either 15 min or 1 h after the injection. The organs of interest were removed, weighed, and counted in a NaI(T1) γ -well counter. Care was exercised to avoid cross contamination of the samples. Blood (3 mL) was obtained from a vein in the thoracic cavity. The activity that was observed in each organ was converted to percent of the injected dose per organ (% ID/organ) and/or per gram of organ (% ID/g).

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Diethyl (4bα,4cα,9aα,9bα)-3,6-Dichlorocyclobuta[1,2-b:3,4-b]bisbenzofuran-9a,9b(4bH,4cH)-dicarboxylate: the Cis,syn Photodimer of Ethyl 5-Chlorobenzofuran-2-carboxylate, an Analogue Related to the Antilipidemic Drug Clofibrate^{1,2}

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The antilipidemic properties of diethyl $(4b\alpha, 4c\alpha, 9a\alpha, 4b\alpha)$ -3,6-dichlorocyclobuta[1,2-b:3,4-b]bisbenzofuran-9a,9b(4bH,4cH)-dicarboxylate, herein termed dimer 8, were studied in sucrose-fed and in Triton-induced hyperlipidemic rats. Whereas clofibrate (0.4 mmol/kg) exhibited both anticholesterolemic and antitriglyceridemic activity, dimer 8 showed only antitriglyceridemic properties at the lower dose (0.2 mmol/kg) in sucrose-fed rats. Dimer 8 only lowered serum triglycerides levels in Triton WR-1339 hyperlipidemic rats, whereas clofibrate lowered both cholesterol and triglyceride levels. In the chronic sucrose-fed model, dimer 8 and clofibrate lowered hepatic HMG-CoA reductase activity and produced significant elevations in several parameters of hepatic drug metabolism. No positive relationship between serum cholesterol lowering and reduction of hepatic HMG-CoA reductase activity was observed by these agents in sucrose-fed rats.

Previous reports from these laboratories have established that the ethyl 5-chloro- and 5-phenyl-2,3-dihydrobenzofuran-2-carboxylate analogues (1 and 2) of clofibrate (3) lowered elevated serum cholesterol levels but had no effect on elevated serum triglyceride concentrations in Triton WR-1339 induced hyperlipidemic rats.^{3,4} The converse was true for the unsaturated 5-chlorobenzofuran analogue 4.⁴



Since each half of the cyclobutane "2 + 2" photodimer of

4 is structurally similar to either 1 or 4, it was of interest to examine the antilipidemic properties of this compound. In this study we discuss the characterization and pharmacological properties of the photodimer compared with clofibrate in both the acute Triton hyperlipidemic⁵ and chronic sucrose-fed rat⁶ models. This study is part of an extensive program designed to utilize clofibrate-related analogues as enzyme and lipid interactive probes in a variety of animal models.⁷ The Triton hypertriglyceridemic model presumably reflects inhibition of triglyceride catabolism,⁸ whereas the sucrose-fed rat model is a reflection of precursor-produced hypertriglyceridemia.9 Further, chronic administration of aryloxy analogues is required to probe the observable effects on the modification of hepatic microsomal enzymes involved in cholesterol biosynthesis and drug oxidation.^{10,11} Investigations of such parameters should provide insight into structural requirements relative to metabolic actions and antilipidemic effects.

Table I. Comparative Effects of Clofibrate (3) and Dimer 8 on Plasma Cholesterol and Triglyceride Levels (mg %) in Triton-Induced Hyperlipidemic Male Sprague-Dawley Rats

lipid analyzed	compd ^a	control group (I) ⁶	drug-treated control $(II)^b$	Triton hyper- lipidemic (III) ^b	drug-treated Triton hyperlipidemic $(IV)^b$
cholesterol	clofibrate (3) dimer 8	88.7 ± 6.9 66.7 ± 12.4	87.0 ± 8.1 63.9 ± 11.6	166 ± 17.8 163 ± 99.2	93.2 ± 8.9^{c} 176.7 ± 148.9 ^d
triglycerides	clofibrate (3) dimer 8	$\begin{array}{r} 20.8 \pm 5.4 \\ 23.6 \pm 5.5 \end{array}$	24.1 ± 7.4 24.3 ± 4.5	$\begin{array}{r} 98.5 \pm 16.9 \\ 188 \pm 189.8 \end{array}$	$22.3 \pm 8.3^{c} \\ 33.0 \pm 11.2^{c,d}$

