Table XIII. Summary of Solutions

site[a]	site configns	mode combinations	solutions with σ = 4.1	1	0
s1	1	16	1	3	0
s1 s2	4	49	4	12	0
s1—s2	1	169	1	12	0
s3 s1 s2	60	100	60	540	0
s3 s1—s2	15	256	15	225	0
s3 / \ s1—s2	1	256	1	9	36

[a] See footnote a of Table XII.

involves a carefully coordinated choice of site point coordinates and proposed binding modes. The odds are that the initial choice of modes will not lead to a solution, so that some provision for altering the initial choice is necessary.[11] Note that the mere availability of nine energy parameters to fit two binding energies in the case of three site points was not sufficient; correct binding modes had to be proposed. Distance geometry binding studies cannot be judged by the same criteria as those used for more conventional QSAR methods.

Registry No. 1, 85304-87-6; 2, 70579-32-7; 3, 17740-29-3; 4, 90-08-4; 5, 85304-88-7; 6, 70579-34-9; 7, 70650-62-3; 8, 4022-58-6; 9, 47071-11-4; 10, 3567-84-8; 11, 17711-73-8; 12, 21316-30-3; 13, 46781-41-3; 14, 4653-75-2; 15, 15233-37-1; 16, 3850-94-0; 17, 1542-59-2; 18, 70743-55-4; 19, 70579-41-8; 20, 4038-60-2; 21, 70579-39-4; 22, 70579-36-1; 23, 13351-02-5; 24, 70579-38-3; 25, 70606-63-2; 26, 24849-96-5; 27, 51012-14-7; 28, 1492-81-5; 29, 4653-73-0; 30, 19161-84-3; 31, 70579-31-6; 32, 85304-89-8; 33, 17944-10-4; 34, 253-82-7; 35, 50440-89-6; 36, 86-96-4; 37, 50828-14-3; 38, 13741-90-7; 39, 1899-48-5; 40, 18917-68-5; 41, 52979-06-3; 42, 55096-56-5; 43, 55096-39-4; 44, 52979-00-7; 45, 55096-41-8; 46, 52979-11-0; 47, 51123-28-5; 48, 51123-83-2; 49, 15018-66-3; 50, 491-36-1; 51, 33081-07-1; 52, 50440-88-5; 53, 50440-83-0; 54, 50828-13-2; 55, 50440-87-4; 56, 20198-19-0; 57, 50440-86-3; 58, 50828-19-8; 59, 49873-59-8; 60, 50440-82-9; 61, 50440-85-2; 62, 52979-15-4; 63, 50440-84-1; 64, 50828-20-1; 65, 53159-19-6; 66, 50828-21-2; 67, 27023-77-4; 68, 52979-04-1; 69, 38944-10-4; 70, 52979-02-9; 71, 52979-05-2; 72, 1899-40-7; 73, 52979-03-0; 74, 50828-17-6; 75, 18671-95-9; 76, 53159-19-6; 77, 52978-97-9; L-78, 58724-36-0; 79, 50440-75-0; 80, 1955-61-9; 81, 1899-41-8; 82, 55096-64-5; 83, 27018-14-0; 84, 52979-13-2; 85, 50930-12-6; 86, 17511-20-5; L-87, 27069-81-4; 88, 17511-21-6; L-89, 58724-37-1; 90, 50828-08-5; 91, 55096-66-7; 92, 52978-99-1; L-93, 5854-11-5; 94, 50546-08-2; 95, 50828-12-1; 96, 52979-09-6; 97, 41934-85-4; 98, 55096-67-8; 99, 55096-15-6; 100, 55096-42-9; 101, 53667-27-9; 102, 55096-44-1; 103, 55096-55-4; 104, 52979-08-5; 105, 52979-10-9; 106, 51123-99-0; 107, 55096-54-3; 108, 55096-57-6; 109, 55096-50-9; 110, 55096-52-1; L-111, 58724-38-2; L-112, 18921-68-1; 113, 55096-40-7; 114, 55096-62-3; 115, 55096-48-5; 116, 55096-43-0; 117, 55096-61-2; L-118, 24205-32-1; 119, 50828-16-5; 120, 50828-09-6; 121, 13794-65-5; 122, 55096-63-4; 123, 55096-59-8; 124, 55096-58-7; 125, 55096-51-0; 126, 55096-49-6; 127, 51124-31-3; 128, 43170-98-5; 129, 55096-53-2; 130, 51124-09-5; 131, 51123-77-4; L-132, 32043-09-7; 133, 55096-60-1; 134, 55096-45-2; 135, 55096-46-3; 136, 55096-47-4; dihydrofolate reductase, 9002-03-3.

