Spatial Requirements of the Na Channel Binding Site for Class I Antiarrhythmics As Derived from the Crystal Structures of 4-Substituted 2,6-Bis(1-pyrrolidinylmethyl)phenols

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The molecular structures of four class I antiarrhythmic agents containing a bis(1-pyrrolidinylmethyl)phenol framework were determined by X-ray diffraction methods and their conformations were analyzed by molecular mechanics. Each structure has an intramolecular hydrogen bond between the phenol OH group and one pyrrolidine N atom; this bond determines the orientation of the pyrrolidine ring. Only two distinct orientations are observed for the other pyrrolidine ring; the combination of the pyrrolidine ring positions produces molecular conformers that either have the two N atoms on the same side of the phenol ring plane or have the N atoms nearly coplanar with the phenol ring. Crystallographic conformations of benzamides as well as those calculated with molecular mechanics find a preferred conformation that has the three planar regions tilted by ca. 30° with respect to one another. The combined restrictions of the hydrogen bond, the conformational preferences for the bis(pyrrolidinylmethyl)phenol moiety, and a consistent conformation for the central benzamide portion provide a suggestion of the active shape of class Ic antiarrhythmic drugs. The distance between two potential recognition groups—a phenyl ring (lipophilic group) separated by 3.8 Å from an oxygen atom and a secondary or tertiary amine—is found to be unique for class Ic antiarrhythmics as compared to other class I antiarrhythmics, such as lidocaine, quinidine, procainamide, and disopyramide, and suggests a correlation between the molecular structures and the activity of class I antiarrhythmics.

Sudden cardiac death claims one life almost every minute in North America alone[2] and ventricular arrhythmias play a major role in these deaths. One of the ways that cardiac arrhythmias can be corrected is to block the conduction of abnormal impulses by slowing electrical conduction in ventricular muscle and conducting tissue. A class of drugs possessing this property are the local anesthetic, class I antiarrhythmics;[3] these drugs bind specifically to sodium channels and interfere with interconversions of the channel protein to different conducting and nonconducting states.[4] Class I agents may be further divided into three subcategories on the basis of their effects on action potential duration[5] and the time constant for association/disassociation with open and/or activated sodium channels.[6] According to this subclassification, subclass Ib, represented by lidocaine and mexilitine, has time constants for recovery from Na-channel blockade of well below 1 s, while quinidine, procainamide, and disopyramide are in subclass Ia with time constants of 1–10 s.[6] During the last decade, many new antiarrhythmic agents, most of them characterized by time constants greater than 10 s (subclass Ic) have been identified; for example, Stout and co-workers[7a-c] introduced a new family of antiarrhythmics developed from changrolin (1) which belong to the Ic subclass.

Three structural units are characteristic of class I antiarrhythmics: (i) an aliphatic amine group, (ii) a lipophilic group, and (iii) an interconnecting chain that contains a hydrogen-bond acceptor, usually a carbonyl group. Although these features are known to be important for antiarrhythmic activity and the X-ray structures of several antiarrhythmics (lidocaine,[8] quinidine,[9] procainamide,[10] and disopyramide[11]) are known, the conformational flexibility of these molecules precludes unambiguous prediction of the common spatial arrangement of the pharmacophores of class I antiarrhythmics. In contrast, the new Ic subclass compounds,[7c] 2,6-bis(1-pyrrolidinylmethyl)-4-benzamidophenol (1), *N*-benzoyl-3,5-bis(*N*-pyrrolidinylmethyl)-4-hydroxyaniline (2), and *N*-[4-hydroxy-3,5-bis(1-pyrrolidinylmethyl)phenyl]-2-methylbenzamide (3), are more suitable for modeling studies due to their more constrained conformations. Structure–activity studies of changrolin (1) derivatives[7a-c] have defined the regions of activity in the molecular structure. These studies show that the quinazoline can be replaced by several other ring systems without loss of antiarrhythmic activity; thus, compounds 3 and 4 are nearly equipotent. It was found that the 4-substituted 2,6-bis(pyrrolidinylmethyl)phenol has the best activity and that the linkage region between these two groups needs a carbonyl group for high antiarrhythmic activity and low toxicity. In the linkage region, amides are especially effective and reversal of the amide has little effect on activity;[7c] thus, compounds 2 and 3 are approximately equipotent. Compounds 2–4 all have twice the antiarrhythmic potency of changrolin (1). Therefore,

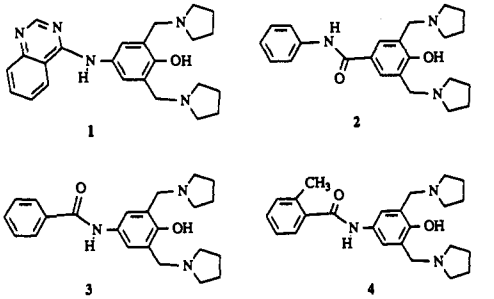

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Table I. Crystallographic Data for 4-Substituted 2,6-Bis(1-pyrrolidinylmethyl)phenols 2–4

	2	3	4
mol formula	$C_{23}H_{29}N_3O_2$	$C_{23}H_{29}N_3O_2$	$C_{24}H_{31}N_3O_2 \cdot HCl$
mol wt	379.51	379.51	429.99
space group (Z)	$I4_1/a$ (16)	$Pbca$ (16)	$Aba2$ (8)
a (esd), Å	23.461 (1)	10.2513 (4)	13.882 (2)
b, Å	$=a$	53.275 (3)	29.115 (3)
c, Å	15.298 (1)	15.149 (2)	11.092 (1)
V, Å^3	8420.3	8273.4	4483.1
density (calcd), g cm^{-3}	1.20	1.22	1.27
crystal color	colorless	colorless	colorless
crystal size, mm	0.40 × 0.20 × 0.20	0.24 × 0.12 × 0.10	0.20 × 0.12 × 0.08
crystallization solvent	ethanol	ethyl acetate	ethanol/ethyl acetate
maximum θ (deg)	75	55	75
no. of unique data	3563	5176	2431
no. of observed data	2308	2645	2018
no. of refined data	3040	4470	2308
R	0.034	0.064	0.041
R_w	0.041	0.057	0.042
weights, w	$[\sigma^2(F_o) + 0.004F_o^2]^{-1}$	$[\sigma^2(F_o) + 0.001F_o^2]^{-1}$	$[\sigma^2(F_o) + 0.0001F_o^2]^{-1}$

the structures of 2–4 were determined by X-ray crystallography and the molecular conformations were explored by molecular mechanics calculations to establish the spatial arrangement of the pharmacophoric groups that contributes to antiarrhythmic activity.

