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(13) In the synthesis of 12 and 13, sodium hydrogen carbonate (5 g) was used in place of triethylamine as base, and DMF (8 mL) was used as solvent. Sodium iodide (50 mg) was added to the reaction mixture for the synthesis of 13, and the temperature was reduced to 60–70 °C for the synthesis of 12.

Synthesis and Pharmacological Evaluation of *cis*-3,4,4a,9a-Tetrahydro-1*H*-pyrano[3,4-*b*]benzofuran-1-ones. Tricyclic Analogues Related to the Antilipidemic Drug Clofibrate[1]

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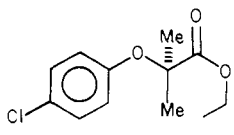
The chemistry and pharmacology of two δ-lactones, *cis*-6-chloro-9a-methyl-3,4,4a,9a-tetrahydro-1*H*-pyrano[3,4-*b*]benzofuran-1-one (2) and the 9a-demethyl analogue 3, are reported. Lactones were prepared from dihydrobenzofuran precursors possessing geometrical configurations confirmed both by synthesis and ^{1}H NMR spectroscopy. All *cis*-dihydrobenzofurans exhibited J_{vic} = 9.0–10.8 Hz, whereas their trans isomers exhibited J_{vic} = 5.0–6.0 Hz in agreement with predictions based on the Karplus equation. The pharmacological profiles for 2 and 3 were compared to that of clofibrate (1) in normal male Sprague–Dawley rats. Using equimolar doses (0.4 mmol/kg, po, twice daily for 7 days), 1 exhibited both anticholesterolemic and antitriglyceridemic activity, lactone 2 exhibited only antitriglyceridemic activity, and 3 was inactive as an antilipidemic agent. No correlation was observed for inhibition of hepatic HMG-CoA reductase activity and serum cholesterol lowering.

As part of a continuing study concerned with antilipidemic drug development,[3-6] we describe in this article the synthesis and pharmacological properties of *cis*-6-chloro-9a-methyl-3,4,4a,9a-tetrahydro-1*H*-pyrano[3,4-*b*]-benzofuran-1-one (2) and its demethyl analogue 3. These



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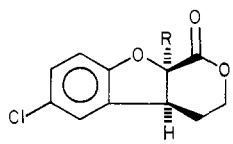
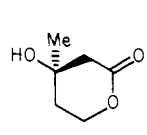


2, R = Me
3, R = H



(*R*)-4

compounds are 6-5-6 tricyclic δ-lactones structurally related to clofibrate (1). Analogue 2 differs from the molecular weight of 1 by 4 H atoms and may be visualized as a compound in which one of the *gem*-dimethyl groups of 1 is concomitantly bonded ortho on the phenyl ring and to the β-carbon of the ethyl function. Furthermore, one enantiomorph of each of these tricyclic lactones 2 and 3 also has a structural resemblance to mevalonolactone [(*R*)-4]. Thus, these molecules were constructed in the hope that they would have increased affinity over 1 for HMG-CoA reductase[7] or other enzymes involved in the biochemical transformation of (*R*)-4 and its precursors to cholesterol.

Synthetic Aspects. Key intermediate diacid *trans*-11 served as the precursor to *cis*-11 and was prepared from aldehyde 5 according to methods similar to those reported by Koelsch[8,9] and Shimizu[10,11] in the preparation of the dechloro analogue. In large scale preparations, diacid 6 (282 g, 1 mol) was converted to the isomeric mixture 9 in 69% yield without isomer separation. Whereas *cis*- and *trans*-9 could not be separated by conversion to and fractional crystallization of their respective isomeric nitrile acids 10, diacid *trans*-11 could be isolated as a high-melting crystalline product devoid of contamination by the cis isomer. A small sample of the cis isomer was obtained from the mother liquor resulting from crystallization of *trans*-11.

Diacid *trans*-11 was isomerized to *cis*-11 using Shimizu's[11] method. Thus, treatment of *trans*-11 with NH_4OH afforded epimerized imide 12 in 46% yield. Yields severely decreased when more than 2 g of *trans*-11 was employed. Conditions for hydrolysis of 12 to *cis*-11 exclusive of epimerization are critical. Hydrolysis in 10% aqueous NaOH solution for 1 h afforded *cis*-11 in 74% yield. Isomerization under longer reaction conditions yielded appreciable

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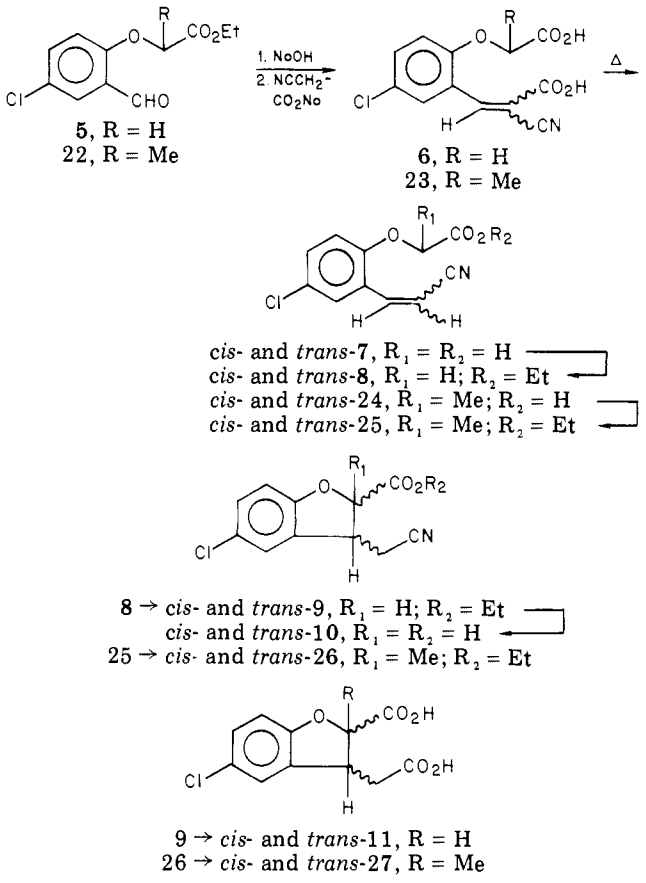
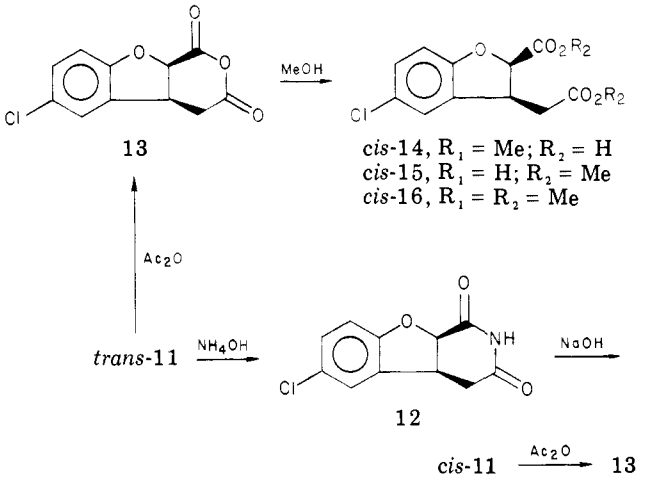


