of water to the residue afforded a yellow precipitate, which was collected by filtration and was recrystallized from ethyl acetate–ethanol (1:3) to yield **19** (Table II) which was used without further purification.

***trans*-4′-(Hydroxymethyl)-4-nitrostilbene (20).** A mixture of $CaCl_2$ (0.159 g, 1.41 mmol) and $NaBH_4$ (0.109 g, 2.86 mmol) in dry THF (60 mL) was stirred at room temperature for 1 h. A solution of **19** (0.28 g, 0.955 mmol) in 30 mL of dry THF was added in one portion, and the mixture was stirred at room temperature for 18 h, cooled in an ice bath, and 3.5 mL of water was added dropwise. The solution was acidified to pH 1.5 by the dropwise addition of cold 6 N HCl, and the resulting mixture was stirred at room temperature for 1 h. The THF was evaporated to afford 0.2 g of a solid material, which was purified by column chromatography on silica gel. Elution with $CHCl_3$ yielded **20** (Table II).

***trans*-4′-Substituted-4-aminostilbenes (10–13 and 21; Table II).** A solution of 0.17 mol of $SnCl_2$ in 40 mL of concentrated HCl was added dropwise to a solution of 0.02 mol of the *trans*-4′-substituted-4-nitrostilbene in 125 mL of glacial acetic acid with constant stirring (**20** was dissolved in warm THF) and the reaction mixture was stirred overnight at room temperature. A precipitate formed which was collected by filtration, washed with 50 mL of glacial acetic acid, and suspended in 1.4 L of H_2O. The aqueous suspension was adjusted to pH 9–10 with NaOH pellets and was extracted with $CHCl_3$. The combined $CHCl_3$ extracts were washed with 150 mL of water and dried over $MgSO_4$, and the $CHCl_3$ was evaporated under reduced pressure. Compounds **10**, **11**, and **13** were recrystallized from ethanol, while compounds **12** and **21** were recrystallized from hexane and benzene, respectively.

***trans*-4′-Substituted-4-acetamidostilbenes (2–5 and 22; Table I).** The appropriate *trans*-4′-substituted-4-aminostilbene (0.5 mmol) was dissolved in 50 mL of benzene. Acetic anhydride (0.5 mmol) was added and the reaction mixture was stirred overnight at room temperature. The precipitate was collected by filtration and recrystallized to a constant melting point, constant specific activity, and homogeneity by TLC.

***trans*-4′-Substituted-4-(*N*-hydroxyacetamido)stilbenes (14–17; Table III).** The appropriate *trans*-4′-substituted-4-nitrostilbene (0.01 mol) was dissolved in 80 mL of DMF, 40 mL of ethanol, and 20 mL of water. Ammonium chloride (0.04 mol) and zinc dust (0.04 mol) were added and the mixture was stirred for 1 h at room temperature. The reaction mixture was filtered and the filter cake was washed with two 50-mL portions of DMF. The washes and the filtrate were combined and poured into 30 mL of water. The hydroxylamine was extracted from the aqueous phase with three 100-mL portions of ether, which were combined and washed with three 50-mL portions of saturated NaCl. The ether solution was cooled to 0 °C and 5 mL of saturated sodium bicarbonate was added. Acetyl chloride (0.01 mol) in 25 mL of ether was added dropwise over 20 min to the reaction mixture. The mixture was stirred for 30 min and the ether was removed under reduced pressure. The residue was stirred with 150 mL of saturated sodium bicarbonate solution for 1 h at 0 °C. The hydroxamic acid was collected by filtration, washed with 100 mL of water, and recrystallized from benzene. The ^{14}C-labeled hydroxamic acids were synthesized using 0.05 mmol of the nitrostilbene and 0.001 mCi of [1-^{14}C]acetyl chloride. The products were recrystallized to constant melting points, constant specific activity, and homogeneity by TLC.

