A Cardioselective, Hydrophilic N,N,N-Trimethylethanaminium α-Tocopherol Analogue That Reduces Myocardial Infarct Size

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The α-tocopherol analogue 3,4-dihydro-6-hydroxy-*N,N,N*,2,5,7,8-heptamethyl-2*H*-1-benzopyran-2-ethanaminium 4-methylbenzenesulfonate (**1a**, MDL 73404) and its *O*-acetate **1b** (MDL 74270) were synthesized. Compound **1a** was found to be hydrophilic (log $P = -0.60$) and to prevent lipid autoxidation in rat brain homogenate with an IC_{50} of 1.7 ± 0.9 μM. Tissue distribution studies with [^{14}C]-**1b** in rats (1 mg/kg iv) showed that radioactivity accumulates in the heart (ratio 20:1 vs blood after 1 h). Infusion of 1 mg/kg per h of **1b** bromide reduced infarct size by 54% in rats subjected to coronary artery occlusion for 60 min followed by reperfusion for 30 min, compared to saline-infused controls. By comparison, the tertiary amine analogue **5** was found not to accumulate in heart tissue, to be an equally effective free-radical scavenger in vitro, but to require a higher dose to reduce infarct size in rats. This shows that the cardioselectivity of compound **1** contributes to its potency in salvaging myocardial tissue in rats after ischemia and reperfusion.

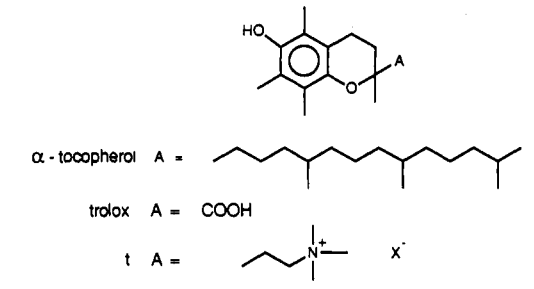
When the blood supply to parts of the heart is blocked, a myocardial infarct (heart attack) results and the deprived muscle tissue dies, resulting in permanent heart damage. If the blood supply can be reestablished within hours after infarction, the heart tissue remains viable and permanent damage can be reduced. This can be accomplished by surgical as well as pharmacologic (thrombolysis) procedures that are now widely and successfully applied.[1] However, reperfusion also poses problems. Oxygen-deprived (ischemic) tissue is in an abnormal state and is vulnerable to damage when suddenly exposed to oxygen-rich blood. This has been termed the "oxygen paradox" and leads to reperfusion damage. It has been postulated that this damage is due to oxygen-derived free radicals (for reviews see refs 2-4).

Oxygen-derived free radicals are highly reactive, short-lived species[5] that under normal conditions are scavenged by endogenous defense mechanisms[6] such as α-tocopherol (vitamin E), a natural antioxidant.[7] α-Tocopherol acts mainly by being incorporated into cell membranes and thereby protects against free-radical attack in the lipid phase. It can be regenerated by antioxidants, e.g. ascorbic acid in the aqueous phase.[7] Under conditions of oxidative stress myocardial concentrations of α-tocopherol are reduced,[8] and pretreatment with α-tocopherol reduces membrane-related alterations resulting from ischemia and reperfusion.[9] A water-soluble α-tocopherol analogue, trolox (3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-carboxylic acid), has been shown to reduce infarct size in dogs subjected to myocardial ischemia followed by reperfusion.[10] A number of other free-radical scavengers have been shown to be effective in this model,[3] including the enzyme superoxide dismutase (SOD). It has been noted in studies with SOD that the myocardial site of action, as well as the duration of free-radical scavenger presence, is essential for its effectiveness.[4,11]

Thus, our aim was to design a compound which would retain the α-tocopherol moiety responsible for its antioxidant properties while replacing the 2-position lipophilic moiety with a trimethylethanaminium chain to impart hydrophilicity and, perhaps, greater affinity for cardiac tissue (Chart I).

