407.
12 Nilaver, G., Zimmerman, E.A., Defendini, R., Liotta, A.S., Krieger, D.T., and Brownstein, M.J., J. Cell Biol. *81* (1979) 50.
13 Ragavan, V.V., and Frantz, A.G., Endocrinology *109* (1981) 1769.
14 Schulz, R., Wilhelm, A., Rirke, K.M., Gramsch, C., and Herz, A., Nature *294* (1981) 757.
15 Gilman, S.C., Schwartz, J.M., Milner, R.J., Bloom, F.E., and Feldman, J.D., Proc. natn. Acad. Sci. *79* (1982) 4226.
16 K. Nakao, Y. Nakai, S. Oki, K. Horrii, and H. Imura, J. clin. Invest. *62* (1978) 1395.
17 Wardlaw, S.L., and Frantz, A.G., J. clin. Endocr. Metab. *48* (1979) 176.
18 Goland, R.S., Wardlaw, S.L., Stark, R.J., and Frantz, A.G., J. clin. Endocr. Metab. *52* (1981) 74.
19 McIntosh, T.K., Vallano, M.L., and Barfield, R.J., Pharmac. Biochem. Behav. *13* (1980) 435.
20 Schwartz, J.C., and Roques, B.P., Biomedicine *32* (1980) 169.
21 McKnight, A.T., and Kosterlitz, H.W., J. Reprod. Fertil. *58* (1980) 513.
22 Motta, M., and Martini, L., Acta endocr. *99* (1982) 321.
23 Grossman, A., West, S., Williams, J., Evans, J., Rees, L.H., and Besser, G.M., Clin. Endocr. *16* (1982) 317.
24 Vribicky, K.W., Baumstark, J.S., Wells, J.C., Hilgers, T.W., Kable, W.T., and Elias, C.J., Fert. Steril. *38* (1982) 701.
25 Schill, W.B., Fert. Steril. *25* (1974) 703.
26 Singer, R., Barnet, M., Sagiv, M., Allalouf, D., Landau, B., Segenreich, E., and Servadio, C., Archs Androl. *8* (1982) 61.
27 Singer, R., Sagiv, M., Allalouf, D., Levinsky, H., Barnet, M., Landau, B., Segenreich, E., and Servadio, C., Int. J. Fert. *27* (1982) 176.

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Conversion of clionasterol into both fucosterol and isofucosterol by the insect *Tenebrio molitor*

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Summary. [7,7-$^3H_2$]Clionasterol was synthesized and fed, together with [4-^{14}C]sitosterol, to *Tenebrio molitor* larvae; fucosterol and isofucosterol, recovered from the sterol fraction, were found to be doubly labeled, indicating that clionasterol is converted into both the ethylidenic compounds.
Key words. Tenebrio molitor; clionasterol; fucosterol; isofucosterol.

Most phytophagous insects convert C_{29} phytosterols into cholesterol (**3**)[1] through a sequence (scheme 1) involving dehydrogenation, epoxidation and rearrangement with loss of a C_2 fragment followed by reduction of the obtained double bond. In *Tenebrio molitor* both sitosterol (**1a**) and its C-24 epimer clionasterol (**1b**) are metabolized[2] and we have shown[3] that the same insect transforms the former compound into both the E and Z $\Delta^{24(28)}$-ethylidenic intermediates fucosterol (**2a**) and isofucosterol (**2b**). The question whether the dealkylation process of clionasterol (**1b**) shows the same lack of stereospecificity, i.e. whether (**1b**) ist also converted into both the double bond compounds (**2a**) and (**2b**), is still unanswered[4].

In order to clarify this metabolic aspect, we synthesized [7,7-$^3H_2$] clionasterol and tested its conversion into (**2a**) and (**2b**) by *Tenebrio molitor* larvae.

Poriferasterol (**4**), obtained from *Ochromonas malhamensis*[5], was transformed[6] into (22E,24R)-3α,5α-cyclostigmast-22-en-6-one (**5**)[7], from which (24S)-3α,5α-cyclostigmastan-6-one (**6**)[7] was obtained by catalytic hydrogenation on Pd/C. The introduction of tritium at C-7 of (**6**), the reduction of the 6-oxo function, and the treatment with $Zn(OAc)_2$/AcOH were carried out according to the usual procedure[6], and afforded [7,7-$^3H_2$]clionasteryl acetate (7a) which was hydrolyzed to [7,7-$^3H_2$]clionasterol (**7b**) (spec. act. 2.48×10^6 dpm of ^{3}H/mg).