***trans*-4′-Substituted-4-(2-hydroxyacetamido)stilbenes (23–25; Table III).** The appropriate *trans*-4′-substituted-4-aminostilbene (1.0 mmol) was dissolved in 15 mL of dry benzene along with 10 mmol of ethyl glycolate. The solution was heated under reflux for 42 h, cooled to room temperature, and 25 mL of petroleum ether was added. The glycolamide was collected by filtration and was recrystallized from benzene.

Acknowledgment. The authors express their gratitude to Professor M. W. Anders for his advice and encouragement. This research was supported in part by American Cancer Society Grant BC-190. Richard E. Gammans was the recipient of NIH postdoctoral fellowship CA 05746.

Resolution, Absolute Configuration, and Antiarrhythmic Properties of the Enantiomers of Disopyramide, 4-(Diisopropylamino)-2-(2-pyridyl)-2-phenylbutyramide

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Received April 7, 1980

Disopyramide was resolved by fractional crystallization of its diastereomeric bitartrate salts from methanol–acetone. Diastereomeric sulfonamides prepared from (+)-camphor-10-sulfonyl chloride and the primary amines obtained by $LiAlH_4$ reduction of the resolved bases were separable by high-performance LC and were used to show that within experimental error resolution of disopyramide was complete. The absolute configuration was determined by X-ray crystallography. (+)-[(2*R*)-(−)-4-(Diisopropylamino)-2-(2-pyridyl)-2-phenylbutyramide (+)-(2*R*,3*R*)-bitartrate] crystallizes in space group $P2_12_12_1$: a = 14.789 (4), b = 18.151 (4), c = 9.878 (2) Å; Z = 4; D_x = 1.225, D_m (flotation C_6H_6/CCl_4) = 1.226 g cm^{-3}. The structure was solved by direct methods. The enantiomeric bitartrates were tested for antiarrhythmic properties. The enantiomeric bitartrate salts were equally effective in prolonging the effective refractory period (ERP) of rabbit ventricle. At 3×10^{-6} M, the (−)-bitartrate [from (2*S*)-(+)-disopyramide] increased the ERP 21.8 ± 6.3 ms and the (+)-bitartrate [from (2*R*)-(−)-disopyramide] increased the ERP 25.8 ± 3.6 ms. At 1.5×10^{-5} M no significant difference was noted, as the increases in the ERP were 41.2 ± 8.9 and 50.5 ± 6.3 ms for the (−)- and (+)-bitartrate, respectively.

Disopyramide [1; 4-(diisopropylamino)-2-(2-pyridyl)-2-phenylbutyramide][1] is a recently available, orally active, quinidine-like agent used in the treatment of cardiac dysrhythmias.[2,3] Interest in its pharmacokinetic properties

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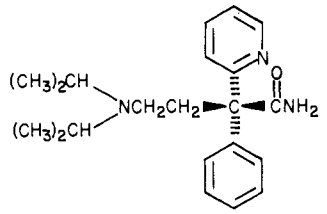


1, shown as the 2*R* enantiomer

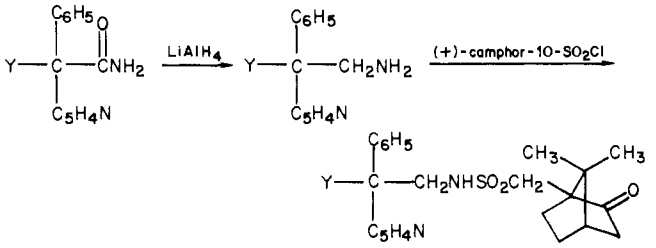
and metabolic fate has greatly increased as this agent has become more widely used.[4-10] Our interest in the relationships between stereochemistry and the biological effects and metabolism of drugs led us to attempt to resolve it into its enantiomers. In this paper, we report a facile resolution of the disopyramide enantiomers as their bitartrate salts, the determination of the absolute configuration of the enantiomers by X-ray crystallographic examination of one of the bitartrates, and the antiarrhythmic properties of these enantiomers.[11]

