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Hypocholesterolemic Action of Tricyclic Diterpenoids in Rats[1]

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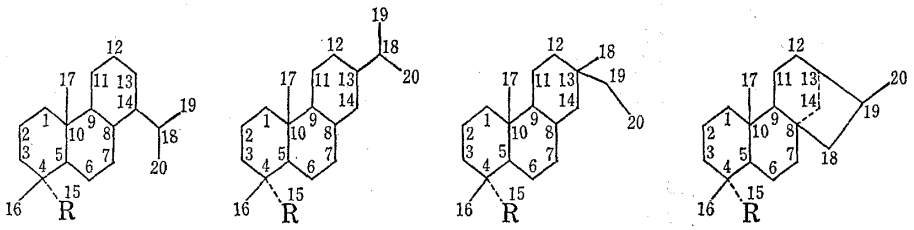
Various tricyclic diterpenoid derivatives of totarol, abietane and pimarane groups were found to have serum cholesterol lowering action in rats. Inhibition of intestinal cholesterol absorption was suggested to be involved in the main mechanism of hypocholesterolemic action of totarol and abietic acid.

Keywords——blood cholesterol; cholesterol lowering; cholesterol absorption; rat; tricyclic diterpenoids; abietic acid; totarol

In the course of a search for pharmacological activities of plant extracts it was discovered that the methanol extract of *Thujopsis dolabrata* Sieb. et Zucc. reduces serum cholesterol in rats. Totarol was isolated as one of the active principles. This discovery led us to examine hypocholesterolemic activities of various other natural tricyclic diterpenoids and their synthetic derivatives. These compounds are roughly classified into four groups; I. totarol group, II. abietane group, III. pimarane group and IV. phyllocladane group. Preliminary studies were also made on the mode of action of totarol and abietic acid, the latter of which can be easily obtained from rosin.



I. totarol Gr. II. abietane Gr. III. pimarane Gr. IV. phyllocladane Gr.

Chart 1

Materials and Methods

Totarol, 7-hydroxytotarol and podototarin were isolated from leaves of *Thujopsis dolabrata* Sieb. et Zucc. Sugiol and acetylferruginol, acetylcryptojaponol, isopimaric acid and phyllocladanol were isolated from barks of *Cryptomeria japonica* D. Don. Abietic acid and sandaracopimaric acid were isolated respectively from resin of Chinese pine and *Chamaecyparis obtusa* Endl. The other derivatives were synthesized from their parent terpenoid compounds.

Hypocholesterolemic activity was assayed in male Wistar rats aged 4 weeks. The animals were fed synthetic basal and control diets *ad libitum* for 3 days and bled following an overnight fast to determine the serum total cholesterol with Technicon Autoanalyzer. The basal diet contained: (in w/w%) sucrose, 63.16; casein, 22; coconut oil, 5; cod liver oil, 0.1; cellulose powder, 5; McCollum's salt mixture, 4; choline chloride, 0.24; and vitamin mixture, 0.5. The control diet contained 1% cholesterol and 0.25% sodium cholate in place of the same amount of sucrose. Test compounds were added in a concentration of 0.1% in

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TABLE I. Hypocholesterolemic Activities of Tricyclic Diterpenoid Derivatives

