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MIGRATION OF C-25 HYDROGEN OF SITOSTEROL TO C-24 DURING THE CONVERSION INTO DESMOSTEROL IN THE SILKWORM *BOMBYX MORI*[1]

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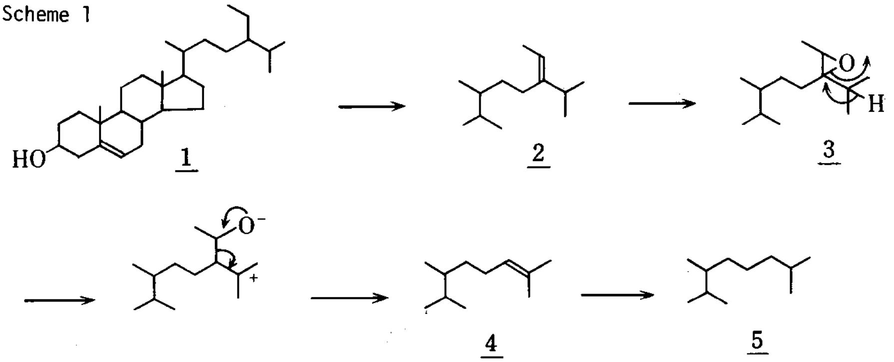
We have recently presented[2] the evidence that fucosterol-24,28-epoxide 3 is a probable intermediate in the conversion of sitosterol 1 to cholesterol 5 in the silkworm, *Bombyx mori*[3], and proposed scheme 1 as one of the main dealkylation routes of C_{29} plant sterol in phytophagous insects. In this scheme, C-25 hydrogen of 3 is postulated, by analogy with chemical reaction of 3 with BF_3-etherate[4], to migrate to C-24 position during its conversion to desmosterol 4[5].

Described here is an experimental proof of this hypothesis. The substrate, [25-^{3}H]-sitosterol 1 was synthesized from fucosterol 2 as shown in scheme 2. As reported previously[6], the side chain double bond of 2-acetate was isomerized

