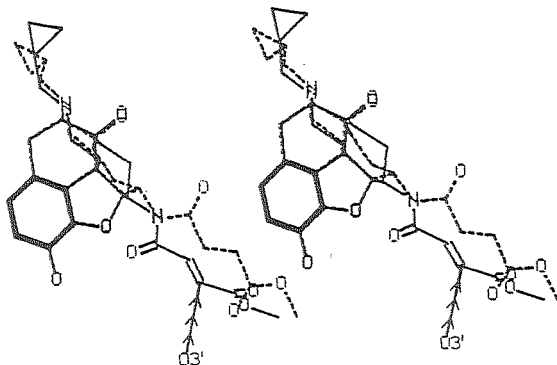


The addition of a nucleophile to the fumarate double bond is an  $S_N2$  type addition. There is a close intermolecular contact between the C23 carbon of the fumarate double bond in  $\beta$ -FNA and the O3-phenolic oxygen on a neighboring  $\beta$ -FNA molecule, which can serve as a model for nucleophilic attack on the fumarate group. After a least-squares fit of the fused ring moieties of  $\alpha$ - and  $\beta$ -FNA, the C23 of the  $\alpha$ -epimer is more than 2Å away from the C23 of the  $\beta$ -compound, too far away and in the wrong orientation for alkylation to take place.



(iii) The use of distance-matrices (2) to define the substrate conformations and orientations which are compatible with the geometrical features previously defined for the receptor sites.

Some examples of the docking of polypeptide substrates into the active sites of enzymes of known structures are given.

- (1) BUSETTA, B., TICKLE, I.J. & BLUNDELL, T.L. (1983), *J. Appl. Cryst.* **16**, 432-437.
- (2) HAVEL, T.F., KUNTZ, I.D. & CRIPPEN, G.M. (1983), *Bull Math. Biol.* **45**, 665-720.

#### 03.4-7 DOCKER : AUTOMATIC ALGORITHMS FOR SIMULATING PROTEIN RECEPTOR AND SUBSTRATE INTERACTIONS.

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If interactive computer graphics programs seem to be the best approach to study the docking of flexible substrates into a protein receptor of known three-dimensional structure (1), they cannot be limited to the geometric operations and facilities in refining conformational energies. Automatic algorithms must be designed to avoid fastidious and somewhat subjective manipulations so as to make the users' task easier and more reliable.

To correlate the results of the conformational analysis at the level of the receptor sites, with the biological observations a complete inspection of the different possible interactions must be assumed. A three-step algorithm to perform an automatic study of the docking of a flexible substrate is proposed with :

- (i) A bitwise use of the computer memory to represent the three-dimensional accessible volume of the receptor site as a fine grid sampled at regular small intervals and a subsequent partition of the set of these accessible points into "hydrophobic" pockets and "hydrophilic" zones.
- (ii) The generation of all the possible "ab initio" conformations of the flexible substrate. For sequential substrates such as peptides, nucleic acids, polysaccharides this task may be done once only, and preserved in a fast accessible data bank.

#### 03.4-8 SMALL MOLECULE + ELASTASE BINDING AS MODELS FOR DRUG + RECEPTOR INTERACTIONS: METHODS AND RESULTS

By E. Meyer, G. Cole, R. Radhakrishnan, L. Presta, G. Carlson, and S. Swanson, Dept. of Biochem. and Biophys., Texas A&M University, College Station, TX, USA

Due to the sum of weak forces on the resulting structural and functional specificity of drug+receptor interactions, it is essential that the receptor architecture be known to the highest resolution possible, that the ubiquitous water molecule be included appropriately, that the internal flexibility of functional groups be considered and that the composite picture be evaluated quantitatively. While molecular modelling via computer graphics makes much of the above both possible and even comprehensible, it is overly subjective.

In order to put such studies on a solid basis and better define the spatial geometry of a receptor, crystallographic investigations have been initiated: the 2.5Å resolution structure of porcine pancreatic elastase (PPE Sawyer, et al., *JMB*(1978)118,137) has been extended to 1.65Å resolution ( $R=0.18$ ).

Next, a crystal of PPE was given excess substrate (Ac-AlaProAla-pNA) and the reaction allowed to reach equilibrium; 1.65Å resolution data sets at pH 5.0 and 7.5 were measured and refined (currently,  $R=0.19$ , pH5). A comparison of the results of the refined structures will be presented.

In order further to probe the model of binding of inhibitors, crystals of PPE have been soaked in solutions of select compounds and low-resolution (4.5Å) data used for difference Fourier calculations to establish binding prior to high-resolution studies. Concurrently, these compounds have been graphically modelled

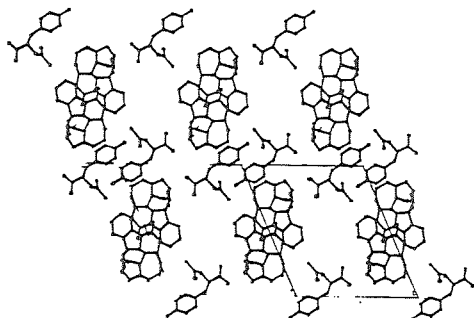
into the extended binding site of PPE and the models subjected to force-field refinement and evaluation. The PPE+PAPY complex may provide proof of the reaction mechanism for serine proteases involving amide N atom inversion (R to S) at the scissile bond. Although we are still evaluating these methods, they surely should be incorporated into a drug-design methodology at the molecular level.

