Table II. Phosphorylation of Compounds by Purified VZV Thymidine Kinase

substrate	kinase activity,[a] pmol/min per mL	
	monophosphate	diphosphate
thymidine	585	174
13a	63	ND[b]
13	1279	ND
15	736	ND
16	333	ND
BVaraU (12)	140	95
acyclovir (9)	13	ND

[a] Initial reaction velocities for mono- and diphosphate production were determined by HPLC using 100 μM substrate and purified VZV (strain Ellen) thymidine kinase. Details of the reaction are described in the Experimental Section. [b] ND = not detectable.

derived from ppIIa. Both were provided by Dr. J. Ostrove, NIH. VZV strain 9021 is a recent clinical isolate provided by Dr. L. Frenkel, Robert Wood Johnson Viral Diagnostic Laboratory. VZV strains Ellen (VR-58) and Oka (VR-795) were obtained from ATCC. WI-38 (CCL75) and Vero (CCL81) cells were obtained from ATCC and were grown in Eagles minimim essential medium with Earle's salts (EMEM) supplemented with 2 mM L-glutamine, 100 units/mL penicillin, 11 μg/mL streptomycin, and 10% FBS (Gibco Laboratories, Grand Island, NY).

Viruses were assayed on WI-38 cell monolayers. Viruses were absorbed to cell monolayers in 6-well culture plates (Costar, Cambridge, MA) for 1–2 h prior to addition of maintenance medium (EMEM plus supplements, 1% (carboxymethyl)cellulose, 2.5% FBS ± drug) containing duplicate dilutions of the test compound. Inhibition of plaque development for all viruses was evaluated after 4–6 days incubation at 37 °C. ID_{50} values were determined from the drug concentration which conferred 50% plaque reduction compared to virus controls. All titrations were done in duplicate and expressed as a range in repeat assays.

Cell Growth Inhibition. WI-38 were planted at 1×10^5 cells/mL in 12-well Costar cell culture plates. Twenty four hours later, the cell cultures were refed with growth medium containing serial dilutions of the antiviral compound. At 24-h intervals, quadruplicate cell cultures at each concentration were resuspended by trypsinization and counted for viable and dead cells using the criterion of trypan blue exclusion. Control cultures were similarly evaluated, and over the 96-h evaluation increased 3- to 5-fold. The ID_{50} for each compound was calculated as the concentration which inhibited growth by 50% relative to control cell cultures.

Varicella-Zoster Thymidine Kinase Assay. VZV thymidine kinase was purified from VZV-infected WI-38 cells by sequential DEAE-cellulose and thymidine-affinity chromatography.[16] The final product was >90% pure as judged by SDS PAGE and approximately 98% free of cellular kinase activities. The enzyme was assayed in a reaction mixture containing 200 mM Tris-Cl (pH 7.5), 6 mM $MgCl_2$, 3 mM ATP, 1 mM DTT, 100 μg/mL BSA, 100 μM nucleoside substrate, and 50 ng/mL purified VZV thymidine kinase. Reactions were incubated at 25 °C for intervals of up to 30 h and then quenched by heating at 80 °C for 2 min. Reaction products were analyzed and quantitated by HPLC. The values shown in Table II represent initial reaction velocities, which were linear for both the nucleoside and nucleoside-monophosphate kinase activities.

Acknowledgment. We thank Dr. Joan Newburger and her staff for the chiral HPLC analysis and Mr. Terry McCormick and his staff for the elemental analyses.

(15) Spectral data were identical to those of the corresponding racemate.

(16) Fyfe, J. A. Differential Phosphorylation of (E)-5-(2-Bromovinyl)-2′-deoxyuridine Monophosphate by Thymidylate Kinases from Herpes Simplex Viruses Types 1 and 2 and Varicella Zoster Virus. *Mol. Pharmacol.* 1982, *21*, 432–437.

Molecular Modeling and Crystallographic Studies of 4-Amino-*N*-phenylbenzamide Anticonvulsants

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Received August 22, 1991

The molecular structures of five different MES-active *N*-phenylbenzamides were determined by X-ray diffraction methods, and the conformations of a series of active and inactive benzamides were analyzed by molecular mechanics calculations. The most active compounds adopt a similar, consistent conformation in both the experimentally determined crystallographic structures and in the calculated molecular mechanics structures. This conformation places one *o*-methyl group proximal to the NH group of the central amide plane and orients the methyl-substituted phenyl ring at an angle of 90° to 120° to the central amide plane. Intermolecular interactions in the crystal structures indicate that hydrogen bonding to the central amide group is the important interaction. The observed consistent conformation facilitates formation of hydrogen bonds to the carbonyl oxygen atom. The conformations of inactive compounds obstruct this interaction. These findings help to outline a model of some of the structural features which this series of benzamides must possess in order to demonstrate MES anticonvulsant activity.

Approximately 1% of the population suffers from epilepsy, but less than 50% of these people achieve seizure control with drug therapy, and only 70–80% experience partial seizure control.[1] Since current anticonvulsant drugs are often inadequate in the control of epileptic seizures, the search continues for different and more active compounds. With the exception of the benzodiazepines and NMDA receptor antagonists, receptor sites have not been identified for anticonvulsants; therefore, drug identification is usually conducted via in vivo screening tests. Two tests commonly used are the maximal electroshock (MES) test and the subcutaneous metrazole (scMET) test.[2] A new series of potent 4-aminobenzamide anticonvulsants

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(1) Jones, G. L.; Woodbury, D. M. Principles of Drug Action: Structure Activity Relationships and Mechanisms. In *Antiepileptic Drugs*, 2nd ed.; Woodbury, D. M., Perry, J. K., Pippenger, C. E., Eds.; New York: Raven Press, 1982; pp 83–109.