Compound **1**[12] was found to have these properties and was evaluated in rats subjected to coronary artery ligation

Chart I



Scheme I

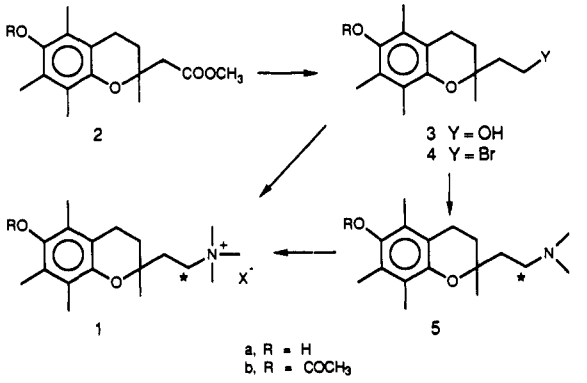


Table I. Partition Coefficients

compd	log p^a	compd	log p^a
1a[b]	-0.60 ± 0.02 ($n = 2$)	**5b**	1.56 ± 0.02 ($n = 4$)
1b[b]	-0.18 ± 0.01 ($n = 4$)	trolox	-1.02 ± 0.01 ($n = 4$)
5a	1.15 ± 0.01 ($n = 4$)		

[a] Octanol and 0.15 M NaCl solution in 0.1 M phosphate buffer, pH 7.4 (see the Experimental Section). [b] Tosylate salt.

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(12) Compound **1a** has the code number MDL 73404 and **1b** is MDL 74270.

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Table II. Prevention of Lipid Autoxidation in Rat Brain Homogenate

compd	IC_{50},[a] μM	compd	IC_{50},[a] μM
1a	1.7 ± 0.9 (5)	trolox	12 ± 1 (3)
5a	0.5 ± 0.3 (3)	d,l-α-tocopherol	12 ± 3 (3)[b]

[a] Mean values ± SD, number of individual determinations in parentheses. [b] In presence of 0.5 mM sodium dodecylsulfate.

followed by reperfusion in comparison with the nonquaternary (tertiary) amine analogue 5.[13]

Chemistry

Compound 1 was synthesized from the 3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-acetic acid ester 2[14] as shown in Scheme I. Reduction with lithium aluminum hydride followed by treatment of the resulting alcohol 3 with bromotriphenylphosphonium bromide gave 4a, which was converted either directly to the quaternary ammonium compound 1a as the bromide salt or via the tertiary amine 5a and treatment with methyl 4-methylbenzenesulfonate to give 1a as its tosylate salt. The *O*-acetates were obtained likewise via bromide 4b.

Results and Discussion

Partition Coefficients. To determine hydrophilicity of compounds 1, 5, and trolox, partition coefficients[15] between *n*-octanol and isotonic phosphate buffer (pH = 7.4) were determined and are shown in Table I. For the quaternary ammonium compounds 1a and 1b it was found that the counterion affected the log *P* values without addition of NaCl; this effect was diminished by determining the partition coefficients in isotonic solution[16] (see the Experimental Section). It can be seen from Table I that the quaternary ammonium compound 1a is nearly as hydrophilic as trolox while the tertiary amine 5a is more lipophilic at pH 7.4.

Prevention of Lipid Peroxidation. Prevention of spontaneous lipid peroxidation in rat brain homogenate is shown in Table II. Compounds 1a and 5a scavenged lipoperoxy radicals with comparable potency and more strongly than trolox and α-tocopherol, solubilized by addition of 0.5 mM sodium dodecylsulfate. The inhibition of lipid autoxidation does not correlate with the lipophilicity of the inhibitors, although it should be noted that the addition of a detergent may diminish the efficacy of α-tocopherol.

Tissue Distribution. ^{14}C side-chain labeled 1b and 5b were synthesized (position of label indicated by * in Scheme I). One hour after iv administration of 1 mg/kg of base resulted in the radioactivity distribution shown in Table III. Compound 1b resulted in heart tissue accumulation of radioactivity compared to skeletal muscle or blood, while 5b did not. Blood concentrations of the two compounds were similar, which shows that the high ratio is not due to low blood levels but to high concentration in heart tissue.[17] Bretylium and dimethylpropranolol have been reported to also accumulate in heart tissue.[18,19] This property thus appears to be quite general for quaternary ammonium compounds, but the underlying mechanism is poorly understood.

Table III. Comparison of Total Radioactivity in Heart, Skeletal Muscle, and Blood and Tissue/Blood Ratios 1 h after Iv Administration of 1 mg/kg of [^{14}C]-1b and 1 mg/kg of [^{14}C]-5b in Rats

compd	tissue	total radioactivity[a]	ratio tissue/blood
1b	heart	2.28 ± 0.24	20.7
	skeletal muscle	0.19 ± 0.04	1.7
	blood	0.11 ± 0.01	1
5b	heart	0.28 ± 0.03	1.6
	skeletal muscle	0.27 ± 0.07	1.5
	blood	0.18 ± 0.03	1

[a] Mean values ± SD, $n = 3$; expressed as μg equiv/g of tissue or mL of blood.