Compound	C=C	Substituent at position 7	11	12	13	14	19	R	Hypocholesterolemic activity (% inhibition)
I. Totarol group									
Totarol (1)	8, 11, 13	—	—	—	OH	C_3H_7	—	CH_3	27[a]
7-Hydroxytotarol (2)	8, 11, 13	OH	—	—	OH	C_3H_7	—	CH_3	6
Totaryl methylether (3)	8, 11, 13	—	—	—	OCH_3	C_3H_7	—	CH_3	14
Totaryl acetate (4)	8, 11, 13	—	—	—	$OCOCH_3$	C_3H_7	—	CH_3	36[a]
Totaryl benzoate (5)	8, 11, 13	—	—	—	$OCOC_6H_5$	C_3H_7	—	CH_3	38[a]
Podototarin (6)	8, 11, 13	—	—	12, 12-dimer		C_3H_7	—	CH_3	8
7-Oxototaryl acetate (7)	8, 11, 13	=O	—	—	$OCOCH_3$	C_3H_7	—	CH_3	24[a]
6-Dehydrototaryl methyl ether (8)	6, 8, 11, 13	—	—	—	OCH_3	C_3H_7	—	CH_3	24[a]
6-Dehydrototaryl acetate (9)	6, 8, 11, 13	—	—	—	$OCOCH_3$	C_3H_7	—	CH_3	−3
II. Abietane group									
Tetrahydroabietic acid (10)	—	—	—	—	$C_3H_7(\beta)$	—	—	COOH	22[a]
Methyl tetrahydroabietate (11)	—	—	—	—	$C_3H_7(\beta)$	—	—	$COOCH_3$	29[a]
Tetrahydroabietyl alcohol (12)	—	—	—	—	$C_3H_7(\beta)$	—	—	CH_2OH	−2
Δ^8-Dihydroabietic acid (13)	8	—	—	—	$C_3H_7(\beta)$	—	—	COOH	26[a]
Methyl Δ^8-dihydroabietate (14)	8	—	—	—	$C_3H_7(\beta)$	—	—	$COOCH_3$	21[a]
Ethyl Δ^8-dihydroabietate (15)	8	—	—	—	$C_3H_7(\beta)$	—	—	$COOC_2H_5$	8
Isopropyl Δ^8-dihydroabietate (16)	8	—	—	—	$C_3H_7(\beta)$	—	—	$COOC_3H_7$(iso)	12
Butyl Δ^8-dihydroabietate (17)	8	—	—	—	$C_3H_7(\beta)$	—	—	$COOC_4H_9(n)$	−5
Octyl Δ^8-dihydroabietate (18)	8	—	—	—	$C_3H_7(\beta)$	—	—	$COOC_8H_{17}(n)$	5
Benzyl Δ^8-dihydroabietate (19)	8	—	—	—	$C_3H_7(\beta)$	—	—	$COOCH_2C_6H_5$	5
N,N-Diethylaminoethyl Δ^8-dihydroabietate (20)	8	—	—	—	$C_3H_7(\beta)$	—	—	$COOC_2H_4N(C_2H_5)_2$	44[b]
Morpholinoethyl Δ^8-dihydroabietate (21)	8	—	—	—	$C_3H_7(\beta)$	—	—	$COOC_2H_4N$⟨O⟩	6
N-Methyl-4-piperidyl Δ^8-dihydroabietate (22)	8	—	—	—	$C_3H_7(\beta)$	—	—	COO-⟨NCH_3⟩	66[b]
Δ^8-Dihydroabietic anhydride (23)	8	—	—	—	$C_3H_7(\beta)$	—	—	15, 15-anhydride	2
$\Delta^{8(14)}$-Dihydroabietic acid (24)	8 (14)	—	—	—	$C_3H_7(\beta)$	—	—	COOH	21[a]
Methyl $\Delta^{8(14)}$-dihydroabietate (25)	8 (14)	—	—	—	$C_3H_7(\beta)$	—	—	$COOCH_3$	21[a]
$\Delta^{8(14)}$-Dihydroabietyl alcohol (26)	8 (14)	—	—	—	$C_3H_7(\beta)$	—	—	CH_2OH	17
Dihydroabietic γ-lactone (27)	—	—	—	—	$C_3H_7(\beta)$	10,15-γ-lactone(methyl, 10→9)			3
Abietic acid (28)	7, 13	—	—	—	C_3H_7	—	—	COOH	23[a]
Methyl abietate (29)	7, 13	—	—	—	C_3H_7	—	—	$COOCH_3$	18
Ethyl abietate (30)	7, 13	—	—	—	C_3H_7	—	—	$COOC_2H_5$	29[a]
Abietic anhydride (31)	7, 13	—	—	—	C_3H_7	—	—	15, 15-anhydride	27[a]
Abietyl alcohol (32)	7, 13	—	—	—	C_3H_7	—	—	CH_2OH	17
Dehydroabietic acid (33)	8, 11, 13	—	—	—	C_3H_7	—	—	COOH	20
Methyl dehydroabietate (34)	8, 11, 13	—	—	—	C_3H_7	—	—	$COOCH_3$	21[a]
12-Sulfodehydroabietic acid (35)	8, 11, 13	—	—	SO_3H	C_3H_7	—	—	COOH	6
Dehydroabietylamine (36)	8, 11, 13	—	—	—	C_3H_7	—	—	CH_2NH_2	4
Sugiol (37)	8, 11, 13	=O	—	OH	C_3H_7	—	—	CH_3	−10
Acetyl ferruginol (38)	8, 11, 13	—	—	$OCOCH_3$	C_3H_7	—	—	CH_3	−28
Acetyl cryptojaponol (39)	8, 11, 13	=O	$OCOCH_3$	—	C_3H_7	—	—	CH_3	1
III. Pimarane group									
Isopimaric acid (40)	7	—	—	—	$\begin{cases}CH_3(\beta)\\CH=CH_2\end{cases}$	—	—	COOH	−1
Sandaracopimaric acid (41)	8 (14)	—	—	—	$\begin{cases}CH_3(\beta)\\CH=CH_2\ (\alpha)\end{cases}$	—	—	COOH	−16
Δ^8-Dihydropimaric acid (42)	8	—	—	—	$\begin{cases}CH_3(\alpha)\\C_2H_5(\beta)\end{cases}$	—	—	COOH	48[b]
$\Delta^{8(14)}$-Dihydropimaric acid (43)	8 (14)	—	—	—	$\begin{cases}CH_3(\alpha)\\C_2H_5(\beta)\end{cases}$	—	—	COOH	40[b]
IV. Phyllocladane group									
Phyllocladanol (44)	—	—	—	—	—	—	$\begin{cases}OH(\alpha)\\CH_3(\beta)\end{cases}$	CH_3	−19
Kaurene (45)	9	—	—	—	—	—	$=CH_2$	CH_3	−24

