03.4–4 CARACURINE-II DIMETHOCHLORIDE OCTAHYDRATE, A POTENT NEUROMUSCULAR BLOCKING AGENT.
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$[C_{40}H_{44}N_4O_2]^{2+}\cdot 2Cl^-\cdot 8H_2O$, $P2_1$, a=12.695(4), b=7.424(2), c=21.762(6) Å, β=98.03(5)°, Z = 2. The structure could not be solved by the heavy atom Patterson method; it was solved by direct methods, and refined by least squares to R=.10.

The alkaloid cation, with two-fold molecular symmetry, has a highly fused ring system and is structurally rigid. This determination gives accurate stereochemical parameters for those atoms and groups (N^+ centers, aromatic rings, and hydrogen bond acceptors) postulated by various theories as involved in binding to the acetylcholine receptor.

In the crystal, layers of alkaloid cations parallel to the bc plane alternate with layers containing two chloride ions and eight water molecules distributed almost randomly over ten sites. Electrostatic attractions between N^+ and Cl^- bind the alternating layers together. Each of the ten sites is, on average, 4.60 Å from one or two N^+, allowing the two Cl^- ions to be disordered. Binding interactions within the alkaloid layers are solely van der Waals attractions. Within each H_2O/Cl^- layer there is a complex hydrogen bond system, including four infinite spirals parallel to the b axis, with an average bonding distance of 2.94 Å. There are no hydrogen bonds between layers. The possible relevance to the activity of the alkaloid of its ability to organize large amounts of water is noted and discussed.

03.4–5 CONFORMATIONAL FEATURES OF CALCIUM CHANNEL AGONIST AND ANTAGONIST ANALOGS OF NIFEDIPINE.
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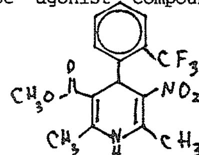
Nifedipine analogs are 2,6-dimethyl-3,5-dicarboalkoxy-4(aryl-substituted)-1,4-dihydropyridine compounds which frequently exhibit important cardiovascular activity in that these drugs inhibit cardiac and smooth muscle contraction by blocking the flow of calcium ions through plasma membrane channels into the muscle cell. All such active nifedipine antagonists appear to act at a common dihydropyridine (DHP) plasma membrane binding site and correlations between pharmacologic and membrane binding activities establish that these binding sites are pharmacologically relevant. Quantitative structure-activity relationships have been derived for nifedipine analogs which correlate antagonist potency with large values of the minimum width steric Verloop parameter of the ortho- or meta- aryl substituent and lipophilic and steric factors of the ester groups (R. Rodenkirchen et al., Naunyn-Schied. Arch. Pharmacol. 310, 69-78 (1979)). Subsequent structural studies appeared to indicate a correlation between DHP ring flatness and activity for these ortho- and meta- substituted nifedipine antagonists (A.M. Triggle et al., J. Med. Chem. 23, 1442-1445 (1980); R. Fossheim et al., J. Med. Chem. 25, 126-131 (1982)).

Our present understanding of these physiochemical and conformational prerequisties for DHP receptor binding and calcium channel inhibition have been further complicated by the marked tissue selectivity shown by certain of the nifedipine analog antagonists which have dissymmetric ester groups. Such tissue selectivities are not shown by any of the symmetric ester analogs. In addition these dissymmetric ester analogs often show a chiral preference for receptor binding and calcium channel inhibition which underscores the probable chiral nature of the putative endogenous hormone, which has yet to be discovered.