quantities of the thermodynamically more stable isomer; reaction for 5 h afforded a 5:1 mixture of *cis*- and *trans*-**11**.

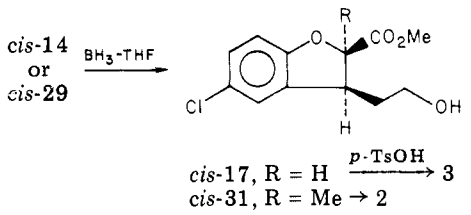
The monomethyl ester *cis*-**14** was prepared from either



cis-**11** $\xrightarrow{Ac_2O}$ **13**

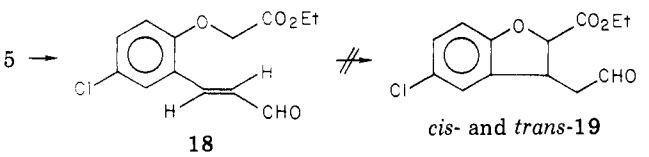
cis- or *trans*-**11** in 76 and 72% yields, respectively. Reaction with Ac_2O, followed by distillation to remove solvent, afforded intermediate anhydride **13**, which was not isolated. Subsequent treatment with MeOH afforded *cis*-**14** regioselectively. Diester *cis*-**16** was isolated in 10 and 11% yields from either *cis*- or *trans*-**11**. A trace of the isomeric monomethyl ester *cis*-**15** could be detected (TLC) in both reaction mixtures.

Selective reduction of the carboxylic acid function in *cis*-**14** using the BH_3–THF complex,[12] followed by *p*-TsOH-catalyzed cyclization in toluene, afforded lactone **3** in 86% yield based on *cis*-**14**. The intermediate hydroxyethyl analogue *cis*-**17** could not be purified by distillation owing to thermal cyclization to **3**. This lactone exhibited a molecular ion (base peak) at m/e 224 in the mass spectrum, and a peak at m/e 152 was in agreement



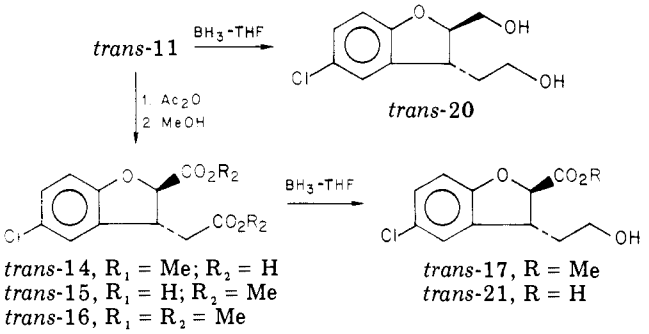
with fragmentation affording the 5-chlorobenzofuran cation.

An alternative approach to the synthesis of **3** from α,β-unsaturated aldehyde **18**, prepared from **5** using the



Wittig reaction,[13] failed to afford **19** under a variety of reaction conditions designed to effect intramolecular Michael cyclization.

Whereas the reaction of diacid *trans*-**11** with Ac_2O, followed by distillation to remove solvent, afforded an epimerized cyclic intermediate anhydride **13**, reaction of *trans*-**11** with Ac_2O, in the absence of solvent distillation, followed by reaction with MeOH, afforded a mixture of monomethyl and dimethyl esters *trans*-**14**, -**15** and -**16** via



mixed anhydride formation. Regioselective formation of *trans*-**14** (59% yield) with only 5% *trans*-**15** was observed. Diester *trans*-**16** was formed in 10% yield. Isolation of starting diacid *trans*-**11** in 8% yield provided evidence for the formation of a mixed anhydride intermediate. The structure of diester *trans*-**16** was confirmed by independent synthesis using *trans*-**11** and excess CH_2N_2. Monoester *trans*-**14** was obtained free of *trans*-**15** and -**16** by column chromatography on silica gel (see Experimental Section).

Reduction of monoester *trans*-**14** using BH_3–THF complex[12] afforded hydroxy ester *trans*-**17** in 77% yield. In the presence of excess BH_3–THF complex, the diol *trans*-**20** was obtained from *trans*-**11**. Attempts to convert hydroxy ester *trans*-**17** or hydroxy acid *trans*-**21** to the trans analogue of **3** were unsuccessful under conditions reported[14,15] to yield strained ring systems. Thus, *trans*-**17** afforded a complex mixture of products in benzene, toluene, or xylene containing a catalytic amount of *p*-TsOH. Hydroxy acid *trans*-**21** in the presence of DCC in pyridine or *p*-TsOH in toluene or xylene also failed to yield the strained lactone.

