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클로람페니콜의 Conformation에 관한 분자궤도론적인 연구

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Molecular Orbital Consideration of the Conformation of Chloramphenicol

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요 약 Chloramphenicol分子에는 threo 및 erythro의 conformational isomer가 있고 biological activity가 아주 상이하여 threo(—)꼴만 biological activity가 있다.

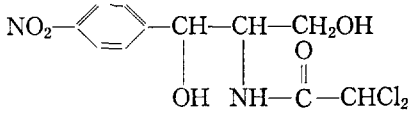
extended Hückel 이론을 사용 이들 分子들의 conformation을 계산하여 상기의 차이점의 이론적 설명을 꾀하였다.

Abstract Chloramphenicol molecule has an asymmetric carbon, so it has conformational isomer, namely, threo and erythro form. These two forms have great difference in biological activity, that is, only threo(—) form has biological activity.

Semiempirical quantum mechanical treatments are applied to these molecules to explain the difference, using the extended Hückel theory. The theoretical predictions for the preferred conformation are in good agreement with the experimental results.

Introduction

Chloramphenicol is an antibiotic produced by Streptomyces venezuelae[1] and it has the following structure.



Chloramphenicol possesses a fairly wide spectrum of antimicrobial activity.[2] The mode of action of chloramphenicol is that it inhibits protein synthesis on the 50s ribosome in bacteria and in cellfree systems. It acts primarily on the 50s ribosomes and supressed the peptidyl transferase activity which directly forms a peptide bond[3] Chloramphenicol is unique among natural compounds in that it contains a nitrobenzene moiety and is a derivatives of dichloroacetic acid. This molecule has two asymmetric carbon, so as synthetic chloramphenicol has two conformational isomer, namely, threo and erythro form, each conformational isomer has a levo and dextroratory optical active enantiomers.[4] Among those four isomers only the threo levo form is biologically active. The activity of this molecule not only depends upon to its conformational, but also the substiuent effect must be considered since

certain enzymes inactivate the chloramphenicol through reducing the nitro group or hydrolize the amide linkage so that dichloroacetic acid and the corresponding primary aliphatic amine are formed.[5]

We have undertaken a detailed study of the conformational differences between those four isomers by using the extended Hückel theory and correlate with the drug receptor events.

Calculation

In previous studies a semiemiprical molecula orbital theory has been successfully employed to predict molecular conformation. The method we have used is known as extended Hückel theory (EHT) developed by Hoffmann[6] The expansion of a molecular orbital as a linear combination of atomic orbitals yields

$$\Psi_i=\sum C_{ij}\phi_j$$

Upon minimization of the total energy, a set of Hückel equations arises

$$\sum_{n=1}^{j}(H_{ij}-ES_{ij})C_{ij}=0 \quad (i=1,2,\cdots\cdots n)$$

where Ψ is the molecular orbital wave function, H, the Hamiltonian operator, E, the energy, S, the overlap integral, and C, the orbital coefficient. The calculations are performed for the valence electrons in s and p orbitals using Slator orbitals as a basis set.

The input parameters consist of the Slater exponent and the Coulomb integral, approximated as the valence state ionization potential. All overlap integrals are considered. The resonance integrals are approximated by the equation

$$H_{ij}=0.5\,K(H_{ii}+H_{jj})\,S_{ij}$$

with K set at 1.75, the value prefrered by Hoffmann.[6]

The total electronic energy is computed to be the sum of the doubly occupied molecular orbitals.

$$E_t=2\sum E_i$$

Since all verlap integrals and all non-bonded interactions are explicitly considered, the computed total energy Et is sensitive to the molecular geometry. Thus, by varying the geometry and calculating the total energy, Et, for each, the minimum energy is found, which corresponds to the preferred conformation.

It is, therefore, necessary to include the three-dimensional coordinates of each atom in the conformation selected for calculation. These are derived by use of a vector program that locates each atom in space in Cartesian coordinates.

