Stereochemical Aspects of the Metabolism of 5-(4′-Fluorophenyl)-5-phenylhydantoin in the Rat

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Racemic 5-(4′-fluorophenyl)-5-phenylhydantoin was synthesized to examine its metabolism in rat. This compound differs from the antiepileptic agent 5,5-diphenylhydantoin in that the normal site of hydroxylation in 5,5-diphenylhydantoin is blocked on one of the phenyl groups by a fluorine atom. The 4′-fluoro analogue gives a major metabolite, which was isolated and identified as (*R*)-(−)-5-(4′-fluorophenyl)-5-(4′-hydroxyphenyl)hydantoin of 37% enantiomeric purity. The absolute configuration and enantiomeric purity of the metabolite was determined by chemical conversion to (*S*)-(−)-5-(4′-hydroxyphenyl)-5-phenylhydantoin. A second metabolite of the catechol type, possibly as a mixture of 5-(3′,4′-dihydroxyphenyl)-5-(4′-fluorophenyl)hydantoin, and the corresponding *O*-3′-methyl derivative was detected by gas chromatography–mass spectrometry after methylation.

The metabolic fate of 5,5-diphenylhydantoin (DPH, **1**), a widely prescribed anticonvulsant,[1] has received considerable attention in recent years.[2] The main pathway of its biotransformation in man appears to be the hydroxylation by the hepatic microsomal enzymes system[3] of one of the phenyls to give (−)-5-(4′-hydroxyphenyl)-5-phenylhydantoin [HPPH, (−)-**2**] of the *S* configuration.[4]

The preparation of the *p*-fluoro analogues of the above compounds, 5-(4′-fluorophenyl)-5-phenylhydantoin (**3**) and 5-(4′-fluorophenyl)-5-(4′-hydroxyphenyl)hydantoin (**4**) and its enantiomers [(+)-**4** and (−)-**4**] was undertaken to examine the modifications of the metabolism of DPH when one site of hydroxylation is blocked by a fluorine substituent.

Chemistry. 4-Fluoro-4′-methoxybenzophenone (**5**) was obtained by refluxing a mixture of *p*-fluorobenzoyl chloride and anisole in the presence of iodine.[5] Under the conditions of the Büchere–Berg[6] reaction (as modified by Henze[7]), **5** was transformed into the corresponding hydantoin **7**;[8] similarly, **6** was converted into **3**. The hydantoin **7** was O-demethylated to **4**, which was resolved by fractional crystallization of the brucine salt in methanol to give (+)-**4** ($[\alpha]^{25}_{546}$ +120° (*c* 1, 0.5 N NaOH)) and (−)-**4** ($[\alpha]^{25}_{546}$ −120° (*c* 1, 0.5 NaOH)). The removal of the *p*-fluoro substituent in (+)-**4** was performed using a modification of the procedure of Buu-Hooi et al. A sample of (+)-**4** was refluxed in 2.5 N NaOH for 5 h in the presence of five times its weight of Ni–Al alloy, and, after filtration, acidification and preparative TLC yielded (*R*)-(+)-HPPH [(+)-**2**], $[\alpha]^{25}_{546}$ +102° (*c* 1, 0.5 N NaOH), which was identical (TLC, MS, and IR) with an authentic sample.[4] A specific rotation of +108° under the same conditions for (+)-**2** represents 100% enantiomeric purity;[10] (+)-**4** had an enantiomeric purity of 94.4%. Moreover, since the priorities according to the sequence rules of Cahn, Ingold, and Prelog[11] are modified in this transformation, (+)-**4** is of the *S* configuration.

Metabolism Study. Upon intraperitoneal administration of **3**, either as the free acid or the sodium salt, two compounds bearing a fluorophenyl moiety were detected by GC–MS analysis of the rat urine extracts after acidic deconjugation and permethylation with methyl iodide and tetramethylammonium hydroxide. These two metabolites were identified by comparison of their mass spectra with synthetic samples of trimethylated **4** (major component) and dimethylated 5-(3′,4′-dimethoxyphenyl)-5-(4′-fluorophenyl)hydantoin (**9**). Owing to the prior methylation process, it was not possible to decide whether this compound exists per se in urine or indicates the presence of 5-(3′,4′-dihydroxyphenyl)-5-(4′-fluorophenyl)hydantoin (**10**) or/and the corresponding 3′-*O*-methyl derivative **11**.

