J. Am. Chem. Soc. **1985**, *107*, 4768–4772

Antileprosy Dihydrophenazines. Structural Characterization of Two Crystal Forms of Clofazimine and of Isoclofazimine, B.3857

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Abstract: The crystal and molecular structures of two modifications of the antileprosy drug clofazimine, 3-(*p*-chloroanilino)-10-(*p*-chlorophenyl)-2,10-dihydro-2-(isopropylimino)phenazine, $C_{27}H_{22}Cl_2N_4$, and that of the inactive isomeric compound isoclofazimine, 2-[(*p*-chlorophenyl)imino]-10-(*p*-chlorophenyl)-2,10-dihydro-3-(isopropylamino)phenazine, have been determined from three-dimensional single-crystal counter X-ray data by using Mo Kα radiation. The monoclinic modification of clofazimine crystallizes in the space group $P2_1/a$ with four molecules in a cell of dimensions a = 7.788 (14) Å, b = 22.960 (13) Å, c = 13.362 (7) Å, β = 98.58 (12)°; the structure has been refined to a final value of the weighted R factor of 0.036 based on 2180 independent data with $I > 3\sigma(I)$. The triclinic form crystallizes in space group $P\bar{1}$ with two molecules in a cell of dimensions a = 10.507 (4) Å, b = 12.852 (12) Å, c = 9.601 (2) Å, α = 95.96 (4)°, β = 97.22 (1)°, γ = 69.73 (6)° and has been refined to a weighted R factor of 0.062 based on 1462 independent data with $I > 2\sigma(I)$. Crystals of isoclofazimine are also in the triclinic space group $P\bar{1}$, with a = 12.849 (3) Å, b = 13.252 (5) Å, c = 7.598 (2) Å, α = 96.20 (3)°, β = 104.80 (2)°, γ = 105.44 (3)°; the structure has been refined to a weighted R factor of 0.043 based on 1597 independent intensities with $I > 3\sigma(I)$. The geometric differences between clofazimine and isoclofazimine have been probed by CNDO/2 molecular orbital calculations. The geometry at the exocyclic amino nitrogen atom N(3) is significantly different in isoclofazimine from that in both forms of clofazimine and in other active analogues, which leads to the suggestion that the value of the intramolecular angle α at N(3) [defined by C(3)–N(3)–C(21) in clofazimine] may be indicative of in vitro activity in these systems; molecules with values of α in the range 125.5 ± 1° are inactive, while those with expanded α angles [i.e., 131 ± 1°] are active in vitro.

Leprosy, a bacterial disease which has plagued mankind for thousands of years, continues to be common in many parts of the world. The three drugs most commonly used in leprosy therapy programs are 4,4′-diaminodiphenyl sulfone (dapsone, DDS), the antiobiotic rifampin, and the substituted dihydrophenazine clofazimine, 3-(*p*-chloroanilino)-10-(*p*-chlorophenyl)-2,10-dihydro-2-(isopropylimino)phenazine (I); the latter has been in use for more than 20 years.[2-6] Although it has been shown to be effective against a number of diseases related to the autoimmune system,[7-10] clofazimine does have some serious drawbacks; these include crystal desposition in the spleen and elsewhere,[11-13] which can prove fatal, and a pronounced tendency to discolor the skin. Moreover, as has been the case for the sulfone dapsone for many years,[14,15] bacterial resistance to clofazimine has now been observed.[16] Consequently, there is a pressing need for the development of suitable clofazimine analogues for use in leprosy therapy programs.

The precise mode of action of clofazimine is not yet well understood. Morrison has shown that clofazimine binds to the G/C regions of DNA in a nonintercalative manner,[17] but the molecular basis for the differences in activity among various iminodihydrophenazines is not apparent. On the basis of a pharmacodynamic study (using animal models) of four related substituted dihydrophenazines, Levy[18] has noted the importance of the two *p*-chloro substituents in clofazimine, but the absence of such substituents in the active analogue B.1912 (II) demonstrates that their presence is not required for activity.[18,19] In an attempt to understand the molecular basis for activity in these related species, we have recently initiated a comprehensive examination of the structural and electronic properties of these molecules.[20-22] We here report the structures of two different modifications of clofazimine (I) itself and of its inactive isomer 2-[(*p*-chlorophenyl)imino]-10-*p*-chlorophenyl-2,10-dihydro-3-(isopropylamino)phenazine (III), B.3857, hereinafter isoclofazimine, in which the isopropyl group is at N(3)H rather than at N(2). In an attempt to interpret the relative inactivity of isocloazimine, we have also performed molecular orbital calculations on these three systems.

