4-Isoxazolyl-1,4-dihydropyridines: Biological, Theoretical, and Structural Studies

N. R. Natale,*,† David J. Triggle,‡ Robert B. Palmer,† Barbara J. Lefler,† and W. Daniel Edwards†

School of Pharmacy, State University of New York at Buffalo, C126 Cooke-Hochstetter Complex, Buffalo, New York 14260, and Department of Chemistry, University of Idaho, Moscow, Idaho 83843. *Received September 29, 1989*

Biological activity was determined for a series of seven isoxazolyldihydropyridines (IDHPs). The highest biological activity was observed for 5-alkyl-3-phenyl-IDHP (1), for which the O-endo conformation at the ring juncture between the heterocyclic rings is known in the solid state. The 3,5-dialkyl-IDHPs were intermediate in overall activity. A theoretical study of rotation about this ring juncture was performed to estimate the relative energy and barrier to rotation for the different conformers as a function of both the ring juncture between the heterocyclic rings and the esters in the 3- and 5-position of the dihydropyridine. Molecular mechanics predicts the minimum energy conformer to be O-exo-*ap,ap*, while quantum mechanical calculations predict O-exo-*sp,sp* as the minimum-energy conformer. Both methods indicate that the barrier to rotation about the heterocyclic ring juncture should be relative low, but both methods appear to overestimate the difficulty of ester rotation. A single-crystal X-ray diffractometry study of the (3,5-dimethylisoxazolyl)dihydropyridine **2** was carried out, and shows the O-endo ring juncture and *sp,sp* ester conformation. 2D NOESY NMR spectroscopy indicates the presence of both conformations about the ring juncture, at room temperature, as evidenced by correlations for both alkyl groups on the isoxazole with the C-2 methyl on the DHP moiety. The *ap* ester conformer was also evidenced by NOESY, indicating that ester interconversion must take place.

Introduction

4-Aryl-1,4-dihydropyridines are important cardiovascular drugs which exhibit calcium channel antagonist activity.[1] Interest in the synthesis of this class of compounds continues, both to elucidate the molecular basis of action and to improve their pharmacological profile.[2] We have found that replacement of the 4-aryl substituent with the isoxazole moiety leads to compounds which retain biological activity.[3]

The hypothesis has been advanced that the 1,4-dihydropyridine ring of active antagonists adopts a boat-shaped conformation[4a-c] and that ring planarity is associated with enhanced activity.[4b] NMR investigations support a preferred solution equilibrium conformation in which the 4-aryl substituent is in a pseudoaxial orientation perpendicularly bisecting the plane of the 1,4-dihydropyridine.[5] We suggest, for the purpose of clarity, that the conformation at the juncture between the DHP and 4-isoxazolyl substituent be denoted O-endo or O-exo, to readily distinguish this conformational feature from the ester moieties in the 3- and 5-positions which can attain antiperiplanar (*ap*) and synperiplanar (*sp*) conformations. Studies on 1,3-benzothiazocines[2c] convincingly ruled out boat to boat interconversion (which would provide a pseudoequatorial 4-aryl substituent), and Baldwin et al. favored an exo geometry of the 4-aryl substituent with respect to the dihydropyridine moiety. However, the sulfur bridge which provides the covalent conformational "lock" of these compounds both seriously perturbs the electronic parameters germane to drug–receptor interaction and was always present in the "endo" geometry. Furthermore, biological activity was observed even in the absence of other substituents on the locked benzene ring. Finally, the most active analogue in the aryl thiol series was not conformationally locked. More recently NOE studies of 4-aryl-DHPs in solution, by Rovnyak and co-workers, indicate that both exo and endo conformers were present in solution.[5a]

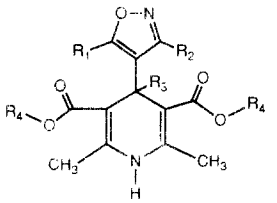
Theoretical calculations have previously addressed the conformational mobility of dihydropyridines. Hofmann and Cimiraglia[6] concluded that a highly flexible boat conformation, with a low barrier between planar and boatlike conformers, was most likely. Mahmoudian and Richards[7a] used semiempirical MO theory to study the functional groups in the C-3- and C-5-positions. They concluded that an *ap* conformation of the ester nonco-

† University of Idaho.
‡ State University of New York at Buffalo.