Lactone **2** was prepared by utilizing procedures similar to those outlined for the preparation of **3**. Intramolecular Michael addition of esters *cis*- and *trans*-**25** with subsequent hydrolysis afforded cis and trans diacids **27** in the ratio 1:2.6 (^1H NMR integration of CH_3 peak). The trans isomer was isolated by fractional crystallization from water. Repeated recrystallizations of the mother liquor residue

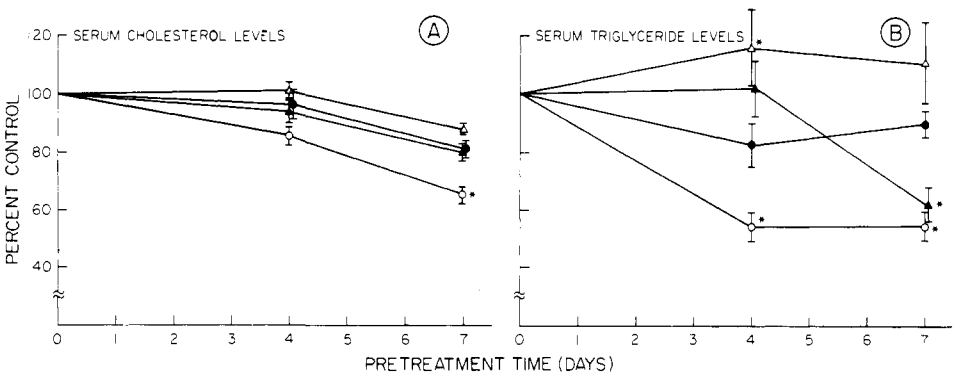
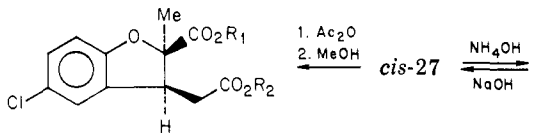
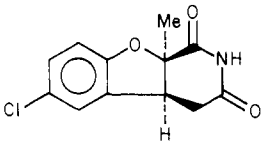


Figure 1. Comparative effects of clofibrate (1) and lactones 2 and 3 on serum cholesterol (frame A) and triglyceride (frame B) levels in normal male Sprague–Dawley rats. Key: control (●–●); clofibrate (○–○); lactone 2 (▲–▲); lactone 3 (△–△). Serum cholesterol and triglycerides were analyzed 12 h after the last dose (at 4 or 7 days). Values reported are the mean ± SE of $N = 9$ or 10. The asterisk indicates a significant difference ($p < 0.05$) between drug- and non-drug-treated groups. Individual values were expressed as a percent of the mean value obtained for the corresponding zero time group.

afforded only mixtures of *cis*- and *trans*-**27** in an approximate ratio 1:1.2 (^{1}H NMR). However, reaction of this mixture with NH_4OH afforded imide **28** (78% yield based



cis-**29**, R_1 = Me; R_2 = H
cis-**30**, R_1 = H; R_2 = Me



28

on *cis*-**27**). Diacid *trans*-**27** did not yield **28** under similar conditions. Hydrolysis of **28** afforded diacid *cis*-**27**, thus establishing the stereochemical assignments for both *cis*- and *trans*-**27**. Whereas the ^{1}H NMR spectra of *cis*- and *trans*-**27** were consistent with assigned structures, such spectra were of little value for assignment of stereochemical configuration. Facile conversion of *cis*-**27** to lactone **2** was carried out according to the procedure outline for the preparation of **3**.

Structure Confirmation (^{1}H NMR Studies). Whereas vicinal coupling constants of H-2 and -3 protons in 2,3-disubstituted 2,3-dihydrobenzofurans commonly have been reported to be larger for cis than trans isomers[16-23] and are in agreement with assignments made on the basis of the Karplus equation,[24] exceptions may be found in the literature[25,26] where $J_{cis} \leq J_{trans}$. In our studies trans-substituted dihydrobenzofurans exhibited J_{vic} = 5.0–6.0 Hz, whereas cis isomers exhibited J_{vic} = 9.0–10.8 Hz. Cyclic intermediates served to confirm the stereoisomeric relationships to predicted J values which were found to be consistent within this series (Table I).

The site of esterification in monoesters *trans*-**14** and -**15** was established through analysis of chemical-shift differences of H-2 (methine) and methylene proton resonance signals in diester *trans*-**16** vs. diacid *trans*-**11** (Table I). α-Proton resonance signals of carboxylic acids generally absorb at slightly lower field (ca. 5 Hz) than their corresponding esters. However, since these signals are affected by both the high diamagnetic anisotropic effect of C=O functions as well as small changes in conformation, exceptions to this generality have been observed.[27,28] In these studies, α-proton resonance signals of acids were consistently found to be upfield (3.6–9.0 Hz) from those observed for the corresponding ester (Table I). The site of esterification in monoester *cis*-**14** was established utilizing analogous reasoning.

Table I. Comparative ^{1}H NMR[a] Data for Stereoisomeric Dihydrobenzofuran Intermediates and Lactone 3

	$J_{vic\ 2,3}$, Hz		δ	
compd	cis[b]	trans	H-2[b]	$-CH_2CO_2-$[b] (methylene H)
3	10.8[f]			
			5.45 (H-9a)	
9	9.0	5.5	5.15 (trans)	3.11 (trans; for CH_2CN)
			5.52 (cis)	2.94 (cis; for CH_2CN)[c]
11	9.6	6.0	5.00 (trans)	2.75 (trans)
			5.32 (cis)	2.20–3.00 (cis)[d]
12	9.4[f]			
			5.30 (H-9a)	2.90–3.20 (for CH_2CON)
14	9.7	5.8	5.12 (trans)	2.78 (trans)
			5.45 (cis)	2.41 and 2.82 (cis)[e]
15		5.5	4.98 (trans)	2.84 (trans)
16		5.8	5.12 (trans)	2.88 (trans)
17		5.0	5.03 (trans)	

[a] Taken in Me_2SO-d_6 with Me_4Si as internal standard. [b] Unless otherwise indicated. [c] The cis isomer was only detected as a trace component in the trans compound. [d] Peaks partially obscured by Me_2SO-H resonance signals. [e] In this case, geminal coupling was observed. In trans isomers doublets observed for methylene protons may be deceptively simple. [f] $J_{vic\ 4a,9a}$.