Substituent Effects in Cephalosporins as Assessed by Molecular Orbital Calculations, Nuclear Magnetic Resonance, and Kinetics

Donald B. Boyd

Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285. *Received October 28, 1982*

For cephalosporins with different side chains at position 3, the quantum mechanically computed charge distribution in the β-lactam carbonyl group can be correlated with observables, such as carbon-13 chemical-shift differences at C_3 and C_4 of the dihydrothiazine ring and alkaline rates of hydrolysis of the β-lactam. The relationship of these properties and the theoretical transition-state energy (TSE) corroborate the fact that chemical reactivity is one important determinant affecting inhibitory activity of cephalosporins against peptidoglycan-regulating enzymes.

Several physicochemical properties of cephalosporins have now been identified that can be related to antibacterial activity. These properties reflect the effects on the rest of the molecule that derive from the different substituents at position 3 of the 3-cephem nucleus. For instance, molecular orbital calculations can be used to evaluate the ease of approach of a nucleophile to a 3-substituted 3-cephem in a model reaction.[1-4] The calculations yield a transition-state energy (TSE), which is defined as the change in the CNDO/2 total energy of the 3-cephem–OH^- complex (formed by placing OH^- 1.5 Å from the α face of the β-lactam carbonyl carbon) with respect to the sum of the energies of the separated 3-cephem and OH^- reactants.[3] TSE values for cephems with 13 different R groups are given in Table I. Depending on the 3-substituent, those cephalosporins with a more favorable energy of interaction in the complex tend to exhibit better in vitro Gram-negative activity.

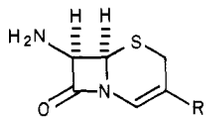
Another property more recently found to correlate with minimum inhibitory concentrations (MICs) of cephalosporins is the difference in ^{13}C chemical shifts for carbons 3 and 4 of the dihydrothiazine ring of the 3-cephem nucleus.[5,6] This correlation was discovered by making note of the fact that cephalothin and cephaloridine had been reported to have larger $\Delta\delta(4-3)$ quantities than cephalexin.[7] Not only does $\Delta\delta(4-3)$ correlate with MICs, but it also correlates linearly with TSEs and inductive substituent constants σ_I for the 3-position side chains.[6]

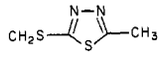
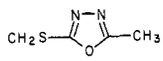
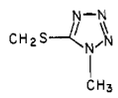
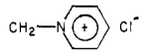
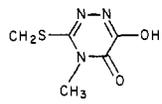
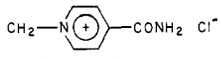
Also, it is known that the TSEs and antibacterial activities each correlate linearly with alkaline hydrolysis rates for a series of 7-(thien-2-ylacetyl)cephalosporins.[8] The

(1) Boyd, D. B.; Hermann, R. B.; Presti, D. E.; Marsh, M. M. *J. Med. Chem.* **1975**, *18*, 408.
(2) Boyd, D. B.; Lunn, W. H. W. *J. Antibiot.* **1979**, *32*, 855.
(3) Boyd, D. B.; Herron, D. K.; Lunn, W. H. W.; Spitzer, W. A. *J. Am. Chem. Soc.* **1980**, *102*, 1812.
(4) Boyd, D. B. *Ann. N.Y. Acad. Sci.* **1981**, *367*, 531.
(5) Tori, K.; Nishikawa, J.; Takeuchi, Y. *Tetrahedron Lett.* **1981**, *22*, 2793.
(6) Nishikawa, J.; Tori, K. *J. Antibiot.* **1981**, *34*, 1641.
(7) Paschal, J. W.; Dorman, D. E.; Srinivasan, P. R.; Lichter, R. L. *J. Org. Chem.* **1978**, *43*, 2013.