Resolution. Disopyramide was resolved by repeated crystallization of the salt formed by the addition of 1 equiv of (2*R*,3*R*)-(+)-tartaric acid to a solution of disopyramide in methanol–acetone. Repeated crystallization from absolute methanol–acetone (1:10) afforded one diastereoisomeric salt, $[\alpha]^{25}_D$ +36.0°. Decomposition of the salt and crystallization (*n*-hexane) afforded the free base, $[\alpha]_D$ −19.4°. Disopyramide enriched in the (+) enantiomer was recovered from the filtrates of the initial resolution. The enantiomeric bitartrate, formed using (2*S*,3*S*)-(−)-tartaric acid, was obtained by crystallization from methanol–acetone (1:10), $[\alpha]_D$ −36.0°, and subsequently converted to the enantiomeric free base, $[\alpha]_D$ +18.9°.

To establish optical purity, the enantiomers were reduced with lithium aluminum hydride, and the resulting primary amines were converted to their diastereomeric sulfonamides by reaction with (+)-camphor-10-sulfonyl chloride (Scheme I). The diasteriomeric sulfonamides were separable by high-performance LC, and no contamination of one by the other was observed (Figure 1).

Crystallography. The X-ray structure and determination of absolute configuration was accomplished using

Scheme I[a]



[a] Y = $-CH_2CH_2N(i\text{-}Pr)_2$

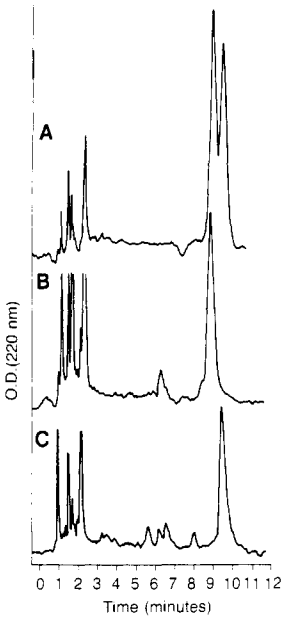


Figure 1. (A) High-performance LC trace of the products obtained by treatment of (+)-camphor-10-sulfonyl chloride with the primary amines obtained by $LiAlH_4$ reduction of racemic 1, (B) of (+)-1, and (C) of (−)-1. Separation conditions are given under Experimental Section.

the (+)-disopyramide (2*R*,3*R*)-bitartrate salt. It crystallizes from methanol–acetone (1:10), in orthorhombic space group $P2_12_12_1$: a = 14.789 (4), b = 18.151 (4), c = 9.878 (2) Å; Z = 4; D_x = 1.225, D_m (flotation C_6H_6/CCl_4) = 1.226 g cm^{-3}. The structure was solved by direct methods.[14-17]

Of critical importance to the determination of absolute configuration of the disopyramide molecule was the unambiguous discrimination of the phenyl and 2-pyridyl groups. The position of N(3) was assigned on the basis of the short bond distance, 1.345 (7) Å, from aromatic ring C(11) to one of the adjacent ring atoms.[15] The other interatomic bond distances from the aromatic ring carbons to adjacent ring positions varied from 1.377 to 1.396 Å. In the earlier stages of refinement, this position had also been included as a carbon atom. In the final stages of refinement, positional and isotropic thermal parameters for hydrogen atoms were allowed to vary along with positional

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Table I. Changes in Effective Refractory Period (ΔERP) Induced in Rabbit Ventricle by the Enantiomeric Disopyramide Bitartrates

disopyramide concn, M	ΔERP, ms, ± SEM (2R)-1[a]	N	(2S)-1[a]	N
3.0×10^{-6}	+25.8 ± 3.6	6	+21.8 ± 6.3[b]	5
1.5×10^{-5}	+50.5 ± 6.3	4	+41.2 ± 8.9[b]	5

[a] (2R)-1 is the (+)-(2R,3R)-bitartrate of (-)-(2R)-1. (2S)-1 is the (-)-(2S,3S)-bitartrate of (+)-(2S)-1. [b] Effect on ΔERP is not statistically different from (2R)-1 by one-tailed Student's t test ($p \geq 0.50$).

and anisotropic thermal parameters of the heavy atoms until convergence at $R = 0.066$. A final difference map was calculated omitting H(C6). This atom appeared as a peak much larger than other randomly distributed peaks (0.43 vs. 0.25 e/Å^3). None of the randomly distributed peaks were within bonding distance of N(3), thus confirming the identification of this nitrogen atom and the pyridine ring.

