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MECHANISM OF CAMPESTEROL DEMETHYLATION IN INSECT

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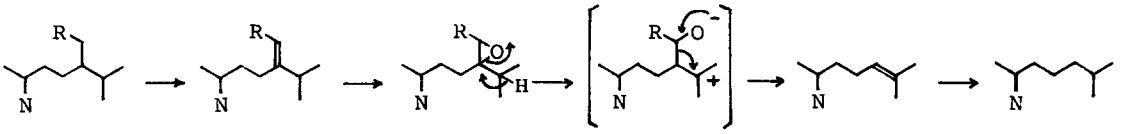
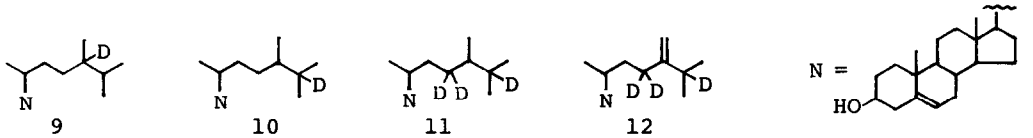
Summary: [24ξ-^{2}H]-, [25-^{2}H]-, and [23,23,25-$^2H_3$]-24ξ-methylcholesterol as well as [23,23,25-$^2H_3$]-24-methylenecholesterol were metabolized in the silkworm Bombyx mori to cholesterol containing zero, one, three and three deuterium, respectively.

Phytophagous insect are capable of dealkylation of plant sterols, e.g. sitosterol(1) and campesterol(2) to convert into cholesterol(8). We have previously shown that deethylation of sitosterol in the silkworm Bombyx mori proceeds through fucosterol(3), its epoxide(5) and desmosterol(7), with migration of 25-hydrogen to the C-24 position.[1,2] There is evidence that 24-methylenecholesterol (4) and desmosterol(7) are intermediates in the transformation of campesterol into cholesterol in Manduca sexta.[3] However, 24-methylenecholesterol epoxide(6) was shown to be an inadequate nutrient for the silkworm growth and development, inducing a doubt on the intermediacy of this epoxide in campesterol demethylation.[4]

In order to obtain a further insight into campesterol demethylation, we have now synthesized [24-^{2}H]-, [25-^{2}H]-, and [23,23,25-$^2H_3$]-24ξ-methylcholesterol(9, 10, and 11) as well as [23,23,25-$^2H_3$]-24-methylenecholesterol(12), and their metabolism in the silkworm was examined.



