Role of Cholesterol 10-Methyl Group and Effect of "Extra" 14-Methyl Group on Silkworm Growth and Development

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In order to establish the functional importance of the 10-methyl group of cholesterol and the planarity of the steroid ring, silkworms (*Bombyx mori*) were reared on an artificial diet containing 19-norcholesterol (1), 14α-methylcholesterol (3) or 19,19-difluorocholesterol (2). The former two sterols (1 and 3) only partially satisfied the silkworm sterol requirement; growth and development were seriously retarded. The fluorinated sterol (2) was much more deleterious and was totally inadequate in meeting the sterol requirement.

Keywords cholesterol; silkworm; structure–function relationship; 19-norcholesterol; 19,19-difluorocholesterol; 14-methylcholesterol

Cholesterol occurs widely in animal cells and act as a biogenetic precursor of various steroid hormones and bile acids. It also functions as a modulator of the properties of membranes and/or a regulator of cell growth.[1] These biological functions of cholesterol should be intimately related to its unique chemical structure. For studies on the structure–function relationship of cholesterol, the silkworm *Bombyx mori* is convenient because it is incapable of *de novo* sterol synthesis and hence requires appropriate sterols for normal growth and development. Our previous studies along these lines have indicated the structural importance of the 3β-hydroxy-5-ene moiety,[2] the length of the side chain[3] and the stereochemistry at the carbon 20 position.[4] In the present experiments, we have tested three sterols, 19-norcholesterol (**1**), 19,19-difluorocholesterol (**2**) and 14α-methylcholesterol (**3**) in order to examine the functional role of the 10-methyl group of cholesterol and the effect of an "extra" methyl group at the carbon 14 position. The 14α-methyl group of 14α-methylcholesterol protrudes from the planar steroid ring system, and, in the cell membrane, might prevent interaction with the fatty acyl chain, resulting in some deleterious effect on the silkworm growth and development.

19-Norcholesterol (**1**)[5] and 14α-methylcholesterol (**3**)[6] were prepared by the known methods. 19,19-Difluorocholesterol (**2**) was synthesized by fluorination of 19-oxocho-

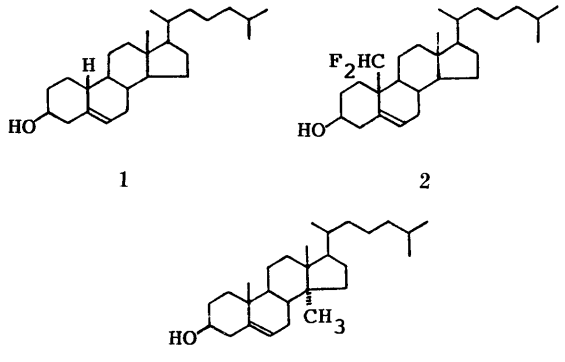

Chart 1

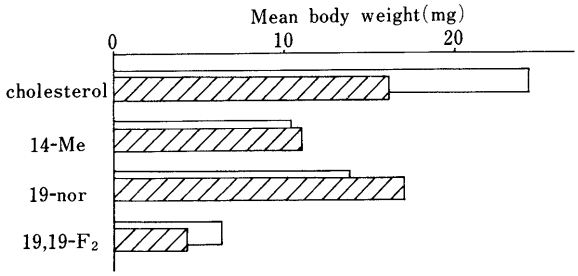

Fig. 1. Mean Body Weight of Silkworm Larvae 11 d after Hatching (▨), and on 22 d after Hatching (□).

