CORRELATIONS BETWEEN CNDO/2 CHARGE DISTRIBUTION AND ^{13}C NMR CHEMICAL SHIFT IN 7-ACYLAMINO SIDE CHAINS OF CEPHALOSPORINS†

BERNARD J. GRAVES and DONALD B. BOYD*

Lilly Research Laboratories, Eli Lilly and Company,
Indianapolis, Indiana 46285, U.S.A.

KENNY B. LIPKOWITZ

Department of Chemistry, Indiana University-Purdue University at Indianapolis,
Indianapolis, Indiana 46223, U.S.A.

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Molecular orbital calculations by the CNDO/2D method yield charge distributions which correlate well with the observed ^{13}C NMR chemical shift for the amide carbon of acylamino side chains of cephalosporins. Acyl groups that withdraw electrons from the amide C–N bond and concomitantly make the amide nitrogen more negatively charged increase the chemical shift. The trends are related to the degree of amide resonance. No direct correlation was found between the chemical shift of the amide carbon and the antibacterial activity of the cephalosporins.

Solution of the SCHRÖDINGER equation can, in principle, lead to detailed information about molecular properties. The insight gained from quantum chemical studies can be especially enlightening when used in conjunction with experiment. As part of a long term analysis of β-lactam antibiotics, we have been exploring their structural and physico-chemical properties in order to identify those that are important to the biological activity of the compounds[1,2]. In this paper we present our findings which correlate electronic properties of the acylamino side chain at position 7 of cephalosporins with ^{13}C NMR chemical shifts.

NISHIKAWA and TORI established the existence of linear relationships involving the ^{13}C NMR chemical shift of the amide carbon of the acylamino substituent of cephalosporins, δ(CONH)[3]. For instance, they found rough correlations of δ(CONH) with ^{13}C NMR chemical shifts for the β-lactam carbonyl carbon, δ(C-8), and with the difference in shifts of the ethylenic carbons of the cephem nucleus, $\Delta\delta$(4-3). The latter quantity has been shown to be relatable to Gram-negative *in vitro* antibacterial activity[4] and various theoretical and measured physico-chemical properties of cephalosporins[1,5,6]. Also, NISHIKAWA and TORI reported that δ(CONH) is proportional to the base-catalyzed rate of β-lactam hydrolysis of cephalosporins[3]. A shift of δ(CONH) to lower magnetic field parallels an increase in β-lactam reactivity. In addition, δ(CONH) is very roughly correlated to the inductive substituent constant δ_I of the acyl groups, suggesting that more electronegative groups tend to shift the amide carbon resonance to lower field (higher δ)[3].

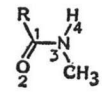
Methodology

This study involved computing three-dimensional atomic coordinates for a set of acylamino structures, using the atomic coordinates to calculate molecular orbitals (MO) and charge distributions for the structures, and then statistically analyzing the calculated and experimental data to uncover correlations.

The atomic coordinates were generated as described below using a computer program called

† Part 16 of the series "Electronic structures of cephalosporins and penicillins". See ref 1 for Part 15.

Table 1. Acyl monomethyl amide model structures.



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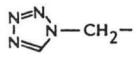
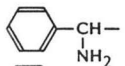
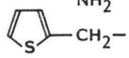
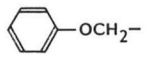
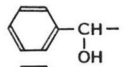
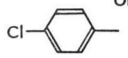
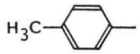
1	
2	
3	
4	
5	
6	
7	
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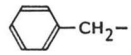
9	
10	CH_3–
11	H–
12	$HOOCCH(CH_2)_3$– 

Table 2. CNDO/2D charge distribution quantities and their correlation with δ(CONH) for all twelve structures of Table 1 and for the nine aryl-containing structures.

	r (n=12)	r (n=9)
Net atomic charges		
$Q(C_1)$	0.35	0.30
$Q(O_2)$	−0.43	0.11
$Q(N_3)$	−0.85	−0.92
$Q(H_4)$	−0.02	0.80
Super-delocalizabilities		
$S_E(C_1)$	−0.62	−0.21
$S_E(O_2)$	−0.37	0.14
$S_E(N_3)$	−0.72	−0.49
$S_E(H_4)$	0.15	0.55
$S_N(C_1)$	−0.75	−0.46
$S_N(C_2)$	−0.47	−0.63
$S_N(N_3)$	−0.69	−0.66
$S_N(H_4)$	−0.59	−0.67
Overlap populations for bonds		
$n(C_1=O_2)$	−0.68	−0.25
$n(C_1-N_3)$	−0.85	−0.97
$n(N_3-H_4)$	0.44	0.97
$2p_\pi$ Orbital population		
$N(C_1 2p_\pi)$	−0.63	−0.90
$N(O_2 2p_\pi)$	0.84	0.86
$N(N_3 2p_\pi)$	0.79	0.88

