Hypolipidemic Activity of Indan-1,3-dione Derivatives in Rodents

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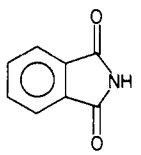
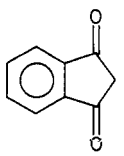
A series of 2-substituted indan-1,3-dione derivatives, including alkyl (C-1–C-5), mono- and disubstituted phenyl, and other 2-aryl derivatives, were tested for hypolipidemic activity of CF_1 male mice at 20 mg/kg per day. These derivatives reduced both serum cholesterol and triglycerides after 16 days of administration intraperitoneally. 2-(4-Methoxyphenyl)indan-1,3-dione was one of the more active compounds with 41% reduction of serum cholesterol and 58% reduction of serum triglyceride levels on day 16. This activity was confirmed in the rat after oral administration. 2-(2-Methylphenyl)- and 2-(4-chlorophenyl)indan-1,3-dione were effective in reducing serum triglyceride levels 58% and 53%, respectively, in mice. Serum cholesterol on day 16 was effectively reduced 46% by 2-(2,4-dimethylphenyl)indan-1,3-dione. The indan-1,3-dione derivatives were more effective than clofibrate in lowering lipid levels in mice. A more detailed study on the effects of 2-(4-methoxyphenyl)indan-1,3-dione demonstrated that key enzymes in the de novo synthesis of lipids were inhibited by the drug lowering tissue levels of lipids but raising those in the feces. The alterations in lipid content of rat lipoprotein fractions by the drug appeared favorable.

The hypolipidemic activity of a series of N-substituted phthalimides and many other cyclic imides like 1,8-naphthalimide, diphenimide, etc. has been extensively investigated in our laboratory.[1-4] Most of these compounds have exhibited potent activity in reducing serum cholesterol and triglyceride levels at the optimum dose of 20 mg/kg per day intraperitoneally in CF_1 male mice. Further investigation into this class of compounds led us to replace the nitrogen atom in phthalimides with a carbon to obtain indan-1,3-dione (1) since the carbon atom is isoteric to the nitrogen atom. Included are several of the 2-substituted analogues of indan-1,3-dione on the basis of those N-substituted derivatves of phthalimide that demonstrated hypolipidemic activity.



phthalimide



indan-1,3-dione (1)

Chemistry. Synthetic methods to prepare 2-substituted indan-1,3-diones are reported in the literature.[5-8] Though the parent compound indan-1,3-dione (1) is commercially available, all attempts to introduce alkyl groups on the active methylene carbon by treatment with base accompanied by the addition of alkyl halide were unsuccessful. However, 2-methyl- and 2-ethylindan-1,3-diones (**2**, **3**) were prepared according to the procedure of Mosher[5] by the condensation of dimethyl phthalate with 3-pentanone and 4-heptanone, respectively, in the presence of sodium hydride. The same method has been extended to synthesize higher homologues (**4–6**) and 2-phenylindan-1,3-dione (**7**) by using 5-nonanone, 6-undecanone, di-*n*-hexyl ketone, and dibenzyl ketone, respectively. All substituted phenyl analogues (**8–30**) were synthesized following the method of Freedman et al.[6] Thus, phthalide was reacted with suitably substituted benzaldehyde in the presence of sodium ethoxide to afford 2-(substituted phenyl)indan-1,3-dione (**8–26**). Similarly, α-naphthaldehyde, β-naphthaldehyde, 4-biphenylcarboxaldehyde, and 9-anthraldehyde afforded the respective 2-arylindan-1,3-diones (**27–30**).

Results and Discussion

The parent compound indan-1,3-dione (1) demonstrated significant activity in reducing sodium cholesterol and triglyceride levels by 40% and 20%, respectively, after 16 days of administration of CF_1 male mice at 20 mg/kg per day, ip. Whereas the introduction of alkyl groups at the 2-position (**2–6**) did not change the hypocholesterolemic activity significantly, serum triglyceride levels were reduced more effectively, affording reduction of 30–48%. The hypotriglyceridemic activity of 2-*n*-pentylindan-1,3-dione was reduced considerably when compared to **4**. Since 2-phenylindan-1,3-dione (**7**) showed potent hypolipidemic activity by reducing serum cholesterol and triglyceride levels by 40%, we elected to study the influence of the substitution on the phenyl ring with methoxy (**8–10**), methyl (**11–13**), halogen (**14–16**), and carboxyl (**24**, **25**). Substitutions in the ortho, meta, and para positions of the phenyl ring were evaluated for steric effects. The methyl and methoxy groups were assessed because they had electron-releasing effects whereas the halogens had electron-withdrawing effects. The chloride was compared to bromine because of an increased steric size. The carboxyl groups were examined because of acidic function of the moiety. Of all these compounds, 2-(4-methoxyphenyl)-indan-1,3-dione (**10**) was shown to be the most potent hypolipidemic agent tested, reducing cholesterol by 41% and triglyceride levels by 58%. In general, monosubstitution on the phenyl ring (**8–25**) did not improve the hypochlolesterolemic activity. However, every compound, with the exception of **15** and **25**, demonstrated potent hypotriglyceridemic activity, i.e., >58–34%. The replacement of chlorine in compound **16** by bromine (**17**) improved hypocholesterolemic activity by 6% but suppressed the hypotriglyceridemic activity by 21%. Changing the methoxy group in compound **10** to ethoxy (**26**) retained the hypocholesterolemic activity but lowered the hypotriglyceridemic activity markedly for 58% to 19%, indicating steric hindrance of the larger ethoxy group. Disubstitution on the phenyl ring (**18–23**), in general, resulted in the loss of hypolipidemic activity. However, 2-(2,4-dimethylphenyl)indan-1,3-dione (**21**) exhibited significant activity by lowering serum cholesterol levels by

