The infrared spectrum of a dilute solution of tetramethylenecyclobutane in carbon tetrachloride shows a sharp intense peak at 880 cm.$^{-1}$ which is near the characteristic region for the hydrogen out of plane deformations in terminal disubstituted olefins.[12] This band is replaced by one at 855 cm.$^{-1}$ when the carbon tetrachloride solution is allowed to stand for 24 hours at room temperature. The latter band may arise from terminal methylenic groups in low molecular weight polymers. Other characteristic bands in the infrared spectrum of tetramethylenecyclobutane appear at 3095 (w), 1705(w) and 1400 (m-w) cm.$^{-1}$. The absorption band at 1705 cm.$^{-1}$ also diminishes on polymerization while new bands appear at 1675, 1730 and 1640 cm.$^{-1}$.

Difficulties were encountered in all attempts to determine accurately the degree of unsaturation in Ia, by hydrogenation, because of problems associated with establishing accurately the concentration of the tetramethylenecyclobutane in dilute solution. Catalytic deuteration and mass spectral analysis were seen as a possible means of circumventing this problem. All experiments in this direction, however, were complicated by deuterium–hydrogen exchange. Similar exchange under a variety of solvent and catalyst conditions was noted in control experiments with cyclooctatetraene.

On warming to room temperature in air, or on prolonged storage at 0°, there deposited from a hexane solution of Ia polymeric material whose combustion analysis indicated incorporation of oxygen. Similar observations have been made by Blomquist on dimethylenecyclobutene, a related system.[11] Furthermore, the tendency to polymerize is in qualitative agreement with calculations of the "free valence index" for Ia.[2] A value of 0.88 was predicted at the termini of the methylene groups. For comparison, the predicted index for *p*-xylylene is 0.92, and while the latter is stable in the gas phase, it also polymerizes rapidly in solution.[2,13]

Pyrolysis of the tetraquaternary salt IIf[1] at temperatures between 120–250° provided low yields of a mixture which, from its ultraviolet spectrum, appears to contain Ia possibly contaminated with a dimethylenecyclobutene such as IVb. Similarly, the phosphonium salt IIg affords Ia on pyrolysis at 155°.[14] The product (Ia) which, from its ultraviolet spectrum, appears homogeneous, is obtained in only trace amounts from this precursor. Other studies on the physical and chemical properties of Ia are in progress.

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(13) C. A. Coulson, *Discussions Faraday Soc.*, **2**, 7 (1947); *J. Chim. Phys.*, **45**, 243 (1948).

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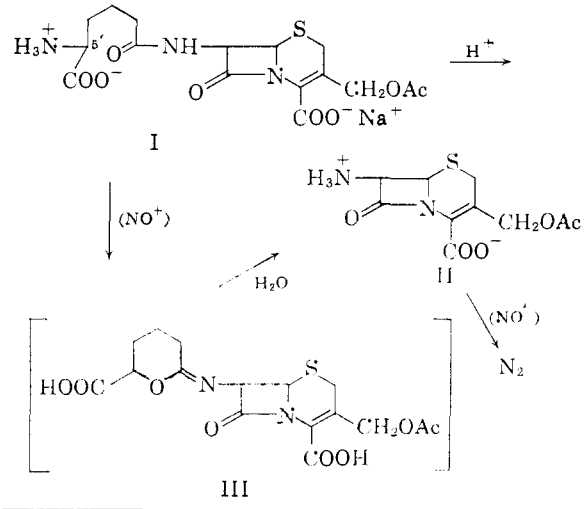
CHEMISTRY OF CEPHALOSPORIN ANTIBIOTICS. I. 7-AMINOCEPHALOSPORANIC ACID FROM CEPHALOSPORIN C

Sir:

Cephalosporin C (I),[1] an antibiotic isolated in 1956[2] from a species of *Cephalosporium*, possesses greater acid and penicillinase stability than other β-lactam containing antibiotics but much weaker antibacterial action. The antimicrobial activity can be enhanced greatly by N-acylation of 7-aminocephalosporanic acid (7-ACA, II) which has been obtained in a low yield by mild acid hydrolysis of cephalosporin C.[3] However, 7-ACA has not been sufficiently available to evaluate fully this interesting new class of antibiotics.[4]

We wish to report a convenient method of converting cephalosporin C to 7-ACA.