0022-2623/83/1826-1010$01.50/0

Table I. Transition-State Energies[a] and Charge Distribution Quantities[b] from CNDO/2D Molecular Orbital Calculations on 3-Substituted 7-Amino-3-cephems



no.	R	−TSE	$n(C_8{=}O_9)$	$Q(O_9)$
1	CH_3	129.6	0.7910	−0.3512
2	CH_2OH	131.0	0.7919	−0.3491
3	CH_2SCH_3	132.2	0.7913	−0.3501
4	CH_2OCONH_2	132.7	0.7921	−0.3484
5	CH_2OCOCH_3	133.1	0.7922	−0.3483
6		133.7	0.7918	−0.3495
7		135.0	0.7921	−0.3482
8		135.2	0.7926	−0.3469
9	OSO_2CH_3	136.1	0.7948	−0.3404
10		136.6	0.7925	−0.3474
11		137.7	0.7925	−0.3477
12	Cl	138.5	0.7950	−0.3411
13		139.0	0.7930	−0.3463

[a] In kilocalories per mole. [b] In electrons.

regression equation involving TSEs and hydrolysis rates[8-12] is shown in Table II. For the 11 7-(thien-2-ylacetyl)cephalosporins with 3-substituents, 1–3 and 5–12, 52% of the variance in log k_{obsd} is explained by the TSEs (Figure 1a). Similarly, the log k_{obsd} values are able to explain more than half of the variance in average Gram-negative minimum inhibitory concentrations (MIC as defined in ref 3). The value of r^2 and the other statistics are better if a parabolic, rather than linear, curve is fit to the data.[13]

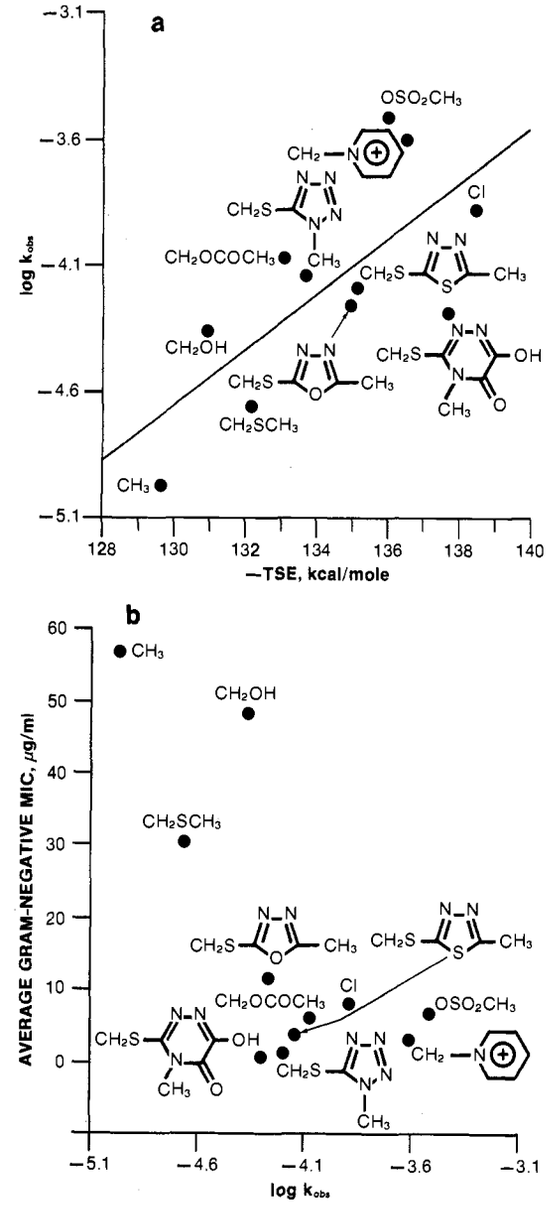


Figure 1. Relationships of (a) TSEs and (b) MICs to alkaline hydrolysis rates for 7-(thien-2-ylacetyl)cephalosporins with the 3-substituents as shown.

$$n = 11,\ r = 0.72,\ s = 14.6,\ p = 0.0126$$

$$\text{MIC} = -33.84 \log k_{obsd} - 125.2$$

$$\text{MIC} = 36.239(\log k_{obsd})^2 + 271.04 \log k_{obsd} + 510.1$$

$$n = 11,\ r = 0.82,\ s = 12.8,\ p = 0.0116$$

(8) Boyd, D. B. In "Chemistry and Biology of β-Lactam Antibiotics"; Morin, R. B.; Gorman, M., Eds.; Academic Press: New York, 1982, Vol. 1, Chapter 5. Paper 13 of the series on Electronic Structures of Cephalosporins and Penicillins, of which the present paper is part 14.