Myocardial Infarct/Reperfusion Studies in Rats. Infusion with 1b bromide commencing 10 min before coronary artery ligation (60 min) until the end of reperfusion (30 min) significantly reduced infarct size at 1 mg/kg per h. The percentage of the area at risk infarcted was 70.8 ± 25.1% and 31.5 ± 26.5% ($p < 0.025$; $x \pm SD$, $n = 6$) after saline and compound 1b, respectively. A 10-fold higher concentration was required to produce a significant and equivalent reduction in infarct size with compound 5b. The percentage of the area at risk infarcted was 78.9 ± 26.2% and 38 ± 32.9% ($p < 0.02$, $n = 7$) after saline and compound 5b, respectively. Thus, the cardioselectivity of 1b accounts for its greater potency compared to that of 5b. A preliminary account of these studies has been published,[13] and a complete description will follow.

Conclusions

An analogue of α-tocopherol in which the lipophilic side chain that is responsible for its intercalation into lipid layers[7] is replaced by a trimethylethanaminium chain (compound 1) was found to accumulate in heart tissue, to prevent lipid peroxidation in vitro, and to reduce myocardial infarct size in rats subjected to coronary ligation followed by reperfusion. It is reasonable to conclude that the coronary protection provided by compound 1 is due to its free-radical-scavenging properties. This conclusion is strengthened by the comparison with compound 5, which also reduces infarct size but only at the 10-fold dose (corresponding to its lower heart tissue concentration), both compounds being equally effective free-radical scavengers in vitro. The cardioselectivity of compound 1 provides high concentrations of scavenger in the heart capable of preventing oxygen-derived free-radical-induced tissue damage from occurring during reperfusion. Compound 1 has potential for clinical use during acute reperfusion of the myocardium.

Experimental Section

Melting points are uncorrected. Elemental analyses for the elements indicated were within ±0.4% of calculated values. ^1H NMR spectra were obtained at 360 MHz, ^{13}C NMR were obtained at 90.5 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.

3,4-Dihydro-2-[2-(dimethylamino)ethyl]-2,5,7,8-tetramethyl-2*H*-1-benzopyran-6-ol (5a). To 55.08 g (0.21 mol) of triphenylphosphine in 400 mL of dichloromethane was added dropwise 19.6 mL (32.76 g, 0.205 mol) of bromine. The resulting slurry was stirred for 30 min and a suspension of 50 g (0.2 mol)

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of **3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-ethanol**[20] in 100 mL of dichloromethane was added and the mixture was stirred at reflux temperature for 5 h. The solution was allowed to cool, was washed with 2 × 250 mL of 2 N Na_2CO_3 and saturated NaCl solution, and was dried (Na_2SO_4). The oil obtained after evaporation of the solvent (129 g) was dissolved in 50 mL of methanol and allowed to cool in a refrigerator overnight. The resulting crystals were recrystallized from methanol to give 34–43 g (55–70%) of **2-(2-bromoethyl)-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyran-6-ol (4a)**, mp 120 °C.

To a solution of 12.53 g (0.04 mol) of **4a** in 80 mL of DMF was added a solution of ca. 10 g of dimethylamine gas in 40 mL of DMF (volume increase to 50 mL). The flask was stoppered and the mixture was stirred at room temperature overnight. To the flask was added 400 mL of water and 100 mL of saturated $NaHCO_3$ solution, the mixture was extracted with ether (2×), and the extract was washed with water and NaCl solution, dried (Na_2SO_4) and evaporated. The residue (13.24 g) was dissolved in 50 mL of 2-propanol and 3.5 mL of concentrated HCl was added (to pH < 3). The resulting salt was collected and recrystallized twice from 2-propanol/water to give, after drying at 80 °C at 0.1 mm, 11.30 g (90%) of **5a**, mp 295–297 °C. Anal. C, H, N.

