STRUCTURE-ACTIVITY CORRELATIONS IN THE CEPHALOSPORIN C SERIES USING EXTENDED HÜCKEL THEORY AND CNDO/2

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(Received for publication December 11, 1972)

Using two different molecular orbital methods which treat all valence electrons, namely extended HÜCKEL theory and CNDO/2, a correlation has been found between the biological activity of cephalosporin derivatives and certain aspects of the electronic structure of the β-lactam ring. Electron populations of the carbonyl carbon and bond strength indices of both the carbonyl carbon-nitrogen bond and the carbonyl carbon-oxygen bond in a number of cephalosporin derivatives are correlated with the inhibition of seven strains of gram-negative bacteria by the cephalosporin derivatives. The agreement is more consistent using CNDO/2, which accounts better for long-range inductive effects. It has been found that side chains on the six-membered ring having a positive inductive effect corresponding to the withdrawal of electrons enhance biological activity.

Numerous attempts have been made to modify the molecular structure of penicillin G and cephalosporin C in order to increase their biological activity. For the most part, these attempts have been programs of experimental chemical modifications and testing. Fewer attempts have been made to predict active compounds through an understanding of necessary basic physical or chemical properties. As an example of the latter, recently HANSCH[1] has been able to correlate the nature of a number of side chains at position R with the biological activity of the corresponding penicillin (**I**). This was done using an empirical relationship which makes use of an actanol-water partition function, π, characteristic of the side chain. Thus, the transport properties of the particular side chain R have been shown to be an important factor in the biological activity of these substances.

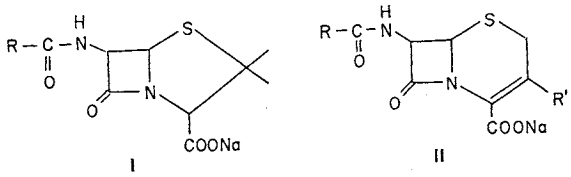
This paper represents a preliminary investigation of the possibility of treating structure-activity relationships within a series of cephalosporins with quantum chemical techniques. In this first paper we are interested in modifying the R′ side chain of a cephalosporin (**II**). While such modified side chains may also have a number of properties affecting the biological activity of the molecule, we attempt here to single out only certain properties describably in terms of current quantum chemical techniques, and correlate these with biological activity. Since the possible side chains are not generally aromatic, it is necessary to go beyond the usual π-electron molecular orbital methods and instead treat all the valence electrons. Two recently developed methods for this are used here, namely extended HÜCKEL theory (EHT)[2] and Complete Neglect of Differential Overlap (CNDO/2).[3]

Biological Action and Significant Molecular Properties

In order to determine which properties might be pertinent with regard to biological activity, we first consider a suggested mechanism of biological action of a cephalosporin and a penicillin.[4]

According to this mechanism, cephalosporins, like penicillins, are biologically active against bacteria due to their interference with the cell wall building process of the organism. At one stage in the process, cross-linking of linear peptidoglycan strands occurs. This cross-linking is a transpeptidation in which two linear peptidoglycan strands interact to form an interpeptide cross bridge and to eliminate D-alanine. An enzyme, transpeptidase, ordinarily brings about cross-linking by first combining with the D-alanyl D-alanine end of the chain and eliminating D-alanine. The acylated enzyme then reacts with a second chain. Since penicillin and cephalosporin are believed to be structural analogs of the D-alanyl D-alanine end of the pentapeptide chain and have a reactive β-lactam, they permanently acylate the transpeptidase and thereby inactivate it.

Fig. 1.



The above mechanism suggests that the biological activity is related to the reactivity of the β-lactam C-N bond. Ideally, we should calculate for each cephalosporin derivative the free energy change in going from the ground state system, which is the enzyme plus the cephalosporin derivative, to the transition state complex. Due mainly to a lack of enzyme structure information, we consider factors affecting the reactivity of the β-lactam bond under some possible conditions of hydrolysis.

In one possible mechanism of hydrolysis the rate determining step is the attack of a nucleophilic agent on the carbonyl carbon of the lactam ring. A second possible mechanism could be one in which the rate determining step is the rupture of the β-lactam C-N bond. We consider both mechanisms here since the actual mode of participation of the enzyme is unknown; likewise, the more representative mechanism is also unknown. Not considered is a mechanism having as a primary slow step an electrophilic attack on nitrogen or oxygen, since examples of this, such as protonations, are generally fast.

