these three reactions proceed simultaneously at equilibrium. The hydrolysis reaction and ^{18}O-exchange reaction are subject to ionic strength and salt effects. Although the pseudobase tautomer **2** of the 2,4,6-trimethylpyrylium salt appears to be so reactive that it has not yet been identified, pseudobases of other derivatives such as the flavylium ion have been observed.[31,35] The present study provides information about the existence and reactivity of intermediates such as **3** and **4**. Starting with the recently reported compound 4-methyl-4-hydroxy-2,6-heptanedione,[36] a direct synthesis of the diketone hydrolysis product might be achieved by dehydration. Further insight into the details of the reactions of the pyrylium salt, including its formation, should be obtainable.

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Registry No. 2,4,6-Trimethylpyrylium perchlorate, 940-93-2; 2,4,6-trimethyl[2,6-$^{13}C_2$]pyrylium perchlorate, 93111-97-8; 2,4,6-trimethyl[2-^{13}C]pyrylium perchlorate, 93111-99-0; [1-^{13}C]acetyl chloride, 1520-57-6; *tert*-butyl chloride, 507-20-0; aluminum chloride, 7446-70-0; mesityl oxide, 141-79-7; oxygen-18, 14797-71-8.

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Molecular Recognition in Model Crystal Complexes: The Resolution of D and L Amino Acids

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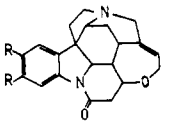
Abstract: The structures of the molecular complexes of *N*-benzoyl-L-alanine + strychnine and *N*-benzoyl-D-alanine + brucine have been determined by X-ray crystallography. The crystal structures of these two complexes provide for the first time some insight into the mechanism of the resolution of racemic mixtures of amino acids. The two methoxy groups on brucine produce a dramatic difference in its packing arrangement compared to that of strychnine. Wedge-shaped brucine molecules form corrugated monolayer sheets which concertina together to form a inclusion complex. In contrast, the strychnine molecules are arranged in bilayers. Despite the very different molecular environments, the L and D enantiomers of benzoylalanine adopt conformations closely related by mirror symmetry.

Separation of racemic mixtures was first achieved by Pasteur in 1853,[1] and the principles he developed are still commonly used today. An optically pure enantiomeric base is added to a racemic mixture of D and L acid. In a large number of cases it is found that one of the diastereomeric salts (base + L acid or base + D acid) is very much less soluble and preferentially crystallizes out of solution. The first amino acids were resolved by Emil Fischer in 1899[2] using the naturally occurring alkaloids strychnine and brucine (I) to separate the racemic *N*-benzoyl derivatives of alanine, glutamic acid, and aspartic acid. Approximately 2000 organic compounds have been reported in the literature[3,11] as being resolvable by the formation of diastereomeric salts, and for compounds with acid functional groups, strychnine and particularly brucine have been widely used as resolving agents. Successful resolutions can only the achieved by a time-consuming trial-and-error procedure in which various resolving agents and solvents are tested. Despite the practical importance of this technique, there is no explanation or rationalization of any of the many experimental results. Apart from work in this laboratory,[7,10] there are only three published crystal structures involving strychnine or brucine: strychnine bromide,[8] strychnine sulfate,[9] and the 1:1 complex brucine + 1-(*O*-bromophenyl)-1-phenyl-2-propynol.[5]



I

strychnine, R = H
brucine, R = OMe

Crystal Structure of Strychnine and *N*-Benzoyl-L-alanine

Crystals of this complex were prepared by using the method described by Pope[4] and were found to be orthorhombic with space group $P2_12_12_1$ and unit cell dimensions a = 10.751 Å, b = 30.366 Å, c = 8.608 Å. All hydrogen atoms were located, and the structure was refined to give a final R = 0.04; full details of the structure determination will be published elsewhere. A view of the unit cell is shown in Figure 1.