Experimental Section

X-ray Data Collection. (a) Monoclinic Modification of Clofazimine. A powdered sample of clofazimine was recrystallized from acetone, yielding red-orange prisms of the *monoclinic* modification of I. The cell

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0002-7863/85/1507-4768$01.50/0

Table I. Atomic Positional Parameters for Monoclinic Clofazimine (B.663)

atom	*X*	*Y*	*Z*
Cl(18)	0.3867 (1)	−0.07310 (4)	0.39675 (8)
Cl(24)	−0.3646 (1)	0.44783 (4)	−0.29394 (8)
N(2)	−0.1755 (3)	0.12955 (10)	0.0017 (2)
N(3)	−0.1654 (3)	0.23558 (10)	−0.0572 (2)
N(5)	0.1451 (3)	0.29139 (10)	0.2623 (2)
N(10)	0.2074 (3)	0.17437 (9)	0.3141 (2)
C(1)	0.0205 (4)	0.1471 (1)	0.1611 (2)
C(2)	−0.0829 (4)	0.1628 (1)	0.0674 (2)
C(3)	−0.0834 (4)	0.2253 (1)	0.0380 (2)
C(4)	−0.0044 (4)	0.2651 (1)	0.1033 (2)
C(6)	0.2929 (4)	0.3195 (1)	0.4251 (2)
C(7)	0.3869 (4)	0.3069 (1)	0.5174 (2)
C(8)	0.4306 (4)	0.2496 (1)	0.5416 (2)
C(9)	0.3733 (4)	0.2055 (1)	0.4749 (2)
C(11)	0.2731 (4)	0.2177 (1)	0.3825 (2)
C(12)	0.2350 (4)	0.2761 (1)	0.3547 (2)
C(13)	0.0842 (4)	0.2500 (1)	0.2004 (2)
C(14)	0.1025 (4)	0.1876 (1)	0.2247 (2)
C(15)	0.2523 (4)	0.1145 (1)	0.3365 (2)
C(16)	0.1649 (4)	0.0819 (1)	0.3996 (2)
C(17)	0.2088 (4)	0.0249 (1)	0.4188 (2)
C(18)	0.3372 (4)	0.0003 (1)	0.3740 (2)
C(19)	0.4293 (4)	0.0318 (1)	0.3130 (2)
C(20)	0.3850 (4)	0.0896 (1)	0.2942 (2)
C(21)	−0.2045 (4)	0.2874 (1)	−0.1107 (2)
C(22)	−0.2449 (4)	0.3379 (1)	−0.0635 (2)
C(23)	−0.2930 (4)	0.3877 (1)	−0.1209 (3)
C(24)	−0.2989 (4)	0.3860 (1)	−0.2227 (2)
C(25)	−0.2590 (5)	0.3365 (2)	−0.2700 (2)
C(26)	−0.2115 (5)	0.2872 (1)	−0.2139 (2)
C(27)	−0.1948 (4)	0.0677 (1)	0.0218 (2)
C(28)	−0.3328 (4)	0.0592 (1)	0.0886 (3)
C(29)	−0.2419 (5)	0.0377 (1)	−0.0800 (3)

constants, obtained by least-squares methods, are a = 7.788 (14) Å, b = 22.960 (13) Å, c = 13.362 (7) Å, β = 98.58 (12)°, V = 2368 (8) Å^3; the observations were made at 293 K. The density of 1.30 (2) g cm^{-3} observed by flotation in aqueous potassium iodide agrees well with a value of 1.331 g cm^{-3} calculated for four molecules in the cell. Systematic absences uniquely define the space group as $P2_1/a$, a nonstandard setting of $P2_1/c$ (C_{2h}^5, no. 14).

Intensity data were collected on an Enraf-Nonius CAD-4 diffractometer by using Mo Kα radiation out to a maximum value of 2θ (Mo) = 50°. A total of 4491 unique reflections was collected. A weighting scheme of the type described by Ibers and co-workers[23] was applied, the weighting factor p being defined as 0.01. Data were corrected for Lorentz polarization effects and backgrounds but not for absorption (μ = 3.0 cm^{-1}). Of the 4491 independent data, only the 2180 with $I > 3\sigma(I)$ were considered to be observed and used in the subsequent analysis.

(b) Triclinic Modification of Clofazimine. Recrystallization of the powdered sample used above from 1:2 *N*-methylformamide/acetone solution gave red needles of the *triclinic* modification of I. Preliminary diffractometry indicated a triclinic cell, space group $P\bar{1}$ or $P1$, and the absence of additional symmetry was confirmed by a Delauney reduction. Cell constants are a = 10.507 (4) Å, b = 12.852 (12) Å, c = 9.601 (2) Å, α = 95.96 (4)°, β = 97.22 (1), γ = 69.73 (6)°, V = 1204(1) Å^3. The measured density of 1.29 (2) g cm^{-3} is in agreement with the value of 1.306 g cm^{-3} calculated for two molecules per cell. The space group was assumed to be $P\bar{1}$ (C_i^1, no 2), and this was confirmed by the successful refinement. Data were collected as above, with a maximum value of 2θ (Mo) of 46°. A total of 3310 data was collected, of which 1462 with $I > 2\sigma(I)$ were used in the calculations.