^a All animals were given a total screening dose of 0.124 mM/kg of compound. ^b Mean ± SD; ten rats per group. ^c Statistically significant; comparison of groups III and IV. The large standard deviations for the data in groups III and IV dictate that the rank sum nonparametric statistical treatment should be employed. Results summarized for clofibrate cholesterol show $T_1 = 55$, $T_2 = 155$, $\alpha = 0.000$; clofibrate triglycerides show $T_1 = 55$, $T_2 = 155$, $\alpha = 0.000$; dimer 8 cholesterol show $T_1 = 90$, $T_2 = 120$, $\alpha = 0.14$; dimer 8 triglycerides show $T_1 = 145$, $T_2 = 65$, $\alpha = 0.001$. ^d Statistically significant; p < 0.05; comparison of groups I and IV.

Chemistry. Monomer ethyl 5-chlorobenzofuran-2carboxylate (4) was synthesized by a modification of the procedure of Tanaka.¹² Intermediate 7, initially formed during condensation of 5-chlorosalicylaldehyde (5) with 6, had not previously been isolated. Only when dry sol-



vents and molar equivalent amounts of K_2CO_3 and 6 are employed can 7 be isolated in 70% yield. In the presence of >2 equiv of K_2CO_3 , diester 7 undergoes monoester hydrolysis followed by decarboxylative elimination of hydroxide ion in situ yielding 4 in 73% yield based on starting 6. Similar decarboxylative eliminations have been reported for the synthesis of 2-phenylbenzofuran.^{13,14}

Krauch et al.¹⁵ and DeBoer¹⁶ were first to report photodimerization of benzofuran and methyl benzofuran-2-carboxylate, respectively, but product mixtures were not rigorously characterized. In these laboratories, irradiation of 4 during 14–20 h in absolute ethanol using a 450-W Hanovia high-pressure mercury arc in a water-cooled immersion well fitted with either a Vycor or Pyrex filter resulted in precipitation of a single crystalline dimer [MW = 441 (Rast camphor)] isolated in 45–50% yield and characterized as the *cis,syn*-8 isomer.



Ester hydrolysis and anhydride formation of the resulting diacid in refluxing Ac₂O established the cis relationship of the carbethoxy groups. Anhydride 9 exhibited

two carbonyl stretching vibrations at 1885 and 1795 cm⁻¹ with the lower frequency resonance the more intense of the two bands. Anhydride 8 was unstable to ice-cold 5% NaHCO₃ solution and in EtOH underwent solvolysis affording cis,syn monoester 10.

Characterization of 10 provided further evidence for the cis, syn structural assignment for photoproduct 8. The infrared spectrum for 10 exhibited two carbonyl absorption resonances at 1738 and 1720 cm⁻¹ corresponding to carbethoxy and carboxylic acid carbonyl functions, respectively. The parent ion as well as ions attributable to the ester and acid halves of the dimer was observed in the CI (5 eV) mass spectrum (see the Experimental Section). This and all related compounds assaved show the characteristic chlorine isotope distribution pattern in the mass spectrum. Additionally, reaction of 9 in the presence of a stoichiometric quantity of aniline followed by ring closure afforded cis.syn imide 11 in 52% vield. Imide 11 exhibited two carbonyl stretching vibrations in the infrared at 1795 and 1730 cm⁻¹. The lower frequency absorption is more intense indicating the presence of a cyclic imide. These absorption bands are consistent with a strained five-membered cyclic N-phenylimide. Expected values¹⁷ are 1790-1735 and 1745-1680 cm⁻¹.