a) $p < 0.05$, b) $p < 0.01$

the control diet. The hypocholesterolemic activity was expressed as the % inhibition of serum cholesterol elevation induced by cholesterol feeding.

Intestinal cholesterol absorption was measured by the dual isotope method of Zilversmit.[3] In the hypocholesterolemic assay system, radioisotopes were given on the 3rd day; ^{3}H-cholesterol (1.24×10^{7} dpm/rat) orally by means of stomach tube and ^{14}C-cholesterol (7.85×10^{6} dpm/rat) intravenously. The fraction of oral dose absorbed can be given by the ratio of ^{3}H/^{14}C in the serum collected more than 24 hours after radioisotope dosage, when adjusted for the equal radioacitvity in the oral and intravenous dose.

Results and Discussion

Hypocholesterolemic activities of nine natural diterpenoids and their thirty-five derivatives, added 0.1% in diet, are shown in Table I. Totarol (**1**) reduces the elevated serum cholesterol level by 27%. Among totarol derivatives acetate (**4**) and benzoate (**5**) are more active than totarol. Sugiol analogs (**37, 38, 39**) and phyllocladanol analogs (**44, 45**) do not reduce or rather increase the cholesterol level. Abietic acid (**28**), tetrahydroabietic acid (**10**), dihydroabietic acids (**13, 24**) and dehydroabietic acid (**33**) decrease the serum cholesterol to nearly the same extent. Pimaric acid derivatives (**42, 43**) are more active than abietic acid, while isopimaric acid derivatives (**40, 41**) are less active. γ-Lactone (**27**) and anhydride (**23**) of dihydroabietic acid, abietyl, tetrahydro- or dihydroabietyl alcohols (**32, 12, 26**), and dehydroabietylamine (**36**) are in general less active than abietic acid. Esterification does not potentiate or rather decreases the hypocholesterolemic activity, except for the case of N,N-diethylaminoethyl (**20**) and N-methyl-4-piperidyl (**22**) ester, as principally exemplified in Δ^{8}-dihydroabietic acid.

When compared in terms of the 40% inhibitory dose, both totarol and abietic acid are comparable to clofibrate and more active than β-sitosterol, cholestyramine, nicotinic acid or pectin. Neiter totarol nor abietic acid reduces the serum cholesterol in normocholesterolemic rats fed a normal diet.