(c) Isoclofazimine. Recrystallization of a powdered sample of III from acetone gave red rectangular plates of III. The crystals are triclinc, $P\bar{1}$, with a = 12.849 (3) Å, b = 13.252 (5) Å, c = 7.598 (2) Å, α = 96.20 (3)°, β = 104.80 (2)°, γ = 105.44 (3)°, V = 1184 (2) Å^3. The observed and calculated (for Z = 2) densities are 1.30 (2) g and 1.328 g cm^{-3}, respectively. Data were collected as above, with 2θ(Mo) ≤ 48°. A total of 3550 independent data was collected, of which 1597 had $I > 3\sigma(I)$ and were considered observed.

Solution and Refinement of the Structures. All three structures were solved by direct methods by using the multiple solution program MULTAN.[24] All least-squares analyses were on F, the function minimized being $\sum w(|F_o| - |F_c|)^2$ where the weights w are $4F_o^2/\sigma^2(I)$. Scattering factors and anomalous dispersion corrections were taken from ref 25. The programs used were those provided by Enraf-Nonius in the CAD-4/SDP package.

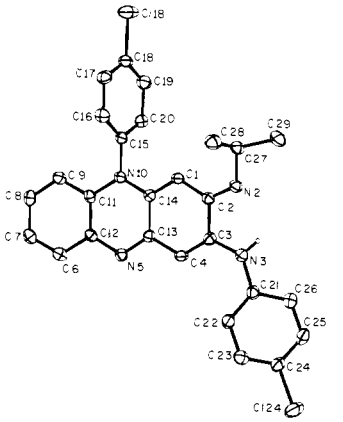

Figure 1. View of one molecule of the monoclinic form of clofazimine. Atomic thermal ellipsoids are drawn at the 25% probability level; atom H(3) is shown as a sphere of arbitrary size, and other hydrogen atoms are omitted for clarity.

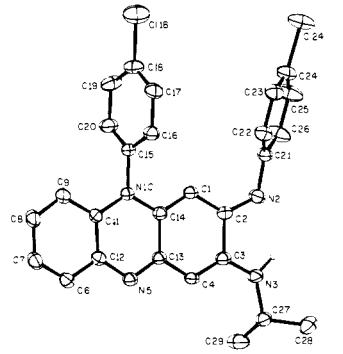

Figure 2. View of the isoclofazimine molecule, drawn as in Figure 1.

(a) Monoclinic Clofazimine. All hydrogen atoms were refined isotropically, non-hydrogen atoms anisotropically. A correction for secondary extinction was included, the value of the extinction coefficient being 8.2 (2) × 10^{-7}. A final difference Fourier was featureless, with no peak higher than 0.11 e Å^{-3}. The final value of the agreement factors $R_1 = \sum||F_o| - |F_c||/\sum|F_o|$ and $R_2 = |\sum w(|F_o| - |F_c|^2/\sum w(F_o)^2]^{1/2}$ were 0.044 and 0.036, respectively. Positional parameters are in Table I; hydrogen atom parameters, atomic thermal parameters, and observed and calculated structure amplitudes are avaialble as supplementary material.

(b) Triclinic Clofazimine. The isopropyl group exhibits a 50:50 rotational disorder around the N(2)–C(27) bond. This disorder precluded the location of hydrogen atoms on the isopropyl carbon atoms [C(27), C(28), and C(29)]; other hydrogen atoms were placed in calculated positions and not refined. A final difference Fourier showed peaks of 0.25 and 0.17 e Å^{-3} near atom Cl(24), with no other peak higher than 0.10 e Å^{-3}. Final agreement factors are R_1 = 0.081 and R_2 = 0.062. Positional parameters are in Table II; other information is in supplementary material as before.

(c) Isoclofazimine. Hydrogen atoms were placed in calculated positions, but only the amino hydrogen atom H(3) was refined isotropically. A final difference Fourier showed peaks as high as 0.20 e Å^{-3} in chemically meaningless locations. Final agreement factors were 0.049 and 0.043, respectively. Positional parameters are in Table III; other material was deposited as above.

Molecular Orbital Calculations. The MO calculations were performed by the CNDO/2 self-consistent field method,[26] using a local modification of the program by Pople. Molecular geometries were taken from the present X-ray studies, except that all C–H and N–H bond lengths were changed to 1.08 and 1.01 Å, respectively, keeping their bond directions

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Table II. Atomic Positional Parameters for Triclinic Clofazimine (B.663)

