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# *Ab Initio* Calculations on Large Molecules Using Molecular Fragments. Structural Correlations between Natural Substrate Moieties and Some Antibiotic Inhibitors of Peptidyl Transferase

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Lincomycin, chloramphenicol, and erythromycin are antibiotic inhibitors of bacterial protein synthesis which are postulated to act by blocking the peptidyl transferase site at the 50S ribosomal subunit. Correlations between structural features of these antibiotics and the terminal moieties of the natural substrate molecules, peptidyl and aminoacyl tRNA, aid in rationalizing the observed competitive binding of these species at the catalytic site for peptide bond formation. Investigations of the electronic and geometric structure of the terminal moiety of peptidyl tRNA have been carried out using the *ab initio* SCF-FSGO method with 3-*O*-(*N*-glycylglycyl)ribose as a model. These studies give insight into the relationship between chemical structure and biological activity for the natural substrate as well as the antibiotic inhibitors of peptidyl transferase. The results of the studies indicate that it is not necessary to postulate allosteric mechansims of action to explain the effects of the drugs.

Lincomycin, chloramphenicol, and erythromycin are antibiotics with diverse molecular structures which, nevertheless, produce similar effects in various experiments designed to study their mode of action. All three antibiotics inhibit ribosomal protein synthesis while allowing RNA and DNA synthesis to continue.[1-3] Each of the drugs forms a reversible one-to-one complex with the 50S subunit of the bacterial ribosome.[4-7] Furthermore, competition for binding at the ribosome occurs when any two of the three antibiotics are present in the system.[7,8] In studies[9-13] using the puromycin reaction to model ribosome-catalyzed peptide bond formation, the drugs produce marked inhibitory effects, although the extent of inhibition depends on the assay employed. These facts led Monro, *et al.*,[14] to suggest that the antibiotics bind at overlapping sites at the peptidyl transferase center of the 50S ribosomal subunit.

Interesting differences in the actions of lincomycin, chloramphenicol, and erythromycin have been noted by various workers. Certain erythromycin-resistant strains of *Staphylococcus aureus* display cross resistance toward lincomycin but not chloramphenicol.[15,16] The three antibiotics also exhibit different effects on substrate binding at the peptidyl transferase center of the 50S subunit.[17-20] For example, erythromycin appears to stimulate binding of CACCA-Leu and CACCA-(Ac-Leu) while lincomycin inhibits the binding of both fragments. On the other hand, chloramphenicol apparently stimulates binding of the Ac-Leu substrate and inhibits the binding of the Leu substrate. As stated by Monro, *et al.*,[14] different effects, such as these, could be observed even if the antibiotics bind at overlapping sites since the molecules differ in size, shape, and other properties. However, some investigators[21,22] have suggested that the drugs must act through allosteric mechanisms on account of these dissimilar effects.

In the current study, various structural relationships are noted between lincomycin, chloramphenicol, erythromycin, and a moiety of peptidyl tRNA which is postulated to bind at the locus of peptidyl transferase. The structural correlations are employed to develop a simple chemical model for the mechanism of peptide bond formation at the ribosome and the interactions of the drugs at the peptidyl transferase center. Many similarities and differences in the effects of the three antibiotics may be rationalized in terms of the model. In addition, the model provides a basis for understanding relationships between biological activity and features of the chemical structure exhibited by several drug analogs.

**Description of Model.** The process of bacterial protein synthesis requires binding of peptidyl tRNA at the P site and aminoacyl tRNA at the A site of the 70S ribosome.[23,24] Since template mRNA is bound at the 30S subunit, the interaction with tRNA in that portion of the ribosome involves codon-anticodon recognition which determines the sequence of amino acids in the protein. Specific binding to the 50S ribosomal subunit occurs in the region of the peptidyl transferase catalytic center. Monro, *et al.*,[14] have proposed that interactions at the P site of peptidyl transferase involve the 3′-terminal nucleotide grouping CCA, which is common to all species of tRNA, and are favored by acylation of the $\alpha$-$NH_2$ group of the attached amino acid. They also suggested that the terminal CA binds specifically at the A site of peptidyl transferase.

The reaction to form the peptide bond may be postulated to occur as shown in Scheme I where $T_nOH$ is the carrier tRNA molecule for the amino acid with side chain $R_n$ and X denotes the previously synthesized segment of the protein chain. Although the species designated as III may only have a transitory existence, the primary function of peptidyl transferase would be to facilitate its formation and direct its break-up into the elongated peptidyl tRNA molecule, IV, and the released tRNA molecule, V. Thus, it would be possible to make useful inferences regarding the nature and relative positions of ribosomal binding sites at the peptidyl transferase center if the detailed structural features of III were known. By considering a system consisting only of the terminal adenosine nucleotide of tRNA and the attached peptidyl or aminoacyl groups, a simple model for species III is developed in succeeding paragraphs.

If lincomycin, chloramphenicol, and erythromycin are specific inhibitors of peptidyl transferase, as indicated by

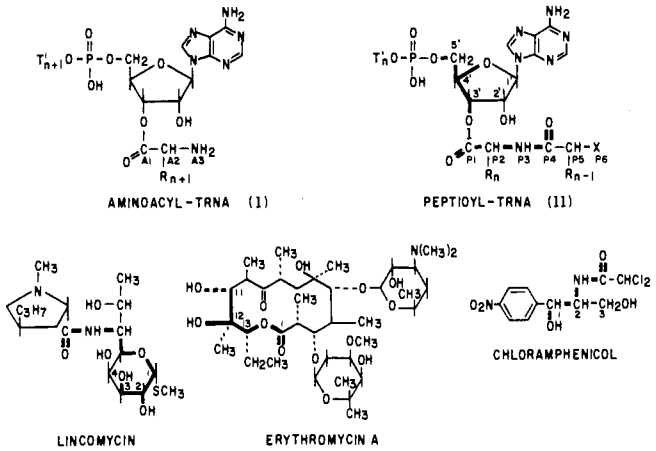
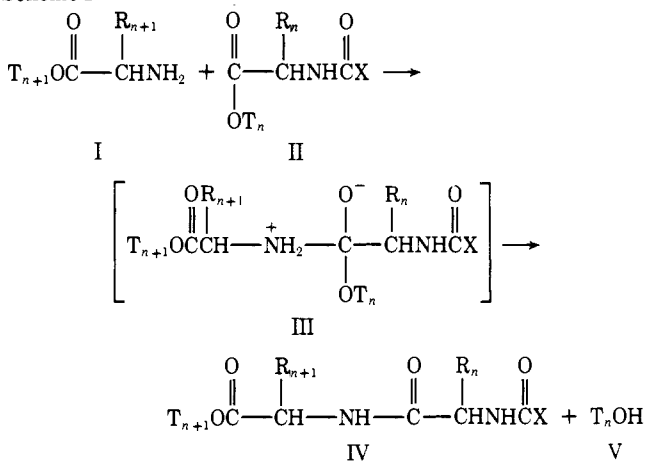



**Figure 1.** Natural substrate moieties and antibiotic inhibitors of peptidyl transferase. $T'_n$ and $T'_{n+1}$ are segments of tRNA chains excluding the final adenosine nucleotides.

