ethylene glycol. The mixture was refluxed for 24 h using a Dean-Stark trap to remove the water which formed during condensation. The mixture was then extracted with 5 ml of 10% $NaHCO_3$. The benzene layer was separated, washed with 5 ml of water, dried ($MgSO_4$), and removed under vacuum to leave the desired compound.

4-Pyrrolidino-2-formylpyridine Ethylene Acetal (27). To 0.93 g (0.005 mol) of **26** was added 3 ml of pyrrolidine and the solution was refluxed for 2 h. Excess pyrrolidine was removed under vacuum, the residue was extracted with Et_2O, the solvent removed, and the residue was crystallized from Et_2O and petroleum ether.

4-Bis(hydroxyethyl)amino-2-formylpyridine Ethylene Acetal (29). To 0.93 g (0.005 mol) of **26** was added 3 g of diethanolamine. This mixture was heated at 140° for 8 h, extracted with 100 ml of $CHCl_3$, treated with activated charcoal, and filtered, and the solvent was removed under vacuum. The residue was crystallized from acetone.

4-Morpholino-3-methyl-2-formylpyridine Ethylene Acetal (36). This compound was synthesized by a procedure similar to that described for **27** except that heating of **35** with morpholine was carried out at 140° for 40 h.

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Synthesis and Antilipidemic Properties of *cis*-7-Chloro-3a,8b-dihydro-3a-methylfuro[3,4-*b*]benzofuran-3(1*H*)-one, a Tricyclic Clofibrate Related Lactone Having a Structural Resemblance to Mevalonolactone[1]

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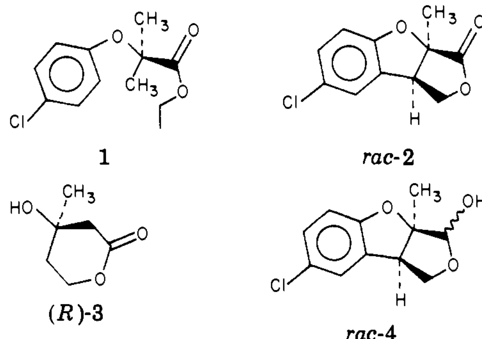
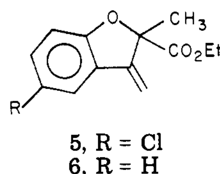
The synthesis for the title lactone **2**, designed to be an antagonist of the enzyme HMG-CoA reductase (E.C. 1.1.1.34), is described. Lactone **2**, its synthetic tricyclic hemiacetal precursor **4**, and clofibrate were investigated for their antilipidemic activity in 7-day treated normal and in Triton WR-1339 induced hyperlipidemic male Sprague–Dawley rats. After 7-day drug administration to normal rats, lactone **2** was less effective than clofibrate in lowering HMG-CoA reductase activity and serum cholesterol; however, unlike clofibrate, lactone **2** did not increase liver weight or liver–body weight ratio or lower serum triglycerides. Since hemiacetal **4** selectively influenced triglycerides in normal animals, lactone **2** and hemiacetal **4** appear to have differential hypolipidemic effects. In the Triton hyperlipidemic model **2** and **4** lowered elevated triglycerides; only **4** significantly reduced elevated cholesterol levels; but neither **2** nor **4** was as effective as clofibrate. Differences in the observed antilipidemic properties for clofibrate, **2**, and **4** in the two animal models are discussed. On the basis of preliminary biological data described in this article it is concluded that tricyclic analogues **2** and **4** represent reasonable leads for the development of new antilipidemic agents.

A major action of clofibrate (**1**) involves its hypotriglyceridemic activity, and for these reasons this drug is mainly effective in the treatment of patients with hyperlipoproteinemia types III, IV, and V.[2] However, clofibrate also inhibits 3-hydroxy-3-methylglutaryl-CoA reductase activity (HMG-CoA reductase, E.C. 1.1.1.34) in vivo[3] and its hypocholesterolemic activity, in part, may be related to its ability to block cholesterol biosynthesis. For purposes of developing new compounds which might retain the hypotriglyceridemic properties of clofibrate, but exhibit

enhanced hypocholesterolemic activity, we developed a synthesis for the "clofibrate-related" lactone **2**. One enantiomorph of racemic lactone **2** is also related to (*R*)-mevalonolactone (**3**); therefore, we rationalized that **2** might exhibit greater affinity for HMG-CoA reductase or other enzymes involved in the conversion of **3** and its precursors to cholesterol. Owing to the similar structural relationship of hemiacetal **4** to **1** and **2**, this synthetic precursor to lactone **2** was also investigated for antilipidemic activity and its effect on HMG-CoA reductase activity.



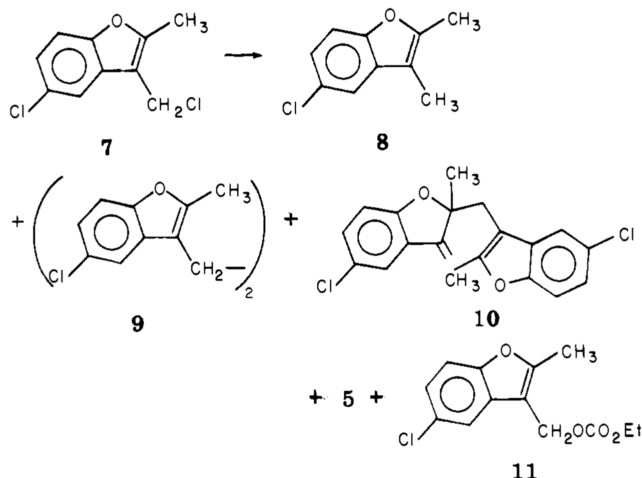
Synthetic Aspects. The key intermediate, 3-methylenedihydrobenzofuran ester **5**, was prepared according to methods similar to those employed by Gaertner[4] in the preparation of deschloro analogue **6**. Whereas Gaertner was unable to obtain pure **6**, we obtained pure **5** in approximately 56% yield from intermediate **7** after column chromatography of the reaction mixture.