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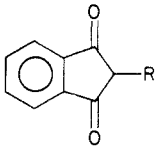
Table I. Physical and Spectral Characteristics of 2-Substituted Indan-1,3-diones



compd	R	mp (lit.), °C	% yield	formula
1	H			
2	methyl[g]	81–82 (83–84)[a]	60	
3	ethyl[g]	51–53 (53–54)[a]	48	
4	propyl[g]	47–48 (48–49.5)[e]	36	
5	*n*-butyl[g]	32–34 (35)[e]	27	
6	*n*-pentyl	oil	22	$C_{14}H_{16}O_2$
7	phenyl[h]	147–148 (148–149)[b]	53	
8	2-methoxyphenyl[g]	156–159	54	$C_{16}H_{12}O_3$
9	3-methoxyphenyl[g]	134–136	82	$C_{16}H_{12}O_3$
10	4-methoxyphenyl[g]	143–146 (152–154)[c]	47	
11	2-methylphenyl[i]	176–178 (179–180)[d]	60	
12	3-methylphenyl[i]	132–134 (134–135)[f]	62	
13	4-methylphenyl[i]	135–138	36	$C_{16}H_{12}O_2$
14	2-chlorophenyl[i]	180–181 (183–184)[b]	7	
15	3-chlorophenyl[h]	151–152 (153–155)[b]	26	
16	4-chlorophenyl[j]	142–144 (142–144)[b]	10	
17	4-bromophenyl[k]	201–202 (203–204)[b]	8	
18	2,4-dichlorophenyl[j]	142–143 (143–145)	19	
19	2,6-dichlorophenyl[b]	144–146	22	$C_{15}H_8O_2Cl_2$
20	3,4-dichlorophenyl[j]	222–225	16	$C_{15}H_8O_2Cl_2$
21	2,4-dimethylphenyl[j]	122–125	18	$C_{17}H_{14}O_2$
22	2,4-dimethoxyphenyl[i]	176–178	9	$C_{17}H_{14}O_2$
23	3,4-dimethoxyphenyl	181–184	22	$C_{17}H_{14}O_4$
24	2-carboxyphenyl[i]	242–246 (248–254)[b]	32	
25	4-carboxyphenyl[i]	221–225	10	$C_{16}H_{10}O_4$
26	4-ethoxyphenyl[m]	132–135	30	$C_{17}H_{14}O_3$
27	α-naphthyl[n]	213–215 (213–215)[b]	17	
28	β-naphthyl[h]	171–172 (172–174)[b]	20	
29	biphenylyl[i]	180–184	68	$C_{21}H_{14}O_2$
30	9-anthryl[i]	186–190	5	$C_{23}H_{14}O_2$

[a] Reference 5. [b] Reference 6. [c] Reference 8. [d] Reference 9. [e] Reference 7. [f] Reference 10. [g] Benzene. [h] 2-Propanol. [i] Ethanol. [j] Methanol. [k] Chloroform/hexanne. [l] H_2O/HCl. [m] Ether. [n] Methyl ethyl ketone.

46% and triglyceride levels by 42%. The replacement of phenyl in compound **7** by α-naphthyl (**27**), β-naphthyl (**28**), biphenylyl (**29**), and 9-anthranyl (**30**) did not afford any improvement in the hypolipidemic activity, indicating that additional bulk in the 2-substitution position or flatness of the rings did not improve the activity. Of all compounds tested for hypolipidemic activity, only 2-(2-chlorophenyl)-, 2α-naphthyl, and 9-anthyrlindan-1,3-dione (**14**, **27**, and **30**) were toxic in CF_1 male mice at 20 mg/kg per day. All of the 2-substituted indan-1,3-dione derivatives were more active at 20 mg/kg per day than the commercial agent clofibrate, which at 150 mg/kg per day lowered serum cholesterol levels 13% and serum triglyceride level 25% on day 16 in CF_1 male mice (Table II).

