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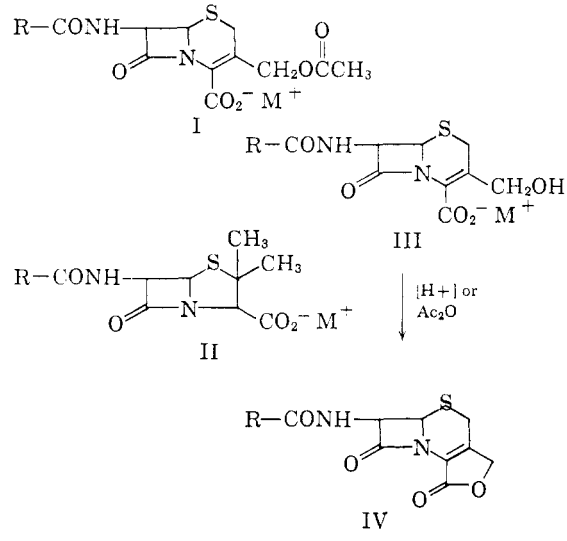
CHEMISTRY OF CEPHALOSPORIN ANTIBIOTICS. II. PREPARATION OF A NEW CLASS OF ANTIBIOTICS AND THE RELATION OF STRUCTURE TO ACTIVITY

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).

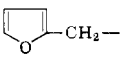
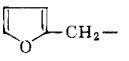
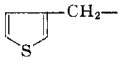
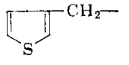
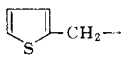
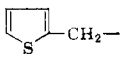
(5) Results with series III and IV will be reported later.

(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.

TABLE I

ANTIBACTERIAL ACTIVITY OF CEPHALOSPORANIC ACIDS AND PENICILLINS IN A GRADIENT PLATE TEST[8,a]

Compound	Organism,[b] μg. to inhibit						
	N9	N10	N26	X26	X68	K1	S1
II, R = $C_6H_5CH_2$–	32	48	42	109	42	13	44
I, R = $C_6H_5CH_2$–	33	37	24	2	15	27	27
II, R = $C_6H_5OCH_2$–	112	144	110	138	>200	>200	105
I, R = $C_6H_5OCH_2$–	107	132	101	7	110	>200	102
II, R = 	31	59	41	>200	84	13	40
I, R = 	15	36	10	7	8	11	17
II, R = 	15	33	25	105	38	10	26
I, R = 	31	33	17	2	7	20	8
II, R = 	28	46	38	122	56	10	39
I, R = 	11	15	8	6	5	8	11
I, R = D-$HO_2CCH(NH_2)(CH_2)_3$	75	104	79	38	48	37	73

[a] Results of this type test should be interpreted on a comparative basis only and require use of an internal standard for accuracy. [b] N9 = *Shigella sp.*; N10, N26 = *E. coli*; X26, K1 = *Klebsiella sp.*; X68 = *Aerobacter sp.*; S1 = *Sh. sonnei*.

several Gram negative organisms.[8] In the group of penicillins (Table I) no increase in Gram negative activity was observed over that exhibited by benzylpenicillin.[9] By contrast, cephalosporins with either thiophene-2-acetyl or furan-2-acetyl side-chains at position 7 showed at least a threefold enhancement of activity over benzylpenicillin. Further, *all* of the cephalosporanic acids showed as good or better action than the corresponding penicillins. It appears, therefore, that greater potential for Gram negative activity resides in the cephalosporin structure.

The sodium salt of 7-(thiophene-2-acetamido)-cephalosporanic acid, which has been given the generic name cephalothin, has received extensive clinical evaluation as a broad spectrum antibiotic. Results of this work will be reported later.

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(8) C. W. Godzeski, C. Brown, D. E. Pavey and J. McGowan in "Antimicrobial Agents and Chemotherapy—1961," Amer. Soc. for Microbiology, 1962, p. 547. See also C. W. Godzeski, G. Brier and D. E. Pavey, in preparation.

(9) A penicillin (II, R = D-$C_6H_5CH(NH_2)$ has been reported to have greater Gram negative activity than benzylpenicillin but to be ineffective vs. penicillin resistant staphylococci. See G. N. Rollinson and S. Stevens, *Brit. Med. Jour.*, (2), 191 (1961). We have prepared the analogous cephalosporanic acid (I, R = D-$C_6H_5CH(NH_2)$-) and find that it has substantial broad spectrum activity. However, the compound decomposed rapidly in aqueous solution so that quantitative comparisons of activity were unreliable.

CONJUGATE ANION FORMATION AND ALKYLATION OF α,β-UNSATURATED KETONES[1]

Sir:

Sodium and potassium salts of tertiary alcohols are efficient bases for the alkylation of α,β-unsaturated ketones in the α-position.[2] The system methyl iodide–potassium *t*-butoxide *t*-butyl alcohol, has been utilized extensively for the conversion of Δ^4-3-keto steroids (I),[3] as well as simple bicyclic derivatives,[4] into 4,4-dimethyl-Δ^5-3-ketones (VI), but certain aspects of this reaction are poorly understood. Alkylation even with a limited amount of base and alkyl halide leads to the 4,4-dimethyl compound (VII) as the major product and the 4-monomethyl-Δ^4-3-ketone (VI) as a minor product, indicating that the second alkylation step and/or tertiary carbanion formation proceeds more rapidly than the first alkylation step and/or secondary carbanion formation. Under special reaction conditions (slow addition of 1.2 equiv. of methyl iodide to the steroid and 1.5 equiv. of potassium *t*-butoxide in boiling *t*-butyl alcohol), Atwater[5] found that product formation can be reversed with monomethylation (VI) greater than dimethylation (VII) and suggested that the increased steric hindrance of methyl at high temperature was the deciding factor.

(1) Supported in part by grants A-4044 and CY-4550, U. S. Public Health Service.

(2) *Cf.* J. M. Conia and M. A. Le Craz, *Bull. Soc. Chim.*, 1327 (1960).

(3) (a) R. B. Woodward, A. A. Patchett, D. H. R. Barton, D. A. J. Ives and R. B. Kelly, *J. Am. Chem. Soc.*, **76**, 2852 (1954); (b) G. Cooley, B. Ellis and V. Petrow, *J. Chem. Soc.*, 2998 (1955); (c) H. J. Ringold and G. Rosenkranz, *J. Org. Chem.*, **22**, 602 (1957); (d) F. Sondheimer and Y. Mazur, *J. Am. Chem. Soc.*, **79**, 2906 (1957).

(4) M. Yanagita, M. Hirakura and F. Seki, *J. Org. Chem.*, **23**, 841 (1958).

(5) N. W. Atwater, *J. Am. Chem. Soc.*, **82**, 2847 (1960).