H_2O. The chloroform layer was evaporated to afford a pale yellow powder. This was recrystallized from ether–ethyl acetate to give product 4 (0.55 g, 96%): mp 165 °C dec; TLC R_f 0.58 (solvent A), 0.06 (EtOAc); UV λ_{max} (EtOH) 380, 290, 255 nm ($\epsilon \times 10^{-4}$ 1.92, 1.20, 2.13); NMR δ 7.67 (s, 1 H, H-8), 7.38 (d, 1 H, $J_{11,12}$ = 10.1 Hz, H-12), 7.14 (d, 1 H, H-11), 6.79 (d, 1 H, $J_{7,13}$ = 7.1, NH-13), 5.52 (br s, 1 H, NH-15), 4.66 (m, 1 H, H-7), 3.95, 3.91, 3.68 (3 s, 3 H each, OCH_3 of C-2, C-3, and C-1), 2.70 (s, 3 H, NCH_3), 2.46 (s, 3 H, SCH_3), 2.49, 2.33 (br m, 2 H each, CH_2-6 and CH_2-5). Anal. ($C_{22}H_{26}N_2O_5S$) C, H, N; C: calcd, 61.37; found, 59.88.

***N*-Deacetyl-*N*-(*N*-methyl-*N*-nitrosocarbamoyl)methylthiocolchicine (5).** Sodium nitrite (0.20 g, 2.9 mmol) was added slowly to a solution of 4 (0.48 g, 1.1 mmol) in 40 mL of 50% aqueous acetic acid at 0 °C. The reaction mixture was neutralized with sodium bicarbonate solution and extracted with chloroform (3 × 50 mL). The chloroform layer was washed with water, dried over anhydrous magnesium sulfate, and then evaporated to dryness. This was dissolved in a minimum amount of ethyl acetate to yield pale yellow rectangular prisms of 5 (0.43 g, 85%) on standing at room temperature overnight: mp 177 °C dec; TLC R_f 0.88 (solvent A), 0.75 (EtOAc); UV λ_{max} (EtOH) 380, 285, 253 nm ($\epsilon \times 10^{-4}$ 1.95, 1.23, 2.58); NMR δ 7.56 (d, 1 H, $J_{7,13}$ = 6.6 Hz, NH-13), 7.32 (d, 1 H, $J_{11,12}$ = 7.1 Hz, H-12), 7.31 (s, 1 H, H-8), 7.07 (d, 1 H, H-11), 6.58 (s, 1 H, H-4), 4.75 (m, 1 H, H-7), 3.96, 3.92, 3.70 (3 s, 3 H each, OCH_3 of C-2, C-3, and C-1), 3.10 (s, 3 H, NCH_3), 2.61, 2.50, 2.36, 2.03 (4 m, 1 H each, CH_2-6 and CH_2-5), 2.44 (s, 3 H, SCH_3). Anal. ($C_{22}H_{25}N_3O_6S$) C, H, N.

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Crystal Structures of Calcium Channel Antagonists: 2,6-Dimethyl-3,5-dicarbomethoxy-4-[2-nitro-, 3-cyano-, 4-(dimethylamino)-, and 2,3,4,5,6-pentafluorophenyl]-1,4-dihydropyridine

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The crystal structures of 2,6-dimethyl-3,5-dicarbomethoxy-4-(2-nitrophenyl)-1,4-dihydropyridine (Nifedipine) and the 3-cyano-, 4-(dimethylamino)- and 2,3,4,5,6-pentafluorophenyl derivatives were determined. The 1,4-dihydropyridine ring in all four compounds has a boat-type conformation with varying degrees of puckering at the C4 position. Increasing distortion from planarity at this position shows a limited correlation with decreasing biological activity, determined as the ability to inhibit the Ca^{2+}-dependent muscarinic-induced mechanical responses of guinea pig ileal longitudinal smooth muscle.

