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THE STRUCTURE OF MK-571 (FORM I) AT 170 K AND CONFORMATIONAL ANALYSIS BY MOLECULAR MODELING

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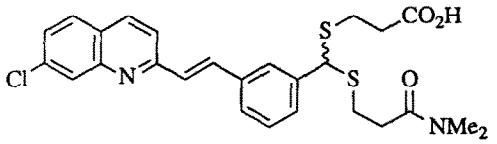
Abstract: The crystal structure of a potent leukotriene D_4 receptor antagonist, MK-571, (±)-(*E*)-3-[[[3-[2-(7-chloro-2-quinolinyl)ethenyl]phenyl][[3-(dimethylamino)-3-oxopropyl]thio]methyl]thio]propanoic acid, reveals that the molecules form hydrogen-bonding dimeric pairs of enantiomers. Molecular modeling, conformational studies, and energy minimizations were performed and compared with those of the *R*-(–)-enantiomer, MK-679.

Leukotriene D_4 (LTD_4), a cysteine-containing leukotriene and a slow-reacting substance of anaphylaxis (SRS-A), has been shown to be a potent brochoconstrictor in humans as well as several other species both *in vitro* and *in vivo*.[1] Because of this, a number of LTD_4 antagonists have been synthesized[2–14] and a model of the LTD_4 receptor was developed[15,16] to produce a rational approach to leukotriene D_4 receptor antagonists. This model indicates that the receptor contains: (1) a planer, lipophilic pocket; (2) a hydrophilic–ionic binding site; and (3) a hydrophilic-polar binding site. Through the use of this model, the racemic compound MK-571, **1**, and the *R*-(-)-enantiomer, MK-679, **2**, were developed[17,18] and have been shown to be an extremely potent, orally active LTD_4 receptor antagonist *in vitro* and *in vivo*.[19]



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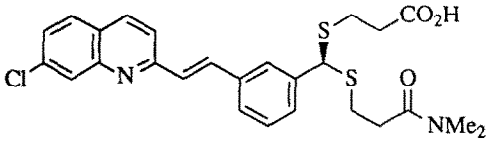

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MK-571, (±)-(*E*)-3-[[[3-[2-(7-chloro-2-quinolinyl)ethenyl]phenyl][[3-(dimethylamino)-3-oxopropyl]thio]-methyl]thio]propanoic acid, has been synthesized from a monothioacetal by selective carbon–oxygen bond cleavage.[20] Because enatiomers may have differing pharmacological properties, the pure enantiomers were prepared from asymmetric dithioacetal intermediates whose diatereomeric esters were separated by flash chromatography,[21] or by enzymatic hydrolysis of prochiral diesters,[22] or by a synthesis involving separation of diasteriomeric dithioacetals.[23] Studies have shown that although the *R*- and the *S*-enantiomers exhibit species–dependent pharmacological activity the difference is only slight in humans.[24,25] In this paper we compare the conformation of this LTD_4 antagonist in both the racemic (MK-571) and chiral (MK-679) crystals.[26] This study provides important information on the conformational flexibility of this molecule.

MK-571 (the racemate) has been shown to exist in two distinct crystalline polymorphic forms (Form I and Form II) with Form I being the more stable polymorph.[27] It is important to understand the solid-state properties and conformation of this drug since these properties and conformation relate to both its solid–state chemistry and mode of action.[28] The crystal structure and molecular conformation studies of MK-571 (Form I) are reported herein and compared with those of MK-679.[26] In addition, the conformational energies of (*R*)-(–)-(*E*)-3-[[[3-[2-(7-chloro-2-quinolinyl)ethenyl]phenyl][[3-(dimethylamino)-3-oxopropyl]thio]methyl]thio]propanoic acid in the

crystals lattice of both the racemate (MK-571) and enantiomer (MK-679) are compared to provide information on the influence of the crystal environment on the conformation of the molecule.

