The Metabolism of Carbamazepine in Humans: Steric Course of the Enzymatic Hydrolysis of the 10,11-Epoxide[1]

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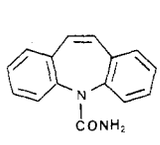
Carbamazepine 10,11-oxide (1a,10b-dihydro-6*H*-dibenzo[*b*,*f*]oxireno[*d*]azepine-6-carboxamide), a key intermediate in carbamazepine metabolism, was found to be unusually resistant to enzymatic hydrolysis when incubated with microsomal and cytosolic fractions from rabbit, rat, and guinea pig livers. However, its hydrolysis product, *trans*-10,11-dihydro-10,11-dihydroxy-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide, was excreted, as previously reported, both in the free and in conjugated forms, as the main metabolite in the urine of humans under carbamazepine treatment. The free diol and that obtained after treatment with β-glucuronidase/arylsulfatase were both found by Mosher's method to be formed in an enantiomeric excess of 80%, the prevalent enantiomer having the (−)-10*S*,11*S* absolute configuration, as determined by applying the CD exciton coupling method to its bis[*p*-(dimethylamino)benzoyl] ester. This finding confirms the pronounced enantioselectivity of the microsomal epoxide hydrolase toward meso and racemic substrates, but is in contrast with the prevalent formation of (*R*,*R*)-diols in most other known cases of enzymatic hydrolysis of epoxides. Preparatively useful syntheses of the racemic *trans*-10,11-dihydro-10,11-diol and of 9-(hydroxymethyl)-10-carbamoylacridan, another carbamazepine metabolite, are reported for the first time.

Carbamazepine (1, 5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide), the active principle of Tegretol, is a widely used agent for the treatment of epilepsy and trigeminal neuralgia. An exact knowledge of its metabolism is particularly important owing to the fact that it may be administered in large amounts (up to 1 g/day) for extensive, even lifelong, periods, a low toxicity of the drug and of its metabolites being an essential feature for its protracted employ. A large number of papers dealing with its pharmacokinetics and metabolism have therefore appeared since its introduction in therapy during the early 1960s.[2] Metabolites accounting for over 70% of the administered dose are recovered from human urine and over 30 different compounds were identified after enzymatic hydrolysis of conjugates.[3] Most of them derive from intermediate cytochrome P-450 dependent monooxygenase oxidation products, the main pathway being the one involving formation of 10,11-dihydro-10,11-epoxycarbamazepine (**2**) and giving rise to about 40% of the urinary metabolites. Only minimal amounts (ca. 1%) of **2**, however, are found as such,[2] since it is further converted into *trans*-10,11-dihydro-10,11-dihydroxycarbamazepine (**3**) by an epoxide hydrolase promoted hydrolysis.[4] This diol and the corresponding glucuronide[5] are therefore the main metabolites of carbamazepine. On the other hand, when epoxide **2** is administered to humans, over 90% of the dose is recovered as **3** and its glucuronide.[6]



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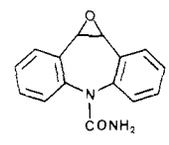

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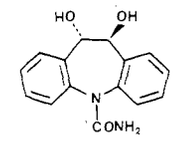

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In connection with a research program on the substrate selectivity and stereoselectivity of microsomal epoxide hydrolase (MEH), we have investigated the steric course of the enzymatic hydration of epoxide **2** to diol **3**. The initial intention was to subject **2** to the action of MEH from animal liver in vitro, as done in our previous studies, but attempts with microsomes from rabbit, rat, and guinea pig and with cytosolic fractions from rat and guinea pig showed that their hydrolytic activity on **2** was extremely low, only traces of the diol **3** being formed after protracted incubations, the amounts being too small for isolation on a scale sufficient for a stereochemical characterization. The fact that the level of MEH activity toward **2** in rat liver is about $^1/_{100}$ with respect to that in human liver has been previously reported.[6] Because of difficulties in securing human liver microsomes and of risks involved in the administration of epoxides, we decided to isolate diol **3** from the urine of patients under carbamazepine treatment.

Results

Preparation and Characterization of Reference Compounds. Reference samples of epoxide **2** and of racemic *trans*-diol **3** were needed. The previously reported low yield (30–35%)[7,8] of the epoxidation of carbamazepine was increased to 65% by longer reaction times. The acid-catalyzed hydrolysis of **2** to **3** has been reported[3] to take place under very mild conditions (6% acetic acid in 1:1 THF/H_2O), but we were unable to duplicate this procedure, unchanged epoxide being quantitatively recovered. Longer reaction times or increases in acidity and temperature caused slow conversion of **2** into the rearrangement and elimination products 9-formyl-10-carbamoylacridan (**4**) and 9-formylacridine (**5**). Formation of the latter from **2** in boiling acetic acid had been previously reported.[9]

We also obtained **4** by treatment of epoxide **2** with $BF_3{\cdot}Et_2O$, but attempts at its purification by recrystallization or through chromatographic methods failed owing to its high tendency to be converted into **5**, the elimination of formamide probably being favored by the formation of

