Cardioselective Ammonium, Phosphonium, and Sulfonium Analogues of α-Tocopherol and Ascorbic Acid That Inhibit *in Vitro* and *ex Vivo* Lipid Peroxidation and Scavenge Superoxide Radicals

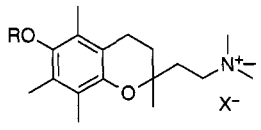
J. Martin Grisar, Gilbert Marciniak,* Frank N. Bolkenius, Joëlle Verne-Mismer, and Eugene R. Wagner

Marion Merrell Dow Research Institute, 67080 Strasbourg-Cedex, France

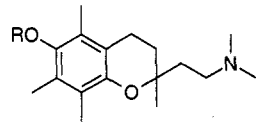
Received March 14, 1995[⊗]

Analogues of α-tocopherol and ascorbic acid with permanently cationic substituents, i.e., phosphonium (**8**, **9**), sulfonium (**11**), acylhydrazinium (**13**, **14**), and ammonium (**1**, **16**, **21**), were synthesized, and the 2*R* and 2*S* enantiomers of the α-tocopherol analogues **1**, **8**, **11**, and **13** were separated. The compounds were found to scavenge lipoperoxyl and superoxide radicals *in vitro* and accumulate in heart tissue (cardioselectivity) as demonstrated by measurement of *ex vivo* inhibition of lipid peroxidation in mouse heart homogenates and confirmed by HPLC determination of drug concentrations for **1** and **11**. The 2*R* and 2*S* enantiomers of **1** inhibited *ex vivo* lipid peroxidation to an equal extent. Thus the *in vivo* uptake into myocytes (cardioselectivity) is independent of the geometry at the chiral center and common to permanently cationic compounds.

Free radical reactions have been implicated in the pathology of more than 50 human diseases.[1] Radicals and other reactive oxygen species are formed constantly in the human body both by deliberate synthesis (e.g., by activated leukocytes of the immune system) and by chemical side reactions. They are removed by enzymatic and nonenzymatic antioxidant defense systems. Oxidative stress, occurring when antioxidant defenses are inadequate, can damage lipids, proteins, carbohydrates, and nucleic acids. A few clinical conditions are caused by oxidative stress, but more often the stress results from the disease and can make a significant contribution to the disease pathology. Vitamins C and E are very effective chain-terminating, natural antioxidants,[2,3] and their structures have often served as starting points for the synthesis of therapeutically active analogues.[4–7,22]



1 R = H, X⁻ = OTs
2 R = $COCH_3$, X⁻ = OTs



3 R = H
4 R = $COCH_3$,

The α-tocopherol analogue **1**, in which the lipophilic phytyl ($C_{16}H_{33}$) side chain was replaced by a hydrophilic trimethylethanaminium moiety,[8] was found to reduce myocardial infarct size in rats rendered ischemic by coronary artery ligation followed by reperfusion and was also found to restore contractility of isolated rat hearts subjected to anoxia and reoxygenation.[9] Compound **1** inhibits lipid peroxidation and scavenges superoxide and hydroxyl radicals more effectively than α-tocopherol[10] and prevents the leukocyte-mediated oxidative inactivation of α_1-proteinase inhibitor.[11] In rats, **1** and its O-acetylated prodrug **2** accumulate in heart tissue where concentrations were 10–30 times higher than in blood and other tissues after intravenous administration.[12] This cardioselectivity contributes significantly to the potency of **2** compared to that of the noncardioselective analogue **4**, which requires 10–30 times higher doses to protect against myocardial reperfusion injury.[13,14]

The potential usefulness of the trimethylethanaminium side chain has also been demonstrated in ulcerative colitis, which is known to involve oxygen-derived free radicals.[15–17] Upon oral administration to mice and dogs, compound **1** is not absorbed from the gastrointestinal tract and reaches the colon unchanged and undiluted.[18] Hence compound **1** was shown by Murthy et al. to be orally effective in a colitis model in mice at doses as low as 1–10 mg/kg po[19,20] and also iv in a model of colonic ischemia in rats.[21] This therapeutic efficacy was enabled by targeting a free radical scavenger to a particular tissue or compartment. Because of these promising results, we synthesized additional water-soluble, permanently cationic analogues of α-tocopherol and ascorbic acid. Since the α-tocopherol analogues have a chiral center at the 2-position, we also synthesized and evaluated the enantiomers.

Chemistry

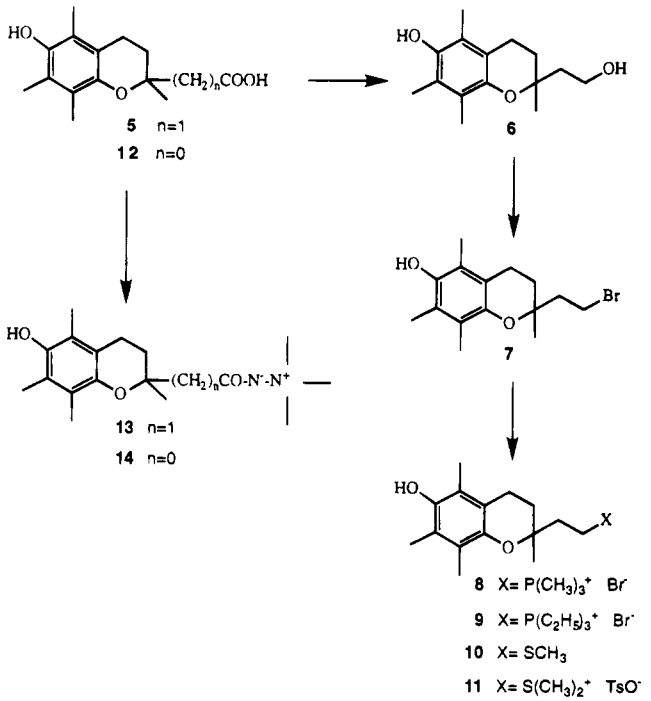
The synthesis of the α-tocopherol analogues is described in Scheme 1. Two phosphonium analogues, **8** and **9**, were obtained by reaction of **7**[8] with trimethyl- and triethylphosphine, respectively. The sulfonium analogue **11** was obtained by treatment of the sulfide **10** with methyl *p*-toluenesulfonate in refluxing acetonitrile. The sulfide **10** was obtained from **7**[8] by reaction with excess $NaSCH_3$ in DMF. The acylhydrazinium compounds **13** and **14** were obtained by treatment of the unsymmetrical dimethylhydrazides with methyl *p*-toluenesulfonate in refluxing acetonitrile. Treatment of the resulting tosylates with aqueous NaOH gave the inner salts.