GEOM[7]. The molecular orbital calculations were obtained in the CNDO/2 approximation[8,9] because of its ability to reasonably describe long range inductive effects[10]. The MO coefficients were subjected to a deorthogonalization transformation, and the resulting CNDO/2D MOs were used to do a MULLIKEN population analysis[11~13]. The GEOM and CNDO/2D calculations were done in double precision using both IBM 3083 and VAX 11/780 computers. Regression analysis[14,15] was done using a program called STATPACK (R. Houchard, Western Michigan University, Kalamazoo, Michigan, U.S.A., 1974) running on a DEC 10 computer.

^{13}C NMR spectral data are available for twenty cephalosporins[3,16]. The compounds fall into two series: those with a methyl substituent at the 3-position of the cephem ring and those with acetoxymethyl at this position. The δ (CONH) values (ranging from 168 to 176 ppm) for compounds with 7-position side chains common to the two series are very similar. In fact, for the six acylamino groups appearing in both series, regression analysis gives

$$\delta(\text{CONH};\ 3\text{-}CH_2OAc) = (0.970 \pm 0.051)\ \delta(\text{CONH};\ 3\text{-}CH_3) + 5.1 \quad (1)$$

$$n=6,\ r=0.9945,\ r^2=0.989,\ s=0.34,\ P=0.0000$$

Per the usual notation, n is the number of observations, r^2 is the coefficient of determination (the amount of variance explained by the regression), s is the standard error of the estimate, and P is the probability that the null hypothesis is satisfied ($P=1$ means the correlation is purely by chance). Note that the slope of the regression line in Equation (1) is close to 1.0, and that the correlation coefficient r is very close to 1.0. Hence it is justified in this work to use an average of the reported[3] δ(CONH) values for each acylamino group.

The effect of the rest of the antibiotic molecule on the acylamino group chemical shift is relatively constant as demonstrated by the above equation. It is therefore adequate to replace the cephem nucleus by simply a methyl group for purposes of computing charge distributions. In other words, the trends in the predicted charge distribution should be the same regardless of whether the calculations are done on

the full cephalosporin or the simple model shown in Table 1. By doing calculations on a model with a methyl group in place of the attachment to the β-lactam ring, the computer times are greatly reduced.

Calculations were made on twelve acyl groups R from ref 3 (Table 1). A phthalimido derivative, for which data are given in ref 3, does not strictly fit the structural series in the present paper and was excluded.

The atomic coordinates for the twelve structures in Table 1 were obtained by combining available X-ray crystallographic data and standard bond lengths and bond angles. The latter were taken from a frequently used set[17]. An obvious source of coordinates for the tetrazolylacetyl **1** is cefazolin, but due to the low resolution of that determination[18], the model structure was constructed from the methyltetrazole coordinates of the 3-position side chain of a latamoxef (moxalactam) salt[19]. The phenylglycyl structure **2** was derived from amoxicillin[20] because the published coordinates for ampicillin[21] contain errors. Thienylacetyl coordinates were obtained from molecule 2 of ref 22, rather than from cephalothin, which was not as well resolved[23]. Crystalline state coordinates for the α-aminoadipyl model **12** were obtained from the LL isomer of diaminopimelic acid[24]. In addition, several conformers and ionization states of **12** were built up from standard geometrical data[17]. The structures of **4**~**7** and **9** were constructed by placing atoms with standard bond lengths and angles onto crystalline phenyl ring coordinates[20]. Atomic coordinates for **8**, **10** and **11** were constructed using all standard geometrical data[17].

Calculations were repeated on several of the structures using different geometries. It was found that none of the geometrical assumptions had a major effect on the final correlations that were obtained.

Eighteen different theoretically computed quantities (Table 2) for structures **1**~**12** were used to search for correlations with δ(CONH) values[3]. The quantities were all obtained from the CNDO/2D MOs and pertain to the amide atoms numbered in Table 1. Previous work has shown that the net atomic charges (*i.e.*, nuclear core charge minus electronic population) computed by CNDO/2D and CNDO/2 parallel each other[2]. Mulliken overlap populations[13] are defined within the context of the CNDO/2D method (but not CNDO/2) and are proportional to covalent bond strength[2].