The bond lengths used were
C—H=1.09 N—N=1.04 O—H=0.94 C=O=1.23 C—O=1.43 C(SP^3)—C(SP^2)=1.50 C(SP^3)—C(SP^3)=1.54 C(SP^2)—N(SP^3)=1.32 C(SP^3)—N(SP^3)=1.47 and C—C(benzene)=1.40, all in angstroms.

All bond angles were assumed to be tetrahedral, except in the following:
C—C=0;120° N—C=0;120° H—N—;106.6° C—O—H;106° H—N—H;106.6° and ;120°
the input parameters are as follows:

Coulomb Integrals

Electron	Value (eV)	Electron	Value (ev)
C 2*s*	−21.4	C 2*p*	−11.4
N 2*s*	−26.0	N 2*p*	−13.4
O 2*s*	−35.3	O 2*p*	−17.76
H 2*s*	−13.6		

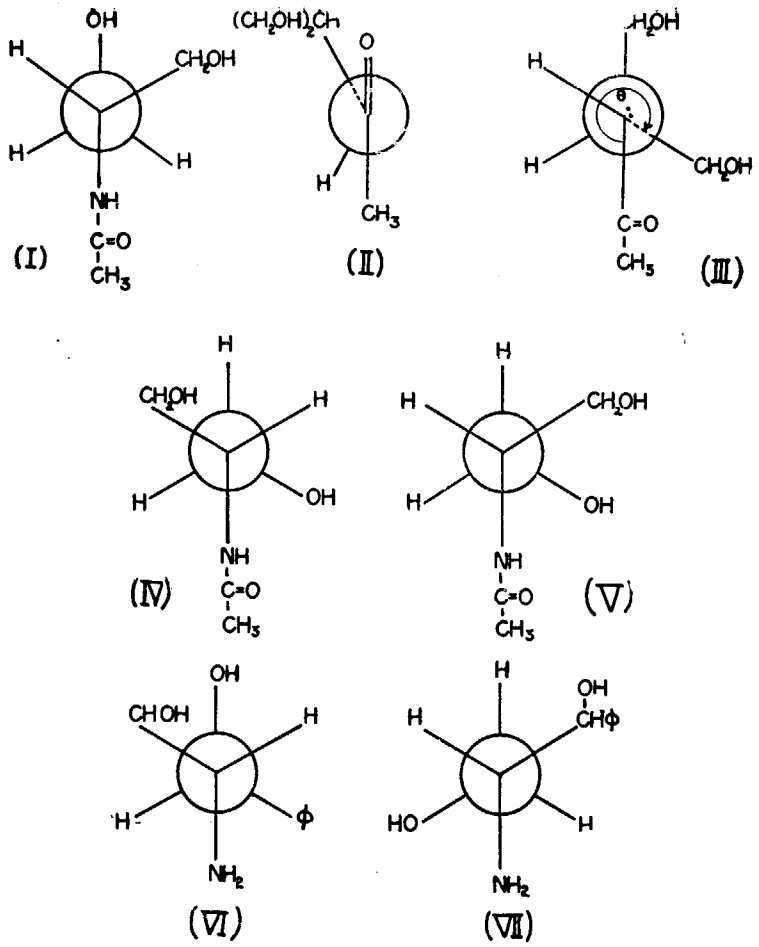


Fig. 1. *a.* Initial conformation of simplified chloramphenicol molecule type (A)
b. Initial conformation of simplified chloramphenicol molecule type (B)
c. Initial conformation of chloramphenicol in erythro Form
d. Initial conformation of chloramphenicol in threo form