atom	X	Y	Z
Cl(18)	0.5111 (2)	0.3001 (2)	0.4422 (2)
Cl(24)	1.9675 (3)	0.1179 (3)	1.2332 (3)
N(2)	1.2884 (5)	0.1364 (4)	0.6822 (6)
N(3)	1.4514 (5)	0.1463 (4)	0.9018 (5)
N(5)	1.0751 (5)	0.3800 (4)	1.1537 (6)
N(10)	0.8925 (5)	0.3605 (4)	0.9231 (6)
C(1)	1.0810 (6)	0.2469 (5)	0.7970 (6)
C(2)	1.2271 (6)	0.1933 (5)	0.7880 (7)
C(3)	1.3175 (6)	0.2055 (5)	0.9184 (7)
C(4)	1.2599 (6)	0.2663 (5)	1.0364 (6)
C(6)	0.8902 (7)	0.4923 (6)	1.2790 (7)
C(7)	0.7546 (8)	0.5483 (6)	1.2909 (8)
C(8)	0.6623 (7)	0.5407 (6)	1.1803 (8)
C(9)	0.7031 (7)	0.4775 (6)	1.0550 (8)
C(11)	0.8423 (7)	0.4240 (5)	1.0462 (7)
C(12)	0.9371 (6)	0.4304 (5)	1.1583 (7)
C(13)	1.1189 (6)	0.3200 (5)	1.0393 (7)
C(14)	1.0302 (6)	0.3076 (5)	0.9146 (7)
C(15)	0.7998 (6)	0.3481 (5)	0.8068 (7)
C(16)	0.7528 (7)	0.2623 (6)	0.7996 (7)
C(17)	0.6633 (7)	0.2459 (6)	0.6906 (7)
C(18)	0.6252 (6)	0.3183 (6)	0.5838 (7)
C(19)	0.6736 (7)	0.4028 (6)	0.5866 (8)
C(20)	0.7598 (7)	0.4201 (6)	0.6996 (8)
C(21)	1.5668 (6)	0.1425 (6)	0.9894 (7)
C(22)	1.6794 (7)	0.0469 (6)	0.9752 (8)
C(23)	1.8029 (7)	0.0411 (6)	1.0529 (8)
C(24)	1.8113 (7)	0.1265 (7)	1.1417 (8)
C(25)	1.7020 (7)	0.2212 (7)	1.1558 (7)
C(26)	1.5794 (6)	0.2286 (6)	1.0810 (7)
C(27)	1.2055 (6)	0.1208 (6)	0.5482 (6)
C(28)A	1.1903 (19)	0.2202 (14)	0.4613 (16)
C(29)A	1.2923 (16)	0.0167 (13)	0.4785 (16)
C(28)B	1.1848 (15)	0.0083 (11)	0.5590 (16)
C(29)B	1.3024 (14)	0.1073 (12)	0.4301 (14)

Table III. Atomic Positional Parameters for Isoclofazimine (B.3857)

atom	X	Y	Z
Cl(18)	0.3290 (1)	0.4127 (1)	1.1026 (2)
Cl(24)	0.7996 (1)	0.5352 (1)	1.5979 (2)
N(2)	0.8613 (3)	0.3128 (3)	0.9329 (4)
N(3)	0.9238 (3)	0.2047 (3)	0.6921 (5)
N(5)	0.5451 (3)	0.0628 (3)	0.3014 (4)
N(10)	0.4734 (3)	0.1867 (3)	0.5412 (4)
C(1)	0.6646 (3)	0.2570 (3)	0.7384 (5)
C(2)	0.7799 (3)	0.2593 (3)	0.7836 (5)
C(3)	0.8131 (3)	0.1953 (3)	0.6456 (5)
C(4)	0.7320 (3)	0.1316 (3)	0.4920 (5)
C(6)	0.3546 (4)	−0.0067 (4)	0.1036 (6)
C(7)	0.2438 (4)	−0.0129 (4)	0.0619 (6)
C(8)	0.2054 (4)	0.0469 (4)	0.1778 (6)
C(9)	0.2804 (4)	0.1141 (4)	0.3366 (6)
C(11)	0.3936 (3)	0.1203 (3)	0.3808 (5)
C(12)	0.4341 (3)	0.0604 (3)	0.2654 (5)
C(13)	0.6171 (3)	0.1255 (3)	0.4525 (5)
C(14)	0.5862 (3)	0.1930 (3)	0.5841 (5)
C(15)	0.4363 (3)	0.2426 (3)	0.6758 (5)
C(16)	0.4168 (4)	0.1955 (4)	0.8213 (6)
C(17)	0.3839 (4)	0.2495 (4)	0.9538 (6)
C(18)	0.3728 (4)	0.3476 (4)	0.9387 (6)
C(19)	0.3919 (4)	0.3940 (4)	0.7935 (7)
C(20)	0.4245 (4)	0.3407 (4)	0.6596 (6)
C(21)	0.8387 (3)	0.3651 (3)	1.0832 (5)
C(22)	0.7579 (4)	0.3176 (4)	1.1614 (6)
C(23)	0.7447 (4)	0.3701 (4)	1.3191 (6)
C(24)	0.8138 (4)	0.4698 (4)	1.3966 (6)
C(25)	0.8972 (5)	0.5186 (4)	1.3250 (7)
C(26)	0.9092 (4)	0.4653 (4)	1.1674 (6)
C(27)	0.9756 (3)	0.1425 (4)	0.5948 (6)
C(28)	1.0840 (4)	0.1422 (4)	0.7251 (8)
C(29)	0.9939 (4)	0.1820 (4)	0.4234 (7)

the same as those observed in the X-ray study. Initially, the energies showed a tendency to diverge. This problem was overcome by a procedure which essentially dampened the calculated shifts in each iteration by 50%; while we are unaware of any previous use of a dampening procedure in conjunction with CNDO calculations, the validity of this approach has been demonstrated for ab initio calculations by Pedersen and Morokuma.[27]