The crystal structure determination was conducted to characterize the crystal packing and conformation of the drug. Colorless needles of MK-571, $C_{26}H_{27}ClN_2O_3S_2$, M_r = 515.10, were grown from an ethanol–water solution by slow evaporation. A crystal having approximate dimensions of 0.28 × 0.13 × 0.11 mm was cooled to 170 K on an Enraf—Nonius CAD_4 diffractometer equipped with a low temperature device. The crystal belongs to the orthorhombic space group $P2_1/c$ (No. 4) with a = 12.521(8), b = 23.621(8), c = 8.464(7) Å; β = 97.49(3)°, V = 2481(5) Å^3, Z = 4, ρ_{calc} = 1.378 g·cm^{-3}, λ(Mo$K\alpha$) = 0.71073 Å, μ = 3.44 cm^{-1}, and F(000) = 1080.0. A total of 3329 unique reflections were measured of which 1858 had $I > 3.0\ \sigma(I)$. The structure was solved by direct methods using *SHELXS86*,[29] refined to a final R of 0.065 and R_w of 0.085, and exhibited no unusual bond lengths or angles. The structure and numbering scheme is shown in Fig. 1; Fig. 2 provides stereoviews of (a) the conformation and hydrogen bonding of both the MK-571 (racemate) and (b) the MK-679 (*R*-enantiomer) crystals. The similarities in the crystal conformations of MK-571 and MK-679 include: (1) the carboxylic acid as the hydrogen–bond donor and the nitrogen of the quinoline ring as the hydrogen–bond acceptor; (2) the substituents of the ethene moiety (the quinoline ring and the phenyl ring) are in an *E*-conformation; and (3) the carboxylic acid side chain and the amide side chain are positioned on either side of the least-squares plane formed by the quinoline–ethene–phenyl conjugated system. The crystal packing of MK-571 is dictated by hydrogen-bonded *R*,*S*-dimers having an Etter–Bernstein[30] graph set notation of $R_2^2(28)$ which is facilitated by positioning the nitrogen of the quinoline ring system *towards* the carboxylic acid group of the same molecule The crystal packing of MK-679 is dictated by hydrogen–bonded linear chains of the molecule having an Etter–Bernstein graph set notation of C(14) which is facilitated by positioning the nitrogen of the quinoline ring system *away from* the carboxylic acid group of the same molecule. A significant difference between the structures of MK-571 and MK-679 is the coplanarity of the quinoline and phenyl rings. In MK-571 the dihedral angle between these two rings is 22.8° but in MK-679, this angle is 5.4°, much closer to the expected value of 0°

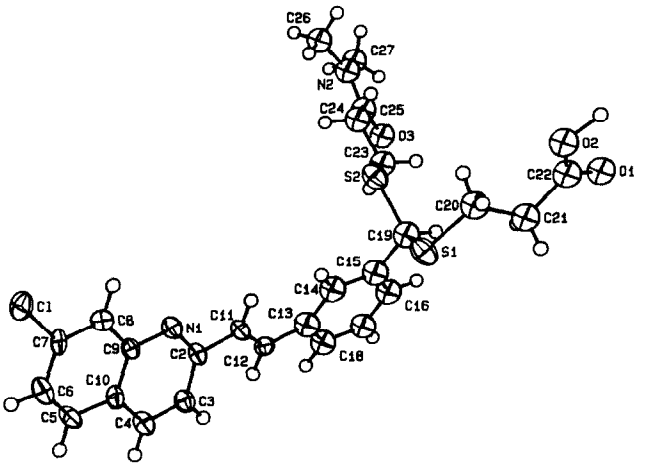

Figure 1. View of MK-571 showing the atom-labeling scheme. Thermal ellipsoids are scaled to the 50% probability level.

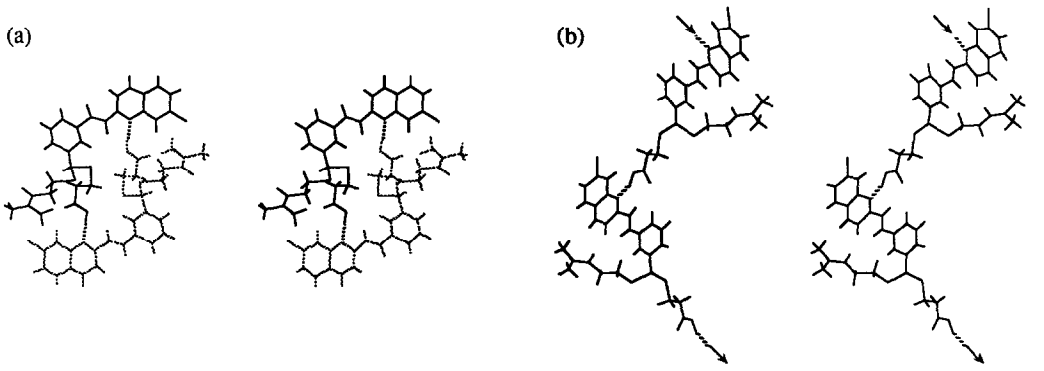

Figure 2. Stereoview of (a) an MK-571 *R,S*-dimer (the *S*-enantiomer is represented in grey) showing the intermolecular hydrogen bonding (dashed lines); and (b) an MK-679 dimer showing the intermolecular hydrogen bonding (dashed lines—including a hydrogen bond from a previous molecule and a hydrogen bond to the next molecule of the chain).

for an extended conjugated system.