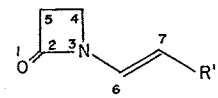
We now wish to compare relative reactivities of a series of similar compounds by examining their calculated ground state properties alone. Molecular orbital theory is used to determine such properties as charge densities, overlap populations, and bond energies. We assume that, other things being equal, the carbonyl carbon atom of lesser electronic charge density is more prone to nucleophilic attack within our series, and that the C-N bond having the highest overlap population is the stronger and less reactive bond. These assumptions find some justification in much of the work done on π-electron systems.[5] However, it must be emphasized that the presumed meanings of charge densities and overlap populations stated above, as well as the relevance of the proposed chemical mechanisms, are not actually necessary for our conclusions but serve as a useful guide to suggest parameters for our correlations.

Description of Calculations

Due to computer size limitations and degree of sophistication of the molecular orbital methods used here, it was impossible to treat the entire cephalosporin molecule. It is assumed that the neglected portions of the molecule would influence the interaction of the side chain R′ with the β-lactam moiety in a generally constant manner for the whole series, provided the series differs

among its members by only the nature of the side chain R′. Therefore the structure **III** was the subject of theoretical investigation. The conformations chosen, where several choices were possible, were the ones most likely to be a portion of the stable cephalosporin derivative.

Fig. 2.



III

Bond lengths and angles in the β-lactam ring were taken from CROWFOOT's structure determination of penicillin.[6]* In the remaining portion of the molecule, standard bond lengths[7] were used where possible. The C_6-N bond was bent out of the plane of the ring so that angle C_6-N-C_4 and angle C_6-N-C_2 were both 120°. The atoms N, C_6, C_7 and C_8 lie in the same plane. Angles where C_6 and C_7 form vertices are all 120°. Other bond lengths are C_6-N, 1.39 A; C_6-C_7, 1.34 A; C_7-C_8, 1.51 A; C_6-H and C_7-H, 1.07 A; C_8-H, 1.09 A. A case can be made for using a set of standard bond lengths and angles. Bond lengths and angles are determined by the electronic structure rather than the other way around, and differences in electronic structure between molecules show up better if certain geometrical features are kept constant.[8]

Reactivity Indices

For the extended HÜCKEL theory treatment, the atomic charge densities were computed in the usual manner.[2] We use the MULLIKEN gross atomic population to be an indication of the charge density on an atom, and the overlap population is taken to be an indication of bond strength.[9]

For the CNDO/2 method, the atomic charge densities were computed in the usual manner.[8] The interaction energies between atoms is more complicated than in the case of extended HÜCKEL theory due to the explicit inclusion of electron-electron interactions. This total interaction energy

Table 1[a]

R′	EHT, π			EHT, Total			CNDO/2			Biological activity[b]						
	C*	CN**	CO**	C*	CN**	CO**	C*	CN*	CO*							
CH_3	.5652	.1445	.1741	2.8951	.8452	.8203	3.5961	−1.0742	−1.4496	>50	>50	>50	16	>50	>50	>50
CH_2NH_2	.5628	.1430	.1747	2.8917	.8428	.8212	3.5952	−1.0743	−1.4495							
CH_2OH	.5618	.1424	.1750	2.8905	.8418	.8215	3.5952	−1.0724	1.5503	98	134	89	9	18	16	62
CH_2OAc	.5618	.1424	.1750	2.8904	.8416	.8216	3.5949	−1.0709	−1.5509	20	6	6	1	8	20	7
CHO	.5554	.1365	.1770	2.8749	.8275	.8257	3.5920	−1.0660	−1.5526							
CH_2Pr^+	.5650	.1444	.1740	2.8950	.8454	.8203	3.5926	−1.0545	−1.5569	7	6	4.8	8	6	26	5
$CH_2NH_3^+$	.5649	.1444	.1742	2.8950	.8454	.8204	3.5907	−1.0471	−1.5598							

* charge density. ** overlap population. * bond energy.

[a] R=2-thienyl. [b] =relative *weights* necessary to inhibit 7 strains of gram-negative bacteria in gradient plate test.

