PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 12–17 (1974)

The Effect of Two Substituted Valeric Acid Derivatives on Cholesterol Metabolism in Rats[1] (37738)

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The widely accepted use of ethyl *p*-chlorophenoxyisobutyrate (clofibrate) as a hypolipidemic agent has prompted the synthesis and testing for hypolipemic activity of many other aryloxy aliphatic acids or similar compounds. Several such compounds have been reported (1–3).

This report deals with two substituted valeric acids, 4-[2-carboxyethyl]-7-methyl-5-oxoindan-1β-yl valeric acid (compound X) and 4-(Decahydro-6-methyl-3-oxo-cyclopenta [*f*]quinolin-7β-yl) valeric acid (compound Y) (Fig. 1) and their influence on serum and liver lipid levels in rats, as well as cholesterol absorption, synthesis, oxidation, and 7α-hydroxylation.

Methods and Materials. Male, Wistar rats (150–160 g) were maintained for 14 days on a semisynthetic diet containing mixed cereal (70%), skim milk powder (22%), wheat germ (7%) and vitamin mix (1%). This diet has been used previously and is readily accepted by the rats (4). Compounds X and Y and clofibrate were added (0.3%) at the expense of the cereal. On Day 12, each animal was given an oral dose (1 μCi) of [4-^{14}C]cholesterol in 0.2 ml propylene glycol. On Day 14 the animals were killed by decapitation. Serum cholesterol was determined by the method of Mann (5) and triglycerides by the Van Handel-Zilversmit technique (6). A portion of the liver was extracted and liver cholesterol (5) and triglycerides (6) were determined from aliquots of this extract. Levels of radioactive cholesterol in serum and liver were determined by preparation of digitonides (7) and assay by liquid scintillation spectrometry (8).

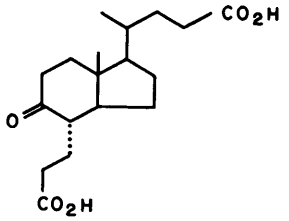
Pooled feces (Days 12–14) were weighed and suspended in water. Radioactivity was recovered from these suspensions by saponification, extraction of neutral lipids with petroleum ether, extraction of acidic lipids with ethyl ether after acidifying with HCl, and determination of radioactivity by liquid scintillation spectrometry (9).

For lipid biosynthesis experiments, 0.5 g slices of liver were incubated at 37° for 3 hr in 5 ml phosphate buffer (pH 7) containing 0.0006 *M* $MgCl_2$, 0.03 *M* nicotinamide, and either 1 μCi [1-^{14}C]acetate or 0.5 μCi [2-^{14}C]mevalonic acid. The reaction was stopped by addition of 15% alcoholic KOH. Cholesterol was extracted from the saponification mixture and isolated as the digitonide (7). The aqueous residue was acidified to pH 1 with strong mineral acid and fatty acids were extracted into ether. Cholesterol digitonides were dissolved in methanol (8) and assayed by liquid scintillation spectrometry. The fatty acids were counted directly.

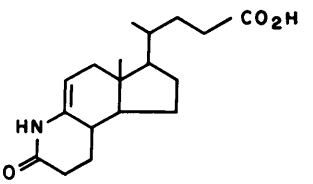
Mitochondrial suspensions were prepared as described previously (10, 11). Incubations were carried out in stoppered 125 ml Erlenmeyer flasks containing center wells. The incubation mixture consisted of 1 ml of mitochondrial preparation; 1 ml of a solution containing adenosine triphosphate (ATP, 25 mg), nicotinamide adenine dinucleotide

[1] Presented, in part, at the FASEB meeting, Apr. 1973.

[2] Supported, in part, by Public Health Service Research Grant HL-03299 and Research Career Award HL-00734 from the National Heart and Lung Institute, GM-00142 from Nat. Inst. of Gen. Med. Sciences, RR-05540 from the Division of Research Resources, and funds from the Commonwealth of Pennsylvania.



(4R)-4-[4α-(2-Carboxyethyl)-3aα-perhydro-7aβ-methyl-5-oxoindan-1β-yl]valeric acid, (Compound X).



(4R)-4-(2,3,4,6,6aβ,7,8,9,9aα,9bβ-Decahydro-6aβ-methyl-3-oxo-1H-cyclopenta[f]quinolin-7β-yl) valeric acid, (Compound Y).

FIGURE 1

(NAD, 5 mg), adenosine monophosphate (AMP, 8 mg), reduced glutathione (15 mg), sodium citrate monohydrate (30 mg), magnesium nitrate hexahydrate (10 mg), potassium penicillin G (2000 units) and streptomycin sulfate (1 mg); 5 ml of labeled substrate in 0.25 *M* tris(hydroxymethyl)aminomethane HCl, pH 8.5; and 5 ml of boiled supernatant.