Pharmacological Results. The antilipidemic actions of clofibrate (1) and lactones **2** and **3** on serum cholesterol and triglyceride concentrations in Purina Chow fed male Sprague–Dawley rats are shown in Figure 1. Clofibrate administration (0.4 mmol/kg, twice daily) for 4 and 7 days significantly reduced serum triglyceride levels while serum cholesterol levels were reduced only after 7 days of pretreatment. At the same dose, analogues **2** and **3** did not show any serum cholesterol lowering effects (Figure 1A). Analogue **2** was equally as effective as clofibrate on serum triglyceride lowering after the 7-day pretreatment period, whereas **3** failed to show any antitriglyceridemic activity (Figure 1B). Analogue **3** showed a significant increase in serum triglyceride levels only after the 4-day pretreatment period.

Results of chronic treatment (0.4 mmol/kg, twice daily for 7 days) with these analogues on various hepatic parameters are presented in Table II. Each of the compounds exhibited a significant increase in hepatic tri-

Table II. Comparative Effects of Clofibrate (1) and Lactones 2 and 3 on Various Hepatic Parameters after Chronic Administration to Purina-Fed Male Sprague-Dawley Rats

parameters[a]	experimental group			
	control	1	2	3
liver cholesterol, mg/g	2.96 ± 0.42	2.59 ± 0.50	3.19 ± 0.46	2.93 ± 0.50
liver triglyceride, mg/g	3.87 ± 0.59	4.65 ± 0.25[b]	7.75 ± 0.69[b]	5.66 ± 1.01[b]
HMG-CoA reductase, (nmol/mg)/h	13.2 ± 3.30	11.3 ± 5.40	8.20 ± 1.10[b]	11.0 ± 3.60
liver/body wt, %	3.96 ± 0.20	4.84 ± 0.16[b]	4.56 ± 0.27[b]	4.03 ± 0.30
liver wt, g	11.8 ± 0.93	15.1 ± 0.93[b]	14.1 ± 1.37[b]	12.1 ± 0.89
microsomal protein, mg/g	25.8 ± 1.17	29.0 ± 2.14[b]	31.9 ± 4.47[b]	25.2 ± 2.44
ethylmorphine *N*-demethylase, nmol of HCHO formed mg^{-1} min^{-1}	6.48 ± 0.70	7.82 ± 1.07[b]	11.1 ± 1.87[b]	7.96 ± 0.57[b]
NADPH:cyt *c* reductase, nmol mg^{-1} min^{-1}	164 ± 16.7	246 ± 51.9[b]	315 ± 18.7[b]	210 ± 38.2[b]
cyt b_5, nmol/mg of protein	0.310 ± 0.052	0.293 ± 0.241	0.421 ± 0.042[b]	0.292 ± 0.039
cyt P-450, nmol/mg of protein	0.833 ± 0.064	1.06 ± 0.180[b]	1.62 ± 0.286[b]	0.962 ± 0.043[b]

[a] Values of the parameters are reported as the mean ± SD for $N = 5$. These animals were randomly selected from the treatment groups indicated in Figure 1. [b] Significant differences from the control ($p < 0.05$) for drug treated vs. control animals.

glyceride concentration after the 7-day pretreatment period. Elevations of 20, 100, and 46% were observed with **1**, **2**, and **3**, respectively. In contrast, none of these analogues modified liver cholesterol content. However, **2** significantly reduced hepatic HMG-CoA reductase activity by 38% after the 7-day pretreatment period. Lesser reductions ($p > 0.05$) in hepatic HMG-CoA reductase activity were noted after treatment with **1** and **3**. Of the remaining hepatic parameters, **1** was found to elevate liver/body weight ratio, liver weight, microsomal protein content, ethylmorphine *N*-demethylase and NADPH-cytochrome *c* reductase activities, and cytochrome P-450. Analogues **2** and **3** produced significant elevations in ethylmorphine *N*-demethylase and NADPH-cytochrome *c* reductase activities and cytochrome P-450 content. Analogue **3** did not modify liver weight, liver/body weight ratio, or microsomal protein, whereas **2** produced significant elevations in each of these hepatic parameters (151–124% of the control values). Additionally, pretreatment with **2** produced a significant elevation ($p < 0.05$) in liver microsomal cytochrome b_5 concentrations (136% of the control).

Discussion

The hypocholesterolemic action of **1** has been proposed[7] to be associated with an inhibition of hepatic cholesterol biosynthesis by specifically blocking HMG-CoA reductase activity. Previous reports[29,30] from this laboratory have demonstrated that inhibition of hepatic HMG-CoA reductase activity by **1** and related analogues in sucrose-fed rats did not correlate with serum-cholesterol lowering. Although HMG-CoA reductase activity varied directly with cholesterol lowering in normolipidemic rats treated with **1** and related analogues,[6] in the present study, **1** reduced serum cholesterol concentrations without a significant inhibition of HMG-CoA reductase activity (Table II). By contrast, analogue **2** showed an inhibitory effect on hepatic HMG–CoA reductase activity at a dose equal to **1** but did not exhibit any serum cholesterol lowering effect in normolipemic rats. Therefore, these findings support the hypothesis that blockade of hepatic cholesterol biosynthesis by clofibrate and related cyclic analogues is not necessarily associated with hypocholesterolemia.[29,30]

The increase in liver triglyceride content after chronic administration of **1–3** may reflect an inhibition of the release of triglycerides from the liver bound to VLDL.[31,32] That this increase is not always associated with a reduction in serum triglyceride is evidence that this hypothesis does not completely explain the liver–serum triglyceride relationship. An increased catabolism of serum lipoproteins may be another aspect of the triglyceride lowering mechanism of clofibrate in normolipemic rats. In fact, Segal et al.[33] have reported that there is an increased catabolism of VLDL during chronic clofibrate treatment in sucrose-fed rats.

Previous findings from these laboratories[29,30,34] have shown little correlation between enhancement of hepatic microsomal drug metabolism and antilipidemic activity. In the present study, chlorine-containing analogues **2** and **3**, like **1**, produced the expected elevations in parameters of hepatic drug metabolism and confirm the observation that there is a dissociation between these biological effects.

Experimental Section

Melting points were determined with a Thomas-Hoover "Unimelt" apparatus and are uncorrected. ^1H NMR spectra were recorded with a Varian A-60A spectrophotometer or, unless otherwise stated, with a Bruker Model HX-90E spectrophotometer using tetramethylsilane as internal standard. Chemical shifts are reported on the δ scale, and the J values recorded are first-order spacings. Gas–liquid partition chromatography was performed with a Hewlett Packard F&M Scientific Model 402 gas chromatograph, and mass spectra were recorded with a DuPont 21-491 mass spectrometer by electron impact at an ionization potential of 70 eV. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Where analyses are reported by symbols of the elements, analytical results were within ±0.3% of the theoretical values.