(2) Porter, R. J.; Pitlick, W. H. Antiepileptic Drugs. In *Basic and Clinical Pharmacology*, 3rd ed.; Katzung, B. G., Ed.; Appleton and Lange: East Norwalk, CT, 1987; pp 262–278.

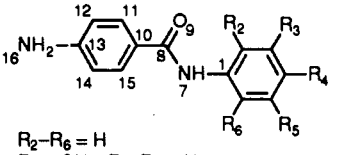
0022-2623/92/1835-1806$03.00/0

Table I. Crystallographic Data for the 4-Amino-*N*-phenylbenzamides

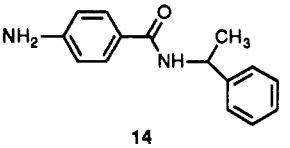
	1	2	3	8	14
molecular formula	$C_{13}H_{12}N_2O$	$C_{14}H_{14}N_2O$	$C_{14}H_{14}N_2O$	$C_{15}H_{16}N_2O$	$C_{15}H_{16}N_2O$
molecular weight	212.25	226.28	226.28	240.31	240.31
crystal size (mm)	0.10 × 0.18 × 0.28	0.30 × 0.30 × 0.40	0.07 × 0.18 × 0.20	0.41 × 0.48 × 0.51	0.26 × 0.26 × 0.36
solvent	acetone	acetone	acetone	ethyl acetate	$^2/_3$EtOH/$^1/_3H_2O$
crystal system	monoclinic	monoclinic	orthorhombic	monoclinic	orthorhombic
space group; Z	Cc; 4	$P2_1$; 8	$P2_12_12_1$; 4	$P2_1/c$; 8	$P2_12_12_1$; 4
a (Å)	6.815 (4)	8.837 (1)	11.9261 (5)	8.9013 (5)	11.3497 (5)
b (Å)	30.622 (2)	16.5433 (6)	13.0779 (8)	16.722 (2)	22.223 (2)
c (Å)	9.903 (7)	17.129 (4)	7.7419 (7)	18.390 (2)	5.1067 (3)
β (°)	148.74 (2)	99.88 (1)	90	95.364 (5)	90
volume (Å)	1072.6 (10)	2467.0 (7)	1207.5 (1)	2725.3 (4)	1288.1 (1)
ρ_c (g/cm^3)	1.314	1.218	1.245	1.171	1.239
total reflections	1847	8504	2937	12,277	3135
unique reflections	1025	5242	1353	5147	1449
observed reflections (>2.5 σ)	1006	4939	1053	4505	1376
R	0.030	0.050	0.048	0.053	0.042
R_w	0.039	0.076	0.057	0.094	0.057
S	1.03	1.07	1.04	1.02	1.07
highest residual electron density (e$^-$/Å^3)	0.118	0.219	0.149	0.304	0.152

have been reported;[3-10] included are 4-amino-*N*-(2,6-dimethylphenyl)benzamide, **8**, a potent MES-active drug (MES ED_{50} = 2.6 mg/kg in mice[3]), and 4-amino-*N*-(1-phenylethyl)benzamide, **14**, a drug active in both of the above screening tests (MES ED_{50} = 10.3 mg/kg, scMET ED_{50} = 41.7 mg/kg in mice).[5,9] Both **8** and **14** are as active or more active than the commonly prescribed anticonvulsants phenobarbital, diphenylhydantoin, and valproic acid; in addition, the protective indices of these *N*-phenylbenzamides compare favorably with those of the prescribed drugs.

Structure–activity relationship (SAR) studies on the benzamide framework reveal that several features of **8** and **14** are required for optimum activity. 4-Aminobenzamide is MES-inactive,[5] but the introduction of an alkyl or aryl substituent onto the amide nitrogen atom confers MES activity. Also a cyclic substituent, such as a cyclohexyl or a phenyl ring,[5] yields greater MES potency than does a noncyclic analogue such as a saturated *n*-hexyl chain. For the benzamide phenyl ring, an amino substituent is required for optimal MES activity, and drug potency follows the order of para > meta > ortho[6] for the amino position. This order suggests that the substituent is not acting purely via electronic effects, but that the amino group must occupy a particular position with response to the remainder of the molecule. Moreover, a primary amino group is preferred; methylation at the nitrogen reduces MES activity,[6] and N-acetylation destroys the anticonvulsant activity of **14** altogether.[8]



1 R_2–R_6 = H
2 R_2 = CH_3, R_3–R_6 = H
3 R_3 = CH_3, R_2 = R_4–R_6 = H
4 R_4 = CH_3, R_2 = R_3 = R_5 = R_6 = H
5 R_2 = R_3 = CH_3, R_4–R_6 = H
6 R_2 = R_4 = CH_3, R_3 = R_5 = R_6 = H
7 R_2 = R_5 = CH_3, R_3 = R_4 = R_6 = H
8 R_2 = R_6 = CH_3, R_3–R_5 = H
9 R_3 = R_4 = CH_3, R_2 = R_5 = R_6 = H
10 R_3 = R_5 = CH_3, R_2 = R_4 = R_6 = H
11 R_2 = $CH(CH_3)_2$, R_3–R_6 = H
12 R_2 = $CH(CH_3)_2$, R_3–R_5 = H, R_6 = CH_3
13 R_2 = $CH(CH_3)_2$, R_3–R_5 = H, R_6 = CH_2CH_3



14

The MES activity of 4-amino-*N*-phenylbenzamide, **1**, is increased by the addition of an *o*-methyl substituent on the phenyl ring (**2**); the addition of a second *o*-methyl group (**8**) yields an even more MES-potent anticonvulsant. The superior activity of **8** is not simply a result of two methyl substituents being present, but is closely related to the positions of these groups on the phenyl ring, as can be observed by comparison to both compounds **5** and **9**. The order of MES activity for these benzamides is[3] **1** ≈ **3** ≈ **9** < **5** ≤ **2** < **8**. Insertion of a methylene group between the amide carbonyl group and the adjacent phenyl group (between atoms C(8) and C(10)) results in a distinct loss of MES activity.[3,4] In contrast, placing a methylene group between the amide nitrogen atom and the unsubstituted phenyl ring of **1** (between atoms C(1) and N(7)) enhances MES activity; however, extending the length of this spacer reduces MES and scMET activity, thus the structure of **14** is optimal for activity.