The crystal is composed of bilayers of strychnine molecules separated by hydrogen-bonded sheets comprising the carboxyl oxygen atom and the two solvent water molecules. Crystal

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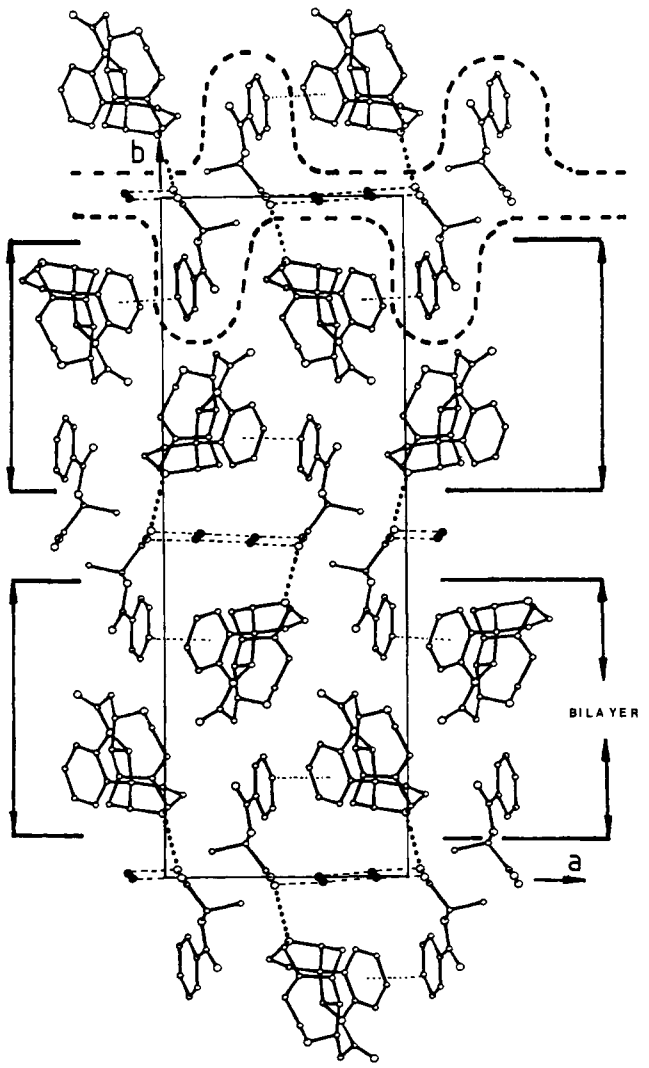

Figure 1. View of the unit cell of strychnine–benzoyl-L-alanine projected along *c*. Hydrogen bonds and the interaction between aromatic groups are indicated by thin dashed lines. The salt bridge between the carboxyl group and the charged nitrogen is shown by a dotted line. The heavy dashed lines indicated the hydrogen-bonded sheet of peptide and water molecules highlighted in Figure 3, parts a and b.

structures of the simpler strychnine salts (NO_3^-, SO_4^{2-}, Cl^-, Br^-, I^-) all show a similar conserved bilayer packing pattern[7] in which the strychnine molecules pack back to back to allow the protonated amine nitrogen to hydrogen bond with the water/anion sheets. The larger benzoylalanine anion in this complex still preserves this scheme with the benzoyl group managing to intercalate between the strychnine molecules. A salt bridge between the ionized carboxyl group of the amino acids and the protonated amine nitrogen atom on strychnine provides the strongest and most specific intermolecular interaction. Apart from the hydrogen-bonded sheets, the only other significant intermolecular interaction is between the benzoyl group of the amino acid and the indole group of the strychnine. Hydrogen atoms of the benzoyl group point into the center of the indole, and there is a reciprocal interaction with indole hydrogen atoms pointing into a benzoyl ring in an adjacent unit cell. The interplanar angle between the aromatic groups is 56° with H···C nonbonded contacts of 2.6 Å.

