GANODERIC ACID AND ITS DERIVATIVES AS CHOLESTEROL SYNTHESIS INHIBITORS

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Oxygenated lanosterol derivatives, which were isolated from Ganoderma lucidum (Polyporaceae) or their derivatives obtained by chemical conversion, were tested for their effect on cholesterol biosynthesis from 24,25-dihydrolanosterol by rat hepatic subcellular 10,000 x g supernatant fraction. The sterol (VI, 40 μM) with 7-oxo and 15α-hydroxy groups potently inhibited the synthesis of cholesterol from [24,25-^{3}H]-24,25-dihydrolanosterol (18 μM).

KEYWORDS cholesterol synthesis; Ganoderma lucidum; oxygenated lanosterol derivative; [24,25-^{3}H]-24,25-dihydrolanosterol; ganoderic acid derivative; inhibitory activity; chemical conversion

Cholesterol biosynthesis is regulated by a variety of compounds. The mechanism of the regulation has been extensively studied, especially to develop an agent for the treatment of atherosclerosis and heart disease. Many oxygenated sterols have been found to be potent inhibitors of sterol synthesis in animal cells in culture, and the main site of action appears to be at the level of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase.[1] However, since mevalonic acid (MVA) is a common precursor for many isoprenoids, blocking of MVA formation may induce undesired side effects besides inhibiting sterol synthesis. More specific inhibition of cholesterol synthesis may be attained by inhibition at some later stage of cholesterol synthesis.

Recently, we reported the effects of lanosterol derivatives and cholesterol derivatives[2] on cholesterol biosynthesis from lanosterol or 24,25-dihydrolanosterol. Among the tested compounds 7-oxolanost-8-en-3β-ol (7-oxo-DHL), 14α-methylcholest-7-ene-3β,15α-diol, and lanost-7-ene-3β,15α-diol have been shown to be potent inhibitors of cholesterol biosynthesis from lanosterol or 24,25-dihydrolanosterol in rat hepatic subcellular fraction. The results suggested that the site of inhibition is lanosterol 14-demethylation, which is catalyzed by a cytochrome P-450$_{14DM}$.[3] We showed that 7-oxo-DHL was the inhibitor of DHL 14-demethylation by a reconstituted system containing a partially purified cytochrome P-450$_{14DM}$ from rat liver microsomes.[4] Further, we demonstrated with yeast cytochrome P-450$_{14DM}$ that 7-oxo-DHL has two characteristics as lanosterol 14-demethylase inhibitor: it is a typical competitive inhibitor and it acts as an inhibitor of electron trasfer to the oxyferro intermediate.[5]

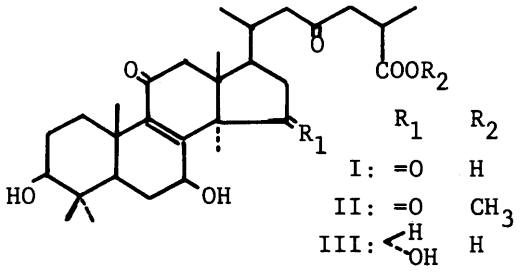
It seems that 7-oxo or 15α-hydroxy groups may be very important functional groups with potent inhibitory activity. We have been interested in the importance of these oxygenated groups. Therefore, we intended to test the biological activity of the compounds that have the oxygenated groups on both 7 and 15 positions on the same skeleton.

On the other hand, the fungus Ganoderma lucidum (Polyporaceae) has various pharmacological effects and has long been used as a home remedy. Recently, a lot of highly oxygenated lanostanoid triterpenes have been isolated from G. lucidum.[6-32] Most of them taste bitter. Some of them are cytotoxic,[7] inhibit histamine release from rat mast cells,[9] or inhibit angiotensin converting enzyme.[18] Many triterpenes of G. lucidum, including ganoderic acid B (I) and ganoderic acid C (III), have oxygenated groups on both the 7 and 15-positions. Therefore, I and the derivatives of I and III obtained by chemical conversion were selected to test their inhibitory effects on cholesterol biosynthesis from 24,25-dihydrolanosterol.

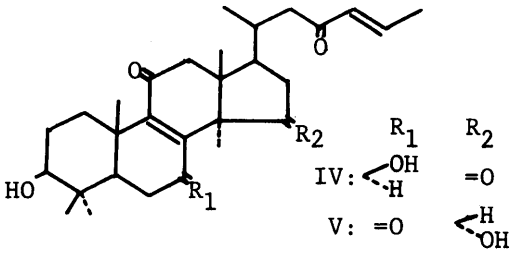
SYNTHESIS OF GANODERIC ACID DERIVATIVES

Ganoderic acid B methyl ester (II) was obtained by treating I with ethereal diazomethane.[9] Decarboxylated compounds (IV) and (V) were synthesized by the reaction of I and III with lead tetraacetate in the presence of cupric acetate containing a drop of pyridine in refluxing benzene. In the case of V derived from III, the 7β-hydroxyl group was further oxidized to the carbonyl group. IV, colorless plates (ethyl acetate), mp 193-195°C, $C_{29}H_{42}O_5$, m/z 470 (M^+). ^{1}H-NMR ($CDCl_3$, δ, J in Hz), 6.85 (1H, double q, J=6.6 and 15.5, 25-H), 6.13 (1H, double q, J=1.7 and 15.5, 24-H, 4.80 (1H, 7-H), 3.21 (1H, 3-H) and 1.91(3H, double d, J=1.7 and 6.6, 27-H). V, pale yellow needles (ethanol), mp 184-186°C, $C_{29}H_{42}O_5$, m/z 470 (M^+), ^{1}H-NMR ($CDCl_3$, δ, J in Hz), 6.84 (1H, double q, J=6.9 and 15.8, 25-H), 6.12 (1H, double q, J=1.7 and 15.8, 24-H), 4.35 (1H, 15-H), 3.28 (1H, 3-H) and 1.90 (3H, double d, J=1.7 and 6.9, 27-H).