In order to determine the effect of totarol and abietic acid on the intestinal cholesterol absorption one oral ^{3}H- and one *intravenous* ^{14}C-cholesterol were simultaneously given to cholesterol fed rats with or without the addition of these terpenoids. As shown in Table II, both totarol and abietic acid significantly decrease the serum radioactivity of ^{3}H- and ^{14}C-cholesterol to about half and two thirds of the control respectively. The cholesterol absorption, as given by the adjusted ^{3}H/^{14}C ratio, is reduced by 25% ($p<0.01$) in both totarol and abietic acid treated groups. Since the rate of entero-hepatic circulation of cholesterol is considered to be several times per day, continuing inhibition of reabsorption of endogenous as well as exogenous cholesterol throughout the experimental period would apparently magnify the effect on net absorption of cholesterol. This might explain the rather big difference between the observed hypocholesterolemic effects (72% for totarol and 40% for abietic acid) and the inhibitory effects on cholesterol absorption. Significant reduction of serum radioactivity of

Table II. Effect of Totarol and Abietic Acid on the Intestinal Absorption of Cholesterol in Cholesterol Fed Rats

Group	No. of rats	Serum total cholesterol (mg%)	Serum radioactivity		
			^{3}H ($\times10^{5}$ dpm/ml)	^{14}C ($\times10^{5}$ dpm/ml)	^{3}H/^{14}C (%)
control	11	507±34[a]	3.66±0.27	4.19±0.33	56±2
0.3 % totarol	10	242±17[b]	1.79±0.20[b]	2.71±0.23[b]	42±2[b]
0.3 % abietic acid	9	305±28[b]	1.83±0.20[b]	2.56±0.32[b]	42±2[b]

a) mean ± S.E.
b) significantly different from control ($p<0.01$)

3) D.B. Zilversmit, *Proc. Soc. Exptl. Biol. Med.*, **140**, 862 (1972).

intravenously administered ^{14}C-cholesterol would also support this assumption, or, otherwise, suggests possible stimulation of excretion or catabolism of endogenous cholesterol by these compounds.

In this connection the effect of propylthiouracil was studied on the hypocholesteromic activity of abietic acid in cholesterol fed rats of different ages. As illustrated in Fig. 1, addition of abietic acid 0.3% in the diet significantly reduces the serum cholesterol only in weanling rats in the absence of propylthiouracil. However, in the hypothyroid rats treated with propylthiouracil significant reduction of serum cholesterol could be demonstrated in both weanling and adult rats. Considering the fact that weanling rats do not have the sufficient cholesterol catabolizing activity, a possibility would not be excluded that abietic acid stimulates cholesterol catabolism.

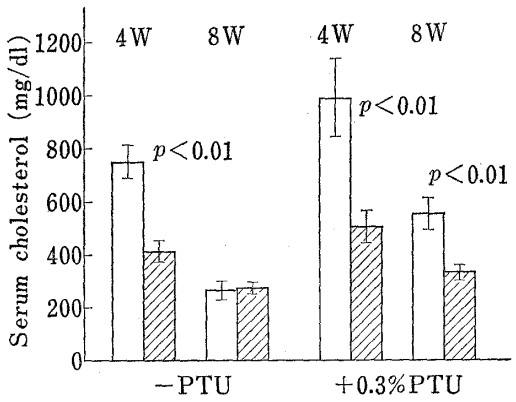

Fig. 1. Effect of Propylthiouracil (PTU) on Hypocholesterolemic Action of Abietic Acid in Cholesterol Fed Rats of Different Ages

4 W: 4 weeks old, 8 W: 8 weeks old. Each bar represents mean ±S.E.
□: 1% cholesterol
▨: 1% cholesterol+0.3% abietic acid

Stimulation by propylthiouracil of hypocholesterolemic action of abietic acid, or reported estrogenic activity of podocarpic acid and podocarpinol[4] also suggests the possible involvement of some hormonal actions. However, abietic acid was found to have far less than 1/3000 thyromimetic activity of D-thyroxine, less than 1/300 estrogenic activity of estradiol and far less than 1/300 androgenic activity of testosterone. Therefore, it may be asserted that the hypocholesterolemic action of abietic acid is independent of these hormonal activities, if any.

The above results suggest that abietic acid and totarol exert their hypocholesterolemic action mainly by way of inhibition of cholesterol absorption from the intestine and also possibly by way of stimulation of cholesterol catabolism or excretion. Since this type of pharmacological action seems to be potentially common to various tricyclic diterpenoid compounds, an extensive work is expected to find the more active and valuable one which can be used for the treatment of human hypercholesterolemia or atherosclerosis.

4) C.W. Brandt and D.J. Ross, *Nature*, **161**, 892 (1948).