3,4-Dihydro-2-[2-(dimethylamino)ethyl]-2,5,7,8-tetramethyl-2*H*-1-benzopyran-6-ol Acetate Hydrochloride (5b). To a solution of 39.16 g (0.125 mol) of **4a** in 200 mL of 2,6-lutidine was added 75 mL of acetic anhydride. The solution was stirred at room temperature overnight, ice/water was added, and the warm (30 °C) solution was stirred for 1 h. The precipitate formed on addition of more ice/water was collected, washed with H_2O, and dried over P_2O_5 at 80 °C under high vacuum. Recrystallization from ethyl acetate/hexane gave 37–40 g (85–90%) of **4b**, mp 102–103 °C. Anal. C, H.

From this bromide (20.36 g), **5b** was obtained by the procedure described above (16.93 g, 83%): mp 263–270 °C; ^{1}H NMR (DMSO-d_6) δ (ppm/TMS) 1.76 (1 H, t, J = 6.7 Hz), 1.90 (3 H, s), 1.92 (3 H, s), 1.97 (1 H, t, J = 8.5), 2.02 (3 H, s), 2.29 (3 H, s), 2.59 (1 H, m), 2.73 (6 H, s), 3.17 (1 H, m), 10.31 (1 H, s); UV (H_2O) λ_{max} 281 nm (ε = 1578), 276 (1478). Anal. C, H, N.

3,4-Dihydro-6-hydroxy-*N*,*N*,*N*,2,5,7,8-heptamethyl-2*H*-1-benzopyran-2-ethanaminium 4-Methylbenzenesulfonate (1a). A solution of 6.30 g (0.0227 mol) of **5a** (free base) and 4.37 g (0.032 mol) of methyl *p*-toluenesulfonate in 60 mL of acetonitrile was refluxed for 4 h. The salt crystallized on cooling and was recrystallized from acetonitrile to give 5.20 g (50%) of **1a**: mp 180 °C dec; ^{1}H NMR (DMSO-d_6) δ (ppm/TMS) 1.20 (3 H, s), 1.76 (1 H, t, J = 6.8 Hz), 2.00 (1 H, m), 2.01 (3 H, s), 2.03 (3 H, s), 2.06 (3 H, s), 2.29 (3 H, s), 2.55 (1 H, m, J = 6.8), 3.06 (9 H, s), 3.48 (1 H, m), 7.11 (2 H, d, J = 7.5), 7.44 (1 H, s), 7.49 (2 H, d, J = 7.5); ^{13}C NMR (DMSO-d_6) δ (ppm/TMS) 11.8, 12.7, 12.9, 19.8, 20.7, 30.9, 31.7, 52.0, 61.3, 72.8, 116.5, 120.3, 121.0, 122.7, 125.4, 128.0, 137.5, 143.7, 145.5, 145.7, UV (H_2O) λ_{max} 287 nm (ε = 2714), 219 (21 910). Anal. C, H. N.

The bromide salt was prepared by reaction of 2.90 g of **4a** with ca. 6.7 g of trimethylamine in 80 mL of 2-butanone at 120–125 °C for 60 h in a closed, stainless steel vessel. The residue, after evaporation and one recrystallization from ethanol, gave 1.99 g (58%) of the bromide salt, mp 249 °C dec. Anal. C, H, N.

The chloride salt was prepared by treating 5.06 g of **1b**-tosylate with ca. 5 mL of strongly basic resin (AG-1-X2) in 200 mL of water overnight, passing the slurry through a column packed with the same resin, and eluting with water. The eluate was evaporated, 1 equiv of 2 N HCl was added, and the mixture was again evaporated. Two recrystallizations from ethanol/ethyl acetate and from acetonitrile, respectively, gave 0.9 g (27%) of the chloride salt, mp 260–265 °C dec. Anal. C, H, N.