Resolution of **5**[22] with (*S*)-(−)- and (*R*)-(+)-α-methylbenzylamine gave the two enantiomers in >99% enantiomeric excess. From these, the enantiomers of **8** and **11** were synthesized by the route shown in Scheme 1. The enantiomers of **3** were obtained by resolution of the

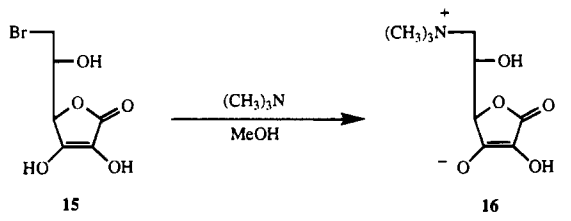
* Author to whom correspondence should be addressed.
[⊗] Abstract published in *Advance ACS Abstracts*, July 1, 1995.

0022-2623/95/1838-2880$09.00/0

Scheme 1



Scheme 2



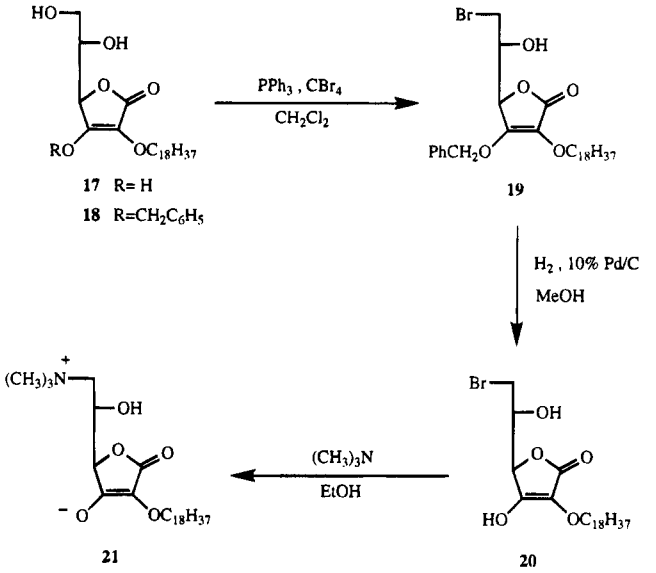
salts formed with (1*R*)-(−)- and (1*S*)-(+)-10-camphorsulfonic acid. X-ray crystallography of the pure diastereomeric salt of **3** with (1*S*)-(+)-10-camphorsulfonic acid showed **3** to have the 2*S* configuration. The same enantiomer of **3** was obtained from one of the enantiomers of **5** which was therefore assigned the *S* configuration. The enantiomers of **1** were obtained from the enantiomers of **3** by treatment with methyl *p*-toluenesulfonate in refluxing acetonitrile. Due to the nomenclature rules for assignment of absolute configuration, the 2*S* configuration of compounds **1**, **3**, **5**, **8**−**11**, and **13** corresponds to the 2*R* configuration of natural α-tocopherol.

The ascorbic acid analogue **16** was obtained from **15**[23] as shown in Scheme 2, and analogue **21** was obtained as shown in Scheme 3. Bromination of **18**[7] with $P(C_6H_5)_3$ and CBr_4 gave **19** in 75% yield, which was quantitatively debenzylated (H_2, 10% Pd/C) to **20**. Treatment of **20** with trimethylamine in EtOH afforded **21** in 31% yield. Both compounds are made from natural L-ascorbic acid and are therefore enantiomerically pure.

Biological Evaluation

The *in vitro* free radical scavenger activity was evaluated by measuring inhibition of lipid autoxidation in rat brain homogenate and measuring their relative reaction rate constants with superoxide radicals generated by xanthine oxidase/xanthine. Their competition with the detector molecule nitro blue tetrazolium was determined.

Scheme 3



To estimate their cardioselectivities and potential antioxidant effects *in vivo*, compounds were administered subcutaneously to mice. The hearts were excised 1 h postadministration, and inhibition of *ex vivo* lipid peroxidation was measured in the heart homogenates as compared to vehicle-treated controls. The method has been previously described for brain-directed analogues of α-tocopherol.[4] The ratio of *in vitro* IC_{50} to *ex vivo* ID_{50} served as an indicator of cardioselectivity. With compounds **1** and **11**, drug concentration was also measured directly by HPLC in the heart tissue.

Results and Discussion

In the present paper we report on the synthesis of the phosphonium analogues **8** and **9** and the sulfonium analogue **11**, as well as the two acyl hydrazinium compounds **13** and **14** and the two quarternary ammonium analogues **16** and **21** of ascorbic acid (vitamin C). While ammonium and phosphonium compounds have previously been reported to be cardioselective, i.e., to accumulate in heart tissue,[24−29] this property, to our knowledge, has never been reported for sulfonium compounds. The dimethylsulfonium analogues of dopamine, norepinephrine, chlorpromazine, and sulpiride have been synthesized but were only studied *in vitro* for their interactions with dopaminergic receptors.[30−33] Little is known about the pharmacokinetics of acylhydrazinium compounds, although their chemistry has been reviewed.[34] Ascorbic acid (vitamin C) also plays an important role in the physiological protection of cells and tissues against oxygen-derived free radicals.[35,36] It is capable of reducing the α-tocopheroxyl radical.[37] Although being a potent scavenger of superoxide radicals,[36] ascorbic acid did not inhibit lipid peroxidation. Kato et al. synthesized a series of 2-*O*-alkylascorbic acids and found the octadecyl ether **17** to be a potent inhibitor of lipid peroxidation,[7] a result confirmed in the present study (Table 1).

The compounds were evaluated *in vitro* for inhibition of spontaneous lipid peroxidation in rat brain homogenate (Table 1). The phosphonium derivatives **8** and **9** and the sulfonium derivative **11** were as effective as the ammonium derivative **1**, while the acylhydrazonium derivatives **13** and **14** were somewhat less active. The

Table 1. Inhibition of Lipid Autoxidation in Rat Brain Homogenate

compd	IC_{50}, μM^a (n)	compd	IC_{50}, μM^a (n)
1[b]	2.6 ± 0.8 (7)	**11**[e]	2, 3
S-(−)-**1**[c]	2.3 ± 0.2 (3)	*S*-(−)-**11**	2
R-(+)-**1**[d]	2.2 ± 0.2 (3)	*R*-(+)-**11**	2
3	0.7 ± 0.4 (4)	**12**[f]	14 ± 4 (4)
S-(−)-**3**	1	*S*-(−)-**12**	not determined
R-(+)-**3**	1	*R*-(+)-**12**	not determined
5	12	**13**	6, 8
S-(−)-**5**	17	*S*-(+)-**13**	9
R-(+)-**5**	14, 16	*R*-(−)-**13**	8
8	2	**14**	14
S-(−)-**8**	2	**16**	>200
R-(+)-**8**	2	**17**[g]	6
9	2	**21**	>20
10	3	*d,l*-α-tocopherol[h]	14 ± 4 (4)

[a] Concentration that inhibits formation of thiobarbituric acid-reactive substances (TBARS) by 50%, see the methods section. Single or duplicate determinations or means ± SD of the number of determinations in parentheses. [b] MDL 73,404. [c] MDL 74,405. [d] MDL 74,406. [e] MDL 73,473AQ. [f] Trolox. [g] CV-3611. [h] In the presence of sodium dodecyl sulfate as detergent.