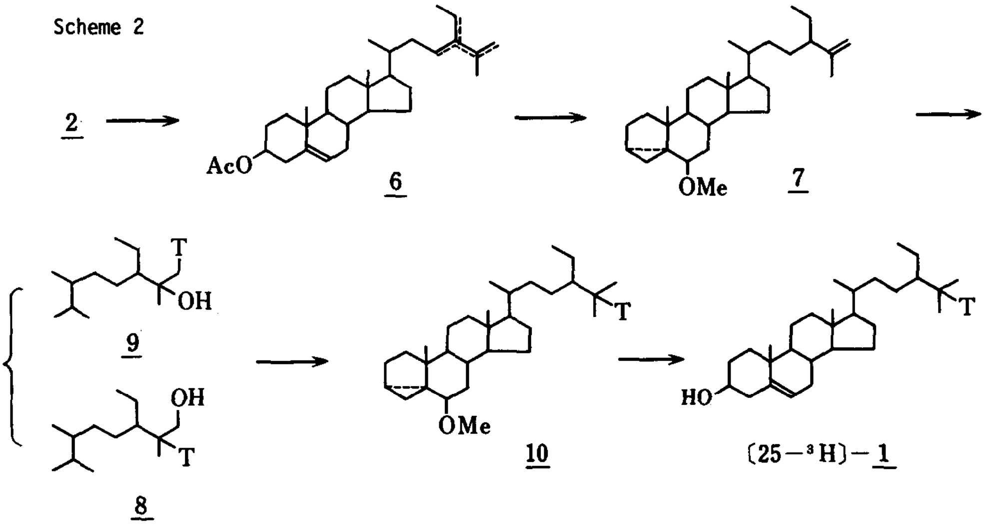
Scheme 1



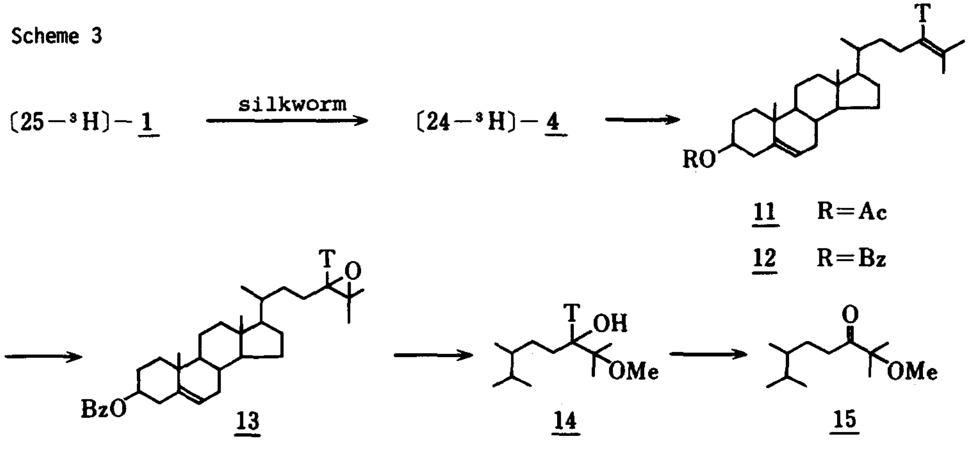
on the action of iodine to give a complex mixture of products. After removing most of $\Delta^{24(25)}$-isomer by several crystalization, the mother liquor containing the desired $\Delta^{25(26)}$-isomer (70 % by glc analysis) was saponified, tosylated and solvolyzed to give i-methylether. On a controlled ozonolysis by which the other double bond isomers were destroyed, the exomethylene was remained intact and thence pure $\Delta^{25(26)}$-i-methylether 7[7] was obtained in an overall yield of 18 % from 2.

Hydroboration[8] of 7 was performed with tritiated diborane generated *in situ* by treatment of [^{3}H]-$NaBH_4$ (25 mCi) with BF_3-etherate. The subsequent

Scheme 2



Scheme 3



oxidation with alkaline H_2O_2 afforded a mixture of [25-^{3}H]-26-ol 8 and [26-^{3}H]-25-ol 9 in a ratio of 3:1. This was, without separation, treated with mesylchloride/pyridine and then with $LiAlH_4$. Column chromatography of the products gave the recovered 9 and [25-^{3}H]-i-methylether 10 (30 % yield from 7). Solvolysis of 10 with KOAc/AcOH, followed by saponification afforded [25-^{3}H]-sitosterol[9] (9.7 μCi/mg).

The silkworm larvae of B. mori (50 species) were reared on a synthetic diet[2] containing 0.1 % of [25-^{3}H]-1 (57 μCi)[10]. On 11th day, the insects were homogenized with $CHCl_3$-MeOH (2:1), and the lipid extract was saponified. The unsaponifiable fraction (7.4 μCi) was treated with Ac_2O/pyridine to give sterol acetates, from which desmosterol acetate 11 (0.40 μCi) was isolated by means of $AgNO_3$-impregnated tlc. Recrystalization with carrier (50 mg) gave a constant specific activity (6300 cpm/mg).

An aliquot of this sample, with a further addition of carrier, was successively transformed (scheme 3) to benzoate 12 (saponification and BzCl/ pyridine), 24,25-epoxide 13 (m-chloroperbenzoic acid), 25-methoxy-24-ol 14 (methanolysis with $HClO_4$) and finally to 25-methoxy-24-one 15[11] (Jones oxidation).

The data in the table indicates that more than 85 % of ^{3}H in 12 is located at C-24, and therefore that C-25 hydrogen of sitosterol 1 migrates to C-24 during its conversion into desmosterol 4 in B. mori[12].