Formation of cis,syn-8 is in general agreement with results and conclusions reached with coumarin photochemical reactions.¹⁸⁻²⁰ Dimer 8 was the only isolated product after photolysis of 4 for 14 h in 200 mL of benzene. From 0.06 mol of 4 the yield of dimer 8 was 0.24 g (8.9%) after solvent removal and recrystallization from ethanol. These results support the proposal that polar media and high concentrations of monomer are expected to increase the yield of cis products.^{19,20}

Pharmacology. Antilipidemic Effects in the Triton **Rat Model.**⁵ The antilipidemic effects of clofibrate (3)and dimer 8 on plasma triglyceride and cholesterol concentrations were tested in control male Sprague-Dawley Purina chow fed and Triton-induced hyperlipidemic rats (Table I). In Triton-hyperlipidemic animals (group IV), clofibrate significantly reduced plasma cholesterol and triglyceride levels by 44 and 77%, respectively, but the dimer only lowered the serum triglyceride concentrations significantly (82%) when compared to the corresponding Triton-hyperlipidemic controls (group III). In this acute drug treatment model (43 h post-Triton administration), neither clofibrate (3) nor dimer 8 was able to modify plasma lipid levels in normolipidemic animals (compare groups I and II in Table I). Moreover, in order to define and evaluate the relative effectiveness of clofibrate and dimer 8 on lowering plasma cholesterol and triglycerides in the Triton-induced hyperlipidemic animals, groups I and IV were tested for significant differences. When analyzed in this manner, only dimer 8 showed a significant difference between these treatment groups. The observed

Table II. Comparative Effects of Clofibrate and Dimer 8 on Serum Cholesterol and Triglyceride Levels (mg/dL) in Sucrose-Fed Male Sprague-Dawley Rats

evntl		cholesterol ^a		triglyceride ^a	
group	drug	0 day	7 day	0 day	7 day
I	control	74.4 ± 16 (9)	61.7 ± 11.6	$113 \pm 25.4(7)$	91.9 ± 19.8
II	0.4 mmol/kg of clofibrate	79.4 ± 13 (9)	31.7 ± 6.9^{b}	104 ± 49.2 (9)	26.9 ± 18.4^{b}
III	0.2 mmol/kg of dimer 8	76.0 ± 15	69.5 ± 9.7	121 ± 31.2	70.2 ± 24.6^{b}
IV	0.4 mmol/kg of dimer 8	81.1 ± 14	81.0 ± 17.6^{c}	98.1 ± 33.7 (9)	102.7 ± 37.1 (9)

^a Values reported as mean \pm SD for ten rats unless otherwise noted in parentheses. ^b Significant at p < 0.05 for drug vs. 0. or 7-day control. ^c Significant at p < 0.05 for drug vs. 7-day control.

Table III. Comparative Effects of Clofibrate and Dimer 8 on Various Hepatic Parameters after Chronic Administration to Sucrose-Fed Male Sprague-Dawley Rats

