inversion of configuration. Allowing a 4.8% racemization for the chemical degradation of complex **9**, the stereospecificity of the oxidation step (**7** → **9**) was determined to be approximately 95% (compare the last three entries in Table I).

Inversion of configuration at the chiral carbon during the oxidative addition of **7** to either **1** or **4** suggests an SN2-type mechanism in which palladium(0) serves as a nucleophile. In the reaction of **7** and **4**, an alternative mechanism involving direct nucleophilic attack by the carbonyl group seems unlikely since metal carbonyls are known to be reactive toward bases.[41] Finally, invocation of a three- or four-centered mechanism which also allows pseudorotation to give configurational inversion is considered improbable on steric grounds.

Acknowledgment. This research was supported in part by a grant (GP 41267 X) from the National Science Foundation and in part by the donors of the Petroleum Research Fund, administered by the American Chemical Society. Grateful acknowledgment is made to the donors of this fund.

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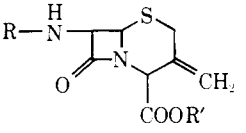
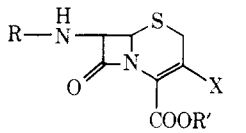
Chemistry of Cephalosporin Antibiotics. XXIX.[1] 3-Halo- and 3-Methoxy-3-cephems

Sir:

We have recently reported on the preparation of 3-methylenecephams[2] (**1a**) from cephalosporanic acids. Esters of 3-methylenecephams are isomerized to 3-methyl-3-cephems (**2a**), intermediates in syntheses[3] of cephalexin (**2b**, R = $C_6H_5CH(NH_2)CO$, R′ = H). Further exploration of the chemistry of 3-methylenecephams has led to the preparation of a new series of potent antibiotics having in common an electronegative heteroatom substitutent directly attached to the 3-position of the 3-cephem ring system.



1a, b



2a–c, X = CH_3 **5a, b**, X = Br
3a, b, X = OH **6a–c**, X = OCH_3
4a–c, X = Cl **7a**, X = CH_2OCOCH_3

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The key step in the preparation of these new antibiotics is a low temperature ozonolysis of the 3-exomethylene function in esters of 3-methylenecephams to produce a 3-"oxo"-cephalosporin.[4] Thus, *p*-nitrobenzyl 7-amino-3-methylenecepham-4-carboxylate hydrochloride (**1b**, R = H·HCl, R′ = *p*-NB) with ozone in CH_3OH at −80°, followed by addition of sulfur dioxide, afforded a 75% yield of a crystalline (from acetone) 3-"oxo"-nucleus ester hydrochloride, mp 150–180° dec. The free amino ester (crystalline from EtOAc) was characterized as *p*-nitrobenzyl 7-amino-3-hydroxy-3-cephem-4-carboxylate (**3a**, R = H, R′ = *p*-NB) by elemental analysis and the following data. Uv spectrum (pH 7 phosphate buffer) showed λ_{max} 278 nm (ϵ 13,700) consistent with 3-cephem unsaturation or enol form of the 3-"oxo" derivative. In further support of an enol–keto equilibrium, this uv maximum reversibly decreased in intensity at low pH. The nmr spectrum (DMSO-d_6) showed signals at τ 6.22 (ABq, 2 H, C2–H_2) as well as all other signals expected for the proposed 3-hydroxy-3-cephem structure. Electrometric titration (66% aqueous DMF) showed pK_a values of 4.0 and 6.3, consistent with amino and enolic hydroxyl groups.

Acylation of the 3-hydroxy nucleus ester hydrochloride (**3a**) with thiophene-2-acetyl chloride in aqueous acetone solution containing excess $NaHCO_3$ gave *p*-nitrobenzyl 7-(thiophene-2-acetamido)-3-hydroxy-3-cephem-4-carboxylate (**3b**, R = thiophen-2-ylacetyl, R′ = *p*-NB) in nearly quantitative yield. The uv spectrum of **3b** (CH_3CN) showed λ_{max} 238 and 268 nm (ϵ 16,850 and 14,750, respectively). The nmr spectrum ($CDCl_3$) showed signals at τ 6.60 (s, 2 H, C2–H_2), 6.13 (s, 2 H, α-CH_2), 4.96 (d, 1 H, C6–H), 4.62 (d, 2 H, ester–CH_2), 4.46 (q, 1 H, C7–H), and 3.1–1.7 (m, 8 H, aromatic H and C7–NH). The pK_a of the 3-enolic OH was found to be 5.9 by titration (66% aqueous DMF).

Compound **3b**, with freshly distilled $SOCl_2$ in dry DMF at room temperature, gave *p*-nitrobenzyl 7-(thiophene-2-acetamido)-3-chloro-3-cephem-4-carboxylate (**4a**, R = thiophen-2-ylacetyl, R′ = *p*-NB) in 61% yield, mp 164–166°, crystallized from EtOAc–ether. Similarly, compound **3b** reacted with PBr_3 in DMF to give the corresponding 3-bromo-3-cephem derivative (**5a**, R = thiophen-2-ylacetyl, R′ = *p*-NB).