In the comparison of similar derivatives of indan-1,3-dione with the appropriate derivatives of phthalimide, which has been previously published,[1] the 2-methyl, 2-ethyl, 2-propyl, and 2-butyl derivatives of the indan-1,3-dione were more active in both the hypocholesterolemic and hypotriglyceridemic screens than the corresponding derivatives of phthalimide.[1] The *n*-pentyl derivative (**6**) was more active in the hypotriglyceridemic screen but not as active in the hypocholesterolemic screen when compared to *N*-*n*-pentylphthalimide.[1] The 2-phenylindan-1,3-dione, when compared to *N*-phenylphthalimide,[2] exhibited approximately equal hypolipidemic activity. The 2-(4-methoxyphenyl)indan-1,3-dione derivative was a more active hypotriglyceridemic agent than *o*-, *m*-, or *p*-phthalimidoacetophenone;[2] however, the (methoxyphenyl)indan-1,3-diones (**8**–**10**) were not active hypocholesterolemic agents in mice when compared to the appropriate phthalimidoacetophenone.[2]

When the 2-(4-methoxyphenyl)indan-1,3-dione derivative was examined in more detail, the agent was shown to be active at 20 mg/kg per day in Sprague–Dawley rats when administered orally, lowering serum cholesterol levels 45% and serum triglyceride levels 41%. A dose–response study in CF_1 male mice showed the optimum dose required to reduce both serum cholesterol and triglyceride levels was 20 mg/kg even though the maximum reduction, 52% of cholesterol, was at 40 mg/kg per day (Table III). In hyperlipidemic mice, the serum cholesterol levels were elevated to 375 mg/dL whereas the serum triglycerides were elevated to 367 mg/dL. Drug treamtent at 20 mg/kg per day for 14 days lowered the cholesterol level 68% to 120 mg/dL while triglyceride levels were reduced 61% to 143 mg/dL, indicating not only was the drug effective in lowering normal lipid levels but also was effective in elevated levels of lipid, which would be the case with hyperlipidemic patients. The LD_{50} for compound **10** was 200 mg/kg ip.

Compound **10** appeared to lower triglyceride levels by interfering with regulatory enzymes in the de novo synthetic pathway. Both *sn*-glycerol-3-phosphate acyl transferase and phosphidate phosphohydrolase activities were suppressed in vitro and in vivo (Tables IV and V). Lamb et al.[23] have shown a positive correlation between

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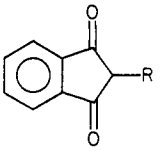
Table II. Hypolipidemic Effects of 2-Substituted Indan-1,3-diones in CF_1 Male Mice at a Dose of 20 mg/kg per Day Intraperitoneally



compd (N = 6)[e]	R	% control ($X \pm$ SD) serum cholesterol day 9	serum cholesterol day 16	serum triglyceride: day 16
1	H	74 ± 5*[d]	60 ± 5*	79 ± 6
2	methyl	74 ± 7	58 ± 2	61 ± 5*
3	ethyl	63 ± 5*	60 ± 3*	63 ± 3*
4	propyl	68 ± 9*	66 ± 4*	52 ± 7*
5	*n*-butyl	74 ± 9*	58 ± 2*	63 ± 5*
6	*n*-pentyl	65 ± 5*	64 ± 3*	70 ± 3*
7	phenyl	64 ± 6*	60 ± 2*	60 ± 4*
8	2-methoxyphenyl	79 ± 8*	72 ± 2*	48 ± 3*
9	3-methoxyphenyl	75 ± 6*	67 ± 5*	55 ± 4*
10	4-methoxyphenyl	58 ± 6*	59 ± 3*	42 ± 4*
11	2-methylphenyl	73 ± 5*	69 ± 3*	49 ± 1*
12	3-methylphenyl	75 ± 6*	71 ± 3*	51 ± 4*
13	4-methylphenyl	68 ± 7*	65 ± 3*	42 ± 2*
14	2-chlorophenyl	75 ± 7*	56 ± 4*	55 ± 3*
15	3-chlorophenyl	70 ± 5*	60 ± 4*	70 ± 3*
16	4-chlorophenyl	64 ± 6*	63 ± 3*	47 ± 2*
17	4-bromophenyl	72 ± 7*	56 ± 3*	66 ± 6*
18	2,4-dichlorophenyl	89 ± 3	73 ± 6*	87 ± 5*
19	2,6-dichlorophenyl	71 ± 8*	68 ± 2*	61 ± 1*
20	3,4-dichlorophenyl	93 ± 9	84 ± 5	89 ± 4
21	2,4-dimethylphenyl	61 ± 7*	54 ± 3*	58 ± 2*
22	2,4-dimethoxyphenyl	89 ± 8	74 ± 2	87 ± 3
23	3,4-dimethoxyphenyl	75 ± 8*	69 ± 3*	66 ± 8*
24	2-carboxyphenyl	79 ± 9	78 ± 2	52 ± 4
25	4-carboxyphenyl	95 ± 8	78 ± 2	95 ± 2
26	4-ethoxyphenyl	66 ± 5*	59 ± 2*	81 ± 4*
27	α-naphthyl			
28	β-naphthyl	72 ± 7*	66 ± 3*	66 ± 5*
29	biphenylyl	78 ± 8*	64 ± 2*	63 ± 4
30	9-anthryl			
31	clofibrate (150 mg/kg)	88 ± 7	87 ± 2	75 ± 3*
32	control, 1% (carboxymethyl)cellulose	100 ± 5[a]	100 ± 6[b]	100 ± 6[c]

[a] 125 mg/dL. [b] 128 mg/dL. [c] 137 mg/dL. [d] Asterisk indicates $p \leq 0.001$. [e] N = number of mice per test group.