(9) Indelicato, J. M.; Norvilas, T. T.; Pfeiffer, R. R.; Wheeler, W. J.; Wilham, W. L. *J. Med. Chem.* **1974**, *17*, 523.

(10) Indelicato, J. M.; Dinner, A.; Peters, L. R.; Wilham, W. L. *J. Med. Chem.* **1977**, *20*, 961.

(11) Indelicato, J. M., unpublished data. For 3-substituted 7-(thien-2-ylacetamido)-3-cephem-4-carboxylic acid or its sodium or lithium salt, rates of hydrolysis of the β-lactam ring were measured at pH 10 and 35 °C by using high-performance liquid chromatography (HPLC) to follow the reaction. A carbonate buffer was used with **2** and **5**, whereas a pH stat was used for **6–8**, **10**, and 11. When k_{obsd} is in units of reciprocal seconds, −log k_{obsd} values are 4.36 (**2**), 4.07 (**5**), 4.14 (**6**), 4.26 (**7**), 4.19 (**8**), 3.60(**10**), and 4.29 (11) for the side chains shown in Table I. These have an experimental uncertainty less than or equal to ±0.22 log unit. By an earlier UV method (ref 9 and 10) at the same temperature and pH, −log k_{obsd} values of 4.58 (**2**), 4.66 (**3**), 4.01 (**5**), 3.51 (**9**), 3.73 (**10**), and 3.88 (**12**) were obtained. A value of 4.97 for 1 was obtained by titration (ref 9). As pointed out in ref 12, the rate for the acetoxymethyl-containing cephalothin (**5**) needs to take into account the fact that the deacetyl derivative (**2**) is also formed as an intermediate during decomposition. The HPLC value for **5** above does do this by using the HPLC decomposition rate for an authentic sample of deacetylcephalothin (**2**) and the assumption that the rate of decomposition of **5** is the sum of the rates for β-lactam ring hydrolysis and side chain hydrolysis.

(12) Yamana, T.; Tsuji, A. *J. Pharm. Sci.* **1976**, *65*, 1563.

(13) There are two fundamental reasons for preferring to use MICs in these regression equations instead of log (1/MIC). One is that the MIC represents the inhibition of the exponential growth rate of cells. Not only are the target enzymes being inactivated, they are also being synthesized exponentially (Blumberg, P. M.; Strominger, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 2814). In contrast, in quantitative structure–activity studies not involving target enzymes being newly generated during the biological assay, it is appropriate to take the logarithm of the drug concentration because the biological process affected by the drug is a simple first-order process. The second reason is that taking logarithms emphasizes small differences in the small magnitude data relative to data of larger magnitude. However, such small differences are often beyond the accuracy of the biological assay.

Table II. Linear Regression Analysis

equation	n^a	r^b	s^c	p^d	ref[e]
$\log k_{obsd} = 0.1092(-TSE) - 18.85$	11	0.72	0.31	0.0129	
$\log k_{obsd} = 253.36[n(C_8{=}O_9)] - 204.97$	11	0.76	0.29	0.0062	
$\log k_{obsd} = 92.951[Q(O_9)] + 28.10$	11	0.76	0.29	0.0069	
$\Delta\delta(4\text{-}3) = 2.0286(-TSE) - 257.55$	9	0.93	2.46	0.0003	6
$\Delta\delta(4\text{-}3) = 8451.20[n(C_8{=}O_9)] - 6680.12$	9	0.85	3.50	0.0036	6
$\Delta\delta(4\text{-}3) = 3329.88[Q(O_9)] + 1174.33$	9	0.84	3.65	0.0048	6
$\ln k_{obsd} = 0.4295(-TSE) - 59.56$	5	0.95	0.44	0.0146	14
$\ln k_{obsd} = 1576.90[n(C_8{=}O_9)] - 1251.10$	5	0.86	0.69	0.0584	14
$\ln k_{obsd} = 565.93[Q(O_9)] + 195.15$	5	0.83	0.77	0.0855	14

[a] Number of different side chains. [b] Correlation coefficient. [c] Standard estimate of error. [d] Probability out of 1.0 that the null hypothesis is satisfied. [e] Source of experimental data. In cases of several compounds with the same side chain at position 3, the $\Delta\delta(4\text{-}3)$ data from ref 6 and 14 were averaged because they are quite similar. In other words, of the cephalosporins reported in ref 6 and 14, the 7-acylamino side chain has a relatively minor influence.

