Hypolipidemic Arylthioalkanoic Acids[1]

Eugene R. Wagner,* Robert G. Dull, Larry G. Mueller, Bobbie J. Allen,

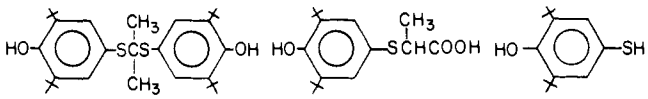
Pharmaceutical Chemistry Department

Alfred A. Renzi, Darrel J. Rytter, James W. Barnhart, and Carol Byers

Pharmacology Department, The Dow Chemical Company, Midland, Michigan 48640. *Received March 4, 1977*

A series of arylthioalkanoic acids related to probucol was studied for hypolipidemic activity. Homologation of the alkyl side chain led to marked changes in the serum cholesterol and serum triglyceride lowering activity in rats with the best combination of properties appearing in compound 7, 2-[(3,5-di-*tert*-butyl-4-hydroxyphenyl)thio]hexanoic acid. Modification of the ring substitution failed to improve the activity despite the empirical observation that lipophilic substitution was necessary. Removal of the phenolic hydroxyl produced compound 23 with properties similar to 7 but of somewhat lower activity. Replacement of the sulfur by oxygen increased the toxicity of the series. Resolution of racemic 7 did not change the activity of the compound. The LD_{50} in mice of 7 was between 5000 and 10000 mg/kg and compound 7 has been submitted for human clinical evaluation.

During an investigation of possible metabolites of the clinically active hypocholesteremic drug, probucol (DH-581) [4,4'-(isopropylidenedithio)bis(2,6-di-*tert*-butylphenol)] (1),[2] it was found that the hydroxyphenylthiopropionic acid 2 possessed significant serum cholesterol and triglyceride lowering activity in rats. Since 2 contained structural similarities to both probucol and clofibrate, it was hoped that compounds of this type might be useful hypolipidemics. Unfortunately, 2 itself was not of interest because it caused marked liver enlargement in both rats and mice. Therefore, a synthetic program was undertaken to prepare analogues of 2 which would have less toxicity.

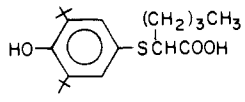


1 2 3

Results and Discussion

Side Chain Homologation. The first approach to modify structure 2 was to systematically vary the length of the alkyl side chain by alkylating 2,6-di-*tert*-butyl-4-mercaptophenol (3) with the homologous series of α-bromo acids and esters. Table I lists the compounds prepared in this series. One group (compounds 4–8, 15, and 16) represented a homologation of the α-methyl group of 2, while a second (9–14 and 17) consisted of replacement of the α-hydrogen atom by homologous alkyl chains, retaining the α-methyl.

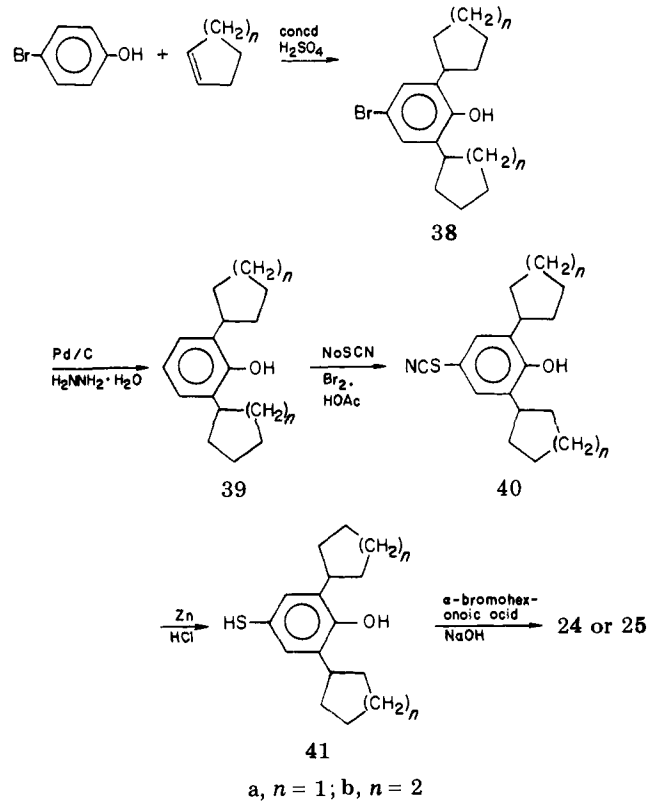
The biological effect of increasing the length of the alkyl side chains is seen in Table I. The principal beneficial effect of this homologation in both groups was reduction of the amount of the rat liver weight increase. In the series of compounds with the α-hydrogen (4–8), the hypocholesterolemic and hypotriglyceridemic effect remained as the liver weight elevation was reduced to more acceptable levels (i.e., similar to that caused by clofibrate, Table V). However, in the group with the α-methyl intact (9–14) as the liver weight dropped, the ability of the compounds to significantly reduce rat serum cholesterol was destroyed. From these results, 7 was selected for further study, since it possessed the best combination of properties, high lipid-lowering activity with minor effect on liver weight.



7

Ring Substituent Modification. Having selected hexanoic acid analogue 7 as the most promising member of the series of homologues, a study of the effect of changes in ring substitution on this structure was undertaken. The compounds prepared are listed in Tables II and III.