Table 2. Relative Reaction Rate Constants with Superoxide Radicals

compd[a]	k, 10^4 M^{-1} s^{-1} [b] (n)
1	2.4 ± 0.2 (3)
S-(−)-**1**	2.1 ± 0.2 (3)
R-(+)-**1**	1.8 ± 0.2 (3)
3	2.7 ± 0.2 (3)
8	2.4 ± 0.1 (3)
9	2.2 ± 0.2 (3)
10	0.08 ± 0.03 (3)
11	2.1 ± 0.5 (3)
12	2, 4[c]
13	2.8 ± 0.3 (3)
16	60 ± 6 (3)
17	2.1 ± 0.2 (3)
21	not determined
ascorbic acid	35 ± 5

[a] For code numbers of some compounds, see Table 1. [b] Competition with nitro blue tetrazolium of superoxide radicals formed by xanthine oxidase/xanthine; see ref 9. Means ± SD (number of determinations in parentheses). [c] Duplicate determination.

ascorbic acid analogue **16** was inactive, as was found for the parent compound; however, the effect of the octadecyl ether derivative **21** was in the same order as **17**. No difference in activity was found among the 2*R* and 2*S* enantiomers of the α-tocopherol analogues. Superoxide radical ($O_2^{\cdot-}$)-scavenging activity (Table 2) was found to be similar for all α-tocopherol analogues, while the ascorbic acid analogue **16** reacted more than 20 times faster. As previously reported,[11] the superoxide-scavenging effect of the α-tocopherol analogues appears to involve covalent binding of the oxygen at the expense of hydrogen peroxide formation. Again, no difference was found between the 2*R* and 2*S* enantiomers of the α-tocopherol analogues. We have previously studied the rate of reaction of **1** with hydroxyl radicals and found it to be an effective scavenger also of that radical.[10]

The cardioselectivity of **2** was studied earlier using the ^{14}C-labeled compound.[12] The concentration of **1** was found to be 10−30 times higher in heart tissue than in blood and other tissues. More recently we have obtained similar data with ^{14}C-labeled **1** (J. Dow, et al., unpublished results). These studies also indicated that there is no advantage to the use of **2** as a prodrug of **1**. Using the *ex vivo* inhibition of lipid peroxidation in mouse heart homogenates, we now have an assay that allows to determine the cardioselectivity without ^{14}C-labeling (Table 3). With the cardioselective, permanently cationic α-tocopherol analogues **1**, **8**, **9**, and **11**, ratios of effective antioxidant concentration *in vitro* (IC_{50}) versus effective dose *in vivo* (ID_{50}) of greater than one were obtained. By contrast, **3** (studied earlier with ^{14}C-labeled **4**),[12] as well as **10**, in which the cationic charge is subject to a dynamic process at physiological pH, gave IC_{50}/ID_{50} ratios of below 0.2 (Table 3). The *ex vivo* effects of the enantiomers of the ammonium derivative **1** were not different, as shown in Figure 1. Furthermore, the *ex vivo* effect of the racemate of the sulfonium derivative **11** was undistinguishable from its enantiomers, producing 73 ± 5% inhibition 1 h after a sc dose of 20 μmol/kg (not shown). The HPLC determination of **1** and **11** revealed an up to 3-fold concentration above the dose in the heart tissue (Figure 2). The cardioselectivity of **11** appears to be slightly higher than that of **1**.

Table 3. *In Vitro and ex Vivo* Inhibition of Lipid Autoxidation in Mouse Heart Homogenate

compd	*in vitro*[a] IC_{50}, μM	*ex vivo*[b] ID_{50}, μmol/kg	IC_{50}/ID_{50}
1	19 ± 1 (3)	11	1.7
3	19, 20[c]	>150	>0.2
8	10 ± 4 (3)	8	1.3
9	10 ± 6 (3)	7	1.4
10	18, 20	>150	<0.2
11	7, 8	6	1.3

[a] Concentration that inhibits TBARS formation by 50%; see the methods section. Duplicate determinations or means ± SD of the number of determinations in parentheses. [b] Dose that inhibits TBARS formation by 50% 1 h after sc administration; see the methods section. [c] Duplicate determinations.

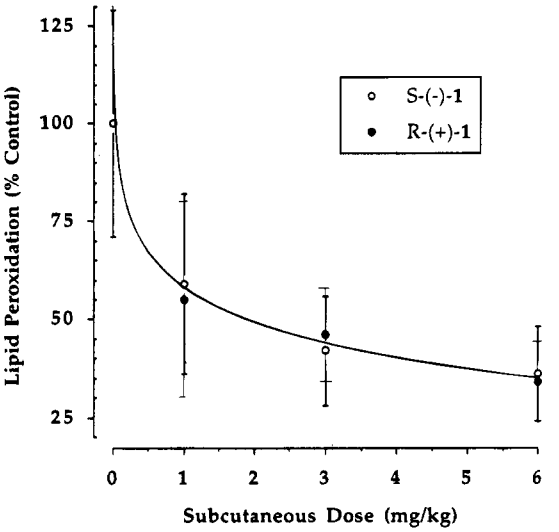


Figure 1. Dose-dependent inhibition of *ex vivo* lipid peroxidation in mouse heart homogenate 1 h after sc administration of *S*-(−)-**1** or *R*-(+)-**1**. See the methods section. Values represent means ± SD of five mice.

Therefore, it appears that the cardioselectivity is not related to the molecular geometry at the chiral center. An active transport mechanism, as demonstrated for L-carnitine,[38] would be unlikely. This is in accordance with the finding that coadministration of L-carnitine did not affect the *ex vivo* inhibition of lipid peroxidation in mouse hearts produced by **1** (F. Bolkenius, unpublished observation). It has been suggested that quaternary

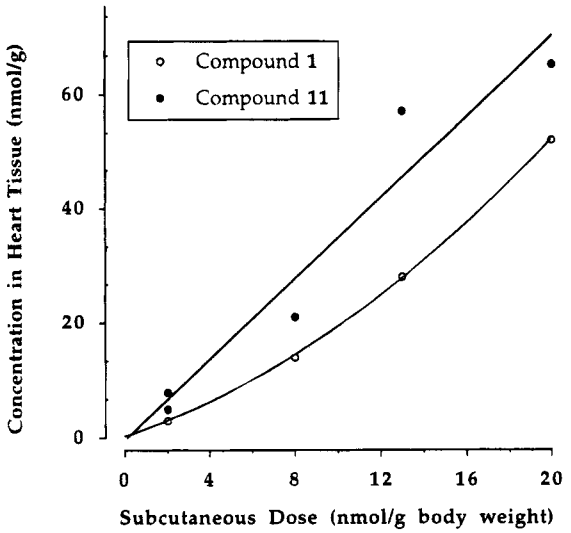


Figure 2. HPLC determination of compounds **1** and **11** in mouse hearts 1 h after sc administration. See the methods section. Values are from single animals.

ammonium compounds concentrate in myocytes due to the attractive forces of the transmembrane potential, the inner part of the cell membrane being charged with negative ions.[14] Another compound of this type, the more lipophilic tetraphenylphosphonium cation, was shown earlier[39] to mimic calcium uptake through the sodium–calcium exchange system in sarcolemmal membrane vesicles. The cardioselective, permanently cationic α-tocopherol analogues, presented here, may similarly act as substrates of this transporter and can thus accumulate in the myocytes. Our present findings may strengthen this view. The ascorbic acid analogues, unfortunately, did not inhibit lipid peroxidation sufficiently (see Table 1) to be evaluated by this assay.