Because of the structural (and functional) homology exhibited by the serine proteases, we have extended modelling methodologies to permit us to predict the tertiary structures of analogous elastases (rat I=84% homologous, rat II=58%, with one insertion). The novel aspects of our method include the inclusion of conserved molecules of hydration and the refinement of graphics generated models by means of force-field calculations. As these methods too should be part of drug design efforts, they will be discussed, together with the results they have yielded. As appropriate, a motion-picture film will be shown to illustrate these various techniques.

We acknowledge Prof. R. Huber, Dr. W. Bode, Dr. M. Zimmerman, Prof. J. Powers, Dr. E. Czerwiński, Dr. R. MacDonald, Prof. A. McCammon, the Robert A. Welch Foundation, the Petroleum Research Fund, and the Texas Agricultural Experiment Station.

**03.4-9** A CRYSTAL COMPLEX OF STRYCHNINE AND N-ACETYL-L-TYROSINE. By S.B.B. Glover, R.O. Gould and M.D. Walkinshaw, Chemistry Department University of Edinburgh, EH9 3JJ, U.K.

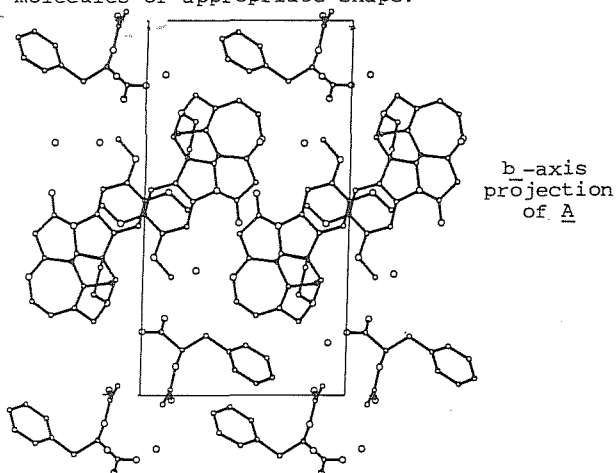
Strychnine is less used as an agent to resolve racemates than is brucine, an alkaloid similar except for two -OMe substituents on the indole ring. This difference affects the packing of their salts profoundly. Strychnine salts form alkaloid bilayers separated by solvent sheets, with rather restricted "holes" for guest molecules. An interesting example is the salt of strychnine with N-acetyl-L-tyrosine. It crystallises in space group  $P2_1$ , with  $a = 16.544$ ,  $b = 7.866$ ,  $c = 15.384$  Å,  $\beta = 115.71^\circ$ . The diagram shows the  $b$  projection of the ordered strychnine moieties with one of two alternative tyrosine arrangements, each having 50% site occupancy. The alternative positions and their differing hydrogen bonding will be discussed.



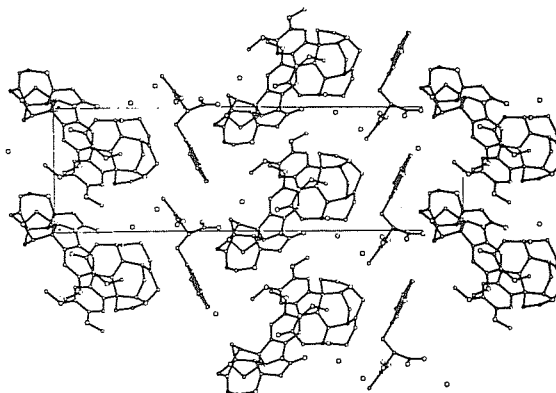
**03.4-10** TWO BRUCINE-AMINO ACID COMPLEXES. By R.O. Gould, P. Taylor and M.D. Walkinshaw, Chemistry Department, University of Edinburgh, Edinburgh EH9 3JJ, U.K.

Brucine is widely used in the resolution of racemates of N-protected amino acids. We are undertaking a systematic study of such peptide alkaloid complexes in order to explain the preferential co-crystallisation of D- or L- forms.

Brucinium N-acetyl-D-phenylalaninate.  $4H_2O$  (A) crystallises in space group  $P2_1$ , with  $a = 11.080$ ,  $b = 7.526$ ,  $c = 20.137$  Å,  $\beta = 91.24^\circ$ . The main packing feature is the corrugated monolayer sheets of brucine normal to the  $c$ -axis which provide cavities to trap preferentially chiral molecules of appropriate shape.



A more unusual situation is illustrated by the 2:1 adduct of brucine with N-acetyl-L-tryptophan. Brucinium N-acetyl-L-tryptophanate. brucine.  $5H_2O$  (B) is monoclinic,  $P2_1$ , with  $a = 9.573$ ,  $b = 31.775$ ,  $c = 9.173$  Å,  $\beta = 97.96^\circ$ . Like A, B shows the conserved, corrugated monolayer of brucine, although in B one of the crystallographically independent brucine moieties is cationic, the other neutral.



Both structures were solved using the DIRDIF procedure, linked with a Patterson orientation search, techniques ideally suited to crystals containing large, rigid groups. The solution of the structures and their hydrogen bonding will be presented.