The regulation of intracellular Ca^{2+} concentration is of fundamental significance to a host of cellular processes, including excitation–contraction and stimulus–secretion coupling.[1-3] Entry of extracellular Ca^{2+} can occur through a variety of mechanisms, including the use of Ca^{2+} channels. These channels can be defined in terms of ion selectivity, electrophysiological properties, and through the use of antagonists.[2,4] Amongst these antagonists are 1,4-dihydropyridines, including Nifedipine [2,6-dimethyl-3,5-dicarbomethoxy-4-(2′-nitrophenyl)-1,4-dihydropyridine], which is one of the most potent of the Ca^{2+} antagonists and is a powerful negative inotropic and smooth-muscle relaxant species.[2,4-6] Many 1,4-dihydropyridines related to Nifedipine have been synthesized, but few attempts have been made to generate a quantitative structure–activity relationship.[2,7,8] In this paper we report the solid-state structure of Nifedipine and three related compounds as a contribution toward the definition of a structure–activity relationship for this important class of compounds.

Experimental Section

Crystals suitable for single-crystal diffraction studies of these compounds were grown from methanol. The crystallographic parameters measured for these crystals are listed in Table I. X-ray diffraction intensity data were collected by the stationary counter–stationary crystal technique using Ni-filtered Cu Kα radiation and balanced Ni–Co filters. The data collection extended to 100 or 110° in 2θ. The measured intensities were corrected for α_1–α_2 splitting, absorption, and Lorentz-polarization effects. The number of reflections measured for each compound and the number whose intensities were significantly above background (noted as observed reflections) are given in Table I.

The structures were solved by "direct methods" using the program MULTAN.[9] They were refined by difference electron-density maps and by lease-squares analysis using a block diaganol approximation to the normal equations. The hydrogens were included in the final stages of least-squares refinement. The nonhydrogen atoms were refined with anisotropic thermal pa-

(1) J. W. Putney, *Pharmacol. Rev.*, 30, 209 (1978).
(2) L. Rosenberger and D. J. Triggle, in "Calcium and Drug Action", G. B. Weiss, Ed., Plenum Press, New York, 1978, p 1.
(3) P. A. van Zwieten and E. Schönbaum, Eds., *Prog. Pharmacol.*, 2(1), 39 (1978).
(4) A. Fleckenstein, *Annu. Rev. Pharmacol. Toxicol.*, 17, 149 (1977).
(5) W. Lochner, W. Braasch, and G. Kroneberg, Eds., 2nd International Adalat Symposium. New Therapy of Ischemic Heart Disease, Springer-Verlag, Berlin, 1975.
(6) S. Imai, *Trends Pharmacol. Sci.*, 2, 81 (1979).
(7) B. Loev, M. M. Goodman, K. M. Snader, R. Tedeschi, and E. Macko, *J. Med. Chem.*, 17, 956 (1974).
(8) R. Rodenkirchen, R. Bayer, R. Steiner, H. Meyer, and E. Möller, *Naunyn-Schmiedebergs Arch. Pharmacol.*, 310, 69 (1979).
(9) G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr., Sect. A*, 27, 368 (1971).

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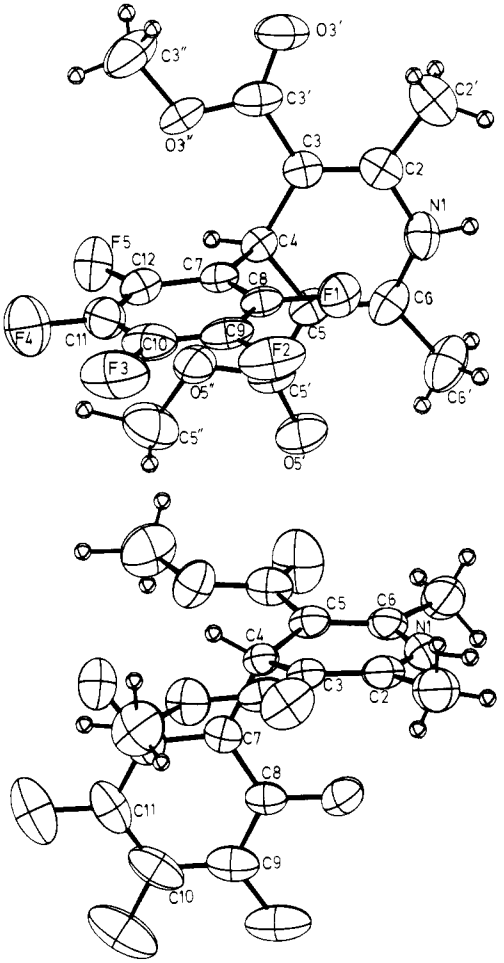

