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Effects of Cholesterol Analogs on Cholesterol Biosynthesis from Lanosterol[1)]

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Cholesterol biosynthesis was examined with rat hepatic subcellular $10000 \times g$ supernatant fraction incubated with [24-^{3}H]-lanosterol in the presence of one of twelve cholesterol analogs (**1**—**12**) including sitosterol. Cholesterol analogs (40 μM) with different sizes of side chains exhibited very minor inhibitory effects (2—7%) compared with that of cholesterol (21%) on the synthesis of cholesterol from [24-^{3}H]-lanosterol (18 μM). The structure–inhibitory activity relationship of cholesterol analogs is discussed.

Keywords——cholesterol biosynthesis; [24-^{3}H]-lanosterol; cholesterol analog; rat hepatic subcellular preparation; inhibitory activity

In the previous paper[2)] of this series, we reported that cholesterol biosynthesis from lanosterol in rat hepatic subcellular preparations is strongly inhibited by some lanosterol analogs with shorter side chains. Among the tested compounds, 27-nor-24,25-dihydrolanosterol, which can be considered as one of the least modified analogs of 24,25-dihydrolanosterol, was the most potent inhibitor. Inhibitory activities decreased successively with shortening of the chain length, and the analog without a side chain, octanordihydrolanosterol, had almost no effect. These results strongly suggested the importance of the side chain structure for inhibitory activity in the lanosterol analogs. In order to determine whether chemical structures other than the side chain could also be involved in the inhibitory activity, side-chain-modified cholesterol analogs, whose ring structure is different from that of lanosterol, are intriguing candidates for testing.

This paper describes studies on the effects of twelve cholesterol analogs (**1**—**12**) with shorter or longer side chains than that of cholesterol on cholesterol biosynthesis from [24-^{3}H]-lanosterol in rat hepatic subcellular $10000 \times g$ supernatant (S_{10}) fraction. It is demonstrated that cholesterol itself has a marked inhibitory effect (21%), but the cholesterol analogs showed no significant inhibitory effects compared with those of cholesterol and the previously described[2)] lanosterol analogs.

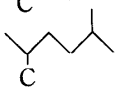
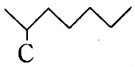
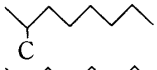
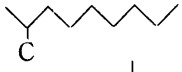
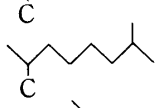
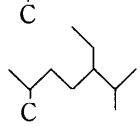
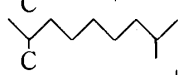
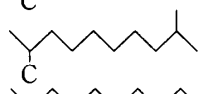
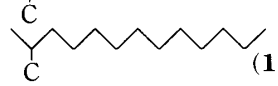
Materials and Methods

The tested cholesterol analogs[3)] and the substrate, [24-^{3}H]-lanosterol,[2)] were prepared as described previously. Experiments for examining the effects of the test compounds on cholesterol biosynthesis from 18 μM [24-^{3}H]-lanosterol were performed at a concentration of 40 μM cholesterol analog in rat hepatic subcellular fraction as described previously.[2)]

Results and Discussion

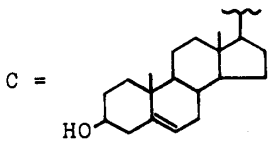
Twelve cholesterol analogs (**1**—**12**) including sitosterol were investigated to determine their possible inhibitory effects on the synthesis of cholesterol (as digitonin-precipitable sterols) from labeled lanosterol. The results, shown in Table I, indicate that cholesterol analogs with various sizes of side chain were less active (2—7%) than cholesterol (21%) in inhibiting the conversion of lanosterol to cholesterol, and their effects were minor regardless of the size of the side chain. This result suggests that the eight-carbon side chain structure of

TABLE I. Cholesterol Biosynthesis during Incubation of S_{10} Fraction of Rat Liver Homogenate with [24-^{3}H]-Lanosterol in the Presence of Cholesterol Analogs

Compound		Lanosterol Fr. (%)	Cholesterol Fr. (%)	Inhibition (%)
None (control)		23.0	22.9	—
	(**1**)	22.1	21.5	6
	(**2**)	22.4	22.1	3
	(**3**)	22.4	21.7	5
	(**4**)	20.8	21.4	7
	(**5**)	24.8	21.5	6
	(**6**)	23.3	22.5	2
	(**7**)	19.2	22.5	2
	(**8**)	19.6	22.0	4
	(**9**)	19.3	21.6	6
	(**10**)	18.4	21.5	6
	(**11**)	18.8	21.5	6
	(**12**)	21.4	21.8	5
Cholesterol[a]		35.2	18.2	21

[24-^{3}H]-Lanosterol (90600 dpm; 0.43 μCi/μmol) was incubated with rat liver S_{10} fraction (19.5-20.5 mg protein/ml) at 37 °C for 3 h. The incubation mixture contained, in a total volume of 5 ml, 4 ml of S_{10} fraction and cofactors. Incubation was started by the addition of the substrate and test compounds as an emulsion (0.1 ml) with Tween 80 (3 mg).[7] Analytic methods for incubation products and the calculation of the percentage inhibition were described previously.[2] Each incubation was carried out in triplicate and the standard deviation of each value listed was less than 5 percent.

a) This compound was tested as a reference; the result was somewhat different from that reported[2] previously.



cholesterol is critical for the inhibitory activity and even slight deviations from this structure markedly decrease the inhibitory activity.

When compared to the previous results,[2] it is evident that cholesterol analogs (**1**, **2**, and **5**) are less inhibitory than the corresponding lanosterol analogs. For example, 27-nor-24,25-dihydrolanosterol[2] showed 81% inhibition but 27-norcholesterol (**5**) showed only 6% inhibition. These results suggested that the steroid skeleton structure is also important for the inhibitory effect. Raulston *et al.*[4] have shown that in cell-free homogenate preparations of rat liver,

14α-ethyl-5α-cholest-7-ene-3β,15α-diol inhibits cholesterol biosynthesis from acetate, resulting in a marked accumulation of lanosterol and 24,25-dihydrolanosterol, and Miller *et al.*[5] have proved that various cholesterol analogs without the 14α-ethyl group have no effect on cholesterol biosynthesis from acetate upon incubation with cell-free homogenate preparations of rat liver, though they are potent inhibitors of sterol synthesis in cultured mammalian cells.[6]

In summary, it may be concluded that both the side chain structures and 14α-alkylated steroidal skeleton structure are critically important for inhibitory effect on cholesterol synthesis from lanosterol.

References and Notes

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