Conclusions

The present study demonstrates that cardioselectivity may be a common feature of permanently cationic derivatives of α-tocopherol, irrespective of the heteroatom involved, and that the molecular geometry at the chiral center is probably not a determining factor for their radical-scavenging as well as cardioselective properties. The pharmacological value of the ammonium derivative as a cardioprotective agent has been demonstrated during the past. Such an effect is also expected for the present compounds which may enhance the choice for selected targets. This and the compartmentalized anti-inflammatory effect, e.g., as in colitis models, remain under further investigation.

Experimental Section

Melting points are uncorrected. Elemental analyses for the elements indicated were within ±0.4% of calculated values. ^{1}H NMR spectra were obtained at 360 MHz with an AM-360 Bruker spectrometer, IR spectra were recorded with a Bruker IFS 48 FTIR spectrometer, and UV spectra were recorded with a Beckman DU-7 spectrometer. Enantiomeric excess (ee) was determined on an Ultron ES-OVM column (150 × 4 mm i.d., 5 μm particle size) supplied by Liquid Chromatography Columns, Rockland Technologies, IL. The mobile phase consisted of 0.025 M phosphate buffer at pH 6.5, containing 9% acetonitrile (v/v) at a flow rate of 1 mL/min. UV detection was used at 210 nm.

[2-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethyl]trimethylphosphonium Bromide (8). A solution of **7**[8] (3.13 g, 10 mmol) in 25 mL of 2-butanone and 20 mL of 1 M trimethylphosphine in THF was stirred in a closed stainless steel vessel at 100 °C for 44 h. After cooling the bomb was opened, the content was transferred to a round-bottom flask with 2-butanone, and the solvents were evaporated. Crystallization and recrystallization of the residue from acetonitrile gave 3.01 g (77%) of **8**: mp 230–3 °C; ^{1}H NMR (CD_3OD) δ (ppm/TMS) 1.28 (3H, s, 2-C-CH_3), 1.72–1.76 (9H, 3s, P-CH_3), 1.90 (4H, m, 2-CH_2, β-CH_2), 2.10–2.15 (9H, 3s, Ar-CH_3), 2.25–2.50 (2H, m, α-CH_2), 2.68 (2H, t, 4-CH_2); UV (EtOH) λ_{max} 291 (ε = 3095), 220 (10 475), 204 (36 540) nm. Anal. C, H.

The 2*S* (−)-enantiomer of **8** was similarly prepared from 2*S*-(−)-**7**: mp 244–8 °C; ee = 99.9%; $[\alpha]^{25}_D$ = −12.33° (*c* = 0.94 in MeOH). Anal. C, H. The 2*R* (+)-enantiomer of **8** was prepared from 2*R*-(+)-**7**: mp 245–8 °C; ee = 99.3%; $[\alpha]^{25}_D$ = +12.75° (*c* = 1.15 in MeOH). Anal. C, H.

[2-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethyl]triethylphosphonium Bromide (9). Heating **7** and triethylphosphine in 2-butanone at 100 °C in a closed vessel for 44 h, as described for **8**, gave **9**: mp 187–8 °C. Anal. C, H.

3,4-Dihydro-2,5,7,8-tetramethyl-2-[2-(methylthio)ethyl]-2*H*-1-benzopyran-6-ol (10). A mixture of **7** (6.26 g, 20 mmol) and $NaSCH_3$ (2.8 g, 40 mmol) in 50 mL of 4 Å molecular sieve-dried DMF was stirred at 70 °C for 16 h. Water and 2 N HCl were added. The mixture was extracted with EtOAc (2×), washed with H_2O, $NaHCO_3$, and NaCl solution, dried (Na_2SO_4), and evaporated. The residue was slurried in hot hexane, decanted to separate a dark oil, concentrated, and allowed to cool in a refrigerator to give 3.95 g (70%) of **10**: mp 66.5–67 °C. Anal. C, H.

The 2*S* (−)-enantiomer of **10** was prepared from 2*S*-(−)-**7**: mp 63–5 °C; $[\alpha]^{25}_D$ = −11.8° (*c* = 0.92 in MeOH). Anal. C, H. The 2*R* (+)-enantiomer of **10** was prepared from 2*R*-(+)-**7**: mp 64.5–65 °C; $[\alpha]^{25}_D$ = +9.7° (*c* = 0.99 in MeOH). Anal. C, H.

[2-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)ethyl]dimethylsulfonium 4-Methylbenzenesulfonate (11). A solution of **10** (2.9 g, 10.3 mmol) and methyl *p*-toluenesulfonate (2.12 g, 11.4 mmol) in 30 mL of acetonitrile was refluxed for 24 h. The product crystallized on cooling and was recrystallized from acetonitrile to give 4.19 g (87%): mp 156–8 °C; ^{1}H NMR (DMSO-d_6) δ (ppm/TMS) 1.32 (3H, s, 2-C-CH_3), 1.90 (2H, m, 3-CH_2), 2.13–2.18 (11H, m, Ar-CH_3, β-CH_2), 2.38 (3H, s, tosyl-CH_3), 2.20 (2H, t, 4-CH_2) 2.95–3.00 (6H, 2s, S-CH_3), 3.4–3.6 (2H, m, α-CH_2), 7.20 (2H, m, ArH), 7.57 (2H, m, ArH); UV (EtOH) λ_{max} 290 (ε = 3310), 217 (22 155), 207 (31 815) nm. Anal. C, H.

The 2S (−)-enantiomer of **11** was prepared from 2*S*-(−)-**10**: mp 172–3 °C; $[\alpha]^{25}_D$ = −18.74° (*c* = 0.95 in MeOH); ee = 99.9%. Anal. C, H. The 2*R* (+)-enantiomer of **11** was prepared from 2*R*-(+)-**10**: mp 171–2 °C; $[\alpha]^{25}_D$ = +18.24° (*c* = 1.19 in MeOH); ee = 99.9%. Anal. C, H.

To test the stability of **11** in aqueous solution, a sample of **11** was dissolved in D_2O and the ^{1}H NMR spectrum was taken immediately and after 48 h of standing at room temperature. The two spectra were identical.

[2-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)acetyl]-1,1,1-trimethylhydrazinium, Inner Salt (13). To a solution of **5** (10.6 g, 40 mmol) and Et_3N (4.05 g, 40 mmol) in 50 mL of THF at 0 °C were added dropwise ethyl chloroformate (4.34 g, 40 mmol) and, after stirring at 0 °C for 1 h, unsymmetrical dimethylhydrazine (2.40 g, 40 mmol) in 30 mL of THF. The solution was stirred at room temperature overnight and evaporated, and the residue was taken up in EtOAc, washed with H_2O and $NaHCO_3$ solution, dried (Na_2SO_4), and evaporated. The residue was crystallized and recrystallized from EtOAc/heptane to give 7.30 g (60%) of unsymmetrical dimethylhydrazide of **5**: mp 151–2 °C. Anal. C,H,N.

