## Synthesis and Anticholinergic Properties of the Enantiomers of 4-(Isopropylamino)-2-(2-pyridyl)-2-phenylbutyramide, the Mono-N-dealkylated Metabolite of Disopyramide

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The 2R and 2S enantiomers of 4-isopropyl-2-(2-pyridyl)-2-phenylbutyramide [(2R)-2 and (2S)-2] were prepared from the respective 2R and 2S enantiomers of disopyramide [(2R)-1 and (2S)-1] by oxidation with peracid, Cope elimination, and subsequent zinc/HCl reduction of the resulting hydroxylamines (2R)-3 and (2S)-3. The enantiomers were tested as antagonists to the contraction of guinea pig ileum longitudinal muscle produced in response to electrically stimulated release of acetylcholine. The enantiomers showed IC<sub>50</sub> values of  $5.0 \times 10^{-6}$  and  $14 \times 10^{-6}$  M for (2S)-2 and (2R)-2 respectively, about a 3-fold difference between enantiomers. Data are presented showing direct antagonism of acetylcholine in the guinea pig ileum assay. In a comparison of the anticholinergic effects of 2 and 1, the metabolite (2) was slightly less potent than disopyramide (1).

Disopyramide,<sup>1</sup> 4-(diisopropylamino)-2-(2-pyridyl)-2phenylbutyramide (1), is an agent used in the treatment





of cardiac dysrhythmias.<sup>2,3</sup> It has electrophysiologic properties similar to quinidine but is structurally unrelated to it or to any other major antiarrhythmic drug.<sup>4-6</sup> Even when the drug is effective in controlling arrhythmias, unwanted side effects limit its use.<sup>7-9</sup> Among its major untoward effects are those related to its anticholinergic properties, including dry mouth, gastrointestinal symptoms, and urinary retention.<sup>9,10</sup> Because of these anticholinergic side effects, disopyramide is contraindicated in the presence of glaucoma and myasthenia gravis.<sup>11</sup>

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Other side effects have been reported, including negative inotropic activity, which can result in congestive heart failure.12,13

Metabolically, in man, the major product is 4-(isopropylamino)-2-(2-pyridyl)-2-phenylbutyramide (2), formed by mono-N-dealkylation with loss of one N-isopropyl group from 1. This metabolite represents about 20-30% of the dose, with about 50-60% of the dose being excreted unchanged.<sup>14,15</sup> The metabolite (2) has been reported to be a less potent antiarrhythmic agent and to possess only slightly different pharmacological properties.<sup>16,17</sup> Although it is usually present in the blood in a concentration of one-third to one-fourth that of the parent drug, patients with high plasma concentrations of the metabolite (and correspondingly low levels of disopyramide) have been reported.<sup>18</sup>

The metabolite has been reported to possess significantly greater anticholinergic activity, about 25 times greater than disopyramide in a guinea pig ileum assay (acetylcholine as the agonist), and to have about  $1/_{50}$  of the molar potency of atropine.<sup>19</sup> In the same assay, disopyramide was about  $1/_{1200}$  as potent as atropine but was reported to be  $1/_{30}$   $-1/_{300}$ as active in vivo in anesthesized dogs.<sup>19</sup> In accordance with these findings, it has been suggested that at least a portion of the undesirable pharmacological effects could result from the presence of metabolite 2.

Differences in the dispositional behavior of the enantiomers of disopyramide have been recently reported to occur in dogs and rats.<sup>20,21</sup> Giacomini et al.<sup>20</sup> reported an

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Scheme I



ca. 50% shorter half-life for the (2S)-(+)-enantiomer in dogs after iv administration of the drug, with a corresponding difference in clearance. Kook et al.<sup>21</sup> reported a more than 2-fold difference in  $C_{\max}$  and AUC<sub> $\infty$ </sub> after oral administration in dogs, with lower values for the (+)-enantiomer, corresponding to greater metabolism of it. Similar differences were reported in rats following oral administration of the enantiomers of disopyramide.<sup>21</sup>