**Scheme I**



I II III IV V

results of the puromycin reaction,[9-13] the antibiotic molecules should possess certain structural features analogous to those of the natural substrate in order to bind at the catalytic site. Functional groups which can participate in relatively weak interactions, such as hydrogen bonds and charge-transfer complexes, would be key items to note in discerning similarities between the drugs and the natural substrate. Although obvious structural similarities among these molecules are not revealed directly by the molecular diagrams shown in Figure 1, possible correlations exist among the groups connected by heavy lines. There are amide linkages in peptidyl tRNA, lincomycin, and chloramphenicol which have been shown to be necessary in ribosomal binding studies[14] or other tests of biological activity.[25,†,‡] Furthermore, O(1), O(2), and O(3) in lincomycin could correspond with O(3′), O(1′), and O(5′) in the peptidyl tRNA moiety as determined by the number of bonds separating these atoms from the amide group common to both molecules. Alternatively, one could match O(7) with O(3′), but this possibility is discounted since it would preclude any correspondence between other oxygen atoms in the two molecules. Chloramphenicol possesses an oxygen comparable to O(3′). Erythromycin lacks an amide group but exhibits a lactone moiety similar to the ester linkage of peptidyl tRNA. Hence, it is possible to relate

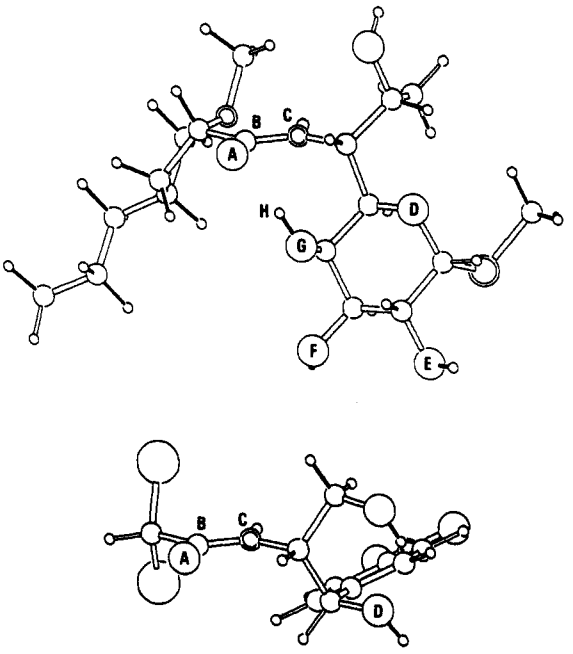



**Figure 2.** Top: lincomycin free base. Bottom: chloramphenicol. Since the postulated interaction of each key group at its ribosomal binding site is relatively weak and reversible, formation of the drug–ribosome complex should produce only minor deviations from the favored conformation of lincomycin or chloramphenicol. Therefore, the spatial relationships among the key groups are expected to remain essentially unchanged during engagement with the peptidyl transferase center.

O(1), C(1), O(11), O(12), and O(13) in erythromycin with O(P1), C(P1), O(5′), 0(1′), and O(3′) in the peptidyl tRNA moiety. Studies conducted with lincomycin,[26,†,§] erythromycin,[6] and chloramphenicol[8,25] have indicated that these key oxygen atoms are required for binding at the ribosome as well as antibacterial activity. On the basis of these comparisons, the three antibiotics are postulated to mimic the binding of peptidyl tRNA at the peptidyl transferase center.

The spatial relationships, obtained by experimental and theoretical techniques, of the key groups in lincomycin[27,28] and chloramphenicol[29,30] are portrayed in Figure 2. Although X-ray crystallographic analysis has elucidated the structure and stereochemistry of erythromycin A hydroiodide dihydrate,[31] the molecule is not included in Figure 2 since the crystal conformation yields a different spatial arrangement of the key oxygen atoms from that of lincomycin and chloramphenicol. However, the flexibility of the macrolide ring is expected to permit adoption of the proper conformation at little or no cost in energy. In lincomycin, the key atoms which constitute possible foci for binding interactions at the ribosome are the amide group (A, B, C) and the oxygen atoms D, E, and F. In chloramphenicol, only the oxygen designated as D is present in addition to the amide group. The 4-hydroxyl group of lincomycin does not have a counterpart in the peptidyl tRNA moiety. Nevertheless, it has been shown to be necessary for antibiotic activity.[26] Furthermore, it possesses a unique relationship to atoms A–F which leads to its inclusion among the key atoms involved in binding. Since the relationship depends on the postulated mechanism for transpeptidation, a brief description of the reaction se-

†J. Davies, The University of Wisconsin, private communication.

‡Removal of the amide bridge in lincomycin by reducing C=O to $CH_2$ destroys the antibacterial activity as well as the ability of the molecule to inhibit protein synthesis in a cell-free system.

§The necessity of the hydroxyl groups at C(2) and C(3) of lincomycin is indicated by the fact that inversion of configuration at those positions results in complete loss of capacity to inhibit protein synthesis in a cell-free system. In addition, the 2-deoxy analog is much less effective than lincomycin.

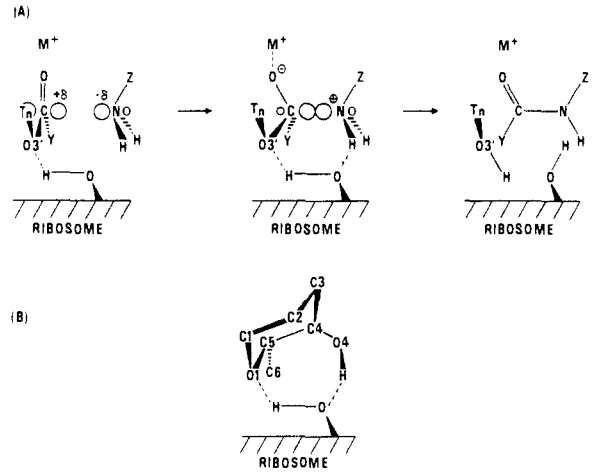



Figure 3. (a) Hypothetical mechanism for ribosome-catalyzed peptide bond formation, where $T_nOH$ is the tRNA specific for nth amino acid, Z = $-CHR_{n+1}C(=O)OT_{n+1}$, Y is the segment of previously synthesized protein, and $M^+$ is a cation. Although the model requires an unprotonated $NH_2$ group in aminoacyl tRNA, the $pK_a$ value is low enough (~8.3) to allow a significant population of this form within the commonly observed physiological pH range. (B) Postulated binding of lincomycin in imitation of the bound substrate at the catalytic site of peptidyl transferase.

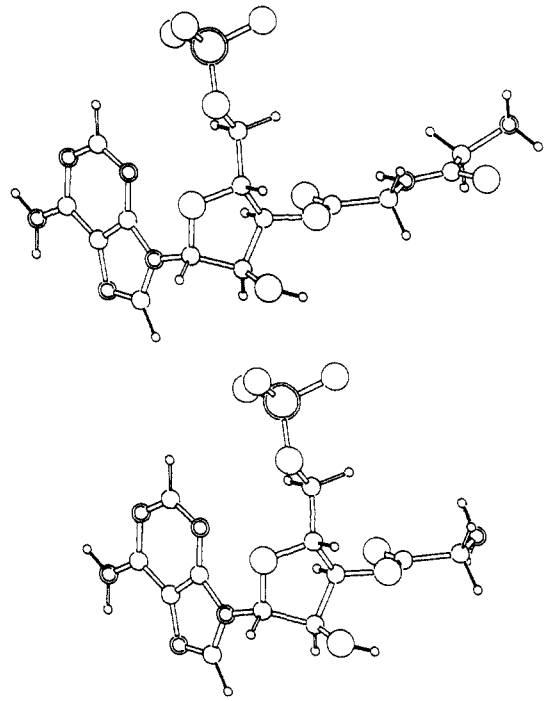


Figure 4. Top: A-Gly-Gly model for unbound P-site substrate of peptidyl transferase. Bottom: A-Gly model for unbound A-site substrate of peptidyl transferase.

quence employed in the model is given in the next paragraph.