Table III. Hypolipidemic Effects of 2-(4-Methoxyphenyl)indan-1,3-dione in Rodents

compd (N = 6)	% control ($X \pm$ SD): CF_1 male mice (orally) serum cholesterol day 9	serum cholesterol day 16	serum triglyceride: day 16
1% CMC, control	100 ± 5[a]	100 ± 6[b]	100 ± 6[c]
2-(4-methoxyphenyl) indan-1,3-dione			
10 mg/kg	82 ± 4	63 ± 3*	64 ± 5*
20 mg/kg	67 ± 3*[h]	62 ± 3*	48 ± 4*
40 mg/kg	79 ± 4*	48 ± 2*	73 ± 4*
60 mg/kg	71 ± 5*	62 ± 4*	67 ± 4*

compd (N = 6)	% control ($X \pm$ SD: Sprague–Dawley rats (orally) serum cholesterol day 9	serum cholesterol day 16	serum triglyceride day 9	serum triglyceride day 16
1% CMC, control	10 ± 5[d]	10 ± 6[e]	100 ± 4[f]	100 ± 6[g]
2-(4-methoxyphenyl)-indan-1,3-dione, 20 mg/kg per day	74 ± 5*	55 ± 4*	73 ± 3*	59 ± 5*

[a] 126 mg/dL. [b] 127 mg/dL. [c] 136.5 mg/dL. [d] 75 mg/dL. [e] 78 mg/dL. [f] 110 mg/dL. [g] 112 mg/dL. [h] Asterisk indicates $p \leq 0.001$.

the inhibition of the activities of these two regulatory enzymes and the lowering of serum triglyceride levels. Similar observations have also been made for phthalimide and saccharin derivatives.[1] The reduction of cholesterol levels appeared to be brought about by the inhibition of mitochondrial citrate exchange and ATP-dependent citrate lyase activity (Tables IV and V). Blocks at these two sites would lead to a reduction in cytoplasmic acetyl-CoA, which is required in the initial steps of fatty acid and cholesterol synthesis. The rate-limiting enzyme of cholesterol de novo synthesis HMG CoA reductase was not affected by the drug as seen with the cyclic imides. The enzyme that regulates cholesterol ester formation, acyl-CoA cholesterol acyl transferase, was suppressed significantly both in vitro and in vivo. The rate-limiting enzyme of fatty acid synthesis and heparin-induced hepatic lipoprotein lipase was not markedly affected by the drug. Interestingly, there was no accumulation of cholesterol or triglycerides (Tables V and VI) in the liver or small intestine tissue as observed with the hypolipidemic agent nicotinic acid. Fecal collections demonstrated an increase in cholesterol and triglyceride content in rats after 14 days of administration of the drug. [^{3}H]Cholesterol distribution studies confirm these findings in that brain, heart, liver, kidney, spleen, small intestine, and plasma demonstrated less radioactivity than the control. The stomach demonstrated a 155% increase in [^{3}H]cholesterol compared to that in the control rats whereas the large intestine showed a 32% increase and

Table IV. In Vitro Effects of 2-(4-Methoxyphenyl)indan-1,3-dione on CF_1 Liver Enzyme Activity

	% control (X = ± SD)			
enzyme assay (N = 6)	control	50 μM	100 μM	200 μM
mitochondrial citrate exchange	100 ± 5[a]	36 ± 3	38 ± 2	36 ± 3
ATP-dependent citrate exchange	100 ± 5[b]	72 ± 4	51 ± 3	52 ± 3
acetyl-CoA synthetase	100 ± 7[c]	75 ± 6	75 ± 5	74 ± 4
HMG CoA reductase	100 ± 6[d]	107 ± 5	109 ± 4	105 ± 5
acyl cholesterol acyl transferase	100 ± 5[e]	94 ± 4	63 ± 4	60 ± 4
cholesterol 7α-hydroxylase	100 ± 4[f]	106 ± 5	144 ± 5	113 ± 7
acetyl-CoA carboxylase	100 ± 6[g]	118 ± 3	93 ± 3	67 ± 5
sn-glycerol-3-phosphate acyl transferase	100 ± 6[h]	85 ± 6	61 ± 2	47 ± 3
phosphatidate phosphohydrolase	100 ± 6[i]	36 ± 2	29 ± 3	11 ± 1
hepatic lipoprotein lipase	100 ± 5[j]	86 ± 5	94 ± 6	93 ± 5

[a] 30.8 mg % exchange of mitochondrial citrate. [b] 30.5 mg of citrate lyased/g of wet tissue per 30 min. [c] 28.5 mg of acetyl-CoA formed/g of wet tissue per 30 min. [d] 384 900 dpm cholesterol formed/g of wet tissue per 60 min. [e] 4808 dpm/mg of microsomal protein per 20 min. [f] 224 000 dpm/μg of microsomal protein. [g] 32 010 dpm/g of wet tissue per 30 min. [h] 537 800 dpm/g of wet tissue per 20 min. [i] 16.7 mg of P_i/g of wet tissue per 15 min. [j] 278 503 dpm/g of wet tissue.