6-(Acetyloxy)-3,4-dihydro-*N*,*N*,*N*,2,5,7,8-heptamethyl-2*H*-1-benzopyran-2-ethanaminium, 4-Methylbenzenesulfonate (1b). A solution of 25.47 g (0.0797 mol) of **5b** (free base) and 16.34 g (0.0877 mol) of methyl *p*-toluenesulfonate in 400 mL of acetonitrile was refluxed for 4 h. The solvent was evaporated and the residue was crystallized and recrystallized from ethyl acetate to give 34.7 g (86%) of the title compound: mp 106–108 °C; ^{1}H NMR (DMSO-d_6) δ (ppm/TMS) 1.21 (3 H, s), 1.81 (1 H, m), 1.91 (3 H, s), 1.93 (3 H, s), 2.0 (1 H, m), 2.03 (3 H, s), 2.25 (3 H, s), 2.27 (3 H, s), 2.61 (1 H, m), 3.07 (9 H, s), 7.07 (2 H, d, J = 7.5 Hz), 7.44 (2 H, d, J = 7.5 Hz); ^{13}C NMR (DMSO-d_6) δ (ppm/TSP) 11.7, 11.9, 12.7, 19.5, 20.2, 20.7, 23.0, 30.3, 32.0, 52.0, 61.1, 73.7, 117.1, 122.0, 125.0, 125.5, 126.5, 128.0, 137.0, 140.8, 145.8, 147.8, 169.1; UV (H_2O) λ_{max} 281 nm (ε = 1494), 273 (1415), 262 (996), 218 (21 012), 200 (53 862); IR (KBr) 1754, 1214, 1120 cm^{-1}. Anal. C, H, N.

The bromide salt was prepared by reaction of 6.71 g of **4b** with ca. 10 g of trimethylamine in 80 mL of 2-butanone at 100–110 °C for 60 h in a closed, stainless steel vessel. The residue, after evaporation and two recrystallizations from ethanol, gave 5.18 g (66%) of the bromide salt, mp 285 °C dec. Anal. C, H, N.

Synthesis of ^{14}C-Labeled 1b and 5b. 6-(Acetyloxy)-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-acetic-*carboxy*-^{14}*C* Acid Methyl Ester. To a slurry of 183 mg (4.6 mmol) of 60% NaH in a mineral oil dispersion in 10 mL of dry THF under argon was added dropwise 316 mg (50 mCi at 28.8 mCi/mmol) of trimethyl phosphonoacetate-*1*-^{14}*C*, obtained from Sigma Chemical Co. The labeled reagent was rinsed with 5 mL of dry THF, and 43 μL (0.26 mmol) of unlabeled trimethyl phosphonoacetate was added. The yellow, cloudy solution was stirred for 30 min. A solution of 530 mg (2.01 mmol) of 6-(acetyloxy)-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-ol[14] in 5 mL of THF was added dropwise and rinsed with 1 mL of THF. The green solution was stirred at room temperature overnight. The reaction was quenched with 1 mL of water, THF was removed under N_2 in a warm-water bath, 5 mL of water was added, and the mixture was extracted three times with ether. The extract was washed with water and with saturated NaCl solution, dried by filtration through $Na_2SO_4/MgSO_4$, and evaporated. The resulting oil was flash chromatographed on silica gel with 25% EtOAc in hexane. The fractions were analyzed by TLC (I_2) using 50% ethyl acetate in hexane. The product-containing fractions were combined and evaporated, giving 247 mg (38% yield) of an orange oil after drying in vacuo. The oil showed a single spot on TLC.

3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-ethanol-α-^{14}*C*. To a suspension of 155 mg of $LiAlH_4$ in 5 mL of dry THF was added dropwise a solution of 247 mg of the ester described above in 3 mL of dry THF, rinsed with 2 mL of THF. The mixture was refluxed for 2.5 h, cooled, and quenched by careful addition of 2 mL of H_2O and 5 mL of 2 N HCl. After stirring to complete the decomposition, the THF was evaporated under N_2 and the mixture was extracted twice with ether. The extract was washed with 1 N HCl, H_2O, saturated $NaHCO_3$, H_2O, and saturated NaCl solution, dried ($Na_2SO_4/MgSO_4$), and evaporated under N_2 to leave an orange oil weighing 266 mg. TLC analysis showed one major spot. The product was recrystallized from a minimum amount of ethyl acetate with hexane added as crystals formed. After refrigeration overnight, the solvent was decanted, and the tan crystals were dried in vacuo to leave 206 mg of the title compound.

^{14}C-Labeled 5b. Scaling down the procedure described for the synthesis of unlabeled **5b**, 126 mg of crude [^{14}C]-**5b** (base) was obtained; it was diluted with 126 mg of unlabeled material and chromatographed on silica gel using 0.5% NH_4OH and 5% MeOH in CH_2Cl_2. The pure product fractions were combined and the solvent was evaporated, leaving 220 mg of colorless oil. This was dissolved in a mixture of methanol and ether, and excess ethereal HCl was added. After dilution with 50 mL of ether, the solid was collected on a filter and dried in vacuo to give 220 mg of **5b**·HCl as a white powder (0.62 mmol, 16% overall chemical yield). This material was recrystallized from acetonitrile to give 146 mg of pure [^{14}C]-**5b**.