1 R=Me 3 R=Me 5 R=Me 7 8
2 R=H 4 R=H 6 R=H



9 10 11 12

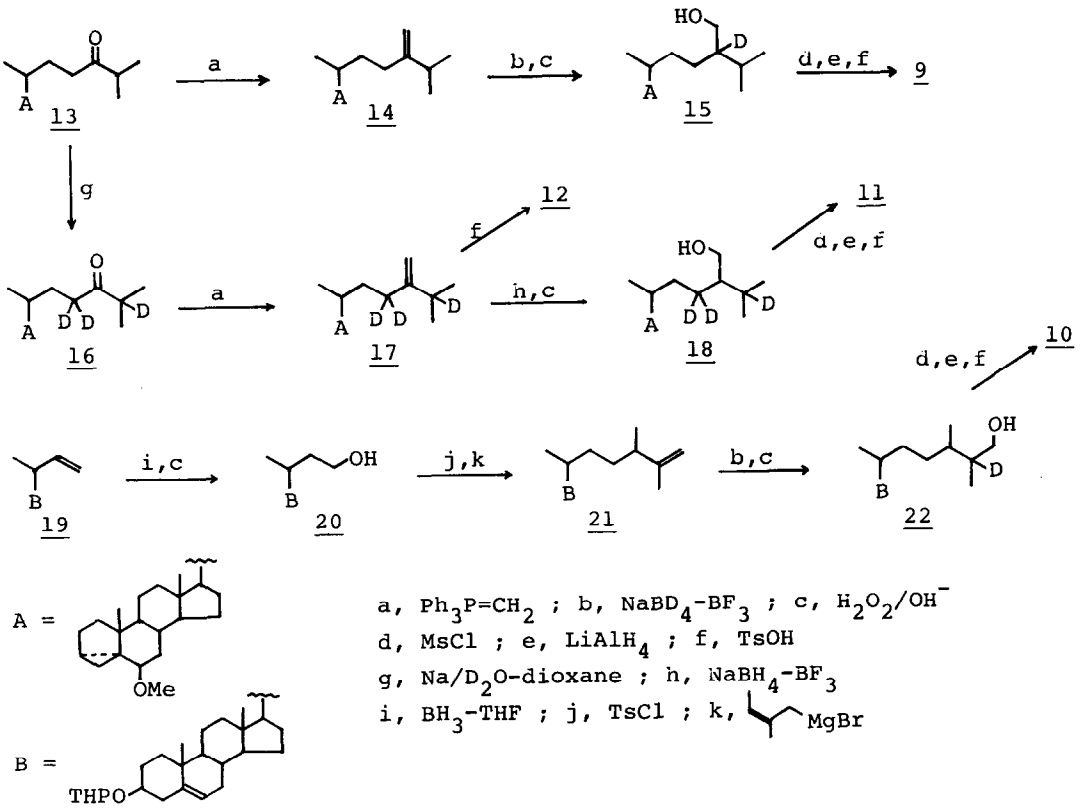
The 3,5-cyclo-6-methoxy derivative of 24-oxocholesterol(13) was reacted with the ylide prepared from triphenylmethylphosphonium iodide with BuLi in tetrahydrofuran(THF) in a sealed tube(100°C, 1h) to give the 24-methylene compound 14 in 45% yield. This was submitted to deuteroboration($NaBD_4/BF_3$ etherate in THF, 0°C, 10 min and then 15°C, 2.5h)-oxidation(30% H_2O_2/10% NaOH, 15°C, 1h) to afford the 24-D-28-ol 15 in 54% yield. Deoxygenation of 15 was effected by mesylation(MsCl/pyridine, 0°C, 15h) followed by reduction with $LiAlH_4$ in THF (reflux, 1.5h). The subsequent acid treatment(p-TsOH/H_2O/dioxane, reflux, 2h) produced [24-^{2}H]-24ξ-methylcholesterol(9, 30%), mp 155.5-156.5°C, m/z 401(M^+). That the compound 9 is an almost 1 : 1 mixture of the C-24 epimers was indicated by ^{1}H NMR[5] and also by ^{13}C NMR[6], in which a pair of signals appeared at 35.9, 36.2 ppm(C-20);18.7,18.9(C-21);30.1,30.5(C-23);31.3,32.3(C-25);17.5,18.2(C-26) and 20.2, 20.5(C-27). ^{13}C NMR also ascertained the location of deuterium by the absence of signal at 38.9 ppm which is assignable to C-24 of campesterol.

Introduction of deuterium at C-23,25 was accomplished by refluxing 13 with a mixture of sodium, D_2O and dioxane for 15h to afford 16, m/z 417(M^+). Wittig methylenation followed by acid treatment in the same manner as described for 13 afforded [23,23,25-$^2H_3$]-24-methylenecholesterol(12), mp 139.5-141°C. The corresponding trimethylsilyl(TMS) ether showed m/z 473(M^+) and the molecule containing three atoms of deuterium was more than 95%.

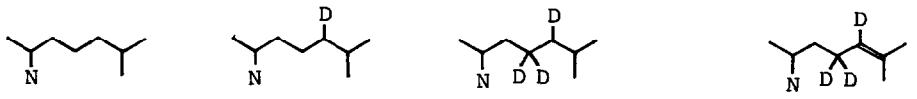
Catalytic hydrogenation(10% Pd-C in ethyl acetate) of 17 induced a loss of deuterium, and therefore hydroboration-oxidation and the subsequent deoxygenation in the same manner as described for 14 were used to prepare [23,23,25-$^2H_3$]-24ξ-methylcholesterol(11, 67%), mp 156.5-157.5°C. Its TMS ether showed m/z 475(M^+).