Scheme I



38 39 40 41

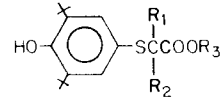
a, $n = 1$; b, $n = 2$

Chemistry. Compounds 18–22 were synthesized via direct alkylation using commercially available starting materials. The synthesis of compound 23 and 26–29 required 3,5-di-*tert*-butylbenzenethiol as a precursor. This was prepared from 3,5-di-*tert*-butylphenol by the method of Newman and Karnes.[3]

Compounds 24 and 25 were prepared from 2,6-dicyclopentyl-4-mercaptophenol (41a) and 2,6-dicyclohexyl-4-mercaptophenol (41b), which were obtained as shown in Scheme I using a modification of the procedure described by Pajeau and Begue.[4] The substituted bromophenols 38a,b were isolated by chromatography as crystalline products in low yield. They were debrominated in high yield with hydrazine hydrate and palladium on carbon in ethanol.[5]

Treatment of the dicycloalkylated phenol 39 with NaSCN and Br_2 in glacial HOAc produced the thiocyanate 40.[6] Reduction with Zn and HCl gave the free mercap-

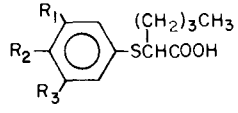
Table I. Homologous 2-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)thioalkanoic Acids and Esters and Their Hypolipidemic Profiles in Rats



Compd	R_1	R_2	R_3	Mp, °C	% yield	Recrystn solvent	Emp formula[a]	% in diet[b]	Serum cholesterol			Serum triglycerides			Liver wt change from controls[d]
									Control, mg %	Treated, mg %	% change	Control, mg %	Treated, mg %	% change	
4	H	H	H	138-140	67	Acetone-hexane	$C_{16}H_{24}O_3S$	0.25	57 ± 10	47 ± 15	−16				1/6 died
2	H	CH_3	H	111-112	64	CH_2Cl_2-hexane	$C_{17}H_{26}O_3S$	0.25	59 ± 8	40 ± 9[c]	−32	69 ± 14	15 ± 4[c]	−78	++++ +++
5	H	CH_2CH_3	H	109-111	39	CH_2Cl_2-hexane	$C_{18}H_{28}O_3S$	0.25	59 ± 8	37 ± 9[c]	−37	69 ± 14	18 ± 4[c]	−74	++++
6	H	$(CH_2)_2CH_3$	H	90-91	38	Petr ether	$C_{19}H_{30}O_3S$	0.125	72 ± 6	70 ± 6	−3	78 ± 15	22 ± 8[c]	−72	+++
7	H	$(CH_2)_3CH_3$	H	140-142	85	CH_2Cl_2-hexane	$C_{20}H_{32}O_3S$	0.125	77 ± 9	52 ± 10[c]	−32	32 ± 10	11 ± 4[c]	−66	+
								0.25	73 ± 5	51 ± 9[c]	−30	64 ± 21	20 ± 7[c]	−69	++
8	H	$(CH_2)_4CH_3$	H	85-87	83	Hexane	$C_{21}H_{34}O_3S$	0.25	55 ± 13	42 ± 6[c]	−24	59 ± 19	28 ± 8[c]	−53	+
9	CH_3	CH_3	H	133-134	79	CH_2Cl_2-hexane	$C_{18}H_{28}O_3S$	0.25	75 ± 10	59 ± 9	−21	29[e]	10[e]	−66	++++ ++++
10	CH_3	CH_2CH_3	H	152-153	41	Hexane	$C_{19}H_{30}O_3S$	0.25	73 ± 5	50 ± 11[c]	−32	64 ± 21	13 ± 4[c]	−80	++++
11	CH_3	$(CH_2)_2CH_3$	H	130-131	86	CH_2Cl_2-hexane	$C_{20}H_{32}O_3S$	0.125	78 ± 9	87 ± 8	+12	69 ± 28	31 ± 9[c]	−55	+++
12	CH_3	$(CH_2)_3CH_3$	H	128-129	29	CH_2Cl_2-hexane	$C_{21}H_{34}O_3S$	0.125	79 ± 5	73 ± 7	−8	41 ± 8	14 ± 4[c]	−66	++
13	CH_3	$(CH_2)_4CH_3$	H	95-96.5	66	CH_2Cl_2-hexane	$C_{22}H_{36}O_3S$	0.125	77 ± 6	69 ± 6[c]	−10	30 ± 7	12 ± 2[c]	−60	+
14	CH_3	$(CH_2)_5CH_3$	H	103-105	87	CH_2Cl_2-hexane	$C_{23}H_{38}O_3S$	0.125	77 ± 6	64 ± 8[c]	17	30 ± 7	8 ± 3[c]	−73	+
15	H	CH_2CH_3	C_2H_5	60-62	54	Acetone	$C_{20}H_{32}O_3S$	0.25	55 ± 13	52 ± 8	−5	59 ± 19	26 ± 6[c]	−55	+
								0.125	78 ± 9	88 ± 15	+13	69 ± 28	39 ± 8[c]	−43	
16	H	$(CH_2)_2CH_3$	C_2H_5	65-66	52	EtOH	$C_{21}H_{34}O_3S$	0.25	55 ± 13	55 ± 11	0	59 ± 19	27 ± 8[c]	−54	0
17	CH_3	CH_3	C_2H_5	77-79	72	EtOH	$C_{20}H_{32}O_3S$	0.25	74 ± 13	57 ± 11[c]	−23	63 ± 27	25 ± 7[c]	−60	+++ ++

[a] Analyses for C, H, and S were within 0.4% of theoretical values. [b] Six rats per group. [c] Significant at $p < 0.05$. [d] Each + represents approximately 10% increase over control levels. [e] Pooled samples.

