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9-Chloro-2,3-dihydro-5*H*-1,4-dioxepino[6,5-*b*]benzofuran, a Novel Antilipidemic Agent Structurally Related to Clofibrate†

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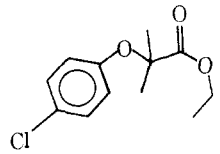
The synthesis and antilipidemic activity of 9-chloro-2,3-dihydro-5*H*-1,4-dioxepino[6,5-*b*]benzofuran (**3**), a novel enol lactone which is considerably more resistant to serum esterase hydrolysis than clofibrate (**1**), are discussed. Whereas both **3** and **1** reduced hypercholesterolemic and hypertriglyceridemic serum levels in the Triton WR-1339 induced hyperlipidemic Sprague–Dawley rat to normal, the hydrolysis product of **3**, namely 5-chloro-3-(2′-hydroxyethoxy)-2-benzofurancarboxylic acid (**4**), was found to be inactive. Further, **3** is comparable to the hydrolysis product of **1** when both were assessed for their ability to block norepinephrine (NE) induced lipolysis in vitro. **4** is inactive at comparable concentrations (5×10^{-4}–10^{-3} *M*). The antilipidemic action of **3** and **1** may, in part, be due to their ability to block NE-induced lipolysis.

In previous reports from our laboratory we have considered the differential biological effects of certain benzodioxane, chroman, and dihydrobenzofuran analogs of clofibrate (**1**) on inhibition of lipolysis and cholesterol biosynthesis in vitro,[1,2] inhibition of lipoprotein lipase in vitro,[3] hypolipidemic activity in a Triton WR-1339 induced hyperlipidemic rat model,[4,5] and hepatic drug metabolism.[6] In all cases the ethyl esters of the various analogs were employed for biological studies in rats since we were interested in assessing new compounds synthesized relative to **1** which is administered as an ethyl ester. For studies in vitro, we investigated the corresponding free carboxylic acids since **1** and related ester analogs are known to undergo rapid hydrolysis by serum esterases and the carboxylic acids are presumed to be the active antilipidemic agents.[7–10]

In this article we describe the synthesis and antilipidemic activity of 9-chloro-2,3-dihydro-5*H*-1,4-dioxepino[6,5-*b*]benzofuran (**3**), a novel enol lactone, which possesses a conformationally constrained ethyl group and is considerably more resistant to serum esterase hydrolysis. Tricyclic enol lactone **3** may be visualized as a cyclic analog of dihydrobenzofuran **2** where the β-carbon of the ethyl function is covalently bonded to the benzofuran ring at position 3 through an enol ether linkage. Dihydrobenzofuran **2**, which only exhibits cholesterol lowering activity,[4] in turn, represents a molecular modification of **1**, an analog which decreases to normal concentrations both cholesterol and triglycerides in the hyperlipidemic rat model.[4] The antilipidemic activity of **3** and its hydrolysis product **4** are discussed in light of their antilipolytic properties in vitro and compared to the biological activity of **1** and **2** and their corresponding hydrolysis products.



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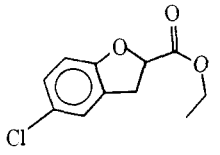

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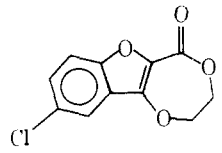

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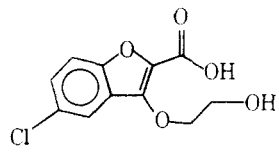

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Chemistry. The tetrahydropyranyl (THP) protected derivative of β-bromoethanol (**5**), namely **7**, was prepared in 90% yield by condensation of **5** with dihydropyran (**6**) in the presence of a catalytic amount of *p*-TsOH and served as the source of the β-hydroxyethyl side chain of **4**. Starting ethyl-5-chloro-2-carbethoxy-3(2*H*)-benzofuranone (**9**)[11,12] was prepared in 88% yield by Dieckmann condensation of ethyl 4-chloro-2-carbethoxyphenoxyacetate (**8**)[13] with NaOEt in dry benzene.[14] The yield reported here is greater than the one reported by Schroeder and coworkers[12] owing to a longer reflux time. Reaction of anion **10** generated from **9** using NaH in dry diglyme with **7** for 5 hr at 150° afforded **11** as part of an uncharacterized mixture of products (GLC, see the Experimental Section). Hydrolysis of the mixture containing **11** in refluxing 10% ethanolic KOH for 1 hr, followed by cooling, acidification with 25% H_2SO_4, and THP protecting group removal under reflux for 10 min, afforded 5-chloro-3-(2′-hydroxyethoxy)-2-benzofurancarboxylic acid (**4**) in 52% yield based on starting **9**. Hydroxy acid **4** was converted to the desired enol lactone **3** in 90% yield by refluxing in benzene containing a catalytic amount of *p*-TsOH.