Incubations were carried out at 37° for 18 hr. At the end of this period 2.5 ml of a 1 *M* methanolic solution of Hyamine 10X (*p*-diisobutyl-cresoxyethoxyethyl) dimethylbenzylammonium hydroxide) was injected into the center well. The solution was acidified with 1 *N* H_2SO_4 (2.5 ml) and the flasks were shaken for 3 hr at 37° to displace $^{14}CO_2$. The Hyamine solution was removed from the center well and a sample was taken for radioactive assay by liquid scintillation spectrometry.

Microsomal suspensions were prepared as described by Shefer, Hauser and Mosbach (12). Incubation was carried out in a 25 ml Erlenmeyer flask containing [1,2-^{3}H]cholesterol (0.5 μmole, 8.25 × 10^5 cpm/μmole) solubilized with 6.7 mg Tween 20; potassium phosphate buffer (pH 7.4), 0.167 mmole; $MgCl_2$, 11 μmoles; $NADP^+$, 3.0 μmoles; glucose-6-phosphate, 6.0 μmoles; glucose-6-phosphate dehydrogenase, 1 IU; and 1 ml microsomal suspension. The final volume was 2.3 ml. Incubation was carried out at 37° with shaking. The reaction was stopped by the addition of 7.5 ml methylene dichloride–ethanol (5:1) to a 0.5 ml aliquot of the reaction mixture. Steroids were separated by thin layer chromatography on silica gel G with ethyl acetate–hexane (8:2). The bands were visualized with iodine vapor (13), scraped from the plates, and assayed for radioactivity by liquid scintillation spectrometry.

All radioactive substrates were purchased from New England Nuclear Corporation, Boston, MA, and the cholesterol was purified by thin layer chromatography prior to use.

We are indebted to Dr. S. Hayakawa (Shionogi and Co., Ltd., Osaka, Japan) for gifts of the two valeric acid derivatives and to Dr. J. Nobel, Ayerst Laboratories, New York, NY, for the clofibrate.

Results and Discussion. Table I contains the results of two experiments comparing the effects of feeding compounds X and Y and clofibrate. In Expt 1, compound Y was observed to be hepatomegalic, increasing liver weight by 59%. Since such effects had been reported for clofibrate (14–16) the second experiment was designed to allow a direct comparison between Y and clofibrate. Both caused an increase in liver weight, Y by 39% and clofibrate by 38%.

There were few significant changes in serum lipid levels. In Expt 1, Y feeding resulted in serum triglyceride levels significantly higher than control and in Expt 2 clofibrate resulted in a serum cholesterol level significantly lower than any of the other groups. There were no significant changes in hepatic lipids in either experiment. The serum plus liver pool of cholesterol and of triglycerides were significantly increased by the feeding of Y in Expt 1 and clofibrate in

TABLE I. Effects of Valeric Acid Derivatives and of Clofibrate on Serum and Liver Lipids in Rats.

	Survival ratio	Wt gain (g)	Liver wt		Cholesterol			Triglycerides		
			(g)	(% body wt)	Serum (mg/dl)	Liver (mg/100 g)	Serum and liver (mg)	Serum (mg/dl)	Liver (mg/100 g)	Serum and liver (mg)
Expt 1										
Cpd X[g]	7/7	73 ± 3^{a}	9.1 ± 0.3^{b}	3.9 ± 0.1^{b}	20.4 ± 1.4	205 ± 8	20.0 ± 1.0^{b}	42.3 ± 5.7	318 ± 10	31.7 ± 1.0^{b}
Cpd Y[h]	7/7	62 ± 7	13.2 ± 0.5^{bc}	6.1 ± 0.1^{bc}	23.2 ± 1.4	194 ± 10	27.2 ± 1.8^{bc}	56.6 ± 4.2^{c}	315 ± 11	45.4 ± 2.2^{bc}
Control	6/7	71 ± 2	8.3 ± 0.1^{e}	3.6 ± 0.1^{e}	19.9 ± 1.9	177 ± 16	16.0 ± 1.1^{e}	42.7 ± 3.5^{c}	296 ± 8	27.5 ± 0.9^{e}
Expt 2										
Clofibrate 4[i]	6/6	50 ± 3^{b}	12.3 ± 0.6^{ce}	5.2 ± 0.2^{ce}	28.5 ± 2.3^{cef}	195 ± 23	25.3 ± 2.3^{c}	40.8 ± 7.7	755 ± 65	95.1 ± 7.4^{c}
Cpd X[g]	6/6	47 ± 7	9.2 ± 0.5^{ef}	3.9 ± 0.1^{ef}	45.0 ± 3.3^{e}	173 ± 14	19.2 ± 1.9^{f}	72.7 ± 14.6	608 ± 99	61.7 ± 10.0
Cpd Y[h]	6/6	31 ± 3^{bd}	12.5 ± 0.6^{df}	5.7 ± 0.1^{df}	53.3 ± 4.2^{f}	178 ± 8	25.7 ± 1.3^{df}	38.3 ± 7.4	632 ± 68	82.5 ± 11.2
Control	6/6	51 ± 5^{d}	9.0 ± 0.3^{cd}	3.8 ± 0.1^{cd}	44.0 ± 3.8^{c}	174 ± 9	18.7 ± 1.1^{cd}	61.7 ± 16.1	630 ± 74	59.9 ± 4.8^{c}