the fecal collection showed a 97% increase in radioactivity. The organ weight showed no increase over the control animal, indicative also of no lipid deposition in the organ. Furthermore, the daily food consumption was not altered by drug therapy for 14 days. The lipid distribution in the rat serum lipoproteins after 14 days of dosing looked promising (Table VII). Firstly, the triglyceride content was reduced in the VLDL and LDL fraction. Since these are the lipoproteins that carry lipids to the tissues and atherogenic plaques, it is important that the content of these lipids be reduced in hyperlipidemic states. Secondly, the cholesterol content of the HDL fraction was elevated markedly. Supposedly the HDL lipoprotein clears cholesterol from the peripheral tissues and plaques and returns it to the liver for excretion from the body. If the HDL returns cholesterol to the liver, there should be a suppression of the regulatory enzyme, acyl-CoA cholesterol acyl transferase, which was suppressed by the drug, indicating no cholesterol ester storage. This is important since this enzyme regulates ester formation and deposition in atherogenic plaques and thus the growth of the plaque. Whereas it is difficult to draw direct analogy to human lipoprotein levels from rodent lipoprotein fraction, high cholesterol content in the HDL fraction in man protects against cardiac infarction.[34] Thus this agent may warrant clinical trials at some time in the future.

Experimental Section

All starting materials were obtained from Aldrich Chemical Co. and were used as such. Melting points were obtained with a Thomas-Hoover melting point apparatus and were uncorrected. Elemental analyses were obtained from MHW Laboratories, Phoenix, AZ, and are correct within ±0.4% of theoretical values. Column chromatography was performed on silica gel 60 (70–230 mesh). ^1H NMR spectra were recorded on a JEOL FX-60 Fourier-transform spectrometer.

2-Alkyl- and 2-Phenylindan-1,3-diones 2–7. General Procedure. These compounds were prepared following the method of Mosher.[5] To a suspension of sodium hydride (60% in mineral oil, 0.1 mol) in anhydrous benzene (50 mL) was added slowly a mixture of the appropriate ketone (0.05 mol) and dimethyl phthalate (0.05 mol) and the mixture was refluxed for 15 h. The deep red solid that formed on cooling was collected by filtration, dried in vacuo, and dissolved in water (75 mL). The solution was acidified with concentrated HCl to afford the indan-1,3-diones 2–7.

2-(Substituted phenyl)indan-1,3-diones 8–30. General Procedure. All compounds were synthesized by the method of Freedman.[6] Sodium ethoxide (5.5 g, 0.08 mol) was prepared by dissolving sodium metal (1.85 g) in absolute ethanol (80 mL). To this solution was added a mixture of the appropriate benzaldehyde (0.075 mol) and phthalide (10 g, 0.075 mol) and the reaction was heated under reflux for 1.5 h. The solvent was removed and the residue was dissolved in ice water (500 mL). The aqueous portion was washed with two 80-mL portions of ether. After acidification with hydrochloric acid, the product was extracted into 100 mL of ether (in case the product is not soluble in ether, it is filtered and crystallized from the specified solvent) and then reextracted into aqueous sodium bicarbonate and acidified with hydrochloric acid to pH 2. The precipitate was filtered and purified by recrystallization to obtain the desired product (**8–30**).

Hypolipidemic Screen in CF_1 Male Mice. All compounds were tested at 20 mg/kg per day and administered intraperitoneally to CF_1 male mice (~30 g) or orally to Sprague–Dawley male rats (~350 g) at 11:00 a.m. On days 9 and 14 or 16, the blood was collected via the tail vein. The blood samples were collected between 8:00 and 9:30 a.m. in alkali-free nonheparinized microcapillary tubes, which was centrifuged for 3 min to obtain the serum. Duplicate 25-μL samples of nonhemolyzed serum were used to determine the milligram percent serum cholesterol levels by a modification of the Liebermann–Burchard reaction.[11] With use of a separate group of mice, which were bled on day 16, serum triglyceride levels were measured, using duplicate samples of 50 μL.[12] The "p" values were obtained by a Student's "t" test.

Dose Response. CF_1 male mice were administered 2-(4-methoxyphenyl)indan-1,3 dione orally from 10 to 60 mg/kg per day. On day 9 and 16 blood was collected for the serum cholesterol[11] and serum triglyceride assays.[12]

Testing in Hyperlipidemic Induced Mice. CF_1 male mice (~25 g) were placed on a commercial diet (U.S. Biochemical Corp. basal atherogenic test diet) for 14 days, which produced a "hyperlipidemic" state.[13] After the serum cholesterol and triglyceride levels were observed to be elevated, the mice were administered test drugs at 20 mg/kg per day, orally, for an additional 14-day period. Serum cholesterol and triglyceride levels were measured at that time.