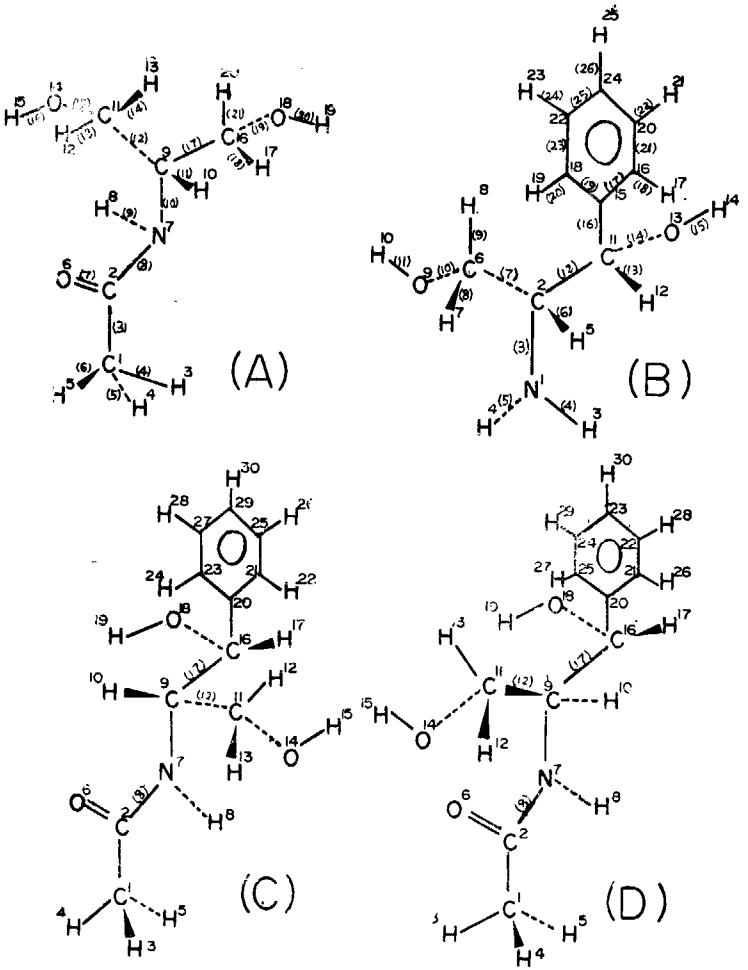


Fig. 2. A Series of rotation about each axis
(I)—(V) Simplified chloramphenicol molecule in form (A)
(VI)—(VII) Simplified chloramphenicol molecule in form (B)
(VIII)—(X) Simplified chloramphenicol molecule in erythro form
(XI)—(XIII) Simplified chloramphenicol molecule in threo form

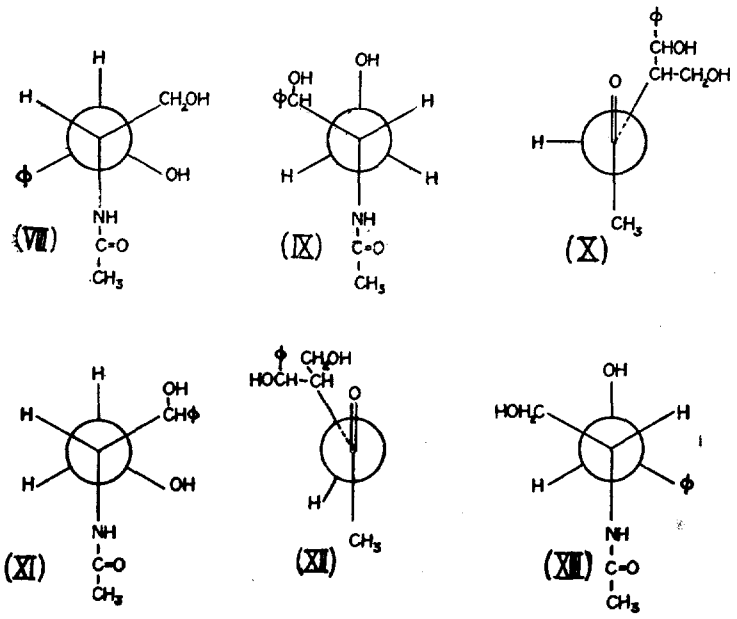


Fig. 2. Continued

Slater Exponents

Atom	Value
C	1.625
N	1.950
O	2.275
H	1.000

Results and Discussion

Four separate series of calculations were performed on the chloramphenicol in two simplified, erythro and threo forms. These simplified forms were introduced to predict the preferred conformation and to simplify the calculation of the total energy of chloramphenicol molecule.

