COMPARATIVE STABILITIES OF CEPHALOSPORINS IN AQUEOUS SOLUTION

Sir:

Penicillins and cephalosporins have been known to undergo remarkably facile cleavage of their β-lactam bonds in aqueous solution.[1~3] Recent work[4] concerning the immunochemical mechanisms underlying allergic reactions to the β-lactam antibiotics has caused a renewed interest in the stability of aqueous solutions of these antibiotics. The kinetics of the hydrolysis of penicillins have been extensively investigated and recently reviewed[1,2], but no kinetic studies except for cephalosporin C[5] have been reported on the hydrolysis of cephalosporins.*

In our recent investigation intended to explore the stability of β-lactam antibiotics in aqueous solution, we reported the kinetics and mechanism of the hydrolyses of semisynthetic penicillins and 6-aminopenicillanic acid (6-APA).[6] The present communication describes a comparative study on the stability of a series of cephalosporins in broad pH ranges.

The hydrolyses of cephalosporins were carried out at 35°C and ionic strength 0.5 by using appropriate buffer systems and by means of a pH-stat. The rates of hydrolysis were determined by the iodometric titration method[7] and/or by following the loss of the characteristic UV absorbance at *ca.* 260 nm due to the β-lactam system. Extrapolation of the experimentally determined first-order rate constants, obtained at constants pH's but with varying buffer compositions, to zero buffer concentration afforded pseudo-first-order rate constants (k_{pH}) which are functions of only pH.

The log k_{pH}—pH profiles for the hydrolyses of cephalothin (**I**), cephaloridine (**II**), cephaloglycin (**III**), cephalexin (**IV**), 7-aminocephalosporanic acid (7-ACA) and 7-aminodeacetoxycephalosporanic acid (7-ADCA), and those for the substituted phenylcephalosporins (**V**) are shown in Fig. 1.

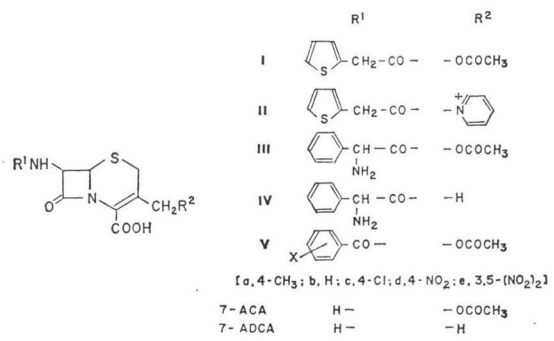
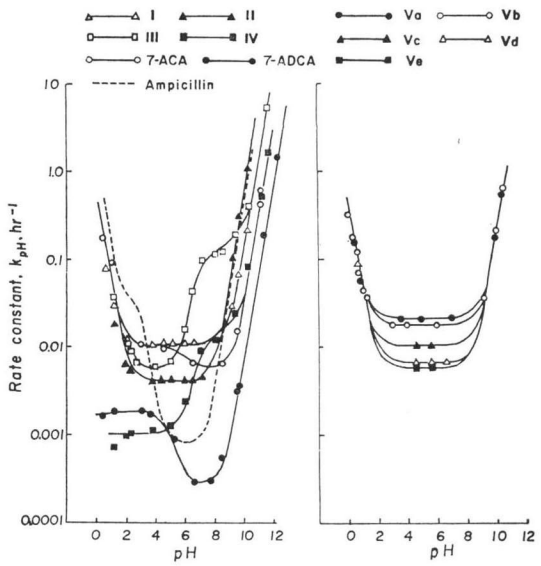


Fig. 1. Log k_{pH}—pH Profile for the hydrolysis of **I**, **II**, **III**, **IV**, 7-ACA, 7-ADCA, **Va**, **Vb**, **Vc**, **Vd**, **Ve** and ampicillin* at 35°C and μ=0.5.

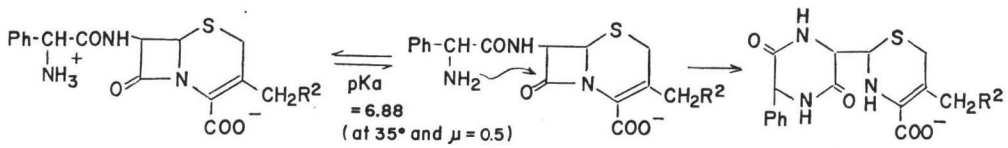
* The log k_{pH}—pH profile was taken from J.P. HOU & J. W. POOLE.[8]



It may be seen that below pH 2 all cephalosporins except **IV** and 7-ADCA undergo hydrolysis at almost the same rate regardless of the side-chain structure (R^1) and that they are *ca.* 3 times more stable than the most acid-stable penicillin, ampicillin (see Fig. 1). The hydrolyses exclusively consist of a specific hydrogen-ion catalyzed reaction. The acidic hydrolyses of **IV** and 7-ADCA were found to

* After this manuscript had been submitted for publication, a report on the alkaline hydrolysis of several cephalosporins (all rate constants were determined at only pH 10 and 35°C) has appeared.[12] INDELICATO, *et al.*[12] proposed the same mechanism that we suggested in this paper (see Chart 1), but were unsuccessful in attempts to isolate the compound, **VI**, from aqueous hydrolyses of **III** and **IV**.