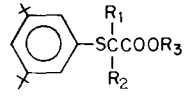
Table II. Aryl-Substituted Phenylthiohexanoic Acids and Their Hypolipidemic Profiles



Compd	R_1	R_2	R_3	Mp or bp (mm), °C	% yield	Recrystn solvent	Emp formula[a]	% in diet[b]	Serum cholesterol: Control, mg %	Serum cholesterol: Treated, mg %	Serum cholesterol: % change	Serum triglycerides: Control, mg %	Serum triglycerides: Treated, mg %	Serum triglycerides: % change	Liver wt change from controls[d]
18	H	H	H	25-28	27	Hexane	$C_{12}H_{16}O_2S$[e]	0.125	75 ± 5	63 ± 7[c]	−16	70 ± 13	44 ± 15[c]	−37	0
19	H	Cl	H	69-72	67	Heptane	$C_{12}H_{15}ClO_2S$	0.125	75 ± 5	63 ± 6[c]	−16	70 ± 13	41 ± 10[c]	−41	0
20	H	CH_3	H	49-52	63	Hexane	$C_{13}H_{18}O_2S$	0.125	81 ± 4	72 ± 7[c]	−11	31 ± 15	24 ± 10	−23	0
21	H	CH_3O	H	184 (1)	62		$C_{13}H_{18}O_3S$	0.125	81 ± 4	71 ± 9[c]	−12	31 ± 15	17 ± 13	−45	0
22	CH_3	Br	H	180 (0.5)	47		$C_{13}H_{17}BrO_2S$	0.125	75 ± 5	62 ± 6[c]	−17	70 ± 13	36 ± 7[c]	−49	++
23	$C(CH_3)_3$	H	$C(CH_3)_3$	90-91	56	Hexane	$C_{20}H_{32}O_2S$	0.125	84 ± 4	65 ± 9[c]	−23	31 ± 15	13 ± 7[c]	−58	++
								0.25	64 ± 5	51 ± 9[c]	−20	27 ± 8	16 ± 7[c]	−41	
24	c-C_5H_9	OH	c-C_5H_9	70-72	83	CH_2Cl_2-hexane	$C_{22}H_{32}O_3S$	0.125	80 ± 8	71 ± 5[c]	−11	43 ± 14	36 ± 8	−16	−
25	c-C_6H_{11}	OH	c-C_6H_{11}	103-106	86	CH_2Cl_2-hexane	$C_{24}H_{36}O_3S$	0.125	80 ± 8	78 ± 6	−3	43 ± 14	45 ± 9	+5	−

[a] Analyses for C, H, and S were within 0.4% of theoretical values. [b] Six rats per group. [c] Significant at $p < 0.05$. [d] See corresponding footnote in Table I. [e] C: calcd, 64.25; found, 64.78. S: calcd, 14.30; found, 13.67.

Table III. 2-(3,5-Di-*tert*-butylphenyl)thioalkanoic Acids and Esters and Their Hypolipidemic Profiles



Compd	R_1	R_2	R_3	Mp, °C	% yield	Recrystn solvent	Emp formula[a]	% in diet[b]	Serum cholesterol: Control, mg %	Serum cholesterol: Treated, mg %	Serum cholesterol: % change	Serum triglycerides: Control, mg %	Serum triglycerides: Treated, mg %	Serum triglycerides: % change	Liver wt change from controls[d]
26	CH_3	CH_3	H	115-116	82	Benzene-petr ether	$C_{18}H_{28}O_2S$	0.125	77 ± 6	55 ± 5[c]	−31	30 ± 7	11 ± 2[c]	−63	++++ +++
27	CH_3	$(CH_2)_2CH_3$	H	116-118	90	Hexane	$C_{20}H_{32}O_2S$	0.125	77 ± 6	55 ± 5[c]	−29	30 ± 7	6 ± 3[c]	−80	++++
28	CH_3	$(CH_2)_4CH_3$	H	97-99	89	Hexane	$C_{22}H_{36}O_2S$	0.125	77 ± 6	56 ± 4[c]	−27	30 ± 7	8 ± 4[c]	−73	++
29	CH_3	$(CH_2)_5CH_3$	H	79-81	57	Hexane	$C_{23}H_{38}O_2S$	0.125	79 ± 5	49 ± 4[c]	−38	41 ± 8	13 ± 2[c]	−68	+++

[a-d] See corresponding footnotes in Tables I and II.

Table IV. Substituent Constants and ΔR_m Values Compared with Percent Lowering of Lipids

Compd	Calcd[a] π value	Determined[b] ΔR_m value	% lowering serum cholesterol	% lowering serum triglycerides
21	−0.02	0.08 ± 0.02	−12	−45
18	0.00	0.00	−16	−37
20	0.56	0.23 ± 0.03	−11	−23
19	0.71	0.25 ± 0.03	−16	−41
22	1.42	0.51 ± 0.05	−17	−49
7	3.29	0.96 ± 0.02	−32	−66
24	3.61	0.94 ± 0.04	−11	−16
23	3.96	1.18 ± 0.04	−23	−58
25	4.35	1.10 ± 0.04	−3	+5

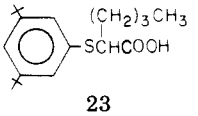
[a] Values were calculated from the chart of substituent constants from C. Hansch, A. Leo, S. Unger, K. H. Kim, D. Nikaitani, and E. Lien, *J. Med. Chem.*, 16, 1207 (1973). [b] ΔR_m was determined as described in ref 7.

tophenols **41a,b** which were alkylated with α-bromohexanoic acid to produce compounds **24** and **25**, respectively.

Structure–Activity Studies. Compounds **18–25** of Table II were prepared with a wide range of calculated π-substituent constants. There was not much change in the rat serum cholesterol lowering abilities of these compounds within the series (see Table IV). A stronger tendency toward greater reductions in serum triglyceride levels was evident as the π values increased, but the alicyclic substituted analogues, **24** and **25**, with the largest π values were completely inactive.