Crystal Complex of Brucine + *N*-Benzoyl-D-alanine·4.5 Water

Large crystals of the complex were grown from an aqueous solution and were found to be monoclinic, space group $P2_1$, with cell dimensions a = 12.423 Å, b = 33.343 Å, c = 8.222 Å, β = 97.46°. There are two crystallographically unrelated brucine–benzoylalanine ion pairs and nine solvent water molecules per

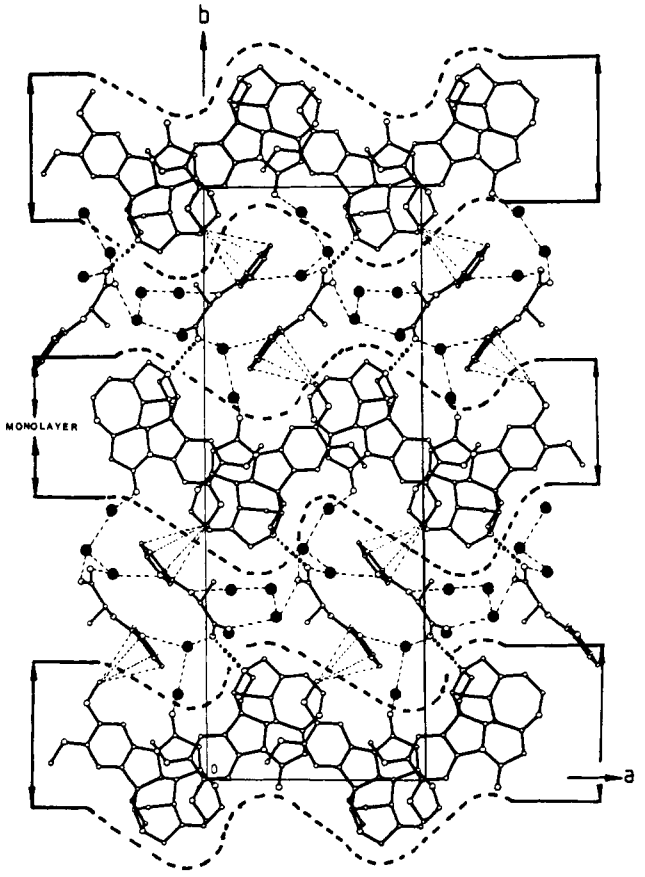

Figure 2. View of the unit cell of brucine–benzoyl-D-alanine projected along *c*. Hydrogen bonds and the interactions between the methoxy group and the benzoyl group are indicated by thin dashed lines. The salt bridges between the carboxyl groups and charged nitrogen atoms are shown by a dotted line. The heavy dashed lines indicate the boundary between the brucine monolayer and the hydrophilic water–peptide channels.

asymmetric unit, providing a substantial crystallographic problem which was solved by using a Patterson rotation function (Beurskens, Gould, and Walkinshaw, to be submitted). All hydrogen atoms were located and the final R factor was 0.048.

With the exception of the prominent methoxy groups lying in the plane of the indole ring, no bond lengths, angles, and torsion angles for brucine are significantly different from those in strychnine. Torsion angles defining the conformation of *N*-benzoyl-D-alanine only differ by ±6° from the mirror image of the L isomer found in the strychnine complex and suggest that the amino acid conformation is little influenced by intermolecular interactions.

The crystal structure (Figure 2) is composed of corrugated monolayer sheets of brucine which are separated by solvent channels containing the guest amino acid and water of crystallization. Brucine molecules stack with the plane of the indole group perpendicular to the plane of the corrugated monolayer sheet, allowing the protruding methoxy groups to pack efficiently. Crystal structures of complexes of brucine:ethanol[10] (space group $P2_12_12_1$ and cell dimensions a = 12.34 Å, b = 25.21 Å, c = 7.72 Å) and brucine:1-(bromophenyl)-1-phenyl-2-propynol[5] (space group $P2_12_12_1$ and cell dimensions a = 12.45 Å, b = 33.45 Å, c = 7.72 Å) have similar a and c cell dimensions to the brucine:*N*-benzoylalanine complex. These three structures also have very similar monolayer packing arrangements for brucine molecules, indicating that the conserved corrugated sheets of brucine can have a variable separation along the b direction depending on the amount of solvent and the size of the guest molecule.