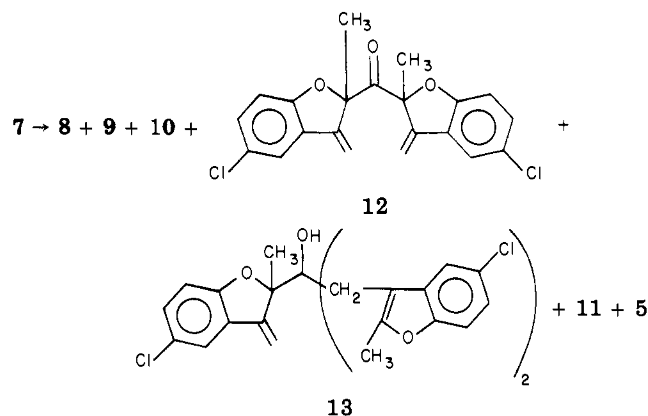
5, R = Cl
6, R = H

The products resulting from attempts to convert **7** to **5** under Grignard conditions using two entirely different reaction conditions were investigated. One involved reaction conditions virtually identical with those reported by Gaertner[4,5] (Mg, $ClCO_2Et$, THF, dry ice–acetone bath), whereas the other involved reaction conditions similar to those employed in the carbonation of α-naphthylmagnesium bromide[6] [$(EtO)_2CO$, THF, room temperature]. Reaction products resulting from both methods were separated by column chromatography on silica gel by elution with solvent mixtures of increasing polarity (see Experimental Section). Under Gaertner's conditions **5** eluted from the column last and was contaminated with a trace of carbonate **11** (GLC). Distillation afforded pure **5** which exhibited characteristic vinyl proton resonance signals at δ 5.23 and 5.48. Elution of **5** was preceded by dimethylbenzofuran **8**[7] (2.2%), followed by dimers **9** (6.5%) and **10** (5.4%), respectively. Dimers **9** and **10** exhibited the same molecular ion (*m/e* 358) in their mass spectra and their structures were confirmed by NMR and uv analysis. The uv spectra for **9** and **10** when analyzed in light of the spectra for **5** and **8**, were particularly diagnostic. The uv spectrum for **9** showed λ_{max} (MeOH) 294 nm (ϵ 7240) and 287 (7360) which are similar to those observed for **8** [λ_{max} (MeOH) 293 nm (ϵ 3400), 285 (3630)], whereas **10** exhibited λ_{max} (MeOH) [339 nm (ϵ 6730), 326 (6440), 293 (4050), 285 (3830)] attributable to the mixture of chromophores found in **8** and **5** [λ_{max} (MeOH) 335 nm (ϵ 7350) and 322 (7350)].

Reaction of **7** at room temperature in the presence of Mg and $(EtO)_2CO$ afforded a considerably different



spectrum of isolable products. Only a trace of **5** could be detected by GLC as a contaminant of **11** following elution from the column. Again, **8** (9.2%), **9** (2.2%), and **10** (3.4%) eluted from the column first. These were followed by the new ketone dimer **12** (5.1%) and tertiary alcohol trimer **13** (25.8%) with the mixture of **11** (7.3%) and **5** (trace) eluting last. Although ketone **12** did not exhibit a molecular ion in the mass spectrum, its NMR, ir (1725 cm^{-1}, C=O), and uv [λ_{max} (MeOH) 335 nm (ϵ 12700), 322 (13300)] spectra were in agreement with the assigned structure; i.e., the uv spectrum for **12** was similar to the spectrum observed for **5**. The major product, trimer **13**, exhibited a molecular ion at *m/e* 566 and NMR and ir spectra consistent with the structural assignment. The uv spectrum of **13** [λ_{max} (MeOH) 338 nm (ϵ 6700), 325 (6440), 293 (6700), 285 (7090)] supported the presence of the 2,3-dihydro-3-methylenebenzofuran chromophore and two benzofuran functions. Carbonate **11** was characterized by ir, NMR, and uv analysis and by conversion to **16** upon hydrolysis in alcoholic KOH. Since **12** and **13** likely arise by attack of the Grignard reagent on **5** at the higher temperature, only traces of the desired product were obtained. Reverse addition and cold temperatures favor formation of the desired intermediate. Owing to the complex nature of Grignard reagents, further explanation for product formation under these conditions is not warranted.



Conversion of **5** to **4** is consistent with the assigned structure for **5**. Further, the properties for Grignard product **5** were different than those observed for an authentic sample of the isomeric analogue **15** prepared from **7** by reaction with NaCN followed by acid-catalyzed solvolysis of product **14** in ethanol. The NMR and ir spectra were consistent with the assigned structure for **15** and the uv spectrum showed the presence of the characteristic benzofuran chromophore.

Table I. Comparative Effects of Clofibrate (1) with Tricyclic Analogues 2 and 4 on Plasma Cholesterol Levels (mg %) in Triton-Induced Hyperlipidemic Male Sprague-Dawley Rats

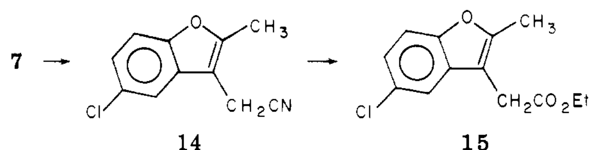
Compd[a]	Control group (I)	Drug-treated control (II)	Triton hyperlipidemic (III)	Drug-treated Triton hyperlipidemic (IV)
Clofibrate (1)	88.7 ± 6.9[b]	87.0 ± 8.1	166 ± 17.8	93.2 ± 8.9[c]
2	86.3 ± 13.0	79.2 ± 13.1	142 ± 27.0	127 ± 17.9[d]
4	94.0 ± 10.8	93.0 ± 14.1	157 ± 18.6	119 ± 11.2[c,d]

[a] All animals were given a total screening dose of 0.124 mM/kg of analogue. [b] Mean ± SD; ten rats per group. [c] Statistically significant, $p < 0.05$; Triton hyperlipidemic vs. drug-treated Triton hyperlipidemic (comparison of groups III and IV). [d] Statistically significant; $p < 0.05$; drug-treated hyperlipidemic vs. control (comparison of groups I and IV).