Figure 1. Two views of molecule 1 of 2,6-dimethyl-3,5-dicarbomethoxy-4-(2,3,4,5,6-pentafluorophenyl)-1,4-dihydropyridine (I). Molecule 2 is not illustrated because of its basic similarity to molecule 1.

rameters and the hydrogen isotropically. The R values (usual crystallographic reliability index) for the refined structures are given in Table I (see paragraph at the end of paper concerning supplementary material). The positional parameters for the nonhydrogen atoms are presented in Tables II and II (supplementary material). Other parameters, such as the hydrogen positions and structure factors, can be obtained from the authors on request.

In the crystals of the pentafluoro compound (I) there are two crystallographically independent molecules which are denoted as molecules 1 and 2.

Results and Discussion

The three-dimensional structures of the compounds are illustrated in Figures 1 to 4, together with their atomic labeling schemes. The intramolecular bonding parameters involving the nonhydrogen atoms comprising the molecules are presented in Tables IV and V (supplementary material). Within experimental error, the bond distances and angles about the common molecular framework of the four structures are similar. Though the hydrogen atoms were located in their expected positions, their accuracy precludes any detailed discussion of them.

The distortion from planarity of the atoms comprising the dihydropyridine ring can be clearly seen from the torsion angles calculated about the ring bonds (Table VI, supplementary material). The magnitude of these angles would be zero degrees if the ring atoms were coplanar. The greatest displacement from zero occurs about the bonds from N1 and C4, indicating that the greatest degree of ring puckering occurs at these positions, the distortion being

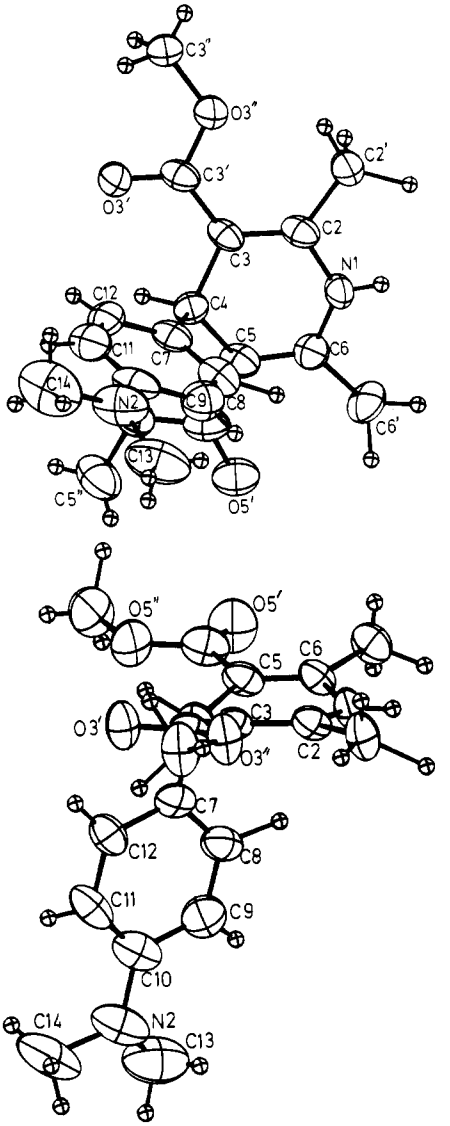

Figure 2. Two views of 2,6-dimethyl-3,5-dicarbomethoxy-4-(dimethylamino)phenyl]-1,4-dihydropyridine (II).

greatest at the C4 position. The magnitude and sign of these torsion angles indicate that both C4 and N1 are displaced from the ring in the same direction, opposite to that of the phenyl ring, which imparts a boat-type conformation to the dihydropyridine ring.