A stereoscopic drawing of (+)-disopyramide (2R,3R)-bitartrate is shown in Figure 2. The (-)-disopyramide enantiomer in this salt has the 2R absolute configuration. Supplementary material available include Figure 3, which contains pertinent angles and bond distances; Table II, which contains atomic positional parameters; and Table III, which contains structure factors and thermal parameters.

Antiarryhthmic Activity. Antiarrhythmic drugs may exert their therapeutic effect on the heart in a variety of ways, dependent in large measure on the nature of the arrhythmia. Reentry arrhythmias may be terminated by prolonging the effective refractory period (ERP) of myocardial cells and/or increasing the velocity of impulse propagation through the myocardium.[19] Disopyramide and quinidine prolong the ERP but slow myocardial conduction velocity.[20]

The enantiomeric bitartrate salts were tested for their effect on the ERP of the ventricular myocardium of rabbits as an estimate of relative antiarrhythmic activity. The results appear in Table I. The enantiomers are nearly equiactive.

Our finding that the effects of disopyramide in prolonging ERP resides in both the enantiomers in essentially equal amounts is in agreement with the lack of stereoselectivity among most antiarrhythmic agents.[21] Kook et al.[12] have shown that the anticholinergic effect of disopyramide, which requires interaction with a specific membrane receptor, is more stereoselective. (+)-1 had 3.6 times the anticholinergic activity of (-)-1. Mirro, Watanabe, and Bailey[13] have examined the effects of racemic 1 and its enantiomers on the action-potential duration (APD) in canine cardiac Purkinje fiber and report that racemic 1 and (+)-1 prolong APD, while (-)-1 shortens APD. These results suggested that refractory periods would be similarly affected, a suggestion that is not supported by our results. The difference could, of course, be due to species and/or preparation differences. On the other hand, refractory period duration may not be absolutely dependent on the APD. For example, Dawson et al.[22] observed that APD increases induced by propranolol were reversed by simultaneous infusions of isoproterenol, whereas the propranolol-induced ERP prolongation was not. Thus, APD effects may be mediated through β-adrenergic receptors, while the ERP effects may not be.

Although our results show no difference in the antiarrhythmic activity of the enantiomers of 1, as measured by change in ERP, differences in other pharmacological properties of the enantiomers and/or of circulating metabolites, e.g., anticholinergic properties,[23] could be important to the therapeutic use of the drug. Similarly, dispositional characteristics, e.g., stereoselectivity in metabolic pathways or in renal clearance, could also contribute to differences in observed effects in vivo. Some differences in the disposition of the enantiomers have been noted in rats and dogs.[12,24] Further work is needed to examine these possibilities in some detail.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Circular dichroism spectra were recorded on a Jobin Yvon Dichrographe R. J. Mark III instrument. CI mass spectral data were obtained on a Biospect mass spectrometer using methane as reagent gas.

Resolution. To a solution of 15.90 g (46.3 mmol) of disopyramide (1) and 6.95 g (46.3 mmol) of (2R,3R)-(+)-tartaric acid [L-(d)-tartaric acid] in 120 mL of absolute methanol was added 600 mL of acetone. After the solution had cooled for 44 h, 9.16 g of a mixture of diastereomeric salts was collected as rosette-like clumps of needles, mp 167–170 °C. This salt was dissolved in 25 mL of hot absolute methanol, 250 mL of acetone was added, and the solution was cooled at room temperature. Repeated crystallization of the resulting salt (five times) from absolute methanol–acetone (1:10) afforded 5.10 g of the (+)-bitartrate salt: mp 174.5–175.5 °C; $[\alpha]_D$ +36.0° (c 1.0, absolute methanol). This salt was used for X-ray crystallography.