To help identify common conformational features of the *N*-phenylbenzamide drugs, and to correlate structure with

(3) Clark, C. R.; Sansom, R. T.; Lin, C. M.; Norris, G. M. Anticonvulsant Activity of Some 4-Aminobenzanilides. *J. Med. Chem.* **1985**, *28*, 1259–1262.
(4) Clark, C. R.; Davenport, T. W. Anticonvulsant Activity of Some 4-Aminophenylacetamides. *J. Pharm. Sci.* **1987**, *76*, 18–20.
(5) Clark, C. R.; Wells, M. J. M.; Sansom, R. T.; Norris, G. N.; Dockens, R. C.; Ravis, W. R. Anticonvulsant Activity of Some 4-Aminobenzamides. *J. Med. Chem.* **1984**, *27*, 779–782.
(6) Clark, C. R.; Lin, C. M.; Sansom, R. T. Anticonvulsant Activity of 2- and 3-Aminobenzanilides. *J. Med. Chem.* **1986**, *29*, 1534–1537.
(7) Clark, C. R.; Davenport, T. W. Synthesis and Anticonvulsant Activity of Analogues of 4-Amino-*N*-(1-phenylethyl)benzamides. *J. Med. Chem.* **1987**, *30*, 1214–1218.
(8) Robertson, D. W.; Leander, J. D.; Lawson, R.; Beedle, E. E.; Clark, C. R.; Potts, B. D.; Parli, C. J. Discovery and Anticonvulsant Activity of the Potent Metabolic Inhibitor 4-Amino-*N*-(2,6-dimethylphenyl)-3,5-dimethylbenzamide. *J. Med. Chem.* **1987**, *30*, 1742–1746.
(9) Robertson, D. W., personal communication, Lilly Research Laboratories, Indianapolis, IN.
(10) Germain, G.; Main, P.; Woolfson, M. M. Application of Phase Relations to Complex Structures III. Optimum Use of Phase Relations. *Acta Crystallogr., Sect. A: Cryst. Phys., Diffr. Theor. Gen. Crystallogr.* **1971**, *A27*, 368–376.

activity for these anticonvulsants, the crystal structures of compounds 1–3, 8, and 14 and the stable molecular mechanics conformations of active and inactive compounds have been determined and are presented herein. Compound 14, which contains one asymmetric carbon atom, is reported as the more MES-potent (*S*)-isomer.[8,9]

Crystallographic Experimental Section

Samples of the drugs were obtained from C.R. Clark of Auburn University, AL,[3-8] and from D.W. Robertson of Lilly Research Laboratories, Indianapolis, IN.[8,9] For all of the crystal structure determinations, data were measured at room temperature using an Enraf-Nonius CAD4F diffractometer, nickel-filtered CuKα radiation (λ = 1.5418 Å), and ω-2θ scans. Three intensity standards were measured every 2000 s of exposure time; one of the five crystals showed significant deterioration during data collection. Both Lorentz and polarization corrections were applied. The crystallographic data for all five of the crystal structures are listed in Table I.

The structures of 1, 3, 8, and 14 were determined via MULTAN78;[10] 2 was solved using SHELXS86.[11] For structures 1–3 and 14, all of the non-hydrogen atoms were located in the original E-synthesis. For structure 1, the origin of the unit cell was defined by fixing the *x* and *z* coordinates of the N(7) atom; for structure 2 by fixing the *y* coordinate of the N(107) atom. For structures 1 and 3, all of the hydrogen atoms were located in difference Fourier syntheses and the hydrogen parameters were included in the refinement. For 2, all amine and amide hydrogen atoms and most of the remaining methyl and phenyl hydrogen atoms were found in difference Fourier syntheses. The amine and amide hydrogen atoms were placed at observed positions, while all of the other hydrogen atoms were placed at calculated positions; all of the hydrogen thermal parameters were assigned as 120% of the *B* (equivalent) value of the attached non-hydrogen atoms, and no hydrogen parameters were refined. For 14, all of the hydrogen atoms except for H(4) were found in difference Fourier syntheses. The phenyl hydrogen atoms H(2)–H(6) were placed at calculated positions, assigned thermal parameters as in 2, and were not refined. All of the remaining phenyl and methyl hydrogen atoms were placed at calculated positions, H(7) and all of the amine and amide hydrogen atoms were placed at observed positions, and for all of these hydrogen atoms both the atomic coordinates and the isotropic thermal parameters were refined.

Structure 8 revealed four fragments of molecules in the E-synthesis; one of the larger fragments was almost complete, but problems with refinement were encountered which indicated that the fragments was misplaced within the unit cell. This fragment was used to find a translation vector via DIRDIF and TRADIR.[12] Application of this vector allowed identification of the remainder of this molecule, and all of the second molecule was then easily identified. Difference Fourier syntheses revealed all of the amine, amide, and aminophenyl hydrogen atoms, but few of the other hydrogen atoms. All of the aminophenyl hydrogen atoms were placed at calculated positions, and their atomic coordinates and isotropic thermal parameters were refined. Amide and amine hydrogen atoms were located at observed positions, but only the atomic coordinates were refined; the thermal parameters were assigned as in 2. All of the methyl and methylphenyl hydrogen atoms were placed at calculated, idealized positions, assigned thermal parameters as above, and were not refined.