The simplified one, in which Cl and $-NO_2$ were replaced by hydrogen atom respectively, is examined firstly. Rotations about 4 axes, 12th, 8th, 17th and axis in *Fig.* 1 (*A*) consecutively, were considered and individual calculations of total energy were made for each rotamer. The most stable calculated conformations about each rotation are expressed in *Fig.* 2 (I)—(V).

A calculated energy difference between θ—60° and θ—300° is 0.674 Kcal/mole in the rotation about the 10th axis. From this face, the rotation about this axis could be abbreviated in next series of calculations, if the value of angle would be fixed as θ=300° in (III). The second series of calculations were made on the simplified one, in which acetyl, $CH_3\overset{\overset{O}{\|}}{C}-$, and nitro, NO_2-, segments were substituted by hydrogen atom respectively.

In this molecule, there was an asymmetric carbon and the effect of phenyl group on conformation was examined. The phenyl segment

Table 1. Distances between specified atoms (Å unit)

	H(4)-O(6)	H(4)-N(7)	H(4)-O(14)	H(4)-O(18)	H(4)-H(30)	O(6)-N(7)	O(6)-O(14)
Threo form	2.519	3.359	4.686	6.754	8.616	2.209	3.794
Erythro form	2.519	3.359	5.676	5.946	8.616	2.209	3.794

O(6)-O(18)	O(6)-H(30)	N(7)-O(14)	N(7)-O(18)	N(7)-H(30)	O(14)-O(18)	O(14)-H(30)	O(18)-H(30)
4.746	7.234	2.855	3.709	5.945	3.432	8.043	6.020
4.746	7.234	3.709	2.855	5.945	3.432	8.043	6.020

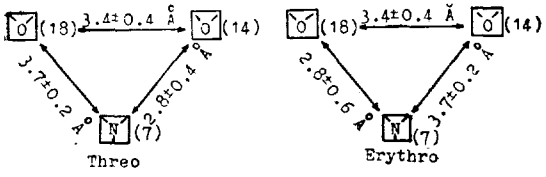
was found to be in a straight line to hydroxyl group in the calculated preferred conformation.

Final two series of calculations were carried out on the chloramphenicol molecules in erythro and threo forms, where Cl and NO_2 segments were replaced by hydrogen atom respectively. Stable conformations could be predicted from the previous instances and preferred ones are depicted in *Fig.* 2 (VIII)—(X) and (XI)—(XIII) conformation.

Comparison of the distance between important atoms for two forms in minimal energy conformations is as follows:

As it can be seen in *Table* 1, the distances separating the H(30) atom, which is replaced for nitro moiety, or H(4) atom, which is simplified for Cl atom, from any other atoms in the threo form is exactly coincide with those of the erythro form, except those from the O(14), O(18) and N(7) atom.

Only the differences between threo and erythro form are due to the distances of O(14)—O(18), O(14)—N(7) and O(18)—N.(7). Important relationships in intermolecular distance between erythro and threo forms were found from the result of calculating the preferred conformations. Triangles among the atoms N(7), O(14) and O(18) might be drawn



They were congruous in geometry but very different in chemical nature. The dissimilarity in biological activiy may arise from this difference in intermolecular distance between N(7)—O(14) and N(7)—O(18) of both forms.

In the energy minimum conformation of chloramphenicol, it was found that NO_2 moiety and dichloroacetyl group has a same conformational arrangement in threo and erythro form. These results indicate that NO_2 and dichloroacetyl groups might play important role in the biological activity of chloramphenicol throhug the charge density in the molecule rather than its conformational effect. For a further justification, NO_2 moiety might be replaced with $-SO_2CH_3$ group without losing its biological activity, which is known as thiamphenicol.

Acknowledgements

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