The mechanism illustrated in (A) of Figure 3 provides a possible explanation of the involvement of the ribosome in catalyzing peptide bond formation. Although little is known about the entities which constitute the peptidyl transferase site, the essential features of the current model can be developed by making inferences from the structural characteristics of the natural substrate molecules. The ribosomal OH (or $NH_2$) group, assumed to be involved in binding at the catalytic center, would facilitate peptide bond formation through its ability to act simultaneously as a donor and acceptor of H atoms in hydrogen bonding. In the first diagram of Figure 3, the bound peptidyl tRNA (II) is portrayed on the left with the incoming aminoacyl tRNA (I) on the right. The optimal direction of approach is assumed to be controlled by the orientation of the p orbital on the carbonyl carbon, C(P1), and the lone-pair $sp^3$ hybrid orbital on the amino nitrogen, N(A3). Formation of the intermediate species III, shown in the middle diagram, requires rehybridization of C(P1) as the new C–N bond is created. Stabilization of the charge separation in III is accomplished by involvement of cations[32-34,‡] present in the system and the formation of a second hydrogen bond to the ribosomal OH ($NH_2$) group. Species III dissociates into the products shown in the third diagram, releasing both the stripped tRNA and the elongated peptidyl tRNA from the peptidyl transferase center in preparation for translocation. Although other mechanisms may be possible, this concerted nucleophilic substitution reaction merits consideration due to its simplicity and reasonable energetic requirements.

The interesting feature with regard to the 4-OH group of lincomycin is shown in (B) of Figure 3. The spatial relationship of the 4-hydroxyl to other key atoms in the molecule is almost precisely the same as that assumed by the amino group of aminoacyl tRNA in the hypothetical bound state (corresponding closely to species III) of the natural substrate. Thus, the 4-OH group could act as a surrogate for the terminal $NH_2$ group of aminoacyl tRNA in binding at the ribosome. According to this hypothesis, lincomycin slightly overlaps the A site and acts as a specific inhibitor of peptidyl transferase by direct blockage of the catalytic center.

Various calculations have been performed on molecules simulating the natural substrate in order to determine whether the key functional groups can indeed conform realistically to the spatial relationships of their assumed counterparts in lincomycin and chloramphenicol. Since the peptidyl tRNA moiety in the region of the peptidyl transferase catalytic center consists of the terminal adenosine nucleotide and a segment of the attached protein chain extending beyond the previously formed peptide linkage, a simple model for the P-site substrate which contains these essential features is 5′-adenylic acid 3′-(*N*-glycylglycinate). Similarly, the model for the A-site substrate may be taken as 5′-adenylic acid 3′-glycinate since it bears a strong resemblance to the terminal moiety of aminoacyl tRNA. Hereafter, the model compounds for the terminal moieties of aminoacyl tRNA and peptidyl tRNA will be designated A-Gly and A-Gly-Gly, respectively. The initial molecular geometries employed in the computations were devised by combining moieties taken from molecules with established conformations. Structural data for the 5′-adenylic acid portions of A-Gly and A-Gly-Gly were taken from the work of Lakshminarayanan and Sasisekharan.[35] In accordance with the findings of Sundaralingam and Jensen,[36] a C(3′)-endo conformation of the ribose ring was employed. Molecular geometries for glycine[37] and β-glycylglycine[38] were obtained from the results of X-ray crystallographic studies. The geometry of the ester linkage in 3′-*O*-acetyladenosine[39] was used as a model for combining these molecules to yield reasonable structures for A-Gly and A-Gly-Gly as illustrated in Figure 4.

‡The requirement of $NH_4^+$, $K^+$, and $Mg^{2+}$ in the process of ribosomal peptide bond formation has been noted by several investigators (ref 32–34). Apparently, $NH_4^+$ and $K^+$ act near the peptidyl transferase center to facilitate binding of the natural substrate and antibiotic inhibitors (ref 4-6, 33). The involvement of a cation in the model to stabilize the charge separation in species III is not necessarily related to the effects of $NH_4^+$ and $K^+$ on substrate binding; however, it does provide a rationale for the necessity of $Mg^{2+}$ which appears to affect catalysis of peptidyl transfer as well as substrate binding.

**Table I.** Relative Positions of Corresponding Atoms in the Peptidyl Transferase Substrate Model, Lincomycin, and Chloramphenicol[a]

| Peptidyl transferase substrate model | | | | Lincomycin | | | | | Chloramphenicol | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Atom[b] | x | y | z | Atom[b] | x | y | z | Δ[c] | Atom[b] | x | y | z | Δ[c] |
| O(P4) | 2.33 | 0.0 | 0.0 | O(amide) | 2.32 | 0.0 | 0.0 | 0.01 | O(amide) | 2.32 | 0.0 | 0.0 | 0.01 |
| C(P4) | 0.0 | 0.0 | 0.0 | C(amide) | 0.0 | 0.0 | 0.0 | 0.0 | C(amide) | 0.0 | 0.0 | 0.0 | 0.0 |
| N(P3) | −1.40 | 2.00 | 0.0 | N(amide) | −1.42 | 2.07 | 0.0 | 0.07 | N(amide) | −1.42 | 2.07 | 0.0 | 0.07 |
| O(1′) | 2.35 | 9.02 | −9.28 | O(2) | 2.71 | 9.14 | −9.11 | 0.41 | | | | | |
| O(3′) | 0.67 | 7.88 | −2.74 | O(1) | 1.15 | 8.02 | −2.41 | 0.61 | O(1) | 0.03 | 8.32 | −2.46 | 0.82 |
| O(5′)[d] | 0.17 | 5.14 | −10.98 | O(3)[d] | 2.80 | 3.76 | −8.80 | 3.68 | | | | | |
| | (2.18) | (3.68) | (−9.02) | | | | | (0.66) | | | | | |
| N(A3) | 3.95 | 3.78 | −3.42 | O(4) | 3.86 | 3.56 | −3.67 | 0.34 | | | | | |
| C(A2) | 4.05 | 1.36 | −5.00 | | | | | | | | | | |
| C(A1) | 6.78 | 0.48 | −5.36 | | | | | | | | | | |

[a] Coordinates expressed in atomic units (1 bohr = 0.529172 Å) with the amide carbon placed at the origin. [b] Atom designations in the substrate model, lincomycin, and chloramphenicol are given in the structural diagrams of Figure 1. [c] Δ is the difference between the spatial positions of corresponding atoms in the drug molecule and substrate model. [d] The data in parentheses pertaining to the relative positions of O(5′) and its lincomycin counterpart, O(3), were determined by application of the Waser procedure. In order to relieve steric strain in the model, the position of O(5′) was adjusted using quantum mechanical calculations as discussed in the text.

By employing the Waser[40] least-squares method in combination with quantum mechanical calculations, the key atoms of A-Gly-Gly were forced to assume a configuration in space as near as possible to the configuration of the corresponding atoms in lincomycin. In carrying out the Waser procedure, which uses interatomic distances as constraints to fit the desired molecular geometry, bond lengths and bond angles were fixed at the values in the initial structure of A-Gly-Gly by specifying the appropriate separations between all 1,2 and 1,3 atom pairs. Most torsional angles were also restricted by giving 1,4 distances in order to maintain the ring conformations. However, rotation about three bonds [C(4′)-C(5′), C(3′)-O(3′), and C(P2)-N(P3)] was allowed so that separations between key atoms could be adjusted to match the distances between corresponding atoms in the lincomycin X-ray structure.[27] Although the resulting conformation produced spatial relationships between key atoms which closely resembled those found in lincomycin, additional alterations were required to relieve severe crowding between a C(5′)-hydrogen and the carbonyl oxygen, O(P1). The orientation about the C(4′)-C(5′) bond was adjusted by means of quantum mechanical calculations using the *ab initio* molecular fragment method developed by Christoffersen, *et al.*[41] Moiety VI was employed in the computations to re-



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produce the major steric interactions experienced by the complete molecule in the spatial region of interest. A minimum in the calculated potential energy function for rotation about the (C4′)-C(5′) bond is obtained when ω [∠O(1′)-C(4′)-C(5′)-O(5′)] ≈ 21° so that O(5′) does not quite eclipse O(1′).

