

Measurement of Quantum Tunneling Between Chiral Isomers of the Cyclic Water Trimer

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A detailed experimental study of the water trimer is reported. A vibration-rotation-tunneling band of the perdeuterated cluster has been measured near 89.6 wavenumbers by tunable far infrared laser absorption spectroscopy. The data indicate a chiral six-membered ring structure with rapid quantum tunneling occurring between the enantiomers. The observed vibration involves torsional motion of the water subunits about each hydrogen bond axis. It is speculated that larger water clusters would also exhibit transient chirality and that similar effects may be found in liquid water.

Despite decades of extensive investigation into the properties of water and its chemical behavior (1), there remain important unanswered questions that may have significant impact on many chemical and biological problems. Two important subjects that remain incompletely understood are the intermolecular forces that act among water molecules, especially with regard to non-pairwise additive interactions, and the concerted hydrogen-bond tunneling motions that occur both in condensed phases and in gaseous water clusters. These are critical elements in understanding the nature of proton transfer in chemical and biological systems as well as the structure of liquid water itself.

Far-infrared (FIR) vibration-rotation-tunneling (VRT) spectroscopy of clusters has recently been developed to address such questions (2). Low-frequency van der Waals vibrations in clusters can be measured with tunable FIR lasers to resolve rotational and tunneling motions; the resulting VRT spectra can be analyzed in terms of permutation-inversion (PI) group theory and scattering theory to yield pair potentials of unprecedented accuracy and detail for important weakly bound systems (3, 4). In addition, FIR-VRT spectroscopy is a powerful probe of the tunneling dynamics that occur in hydrogen-bonded clusters. For example, quantum tunneling of the six equivalent protons in the ammonia dimer among 72 symmetrically equivalent configurations has recently been characterized in great detail by Loeser *et al.* with this method (5).

The water dimer has been the subject of numerous investigations by FIR-VRT spectroscopy (6) and other high-resolution spectroscopic techniques (7). The resulting characterization of the dimer structure and the hydrogen-bond tunneling dynamics among the structurally equivalent forms has been an important accomplishment that

has possible implications for condensed phase problems. From such measurements, an extremely accurate pair potential would eventually be obtained. It should be possible then to investigate the cooperative (nonpairwise) effects in hydrogen bonding through VRT spectroscopy of the water trimer.

In this report we present detailed experimental results for the cyclic water trimer. More than 100 FIR-VRT transitions were measured for the d_6 isotopomer in the frequency range from 86.7 to 92.0 cm^{-1} spectral region with a precision of ~ 1 ppm and analyzed by nonlinear regression. The data are interpreted in terms of a c-type (parallel) VRT band of a near oblate asymmetric rotor, with rotational constants in good agreement with the cyclic structure predicted from ab initio calculations (8, 9). Many of the observed rovibrational features appear as an evenly spaced quartet of lines. The relative intensities within each multiplet correspond to those calculated for transitions between VRT states of F symmetry in the PI group G_{96} , as determined from the relative nuclear spin weights (10) (45:63:36:18) of the rovibrational tunneling states. These results indicate that each of the monomers behaves both as a donor and as an acceptor, as in the bulk phase of water. The most interesting deduction, however, is that the cyclic water trimer is a chiral molecule, with low-barrier quantum tunneling motions interconverting the left- and right-handed stereoisomers.

The Berkeley tunable FIR laser spectrometer has recently been described (11), and only the relevant details of the present experiment are discussed here. A 150-W line tunable CO_2 laser is used to longitudinally pump a fixed-frequency FIR laser. Four FIR laser lines were used in this work: 2,588,361.8 MHz ($^{13}\text{CH}_3\text{OH}$); 2,633,900 MHz ($^{13}\text{CH}_3\text{OH}$); 2,664,085.3 MHz (CH_2DOH); and 2,714,715.1 MHz ($^{13}\text{CH}_3\text{OH}$). Tunable FIR radiation is generated by mixing continuously tunable microwave radiation (2 to 65 GHz) with the fixed frequency FIR laser in a Schottky