[2-(2-Carboxy-2-cyanoethenyl)-4-chlorophenoxy]acetic Acid (6). Treatment of phenoxyacetate[35] **5** (60.9 g, 0.25 mol) with NaOH and sodium cyanoacetate[36] as described[8] afforded acid **6** in 71% yield as yellow crystals, mp 255–257 °C dec (AcOH). Anal. ($C_{12}H_8ClNO_5$) C, H, Cl, N.

***cis*- and *trans*-[4-Chloro-2-(2-cyanoethenyl)phenoxy]acetic acid (*cis*- and *trans*-7)** were prepared according to a modified procedure of Koelsch.[8] Diacid **6** (50 g, 0.18 mol) was added in small portions to freshly distilled quinoline (100 mL) previously heated to 165–170 °C in a 500-mL round-bottomed flask equipped with a magnetic stirrer. The reaction was terminated after evolution of CO_2 ceased (ca. 1 h at 165–170 °C). Quinoline (ca. 75 mL) was removed by distillation under reduced pressure and the residue was treated with dilute HCl solution. The semisolid mixture was triturated in a large mortar and pestle, and the acid was collected by filtration (31.1 g, 74%) and washed with H_2O. Analytical samples of *cis*- and *trans*-**7** were obtained by fractional crystallization. Crystallization from ethanol afforded pure *trans*-**7**, mp 167–169 °C. Anal. ($C_{11}H_8ClNO_3$) C, H, Cl, N.

The mother liquor was concentrated, and crystallization from EtOAc–*n*-heptane afforded *cis*-**7**, mp 143–145 °C. Anal. ($C_{11}H_8ClNO_3$) C, H, Cl, N.

Ethyl *trans*-[4-Chloro-2-(2-cyanoethenyl)phenoxy]acetate (*trans*-8). The mixture of acids *cis*- and *trans*-**7** (47.5 g, 0.2 mol)

was dissolved in hot absolute EtOH (250 mL). Gaseous HCl was bubbled into the EtOH solution and the reaction mixture was refluxed for 2 h. After removal of the solvent under reduced pressure, CH_2Cl_2 (800 mL) was added to the residue. The CH_2Cl_2 solution was washed to neutrality with dilute $NaHCO_3$ solution followed by H_2O (2 × 100 mL), dried ($MgSO_4$), and filtered. The solvent was removed under reduced pressure, affording a white solid which was crystallized twice from 95% EtOH affording *trans*-8. A small amount of *trans*-8 was further obtained by concentrating the mother liquor and crystallization from a minimum amount of EtOH. A combined yield of 37.1 g (70%) of *trans*-8, mp 111–113 °C, was obtained. Anal. ($C_{13}H_{12}ClNO_3$) C, H, Cl, N.

Ethyl *cis*-[4-Chloro-2-(2-cyanoethenyl)phenoxy]acetate (*cis*-8). The mother liquor resulting from crystallization of *trans*-8 was concentrated under reduced pressure and the precipitate was recrystallized from benzene–petroleum ether, affording *cis*-8 contaminated with a trace of *trans*-8. Analogue *cis*-8 was further recrystallized from 95% EtOH, yielding 10.1 g (19%) of white solid, mp 86–89 °C.

Ethyl *cis*- and *trans*-5-chloro-3-(cyanomethyl)-2,3-dihydro-2-benzofurancarboxylate (*cis*- and *trans*-9) was prepared using a method virtually identical to the preparation of the dechloro analogues according to the procedure of Koelsch.[8] Treatment of *trans*-8 (10.63 g, 40 mmol) with concentrated ethanolic NaOEt afforded a colorless oil, bp 158–161 °C (0.2 Torr), and solidified after standing for several weeks. GLC showed the presence of a trace of cis isomer (cis/trans, 1:50), yield 9.87 g (93%).

An identical procedure was used to convert *cis*-8 (5.31 g, 20 mmol) to product *trans*-9 contaminated with cis isomer in the same ratio, yield 4.77 g (90%).

In a large-scale preparation, acid 6 (281.7 g, 1 mol) was converted to cyclized ester *cis*- and *trans*-9 (184.3 g, 69% based on starting 6) without any isomer separation.

***cis*- and *trans*-5-Chloro-3-(cyanomethyl)-2,3-dihydro-2-benzofurancarboxylic Acid (*cis*- and *trans*-10).** A mixture of *cis*- and *trans*-9 (3.32 g, 12.5 mmol), NaOH (510 mg, 12.7 mmol), H_2O (2 mL), and EtOH (15 mL) was heated on a steam bath for 5 min. Crystallization from benzene gave a white product, mp 138–144 °C. Three recrystallizations from EtOAc–*n*-heptane did not remove traces of *cis*-10; *trans*-10 (2.63 g, 89%) contaminated with a trace of cis isomer exhibited mp 144–148 °C.

***trans*-3-(Carboxymethyl)-5-chloro-2,3-dihydro-2-benzofurancarboxylic Acid (*trans*-11).** The ester nitrile *cis*- and *trans*-9 (132.9 g, 0.5 mol) was added to NaOH solution (30%, 600 mL) and was refluxed for 6 h until evolution of NH_3 ceased (litmus paper). The reaction mixture was cooled in an ice bath and acidified with dilute HCl solution. The crystalline precipitate was filtered and recrystallized from H_2O using decolorizing charcoal, affording 100.2 g (78%) of diacid *trans*-11: mp 205–208 °C; ^1H NMR (Me_2SO-d_6) δ 2.75 (2 H, d, J = 7 Hz, $-CH_2CO_2-$), 3.65–4.20 (1 H, deceptively simple quartet, H-3), 5.00 (1 H, d, J = 6.0 Hz, H-2), 6.70–7.40 (3 H, m, aromatic). Anal. ($C_{11}H_9ClO_5$) C, H, Cl.