Differences in the anticholinergic potency of the enantiomers of disopyramide have been noted by Giacomini et al.,<sup>22</sup> with the (2S)-(+)-enantiomer being more active by 3- to 4-fold in assays in guinea pig ileum longitudinal muscle as an antagonist to the electrically stimulated release of acetylcholine or to acetylcholine directly added to the bath. Kook et al.<sup>21,23</sup> noted a similar difference of 3.6-fold [(+) > (-)] in the guinea pig ileum assay (acetylcholine as agonist) and 1.25- to 1.76-fold in a mouse mydrasis assay when the enantiomers of 1 were administered intragastrically, ip, or iv.<sup>21,23</sup> Recently, Mirro et al.<sup>24</sup> noted about a 3-fold difference in potency [(+) > (-)] in competing with tritiated 3-quinuclidinyl benzilate for binding in guinea pig atrial preparations.

In this paper, we report the synthesis of the enantiomers of 2 of known absolute configuration and their activity in a guinea pig ileum assay (electrically stimulated release of acetylcholine). Data are also presented showing the enantiomers of 2 directly antagonize the effects of acetylcholine in the guinea pig ileum assay.

Chemistry. The enantiomers of disopyramide (1) were obtained by selective crystallization of diastereomeric bitartrate salts, as previously reported.<sup>25</sup> The facile Ndealkylation procedure reported by Karim et al.,<sup>26</sup> based on peracid oxidation, Cope elimination, and reduction of the resultant hydroxylamine (3) (Scheme I), was applied to the individual enantiomers. The intermediate hydroxylamines [(2R)-3 and (2S)-3] were not isolated, but reduced with zinc in the presence of HCl to the enan-

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Figure 1. Log concentration-response curves obtained for (2S)-2 (•) and for (2R)-2 (0) in two guinea pig ileum strips. The IC<sub>50</sub> value for (2S)-2 in this experiment is  $3.5 \times 10^{-6}$  M and for (2R)-2 is  $2.5 \times 10^{-5}$  M.

tiomers of 2, which were isolated as their acid oxalate salts:  $[\alpha]_{\rm D}$  +24.6° for (2R)-2 [from (2R)-1] and  $[\alpha]_{\rm D}$  -25.7° for (2S)-2 [from (2S)-1], in ca. 20% overall yield. Their CD spectra were mirror images of each other and are in agreement with those of the corresponding disopyramide enantiomers, as their acid oxalate salts.<sup>27</sup>

Pharmacological Testing. Representative log concentration-response curves of (2S)-2 and (2R)-2 are shown in Figure 1. The curves appeared to be log linear in the range between 20 and 80% of the maximal response. The  $IC_{50}$  value (mean ± SEM) for (2S)-2 (four strips) was 5.0  $\times 10^{-6} \pm 1.2 \times 10^{-6}$  M and for (2R)-2 was  $14 \times 10^{-6} \pm 3.8$  $\times$  10<sup>-6</sup> M (four strips), indicating that the 2S enantiomer of 2 was about 3-fold more potent than the 2R enantiomer (p < 0.05). The observation that (2S)-2 is a more potent anticholinergic agent than the 2R enantiomer is consistent with what has been observed previously for disopyramide.<sup>22</sup> i.e., the 2S enantiomer of disopyramide [(2S)-1] is 3- to 5-fold more potent as an anticholinergic agent than the 2Renantiomer. No effects on contractile tension by sodium oxalate were observed.