A rationale for preferring the latter equation is that those compounds that are especially reactive may undergo side reactions before reaching the active sites in the bacterial cell walls. Some compounds are known to be inactive because they are not stable enough. Hence, biological activity may be greatest when β-lactam reactivity is in a certain range.

Completing the bridge between these various properties is the report that $\Delta\delta(4\text{-}3)$ and TSEs can each be related to alkaline hydrolysis rate constants k_{obsd} for a diverse group of first- and second-generation cephalosporins.[14]

The fact that all of the above physicochemical properties are related to antibacterial activity can be linked with the known mode of action of cephalosporins (and penicillins) against the several transpeptidases and carboxypeptidases in the periplasmic space of bacterial cell walls.[15,16] The β-lactam antibiotics act as acylating agents, thereby reacting with and covalently binding a serine hydroxy group in the active site that normally processes pentapeptide side chains of nascent peptidoglycan strands.[17] A high reactivity of the β-lactam ring, up to the point where stability is still maintained, is helpful for the rapid acylation and inactivation of the target enzymes.

This note is prompted by the fact that other theoretically computed properties of model cephem structures are related to the properties mentioned above. One is $n(C_8{=}O_9)$, which is the Mulliken overlap population for the β-lactam carbonyl bond $C_8{=}O_9$. This quantity reflects the covalent strength of the β-lactam carbonyl bond. The second quantity is $Q(O_9)$, the net atomic charge on the carbonyl oxygen. These quantities (Table I) are obtained from CNDO/2 MO calculations on model reactant structures, 3-substituted 7-amino-3-cephem, followed by a Löwdin deorthogonalization procedure and Mulliken population analysis.[1,18] The molecular geometries are the same as those used in earlier work.[3,18]

The nature of the relationships between the carbonyl charge distributions and the recent literature data[6,8,14] are apparent in Figures 2–4 and Table II. Plots of TSE vs. $\Delta\delta(4\text{-}3)$ and ln k_{obsd} have appeared elsewhere.[6,14] No strong, significant correlations between the observed properties and CNDO/2 charge distributions involving C_3 and C_4, or simple functions thereof, are found. But the two quantities reflecting the charge distribution of the β-lactam carbonyl correlate with the chemical shift and rate data almost as well as do the TSEs. Those compounds with higher $\Delta\delta(4\text{-}3)$ and faster hydrolysis rates have a

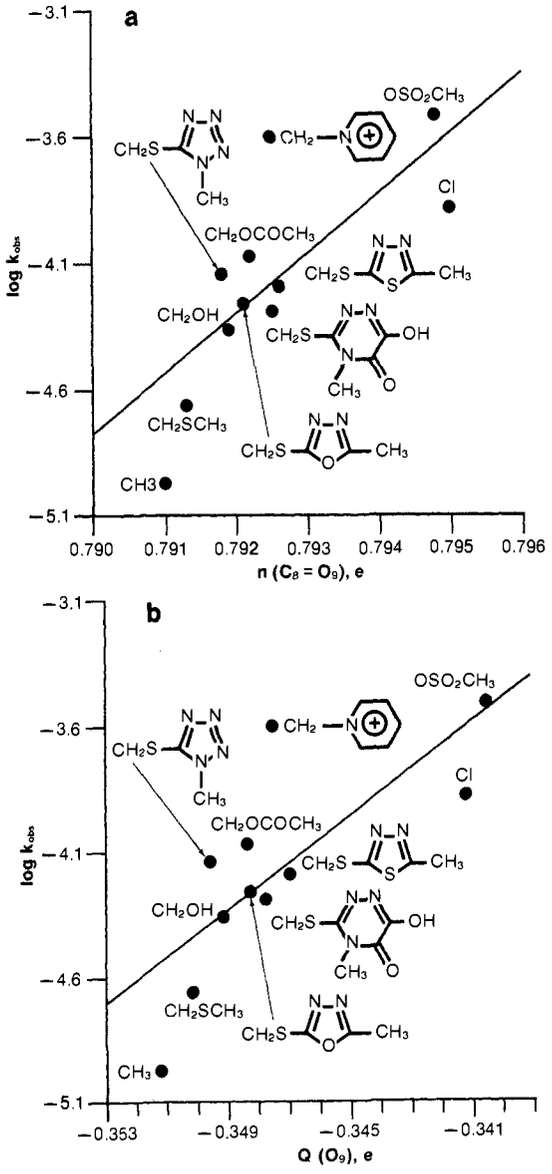


Figure 2. Relationships of (a) $n(C_8{=}O_9)$ and (b) $Q(O_9)$ from CNDO/2D MO calculations on 3-substituted 7-amino-3-cephems to alkaline hydrolysis rates for 7-(thien-2-ylacetyl)cephalosporins with the 3-substituents as shown.