	tal group ^a			
parameters	control, I	clofibrate (0.4 mmol/kg), II	dimer 8 (0.2 mmol/kg), III	dimer 8 (0.4 mmol/kg), IV
liver cholesterol (mg/g) liver triglyceride (mg/g) HMG-CoA reductase (nmol/mg/h) liver/body wt (%) liver wt (g) microsomal protein (mg/g) ethylmorphine N-demethylase	$\begin{array}{c} 1.86 \pm 0.17 \\ 9.74 \pm 1.39 \\ 6.07 \pm 1.73 \\ 4.07 \pm 0.28 \\ 11.76 \pm 1.32 \\ 32.7 \pm 2.2 \\ 6.06 \pm 0.87 \end{array}$	$\begin{array}{c} 1.84 \pm 0.27 \\ 3.21 \pm 0.49^{b} \\ 2.10 \pm 0.60^{b} \\ 5.24 \pm 0.16^{b} \\ 14.30 \pm 0.94^{b} \\ 39.3 \pm 5.1^{b} \\ 10.90 \pm 2.17^{b} \end{array}$	$\begin{array}{c} 1.98 \pm 0.006 \\ 8.09 \pm 1.77^{b} \\ 2.42 \pm 0.34^{b} \\ 3.69 \pm 0.16 \\ 11.24 \pm 0.55 \\ 41.4 \pm 1.4^{b} \\ 9.53 \pm 0.44^{b} \end{array}$	$\begin{array}{c} 2.10 \pm 0.25 \\ 12.34 \pm 4.80 \\ 2.41 \pm 0.27^{b} \\ 4.03 \pm 0.30 \\ 11.99 \pm 1.05 \\ 38.2 \pm 5.2 \\ 11.02 \pm 0.73^{b} \end{array}$
(nmol of HCHO formed/mg/min) NADPH cytochrome c reductase (nmol/mg/min)	148.7 ± 17.7	243.1 \pm 26.9 ^b	168.7 ± 19.4	182.5 ± 15.6^{b}
cytochrome b_s	0.184 ± 0.023	0.200 ± 0.043	0.179 ± 0.005	0.190 ± 0.024
cytochrome P-450 (nmol/mg of protein)	0.361 ± 0.066	0.947 ± 0.160^{b}	0.386 ± 0.044	0.499 ± 0.045^{b}

^a Values of the parameters are reported as mean \pm SD for N = 5. These animals were randomly selected from the treatment groups indicated in Table II. ^b Significant at p < 0.05 for drug treatment vs. control groups.

ability of clofibrate to lower plasma cholesterol and triglyceride levels in Triton-induced hyperlipidemic rats to those of Purina chow fed normolipidemic rats has been reported previously from our laboratory.^{3,21}

Antilipidemic Effects in the Sucrose Rat Model.⁶ Comparisons of serum cholesterol and triglyceride concentrations between controls and experimental treatments (Table II) show that clofibrate treatment was hypocholesterolemic and hypotriglyceridemic (p < 0.05) (group II vs. group I). Dimer 8, administered at 0.4 mmol/kg, appeared to produce a significant increase in cholesterol levels, but this result was not significant relative to zero-time cholesterol concentrations (Table II). However, zero-time cholesterol concentrations for groups I-III were not significantly different from the data obtained for group I after the 7-day pretreatment period. Additionally, no significant difference among 0-day cholesterol or triglyceride concentrations was observed. Dimer administered at 0.2 mmol/kg reduced serum triglyceride concentrations significantly from the control levels (p < 0.05). No such reduction was found in 0.4 mmol/kg of dimer treated rats.

Hepatic Effects in the Sucrose Rat Model.⁶ A comparison of various hepatic parameters after pretreatment with clofibrate or dimer 8 is presented in Table III. No changes were noted in liver cholesterol concentrations even though clofibrate and dimer 8 reduced the activity of HMG-CoA reductase by 60-65%. No dosedependent inhibition of HMG-CoA reductase activity was obtained with dimer 8; both doses afforded a 60% blockade. Additionally, dimer 8 (at 0.2 mmol/kg only) and clofibrate lowered liver triglyceride concentrations by 17 and 67%, respectively.

The influence of these agents on parameters associated with alterations in hepatic drug metabolism is also given in Table III. Increases in liver weight and liver to body weight ratio were obtained after pretreatment with clofibrate but not with dimer 8. Dimer 8 (0.2 mmol/kg) and clofibrate showed a significant elevation in microsomal protein. As an index of hepatic drug oxidation, it was observed that clofibrate and dimer 8 increased the rates of ethylmorphine N-demethylation in these liver microsomal preparations. This activity was increased by 157-180% of the corresponding control value. Concomitant with these changes in ethylmorphine N-demethylation, elevations in microsomal NADPH cytochrome c reductase activity (123-163% of the corresponding control value) and cytochrome P-450 (138-262% of the corresponding control value) were observed after treatment with clofibrate and dimer 8 (0.4 mmol/kg dose only). Cytochrome b_5 levels in hepatic microsomes remained unaltered by either clofibrate or dimer 8 pretreatment.