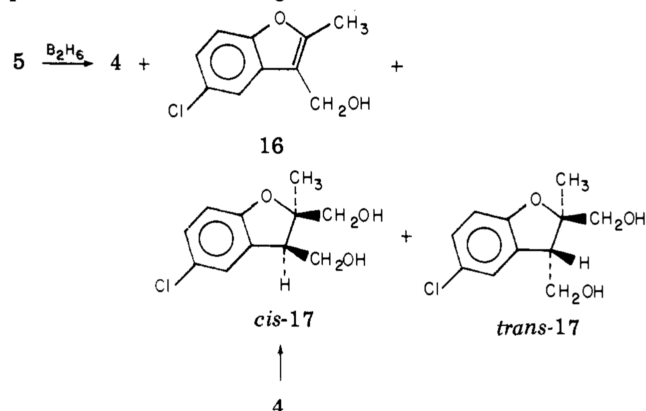
Table II. Comparative Effects of Clofibrate (1) with Tricyclic Analogues 2 and 4 on Plasma Triglyceride Levels (mg %) in Triton-Induced Hyperlipidemic Male Sprague-Dawley Rats

Compd[a]	Control group (I)	Drug-treated control (II)	Triton hyperlipidemic (III)	Drug-treated Triton hyperlipidemic (IV)
Clofibrate (1)	20.8 ± 5.4[b]	24.1 ± 7.4	98.5 ± 16.9	22.3 ± 8.3[c]
2	30.7 ± 11.6	32.3 ± 6.8	94.8 ± 13.4	54.2 ± 9.2[c,d]
4	27.8 ± 5.4	25.2 ± 6.9	112 ± 15.5	34.5 ± 7.1[c,d]

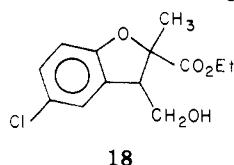
[a] All animals were given a total screening dose of 0.124 mM/kg of analogue. [b] Mean ± SD; ten rats per group. [c] Statistically significant; $p < 0.05$; Triton hyperlipidemic vs. drug-treated Triton hyperlipidemic (comparison of groups III and IV). [d] Statistically significant; $p < 0.05$; drug-treated hyperlipidemic vs. control (comparison of groups I and IV).



Hydroboration–oxidation[8] of **5** (1:1; BH_3-**5**) afforded hemiacetal **4** (47%), alcohol **16** (2.7%), and a mixture of *cis*- and *trans*-diols **17** (3.7 and 26.8%, respectively) which were separated by column chromatography on silica gel. The structure for hemiacetal **4** was confirmed by spectroscopy and by $LiAlH_4$ reduction to *cis*-diol **17**. The NMR spectrum for **4** was in agreement with the assigned structure and the ir spectrum showed no carbonyl stretching vibrations, but broad OH stretching at 3400 cm^{-1}. While these data substantiate the cis ring juncture for **4**, it also should be pointed out that a Dreiding molecular model of **4** indicates *trans*-**4** to be virtually impossible to obtain owing to bond strain.

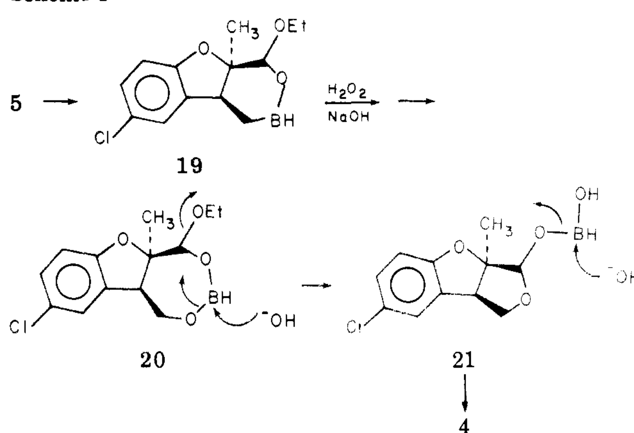


Although hydroxymethyl analogue **18** and isomeric diols **17** were expected to be formed from **5** under the conditions of our experiments, **18** could be detected in the reaction mixture. Hemiacetal formation during this reaction is of



interest since Brown and co-workers[9] could not detect formation of aldehydes during the conversion of esters to

Scheme I



alcohols under a variety of hydroboration reaction conditions. When the molar amount of BH_3 was reduced to one-third that of **5**, starting material (42%) was recovered along with 42% hemiacetal **4** and 4% diol products **17** (mainly trans). Utilization of dicyclohexylborane[10] and dihexylborane gave no reactions. A possible rationale for formation of **4** is found in Scheme I and involves formation of cyclic intermediate **19**. Similar cyclic intermediates have been proposed to account for the double reduction of the amide and ester functions in methyl hippurate.[11] Coordinate covalent bond formation of the ester carbonyl oxygen with boron results in an increase in the electrophilic character of the ester carbonyl carbon.[11] Intramolecular hydride attack on this carbon is thus facilitated, affording **19**. Peroxide oxidation of **19**, followed by ring expansion, ultimately is expected to yield **20**.[8] Nucleophilic attack by ^-OH ion on boron followed by alkoxide migration and loss of ethoxide ion would yield **21**, the likely precursor to hemiacetal **4**.

Jones oxidation[12] of hemiacetal **4** afforded lactone **2** in 73% yield. Lactone **2** exhibited characteristic carbonyl stretching for the five-membered lactone[13] in the ir (1780 cm^{-1}) and its NMR spectrum was in agreement with the assigned structure. The resonance signal for the proton bonded to the hemiacetal C of **4** [δ 5.44 (1 H, d, J = 3 Hz)] was absent in the NMR spectrum of **2**.