Super-delocalizabilities of atoms were computed using the definitions given elsewhere[25]. These values are generated as part of the standard calculation of the CNDO/2 and CNDO/2D MOs by the BNDO program[12]. Super-delocalizability for nucleophilic attack on atom i, $S_N(i)$, is related to how much character the empty MOs have on that atom. The contribution of the linear-combination-of-atomic-orbital (LCAO) coefficients are weighted according to the MO energies, so that the coefficients of the low-lying empty MOs are more important. Conversely, super-delocalizability for electrophilic attack on an atom, $S_E(i)$, is related to the LCAO coefficients of the filled MOs, again weighted according to the eigenvalues, so that the high-lying MOs are more important. $S_E(i)$ is more negative when an atom is supposedly more susceptible to electrophilic attack, and $S_N(i)$ is more positive when an atom is supposedly more susceptible to nucleophilic attack. As with most semi-empirically computed quantities, the $S_E(i)$ and $S_N(i)$ have no absolute meaning and can at best be useful only on a relative basis for the same atom in a closely related series of molecules.

Each of the quantities in Table 2 was used as a single variable in linear regression analysis because some of the quantum mechanical quantities are highly interrelated with each other[2]. Of the 153 possible pairs of quantum mechanical indices, about 20 percent have r values greater than 0.7. The net atomic charges on the amide carbon and hydrogen were two of the indices least correlated with other quantum mechanical indices.

Results

The correlation coefficients in Table 2 reveal that several of the quantum mechanically derived quantities are highly related to ^{13}C NMR chemical shifts for the side chain amide carbon of cephalosporins. Positive values of r approaching 1.0 mean that the two variables are proportional to each other; negative values approaching −1.0 mean that the variables are inversely proportional.

Fig. 1 serves to illustrate the nature of the correlation with the highest absolute r value. The regression equation is given by

Fig. 1. ^{13}C NMR chemical shifts (in ppm from Me_4Si) of cephalosporins and Mulliken overlap populations for the amide C–N bond of model side chain structures **1**~**12** (Table 1).

The solid line is Equation (3); the dashed one is Equation (2).

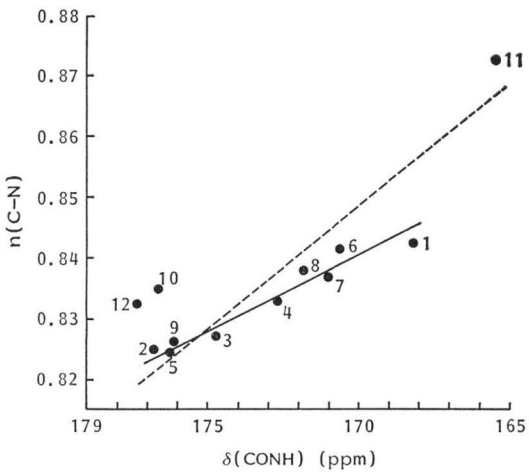
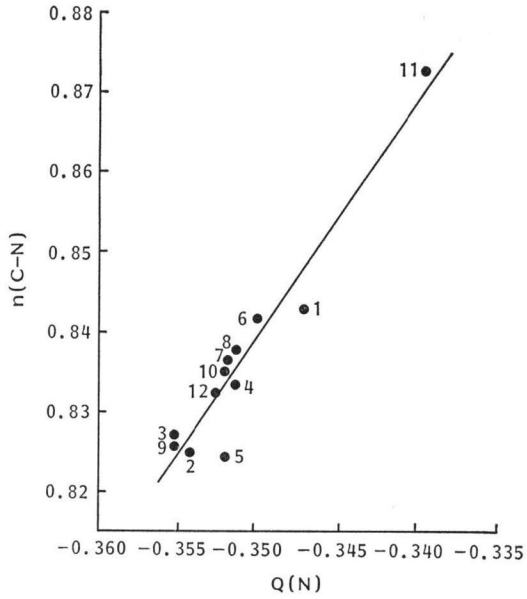


Fig. 2. Net atomic charges on amide nitrogen and Mulliken overlap populations for the amide C–N bond of structures **1**~**12**.



$$\delta(\text{CONH}) = -248.1\ n(\text{C–N}) + 380.6 \quad (2)$$
$$n=12,\ r=-0.8522,\ r^2=0.726,\ s=2.09,\ P=0.0004$$

Fig. 1 shows that as the amide C–N bond becomes stronger, the chemical shift decreases. Excluding the three non-aryl-containing R structures (**10**~**12**) makes the regression equation even better.

$$\delta(\text{CONH}) = -400.8\ n(\text{C–N}) + 506.8 \quad (3)$$
$$n=9,\ r=-0.9729,\ r^2=0.947,\ s=0.74,\ P=0.0000$$

Thus, about 95 percent of the variance in the δ(CONH) values is explained by the C–N overlap population.