Because electrically stimulated contractions result from release of acetylcholine from the cholinergic neurons of the myenteric plexus,<sup>28</sup> it was necessary to determine whether the effect of 2 on antagonizing these contractions was due to direct antagonism of acetylcholine at muscarinic receptors on the muscle fibers. Therefore, acetylcholine stimulation experiments were performed in the presence of racemic 2 and 1. Both compounds directly antagonized acetylcholine-induced contractions. The mean  $K_{\rm D}$  for 1 in the two strips was  $2.2 \times 10^{-6}$  M ( $2.8 \times 10^{-6}$  and  $1.6 \times$ 10<sup>-6</sup> M) and for the metabolite 2 was  $3.8 \times 10^{-6}$  M ( $3.9 \times$  $10^{-6}$  and  $3.8 \times 10^{-6}$  M). Neither compound showed any significant effect against histamine-induced contractions; the compounds, therefore, do not depress ileum smooth muscle contractility at these concentrations. Because the  $IC_{50}$  values for 1 and 2 in inhibiting contractions induced by applied acetylcholine are similar to their  $IC_{50}$  values in inhibiting the response to electrical stimulation, it is likely that the locus of their inhibition of the response to cholinergic neuron activation is blockade of the released acetylcholine at muscarinic receptors located on the smooth-muscle fibers and not a reduction in the output of acetylcholine released from the cholinergic neuron.

The potency ratio of racemic 1 and 2 obtained in the acetylcholine antagonism study is consistent with what was

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observed when 1 and 2 were directly compared in their ability to antagonize electrically stimulated contractions. The mean  $IC_{50}$  value obtained in the two strips for racemic 1 was  $1.5 \times 10^{-6}$  M ( $1.2 \times 10^{-6}$  and  $1.8 \times 10^{-6}$  M) and for 2 was  $3.2 \times 10^{-6}$  M ( $2.5 \times 10^{-6}$  M,  $3.9 \times 10^{-6}$  M). Because both the acetylcholine and the electrically stimulated contractions were performed in a total of only four guinea pig ileum strips, the observed potency differences between 1 and 2 as anticholinergic agents are not conclusive. Both 1 and 2 appear to be about equipotent as anticholinergic agents when the results obtained in a previous study using the enantiomers of 1<sup>22</sup> are compared to those obtained in this study with the enantiomers of 2. Thus, it appears that both 1 and 2 are anticholinergic agents and that 1 may be somewhat more potent than 2. These results are in conflict with the findings of Baines et al.<sup>19</sup> who observed in guinea pig ileum strips that racemic 2 had about 25 times greater anticholinergic potency than racemic disopyramide (1). However, little experimental detail was given in the report of their study, preventing appropriate comparison.

The clinical implications of the observed differences in anticholinergic potencies are unclear, since in vitro data can not be extrapolated directly to humans. However, it is interesting that results from this study and from a previous study<sup>22</sup> indicate that the 2S enantiomers of both the metabolite and parent drug are 3- to 4-fold more potent than the respective 2R enantiomers as anticholinergic agents. Moreover, the mean  $IC_{50}$  values for (2S)-disopyramide  $(4.5 \times 10^{-6} \text{ M or } 1.5 \,\mu\text{g/mL})$  and for (2S)-2 (5 ×  $10^{-6}$  M or  $1.5 \,\mu g/mL$ ) are well within the range of plasma concentrations obtained after chronic therapy with racemic disopyramide.<sup>22</sup> Because of the significant anticholinergic side effects associated with disopyramide administration, these results suggest a need for clinical studies on the pharmacology and pharmacokinetics of single enantiomers of 1.

## **Experimental Section**

Melting points were determined on a Fischer-Johns capillary melting point apparatus and are corrected. Circular dichroism spectra were recorded on a Jobin Yvon Dichrographe R.J. Mark III instrument. Optical rotations were determined on a Jasco DIP-4 digital polarimeter. Chemical ionization mass spectral data were obtained on a Biospect mass spectrometer using methane as reagent gas. Microanalyses were performed by Dr. F. B. Strauss, Oxford, England. Where indicated by the symbols of the elements, analyses were within  $\pm 0.4\%$  of theoretical values.