3(2*H*)-Benzofuranone **9** does not form a 2,4-dinitrophenylhydrazone derivative under the mild conditions described by Pasto and Johnson;[15] however, after refluxing for 4 hr in concentrated HCl containing 2,4-DNPH the hydrazone formed in 40% yield. Keto derivatives are difficult to obtain since **9** exists in equilibrium with its aromatic enol form [ir ($CHCl_3$) C=O stretching at 1670 and 1730 cm^{-1}; OH stretching at 3350 cm^{-1}]. Therefore, we anticipated **10** to be predominantly resonance stabilized as the π-excessive heteroaromatic oxygen anion; O-alkylation by **7** was expected to predominate. In fact, we were unable to isolate any C-alkylated product under a variety of reaction conditions. O-Alkylation was confirmed by the observation that enol lactone **3** failed to give a 2,4-dinitrophenylhydrazone derivative. Further, **3** showed only one C=O stretching

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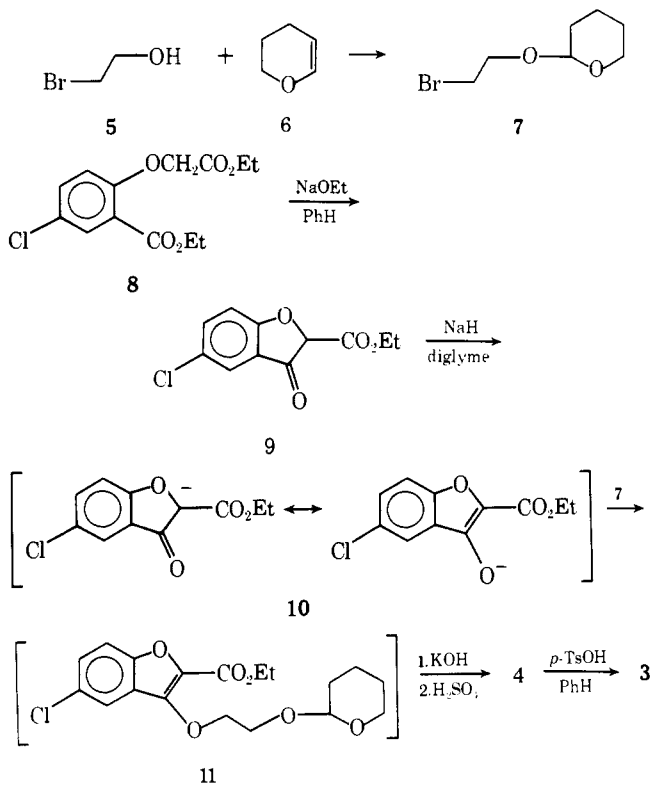
‡ Abstracted in part from a dissertation by G.K.P. presented to the Graduate School of The Ohio State University, 1974.
§ Undergraduate research participant, summer, 1974.

Table I. Hydrolysis of Clofibrate (1) and Enol Lactone 3 by Rat Plasma in Vitro at 37°

Time, min	% hydrolysis ± SD	
	Clofibrate (1)	Enol lactone 3
5	81.44 ± 0.88[a]	8.04 ± 0.47
10	89.99 ± 1.25	12.41 ± 1.36
30	91.56 ± 1.31	12.53 ± 1.34
60	91.20 ± 1.18	11.70 ± 0.91

[a]Mean ± SD; for four determinations.

band (1715 cm^{-1}); the C=O stretching band at 1670 cm^{-1} of **9** was absent. The mass spectrum for **3** is summarized in Table V; the following interpretation is consistent with the assigned structure. The molecular ion (M^+) abundancy is



high owing to the aromatic nature of the compound. Ions *m/e* 210 and 194 arise through loss of C_2H_4 and C_2H_4O, respectively, from the seven-membered ring of M^+. The *m/e* 179 and 167 (base) peaks do not arise by simple bond cleavage; loss of $C_2H_3O_2$ and $C_3H_3O_2$ from M^+ requires transfer of one H atom from the ethylenedioxy function to the respective benzofuran fragments. Loss of CHO from the base peak (*m/e* 167) affords ion *m/e* 138 which loses CO to give ion *m/e* 110.[16] Loss of Cl (observed to be present in all other fragments) from *m/e* 110 affords an ion of mass 75. Successive loss of two CO molecules from the base peak affords ions *m/e* 139 and 111.[16] The ion at *m/e* 75 may also be derived from ion *m/e* 111 through loss of HCl.

