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MECHANISM OF STIGMASTEROL DEALKYLATION IN INSECT

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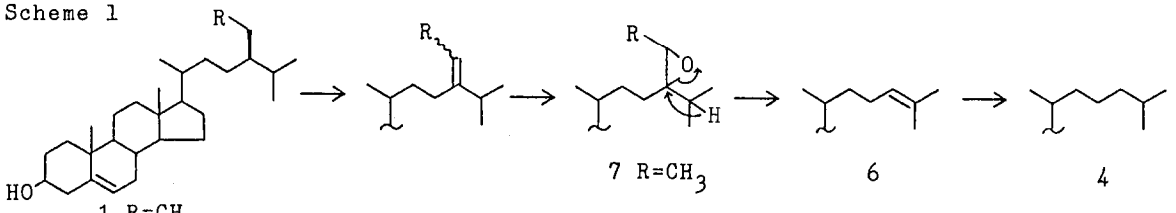
Abstract: Deuterated stigmasterols (10, 11 and 12) were chemically synthesized and fed to silkworm larvae. GC-MS analysis of the metabolites, cholesterol (4) and desmosterol (6), indicates the migration of 25-hydrogen to C-24 position during stigmasterol dealkylation. 22,24(28)-Dienes (8a and 8b) were shown to be converted to 22,24-diene (5), desmosterol and cholesterol.

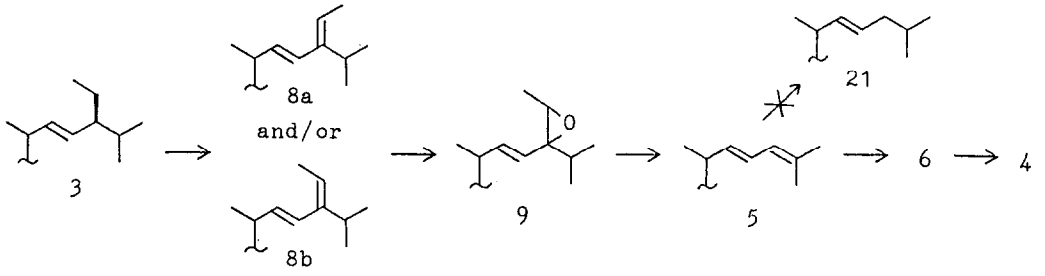
It is well known that phytophagous insects can dealkylatively metabolize plant sterol, e.g. sitosterol (1), campesterol (2), and stigmasterol (3) into cholesterol (4). The dealkylation is one of the essential metabolism in insect which has no capacity of de novo sterol biosynthesis. Conversion of sitosterol into cholesterol has been established to proceed according to scheme 1 ($R=CH_3$),[1] in which a characteristic feature is fragmentation reaction of the 24,28-epoxide (7) involving 25-H migration to C-24.[2] Similar mechanism (scheme 1, R=H) seems also to be operated in the demethylation of campesterol.[3] By analogy, scheme 2 can be considered as a probable deethylation route of stigmasterol (3). However, identification of cholesta-5,22,24-trien-3β-ol[4] (5) and desmosterol[5] (6) from Manduca sexta has, so far been almost the sole evidence for supporting the postulation.

In this paper, the fate of C-25 as well as C-24 and C-23 hydrogens during the dealkylation of stigmasterol in the silkworm Bombyx mori was described. In addition, the $\Delta^{24(28)}$ compounds,