***cis*-6-Chloro-4a,9a-dihydrobenzofuro[2,3-*c*]pyridine-1,3(2*H*,4*H*)-dione (12)** was prepared according to a modified procedure of Shimizu.[11] Diacid *trans*-11 (2.0 g, 7.8 mmol) was dissolved in NH_4OH solution (10%, 4 mL) and the solution evaporated to a thick oil on a steam bath. Subsequently, the oil was heated at 210–220 °C for 1 h, during which time it became dark brown in color. The oil was cooled and treated with 95% EtOH. The solid product was finely pulverized, filtered, and recrystallized from EtOAc–*n*-heptane, affording 855 mg (46%) of 12, mp 200–202 °C. Anal. ($C_{11}H_8ClNO_3$) C, H, Cl, N.

***cis*-3-(Carboxymethyl)-5-chloro-2,3-dihydro-2-benzofurancarboxylic Acid (*cis*-11).** Imide 12 (1.19 g, 5.0 mmol) in a NaOH solution (10%, 7 mL) was refluxed for 1 h. The reaction mixture was cooled in an ice bath, acidified with dilute HCl solution, and filtered. ^1H NMR of this crude sample showed the presence of a trace of the diacid *trans*-11. Recrystallization, using decolorizing charcoal, from H_2O afforded 950 mg (74%) of diacid *cis*-11 as white crystals, mp 213–216 °C: ^1H NMR (Me_2SO-d_6) δ 2.20–3.00 (2 H, m, $-CH_2CO_2-$), 3.80–4.50 (1 H, deceptively simple quartet, H-3), 5.32 (1 H, d, $J_{2,3}$ = 9.6 Hz, H-2), 6.70–7.40 (3 H, m, aromatic). Anal. ($C_{11}H_9ClO_5$) C, H, Cl.

Methyl *cis*-3-(Carboxymethyl)-5-chloro-2,3-dihydro-2-benzofurancarboxylate (*cis*-14). Procedure A. A mixture of diacid *cis*-11 (512 mg, 2.0 mmol), Ac_2O (2 mL), and AcCl (3 drops) was refluxed for 1 h. The solvents were removed by reduced pressure distillation and the residue was cooled in an ice bath. With stirring, absolute MeOH (5 mL) was added by dropwise addition and subsequently stirred for an additional 0.5 h. The MeOH was removed under reduced pressure, affording a thick brown oil which was chromatographed on 70–230 mesh silica gel [$CHCl_3$–EtOAc–HCO_2H (50:50:1)]. Only a trace of the presumed isomeric acid ester *cis*-15 could be detected by TLC. The first fraction gave 56 mg (10%) of *cis*-16 as brown syrup that could not be obtained pure. The second fraction crystallized as a white solid after removal of the solvent. Recrystallization from EtOAc–*n*-heptane afforded 413 mg (76%) of *cis*-14 as crystalline colorless needles, mp 157–159 °C. In subsequent preparations, this ester acid could be isolated pure in comparable yields from the reaction mixture by fractional crystallization without resorting to chromatography: ^1H NMR (Me_2SO-d_6) δ 2.41 (1 H, dd, J = 8 and 18 Hz, $-CH_2CO_2-$), 2.82 (1 H, dd, J = 6.5 Hz, $-CH_2CO_2-$), 3.69 (3 H, s, CH_3), 3.90–4.50 (1 H, deceptively simple quartet, H-3), 5.45 (1 H, d, $J_{2,3}$ = 9.7 Hz, H-2), 6.70–7.35 (3 H, m, aromatic). Anal. ($C_{12}H_{11}ClO_5$) C, H, Cl.

Procedure B. Compound *cis*-14 was prepared directly from *trans*-11 in 72% yield. A mixture of diacid *trans*-11 (12.83 g, 0.05 mol), Ac_2O (25 mL), and AcCl (5 drops) was refluxed for 4 h. The solvents were removed by reduced pressure distillation and the residue was heated at 110 °C for 2–5 min. After cooling, this residue was treated with MeOH and worked up as previously described. TLC of the reaction mixture also showed a trace of the isomeric acid ester *cis*-15. Diester *cis*-16 was isolated in 11% yield (1.57 g) containing a trace of *trans*-16 owing to isomerization during isolation.

Methyl *cis*-5-Chloro-2,3-dihydro-3-(2-hydroxyethyl)-2-benzofurancarboxylate (*cis*-17). To a solution of ester acid *cis*-14 (8.12 g, 30 mmol) in dry THF (40 mL, 0 °C) was slowly added dropwise a solution of BH_3–THF complex[12] (1 M, 30 mL, 30 mmol). With stirring, the solution was allowed to slowly warm to room temperature. After 2 h, the mixture was cooled to 0 °C and H_2O (20 mL, 0 °C) was added. After addition of K_2CO_3 (5 g) to this mixture, the THF phase was separated and the aqueous phase was extracted with Et_2O (3 × 150 mL). The combined Et_2O extract was washed with a saturated NaCl solution (50 mL), dried ($MgSO_4$), and concentrated under reduced pressure to afford crude *cis*-17, which was not further purified but used immediately in the subsequent reaction. Ester *cis*-17 undergoes spontaneous cyclization to 3 during purification by distillation under reduced pressure.

***cis*-6-Chloro-3,4,4a,9a-tetrahydro-1*H*-pyrano[3,4-*b*]benzofuran-1-one (3).** A mixture of toluene (100 mL), *p*-TsOH·H_2O (100 mg), and crude *cis*-17 from the previous reaction was heated at reflux with stirring for 1 h. The reaction mixture was cooled (ice bath), washed with $NaHCO_3$ solution (5%) followed by H_2O, and dried ($MgSO_4$). The dried solution was concentrated under reduced pressure and the residue was crystallized from EtOAc–*n*-heptane to yield 5.82 g (86% based on starting *cis*-14) of 3: mp 109–111 °C; ^1H NMR (90 MHz, Me_2SO-d_6) δ 1.50–2.00 (1 H, m, H-4), 2.25–2.70 (1 H, m, H-4), 3.95–4.60 (3 H, m, H-4a and -3), 5.45 (1 H, d, $J_{4a,9a}$ = 10.8 Hz, H-9a), 6.75–7.45 (3 H, m, aromatic); mass spectrum m/e (rel intensity) 224 (M, 100), 152 (91.2). Anal. ($C_{11}H_9ClO_3$) C, H, Cl.