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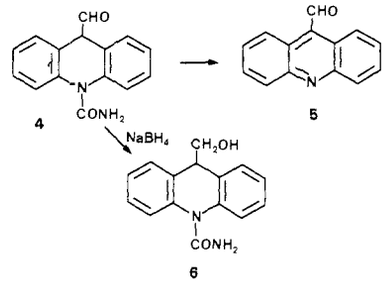
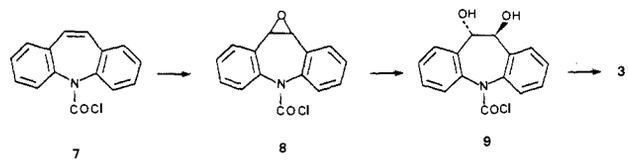
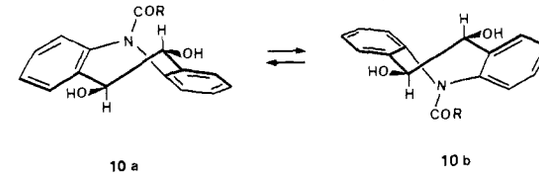


the highly conjugated 9-formylacridine system. A full characterization of **4**, however, was possible through the NMR and mass spectra of the crude product. In addition, its reduction to the more stable hydroxymethyl derivative **6**, which was purified by recrystallization, provided a further confirmation. Compound **6** has been reported as one of the metabolites of carbamazepine on the basis of mass spectrometric data,[3] but was never isolated or synthesized.

Several attempts to find alternative ring-opening conditions for **2** ($HClO_4$ of different concentrations, glacial acetic acid, trichloroacetic acid, KOH in Me_2SO) failed, compounds **4** and **5** always being the only identified products. Available information[9-12] points to a competition between normal anti ring opening and ring restriction to acridan derivatives in reactions of dibenzo[*b,f*]azepine 10,11-oxides under acidic conditions, electron-withdrawing groups on nitrogen favoring the former and alkyl groups or hydrogen the latter course of reaction. Evidently, the carbamoyl substituent of carbamazepine is not sufficiently electron withdrawing to prevent development of positive charge at C-10 and the subsequent rearrangement. A better electron acceptor was therefore required on nitrogen. For this purpose diol **3** was prepared by epoxidation of 5*H*-dibenzo[*b,f*]azepine-5-carbonyl chloride **7** to **8**, followed by acid-catalyzed hydrolysis to **9**, which proceeds without rearrangement, and the acid chloride was finally converted into **3** with ammonia.



Acid-catalyzed epoxide ring openings normally occur in an anti fashion, and other types of reactions of **8** have been found to take this steric course.[9] However, since in several cases opening of aryl-substituted epoxides can occur with partial or total retention of configuration at benzylic positions,[13] a confirmation was needed for the trans configuration of diol **9** (and consequently of **3**). This was given by the NMR spectra of **3** and **9** in which the protons at C-10 and C-11 appear to be nonequivalent, showing up as an AB system with $\Delta\delta$ = 0.9 ppm and J = 9.5–9.7 Hz. These data point to a dihedral angle nearing 180° between these C–H bonds and consequently to a trans-gauche relationship between the hydroxy groups. The conformational situation in 10,11-dihydrodibenzo[*b,f*]azepines is fairly complicated, since up to four independent restricted conformational processes can coexist, of which three have been experimentally observed:[14] (a) rotation around the N–COR bond, (b) pyramidal inversion at nitrogen, (c) twisting on the C-10/C-11 bond, (d) ring inversion by torsion about the C-4a/N-5/C-5a bonds. In the specific case of **3** and **9** process (b) probably has a very low barrier or is irrelevant, owing to the likely planarity of the amide system. The high value of the H-10/H-11 coupling constant further implies a barrier for process (c) that is high enough to keep the hydroxy groups in the gauche (approximately diequatorial) conformation. Intramolecular hydrogen bonding does not seem to be a determinant factor, since the same diequatorial conformation is found for diol **9** both in the low polarity aprotic $CDCl_3$ and in the polar protic CD_3OD solvents. The interference of one of the hydroxy groups with the *N*-carbamoyl group in the alternative anti diaxial conformer could be responsible for the adopted conformation. However, the *trans*-10,11-dibromo derivative of **7** has been shown to prefer the anti diaxial conformation in several solvents.[12] A further observation is that the degenerate process (d) involving identical conformers **10a** and **10b** must also have a fairly



high barrier since, if it were fast at room temperature, the nonequivalence of protons at C-10 and C-11 could not be observed. As pointed out before,[14] this process would require concurrent operation of effect (a) in order to allow the amide group to become perpendicular to the plane C-4a/N-5/C-5a so as to facilitate its passage past H-4 and H-6. One can therefore conclude that diols **3** and **9** exist exclusively in a relatively rigid conformation of type **10**, in which the pronounced nonequivalence of the benzylic protons is well explained by their significantly different orientation with respect to the aromatic rings and to the acyl group.