Table IV. Bond Lengths (Å) in Clofazimine and Isoclofazimine

atoms	monoclinic clofazimine	triclinic clofazimine	isoclofazimine
C(1)–C(2)	1.430 (3)	1.459 (6)	1.424 (4)
C(1)–C(14)	1.354 (3)	1.359 (6)	1.355 (4)
C(2)–C(3)	1.488 (3)	1.502 (6)	1.494 (4)
C(2)–N(2)	1.299 (3)	1.291 (5)	1.307 (4)
C(3)–C(4)	1.347 (3)	1.387 (6)	1.363 (4)
C(3)–N(3)	1.355 (3)	1.369 (5)	1.342 (4)
C(4)–C(13)	1.418 (3)	1.404 (6)	1.408 (4)
N(5)–C(13)	1.302 (3)	1.311 (5)	1.306 (4)
N(5)–C(12)	1.372 (3)	1.372 (6)	1.373 (4)
C(6)–C(12)	1.398 (3)	1.378 (6)	1.409 (4)
C(6)–C(7)	1.368 (3)	1.369 (7)	1.355 (5)
C(7)–C(8)	1.386 (3)	1.364 (8)	1.388 (5)
C(8)–C(9)	1.378 (3)	1.407 (7)	1.377 (4)
C(9)–C(11)	1.388 (3)	1.394 (7)	1.384 (4)
N(10)–C(11)	1.395 (3)	1.414 (6)	1.400 (4)
N(10)–C(14)	1.377 (2)	1.378 (5)	1.379 (4)
N(10)–C(15)	1.438 (3)	1.420 (6)	1.448 (4)
C(11)–C(12)	1.411 (3)	1.388 (6)	1.404 (4)
C(13)–C(14)	1.470 (3)	1.453 (6)	1.468 (4)
C(27)–C(28)	1.508 (4)		1.489 (5)
C(27)–C(29)	1.521 (4)		1.505 (5)
C(27)–C(28A)		1.549 (13)	
C(27)–C(29A)		1.481 (14)	
C(27)–C(28B)		1.551 (13)	
C(27)–C(29B)		1.574 (14)	
C(27)–N(2)	1.457 (3)	1.497 (5)	
C(21)–N(3)	1.398 (3)	1.375 (6)	
C(27)–N(3)			1.443 (4)
C(21)–N(2)			1.405 (4)
C(18)–Cl(18)	1.746 (2)	1.748 (6)	1.730 (4)
C(24)–Cl(24)	1.744 (2)	1.735 (6)	1.750 (4)

Description of the Structures. A view of the clofazimine molecule in the monoclinic crystals is shown in Figure 1; the molecule in the triclinic crystals is similar to this, with the exception of the disorder of the isopropyl group. The isoclofazimine molecule is shown in Figure 2, and bond lengths and angles in the three molecules are compared in Tables IV and V, respectively.

The dihydrophenazine moieties are not aromatic, but the 14-atom ring systems in both triclinic clofazimine and isoclofazimine are approximately coplanar, the maximum deviation from the least-squares plane being only 0.038 (6) and 0.038 (4) Å, respectively. These two 14-atom units are, in fact, more nearly planar than the equivalent moiety recently reported[28] in an unreduced, presumably fully aromatic phenazine, which shows a maximum deviation of 0.046 (8) Å; in another recent study of unreduced phenazine, however, the maximum deviation from planarity is only 0.004 Å, as expected.[29] If the molecules are viewed as two planar units which form a butterfly at the N(5)–N(10) axis, the butterfly angles are only 0.9° in triclinic clofazimine and 0.3° in isoclofazimine. The value of 6.7° in monoclinic clofazimine is significantly larger and is comparable only with the value of 6.3° in 4,9-dichloroclofazimine.[22]

As can be seen in the figures, the *p*-chlorophenyl ring at N(10) is approximately perpendicular to the dihydrophenazine plane in all three molecules, the torsion angles τ_1 defined by C(14)–N(10)–C(15)–C(16) being −98.8 (3)°, 88.6 (9)°, and −80.7 (5)° in monoclinic clofazimine, triclinic clofazimine, and isoclofazimine, respectively. Thus, the deviations of τ_1 from ±90° in the present cases are 8.8°, 1.4°, and 9.3°; deviations in the range 0.2–16.4° have been observed in other derivatives.[20-22,30,31] This approximate perpendicularity of the 10-(*p*-chlorophenyl) and dihydrophenazine ring systems presumably stems from unfavorable interactions between the hydrogen atoms at C(1) and C(9) and