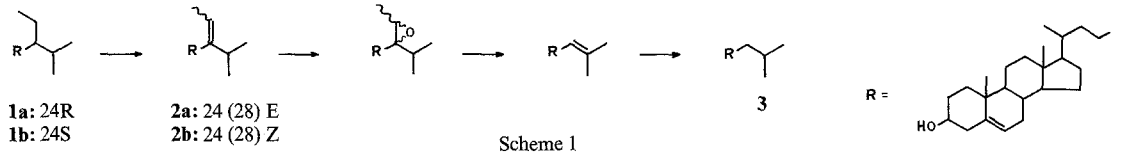
[7,7-$^3H_2$]clionasterol (**7b**) (2.23×10^6 dpm of ^{3}H) was mixed with [4-^{14}C]sitosterol (**8**) (9.50×10^5 dpm of ^{14}C, spec. act. 2.85×10^8 dpm of ^{14}C/mg, the Radiochemical Centre, Amersham) and fed, together with unlabeled fucosterol and isofucosterol as cold traps, to young *Tenebrio molitor* larvae. Two days later the larvae were frozen and from the benzoylated sterol fraction[8] pure fucosteryl (**9a**) and isofucosteryl (**10a**) benzoates were obtained by repeated argentation t.l.c. The 2 benzoates (**9a**) and (**10a**) were diluted with cold material and crystallized to constant specific activity; the free sterols (**9b**) and (**10b**), obtained by alkaline hydrolysis, were also crystallized and counted. The values obtained are summarized in the table.

The data obtained clearly show that clionasterol (**1b**) is converted into fucosterol (**2a**) and isofucosterol (**2b**) as both the compounds were found doubly labeled. Furthermore, from the ^{3}H/^{14}C ratios of the recovered compounds it can be deduced that clionasterol is converted into fucosterol with about the same efficiency as sitosterol, whereas it is transformed into isofucosterol less readily than sitosterol.

Moreover, the different ^{3}H/^{14}C ratios of fucosterol (**2a**) and isofucosterol (**2b**) make it unlikely that the sequential formation of (**2a**) and (**2b**) is the only operative pathway; they seem rather to be in agreement with parallel transformations of the 24-ethyl precursors into the $\Delta^{24(28)}$-ethylidenic intermediates.

Total radioactivities and ^{3}H/^{14}C ratios of the administered precursors and of the recovered products

Compounds	dpm of ^{14}C	dpm of ^{3}H	^{3}H/^{14}C
[7, 7-$^3H_2$] Clionasterol (**7b**) + [4-^{14}C] sitosterol (**8**)	9.50×10^5	2.23×10^6	2.35
[4-^{14}C, 7, 7-$^3H_2$] Fucosteryl benzoate (**9a**)	1.42×10^3	3.58×10^3	2.52
[4-^{14}C, 7, 7-$^3H_2$] Fucosterol (**9b**)	1.48×10^3	3.82×10^3	2.58
[4-^{14}C, 7, 7-$^3H_2$] Isofucosteryl benzoate (**10a**)	0.85×10^3	1.08×10^3	1.27
[4-^{14}C, 7, 7-$^3H_2$] Isofucosterol (**10b**)	0.86×10^3	1.15×10^3	1.34