Isolation and Characterization of Diol 3 from Urine. Free *trans*-diol **3** was isolated from the urine of four patients, two of which were in monotherapy with carbamazepine and the other two under treatment with carbamazepine and phenobarbital. Separation was carried out by extraction with ethyl acetate, followed by chromatography on a silica column; final purification was made by preparative TLC, avoiding recrystallization in order to prevent modifications of enantiomer ratios. Part of diol **3** was present in conjugated form and remained in the aqueous phase. It was obtained separately by concentration of this phase, followed by treatment with β-glucuronidase/arylsulfatase and repetition of the same extraction/purification sequence. The samples of free diol **3** obtained in the direct extraction of all urine samples were highly levorotatory, a fact that indicated a pronounced enantioselection during the enzymatic hydrolysis of the *meso*-epoxide **2**. The optical activity of diol **3** isolated from human urine had been reported at a conference in 1975,[15]

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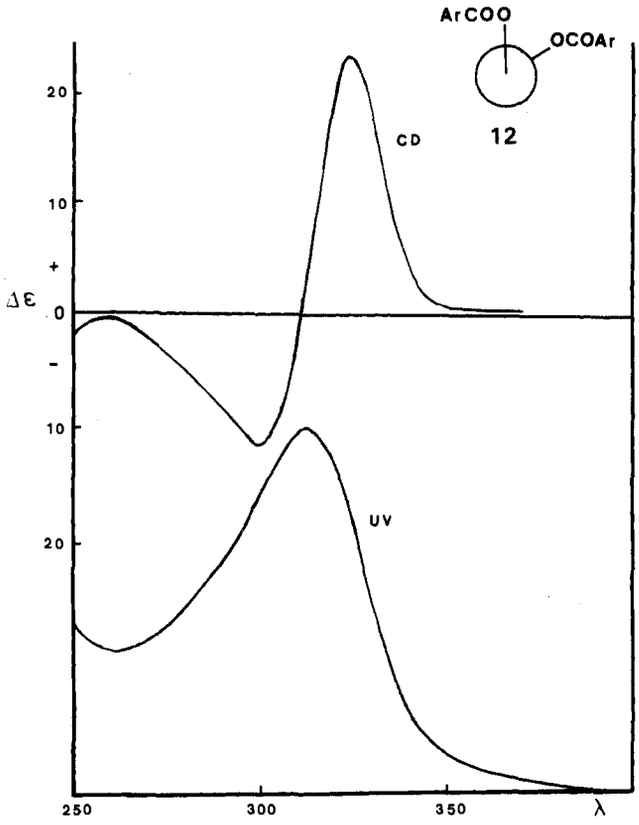
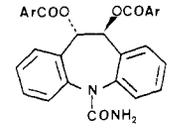


Figure 1. CD and UV spectra of the bis[*p*-(dimethylamino)-benzoate] of (−)-(*S,S*)-*trans*-10,11-dihydro-10,11-dihydroxy-5*H*-dibenzo[*b,f*]azepine-5-carboxamide.

but no indication about its optical purity and absolute configuration was given.

Owing to the small amount of optically active diol available, a chiroptical method was applied by us to deduce its absolute configuration. As far as we know, exciton coupling methods[16] have never been applied to chiral dibenzoazepine derivatives. The diol was therefore converted into its bis[*p*-(dimethylamino)benzoate] 11 in order to have



11 Ar = p-Me_2N-C_6H_4-

13 Ar = (−)-PhC(OMe)(CF_3)

chromophores absorbing at higher wavelength, thus avoiding overlap with the dibenzoazepine system spectrum, and to apply the reliable dibenzoate chirality rule.[16] The CD spectrum of 11 exhibited the expected well-defined exciton splitting with a positive Cotton effect at 324 nm ($\Delta\epsilon$ = 23) and a negative one at 300 nm ($\Delta\epsilon$ = −11.6) (Figure 1), pointing to a right-handed screwness in the disposition of the (arylcarbonyl)oxy groups, as shown in **12**, which corresponds to the 10*S*,11*S* absolute configuration for 11 and consequently for (−)-**3**.[16] Although a deduction of the conformation of 11 from its NMR spectrum was not possible, owing to the overlap between the benzylic and aromatic proton signals, the high $\Delta\epsilon$ values indicate a high preference for the conformation with gauche (arylcarbonyl)oxy groups in the ester, as found by NMR for the free diol.

The enantiomeric excess of the diols extracted from urine was determined through conversion into the bis-MTPA esters **13**[17] and analysis by HPLC of the diastereomeric mixtures. The enantiomeric excess was found to be 80 ± 2% for all the examined cases, although samples had values of specific rotations differing as much as 10% from one another. This may be due to the fact that diol **3** strongly retains solvent (AcOEt, $CHCl_3$) that is difficult to eliminate even after drying in vacuo.

The determination of the enantiomeric excess was also carried out in the same way for the conjugated fraction of diol **3**, after hydrolysis with β-glucuronidase/arylsulfatase. It was found to be practically identical with that of the free diols.

Discussion

Although only traces of epoxide **2** were present in the urine extracts, previous literature provides ample proof that **2**, formed by enzymatic epoxidation of carbamazepine, is the primary intermediate responsible for the formation of *trans*-diol **3**. The resistance of the oxirane ring to chemical hydrolysis and the high optical purity of the isolated **3** further confirm that it is formed in an enzyme-catalyzed process. Our data show that the enzyme has an ca. 90:10 preference for catalyzing attack by water at one of the two enantiotopic oxirane carbons of the *meso*-epoxide, that is, at the *R* carbon, giving rise to the (*S,S*)-diol. A possible alternative hypothesis, namely, an enantioselection favoring the reaction of the (*R,R*)-diol at the stage of glucuronide formation and leaving an excess of free (*S,S*)-diol, was ruled out when it was found that the diol recovered after hydrolysis of the glucuronide had the same sense of rotation and optical purity as the free diol.