All of the refinements varied the positional parameters and the anisotropic thermal parameters of the non-hydrogen atoms and the parameters of the hydrogen atom parameters as described below. In all cases except 2, the refinement was broken into blocks to assure overdetermination of the least squares equations. In 2, the hydrogen atom parameters were not refined. For 1, all non-hydrogen atom parameters were refined in one block; the parameters for all hydrogen atoms and atom N(7) were refined in another block. For 3, atoms C(1)–O(9) were in a block, atoms N(7) and C(10)–C(17) were in another block, and atom N(7) and all hydrogen atoms were in a third block. For 8, the parameters for the non-hydrogen atoms and the positional parameters for the NH hydrogen atoms were refined; molecule 1 made up one block and molecule 2 was in another block. All of the programs used were part of the XRAY76[13] package, with the exception of the structure solution programs mentioned above. The scattering factors were those of Cromer and Mann,[14] except for the hydrogen atom factors, which came from Stewart et al.[15]

The atomic coordinates and thermal parameters for all atoms and complete lists of bond distances and bond angles are available (see paragraph at the end of the paper regarding supplementary material).

Calculation Methodology

The molecular mechanics calculations on the chemical structures which were also studied crystallographically were performed by using MMP2[16] (release 11.0), supplemented with amide parameters from MMX.[17] The phenyl ring (ring C in Table VII) was treated by using the special phenyl parameters contained in MM2P and omitting a π calculation for this ring. The program was implemented on a Honeywell Multics (HIS DPS 8/70M) mainframe computer. The energy differences between all of the observed molecular conformations of compounds 1–3 and 8 were evaluated. To reproduce the crystallographic conformations, the dihedral angles τ_1 (2-1-7-8) and τ_2 (9-8-10-11) were restricted to the crystallographically observed values, and the remainder of the structure was minimized.

MMP2 calculations were used to evaluate the barrier to rotation about the torsion angle τ_1 for compounds 1–3 and 8, and about τ_2 for compound 1. (The molecular fragment that defines τ_2 is the same in each of the compounds studied so only one calculation of the barrier for τ_2 was necessary.) The barrier to rotation was taken as the difference between the highest energy conformation and the minimum energy conformation obtained by varying τ_1 or

(11) Sheldrick, G. SHELXS-86, Institut für Anorganische Chemie der Universität, Tammannstrabe 4, D-3400 Göttingen, Federal Republic of Germany, 1986.
(12) Beurskens, P. T.; Bosman, W. P.; Doesburg, H. M.; Gould, R. O.; van der Hark, Th. E. M.; Prick, P. A. J.; Noordik, J. H.; Beurskens, G.; Parthasarathi, V. DIRDIF: Direct Methods for Difference Structures. Crystallography Laboratory, Toernooiveld, Nijmegan, Netherlands: 1982.
(13) Stewart, J. M. XRAY76: The X-ray System of Crystallographic Programs; Computer Science Centre, University of Maryland, College Park, MD; 1977.
(14) Cromer, D. J.; Mann, J. B. X-ray Scattering Factors Computed from Numerical Hartree-Fock Wavefunctions. *Acta Crystallogr., Sect. A: Cryst. Phys., Diffr. Theor. Gen. Crystallogr.* **1968**, *A24*, 321–324.
(15) Stewart, R. F.; Davidson, E. R.; Simpson, W. T. Coherent X-ray Scattering for the H-atoms in the H molecule. *J. Chem. Phys.* **1965**, *42*, 3175–3187.
(16) Allinger, N. L. MMP2: Molecular Mechanics, Quantum Chemistry Program Exchange, Chemistry Department, Indiana University, Bloomington, IN 47405; 1982.
(17) PCMODEL Molecular Modeling Software, Version 1.0, Serena Software, Box 3076, Bloomington, IN 47402-0823; 1989.

Table II. Selected Bond Distances (Å) and Bond Angles (deg) for the Central Region of the Phenylbenzamides (Atom Numbering as in Figures 1–5)

compound	C10–C8	C8–O9	C8–N7	N7–C1	C8–N7–C1	C10–C8–N7
1	1.493 (8)	1.219 (7)	1.363 (2)	1.419 (5)	127.3 (3)	115.1 (4)
2 (molecule 1)	1.490 (4)	1.236 (3)	1.334 (3)	1.440 (4)	122.9 (2)	117.8 (2)
2 (molecule 2)	1.498 (4)	1.228 (4)	1.332 (4)	1.434 (4)	124.1 (3)	116.5 (2)
2 (molecule 3)	1.487 (4)	1.243 (3)	1.341 (4)	1.424 (4)	124.6 (2)	118.5 (2)
2 (molecule 4)	1.489 (3)	1.240 (3)	1.329 (4)	1.434 (3)	123.7 (2)	118.1 (2)
3	1.483 (4)	1.235 (4)	1.364 (4)	1.419 (4)	128.8 (3)	117.1 (3)
8 (molecule 1)	1.485 (2)	1.241 (2)	1.343 (2)	1.426 (2)	123.4 (1)	116.7 (1)
8 (molecule 2)	1.496 (2)	1.238 (2)	1.333 (2)	1.429 (2)	123.8 (1)	116.7 (1)
14	1.486 (3)	1.246 (2)	1.342 (2)	1.460 (2)[a]	122.5 (2)[a]	116.8 (2)

[a] The carbon atom in the C1 position has sp^3 hybridization in compound 14; in all of the other compounds, C1 is sp^2 hybridized.