It was felt that the yield of 7-ACA from an acid or base catalyzed hydrolysis is limited by the lability of the molecule under these conditions. A mild, selective cleavage of the aminoadipyl amide might result from a reaction in which an intramolecular interaction of the amide with the C_5' center in cephalosporin C could occur.[5] Deamination of cephalosporin C causes a cleavage of the amide under mild conditions since treatment of the antibiotic as the sodium salt with excess nitrous acid in acetic acid–water solution gives two moles of nitrogen per mole of cephalosporin C; the second

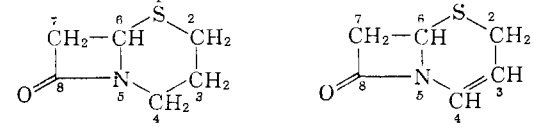


(1) E. P. Abraham and G. G. F. Newton, *Biochem. J.*, **79**, 377 (1961); D. C. Hodgkin and E. N. Maslen, *ibid.*, **79**, 393 (1961).

(2) G. G. F. Newton and E. P. Abraham, *ibid.*, **62**, 651 (1956).

(3) B. Loder, G. G. F. Newton and E. P. Abraham, *ibid.*, **79**, 408 (1961).

(4) As a convenience in naming new members of this series, we suggest, in collaboration with Dr. E. P. Abraham, the terms cepham and cephem for the structures i and ii.



i, cepham ii, Δ^3-cephem

This system is in accord with that generally accepted for the penicillins, see footnote 2, J. C. Sheehan, K. R. Henery-Logan and D. A. Johnson, *J. Am. Chem. Soc.*, **75**, 3293 (1953).

(5) For a review of facile cleavages of amide bonds by reactions which involve neighboring group participation see L. A. Cohen and B. Witkop, *Angew. Chem.*, **73**, 253 (1961).

mole of nitrogen presumably arises from reaction of the reagent with II, formed by hydrolysis of the iminolactone (III).[6] With anhydrous acetic acid and nitrosyl chloride the hydrolysis during deamination is avoided, and 7-ACA (II) can be isolated in 7% yield. The yield is raised to 40% by carrying out the reaction in formic acid which has the additional advantages of being a better solvent for I and having a greater volatility. Crystalline 7-ACA[7] is obtained in nearly analytical purity by evaporation of the reaction medium, addition of water and adjustment of the pH to 3.5 with dilute base. α-Hydroxyadipic acid can be isolated from the reaction in a yield corresponding to that of the amide-free product.

A detailed discussion of this work and of other products formed in this reaction will be reported in a subsequent publication. The present procedure provides practical quantities of 7-ACA for preparation of acylamidocephalosporanic acids, which are being evaluated as antibiotics.[8]

(6) We are indebted to E. E. Logsdon of these laboratories for this determination. For an analogous result with glutamine see A. T. Austen and J. Howard, *J. Chem. Soc.*, 3593 (1961).

(7) The identity of this was established through comparison with physical and chromatographic data supplied by Dr. E. P. Abraham in a personal communication. See also reference 3.

(8) See R. R. Chauvette, *et al.*, *J. Am. Chem. Soc.*, **84**, 3401 (1962).

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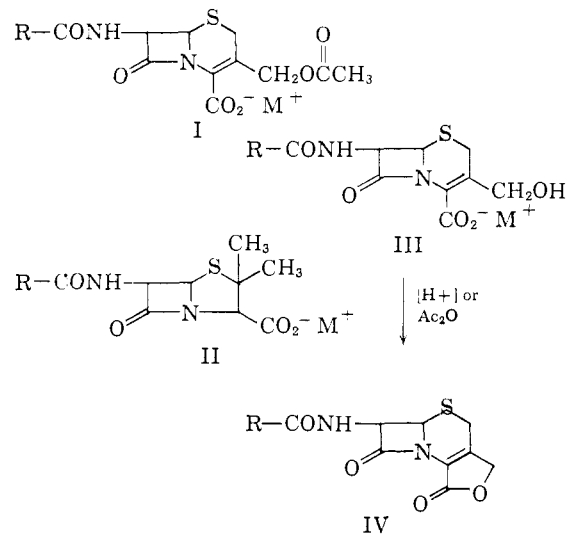
CHEMISTRY OF CEPHALOSPORIN ANTIBIOTICS. II. PREPARATION OF A NEW CLASS OF ANTIBIOTICS AND THE RELATION OF STRUCTURE TO ACTIVITY