(2R)-4-(Isopropylamino)-2-phenyl-2-(2-pyridyl)butyramide [(2R)-2]. To a stirred solution of (2R)-(-)-disopyramide [(2R)-1],<sup>25</sup> mp 83-85 °C,  $[\alpha]_D$ -19.4° (c 1.0, absolute methanol), 3.40 g (10.0 mmol), in 65 mL of CH<sub>2</sub>Cl<sub>2</sub> at 5 °C, was added 2.1 g of 85% *m*-chloroperoxybenzoic acid (10.3 mmol) in several portions over 10 min. The reaction mixture was stirred at room temperature for 35 min and washed with a solution prepared from 3.0 g of Na<sub>2</sub>SO<sub>4</sub>, 5 mL of concentrated aqueous NH<sub>3</sub>, and 30 mL of H<sub>2</sub>O. The aqueous solution was washed with CH<sub>2</sub>Cl<sub>2</sub> (2 × 25 mL), and the combined organic extracts were dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to dryness. The residue was dissolved in 25 mL of benzene, heated to reflux for 10 min, and evaporated to dryness to afford the crude hydroxylamine (2R)-3 as an oil.

To a stirred solution of the crude hydroxylamine [(2R)-3] in 75 mL of EtOH (95%) was slowly added concentrated aqueous HCl (10 mL). Zinc dust, 4.00 g (61.1 mg-atoms), was added in several portions over 20 min with stirring. After an additional 20 min, granular K<sub>2</sub>CO<sub>3</sub> (5.0 g) was added and the mixture stirred for 30 min. The mixture was filtered and residue extracted with several portions of EtOH. The combined EtOH extracts were neutralized (pH ~7) with aqueous 4% K<sub>2</sub>CO<sub>3</sub> solution, evaporated to near dryness, made alkaline with aqueous 10% NaOH solution, and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 25 mL). Evaporation of the combined CH<sub>2</sub>Cl<sub>2</sub> extracts afforded an oil, which showed four spots by silica gel TLC:  $R_f$  0.60, 0.48, 0.35, and 0.20 (EtOAc-MeOH- concentrated aqueous NH<sub>3</sub>, 80:20:2). The slowest running spot corresponds to 2. Column chromatography on 30 g of silica gel was performed, eluting with 250 mL of CHCl<sub>3</sub>, with 1500 mL of CHCl<sub>3</sub>-MeOH-concentrated aqueous NH<sub>3</sub> (90:10:2), and then with 100 mL of CHCl<sub>3</sub>-MeOH-concentrated aqueous NH<sub>3</sub> (90:10:2), and then with 100 mL of CHCl<sub>3</sub>-MeOH-concentrated aqueous NH<sub>3</sub> (70:30:5). (2R)-2 was eluted in the most polar solvent system. To the oil obtained, 800 mg (27% of theory), in EtOH was added oxalic acid dihydrate (325 mg, 1.1 equiv) and the salt crystallized from EtOH-Et<sub>2</sub>O, affording 750 mg of the acid oxalate salt of (2R)-2, mp 172-173 °C;  $[\alpha]_D 24.6^\circ$  (c 1.0, EtOH); CIMS, m/z 298 (QM); CD (EtOH)  $[\theta]_{285}$  0,  $[\theta]_{287}$  -7900,  $[\theta]_{285}$  -4000,  $[\theta]_{287}$  -5390,  $[\theta]_{292}$  0,  $[\theta]_{210}$  +7400. The CD spectrum of (2R)-disopyramide acid oxalate (EtOH) shows a maximum at  $[\theta]_{285}$  0,  $[\theta]_{285}$  -15800,  $[\theta]_{252}$  -11300,  $[\theta]_{224}$  -15000,  $[\theta]_{224}$  0,  $[\theta]_{210}$  = +24000. Anal. (C<sub>20</sub>H<sub>25</sub>N<sub>3</sub>O<sub>5</sub>) C, H, N.