The conversion of carbamazepine into its 10,11-epoxide **2** and its hydrolysis to diol **3** most probably takes place in the liver, the capability of isolated human liver microsomes to catalyze this hydrolysis having previously been demonstrated.[6] The much lower hydrolytic activity toward epoxide **2** found for microsomes from other species (rat, rabbit, guinea pig, etc.) could perhaps point to the presence of a hydrolase specific for **2** in humans, since species-related differences in reactivity usually are much smaller than those found in this case,[18] but much more extensive testing, possibly with purified epoxide hydrolases from different species, would be necessary to legitimate this hypothesis.

The enantioselectivity of MEH has been extensively investigated for substrates in which the epoxide ring is fused to a six-membered ring, such as cyclohexene and polycyclic arene oxides, and to a smaller extent for epoxides derived from noncyclic alkenes.[19] but, as far as we know, no data are available for oxiranes fused to seven-membered rings. Some other cases of *meso*-epoxides giving rise to an excess of one of the corresponding enantiomeric *trans*-diols are known, such as benzene oxide[20] and a close

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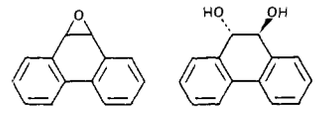
analogue of **2**, *cis*-stilbene oxide.[21] With these substrates, attack by water in the MEH-catalyzed hydrolysis occurs preferentially at the *S* carbon to give excesses of the *R,R* enantiomers of the *trans*- or *threo*-diols. The same tendency to the formation of *R* or *R,R* enantiomers by preferential attack at the *S* oxirane carbons was also observed in most cases of racemic substrates, such as styrene oxide,[22] several substituted epoxycyclohexanes[23] and epoxytetrahydropyrans,[24] and polycyclic arene oxides.[25] A hypothesis accounting for most of these results was based on the assumption of a topology of the MEH active site requiring preferential binding of that enantiomer of the substrate in which the bulkier lipophilic substituent fits into a large hydrophobic pocket that is asymmetrically situated with respect to the site of binding of the epoxide oxygen and attack by water (catalyzed by a specific histidine residue) occurs at the oxirane carbon that is more distant from the lipophilic moiety.[23]

The MEH-catalyzed hydrolysis of epoxide **2**, in which a high preference for nucleophilic attack by water at the *R* carbon is observed, therefore differs in its steric course from most of the known reactions of this type. Apart from the totally untested hypothesis of a carbamazepine-specific epoxide hydrolase, with a particular steric demand, this may be explained by the absence of a dissymmetric distribution of substituents with respect to the oxirane ring. Also in a substrate that bears a structural analogy to **2**, phenanthrene 9,10-oxide (**14**), a definite even if low preference (ca. 25% ee) for the formation of (*S,S*)-diol **15**, was observed.[25b,d] The very high opposite product enan-



14 **15**

tioselectivity in the MEH-promoted hydrolysis of *cis*-stilbene oxide, yielding almost optically pure (*R,R*)-diol,[21] may be attributed to the much lower conformational rigidity of the latter substrate with respect to **2** and **14**, the rotameric freedom of the phenyl groups probably allowing it to find a better fit into the enzyme active site in a conformation, not available to **2** and **14**, that exposes the *S* oxirane carbon to backside attack by water.

Experimental Section

Melting points (uncorrected) were determined on a Kofler apparatus. IR and UV–vis spectral data were respectively obtained with Pye-Unicam SP3-300 and Pye-Unicam SP4-800 spectrophotometers. NMR spectra were determined with a Varian CFT-20 or with a Varian EM-360 A spectrometer with Me_4Si as the internal standard. Mass spectra were obtained with a VG Analytical 70-70 E spectrometer; fast atom bombardment (FAB) technique was used. CD spectra were recorded with a JASCO J 500 C spectropolarimeter.

Kieselgel (150–230 mesh, ASTM, Merck) was used for column chromatography. Analytical TLC was run on DC-Alufolien Kieselgel 60 F254 (Merck) and preparative TLC was performed on PSC-Fertigplatten Kieselgel 60 F254 (Merck) 1- or 2-mm thickness). HPLC analyses were carried out under isocratic conditions with a Waters 6000 A apparatus equipped with a Lambda-Max 480 UV detector: (a) reverse phase, 30 cm, 10 μm, C_{18} Bondapack column (Waters), 65:35 MeOH/H_2O, 1.5 mL/min, 240 nm; (b) normal phase, 25 cm, 10 μm, Techsil 10 silica column (HPLC), 90:10 hexane/EtOAc, 1 mL/min, 260 nm.

Materials. All the solvents were reagent grade. 5*H*-Dibenzo[*b,f*]azepine-5-carboxamide (**1**) (carbamazepine, Sigma) was pure to HPLC. 5*H*-Dibenzo[*b,f*]azepine (iminostilbene, Ega) was >97% pure and was used without purification. Gaseous phosgene (Matheson) was >99% pure. *m*-Chloroperoxybenzoic acid (85% pure) was purchased from Fluka. β-Glucuronidase/arylsulfatase from Helix pomatia, 100000 Fishman units/mL (Boehringer), was used as a stabilized solution.