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Table V. Bond Angles (deg) in Clofazimine and Isoclofazimine

atoms	monoclinic clofazimine	triclinic clofazimine	isoclofazimine
C(2)–C(1)–C(14)	121.8 (2)	121.9 (5)	121.6 (3)
C(1)–C(2)–C(3)	117.0 (2)	115.9 (5)	117.2 (4)
N(2)–C(2)–C(1)	128.8 (2)	128.1 (4)	127.1 (3)
N(2)–C(2)–C(3)	114.1 (2)	116.0 (5)	115.7 (4)
C(2)–C(3)–C(4)	120.0 (2)	119.7 (5)	119.4 (4)
N(3)–C(3)–C(2)	113.4 (2)	111.2 (4)	114.3 (4)
N(3)–C(3)–C(4)	126.6 (2)	129.1 (5)	126.2 (4)
C(3)–C(4)–C(13)	122.7 (2)	122.7 (4)	123.1 (3)
C(13)–N(5)–C(12)	118.3 (2)	118.2 (4)	117.4 (3)
C(12)–C(6)–C(7)	122.2 (3)	122.4 (6)	121.1 (4)
C(6)–C(7)–C(8)	119.4 (3)	118.8 (6)	120.8 (4)
C(7)–C(8)–C(9)	120.1 (3)	121.7 (6)	120.0 (4)
C(8)–C(9)–C(11)	120.9 (3)	117.5 (5)	119.6 (4)
C(11)–N(10)–C(14)	121.5 (2)	121.3 (5)	121.8 (3)
C(11)–N(10)–C(15)	119.1 (2)	119.8 (5)	119.7 (3)
C(14)–N(10)–C(15)	119.4 (2)	118.9 (5)	118.2 (3)
N(10)–C(11)–C(9)	122.8 (2)	121.3 (6)	122.0 (3)
N(10)–C(11)–C(12)	117.6 (2)	117.4 (5)	116.7 (4)
C(9)–C(11)–C(12)	119.6 (2)	121.3 (6)	121.3 (4)
C(11)–C(12)–C(6)	117.8 (2)	118.2 (6)	117.3 (4)
C(11)–C(12)–N(5)	122.6 (2)	123.2 (5)	124.2 (4)
N(5)–C(12)–C(6)	119.6 (2)	118.5 (6)	118.5 (4)
N(5)–C(13)–C(4)	118.9 (2)	118.0 (5)	119.0 (3)
N(5)–C(13)–C(14)	123.8 (2)	123.8 (5)	123.9 (3)
C(4)–C(13)–C(14)	117.3 (2)	118.2 (5)	117.1 (4)
C(13)–C(14)–C(1)	120.6 (2)	121.5 (5)	121.4 (3)
C(13)–C(14)–N(10)	115.6 (2)	116.1 (5)	116.0 (3)
C(1)–C(14)–N(10)	123.8 (2)	122.4 (5)	122.6 (3)
N(2)–C(27)–C(28)	109.8 (2)		
N(2)–C(27)–C(29)	107.1 (2)		
N(2)–C(27)–C(28A)		105.5 (6)	
N(2)–C(27)–C(29A)		104.5 (6)	
N(2)–C(27)–C(28B)		104.5 (6)	
N(2)–C(27)–C(29B)		104.3 (6)	
N(3)–C(27)–C(28)			108.6 (3)
N(3)–C(27)–C(29)			112.7 (3)
C(28)–C(27)–C(29)	111.8 (3)		111.2 (3)
C(28A)–C(27)–C(29A)		109.4 (1.1)	
C(28B)–C(27)–C(29B)		109.0 (1.0)	
C(3)–N(3)–C(21)	131.6 (2)	129.9 (5)	
C(3)–N(3)–C(27)			125.4 (3)
C(2)–N(2)–C(27)	120.7 (2)	119.2 (4)	
C(2)–N(2)–C(21)			121.2 (3)

those at the phenyl ortho carbon atoms C(16) and C(20) and may provide the structural basis for the observation[17] that clofazimine does not intercalate into polynucleotides.

The major difference between isoclofazimine (III) and all other clofazimine analogues which have been structurally characterized [i.e., I, II, and their derivatives] is the replacement of the aromatic substituent at N(3) by an alkyl group in III. As anticipated, one effect of this substitution is to lengthen the N(3)–C(27) bond (to 1.443 (4) Å) as compared to the N(3)–C(21) distances (of 1.398 (3) and 1.375 (6) Å) in monoclinic and triclinic clofazimine, respectively, and the range of 1.375 (6) to 1.423 (7) Å (average value 1.401 (12) Å) found[20-22,30,31] in other derivatives. This effect, which presumably reflects the change from sp^2 to sp^3 hybridization at the substituent, can also be seen in the MO calculations; the presence of an aromatic substituent in I leads to significant multiple bonding in the N(3)–C(21) bonds, the average bond order for this bond being 11.4% higher than that of the N(3)–C(27) bond in III. It is also noteworthy that this lengthening of the N(3)–C(27) bond is accompanied by a shortening of the C(3)–N(3) bond to 1.342 (4) Å in III as opposed to the values of 1.355 (3) and 1.369 (5) Å in monoclinic and triclinic clofazimine, respectively. In 12 derivatives[20-22,30,31] containing aromatic substituents at N(3), the corresponding values are 1.355 (3) to 1.375 (4) Å, with an average of 1.366 (8) Å. The shortening of the C(3)–N(3) bond in III is brought about by a small but significant increase in the C(3)–N(3) bond order of 3.4% over the average of the bond orders for this bond in the clofazimines. Presumably, this increased bond order reflects the lack of competition for the π electrons at N(3) in III. Another manifestation of this change is a small increase in the residual electron density at N(3) in III, from −0.16 e^- in I to −0.18 e^-.