(2S)-4-(Isopropylamino)-2-phenyl-2-(2-pyridyl)butyramide [(2S)-2]. The 2S enantiomer of 2 was prepared from (2S)-(+)-disopyramide [(2S)-1],<sup>25</sup> mp 83-85 °C,  $[\alpha]_D$  +18.9° (c 1.0, EtOH), by an analagous procedure to afford (2S)-2 as the acid oxalate salt (21% overall yield): mp 171-172 °C;  $[\alpha]_D$  25.7° (c 1.0, EtOH); CIMS, m/z 298 (QM); CD (EtOH) [ $\theta$ ]<sub>285</sub> 0,  $[\theta]_{286}$ +7900,  $[\theta]_{250}$  +4250,  $[\theta]_{235}$  +5830,  $[\theta]_{225}$  0;  $[\theta]_{210}$  -6500. Anal. (C<sub>20</sub>H<sub>25</sub>N<sub>3</sub>O<sub>5</sub>) C, H, N.

Pharmacological Testing. To assess the anticholinergic properties of the enantiomers of 2, eight longitudinal muscle strips with the myenteric plexus attached were obtained from two male Hartley guinea pigs. Each was studied in a 5-mL organ bath according to the method of Goldstein and Schulz.<sup>29</sup> The baths contained Krebs-bicarbonate buffer with the following composition (mM): NaCl, 118; KCl, 4.75; CaCl<sub>2</sub>, 2.54; MgSO<sub>4</sub>, 1.20; KH<sub>2</sub>PO<sub>4</sub>, 1.19; NaHCO<sub>3</sub>, 25.0. The baths also contained glucose, 11.1 mM, choline chloride 20  $\mu$ M, and pyrilamine maleate, 125 nM. The baths containing the strips were oxygenated with 95% O<sub>2</sub> and 5% CO<sub>2</sub> and maintained at 37 °C. Contractions were induced by field stimulation at 0.1 Hz with 25-ms pulses at maximal voltage (80 V). Isometric responses were recorded on a Grass Model 7B polygraph. After equilibration for approximately 1-2 h, during which the bath solutions were changed frequently, cumulative dose–response curves for (2R)- and (2S)-2 (oxalate salts) were obtained. Four strips (two from each guinea pig ileum) were tested with (2R)-2 oxalate and the other four strips with (2S)-2 oxalate. Each dose of the enantiomer was left in contact with the tissue for at least 3 min before the next incremental dose was added. Concentrations of (2R)- and (2S)-2 ranged from  $2 \times 10^{-7}$  to  $2 \times 10^{-4}$  M. In order to determine whether the oxalate anion (present as the acid oxalate salts of the enantiomers of 2) had an effect on contractile force, sodium oxalate was added to four ileum strips to achieve a concentration in the bath of 2  $\times$  10<sup>-4</sup> M.

To assess the relative potencies of racemic disopyramide (1) and its monodealkylated metabolite (2) in antagonizing electrically stimulated contractions, two additional guinea pig ileum strips were obtained from a third male Hartley guinea pig. The study was conducted similarly to the study determining the anticholinergic potencies of the enantiomers of 2. One strip initially received racemic disopyramide while the other received racemic metabolite. The bath concentration of drug was increased progressively. Each concentration of drug was left in contact with the tissue for at least 3 min. After contractions had been reduced to 20% or less of initial control levels, the tissues were washed repeatedly for 60 min and then the dose-response curve was repeated. The first strip then received the metabolite and the second disopyramide.

On two additional strips obtained from the third male Hartley guinea pig were constructed dose-response curves for acetylcholine and histamine in the presence of racemic disopyramide (1) or racemic monodealkylated metabolite (2). In these strips no electrical stimulation was used to induce contractions. Instead, contractions were induced by applying appropriate concentrations of acetylcholine or histamine to the tissues for periods of 30 s, after which the tissues were washed with bath solution. Contractions were recorded using an isotonic transducer (Ealing Co.). Two minutes were allowed between each drug (acetylcholine or

<sup>(29)</sup> A. Goldstein and R. Schulz, Br. J. Pharmacol., 48, 655 (1973).