Experimental Section

Crystallography. Samples of 2–4 were provided by Dr. David M. Stout of American Critical Care.[12] The crystallographic and experimental data for all three compounds are given in Table I. The unit-cell parameters and orientation angles for each crystal were obtained by the method of least squares from the positional parameters of reflections centered on an Enraf-Nonius CAD4F diffractometer using Cu Kα radiation and a Ni filter (λ = 1.5418 Å). The diffracted intensities were collected with an ω–2θ scan at room temperature for 2 and 3 and at −50 °C for 4. The data were corrected for Lorentz and polarization effects; corrections for absorption were deemed unnecessary.

The structures were solved with the direct methods program MULTAN[13] and were refined by least-squares calculations. Hydrogen atoms were identified in difference Fourier syntheses, were added to the models in calculated, idealized positions, and were included in the refinement. For all structures, the final cycles of refinement included the coordinates of all atoms, the anisotropic thermal parameters of the non-hydrogen atoms, and the isotropic thermal parameters of the hydrogen atoms. Refinement of 3 differed in two respects: the two unique molecules were refined in separate cycles to assure that the least-squares calculation was overdetermined and H112 was ill-behaved in refinement and was, therefore, fixed at the position identified in the difference Fourier synthesis. The data included in the refinement were the observed reflections ($I_o > 2.5\sigma(I_o)$) and those unobserved reflections with $|F_c| > 2.5\sigma(F_o)$; the number of data and the final agreement factors are given in Table I. The scattering factors used in the calculations were those of Cromer and Mann[14] for the non-hydrogen atoms and of Stewart et al.[15] for the hydrogen atoms; the calculations were done with the XRAY76[16] suite of programs.

Modeling. Molecular mechanics calculations for the benzanilide and the 2-(1-pyrrolidinylmethyl)phenol fragments were performed using the MMP2(87)[17] program of Allinger. The potential energy profiles of the neutral molecules were obtained by a driver calculation in 10° incremental steps for two selected torsion angles followed by unrestricted optimization of the structure at each step. Evaluation of the stability of the protonated species was done with torsion angle steps of 30° and the rigid-body side chain driver option of MMP2.

Table II. The Fractional Coordinates (×10^5) and Equivalent Isotropic Vibration Parameters (×10^2) for the Non-Hydrogen Atoms of Compound 2[a]

atom	x/a	y/b	z/c	B_{eq}
C001	93762 (8)	50546 (8)	12525 (14)	470 (9)
C002	99425 (9)	51043 (10)	14928 (18)	600 (12)
C003	101379 (9)	48675 (10)	22504 (17)	605 (12)
C004	97611 (10)	45977 (12)	27814 (18)	657 (13)
C005	91902 (8)	45400 (10)	25560 (15)	528 (10)
C006	89969 (6)	47687 (7)	17848 (11)	329 (7)
N007	84243 (5)	47401 (6)	14933 (9)	324 (6)
C008	79873 (6)	44423 (6)	18506 (10)	399 (5)
O008	80469 (5)	41251 (5)	24851 (8)	399 (5)
C009	74190 (6)	45214 (6)	14352 (10)	310 (7)
C010	72525 (6)	50286 (7)	10377 (11)	330 (7)
C011	67150 (7)	50905 (7)	6791 (11)	335 (7)
C012	63327 (7)	46336 (7)	7312 (11)	351 (7)
O012	57977 (5)	46769 (5)	3844 (9)	458 (6)
C013	64842 (7)	41247 (7)	11399 (11)	337 (7)
C014	70268 (6)	40747 (7)	14860 (11)	330 (7)
C015	60590 (7)	36401 (8)	11536 (12)	412 (8)
N016	61948 (6)	31864 (6)	17661 (9)	374 (6)
C017	60405 (9)	33137 (9)	26712 (13)	509 (10)
C018	60296 (11)	27417 (10)	31244 (16)	669 (13)
C019	59513 (15)	23083 (11)	23913 (19)	701 (15)
C020	58869 (10)	26603 (9)	15709 (15)	571 (11)
C021	65506 (8)	56346 (8)	2136 (14)	404 (9)
N022	59648 (6)	58071 (6)	4155 (9)	378 (6)
C023	58964 (9)	60051 (10)	13166 (14)	476 (10)
C024	53227 (11)	62993 (12)	13072 (19)	642 (13)
C025	52439 (11)	64957 (12)	3736 (19)	641 (13)
C026	57720 (9)	62949 (9)	−1032 (13)	525 (10)

[a] Estimated standard deviations are given in parentheses. B_{eq} is defined as one-third the trace of the U_{ij} matrix.

Results

Crystallography. The solid-state, molecular conformations and the atomic labeling schemes for compounds 2–4 are shown in Figure 1a–c. Compound 3 crystallized with two molecules (**3a** and **3b**) in the asymmetric unit, both are shown in Figure 1c. The atomic coordinates of all three structures are given in Tables II–IV.