Ethyl *trans*-[4-Chloro-2-(3-oxo-1-propenyl)phenoxy]-acetate (18). A solution of phenoxyacetate 5 (7.28 g, 30 mmol) and (triphenylphosphoranylidene)acetaldehyde[13] (9.40 g, 31 mmol) in dry benzene (200 mL) was refluxed for 24 h. The solvent was removed under reduced pressure and the residue was extracted with cold Et_2O (250 mL). The Et_2O solution was allowed to stand overnight, during which time the triphenylphosphine oxide precipitated. The oxide was removed by filtration and the filtrate was concentrated under reduced pressure, affording 4.55 g (56%) of a yellow solid, mp 70–72 °C, after recrystallization from EtOH. Anal. ($C_{13}H_{13}O_4Cl$) C, H, Cl.

Reaction of Diacid *trans*-15 with Ac_2O and MeOH. A mixture of diacid *trans*-11 (2.57 g, 10 mmol), Ac_2O (5 mL), and AcCl (3 drops) was heated at 100 °C in an oil bath for 4 h. The solution was cooled to 0 °C in an ice bath and absolute MeOH

(20 mL) was added slowly by dropwise addition; the solution was stirred at room temperature for 0.5 h and subsequently heated at reflux for 0.5 h. After removal of the solvent mixture under reduced pressure, $CHCl_3$ and H_2O were added. Insoluble starting *trans*-11 was removed by filtration (203 mg, 8%). The $CHCl_3$ layer was separated, washed with H_2O, dried ($MgSO_4$), and concentrated under reduced pressure. The residual oil was chromatographed on 70–230 mesh silica gel [$CHCl_3$–EtOAc–HCO_2H (100:100:1)].

Fraction 1 yielded **methyl *trans*-5-chloro-2,3-dihydro-3-[(methoxycarbonyl)methyl]-2-benzofurancarboxylate (*trans*-16)** (289 mg; 10%) as a thick oil identical with that prepared from diacid *trans*-11 using CH_2N_2.

Fraction 2 afforded 1.60 g (59%) of white solid **methyl *trans*-3-(carboxymethyl)-5-chloro-2,3-dihydro-2-benzofurancarboxylate (*trans*-14)**, mp 137–139 °C, after recrystallization from EtOAc–*n*-heptane. Anal. ($C_{12}H_{11}ClO_5$) C, H, Cl.

Fraction 3 afforded 132 mg (5%) of white solid **trans-5-chloro-2,3-dihydro-3-[(methoxycarbonyl)methyl]-2-benzofurancarboxylic Acid (*trans*-15)**, mp 117–119 °C, after recrystallization from EtOAc–*n*-heptane. Anal. ($C_{12}H_{11}ClO_5$) C, H, Cl.

Methyl *trans*-5-Chloro-2,3-dihydro-3-[(methoxycarbonyl)methyl]-2-benzofurancarboxylate (*trans*-16). Diacid *trans*-11 (1.03 g, 0.04 mol), dissolved in a minimum quantity of absolute MeOH, was added dropwise to a solution of CH_2N_2[37] in Et_2O. Excess CH_2N_2 was decomposed by dropwise addition of glacial AcOH and the Et_2O layer was washed successively with $NaHCO_3$ solution (5%), H_2O, and saturated NaCl solution. The Et_2O extract was dried ($MgSO_4$), filtered, and concentrated under reduced pressure, affording an oil which was distilled by short-path distillation yielding 1.01 g (88%) of *trans*-16 as a colorless oil, bp 140–142 °C (0.025 Torr), which solidified on standing over several days. Anal. ($C_{13}H_{13}ClO_5$) C, H, Cl.

Methyl *trans*-5-Chloro-2,3-dihydro-3-(2-hydroxyethyl)-2-benzofurancarboxylate (*trans*-17). Reduction of ester acid *trans*-14 (1.62 g, 6.0 mmol) as in the preparation of *cis*-17, afforded 1.18 g (77%) of *trans*-17, bp 145–150 °C (0.005 Torr). Anal. ($C_{12}H_{13}ClO_4$) C, H, Cl.

***trans*-5-Chloro-3-(2-hydroxyethyl)-2-(hydroxymethyl)-2,3-dihydrobenzofuran (*trans*-20).** Diacid *trans*-11 (2.00 g, 7.8 mmol) was reduced using BH_3–THF complex (0.94 M, 20 mL, 18.8 mmol) employing a procedure similar to the one used for *trans*-14. Distillation of the crude diol afforded 1.68 g (94%) of *trans*-20, bp 135–141 °C (0.025 Torr). Anal. ($C_{11}H_{13}ClO_3$) C, H, Cl.

***trans*-5-Chloro-2,3-dihydro-3-(2-hydroxyethyl)-2-benzofurancarboxylic Acid (*trans*-21).** Ester *trans*-17 (220 mg, 0.86 mmol) in NaOH solution (10%, 2 mL) was warmed on a steam bath for 10 min. Acidification with dilute HCl solution afforded 172 mg (83%) of the *trans*-21, which after several recrystallizations (mp 141–149 °C) from H_2O appeared homogeneous by TLC.

Ethyl 2-(4-Chloro-2-formylphenoxy)propionate (22). A mixture of 5-chlorosalicylaldehyde (156.6 g, 1.0 mol), ethyl 2-bromopropionate (190 g, 1.05 mol), and anhydrous K_2CO_3 (190 g, 1.37 mol) in DMF (300 mL) was heated at 80–85 °C for 1 h. After 1 h, the yellow solution turned colorless; H_2O (3.0 L) was added and the mixture was extracted with Et_2O. The Et_2O extract was washed with H_2O, dried ($MgSO_4$), and concentrated under reduced pressure, affording a colorless liquid, bp 122–125 °C (0.05 Torr), which immediately solidified. Recrystallization from 95% EtOH afforded 231.8 g (90%) of **22**, mp 46–48 °C. Anal. ($C_{12}H_{13}ClO_4$) C, H, Cl.

2-[2-(2-Carboxy-2-cyanoethenyl)-4-chlorophenoxy]propionic Acid (23). This acid, mp 224–226 °C dec (AcOH), was prepared in 72% yield from phenoxypropionate **22** (192.5 g, 0.75 mol) employing the procedure outlined for **6**. Anal. ($C_{13}H_{10}ClNO_5$) C, H, Cl, N.