1a,10b-Dihydro-6*H*-dibenzo[*b,f*]oxireno[*d*]azepine-6-carboxamide (2). *m*-Chloroperoxybenzoic acid (3.8 g, 18.7 mmol) was added portionwise to a stirred solution of **1** (3.0 g, 12.7 mmol) in $(CH_2Cl)_2$ (50 mL). After being allowed to stand at room temperature for 4 days, the mixture was cooled at 0 °C and the *m*-chlorobenzoic acid was filtered off. The solution was washed with 5% aqueous $NaHSO_3$ and with saturated $NaHCO_3$ solutions; it was dried ($MgSO_4$) and was evaporated in vacuo. The crude residue (2.9 g) was crystallized from THF, leading to **2** (2.1 g, 63% yield): mp 204–206 °C (lit.[8] mp 205–207 °C); UV λ_{max} (7:3 MeOH/H_2O) 210 nm (ε 36500); IR (Nujol) 3450–3100 (NH_2), 1660 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.26 (s, 2 H, H-10,11), 4.51 (s, 2 H, NH_2). Reverse-phase HPLC analysis showed that the sample was about 99% pure, a trace of **1** being the only detectable impurity. Retention times of **2** and **1** were 84 and 128 s, respectively, R_s = 8.8.

Attempts To Open the Oxirane Ring of 2. Several attempts were made to convert epoxide **2** into *trans*-diol **3** through acid-catalyzed hydrolysis. Reaction mixtures were extracted with EtOAc and analyzed by TLC (elution with 95:5 $CHCl_3$/MeOH), which revealed at most three compounds in all cases: unchanged **2** (R_f 0.35) and aldehydes **4** (R_f 0.45) and **5** (R_f 0.60, blue fluorescence). The latter two compounds were separated by preparative TLC and compared with authentic samples (IR and NMR). There was some conversion of **4** into **5** during preparative TLC. The reported procedure[3] (6% acetic acid in 1:1 THF/H_2O, 24 h at room temperature) led to total recovery of the starting material. More acidic conditions (1:1 acetic acid/THF, 0.1 M trichloroacetic acid in CH_3CN or $(CH_2Cl)_2$, $HClO_4$ in aqueous THF, at pH values ranging from 2 to 0) failed to produce any **3**. Also, when epoxide **2** was kept at 40 °C for 4 h with 2 N KOH in Me_2SO, no diol **3**, but unidentified products were formed.

9-Formylacridine (5): crystallized from EtOH, mp 146–148 °C (lit.[8] mp 145–146 °C); IR (Nujol), 1680 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.6–8.9 (m, 8 H, aromatic protons), 11.5 (s, 1 H, CHO).

9-Formyl-10-carbamoylacridan (4). BF_3·Et_2O (0.13 mL, 1 mmol) was added to a solution of **2** (0.126 g, 0.5 mmol) in anhydrous CH_2Cl_2 (10 mL). After being allowed to stand at room temperature for 30 min, the mixture was washed with aqueous saturated $NaHCO_3$. The organic layer was dried ($MgSO_4$) and evaporated in vacuo to yield a 75:25 mixture of **4** and **5** (0.112 g): ^1H NMR ($CDCl_3$) δ 4.63 (d, 1 H, J = 1.9 Hz, C*H*CHO), 5.18

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(s, 2 H, NH_2), 7.35 (m, 8 H, aromatic protons), 9.4 (d, 1 H, J = 1.9 Hz, CHO); FAB MS, m/e 253 (M^+ + 1, 15), 252 (M^+, 7), 180 (base peak); IR (Nujol) 3450–3150 (NH_2), 1710 (aldehyde C=O), 1660 (amido C=O) cm^{-1}. Attempts at purification by crystallization or chromatography caused further conversion to **5**, but the NMR spectrum of the crude product proved its structure.

9-(Hydroxymethyl)-10-carbamoylacridan (6). $NaBH_4$ (35 mg, 0.9 mmol) was added to a solution of **4** (152 mg, 0.6 mmol) in 15 mL of absolute EtOH. After being allowed to stand at room temperature for 2 h, the solution was diluted with water and extracted with Et_2O. The organic layer was dried ($MgSO_4$) and evaporated. The crude residue was crystallized from $CHCl_3$/hexane, leading to pure **6**: mp 183–185 °C; ^1H NMR (Me_2SO-d_6) δ 3.38 (d, 2 H, J = 7.5 Hz, CH_2OH), 3.87 (t, 1 H, J = 7.5 Hz, $CHCH_2OH$), 6.24 (s, 2 H, NH_2), 7.3 (m, 8 H, aromatic protons); FAB MS, m/e 255 (M^+ + 1, 61), 238 (25), 223 (18), 194 (60), 180 (base peak). Anal. ($C_{15}H_{14}N_2O_2$) C, H, N.

5*H*-Dibenzo[*b*,*f*]azepine-5-carbonyl Chloride (7). Gaseous phosgene was bubbled into a vigorously stirred suspension of iminostilbene (4 g, 20.7 mmol) in anhydrous toluene (100 mL) until the initial orange color turned to pale yellow. After further stirring for 5 h, excess phosgene was removed by a nitrogen stream and the iminostilbene hydrochloride (2.3 g) was filtered off. After concentration in vacuo to a small volume, pure **7** precipitated on cooling: mp 158–160 °C after crystallization from toluene (lit.[26] mp 168–169 °C); ^1H NMR ($CDCl_3$) δ 6.83 (s, 2 H, CH=CH), 7.32 (m, 8 H, aromatic protons).