histamine) administration. Three concentrations of acetylcholine and histamine were applied, each being repeated once, in a random sequence. A 10  $\mu$ M concentration of disopyramide was applied to bath 1 and a similar concentration of the dealkylated metabolite to bath 2. The tissues were left in contact with the drug for 20 min. The cycle of acetylcholine and histamine doses was then repeated, with disopyramide and the metabolite being added to the bath after each wash of the tissues. At the completion of the dose cycle, the tissues were washed frequently for 60 min before the control cycle of acetylcholine and histamine doses was repeated. Then, 10  $\mu$ M disopyramide was applied to bath 2 and 10  $\mu$ M metabolite was applied to bath 1. After 20 min in contact with the drug, the acetvlcholine and histamine dose cycle was again repeated, with the drug or metabolite readministered after each wash.

Dose-response curves were constructed by plotting the percentage inhibition of contractile tension against the  $\log_{10}$  of the cumulative concentration of the isomer in the bath. The concentration which resulted in 50% inhibition of contractile tension  $(IC_{50})$  was determined from the plots after the slope and intercept of the linear portions were obtained by log-linear least-squares

regression analysis. The statistical significance of the difference between the IC<sub>50</sub> values of the two isomers in inhibiting electrically stimulated contractions were evaluated with a Wilcoxon test. No statistical analysis was performed on the two strips where the relative potencies of the metabolite and disopyramide were evaluated.

For the acetylcholine and histamine studies, contraction heights were measured for each response to both compounds in the presence and absence of racemic disopyramide and monodealkylated disopyramide. Dose ratios were calculated as the ratio of stimulant concentration required to give a defined effect in the presence of drug (disopyramide or metabolite) to the equivalent concentration in the absence of drug. From the dose ratio, an estimate of the antagonist dissociation concentration was determined  $[K_D = antagonist concentration/(dose ratio)].$ 

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## Synthesis of Spiro[isobenzofuran-1(3H),4'-piperidines] as Potential Central Nervous System Agents. 6. Synthesis, <sup>13</sup>C NMR, and Biological Evaluation of *cis*and trans-4-Amino-3'-arylspiro[cyclohexane-1,1'(3'H)-isobenzofuran] Derivatives<sup>1</sup>

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4-(Dimethylamino)- and 4-(methylamino)-3'-arylspiro[cyclohexane-1,1'(3'H)-isobenzofuran] derivatives were prepared as analogues of previously reported 3-arylspiro[isobenzofuran-1(3H),4'-piperidines]. Metalation of benzanilide with n-butyllithium, addition of 4-(dimethylamino)cyclohexanone, and acidification afforded a mixture of cis- and trans-4-(dimethylamino)spiro[cyclohexane-1,1'(3'H)-isobenzofuran]-3'-ones (1a,b), which were separated by fractional crystallization. Addition of aryllithium or aryl Grignard reagents to 1a,b and formic acid reduction afforded cisand trans-4-(dimethylamino)-3'-arylspiro[cyclohexane-1,1'(3'H)-isobenzofurans] 3a-f, which were converted to secondary amine analogues 5a-e. Tentative stereochemical assignments are based on chemical arguments and are supported by <sup>13</sup>C NMR chemical shift data. Marked inhibition of tetrabenazine-induced ptosis is a property of most antidepressants, and significant antitetrabenazine activity is observed for several of these compounds. Optimal antitetrabenazine activity is associated with the cis-3'-phenyl series, and the cis secondary amine 5a is approximately twice as potent as the cis tertiary amine 3a. The various compounds are relatively weak with respect to potentiation of L-5-hydroxytryptophan-induced seizures.

We previously reported the synthesis, pharmacology, and biochemical properties of 3-arvlspirolisobenzofuran-1(3H),4'-piperidines] and conformationally mobile analogues.<sup>1,3–13</sup> Many of these compounds display strong

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Scheme I



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