Sir:

The coign of vantage resulting from discovery of a practical procedure for the preparation of 7-aminocephalosporanic acid (7-ACA) from cephalosporin C[1] has permitted synthesis of a large number of 7-acylamidocephalosporanic acids (I). This new class of antibiotics possesses a number of desirable attributes. They are very nontoxic (acute toxicity is less than, *e.g.*, benzylpenicillin; chronic toxicity studies, limited to two members, have borne out the lack of toxicity implied by acute studies), acid-stable, and unaffected by penicillinase. Two of the compounds described here have broad spectrum antibiotic activity as evidenced by tests with both Gram positive and Gram negative microörganisms *in vitro*, in animals,[2] and in human infections.

N-Acyl derivatives were prepared by the reaction of 7-ACA with the appropriate acid as its acid chloride, or as the mixed anhydride. N,N′-Dicyclohexylcarbodiimide also was used as mediating agent.[3] Products were isolated either as the sodium or potassium salts or, as a consequence of increased acid stability, as the free acids. The new derivatives (I) were converted by means of orange peel acetyl esterase[4] to the corresponding O-desacetates (III) which in turn afforded the lactones (IV) by treatment with acid or acetic anhydride.[5]



The cephalosporins were characterized by satisfactory analyses and titration (pK_a 4.8 ± 0.1). Each has an ultraviolet maximum near 260 mμ apparently attributable to the O=C–N–C=C– chromophore[6] of the ring. Infrared spectra of the salts in mineral oil mull show an N–H stretching band at about 3300 cm.$^{-1}$ and four carbonyls at about 1760 cm.$^{-1}$ (β-lactam), 1735 cm.$^{-1}$ (acetate—often not resolved from 1760 peak), 1650 cm.$^{-1}$ (amide) and 1600 cm.$^{-1}$ (carboxylate). The n.m.r. spectra were characteristic, well resolved, and readily interpretable. Details will be reported later.

A comparison of the new cephalosporanic acids (I) with cephalosporin C itself (I, R = D-$HO_2CCH(NH_2)(CH_2)_3$-) showed that replacement of the α-aminoadipyl radical with variously substituted acetyl groups led to greatly enhanced activity.

Of considerable interest was a comparison of the cephalosporanic acids with their penicillin congeners (II) (Table I). The penicillins (II) inhibited growth of typical *S. aureus* strain 209P at concentrations of 0.006–0.012 μg./ml., while the cephalosporins (I) were active at 0.02–0.04 μg. However, when four clinical isolates of penicillinase-producing *S. aureus* were used, the cephalosporins were active at 0.2–0.6 μg./ml., while greater than 50 μg. of benzylpenicillin was required.[7]

Perhaps of greatest moment was a gradient plate comparison using typical clinical isolates of

(1) R. B. Morin, B. G. Jackson, E. H. Flynn and R. W. Roeske, *J. Am. Chem. Soc.*, **84**, 3400 (1962).

(2) W. S. Bonnece, W. E. Wick, D. H. Holmes and C. E. Redman, in preparation.

(3) Y. G. Perron, W. F. Minor, C. T. Holdredge, W. J. Gottstein, J. C. Godfrey, L. B. Crast, R. H. Babel and L. C. Cheney, *J. Am. Chem. Soc.*, **82**, 3934 (1960).

(4) J. D'A. Jeffery, E. P. Abraham and G. G. F. Newton, *Biochem. J.*, **81**, 591 (1961).

(5) Results with series III and IV will be reported later.

(6) Evidence exists that the carboxyl group is not an essential feature of the chromophore.

(7) Studies reported by B. Loder, G. G. F. Newton and E. P. Abraham, *Biochem. J.*, **79**, 408 (1961), indicated enhancement of activity against *S. aureus* when the aminoadipyl function was replaced by, *e.g.*, phenylacetyl. Quantitative evaluation in a broad sense was prohibited by the small amounts of 7-ACA available.