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Table III. The Fractional Coordinates ($\times 10^5$) and Equivalent Isotropic Vibration Parameters ($\times 10^2$) for Non-Hydrogen Atoms of Compound **3**[a]

atom	x/a	y/b	z/c	B_{eq}
C101	30101 (53)	9211 (10)	139318 (37)	475 (32)
C102	31377 (60)	10322 (11)	147511 (40)	546 (36)
C103	43164 (67)	10299 (11)	151771 (37)	524 (35)
C104	53841 (58)	9185 (11)	147895 (37)	499 (33)
C105	52562 (46)	8063 (10)	139638 (33)	378 (27)
C106	40808 (47)	8094 (9)	135304 (32)	328 (25)
C107	38724 (51)	6990 (9)	126176 (35)	384 (28)
O107	27608 (31)	6619 (7)	123274 (21)	468 (19)
N108	49616 (37)	6449 (8)	121606 (25)	377 (22)
C109	50028 (47)	5489 (9)	112757 (31)	331 (25)
C110	60251 (49)	3848 (9)	110746 (31)	346 (26)
C111	61616 (50)	2913 (9)	102234 (34)	399 (29)
C112	52964 (49)	3659 (9)	95655 (32)	415 (28)
O112	54445 (36)	2728 (7)	87291 (22)	559 (22)
C113	42719 (48)	5237 (9)	97735 (32)	371 (27)
C114	41231 (46)	6171 (9)	106284 (32)	373 (27)
C115	32740 (52)	5897 (10)	90751 (35)	461 (30)
N116	32918 (43)	8557 (9)	88356 (27)	462 (25)
C117	45577 (62)	9390 (11)	85005 (40)	603 (37)
C118	42443 (78)	11827 (13)	79893 (42)	759 (45)
C119	27686 (85)	11831 (15)	78518 (48)	933 (53)
C120	23858 (58)	9150 (13)	81113 (37)	644 (37)
C121	72040 (53)	979 (9)	100374 (35)	484 (31)
N122	78084 (42)	1351 (8)	91634 (27)	435 (24)
C123	86635 (61)	3508 (11)	91062 (38)	578 (36)
C124	96880 (65)	2908 (13)	84402 (46)	766 (45)
C125	94128 (63)	247 (12)	81322 (40)	699 (40)
C126	86085 (57)	-799 (9)	88623 (36)	590 (34)
C201	12002 (48)	24518 (9)	8663 (34)	382 (28)
C202	14757 (51)	25276 (10)	136 (36)	415 (29)
C203	24838 (56)	24230 (10)	-4533 (33)	426 (30)
C204	32183 (52)	22400 (10)	-636 (34)	433 (30)
C205	29968 (48)	21609 (9)	7954 (32)	356 (27)
C206	19711 (44)	22885 (8)	12635 (29)	267 (23)
C207	16066 (50)	21828 (8)	21829 (31)	302 (25)
O207	4777 (30)	22033 (6)	24477 (22)	373 (17)
N208	25637 (36)	20757 (7)	26496 (26)	335 (20)
C209	24514 (47)	19459 (8)	34809 (29)	307 (24)
C210	35220 (44)	19350 (9)	40320 (30)	302 (24)
C211	34493 (47)	18072 (9)	48203 (29)	304 (24)
C212	22940 (47)	18897 (8)	50585 (27)	315 (25)
O212	21879 (31)	15698 (6)	58604 (19)	409 (18)
C213	12179 (45)	16930 (8)	44955 (31)	320 (25)
C214	13069 (43)	18199 (8)	37052 (28)	286 (23)
C215	-22 (51)	15579 (9)	47797 (32)	398 (28)
N216	-10222 (38)	15556 (7)	41108 (27)	353 (21)
C217	-8735 (53)	13617 (10)	34213 (38)	492 (32)
C218	-22304 (68)	13488 (11)	29986 (42)	661 (40)
C219	-31519 (54)	14702 (11)	36544 (43)	573 (36)
C220	-23221 (50)	15042 (9)	44827 (37)	458 (30)
C221	46462 (46)	17930 (9)	54136 (31)	362 (26)
N222	43039 (39)	18501 (7)	63287 (24)	353 (21)
C223	39019 (54)	21125 (10)	64557 (35)	473 (30)
C224	40293 (64)	21592 (10)	74453 (42)	633 (37)
C225	49587 (71)	19608 (13)	77579 (40)	825 (47)
C226	53993 (53)	18209 (11)	69560 (38)	532 (33)

In all four of the observed molecular conformations, there is an intramolecular hydrogen bond between the hydroxyl group of the phenol ring and the unprotonated N atom of one pyrrolidine ring; the geometries of these hydrogen bonds are given in Table V. This hydrogen bond constrains the torsion angle ϕ_1 = C12–C11–C21–N22 to either + or −40° and thus defines the orientation of one of the pyrrolidine rings. The other pyrrolidine ring is found in two orientations in the crystal; the torsion angle ϕ_2 = C12–C13–C15–N16 that defines the orientation is ca. 180° in **2** and **3b** and ca. −100° in **3a** and **4**. When the bis(pyrrolidinylmethyl)phenol moiety is considered as a whole and the centrosymmetric molecules in structures **2** and **3** are included in the consideration, we find that only four combinations of the pair of torsion angles ϕ_1 and ϕ_2 are present in the crystals.[18] These are two centrosymmetrically related sets of angles: set 1, $\phi_1 = \pm 40°$ and $\phi_2 = 180°$; set 2, $\phi_1 = +40°$ and $\phi_2 = -100°$, $\phi_1 = -40°$ and $\phi_2 = +100°$. The possible combinations related to set 2 (ϕ_1, ϕ_2 both positive or ϕ_1, ϕ_2 both negative) are not observed.