On treatment with PCl_5 and dry pyridine in CH_2Cl_2 at room temperature, compound **4a** undergoes imino–chloride formation at C7-amide and subsequent side-chain cleavage.[5] The resulting 3-chloro nucleus ester (**4c**, R = H·HCl, R′ = *p*-NB) crystallized as a hydrochloride directly from the reaction mixture in 49% yield, mp 168° dec. Compound **4c** with a variety of acid chlorides or mixed anhydrides gave a series of 3-chloro-3-cephem derivatives.[6]

The 3-hydroxy nucleus ester hydrochloride (**3a**) reacted with diazomethane[4] at room temperature in THF solution containing 1 mol of triethylamine. In this

(4) Since submission of this publication, a report on the preparation of 3-"oxo"-cephalosporins and their reaction with diazomethane has appeared in the patent literature: *Chem. Abstr.*, 80, 83019 and 83019 (1974).
(5) (a) B. Fechtig, H. Peter, H. Bickel, and E. Vischer, *Helv. Chim. Acta*, **51**, 1108 (1969); (b) F. M. Huber, R. R. Chauvette, and B. G. Jackson in "Cephalosporins and Penicillins: Chemistry and Biology," E. H. Flynn, Ed., Academic Press, New York, N. Y., Chapter 2, 1972.
(6) A forthcoming publication will detail the preparation of these and other 3-halo- and 3-methoxy-3-cephems, their characterization and antimicrobial properties.

Table I. Gradient Plate Assay, Minimum Inhibitory Concentrations (MIC) Expressed in μg/ml

Compound	*Shigella* sp. N9	*Escherichia coli* N10	*Klebsiella pneumoniae* X26	*Aerobacter aerogenes* X68	*Salmonella heildelberg* X514	Penicillin resistant *Staphylococcus* V41	Penicillin resistant *Staphylococcus* V84
7a	14	15	1.0	3.0	1.0	0.5	0.5
4b	17.5	21.2	0.8	0.9	0.9	11.4	0.5
5b	19.8	20.8	1.0	1.0	1.0	12.0	0.5
6c	49.0	57.5	11.2	9.9	9.8	>20	4.0
2c	120	116	10	30	29	10	3.3

way the 3-methoxy nucleus ester (**6a**, R = H, R′ = *p*-NB) was obtained in 60% yield, mp 163–164°. The uv spectrum of **6a** (EtOH) showed λ_{max} 268 nm (ε 14,600). The nmr spectrum (DMSO-d_6) showed in addition to signals expected tor a 3-cephem, a 3-proton singlet at τ 6.20 for a methoxyl grouping at C3.

The 3-methoxy nucleus ester (**6a**) provided a variety of 7-acylamido derivatives[6] upon acylation. With thiophene-2-acetyl chloride for example, in aqueous acetone containing excess $NaHCO_3$, **6a** converted to **6b** (R = thiophen-2-ylacetyl, R′ = *p*-NB), mp 171–172°, in 60% yields. Compound **6b** crystallized from EtOAc and gave satisfactory physical data and elemental analysis.

The *p*-nitrobenzyl ester at C4 in **4a**, **5a**, and **6b** was removed by hydrogenolysis using an equal weight of 5% palladium on carbon in CH_3OH solution, at 50–60 psi and room temperature. Products **4b** (R = thiophen-2-ylacetyl, R′ = H), **5b** (R = thiophen-2-ylacetyl, R′ = H), and **6c** (R = thiophen-2-ylacetyl, R′ = H) crystallized upon trituration with ether. Physical data and elemental analyses for **4b**, **5b**, and **6c** were in agreement with the proposed structures. Compound **3b** could not be isolated as a 4-carboxylic acid due to spontaneous decarboxylation upon ester removal.

Table I compares the antimicrobial activity of compounds **4b**, **5b**, and **6c** with that of cephalothin (**7a**, R = thiophen-2-ylacetyl, R′ = H) and (**2c**, R = thiophen-2-ylacetyl, R′ = H). All three new compounds are especially more potent than the deacetoxy derivative **2c** with regard to inhibiting gram negative bacteria.

Since irreversible acylation of a transpeptidase enzyme important to bacterial cell wall synthesis[7] has been implicated as the mode of action of β-lactam antibiotics, it follows that the chemical reactivity of the β-lactam is an important parameter of biological effectiveness.[8] Reactivity of the penicillin β-lactam has been attributed to the strain caused by the 4–5 ring system.[9] In cephalosporanic acids, such as cephalothin, **7a**, reactivity of the β-lactam is enhanced by stabilization of the possible "enamine" resonance forms of the molecule.[9] The deacetoxy cephalosporins generally possess reduced antibiotic activity.

This new group of antibiotics with direct attachment of an electronegative substituent at C3 has equivalent biological activity to cephalothin (**7a**) and increased activity over the deacetoxy compound (**2c**). This activity can be attributed to an inductive effect on the β-lactam nitrogen causing a weakening of the β-lactam carbonyl nitrogen bond.

The potential utilization of these new cephalosporins for the treatment of human infections depends on many factors in addition to antibacterial potency and these are currently under investigation.

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(9) R. M. Sweet, ref 5b, pp 302–306.

Role of Acids in Reduction of Acetylene Catalyzed by Molybdenum–Thiol Complexes

Sir:

Recently Schrauzer, *et al.*, discovered that systems which consist of $NaBH_4$, molybdate, and a thiol compound (cysteine, glutathione, or thioglycerol) (designated as Mo–SH systems) can reduce unsaturated compounds such as C_2H_2, N_3^-, and N_2O.[1–6]

These systems were proposed[1–6] as close models of nitrogenase although they are much less active toward N_2[3] than are simpler heterogeneous[7] and homogeneous[8] Mo- or V-containing systems which reduce nitrogen to hydrazine and ammonia in water and water–alcohol solutions. (In agreement with the claims of ref 9, we were also unable to detect any reduction of N_2 to NH_3 with the Mo–SH systems.)

One characteristic feature of the Mo–SH systems is the stimulating effect of adenosine triphosphate (ATP) and other nucleoside phosphates on the reaction rate and yields of products. ATP is known to be a necessary participant of nitrogenase function, the hydrolysis of the macroergic O–P bond being coupled with electron transfer from reducing agent to a substrate. The mechanism of ATP function in nitrogenase is still obscure. Therefore Schrauzer's papers attracted much

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