Pharmacology. The percent hydrolysis observed for clofibrate (1) and enol lactone **3** during incubation with rat plasma at 37° after 5, 10, 30, and 60 min is found in Table I. After 5 min 1 was >80% hydrolyzed. Enol lactone **3** was only 8% hydrolyzed after 5 min; after 60 min no greater than 13% conversion to hydroxy acid **4** was observed.

The antilipidemic activity of 1, enol lactone **3**, and hydroxy acid **4** was determined in normal and Triton WR-1339 induced hyperlipidemic male Sprague–Dawley rats fed Purina Chow ad libitum. This animal model may be employed to predict antilipidemic activity in man.[17] The hypocholesterolemic and hypotriglyceridemic results obtained are found in Tables II and III, respectively. At doses of 0.124 mmol/kg, clofibrate (1)[4] and enol lactone **3** significantly reduced plasma cholesterol and triglyceride levels relative to hyperlipidemic controls (comparison of groups III and IV). Like clofibrate (1), enol lactone **3** also reduced plasma lipid in hyperlipidemic rats back to normal (no significant difference between groups I and IV) and had no effect in normal lipidemic rats (comparison of groups I and II). At 0.5 the dose (0.062 mmol/kg of total screening dose) **3** and 1 lowered only plasma triglyceride levels. Conversely, the hydroxy acid hydrolysis product **4** had no antilipidemic activity in this animal model.

The antilipolytic actions of 1 and related analogs are well recognized[1,2,18] and are proposed to be responsible in part for the hypotriglyceridemic effect of 1 in vivo. A comparison of **3** and **4** as antagonists of norepinephrine (NE) induced glycerol release from isolated fat cells is presented in Table IV. From the results, it was evident that the enol lactone **3** possessed significant antilipolytic activity in the concentration range 0.5–5 × 10^{-4} *M*, whereas **4** was unable to modify the lipolytic response of NE (see experiment III in Table IV). Moreover, the acidic hydrolysis product of 1 was observed to be comparable to **3** as antagonists of glycerol release in adipose tissue (Table IV).

Discussion

At doses of 0.124 and 0.062 mmol/kg, clofibrate (1) significantly reduced elevated serum triglyceride levels in the Triton hyperlipidemic model. At the lower dosage clofibrate exhibited no significant effect on elevated cholesterol levels in hyperlipidemic rats but caused a small significant

Table II. Effect of Enol Lactone 3 and Hydroxy Acid 4 on Plasma Cholesterol Levels in Male Sprague-Dawley Rats

Compd[a]	Control group I	Drug-treated control II	Triton hyperlipidemic III	Drug-treated Triton hyperlipidemic IV
1[b]	60.6 ± 7.2	62.4 ± 8.5	267 ± 85.0	59.3 ± 12[c]
1 (0.5 dose)	90.1 ± 6.5[d]	81.3 ± 10.8[e]	113.8 ± 8.2	112.9 ± 13.1[f]
3	85.9 ± 7.6[d]	85.6 ± 10.5	233.2 ± 105.3	95.0 ± 16.1[c]
3 (0.5 dose)	84.5 ± 3.6[d]	84.0 ± 5.5	122.4 ± 8.9	112.6 ± 13.7[f]
4	78.1 ± 14.7[d]	84.5 ± 16.1	406.0 ± 184.6	334.4 ± 172.6[f]

[a]All animals were given a total screening dose of 0.124 mmol/kg of analog except for 3 (0.5 dose) and 1 (0.5 dose) which represents 0.062 mmol/kg of total screening dose. [b]Data taken from a previous publication (Newman et al.[4]). [c]Statistically significant, $p < 0.05$; Triton hyperlipidemic vs. drug-treated Triton hyperlipidemic (comparison of groups III and IV). [d]Mean ± SD; ten rats per group. [e]Statistically significant, $p < 0.05$; control vs. drug-treated control (comparison of groups I and II). [f]Statistically significant, $p < 0.05$; drug-treated hyperlipidemic vs. control (comparison of groups I and IV).