Decomposition of the (+)-bitartrate salt was accomplished by adding 100 mL of aqueous 5% sodium hydroxide to a solution of 5.10 g of the salt in 100 mL of water and extracting the free base into ether (2 × 100 mL). Evaporation of the solvent and crystallization from 40 mL of n-hexane afforded 3.00 g (41% yield from disopyramide) of the free base [(2R)-1]: mp 82.5–84 °C; $[\alpha]_D$ -19.4° (c 1.0, absolute methanol); CD (MeOH) $[\theta]_{325}$ 0, $[\theta]_{296}$ +44, $[\theta]_{289}$ 0, $[\theta]_{285}$ −71, $[\theta]_{282}$ 0, $[\theta]_{274}$ +11 190, $[\theta]_{270}$ +6710, $[\theta]_{267}$ +10 290, $[\theta]_{261}$ +7600 (shoulder), $[\theta]_{245}$ 0, $[\theta]_{224}$ −17 000, $[\theta]_{210}$ 0.

The (+) enantiomer of disopyramide was obtained from the filtrates of the resolution of the (+)-bitartrate. The combined filtrates from the initial resolution were evaporated. The resulting salt was decomposed, affording 12.04 g of disopyramide, enriched in the (+) enantiomer. Repeated crystallization of the bitartrate salt formed from (2S,3S)-(-)-tartaric acid [D-(l)-tartaric acid] (methanol–acetone, 1:10) afforded 5.97 g of (-)-bitartrate salt: mp 174–175 °C; $[\alpha]_D$ -36.0° (c 1.0, absolute methanol).

Decomposition of the (-)-bitartrate salt (5.97 g) as described for the (-) enantiomer of disopyramide [(+)-bitartrate] afforded 3.34 g of (2S)-(+)-disopyramide [(2S)-1]: mp 83–85 °C; $[\alpha]_D$ +18.9° (c 1.0, absolute methanol); CD (MeOH) $[\theta]_{325}$ 0, $[\theta]_{296}$ −38, $[\theta]_{289}$ 0, $[\theta]_{285}$ +60, $[\theta]_{282}$ 0, $[\theta]_{274}$ −12 080, $[\theta]_{270}$ 7520, $[\theta]_{267}$ −10 520, $[\theta]_{261}$ −7270 (shoulder), $[\theta]_{245}$ 0, $[\theta]_{224}$ = +15 330, $[\theta]_{210}$ 0.

$LiAlH_4$ Reduction. A mixture of 100 mg (0.29 mmol) of racemic disopyramide (1) and 40 mg (1.05 mmol) of $LiAlH_4$ in 5 mL of anhydrous ether were stirred at reflux for 24 h. Excess hydride reagent was decomposed by the addition of 5–10 drops of H_2O (until liberation of gas stopped) and the suspension was filtered (Celite). The solvent was removed under nitrogen, affording 77 mg of clear, yellow oil.

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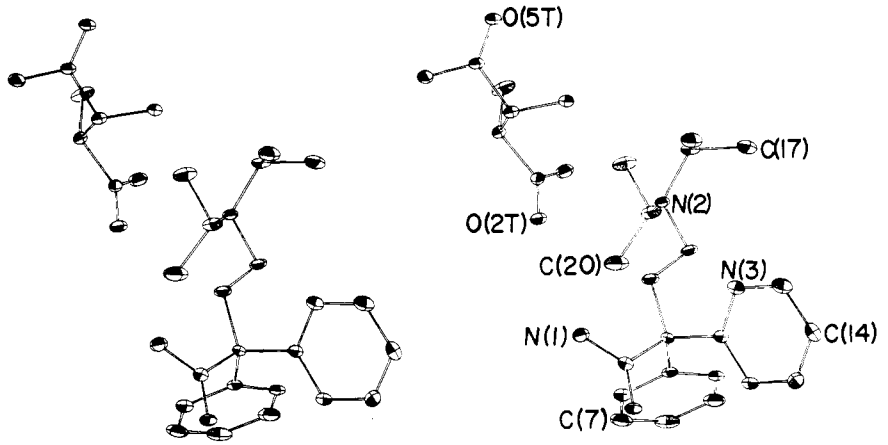