(1) (a) Triggle, D. J.; Langs, D. A.; Janis, R. A. *Med. Res. Rev.* **1989**, *9*, 123–180. (b) Janis, R. A.; Silver, P. J.; Triggle, D. J. *Adv. Drug Res.* **1987**, *16*, 309–591. (c) Janis, R. A.; Triggle, D. J. *J. Med. Chem.* **1983**, *26*, 775.
(2) (a) Frigerio, M.; Zaliani, A.; Riva, C.; Palmisano, G.; Pilati, T.; Gandolfi, C. A. *Tetrahedron Lett* **1989**, *29*, 6335. (b) Davis, R.; Kern, J. R.; Kurz, L. J., Pfister, J. R. *J. Am. Chem. Soc.* **1988**, *110*, 7873. (c) Baldwin, J. J.; Claremon, D. A.; Lumma, P. K.; McClure, D. E.; Rosenthal, S. A.; Winquist, R. J.; Faison, E. P.; Kaczorowski, G. J.; Trumble, M. J.; Smith, G. M. *J. Med. Chem.* **1987**, *30*, 690. (d) Arrowsmith, J. E.; Campbell, S. F.; Cross, P. E.; Stubbs, J. K.; Burges, R. A.; Gradiner, D. G.; Blackburn, K. J. *J. Med. Chem.* **1986**, *29*, 1696.
(3) (a) McKenna, J. I.; Schlicksupp, L.; Natale, N. R.; Willett, R. D.; Maryanoff, B. E.; Flaim, S. F. *J. Med. Chem.* **1988**, *31*, 473. (b) Knerr, G.; McKenna, J. I.; Quincy, D. A.; Natale, N. R. *J. Heterocycl. Chem.* **1987**, *24*, 1429. (c) Schauer, C. K.; Anderson, O. P.; Natale, N. R.; Quincy, D. A. *Acta Crystallogr.* **1986**, *C42*, 884. (d) Natale, N. R.; Quincy, D. A. *Synth. Commun.* **1983**, *13*, 817. (e) Kovacic, P.; Edwards, W. D.; Natale, N. R.; Sridhar, R.; Kiser, P. F. *Chem.-Biol. Interact.*, in press.
(4) (a) Fossheim, R. *J. Med. Chem.* **1986**, *29*, 305. (b) Fossheim, R.; Svarteng, K.; Mostad, A.; Romming, C.; Shefter, E.; Trigle, D. J. *J. Med. Chem.* **1982**, *25*, 126. (c) Seidel, W.; Meyer, H.; Born, L.; Kasda, S.; Dompert, W. In *Abstracts of Papers*, Division of Medicinal Chemistry; 187th National Meeting of the American Chemical Society, St. Louis, MO, April 8–13, 1984; American Chemical Society: Washington, DC, 1984. (d) Fossheim, R. *Acta Chem. Scand.* **1987**, *B41*, 581.
(5) (a) Rovnyak, G.; Andersen, N.; Gougoutas, J.; Hedberg, A.; Kimball, S. D.; Malley, M.; Moreland, S.; Porubcan, M.; Pudzianowski, A. *J. Med. Chem.* **1988**, *31*, 936. (b) Goldman, S.; Geiger, W. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 301. (c) Berntsson, P.; Carter, R. E. *Acta Chem. Suec.* **1981**, *18*, 221.
(6) Hoffmann, H. J.; Cimiraglia, R. *FEBS Lett.* **1988**, *241*, 38.
(7) (a) Mahmoudian, M.; Richards, W. G. *J. Chem. Soc., Chem. Commun.* **1986**, 739. (b) Holtje, H. D.; Marrer, S. *J. Comput. Aided Mol. Des.* **1987**, *1*, 23. (c) Turi, Nagy, N.; Jergelova, M. *Chem. Papers* **1989**, *43*, 279; *Chem. Abstr.* **1989**, *111*, 49918.
(8) Meier, B. H.; Ernst, R. R. *J. Am. Chem. Soc.* **1979**, *101*, 6441.
(9) Natale, N. R.; McKenna, J. I.; Niou, C. S.; Borth, M.; Hope, H. *J. Org. Chem.* **1985**, *50*, 5660.
(10) (a) Burkert, U.; Allinger, N. L. *Molecular Mechanics*; American Chemical Society: Washington, DC, 1982. (b) Ponder, J. W.; Richards, F. M. *J. Comput. Chem.* **1987**, *8*, 1016.
(11) Seager, M. J.; Takahashi, M.; Catterall, W. A. *Ann. N. Y. Acad. Sci.* **1988**, *522*, 162.
(12) (a) Bolger, G. T.; Gengo, P.; Klowckowski, R.; Luchowski, E.; Siegel, H.; Janis, R. A.; Triggle, A. M.; Triggle, D. J. *J. Pharmacol. Exp. Ther.* **1983**, *225*, 291. (b) Janis, R. A.; Sarmiento, J. G.; Maurer, S. C.; Bolger, G. T.; Triggle, D. J. *Pharmacol. Exp. Ther.* **1984**, *231*, 291.

0022-2623/90/1833-2255$02.50/0

Chart I. Structures of Isoxazolyldihydropyridines



entry	R_1	R_2	R_3	R_4
1	CH_3-	C_6H_5-	H	CH_3CH_2-
2	CH_3-	CH_3-	H	CH_3CH_2-
3	CH_3-	CH_3-	D	CH_3CH_2-
4	CH_3CH_2-	CH_3-	H	CH_3CH_2-
5	CH_3-	CH_3-	H	CH_3-
6	n-C_5H_{11}-	C_6H_5-	H	CH_3CH_2-
7	i-C_3H_7-	C_6H_5-	H	CH_3CH_2-