Ethyl *cis*- and *trans*-2-[4-Chloro-2-(2-cyanoethenyl)phenoxy]propionate (*cis*- and *trans*-25). Esters *cis*- and *trans*-**25**, bp 160–171 °C (0.1 Torr), were prepared in 73% yield from acid **23** (147.8 g, 0.5 mol) employing the procedure outlined for the preparation of *cis*- and *trans*-**8**. ^1H NMR analysis showed the oil (101.7 g, 73%) to consist of a mixture of *cis*- and *trans*-**25** in the ratio 1:1.4.

Ethyl *cis*- and *trans*-5-Chloro-3-(cyanomethyl)-2,3-dihydro-2-methyl-2-benzofurancarboxylate (*cis*- and *trans*-26). Esters *cis*- and *trans*-**26**, bp 205–211 °C (0.05 Torr), were prepared in 92% yield from nitrile *cis*- and *trans*-**25** (100.0 g, 0.36 mol) employing the procedure outlined for the preparation of *cis*- and *trans*-**9**.

***trans*-3-(Carboxymethyl)-5-chloro-2,3-dihydro-2-methyl-2-benzofurancarboxylic Acid (*trans*-27).** Ester nitriles *cis*- and *trans*-**26** (90.0 g, 0.32 mol) were hydrolyzed employing the procedure outlined for the preparation of *cis*- and *trans*-11, affording 28.2 g (32%) of diacid *trans*-**27**, mp 182–184 °C (H_2O). Anal. ($C_{12}H_{11}ClO_5$) C, H, Cl.

The mother liquor from the crystallization of diacid *trans*-**27** was concentrated under reduced pressure and the residue was recrystallized twice from H_2O, affording 43.1 g (49%) of a mixture of *cis*- and *trans*-**27** (1:1.2, by ^1H NMR integration of CH_3 peaks). The isomeric ratio remained unaltered by further recrystallizations from H_2O and the mixture was used as such in the next reaction.

***cis*-6-Chloro-9a-methyl-4a,9a-dihydrobenzofuro[2,3-*c*]pyridine-1,3(2*H*,4*H*)-dione (28).** The mixture of *cis*- and *trans*-**27** (8.12 g, 30 mmol; 1:1.2) was dissolved in NH_4OH solution (15%, 25 mL) and heated to evaporate the H_2O. The resulting oily residue was heated at 180 °C (20 min), EtOH was added, and the product was filtered and crystallized from EtOAc–*n*-heptane, affording 2.68 g (78%, based on *cis*-**27**) of imide **28**, mp 206–209 °C. Anal. ($C_{12}H_{10}ClNO_3$) C, H, Cl, N.

***cis*-3-(Carboxymethyl)-5-chloro-2,3-dihydro-2-methyl-2-benzofurancarboxylic Acid (*cis*-27).** Imide **28** (2.52 g, 10 mmol) was hydrolyzed with NaOH, as described for 12 → *cis*-11, to afford 2.48 g (92%) of pure diacid *cis*-**27**, mp 192–194 °C (H_2O). Anal. ($C_{12}H_{11}ClO_5$) C, H, Cl.

Methyl *cis*-3-(Carboxymethyl)-5-chloro-2,3-dihydro-2-methyl-2-benzofurancarboxylate (*cis*-29). A mixture of diacid *cis*-27 (1.35 g, 5 mmol), Ac_2O (5 mL), and AcCl (2 drops) was refluxed for 1.5 h. The solvents were removed at room temperature under reduced pressure, cooled to 0 °C, and treated with absolute CH_3OH (5 mL). A white crystalline material immediately appeared. The mixture was stirred for 15 min at room temperature. A trace of the isomeric monomethyl ester *cis*-**30** could be detected (TLC). Concentration under reduced pressure followed by crystallization of the residue from EtOAc–*n*-heptane afforded 1.12 g (79%) of *cis*-**29**, mp 156–158 °C. Anal. ($C_{13}H_{13}ClO_5$) C, H, Cl.

***cis*-6-Chloro-9a-methyl-3,4,4a,9a-tetrahydro-1*H*-pyrano[3,4-*b*]benzofuran-1-one (2).** Lactone **2**, mp 79–81 °C (EtOAc–*n*-heptane) was prepared in 81% yield from *cis*-**29** (1.0 g, 3.5 mmol) via *cis*-**31** employing the procedure outlined for **3**: ^1H NMR (90 MHz, Me_2SO-d_6) δ 1.66 (3 H, s, CH_3), 1.65–2.10 (1 H, m, H-4), 2.10–2.65 (1 H, m, H-4), 3.60 (1 H, t, $J_{4,4a}$ = 6.3 Hz, H-4a), 4.30 (2 H, dd, $J_{3,4}$ = 4.5 and 5.7 Hz, H-3), 6.70–7.30 (3 H, m, aromatic); mass spectrum *m/e* (rel intensity) 238 (M^+ 63.6), 166 (100). Anal. ($C_{12}H_{11}ClO_3$) C, H, Cl.

Biological Methods. Normolipemic Rat Model. Methods utilized were identical to those previously reported.[6]

Preparation of Microsomes. Animals were anesthetized (Et_2O) to permit blood removal from the abdominal aorta and livers were excised immediately thereafter. The homogenization of liver and preparation of microsomes were carried out as previously described.[3,6]

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

Ethylmorphine *N*-Demethylase Assay. The assay of formaldehyde liberated from ethylmorphine was carried out by procedures described previously.[38] Incubation mixtures contained 5 mg of microsomal protein, 10 μmol of ethylmorphine, a NADPH-generating system, and 60 μmol 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.[39]

Methods of Analysis in Liver. Hepatic microsomal cytochrome P-450 and cytochrome b_5 were estimated by the procedure of Kinoshita and Horie.[40] Microsomal protein was assayed by

the method of Lowry et al.[41] Cholesterol and triglycerides were extracted from liver by the method of Abell et al.[42] Triglyceride content was determined by the method of Soloni.[43] Student's *t* test was used to make comparisons between means of treatment groups.

References and Notes

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