At N(2), of course, the substitution pattern is reversed, with III having an aromatic substituent while I and most other analogues have alkyl groups. The most dramatic impact of this change is on the torsion angle τ_4 [defined by C(1)–C(2)–N(2)–C(21) in III] around the C(2)–N(2) double bond. This angle is 4.7° and 0.5° in monoclinic and triclinic clofazimines and is in the range of 0.3–4.7° (average value 2 ± 2°) for other derivatives with alkyl substituents at N(2). In isoclofazimine, the value is 9.0°, while in the only other derivative studied[32] which has an aromatic substituent at N(2) the value is 7.1°. This increased torsion in III is permitted as a result of a weakening of the C(2)–N(2) bonds, the calculated bond order for this bond in III being 3.2% lower than the average value for the other systems. The residual electron density at N(2) in III is again enhanced to −0.23 e^- from −0.20 e^- in I. The presence of the aromatic ring at N(2) in III might, in principle, have permitted an intramolecular stacking interaction between the two *p*-chlorophenyl rings. As is apparent in Figure 2, however, such an interaction is not observed. The torsion angle C(2)–N(2)–C(21)–C(22) is 49.5°, which brings about an interplanar angle of 50.0° between the two *p*-chlorophenyl ring planes in III.

The present structural study, when combined with earlier results, does allow the proposal of a simple structure–activity relationship for these dihydrophenazines and the suggestion of a possible mode of action of these molecules which is consistent with the results. At N(3), we define the angle C(3)–N(3)–C(21) (or C(27) in III) as α, C(3)–N(3)–H(3) as β, and C(21)–N(3)–H(3) as γ; of these three angles, only α is precisely determined in X-ray structural studies, since both β and γ are dependent on the coordinates of hydrogen atom H(3). As can be seen in Table V, the value of α in the inactive isoclofazimine III molecule is 125.4 (3)°, while in clofazimine α has values of 131.6 (2)° and 129.9 (5)°. An examination of the structures[20-22] of eight in vitro active analogues shows α values in the range 129.7 (6)° to 132.7 (4)°, with an average value of 131.1 (10)°. Fewer data are available for inactive analogues, but the three structures available[30,31] show a range of 125.1 (3)° to 126.4 (5)° with an average of 125.6 (7).

The geometry at N(3) has been examined in an important related series of molecules; these species include 8-anilino-1-naphthalenesulfonate (ANS),[32,33] 2-(*p*-toluidinyl)-6-naphthalenesulfonate (TNS),[34] and 1-methyl-4′-methoxy-3,5-diiododiphenylamine (DN14).[35] In one molecule of ANS and in DN14, the geometry at N(3) is severely distorted toward pyramidal, the sum of the three bond angles being 335.7° and 345.1° in the two cases, respectively.[32,35] In two other molecules of ANS and in TNS, however, the geometry is trigonal, the bond angle sums being 357.6°, 358.7°, 359.7°.[32-34] In compounds I and III here, in which the position of H(3) was refined, the bond angle sums are 359.6° and 360.0°, respectively, and the geometry is trigonal.

In ANS and its analogues, the N(3)–H unit is involved in intramolecular hydrogen bonding to form a seven-membered ring. In compounds I, II, and III, however, any intramolecular hydrogen bonding would involve N(3)–H···N(2) interactions and would lead to a five-membered ring; such hydrogen bond formation leading to an approximately planar five-membered ring has been suggested in a variety of systems on the basis of neutron diffraction, X-ray diffraction, and infrared spectroscopy.[22,36-38] The effect of such an interaction would be to attract H(3) toward N(2), thereby reducing the angle β and increasing γ and α. The X-ray evidence for the presence or absence of hydrogen bonding in these compounds is far from convincing, but the N(2)···N(3) separations of 2.563 (3) and 2.565 (5) Å in the monoclinic and triclinic forms of clofazimine are significantly shorter than the value of 2.615 (4) Å in isoclofazimine, which supports the contention that an intramolecular hydrogen bond is present in I and II but absent in III; the relative imprecision of the hydrogen atom location [this atom was not refined in the structure of triclinic clofazimine] precludes our checking for any significant differences in the N(3)–H(3)···N(2) angles or the N(2)···H(3) separations. In general, however, a possible conclusion is that the compounds with large α values exhibit intramolecular hydrogen bonding in the crystal and are therefore capable of participating in intermolecular hydrogen bonding in solution in the presence of suitable donor–acceptor pairs. Obviously, if the mode of action of active molecules involves hydrogen bond formation at N(3)–H and N(2) to a suitable acceptor and donor [e.g., O(6) and N(1)–H of guanine], it follows that molecules with large α angles are predicted to be active.