[a] Mean ± standard error. Any two means with same superscript (*b–f*) are significantly different ($P < 0.05$).

[g] 4-[2-Carboxyethyl]-7-methyl-5-oxoindan-1β-yl valeric acid.

[h] 4-(Decahydro-6-methyl-3-oxo-cyclopenta[*f*] quinolin-7β-yl valeric acid.

[i] Ethyl *p*-chlorophenoxyisobutyrate.

TABLE II. Influence of Valeric Acid Derivatives (0.3%) on Lipid Biosynthesis by Rat Liver Slices.

Precursor	Product	Cpd X[d]	Cpd Y[e]	Control
[1-^{14}C]Acetate	Cholesterol (dpm × 10^3/mg)	16.34 ± 2.26[ab]	1.12 ± 0.17[bc]	10.54 ± 2.48[c]
	Fatty acids (dpm × 10^4)	5.03 ± 0.57[b]	1.33 ± 0.40[bc]	3.68 ± 0.78[c]
[2-^{14}C]Mevalonate	Cholesterol (dpm × 10^3/mg)	7.89 ± 0.53	6.03 ± 0.71[c]	8.31 ± 0.53[c]

[a] Standard error. Any two means with same superscript (b, c) are significantly different ($P < 0.5$).

[d] 4-[2-Carboxyethyl]-7-methyl-5-oxoindan-1β-yl valeric acid.

[e] 4-(Decahydro-6-methyl-3-oxo-cyclopenta[f] quinolin-7β-yl valeric acid.

Expt 2. Y feeding resulted in a significantly higher cholesterol pool and a larger, but not significant, pool of triglycerides in Expt 2. Increased pool size has been observed previously with clofibrate (16).

Hepatomegaly is the reason for the higher serum plus liver cholesterol and triglyceride pools observed in rats fed compounds Y or clofibrate. The livers of rats fed Y were 36–45% larger than those of rats fed compound X and 40–60% larger than controls. Livers of clofibrate fed rats were 34 and 37% heavier than livers of rats fed compound X or control diet, respectively. The effect of liver size apparently overcomes the metabolic effects of compound Y on cholesterol synthesis or degradation.

TABLE III. Influence of Valeric Acid Derivatives (0.3%) on Oxidation of [26-^{14}C]Cholesterol by Rat Liver Mitochondria.

Compound	Cytosol	Oxidation: Gross (pmoles)	Oxidation: Corrected (pmoles/mg N)
Cpd X[h]	+	7.4 ± 1.2[a]	2.6 ± 0.4[d]
	—	2.3 ± 0.4	0.8 ± 0.1[d]
Cpd Y[i]	+	24.1 ± 3.1[b]	4.4 ± 0.3[ef]
	—	17.1 ± 2.8[c]	3.1 ± 0.4[eg]
Control	+	7.7 ± 2.2[b]	3.0 ± 0.9[f]
	—	2.8 ± 0.6[c]	1.1 ± 0.2[g]

[a] Standard error. Any two means with same superscript (b–g) are significantly different ($P < 0.5$).

[h] 4-[2-Carboxyethyl]-7-methyl-5-oxoindan-1β-yl valeric acid.

[i] 4-(Decahydro-6-methyl-3-oxo-cyclopenta[f] quinolin-7β-yl valeric acid.

Administration of Y resulted in significantly lowered cholesterol synthesis (Table II) from both acetate (89%) and mevalonate (27%). Fatty acid synthesis from acetate was also decreased in Y fed animals (64%). Feeding of X resulted in no significant changes in synthesis of cholesterol or fatty acids, although acetate incorporation into either class of lipids was higher than in controls.