A solution of the hydrazide (6.61 g, 21.6 mmol) and methyl *p*-toluenesulfonate (4.02 g, 21.6 mmol) in 75 mL of acetonitrile was refluxed for 3 h. After cooling overnight, the crystalline material was collected and recrystallized from acetonitrile: 7.8 g (73%); mp 182–3 °C. Anal. C,H,N.

To a hot solution of the tosylate (7.1 g, 14.5 mmol) in 25 mL of H_2O and 50 mL of EtOH was added 7.2 mL of 2 N NaOH. Most of the EtOH was evaporated, and the crystalline material was recrystallized from water, dried, and equilibrated in moist air to give 4.09 g (85%) of **13** containing 0.75 mol of H_2O: mp 219 °C dec; ^{1}H NMR (DMSO-d_6) δ (ppm/TMS) 1.45 (3H, s, 2-C-CH_3), 1.8–2.05 (2H, m, 3-CH_2), 2.08–2.12 (9H, 3s, $ArCH_3$), 2.0–2.2 (2H, m, α-CH_2), 2.48–2.80 (2H, m, 4-CH_2), 3.33 (9H, s, $^+$N-CH_3); UV (CH_3CN) λ_{max} 294 (ϵ = 3665), 225 (10 440), 202 (48 730) nm; IR (KBr) 1693, 1571 cm^{-1}. Anal. ($C_{18}H_{28}N_2O_3 \cdot 0.75H_2O$) C,H,N.

The 2*S* (+)-enantiomer of **13** was prepared from 2*S*-(−)-**5**: $[\alpha]^{25}_D$ = +2.30° (*c* = 1 in MeOH); ee = 99.4%. Anal. C,H,N (for salt containing 0.25H_2O). The 2*R* (−)-enantiomer of **13** was prepared from 2*R*-(+)-**5**: $[\alpha]^{25}_D$ = −3.07° (*c* = 1 in MeOH); ee = 99.6%. Anal. C,H,N.

[(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)carbonyl]-1,1,1-trimethylhydrazinium, Inner Salt (14). **14** was prepared as described for **13** from 3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-carboxylic acid (**12**): mp 187 °C dec. Anal. C,H,N.

Resolution of 3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-acetic Acid (5). To a hot solution of racemic **5**[22] (132.2 g, 0.5 mol) in 700 mL of *i*-PrOH were added (*S*)-(−)-α-methylbenzylamine (60.6 g, 0.5 mol) and 100 mL of EtOAc. Slow crystallization overnight in a refrigerator gave somewhat more than one-half the amount of crystalline material (checked by evaporating filtrate to dryness). This material was recrystallized in like manner three times. (To dissolve the material, it was necessary to add a small amount of water that was subsequently removed by azeotroping.) The resulting diastereomeric salt was added to 200 mL of 2 N HCl and 400 mL of ethyl acetate, and the mixture was shaken until all solid had dissolved. The aqueous phase was separated and extracted with EtOAc. The combined organic phase was washed with 2 N HCl, H_2O, and NaCl solution, dried (Na_2SO_4), and evaporated. The residue was recrystallized from EtOAc/heptane to give 40.85 g (62%) of *S*-(−)-**5**: mp 148–9 °C; $[\alpha]^{25}_D$ = −9.61° (*c* = 1.05 in MeOH); ee = 99.9%. Anal. C,H.

The combined filtrates were evaporated and converted to free acid as described to give 92.02 g of material. It was dissolved in 600 mL of *i*-PrOH, and (*R*)-(+)-α-methylbenzylamine (42.2 g, 0.35 mol) was added, as well as 200 mL of EtOAc. Two recrystallizations, conversion to free acid, and one final recrystallization gave 41.50 g (63%) of *R*-(+)-**5**: mp 148–9 °C; $[\alpha]^{25}_D$ = +9.70° (*c* = 1.02 in MeOH); ee = 99.9%. Anal. C,H. Workup of the remaining filtrates established a material balance of 97%. The resolving agents were not recovered.

(2*S*)-(−)- and (2*R*)-(+)-3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-ethanol (6). To a stirred solution of *S*-(−)-**5** (38.9 g, 0.147 mol) in 500 mL of THF was added 30 mL of 10 M $BH_3 \cdot Me_2S$ over 30 min, and the mixture was stirred at reflux temperature for 3 h. After cooling 120 mL of MeOH was added dropwise, and the resulting solution was evaporated to dryness. The residue was taken up in EtOAc, washed with 2 N HCl, H_2O, $NaHCO_3$, and NaCl solution, dried (Na_2SO_4), and evaporated. The residue was recrystallized from EtOAc/heptane to give 30.69 g (83%) of *S*-(−)-**6**: mp 153–6 °C; $[\alpha]^{25}_D$ = −6.44° (*c* = 0.90 in MeOH). Anal. C,H.

In like fashion, *R*-(+)-**5** gave *R*-(+)-**6**: mp 155–7 °C; $[\alpha]^{25}_D$ = +6.00° (*c* = 1.01 in MeOH). Anal. C,H.

(2*S*)-(−)- and (2*R*)-(+)-2-(2-Bromoethyl)-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyran-6-ol (7). To a solution of 2*S*-(−)-**6** (30.2 g, 0.12 mol) and CBr_4 (44 g, 0.13 mol) in 120 mL of DMF, cooled in ice, was added in portions over 30 min triphenylphosphine (33.2 g, 0.13 mol). The mixture was stirred at room temperature overnight. Water was added, the product was extracted with Et_2O (3×), and the extract was washed with H_2O and NaCl solution, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel, eluted with EtOAc/heptane, 1:3. The product-containing fractions were combined, evaporated, and recrystallized from EtOAc/heptane to give 28.2 g (75%) of 2*S*-**7**, which was used to prepare 2*S*-(−)-**8**, 2*S*-(−)-**10**, and 2*S*-(−)-**11**. The 2*R* enantiomer was likewise obtained from 2*R*-(+)-**6** and used to prepare 2*R*-(+)-**8**, 2*R*-(+)-**10**, and 2*R*-(+)-**11**.