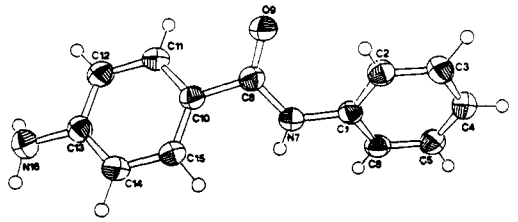


Figure 1. Molecular conformation and atomic labeling scheme for compound 1. The drawing was made with the computer program ORTEP.[19]

τ_2. The torsion angles were varied in 10° increments by using the torsion angle driver option of MMP2. At each step, the input coordinates were taken from the geometry of the preceding rotamer.

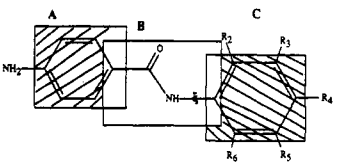
The MM2 molecular mechanics force field (with extensions as provided in the MACROMODEL software package[18]) and Monte Carlo searching (MACROMODEL) of conformational space were used to calculate all possible low energy conformers of compounds 2–8. In each calculation, 3000 conformers were sought, and the torsion angles that define the orientation of the NH_2 group, the aminophenyl ring, and the methylphenyl ring were varied.

Results

For the crystal structures of 1, 3, and 14 there was one molecule per asymmetric unit; compound 8 had two molecules and compound 2 had four molecules per asymmetric unit. The molecular conformations for all unique molecules are shown in Figures 1–5. Selected bond distances and bond angles are presented in Table II.

Extensive hydrogen bonding to the central amide occurs in all of the crystal structures. In eight of the nine independent observations of intermolecular contacts, the hydrogen bonding pattern is the same: the carbonyl oxygen atom forms two interactions, one to the central amide NH of a neighboring molecule and one to the terminal amino group of a second adjoining molecule. The central amide NH groups participate in one or two hydrogen bonds; thus, the center of each molecule has intermolecular contacts in two directions. The exception is compound 3, which has a nearly planar molecular structure; in 3, the carbonyl O atom has only one hydrogen bond, to the terminal amino group. In addition to the hydrogen bonds, there are also electrostatic interactions involving the amino H atom or the amide H atom which provide additional stabilization to the observed crystal structure packing of the molecules.

Table III. The Acute Angles between the Three Planar Regions of the Benzamides, As Found in the Crystal Structures



compd	angle A–B (°)	angle B–C (°)	Angle A–C (°)
1	34.9	27.8	61.7
2 (1)	24.1	70.3	46.5
(2)	23.0	87.8	71.0
(3)	4.3	55.4	53.5
(4)	19.7	59.2	77.8
3	18.2	2.3	20.5
8 (1)	29.8	65.1	35.5
(2)	37.2	89.9	55.6
14	24.2	84.2	75.8

In all of the crystal structures, the amide region and each of the two phenyl rings are individually planar, but these three groups are not necessarily coplanar; hence, the degree of conjugation among these aromatic fragments varies from structure to structure. A list of the acute angles between these planes for all of the crystal structure conformations is given in Table III. Examination of Table III reveals that the methylphenyl ring is twisted farther from the plane of the amide than is the aminophenyl ring.

The energy differences between the individual crystallographic conformations were evaluated using molecular mechanics. The conformations are calculated to be within 3.2 kcal/mol of one another for 2 and within 1.7 kcal/mol for 8, in each case, an energy difference that could be easily regained by the formation of one hydrogen bond. In each case, full geometry optimization produces a single low-energy conformer, regardless of which crystallographic conformation was used as a starting point. The fully optimized structures were not the same for the various compounds, and varied from each other mainly with respect to τ_1. Energy differences between the crystallographic conformers and the MMP2 minima were 1: 3.3 kcal/mol, 2: 0.3–3.5 kcal/mol, 3: 0.7 kcal/mol and 8: 3.2–5.2 kcal/mol.

The MMP2 minimizations of the crystal structures find that the dihedral angle τ_2 of the fully minimized structures demonstrates a strong tendency for the aminophenyl ring to remain coplanar with the adjacent carbonyl group; the calculated barrier to rotation about τ_2 is 15.3 kcal/mol. The torsion angle τ_1, however, exhibits more variability; the calculated barrier to rotation is in the range of only 1.1–4.0 kcal/mol (Figure 6), and is thus much lower than that for τ_2.

The conformational preferences for the orientation of the *o*-methyl group was investigated for the compounds

(18) Mohamadi, F.; Richards, N. G. J.; Guide, W. C.; Liskamp, M. L.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. Macromodel—An Integrated Software System for Modeling Organic and Bioorganic Molecules Using Molecular Mechanics. *J. Comput. Chem.* **1990**, *11*, 440–467.

(19) Johnson, C. K. ORTEP-II: A Fortran Thermal Ellipsoid Plot Program for Crystal Structure Illustrations. Oak Ridge National Laboratory, Oak Ridge, TN; report number ORNL-5138, 1976.