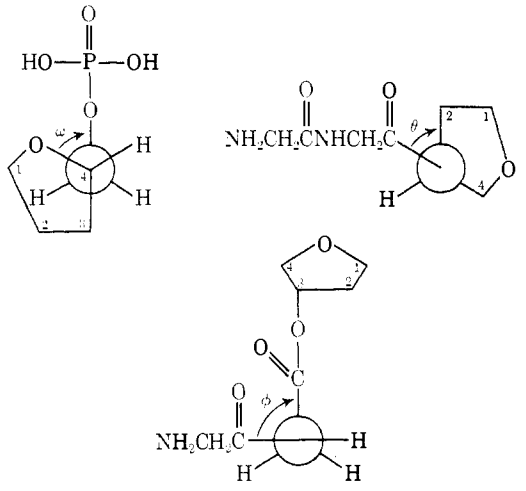
Table I lists the final coordinates of the principle atoms in the peptidyl transferase substrate model together with those of lincomycin and chloramphenicol. Although O(5′) is removed from the position of its lincomycin counterpart O(3) by 3.68 bohr, a possibility remains for the two atoms to form hydrogen bonds at the same ribosomal site. Since the spatial locations of other corresponding atoms in the substrate model and lincomycin differ by less than 0.75 bohr (~0.4 Å), the substrate and antibiotic molecules could engage the peptidyl transferase center in a remarkably similar manner. According to this model, effective binding at the peptidyl transferase center would require the peptidyl tRNA substrate to undergo a conformational change which may be described, for the most part, by variations in the three torsional angles (ω, θ, and φ) given in Table II. In subsequent discussion, the conformation assumed to be required for effective binding will be denoted the B form, while that of the isolated or "unbound" molecule will be designated as the U form.

Owing to the fact that lincomycin and chloramphenicol do not appear to significantly overlap the A site of peptidyl transferase, there is no direct way to predict the binding conformation of A-Gly as has been done for A-Gly-Gly. However, the mechanism described in Figure 3 provides several guidelines which aid in developing the peptidyl transferase substrate model to include portions of aminoacyl tRNA. First, atom N(A3) of the amino group in A-Gly must occupy the same region of space at the ribosome as its postulated counterpart, O(4), in lincomycin. Second, the nitrogen lone-pair orbital is to be directed along the line between N(A3) and C(P1) of A-Gly-Gly. Third, one of

**Table II.** Values Determined for the Torsional Angles $\omega$, $\theta$, and $\phi$ in Structures of A-Gly-Gly Corresponding to the "Unbound" (U) and "Binding" (B) Conformations of the Model[a]

| Conformation | $\theta$, deg | $\phi$, deg | $\omega$, deg |
|---|---|---|---|
| U | 120 | 180 | 63 |
| B | 198 | 98 | 21 |

[a]The definitions of $\omega$, $\theta$, and $\phi$ are



where those groups attached to the ribose ring of A-Gly-Gly which are unessential in specifying the angles have been omitted from the diagram.

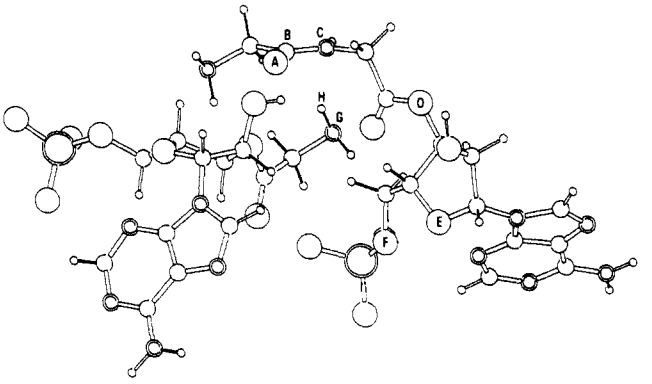
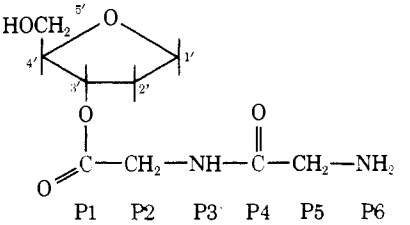


**Figure 5.** Substrate model for peptide bond formation showing the key atoms, A-H, in the terminal peptidyl tRNA moiety (A-Gly-Gly located in upper right) and the final aminoacyl tRNA moiety (A-Gly). The molecules are portrayed in the conformations and relative orientations required for the initial stage of peptide bond formation at the ribosome.



VII

the hydrogens of the amino group must be located in an exposed position near Q(3′) of the peptidyl tRNA moiety. Fourth, the aminoacyl tRNA molecule has to be oriented so that steric conflicts with peptidyl tRNA are minimized. The last restriction is especially important for amino acids other than glycine, which have bulky side chains. These four conditions permit the locations of N(A3), C(A2), and C(A1) to be specified within rather narrow limits relative to the peptidyl tRNA moiety. Hence, the coordinates of N(A3) were determined by the Waser procedure with distances from O(4) to other key atoms in lincomycin serving as the necessary constraints. Guidelines 2-4 then provided the necessary conditions to fix the positions of C(A1) and C(A2) as given in Table I. Fixation of the last three atoms in the amino acid chain still leaves a great deal of conformational freedom in the A-Gly structures.** However, the general orientations of the aminoacyl tRNA and peptidyl tRNA moieties relative to one another are quite clearly indicated, as shown in Figure 5.

By means of the molecular fragment SCF technique,[41] quantum mechanical calculations have been performed on the terminal peptidyl tRNA moiety, 3′-*O*-(*N*-glycylglycyl)-ribose†† (VII), in order to analyze various energetic and electronic aspects of the substrate model in greater detail. A summary of the molecular fragments and types of basis functions employed to describe VII is given in Table III. The computations were performed with a basis set of 85 functions, including eight p-type orbitals composed of two lobe functions and 77 single Gaussian orbitals representing inner shells, $\sigma$ bonds, and $\sigma$-type lone pairs. Tables IV and V contain lists of the nuclear coordinates used in the calculations for conformations U and B, respectively.

From the results of the computations, the energy required to force the molecule from the U into the B form is roughly 0.0477 Hartree (29.9 kcal/mol). Since the molecular fragment method often exaggerates conformational energy differences‡‡ and no relaxation of bond lengths or bond angles was permitted in determining the conformational changes, the calculated energy difference must be regarded as an upper limit. Based on results obtained from a model peptide system,[43] the energy change associated with variation of $\phi$ from 180 to 98.4° is estimated to be less than 5 kcal/mol. Hence, the changes in $\theta$ and $\omega$ are considered to be responsible for the major fraction of the energy difference between the U and B conformations. In the B form, significant nonbonded repulsive interactions occur between O(P1) and the C(5′)-methylene group. Such interactions could constitute part of the driving force to create the intermediate species III during peptidyl transfer since the change from $sp^2$ to $sp^3$ hybridization at C(P1) would yield a more favorable geometry with greater separation between O(P1) and the hydrogen atoms bonded to C(5′).

Many theoretical studies[44] have emphasized the importance of overlapping between the highest occupied molecular orbital (HOMO) of an electron donor and the lowest unoccupied orbital (LUMO) of an electron acceptor in determining the favorable position and spatial direction of a

**Although no definitive evidence is provided by the substrate model, the conformation of bound A-Gly is assumed to be different from that of the isolated molecule. Experimental results supporting this assumption were obtained by Nathans and Neidle[42a] as well as Symons, *et al.*,[42b] who found that the puromycin analog containing glycyl was ineffective as a peptidyl acceptor although puromycin itself with *p*-methoxy-L-phenylalanyl is active. Yathindra and Sundaralingam[42c] have performed potential energy calculations on the puromycin molecule which yield a favored conformation different from that of A-Gly with respect to rotation about the C(A1)-C(A2) bond. A C(A1)-C(A2) rotational angle within the range determined by these workers is compatible with the constraints of the model.