planar with the DHP ring is associated with antagonists, whereas a nearly coplanar arrangement is associated with agonist activity. Holtje and Marrer considered both rotation about the ring juncture between the 4-aryl substituent and the DHP ring and the ester conformation by using force field and quantum chemical calculations; they concluded that, for the 4-aryl substituents studied, the exo geometry is favored at the ring juncture (by 3–4 kcal) and the *ap* ester rotamer is energetically favored in five of six cases (by 2–3 kcal).[7b] In contrast Rovnyak and co-workers, using AM1 methods,[5a] had concluded that there was not a large intrinsic difference between exo and endo at the ring juncture (less than 1 kcal) and, in fact, calculated that in five of six structures studied that endo would be preferred. In a recent study, Turi Nagy and Jergelova using quantum mechanical calculations[7c] estimated that the barrier to rotation at the ring juncture for nifedipine was "relatively high", on the order of over 7000 kcal.

We were interested in the role that different conformations play in determining the biological activity of isoxazolyldihydropyridine calcium antagonists. The isoxazole systems presented herein have very similar electronic and resonance components, if one assumes that the 4-aromatic ring always has the same conformation. Different 4-aryl conformations, however, arise from apparent steric effects as reflected in conformational variance in the solid state. Some features of the conformation in the crystal structure result from intermolecular hydrogen bonding of the DHP N–H in the unit cell and may be of limited usefulness in making predictions for SAR (structure–activity relationships). Theoretical calculations are so-called "gas phase", since they concern only one molecule in the absence of solvent effects. Solution studies can also shed light on equilibrium conformational preferences. We report in this study the three dimensional shape of isoxazolyldihydropyridines, (IDHPs) using solid-state, solution, and theoretical methods, and interpret the data with the corresponding biological activities.

Table I. Inhibition of [^{3}H]PN 200 110 Binding by Isoxazolyl-DHPs in Guinea Pig Heart and Ileum

entry	K_I, M (SEM)[a]	n_H
	Heart	
1	$1.39 \pm 0.27 \times 10^{-8}$	1.14 ± 0.07
2	$2.71 \pm 0.31 \times 10^{-8}$	1.06 ± 0.02
3	$2.85 \pm 0.19 \times 10^{-8}$	1.04 ± 0.04
4	$5.20 \times 0.35 \times 10^{-8}$	1.07 ± 0.03
5	$1.22 \pm 0.17 \times 10^{-7}$	1.01 ± 0.03
6	$7.90 \pm 1.67 \times 10^{-8}$	1.38 ± 0.05
7	$4.17 \pm 0.38 \times 10^{-7}$	1.18 ± 0.09
	Ileum	
1	$2.17 \pm 0.25 \times 10^{-8}$	1.01 ± 0.03
2	$3.28 \pm 0.16 \times 10^{-8}$	1.08 ± 0.04
3	$2.99 \pm 0.20 \times 10^{-8}$	1.05 ± 0.02
4	$6.25 \pm 0.17 \times 10^{-8}$	0.97 ± 0.03
5	$1.73 \pm 0.24 \times 10^{-7}$	1.02 ± 0.03
6	$7.84 \pm 0.72 \times 10^{-8}$	1.46 ± 0.10
7	$2.54 \pm 0.33 \times 10^{-7}$	1.55 ± 0.21

[a] Each value is the mean of six to eight separate determinations.

Table II. Effect of Ester Conformation on the Calculated Energy of IDHPs at Different C(1)–C(5)–C(7)–H(7) Dihedral Angles

ester conformn	O-exo	90 °[b]	barrier, kcal	O-endo	Δ endo–exo, kcal
ap,ap	31.194	40.413[c]	9.2	32.984	1.8
ap,sp	32.946	43.903[d]	10.9	33.880	0.9
sp,sp	36.015	47.611	11.6	36.215	0.2

[a] Numbering corresponds to that shown in Figure 2. [b] Initial geometry was set at 90° for the C(1)–C(5)–C(7)–H(7) dihedral angle, in the cases noted this angle changed by more than ±2° during the energy minimization. [c] Final dihedral angle was 75°. [d] Final dihedral angle was 83°.

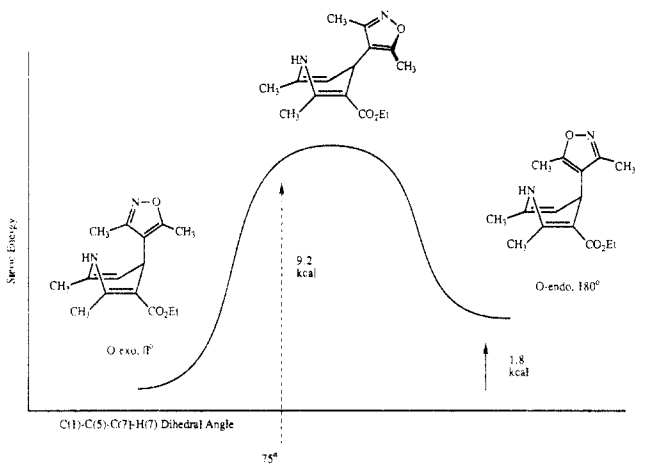


Figure 1. Schematic representation of the molecular mechanics study of rotation about the ring juncture–C(1)–C(7) bond. Results shown are for the *ap,ap* ester conformation. The C-3 ester is omitted for clarity. Numbering corresponds to the crystal structure (Figure 2).

