a somewhat compact shape inspite of the positive charges on the two bases; the adenine and nicotinamide rings are about 9.6Å apart. The planes through the two bases are parallel and are at 4.08Å apart. The C-NH_2 of the carboxamide group is trans to C(3)-C(4) of the nicotinamide whereas it is cis in the Li^+-complex. Both the nucleotides adopt the preferred conformation usually found for nucleotides, viz: anti, g^+ for C(4')-C(5') and C(2')-endo. Both the sugars exhibit a very pronounced bond-shortening anomeric effect (i.e. C(1')-O(4') < C(4')-O(4') by 0.078Å and 0.039Å respectively for the sugars attached to nicotinamide and adenosine. The pyrophosphate group exhibits what appears to be an inherent asymmetry in the P-O bonds of the P-O-P link; P_N-O greater than P_A-O by 0.04Å. The P_N-O-P_A angle has widened considerably to 133.3(1)°, allowing a variety of possible conformations across the two P-O bonds. Looking along the P...P virtual bond, the phosphate groups are staggered, a conformation quite different from that in the Li^+-complex. There is no intramolecular stacking, but this structure exhibits a novel intermolecular stacking giving rise to sandwiching of nicotinamide half way between adenine and water molecules 6.97Å apart. It is interesting to observe that the binding sites in different enzymes for such a chameleonic coenzyme is so similar.

x)Work supported by a grant from the National Institutes of Health, GM24864.

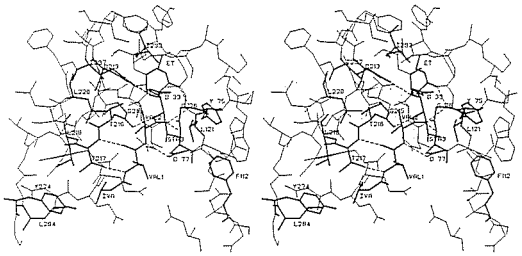
02.11–1 STRUCTURAL TRENDS IN N-ACYLTHIOESTERS OF RELEVANCE TO ACYLPAPAINS. By C. P. Huber and K. I. Varughese, Div. of Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A 0R6.

We are determining the crystal structures of a series of dithio- and thiol-esters which are closely related to the ester groups in the active site of acylpapains. The conformation of eight of these compounds is characterized by small N-C-C-S (ψ') torsional angles, with short N···S(thiol) distances in the range 2.83 - 2.93 Å, and by nearly orthogonal amide and thioester planes, leading to an N···S interaction. The C-N-C-C (ϕ') torsional angles are in the range -75.4 to -97.1° and the ψ' angles are in the range 9.5 to -23.0°. In a Ramachandran-type plot, the values of ϕ', ψ' for four N-acylglycine ethyl dithioesters lie essentially along a straight line which seems to represent the conformational pathway with maximal nitrogen-sulfur orbital interaction. Values of ϕ', ψ' for two N-acylalanine ethyl dithioesters and for two N-acylglycine ethyl thiolesters, while in the same range, show some deviations from the straight line. Resonance Raman spectroscopic studies of transient dithioacylpapains (Ozaki, Pliura, Carey and Storer, (1982), Biochemistry, 21, 3102) indicate that in the major population of the acyl-enzyme the dithioacyl group assumes a comparable conformation with N···S interaction. The fact that the conformations of the thiolesters in our series are very similar to those of the dithioesters suggests that information obtained for the dithioacylenzymes may be transferable to the natural thiol-intermediates.

02.11–2 A STEREOCHEMICAL ANALYSIS OF THE ASPARTYL PROTEINASE HYDROLYTIC MECHANISM. Michael N.G. James and Anita R. Sielecki, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

The crystal and molecular structure of penicillopepsin, the aspartyl proteinase from *Penicillium janthinellum*, has been refined at 1.8 Å resolution to an R-factor of 0.136 for the 21,962 reflections with $I \geq 1\sigma(I)$ (James & Sielecki, J. Mol. Biol. 163, 299 [1983]). The close proximity of the two catalytically important carboxyl groups of Asp33(32) and Asp213(215) suggests that they share a proton (d_{O-O} = 2.87 Å) in a tight hydrogen-bonded environment (residue numbers in parentheses refer to those of porcine pepsin). Hydration of this active site region suggests that a specific water molecule hydrogen-bonded to Asp33(32) plays the role of the attacking nucleophile (OH^-) in the hydrolytic mechanism. A plausible substrate binding mode to penicillopepsin has been deduced on the basis of the observed binding of a pepstatin analogue (James *et al.*, Proc. Natl. Acad. Sci. 79, 6137 [1982]). Crystallographic refinement of this molecule, Isovaleryl-valyl-valyl-statyl ethyl ester, in the complex [R = 0.131 for 21,197 reflections, $I \geq 1\sigma(I)$] shows the detailed binding interactions responsible for its inhibitory character ($K_I = 2.4 \times 10^{-8}$M). This inhibitor is taken as a model for the tetrahedral intermediate in the catalytic pathway of a good substrate. The statyl residue has a secondary hydroxyl group on a tetrahedral carbon atom analogous to the C=O group of the scissile bond. The figure below shows the refined structure of the pepstatin analogue bound to penicillopepsin. The hydroxyl group is bound between the two aspartyl carboxyl groups and replaces a strongly bound solvent from the native enzyme.

In spite of the remarkable 2-fold symmetric arrangement of the active site region of the aspartyl proteases, the



interaction with substrates is decidedly asymmetric. Residues in penicillopepsin involved in hydrophobic binding of P_1 residues are tyrosine-75(75), phenylalanine-112(111) and leucine-121(120); those most important for binding P_1' residues of a substrate are: phenylalanine-190(189), isoleucine-211(213), phenylalanine-295(299), isoleucine-297(301) and isoleucine-293(297).

It is proposed that the electrophile is the shared proton between Asp213(215) and Asp33(32); the nucleophile is a water that is activated to an OH^- by the proximity of Asp33(32); the leaving group is protonated by the acidic solution in the cases of those proteases with pH optima in the range 1-4. Analysis of the stereochemistry of this proposed reaction pathway suggests a tetrahedral intermediate with opposite hand to that proposed for the serine proteinases.

Research supported by the Medical Research Council of Canada.