A comparison of the calculated π values with ΔR_m[7] values determined on these compounds indicated very good correspondence (see Figure 1). This implies that any correspondence of biological activity with lipophilicity is highly structure dependent. Apparently the bulky *tert*-butyl groups in **7** and **23** are responsible for the activity seen in these compounds.

Table III lists some analogues of **23**. Compound **28** showed excellent serum cholesterol and serum triglyceride lowerings, but it caused marked elevations of liver triglycerides and was not studied further.

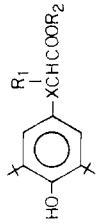


23

Oxygen Analogues. In order to determine the effect of allosteric replacement of the sulfur atom in **7** with oxygen, the first series of compounds listed in Table V was prepared. Although these compounds are mentioned in a U.S. Patent,[8] only the acetic acid analogue **30** could be prepared by the method described. It was necessary to use NaOMe in MeOH as the base to prepare **31** and **32**. For compound **33** (directly analogous to **7**), a good yield could only be obtained via hydrolysis of the ester **34** which had to be prepared using NaH in Me_2SO as the base. The biological activity was surprising in that high toxicity was seen in rats, particularly in the lower members of the homologous series. This toxicity was not seen in compound **33**, but **33** also had little effect on serum cholesterol levels and only weak activity in lowering serum triglyceride levels.

Sulfone. Oxidation of **7** with H_2O_2 in HOAc gave the crystalline sulfone **35** in excellent yield. The hypolipidemic activity was reduced from that observed with the thioether **7**.

Table V. Alkanoic Acids Containing Modification of Link between Aromatic and Alkyl Portions and Their Hypolipidemic Profiles



Compd	X	R_1	R_2	Mp or bp (mm), °C	% yield	Recrystn solvent	Emp formula[a]	% in diet[b]	Serum cholesterol: Control, mg %	Serum cholesterol: Treated, mg %	Serum cholesterol: % change	Serum triglycerides: Control, mg %	Serum triglycerides: Treated, mg %	Serum triglycerides: % change	Liver wt change from controls[d]
30	O	H	H	162–163	34	CH_2Cl_2–hexane	$C_{16}H_{24}O_4$	0.125	84 ± 8	95 ± 15[c]	+13	28 ± 9	14 ± 10[c]	−50	1/6 died
31	O	CH_3	H	136–137	42	Hexane	$C_{17}H_{26}O_4$	0.125	Toxic						6/6 died
32	O	CH_2CH_3	H	130–133	20	Hexane	$C_{18}H_{28}O_4$	0.125	83 ± 10	88 ± 11	+6	29 ± 9	40 ± 29	+38	2/6 died
33	O	$(CH_2)_3CH_3$	H	87–91	40	Pentane	$C_{20}H_{32}O_4$	0.125	81 ± 9	75 ± 2	−7	40 ± 15	25 ± 3[c]	−38	++
34	O	$(CH_2)_3CH_3$	C_2H_5	195–200 (2.0)	76		$C_{22}H_{36}O_4$	0.125	83 ± 10	72 ± 5[c]	−13	29 ± 9	21 ± 6	−28	+
35	SO_2	$(CH_2)_3CH_3$	H	161–162	91	CH_2Cl_2–hexane	$C_{20}H_{32}O_5S$	0.125	86 ± 10	74 ± 7	−14	25 ± 6	16 ± 6	−36	++
								0.25	86 ± 10	71 ± 11	−17	25 ± 6	14 ± 3	−44	
36		$(CH_2)_3CH_3$	H	104–105	90	Hexane	$C_{20}H_{32}O_3$	0.125	64 ± 12	51 ± 9[c]	−20	49 ± 17	37 ± 10	−24	
37		$(CH_2)_3CH_3$	C_2H_5	78–80	76	EtOH	$C_{22}H_{36}O_3$	0.125	64 ± 12	63 ± 11	−2	49 ± 17	44 ± 17	−10	
1	Probucol							0.25[e]	64 ± 12	40 ± 3[c]	−38	49 ± 17	33 ± 10[c]	−33	0
38	Clofibrate							0.25	68 ± 10	49 ± 7[c]	−28	30 ± 8	15 ± 3[c]	−50	++

[a–d] See corresponding footnotes in Table I. [e] Eight animals used per group.

Table VI. Oral Dose-Response Study of 7 in Rats[a]

Dose, % in diet	Serum cholesterol			Serum triglycerides			Liver wt change from controls[c]
	Control, mg %	Treated, mg %	% change	Control, mg %	Treated, mg %	% change	
0.03	77 ± 9	61 ± 8[b]	−21	32 ± 10	21 ± 8[b]	−35	−
0.06	77 ± 9	56 ± 5[b]	−28	32 ± 10	12 ± 4[b]	−61	0
0.125	77 ± 9	52 ± 10[b]	−33	32 ± 10	11 ± 4[b]	−67	+
0.25	77 ± 9	55 ± 11[b]	−29	32 ± 10	5 ± 2[b]	−84	++

[a] Eight rats per group. [b] Significant at $p < 0.05$. [c] Each + or − sign represents a change of about 10%.

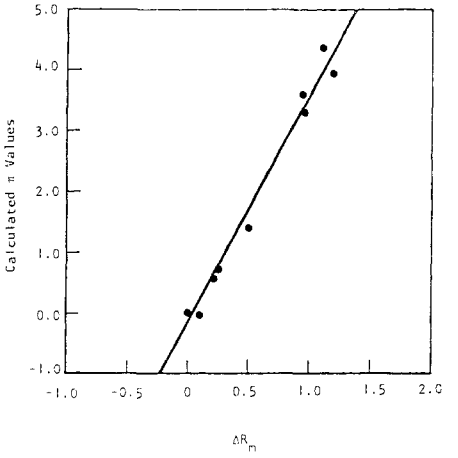


Figure 1. Comparison of ΔR_m with calculated substituent constants.