(14) Schanck, A.; Coene, B.; Dereppe, J. M.; Van Meerssche, M., unpublished data; *Bull. Soc. Chim. Belg.* **1983**, *92*, 81. Rates were measured by a UV method at pH 9.6 in reciprocal hours.
(15) Mirelman, D. In "β-Lactam Antibiotics: Mode of Action, New Developments, and Future Prospects"; Salton, M. R. J.; Shockman, G. D., Eds.; Academic Press: New York, 1981; p 67.
(16) Kelly, J. A.; Moews, P. C.; Knox, J. R.; Frere, J.-M.; Ghuysen, J.-M. *Science* **1982**, *218*, 479.
(17) Waxman, D. J.; Yocum, R. R.; Strominger, J. L. *Philos. Trans. R. Soc. London, Ser. B* **1980**, *289*, 257.
(18) Boyd, D. B. *J. Phys. Chem.* **1974**, *78*, 2604.

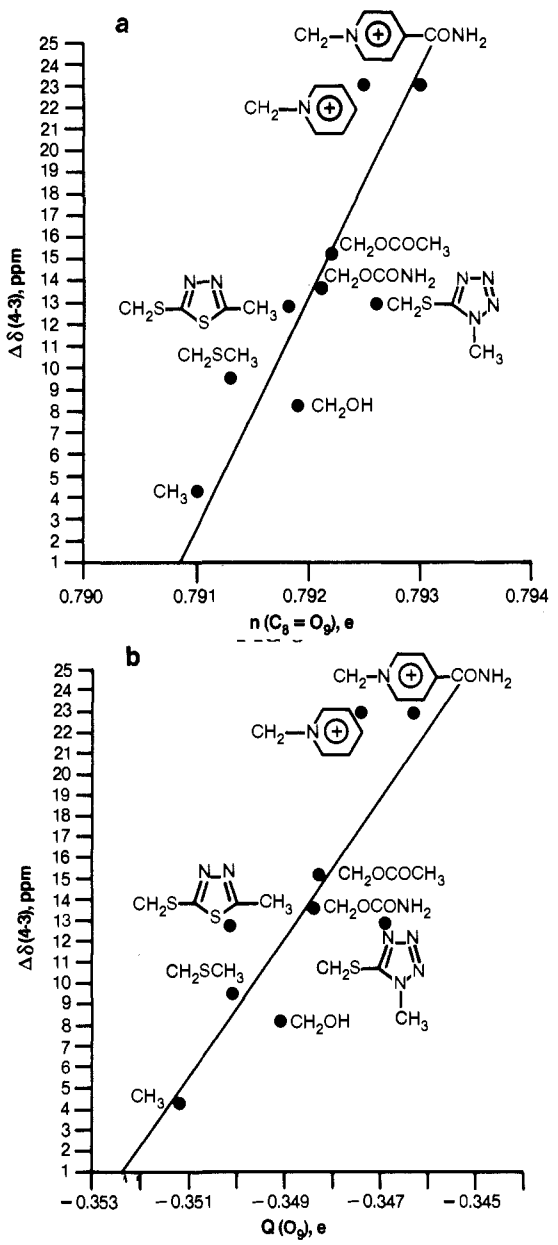


Figure 3. Relationships of (a) $r(C_8{=}O_9)$ and (b) $Q(O_9)$ from CNDO/2D MO calculations on 3-substituted 7-amino-3-cephems to $\Delta\delta(4\text{–}3)$ for the nine cephalosporin salts in ref 6 with the 3-substituents as shown.