††The primed notation is retained for numbering the ribose ring atoms in species VII in order to facilitate discussion.

‡‡Molecular fragment SCF calculations of rotational barriers about various types of bonds have generally yielded magnitudes which are roughly 20-80% too high. Nevertheless, the calculations have proven to be useful for conformation studies since qualitative features of the rotational potential curve, such as overall shape and locations of extrema, have been consistently reproduced.

**Table III.** Molecular Fragment Data

| Fragment type[a,b] | FSGO type[c] | FSGO distance from "heavy" atom[b] | FSGO radius[b] |
|---|---|---|---|
| $CH_4$ ($sp^3$), $R$(CH) = 2.05982176 | b(CH) | 1.23379402 | 1.67251562 |
| | s(C) | 0.0 | 0.32784375 |
| $\cdot CH_3$ ($sp^2$), $R$(CH) = 1.78562447 | b(CH) | 1.13093139 | 1.51399487 |
| | $p_\pi$(C) | ±0.1 | 1.80394801 |
| | s(C) | 0.0 | 0.32682735 |
| NH ($sp^3$), $R$(NH) = 1.9313912 | b(NH) | 0.88573239 | 1.53557305 |
| | l(N) | 0.25630919 | 1.58812372 |
| | s(N) | 0.00101043[d] | 0.27735920 |
| $NH_3$ ($sp^2$), $R$(NH) = 1.93131910 | b(NH) | 0.75201903 | 1.39424495 |
| | $p_\pi$(N) | ±0.1 | 1.50625972 |
| | s(N) | 0.0 | 0.27684894 |
| $H_2O$ ($sp^3$),[e] $R$(OH) = 1.81415494 | b(OH) | 0.74365356 | 1.35682617 |
| | l(O) | 0.43956044 | 1.30568359 |
| | s(O) | 0.00077105[d] | 0.24051208 |
| $H_2O$ ($sp^2$), $R$(OH) = 1.81415494 | b(OH) | 0.79688650 | 1.37661071 |
| | $p_\pi$(O) | ±0.1 | 1.13699151 |
| | l(O) | 0.23857697 | 1.36874695 |
| | s(O) | 0.00083437[d] | 0.24089701 |
| $\cdot OH$ (sp), $R$(OH) = 1.54774058 | b(OH) | 0.76467773 | 1.23671871 |
| | $p_\pi$(O) | ±0.1 | 1.12242182 |
| | $p_l$(O) | ±0.1 | 1.19741696 |
| | l(O) | 0.21614258 | 1.28753780 |
| | s(O) | 0.00057129[d] | 0.24028227 |

[a]The symbols $XH_n$ and $\cdot XH_{n-1}$ indicate the chemical structures of the molecular fragments used to obtain optimized FSGO basis functions for describing large molecules. Arrangement of the FSGO about the central atom of the fragment simulates the hybridization state given in parentheses following the symbol. $R$(XH) is the X–H internuclear distance. [b]Distances expressed in atomic units (1 bohr = 0.529172 Å). [c]The convention for designating FSGO types is as follows: b(XY) = a bonding orbital located between atoms X and Y; l(X) = an $sp^n$ hybrid lone-pair orbital on atom X; $p_\pi$(X) = a p orbital on X oriented for maximal interaction in a $\pi$-bonding system; $p_l$(X) = a p orbital on X containing lone-pair electrons which have minimal involvement in $\pi$ or $\sigma$ bonds, and s(X) = an inner-shell orbital on atom X. [d]Distance measured along $C_n$ symmetry axis of the fragment toward the region where the H atoms are located. [e]Polarization of the lone-pair orbitals causes deviations from $sp^3$ hybridization. This is accounted for by varying the angle, ∠lOl, formed by lines from the center of the lone-pair orbitals to the oxygen nucleus. The value of ∠lOl depends on the value of ∠HOH according to the following approximate relationship: ∠lOl = 215.5629 − 1.083117∠HOH − 0.003312919∠HOH².

**Table IV.** Nuclear Coordinates of Peptidyl tRNA Moiety VII in Conformation U[a]

| Nucleus | X | Y | Z |
|---|---|---|---|
| H(5′–0) | −4.26649413 | 13.44589892 | −8.27571600 |
| O(5′) | −3.75286224 | 13.75876172 | −6.56415020 |
| C(5′) | −4.07800027 | 11.71454535 | −4.71133591 |
| H(5′–1) | −6.07066220 | 11.20870212 | −4.58372188 |
| H(5′–2) | −2.98821566 | 10.06886728 | −5.30038732 |
| C(4′) | −3.15159430 | 12.61481656 | −2.15862693 |
| H(4′) | −1.17207852 | 13.16826878 | −2.29307064 |
| O(1′) | −4.68983719 | 14.72744231 | −1.33465131 |
| C(1′) | −4.85526850 | 14.71314603 | 1.35686310 |
| H(1′–1) | −4.13269680 | 16.48418522 | 2.12117647 |
| H(1′–2) | −6.81271521 | 14.45908892 | 1.94569524 |
| C(2′) | −3.26066150 | 12.52366914 | 2.27049646 |
| H(2′) | −4.03430854 | 11.71934377 | 4.00179586 |
| O(2′) | −0.74225207 | 13.43858777 | 2.67491369 |
| H(2′–0) | 0.23397468 | 11.93525333 | 3.27284209 |
| C(3′) | −3.34980461 | 10.72168986 | 0.00000117 |
| H(3′) | −5.20603828 | 9.84863654 | 0.18718541 |
| O(3′) | −1.51450857 | 8.80570884 | 0.00000108 |
| C(P1) | −2.39417082 | 6.56669473 | 0.0 |
| O(P1) | −4.62093073 | 6.05022556 | 0.0 |
| C(P2) | −0.30256674 | 4.56667682 | 0.0 |
| H(P2–1) | 0.86225447 | 4.80643066 | −1.68183730 |
| H(P2–2) | 0.86225365 | 4.80642986 | 1.68183858 |
| N(P3) | −1.39614276 | 1.99298011 | 0.0 |
| H(P3) | −3.31689661 | 1.79124367 | 0.0 |
| C(P4) | 0.0 | 0.0 | 0.0 |
| O(P4) | 2.32782324 | 0.0 | 0.0 |
| C(P5) | −1.48572139 | −2.47463614 | 0.0 |
| H(P5–1) | −2.67200304 | −2.55845039 | 1.68183682 |
| H(P5–2) | −2.67200342 | −2.55845018 | −1.68183859 |
| N(P6) | 0.31213293 | −4.68985326 | 0.0 |
| H(P6–1) | 1.41384601 | −4.61671225 | 1.56148424 |
| H(P6–2) | 1.41384530 | −4.61671295 | −1.56148623 |

[a]Coordinates expressed in atomic units (1 bohr = 0.529172 Å).

chemical reaction. Therefore, it is interesting to note that the LUMO of structure VII, in both U and B forms, is an antibonding $\pi$ orbital localized in the region of the ester unit. Furthermore, $p_\pi$[C(P1)] (see footnote *c* in Table III for explanation of notation) is the site of highest density in the LUMO, as expected for the focus of nucleophilic attack. Since other calculations indicate that l[N(A3)] is the location of greatest electron density in the HOMO of the terminal aminoacyl tRNA moiety, the electronic requirements for the nucleophilic substitution reaction at C(P1) are apparently well satisfied. In the construct of the initial stage of interaction, shown in Figure 5, the spatial relationship between $p_\pi$[C(P1)] and l[N(A3)] is nearly ideal for maximal overlap since the N(A3)–C(P1) axis is almost perpendicular to the plane formed by O(3′), C(P1), O(P1), and C(P2). Thus, the geometric requirements for the reaction are also satisfied by the model.