Though each of the compounds exhibit the same characteristic puckering, significantly different degrees of distortion do exist. The dihydropyridine ring of I has the least amount of distortion (magnitude of deviation of torsion angles from zero), while the greatest distortion is found for compounds II and IV. The *o*-nitrophenyl analogue (III) exhibits an intermediate degree of puckering. The puckering of the four compounds is much greater than that found in *N*-benzyl-1,4-dihydronicotinamide (V),[10] which also shows substantial puckering at C4 but very little at N1 (see Table VI). The magnitude of the ring distortion at C4 of compound V, as measured by the magnitude of the torsion angles, is much less than that of the phenyl-substituted analogues.

There is a considerable amount of strain in the compounds at C4, due to nonbonded interactions. These take place between the ortho substituents on the phenyl ring with the carbomethoxy oxygens (at positions C3 and C5),

(10) I. L. Karle, *Acta Crystallogr.*, 14, 497 (1961).

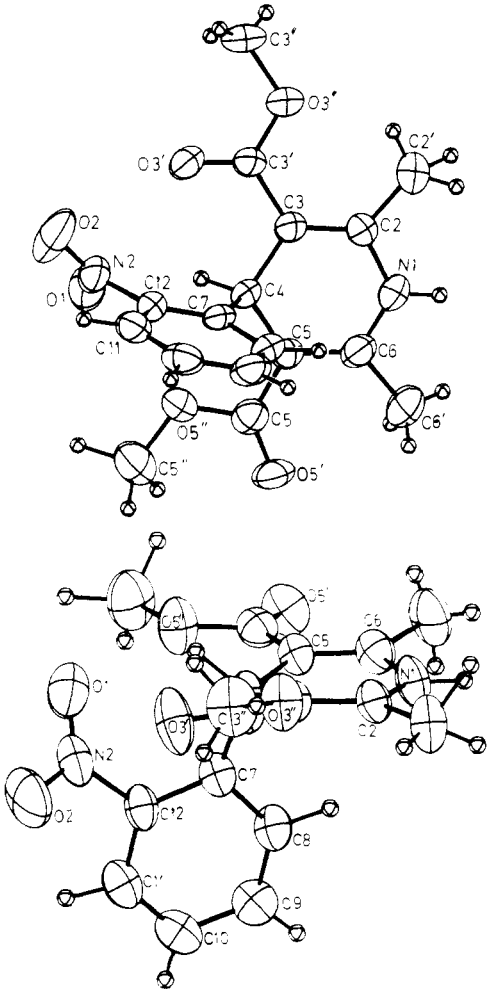

Figure 3. Two views of 2,6-dimethyl-3,5-dicarbomethoxy-4-(2-nitrophenyl)-1,4-dihydropyridine (Nifedipine, III).

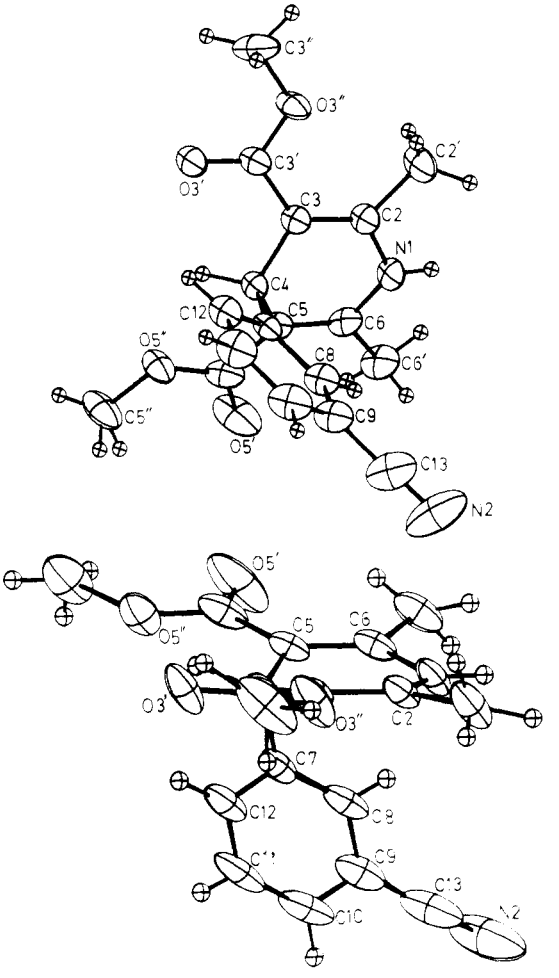

Figure 4. Two views of 2,6-dimethyl-3,5-dicarbomethoxy-4-(3-cyanophenyl)-1,4-dihydropyridine (IV).