Table III. Effect of Enol Lactone 3 and Hydroxy Acid 4 on Plasma Triglyceride Levels in Male Sprague-Dawley Rats

Compd[a]	Control group I	Drug-treated control II	Triton hyperlipidemic III	Drug-treated Triton hyperlipidemic IV
1[b]	20.9 ± 6.2	30.5 ± 8.2	774.0 ± 329.0	27.6 ± 15.0[c]
1 (0.5 dose)	34.6 ± 5.7[d]	34.7 ± 6.8	100.8 ± 26.4	44.0 ± 13.9[c]
3	24.7 ± 9.6[d]	27.1 ± 8.0	204.7 ± 69.9	35.0 ± 32.8[c]
3 (0.5 dose)	19.9 ± 6.2[d]	21.3 ± 4.8	100.2 ± 13.7	34.3 ± 10.1[c,e]
4	42.2 ± 13.0[d]	29.7 ± 9.4	547.5 ± 261.5	473.3 ± 279.4[e]

[a]All animals were given a total screening dose of 0.124 mmol/kg of analog except for 3 (0.5 dose) and 1 (0.5 dose) which represents 0.062 mmol/kg of total screening dose. [b]Data taken from a previous publication (Newman et al.[4]). [c]Statistically significant, $p < 0.05$; Triton hyperlipidemic vs. drug-treated Triton hyperlipidemic (comparisons of groups III and IV). [d]Mean ± SD; ten rats per group. [e]Statistically significant, $p < 0.05$; drug-treated hyperlipidemic vs. control (comparisons of groups I and IV).

Table IV. Effect of Enol Lactone 3 and Hydroxy Acid 4 on the Release of Glycerol from Isolated Fat Cells in Response to Norepinephrine (NE, 2×10^{-6} *M*) in Vitro

			Glycerol release	
Expt	Compd	Concn, *M*	μmol/0.2 ml/hr[a] ± SEM	% inhibition
I	NE alone		0.44 ± 0.07	0
	Plus 3	10^{-3}	0.16 ± 0.01[b]	64
		5×10^{-3}	0.19 ± 0.01[b,c]	57
II	NE alone		0.33 ± 0.01	0
	Plus 3	5×10^{-4}	0.23 ± 0.02[b]	30
		10^{-3}	0.13 ± 0.01[b]	61
III	NE alone		0.20 ± 0.02	0
	Plus 3	5×10^{-4}	0.16 ± 0.00[b]	20
		10^{-3}	0.10 ± 0.01[b]	50
	Plus 4	5×10^{-4}	0.22 ± 0.01	NC[d]
		10^{-3}	0.21 ± 0.01	NC[d]
IV	NE alone		0.46 ± 0.02	0
	Plus CPIB[e]	10^{-3}	0.15 ± 0.03[b]	67
	Plus 3	10^{-3}	0.18 ± 0.05[b]	61

[a]Each value represents the mean ± SEM of $N = 2$–4. [b]Significant difference from the NE control ($p < 0.05$). [c]Compound did not completely dissolve at this concentration. [d]The changes were not significantly different ($p > 0.05$) when compared to control values. [e]CPIB = 2-(4-chlorophenoxy)isobutyric acid, the acidic hydrolysis product of 1.

hypocholesterolemic effect in normal rats. These results are consistent with the observation that clofibrate is most effective against elevated serum triglyceride levels in humans. Since enol lactone 3 exhibits biological properties similar to those observed for 1 in the Triton-induced hyperlipidemic rat and in its ability to block NE-induced lipolysis in vitro,[1,2] we anticipate 3 would exert antilipidemic actions similar to 1 in man. From our preliminary results, however, it seems most likely that enol lactone 3 is active as the unhydrolyzed lactone whereas clofibrate first undergoes hydrolysis to the free carboxylic acid which is presumed to be the active antilipidemic agent.[10]

Although it might be anticipated that neutral enol lactone 3 should have a different mode of action than the acidic hydrolysis product of 1, our initial studies in vivo and in vitro suggest that the modes of action of these two compounds may, in fact, be similar. Previous attempts, using a limited series of clofibrate related analogs to correlate the serum triglyceride lowering effects of various esters with the antilipolytic activity in vitro of the corresponding hydrolysis products, were unsuccessful.[4] However, the excellent correlation between data obtained in vivo and in vitro for 3 and 4 lends support to the hypothesis that clofibrate related analogs do indeed exhibit an antilipolytic action in vivo. A precise linear correlation between data obtained in vivo and in vitro is not to be expected since there is considerable variability in the Triton model and the in vivo results are probably complicated by differential absorption distribution and metabolism of the various analogs.