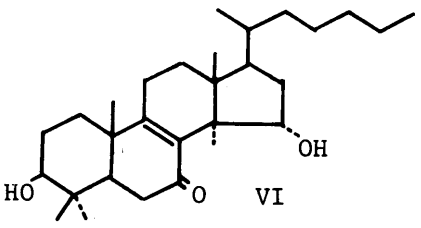
The hydroxyl groups of ganoderic acid C methyl ester[7] were protected to yield the tri MEM derivative. The tri MEM derivative reacted under Wolff-Kishner conditions (i, 80% hydrazine hydrate/diethylene glycol, 190°C, 1 h; ii, Na, 200-210°C, 3 h) to afford the 7-oxo-11-deoxo derivative, which was further treated successively by decarboxylation under the same conditions described above, catalytic reduction (palladium charcoal, ethanol, r.t., 7 h), and deprotection of the hydroxyl groups ($TiCl_4$, CH_2Cl_2, 0°C, 1/2 h) to give the final product (VI), colorless plates, mp 155-157°C, $C_{29}H_{48}O_3$, m/z 444 (M^+), ^{1}H-NMR ($CDCl_3$, δ, J in Hz), 4.35 (1H, double d, J=5.0 and 9.0, 15-H), 3.24 (1H, double d, J=5.4 and 11.0, 3-H), 3.01 (1H, double t, J=3.4 and 13.5, 11-H), 2.72 (1H, broad d, J=16.6, 6-H) and 2.43 (1H, d, J=16.6, 6-H).



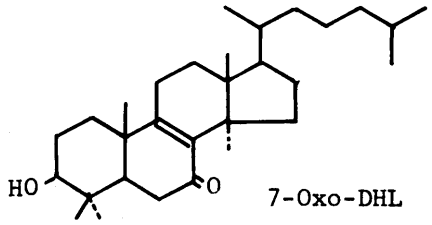
	R_1	R_2
I:	=O	H
II:	=O	CH_3
III:	H, OH	H



	R_1	R_2
IV:	OH, H	=O
V:	=O	H, OH



VI



7-Oxo-DHL

EFFECTS OF GANODERIC ACID DERIVATIVES ON CHOLESTEROL SYNTHESIS

The inhibitory effects of ganoderic acid derivatives were tested at a concentration of 40 μM, except VI in which 20 and 40 μM were used. The results are shown in Table I.

The compounds (I) and (II), which have carboxyl groups at C-25 in the side chain, showed almost no inhibitory effects even though they have 7β-hydroxy and 15-oxo groups. The decarboxyl compounds (IV) and (V) had moderate inhibitory effects. These results suggested that the compounds with a terminal carboxyl group in the side chain do not interfere with the enzyme activity.

The compound (VI), which has no functional group in the side chain and has both 7-oxo and 15α-hydroxy groups on the same skeleton, showed potent inhibitory effect. However, it was a little less potent than 7-oxo-DHL, 14α-methylcholest-7-ene-3β,15α-diol and lanost-7-ene-3β,15α-diol. This indicates that VI did not have the additive effect of the 7-oxo and 15α-hydroxy groups for the inhibitory enzymatic conversion of 24, 25-dihydrolanosterol through the 14-demethylation step into cholesterol. This suggests that since stereochemically 7-oxo and 15α-hydroxy groups of VI is located very close, the functional groups could interact with each other and consequently the contribution of the groups in their interaction with the enzyme would be decreased.

Table I. Cholesterol Biosynthesis during Incubation of the S-10 Fraction of Rat Liver Homogenate with [24,25-^{3}H]-24,25-Dihydrolanosterol in the Presence of Lanosterol Derivatives

Compounds	24,25-Dihydro-lanosterol fr. (%)	Cholesterol fr. (%)	Inhibition[c] (%)
None (control)	31.2	32.8	------
Ganoderic acid B (I) (40 μM)[a]	32.1	32.5	1
Ganoderic acid B methyl ester (II) (40 μM)[a]	31.9	31.4	4
Compound IV (40 μM)[a]	40.3	26.9	18
Compound V (40 μM)[a]	43.7	22.8	30
Compound VI (40 μM)[b]	79.9	5.3	84
(20 μM)[a]	69.3	9.1	72
7-Oxo-DHL (40 μM)[b]	94.3	1.6	95
(20 μM)[a]	85.4	3.2	90

The methods of incubation and fractionation of the incubation products were exactly the same as described previously.[33] a) Each incubation was carried out in duplicate. b) Each incubation was carried out in triplicate and the relative standard deviation of each value listed was less than 5 percent. c) Percent inhibition of cholesterol synthesis = [(percent yield of cholesterol in control - percent yield in run with test compound)/percent yield in control] x 100.

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