Figure 2. Stereoscopic drawing of (+)-[(2*R*)-(−)-4-(diisopropylamino)-2-(2-pyridyl)-2-phenylbutyramide (2*R*,3*R*)-(+)-bitartrate] showing the conformation and relative sizes and shapes of the thermal motion ellipsoids (50% probability), viewed along the *y* axis.

Thin-layer chromatography [petroleum ether (bp 30–60 °C)/MeOH (1:1)] showed starting material at R_f 0.44 as a light spot and product at R_f 0.27 as a dark spot. A less intense third spot appeared at R_f 0.61. High-performance LC separation [Zorbax CN column, using 95% cyclohexane and 5% strong solvent (MeOH–*i*-PrOH–NH_4OH, 70:30:1), flow rate 3 mL min^{-1}, detection at 220 nm] showed starting material eluting at 7.0 min and product at 12.3 min. Peak identification was done on the basis of mass spectral examination of the appropriate fractions, disopyramide QM = 340 amu and the amine QM = 326 amu. The optical isomers (2*R*)-1 and (2*S*)-1 were treated in the same way.

Preparation of Diasteriomeric Sulfonamides. The following procedure was performed on amines from racemic disopyramide (1) and its (+) and (−) enantiomers. A solution of 33 mg (0.10 mol) of the crude amine in 5 mL of CH_2Cl_2 containing 0.5 mL of NEt_3 and 250 mg (1.0 mmol) of (+)-camphor-10-sulfonyl chloride was refluxed overnight. The solvent was evaporated (nitrogen) and the residue was stirred with 5 mL of aqueous 2 N HCl for 5 min. The aqueous acidic layer was washed with 1 × 20 mL of ether, made alkaline (pH ≥10) by the addition of solid NaOH, and extracted with 20 mL of ether. Evaporation of the ether (nitrogen) afforded 52 mg of clear light brown syrup. Thin-layer chromatography [ether–MeOH (5:1)] showed one predominant spot, R_f 0.67.

High-performance LC separation of diastereomers was performed as described above. The diastereomeric camphor-10-sulfonamide from the (−) enantiomer [(2*R*)-1] eluted at 9.4 min, and the one from the (+) enantiomer [(2*S*)-1] at 8.7 min (Figure 1). The composition of the peaks was confirmed by mass spectral analysis of collected fractions, QM = 540 amu.