Table IV. The Fractional Coordinates ($\times 10^5$) and Equivalent Isotropic Vibration Parameters ($\times 10^2$) for the Non-Hydrogen Atoms of Compound **4**[a]

atom	x/a	y/b	z/c	B_{eq}
Cl	39548 (7)	11080 (3)	95611 (13)	318 (3)
C1	77169 (30)	18235 (15)	9757 (43)	394 (22)
C01	68141 (30)	20256 (13)	4277 (40)	296 (17)
C02	88907 (33)	22913 (15)	-6134 (47)	400 (22)
C03	60887 (38)	24952 (17)	-11298 (43)	419 (22)
C04	51858 (33)	24330 (14)	-6301 (44)	365 (21)
C05	50968 (29)	21575 (12)	3850 (40)	297 (17)
C06	58974 (26)	19568 (12)	9177 (35)	232 (15)
C07	57677 (25)	18827 (11)	20554 (36)	227 (14)
O07	62211 (19)	17721 (9)	29806 (28)	314 (12)
N08	50951 (21)	13473 (10)	19692 (30)	233 (12)
C09	47690 (23)	14029 (11)	28834 (34)	208 (14)
C10	42473 (26)	6571 (12)	25275 (34)	237 (14)
C11	39030 (25)	3403 (12)	33494 (37)	240 (15)
C12	40797 (25)	4148 (11)	45858 (42)	249 (14)
O12	37544 (20)	1134 (9)	54329 (27)	320 (12)
C13	45943 (27)	8024 (13)	49560 (33)	235 (15)
C14	49557 (26)	11090 (12)	41036 (35)	234 (14)
C15	47765 (27)	8732 (13)	62835 (35)	268 (16)
N16	40719 (21)	11940 (10)	68790 (29)	246 (13)
C17	30196 (27)	11128 (14)	66103 (40)	304 (17)
C18	25368 (41)	15585 (15)	69937 (66)	521 (24)
C19	33362 (37)	19090 (14)	71821 (51)	449 (24)
C20	42299 (33)	16924 (14)	66605 (44)	369 (20)
C21	34172 (30)	-942 (12)	29132 (39)	297 (17)
N22	27091 (22)	-2791 (10)	37623 (33)	279 (14)
C23	24348 (34)	-7562 (12)	35137 (48)	387 (21)
C24	15711 (33)	-8271 (15)	43278 (56)	463 (24)
C25	10816 (29)	-3598 (15)	43752 (51)	398 (21)
C26	17800 (32)	-316 (15)	37335 (46)	360 (19)

[a] Estimated standard deviations are given in parentheses. B_{eq} as defined in Table II.

Table V. Hydrogen-Bonding Geometry for Compounds **2**–**4**

Intramolecular O12–H12···N22

compd	O–H, Å	O···N, Å	H···N, Å	<O–H···N, deg
2	0.94 (2)	2.681 (2)	1.83 (2)	149 (2)
3a	1.17[a]	2.641 (7)	1.63[a]	141 (3)
3b	1.00 (4)	2.723 (6)	1.83 (4)	153 (3)
4	0.88 (4)	2.617 (4)	1.84 (4)	147 (4)

Intermolecular, Compound **4**

bond	N–H, Å	N···Cl, Å	H···Cl, Å	<N–H···Cl, deg
N16–H16···Cl	0.89 (5)	2.989 (4)	2.10 (5)	169 (4)
N08–H09···Cl[b]	0.85 (3)	3.182 (3)	2.33 (3)	177 (3)

[a] Atom H112 was not refined. [b] At equivalent position: $(x, y, z-1)$.

In the crystal structures, the C=O bond was consistently found to be antiparallel to the phenol O—H bond; this antiparallel orientation produces a molecular edge that contains both the carbonyl oxygen atom and the free (non-hydrogen-bonded) pyrrolidine N atom as evident in Figure 1a–c.

The conformation of the benzanilide fragment in the center of these molecules affects the shape of the molecule and the orientation of the carbonyl group. The benzanilide fragment can be regarded as three connected, planar units: two terminal phenyl rings and a central amide group. The torsion angles between these three planes are given in Table VI along with similar torsion angles obtained from

(18) The authors wish to acknowledge the helpful comments of one reviewer who noted this conformational restriction.