Pharmacological Results. The antilipidemic activity of **1**, **2**, and **4**, at equivalent dosages (mmol/kg), was first determined in fasted normal and Triton WR-1339 induced

Table III. Effect of Pretreatment with 1, 2, and 4 on Hepatic β-Hydroxy-β-methylglutaryl-CoA Reductase Activity and on Levels of Cholesterol and Triglycerides in Normolipemic Rats

Parameter[a] (7-day treatment)	Clofibrate 1		Lactone 2		Hemiacetal 4	
	Control[b] group	Treated[b,c] group	Control[b] group	Treated[b,c] group	Control[b] group	Treated[b,c] group
Serum cholesterol (mg %)	68.2 ± 16.0	43.9 ± 4.0[d]	93.8 ± 21.2	69.7 ± 14.1	88.7 ± 14.7	93.9 ± 20.1
Serum triglycerides (mg %)	91.0 ± 23.4	43.9 ± 20.9[d]	100.0 ± 63.6	139.0 ± 59.1	96.1 ± 38.0	28.6 ± 16.8[d]
Liver cholesterol (mg/g)	2.64 ± 0.29	2.22 ± 0.14[d]	2.07 ± 0.57	1.71 ± 0.29	1.90 ± 0.20	1.90 ± 0.33
Liver triglycerides (mg/g)	3.70 ± 0.54	6.15 ± 0.49[d]	4.52 ± 0.86	5.65 ± 0.60[d]	4.45 ± 0.62	4.91 ± 0.73
HMG-CoA reductase (nmol/mg/h)[e]	77.1 ± 16.3	61.0 ± 9.20[d]	20.4 ± 4.70	16.8 ± 2.90	14.2 ± 2.00	15.6 ± 3.00
Liver/body wt (%)	4.29 ± 0.37	5.62 ± 0.35[d]	4.18 ± 0.29	4.48 ± 0.22	4.06 ± 0.32	4.74 ± 0.10
Liver wt (g)	9.88 ± 0.99	13.5 ± 1.97[d]	8.31 ± 1.46	9.18 ± 0.49	8.54 ± 1.11	9.62 ± 0.68[d]
Microsomal protein (mg/g of liver)	22.8 ± 2.36	26.5 ± 1.50[d]	31.3 ± 0.78	36.6 ± 2.55[d]	30.8 ± 2.26	34.0 ± 4.78

[a] The measurements were determined before (day 0) and after completion of the 7-day drug pretreatment period. [b] Values represent the mean ± SD of $N = 5-6$. [c] Treated animals were given the drug orally twice daily at a dose level of 0.4 mmol/kg; control animals received a requisite volume of vehicle. [d] Significant difference from the control ($p < 0.05$). [e] mmol/mg/h refers to millimoles of mevalonate formed per milligram of microsomal protein per hour of incubation.

hyperlipidemic male Sprague–Dawley rats according to the method of Schurr and co-workers.[14] The results are found in Tables I (serum cholesterol levels) and II (serum triglyceride levels). Clofibrate significantly reduced both elevated serum cholesterol and triglyceride levels to those found in controls; these data are in agreement with results obtained by us in an earlier structure–activity study involving clofibrate and related cyclic analogues.[15] Lactone 2 did not reduce elevated cholesterol levels under these conditions but did reduce elevated serum triglyceride levels (comparison of groups III and IV). Hemiacetal 4 reduced both elevated serum cholesterol and triglyceride levels, but neither 2 nor 4 lowered elevated cholesterol or triglycerides to serum concentrations found in control animals (comparison of groups I and IV). Under the conditions of these experiments (0.124 mmol/kg total dose) neither 1, 2, or 4 lowered cholesterol or triglyceride levels in normal-fasted animals (comparison of groups I and II).

The hypolipidemic activity of cyclic analogues 2 and 4 of clofibrate (1) was also investigated in nonfasted normal male Sprague–Dawley rats weighing 180–200 g. In this model, a 7-day administration of clofibrate significantly lowered serum triglycerides and liver cholesterol concentrations, elevated liver triglyceride concentrations, and inhibited hepatic HMG-CoA reductase activity (Table III). Lactone 2 appeared to have its major effect on cholesterol parameters; the effects on liver and serum cholesterol concentrations and HMG-CoA reductase activity were less than that observed after clofibrate administration. Although the effect of lactone 2 on liver triglyceride was similar to that observed for clofibrate, 2 did not lower the serum triglyceride level. Hemiacetal 4 appeared to have its major effect on the triglyceride components. Clofibrate treatment increased liver triglyceride concentrations whereas no change in triglyceride content was observed following administration of hemiacetal 4. However, serum triglyceride levels were markedly reduced by 4, whereas this hemiacetal did not modify any of the cholesterol parameters.

Several investigators[16–19] have demonstrated that clofibrate pretreatment results in an increase of liver weight along with a marked change in the liver cells. In our studies, increases were observed in hepatic microsomal protein content (mg of protein/g of liver) of 116% in the 7-day treatment period with 1. These observations are supported by Platt and Thorpe[16] who have also demonstrated increases in hepatic protein concentration in rats. Lactone 2 was comparable in this respect; pretreatment significantly increased hepatic microsomal protein content to 117% that of controls. However, 2 had no significant effect in increasing the liver weight or liver–body weight ratio. Hemiacetal 4 increased the liver weight and liver–body weight ratio but had no effect on hepatic microsomal protein content.

Discussion

Whereas blocking NE-induced lipolysis in vitro seems to correlate with a lowering of serum triglyceride levels in the Triton hyperlipidemic rat model,[20] results reported in this article indicate inhibiting HMG-CoA reductase activity in normal rats after prefeeding correlates with the ability of an analogue to lower serum cholesterol levels. Results previously communicated[21] utilizing other clofibrate-related analogues also corroborate the positive correlation between the reduction of HMG-CoA reductase activity and cholesterol levels in normal rats chronically administered various compounds. No correlation exists between the effect of these analogues (1, 2, and 4) on serum cholesterol and triglyceride levels in Triton-induced hyperlipidemic rats and chronically treated normal rats. This may be expected since the Triton hyperlipidemic model relies on elevated endogenous lipid levels, whereas the 7-day nonfasted animal model relies on both endogenous and exogenous levels of circulating lipids. Further, there is a definite difference in serum triglyceride levels observed in these two models; this may be directly attributable to the influx of chylomicron triglyceride into the blood stream of the nonfasted animals. For clofibrate, lowered serum cholesterol levels have been attributed to an inhibition of hepatic cholesterol biosynthesis.[3] Additionally, its mode of action in lowering serum lipids could be due to the facilitation of the conversion of very low density lipoproteins (VLDL) to low density lipoproteins (LDL) leading to removal of triglyceride and cholesterol.[22,23] The observation that lactone 2 apparently has an effect on cholesterol parameters in normal animals, whereas hemiacetal 4 only lowered serum triglycerides in this model, lends support to the rationale employed in drug design leading to the synthesis of 2. However, 2 is less effective than 1 in both animal models; this suggests that further structural modification is necessary to amplify the antilipidemic action of this lactone. Interestingly, the small structural difference between 2 and 4 has resulted in a marked change in the lipid-lowering profile in both animal models.