Results and Discussion

The structures of isoxazolyldihydropyridines 1–7 used in this study are shown in Chart I. Competition data for inhibition of (+)-[^{3}H]PN 200 110 binding in guinea pig heart and ileum membranes are summarized in Table I. There is a very good correlation, as expected,[1a] between the two sets of data. The Hill coefficients (n_H) were unity in nearly all cases; however, compounds **6** and **7** show values greater than unity. The explanation may be that these are agents with the most hydrophobic substituents. For comparison purposes, the K_I values for nifedipine

(13) Campana, C. F.; Shepard, D. F.; Litchman, W. M. *Inorg. Chem.* **1980**, *20*, 4039.
(14) Sheldrick, G. SHELXTL, revision 4.1 (1984), Nicolet XRD Corp., Madison, WI.
(15) (a) Ridley, J. E.; Zerner, M. C. *Theor. Chim. Acta* **1973**, *32*, 111. (b) Ridley, J. E.; Zerner, M. C. *Theor. Chim. Acta* **1976**, *42*, 223. (c) Pople, J. A.; Beveridge, D. L.; Dobosh, P. A. *J. Chem. Phys.* **1967**, *47*, 2026.
(16) Zerner, M. C. *Ann. N. Y. Acad. Sci.* **1981**, *367*, 35.
(17) (a) Wheeler, D.; Czuchajowski, L.; Edwards, W. D. *Int. J. Quantum Chem.* **1989**, *S23*, 473. (b) Strekowski, L.; Mokrosz, J. L.; Tanious, F. A.; Watson, R. A.; Harden, D.; Mokrosz, M.; Edwards, W. D.; Wilson, W. D. *J. Med. Chem.* **1988**, *31*, 1231. (c) Strekowski, L.; Chandrasekaran, S.; Wang, Y.-H.; Edwards, W. D.; Wilson, W. D. *J. Med. Chem.* **1986**, *29*, 1311.

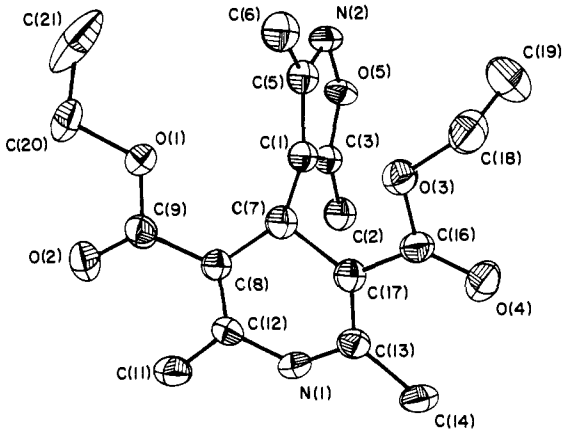


Figure 2. Thermal ellipsoids for **2**.

binding in guinea pig heart and ileum preparations are 5.0 × 10^{-10} and 8.5 × 10^{-10} M, respectively.[1a,b]

We wished to determine whether there was a correlation between solid-state conformation at the heterocyclic ring juncture and biological activity and to estimate by using theoretical methods the energetics of this process (shown schematically in Figure 1). A molecular mechanics study was undertaken, and the O-exo to O-endo conformational change were studied with respect to the three possible ester conformations; the results are shown in Table II. The O-exo conformation was found to be the lowest in energy of all ester conformations studied; however, in the *sp,sp* ester conformation, the difference between the O-exo and O-endo conformations is only about 0.2 kcal/mol. Quantum chemical calculations were performed for selected conformers to compare the barrier to rotation about the ring juncture, as well as the relative energy of different ester conformers. The O-exo-*sp,sp* was found to be lowest in energy of the structures studied, the barrier to rotation about the heterocyclic ring juncture (as estimated by the presumed "saddle point" at 90°) was found to be 4.49 kcal. The O-endo-*sp,sp* conformer was found to be 1.059 kcal higher in energy than the O-exo-*sp,sp* conformer. Neither the magnitude of the barrier nor the difference in energy of the O-exo versus O-endo conformers preclude the possibility of the presence of both isomers in solution. However, force field theory predicts that the *ap,ap* ester conformation is lowest in energy, where X-ray diffractometry usually shows the *ap,sp* or *sp,sp* conformation.[4a] One notable exception is found in the crystal structure of the 4-*o*-chloro DHP, which crystallized with *two* distinct structures in the unit cell, exo-*ap,sp* and endo-*ap,ap*.[5a] Fossheim had previously commented that the ester conformation observed in the crystal structure was probably a result of intermolecular hydrogen bonding and crystal-packing interactions.[4a]

The quantum chemical calculations indicate the *sp,sp* ester arrangement as the minimum-energy conformer, with *ap,sp* 3.5 kcal higher in energy.