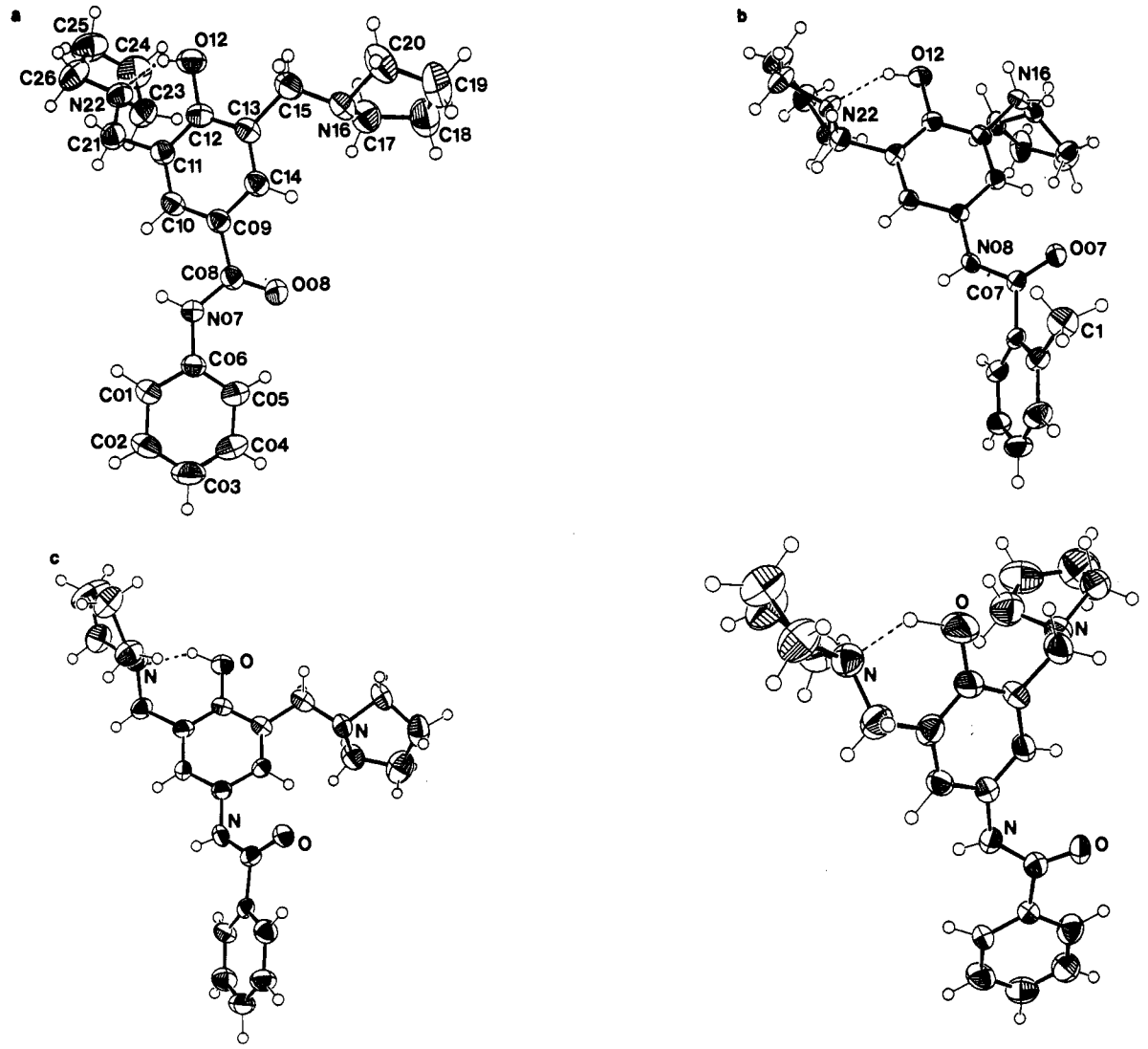


Figure 1. The molecular conformations of compounds **2** (panel a), **3a** and **3b** (panel c), and **4** (panel b). The atomic labeling schemes follow that given in panel a, with differences for the reversed amides given in panel b. The two molecules of **3** are differentiated by a numbering that identifies one molecule with numbers beginning with 101 and the other with numbers beginning with 201 and following the same numerical sequence as shown in a and b. The drawings were made with the computer program ORTEP.[30]

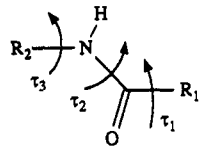
X-ray studies of unsubstituted and para-substituted benzanilides (benzanilide,[19a] *N*-phenylnicotinimide,[19b] *p*-nitrobenzanilide and *p*-methoxybenzanilide,[19c] *N,N'*-dibenzoyl-*p*-phenylenediamine,[19d] and *N,N'*-diphenylterephthalamide[19e]). Examination of Table VI shows that in the solid state, a consistent conformation for the benzanilide is found for all of the fragments found in the Cambridge Crystallographic Database.[20] In this conformation, the phenyl ring connected to the carbon atom forms an angle of 25–33° to the central amide plane and the phenyl ring connected to the nitrogen atom forms an angle of 30–40° to the central plane. In all examples found in the database and those reported here except for **3a**, the two phenyl rings are tipped in opposite directions with respect to the central amide plane. It is not clear why the complex molecules presented herein show the largest deviation from the consistent conformation; one possible explanation could be the complicated packing interactions caused by accomodation of the bulky bis(pyrrolidinylmethyl)phenol group rather than a simple phenyl ring as found in the compared structures. Steric interactions due to the ortho substituent on the phenyl ring in compound **4** distorts the conformation in this molecule so that the phenyl–amide torsion angle is 53.5°; thus, this structure is not part of the set of unsubstituted and para-substituted fragments summarized above.

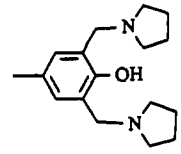
Modeling. The conformational properties of fragments of these molecules were explored with MMP2(87)[17] calculations. For the 2,6-bis(pyrrolidinylmethyl)phenol fragment, the free (non-hydrogen-bonded) pyrrolidine ring was found to adopt two stable orientations relative to the phenyl ring; these orientations are the same as those found in the crystal structures which have C12–C13–C15–N16 torsion angles of ca. 180° and ±100°. (The +100° and −100° are indistinguishable due to symmetry, clearly the effectiveness of the two different conformations might be important at a chiral recognition site.) The stable positions of the

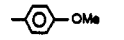
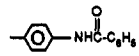
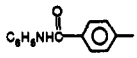
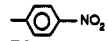
(19) (a) Kashino, S.; Ito, K.; Haisa, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 365–369. (b) Gdaniec, M.; Jaskólski, M.; Kosturkiewicz, Z. *Pol. J. Chem.* **1979**, *53*, 2563–2569. (c) du Plessis, M. I.; Modro, T. A.; Nassimbeni, L. R. *J. Cryst. Mol. Struct.* **1983**, *13*, 179–189. (d) Adams, W. W.; Fratini, A. V.; Wiff, D. R. *Acta Crystallogr.* **1978**, *B34*, 954–956. Harkema, S.; Gaymans, R. J. *Acta Crystallogr.* **1977**, *B33*, 3609–3611. (e) Harkema, S.; Gaymans, R. J.; van Hummel, G. J.; Zylberlicht, D. *Acta Crystallogr.* **1979**, *B35*, 506–508.

(20) Allen, F. H.; Bellard, S.; Brice, M. D.; Cartwrite, B. A.; Doubleday, A.; Higgs, H.; Hummelink, T.; Hummelink-Peters, B. G.; Kennard, O.; Motherwell, W. D. S.; Rogers, J. R.; Watson, D. G. *Acta Crystallogr.* **1979**, *B35*, 2331–2339.