Discussion

In a previous report we found that the hypocholesterolemic action of clofibrate in normolipidemic rats (0.4)mmol/kg, b.i.d., for 4 or 18 days) was correlated to an inhibition of hepatic HMG-CoA reductase activity.⁷ A qualitatively similar change in serum cholesterol and hepatic HMG-CoA reductase activity was observed with clofibrate in sucrose-fed rats but not after chronic administration with dimer 8 (Tables II and III). In fact, dimer 8 at the 0.2 and 0.4 mmol/kg dose levels did not modify serum cholesterol levels. However, the dimer, at both doses, showed an inhibition of hepatic HMG-CoA reductase activity even though liver cholesterol was unchanged via administration of both agents. Therefore, dimer 8 and clofibrate exert differential effects on serum cholesterol levels and HMG-CoA reductase activity in the sucrose-fed model. These findings support the thesis that cholesterol lowering may be operating by a mechanism independent of an inhibition of hepatic HMG-CoA reductase activity in this hyperlipidemic model. Since there is no dietary source of cholesterol in the sucrose-fed animal and no change in liver cholesterol concentration (Table III), it would appear that the hypocholesterolemic action of clofibrate involves increased catabolism of cholesterolcontaining lipoproteins. Using a sucrose-fed rat, Segal et al.²² reported an increased turnover of VLDL. Therefore, it is likely that the hypocholesterolemic action of clofibrate in sucrose-fed rats is mediated by an increased catabolism of cholesterol containing lipoproteins and not via inhibition of hepatic HMG-CoA reductase activity. Preliminary semiquantitative studies in our laboratories using densitometric scans of Fat Red 7B³ stained electrophorograms from sera of control-, clofibrate-, and dimer 8-treated rats demonstrated that clofibrate produced significant changes in the serum lipoprotein band pattern. On the other hand, dimer 8 did not significantly alter the serum lipoprotein band distribution when compared to that found in the sera of control animals (unpublished observations).

Chronic clofibrate treatment in normolipidemic rats is associated with an elevation in liver to body weight ratio and in hepatic microsomal protein, cytochrome P-450, NADPH cytochrome c reductase, and ethylmorphine N-demethylase activities.^{10,11} The present study indicates that these hepatic parameters are also elevated after clofibrate administration in sucrose-fed rats (Table III). With the exception of microsomal protein and liver to body weight ratios, dimer 8 (at the 0.4 mmol/kg dose only) also produced significant elevations in all of these parameters. At the lower dose of 8, the increased activity of microsomal ethylmorphine N-demethylase was not associated with concomitant elevations in cytochrome P-450 and/or NADPH cytochrome c reductase activity (as was the case with clofibrate and the higher dose of 8).

These observations with dimer 8 support our previous findings which show that hepatic microsomal drug oxidations and antilipidemic activities possessed by cyclic analogues of clofibrate (e.g., ethyl 6-cyclohexyl- and 6phenylchroman-2-carboxylate) are unrelated.⁷ The presence of a *p*-chloro substituent, in a series of benzodioxane, chroman, and dihydrobenzofuran analogues of clofibrate, was associated with an enhancement of hepatic microsomal drug metabolism.²³ In this study, dimer 8 exhibited significant elevations in activities of components of the hepatic microsomal drug-metabolizing system and was ineffective as an antilipidemic agent in sucrose-fed animals. In this regard, the presence of a *p*-chlorophenyl substituent, as in dimer 8, may be associated with a propensity of cyclic clofibrate analogues to enhance hepatic drug metabolism.7,23

Experimental Section

Chemistry. General. Melting points were determined using a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were obtained using either a Perkin-Elmer 257 or a Beckman Model 4230 spectrophotometer. NMR spectra were obtained with a Varian A-60A NMR spectrometer at 60 MHz or with a Bruker Model HX-90E (90 MHz) instrument equipped with Fourier transform capability. Mass spectra were determined using a Du Pont Model 21-491 instrument by direct probe insertion either by electron impact (EI mode, 70 eV) or by chemical ionization (CI mode) using isobutane as the reagent gas (5 eV). Chemical analyses were determined by Galbraith Laboratories, Knoxville, Tenn.