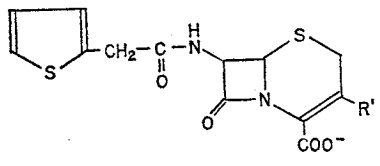
* HODHKIN, D.C.; C.W. BUNN, B.W. ROGERS-LOW, and A. TURNER-JONES in "Chemistry of penicillin," Princeton University Press, Princeton, N.J., 1949, p. 310. While the structure of cephalosoprin C has been determined, it was not felt that the accuracy at that stage was great enough to warrant using this information in constructing the test molecule, (III). The use of refined bond lengths and angles in the test molecule instead of standard ones should at any rate have little effect on the differences among derivatives and would not upset the order found. This was found to be true by actual calculation in the case of the most uncertain parameter of all — the angle between the side chain and the β-lactam ring. For all but the last two derivatives in Table 1, calculations were carried out, keeping the side chain planar with the ring. This extreme deviation did not affect the order of calculated activity of the compounds.

V_{AB} for the interaction of the electronic charge density and nucleus of atom A with the charge density and nucleus of atom B has already been discussed.[3]

Results of the Structure-Activity Correlation

The results of overlap population calculations for the C-N bond separated into σ and π contributions together with the carbonyl carbon charge densities are given in Table 1. The choice of compounds selected was dictated primarily out of simplicity, limitations of the computer programs, and in some cases, availability of experimental data. The compounds for which experimental data was available all had the general structure **IV.**

Fig. 3.



IV

All were available as the sodium salt except the pyridinium derivative, cephaloridine, which exists as the betaine. It can be seen that the CNDO/2 method, which considers electrostatic interactions explicitly, and therefore correctly accounts for charged species, indicates a high degree of activity of cephaloridine. This is in line with the idea that the high activity of this compound is partially due to a heightened degree of reactive opening of the β-lactam ring. The agreement using EHT is not good but EHT does not always make correct predictions for charged species.[10] No theoretical justification of calculated binding energies has been given for charged species, as has been done for neutral species.[11] In general, it may be said that where biological data are available, the order of activity parallels the calculated overlap populations of the C-N bonds and the lowered carbonyl carbon electronic charge density, due to side chains which can withdraw electrons. As a corollary, a cephalosporin that contains any side chain carrying a formal positive charge should be an active acylating agent, other things being equal. A drawback to the usefulness of such a rule is that a weakened β-lactam bond may in certain cases give rise to compound instability.

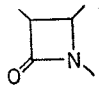
As stated above, other aspects of the activity have not been considered. The lipid solubility of the side chain R is quite important and not considered here. Likewise, the lipid solubility of the side chain R′ that is being changed in our series has not been considered.

The series of compounds treated here are admittedly limited in number. As mentioned in the introduction, however, this work represents a beginning of a program in which a larger series of compounds is being investigated, and all atoms in the parent system are being included. Comparisons between several parent systems such as 7-methoxy cephem, 7-methyl cephem and cephem will be made.

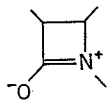
Correlation of β-lactam Carbonyl Stretching Frequency and Biological Activity

The stronger the bond between a given pair of atoms, the larger the force constant is for the bond stretching vibration and hence the higher the observed infrared frequency. Carbonyl bond orders and infrared absorption frequencies have been carrelated before usieg HÜCKEL theory.[12a,b] Since bond orders would be expected to correlate with bond strengths, the I. R. carbonyl stretching frequency is related to the biological activity. This relationship was first pointed out by MORIN.[13] A simple explanation for the inverse correlation between the C-O bond overlap population and the C-N bond overlap population is that, in terms of valence bond theory, the β-lactam which is

represented by the structure



will contain contributions from



depending on the nature of the substituents, so that any factor strengthening the C-N bond by giving it double bond character, weakens the C-O bond by removing double bond character and *vice versa*.

Acknowledgement

I wish to thank Dr. JOHN OTT of Eli Lilly and Company for making his biological activity data available. I also wish to thank MAX M. MARSH, Dr. MARVIN GORMAN, and Dr. E. H. FLYNN for reading the manuscript and making a number of important suggestions, and Dr. R. MORIN for some helpful discussions. The cooperation of the Indiana University Computation Center is gratefully acknowledged.

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