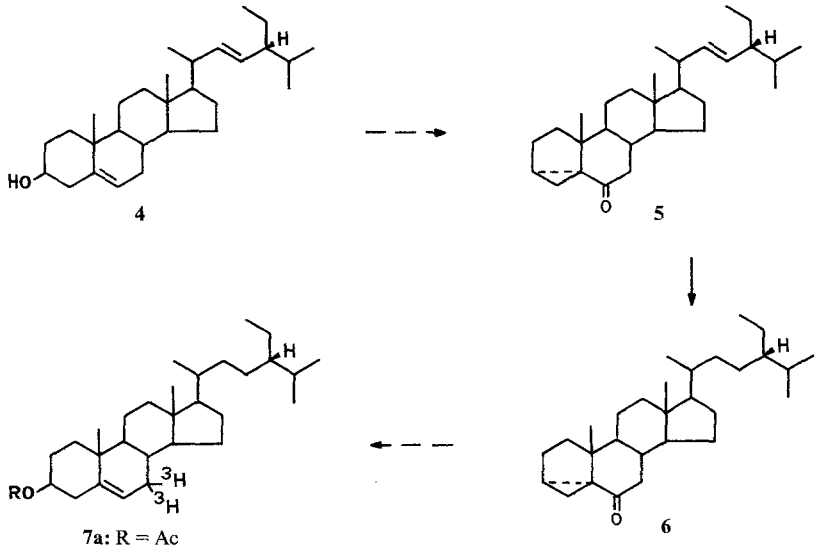
1a: 24R
1b: 24S

2a: 24 (28) E
2b: 24 (28) Z

3

R =

Scheme 1



4 **5** **6**

7a: R = Ac
7b: R = H

Scheme 2

1 Svoboda, J.A., Thompson, M.J., Robbins, W.E., and Kaplanis, J.N., Lipids *13* (1978) 742.
2 Pettler, P.J., Lockley, W.J.S., Rees, H.H., and Goodwin, T.W., Biochem. J. *74* (1978) 397.
3 Nicotra, F., Ronchetti, F., and Russo, G., Experientia *34* (1978) 699.
4 Mulheirn, L.J., in: Biosynthesis, vol.6, p.130. The Chemical Society, London 1980.
5 Nicotra, F., Ranzi, B.M., Ronchetti, F., Russo, G., and Toma, L., J. chem. Soc. chem. Commun. (1980) 752.
6 Knapp, F.F., Goad, L.J., and Goodwin, T.W., Phytochemistry *16* (1977) 1683.
7 Compound (**5**): oily product, $\delta_H(CDCl_3)$ 0.2–0.6 (m, cyclopropyl protons), 0.74 (s, 18-Me), 0.83 (t, J 6 Hz, 29-Me), 0.85 (d, J 6 Hz, 26- and 27-Me), 1.01 (s, 19-Me), 1.04 (d, J 6 Hz, 21-Me), 5.01 (m, 22- and 23-CH). Compound (**6**): oily product, $\delta_H(CDCl_3)$ 0.2–0.6 (m, cyclopropyl protons), 0.68 (s, 18-Me), 0.83 (d, J 6 Hz, 26- and 27-Me), 0.85 (t, J 6 Hz, 29-Me), 0.94 (d, J 6 Hz, 21-Me), 1.02 (s, 19-Me).
8 Nicotra, F., Ronchetti, F., Russo, G., and Toma, L., J. chem. Soc. Perkin Trans. *1* (1983) 787.

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Glutathione-related enzyme activities in pregnant rat liver

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Summary. The levels of GSH-related enzyme activities during pregnancy were determined. A significant increase in Selenium-dependent GSH peroxidase and GSH S-transferase E activity was observed. A concomitant increase in γ-glutamylcysteine synthetase was measured, which indicated an increased ability to synthesize the tripeptide.
Key words. Rat liver; liver, rat; pregnancy, rat; glutathione-related enzymes; enzymes, glutatione-related.

Glutathione (GSH) exerts a protective role in cells by forming conjugate derivatives and by acting as hydrogen donor for the reduction of peroxides. The conjugation process is catalyzed by a class of enzymes known as GSH S-transferases. These enzymes have been purified and characterized from several sources[1,2]. The removal of H_2O_2 and other peroxides is mediated by the action of GSH peroxidase[3]. The intracellular reduced GSH is formed from its precursor amino acids glutamate, cysteine and glycine. γ-Glutamylcysteine synthetase catalyses the dipeptide formation whilst GSH synthetase catalyses the tripeptide formation. The activity of γ-glutamylcysteine synthetase is rate-limiting, and the synthesis of GSH may be regulated by feedback inhibition of this enzyme[4]. Several investigations have indicated that the enzymes related to GSH metabolism gradually develop with age and can also be induced by numerous endogenous and exogenous substances[2,5–8]. In this context it was considered important to determine whether hepatic levels of GSH-related enzymes are in-