Table VI. Torsion Angles for Para-Substituted and Unsubstituted Benzanilide Fragments



py = 

	R_1	R_2	τ_1	τ_2	τ_3
(19a)	Ph	Ph	29.5	179.4	37.3
(19b)		Ph	33.2	178.4	33.2
(19c)	Ph		31.2	178.6	39.8
(19d) [i]	Ph		24.7	176.5	56.3[a]
[ii]			31.4	178.5	36.5
(19e)		Ph	32.2	177.8	32.1
(19c)	Ph		29.4	178.5	36.4
2	py	Ph	31.3 (2)	-176.2 (2)	7.7 (2)
3a	Ph	py	-14.1 (8)	-177.1 (5)	34.9 (8)
3b			7.7 (2)	-170.3 (5)	29.6 (8)
4		py	53.5 (4)	-176.9 (3)	14.2 (5)

[a] This value was not included in the range of values reported in the text because this conformation was constrained by a short intramolecular hydrogen bond; with a longer hydrogen bond the molecule adopts the angles given in ref 19d [ii].

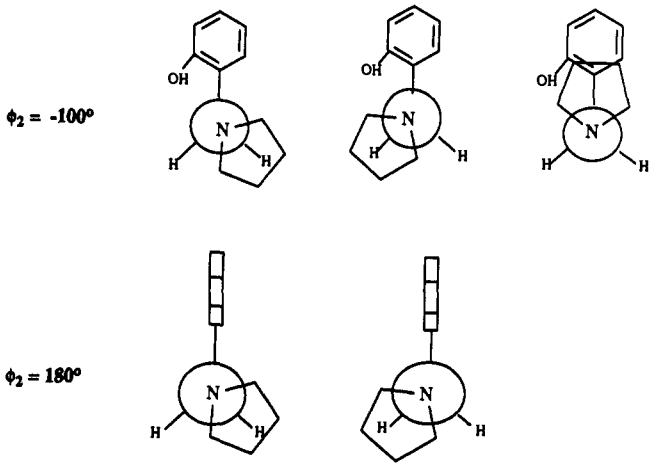


Figure 2. Newman projections of the calculated low-energy conformations of the pyrrolidinylmethyl phenol fragment, for two values (−100° and 180°) of the torsion angle ϕ_2 = C12–C13–C15–N16.

pyrrolidine ring differed in the two orientations. For the 180° orientation, the N atom is coplanar with the phenyl ring and only two of the three gauche conformations are of low energy. The two favored conformations direct the lone pair of the unprotonated N atom toward the phenyl ring, as shown schematically in Figure 2. For the ±100° orientation, the N atom is not coplanar with the phenyl ring and steric clashes are reduced. Thus, all three gauche forms are stable and within 2 kcal/mol of one another; each is more stable, by 3–4 kcal/mol, than the gauche forms found in the 180° orientation. The same pattern of stable conformations was obtained for the fragment with a protonated pyrrolidine ring; however, this result must be regarded with caution. The MMP2(87) force field does not provide complete parameterization for the protonated amine: several key torsion angles (C14–C13–C15–N^+16, C13–N^+16–C17–C18, and C13–C15–N^+16–H) had values of 0.0 for all torsional constants.

Calculation of the conformation of the benzanilide fragment found that the favored conformation had the phenyl rings tipped ca. 30° relative to the plane of the central amide, just as was found in the crystal structures and tabulated in Table VI. The minima in the energy surface calculated for rotation of the phenyl rings are broad and only the orthogonal conformer is strongly disfavored. All of the observed, crystallographic conformations (Table VI) are within 1 kcal/mol of the minimum-energy conformer. Duke[21] used molecular mechanics calculations to establish the effect of *o*-phenyl substituents on the potential energy surface; she found that ortho substituents shifted the minimum conformation toward a mutually perpendicular arrangement of phenyl ring and central amide plane; thus, the torsion angle found in structure 4 is consistent with molecular mechanics calculations.

Discussion

The observed structures of the 2,6-bis(pyrrolidinylmethyl)phenols provide suggestions for the active shape of these compounds and a guide as to which functional groups are important. The features of the molecules which define the active shape are the intramolecular hydrogen bond, the preferred conformation for the benzanilide fragment, the preferred orientation of the free pyrrolidine ring that contains the putative receptor-binding amine, and the restricted set of torsion angles that define the mutual orientation of the two pyrrolidine rings.

In the crystals, an intramolecular hydrogen bond is formed between the phenol and the nitrogen atom of one of the two pyrrolidine rings. This stable hydrogen bond is present in all the crystallographic examples of either the monoprotonated or the free-base compounds. This hydrogen bond is also found in the molecular mechanics minimum-energy conformation. If, as has been observed for the antiarrhythmic disopyramide, this intramolecular hydrogen bond persists in solution[22] and at the receptor, it would limit the interactions of one nitrogen atom with the receptor thereby reducing the molecule to a mono-

(21) Duke, N. E., Ph.D. Thesis, The University of Calgary, Calgary, Alberta, Canada, 1989.
(22) Czeisler, J. L.; El-Rashidy, R. M. *J. Pharm. Sci.* **1985**, *74*, 750–752.