We had previously reported the solid-state structure for **1**,[3b] which was O-endo with respect to the juncture between the heterocyclic rings and *sp,sp* with respect to the esters. The solid-state conformation of **7**,[3a] in contrast, was O-exo and *ap,sp*. The solid-state conformation for (3,5-dimethylisoxazolyl)-DHP **2** now obtained is shown in Figure 2, which shows the thermal ellipsoids and atom-numbering scheme. Crystallographic data are summarized in Table III. The conformation is O-endo at the ring juncture between the heterocyclic rings, in which the oxygen of the isoxazole moiety presented over the dihydropyridine ring. The ester conformations are both synperiplanar (*sp,sp*);

Table III. Crystallographic Data for **2**

empirical formula	$C_{18}H_{24}N_2O_5$
molecular weight	348
diffractometer system	Nicolet R3m/E
crystal class	monoclinic
space group	C_2/c
lattice constants	
a	20.088 (8)
b	8.936 (2)
c	23.691 (6)
β	122.94 (2)
V	3569
$F(000)$	1415.77
crystal size (mm)	0.10 × 0.15 × 0.52
absorption coefficient	7.45
calculated density	ρ = 1.26 (Z = 8)
temperature	−130 °C
scan speeds	6–60°/min
total reflections	2511
2θ	110°
unique reflections	2247, 1732 with $F > 3\sigma(F)$
R for equivalent reflections	0.0350
h,k,l	$0 < h < 21$
	$0 < k < 8$
	$0.25 < l < 16$
R	0.0691
R_w	0.0772, g = 0.00130
goodness of fit	1.844
$\lvert\Delta/\sigma\rvert_{mean}$	0.007
$\lvert\Delta/\sigma\rvert_{max}$	0.026
total parameters refined	204

however, one ester is almost planar, with the C(13)–C(17)–C(16)–O(4) dihedral angle of 5.3°, while the second ester is out-of-plane with the C(8)–C(12)–O(9)–O(2) of 18.6°. Intermolecular hydrogen bonding is observed between the isoxazole N(2) and the DHP N(1)-H in the unit cell. The DHP ring is in a flattened boat conformation in which N(1) and C(7) are 0.0923 and 0.1158 Å from the mean plane defined by C(8), C(12), C(13), and C(17). Previously, increasing pharmacological activity has been found to correlate with increasing 1,4-dihydropyridine planarity for both the nifedipine and nisoldipine series.[18b] The DHP ring puckering parameter is usually defined as the sum of the six intraring torsion angles in the DHP ring. The sum of the six torsion angles in the DHP ring of **2**, $\sum|\rho|$, is 65.5°. For comparison purposes the $\sum|\rho|$ value for **1** is 65.7°, for **7** is 72.6°, and for nifedipine is 72.1°. The relative position of the 4-isoxazolyl ring, as reflected by $\sum|\tau|$, the average magnitude of the C(13)–C(17)–C(7)–C(1) and C(12)–C(8)–C(7)–C(1) torsion angles, is 107.55°. Atomic coordinates, bond lengths, bond angles, and torsion angles are given in the supplementary material. The highest biological activity was observed for 5-alkyl-3-phenyl-IDHP (**1**), for which the O-endo conformation at the ring juncture between the heterocyclic rings is known in the solid state. The 3,5-dialkyl-IDHPs were intermediate in overall biological activity.

The molecular mechanics force field calculations predict that IDHP **2** (O-endo-*sp,sp*) upon solvation may have a reasonably strong intrinsic driving force for interconversion at both conformationally mobile bonds, while the quantum mechanical calculations predict ready conversion at the ring juncture, but a very minor population of the *ap* ester conformer. 2D NOESY NMR spectroscopy (Figure 3) is consistent with the presence of both O-exo and O-endo conformations with respect to the ring juncture in **2**, at

(18) (a) Coburn, R. A.; Wierzba, M.; Suto, M. J.; Solo, A. J.; Triggle, A. M.; Triggle, D. J. *J. Med. Chem.* **1988**, *31*, 2103. (b) Fosheim, R.; Joslyn, A.; Solo, A. J.; Luchowski, E.; Rutledge, A.; Triggle, D. J. *J. Med. Chem.* **1988**, *31*, 300.

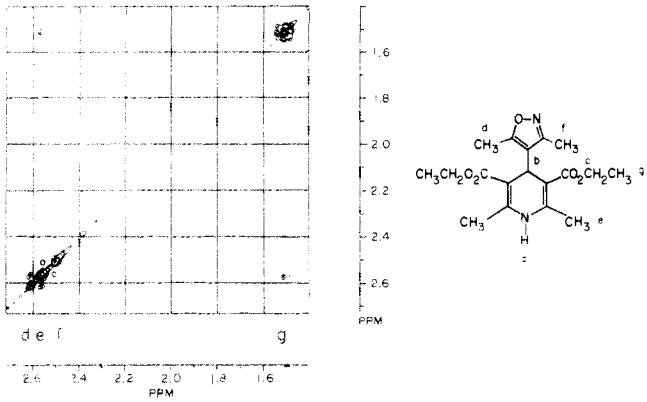


Figure 3. 2D NOESY spectrum for **2**.