Multiple regression analysis using n(C–N) with either Q(C) or Q(H) as the two independent variables gave no significant improvement beyond that achieved by Equation (2).

Essentially equally successful at explaining the variance in δ(CONH) is the net atomic charge on the amide nitrogen.

$$\delta(\text{CONH}) = -761.4\ Q(\text{N}) - 94.1 \quad (4)$$
$$n=12,\ r=-0.8490,\ r^2=0.721,\ s=2.11,\ P=0.0005$$

However, n(C–N) and Q(N) are highly interrelated (Fig. 2). As the charge on nitrogen becomes more negative, the overlap population for the amide C–N bond decreases.

$$n(\text{C–N}) = 2.936\ Q(\text{N}) + 1.867 \quad (5)$$
$$n=12,\ r=0.9532,\ r^2=0.909,\ s=0.004,\ P=0.0000$$

Since n(C–N) and Q(N) are not independent variables, Equation (4) does not add to our understanding of δ(CONH) beyond that achieved by Equation (2).

Other highly interrelated quantum mechanical indices are indicated by the regression equations below.

Fig. 3. Super-delocalizabilities of the amide nitrogen and carbon for electrophilic and nucleophilic reactivity, respectively, of structures **1**~**12**.

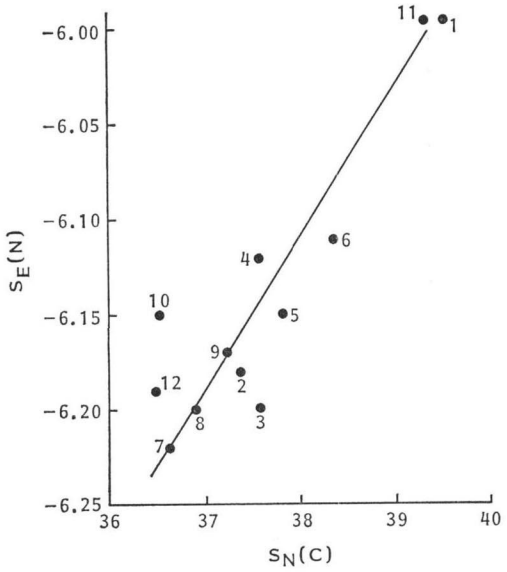
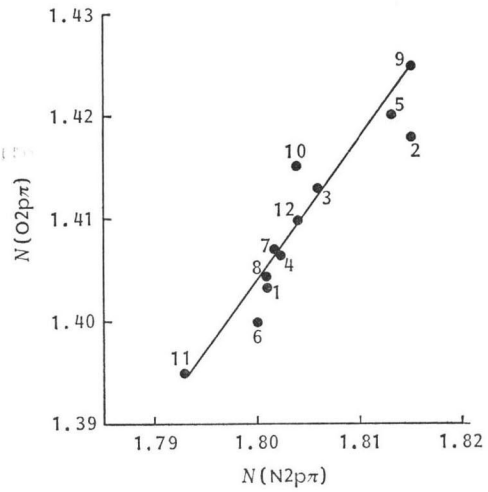


Fig. 4. Mulliken populations of the $2p_\pi$ atomic orbitals on the amide oxygen and nitrogen of structures **1**~**12**.



$$S_N(C) = 12.346\ S_E(N) + 113.36 \quad (6)$$

$n=12$, $r=0.9187$, $r^2=0.844$, $s=0.43$, $P=0.0000$

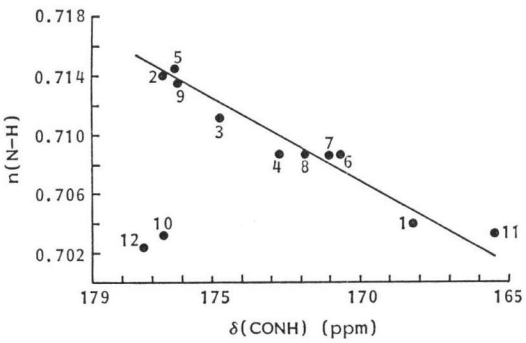
$$N(N2p_\pi) = 0.720\ N(O2p_\pi) + 0.789 \quad (7)$$