Assessment of data[1,2,4,5] obtained in our laboratories utilizing other clofibrate (1) related analogs also suggests that inhibition of lipolysis may be an important component of the antilipidemic action of these analogs. In this regard we have recently observed that ethyl 6-chlorochromanone-2-carboxylate is inactive in vivo;[5] previous studies showed that the free acid of this analog was unable to block NE-induced lipolysis in vitro at the 5×10^{-3} *M* concentration.[2] In addition to the satisfactory correlation between in vivo and in vitro data for clofibrate,[1,4] ethyl chroman-2-carboxylate, which exhibited no serum cholesterol reducing activity at an equivalent dose, did reduce elevated serum triglyceride levels approximately 50%;[4] its free acid inhibited NE-induced lipolysis (42%) in vitro at the 5×10^{-3} *M* concentration.[2] Insertion of Cl into the 6 position of the latter analog enhanced both the in vivo and in vitro biological activity; i.e., at equivalent doses the ester reduced elevated serum triglyceride levels nearly to those found in controls (approximately 90% reduction)[4] and the corresponding acid inhibited NE-induced lipolysis in vitro by 78%.[2] Further, ethyl 1,4-benzodioxane-2-carboxylate, at equivalent doses, reduced elevated serum triglyceride levels to those found in control rats[4] and blocked NE-induced lipolysis in vitro by 38% at the 5×10^{-3} *M* concentration.[1] However, two additional compounds, ethyl 2-(4-chlorophenoxy)propionate and its cyclic analog ethyl 5-chloro-2,3-dihydrobenzofuran-2-carboxylate, were unable to significantly reduce elevated serum triglyceride levels in the Triton model,[4] although they inhibited NE-induced lipolysis about 50% at 5×10^{-3} *M*. Since clofibrate related analogs likely exert their antilipidemic effect by more than one mechanism, further work is necessary before firm conclusions can be drawn.

Experimental Section

Elemental analyses were performed by Clark Microanalytical Laboratory, Urbana, Ill. Infrared spectra were recorded on a Perkin-Elmer Model 257 grating spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian A-60A spectrophotometer. Mass spectra were recorded utilizing the Du Pont 491 mass spectrometer. All melting points were taken with a calibrated Thomas-Hoover capillary melting point apparatus.

A. Synthetic Methods. 2-Bromo-1-(2′-tetrahydropyranyloxy)ethane (7). Dihydropyran (6, 9.3 g, 0.11 mol) was added to 2-bromoethanol (5, 12.5 g, 0.1 mol) in the presence of the catalytic amount of *p*-TsOH at 0°. The reaction mixture was warmed

to room temperature and stirred for 1 hr. Distillation of the mixture afforded 18.8 g (90%) of a colorless liquid: bp 67–68° (0.70 mm); NMR (neat) δ 1.3–1.9 (6 H, m, 3 tetrahydropyran methylene groups remote to the ring oxygen), 3.38–4.22 (6 H, m, 2 methylene groups of the alkyl chain and one methylene adjacent to the tetrahydropyran ring), and 4.56–4.72 (1 H, m, methine proton). Anal. ($C_7H_{13}O_2Br$) C, H, Br.

Ethyl-5-chloro-2-carbethoxy-3(2*H*)-benzofuranone (9). Ethyl 4-chloro-2-carbethoxyphenoxyacetate[12] (8, 8.58 g, 0.03 mol), dissolved in dry benzene (40 ml), was added dropwise with stirring to NaOEt (2.1 g, 0.03 mol) in 40 ml of dry benzene.[14] Stirring was continued under reflux for 16 hr. After cooling to room temperature, the reaction mixture was poured with stirring into H_2O (200 ml) and made alkaline to litmus with dilute NaOH solution. The aqueous layer was made acidic with dilute HCl and extracted with Et_2O. The organic layer was dried (Na_2SO_4) and filtered and the solvent removed under reduced pressure. The resulting solid was recrystallized from EtOH affording 6.4 g (88%) of white crystals: mp 128–129° (lit.[12] mp 126–127°); NMR ($CDCl_3$) δ 1.28–1.58 (3 H, t, CH_3), 4.25–4.68 (2 H, q, CH_2), 7.10–7.74 [4 H, m, aromatic (3) and methine (1)].