the C4 hydrogen, and the dihydropyridine ring atoms C6, N1, and C2. The strain is relieved in a number of ways, the puckering of the dihydropyridine being only one manifestation of these interactions. There is also a significant degree of distortion of the angles about C4 from their tetrahedral value at 109° (see Table V). The C4–C7 bond is also longer than exocyclic phenyl–C (tetrahedral) bonds, which are usually 1.51 Å.[11]

The conformation of the phenyl ring relative to the dihydropyridine ring is constrained by the carbomethoxy groups at C3 and C5. This conformation is best described by the torsion angles about the C4–C7 bond (see Table VI). The phenyl ring for the two unique pentafluoro-substituted molecules (I) bisects the dihydropyridine ring, such that C8 (phenyl) resides nearly over the center of the dihydropyridine ring. The other compounds are twisted from this position by varying amounts. In compounds II, III, and IV the phenyl ring is twisted such that C8 lies closer to the C5–C6 side of the dihydropyridine ring. This is made clear from consideration of the torsion angles about the C4–C7 bond. For example, the closer the torsion angle C8–C7–C4–C5 is to 60° the closer the phenyl ring comes to bisecting the dihydropyridine ring. The deviation of this angle from 60° is directly correlated with the degree of ring puckering.

There are also differences in the spatial arrangement of the carbomethoxy groups at C3 and C5 of the dihydropyridine ring. In compound I the two carbomethoxy groups have similar orientations with respect to the molecule, the carbonyl groups being *antiperiplanar* to the C3–C4 and C5–C4 bonds (see torsion angles listed in Table VI). However, in compounds II–IV, the two carbomethoxy groups do not enjoy equivalent positions, and in the C3 carbomethoxy group the carbonyl group is *synplanar* to the C3–C4 bond, but the carbonyl group of the C5 carbomethoxy group is *antiperiplanar* to the C5–C4 bond. This difference in the conformation of the carbomethoxy group is compounds II–IV imparts chiral character to the 1,4-dihydropyridine ring.

The 2-nitro substituent on the phenyl ring is twisted about the C12–N2 bond because of nonbonded interactions. It is rotated away from the coplanarity with the phenyl ring by approximately 37° (see torsion angles about C12–N2 in Table VI). The 4-(dimethylamino) group shows a slight degree of deviation from coplanarity with the phenyl ring, in part because of twist about the C10–N2 bond and in part due to hybridization.

In each of the four structures, the N1 hydrogen atom is involved in an intermolecular hydrogen bond. The acceptor atom in all cases is a carbonyl oxygen. The parameters associated with these intermolecular hydrogen bonds are listed in Table VII (supplementary material). Aside from these hydrogen bonds, there do not appear to be any short intermolecular contacts suggestive of directional bonding.

The pharmacologic activities, determined as the concentrations to produce 50% inhibition of the muscarinic receptor-mediated Ca^{2+}-dependent contraction of guinea

(11) L. E. Sutton, Ed., *Spec. Publ. Chem. Soc.*, no. 18, P.S. 15S. (1978).

Table VIII. Inhibition of Muscarinic Responses of Guinea Pig Ileal Longitudinal Smooth Muscle by 1,4-Dihydropyridines

no.	ID_{50}, M[a]	act. (III = 1.0)	torsion angle,[b] deg
I	9.0×10^{-10}	6.0	16
II	5.0×10^{-5}	0.0001	28
III (nifedipine)	5.1×10^{-9}	1.0	20
IV	3.0×10^{-7}	0.017	30

[a] Measured against tonic (slow) response to the muscarinic agonist, *cis*-2-methyl-4-[(dimethylamino)methyl]-1,3-dioxolane methiodide (CD), according to our previously described method.[12] [b] Average of the two torsion angles (C_2-C_3-C_4-C_5, C_3-C_4-C_5-C_6, Table VI) describing the distortion from planarity of C_4 of the 1,4-dihydropyridine ring.

pig ileal longitudinal smooth muscle,[2,12] are given in Table VIII. The order of activity, I > III > IV > II, demonstrates, as noted previously,[2,7,8] the highly detrimental effects of 4-substitution in the phenyl ring. The high activity of the pentafluoro derivative (I), which contains a 4-F substituent, may be due to the activity-enhancing effects of the two ortho and two meta substituents, which are sufficient to overcome any detrimental effects of 4-substitution. Structural studies are continuing to test this point and to extend the structure–activity correlation. 2-Substituted compounds are generally somewhat more active than 3-substituted compounds[2,7,8] and consistent with this the 3-cyano derivative (IV) is less active than the 2-nitro derivative (III).