Clofibrate has also been shown to produce a marked increase in liver triglyceride content in the nonfasted rat model, presumably by a decreased release of liver triglycerides into serum.[24] Although the effect of lactone 2 on liver triglyceride was similar to that observed for

clofibrate, this increased liver concentration was not followed by a lowering of serum triglyceride. These findings would imply that the effect of **2** was at a site other than triglyceride synthesis or release from the liver. A negative correlation between liver and serum triglyceride levels is to be expected since others have observed that an increase in liver triglyceride concentration may not be directly related to a decrease in serum triglyceride level.[24,25] The high degree of variability observed in the serum triglyceride data in these studies has also been noted elsewhere for clofibrate.[26-28] This variability, in part, may be attributable to influx of exogenous triglycerides in the nonfasted animals utilized in this study. Nonfasted animals were used in order to parallel the studies of White on the inhibition of hepatic cholesterol biosynthesis by clofibrate.[3] While the degree of variability was greater for drug **2** than **1** or **4**, the variability is likely not related to the drug per se; the serum triglyceride levels from both groups of animals were nearly identical prior to drug treatment. Since hemiacetal **4** seems to have its major effect on serum triglycerides, lactone **2** and hemiacetal **4** appear to have differential hypolipidemic effects in normal animals.

If, in fact, the Triton model is predictive[14,15] of hyperlipoproteinemia types IV and V,[2] it may be anticipated that analogues **2** and **4** would have clinical value in the treatment of elevated triglyceride levels. However, there is greater need for drugs effective in type IIa hyperlipoproteinemia and for these reasons the cholesterol lowering effects of lactone **2** in normal animals are of particular interest. Lactone **2** is putatively useful because unlike **1** and **4** this analogue did not increase the liver weight or liver–body weight ratio during 7-day administration to normal animals.

Experimental Section[29]

A. Synthetic Methods. 5-Chloro-3-chloromethyl-2-methylbenzofuran (7) was prepared according to the method of Gaertner.[4] To a solution of 10.5 g (0.12 mol) of trioxane in 200 ml of concentrated HCl was added dropwise at room temperature 17.2 g (0.11 mol) of 5-chloro-2-methylbenzofuran.[30] After stirring for 6 h, the mixture was dissolved in benzene, washed (H_2O), dried (Na_2SO_4), and concentrated under reduced pressure. The solid residue was recrystallized from hexane affording 14.5 g (65.3%) of white needles: mp 98–99°; NMR ($CDCl_3$) δ 2.45 (3 H, s, $-CH_3$), 4.62 (2 H, s, $-CH_2Cl$), and 7.1–7.6 (3 H, m, aromatic). Anal. ($C_{10}H_8OCl_2$) C, H, Cl.

Grignard reaction of 5-chloro-3-chloromethyl-2-methylbenzofuran (7) with ethyl chlorocarbonate was carried out by a modification of the method of Gaertner.[4] A solution of Grignard reagent prepared from 2.4 g (0.1 g-atom) of Mg and 2 g (0.01 mol) of **7** in 70 ml of THF was added to a solution of 44 g (0.4 mol) of ethyl chlorocarbonate in 50 ml of anhydrous THF cooled in a dry ice–Me_2CO bath. The cooling bath was removed and the mixture was allowed to warm to 0°. H_2O (20 ml) was added followed by a solution of NaOH (12 g) in H_2O (50 ml). After stirring at 0° for 20 min, the reaction mixture was extracted with benzene, and the benzene layer was washed (H_2O), dried (Na_2SO_4), and concentrated under reduced pressure. The residue (24 g) was chromatographed on silica gel (31 × 650 mm) by elution with 300 ml of hexane followed by 750 ml of hexane–benzene (2:1) and 1200 ml of hexane–benzene (1:1). Fractions were monitored by TLC [hexane-benzene (3:1)].

(A) **5-Chloro-2,3-dimethylbenzofuran (8)**, bp 80–82° (0.4 mm) [lit.[7] bp 135–137° (20mm)], was obtained from the first 100 ml of the hexane–benzene (2:1) eluate in a yield of 0.4 g (2.2%) and characterized by NMR, ir, and uv [(MeOH), λ_{max} 293 nm (ϵ 3400), 285 (3630)].

(B) **3,3′-Ethylenebis(5-chloro-2-methylbenzofuran) (9)**, mp 157–158°, was obtained from the eluate resulting from elution with an additional 300 ml of hexane–benzene (2:1). Compound **9** was isolated in a yield of 1.2 g (6.5%) after crystallization from benzene: NMR ($CDCl_3$) δ 2.00 (6 H, s, $-CH_3$), 2.87 (4 H, s, $-CH_2-$), 6.9–7.4 (6 H, m, aromatic); uv (MeOH) λ_{max} 294 nm (ϵ 7240), 287 (7360). Anal. ($C_{20}H_{16}O_2Cl_2$) C, H, Cl.

(C) **5-Chloro-2-[(5-chloro-2-methyl-3-benzofuranyl)methyl]-2,3-dihydro-2-methyl-3-methylenebenzofuran (10)** eluted with the hexane–benzene (2:1) mixture following compound **9**. Concentration of the eluate under reduced pressure followed by recrystallization of the residue from cyclohexane afforded 1.0 g (5.4%) of white plates: mp 113–114°; NMR ($CDCl_3$) δ 1.48 (3 H, s, $-CH_3$), 2.33 (3 H, s, $-CH_3$), 2.90 (2 H, s, $-CH_2-$), 4.94 (1 H, s, vinyl H), 5.39 (1 H, s, vinyl H), 6.5–7.6 (6 H, m, aromatic); uv (MeOH) λ_{max} 339 nm (ϵ 6730), 326 (6440), 293 (4050), 285 (3830). Anal. ($C_{20}H_{16}O_2Cl_2$) C, H, Cl.