Dethio Analogue. Removal of the sulfur atom from this structural type to give **36** and **37** required the direct alkylation[9] of 2,6-di-*tert*-butylphenol with ethyl 2-bromohexanoate using *t*-BuOK in *t*-BuOH and 1,2-bis-(methoxyethoxy)ethane. The carboxylic acid **36** was obtained by hydrolysis of the ester **37**. Hypolipidemic activity was essentially eliminated by this change, indicating that the sulfur atom is a requirement for activity.

Optical Resolution of 7. Attempts to resolve the enantiomers of **7** with dehydroabietylamine failed to give a diastereomeric salt that could be fractionally recrystallized successfully. The *l*-cinchonidine salt could be recrystallized from CCl_4, but the crystals contained solvent of crystallization and had to be carefully dried under vacuum. Regeneration of the resolved acid gave a compound with a specific rotation of $[\alpha]^{25}_D$ +7.7 ± 0.1° (*c* 0.012, MeOH). This compound showed no significant difference in biological activity from unresolved **7**.

Summary of Structure–Activity Requirements. It is apparent from the data presented that several requirements can be defined for optimal activity and low toxicity for the phenylthioalkanoic acids in the normolipemic rat. The thioether group α to a carboxyl and attached to a phenyl ring is necessary. A four-carbon aliphatic chain on the α carbon gives maximal activity with acceptably low effect on liver weight. The 3,5-di-*tert*-butyl-substituted aromatic ring gives the best hypotriglyceridemic properties, and a phenolic hydroxyl in the para position maximizes the hypotriglyceridemic effect while maintaining good cholesterol lowering.

In spite of considerable effort to find more effective analogues, compound **7**, 2-[(3,5-di-*tert*-butyl-4-hydroxyphenyl)thio]hexanoic acid (DL-990), remained the most promising candidate in this series for further evaluation. It was determined to have an LD_{50} in mice of 5000–8000 mg/kg and in rats from 7200 to 10 000 mg/kg. Table VI shows a dose–response study of **7** in rats. There is little change in the serum cholesterol lowering with dose but a relatively good response with serum triglycerides. Details of the pharmacological profile of this compound and its hypolipidemic properties in other species will be reported elsewhere. The compound has been submitted for human clinical evaluation.

Experimental Section

Chemical. Elemental analyses were performed by Midwest Micro Laboratories, Indianapolis, Ind., and the Analytical Laboratories of the Dow Chemical Co., Midland, Mich. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer Model 727 spectrophotometer as Nujol mulls. NMR spectra were obtained in $CDCl_3$ solution (Me_4Si) using a Varian A-60 spectrometer. All IR and NMR spectra were consistent with the structures assigned. Optical rotations were taken in a 10-cm cell using a Perkin-Elmer Model 141 polarimeter.

General Procedure for the Alkylation of the Mercaptophenols. Preparation of 2-[(3,5-Di-*tert*-butyl-4-hydroxyphenyl)thio]hexanoic Acid (7). The 2,6-di-*tert*-butyl-4-mercaptophenol was prepared by the method of Laufer.[10] To a solution of 64.3 g (0.27 mol) of 2,6-di-*tert*-butyl-4-mercaptophenol (**3**) in 540 mL of EtOH was added a solution of 22 g (0.55 mol) of NaOH in 41 mL of H_2O with stirring and cooling under N_2. Then 52.8 g (0.27 mol) of 2-bromohexanoic acid (Eastman) in 27 mL of EtOH was added. The mixture was stirred at room temperature for 6 h and then allowed to stand overnight. The reaction was diluted with 400 mL of H_2O and acidified with cold 6 N HCl. The yellow-brown solid was filtered, washed with H_2O, and recrystallized from CH_2Cl_2–hexane giving 81 g (85%) of white crystals. Alternatively the acid could be prepared by alkaline hydrolysis of the corresponding ester which is formed in the same manner as above, except utilizing ethyl 2-bromohexanoate and 1 equiv of NaOH.

3,5-Di-*tert*-butylbenzenethiol. To a solution of 227 g (1.10 mol) of 3,5-di-*tert*-butylphenol in 1000 mL of dry benzene was added 23 g (1.0 mol) of Na metal chips over a 1-h period. An additional 200 mL of benzene was added and the reaction stirred at room temperature overnight. It was then refluxed for 72 h, cooled, and filtered. The sodium phenolate was briefly dried in air and stored over P_2O_5.

To a 15 °C solution of 60 g (0.49 mol) of dimethylthiocarbamoyl chloride in 300 mL of DMF was added portionwise 82 g (0.36 mol) of phenolate above. The reaction was warmed to 50 °C and stirred overnight. It was poured into 500 mL of ice water and a precipitate formed immediately. After filtering and washing, the precipitate was dissolved in benzene, dried over $MgSO_4$, and evaporated to yield 194 g of crude 3,5-di-*tert*-butylphenol dimethylthiocarbamate (62%). Recrystallized from methanol, the product melted at 117 °C.

This thiocarbamate (52.4 g, 0.18 mol) was pulverized and heated at 300 °C for 2 h. Recrystallization (pentane) gave 36 g (70%) of the 3,5-di-*tert*-butylphenyl ester of dimethylthiocarbamic acid.

To a solution of this ester (28.3 g, 0.096 mol) in 200 mL of MeOH was added a solution of 6 g (0.15 mol) of NaOH in 60 mL of H_2O and the reaction was refluxed under N_2 for 5 h. The cooled reaction was poured into ice water and neutralized with 10% HCl. The product was extracted into benzene and, after washing with H_2O, was dried over $MgSO_4$ and the benzene evaporated. The crude product was recrystallized from 95% EtOH to yield 17.85 g of 3,5-di-*tert*-butylbenzenethiol, mp 55–56 °C.