There is a little supporting evidence for this model. Morrison[17] has shown that clofazimine interacts with poly dG but not with poly dA,

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which suggests the need of specific hydrogen bonding. Moreover, we[20] have shown in the 1:1 complex of clofazimine and DMF that intermolecular N(3)-H···O hydrogen bonds are formed to a ketonic oxygen acceptor atom. We hope to investigate the model further in future studies and shall attempt to isolate 1:1 complexes of clofazimine with polynucleotides.

Acknowledgment. We are grateful to Dr. M. L. Conalty (Medical Research Council of Ireland) and to Dr. A. C. McDougall (Slade Hospital, Headington, England) both for the donations of the samples used in this study and for stimulating our interest in this field and to our colleague Prof. L. G. Pedersen for assistance with the MO calculations. This research was supported by a research grant from the Heiser Program for Research in Leprosy.

Registry No. Clofazimine, 2030-63-9; isoclofazimine, 96728-46-0.

Supplementary Material Available: Hydrogen atom positional parameters, atomic thermal parameters, and observed and calculated structure amplitudes for all three molecules (48 pages). Ordering information is given on any current masthead page.

Solvent Isotope Effects on Formation of Protease Complexes with Inhibitory Aldehydes, with an Appendix on the Determination of Deuterium Fractionation Factors by NMR†

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Abstract: In both CCl_4 and water, equilibria of addition of thiols to acetaldehyde are strongly favored by the presence of deuterium at exchangeable positions, whereas equilibria of addition of hydroxylic compounds are hardly affected. To test the possibility of using solvent isotope effects for diagnosing the structure of enzyme-inhibitor complexes, we examined the influence of D_2O on the interaction of proteases with inhibitory aldehydes. Equilibrium constants for binding of inhibitory aldehydes showed enhancements in D_2O of 1.32 ± 0.12 for α-chymotrypsin and 2.65 ± 0.14 for papain, slightly in excess of those expected for covalent addition of an alcohol or thiol, respectively. In an Appendix, hydroxylic solutes are shown to alter the magnetic susceptibilities of solutions, resulting in systematic errors in apparent fractionation factors of their exchangeable protons as determined by conventional NMR procedures. As a result, apparent fractionation factors change with the orientation of the magnetic field. Corrected values for hydroxylic protons, determined by other methods, differ only slightly from unity.

Many enzymes that catalyze acyl-transfer reactions are reversibly inhibited by substrate-related aldehydes that can generate complexes resembling intermediates in substrate hydrolysis.[1,2] Other enzyme reactions, such as those involved in the oxidative phosphorylation of aldehydes, are believed to proceed by way of thiohemiacetal derivatives that serve as immediate substrates for dehydrogenation.[3] When the mechanism of action of such an enzyme has not been established, it would be of interest to know how an aldehyde inhibitor is bound: intact, or as an adduct formed by addition of an oxygen or a sulfur nucleophile. This information may also be useful in considering further improvements in inhibitor design. The form in which an aldehyde is bound to a specific enzyme can in principle be determined by using the diffraction of X-rays from single crystals of enzyme ligand complexes,[4] secondary deuterium isotope effects on equilibrium binding,[5,6] or nuclear magnetic resonance spectroscopy.[7-11] These methods require suitable enzyme crystals and highly purified aldehydes, incorporating isotopes at appropriate positions.

This paper explores the feasibility of an independent approach, based on differing affinities of reactants and products for deuterium at exchangeable sites.[12-14] If equilibrium constants (or fractionation factors, designated ϕ in eq 1) can be established for exchange of solute protons with deuterons from solvent water, then the effect of D_2O on the equilibrium constant of a reaction can be predicted (eq 2). The ratio of the equilibrium constant in D_2O to the equilibrium constant in water is equivalent to the arithmetic product of the fractionation factors of the products divided by the arithmetic product of the fractionation factors of the reactants. Conversely, it should be possible to infer, from the influence of D_2O on the equilibrium binding of a competitive inhibitor, what changes in structure occur as an inhibitor is taken up by an enzyme. In cases where the structure of an enzyme-inhibitor complex is already available, it might also be possible to obtain structural information about states of the enzyme and inhibitor before reaction.

$$\text{R-H} + \text{HOD} \overset{\phi}{\rightleftharpoons} \text{R-D} + \text{HOH} \qquad (1)$$

$$K_{eq}(D_2O)/K_{eq}(H_2O) = \Pi\phi(\text{products})/\Pi\phi(\text{reactants}) \qquad (2)$$

In attempting to evaluate this possibility, we became aware of apparent discrepancies between average values of 1.25 for fractionation factors of hydroxylic protons in *gem*-diols and hemiacetals determined by NMR[15] and values in the neighborhood

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