As in the strychnine complex, the protonated amine nitrogen atoms on the brucine form a salt bridge with the carboxyl oxygen atoms on the amino acid. The nine water molecules form a complex hydrogen-bonded network shown in Figure 2. Both

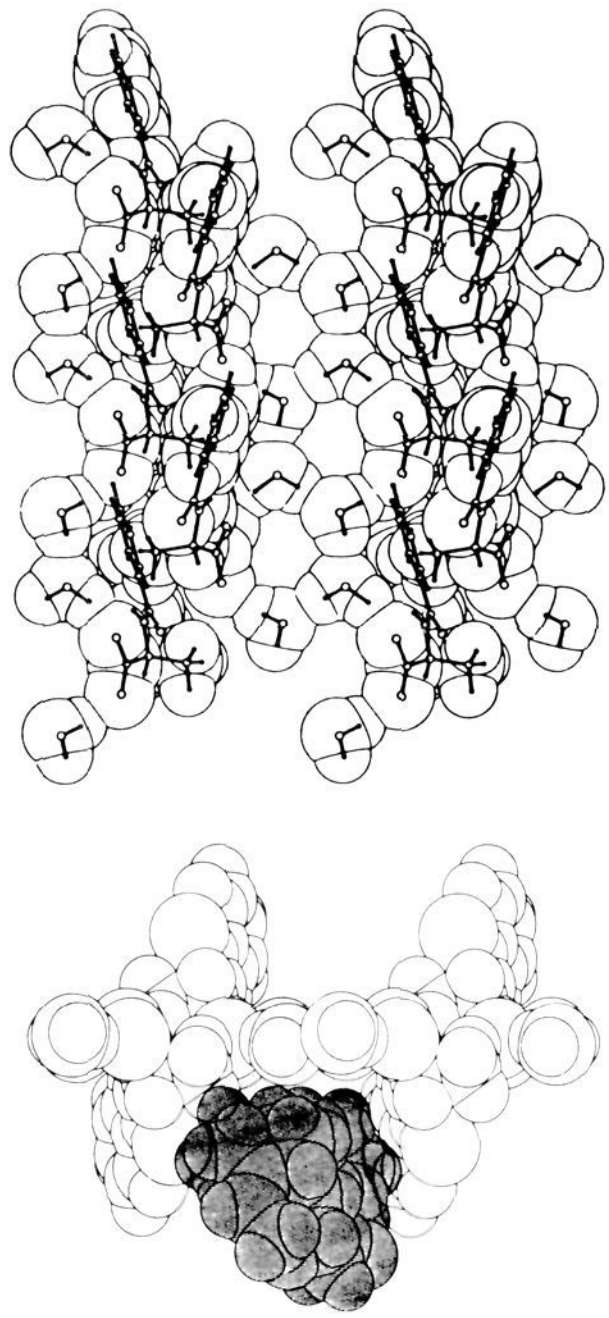

Figure 3. Top (a): A model receptor surface. View along *b* of the portion of Figure 1 outlined by heavy dashed lines. Water molecules and the carboxyl groups form an infinite hydrogen-bonded sheet from which the benzoyl-L-alanine molecules protrude. Atom radii are H 1.2 Å, C 1.5 Å, O and N 1.6 Å. Bottom (b): A model drug–receptor binding site. Projection down *c* of the water–benzoylalanine sheet shown in part a. The shaded strychnine molecule fits into the grooves formed by the protruding benzoyl groups.

benzoyl groups have similar atomic environments with oxygen atoms tending to lie in the planes of the aromatic groups. There are more than eight phenyl carbon···O contacts between 3.1 and 3.6 Å. Hydrogen atoms from the brucine methoxy groups sit about 2.8 Å above the plane of the benzoyl groups and again reflect the electron-rich faces and electron-deficient edges of the aromatic groups.