^{14}C-Labeled 1b. The mother liquors of the acetonitrile recrystallization of [^{14}C]-**5b** were evaporated to leave 74 mg of crude [^{14}C]-**5b**·HCl. This was suspended in 5 mL of ethyl acetate and basified with excess saturated $NaHCO_3$ solution. The aqueous layer was extracted twice with EtOAc and the combined ethyl acetate phase was washed with H_2O and NaCl solution, dried ($Na_2SO_4/MgSO_4$), and evaporated to give 64 mg of yellow oil. This was dried under reduced pressure for 0.5 h and dissolved in 1.4 mL of acetonitrile. To this solution, under argon, was added a solution of 40 mg of methyl tosylate in 0.5 mL of acetonitrile and

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the solution was refluxed for 3 h. The solvent was evaporated and the residue was triturated with ether until it solidified. After refrigeration overnight, the solid was collected and washed with ether. The product was recrystallized from 2 mL of ethyl acetate refrigerated for 3 h, filtered, washed, and dried in vacuo. The resulting pure [^{14}C]-**1b**·tosylate weighed 90.14 mg (0.178 mmol, 89%).

Analysis of Radiolabeled 1b and 5b. The labeled compounds were compared with unlabeled compounds by HPLC, TLC, and mass spectrometry and were found to be identical. A Spherisorb ODS-1 10-μm C18 column (4.6 × 250 mm) at 2 mL/min with detection by UV at 254 nm was used for HPLC with the following eluants: (**5b**) 25% 0.05M NaH_2PO_4 with 1 mL of H_3PO_4 per liter and 75% CH_3CN; (**1b**) 38% 0.05M sodium 1-heptanesulfonate and 62% CH_3CN. Radiochemical purity was determined by HPLC using the same systems. Forty-nine 18-s fractions were collected and counted by liquid scintillation using Bio-Safe II R scintillation cocktail. The samples were counted for 2 min or 0.2σ and the disintegrations per minute (dmp) for each were determined by the external standard ratios. [^{14}C]-**5b** was shown to be 99.51% radiochemically pure and the specific activity was 52.8 μCi/mg. [^{14}C]-**1b**·tosylate was shown to be 99.87% radiochemically pure and the specific activity was 35.9 μCi/mg.

Determination of the Partition Coefficients. The partition coefficients of the different compounds between 1-octanol and two aqueous phases were determined by the shake-flask method.[15,16] The aqueous media used consisted of (a) a 0.1 M sodium phosphate buffer, pH = 7.4, and (b) of an isotonic buffer, pH = 7.4, prepared by adding 2.3 g of $NaH_2PO_4 \cdot 7H_2O$ and 0.19 g of $NaH_2PO_4 \cdot H_2O$ to 100 mL of a 0.15 M NaCl solution. Aqueous and organic phases were previously saturated with each other. Five milliliters of a 10^{-3} M solution of **1a**, **5a**, and **5b** in the buffer or of **1b** and trolox in octanol were mixed with an equal volume of octanol and buffer, respectively, and shaken on a reciprocal shaker at room temperature for 1 h. After centrifugation at 4000 rpm for 10 min, the two phases were carefully separated and the concentrations of the test compounds were determined by HPLC analysis. The chromatographic system consisted of a Model 6000A pump, a WISP automatic injector from Waters, and a Model SF 773 UV detector from Kratos with wavelength set at 290 nm. The column was an Ultrasphere (250 × 4.6 mm, particle size 5 μm) from Beckman thermostated at 25 °C. Isocratic conditions were used with the two following eluents at a flow rate of 1 mL/min; (A) 700 mL of 0.1 M NaH_2PO_4, 300 mL of acetonitrile, 1 mM tetramethylammonium chloride and the pH adjusted to 6.00 by addition of 1 N NaOH; (B) 300 mL of 0.1 M NaH_2PO_4, 700 mL of acetonitrile, 1 mM tetramethylammonium chloride, pH = 6.00 by addition of 1 N NaOH. Solutions of compounds **1b**, **5b**, and trolox were analyzed with eluent A, an their retention times were 8.1, 8.9, and 3.2 min, respectively. Compounds **1a** and **5a** were analyzed with eluent B and had retention times of 9.5 and 12.7 min.