Crystal Structure. A single crystal (0.1 × 0.1 × 0.2 mm) of the (+)-bitartrate salt ($C_{25}H_{35}N_3O_7$) derived from (2*R*,3*R*)-(+)-tartaric acid was obtained by slow crystallization from acetone–methanol. Systematic absences ($h00$, $h \neq 2n$; $0k0$, $k \neq 2n$; $00l$, $l \neq 2n$) observed in Weissenberg photographs uniquely defined the space group as $P2_12_12_1$ with four molecules per unit cell. Lattice parameters [a = 14.789 (4), b = 18.151 (4), c = 9.878 (2) Å] and the orientation matrix for data collection were determined by least-squares refinement of 14 reflections (30° > 2θ > 25°) automatically centered on a Picker FACS-I diffractometer using zirconium-filtered Mo Kα radiation (λ 0.7107 Å). The experimentally determined and theoretical crystal densities are 1.225 (flotation C_6H_6–CCl_4) and 1.226 g cm^{-3}. Intensity data were collected using the ω-scan mode. The intensities of three standard reflections remained constant [$+3\sigma(I_0)_{av}$] throughout data collection. Intensity data were corrected for Lorentz and polarization effects, but an absorption correction was not applied. Of 2644 unique reflections, 2059 were considered observed according to the criterion $I_0 \geq 3\sigma(I)$. The structure was solved by direct methods using the program MULTAN with magic integers.[14] An *E* map revealed the positions of 32 of the 35 nonhydrogen atoms, and a difference Fourier map revealed the positions of the three remaining atoms. The structure was refined using the CRYLSQ link of the X-RAY 72 system.[12] The atomic scattering factors for nonhydrogen atoms were obtained from Cromer and Waber,[16] and the atomic scattering factors for hydrogen were obtained from Stewart, Davidson, and Simpson.[17] The quantity minimized in the least-squares calculations was $\Sigma_w(|F_o| - |F_c|)^2$. Isotropic refinement of all nonhydrogen atoms was followed by anisotropic refinement and calculation of bond distance in the aromatic rings. Based upon the short bond distances from C(11) to one of the adjacent ring positions (Figure 3), it was possible to assign the position of N(3) which to this point had been included in the refinement as a carbon atom. A difference electron density synthesis revealed the positions of all 35 hydrogen atoms. In all subsequent refinements, the positional and isotropic thermal parameters were allowed to vary along with the positional and anisotropic thermal parameters of the heavier atoms. The final cycles of least-squares refinement were weighted by $1/\sigma^2$, where $\sigma = 0.0012|F|^2 - 0.075|F| + 2.25$. Block-diagonal least-squares refinement converged with $R = \Sigma(|F_o| - |F_o|)/\Sigma|F_o|$ and $R_w = [\Sigma(w|F_o| - |F_o|)^2/\Sigma_w|F_o|^2]^{1/2}$ equal to 0.066 and 0.064, respectively. Refinement was terminated when shifts in all parameters were less than the estimated standard deviations. The error in an observation of unit weight, Σ, was 1.42. A final difference map was calculated omitting H(C6). This atom appeared as a peak of 0.43 e/Å^3, and none of the weaker peaks were within bonding distance of N(3). This confirms the assignment of the position of N(3) based on bond distances in phenyl and pyridyl rings (Figure 3).[18] Figure 2 shows the correct absolute configuration for both (+)-tartaric acid (2*R*,3*R*) and (−)-disopyramide (2*R*). A listing of structure factors and thermal parameters has been deposited.

Pharmacologic Evaluation. Prolongation of the effective refractory period (ERP) of ventricular myocardium in the isolated hearts of New Zealand white rabbits was used for the estimation of antiarrhythmic activities of these compounds. Rabbits were sacrificed by blows on the cervical vertebrae, the hearts were removed, and coronary vessels were cleared of blood with Krebs–Henseleit buffer at room temperature. Perfusion of coronary vessels with buffer at 36.5 °C was performed in the Langendorf apparatus under 53 cm water pressure. After 1 h of perfusion for equilibration, the hearts were driven at 3 Hz with square wave pulses of 5 ms duration at a stimulus strength 30% greater than threshold. The ERP was determined from strength–interval curves as described by Brooks et al.[25] The interval was measured by interposing a test stimulus from a slave stimulator after every 10th drive stimulus and increasing the test stimulus strength until it produced a conducted electrical response. The strength of the stimulus (in milliamperes) which produced this response was calculated by measuring the voltage drop across a 20 ohm resistor in the stimulus circuit. The ERP was taken as that interval after the drive stimulus which required a test pulse exceeding 10 mA to obtain a response.

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Acknowledgment. Crystallographic computations were performed at the University of Connecticut Computer Center.

Supplementary Material Available: Figure 3, torsion angles, bond distances, angles, and pertinent bond distances; Table II, atomic positional parameters; and Table III, a listing of the structure-factor amplitudes and the atomic thermal parameters (29 pages). Ordering information is given on any current masthead page.