(D) **Ethyl 5-chloro-2,3-dihydro-2-methyl-3-methylene-2-benzofurancarboxylate (5)** eluted with the hexane–benzene (1:1) mixture. After concentration of the eluate under reduced pressure, the residue was distilled affording 14.2 g (56.3%) of colorless oil: bp 103–105° (0.1 mm). A small portion of the oil crystallized in petroleum ether (bp 30–60°) to give white plates: mp 38–39°; NMR ($CDCl_3$) δ 1.24 (3 H, t, $-CH_2CH_3$, J = 7 Hz), 1.74 (3 H, s, $-CH_3$), 4.20 (2 H, q, $-CH_2CH_3$, J = 7 Hz), 5.23 (1 H, d, vinyl H, J = 1 Hz), 5.48 (1 H, d, vinyl H, J = 1 Hz), 6.7–7.4 (3 H, m, aromatic); ir (neat) 1740 cm^{-1} (C=O); uv (MeOH) λ_{max} 335 nm (ϵ 7350), 322 (7350). Anal. ($C_{13}H_{13}O_3Cl$) (oil) C, H, Cl.

Grignard Reaction of 5-Chloro-3-chloromethyl-2-methylbenzofuran (7) with Diethyl Carbonate. The Grignard reagent prepared from 11.0 g (0.051 mol) of **7** and 2.4 g (0.1 g-atom) of Mg in 70 ml of THF was added with stirring to a solution of 12.0 g (0.1 mol) of diethyl carbonate in 15 ml of THF at room temperature. After stirring for 15 h at room temperature, the mixture was poured over crushed ice, and 15 g of NH_4Cl was added. The product was extracted with ether and the organic layer was washed (H_2O), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was chromatographed on silica gel [monitored by TLC (hexane–benzene) (3:1)] and eluted with hexane–benzene (3:1) and hexane–benzene (1:1). The early fractions eluted with hexane–benzene (3:1) to afford fraction A, 0.9 g (9.2%) of **8**, fraction B, 0.2 g (2.2%) of **9**, and fraction C, 0.3 g (3.4%) of **10**.

(D) **Bis(5-chloro-2,3-dihydro-2-methyl-3-methylene-2-benzofuranyl) ketone (12)** eluted after **10** with hexane–benzene (3:1) affording 0.5 g (5.1%) of colorless plates: mp 141–143° after recrystallization from benzene; NMR ($CDCl_3$) δ 1.58 (6 H, s, $-CH_3$), 4.95 (2 H, d, vinyl H, J = 1.5 Hz), 5.44 (2 H, d, vinyl H, J = 1.5 Hz), 5.8–7.4 (6 H, m, aromatic); ir (Nujol) 1725 cm^{-1} (C=O); uv (MeOH) λ_{max} 335 nm (ϵ 12700), 322 (13300). Anal. ($C_{21}H_{16}O_3Cl_2$) C, H, Cl.

(E) **α,α-Bis(5-chloro-2-methyl-3-benzofuranylmethyl)-5-chloro-2,3-dihydro-2-methyl-3-methylene-2-benzofuranmethanol (13)** eluted with hexane–benzene (3:1) affording 2.5 g (25.8%) of white plates: mp 205–207° after recrystallization from benzene; NMR (Me_2SO-d_6) δ 1.52 (3 H, s, CH_3), 2.14 (3 H, s, $-CH_3$), 2.34 (3 H, s, $-CH_3$), 2.6–3.1 (4 H, m, 2-CH_2–), 4.95 (1 H, s, OH), 5.34 (1 H, s, vinyl H), 5.63 (1 H, s, vinyl H), 6.6–7.7 (9 H, m, aromatic); ir (Nujol) 3550 cm^{-1} (OH); uv (MeOH), λ_{max} 338 nm (ϵ 6700), 325 (6440), 293 (6700), 285 (7090); mass spectrum m/e (rel intensity) 566 (M^+, 1.2), 387 (13.1), 179 (100). Anal. ($C_{31}H_{25}O_4Cl_3$) C, H, Cl.

(F) **5-Chloro-2-methyl-3-benzofuranylmethyl ethyl carbonate (11)** eluted with hexane–benzene (1:1). Concentration of the eluate under reduced pressure followed by distillation gave a colorless oil: bp 120–123° (0.1 mm). The oil crystallized from petroleum ether (bp 30–60°) affording 1.0 g (7.3%) of white needles: mp 58–59° (recrystallization from petroleum ether); NMR ($CDCl_3$) δ 1.30 (3 H, t, $-CH_2CH_3$, J = 7 Hz), 2.50 (3 H, s, $-CH_3$), 4.20 (2 H, q, $-CH_2CH_3$, J = 7 Hz), 5.21 (2 H, s, $-CH_2-$), 7.2–7.7 (3 H, m, aromatic); ir (neat) 1750 cm^{-1} (C=O). Anal. ($C_{13}H_{13}O_4Cl$) C, H, Cl.

Reaction of Ethyl 5-Chloro-2,3-dihydro-2-methyl-3-methylene-2-benzofurancarboxylate (5) with Diborane in THF. A solution of 15.2 g (0.06 mol) of **5** in 40 ml of anhydrous THF was added dropwise to a cooled solution of 70 ml of 0.95 M diborane (0.067 mol of BH_3) in THF (Ventron, Beverly, Mass.). The reaction mixture was stirred for 1 h at 0° followed by 2 h at room temperature. The excess diborane was decomposed by dropwise addition of 15 ml of H_2O at 0° with stirring. Subse-

quently, 3 N NaOH solution (25 ml) was added followed by dropwise addition of 30% H_2O_2 (30 ml). The mixture was stirred for 1 h at room temperature and was extracted with Et_2O. The Et_2O solution was washed (H_2O), dried (Na_2SO_4), and concentrated under reduced pressure. The residue (approximately 15 g) was chromatographed on silica gel (35 × 720 mm) by elution with 500 ml of benzene, followed by 1000 ml of benzene–EtOAc (3:1), 1200 ml of benzene–EtOAc (2:1), and 800 ml of benzene–EtOAc (1:1).