Animal Weights and Food Intake. Periodic animal weights were obtained during the experiments and expressed as a percentage of the rat's weight on day 0. After dosing for 14 days with test drugs, selected organs were excised, trimmed of fat, and weighed. Food consumption was determined daily.

Toxicity Studies. The acute toxicity (LD_{50} values)[14] was determined in CF_1 male mice (~25 g) by administering the test drug intraperitoneally from 100 mg/kg to 1 g/kg as a single dose. The number of deaths was recorded over a 7-day period for each group.

Enzymatic Studies. In vitro enzymatic studies were determined with use of 10% homogenates of CF_1 male mouse liver with 50–200 μM of test drugs. In vivo enzymatic studies were determined with use of 10% liver homogenates (prepared in 0.25 M sucrose + 0.001 M (ethylenedinitrilo)tetraacetic acid, pH 7.2)

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Table V. In Vivo Effects of 2-(4-Methoxyphenyl)indan-1,3-dione on Activity of Enzyme in Lipid Synthesis in CF_1 Mice after 16 Days of Dosing Orally

compd (N = 6)	ATP-dependent citrate lyase	acetyl-CoA synthetase	HMG CoA reductase	cholesterol 7α-hydroxylase	acyl-CoA cholesterol acyl transferase
1% CMC, control	100 ± 5	100 ± 7	100 ± 6	100 ± 4	100 ± 5
2-(4-methoxyphenyl)indan-1,3-dione					
10 mg/kg	70 ± 4*[a]	83 ± 5	116 ± 5	52 ± 3*	92 ± 6
20 mg/kg	43 ± 3*	72 ± 6*	114 ± 5	45 ± 2*	48 ± 4*
40 mg/kg	38 ± 2*	90 ± 7	114 ± 4	55 ± 4*	42 ± 3*
60 mg/kg	93 ± 7	99 ± 6	126 ± 6	60 ± 5*	48 ± 4*

compd (N = 6)	acetyl-CoA carboxylase	*sn*-glycerol-3-phosphate acyl transferase	phosphatidate phosphohydrolase	hepatic lipoprotein lipase
1% CMC, control	100 ± 6	100 ± 6	100 ± 5	100 ± 5
2-(4-methoxyphenyl)indan-1,3-dione				
10 mg/kg	89 ± 5	71 ± 5*	53 ± 5*	83 ± 6
20 mg/kg	107 ± 6	55 ± 5*	35 ± 4*	82 ± 5
40 mg/kg	98 ± 7	46 ± 6*	57 ± 4*	89 ± 5
60 mg/kg	97 ± 6	75 ± 4*	100 ± 6	89 ± 4

[a] Asterisk indicates $p \leq 0.001$. See Table I for the standard values of enzyme activities of CF_1 liver.

Table VI. Effect of 2-(4-Methoxyphenyl)indan-1,3-dione on CF_1 Male Mouse Liver Lipid Content after 16 Days of Dosing Orally

	% control (X ± SD)					
compd (N = 6)	lipids (mg)	cholesterol	neutral lipids	triglycerides	phospholipids	protein
control (1% CMC)	100 ± 6	100 ± 5[a]	100 ± 6[b]	100 ± 5[c]	100 ± 7[d]	100 ± 4[e]
2-(4-methylphenyl) indan-1,3-dione						
10 mg/kg	103 ± 5	73 ± 4*[f]	58 ± 3*	71 ± 5*	77 ± 8	90 ± 6
20 mg/kg	93 ± 6	84 ± 5	61 ± 4*	64 ± 4*	185 ± 10*	101 ± 4
40 mg/kg	97 ± 4	89 ± 3	60 ± 2*	82 ± 6*	129 ± 7	94 ± 5
60 mg/kg	94 ± 2	105 ± 6	59 ± 3	81 ± 5*	103 ± 6	98 ± 4

[a] 12.24 mg og cholesterol/g of tissue. [b] 28.35 mg of neutral lipids/g of tissue. [c] 4.77 mg of triglycerides/g of tissue. [d] 4.39 mg of phospholipids (P)/g of tissue. [e] 4.5 mg of protein/g of tissue. [f] Asterisk indicates $p \leq 0.001$.

Table VII. Effects of 2-(4-Methoxyphenyl)indan-1,3-dione at 20 mg/kg per Day Orally for 14 Days on Rat Tissue and Lipoprotein Lipid Content

