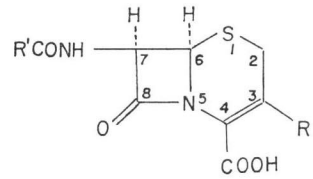
THEORETICAL CRITERIA FOR PREDICTING BIOLOGICAL ACTIVITY OF CEPHALOSPORIN ANTIBIOTICS*

Sir:

Among the many factors which are recognized as influencing antibacterial activity of cephalosporins is the chemical reactivity of the β-lactam ring[1~3]. The reactivity of medium- and small-sized molecules can be predicted fairly well *on a relative basis* using a quantum mechanical approach. An approximate, semiempirical molecular orbital (MO) method would be applicable to molecules as large as cephalosporins and may be adequate for finding trends if the molecules belong to a closely related series. Provided that calculated theoretical reactivity indices for a series of cephalosporins can be shown to correlate with observed biological activity, then it becomes conceivable to screen new members of the series on a computer before they are actually synthesized. It is the purpose of this communication to describe the existence of the requisite correlation.

In vitro antibacterial activity is expressed in terms of minimum inhibitory concentration (MIC) obtained from gradient plate assay[3]. Using average MIC values against some representative Gram-negative microbes, series of 7-acylamino cephalosporins with different substituents R at the 3 position have been compared. The R substituents can be ranked according to their relative contribution to activity (W. H. W. LUNN, unpublished data). The ranking of 3-substituents reported in Table 1 is significant because it holds remarkably well for at least seven different R′ groups. The R′ groups include such familiar acyl functions as phenylglycyl, thienylacetyl, phenylacetyl (G), and phenoxyacetyl (V).

The present application of MO calculations is founded on the premise that cephalosporins (and penicillins) exert their antibacterial activity by interfering with bacterial cell wall biosynthesis through some acylation reaction with cell wall enzymes[1~7]. Because the β-lactam acts as the acylating group, two of the more obvious charge distribution quantities to examine are the net atomic charge on C_8, $Q(C_8)$, and the overlap



population for the C_8–N_5 bond, $n(C_8$–$N_5)$, of the β-lactam ring. The more positive $Q(C_8)$, then the more susceptible one might expect the carbonyl center to be to nucleophilic attack in the initial phase of the acylation step[1,2,8]. Likewise, the lower $n(C_8$–$N_5)$, then the weaker should be this bond which must be broken during acylation[1,2]. These two reactivity indices are calculated for model structures, 7–NH_2–3–R–3-cephems, by the CNDO/2D MO method described earlier[4]. The simplified models lack 4–COOH and the R′ acyl substituent because their electronic contributions to reactivity are roughly constant within a series[9]. A third criterion of β-lactam reactivity is a quantity to be referred to as the transition state energy (TSE). Developed from model studies reported earlier[4], the TSE is expressed as the decrease in CNDO/2 total energy of a complex formed by placing an OH^- 1.50 Å from the α face of C_8 of a given 7–NH_2–3–R–3-cephem compared to OH^- and this cephem structure at infinite separation. The larger the magnitude of the TSE, then the easier it is to form a model transition state for nucleophilic attack. Because of questions regarding the significance of the *absolute* values of semiempirically calculated MO indices of reactivity, only the *relative* rankings are used for comparison to the activity ranking reported in this account.

The results are presented in Table 1. All three quantum mechanical reactivity indices correlate with biological activity to some extent, but distinctly superior to the other two is the TSE. The TSE ranking matches the MIC ranking closely. The only inversion is for the two side chains that yield the highest activity and predicted reactivity: 4-carboxamidopyridiniummethyl and 1-methyl-1H-tetrazol-5-ylthiomethyl. A complicating factor in the pyridinium calculations is that a Cl^- counterion must be placed in the vicinity of the pyridinium ring, so that the side chain as a whole is roughly electrically neutral like the other side chains. With a caveat that electrically charged substituents can be

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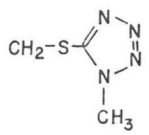
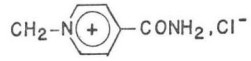
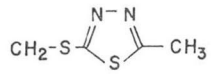
Table 1 Rankings of experimental *in vitro* antibacterial activity of 3-substituted cephalosporins and of predicted reactivity of the β-lactam ring in 3-substituted 3-cephem model structures.

3-R	Activity*	TSE	Q (C_8)	n (C_8–N_5)
	1	2	2	2
	2	1	1	1
	3	3	3	6
CH_2-$OCOCH_3$	4	4	6	3
CH_2-$OCONH_2$	5	5	7	4
CH_2-SCH_3	6	6	4	7
CH_2-OCH_3	7	7	5	5
CH_3	8	8	8	8

* Based on MIC's (μg/ml) averaged over five Gram-negative organisms: N9 (*Shigella sonnei*), N10 (*Escherichia coli*), X26 (*Klebsiella pneumoniae*), X68 (*Enterobacter aerogenes*) and X514 (*Salmonella heidelberg*).

treated with less certainty than other substituents, it may be concluded that a calculationally derived quantity, namely the TSE, has been discovered which correlates qualitatively well with *in vitro* biological activities. Further analysis of the correlation and full numerical details will be forthcoming.

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