Scheme 1



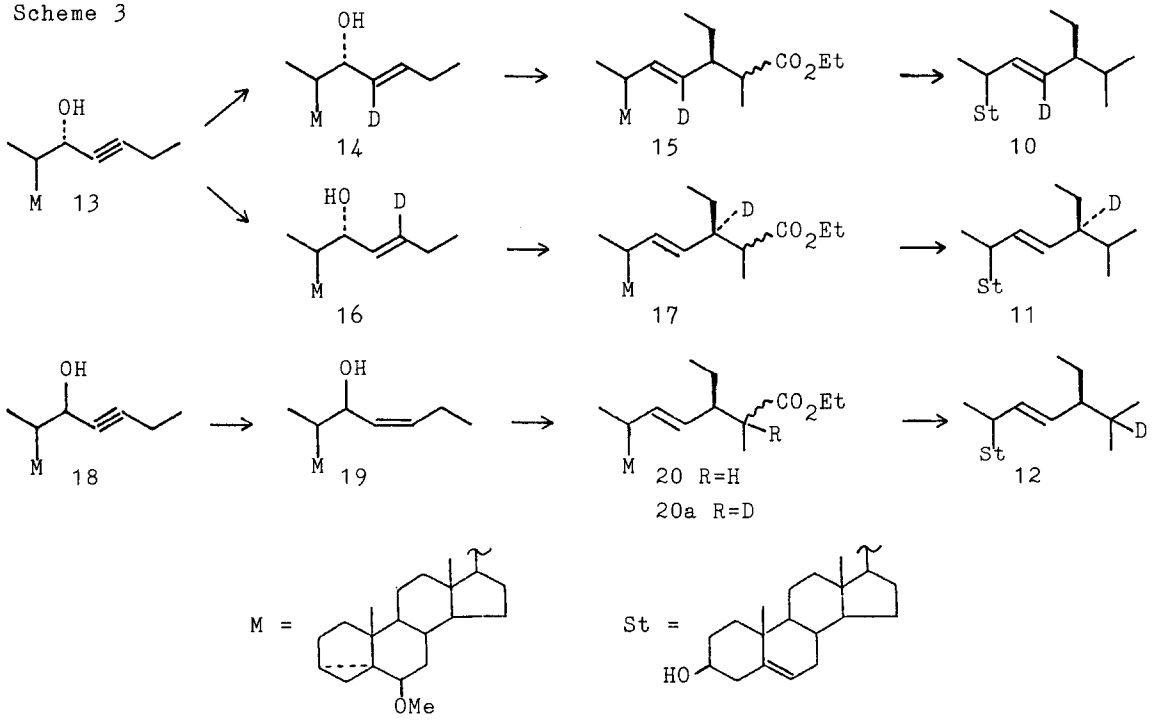
Scheme 2



8a and 8b, possible precursors of the epoxide 9, were examined for supporting the growth and development of the silkowrm.

The required labelled materials, [23-D]-, [24-D]-, and [25-D]-stigmasterols (10, 11 and 12) were synthesized via the orthoester Claisen rearrangement according to scheme 3. The synthesis of 10 and 11 was started with the known (22S)-acetylenic alcohol 13.[6] Reduction of 13 with $LiAlD_4$ in the presence of $NaOCH_3$[7] followed by quenching with H_2O afforded [23-D]-(22R)-allylic alcohol 14 (64%). The position of the deuterium introduced was established by ^1H NMR (5.35, 24-H, broad t, J=7.5 Hz). Compound 14 was rearranged, upon treatment with triethyl orthopropionate/propionic acid, to [23-D]-(24R)-ester 15 as the mixture at C-25 position (98%).[6] Succesive treatment of 15 with $LiAlH_4$, MsCl-pyridine, $LiAlH_4$, and p-TsOH furnished [23-D]-stigmasterol (10) (55% from 14), mp 167-170°C. By changing the deuteration reagent into $LiAlH_4(NaOCH_3)/D_2O$, [24-D]-(22R)-allylic alcohol 16 (^1H NMR, 5.42, 23-H, broad d, J=7.5 Hz) was obtained from 13 in 54% yield. Compound 16 was similarly rearranged to [24-D]-(24S)-ester 17 (79%), which was converted into [24-D]-stigmasterol (11), mp 168-171°C as described above. Deuterium content was determined more than 98% for 10 and 11 by GC-MS analysis. The synthesis of 12 was initiated with the epimeric (22R)-acetylenic alcohol 18.[6] (22S)-Allylic alcohol 19, obtained by hydrogenation of 18 (Lindlar, quinoline, 100% yield), was rearranged to the (24R)-ester 20 (70%) as reported.[6] The introduction of deuterium at C-25 position of 20 was effected by treating with 3 eq. of lithium diisopropylamide in THF at -78°C followed by quenching with D_2O.[8] GC-MS analysis of the deuterated ester 20a showed that the content of deuterium is

Scheme 3



approximately 70%. Transformation of 20a into [25-D]-stigmasterol (12), mp 168-171°C, was carried out as described above.

The artificial diet containing 10, 11 and 12 (0.1%) was prepared and the newly hatched silkworm larvae (30 heads for each group) were reared on them for 17 days as previously reported.[9] They grew to reach the third instar normally as fed with stigmasterol. The insect sterol was extracted and analyzed as reported.[10] The major insect sterols in each run were the dietary deuterated stigamsterol (ca. 50% of total sterol) and the metabolically produced cholesterol (ca. 40%). The molecular ion peaks of the cholesterols (TMS ether) produced from 10, 11, and 12 by insect were found to be m/z 459, 458, and 459, respectively.[11] These values mean that cholesterols contain one, zero, and one deuterium in the molecule, respectively. The fragment ions M-15, M-90, and M-129 also supported the conclusion. These results indicated that the C-24 hydrogen is lost, while the C-23 and C-25 hydrogens remained during this dealkylation. Considering that desmosterol is the intermediate of the dealkylation, the deuterium originally attached to the C-25 position must migrate, probably to C-24 position. This was supported by M^+ of desmosterol (TMS ether, m/z 457, 456, and 457 from the [D]-stigmasterols 10, 11, and 12, respectively), one of the minor insect sterols. The structures of these metabolically produced cholesterols and desmosterols would be the depicted ones in Fig. 1.