Single-point energy calculations to determine the potential energy of the molecular systems were performed for comparison of the two systems using CHARMm®[31] for: (1) one molecule of MK-571 (the *R*-enantiomer) in a vacuum; (2) one molecule of MK-679 in a vacuum; (3) two molecules of MK-571 (an *R,S*-dimeric hydrogen-bonded pair) in a vacuum; and (4) two molecules of MK-679 (the carboxylic acid group of one is hydrogen-bonded to the quinoline ring nitrogen of the other as in the crystal conformation) in a vacuum (Table 1). Then each of these four systems were minimized using an adopted basis Newton–Raphson method (a fixed dielectric of 1.0, nonbonded and hydrogen-bond update frequency of 10) until a tolerance of 0.005 had been attained.

From the single-point energy calculations, it can be seen that the crystal conformation of MK-571 (an isolated *R*-enantiomer in a vacuum and an isolated *R,S*-dimer in a vacuum) is consistently more favorable as compared to the corresponding MK-679 structures (see Table 1). This may be due in part to the temperature at which the x-ray data of the two systems were obtained—170 K for MK-571 and 300 K for MK-679. However, Baum[26], using MM2[32] and removing the hydrogen atoms then adding them back, obtained energies for MK-679 which were slightly lower than MK-571. It is interesting to note that hydrogen bonding stabilizes the structures in both dimeric systems and that the racemic dimer of MK-571 is of lower energy than that of the two

Table 1. Single-point Potential Energies of MK-571 and MK-679 (per Molecule).

Energy Terms (Kcal/mol)	MK-571 monomer[a]	MK-571 dimer[c]	MK-679 monomer[b]	MK-679 dimer[c]
Bond	178.4	178.4	197.4	197.4
Angle	8.6	8.6	6.8	6.8
Dihedral	3.7	3.7	3.0	3.0
Improper Dihedral	10.7	10.7	6.5	6.5
van der Waals	33.3	31.0	40.1	39.4
Electrostatic	-5.4	-9.6	-5.7	-7.9
Hydrogen-bond	0.0	-1.5	0.0	-1.1
Total	229.4	221.4	248.1	244.1

[a] A single isolated *R*-enatiomer in a vacuum [b] A single isolated molecule in a vacuum [c] Average of the two molecules.

Table 2. Fractional Coordinates of Non-hydrogen Atoms and Equivalent Isotropic Temperature Factors.

	x	*y*	*z*	*B*(Å²)		*x*	*y*	*z*	*B*(Å²)
C2	0.0250(6)	0.2516(3)	0.1313(9)	2.2(2)	C19	–0.22419	0.05303	0.53496	4. *
C3	0.0258(6)	0.3105(3)	0.1458(9)	2.7(2)	C20	–0.08005	–0.01568	0.73295	4. *
C4	0.1001(6)	0.3420(3)	0.0797(9)	2.9(2)	C21	–0.14433	–0.01878	0.87680	4. *
C5	0.2561(6)	0.3435(3)	–0.076(1)	2.9(2)	C22	–0.14906	–0.07945	0.93999	4. *
C6	0.3296(6)	0.3145(3)	–0.149(1)	3.3(2)	C23	–0.37670	–0.00639	0.33050	4. *
C7	0.3255(6)	0.2562(3)	–0.1537(9)	2.6(2)	C24	–0.40516	–0.04553	0.18955	4. *
C8	0.2490(6)	0.2249(3)	–0.0886(9)	2.4(2)	C25	–0.52788	–0.05379	0.15370	4. *
C9	0.1728(5)	0.2546(3)	–0.0104(9)	2.0(1)	C26	–0.49687	–0.11882	–0.06609	4. *
C10	0.1763(6)	0.3140(3)	–0.0023(9)	2.5(2)	C27	–0.67742	–0.10263	0.00836	4. *
C11	–0.0510(6)	0.2157(3)	0.2038(9)	2.5(2)	CL	0.4225(2)	0.21975(9)	–0.2450(2)	3.37(4)
C12	–0.1383(6)	0.2337(3)	0.2654(9)	2.4(2)	N1	0.0971(4)	0.2237(3)	0.0547(7)	2.3(1)
C13	–0.20980	0.19768	0.34663	4. *	N2	–0.56375	–0.09119	0.04054	4. *
C14	–0.18580	0.14265	0.38983	4. *	O1	–0.21539	–0.09290	1.01994	4. *
C15	–0.25352	0.11173	0.47899	4. *	O2	–0.07871	–0.11308	0.90721	4. *
C16	–0.34569	0.13729	0.51947	4. *	O3	–0.58827	–0.02748	0.22920	4. *
C17	–0.37051	0.19186	0.47572	4. *	S1	–0.0885(2)	0.0534(1)	0.6417(3)	3.81(5)
C18	–0.30426	0.22244	0.39000	4. *	S2	–0.2305(2)	0.0018(1)	0.3691(3)	3.55(5)