4-Bromo-2,6-dicyclohexylphenol (38b). To a stirred mixture of 275 g (1.59 mol) of pulverized *p*-bromophenol in 250 mL of

concentrated H_2SO_4, cooled and maintained at 15–20 °C, was added, dropwise, 82 g (1.0 mol) of cyclohexene over a 45-min period. The thick red reaction mass was stirred for another hour and then allowed to warm to room temperature over a 2-h period. The dark mass was poured into 1600 mL of ice water and extracted six times with 200-mL portions of benzene. The benzene layers were washed six times with H_2O, dried over Na_2SO_4, and evaporated to leave a blue-black oil. TLC (silica gel 60, 30% $CHCl_3$ in hexane, $R_f \sim 0.25$) showed the presence of product as a bright yellow spot with I_2. The entire residue was chromatographed on 800 g of silica gel 60 (Brinkmann) using 30% $CHCl_3$ in hexane. All fractions containing the desired product (by TLC) were combined to give 55 g of dark brown oil. This was rechromatographed on 600 g of silica gel 60 beginning with hexane and changing gradually to 10% and then 30% $CHCl_3$–hexane. Collecting 150-mL fractions, fractions 22–33 contained a total of 29 g of light green viscous syrup. IR analysis confirmed the identity of the product: MS m/e 336 (M^+). The combined fractions were crystallized from cold pentane to give 18.2 g of **38b**, mp 75–81 °C.

2,6-Dicyclohexylphenol (39b). An 85% aqueous solution of hydrazine hydrate (100 mL) was added to a stirred suspension of 40 g of 5% Pd/C and 25 g (0.074 mol) of **38b** in 1000 mL of EtOH and the mixture heated to reflux for 5–10 min. After cooling and filtering, the filtrate was evaporated and the residue taken up in benzene. The benzene solution was washed with 0.1 N HCl and H_2O, dried (Na_2SO_4), and evaporated to leave 19 g (96%) of a light yellow oil. The phenol **39b** crystallized on standing overnight.

3,5-Dicyclohexyl-4-hydroxyphenyl Thiocyanate (40b). A solution of 12.8 g (0.08 mol) of Br_2 in 50 mL of HOAc was added dropwise, with stirring, to a cold solution of 18.4 g (0.071 mol) of **39b** and 17.4 g (0.21 mol) of NaSCN in 200 mL of glacial HOAc over a 25-min period. The mixture was stirred at room temperature for 3 h. The reaction was poured into 1500 mL of H_2O and an orange solid separated. The solid was suspended in 500 mL of boiling EtOH and the mixture filtered. The filtrate was diluted with H_2O to 800 mL and a crystalline, yellow solid formed. The solid was dried and recrystallized from CH_2Cl_2–hexane. The pale yellow crystals of **40b** weighed 12.1 g: mp 139–142 °C; IR 3400 (OH), 2180 cm^{-1} (SCN).

2,6-Dicyclohexyl-4-mercaptophenol (41b). To a rapidly stirred mixture of 10 g (0.032 mol) of thiocyanate **40b** and 200 g (3.0 g-atoms) of Zn dust (Mallinckrodt) in 250 mL of toluene was added dropwise 250 g of concentrated HCl. The reaction was refluxed gently overnight, then cooled, and filtered and the clear, colorless toluene layer washed twice with H_2O until neutral. The toluene solution was dried (Na_2SO_4), filtered, and evaporated. The resulting product **41b** was used directly for the formation of compound **25** without further characterization. The mercaptophenol had an R_f of 0.55 on silica gel using a 1:1 acetone–hexane solvent. The dicyclopentyl analogues **38a–41a** were prepared in an analogous manner.

Reverse-Phase TLC System for Determining ΔR_m. Silica gel 60 F_{254}, 20 × 20 cm analytical TLC plates (Analabs, Inc.) were developed with a 5% solution of DC 200 silicone oil in ether and air-dried prior to use. The samples were spotted (1 μL of a 10 mg/mL acetone solution) and the chromatogram was developed using a solvent system consisting of 50:50:1 acetone–H_2O–glacial HOAc saturated with DC 200. The R_f values obtained as the average of three runs were used to calculate R_m. The unsubstituted compound **18** was used as the base compound to calculate R_m.[8]

2-(3,5-Di-*tert*-butyl-4-hydroxyphenoxy)propionic Acid (31). 2,6-Di-*tert*-butylhydroquinone (11.1 g, 0.05 mol) was dissolved in 100 mL of N_2 purged MeOH. To this stirred solution was added 8.1 g (0.15 mol) of NaOMe, followed by dropwise addition of 9.1 g (0.05 mol) of ethyl 2-bromopropionate dissolved in 50 mL of MeOH. The dark mixture was heated to reflux under N_2 for 96 h. The cooled reaction was diluted with 100 mL of H_2O and acidified with 6 N HCl. The dark oil was extracted into $CHCl_3$, dried (Na_2SO_4), and evaporated to give 15 g of dark oil. This was crystallized from CH_2Cl_2–hexane to give 4.8 g of off-white crystals.

Compound **32** was prepared in the same manner.