1,1-Dicarbethoxy-2-hydroxy-4-chloro-2,3-dihydrobenzofuran (7). A mixture of 50 g (0.32 mol) of 5-chlorosalicylaldehyde (5), 83.7 g (0.35 mol) of diethyl bromomalonate²⁴ (6), and 48.4 g (0.35 mol) of anhydrous K_2CO_3 was refluxed for 17 h in 800 mL of 2-butanone. The reaction mixture was filtered and concentrated under reduced pressure. The residue was dissolved in Et₂O and washed with 5% NaHCO₃ and H₂O. The organic layer was dried Notes

(Na₂SO₄), filtered, and concentrated under reduced pressure. The resulting liquid crystallized affording 70.5 g (70%) of 7: mp 92-95 °C; NMR (CDCl₃) δ 1.25 (t, 3 H, CH₃), 1.28 (t, 3 H, CH₃), 2.91 (d, 1 H, OH, J = 7.5 Hz), 4.25 (q, 2 H, CH₂), 4.30 (q, 2 H, CH₂), 5.85 (d, 1 H, methine, J = 7.5 Hz), and 6.81-7.32 (m, 3 H, aromatic). Anal. (C₁₄H₁₅O₆Cl) C, H, Cl.

Ethyl 5-Chlorobenzofuran-2-carboxylate (4). A mixture of **5** (50 g, 0.32 mol), **6** (50 g, 0.21 mol), and anhydrous K_2CO_3 (65 g, 0.47 mol) was refluxed in 800 mL of 2-butanone for 15 h. The solution was filtered and the solvent removed under reduced pressure. The residue was dissolved in Et_2O and washed successively with 10% NaOH and H_2O . The Et_2O layer was dried (Na₂SO₄), filtered, and concentrated under reduced pressure. The resulting solid was repeatedly crystallized from 95% EtOH to afford 52.9 g (73.2%) of 4 as white crystals, mp 49.5–50.5 °C (lit.²⁵ mp 65 °C).

Diethyl $(4b\alpha, 4c\alpha, 9a\alpha, 9b\alpha)$ -3,6-Dichlorocyclobuta[1,2-b: 3,4-b]bisbenzofuran-9a,9b(4bH,4cH)-dicarboxylate (8). Ethyl 5-chlorobenzofuran-2-carboxylate (4, 9.50 g, 0.042 mol) was dissolved in 700 mL of absolute EtOH and irradiated for 20 h using a 450-W Hanovia high-pressure mercury arc (Model L, Cat. No. 679A) and a water-cooled immersion well fitted with a Pyrex or Vycor filter. Argon was bubbled through the solution for 1 h preceding the irradiation and for the duration of the reaction. Compound 8 had crystallized on the immersion well walls by the end of the photolysis. The crystals were separated by filtration and the solution was concentrated under reduced pressure. The remaining dimer was separated from the monomer by selective crystallization from absolute EtOH. The combined yield of dimer 8 was 4.7 g (49.5%): mp 165-166 °C (virtually 100% yield based on recovered monomer); NMR (CDCl₃) δ 1.33 (t, 6 H, CH₃), 4.29 (q, 4 H, CH₂), 4.67 (s, 2 H, CH), 6.60-7.20 (m, 6 H, aromatic); mass spectrum (CI mode) m/e (rel intensity) 161 (12.9), 177 (5.0), 179 (2.1), 225 (100.0), 227 (29.7); IR (KBr) 1735 cm⁻¹. Anal. (C₂₂H₁₈O₆Cl₂) C, H, Cl.