(A) **3a,8b-*cis*-7-Chloro-1,3,3a,8b-tetrahydro-3a-methylfuro[3,4-*b*]benzofuran-3-ol (4)** was obtained from the benzene–EtOAc (3:1) eluate and was recrystallized from benzene–hexane or CCl_4 affording 6.4 g (47.0%) of white plates: mp 117–118°; NMR ($CDCl_3$) δ 1.55 (3 H, s, $-CH_3$), 3.19 (1 H, d, OH, J = 3 Hz), 5.44 (1 H, d, -CHO-, J = 3 Hz), 6.6–7.4 (3 H, m, aromatic). The ABC computer simulated spectrum[31] shows for $-CHCH_2-$ δ H_A 4.40, H_B 3.90, H_C 3.54 with J_{AB} = 8.7 Hz, J_{AC} = 6.7 Hz, and J_{BC} = 1.6 Hz; ir (Nujol) 3400 cm^{-1} (OH). Anal. ($C_{11}H_{11}O_3Cl$) C, H, Cl.

(B) **5-Chloro-3-hydroxymethyl-2-methylbenzofuran (16)** was obtained from the mother liquor during crystallization of 4; analogue 16 was recrystallized from benzene affording 0.3 g (2.7%) of white needles: mp 141–142°; NMR ($CDCl_3$) δ 1.68 (1 H, t, OH, J = 5 Hz), 2.43 (3 H, s, $-CH_3$), 4.70 (2 H, d, $-CH_2$, J = 5 Hz), 7.1–7.7 (3 H, m, aromatic); ir (Nujol) 3350 cm^{-1} (OH). Anal. ($C_{10}H_9O_2Cl$) C, H, Cl.

(C) **5-Chloro-2,3-dihydro-*cis*-2,3-bis(hydroxymethyl)-2-methylbenzofuran (*cis*-17)** was obtained from the benzene–EtOAc (2:1) eluate and was recrystallized from benzene affording 0.5 g (3.6%) of white needles: mp 71–73°; NMR ($CDCl_3$) δ 1.47 (3 H, s, $-CH_3$), 3.2–3.5 (3 H, m, -CH- + 2-OH), 3.7–4.1 (4 H, m, 2-CH_2-), 6.5–7.3 (3 H, m, aromatic); ir (Nujol) 3350 cm^{-1} (OH). Anal. ($C_{11}H_{13}O_3Cl$) C, H, Cl.

(D) **5-Chloro-2,3-dihydro-*trans*-2,3-bis(hydroxymethyl)-2-methylbenzofuran (*trans*-17)** was obtained from the benzene–EtOAc (1:1) eluate and was recrystallized from benzene affording 3.7 g (26.8%) of white needles: mp 87–88°; NMR ($CDCl_3$) δ 1.40 (3 H, s, $-CH_3$), 2.5–3.1 (2 H, broad s, -OH), 3.3–4.1 (5 H, m, -CH- + 2-CH_2-), 6.6–7.4 (3 H, m, aromatic); ir (Nujol) 3270 cm^{-1} (OH). Anal. ($C_{11}H_{13}O_3Cl$) C, H, Cl.

Hydrolysis of 5-Chloro-2-methyl-3-benzofuranylmethyl Ethyl Carbonate (11). A solution of 100 mg (0.37 mmol) of 11 in 10 ml of EtOH containing 100 mg (1.8 mmol) of KOH was stirred for 14 h at room temperature. After removal of the solvent under reduced pressure, the residue was dissolved in Et_2O and the Et_2O solution was washed (H_2O), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was recrystallized from benzene affording 55 mg (75.3%) of white needles, mp 141–142°, identical in all respects with 5-chloro-3-hydroxymethyl-2-methylbenzofuran (16) prepared from 5.

5-Chloro-2,3-dihydro-*cis*-2,3-bis(hydroxymethyl)-2-methylbenzofuran (*cis*-17) from Hemiacetal 4. To a cooled (ice bath) solution of 1.0 g (0.026 mol) of lithium aluminum hydride in 20 ml of dry THF was added dropwise with stirring 3.6 g (0.016 mol) of hemiacetal 4 in 10 ml of dry THF. After being stirred for 2 h at room temperature, the excess hydride was decomposed by the addition of H_2O and 10% HCl solution. The product was extracted with Et_2O and the Et_2O layer was washed (H_2O), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was recrystallized from benzene affording 3.5 g (96.4%) of white needles, mp 71–73°, identical in all respects with *cis*-17 prepared from 5.

***cis*-7-Chloro-3a,8b-dihydro-3a-methylfuro[3,4-*b*]benzofuran-3(1*H*)-one (2).** To a solution of 4.1 g (0.018 mol) of hemiacetal 4 in 60 ml of Me_2CO held at 0° was added dropwise with stirring 7.0 ml of Jones reagent[12] (14 g of CrO_3 in 12 ml of concentrated H_2SO_4 diluted to 50 ml with H_2O). The reaction mixture was stirred at room temperature for 25 min. Sufficient *i*-PrOH was added to destroy the reagent, and the mixture was diluted with H_2O. The aqueous mixture was extracted with Et_2O and the Et_2O layer was washed with $NaHCO_3$ solution and H_2O and dried (Na_2SO_4). Concentration under reduced pressure followed by recrystallization of the residue from benzene–hexane or CCl_4 afforded 3.0 g (73.2%) of white plates: mp 80–81°; NMR ($CDCl_3$) δ 1.75 (3 H, s, $-CH_3$), 6.7–7.3 (3 H, m, aromatic). The ABC computer-simulated spectrum[31] shows for $-CHCH_2-$ δ H_A 4.65, H_B 4.43, H_C 4.00 with J_{AB} = 9.3 Hz, J_{AC} = 7.5 Hz, and J_{BC} = 1.1 Hz; ir (Nujol) 1780 cm^{-1} (C=O). Anal. ($C_{11}H_9O_3Cl$) C, H, Cl.

5-Chloro-2-methyl-3-benzofuranacetonitrile (14). A mixture of 11.0 g (0.05 mol) of 7, 5.0 g (0.1 mol) of NaCN, 7.5 g (0.05 mol) of NaI, and 100 ml of MeCOEt was refluxed for 10 h. After cooling, the mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in Et_2O and the Et_2O solution was washed (H_2O), dried (Na_2SO_4), and concentrated under reduced pressure. The solid residue was recrystallized from cyclohexane affording 7.7 g (74.7%) of white needles: mp 93–94°; NMR ($CDCl_3$) δ 2.44 (3 H, s, $-CH_3$), 3.60 (2 H, s, $-CH_2-$), and 7.2–7.6 (3 H, m, aromatic); ir (Nujol) 2265 cm^{-1} (CN). Anal. ($C_{11}H_8NOCl$) C, H, N, Cl.