Ethyl 2-(3,5-Di-*tert*-butyl-4-hydroxyphenoxy)hexanoate (34). Sodium hydride (2.90 g, 0.06 mol) (washed twice with hexane to remove the mineral oil) was suspended in 100 mL of dry Me_2SO in a dry flask with stirring under N_2. The mixture was purged with N_2 for 15 min and then 12.2 g (0.055 mol) of 2,6-di-*tert*-butylhydroquinone in 50 mL of dry, N_2 purged Me_2SO was added dropwise over a 30-min period. The slightly exothermic reaction gave a yellow mixture which was stirred until H_2 evolution ceased. A solution of 12.3 g (0.055 mol) of ethyl 2-bromohexanoate in 50 mL of N_2 purged Me_2SO was added dropwise with stirring at a rate such that the reaction temperature did not exceed 35 °C. The dark green reaction was stirred at room temperature for 3 h, diluted with 400 mL of ice H_2O, and acidified with ice-cold 6 N HCl. The oil was extracted three times into $CHCl_3$, dried over Na_2SO_4, and evaporated. The residue was distilled at 192–200 °C (2 mm) to give 15 g of a yellow oil. IR was consistent with the assigned structure.

2-(3,5-Di-*tert*-butyl-4-hydroxyphenoxy)hexanoic Acid (33). Ester **34**, 11.0 g (0.03 mol), was dissolved in 75 mL of ethanol, 2.4 g (0.06 mol) of NaOH in 25 mL of H_2O was added with stirring, and the deep blue solution was refluxed 2 h. After cooling and dilution with 150 mL of ice H_2O, the mixture was acidified with cold 6 N HCl. The dark brown gum which separated was extracted into $CHCl_3$, and the $CHCl_3$ solution was dried (Na_2SO_4) and evaporated. After sitting for 6 weeks, the crude product had partially crystallized. The crystals were separated from the mass and washed with cold pentane. A second crop was obtained from a cold concentrated pentane solution of mother liquors. The two crops were recrystallized from cold pentane to give 3.9 g of white solid.

2-(3,5-Di-*tert*-butyl-4-hydroxyphenylsulfonyl)hexanoic Acid (35). A solution of 50 g (0.14 mol) of **7** in 500 mL of glacial HOAc was prepared by warming the mixture to 60 °C. To this warm solution was added, with stirring, 80 mL of 30% H_2O_2 and the resulting precipitate redissolved by rewarming to 60 °C. A yellow solution resulted that was stirred overnight at room temperature. The entire reaction was poured into a solution of 100 g of Na_2SO_3 in 500 mL of H_2O and diluted to 2500 mL with ice. The mixture was filtered and the white crystalline precipitate was washed well with H_2O and dried in air. It was recrystallized from CH_2Cl_2–hexane to give 59 g of sulfonyl compound **35**.

2-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)hexanoic Acid Ethyl Ester (37). To a solution of 22.4 g (0.20 mol) of *t*-BuOK in 200 mL of *t*-BuOH stirred under N_2 was added 41.2 g (0.20 mol) of 2,6-di-*tert*-butylphenol. The reaction mixture became green and semisolid. 1,2-Bis(methoxyethoxy)ethane (50 mL) was added and a green solution resulted. To this solution was added a solution of 44.6 g (0.20 mol) of ethyl 2-bromohexanoate in 60 mL of *t*-BuOH dropwise over a 30-min period. The resulting mixture was refluxed for 1 h. The green color changed to yellow and a precipitate of KBr separated. The cooled reaction was poured into 1500 mL of ice water and extracted with ether. The ether solution was washed twice with water, dried (Na_2SO_4), and evaporated to yield 53.8 g of crude ester. Unchanged phenol was removed by distillation. The pot residue was crystallized from ethanol to give 11.23 g of **37**.

2(3,5-Di-*tert*-butyl-4-hydroxyphenyl)hexanoic Acid (36). The ester **37** (18.5 g, 0.053 mol) was dissolved in 115 mL of 95% EtOH, 10 g (0.18 mol) of KOH was added, and the reaction was refluxed with stirring for 22 h. It was cooled and poured into 1400 mL of 5% HCl–ice H_2O to give a brown precipitate. The product was extracted into $CHCl_3$, washed with H_2O, dried (Na_2SO_4), and evaporated to give 17.9 g. It was crystallized from hexane to give 11.28 g of **36** as colorless crystals.

Optical Resolution of 7. To a clear boiling solution of 29.4 g (0.1 mol) of *l*-cinchonidine in 2500 mL of CH_3CN was added a hot solution of 35.2 g (0.1 mol) of *dl*-**7** in 600 mL of CH_3CN. The mixture was filtered and the CH_3CN evaporated completely from the filtrate. The residue was dissolved in 600 mL of CCl_4 and chilled to −14 °C. A gelatinous solid formed. The major part of the solvent was filtered off and the gel was redissolved in CCl_4 and allowed to crystallize more slowly. Careful fractional crystallization gave a 12.4 g of salt with a constant α of −41° and a melting point of 53–55 °C from an original α of −60° for the crude material. This salt was dissolved in 50 mL of EtOH and 200 mL of 1 N HCl and 150 mL of H_2O were added. The mixture was warmed until the precipitated oil solidified. It was filtered and the solid washed with H_2O. The resolved acid was recrys-

tallized from CH_2Cl_2–hexane to give 2.83 g of resolved **7**: mp 138–140 °C; $[\alpha]^{25}_D$ +7.7 ± 0.1° (*c* 0.0121, MeOH).

The crude salt remaining was converted to the free acid, mp 139–141 °C, and showed $[\alpha]_D$ of −1.25 ± 0.15°.

Biological. Male rats (Sprague–Dawley derived) weighing 150–160 g were employed in the study. The compounds were dissolved in acetone and taken up on silica gel (3 × weight of drug) and the solvent was evaporated. The silica gel drug mixture was mixed with commercial ground rat chow to yield concentrations of 0.125 or 0.25% (w/w) of drug in the feed. This treated feed was administered to the rats ad libitum over a 14-day period. Control groups were given feed alone. At the end of the experimental period, the animals were weighed and killed by decapitation. Blood samples were collected; the liver was removed, weighed, and frozen for future analysis. The serum cholesterol and triglyceride levels were measured by methods previously described.[11,12]

The data are presented as milligram percent in the serum along with the standard deviation for each group. The significance level (*p*) was determined using Student's *t* test. The mean and standard deviation for the test groups and control groups was used to calculate *t*.