Oxidation of [26-^{14}C]cholesterol to $^{14}CO_2$ (Table III) was stimulated by feeding compound Y in the presence of the boiled supernatant cofactor (cytosol). In the absence of cytosol the effect was even greater (47% with cytosol and 182% without cytosol). Gross oxidation of cholesterol was greatly increased probably due to the hepatomegalic effects of Y. This effect of cytosol has been observed with clofibrate (15) as well as several other hypolipemic agents which are similar in structure to clofibrate and which are

TABLE IV. Influence of Valeric Acid Derivatives and Clofibrate (0.3%) on 7α-Hydroxylation of Cholesterol by Rat Liver Microsomes.

Compound	7α-Hydroxylation (pmoles/mg protein/min)
Clofibrate[b]	8.59 ± 2.72[a]
Cpd X[c]	7.81 ± 3.50
Cpd Y[d]	5.31 ± 1.91
Control	3.36 ± 2.37

[a] Standard error.

[b] Ethyl p-chlorophenoxyisobutyrate.

[c] 4-[2-Carboxyethyl]-7-methyl-5-oxoindan-1β-yl valeric acid.

[d] 4-(Decahydro-6-methyl-3-oxo-cyclopenta[f] quinolin-7β-yl valeric acid.

TABLE V. Influence of Valeric Acid Derivatives and Clofibrate (0.3%) on Recovery of Radioactivity from Rats Fed [4-^{14}C]Cholesterol.

Cpd	Serum (dpm × 10^4)	Liver (dpm × 10^4)	Feces (dpm × 10^5)	
			Neutral	Acidic
Clofibrate[c]	1.50 ± 0.27[a]	10.85 ± 1.45[b]	8.39	7.54
Cpd X[d]	2.04 ± 0.43	7.14 ± 1.41	9.68	3.55
Cpd Y[e]	2.12 ± 0.44	8.60 ± 1.17	10.97	3.43
Control	2.13 ± 0.26	6.85 ± 0.86[b]	8.94	5.28

[a] Standard error. Any two means with same superscript (*b*) are significantly different.
[c] Ethyl *p*-chlorophenoxyisobutyrate.
[d] 4-[2-Carboxyethyl]-7-methyl-5-oxoindan-1β-yl valeric acid.
[e] 4-(Decahydro-6-methyl-3-oxo-cyclopenta[*f*] quinolin-7β-yl valeric acid.

hepatomegalic (16, 17).

Table IV presents results from the assay of cholesterol 7α-hydroxylase activity. None of the compounds tested had any significant effect on the 7α-hydroxylation of cholesterol, but all three of the test compounds resulted in higher levels of hydroxylating activity, especially clofibrate. Levels of activity were 156% higher from clofibrate, 50% from Y, and 131% from X fed animals.

Recovery of radioactive cholesterol from serum, liver and feces is shown in Table V. Highest levels of radioactivity were recovered from the feces; 72% was recovered from the feces of clofibrate animals and 65%, 60%, and 65% from Y, X and control fed animals indicating the lowest level of cholesterol absorption in the clofibrate fed animals. Recovery of radioactive acidic fecal steroids was higher than that from controls in clofibrate fed animals and lower in X and Y fed groups. This increase in acid fecal steroids, resulting from feeding of clofibrate, parallels the increased 7α-hydroxylation noted earlier. Excretion of neutral fecal steroid radioactivity increased in X and Y fed groups, by 8% and 23%, respectively; feeding of clofibrate did not alter neutral fecal steroid radioactivity.

Cholesterol absorption as measured by total fecal steroid excretion has been reported to be decreased by clofibrate treatment in humans (18, 19). However the increase in acidic steroid excretion is not observed in man (18–20).

Summary. Two valeric acid derivatives (4-[2-carboxyethyl]-7-methyl-5-oxoindan-1β-yl valeric acid [compound X] and 4-(decahydro-6-methyl-3-oxo-cyclopenta[*f*] quinolin-7β-yl) valeric acid [compound Y]) and clofibrate (ethyl *p*-chlorophenoxyisobutyrate) have been studied for their effects on cholesterol absorption, 7α-hydroxylation and oxidation, hepatic lipogenesis, and serum and liver lipid levels in rats. All three test compounds were fed (0.3% of diet) for 2 wk.

Compound Y was similar to clofibrate in its effects. Compound Y administration resulted in decreased hepatic synthesis of cholesterol and fatty acids, increased oxidation of [26-^{14}C]cholesterol to $^{14}CO_2$ and increased 7α-hydroxylation of cholesterol. Compound X had no significant effect on any of these parameters.

Clofibrate exhibited a hypocholesteremic effect and clofibrate and compound Y were hepatomegalic. Cholesterol absorption was decreased by clofibrate but not by the two valeric acid derivatives.

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Received July 24, 1973. P.S.E.B.M., 1974, Vol. 145.