(4bα,4cα,9bα)-3,6-Dichlorocyclobuta[1,2-b:3,4-b]bisbenzofuran-9a,9b(4bH,4cH)-dicarboxylic Acid. Diethyl ester 8 (1.20 g, 0.0027 mol), in 50 mL of 10% ethanolic KOH, was refluxed for 3 h. The mixture was concentrated under reduced pressure, poured into H₂O, and washed with Et₂O. The aqueous layer was acidified with 10% HCl and extracted with three 50-mL portions of Et₂O. The collected Et₂O layers were dried (Na₂SO₄), filtered, and concentrated. The residue was recrystallized from H₂O affording 1.04 g (99%) of white crystals: mp 250-252 °C; NMR (Me₂SO-d₆) δ 4.66 (s, 2 H, CH), 6.67-7.28 (m, 6 H, aromatic). 13.15 (br, 2 H, CO₂H); IR (Nujol) 3370, 1715 cm⁻¹; mass spectrum (CI mode) m/e (rel intensity) 197 (100.0), 199 (34.7), 303 (4.5), 305 (3.6), 393 (24.9), 395 (15.3). Anal. (C₁₈H₁₀O₆Cl₂) C, H, Cl.

 $(4b\alpha,4c\alpha,9a\alpha,9b\alpha)$ -3,6-Dichloro-9a,9b(4b H,4c H)methanoxymethanocyclobuta[1,2-b:3,4-b]bisbenzofuran-11,13-dione (9). A mixture of the diacid (0.30 g, 0.76 mmol) derived from diester 8 and 7 mL of Ac₂O was warmed until the diacid dissolved. The solution was heated at reflux for an additional 0.25 h and concentrated under reduced pressure. The excess Ac₂O and HOAc were removed by addition of dry toluene and reconcentrating under reduced pressure three times. The resultant white powder (0.25 g, approximately 87%) which was not recrystallized exhibited mp 233-235 °C dec: IR (Nujol) 1885, 1795 cm⁻¹; mass spectrum (EI mode) m/e (rel intensity) 123 (4.9), 125 (1.8), 179 (100.0), 180 (9.8), 181 (32.1), 182 (2.8), 302 (3.9), 304 (2.1), 346 (3.8), 348 (2.6), 374 (8.7), 376 (5.7).

Monoethyl Ester of $(4b\alpha, 4c\alpha, 9a\alpha, 9b\alpha)$ -3,5-Dichlorocyclobuta[1,2-*b*:3,4-*b*]bisbenzofuran-9a,9b(4bH,4cH)-dicarboxylic Acid (10). A mixture of 100 mg (0.254 mmol) of the diacid derived from diester 8 and 3 mL of Ac₂O was warmed until the diacid dissolved. The solution was heated at reflux for an additional 0.25 h and concentrated under reduced pressure. The excess Ac₂O and HOAc were removed by sequential addition and reconcentration of two 10-mL aliquots of dry toluene. The resultant white powder was dissolved in 5 mL of absolute EtOH and warmed for 5 min on a steam bath. Concentration under reduced pressure afforded 86 mg (80%) of 14: mp 232-234 °C; IR (Nujol) 1740, 1720, 1230, 1140 cm⁻¹; NMR (CDCl₃) δ 1.24 (t, 3 H, CH₃), 4.27 (q, 2 H, CH₂), 4.96 (br, 1 H, CO₂H), 6.51-7.20 (m, 6 H, aromatic); the AB resonance signals for the cyclobutane methine protons showed δ_A 4.63, δ_B 4.72 with $J_{AB} = 8.16$ Hz; mass spectrum (CI mode) m/e (rel intensity) 196 (19.0), 197 (18.2), 198 (7.4), 199 (6.2), 224 (90.7), 225 (43.0), 226 (33.8), 227 (15.2), 375 (32.3), 377 (25.7), 378 (8.4), 421 (100.0), 423 (69.0), 425 (16.0). Anal. (C₂₀H₁₄O₆Cl₂) C, H, Cl.