Ethyl 5-Chloro-2-methyl-3-benzofuranacetate (15). A mixture of 6.7 g (0.032 mol) of 14, 10 ml of concentrated H_2SO_4, and 30 ml of EtOH was refluxed for 7 h, cooled, and poured into 30 ml of ice water. The product was extracted with Et_2O and the Et_2O layer was washed with $NaHCO_3$ solution and H_2O and dried (Na_2SO_4). After concentration under reduced pressure, the residue was distilled affording 6.3 g (76.8%) of colorless oil, bp 104–106° (0.025 mm). The oil crystallized from petroleum ether (bp 30–60°) affording white needles: mp 37–38°; NMR ($CDCl_3$) δ 1.25 (3 H, t, $-CH_2CH_3$, J = 7 Hz), 2.42 (3 H, s, $-CH_3$), 3.53 (2 H, s, $-CH_2-$), 4.16 (2 H, q, $-CH_2CH_3$, J = 7 Hz), 7.0–7.5 (3 H, m, aromatic); ir (neat) 1735 cm^{-1} (C=O). Anal. ($C_{13}H_{13}O_3Cl$) C, H, Cl.

B. Pharmacological Methods. 1. Triton Hyperlipidemic Rat Model. Compounds 1, 2, and 4 were tested in a hyperlipidemic rat model[14] at the same dosage (mmol/kg) and under the same conditions previously reported from our laboratories,[32] except that animals were stabilized for 1 rather than 2 weeks.

At 43 h after Triton administration the rats were anesthetized with ethyl ether; blood was drawn from the abdominal aorta and allowed to clot over a period of 1 h and serum was obtained after centrifugation at 500g for 10 min. Serum triglyceride was determined by the method of Eggstein;[33] serum cholesterol was analyzed by the method of Holub and Galli.[34] Significant differences in the serum cholesterol and triglyceride concentrations between drug-treated Triton hyperlipidemic (IV) and control (I) as well as between drug-treated (IV) and hyperlipidemic control (III) groups were determined by Student's *t* tests on logarithms of individual data to allow pooling of variances.

2. Normal Rat Model. Male Sprague–Dawley rats weighing 180–200 g were housed in a facility with alternating 12-h light and dark cycles and allowed free access to Purina rat chow and tap water. Groups of animals were given oral injections of drug (0.4 mmol/kg) twice daily for 7 consecutive days. Control animals received corresponding volumes of vehicle (0.25% methyl cellulose). Animals were killed 18 h after the last dose and between 8–9 a.m. Based on zero time serum triglyceride and cholesterol measurements no significant differences could be detected among the groups of animals selected for this study.

a. Preparation of Microsomes.[35] Animals were killed by cervical dislocation and livers quickly excised, weighed, and homogenized in 4 vol of ice-cold 0.1 M Tris–HCl buffer (pH 7.2) containing 1.15% KCl. Homogenates were centrifuged at 9000g for 20 min; the 9000g supernatant was carefully removed and centrifuged at 105 000g for 1 h. The microsomal pellet was resuspended in 0.1 M Tris–KCl buffer solution and recentrifuged at 105 000g for 30 min. The pellet was again resuspended in 0.1 M Tris–KCl buffer (pH 7.2) and kept at 0–4° until further use. All incubations were performed within 3 h after the isolation of microsomes.

b. HMG-CoA Reductase Assay.[36] Incubations were carried out in air at 37° with shaking (120 oscillations/min) in a Dubnoff apparatus for 1 h. The reaction mixture consisted of microsomal protein (62.5 μg), 20 μmol of EDTA, 20 μmol of neutralized cysteine, and 0.0125 μmol of DL-3-hydroxy-3-methylglutaryl-3-^{14}C-CoA (18.5 mCi/mmol) (New England Nuclear) with a NADPH-generating system ($NADP^+$, 0.20 μmol; glucose 6-phosphate, 2.30 μmol; $MgCl_2$, 15.9 μmol; and glucose-6-phosphate dehydrogenase, 1 eu). All reagents were added in 0.1 M Tris–HCl buffer (pH 7.2) containing 1.15% KCl except the DL-3-hydroxy-3-methyl-glutaryl-3-^{14}C-CoA which was dissolved in distilled H_2O (pH 5). The final incubation volume was 0.2 ml. The

reaction was terminated by placement on ice. To each tube was added 0.25 ml of [5-^{3}H]mevalonic acid in methanol (approximately 200 000 dpm) and 0.1 ml of 2 N H_2SO_4. [^{3}H]Mevalonic acid was added to monitor the extent of recovery during the isolation procedure.

Under these conditions, mevalonic acid lactone was formed from the [^{3}H]- and [^{14}C]mevalonic acid which was present in the samples. The procedure of Huber et al.[36] was used for the extraction and chromatographic isolation of mevalonic acid lactone. After dehydration, the pellet was crushed and extracted with four elutions of anhydrous diethyl ether (1 × 4.5 ml, 3 × 2.5 ml). The combined extracts were evaporated to dryness and redissolved in 0.5 ml of demineralized, distilled H_2O (DDW) and then eluted through chromatography columns (21 × 0.7 cm) packed with 8 cm of anion-exchange resin (Bio Rad AG 1-X8, 100–200 mesh, chloride form). Each sample tube was rinsed with 4.5 ml of DDW which was also added to the column. This was followed by another 2-ml rinse of the columns. The total eluent was collected into a 20-ml glass scintillation vial to which 12 ml of 3a40 scintillation cocktail (Research Products International, Elk Grove, Ill.) was later added. The ^{3}H and ^{14}C present were simultaneously determined on a Beckman LS-355 scintillation counter utilizing automatic quench compensation.

c. Analytical Methods. Serum and liver cholesterol was measured by the method of Parekh and Jung.[37] Triglyceride content of serum and liver was measured by the method of Saloni.[38] Cholesterol and triglycerides were extracted from liver according to the method of Abell et al.[39]

d. Statistical Analysis. Student's t test was employed to make comparisons between means.

References and Notes

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