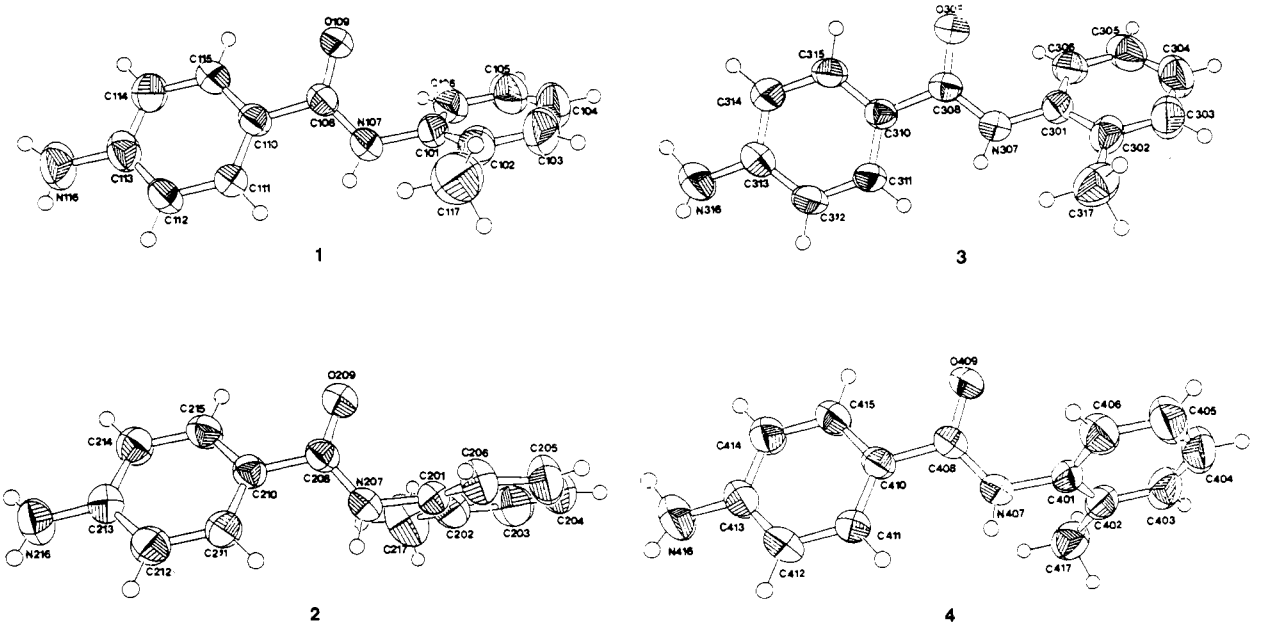


Figure 2. Molecular conformations and atomic labeling schemes for the four unique molecules of compound **2**. The drawing was made with the computer program ORTEP.[19]

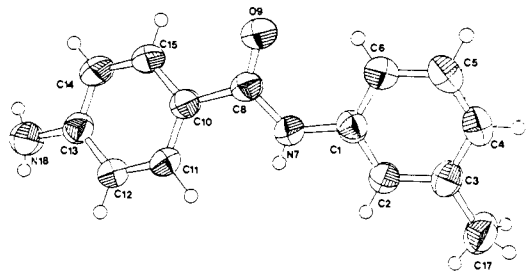


Figure 3. Molecular conformation and atomic labeling scheme for compound **3**. The drawing was made with the computer program ORTEP.[19]

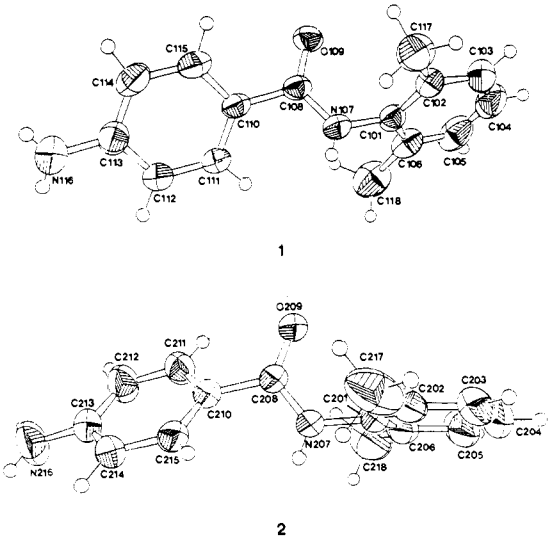


Figure 4. Molecular conformations and atomic labeling schemes for the two unique molecules of compound **8**. The drawing was made with the computer program ORTEP.[19]

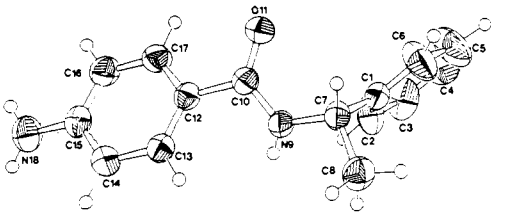


Figure 5. Molecular conformation and atomic labeling scheme for compound **14**. The drawing was made with the computer program ORTEP.[19]

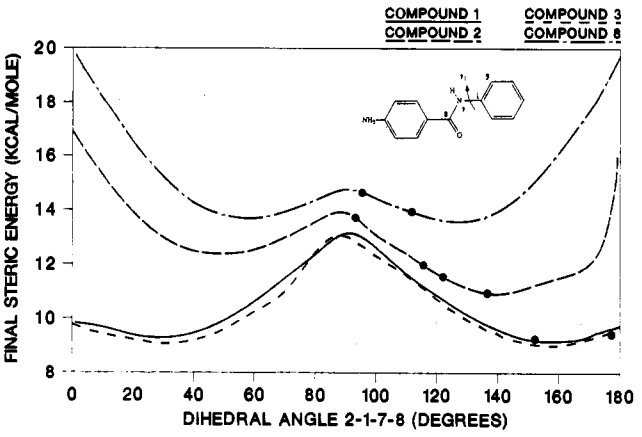


Figure 6. Calculated energy (in kcal/mol) of the barrier to rotation about the bond C(1)–N(7) (τ_1) for compounds **1–3** and **8**. The solid dots mark the torsion angles observed in the crystal structures and tabulated in Table III.

with substituted phenyl rings (**2**, **4–8**, and **10**) by using a Monte Carlo searching procedure. All compounds with a single ortho substituent (**2**, **5–7**) had the lowest energy conformation with the *o*-methyl group oriented toward the NH side of the central amide plane. Analysis of the relative populations of the conformers indicates that the NH side and the C=O side conformers are nearly equally probable. The minimum difference in energy between the NH side and the C=O side conformations varied from 1.8 kcal/mol for **5** to 3.2 kcal/mol for **7**. Compound **8**, with two ortho substituents, had only one orientation for the phenyl ring with a torsion angle C(8)–N(7)–C(1)–C(2) of approximately 121°, which differed by 20° from the average low-energy conformation for the single *o*-methyl compounds (C(8)–N(7)–C(1)–C(2) ≈ 145°). The two compounds within an ortho substituent (**4** and **10**) showed only one orientation for the methylphenyl ring with a torsion

angle between 152 and 158°.