Chart 1.



be independent of pH, and these deacetoxycephalosporins were shown to be fairly acid-stable, *e.g.*, 50 times more stable than the other cephalosporins and 100 times more stable than ampicillin[8] at pH 1.0.

Comparison of the reactivities of several cephalosporins in neutral and basic regions illustrates an apparent dependence on the nature of both substituents, R^1 and R^2.

The principal reaction for **I**, **II**, and **V** in the flat region extending from pH 3 to 8 is probably the result of the competition between the hydrolytic cleavage of the β-lactam bond by direct attack of water and that caused by intramolecular participation of the neighboring side-chain and amido-carbonyl groups. The former spontaneous hydrolysis is apparently *ca.* 10 times faster than that of penicillins. A similar relationship[6] observed for a pair of 7-ACA and 6-APA also reflects this high reactivity of cephalosporins toward water. The fact that the hydrolysis rates of **V** are significantly sensitive to the effects of the substituents supports the assumption that the latter reaction mechanism contributes to the hydrolysis. The introduction of an electron-donating substituent can facilitate the hydrolysis, whereas an electron-withdrawing group can retard it.

The rate profiles for the hydrolyses of **III** and **IV** shown in Fig. 1 are extraordinary for those of cephalosporins. The results are consistent with mechanisms involving intramolecularly catalyzed hydrolysis of the anionic **III** and **IV** at neutral pH, superseded in importance at high pH by hydroxide attack on the anionic antibiotics and at lower pH region from pH 3 to 5 by direct water-catalyzed hydrolysis of the β-lactams. At pH 8, the approximately 10 times higher rate of hydrolysis of **III** as compared with **I** is attributed to facilitation by the neighboring free α-amino group. When **III** was subjected to hydrolysis at pH 8.0, a strongly fluorescent yellow product was formed. The compound with the fluorescence at 433 nm (excitation at 355 nm) was easily extracted into ethyl acetate from the reaction solution which had been adjusted to pH 2.0 with HCl. A similar experiment with **IV** produced essentially identical results. This evidence strongly suggests the mechanism as shown in Chart 1 for intramolecular nucleophilic attack of the side-chain α-amino function on the β-lactam carbonyl to produce a fluorescent product, most probably the diketopiperazine derivative (**VI**). Compounds of this type have recently been isolated from the reaction solutions of cephalosporin esters[9] and cepharadine.[10] However, further kinetic analysis and isolation of the fluorescent product in the hydrolyses of **III** and **IV** will be required for direct confirmation of the suggested mechanism.*

The alkaline hydrolysis of **II** was found to be the fastest of all cephalosporins. The order (**II**>**III**, **I**>**IV**) of the alkaline hydrolysis rates of the therapeutically useful cephalosporins parallels the order of the degree of their antibacterial activities. This finding kinetically supports the idea[11] that high biological activity of β-lactam antibiotics is related to high reactivity of the β-lactam moiety toward nucleophiles.

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* See footnote on page 1000.

References

1) SCHWARTZ, M. A. & F. H. BUCKWALTER: Pharmaceutics of penicillin. J. Pharm. Sci. 51: 1119~1128, 1962
2) HOU, J. P. & J. W. POOLE: β-Lactam antibiotics: Their physicochemical properties and biological activities in relation to structure. J. Pharm. Sci. 60: 503~532, 1971
3) KAISER, R. F. & S. KUKO: Modification of the β-lactam system. *in* Cephalosporins and penicillins, chemistry and biology. E. FLYNN, ed. Academic Press, New York: pp. 125~131, 1972
4) SCHWARTZ, M. A.: Chemical aspects of penicillin allergy. J. Pharm. Sci. 58: 643~661, 1969
5) KONECNY, J.; E. FELBER & J. GRUNER: Kinetics of hydrolysis of cephalosporin C. J. Antibiotics 26: 135~141, 1973
6) YAMANA, T.; A. TSUJI & Y. MIZUKAMI: Kinetic approach to the development in β-lactam antibiotics. I. Comparative stability of semi-synthetic penicillins and 6-aminopenicillanic acid in aqueous solution. Chem. Pharm. Bull. (Tokyo) 22: 1186~1197, 1974
7) ALICINO, J. F.: Iodometric assay of natural and synthetic penicillins, 6-aminopenicillanic acid and cephalosporin C. Anal. Chem. 33: 648~649, 1961
8) HOU, J. P. & J. W. POOLE: Kinetics and mechanism of degradation of ampicillin in solution. J. Pharm. Sci. 58: 447~454, 1969
9) INDELICATO, J. M.; T. T. NORVILAS & W. J. WHEELER: Intramolecular nucleophilic attack in 7α-aminophenylacetoamidocephalosporin esters. J. Chem. Soc., Chem. Comm. 1972: 1162, 1972
10) COHEN, A. I.; P. T. FUNKE & M. S. PUAR: Alkaline degradation product of cepharadine. J. Pharm. Sci. 62: 1559~1561, 1973
11) HERMANN, R. B.: Structure-activity correlation in the cephalosporin C series using extended HUCKEL theory and CNDO/2. J. Antibiotics 26: 223~227, 1973
12) INDELICATO, J. M.; T. T. NORVILAS, R. R. PFEIFFER, W. J. WHEELER & W. L. WIHAM: Substituent effects upon the base hydrolysis of penicillins and cephalosporins. Competitive intramolecular nucleophilic amino attack in cephalosporins. J. Med. Chem. 17: 523~527, 1974