Since an amide group similar to the one in VII is necessary for effective binding at the P site, it is interesting that the next-to-lowest unoccupied MO (NLUMO), the HOMO, and the next-to-highest occupied MO (NHOMO) exhibit significant density in the amide unit. The NLUMO is an antibonding $\pi$ orbital consisting of $p_\pi$ functions on N(P3), C(P4), and O(P4). In the B form of VII, the HOMO is essentially a nonbonding $\pi$ orbital with maximum density in $p_\pi$[N(P3)] and $p_\pi$[O(P4)]. The NHOMO in the B conformation is dominated by contributions from $p_l$[O(P4)] and l[N(P6)]. Aside from a switch in ordering, the two highest occupied molecular orbitals of the U conformation are very similar to those of the B conformation. On the basis of the MO energies exhibited by the NLUMO and HOMO in form B of VII, the amide group could act as either an electron acceptor or an electron donor in forming a charge-transfer complex.[45] Of course, the possibility of other types of binding interactions at the ribosome, such as a hydrogen bond involving O(P4), cannot be excluded from consideration.

As described in the companion paper,[28] a study of the electronic features associated with the amide unit of lincomycin has been performed using various model structures to portray the molecular environment. The highly reactive molecular orbitals localized in the amide units of species VII and the models for lincomycin free base exhibit strong similarities in both composition and energy. However, there are significant differences in energy between amide orbitals of comparable composition in VII and models of protonated (at the pyrrolidine nitrogen) lincomycin. These comparisons of electronic structure indicate that unprotonated lincomycin would resemble the natural substrate in binding to a greater degree than the protonated form. This finding may be significant in view of the experimental observations by Heman-Ackah and Garrett[46] which implicate lincomycin free base as the species responsible for antibacterial activity.

**Table V.** Nuclear Coordinates of Peptidyl tRNA Moiety VII in Conformation B[a]

| Nucleus | X | Y | Z |
|---|---|---|---|
| H(5′-0) | −0.87041334 | 3.93658138 | −11.85618413 |
| O(5′) | 0.16831028 | 5.14178652 | −10.98458091 |
| C(5′) | −0.03297037 | 5.27585502 | −8.21709688 |
| H(5′-1) | −1.96427869 | 5.75824089 | −7.68774223 |
| H(5′-2) | 0.46823131 | 3.45304681 | −7.39915102 |
| C(4′) | 1.75748726 | 7.28638821 | −7.24907973 |
| H(4′) | 3.47277441 | 6.40162196 | −6.52946646 |
| O(1′) | 2.34900646 | 9.02296636 | −9.28444551 |
| C(1′) | 2.78114514 | 11.48049381 | −8.26188151 |
| H(1′-1) | 4.67101138 | 12.13266688 | −8.75780455 |
| H(1′-2) | 1.38386951 | 12.80728824 | −8.98996404 |
| C(2′) | 2.53318941 | 11.24908541 | −5.42352297 |
| H(2′) | 1.79857729 | 12.98962117 | −4.60270282 |
| O(2′) | 4.98143596 | 10.66814502 | −4.41773300 |
| H(2′-0) | 4.72380740 | 10.53441896 | −2.55057111 |
| C(3′) | 0.74492178 | 8.98182176 | −5.15549257 |
| H(3′) | −1.09822595 | 9.80854466 | −5.55823389 |
| O(3′) | 0.66536257 | 7.87989591 | −2.74332171 |
| C(P1) | −0.26715131 | 5.66291351 | −2.67803779 |
| O(P1) | −1.04237254 | 4.52967941 | −4.50560797 |
| C(P2) | −0.30256714 | 4.56667663 | 0.0 |
| H(P2-1) | 1.62484838 | 4.48239964 | 0.72168745 |
| H(P2-2) | −1.43763383 | 5.78366281 | 1.21385088 |
| N(P3) | −1.39614310 | 1.99297925 | 0.0 |
| H(P3) | −3.31689676 | 1.79124261 | 0.0 |
| C(P4) | 0.0 | 0.0 | 0.0 |
| O(P4) | 2.32782387 | 0.0 | 0.0 |
| C(P5) | −1.48572023 | −2.47463666 | 0.0 |
| H(P5-1) | −2.67200226 | −2.55845119 | 1.68183660 |
| H(P5-2) | −2.67200160 | −2.55845048 | −1.68183881 |
| N(P6) | 0.31213483 | −4.68985384 | −0.00000148 |
| H(P6-1) | 1.41384725 | −4.61671313 | 1.56148350 |
| H(P6-2) | 1.41384690 | −4.61671260 | −1.56148695 |

[a] Coordinates expressed in atomic units (1 bohr = 0.529172 Å).

Analysis of the electronic structure of the ribose moiety in VII reveals that the lone-pair basis functions on O(1′), O(2′), O(3′), and O(5′) are dominant contributors to several of the higher occupied molecular orbitals. Since electrophiles may be preferentially directed toward sites of concentrated electron density in these high-energy orbitals, positively charged centers at the ribosome could participate in attractive interactions with the key oxygen atoms [especially O(1′) and O(5′)] of the peptidyl tRNA moiety. Hydrogen bonding is an example of the type of interaction which would serve to stabilize the oxygen lone-pair orbitals. Another possibility is the formation of a complex with a bound cation at the ribosome. Such an interaction could be the basis for the necessity of $K^+$ or $NH_4^+$ to facilitate binding of peptidyl tRNA and the antibiotics under consideration.[4-6,32-34]

## Discussion

In the preceding section, various structural correlations have been drawn between lincomycin, chloramphenicol, erythromycin, and the terminal moiety of peptidyl tRNA which serve to pinpoint key atoms and groups that could be involved in binding at the ribosome. As a result of the correlations, certain conformational requirements were specified for effective binding of the terminal peptidyl tRNA moiety. In addition, a reasonable mechanism for peptide bond formation at the ribosome has been suggested. Some ramifications of the model will be considered in this section.

Due to the high conformational energy associated with the B form of species VII, stabilization of the terminal peptidyl tRNA moiety in the postulated effective conformation requires involvement of several different functional groups if each binding interaction contributes an energy equivalent to that of a hydrogen bond, namely 2–8 kcal/mol. Although the A-Gly-Gly fragment contains various groups capable of such binding interactions, including the key atoms specified in the model, some of the stabilization energy would undoubtedly have to be provided by binding of the immediately preceding nucleotides in the tRNA chain. This conclusion agrees with the results obtained by Monro, *et al.*,[14,47] who found that A-(F-Met) and CA-(F-Met) are inactive as peptidyl donors, whereas CCA-(F-Met) is active.

In view of the foregoing considerations, effective binding of the natural substrate at the P site of the peptidyl transferase center may be considered to proceed in two steps. First, a relatively stable complex is formed between the ribosome and the final three nucleotides of peptidyl tRNA. In this initial stage of the interaction, the peptidyl moiety would remain in the favored U conformation. Second, the conformational change from the U to the B form occurs in the terminal moiety. Since the major readjustment of geometry takes place in the peptidyl fragment, the final three nucleotides of the tRNA carrier would be expected to retain the ribosomal attachments established initially. This step is likely to be rate determining in the overall reaction to create the peptide bond. Without the binding interactions provided by the first amide unit in the peptidyl moiety, the B conformation would be highly inaccessible, and peptide bond formation could not occur at a significant rate.