barrier diode (model no. 1T12, University of Virginia, Charlottesville) mounted in a corner cube mixer. The tunable sidebands ($\nu_{\text{sidebands}} = \nu_{\text{laser}} \pm \nu_{\text{microwaves}}$) are separated from the fixed frequency carrier with a Martin-Puplett diplexer. Further separation is accomplished with two 750-line per inch nickel meshes operating as a tunable Fabry-Perot etalon. The radiation is then multipassed 8 to 16 times through a 4-inch-long (10-cm) continuous wave (cw) planar expansion in which the water trimer is formed and supersonically cooled to a rotational temperature near 5 K. The laser radiation is detected by a liquid helium-cooled Ge:Ga stressed photoconductor (12), and absorption features are measured as a decrease in detected sideband power. The tunable sidebands are frequency modulated at 50 kHz, and absorption signals are processed by lock-in detection at two times the modulation frequency, yielding a second derivative line shape and a minimum detectable fractional absorption of 1 ppm. The water trimer molecular beam is formed by bubbling argon through neat D_2O (99.9% purity) at a pressure of 900 torr. Typical chamber pressures of 300 mtorr are maintained by a 2800-cubic feet per minute Roots blower system. No water trimer lines were detectable if the carrier gas was simply passed over a reservoir of D_2O .

A least squares fit of the observed VRT band yields the following rotational constants (in megahertz): $A'' = 5884.6$ (74); $B'' = 5702.7$ (88); $C'' = 2806.3$ (92); $A' = 5844.9$ (58); $B' = 5719.8$ (73); and $C' = 3018$ (10); and a band origin of 2684979.4 (29) MHz (errors in last digits, in parentheses, are reported as 1σ). These constants can be reproduced with the cyclic trimer structure shown in Fig. 1, which agrees reasonably well with that calculated by high-level ab initio theory (8, 9). This cyclic structure is found to exist as the global minimum on both of these ab initio surfaces. Chain-like structures for the trimer can be ruled out as possible carriers of the measured spectra by examining the rotational constants for these isomers. Both the upper and lower states of the observed rovibrational band are perturbed by the tunneling motions and consequently can only be fit to a rotational Hamiltonian with unrealistically large D_{jk} and D_k distortion constants. A total of 22 P and R branch transitions from the $K_c = 0$ and 1 subbands have been fit to obtain the reported constants. Although the rotational assignment is incomplete at present, the qualitative band structure can be simulated by assuming a c-type transition. Thus, a strong and very compact Q branch of ~ 65 transitions is observed along with the relatively weaker P and R branches. The P and R branches can best be described as evenly spaced

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groups of transitions among the K stacks corresponding to a particular value of ΔJ . Also, the number of transitions for a given $\Delta J = \pm 1$ follows the relation $2J + 1$, as expected for a parallel transition in a near-oblate rotor. The Q branch exhibits strong perturbations and is not incorporated into the present analysis. Since the band structure indicates a parallel transition, the values of the two C rotational constants are difficult to obtain independently with the amount of information included in this fit because the parameters are correlated. However, $(A + B)/2$ is well determined for both the ground and excited states. A representative rovibrational transition, split into a quartet by the tunneling motions, is shown in Fig. 2 along with the stick spectrum of all observed VRT multiplets. The splitting between each of the components of the quartet is ~ 6 MHz and provides a definitive signature of the lines belonging to this particular VRT band.

Although a distribution of dimers, trimers, and larger clusters exists in the supersonic expansion, the identity of the particular cluster responsible for the observed spectrum was established through isotope substitution experiments with H_2O and D_2O . If we assume that the hydrogens in each mixture statistically randomize and neglect kinetic isotope effects, the intensity reduction of a given trimer rovibrational feature due to the equal probability of forming the trimer with protons rather than deuterons follows the simple relation:

$$\ln [\text{fractional intensity}] =$$

$$N \ln [\text{mole fraction of } \text{D}_2\text{O}]$$

Here N would be four or six for a cluster containing two and three D_2O subunits, respectively, and $[\text{fractional intensity}]$ is equal to the ratio of the intensity of the transition observed with the mixture versus that observed with a pure D_2O sample. This relation has been shown to give correct results for observed D_2O dimer transitions ($N = 4$). A plot of mole fraction of D_2O versus fractional intensity of a representative trimer transition (with 5% precision) for five different H_2O - D_2O mixtures yields a straight line with N equal to 5.5 ± 0.4 at the 90% confidence limit. The low value of this slope is mainly due to the successive dilution procedure used and to the assumption that the volume of D_2O remained constant during the course of the experiment.