Resolution of 3,4-Dihydro-2-[2-(dimethylamino)ethyl]-2,5,7,8-tetramethyl-2*H*-1-benzopyran-6-ol (3). To a hot, filtered solution of **3**[8] (50 g, 0.18 mol) in 200 mL of MeCN was added a hot solution of (1*S*)-10-camphorsulfonic acid (Aldrich) (41.8 g, 0.1 mol) in 200 mL of MeCN. The resulting solution was concentrated to 150 mL by distillation of the solvent and then allowed to cool with continued stirring. After several hours, the solid was collected and recrystallized twice from MeCN to give 21.7 g of the diastereomeric salt, mp 212–4 °C. The salt was dissolved in 60 mL of CH_2Cl_2 and the solution stirred vigorously for 30 min each with three successive 30 mL portions of saturated $NaHCO_3$ solution. The CH_2Cl_2 layer was separated, dried ($MgSO_4$), and concentrated to 30 mL, and after hexane was added, the remaining CH_2Cl_2 was distilled off. The resulting hot hexane solution was filtered to remove still unconverted diastereomeric salt. This was dissolved in H_2O, and solid precipitated on addition of $NaHCO_3$. The solid was dissolved in CH_2Cl_2 and added back to the hot hexane solution. Two more filtrations were required as the CH_2Cl_2 was distilled off to give a clear hot hexane solution, which was concentrated to 110 mL and allowed to cool. After standing in a refrigerator overnight, 10.53 g (40%) of 2*S*-(−)-**3** was obtained: mp 102–3 °C; $[\alpha]^{25}_D$ = −7.2° (*c* = 1 in MeOH). Anal. C,H,N.

Of this sample, 500 mg was converted to the HCl salt with ethereal HCl and recrystallized from MeCN/MeOH to give 544 mg of 2*S*-(−)-**3**·HCl: mp 290–1 °C; $[\alpha]^{25}_D$ = −6.4° (*c* = 1 in H_2O); ee > 98%. Anal. C,H,N.

The residues from the mother liquors were converted to free base with CH_2Cl_2 and $NaHCO_3$ as before. The crude material was recrystallized from hexane to give 25.8 g (92.9 mmol) in two crops. These were dissolved in 150 mL of hot MeCN, and a hot solution of (1*R*)-10-camphorsulfonic acid (21.6 g, 92.9 mmol) in 125 mL of MeCN was added. The solution was concentrated to 150 mL and allowed to cool to room temperature with stirring overnight. The solid was collected and recrystallized to give 20.6 g (45%) of the diastereomeric salt, mp 212–4 °C. This salt was dissolved in 300 mL of H_2O, and 200 mL of saturated $NaHCO_3$ solution was added. The solution turned cloudy, and a solid began to precipitate slowly. The pH of the solution was raised to about pH 9 by addition of 1 N NaOH until no further precipitation occurred. The solid was collected, washed with H_2O, and air-dried overnight. Recrystallization from hexane gave 9.85 g (39%) of 2*R*-(+)-**3**: mp 102–3 °C; $[\alpha]^{25}_D$ = +6.8° (*c* = 1 in MeOH). Anal. C,H,N.

Conversion of 600 mg to the HCl salt gave 641 mg of 2*R*-(+)-**3**·HCl: mp 290–1 °C; $[\alpha]^{25}_D$ = +5.6° (*c* = 1 in H_2O); ee = 99%. Anal. C,H,N.

X-ray analysis of the diastereomeric salt of **3** with (1*S*)-10-camphorsulfonic acid allowed the assignment of absolute configuration. The enantiomers of **3** were also prepared from the resolved acids **5**, which proved to be the more convenient route.

(2*S*)-(−)- and (2*R*)-(+)-3,4-Dihydro-6-hydroxy-*N,N,N*-2,5,7,8-heptamethyl-2*H*-1-benzopyran-2-ethanaminium 4-Methylbenzenesulfonate (1). To a solution of methyl tosylate (5.91 g, 32 mmol) in 50 mL of dry MeCN was added 2*S*-(−)-**3** (8.0 g, 29 mmol). The solid dissolved on heating, and the solution was refluxed for 1.5 h. The solvent was evaporated, and the viscous oil remaining was triturated with 100 mL of acetone. The resulting white solid was collected, washed with acetone, dried, and recrystallized from 40 mL of MeCN, to which ether was added to cloudiness, to give 11.8 g (88%) of 2*S*-(−)-**1**: mp 162–3 °C; $[\alpha]^{25}_D$ = −4.0° (*c* = 1 in 1:1 H_2O/MeOH); ee > 98%. Anal. C,H,N. The ^{1}H NMR spectrum corresponded to that of the racemate.[8]

The other enantiomer was similarly prepared from 2*R*-(+)-**3** to give 2*R*-(+)-**1**: mp 162–3 °C; $[\alpha]^{25}_D$ = +3.8° (*c* = 1 in 1:1 H_2O/MeOH); ee > 98%. Anal. C,H,N.

6-(Trimethylammoniumyl)-6-deoxy-L-ascorbic Acid (16). To an ice-cooled solution of **15**[23] (40.7 g, 170.3 mmol) in 150 mL of MeOH was added trimethylamine (50 mL). The flask was well stoppered, and the mixture was stirred for 5 days at

room temperature. The solid formed was collected by filtration, washed with MeOH, and recrystallized from MeOH/H_2O to give 13.5 g (36%) of colorless crystals: mp 180 °C dec; ^{1}H NMR (D_2O) δ (vs TMS) 3.25 (s, 9H), 3.65 (m, 2H), 4.44 (d, J = 2.15 Hz, 1H), 4.58 (dt, J = 8.3 Hz, 1H). Anal. ($C_9H_{15}NO_5 \cdot 0.5H_2O$) C,H,N.

2-*O*-Octadecyl-3-*O*-(phenylmethyl)-6-bromo-6-deoxy-L-ascorbic Acid (19). To a solution of **18**[7] (9.3 g, 17.9 mmol) and CBr_4 (6.23 g, 18.8 mmol) in 90 mL of dry CH_2Cl_2 was added portionwise at 0 °C and under N_2 4.93 g (18.8 mmol) of $P(C_6H_5)_3$. The mixture was stirred overnight at room temperature. The solvent was evaporated to dryness, and the residue was purified by flash chromatography eluting with hexane/EtOAc, 80:20, to give 7.77 g (75%) of **19** as a white solid: ^{1}H NMR ($CDCl_3$) δ (ppm/TMS) 0.88 (3H, t, J = 5.7 Hz), 1.25 (30H, s), 1.66 (2H, m), 3.54 (2H, d, J = 5.7 Hz), 4.05 (3H, m), 4.91 (1H, d, J = 2.8 Hz), 5.5 (2H, s), 7.4 (5H, s).

2-*O*-Octadecyl-6-bromo-6-deoxy-L-ascorbic Acid (20). A solution of **19** (4.3 g, 7.4 mmol) in 75 mL of EtOH was hydrogenated with 10% Pd/C (430 mg) until the theoretical volume of H_2 was absorbed. The catalyst was removed by filtration, and the solvent was evaporated to dryness to give 3.56 g (98%) of **20** as a white solid: ^{1}H NMR (CD_3OD) δ (ppm/TMS) 0.90 (3H, t, J = 5.7 Hz), 1.30 (30H, s), 1.68 (2H, m), 3.55 (2H, m), 3.96 (2H, t, J = 7.1 Hz), 4.08 (1H, dt, J = 7, 2.8 Hz), 4.95 (1H, d, J = 2.8 Hz).