Because of the limited number of structures determined and the presumed importance of electronic, hydrophobic, and other physical chemical factors, any correlation between biological activity and solid-state structure can be, at best, of limited scope. However, there is some indication that activity does vary according to the planarity of the 1,4-dihydropyridine ring, as measured by torsion angles about C4 (Table VIII), activity increasing with increasing ring planarity.

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Supplementary Material Available: Crystallographic data for molecules I–IV (Table I); fractional atomic coordinates for I with estimated standard deviations $\times 10^{-4}$ (Table II); fractional atomic coordinates and estimated standard deviations $\times 10^{-4}$ for compounds II–IV (Table III); bond distances (Å) for compounds I–IV (Table IV); bond angles (degrees) for compounds I–IV (Table V); torsion angles for compounds I–V (Table VI); and intermolecular hydrogen bond data for compounds I–IV (Table VII) (7 pages). Ordering information is given on any current masthead page.

(12) L. B. Rosenberger, M. K. Ticku, and D. J. Triggle, *Can. J. Physiol. Pharmacol.*, **57**, 333 (1979).

Lipophilic and Hydrophilic Esters of 4-Acetyl-2-(2-hydroxyethyl)-5,6-bis(4-chlorophenyl)-2*H*-pyridazin-3-one as Antihypertensive Agents

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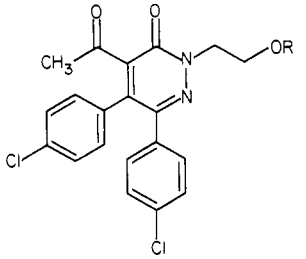
In an attempt to enhance the antihypertensive activity of 4-acetyl-2-(2-hydroxyethyl)-5,6-bis(4-chlorophenyl)-2*H*-pyridazin-3-one, 1, a series of lipophilic and hydrophilic esters was synthesized. These derivatives possessed increased lipid and aqueous solubility, respectively. The esters, in general, cause a larger blood-pressure drop than 1 when tested at high doses in the spontaneously hypertensive rat (SHR) model. At lower doses the antihypertensive activity is the same as with 1.

As described in the accompanying paper,[1] 4-acetyl-2-(2-hydroxyethyl)-5,6-bis(4-chlorophenyl)-2*H*-pyridazin-3-one (1) is active in the spontaneously hypertensive rat (SHR) and the deoxycorticosteroid (DOCA) rat models of hypertension. Evaluation of this compound established that, while a statistically significant reduction in blood pressure does occur following a dose of 3 mg/kg in these models, reduction of blood pressure to less than approximately 140 mmHg does not occur even at doses of 100 mg/kg. Indeed, a plot of the dose–response relationship for this compound plateaus at less than 50 mg/kg. It was also observed that 1 has limited solubility in both water and lipophilic solvents. In order to determine if the efficacy of this compound could be improved, a series of esters of the 2-hydroxy function was synthesized. The initial series which we will describe is a series of alkyl and aryl esters which were designed to have higher solubility in lipid solvents. The second series that we will discuss are amino acid esters of 1 with increased water solubility. The synthesis of amino acid derivatives as potential prodrugs has been reported previously.[2,3]



1, R = H
2, R = $COCH_3$

(1) R. Buchman, J. A. Scozzie, Z. S. Ariyan, R. D. Heilman, D. J. Rippin, L. J. Powers, and R. J. Matthews, *J. Med. Chem.*, under articles section in this issue.

(2) I. M. Kovach, I. H. Pitman, and T. Higuchi, *J. Pharm. Sci.*, **64**, 1070 (1975).

0022-2623/80/1823-1445$01.00/0