Tunneling splittings appear in the rovibrational energy level pattern of the water trimer when quantum tunneling of identical particles can occur by motion through low-barrier pathways. The most facile tunneling motion in the water trimer is a simple rotation of each water subunit about

its hydrogen bond. This motion would flip the out-of-plane deuterons above and below the plane of the cyclic ring, and are referred to as the "flipping" tunneling coordinate (13). In the limit wherein this is the only means of hydrogen tunneling, the molecular symmetry group would be isomorphic to the molecular point group C_{3h} and is called $C_{3h}(\text{M})$ (14). This group contains six tunneling pathways that can be described by PI operations that connect chemically equivalent

structures of the trimer. Three of these flipping motions interconvert right- and left-handed forms, whereas the remaining three preserve the handedness. Figure 3 represents tunneling of the deuteron labeled 5 in the left-handed structure along a pathway that follows a rotation about the $\text{O}_A \cdots \text{D}-\text{O}_C$ hydrogen bond. This operation, defined in the caption for Fig. 3 and in (14) as $(\text{ACB})(153)(264)^*$, is one of the six operations in $C_{3h}(\text{M})$ and it intercon-

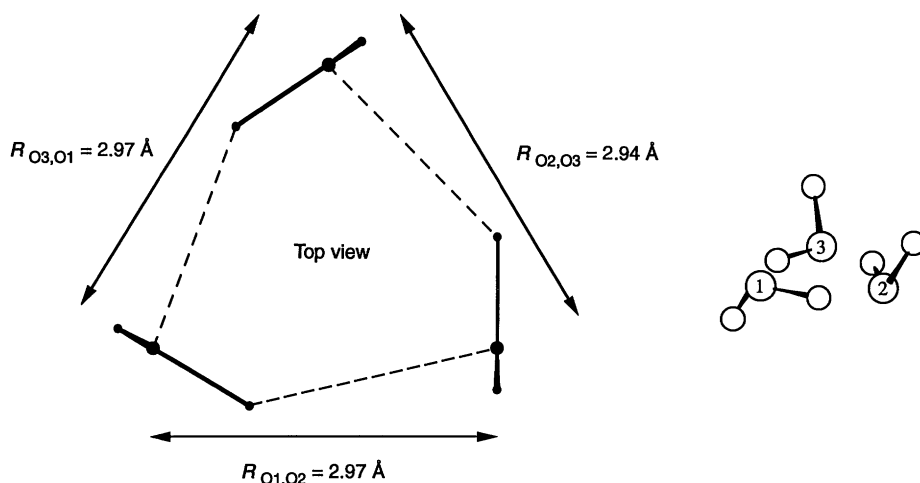
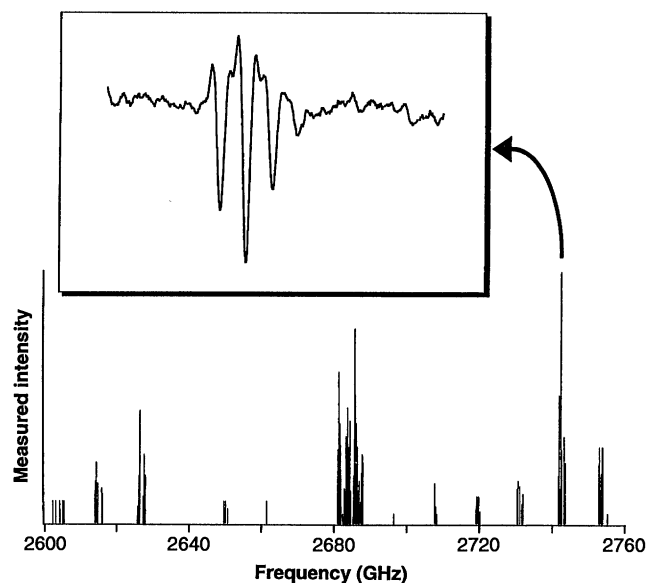