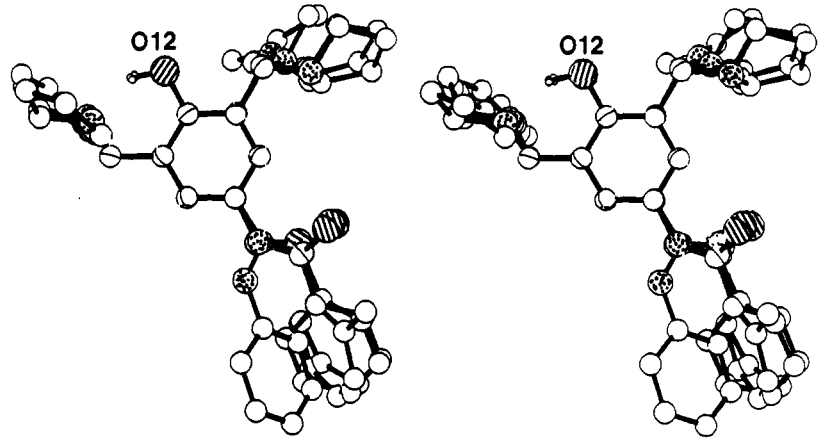


Figure 3. A stereodiagram of the superposition of all four unique molecules (**2**, **3a**, **3b**, and **4**). The oxygen atoms are shown as striped and the nitrogen atoms are shown as speckled. The superposition was obtained by calculating a least squares fit of atoms C9–C14 from the phenol ring of each molecule using the computer program PROFIT.[25] The drawing was made by the program PLUTO.[26]

amine compound. Monoamine compounds are also active antiarrhythmics, including many class I antiarrhythmics and monobasic derivatives of the antiarrhythmic disobutamide, which demonstrate good antiarrhythmic activity;[23] therefore, it is a reasonable hypothesis that the bis(pyrrolidinylmethyl)phenols could act as monoamines due to a persistent intramolecular hydrogen bond.

A preferred, relative orientation of the intramolecular hydrogen bond and the carbonyl bond is observed in all four unique conformation reported here, as well as in two unpublished structures determined in this laboratory. The combination of this preferred orientation of the central amide carbonyl bond and the intramolecular hydrogen bond, which ties up one amine group, places two key pharmacophoric groups, the available amine and the carbonyl oxygen atom, on the same molecular edge in the crystallographic conformations and suggests a mode of recognition at the receptor.

Examination of the observed conformations in the solid state and the stable molecular mechanics conformations, shows that the non-hydrogen-bonded pyrrolidine ring has limited conformational freedom. First, the orientation of the pyrrolidine nitrogen atom is restricted to one of three unique positions: either coplanar with the phenol ring or out of the plane with a torsion angle ϕ_2 (C12–C13–C15–N16) of + or −100°. Second, the intramolecular hydrogen bond and the limited pyrrolidine orientations produce specific shape preferences for the bis(pyrrolidinylmethyl)phenol moiety. We find that only two combinations of torsion angles ϕ_1 and ϕ_2 (and their "enantiomers") are observed, thereby restricting the positions of the two N atoms of the pyrrolidine rings to either (1) the same side of the phenol ring plane as shown on the right in Figure 1c or (2) one in the plane and one in the hydrogen bond as shown on the left in Figure 1c. Third, the orientation of the pyrrolidine ring, relative to the phenol ring, is restricted by steric interactions between the hydrogen atoms on the two rings. The result of these three restrictions is that the position and orientation of the lone pair on the pyrrolidine nitrogen atom can be restricted to a small number of possibilities. These conformational possibilities could be distinguished through construction of a derivative wherein the two N atoms were tied together, perhaps by linking the pyrrolidine rings through an additional ring, and restricted to lie on the same side of the phenol plane as in molecules **3a** (Figure 1c, right).

In crystal structures containing five protonated pyrrolidine rings (**4** and unpublished work in this laboratory), the out of plane conformation predominates, suggesting that steric clashes arising from the additional proton destabilize the coplanar conformation. This supposition cannot be substantiated with MMP2 calculations (see above) which show instead that both the in-plane and out-of-plane conformers of the protonated forms are stable. The MMP2 calculations are hampered by a lack of reliable parameters to describe the torsional barriers for a protonated amine; therefore, the observed structures may represent the true conformational preferences of the protonated forms. Physiological studies[24] indicate that the duration of channel block depends on pH and that the cationic form of a drug dissociates more slowly from the receptor; thus, the conformation preferred by the protonated form (out of plane) may bind more tightly to the receptor and is an important consideration in modeling studies.

The central benzamide portion of these molecules has a preferred conformation which minimizes the steric interaction of the central amide with the phenyl ring hydrogen atoms. The minimum in the potential energy surface for changes in the orientation of the phenyl rings is broad so that deviations from a consistent conformation are observed (as in the structures reported herein; see Table VI); however, the extensive crystallographic data show that an average conformation with angles of ca. 30° is commonly observed and can be considered to be the "resting state" for this fragment.

The general shape of any antiarrhythmic agent can be described by the three-dimensional arrangement of the groups that are important for recognition and binding. In compounds **2**–**4**, we assume that these groups are represented by the nitrogen atom of the free (non-hydrogen-bonded) amine group, the 4-substituent phenyl ring, and the carbonyl group. Given the conformational restraints identified above, all four unique molecules adopt a similar shape in the solid state, as evident in the superposition of the molecules shown in Figure 3. Molecular mechanics calculations, which identify the crystallographic conformations as the stable conformations that are independent of crystal packing forces, support our assumption that the

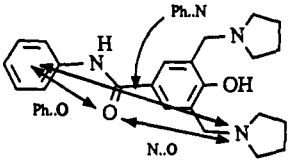
(23) Desai, B. N.; Fowler, K. W.; Chorvat, R. J.; Frederick, L. G.; Hatley, F. R.; Rorig, K. J.; Garthwaite, S. M. *J. Med. Chem.* 1988, *31*, 2158–2164.

(24) Hondeghem, L. M. *Circulation* 1987, *75*, 514–529.

(25) Smith, G. D., PROFIT. Medical Foundation of Buffalo, Buffalo, NY, 1979.

(26) Motherwell, S. PLUTO. University Chemical Laboratory, Cambridge, England, 1979.