If the CCA-peptidyl fragment is able to bind at the P site of peptidyl transferase, the CCA-aminoacyl fragment will also fit the same site since the U forms (see Figure 4) of the terminal moieties are quite similar in the region of the ester linkage. However, some workers[19,48,49] have assumed that aminoacyl oligonucleotide fragments bind only in the A site since peptide bond formation generally does not occur with such fragments. As explained in the foregoing paragraph, this may be due to the inability of the terminal moiety to attain the effective conformation. Under certain conditions, phenylalanyl tRNA has been shown to act weakly as a "peptidyl" donor in the formation of *N*-phenylalanylphenylalanine[17] and phenylalanylpuromycin,[11] indicating that it does bind at the P site as well as the A site. On the basis of these considerations, the results of some binding studies using aminoacyl oligonucleotides as models for the A site substrate may have to be reinterpreted.

The model provides a simple, straightforward rationale for competitive binding of lincomycin, chloramphenicol, and erythromycin since corresponding key atoms in the drugs must occupy the same space at the ribosome. By using the key atoms to orient the antibiotic molecules relative to the natural substrate moieties portrayed in Figure 5, it is possible to visualize the degree of mutual overlap which would occur at the surface of the ribosome. Such a comparison indicates that portions of each drug molecule extend into the A site, although all, except one, of the key binding points revealed in this study are located in the P site. In the case of chloramphenicol, penetration of the A site is limited to a chlorine atom and a section of the *p*-nitrophenyl group. On the other hand, a large segment of the erythromycin macrolide ring, roughly encompassing C(5)–C(8), overlays the area where the terminus of aminoacyl tRNA is expected to bind. In lincomycin, O(4) lies in the region where N(A3) of aminoacyl tRNA would bind prior to peptide bond formation. This analysis indicates that all three antibiotics could affect binding of the natural substrate molecules at both the A and P sites to some degree. Many studies have been reported which support this conclusion.[17-20,48,49]

Although the model only assigns a definite role to a limited number of key atoms in binding at the ribosome, other functional groups in the natural substrate and antibiotic molecules may also be involved in the interactions. In the case of erythromycin, several other portions of the molecule have been implicated in binding,[6] but uncertainties in the molecular geometry preclude definite correlations with features of the natural substrate.§§ Of course, there is a possibility that some groups could participate in fortuitous attractive interactions for which the natural substrate provides no analog. However, without detailed knowledge of the features at the ribosomal binding site, the model cannot be extended to rationalize the participation of substituents other than the key atoms.

Barber and Waterworth[15] found that certain strains of *Staphylococcus aureus* which had become resistant to erythromycin also showed cross resistance toward lincomycin. Since the ribosomes of some resistant bacteria have exhibited a decreased ability to bind erythromycin,[50,51] the mutation giving rise to resistance may involve a subtle change in the ribosomal surface which blocks uptake of the drug but allows the natural substrate to bind. The cross resistance to lincomycin would naturally follow if the topographical modification of the ribosome were to occur within a region occupied by both drug molecules. Weisblum and coworkers[52] have suggested that N-methylation of adenine in a segment of 23S ribosomal RNA causes the resistance to erythromycin and lincomycin in *S. aureus*. Thus, a methyl may be the sterically perturbing group which prevents the drugs from binding. Since uptake of the natural substrate and chloramphenicol[16] proceeds normally in the mutant strains, the perturbing group must be located outside a critical volume filled by these species at the ribosome. As may be ascertained from the constraints of the model in fixing the relative positions and orientations of the molecules at the ribosome, the thiomethyl of lincomycin and 13-ethyl of erythromycin protrude into an otherwise unoccupied region of space beyond the critical volume. Therefore, these groups may possibly be responsible for the reduction in binding to the ribosomes of the mutant bacterial strains. Other groups in the two drug molecules may also satisfy the prescribed criteria but cannot be identified due to uncertainties in the erythromycin geometry.

According to the model, the three antibiotics under consideration occupy a relatively small region at the catalytic center of peptidyl transferase. Since the total size of the P site includes, at least, the area covered by the three terminal nucleotides, CCA, of tRNA as well as a segment of the attached peptidyl chain, the natural substrate may be able to bind in this location whether an antibiotic molecule is present or not. The peptidyl tRNA molecule could have sufficient flexibility to allow displacement of the terminal moiety without seriously disrupting the binding of the preceding nucleotides. A similar argument may be made with regard to substrate binding in the A site, which includes the area overlayed by two, or more, of the final nucleotides in aminoacyl tRNA. In general, the drug molecules would be expected to hinder substrate binding since several important points of attachment to the ribosome are blocked. However, enhanced substrate binding could occur if a relatively stable complex were formed involving the antibiotic, the natural substrate, and the ribosome. In any event, as long as the drug molecule is bound, peptidyl transfer cannot take place due to obstruction of the catalytic center. Thus, the dissimilar effects on substrate binding exhibited by lincomycin, chloramphenicol, and erythromycin are quite compatible with the proposed model and do not require the drugs to act through an allosteric mechanism.

Several workers[53-59] have investigated the effect of lincomycin, chloramphenicol, and erythromycin on the formation of polypeptides at the ribosome in systems designed to simulate the mechanism of natural protein synthesis. In most cases, the antibiotics were found to permit relatively uninhibited formation of dipeptides while preventing the synthesis of longer peptides to a marked extent. These results led Coutsogeorgopoulos[56] to suggest that the antibiotics must interfere with some step other than peptide bond formation *per se*. On the other hand, Mao and Robishaw[58] have suggested that erythromycin does inhibit peptide bond formation through an allosteric mechanism that preferentially blocks the transfer of long peptidyl fragments. Although the model presented in this study is at variance with these proposals, the experimental findings can nevertheless be explained by means of a straightforward kinetic argument which is consistent with the model.

In developing the kinetic argument, the process of polypeptide synthesis is considered only in general terms except for the steps designated by the model as most susceptible to the influence of the antibiotics under consideration. Thus, eq 1 expresses the binding of peptidyl tRNA, $P_i$, at the peptidyl transferase site of the 50S ribosomal subunit, $R_{50S}$, in preparation for creating the $i$th peptide bond. The resulting dissociable complex, $P_iR_{50S}$, involves the favored U form of the peptidyl tRNA terminus. Equation 2 represents the change to the reactive, high-energy B conformation of the peptidyl tRNA moiety. Several steps are implicit in eq 3, namely, binding of aminoacyl tRNA, $A_i$, at the ribosome; transference of the peptidyl fragment; and translocation of the elongated peptidyl tRNA, $P_{i+1}$, from the A to the P site of the ribosome, leaving only the terminal segment unattached at the locus of peptidyl

$$P_i + R_{50S} \underset{k_{-1i}}{\overset{k_{1i}}{\rightleftharpoons}} P_iR_{50S} \quad (1)$$

$$P_iR_{50S} \underset{k_{-2i}}{\overset{k_{2i}}{\rightleftharpoons}} P_i{}^*R_{50S} \quad (2)$$

$$P_i{}^*R_{50S} + A_i \xrightarrow{k_{3i}} P_{i+1} + R_{50S} \quad (3)$$

transferase. The action of the ribosome forces peptidyl transfer and translocation to be channeled in an irreversible direction. Furthermore, each of the steps in eq 3 should proceed rapidly because of the catalytic involvement of the ribosome. As a result, the effective rate constant, $k_{3i}$, for the series of steps is expected to be quite large, providing the proper conditions for ribosomal function prevail. Due to the large energy difference between the B and U forms of $P_i$, $k_{2i}$ should be very small in comparison to $k_{-2i}$ and $k_{3i}$. Hence, the reactive complex, $P_i{}^*R_{50S}$, would be present in extremely low concentration and the steady-state approximation may be used to obtain eq 4 for the rate at which $P_{i+1}$ is formed in the absence of an inhibitor.