Fig. 1. A calculated structure for the water trimer- d_6 is shown from above the plane of the cyclic ring. The three oxygen-oxygen separations are labeled with a vector $\mathbf{R}_{\text{O}_A, \text{O}_B}$ in which each vector points from oxygen A to oxygen B. The distances are calculated by assuming that the hydrogen-bonded D atoms lie in the plane of the cyclic ring and form nonlinear $\text{O}_A-\text{D} \cdots \text{O}_B$ hydrogen bonds, as in the ab initio work of (8). The angles for these bonds are defined as $\text{DO}_1\text{O}_2 = 28^\circ$, $\text{DO}_2\text{O}_3 = 30^\circ$, and $\text{DO}_1\text{O}_3 = 27^\circ$. Also, the out-of-plane D atoms are positioned to lie perpendicular to the plane of the cyclic ring. In this structure, the D on O_1 is below the plane of the ring, whereas those residing on O_2 and O_3 point above the plane. This structure reproduces the ground-state rotational constants presented in this work, such that the error of the distances $R_{\text{O}_A, \text{O}_B}$ is ± 0.03 Å and these values of $R_{\text{O}_A, \text{O}_B}$ are similar to the oxygen-oxygen separation for the water dimer (2.976 Å). Although these separations are sensitive to the positioning of the D atoms, these data indicate that the attractive interaction of three water molecules may be described by the pairwise additive forces present in the water dimer.

Fig. 2. The observed VRT band of the water trimer is displayed as a stick spectrum. A transition within the $J'' = 4 \rightarrow J' = 5$ group of lines, illustrating the quartet structure of the VRT transitions, is shown in the insert. The intensities of this multiplet correspond to the relative nuclear spin weights of states having F symmetry (45:63:36:18) in the full molecular symmetry group G_{96} .



verts the right- and left-handed enantiomers of the cluster. As a result of the flipping tunneling motions, the $J = 0, K = 0$ rotational energy level would split into six VRT states, corresponding to the symmetries (A_1, A_2, E_1 , and E_2) of the irreducible representations of $C_{3h}(M)$. In (8), a barrier of $\sim 40 \text{ cm}^{-1}$ is calculated for this flipping motion for the d_6 isotopomer. The result of such a low barrier would be to generate a large tunneling splitting between the four nondegenerate VRT energy levels described in $C_{3h}(M)$. In this limit, four distinct subbands would arise for every vibrational transition.

Another possible low-barrier motion is the rotation of each water subunit about its C_2 axis, which breaks each hydrogen bond in the tunneling process. More precisely, if the deuterons on a particular water subunit were labeled so as to become distinguishable as in Fig. 3, the effect of

this tunneling motion would exchange the positions of the two deuterons, thus generating a nonsuperimposable but chemically equivalent trimer structure. This coordinate is referred to as " C_2 " tunneling and is described by PI operations of the form (12), (34), and (56). The last tunneling motion to be considered is one that reverses the sense of the hydrogen bonding from clockwise to counterclockwise and is referred to as the "cw-ccw" motion. The operations representing these motions have the form (AB)(13)(24). When all of the flipping, C_2 , and cw-ccw tunneling motions occur, 96 tunneling pathways described by PI operations become possible. Each of these tunneling motions serves as a pathway that converts one labeled form of the trimer into an equivalent labeled form, and all 96 equivalent minima on the potential energy surface can be accessed. In this limit, there exist 48 right-handed

forms of the trimer and 48 left-handed forms. Additional splittings of the VRT levels are introduced, and complete resolution of the rovibrational degeneracy occurs for each $|JK\rangle$ state described in the PI group G_{96} . This is the largest group possible for the cyclic water trimer in the limit wherein the chemical bonds of each monomer remain intact. In this limit, VRT states having A_1 and A_2 symmetry in $C_{3h}(M)$ split into four A states (A_1, A_2, A_3 , and A_4) and four triply degenerate F states (F_1, F_2, F_3 , and F_4). States of E_1 and E_2 symmetry in $C_{3h}(M)$ would split into E_1, E_2 , and another set of F states.

The symmetrical quartet patterns observed in the present VRT spectra are the result of transitions among the F states, for which the relative intensity ratio of 45, 63, 36, and 18 is defined by the nuclear spin statistical weights in G_{96} . Because the F states result from the existence of tunneling coordinates that break the hydrogen bonds, the barriers for these motions are presumed to be fairly high. Thus, small splittings among these VRT energy components are expected. Furthermore, the small values of the measured splittings indicate that this tunneling coordinate is not strongly affected by the observed intermolecular vibrational motion. The rovibrational band of quartets is interpreted as originating from one of the components (A or E states) of the flipping tunneling splitting described by $C_{3h}(M)$. Because the resolution of quartet structure in the VRT spectra indicates that the slower C_2 and cw-ccw tunneling motions are occurring in the molecule, and since the identification of the F states is well established, the appropriate symmetry group for the water trimer must therefore be G_{96} . In this limit, it is probable that the VRT band observed at 89.6 cm^{-1} correlates with a flipping type of vibrational motion. This motion would certainly exhibit a large dipole derivative along the top axis and would result in a c-type band, as observed. The identification of the exact $C_{3h}(M)$ VRT state from which the vibrational transition originates cannot yet be accomplished because all four irreducible representations in this group correlate to F states in G_{96} , and the accompanying A or E states have not yet been assigned.