The observed loss of C-24 hydrogen suggested intermediacy of the $\Delta^{24(28)}$-compound 8. Therefore, the E-diene 8a and Z-diene 8b were synthesized[12] and examined for the ability to support the silworm growth. As expected, the both isomers were found to support normal growth of the larvae. They grew up to the third instar in 12 days. The insect sterol reared on 8a and 8b was analyzed by GC-MS as reported previously.[10] The GLC showed principally the same profile for both cases, one of which is illustrated in Fig. 2. The diet sterol 8a, the metabolically produced cholesterol (4), and more importantly cholesta-5,22,24-trien-3β-ol[13] (5) and desmosterol (6) were identified. The identification of the latter two sterols is parallel to Svoboda's earlier observations.[4,5] The possibility of an alternative pathway (i.e. 5 → 22-dehydrocholesterol (21) → 4) would be excluded since any trace amount of 21 could not

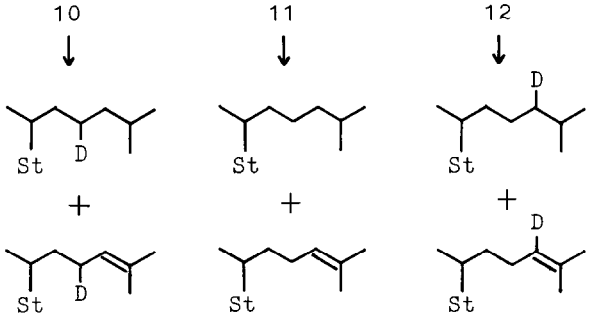


Fig. 1

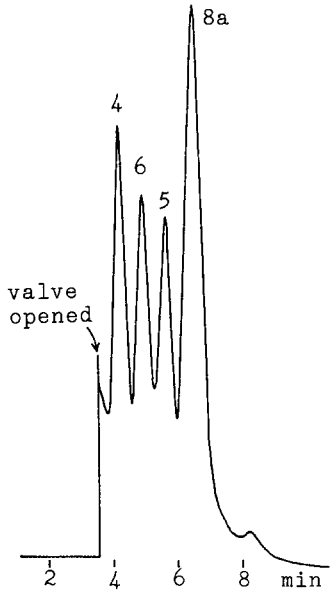


Fig. 2. GC-MS of the insect sterol reared on E-diene 8a (analyzed as the TMS ether).

be detected on mass fragmentgraphic analysis of insect sterol reared on either 3, 8a, 8b or 5.

The present works provide the substantial basis for linking the intermediates of stigmasterol dealkylation and strongly support the mechanism depicted in scheme 2.

References and Notes

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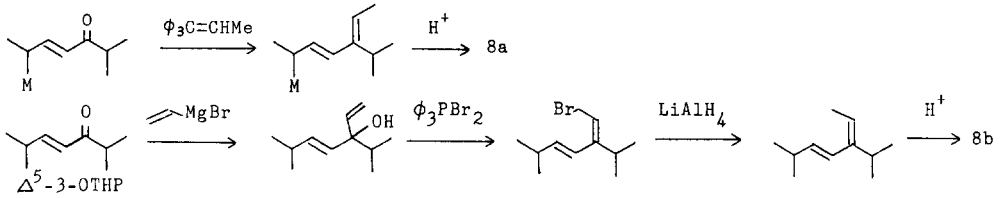
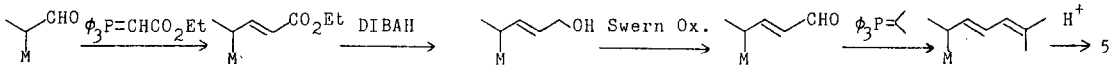
11) Cholesterol TMS ether from 12 contained ca. 70% of deuterium as expected.

12) The olefins were synthesized as follows (Cf. M. Fryberg, A. C. Oehlschlager, A. M. Unrau, Tetrahedron, 27, 1261 (1971); W. Sucrow and B. Raduchel, Chem. Ber., 103, 2711 (1970)).



13) The diene was synthesized as follows. For the previous synthesis see, R. F. N. Hutchins, M. J. Thompson, and J. A. Svoboda, Steroids, 15, 113 (1970). MS m/z of the TMS ether of 5: 454, 439, 372, 343, 262, 255, 253, and 109.



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