1a,10b-Dihydro-6*H*-dibenzo[*b*,*f*]oxireno[*d*]azepine-6-carbonyl Chloride (8). *m*-Chloroperoxybenzoic acid (1.45 g, 7.1 mmol) was added portionwise to a stirred solution of **7** (1.1 g, 4.22 mmol) in $(CH_2Cl)_2$ (30 mL). After being allowed to stand at room temperature for 4 days, the mixture was cooled at 0 °C and the *m*-chlorobenzoic acid was filtered off. The solution was washed (5% $NaHSO_3$ and saturated $NaHCO_3$), dried ($MgSO_4$), and evaporated in vacuo. The crude residue (1.1 g) was crystallized from EtOAc, leading to pure **8** (HPLC) (0.72 g, 63% yield): mp 205–207 °C (lit.[27] mp 205–207 °C); ^1H NMR ($CDCl_3$) δ 4.26 (s, 2 H, $\overline{CH}$–O–$\overline{CH}$), 7.32 (m, 8 H, aromatic protons).

***trans*-10,11-Dihydro-10,11-dihydroxy-5*H*-dibenzo[*b*,*f*]azepine-5-carbonyl Chloride (9).** $HClO_4$ (0.5 M, 6 mL) was added to a solution of **8** (0.31 g, 1.13 mmol) in THF (10 mL). After being allowed to stand at 45 °C for 7 h, the solution was repeatedly extracted with EtOAc. The organic layer, washed with water and aqueous saturated $NaHCO_3$ solution, was dried ($MgSO_4$) and evaporated in vacuo. The crude residue (0.32 g) was crystallized from $CHCl_3$/hexane, leading to pure **9** (HPLC) in 40% yield: mp 163–165 °C; ^1H NMR ($CDCl_3$) δ 2.65 (s, 2 H, 10-OH, 11-OH), 4.43 (d, 1 H, J = 9.7 Hz, $CHOH$), 5.33 (d, 1 H, J = 9.7 Hz, $CHOH$), 7.32 (m, 8 H, aromatic protons); ^1H NMR (CD_3OD) δ 4.37 (d, 1 H, J = 9.6 Hz, $CHOH$), 5.27 (d, 1 H, J = 9.6 Hz, $CHOH$), 7.36 (m, 8 H, aromatic protons). Freshly crystallized product dried 1 h in vacuo at room temperature retained one-third molecule of $CHCl_3$ per molecule of product. Anal. ($C_{15}H_{12}ClNO_3 \cdot {}^1/_3CHCl_3$) C, H, N, Cl. After further drying at 80 °C in vacuo: ($C_{15}H_{12}ClNO_3$) C, H, N.

***trans*-10,11-Dihydro-10,11-dihydroxy-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide (3).** Gaseous ammonia was bubbled into a solution of crude **9** (0.3 g) in MeOH (75 mL). After being allowed to stand at room temperature for 2 h, the solution was evaporated in vacuo. The crude glassy residue was crystallized from $CHCl_3$, giving pure **3** (HPLC) (0.13 g): mp 198–200 °C; IR (Nujol) 3500–3150 (OH, NH_2), 1650 (C=O) cm^{-1}; UV (MeOH) λ 206 (ϵ 31 400). Anal. ($C_{15}H_{14}N_2O_3$) C, H, N (after drying in vacuo at 80 °C).

Microsomal and Cytosolic Preparations and Enzymatic Incubations of 2. Liver microsomes were obtained from male New Zealand white rabbits (2.5–3 kg) and from male Sprague–Dawley rats, both pretreated with sodium phenobarbital for 3 days (35 mg/kg per day) by intraperitoneal injection, and from non-pretreated female guinea pigs. Hepatic cytosolic fractions obtained from rats and guinea pigs were also used.

Livers were removed and homogenized in four volumes of 50 mM Tris·HCl buffer (pH 7.4) containing KCl (1.15% w/v) with a Teflon tissue grinder, and the resulting suspension was centrifuged at 9000*g* for 30 min. The supernatant was further centrifuged at 125000*g* for 1.3 h, leading to the cytosolic and microsomal fractions. The protein concentrations of the cytosolic fractions of rats and guinea pigs were 30–40 and ca. 4 mg/mL, respectively. The microsomal pellets were suspended in the same buffer to a final protein concentration of 8.6, 6.6, and 1.7 mg/mL for the preparations obtained from rabbits, rats, and guinea pigs, respectively. Both cytosolic and microsomal fractions were used directly or stored at −40 °C. The microsomal preparations were tested with safrole oxide[28] and showed V_{max} 0.082, 0.094, and 0.182 μmol (mg of protein)$^{-1}$ min^{-1} for the fractions obtained from rabbits, rats, and guinea pigs, respectively.

The enzymatic incubations were performed by adding to 1–3 mL of microsomal preparations solutions of **2** in EtOH to final concentrations of 0.05–1 mM. The mixtures were incubated with shaking at 37 °C for times ranging from 1 to 23 h. After the prefixed time, the incubations were terminated by cooling below −40 °C. HPLC analysis (reverse phase) of each mixture was performed on the organic phase obtained after thawing, saturation with NaCl, and repeated extractions with EtOAc, followed by drying of the organic layers on $MgSO_4$.