room temperature, as evidenced by correlations for both alkyl groups (C-5 and C-3 methyl) on the isoxazole ring with the C-2 methyl on the DHP moiety. A cross-peak was also observed between the C-2 of the dihydropyridine and the methyl group of the ethyl substituent. This would be observed only for an *ap* ester conformation. The theoretical calculations represent energies for unsolvated, gas-phase molecules at absolute zero and therefore only appropriate the solution-phase energies. The NOESY studies indicate that conformational interconversion from the crystal structure occurs in solution, and both the molecular mechanics and the quantum mechanics methods of calculation suggest that interconversion at the ring juncture should be relatively facile, which is consistent with experimental observation. The fact that only the *ap* ester conformer is evidenced in solution does not rule out the presence of the *sp* ester conformer; however, only the force field calculation predicts a relatively strong driving force for this interconversion, since the relative amount of *ap* conformer predicted by the quantum mechanical method (less than 1%) would probably be difficult to detect under the experimental conditions used.

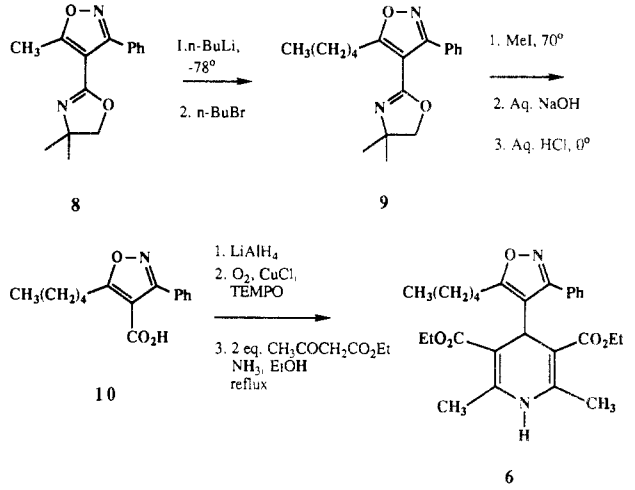
Conclusion

The isoxazolyldihydropyridine systems exhibit a variety of solid-state conformations. On the basis of examination of biological data, solid-state structure, 2D NMR studies, and theoretical calculations, we conclude that conformational mobility about both ester and ring juncture must be considered when interpreting SAR data. In contrast to the calculations of Turi Nagy and Jergelova,[7c] we find that for the 3,5-dialkyl-IDHP system the barriers to rotation do not appear to be substantial, such that conformational adaptation may well occur during the receptor[11] binding process. It has been previously noted that there is a difference observed in binding and pharmacological activity.[18] Furthermore, it has been previously assumed in quantitative SAR analysis that the conformation about the ring juncture of the 4-aryl moiety in DHPs is the same. We suggest that one contributing factor for this difference may be that the combination of electronic and steric factors lead to diverse conformations at ring juncture and ester, which is subsequently reflected in the facility of drug–receptor interaction in vivo.

Experimental Section

Commercial reagents were purified by recrystallization or distillation before use. Reactions conducted under inert atmosphere were done so after several cycles of evacuation and nitrogen purging. Tetrahydrofuran was distilled from sodium and benzophenone. Radial chromatography was performed on a Harrison Associates chromatotron. Preparative HPLC was performed on a Rainin Rabbit system, using a silica gel column unless otherwise noted. All chromatography solvents were distilled. NMR spectra

Scheme I. Representative Synthetic Route to IDHPs



were obtained on an IBM AF300 (300 MHz for ^1H) or a JEOL FX90Q (90 MHz for ^1H) instrument. 2D NOE spectroscopy was performed on the IBM AF300, essentially as described by Meier and Ernst.[8] IR spectra were obtained on a Digilab FTS-80 or Qualimatic spectrophotometers. Mass spectra were obtained on a VG Micromass 70/70 HS mass spectrometer. Combustion analyses were performed by Desert Analytics, Tucson, AZ.

Synthesis. Preparation of isoxazolyldihydropyridines **1–5** and **7** in this study has been previously described.[3,9] The previously unreported compound **6** was prepared in analogous fashion as outlined in Scheme I, and the characterization data for **6** and key intermediate **9** are detailed below.

4-(4′,5′-Dihydro-4′,4′-dimethyl-Δ²-oxazolin-2-yl)-5-*n*-pentyl-3-phenylisoxazole (9). To a solution of 4-(4′,5′-dihydro-4′,4′-dimethyl-Δ²-oxazolin-2-yl)-5-methyl-3-phenylisoxazole (**8**;[9] 2.5 g, 9.77 mmol) in THF (125 mL) at −78 °C was added *n*-butyllithium (4.81 mL, 2.03 M in hexanes). After stirring at low temperature for 2 h, *n*-butyl bromide (1.04 mL, 9.68 mmol) was added dropwise via syringe. The reaction was allowed to warm to room temperature overnight, after which time it was concentrated in vacuo. The residue was chromatographed on silica gel and eluted with chloroform (300 mL). Concentration provided a yellow residue, which was purified by flash distillation on a Kugelrohr apparatus (120–5 °C/0.6 mmHg) and provided the product **9** as an oil (2.19, 72% yield). An analytical sample was provided by preparative thin-layer chromatography on silica gel (solvent system: ethyl acetate–chloroform–hexanes, 1:2:2, R_f = 0.72): ^1H NMR δ 7.5–7.7 (m, 2 H), 7.2–7.4 (m, 3 H), 4.0 (s, 2 H), 3.1 (t, 3 H), 2.6–2.9 (m, 2 H), 1.5 (s, 6 H), 1.3–1.4 (m, 4 H), 1.1 (br t, 3 H); ^{13}C NMR 176.22, 161.44, 155.84, 129.61, 128.84, 104.88, 78.66, 67.7, 31.29, 28.13, 27.0, 26.7, 22.17, 13.83; Mass spectrum *m/z* 313 (100 rel intensity, M + 1⁺). Anal. ($C_{19}H_{24}N_2O_2$) C, H, N.