Scheme I

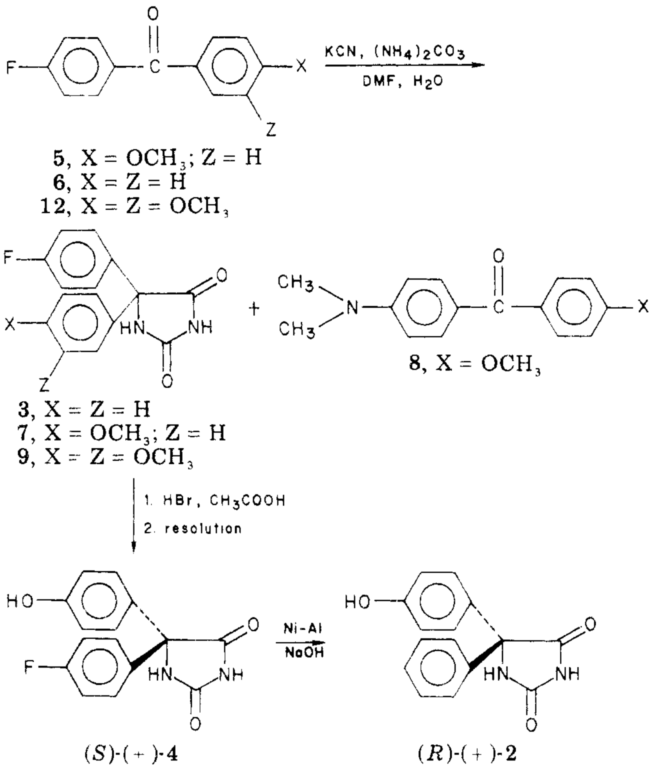

Table I. Comparative Values of the Molecular Rotation, $[\Phi]^{25}$, for (−)-4 at Different Wavelengths and Calculated Enantiomeric Purity (ep)

	$[\Phi]^{25}$, deg		
λ_{max}, nm	100% ep	urinary (−)-4	ep
546	−364	−134	36.8
435	−671	−252	37.5
404	−843	−315	37.3
365	−1184	−421	35.5

Subsequently, (−)-**4** was isolated by preparative TLC, and the above identification was confirmed by MS (direct inlet). An enantiomeric purity of approximately 37% was calculated on the basis of comparative ORD data (Table I). In order to demonstrate that a stereoselective process was involved in the production of **4** rather than an asymmetric destruction of one of the enantiomers of **4**, racemic **4** was administered under the same experimental conditions as **3**. Since **4** was recovered racemic from rat urines, the 37% enantiomeric purity can be related to the in vivo stereoselectivity of the para-hydroxylation process.

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Discussion

Interestingly, the metabolic profile of 3 appears comparable with that of 4-fluorobiphenyl,[12] and the same principal routes of hydroxylation are observed for DPH (1). Nevertheless, it should be noted that, while the urinary (−)-4 showed the same relative configuration (with respect to the position of the hydroxyl group) as urinary HPPH [(−)-2], the degree of stereoselectivity was significantly lowered.[14] This last phenomenon could possibly be explained by an extensive production of 5-(3′,4′-dihydroxy-1′,5′-cyclohexadienyl)-5-(4′-fluorophenyl)hydantoin of configuration opposite to (*R*)-(−)-4.

A communication recently appeared that reported the synthesis and results of a pharmacological evaluation of several fluorinated analogues of DPH,[15] including 3. Our pharmacological data fully confirmed the lack of activity in the pentylenetetrazol assay. Some preliminary pharmacokinetic experiments showed that this can probably be related to low brain levels.