All the incubation mixtures showed, besides the unreacted **2**, at most traces (<1%) of **3**. Retention times of **2** and **3** were 84 and 48 s, respectively, R_s = 3.5.

Isolation of (−)-(*S*,*S*)-*trans*-10,11-Dihydro-10,11-dihydroxy-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide (3) from Human Urine. The 24-h urine from a male epileptic patient (42 years old) under monotherapy with Tegretol (0.8 g/day) were exhaustively extracted with EtOAc. The dried ($MgSO_4$) organic phase was concentrated to one-third of the initial volume, and the precipitated urea was filtered off; the filtrate was evaporated to dryness in vacuo and the residue was dissolved in a small volume of EtOAc and chromatographed on a column of silica gel (ϕ 2 cm, 80 g). Elution was performed with 0.5 L each of $CHCl_3$ and $CHCl_3$ containing 2%, 4%, 6%, 8%, and 10% of MeOH. Eluted fractions (50 mL each) were analyzed by TLC ($CHCl_3$/MeOH, 80:20) and HPLC (reverse phase, MeOH/H_2O, 65:35). The fractions containing **3** were combined and evaporated, and the residue was further purified by preparative TLC ($CHCl_3$/MeOH, 80:20). The product was extracted with EtOAc to give a pure sample of **3** (37 mg) (crystallizing with one molecule of EtOAc), whose IR and NMR spectra were identical with that of the synthetic racemic product: $[\alpha]^{20}_D$ −188° (*c* 1.54, MeOH); CD (MeOH) $\Delta\epsilon_{240}$ = −15.7, $\Delta\epsilon_{211}$ = −25.3, $\Delta\epsilon_{194}$ = +22.7. Anal. ($C_{15}H_{14}N_2O_3 \cdot C_4H_8O_2$) C, H, N. Assay by the MTPA esters method (see below) showed a 90:10 ratio of *S*,*S* to *R*,*R* enantiomers.

The same product, after crystallization from $CHCl_3$ and drying in vacuo at 80 °C, had mp 208–210 °C and gave the following analysis: ($C_{15}H_{14}N_2O_3$) C, H, N.

The aqueous phase remaining after the extraction of the urine was concentrated in vacuo to about 100 mL and extracted again with EtOAc, and the aqueous phase was incubated for 48 h at 37 °C with 3.5 mL of β-glucuronidase/arylsulfatase and then processed according to the above procedure. After chromatography on silica gel, a crude product (15 mg) consisting mainly of diol **3** was directly converted into the bis(MTPA) derivatives that were formed in a *S*,*S*,*S*:*R*,*R*,*S* ratio of 89.5:10.5.

Similar results were obtained with the urine of three other patients: (a) 29-year-old male epileptic in monotherapy with Tegretol (1.4 g/day), (b) 36-year-old male epileptic, and (c) 57-year-old female epileptic in therapy with Tegretol (0.8 and 0.4 g/day, respectively) in association with phenobarbital (0.2 and 0.1 g/day, respectively).

The ratios between free and conjugated **3** were obtained as follows: a 2-mL sample of urine was extracted with EtOAc (3 × 5 mL) and the extract, evaporated in vacuo to dryness, was analyzed by HPLC, with carbamazepine as the internal standard (reverse phase). A preliminary test had shown that carbamazepine

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was completely absent from the urine. The aqueous phase was brought to pH 4.6 with 0.1 M acetate buffer (2 mL) and incubated at 37 °C for 24 h with 200 μL of β-glucuronidase/arylsulfatase. The extraction with EtOAc was repeated and the extract was analyzed by HPLC.

A further 2-mL sample of urine was directly incubated with β-glucuronidase/arylsulfatase and treated and analyzed as above in order to evaluate the total amount of **3** both in the free and in the conjugated form. On the average, the amounts of **3** found in the free and in the conjugated form were respectively 140 ± 10 and 100 ± 10 μg/mL, corresponding to a ratio of about 6:4. The total amount of diol obtained after direct total enzymatic hydrolysis was 260 ± 10 μg/mL.

Determination of the Enantiomeric Excess of Diol 3. The diol (2 mg) was dissolved in pyridine (0.2 mL) and treated with (−)-(*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride ((−)-MTPA chloride, 30 mg). The mixture was stored at room temperature for 4 days, then diluted with H_2O, acidified with 10% HCl, and extracted with EtOAc. The washed (saturated $NaHCO_3$) and dried ($MgSO_4$) solution was evaporated to dryness and analyzed by HPLC (normal phase, hexane/EtOAc, 90:10, retention times of (*R,R,S*)- and (*S,S,S*)-**13**: 732 and 822 s, respectively, R_s = 2.5). When racemic **3** was used, the two diastereoisomeric bis(MTPA) esters were present in a ratio of 50:50.