3,5-Dicarbethoxy-1,4-dihydro-2,6-dimethyl-4-(5′-*n*-pentyl-3′-phenylisoxazol-4′-yl)pyridine (6) were obtained from 5-*n*-pentyl-3-phenylisoxazole-4-carboxylic acid (**10**), as previously described, by (1) reduction with lithium aluminum hydride, (2) oxidation with tetramethylpiperidine-*N*-oxy free radical/copper(I) chloride and oxygen, and (3) Hantzsch pyridine synthesis. The product was obtained by recrystallization from ether–petroleum ether as a solid, mp 159–61 °C; an analytical sample was obtained by preparative thin-layer chromatography (SiO_2, 40% EtOAc in hexanes, R_f = 0.26): ^1H NMR δ 7.386 (br s, 5 H), 5.073 (s, 1 H), 4.485 (br s, 1 H), 3.8–4.1 (m, 4 H, ABX3), 2.844 (t, 2 H, J = 7.4 Hz), 3.0 (m, 2 H), 2.7 (m, 4 H), 1.983 (s, 6 H), 1.157 (t, 6 H, J = 6.9 Hz), 0.94 (br t, 3 H); ^{13}C NMR 169.4, 143.45, 129.56, 128.31, 127.54, 101.43, 59.72, 32.13, 29.27, 26.88, 26.05, 22.54, 19.32, 14.49; IR 3339.7, 3094.8, 2973.3, 2867.2, 1608.3, 1497.7, 1200.4 cm^{-1}. Mass spectrum *m/z* 466 (23.56 rel intensity, M⁺), 465 (23.1, M − 1), 393 (69.62, M − CO_2Et), 290 (100, M − [CO_2Et + PhCN]), 252 (59.35, M − [C-4 − isoxazolyl-substituent]). Anal. ($C_{27}H_{36}N_2O_6$) C, H, N.

Radioligand Binding. Microsomal membranes from guinea pig ileal smooth muscle and rat heart ventricles were prepared

as previously described.[12] Binding of the 1,4-dihydropyridine (+)-[^{3}H]PN 200 110 (isopropyl 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-5-(methoxycarbonyl)-2,6-dimethyl-3-pyridinecarboxylate) and its competition by the isoxazolyl-1,4-dihydropyridines were carried out as previously described. Briefly, membrane protein (40–120 μg) was incubated in 5 mL of 50 mM tris[(hydroxymethyl)amino]methane (Tris) buffer at pH 7.2 for 90 min at 25 °C with 5×10^{-11} (+)-[^{3}H]PN 200 110 and varying concentrations of competing compounds. Duplicate tubes contained 10^{-7} M (+)-PN 200 110 to define nonspecific binding. Tubes were filtered and washed rapidly with two 5-mL portions of ice-cold Tris buffer in a Brandel cell harvester (Model M-24R, Biomedical Research Lab., Gaithesburg, MD). Trapped radioactivity was counted by liquid-scintillation spectrometry at an efficiency of 40–45%. Competing compounds were prepared in ethanol as 10^{-3} stock solutions. Concentrations of ethanol of 0.2% (v:v) did not affect specific binding. Binding data were analyzed by iterative curve-fitting programs (BDATA, CDATA, EMF Software, Knoxville, TN). (+)-[^{3}H]PN 200 110 with a specific activity of 70 Ci/mol [1 Ci = 3.7×10^{10} Bq) was purchased from Du Pont–New England Nuclear (Boston, MA).