Through the detailed study of small water clusters like the trimer, our understanding of the nonpairwise-additive forces operating in hydrogen-bonded clusters (15) and liquids (16) can ultimately be enhanced dramatically. However, the most interesting aspect of the present work is the deduction that the cyclic water trimer is chiral and that quantum tunneling motions of the equivalent deuterons

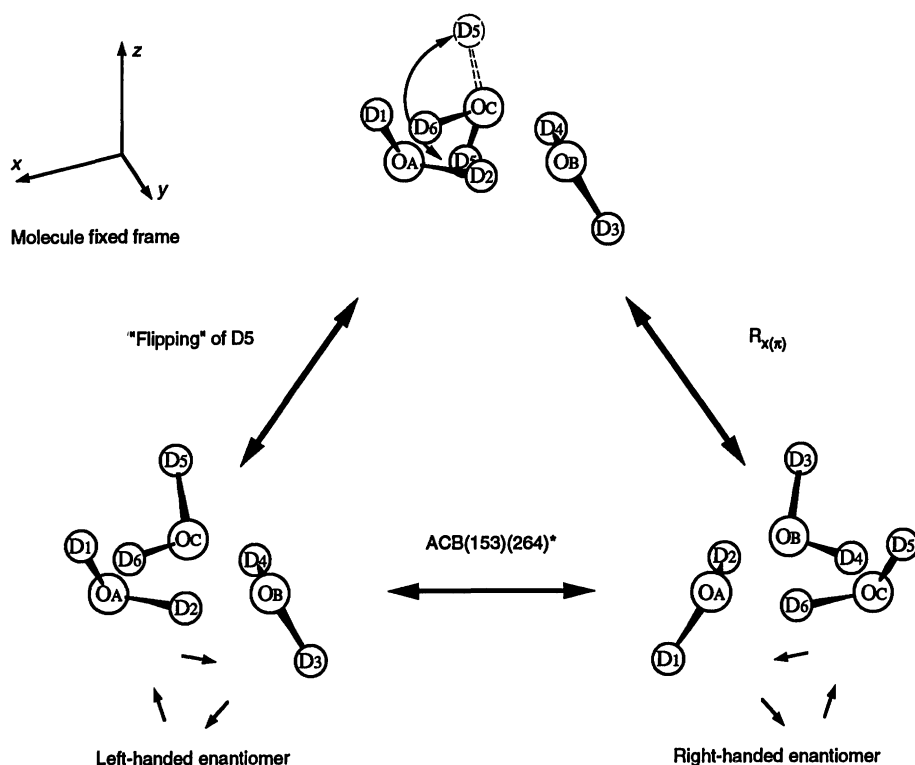


Fig. 3. The chirality of the water trimer- d_6 cluster is illustrated. The handedness of an enantiomer is determined by projecting the dipole moment of each subunit into the plane of the cyclic ring. These projections are positioned beneath the left- and right-handed enantiomers to distinguish between the two forms. The right-handed form is described by following the dipole projections in a right-handed (ccw) sense and by aligning the axis normal to this rotation with the molecule fixed z -axis. The identical nuclei within the cluster are labeled so as to make them distinguishable from one another. An example of an operation which permutes the identical nuclei is written as (ACB)(153)(264)*; for example, (ACB) is a cyclic permutation of identical nuclei such that A moves to position C, C moves to B, and B moves to A, and the * represents the inversion of all particles through the center of mass of the cluster (14). The pathway for this operation is equivalent to flipping D_5 from above to below the plane of the cyclic ring by rotating about the $O_A \cdots D_6 - O_B$ hydrogen bond. This flipping motion is depicted by the dashed D_5 bond, moving along the pathway represented by the arrow in the top portion of the figure. A simple rotation [$R_{x(\pi)}$] about the molecule fixed x -axis orients the cluster so that its mirror image can be easily recognized. Tunneling through this low-barrier pathway is an example of how the left- and right-handed enantiomers of the cluster are interconverted.