Experimental Section

Melting points (corrected) were determined in a Thomas-Hoover capillary melting point apparatus. Elemental analyses are indicated only by symbols of the elements and are within 0.4% of the theoretical values. Optical rotations were measured with a Perkin-Elmer Model 141 spectropolarimeter, at room temperature (*c* 1, 0.5 N NaOH). Routine IR, NMR, and mass spectra were consistent with proposed structures.

4-Fluoro-4′-methoxybenzophenone (5), 4-fluorobenzoyl chloride (120 g, 0.75 mol) and an excess of anisole (300 g, 2.77 mol) under a stream of dry N_2 were refluxed in the presence of iodine (4.5 g) for 4.5 days, after which the dark-red color disappeared. The cooled reaction mixture was poured onto ice and thoroughly extracted with dichloromethane. The organic extract was dried ($MgSO_4$) and concentrated. The residue was distilled [bp 162 °C (0.15 mm)] and recrystallized from 95% C_2H_5OH (98 g, 56% yield), mp 96–98 °C. Anal. ($C_{14}H_{11}FO_2$) C, H.

(*R*,*S*)-5-(4′-Fluorophenyl)-5-(4′-methoxyphenyl)hydantoin (7). A solution of 5 (40 g, 0.174 mol) in 200 mL of DMF, a solution of potassium cyanide (18 g, 0.276 mol) in 30 mL of H_2O, and solid ammonium carbonate (60 g) were placed in a 500-mL steel pressure bottle, which was heated in an oil bath at 120 °C for 5 days. After cooling, the reaction mixture was diluted with hot water (1.5 L). The turbid mixture was acidified (10% HCl), cooled in an ice bath, and filtered. The crude product was washed with cold water and dissolved in 10% NaOH. An insoluble material was filtered and recrystallized from 95% C_2H_5OH to give pure 8,[8] mp 132–133.5 °C. Anal. ($C_{16}H_{17}NO_2$) C, H, N. The basic phase was then extracted with ether and acidified to give, after crystallization from ethanol, 15 g of 7: 29% yield; mp 243–246 °C. Anal. ($C_{16}H_{13}N_2O_3F$) C, H, N.

(*R*,*S*)-5-(4′-Fluorophenyl)-5-(4′-hydroxyphenyl)hydantoin [(±)-4]. To a stirred boiling solution of 7 (11 g, 0.038 mol) in glacial acetic (100 mL) was carefully added 48% hydrobromic acid (100 mL) through the reflux condenser. The reaction mixture was stirred and refluxed for 2 h. Cold water was then added until precipitation occurred. The product was crystallized from 95% ethanol: yield 11 g (95.3%); mp 311–312 °C; MS (*m/e*) 286 (70%), 257 (73%), 214 (100%), 122 (35%), 120 (41%). Anal. ($C_{15}H_{11}FN_2O_3$) C, H, N.

(*R*,*S*)-5-(4′-Fluorophenyl)-5-phenylhydantoin (3). 4-Fluorobenzophenone (6) (9.8 g, 0.049 mol) dissolved in 25 mL of DMF and potassium cyanide (5 g, 0.077 mol) dissolved in 25 mL of water were placed in a 120-mL steel pressure bottle. After addition of ammonium carbonate (18 g, 0.187 mol), the bottle was heated at 120 °C in an oven for 5 days. The procedure was identical with 7. The crude product was dissolved in boiling methanol, treated with charcoal, filtered, and water was added to induce crystallization: yield 4.65 g (35%), mp 280–282 °C.[15] Anal. ($C_{15}H_{11}FN_2O_2$) C, H, N.

(*R*)-(−)-5-(4′-Fluorophenyl)-5-(4′-hydroxyphenyl)hydantoin [(−)-4] and (*S*)-(+)-5-(4′-Fluorophenyl)-5-(4′-hydroxyphenyl)hydantoin [(+)-4]. Anhydrous brucine (34.8 g, 88.3 mmol) was added to a stirred boiling solution of (±)-4 (11 g, 38.4 mmol) in methanol (1.2 L). After stirring the solution for 24 h at room temperature and for 24 h in a refrigerator, a white precipitate was collected (16 g). Decomposition of a sample with 2 N HCl afforded partially resolved (−)-4 ($[\alpha]^{25}_{546}$ −91°). An additional crystallization of the salt and two crystallizations of the free hydantoin from ethanol–water gave 850 mg of (−)-4: $[\alpha]^{25}_{546}$ −120°; mp 319–321 °C. Further crystallizations did not modify the molecular rotation. The dextrorotatory mother liquors were evaporated to dryness. The salt was treated with 2 N HCl and the resulting partially enriched (+)-4 was recrystallized twice from ethanol–water to give a first crop of 500 mg of (+)-4: $[\alpha]^{25}_{546}$ +120°.