Table

Compound	Specific activity	Relative radioactivity
Desmosterol benzoate 12	125 cpm/μmole	100 %
24,25-Epoxide 13	113	91
25-Methoxy-24-ol 14	116	93
25-Methoxy-24-one 15	18	14

REFERENCES AND FOOTNOTES

1. This work was presented at the fall meeting of the American Oil Chemists Society, Philadelphia, October 1, 1974.

2. M. Morisaki, H. Ohtaka, M. Okubayashi, N. Ikekawa, Y. Horie and S. Nakasone, *J. C. S. Chem. Comm.* 1275 (1972); M. Morisaki, H. Ohotaka, N. Awata, N. Ikekawa, Y. Horie and S. Nakasone, *Steroids*, **24**, 165 (1974).

3. Conversion of epoxide **3** into cholesterol **5** has been also observed with the locust, *Locusta migratoria*: J. P. Allais, A. Alcaide and M. Barbier, Experientia, **29**, 944 (1973).

4. N. Ikekawa, M. Morisaki, H. Ohtaka and Y. Chiyoda, *J. C. S. Chem. Comm.*, 1498 (1971); H. Ohtaka, M. Morisaki and N. Ikekawa, *J. Org. Chem.*, **38**, 1688 (1973).

5. Randall *et al.*reported that C-25 hydrogen of isofucosterol was retained during transformation into cholesterol by the yellow mealworm, *Tenebrio molitor*, but the exact position of the migrated hydrogen has not yet been determined: P. J. Randall, J. G. Lloyd-Jones, I. F. Cook, H. H. Rees and T. W. Goodwin, *J. C. S. Chem. Comm.*, 1296 (1972).

6. N. Ikekawa, Y. Honma, N. Morisaki and K. Sakai, *J. Org. Chem.*, **35**, 4145 (1970).

7. M^+, 426.383 ($C_{30}H_{50}O$ requires, 426.386); δ($CDCl_3$), 0.67 (3H, s, 18-Me), 1.01 (3H, s, 19-Me), 1.60 (3H, s, 26-Me), 2.76 (1H, m, C-6-H), 3.32 (3H, s, methoxyl) and 4.75 ppm (2H, m, exomethylene).

8. cf. K. R. Varma, J. A. F. Wickramasinghe and E. Caspi, *J. Biol. Chem.*, **244**, 3951 (1969). They have prepared [25-^{3}H]-cholesterol by hydroboration of Δ^{25}-cholesterol THP ether with disiamylborane. However under the same conditions as described therein, almost no reaction occurred on compound **7**.

9. mp. 127-129°; Nmr spectrum and glc behavior were in complete agreement with those of authentic sitosterol. However, the product should be a mixture of (24R)- and (24S)-isomers in view of the synthetic procedures.

10. The insects were initially fed with triparanol, but any significant accumulation of desmosterol was not observed. However even on rearing the insects without triparanol, desmosterol was comprised of *ca*. 5 % of total sterols (Y. Fujimoto *et al.*,*Steroids*, in press) and this was enough for the present purpose. We thank for Dr. M. J. Thompson, Agricultural Research Center, U. S. Dept. of Agriculture, for sending us triparanol.

11. mp. 179-181°; δ ($CDCl_3$), 0.70 (3H, s, 18-Me), 1.08 (3H, s, 19-Me), 1.28 (6H, s, 26,27-Me), 2.55 (2H, m, C-23-H), 3.21 (3H, s, methoxyl), 4.9 (1H, m, C-3-H), 5.4 (1H, m, C-6-H) and 7.8 ppm (5H, m, aromatic-Hs); m/e, M-C_6H_5COOH, 412.3314 ($C_{28}H_{44}O_2$ requires 412.3341)

12. The nearly identical conclusion was independently obtained with the yellow mealworm, *Tenebrio molitor*: a private communication from Dr. H. H. Rees, University of Liverpool. We thank Dr. Rees for sending us the manuscript before publication.