$$r_{i+1}(0) = (d[P_{i+1}]/dt)_0 = k_{1i}k_{2i}k_{3i}[P_i][A_i][R_{50S}]/\{k_{-1i}k_{-2i} + (k_{-1i} + k_{2i})k_{3i}[A_i]\} \quad (4)$$

The action of a peptidyl transferase inhibitor, D, may

§§A possible correspondence may exist between O(9) of erythromycin and one of the oxygen atoms of the phosphate group in the P site substrate moiety A-Gly-Gly. This tentative correlation is based on geometric relationships exhibited by space-filling models of the molecules.

be treated most simply if the drug concentration is large enough to ensure occupation of every available ribosomal site. In this case peptidyl tRNA must bind according to eq 5, where due allowance is made for simultaneous at-

$$P_i + R_{50S}D \underset{k_{-4i}}{\overset{k_{4i}}{\rightleftharpoons}} P_iR_{50S}D \quad (5)$$

tachment of the antibiotic and substrate to the 50S subunit. The ternary complex, $P_iR_{50S}D$, may dissociate either in the manner described by the reverse reaction of eq 5 or by eq 6, in which the drug is assumed to be expelled irre-

$$P_iR_{50S}D \xrightarrow{k_{5i}} P_iR_{50S} + D \quad (6)$$

versibly since recombination would require intercalation of D between the tightly bound natural substrate and the ribosome. After removal of the antibiotic *via* eq 6, the sequence of events outlined in eq 2 and 3 can take place. If the steady-state approximation is employed, the rate at which $P_{i+1}$ is formed in the presence of D may be expressed as

$$r_{i+1}(D) = (d[P_{i+1}]/dt)_D = k_{2i}k_{3i}k_{4i}k_{5i}[P_i][A_i][R_{50S}D]/(k_{-4i} + k_{5i})\{k_{-1i}k_{-2i} + (k_{-1i} + k_{2i})k_{3i}[A_i]\} \quad (7)$$

It is instructive to compare the values of $r_{i+1}(0)$ and $r_{i+1}(D)$ just after completion of the induction period (at time $t \sim 0$) in two systems where $[P_{i+1}]$ is small and the concentrations of all other materials, except the drug, are identical. If the drug exerts a maximal effect by occupying the binding site of essentially every ribosome in the inhibited system, eq 4 and 7 may be used to make the comparison shown in eq 8. According to eq 8, the presence of the

$$[r_{i+1}(D)/r_{i+1}(0)]_{t\sim 0} \approx k_{4i}k_{5i}/k_{1i}(k_{-4i} + k_{5i}) \quad (8)$$

antibiotic in the system will retard the rate of reaction during the $i$th cycle of the chain elongation process if $k_{4i}k_{5i} < k_{1i}(k_{-4i} + k_{5i})$. In other words, the drug must strongly inhibit binding of $P_i$ and/or resist expulsion from the $P_iR_{50S}D$ complex. These conditions apparently hold when the peptidyl chain exceeds the length of a dipeptide. On the other hand, the drug may actually stimulate synthesis of $P_{i+1}$ if $k_{4i}k_{5i} > k_{1i}(k_{-4i} + k_{5i})$. This will occur in situations where the antibiotic enhances the binding of peptidyl tRNA and the resulting complex preferentially dissociates by eliminating the drug. As a general rule, these conditions seem to be satisfied when the nascent peptidyl fragments are short and erythromycin, or chloramphenicol, is present in the system.

The results of the simple kinetic argument indicate that protein synthesis should proceed through the leakage pathway outlined in eq 5 and 6 when lincomycin, chloramphenicol, or erythromycin is present in high concentration. Thus, the drugs are predicted to retard, but not actually stop, the production of protein in the bacterial cell. This conclusion is consistent with the observation that all three antibiotics are primarily bacteriostatic agents.[60] Of course, the drugs may be bacteriocidal in cases where the rate of protein synthesis is severely retarded.

## Conclusion

Although antibiotic mode of action studies have been extensively employed to discern the processes involved in protein synthesis, a lack of knowledge of the events occurring at the molecular level has often caused investigators to draw contradictory conclusions from the experimental results. The model developed in this study has been designed to resolve some of the conflicts concerning the effect of lincomycin, chloramphenicol, and erythromycin on peptidyl transferase activity by rationalizing the experimental observations from a molecular standpoint. As a result of correlations between the antibiotics and moieties of the natural substrate of peptidyl transferase, the model provides significant insight into the events associated with ribosomal peptide bond formation. Some of the distinctive features specified by the model are: (1) certain key functional groups in the antibiotics and the terminal moiety of peptidyl tRNA that enable the molecules to compete for a binding site at the peptidyl transferase center; (2) the high-energy conformation required for effective binding of the terminal peptidyl tRNA fragment; (3) the relative orientation necessary for effective interaction of the aminoacyl and peptidyl tRNA moieties in the vicinity of the catalytic site; and (4) a feasible mechanism for the catalytic involvement of the ribosome in peptide bond formation. Many similarities and differences in the effects of lincomycin, chloramphenicol, and erythromycin may be rationalized in terms of the model. There does not appear to be any need for postulating allosteric mechanisms to explain the differences in activity exhibited by the antibiotics. Although much experimental evidence supports the model, further tests are being conducted to ascertain the validity of the hypotheses used in its development.

**Acknowledgment.** The author expresses appreciation to T. E. Eble and B. Bannister of the Upjohn Co., as well as R. E. Christofferson of the University of Kansas, for many interesting discussions.

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# Microbial Transformations of Antitumor Compounds. 1. Conversion of Acronycine to 9-Hydroxyacronycine by *Cunninghamella echinulata*

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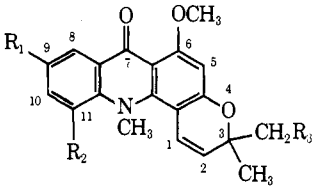
*Received January 24, 1974*

Microbial transformations have been employed as a method for producing quantities of a potentially active metabolite of the antitumor alkaloid acronycine. More than 40 microorganisms were screened for their abilities to convert acronycine to metabolites in small-scale fermentations. Ten cultures were found to accumulate one or more acronycine derivatives in culture media. In larger scale fermentations, *Cunninghamella echinulata* (NRRL 3655) converted acronycine to the phenolic metabolite, 9-hydroxyacronycine, in 30% yield. The extremely insoluble metabolite was acetylated and its structure established by spectral methods. The potential of microbial transformations as a tool for producing synthetically difficult derivatives of antitumor agents is discussed.

The ability of microorganisms to accomplish structural modifications of many types of organic compounds has been well documented.[1-4] With structurally complex compounds such as the steroids and alkaloids, selected microorganisms have been advantageously used to perform single, specific chemical transformations. To date, this technique has found widespread use, especially in the preparation of therapeutically important steroid derivatives. We have initiated a series of studies to establish microbial transformations as a convenient general method for obtaining novel and difficult-to-synthesize analogs of antitumor compounds.

This report is concerned with the antitumor alkaloid, acronycine (**1a**). Acronycine was chosen for study because it has exhibited broad antitumor activity in several experimental tumor systems,[5] and it is currently being studied in the clinic.[6] In addition, the alkaloid is readily available in large amounts from the bark of *Acronychia Baueri* Schott (Rutaceae),[5] and it has been synthesized.[7,8]




| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| acronycine **1a** | H | H | H |
| **1b** | OH | H | H |
| **1c** | H | OH | H |
| **1d** | OH | OH | H |
| **1e** | H | OH | OH |
| **1f** | $OCOCH_3$ | H | H |

**Microbial Transformations.** Preliminary small-scale fermentation experiments were conducted in order to obtain microorganisms capable of metabolizing acronycine. Organisms were selected on the basis of prior experi-