(*R*)-(+)-5-(4′-Hydroxyphenyl)-5-phenylhydantoin [(+)-2]. To a solution of (+)-4 (1 g, 3.72 mmol; $[\alpha]^{25}_{546}$ +120°) in 2.5 N NaOH (150 mL) small portions of Ni–Al alloy (5 g) were added during 2.5 h. The resulting suspension was then refluxed for 5 h. The catalyst was filtered and the filtrate was acidified with 2.5 N HCl. The crude product was contaminated with inorganic material and was purified by preparative TLC (silica gel 60 F; $CHCl_3$–acetone, 80:20, v/v) to give an 82% yield of (+)-2 ($[\alpha]^{25}_{546}$ +102°), which was identical (TLC, MS, and IR) with an authentic sample.[4]

3,4-Dimethoxy-4′-fluorobenzophenone (12). 4-Fluorobenzoyl chloride (40 g, 0.25 mol), veratrol (138 g, 1 mol), and iodine (2 g) were refluxed for 8 h under a slow stream of dry nitrogen. The cooled reaction mixture was washed with 5% K_2CO_3 (200 mL), 5% $Na_2S_2O_3$ (200 mL), and water (200 mL) and dried ($MgSO_4$). After distilling the excess of veratrol, the fraction boiling at 175–200 °C (1 mmHg) was collected (37 g) and recrystallized twice from methanol to give 26 g (40%) of 12, mp 117.5–119.5 °C. Anal. ($C_{15}H_{13}FO_3$) C, H.

(*R*,*S*)-5-(3′,4′-Dimethoxyphenyl)-5-(4′-fluorophenyl)hydantoin (9). Using the same procedure as for 7, 9 was obtained in 22% yield, mp 240–242 °C. Anal. ($C_{17}H_{15}FN_2O_4$) C, H, N.

Identification of Urinary Metabolites by GC–MS. A solution of the sodium salt of 3 in propylene glycol (25 mg/mL) was administered to male rats (Wistar strain, 250–300 g) by ip injection. Urines collected for 96 h were added to the same volume of 6 N HCl. The solution was refluxed for 1 h, brought to pH 4.5 and extracted with diisopropyl ether. The extract, evaporated under a N_2 stream, was dissolved in a DMF–methanol mixture, to which was added an excess of methyl iodide and tetramethylammonium hydroxide. After 3 h at room temperature, the mixture was filtered and the filtrate was used for the GC–MS analysis. Using a 3% OV17 stationary phase, GLC showed two peaks which were not present in the control urine. The first peak was identified as trimethylated 4 on the basis of its retention time and mass spectrum: *m/e* 328 (95%), 233 (100%), 149 (85%), 136 (64%), 107 (38%). The second peak was identified as dimethylated 9: *m/e* 358 (100%), 343 (12%), 330 (65%), 315 (10%), 263 (29%), 221 (10%), 178 (11%), 152 (18%). The presence of trimethylated 2 was not detected.

Isolation of Urinary (−)-4. The above diisopropyl ether extract was applied on preparative TLC plates [2-mm thickness, silica gel G F_{254} (Merck), solvent system CH_3OH–$CHCl_3$–benzene–NH_4OH (66:22:11:1)]. The band between R_f 0.5 and 0.6 was isolated, extracted with methanol, and rechromatographed on 0.5-mm plates. Isolation of the band migrating at the same R_f value as synthetic (±)-4 and methanol extraction gave (−)-4, which was authenticated by comparison with the mass spectrum of synthetic (±)-4.