$n=12$, $r=0.9503$, $r^2=0.9031$, $s=0.002$, $P=0.0000$

Equation (6) shows that the super-delocalizability of the amide carbon for nucleophilic attack is proportional to the super-delocalizability of the nitrogen for electrophilic attack (Fig. 3). This relation just means that as the empty MOs acquire more amide carbon character, the filled MOs give more density on the amide nitrogen. Equation (7) shows that the $p\pi$ orbital populations on nitrogen and oxygen both tend to increase or decrease together according to the electronic effects of the R group (Fig. 4). Thus, when an R group donates π electron density into the amide group, some of it ends up on the nitrogen orbital, and somewhat more goes to the oxygen atomic orbital.

Fig. 5. ^{13}C NMR chemical shifts (in ppm from Me_4Si) of cephalosporins and Mulliken overlap populations for the amide N–H bond of structures **1**~**12**.

The line is Equation (8) and hence excludes the two points **10** and **12**.



Plotting data often reveals relationships not immediately suggested by the correlation statistics. Such was the case with n(N–H). The correlation coefficient between n(N–H) and δ(CONH) was initially poor (Column 1 of Table 2), but a plot of these two variables (Fig. 5) revealed the existence of a strong correlation except for two outliers. With the two points removed, the regression equation is

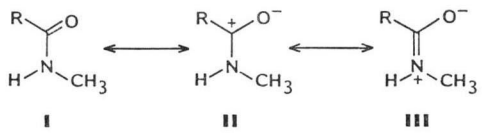
$$\delta(\text{CONH}) = 898.0\ n(\text{N–H}) - 464.7$$
$$n=10,\ r=0.9816,\ r^2=0.963,\ s=0.75,\ P=0.0000 \quad (8)$$

Discussion

CNDO/2 and other molecular orbital methods are known to be sometimes useful in analyzing NMR data[26~36]. The success of this study in finding strong correlations between quantum mechanical indices from CNDO/2D and NMR chemical shifts further establishes this semi-empirical method as a guide to understanding the physical and chemical properties of cephalosporins[2].

The regression equations that are reported here together with CNDO/2D calculations on additional, structurally similar acylamino side chains should facilitate assigning the ^{13}C NMR spectra of other cephalosporins in the future.

The correlations between δ(CONH) and charge distributions in the acylamino structures can be rationalized in terms of amide resonance. The trends evident in Figs. 1, 2 and 5 mean that acyl groups which cause the amide carbon to resonate at lower fields also cause the covalent character of the amide C–N to diminish, the amide nitrogen to become more negative, and the amide N–H bond to strengthen. The principal resonance structure of an amide is **I**[37]. Substituents R that withdraw electrons from the amide system either inductively or by resonance decrease the contribution from resonance structures **II** and **III** relative to **I**. The double bonded form **III** has less electron density on nitrogen (Fig. 2). The strength of the amide N–H bond tends to correlate with δ(CONH) (Fig. 5) because the less electronegative R groups favor **I**, and **I**, in turn, has more electron density available for the N–H σ bonding. Structures **2**, **3**, **5** and **9** (Fig. 1) are relatively poor at withdrawing electrons. Structures **1**, **6**, **7** and **8** are relatively good.



I **II** **III**

It should be noted that the quantum mechanical indices found empirically to correlate well with δ(CONH) all reflect charge distribution in the vicinity of the amide carbon, whereas the charge on this atom itself, Q(C), does not correlate (Table 2). As has been pointed out numerous times before[31,34~36], it is only a rough approximation that electron density is monotonically related to nuclear screening. The magnetic susceptibility of the charge distribution depends mainly on the paramagnetic term which is related to the angular momentum of the electrons in the vicinity of an atom[29,34].

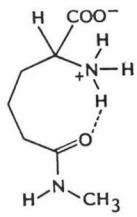
Both Fig. 1 and Fig. 5 suggest that aryl-containing R groups are behaving somewhat differently than structures **10**, **12** and possibly **11**. Among the reasons for this phenomenon are the following.

One likely explanation is that R=H and CH_3 introduce less anisotropy into the amide charge distribution than the other R groups. The α-aminoadipyl R group is highly flexible and exists in many conformations; this situation tends to reduce the average screening of the amide carbon nucleus. Note that points for **10**~**12** all fall to the left of the regression lines in Fig. 1.