Discussion

The nine observations of the *N*-phenylbenzamide framework in these crystal structures display trends which may explain the structure–activity relationships of these compounds. These trends show that a particular conformation is favored for high activity compounds and that the intermolecular bonding is affected by changes in the molecular conformation. The observed molecular conformations show that the aminophenyl ring remains nearly coplanar with the central amide group, but the orientation of the methylphenyl ring (C(1)–C(6)) varies as the number and position of the substituents on the ring change (see Table III). Excluding **14** due to its structural differences, the average angle between the plane of the methylphenyl ring, plane C, and that of the central amide, plane B, is 57.2°, with a large standard deviation (29.6°), indicating the variability of this angle. In contrast, the average angle (including **14**) between the plane of the aminophenyl ring, plane A, and the plane of the central amide, C, is 23.9° (σ = 9.8°). Thus the crystal structures suggest that there is a high barrier to rotation about τ_2 (9–8–10–11) and a lower barrier for τ_1 and that the methylphenyl ring moves farther out of the plane of the amide group as more *o*-methyl substituents are added to the phenyl ring.

The most active compounds, **2**, **8**, and **14**, each have a single crystalline conformation: **2** (2), **8** (2), and **14**, which has approximately the same angles between the three planes (planes A, B, and C in Table III). In particular, the angle between plane B, the central amide, and plane C, the methylphenyl ring, which has a tendency to vary widely (see above), varies minimally in these three structures: 84.2° to 89.9°, average = 87.3° (2.3°). Also, the angle between the central amide (plane B) and the aminophenyl ring (plane A) varies over a slightly narrower range than the full set of conformations, average = 28.1° (6.4°). This consistent conformation places the methylphenyl ring perpendicular to the central amide region of the drug. This conformation (with the methylphenyl ring ca. 90° from the central amide plane) is found only in the crystal structures of the most active compounds.

Molecular mechanics calculations of the barrier to rotation about τ_1 (2–1–7–8) for compounds **1–3** and **8** (see Figure 6) show the effects of changes in the position and number of methyl substituents, namely that (1) the *m*-methyl substituent shows no strong steric interaction with the central amide, thus **3** exhibits the same barrier for τ_1 as does **1**, which has an unsubstituted phenyl ring, (2) the barrier to mutual orthogonality (i.e. τ_1 = 90°) of the methylphenyl ring, plane C, and the central amide group, plane B, decreases as more ortho substituents are added to the phenyl ring, (3) the barrier to coplanarity (i.e. τ_1 = 0° or 180°) of the same two planes increases as more ortho substituents are added, and as a result of (2) and (3), the lowest energy conformation shifts slightly towards τ_1 = 90° with increased substitution in the ortho position. This shift is evident in Figure 6 and is supported by the crystal structure results which are plotted on the curves in Figure 6. The crystal conformations shift from τ_1 values >150° for **3** and **1**, to τ_1 < 120° for **8**.

Due to small distortions arising from the close approach of neighboring molecules, the torsion angles in the crystal structures differ slightly from the ideal values calculated for molecules in a vacuum; the two data together indicate that the effect of ortho substitution is to change the distribution of populations from a widely varying distribution for unsubstituted compounds to a narrow distribution of conformations for the disubstituted compound. Thus, part of the reason that compound **8** has higher activity may be due to a favorable entropic contribution to the free energy of binding, that is, molecules of **8** are more likely to be in the binding conformation.

Analysis of the molecular conformations in the crystals (see Figures 1–5) reveals a strong preference for a conformation with the *o*-methyl group on the same side of the molecule as the NH group of the central amide, and thus opposite the carbonyl oxygen atom. This preference was also found in molecular mechanics calculations which found lower energy for conformations which placed the *o*-methyl group toward the NH of the central amide plane. These findings suggest that the interaction between the aminobenzamides and the acceptor site involves a specific arrangement of the *o*-methyl substituent and the central amide.

The most active compound (2,6-dimethyl, **8**) is symmetrical and has an ortho substituent on each side of the central amide plane; however, this molecule is unique because the low-energy conformation favors a 120° torsion angle for plane C, and the crystalline conformation even displays mutually perpendicular planes, which considerably lessens the steric interaction of the methyl groups and the atoms of the central amide plane. All mono-*o*-substituted compounds, two active compounds (2-methyl, **2**, and 2,3-dimethyl, **5**), and two inactive compounds (2,4-dimethyl, **6**, and 2,5-dimethyl, **7**) show the same conformational preference for the NH side of the central amide plane and show the same torsion angle of 145° for the orientation of plane C. Thus, the conformations of these compounds are determined by the number of ortho substituents and by a preference for orientation of the ortho substituent toward the NH group of the central amide.