Acknowledgment. The authors sincerely appreciate the initial screening results from Phil Shea of the Pharmacology Department and the spectroscopic analyses of Werner Braun of the Pharmacokinetics Group, The Dow Chemical Co.

References and Notes

(1) Presented in part at the 4th International Symposium on Medicinal Chemistry, Noordwijkerhout, The Netherlands, Sept 9–13, 1974, and the 169th National Meeting of the American Chemical Society, Philadelphia, Pa., April 6–11, 1975.
(2) D. T. Nash, *J. Clin. Pharmacol.*, 14 (8 and 9), 470 (1974).
(3) M. S. Newman and H. A. Karnes, *J. Org. Chem.*, **31**, 3980 (1966).
(4) R. Pajeau and J. P. Begue, *Bull. Soc. Chim. Fr.*, 1923 (1962).
(5) W. L. Mosby, *J. Org. Chem.*, **24**, 421 (1959).
(6) J. L. Wood, *Org. React.*, **3**, 240 (1946).
(7) A. N. Tischler, F. M. Thompson, L. J. Libertini, and M. Calvin, *J. Med. Chem.*, **17**, 948 (1974). The principal difference in our TLC system was that acetic acid was added and the ratios were altered in the developing solvent mixture to give rounder shaped spots and less streaking.
(8) J. B. Peterson and M. Dexter, U.S. Patent 3 249 632.
(9) M. Knell, U.S. Patent 3 455 994 (July 1969).
(10) R. J. Laufer, U.S. Patent 3 129 262 (April 1964).
(11) A. A. Henly, *Analyst*, **82**, 286 (1957).
(12) E. E. VanHandel and D. B. Zilversmit, *J. Lab. Clin. Med.*, **50**, 152 (1957); E. E. VanHandel, *Clin. Chem.*, **7**, 249 (1961).

Synthesis and Stereospecific Antipsychotic Activity of (−)-1-Cyclopropylmethyl-4-(3-trifluoromethylthio-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)piperidine

David C. Remy,* Kenneth E. Rittle, Cecilia A. Hunt, Paul S. Anderson, Byron H. Arison, Edward L. Engelhardt, Ralph Hirschmann,

Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486

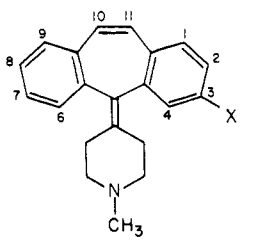
Bradley V. Clineschmidt, Victor J. Lotti, Patricia R. Bunting, Ruby J. Ballentine, Nan L. Papp, Lars Flataker, John J. Witoslawski, and Clement A. Stone

Merck Institute for Therapeutic Research, West Point, Pennsylvania 19486. *Received February 2, 1977*

The synthesis and resolution of 3-iodocyproheptadine [(±)-**5a**] and 1-cyclopropylmethyl-4-(3-iodo-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)piperidine [(±)-**5b**] are described. The resulting atropisomers undergo reaction with trifluoromethylthiocopper to give optically active products without extensive racemization. In this manner, optically pure (+)- and (−)-3-trifluoromethylthiocyproheptadine [(+)-**6a** and (−)-**6a**, respectively] and (+)- and (−)-1-cyclopropylmethyl-4-(3-trifluoromethylthio-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)piperidine [(+)-**6b** and (−)-**6b**, respectively] have been prepared. The influence of a chiral europium shift reagent on the proton and fluorine resonance signals as a diagnostic tool for the determination of the optical purities of these atropisomers is discussed. The four compounds, (+)-**6a**, (−)-**6a**, (+)-**6b**, and (−)-**6b**, were studied in squirrel monkeys for their ability to block conditioned avoidance responding. All of the antiavoidance activity was found to reside solely in the levorotatory compounds (−)-**6a** and (−)-**6b**. Further comparison of the enantiomers (−)-**6b** and (+)-**6b** showed that the ability to antagonize apomorphine-induced stereotyped behavior is confined to the levorotatory isomer (−)-**6b** while weak central anticholinergic activity resides solely in the dextrorotatory isomer (+)-**6b**. Neither (−)-**6b** nor (+)-**6b** has significant peripheral anticholinergic activity.

The introduction of nuclear substituents into the 3 position of cyproheptadine (**1**, X = H) results not only in significant changes in the biological profiles of the resulting compounds but also results in the introduction of atropisomerism into the series. Ebnöther et al.[1] have shown that nonbonded interactions between the protons in the 4 and 6 positions of the aromatic rings and the allylic protons of the piperidine ring restrict the inversion of the central, nonplanar, seven-membered ring of these 3-substituted cyproheptadine derivatives. As a consequence of these interactions, the free-energy barriers to inversion are sufficiently high as to confer a relatively high degree of thermal stability on these chiral conformers (atropisomers).[1]

During the course of investigating structure–activity relationships in a series of 3-substituted cyproheptadine derivatives and analogues, 3-iodocyproheptadine [(±)-**5a**]



1

and 1-cyclopropylmethyl-4-(3-iodo-5*H*-dibenzo[*a*,*d*]-cyclohepten-5-ylidene)piperidine [(±)-**5b**] were prepared and resolved into their optical antipodes.

Trifluoromethylthiocopper, generated in situ by the reaction of bis(trifluoromethylthio)mercury with copper, has recently been shown to react with aromatic iodides and bromides to give aryltrifluoromethyl sulfides.[2] The re-