 $(4b\alpha, 4c\alpha, 9a\alpha, 9b\alpha)$ -3,6-Dichloro-N-phenyl-9a,9b(4bH)-4cH)methanoazomethanocyclobuta[1,2-b:3,4-b]bisbenzofuran-11,13-dione (11). A solution of 100 mg (0.254 mmol) of the diacid derived from diester 8 and 3 mL of Ac₂O was warmed until the dimer dissolved. The solution was heated at reflux for an additional 0.25 h and the concentrated under reduced pressure. The excess Ac₂O and HOAc were removed as previously described. To this residue was added 23.7 mg (0.255 mmol) of aniline in 5 mL of dry toluene. The solution was refluxed for 0.5 h and concentrated under reduced pressure. To the residue was added 3 mL of Ac_2O . After refluxing for 0.5 h the reaction mixture was concentrated and the excess Ac_2O and HOAc were removed as previously described affording 60.2 mg (52.6%) of imide 15: mp 221-224 °C; IR (KBr) 1800, 1730 cm⁻¹; NMR (CDCl₃) δ 4.76 (s, 2 H, CH), 6.73-7.64 (m, 11 H, aromatic). Anal. (C₂₄H₁₃O₄ClN) C, H, Cl, N.

Pharmacology. Triton Hyperlipidemic Rat Model. Clofibrate and dimer 8 were tested in a hyperlipidemic rat model⁵ at the same doses (0.124 mmol/kg total dose) and under the same experimental conditions reported previously²⁶ except that animals were stabilized for 1 rather than 2 weeks. Serum was obtained and analyzed for cholesterol and triglycerides as described previously.²⁶ The rank sum nonparametric statistic test was employed.²⁷

Sucrose-Fed Rat Model. Male Sprague-Dawley rats weighing 240-260 g were housed in a facility with alternating 12-h light and dark cycles. The animals were fed a diet consisting of 63% sucrose, 28% vitamin-free casein, 4% vitamin fortification mixture, 5% salt (USP XIV), and 5% cellulose⁶ and allowed free access to tap water. After a prefeeding period of 4 days, groups of animals were given oral injections of dimer 8 (0.4 and 0.2 mmol/kg) and clofibrate (0.4 mmol/kg) twice daily for seven consecutive days. Control animals were injected with corresponding volumes of vehicle (0.25% methylcellulose). Animals were killed 12 h after the last dose; blood was drawn from the abdominal aorta and allowed to clot at room temperature; and serum was collected by centrifugation at 1000 rpm for 10 min. Serum cholesterol concentrations were determined by the method of Allain et al.²⁸ Serum triglyceride concentrations were determined by the method of Eggstein.²⁹ Significant differences in mean serum cholesterol and triglyceride concentrations between drug-treated groups and controls were determined by the Student's t test at the 5% level of significance.

Preparation of Microsomes. Animals were anesthetized (Et₂O) to permit removal of blood from the abdominal aorta and livers were excised immediately thereafter. The homogenization of liver and preparation of microsomes was carried out as described previously.²¹

HMG-CoA Reductase Assay. The assay of mevalonic acid formation from DL-3-hydroxy-3-methylglutaryl-3-[^{14}C]-CoA was carried out by procedures identical with those described previously.²¹

Ethylmorphine N-Demethylase Assay. The assay of formaldehyde liberated from ethylmorphine was carried out by procedures described previously.¹⁰ Incubation mixtures contained 5 mg of microsomal protein, 10 μ mol of ethylmorphine, a NADPH generating system,¹⁰ and 60 μ mol of Tris (pH 7.4) in a final volume of 3.0 mL. Reactions were terminated after 10 min of incubation at 37 °C.

NADPH Cytochrome c Reductase Assay. The assay of cytochrome c reduction in liver microsomes was carried out by the method of Phillips and Langdon.³⁰

Methods of Analysis in Liver. Hepatic microsomal cytochrome P-450 and cytochrome b_5 were estimated by the procedure of Kinoshita and Horie.³¹ Microsomal protein was assayed by the method of Lowry et al.³² Cholesterol and triglycerides were extracted from liver and analyzed according to methods previously described.²¹ Student's t test was used to make comparisons between means of treatment groups.

References and Notes

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