2-*O*-Octadecyl-6-(trimethylammoniumyl)-6-deoxy-L-ascorbic Acid (21). The 6-bromo derivative **20** (2 g, 4 mmol) was treated with trimethylamine as described for compound **16**. After filtration, the residue was purified by flash chromatography eluting with MeOH/CH_2Cl_2, 50:50, to give after recrystallization from EtOH 0.6 g (31%) of **21** as a white powder: mp 226 °C dec; ^{1}H NMR (CD_3OD) δ (ppm/TMS) 0.90 (3H, t, J = 5.7 Hz), 1.30 (30H, s), 1.68 (2H, m), 3.23 (9H, s), 3.62 (2H, m), 3.82 (2H, t, J = 7.1 Hz), 4.19 (1H, d, J = 2.8 Hz), 4.41 (1H, bd). Anal. ($C_{27}H_{51}NO_5 \cdot 0.25H_2O$) C,H,N.

Biological Methods. Lipid Peroxidation in Rat Brain Homogenate. This was done as previously described.[10,40]

Superoxide Radical Scavenging. This was determined by measuring competition with nitroblue tetrazolium of the superoxide radicals formed by xanthine oxidase/xanthine, as described earlier.[10]

***Ex Vivo* Lipid Peroxidation.** The procedure followed the one previously described in detail.[4] In principle, test compounds were administered subcutaneously to groups of five CD1 mice. One hour later, the hearts were excised. The frozen tissues were homogenized, diluted with potassium phosphate buffer, and incubated at 37 °C for 2 h. The reaction was stopped on ice by addition of $HClO_4$ and the mixture centrifuged. To the supernatant was added thiobarbituric acid, and the samples were heated to 100 °C for 15 min. The chromophore (TBARS) was extracted into *n*-butanol, and the fluorescence was measured at 515 nm (excitation wavelength) and 553 nm (emission wavelength). Malondialdehyde dimethylacetal served as external standard. The dose necessary to inhibit TBARS formation by 50% (ID_{50}) was calculated from semilogarithmic plots (log of sc dose versus percent of control) of at least four different doses.

The *in vitro* lipid peroxidation with mouse heart homogenates followed the same protocol, apart from adding the test compounds at different concentrations prior to incubation.

HPLC Determination of 1 and 11 in Mouse Heart Homogenate. This was done as described before.[4]

Acknowledgment. We thank Dr. J. C. Huffman, Indianapolis, IN, for the single-crystal X-ray determination, C. Cohet, G. Ruba, A. Alak, H. Olivan, E. Wolf, and B. Heintzelman for technical assistance, and C. Froehly for the preparation of the manuscript. We acknowledge with appreciation the advice and encouragement of Drs. M. Petty, J. Dow, J. Wagner, and W. De Jong.

References

(1) Halliwell, B. Drug Antioxidant Effects. A Basis for Drug Selection? *Drugs* **1991**, *42*, 569–605.
(2) Moser, U.; Bendich, A. Vitamin C. In *Handbook of Vitamins*, 2nd ed.; Machlin, L. J., Ed.; Marcel Dekker, Inc.: New York, 1991; pp 195–232.
(3) Machlin, L. J. Vitamin E. In *Handbook of Vitamins*, 2nd ed.; Machlin, L. J., Ed.; Marcel Dekker, Inc.: New York, 1991; pp 99–144.
(4) Grisar, J. M.; Bolkenius, F. N.; Petty, M. A.; Verne, J. 2,3-Dihydro-1-benzofuran-5-ols as Analogues of α-Tocopherol That Inhibit In Vitro and Ex Vivo Lipid Autoxidation and Protect Mice against Central Nervous System Trauma. *J. Med. Chem.* **1995**, *38*, 453–458.
(5) Van Acker, S. A. B. E.; Koymans, L. M. H.; Bast, A. Molecular Pharmacology of Vitamin E: Structural Aspects of Antioxidant Activity. *Free Radical Biol. Med.* **1993**, *15*, 311–328.
(6) Mickle, D. A. G.; Weisel, R. D. Future Directions of Vitamin E and its Analogues in Minimizing Myocardial Ischemia-Reperfusion Injury. *Can. J. Cardiol.* **1993**, *9*, 89–93.
(7) Kato, K.; Terao, S.; Shimamoto, N.; Hirata, M. Studies on Scavengers of Active Oxygen Species. 1. Synthesis and Biological Activity of 2-O-Alkylascorbic Acids. *J. Med. Chem.* **1988**, *31*, 793–798.
(8) Grisar, J. M.; Petty, M. A.; Bolkenius, F. N.; Dow, J.; Wagner, J.; Wagner, E. R.; Haegele, K. D.; De Jong, W. A Cardioselective, Hydrophilic N,N,N-Trimethylethanaminium α-Tocopherol Analogue That Reduces Myocardial Infarct Size. *J. Med. Chem.* **1991**, *34*, 257–260.
(9) Petty, M. A.; Grisar, J. M.; De Jong, W. Protective Effects of an α-Tocopherol Analogue against Myocardial Reperfusion Injury in Rats. *Eur. J. Pharmacol.* **1992**, *210*, 85–90.
(10) Bolkenius, F. N.; Grisar, J. M.; De Jong, W. A. Water-soluble Quaternary Ammonium Analog of α-Tocopherol, that Scavenges Lipoperoxyl, Superoxide and Hydroxyl Radicals. *Free Radical Res. Commun.* **1991**, *14*, 363–372.
(11) Bolkenius, F. N. Leukocyte-mediated Inactivation of α_1-Proteinase Inhibitor is Inhibited by Amino Analogues of α-Tocopherol. *Biochim. Biophys. Acta* **1991**, *1095*, 23–29.
(12) Dow, J.; Petty, M. A.; Grisar, J. M.; Wagner, E. R.; Haegele, K. D . Cardioselectivity of α-Tocopherol Analogues, with Free Radical Scavenger Activity, in the Rat. *Drug Metab. Dispos.* **1991**, *19*, 1040–1045.
(13) Petty, M. A.; Grisar, J. M.; Dow, J.; De Jong, W. Effects of an α-Tocopherol Analogue on Myocardial Ischaemia and Reperfusion Injury in Rats. *Eur. J. Pharmacol.* **1990**, *179*, 241–242.
(14) Petty, M. A.; Dow, J.; Grisar, J. M.; De Jong, W. Effect of a Cardioselective α-Tocopherol Analogue on Reperfusion Injury in Rats Induced by Myocardial Ischaemia. *Eur. J. Pharmacol.* **1991**, *192*, 383–388.
(15) Yamada, T.; Grisham, M. B. Role of Neutrophil-Derived Oxidants in the Pathogenesis of Intestinal Inflammation. *Klin. Wochenschr.* **1991**, *69*, 988–994.
(16) Shiratora, Y.; Aoki, S.; Takada, H.; Kirtyama, H.; Ohto, K.; Hai, K.; Teroaka, H.; Matano, S.; Matsumoto, K.; Kamii, K. Oxygen-Derived Free Radical Generating Capacity of Polymorphonuclear Cells in Patients with Ulcerative Colitis. *Digestion* **1989**, *44*, 163–171.
(17) Salim, A. S. Role of Oxygen-Derived Free Radical Scavengers in the Managaement of Recurrent Attacks of Ulcerative Colitis: A New Approach. *J. Lab. Clin. Med.* **1992**, *119*, 710–717.
(18) Bolkenius, F. N.; Dow, J.; Grisar, J. M.; De Jong, W. MDL 73,-404, a Free Radical Scavenger, Active in a Model of Ulcerative Colitis, is not Orally Absorbed. Presented at the IV. Intern. Symp. Chron. entzündl. Darmerkr., Strasbourg, 1993; Abstract 14.
(19) Murthy, S. N.; Fondacaro, J. D.; Murthy, N. S.; Bolkenius, F.; Cooper, H. S. Effects of MDL 73,404 (MDL) in Experimental Murine Colitis. *Gastroenterology* **1993**, *104*, A752.
(20) Murthy, S. N. S.; Fondacaro, J. D.; Murthy, S. N. S.; Cooper, H. S.; Bolkenius, F. N. Beneficial Effect of MDL 73,404 in Dextran Sulfate (DSS)-mediated Murine Colitis. *Agents Actions* **1994**, *41*, C233–C234.
(21) Sakai, T.; Qi, Q.-H.; Murthy, S. N. S.; Grisar, J. M.; Fondacaro, J. D. Ischemia/reperfusion in the Rat Colon. A Discrete Surgical Model for Biochemical and Pharmacological Studies. *Gastroenterology* **1993**, *104*, A715.
(22) Scott, J. W.; Cort, W. M.; Harley, H.; Parrish, D. R.; Saucy, G. 6-Hydroxychroman-2-carboxylic Acids; Novel Antioxidants. *J. Am. Oil Chem. Soc.* **1974**, *51*, 200–203.
(23) Bock, K.; Lundt, I.; Pedersen, C. Preparation of Some Bromodeoxyaldonic Acids. *Carbohydr. Res.* **1979**, *68*, 313–319.
(24) Namm, D. H.; Wang, C. M.; El-Sayad, S.; Copp, F. C.; Maxwell, R. A. Effects of Bretylium on Rat Cardiac Muscle: the Electrophysiological Effects and Its Uptake and Binding in Normal and Immunosympathectomized Rat Hearts. *J. Pharmacol. Exp. Ther.* **1975**, *193*, 194–208.