Crystal Packing and Molecular Recognition

Of the 15 *N*-acetyl, *N*-formyl, or *N*-benzoyl amino acid derivatives resolved by using brucine, 13 are D enantiomers.[3,6] Strychnine has only been used in four resolutions, three of which gave the L isomer. For other, more general organic acid resolutions, brucine has also proven to be a far more commonly used resolving agent.[3,11] So the apparently small chemical difference between strychnine and brucine of two methoxy groups can both cause a change in preference between D and L isomers and also give brucine a much greater versatility in forming complexes with a wider variety of compounds. It is likely that in the case of brucine there is a conserved packing arrangement of monolayer corrugated sheets. Channels between the sheets can be varied in size depending on the separation of the monolayers and provide anions with specific electrostatic and hydrogen-bonding binding sites in a variability sized cavity. The methoxy groups give the molecule a wedge shape, and the efficient packing of these molecules must be the driving force behind formation of the corrugated monolayers. This contrasts with strychnine which forms a bilayer structure which is conserved in all strychnine salts. The shape and size of the cavity for the guest anion is therefore much more restricted, and this is in keeping with the less general application of strychnine compared to brucine as a resolving agent. Another important consequence of the monolayer packing in brucine is that the carbonyl oxygen is now exposed to the anion cavity and provides additional hydrogen-bonding capability. This may explain why hydrate formation can facilitate the crystallization of complex salts and also provide a reason for the observation that a 96% ethanol/water solvent is a better solvent for effecting resolution than anhydrous ethanol.[3]

Both the strychnine bilayer and the brucine monolayer provide a strong and specific binding site by directing the charged tertiary amino group out into the solvent cavity to form a salt bridge with the acid carboxyl group. All other alkaloid–peptide interactions are essentially van der Waals in nature, and the preference for complexation with the D or L isomer must depend on the shape of the cavity available. (The alkaloids being chiral provide cavities which must also be chiral.) Analysis of the atomic environments of the benzoyl groups in both the D and L benzoylalanine complexes does, however, show that they are quite specific and may play an important role in the recognition procedure. In both structures there is a predominance of oxygen atoms around the edge of the benzoyl aromatic system. In addition, in the strychnine complex there is a pronounced zigzag set of interactions through the crystal in which aromatic hydrogen atoms interact with the indole or benzoyl π-electron clouds. This interaction in the brucine complex is replaced by an apparently specific interaction between the methyl hydrogen atoms of the methoxy group and the π-cloud of the benzoyl group. The conserved packing arrangement in brucine which allows the formation of channel-type inclusion complexes combined with such relatively weak but specific intermolecular interactions may also explain the formation of complexes of brucine with neutral guests devoid of hydrogen-bonding groups such as 2,3-dibromobutane.[12]

A Model for Drug–Receptor Binding

Neurochemical transmission depends on the ability of small transmitter molecules like glycine and acetylcholine to recognize and bind to large trans-membrane receptor proteins. It is interesting to note that most naturally occuring alkaloid resolving agents are also pharmacologically active. Both strychnine and brucine act as competitive antagonists with glycine at postsynaptic inhibitory sites in the central nervous system and also have moderately strong curaremimetic activity with acetylcholine receptor. Large drug–receptor systems have not yet been obtained in a form suitable for high-resolution diffraction studies, and the fine detail of the all-important molecular interactions remains conjecture. However, it is precisely those weak intermolecular bonds involving salt bridges, hydrogen bonds, and van der Waals and electrostatic interactions which can be examined in the model alkaloid–peptide crystal complexes described above.

It is striking that in both brucine and strychnine complexes there is a distinct boundary between the water/peptide molecules and the alkaloid molecules. In the strychnine structure, the water peptide layer forms an impenetrable hydrogen-bonded sheet (Figure 3a) and may be regarded as the model receptor surface. The peptide molecules pack in such a way as to provide a groove formed by the benzoyl groups which is perfectly shaped to fit the drug (strychnine) molecule. The carbonyl group on the peptide provides a specific and strong attachment, and the interaction between the indole and benzoyl groups provides extra stabilization.

Registry No. *N*-Benzoyl-L-alanine strychnine complex, 92818-46-7; *N*-benzoyl-D-alanine brucine water complex, 92818-47-8.

Supplementary Material Available: Tables of atomic positional and thermal parameters for structures shown in Figures 1 and 2 (11 pages). Ordering information is given on any current masthead page.

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