Determination of the Absolute Configuration of (−)-3 via Its Bis[*p*-(dimethylamino)benzoate] 11. *p*-(Dimethylamino)benzoyl chloride (140 mg, 0.75 mmol) was added to a solution of (−)-**3** (20 mg, 0.074 mmol) in pyridine (1 mL) containing 3 mg (0.025 mmol) of *p*-(dimethylamino)pyridine, and the resulting solution was kept at 70 °C for 18 h. After cooling, the mixture was diluted with EtOAc (15 mL), washed with H_2O and aqueous 10% Na_2CO_3, dried ($MgSO_4$), and evaporated in vacuo. The crude residue was purified by preparative TLC ($CHCl_3$/MeOH, 90:10) followed by crystallization from EtOH, giving 8 mg of pure **11** (TLC): ^1H NMR ($CDCl_3$) δ 2.9 (s, 12 H, CH_3), 4.6 (s, 2 H, NH_2), 6.4 and 7.7 (AA′BB′ system, 8 H, aromatic protons ortho and meta to the *N,N*-dimethylamino groups), ~6.8 (m, 2 H, C(10)H, C(11)H), 7.3 (m, 8 H, dibenzoazepine aromatic protons); IR (Nujol) 3450–3150 (NH_2), 1690 (C=O) cm^{-1}; UV λ_{max} (EtOH) 312 nm (ε 24 000); CD (EtOH) $\Delta\epsilon_{324}$ = +23, $\Delta\epsilon_{300}$ = −11.6 (see Figure 1).

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Registry No. **1**, 298-46-4; **2**, 36507-30-9; (−)-**3**, 106758-94-5; (±)-**3**, 106680-78-8; **4**, 106680-74-4; **5**, 885-23-4; **6**, 68011-71-2; **7**, 33948-22-0; **8**, 41359-09-5; **9**, 106680-75-5; **11**, 106680-76-6; **13** (*RRS*), 106680-77-7; **13** (*SSS*), 106759-88-0; (−)-MTPA chloride, 39637-99-5; *p*-$Me_2NC_6H_4COCl$, 4755-50-4; *m*-ClC_6H_4COOOH, 937-14-4; iminostilbene, 256-96-2; epoxide hydrolase, 9048-63-9.

Synthesis and Antiarrhythmic Activity of New 3-[2-(ω-Aminoalkoxy)phenoxy]-4-phenyl-3-buten-2-ones and Related Compounds

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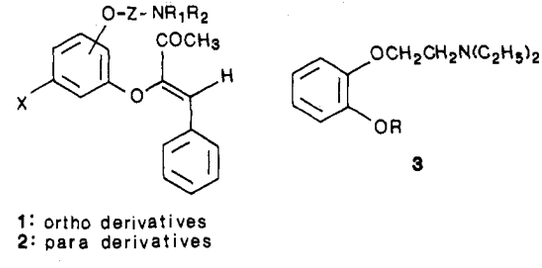
A number of the title compounds (**1**) and a few related hydroquinone derivatives (**2**) have been synthesized and tested for antiarrhythmic activity in vivo (protection against $CaCl_2$-induced ventricular fibrillation in anesthetized rat) and in vitro (ability to reduce the maximum driven frequency of an electrical stimulus in isolated rabbit atria). The effects induced by modification of the enol ether moiety in the parent compound **1a** were also examined. Many of the compounds exhibited antiarrhythmic properties stronger than quinidine and procainamide, associated with a more favorable LD_{50}/ED_{50} ratio. Compounds **1a** (LR-18,460, 3-[2-[2-(diethylamino)ethoxy]phenoxy]-4-phenyl-3-buten-2-one) and **1h** (LR-18,795, 3-[2-[3-(dimethylamino)propoxy]phenoxy]-4-phenyl-3-buten-2-one) were submitted to further antiarrhythmic testing, which confirmed their effectiveness and superiority to quinidine in all the experiments. After safety evaluation studies, both were selected for clinical investigation.

Previous pharmacological screening for new cardiovascular agents led us to discover the antiarrhythmic activity of several basic cyclic ethers of catechol, namely, aminoalkyl-substituted 1,3-benzodioxols and 1,4-benzodioxans.[1] During these studies, 3-[2-[2-(diethylamino)ethoxy]phenoxy]-4-phenyl-3-buten-2-one (**1a**), an open-chain catechol derivative, was also found to possess strong antiarrhythmic activity in an in vivo test ($CaCl_2$ intoxication in the rat). This result prompted us to prepare a series of catechol derivatives of general formula **1** and a few related hydroquinone derivatives **2** (Table I). In this paper, we report their synthesis and some preliminary pharmacological data, with particular reference to antiarrhythmic activity. Modifications of the enol ether function present in the parent compound **1a** were also performed in order to elucidate some structure–activity relationships (compounds of general formula **3**, Table II).

Chemistry. The syntheses of the new compounds **1** and **2**, listed in Table I, were performed according to the following routes (Scheme I), starting from mono enol ethers



3

1: ortho derivatives
2: para derivatives

4a, **4b**, and **5**, respectively: (A) aminoalkylation with the proper ω-chloroalkylamine or (B) alkylation with 1,3-dibromopropane or (C) 1-chloro-2,3-epoxypropane, followed by reaction with the various amines.

Starting catechol derivatives **4a,b** were prepared (Scheme II) by reacting the corresponding dihydroxybenzenes with (*Z*)-3-bromo-4-phenyl-3-buten-2-one according to a modification of a known procedure, which reduces the concurrent ring closure to benzodioxol and benzodioxan compounds.[2] Minor amounts of asymmetrical bis enol ethers **4c,d**, arising from a Michael-type addition of **4a,b** to the acetylenic intermediate originated by dehydrobromination of the substrate,[2] were also ob-

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