5-Chloro-3-(2′-hydroxyethoxy)-2-benzofurancarboxylic Acid (4). NaH emulsion (1.1 g of 57% NaH in mineral oil, 0.022 mol of NaH), purchased from Ventron Chemical Co., was washed with *n*-hexane and transferred with dry diglyme into a three-necked flask fitted with a N_2 inlet, condenser, and a dropping funnel. To this solution was added dropwise ethyl-5-chloro-2-carbethoxy-3(2*H*)-benzofuranone (9, 4.1 g, 0.02 mol) dissolved in diglyme. The mixture was heated with stirring to 75° and 2-bromo-1-(2′-tetrahydropyranylhydroxy)ethane (7, 6.32 g, 0.03 mol) was added dropwise. The mixture was heated at 150° for 6 hr. The solution was cooled to room temperature, the solvent removed under reduced pressure, and the residue poured into H_2O (100 ml) and extracted with Et_2O. The organic layer was dried (Na_2SO_4) and filtered and the solvent removed under reduced pressure. GLC showed three unresolved peaks which could not be separated by column chromatography. Therefore, the mixture was treated with 10% ethanolic KOH and refluxed for 1 hr. After cooling, the mixture was acidified with 25% H_2SO_4 and refluxed for 10 min. The solution was cooled to room temperature, extracted with Et_2O, dried (Na_2SO_4), and filtered, and the solvent was removed under reduced pressure. The residue was recrystallized from Et_2O, affording 2.66 g (51.9%) of crystals; mp 192–193°; NMR (acetone-d_6) δ 3.87 (2 H, t, CH_2CH_2OH), 4.55 (2 H, t, $-OCH_2CH_2OH$), 6.20 (2 H, broad, acidic, hydroxyl), 7.50–7.95 (3 H, m, aromatic); principal ir bands at ν_{max}^{Nujol} 3260, 1680 cm^{-1}; uv λ_{max} [isooctane–ethanol (95:5)] 280 mμ (ε 196). Anal. ($C_{11}H_9O_5Cl$) C, H, Cl.

9-Chloro-2,3-dihydro-5*H*-1,4-dioxepino[6,5-*b*]benzofuran-5-one (3). 5-Chloro-3-(2′-hydroxyethoxy)-2-benzofurancarboxylic acid (4, 2.57 g, 0.1 mol) was dissolved in dry benzene (200 ml). *p*-TsOH (200 mg) was added and the solution was refluxed for 1.5 hr under N_2 using a Dean-Stark apparatus to remove H_2O. The solution was cooled to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in Et_2O and washed with saturated $NaHCO_3$ solution followed by H_2O. The ether solution was dried (Na_2SO_4), filtered, and concentrated under reduced pressure affording 2.1 g (90%) of crystals: mp 201–202°; NMR (DMSO-d_6) δ 4.50–4.90 (4 H, m, lactone methylenes), 7.40–7.95 (3 H, m, aromatic); principal ir band at ν_{max}^{Nujol} 1715 cm^{-1}; mass spectrum (70 eV) see Table V; uv λ_{max} [isooctane–ethanol (95:5)] 229 mμ (ε 54, 154). Anal. ($C_{11}H_7O_4Cl$) C, H, Cl.

B. Biological Methods. 1. Antilipidemic Activity. Compounds 3 and 4 were tested in a hyperlipidemic rat model[17] in which the hyperlipidemia was induced by ip injection of Triton WR-1339 (oxyethylated *tert*-octylphenolformaldehyde polymer, Ruger Chemical Co., Philadelphia, Pa.). Male albino rats (Sprague–Dawley) were housed in groups of five and were fed Purina Laboratory Chow and water ad libitum for a 2-week stabilizing period. After this period, the rats were redistributed by weight into four experimental groups of ten rats each (housed in groups of five). At random two experimental groups (II and IV) were fasted for 24 hr and then injected ip with 225 mg of Triton/kg dissolved in 0.15 *M* NaCl to give a concentration of 62.5 mg/ml. The two control groups (I and III) of comparable weight were also fasted and received only the vehicle (0.25% aqueous methyl cellulose), whereas the remaining groups (II and IV) received test compounds in vehicle. Compounds were dispersed in the vehicle to obtain a concentration of 8.33×10^{-3} mmol/ml, providing a total screening dose of 0.124 mmol/kg for 270 ± 10 g rats in 4 ml. Each rat received two 2-ml doses by gastric intubation, the first immediately after the Triton injection and the second 20 hr later. Fasting was continued during the post-Triton period.