The inactivity of compounds **4**, **6**, and **7** and the similarity of conformations (discussed above) help to define the binding requirements of the recognition site for these compounds. The preferred conformation for binding is assumed to be that adopted by the 2,6-dimethyl compound **8** which places one methyl group above and one below the molecular plane formed by the central amide and the aminophenyl ring. Since low activity is obtained for any compound which lacks an ortho substituent, and since compound **2** is a potent anticonvulsant which can adopt the preferred conformation (e.g. molecule **2** (2)), we postulate that a single methyl group on the NH side of the molecule is recognized by the binding site. This postulate assumes that the lowest energy conformation of compound **2**, which favors the NH orientation, is the preferred binding mode. Since a single methyl substituent in the 4-position produces lower activity than either an unsubstituted (**1**) or *m*-methyl-substituted (**3**) compound,[3] the inactivity of compound **6**, even though the molecule has an ortho substituent and can adopt the same conformation as the active compounds **2** and **5**, is probably due to steric hindrance in the binding site which arises from the 4-substituent. The low activity of compound **7** suggests that the methyl substituent in the 5-position, which would be on the carbonyl side of the central amide plane in the low-energy conformation, interferes with recognition. This leads to a hypothesis that bonding to the carbonyl group of the central amide plane is an important part of the recognition interaction. Thus, compound **7** is inactive because the *m*-methyl group interferes with hydrogen bonding to the carbonyl oxygen atoms, and compound **5** is active because, in it, the *m*-methyl group is on the opposite side of the central amide (oriented toward the NH) and does not affect interactions with the carbonyl group.

The crystal structures show that these conformational effects produce a fundamental change in the bonding in the central amide. Linear regression analysis of the variation in the C(8)–N(7) bond length with changing dihedral angle between planes B and C finds a correlation coefficient of –0.889 which indicates that, as plane C rotates out of the central amide plane, the amide bond length decreases, thus, the π character of the bond increases. This finding is supported by the correlation between the bond distance linking the central amide to the variable phenyl ring; the correlation coefficient between the C(1)–N(7) bond and the dihedral angle is positive (0.726), indicating that the π overlap between the phenyl ring and the amide decreases as the ring rotates out of the central amide plane. The lower value of this correlation indicates that there are other influences on this bond length, including the steric interactions arising from the *o*-methyl substituents. Supporting the indication that the hybridization of N(7) changes as the interplanar angle changes from 2.3° to 89.9°, the correlation between the C(8)–N(7)–C(1) angle and the dihedral angle is strong: –0.890; the bond angle is a maximum for the nearly planar structure (128.8° (3°)) and a minimum for molecule 2 of **2** (see Table II).

These effects on the bonding of the central amide are significant because the main intermolecular interaction observed for these compounds involves hydrogen bonding to this central region (see Results, above). Since the observed intermolecular interactions in crystal structures provide models for the potential molecule to molecule interactions at a recognition site,[20] the sensitivity of the bonding of the central amide to the molecular conformation may influence recognition interactions. Notable in the crystal packing in these compounds are (1) the approximately planar molecule **3** has an altogether different pattern of hydrogen bonding because steric constraints prevent close approach of neighboring central amide groups and (2) the strongest hydrogen bonds are formed by conformer 2 of the most active compound **8**, where the angle between planes B and C is 89.9° (O(9)···N(7), 2.860 (2) Å and O(9)···NH_2, 2.880 (3) Å). Thus, the central amide is "unavailable" for recognition interactions in a planar conformation, and hydrogen bonds are particularly strong in a perpendicular conformation. The effect of molecular conformation on hydrogen bond geometry is supported by the correlation coefficient of –0.874 between the O(9)···NH_2 distance and the dihedral angle between the B and C planes which indicates that as the angle increases, the separation between hydrogen bonded atoms decreases, strengthening the interaction. The correlation is not as strong, but shows the same trend, for the central amide hydrogen bonds to other central amides (r = –0.731).

In summary, a model for the MES-active conformation of these *N*-phenylbenzamide anticonvulsants can be constructed which includes four major factors: (1) an *N*-phenyl ring which is nearly perpendicular to the central amide region, thus facilitating the formation of strong intermolecular hydrogen bonds to the central amide region, (2) an *o*-methyl substituent oriented toward the NH group of the central amide plane, (3) a hydrogen bond acceptor in the central region on the side of the central plane opposite to the *o*-methyl group, and (4) an approximately coplanar orientation of the aminophenyl ring to the central amide plane. Whether the substituent methyl groups play any role other than orienting the phenyl ring with respect to the amide region is uncertain; however, given the preferential orientation of a single *o*-methyl group, we hypothesize that it is recognized in a hydrophobic pocket at the binding site. SAR studies do not suggest that methyl substitution on the *N*-phenyl ring play a role in the metabolism. (However, methyl substituents near the 4-amino group do affect metabolism.[8]) If our hypothesis that a specific orientation of the *o*-methyl group is required for recognition is correct, design of a constrained analogue of **2**, which is restricted to a conformation similar to the *o*-methyl orientation found in **2**(2), should lead to an anticonvulsant with high activity.

Acknowledgment. This work was supported by the Alberta Heritage Foundation for Medical Research (studentship for N.E.C.D. and scholarship for P.W.C.) and the Medical Research Council of Canada. The authors thank Drs. Clark and Robertson for supplying samples of the compounds.

Supplementary Material Available: Anisotropic thermal parameters for the non-hydrogen atoms, positional and isotropic thermal parameters for the hydrogen atoms, and lists of bond distance and bond angles for compounds **1**–**3**, **8**, and **14** (54 pages). Ordering information is given on any current masthead page.

(20) Codding, P. W.; Muir, A. K. S. Molecular Structure of Ro15-1788 and a Model for the Binding of Benzodiazepine Receptor Ligands. *Mol. Pharmacol.* **1985**, *28*, 178–184.