References and Notes

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Antiarrhythmic Activity of Some *N*-Alkylbispidinebenzamides

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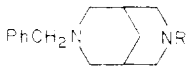
A series of aromatic ring substituted bispidinebenzamides, **2**–**10**, was prepared by condensation of *N*-methyl- or *N*-*n*-butylbispidine with the appropriate acid chlorides. These compounds were initially evaluated in mice for acute toxicity and for their ability to protect against chloroform-induced ventricular fibrillation. All compounds had significant activity, which was optimized in **2**, **3**, and **5**. These last compounds had potencies and LD_{50}/ED_{50} ratios comparable to those of a standard antiarrhythmic, disopyramide. However, their potencies in increasing the effective refactory period in isolated rabbit atria were considerably less than that of disopyramide.

Since the discovery of quinidine as an effective drug for maintaining normal heart rhythm,[1] a structurally diverse group of compounds has been used clinically in management of cardiac arrhythmias.[2] One of the more important structural categories of antiarrhythmic agents is the "amide-type",[3] which includes procainamide, lidocaine, disopyramide, and a large number of experimental compounds.[2,4] Agents in this category are characterized chemically by the presence of amide and amine functional groups.

In a previous paper, we described a series of N,N′-disubstituted bispidines, of which **1a**,**b** are representative.

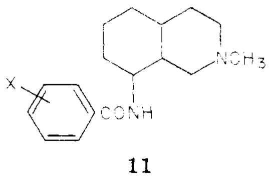


1a, R = CH_3
b, R = n-C_4H_9

These compounds were reasonably potent, but were, in general, quite toxic, with LD_{50}/ED_{50} ratios of less than 2.0.[5] In a preliminary attempt to increase potency and reduce toxicity, we evaluated the effect of replacement of the benzyl group of **1b** with a benzoyl group. The resulting "amide-type" analogue retained antiarrhythmic activity, and its toxicity was decreased more than twofold compared to that shown by **1b**. We wanted to determine the effect of a similar replacement in **1a** and to determine the effect of substitution in the aromatic ring of each of these benzamides. In other series of related antiarrhythmics, increases in potency, as well as the therapeutic index, have been observed as a result of the introduction of appropriate benzene-ring substituents.[6] Thus, we describe here the synthesis and evaluation of antiarrhythmic potencies and acute toxicities of **2**–**10**. Ring-substituted compounds were chosen in accordance with a previously reported operational scheme for finding the optimum substitution on a benzene ring in an active lead compound for maximization of pharmacologic activity.[7]

Results and Discussion

Compounds **2**–**10** were initially evaluated in mice for acute toxicity and for their ability to protect against chloroform-induced ventricular fibrillation. Disopyramide, one of the newer "amide-type" antiarrhythmics, was adopted as a standard. Results are shown in Table I. All of the compounds showed significant antiarrhythmic activity, which ranged over about one order of magnitude. Since the acute toxicities of the compounds did not vary greatly, differences in LD_{50}/ED_{50} ratios were primarily a reflection of differences in potency. In the *N*-methyl compounds **2**–**6**, activity was maximized in the 4-chloro compound **5**, with activity approximating that of disopyramide seen in the 4-methoxy compound **3** and the unsubstituted compound **2**. Multiply substituted compounds **4** and **6** were less active. The activity of **2**, **3**, and **5** was greater than that of their respective *N*-*n*-butyl counterparts. As in the *N*-methyl compounds, activity in the *N*-*n*-butyl compounds was maximized by monosubstitution. This finding is in contrast to results of similar studies of 8-substituted decahydroisoquinolines (**11**) and 6-substituted analogues as well. In these compounds, antiarrhythmic activity was maximal in the 3,4-dichloro- and 3,4,5-trimethoxybenzamides, respectively. Successive removal of substituents resulted in decreases in potencies.[8]



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The favorable potencies of **2**, **3**, and **5** in comparison to that of disopyramide in protection against ventricular fibrillation in the mouse led us to compare these compounds in a test for atrial antiarrhythmic potency. Thus, the compounds were evaluated in isolated rabbit atria for

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