	% control (X ± SD)					
tissue (N = 6)	lipid (mg)	cholesterol	neutral lipids	triglycerides	phospholipids	protein
liver						
control	100 ± 6	100 ± 7[a]	100 ± 5[b]	100 ± 5[c]	100 ± 6[d]	100 ± 5[e]
treated	98 ± 5	69 ± 5	84 ± 4	37 ± 3	111 ± 5	100 ± 4
small intestine						
control	100 ± 5	100 ± 5[f]	100 ± 4[g]	100 ± 8[h]	100 ± 8[i]	100[j]
treated	100 ± 5	87 ± 4	95 ± 5	89 ± 5	136 ± 7	102
feces						
control	100 ± 6	100 ± 6[k]	100 ± 6[l]	100 ± 7[m]	100 ± 7[n]	100 ± 5[o]
treated	103 ± 4	187 ± 7	101 ± 5	118 ± 3	100 ± 6	99 ± 6
lipoprotein chylomicron						
control		100 ± 5[p]	100 ± 6[q]	100 ± 5[r]	100 ± 6[s]	100 ± 6[t]
treated		96 ± 4	99 ± 5	72 ± 4*	135 ± 6*	118 ± 5
VLDL						
control		100 ± 3[u]	100 ± 5	100 ± 6[w]	100 ± 7[x]	100 ± 5[y]
treated		88 ± 4	71 ± 4	72 ± 6	128 ± 6*	106 ± 5
LDL						
control		100 ± 6[z]	100 ± 6[aa]	100 ± 7[bb]	100 ± 7[cc]	100 ± 4[dd]
treated		18 ± 2*	69 ± 3*	40 ± 5*	204 ± 8*	95 ± 5
HDL						
control		100 ± 5[ee]	100 ± 3[ff]	100 ± 5[gg]	100 ± 6[hh]	100 ± 3[ii]
treated		216 ± 7*[jj]	113 ± 4	59 ± 3*	87 ± 4	107 ± 5

[a] 24.03 mg of cholesterol/g of tissue. [b] 44.11 mg of neutral lipid/g of tissue. [c] 6.37 mg of triglyceride/g of tissue. [d] 7.19 mg of phospholipid/g of tissue. [e] 4.5 mg of protein/g of tissue. [f] 7.82 mg/g. [g] 6.98 mg/g. [h] 1.12 mg/g. [i] 2.06 mg/g. [j] 42 mg/g. [k] 28.47 mg/g [l] 33.94 mg/g. [m] 1.86 mg/g. [n] 1.39 mg/g. [o] 6.99 mg/g. [p] 337 μg/mL. [q] 67 μg/mL. [r] 420 μg/mL. [s] 149 μg/mL. [t] 184 μg/mL. [u] 190 μg/mL. [v] 98 μg/mL. [w] 22 μg/mL. [x] 26 μg/mL. [y] 50 μg/mL. [z] 210 μg/mL. [aa] 10 μg/mL. [bb] 45 μg/mL. [cc] 41 μg/mL. [dd] 122 μg/mL. [ee] 544 μg/mL. [ff] 620 μg/mL. [gg] 27 μg/mL. [hh] 153 μg/mL. [ii] 657 μg/mL. [jj] Asterisk indicates $p \leq 0.001$.

from CF_1 male mice obtained after administering the agents for 16 days at a dose ranging from 10 to 60 mg/kg per day, orally. The enzyme activities were determined by following literature procedures:[13] acetyl-coenzyme A synthetase,[15] adenosine triphosphate dependent citrate lyase,[16] mitochondrial citrate exchange,[17,18] cholesterol 7α-hydroxylase,[19] (3-hydroxy-3-methylglutaryl)coenzyme A reductase,[20,21] acyl-coenzyme A carboxylase activity,[22] *sn*-glycerol-3-phosphate acyl transferase activity,[27] phosphatidate phosphohydrolase activity,[24] cholesterol acyl transferase,[25] and heparin-activated hepatic lipoprotein lipase.[26] Protein was determined for all enzyme assays by the Lowry et al.[27] technique.

Liver, Small Intestine, and Fecal Lipid Extraction. In CF_1 male mice that had been administered 2-(4-methoxyphenyl)-indan-1,3-dione for 16 days, the liver, small intestine, and fecal materials (24-h collection) were removed, extracted,[28,29] and an-

alyzed for cholesterol levels.[11] triglyceride levels (Bio-Dynamics/bmc triglyceride kit), neutral lipid content,[30] and phospholipid content.[31] In Sprague–Dawley rats after 14 days of dosing the liver and small intestine, a 24-h fecal collection was extracted for the lipid and analyzed as outlined above.

[^{3}H]Cholesterol Distribution in Rats. Sprague–Dawley rats (~300 g) were administered 2-(4-methoxyphenyl)indan-1,3-dione for 14 days orally. On day 13, 10 μCi of [^{3}H]cholesterol was administered orally by intubation needle to male rats, and according to the procedures described previously,[13] some tissue samples were combusted in a Packard tissue oxidizer or plated on filter paper, dried, and digested for 24 h in Hyamine hydroxide (New England Nuclear) at 40 °C and counted (Fisher Scintiverse in a Packard scintillation counter). Results were expressed as disintegration/min (dpm) per total organ.