Preparation of 25-D-24-methylcholesterol was initiated with hydroboration (BH_3-THF complex, 0°C, 1h)-oxidation(30% H_2O_2/3N NaOH, 0°C, 0.5h) of 24-norchola-5,22-dien-3β-ol tetrahydropyranyl ether(19) which was synthesized by oxydative decarboxylation of 3β-acetoxycholenic acid[7] followed by exchanging the protective group at the C-3 position. The resulting 23-ol 20(91%), mp 139-143°C, was converted into the corresponding tosylate and this was coupled with 2-methyl-2-butenyl magnesium bromide in THF(-20°C, 2h, and then 15°C, 15h) to give the Δ^{25}-olefin 21 in 23% yield. Deuteroboration($NaBD_4/BF_3$ etherate in THF, 0°C, 1h)-oxidation(30% H_2O_2/3N NaOH, 15°C, 1h) of 21 yielded 25-D-26-ol 22 in 55% yield. Mesylation of 22, $LiAlH_4$ reduction and then acid treatment(p-TsOH/CH_2Cl_2/MeOH, 15°C, 0.5h) gave [25-^{2}H]-24ξ-methylcholesterol(10, 73%), mp 154-154.5°C, m/z 401 (M^+). Its ^{13}C NMR showed a similar spectrum as that of 9 except an extra pair of signal being appeared at 38.7; 38.9 ppm(C-24) instead of the C-25 signal.

These deuterated sterols 9-12 were added in 0.1% to an artificial diet, on which newly hatched silkworm larvae were reared.[4] They growed and developed at almost the same rate as the insects fed control diet containing cholesterol or 24ξ-methylcholesterol[8] as the sole sterol source. On day 15th after hatch-



ing, insects were sacrificed and the non-saponifiable fraction of lipid extracts was treated with trimethylsilylimidazole to give sterol TMS ethers. These were analyzed with gas chromatography-mass spectrometry as described previously.[9] The principal insect sterols in each case, were the respective dietary sterol and the metabolically produced cholesterol. Molecular ion peaks of cholesterol TMS ether came from 9, 10, 11 and 12 had m/z 458, 459, 461 and 461, respectively. These values are corresponding to the cholesterol containing zero, one, three and three deuterium atoms in a molecule, respectively. The fragment ions of M-15, M-90, M-15-90 and M-129 also confirmed the results. From the insects fed 11 and 12, a small amount(ca. 5% of total sterol) of desmosterol was detected. The desmosterol contained three atoms of deuterium as evidenced from m/z 459(M^+), 444(M-15), 369(M-90), 354(M-15-90) and 330(M-129). These results indicated that the C-24 hydrogen is lost, while the C-23 and C-25 hydrogens are retained during campesterol demethylation ; the C-25 hydrogen must migrate, probably to C-24. Probable structures of the metabolically produced cholesterol and desmosterol may be as depicted below.



from 9 from 10 from 11 and 12 from 11 and 12

Thus, campesterol would be dealkylated through 24-methylenecholesterol(4), and its epoxide 6, with migration of 25-H to the C-24 position to produce desmosterol(7) and finally to cholesterol(8). The intermediacy of the epoxide 6 has recently been shown in Shistocerca midgut microsomes.[10] Alternative pathway involving successive oxidation of the 24-methyl group followed by deformylation or decarboxylation analogous to lanosterol[11] and androgens demethylation[12], is incompatible with the present results.[13]

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8. 24ξ-Methylcholesterol, mp 151-153°C was prepared by the same method as described for 9, except $NaBH_4/BF_3$ etherate being used instead of $NaBD_4/BF_3$ etherate. It should be noted that this compound was an effective nutrient as 24(R)-methylcholesterol(campesterol). Indiscrimination of the C-24 stereoisomers as the silkworm nutrient has already been observed with 24-ethylcholesterol(sitosterol and clionasterol). See, Y.Fujimoto, M.Morisaki and N.Ikekawa, Biochemistry, 19, 1065(1980).
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13. Our preliminary data indicated that the 24-CH_2OH, 24-CHO and 24-COOH compounds didn't satisfy the silkworm sterol requirement.

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