Calculations. The Molecular Mechanics calculations were performed on a MacIntosh IIcx Computer, using Chem3D (Cambridge Scientific Computing, Inc). Chem3D Plus includes an implementation of the Allinger MM2 force field,[10a] by Ponder.[10b] All structures in Table I were minimized for total steric energy, until termination to root mean square gradients of <0.100. The Cartesian coordinates obtained for these calculations were used in subsequent quantum mechanical calculations. Molecular orbital calculations were of the intermediate neglect of differential overlap (INDO/I) type using the method of Ridley and Zerner.[15–17] Quantum mechanical calculations were run on a Hewlett-Packard 900/350 workstation, a component of the Computational Facility for Theoretical Chemistry in the University of Idaho. Calculations using this method were first applied to nifedipine, and the results obtained [3e] were essentially in agreement with the experimental observations of Rovnyak et al.[5a]

X-ray Data. Suitable crystals of **2** were obtained by slow evaporation from ethyl acetate–hexanes solution. Cell constants were determined by a least-squares fitting of setting angles of the diffractometer from 25 reflections between 55° and 85°. Data were collected by the ω scan technique[13] with graphite-monochromatized Cu Kα radiation (λ = 1.5418 Å). The measured intensities were corrected for Lorentz and polarization effects but not for absorption. The structure was solved with the SHELXTL program.[14] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were constrained to N–H and C–H distances of 0.96 Å; thermal parameters of all hydrogen atoms were set at 0.12 × the equivalent isotropic U of the atom to which it is bonded. Crystallographic data of **2** is listed in Table III. Largest peaks on the final Fourier difference map were 0.4 and −0.45 e/Å^3. The final R value for **2** was 6.91.

Acknowledgment. N.R.N. thanks the Idaho State Board of Education (Grant No. 88-056) and the National Institutes of General Medical Sciences (Grant No. 1-R15-GM42029-01) for generous support of our program. D.J.T. was supported by NIH (Grant No. HL 16003). W.D.E. and N.R.N. thank the National Science Foundation's EPSCoR program for Grant No. R11-8902065. W.D.E. acknowledges a seed grant from the University of Idaho Research Council and generous start-up funds for the components of the Computational Facility for Theoretical Chemistry. We thank Dr. Gary Knerr and Miles Smith of the University of Idaho NMR facility. We also thank the National Science Foundation (Grant No. CHE-8504253) and the M. J. Murdock Charitable Trust of Research Corporation for their part in funding the purchase of the IBM AF-300 NMR spectrometer. We thank Dr. Roger Willett and Brian Scott of the Washington State University X-ray facility. We also thank NSF (Grant No. CHE-8408407) and the Boeing Company for funds which help establish the WSU X-ray facility.

Supplementary Material Available: ^{1}H NMR (300 MHz) of **2**, unit-cell diagram illustrating intermolecular hydrogen bonding in the X-ray of **2**, and X-ray data for **2**: atomic coordinates, bond lengths, bond angles, and torsion angles (7 pages). Ordering information is given on any current masthead page.

Synthesis and Evaluation of 1,2,2-Tris(sulfonyl)hydrazines as Antineoplastic and Trypanocidal Agents

Krishnamurthy Shyam, Philip G. Penketh, Alan A. Divo, Regina H. Loomis, Curtis L. Patton, and Alan C. Sartorelli*

Departments of Pharmacology, and Epidemiology and Public Health and Developmental Therapeutics Program, Comprehensive Cancer Center, and MacArthur Center for Molecular Parasitology, Yale University School of Medicine, New Haven, Connecticut 06510. *Received December 4, 1989*

Several 1,2,2-tris(sulfonyl)hydrazines, conceived as prodrugs of 1,2-bis(sulfonyl)hydrazines, were synthesized and evaluated for antineoplastic and trypanocidal activities in mice. 1-Methyl-1,2,2-tris(methylsulfonyl)hydrazine emerged as an extremely efficacious antitrypanosomal agent, whereas 1-(2-chloroethyl)-1,2,2-tris(methylsulfonyl)hydrazine was inactive. In contrast, 1-(2-chloroethyl)-1,2,2-tris(methylsulfonyl)hydrazine displayed potent antineoplastic activity, producing several 60-day "cures" of mice bearing leukemia L1210, leukemia P388, or Sarcoma 180. Furthermore, the fact that the tris(sulfonyl) derivatives will not generate isocyanates, which contribute to the host toxicity of nitrosoureas like 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), makes them agents of significant promise in trypanosomal and cancer chemotherapy.

Recent studies in our laboratory have identified a number of 1,2-bis(sulfonyl)-1-methylhydrazines with antineoplastic activity.[1–3] The most active compound of this class to emerge from this study, 1,2-bis(methylsulfonyl)-1-methylhydrazine (1), displayed relatively high levels of activity against the L1210 leukemia and the B16 melanoma in mice.[3] More recently, we reported the antitrypanosomal activity of some of these agents against *T. brucei rhodesiense* in mice.[4] Compound 1 also emerged as the most

(1) Shyam, K.; Cosby, L. A.; Sartorelli, A. C. *J. Med. Chem.* **1985**, *28*, 525–527.
(2) Shyam, K.; Furubayashi, R.; Hrubiec, R. T.; Cosby, L. A.; Sartorelli, A. C. *J. Med. Chem.* **1986**, *29*, 1323–1325.
(3) Shyam, K.; Hrubiec, R. T.; Furubayashi, R.; Cosby, L. A.; Sartorelli, A. C. *J. Med. Chem.* **1987**, *30*, 2157–2161.
(4) Penketh, P. G.; Shyam, K.; Divo, A. A.; Patton, C. L.; Sartorelli, A. C. *J. Med. Chem.* **1990**, *33*, 730–732.

0022-2623/90/1833-2259$02.50/0