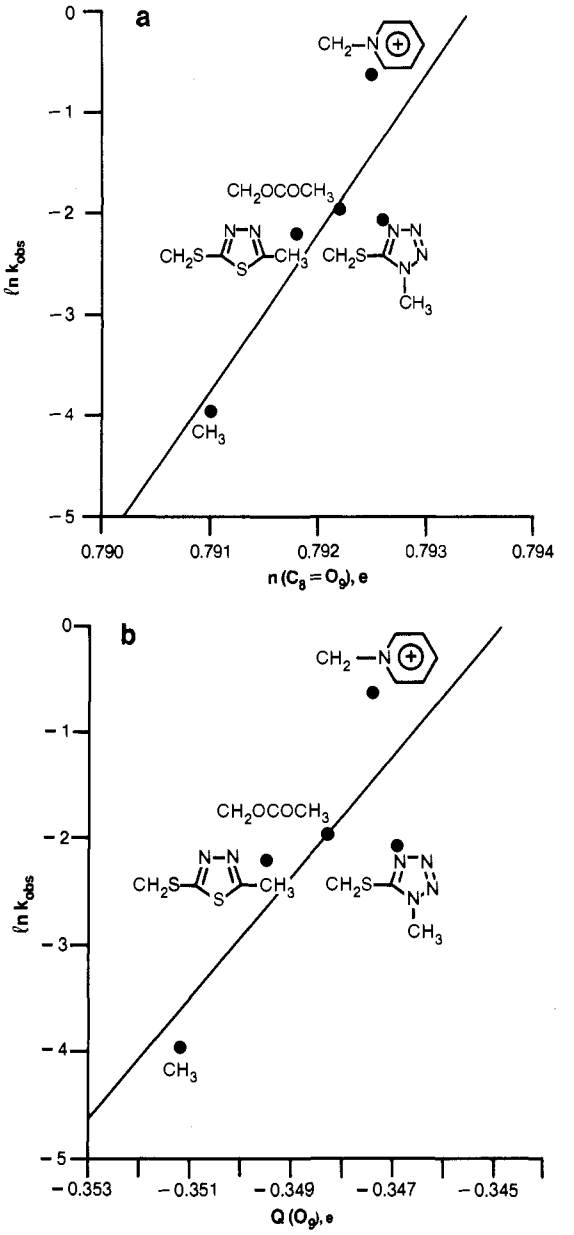
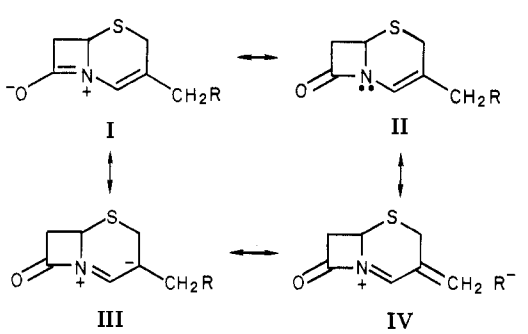


Figure 4. Relationships of (a) $n(C_8{=}O_9)$ and (b) $Q(O_9)$ from CNDO/2D MO calculations on 3-substituted 7-amino-3-cephems to ln k_{obsd} for the ten cephalosporin salts in ref 14 with the 3-substituents as shown.

stronger C=O bond and less negative carbonyl oxygen.

It may be concluded that $n(C_8{=}O_9)$ and $Q(O_9)$ are affected by the substituents at the 3-position and, therefore, reflect the acylating ability of a cephalosporin. Both $n(C_8{=}O_9)$ and $Q(O_9)$ are related to the prevalence of resonance form I compared to II–IV. Structure I strengthens the amide C–N bond and is, therefore, deleterious to reactivity.

An attribute of the quantum mechanically derived, substituent-related parameters is that they can be obtained regardless of whether the compound has been previously synthesized and regardless of whether the substituent is one that appears in one of the familiar tables of quantitative structure–activity substituent constants.



Acknowledgment. Appreciation is expressed to A. Schanck and J. R. Knox who kindly supplied preprints, J. M. Indelicato who generously provided experimental data and helpful discussion, and W. H. W. Lunn and K. F. Koch for comments on the manuscript.

Registry No. 1, 73629-91-1; 2, 73629-92-2; 3, 73629-94-4; 4, 73648-85-8; 5, 55469-86-8; 6, 73629-95-5; 7, 85507-60-4; 8, 73629-96-6; 9, 73630-03-2; 10, 73630-00-9; 11, 73629-97-7; 12, 73630-05-4; 13, 73629-98-8; 7-amino-(6*R*)-*trans*-5-thia-1-azabicyclo[4.2.0]oct-2-en-8-one, 38504-95-9.