Table VII. Intramolecular Distances between the Three Recognition Points in Class I Antiarrhythmics[a]



subclass	compd	Ph···O, Å	Ph···N, Å	N···O, Å
Ia	quinidine[9]	3.710 (4)[b]	5.113 (4)	3.093 (4)
	procainamide[10]	3.691 (8)	7.313 (8)	4.458 (8)
	disopyramide[11]	(i) 3.913 (3)	6.245 (3)	4.175 (3)
		(ii) 2.554 (3)	5.835 (3)	4.385 (3)
	average	3.45 (53)	6.13 (80)	4.03 (55)
Ib	lidocaine[8a]	(i) 3.83 (1)	5.46 (1)	3.59 (1)
		(ii) 3.78 (1)	5.42 (1)	3.57 (1)
	lidocaine[8b]	3.81 (1)	6.15 (1)	2.97 (1)
	lidocaine[8c]	3.799 (6)	6.178 (6)	2.850 (6)
	average	3.80 (2)	5.80 (36)	3.24 (34)
Ic	2	3.988 (1)	8.802 (1)	4.994 (2)
	3a	3.674 (3)	8.424 (4)	5.420 (5)
	3b	3.656 (3)	7.766 (4)	4.541 (5)
	4	3.685 (3)	8.731 (3)	5.517 (4)
	average	3.75 (14)	8.43 (41)	5.12 (39)

[a] Distances taken from X-ray crystallographic structure determinations. Examples of the types of distances are given on the chemical diagram. [b] In quinidine, a quinoline system replaces the phenyl as the lipophilic group; the pyridyl ring of the quinoline was taken as equivalent to the phenyl ring.

molecules adopt the same shape at the binding site as that found in the solid state. Thus, the crystallographically determined structures can be used to define the geometry of the pharmacophore for these compounds and as a model for class I antiarrhythmic drugs.

The distances between the N atom (N16), the center of the phenyl ring (Ph), and the carbonyl oxygen atom (O8), all in the numbering scheme of compound 2, are tabulated in Table VII, which also contains the angle between the phenyl ring and the bond to the oxygen atom. The Ph···O distances are almost all the same value, ca. 3.8 Å, undoubtedly due to the proximity of these two groups in the chemical structures, which suggests that the lipophilic and carbonyl groups can be taken as one pharmacophoric unit and the secondary or tertiary amine as another pharmacophore. The relative orientation of these two groups is not constant in the crystal structures; this variation may well reflect the ease of reorientation of phenyl rings to facilitate crystal packing. Comparison of the distances between these two units with the analogous distances calculated from the X-ray structures of other class I antiarrhythmics,[8-11] also given in Table VII, shows that the Ph···N and N···O distances are correlated with each other and that these distances tend to be correlated with the subclasses. A trend is evident: shorter distances are found for classes Ib and Ia and longer distances for class Ic. The data in Table VII suggest that molecular size, as measured by the distance between these two units, may affect the time constant for recovery from the sodium-channel blockade induced by the drug. This suggestion is tempered by the flexible nature of some of these drugs and needs to be pursued with studies of more rigid compounds.

The disparate separations observed between binding groups in Class I antiarrhythmics challenge our understanding of the receptor site. A receptor that binds all of these agents must undergo structural changes so that the separation between the recognition points for the phenyl–carbonyl groups and the amino nitrogen atom can vary. Binding could be either a one-step, simultaneous recognition of the two sites or recognition of each single site in separate steps. The simultaneous binding of both sites would require that the receptor have a series of intermediate states and that at one particular state the separation between recognition points matches the geometry of the drug; differences in time recovery from channel block might then be correlated to the conformational flexibility of the recognition site and the time the receptor spends in the binding conformation required by a single drug.

Alternatively, stepwise binding of the drug implies a "zipperlike"[27] interaction where one pharmacophore of the drug binds and subsequently the drug undergoes several consecutive conformational changes until it fits into the binding site. In this mode of binding, the variation in the time constant for recovery from blockade might arise from the difference in separation between the two binding pharmacophores or to the height of the energy barriers for the steps in the closing of the "zipper". There could be an optimal separation between the two pharmacophores which would achieve efficient stepwise binding or release; outside of the optimal range, longer separations would require many individual energy barriers to be overcome and would slow the process. Such a model would explain the faster time constants of class Ib which has the shortest separation between Ph···N and N···O and the longer time constants for the larger and more complex molecules in class Ic.

Distinctions between these two possible modes of binding and distinction between classes Ia and Ib will require study of the structures, the conformational energy profiles, and the time constants for recovery of more rigid antiarrhythmic drugs to determine if there is an optimal separation between the pharmacophores or an optimal linkage region between the pharmacophores. Also, additional molecular properties that affect the time constant for recovery, including molecular size[28] and lipophilicity,[29] need to be considered. Clearly, development of useful antiarrhythmic drugs will require optimization of all of these properties and a better understanding of the mode of binding.

Through the correlation between the pharmacophore separation and the time constant for recovery, and through the structural characterization of these more rigid compounds, this work provides a guide for future analysis of structure–activity relationships of class I antiarrhythmics.

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Registry No. 1, 72063-47-9; 2, 90446-66-5; 3, 81079-97-2; 4, 91112-01-5.

Supplementary Material Available: Anisotropic thermal parameters for the non-hydrogen atoms, positional and isotropic thermal parameters for the hydrogen atoms, bond lengths and angles for all atoms (22 pages). Ordering information is given on any current masthead page.

(27) Burgen, A. S. V. *Nature* **1975**, *253*, 753–755.
(28) Courtney, K. R. *J. Mol. Cell. Cardiol.* **1980**, *12*, 1273–1286.
(29) Courtney, K. R. *J. Mol. Cell. Cardiol.* **1983**, *15*, 749–757.
(30) Johnson, C. K. *ORTEP Report ORNL-3794*, 2nd ed.; Oak Ridge National Laboratory: Oak Ridge, TN, 1970.