Without addition of NaCl, the partition coefficients (octanol, 0.1 M phosphate buffer) for the tosylate, bromide, and chloride salts of **1a** were −1.17, −1.82, and −2.05, respectively, while with NaCl (0.15 M) the values were −0.60, −0.71, and −0.72, respectively. Similarly, the partition coefficients for the tosylate and bromide salts of **1b** were −0.76 and −1.64 without NaCl addition and −0.18 and −0.30 with NaCl. For compounds **5a**, **5b**, and trolox, addition of NaCl had little effect on the partition coefficients (less than 0.02 log units).

Lipid Peroxidation. The method employed essentially followed a published procedure.[21] Fresh brains from adult Sprague–Dawley rats were homogenized in ice-cold 40 mM potassium phosphate buffer (pH 7.4, 0.14 M NaCl). The supernatants obtained by 15 min centrifugation of the homogenate at 1000*g* were collected and frozen at −80 °C. For the assay 800 μL of homogenate (diluted 2:5 with the buffer) was incubated in a total volume of 1 mL for 30 min at 37 °C in the presence or absence of scavenger. The reaction was stopped on ice by addition of 200 μL of 35% $HClO_4$ and 200 μL of 1% thiobarbituric acid in water. The color was developed in a boiling water bath for 15 min and the absorbance was read at 532 nm against buffer blanks after cooling to room temperature. Values obtained with nonincubated samples were subtracted from the incubated values obtained in the absence or presence of the scavenger. The scavenger did not interfere with a calibration line obtained with 4–20 nmol of malondialdehyde dimethyl acetal.

Tissue Distribution. Male normotensive Sprague–Dawley rats (Charles River, Cléon, France) weighing 250–300 g were used. Animals were kept under controlled conditions, with respect to temperature, humidity, and light. For injection [^{14}C]-**1b** was diluted with cold material to give a specific activity of 24.8 μCi/mg and a solution of 1 mg of base/mL was prepared in isotonic saline. [^{14}C]-**5b** was diluted to 37.9 μCi/mg and dissolved in saline (1 mg base/mL) for injection (1 mL/kg).

Rats were prepared surgically under ether anesthesia. A cannula was inserted into the femoral vein, exteriorized at the scruff of the neck, and used for the iv administration of drugs. Animals were fasted overnight but had free access to drinking water at all times. The drug solution, prepared as described above, was administered (1 mg of base/kg of body weight) as an iv bolus via the femoral vein (1 mL dose solution/kg of body weight). Rats (n = 3), were sacrificed by decapitation 1 h after drug administration. Whole blood was collected into heparinized tubes and treated with a mixture of Lumasolve/2-propanol (1:2 v/v). Coloration was removed with 500 μL of H_2O_2 (30%), and the samples were left overnight before the addition of 15 mL of scintillation liquid (Aquasol/0.5 N HCl 9:1 (v/v)). Heart and skeletal muscle (thigh or rear leg) were removed and washed with isotonic saline before blotting and weighing. Tissue homogenates (1:2 (w/v)) were prepared in water with a Polytron homogenizer. Aliquots (200 μL) of homogenate were solubilized in 500 μL of Lumasolve at room temperature for 48 h. The solutions were then bleached with 100 μL of H_2O_2 (30% w/v) followed by the addition of 100 μL of 2-propanol. Counting was done in 15 mL of Aquasol/1 N HCl (95:5 (v/v)). Radioactivity was measured with a Beckman LS 1701 scintillation counter, with dpm calculated from quench curves.

Pharmacologic Studies. Myocardial Infarct/Reperfusion in Rats. Coronary artery ligation (60 min) and reperfusion (30 min) was carried out in male, normotensive Sprague–Dawley rats according to the method described previously.[13]

Compounds **1b** (bromide salt) and **5b** were dissolved in saline and were infused into the jugular vein at a rate of 2.3 mL/h. Similarly saline (control) was infused at a rate of 2.3 mL/h. Infusion of each compound commenced 10 min before occlusion until the end of reperfusion.

(21) Stocks, J.; Gutteridge, J. M. C.; Sharp, R. J.; Dormandy, T. L. *Clin. Sci. Mol. Med.* **1974**, *47*, 215.