Table V. Relative Intensities, Fragments, and Empirical Formulas for the Major Ions in the Mass Spectrum of Enol Lactone 3

Peak *m/e*	Rel intensity	Fragment	Empirical formula
238	82.2	M^+	$C_{11}H_7ClO_4$
240	29.3		
210	3.1	$M^+ - C_2H_4O$	$C_9H_3ClO_4$
212	1.7		
194	13.2	$M^+ - C_2H_4O$	$C_9H_3ClO_3$
196	4.7		
179	10.6	$M^+ - C_2H_3O_2$	$C_9H_4ClO_2$
167	100.0	$M^+ - C_3H_3O_2$	$C_8H_4ClO_2$
169	33.8		
138	38.1	167 − CHO	C_7H_3ClO
140	13.4		
110	20.4	138 − CO	C_6H_3Cl
112	7.6		
75	23.5	110 − Cl	C_6H_3

At 43 hr after Triton administration the rats were anesthetized with ethyl ether; blood was drawn from the abdominal aorta and plasma was obtained after centrifugation at 500g for 10 min. Plasma triglyceride was determined by the method of Eggstein[19] and plasma cholesterol was analyzed by the method of Holub and Galli.[20] Significant differences in the plasma 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. Inhibition of Lipolysis in Vitro. The method employed for the isolation, preparation, and incubation of fat cells with the various compounds is similar to that described by Rodbell.[21]

Nonfasted, albino, male Harlan Sprague–Dawley rats (180–250 g) were used. Epididymal fat pads were collected (three to six animals per experiment) and incubated for 1 hr in a 3% albumin buffer solution containing crude collagenase (Worthington). Fat cells were removed and washed several times with Krebs–Ringer bicarbonate (KRB) buffer (pH 7.4). For each test, a 0.2-ml suspension of isolated fat cells was added to a 2.3-ml solution of KRB buffer (pH 7.4) containing 3% bovine albumin fraction V (Nutritional Biochemicals). Compounds tested for inhibitory activity were dissolved in DMSO and a 0.1-ml aliquot was added to the appropriate flask to obtain the desired concentration. In the inhibition studies, all flasks were preincubated for 15 min before addition of NE (2×10^{-6} *M*) in a 0.1-ml volume. Incubations were carried out under air for 1 hr at 37°. After 1 hr, the reactions were terminated by addition of 2.5 ml of 10% CCl_3COOH. Duplicate 1.0-ml aliquots of the supernatant were removed and glycerol was assayed by oxidation to CH_2O[22] followed by colorimetric analysis.[23]

Rates of glycerol released (expressed as μmoles of glycerol/0.2-ml suspension of fat cells/hour of incubation) were calculated by subtracting the production of glycerol in the absence of agonist from the amount released in the presence of NE and/or compounds. A maximal release of glycerol was obtained with 2×10^{-5} *M* NE; 2×10^{-6} *M* NE was used to examine the inhibitory activity of the compounds since this concentration gave a lipolytic response equal to about 80% of the maximal glycerol release rate. Data were analyzed for significance by the Student's t test; p values < 0.05 were considered to represent significant differences between mean values.

3. Hydrolysis of Enol Lactone 3 and Clofibrate (1) by Rat Plasma in Vitro. The hydrolysis of 1 and 3 by rat plasma in vitro at 37° was determined according to a modification of the method of Barrett and Thorp.[24] Blood was collected from the abdominal aorta of anesthetized Sprague–Dawley rats (180–200 g) in Vacutainer tubes containing a small amount of EDTA. The blood was immediately centrifuged for 15 min in an IEC centrifuge and the supernatant plasma was separated and pooled. Compound 1 was dissolved in MeOH while compound 3 was dissolved in DMSO to make 0.05 *M* stock solutions.