(25) Patterson, E.; Stetson, P.; Lucchesi, B. R. Plasma and Myocardial Tissue Concentrations of UM-272 (N,N-Dimethylpropranolol) after Oral Administration in Dogs. *J. Pharmacol. Exp. Ther.* **1980**, *214*, 449–453.
(26) Lindstrøm, T. D.; Murphy, P. J.; Smallwood, J. K.; Wiest, S. A.; Steinberg, M. I. Correlation between the Disposition of [^{14}C]-Clofilium and Its Cardiac Electrophysiological Effect. *J. Pharmacol. Exp. Ther.* **1982**, *221*, 584–589.
(27) Srivastava, P. C.; Knapp, F. F., Jr. (E)-1-[^{123}I]iodo-1-penten-5-yl]triphenylphosphonium Iodide: Convenient Preparation of a Potentially Useful Myocardial Perfusion Agent. *J. Med. Chem.* **1984**, *27*, 978–981.
(28) Srivastava, P. C.; Hay, H. G.; Knapp, F. F., Jr. Effects of Alkyl and Aryl Substitution on the Myocardial Specificity of Radioiodinated Phosphonium, Arsonium, and Ammonium Cations. *J. Med. Chem.* **1985**, *28*, 901–904.
(29) Srivastava, P. C.; Knapp, F. F., Jr.; Callahan, A. P.; Goldstein, B. M. Myocardial Imaging Agents: Synthesis, Characterization and Evaluation of [(Z) and (Z,E)-(1-[^{82}Br]bromo-1-penten-5-yl)]-triphenylphosphonium Cations. *J. Labelled Compd. Radiopharm.* **1990**, *28*, 1161–1169.
(30) Andersen, K. K.; Kuruvilla, A.; Uretsky, N.; Miller, D. D. Synthesis and Pharmacological Evaluation of Sulfonium Analogues of Dopamine: Non Classical Dopamine Agonists. *J. Med. Chem.* **1981**, *24*, 683–687.
(31) Chang, Y.-A.; Ares, J.; Andersen, K.; Sabol, B.; Wallace, R. A.; Farooqui, T.; Uretsky, N.; Miller, D. D. Dopamine Agonists: Effects of Charged and Uncharged Analogues of Dopamine. *J. Med. Chem.* **1987**, *30*, 214–218.
(32) Harrold, M. W.; Chang, Y.-A.; Wallace, R. A.; Farooqui, T.; Wallace, L. J.; Uretsky, N.; Miller, D. D. Charged Analogues of Chlorpromazine as Dopamine Antagonists. *J. Med. Chem.* **1987**, *30*, 1631–1635.
(33) Harrold, M. W.; Wallace, R. A.; Farooqui, T.; Wallace, L. J.; Uretsky, N.; Miller, D. D. Synthesis and D2 Dopaminergic Activity of Pyrrolidinium, Tetrahydrothiophenium, and Tetrahydrothiophene Analogues of Sulpiride. *J. Med. Chem.* **1989**, *32*, 874–880.
(34) McKillip, W. J.; Sedor, E. A.; Culbertson, B. M.; Wawzonek, S. The Chemistry of Aminimides. *Chem. Rev.* **1973**, *73*, 255–281.
(35) *Ascorbic Acid: Chemistry, Metabolism, and Uses.* Seib, P. A., Tolbert, B. M., Eds.; Advances in Chemistry Series 200; American Chemical Society: Washington, DC, 1982.
(36) Halliwell, B.; Gutteridge, M. C. The Antioxidants of Human Extracellular Fluids. *Arch. Biochem. Biophys.* **1990**, *280*, 1–8.
(37) Doba, T.; Burton, G. W.; Ingold, K. U. Antioxidant and Co-Antioxidant Activity of Vitamin C. The Effect of Vitamin C, either Alone or in the Presence of Vitamin E or a Water-Soluble Vitamin E Analogue, upon the Peroxidation of Aqueous Multilamellar Phospholipid Liposomes. *Biochim. Biophys. Acta* **1985**, *835*, 298–303.
(38) Molstad, P.; Böhmer, T.; Eiklid, K. Specificity and Characteristics of the Carnitine Transport in Human Heart Cells (CCL 27) in Culture. *Biochim. Biophys. Acta* **1977**, *471*, 296–304.
(39) Reeves, J. P.; Sutko, J. L. Sodium-Calcium Exchange Activity Generates a Current in Cardiac Membrane Vesicles. *Science* **1980**, *208*, 1461–1464.
(40) Stocks, J.; Gutteridge, J. M. C.; Sharp, R. J.; Dormandy, T. L. Inhibition of Lipid Auto-oxidation by Human Serum and its Relation to Serum Proteins and α-Tocopherol. *Clin. Sci. Mol. Med.* **1974**, *47*, 223–233.

JM9501893