Starred atoms were refined isotropically. Anisotropically refined atoms are given in the form of the isotropic equivalent thermal parameter defined as: $\frac{4}{3}(a\beta_{11} + b\beta_{22} + c\beta_{33} + ab\beta_{12}\cos\gamma + ac\beta_{13}\cos\beta + bc\beta_{23}\cos\alpha)$

*R*enantiomers of MK-679 most likely because of the reciprocating hydrogen-bonds present in MK-571 which are absent in MK-679.

Examining the angles between the normals to the least-squares planes of the unsaturated moieties of MK-571 and MK-679 (the quinoline ring system, the ethene group, and the phenyl ring system), it is noted that the crystal structure of MK-571 exhibits a larger twist in the extended conjugated system (22.7°) than the crystal structure of MK-679 (5.4°) (see Table 3). This contributes to the nearly 10 kcal/mol difference in the van der Waals energy terms of the two systems (see Table 1). The hydrogen bonding between the *R*,*S*-dimers in the crystal structure of MK-571 dictates the conformation of the molecule since the hydrogen of the carboxylic acid group of the *R*-enantiomer is hydrogen bonded to the quinoline nitrogen of the *S*-enantiomer and in turn, the hydrogen of the carboxylic acid group of the *S*-enantiomer is hydrogen bonded to the quinoline nitrogen of the *R*-enantiomer. Although there is some flexibility in this dimeric arrangement, it appears that the quinoline and phenyl rings twist out of planarity to accommodate this hydrogen bonding conformation. The crystal structure of MK-679, however, does not have this constraint since the hydrogen bonding between the molecules is in a head-to-tail chain allowing for greater freedom in its conformation. After the minimizations, the conjugated systems become nearly coplanar. The move to coplanarity is a consequence of the CHARMm® terms used to keep the conjugated system from acting independently and thus greatly twisting out-of-plane. It is interesting to note that

Table 3. Angles Between Normals to the Least-Squares Planes in the Crystal Structure.

Least-Squares Planes	MK-571 monomer	MK-571 dimer	MK-679 monomer	MK-679 dimer
Quinoline—Ethene				
x-ray coordinates	12.09	12.09	4.193	4.193
minimized coordinates	1.110	1.998	–0.2221	–0.6603
Ethene—Phenyl				
x-ray coordinates	10.86	10.86	9.193	9.193
minimized coordinates	0.4352	1.173	–0.7391	–2.858
Quinoline—Phenyl				
x-ray coordinates	22.76	22.76	5.409	5.409
minimized coordinates	1.110	2.525	–0.6720	–3.497

the conjugated systems of MK-679 seem to flatten and then twist in the opposite direction.

Overall, the crystal conformations of MK-571 and MK-679 are quite similar in many regards. The densities of the two crystals are nearly identical (MK-571: 1.378 g·cm^{-3} at 170 K; MK-679: 1.381 g·cm^{-3} at 293 K) and the energy determinations after the minimization are very close. The most significant difference is the hydrogen bonding arrangement and resultant difference in the coplanarity of the phenyl and quinoline rings in the two compounds. Further molecular dynamics studies of these two crystal structures are planned.

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