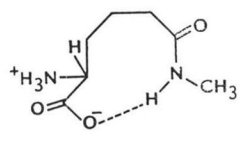
Another reason for structures **10**~**12** being outliers may be that the solvation contributions to the chemical shifts may be different for these structures compared to the aryl-containing side chains.

Another possibility is that the CNDO/2D method does not equally well predict the charge distribution in the two classes of R groups (aryl- and non-aryl-containing). Inductive and resonance effects both come into play in CNDO, but as with every molecular orbital method, the relative importance of these depends on many parameters, such as the basis set orbitals, orbital exponents, *etc.* The charge distributions depend on the complex balance of all the parameters that enter the theory, as well as on how the charges are defined[13].

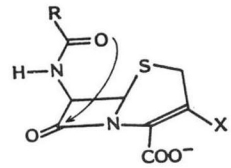
A factor that could be operable in the one case of the α-aminoadipyl structure **12** is the possibility of intramolecular hydrogen bonding. Several geometries of **IV** and **V** and the neutral, zwitterionic, and ionized forms of **12** were investigated computationally. Geometry **IV** with the ammonium group intramolecularly hydrogen bonded to the acyl oxygen gave n(C–N) of *ca.* 0.87 and n(N–H) of 0.705. The latter only slightly improves the fit of **12** to the regression line in Fig. 5, but the fit of **12** to the regression line in Fig. 1 becomes much worse. Geometry **V** with the amide N–H hydrogen bonded to the carboxyl-



IV



V



VI

ate, has n(C–N) of *ca.* 0.85 and n(N–H) of 0.701. These do not improve either correlation. The effect on these overlap populations due to other structural variations was minor.

Early work considered chemical shifts as a parameter in quantitative structure-activity relationships[38]. The possibility of ^{13}C NMR chemical shifts correlating with biological activity of cephalosporins was addressed by NISHIKAWA and TORI[3]. They showed that with a related series of cephalosporins with the same 3-position substituent, δ(CONH) values tend to increase as alkaline hydrolysis rates increase. It is known that for a series of cephalosporins with a common acylamino side chain, hydrolysis rates are related to Gram-negative *in vitro* activity[2,5]. In addition, δ(CONH) is roughly proportional to the difference in ^{13}C NMR chemical shift for C-3 and C-4 of cephalosporins $\Delta\delta$(4-3). NISHIKAWA and TORI also showed that for a series of cephalosporins with a common acylamino side chain, $\Delta\delta$(4-3) is parabolically related to Gram-negative minimum inhibitory concentrations[4].

As appealing as it would be to find a correlation between δ(CONH) and MICs of cephalosporins with *different* acylamino side chains, no such relationship has been reported in the literature, and none is evident in the work here. For instance, the 7-aminocephalosporanic acid (7-ACA) derivatives of tetrazoylacetyl **1** and thienylacetyl **3**, which are well separated points in Fig. 1, give similar, good Gram-positive and Gram-negative MICs[39]. The phenoxyacetyl **4** derivative of 7-ACA has better Gram-positive and much poorer Gram-negative activity, but lies intermediate between the points for **1** and **3** in Fig. 1. Cephalosporin C, which has side chain **12** and which lies at extreme of Fig. 1, has relatively low activity[39].

The lack of a clear correlation between δ(CONH) and MICs does not mean that the charge distribution in the 7-acylamino side chain is irrelevant to activity. Rather it means that there are so many other variable properties (lipophilicity, conformation, steric size) affecting chemical stability, transport through the bacterial outer membrane, β-lactamase susceptibility, and attachment to the target enzymes that it is difficult to correlate a single variable, such as δ(CONH), with antibacterial activity for the set of cephalosporins considered here.

The correlation[3] between alkaline-catalyzed hydrolysis rates of cephalosporins with δ(CONH) is consistent with intramolecular participation[2,40] of the side chain in nucleophilic attack on the β-lactam. For instance, mechanism **VI** may be operable[40]. If it is, then the nucleophilicity of the acyl oxygen, which is under the influence of the inductive and resonance effects of R, will be important. Alternative mechanisms where the amide side chain can participate in hydrogen bonded interactions with hydroxide ion or water, can also be envisioned. The strength of these interactions will be affected by the amount of resonance (**I**~**III**) in the amide group. Therefore, it is not unreasonable to expect that the charge distribution in the amide side chain will play a role in the chemical reactivity of β-lactam antibiotics.

Acknowledgment

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