interconvert the left- and right-handed enantiomers. This result raises interesting possibilities concerning the existence of chiral structures for larger water clusters, such as the stable water clathrates recently studied by Castleman (17, 18). The interaction of such chiral water clusters with another molecule would break the left-right degeneracy and quench the interconversion tunneling, thereby yielding a complex having a well-defined handedness. In this situation, a racemic mixture of chemically equivalent right- and left-handed forms would result. It is also interesting to consider that the smallest repetitive unit of liquid water exhibits a diamond-like tetrahedral lattice (19). The asymmetry of the hydrogen bonding introduces the possibility of a local chirality existing in the liquid on a time scale shorter than the period of hydrogen-tunneling motions or fluctuations that rearrange the hydrogen bonding network (20). It seems that no previous work has

considered either the existence of such transient local chiral structures in liquid water or the possible manifestations of this phenomenon in chemical dynamics.

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Discovery of a Peptide-Based Renin Inhibitor with Oral Bioavailability and Efficacy

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Peptidic renin inhibitors have been poorly absorbed across the intestine or rapidly eliminated by the liver and have been reported to have oral bioavailabilities of less than 2%. A peptide-based renin inhibitor, A-72517 (molecular mass of 706 daltons), was devised that has oral bioavailabilities of 8, 24, 32, and 53% in the monkey, rat, ferret, and dog, respectively. Dose-related reductions in blood pressure, plasma renin activity, and plasma angiotensin II in parallel with increased plasma drug concentrations were observed after oral administration of A-72517 to conscious, salt-depleted dogs. Thus, peptide-based molecules of sizable molecular mass can be absorbed intact into the systemic circulation of animals. These findings support the potential of peptide-based drugs for oral administration.

Little is understood about the requirements necessary to allow a peptidic molecule to be transported intact across the intestine. The problem of devising an orally active renin inhibitor has served as a paradigm for the broader problem of designing peptide-based entities for oral administration (1). The physiologic role of the renin-angiotensin system (RAS) in regulating blood pressure and fluid balance has made the RAS a target for cardiovascular therapy. The wide degree of therapeutic applicability of angiotensin-converting enzyme (ACE) inhibitors in the treatment of diverse populations of hypertensives and individuals with congestive heart failure was unanticipated (2).

However, the ability of ACE to interact with a host of substrates (for example, bradykinin, enkephalins, substance P, neurotensin, and luteinizing hormone-releasing hormone) in addition to angiotensin I (3) raises the possibility that the side effects associated with ACE inhibition may be unrelated to the RAS (4). An alternate approach to the interference of the RAS is through the direct inhibition of the enzyme renin, which catalyzes the first and rate-limiting step in the synthesis of the effector hormone angiotensin II (ANG II). Renin inhibitors offer a high degree of specificity because angiotensinogen is renin's only known natural substrate. The most successful design of renin inhibitors has been based on transition-state analogs of angiotensinogen that are peptidomimetics (5).

The structure of A-72517, a transition-state analog inhibitor of renin with a dipeptide core, is shown in Fig. 1. This compound represents the second generation of dipeptide renin inhibitors discovered in our laboratory. A-72517 is a structural relative of A-64662 (enalkiren), a first-generation renin inhibitor that is intravenously efficacious and has been studied extensively in preclinical and clinical experiments and shown to lack oral bioavailability (6). These two compounds can be classified as peptidic renin inhibitors with comparable molecular masses of 706 and 657 daltons, respectively, and have in common a dipeptide-glycol fragment at the COOH-terminus (7), an alanine residue substituted with a heterocyclic moiety at the P₂-site, a replacement for the P₃-phenylalanine to impart stability to chymotrypsin degradation of the P₂-P₃ peptide bond (8), and a basic group at the NH₂-terminus to improve solubility.

However, they differ in three aspects. First, the P₂-site histidine and NH₂-terminal β -alanine residues of A-64662 are more basic than their counterparts in A-72517 and contain nitrogen-bound protons capable of forming hydrogen bonds. Consequently, A-72517 is the more lipophilic compound with a log P of 4.6 (in octanol-water, pH 7.4), as compared with a log P of 2.6 for A-64662, and the aqueous solubilities of the salts are 10 mg/ml versus 100 mg/ml, respectively. Second, the histidine and NH₂-terminal β -alanine residues of A-64662 are capable of forming conjugates; thus, these groups may play a role in the rapid clearance of A-64662. Finally, the P₃-site residue of A-72517 imparts proteolytic stability, and the sulfonamide moiety improves potency (9) tenfold as compared with A-64662.

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