Plasma Lipoprotein Fractions. Sprague-Dawley male rats (~300 g) were administered test drugs at 20 mg/kg per day, orally. Blood was collected from the abdominal vein, and lipoprotein fractions were obtained by the method of Hatch and Lees[32] and Havel et al.[33] Each of the fractions was analyzed for cholesterol,[13] triglyceride (Bio-Dynamics/bmc triglyceride kit),[12] neutral lipids,[30] and protein levels.[27]

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Registry No. 1, 606-23-5; 2, 876-83-5; 3, 27606-61-7; 4, 14570-43-5; 5, 91909-55-6; 6, 97920-50-8; 7, 83-12-5; 8, 1470-39-9; 9, 6149-23-1; 10, 117-37-3; 11, 15432-97-0; 12, 15432-98-1; 13, 7561-48-0; 14, 1470-42-4; 15, 1470-44-6; 16, 1146-99-2; 17, 1146-98-1; 18, 19055-67-5; 19, 55994-28-0; 20, 6549-60-6; 21, 7561-62-8; 22, 22445-53-0; 23, 1470-38-8; 24, 19147-02-5; 25, 97920-51-9; 26, 27533-97-7; 27, 1786-03-4; 28, 2156-11-8; 29, 2156-14-1; 30, 6549-63-9; dimethyl phthalate, 131-11-3; phthalide, 87-41-2; 3-pentanone, 96-22-0; 4-heptanone, 123-19-3; 5-nonanone, 502-56-7; 6-undecanone, 927-49-1; di-*n*-hexyl ketone, 462-18-0; dibenzyl ketone, 102-04-5; α-naphthaldehyde, 66-77-3; β-naphthaldehyde, 66-99-9; 4-biphenylcarboxaldehyde, 3218-36-8; 9-anthraldehyde, 642-31-9; benzaldehyde, 100-52-7; 2-methoxybenzaldehyde, 135-02-4; 3-methoxybenzaldehyde, 591-31-1; 2-methylbenzaldehyde, 529-20-4; 3-methylbenzaldehyde, 620-23-5; 4-methylbenzaldehyde, 104-87-0; 2-chlorobenzaldehyde, 89-98-5; 3-chlorobenzaldehyde, 587-04-2; 4-chlorobenzaldehyde, 104-88-1; 4-bromobenzaldehyde, 1122-91-4; 2,4-dichlorobenzaldehyde, 874-42-0; 2,6-dichlorobenzaldehyde, 83-38-5; 3,4-dichlorobenzaldehyde, 6287-38-3; 2,4-dimethylbenzaldehyde, 15764-16-6; 2,4-dimethoxybenzaldehyde, 613-45-6; 3,4-dimethoxybenzaldehyde, 120-14-9; 2-carboxbenzaldehyde, 119-67-5; 4-carboxybenzaldehyde, 619-66-9; 4-ethoxybenzaldehyde, 10031-82-0; 4-methoxybenzaldehyde, 123-11-5; acetyl-CoA synthetase, 9012-31-1; HMG CoA reductase, 9028-35-7; acyl cholesterol acyl transferase, 9027-63-8; cholesterol 7α-hydroxylase, 9037-53-0; acetyl-CoA carboxylase, 9023-93-2; *sn*-glycerol-3-phosphate acyl transferase, 9029-96-3; phosphatidate phosphohydrolase, 9025-77-8; lipoprotein lipase, 9004-02-8; citrate lyase, 9012-83-3.

Supplementary Material Available: Analyses of variance followed by Duncan's multiple-range test of serum cholesterol and serum triglyceride levels (2 pages). Ordering information is given on any current masthead page.

Synthesis and Pharmacology of the Potent Angiotensin-Converting Enzyme Inhibitor *N*-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-(*S*)-alanyl-(*S*)-pyroglutamic Acid†

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Structure **3a**, a potent angiotensin-converting enzyme inhibitor, was prepared in five steps from L-(+)-α-amino-4-phenylbutyric acid by construction of the activated side-chain ester **16**, displacement with L-pyroglutamate ester anion, and deblocking. Diastereomer separation was accomplished by chromatography at the diester stage, **17**. Pharmacological assays established that **3a** parallels enalapril in its ability to inhibit converting enzyme and lower blood pressure.

Recent research activity on antihypertensive agents has been concerned with the rational design of angiotensin-converting enzyme inhibitors,[1] the best known examples of which are captopril,[2] **1**, and enalapril,[3] **2**. We were interested in knowing if the L-pyroglutamic acid analogue (**3a**) of enalapril possesses similar biological properties. However, a thorough search of the existing literature did not reveal **3a** to be a known compound. Since we were convinced that **3a** was synthetically accessible, we sought reasons for this omission because we reasoned that it was a novel structure that ought to possess interesting biological properties. While the 2-oxopyrrolidine and pyrrolidine rings are geometrically similar, there are profound differences in acid–base properties, and we sought to determine if this was important to interaction of the proposed non-sulfhydryl inhibitor with converting enzyme. It turned out that the synthetic method[3] used to prepare **2** is not practical for the preparation of **3a** because of the hydrolytic instability of L-alanyl-L-pyroglutamic acid, **4**. Structure **3a** was prepared by an alternative method and found to be a potent angiotensin-converting enzyme inhibitor and hypotensive agent.[4]

Synthesis of Structure 3a

Structure **3a** consists of three units: L-pyroglutamic acid (A), L-alanine (B), and 2-substituted 4-phenylbutyrate (C). Therefore, its assembly has two possible routes: (A + B) + C and A + (B + C), depending on which final connection is made. Enalapril, **2**, was prepared by the (A + B) + C

† Contribution No. 84-P26.

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