Notes

Structure–Activity Relationship of Diphenylthiourea Antivirals

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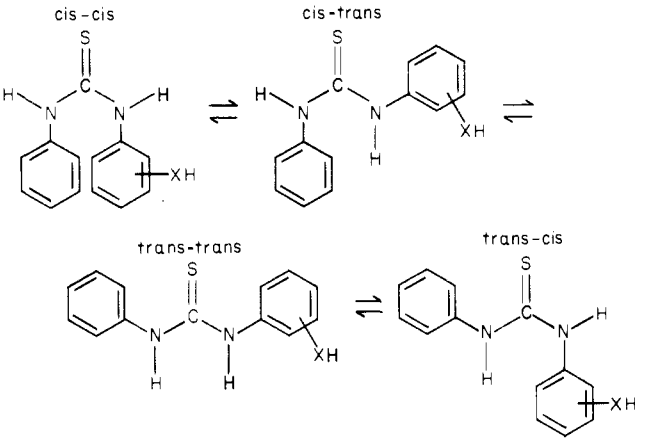
The dependence between chemical structure and antiviral activity of *N,N'*-diphenylthioureas is studied by synthesis and testing of model compounds and use of conformational data. The analysis revealed a number of structural features as essential for the antiviral effect: (1) the presence of an intact -NHC(=S)NH- grouping; (2) the presence of a substituent of the XH type (X = O, NH) in the aromatic ring; (3) the distance between these substituents and the sulfur atom in the 6.68–6.75 Å range for the active compounds; (4) a trans conformation of the -C(=S)NH- group bound to the substituted phenyl ring. The directed synthesis of compounds satisfying the above requirements yielded the derivative *N*-phenyl-*N'*-(*m*-aminophenyl)thiourea exhibiting a very high antipicornavirus activity in vitro and several other active analogues (four out of seven synthesized). The possible mechanism of interaction between the active diphenylthiourea derivatives and the viral target is discussed.

N,N'-Disubstituted thioureas were reported to show a strong antipicornavirus activity both in vitro and in vivo.[1-6] The structure–activity analysis of 54 *N*-phenyl-*N'*-aryl- or -alkylthiourea derivatives on poliovirus 1 (Mahoney) growth in cell cultures revealed a marked antiviral effect in compounds containing a hydroxyl group in the phenyl or alkyl radical bound to one of the nitrogen atoms, the spatial location of the hydroxyl group being of critical importance. The molecular geometry of these compounds shows that the most active among them have one common feature: the distance between the oxygen atom of the hydroxyl group and the nitrogen atom attached to the same ring is within the 4.73–4.81 Å range. Besides, it was found that the electronic effect of the substituents is not significant. Thus, for example, the corresponding methoxy derivatives which have a similar electronic effect are inactive.[1] The governing role of the geometric factor in antiviral activity made us study the conformation of those derivatives, since the -NHC(=S)NH- grouping offers several possible conformations. IR and NMR spectral analysis of diphenylthioureas in neutral medium reveals a state of equilibrium between several conformational forms[7] (Scheme I). Obviously, a systematic structure–activity relationship study should cover all conformational possibilities.

Scheme I



Results and Discussion

For the purpose of a more detailed study of the structure–activity relationship of diphenylthioureas, we carried out the synthesis of a series of model compounds and diphenylthiourea derivatives expected to exhibit an antiviral effect on the basis of data accumulated until now. Antiviral activity was tested in vitro against three human enteroviruses—poliovirus 1 (Mahoney), Coxsackie B1, and ECHO19—using the agar-diffusion plaque-inhibition screening method of Rada and Zâvada,[8,10] which in the case of poliovirus 1 was supplemented with the one-step growth cycle setup (two-stage system).[1,9,10] The two most active

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(10) Details of the methods used for antiviral testing are given under Experimental Section.

0022-2623/80/1823-1048$01.00/0