More recently several dissymmetric nifedipine analogs have been developed which surprisingly exhibit calcium channel agonism and stimulate cardiac and smooth muscle contraction (M. Schramm et al., Nature 303, 535-537 (1983); A. G. Truog, oral presentation at FASEB meeting, Chicago, April 1983). A diffraction study on the first of these agonist compounds, BAY K 8644, has



revealed that this compound has the flattest DHP ring of all the nifedipine analogs examined to date. Thus it appears that this conformational feature is not a characteristic of calcium channel antagonism, but rather a common feature which allows both agonists and antagonists to bind to the same DHP calcium channel receptor. Agonist or antagonist response must be encoded in other stereochemical and electronic characteristics which may be differentiated by the receptor. The crystal and molecular structure of BAY K 8644 suggests that the agonist behavior of this compound may in part be associated with a strong positive charge on the amine group brought about by a delocalization of electrons in the DHP ring as a consequence of the electron withdrawing effect of the 3-nitro substituent. Crystal data : BAY K 8644, $C_{16}H_{15}O_4N_2F_3$, M_r = 356.3, monoclinic, $P2_1/c$, a = 10.769(2), b = 12.762(2), c = 12.603(2) Å, β = 108.61(2)°, V = 1641 $Å^3$, Z = 4, D_x = 1.44 gm cm^{-3}, R = .064 for 4059 data with F > 2σF. Research supported in part by Grant No. HL32303 from the National Heart, Lung, and Blood Institute.

03.4–6 SELECTIVITY AT THE μ OPIATE RECEPTOR: THE STRUCTURES OF α- AND β-FUNALTREXAMINE.
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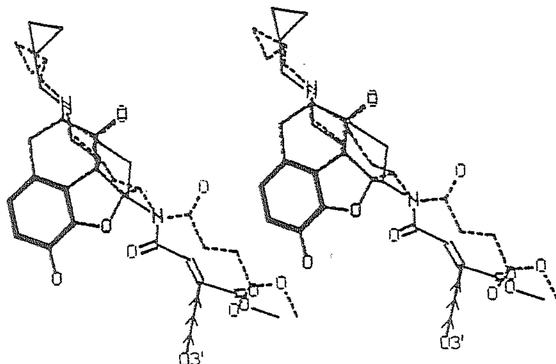
α- and β-Funaltrexamine (α- and β-FNA) are naltrexone derivatives differing only in chirality at C-6. Both α- and β-FNA bind to the μ opiate receptor in guinea pig ileum and mouse vas deferens preparations, but only the β-epimer selectively alkylates this receptor in both preparations. For this reason, βFNA has been used to "knock-out" μ receptors and study the remaining δ sites in these preparations and δ and κ sites in brain homogenate preparations. Sayre et al. (J. Med. Chem., 26, 1229-1235 (1983)) proposed a two step recognition process at the μ site with only the β-epimer in the proper orientation for the second recognition step which results in alkylation. Recent ^{1}HNMR studies of 6α- and 6β-oxymorphamine showed that the conformation of ring C was dramatically influenced by the stereochemistry of the 6-amino group: the 6β-epimer existed in a chair conformation and the 6α-epimer in the twist-boat conformation.

We have now determined by X-ray diffraction studies the molecular structures of both α- and β-FNA. The two epimers have almost identical conformations in the fused ring moiety except for ring C: in α-FNA ring C is observed in a twist-boat conformation, and in β-FNA ring C is in a chair conformation. The ring conformations result in the fumarate chain on C-6 being equatorial to ring C in both compounds. The fumarate moieties are approximately orthogonal to one another in the two structures.

The addition of a nucleophile to the fumarate double bond is an SN2 type addition. There is a close intermolecular contact between the C23 carbon of the fumarate double bond in β-FNA and the O3-phenolic oxygen on a neighboring β-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 α- and β-FNA, the C23 of the α-epimer is more than 2Å away from the C23 of the β-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 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

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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 2.5A resolution structure of porcine pancreatic elastase (PPE Sawyer, et al., JMB(1978)118,137) has been extended to 1.65A resolution (R=0.18).
Next, a crystal of PPE was given excess substrate (Ac-AlaProAla-pNA) and the reaction allowed to reach equilibrium; 1.65A resolution data sets at pH 5.0 and 7.5 were measured and refined (currently, R=.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 (4.5A) data used for difference Fourier calculations to establish binding prior to high-resolution studies. Concurrently, these compounds have been graphically modelled