One-tenth milliliter of the 0.05 *M* solution of 1 and 3 was added

to 8 ml of rat plasma and the mixture was incubated at 37° on a Dubnoff metabolic shaker. Samples (1.0 ml) were removed at 0, 5, 10, 30, and 60 min and assayed for the presence of ester 1 or lactone 3.

The 1.0-ml sample was placed in a 10-ml glass-stoppered centrifuge tube. HCl (0.5 ml, 3 *N*) and 5.0 ml of an isooctane–absolute EtOH mixture (95:5, v/v) were added to the tube. The tube was stoppered, shaken by hand, and allowed to stand. After the layers separated the uv absorption of the extract was determined against a blank treated similarly to that of the sample but not containing the compounds (1 or 3). An aliquot of this isooctane–EtOH extract was then treated with an equal volume of 2% $NaHCO_3$ solution to remove the free acid. The amount of free acid was determined by observing the reduction in the uv absorption of the organic phase at 226 mμ (ε 11,600) for 1 and 229 mμ (ε 54,154) for 3. Similar experiments with 3, carried out in the absence of enzyme, showed no chemical hydrolysis took place at the times indicated. Further, hydrolysis product 4 under similar conditions was shown not to undergo lactonization under identical incubation conditions and during the same intervals of time.

Note Added in Proof. After submission of this manuscript we learned that the title compound had also been prepared in 81% yield from a 4-substituted 3,6-dichlorocoumarin.[25]

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Stereoisomeric 5,9-Dimethyl-2′-hydroxy-2-tetrahydrofurfuryl-6,7-benzomorphans, Strong Analgesics with Non-Morphine-Like Action Profiles

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The eight optically active stereoisomers and the corresponding four racemic forms of 5,9-dimethyl-2′-hydroxy-2-tetrahydrofurfuryl-6,7-benzomorphan (1) have been prepared. Depending on their configurations these compounds are potent analgesics or inactive substances in mice. The analgesics attain potencies up to about a hundred times that of morphine but they do not show morphine-like side effects in mice nor do they suppress abstinence in withdrawn morphine-dependent monkeys. Their therapeutic ratios are favorable and, in the case of 1**a**-1 and 1**a**-2, exceptionally good. Configuration–activity relationships are discussed. *R* configuration of the *N*-tetrahydrofurfuryl group is a major prerequisite for high analgesic potency.

Recently, *N*-furylmethyl substituents have been shown to confer opioid agonist and/or antagonist properties to the molecules of strong analgesics, the action profiles of the novel compounds being closely related to the nature of the *N*-furylmethyl group.[1] In the course of our continuing efforts to develop strong, nonaddicting analgesics further modifications of the *N*-furylmethyl residue of appropriate 2′-hydroxy-6,7-benzomorphans have been accomplished. We now wish to report on the chemistry and pharmacology of the stereoisomeric 5,9-dimethyl-2′-hydroxy-2-tetrahydrofurfuryl-6,7-benzomorphans and to discuss configuration–activity relationships.

Chemistry. According to the well-established stereochemistry of 5,9-dimethyl-2′-hydroxy-6,7-benzomorphan[2] the cis, diaxial fusion of the iminethano system allows only two (racemic) diastereoisomers (the α form with cis and the β form with trans orientation of the methyl groups with respect to the hydroaromatic ring). Consequently, due to the additional chiral carbon atom 2″ introduced into the molecule with the *N*-tetrahydrofurfuryl substituent, the 5,9-dimethyl-2′-hydroxy-2-tetrahydrofurfuryl-6,7-benzomorphan 1 comprises four racemic diastereoisomers and the corresponding eight optically active forms. All these 12 substances which may be subdivided into compounds 1**a** of the α series and compounds 1b of the β series have been prepared.

Syntheses were achieved by alkylation of 5,9-dimethyl-2′-hydroxy-6,7-benzomorphans (2) with tetrahydrofurfuryl bromides (3). As shown in Table I the type of reaction product(s) obtained depends on the stereoisomeric forms of 2 and 3 used in the synthesis. Satisfactory separations of diastereoisomeric mixtures resulting from application of methods B, C, and D could be achieved by fractional crystallization guided by thin-layer chromatography. Racemic compounds 1 were also prepared by mixing equal parts of the corresponding antipodes and crystallization of the mixtures (method E). Further synthetic routes are described in the patent literature.[3]

The absolute stereochemistry of the compounds 1 obtained by method A follows unequivocally from the well-