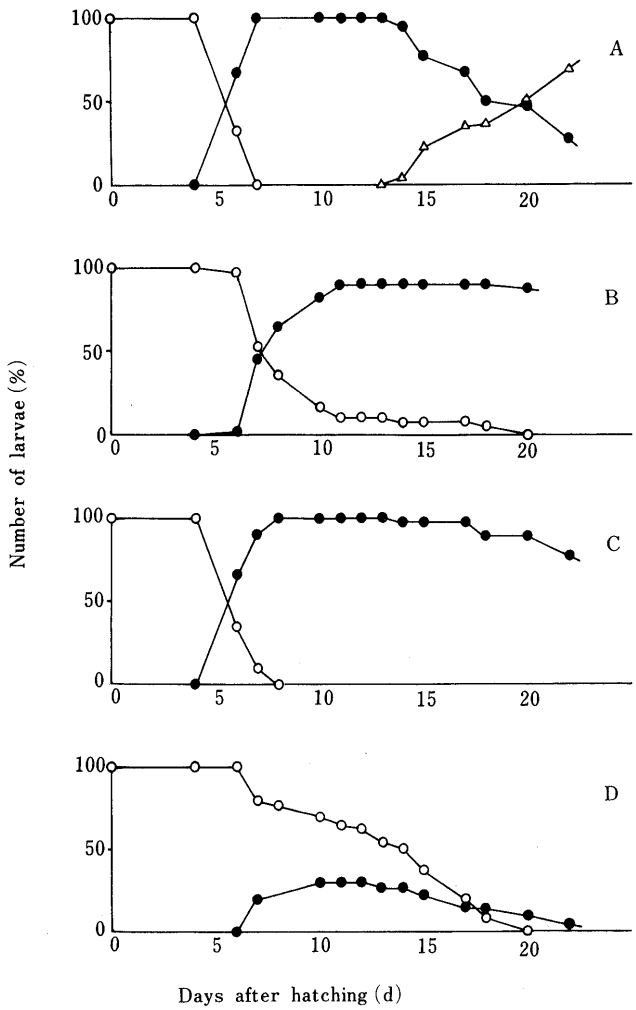

Fig. 2. Development of Silkworm Larvae fed Cholesterol (A), 14α-Methylcholesterol (B), 19-Norcholesterol (C) and 19,19-Difluorocholesterol (D)

The numbers of larvae of the first instar (○), the second instar (●) and the third instar (△) are shown as percent of 40 larvae.

lesteryl acetate with diethylaminosulfur trifluoride,[7] followed by basic hydrolysis. For biological experiments, newly hatched larvae of the silkworm *Bombyx mori* were fed with an artificial diet containing the synthetic sterols (**1**, **2** and **3**), and their growth and development were compared with those of control larvae fed cholesterol, in the same manner as described previously.[2-4]

It can be seen from Fig. 1 that the mean body weights of the larvae reared on the test sterols (**1**, **2** and **3**) were considerably lower than that of the control group. The growth retardation was most evidently observed with 19, 19-difluorocholesterol. The rates of development of the insects fed these sterols are shown in Fig. 2. The larvae fed cholesterol or 19-norcholesterol reached to the second instar 5—8 d after hatching, whereas those fed 14α-methylcholesterol developed to the second instar more slowly, and some of them (*ca.* 15%) remained in the first instar even at 20 d after hatching. 19,19-Difluoro-cholesterol was highly deleterious as indicated by the fact that more than half of the larvae fed this sterol were unable to develop to the second instar and 90% of them died within 20 d after hatching. These results suggest that 19,19-difluorocholesterol and 19-norcholesterol are unable to substitute for cholesterol to maintain the normal growth and development of the silkworm. Thus, the 10-methyl group of cholesterol is important for eliciting its biological function, presumably through attractive van der Waals interaction with fatty acyl chain of membrane phospholipid. The 10-hydrogen atom of 19-norcholesterol and the difluoromethyl group of 19,19-difluorocholesterol may be too small, or too electronegative, respectively. It is also clear that 14α-methylcholesterol only partially satisfies the silkworm sterol requirement. As postulated above, protrusion of the 14α-methyl group from the plane of the steroid ring may account for the observed deleterious effect, suggesting the functional importance of the planarity of cholesterol structure. However, the possibility that the 14α-methyl group blocks 14α-hydroxylation, which is one of the essential steps of ecdysone biosynthesis, should also be considered.

Experimental

Sterols Synthesis of 14α-methylcholesterol (**3**) was described previously.[6] 19-Norcholesterol (**1**) was prepared by the literature method.[5] 19,19-Difluorocholesterol (**2**) was prepared from the corresponding acetate[7] by saponification as follows. A mixture of the acetate (26 mg) and 10% methanolic KOH (1.5 ml) was stirred at ambient temperature for 40 min. Extraction with dichloromethane followed by washing of the extract with water and drying over magnesium sulfate, gave 19,19-difluoro-cholest-5-en-3β-ol (16 mg). mp 116—118 °C (from methanol). ^{1}H-NMR ($CDCl_3$) δ: 0.70 (3H, s, 13-Me), 0.86 (3H, d, J=6.5 Hz, 20-Me), 3.6 (1H, m, 3-H), 5.73 (1H, m, 6-H), 5.91 (2H, t, J=57 Hz, 19-H_2). MS m/z (relative intensity): 422 (100), 404 (11), 371 (57), 353 (71), 309 (14), 267 (32), 249 (14), 213 (42).

Insect Rearing The newly hatched larvae (40 larvae in each group) of the silkworm *Bombyx mori* were reared on an artificial diet containing 0.1% sterol as described previously.[2-4]

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