Notes

Synthesis of 7α-Methoxycephalosporins

H. E. Applegate, J. E. Dolfini, M. S. Puar, W. A. Slusarchyk,* and B. Toeplitz

The Squibb Institute for Medical Research, Princeton, New Jersey 08540

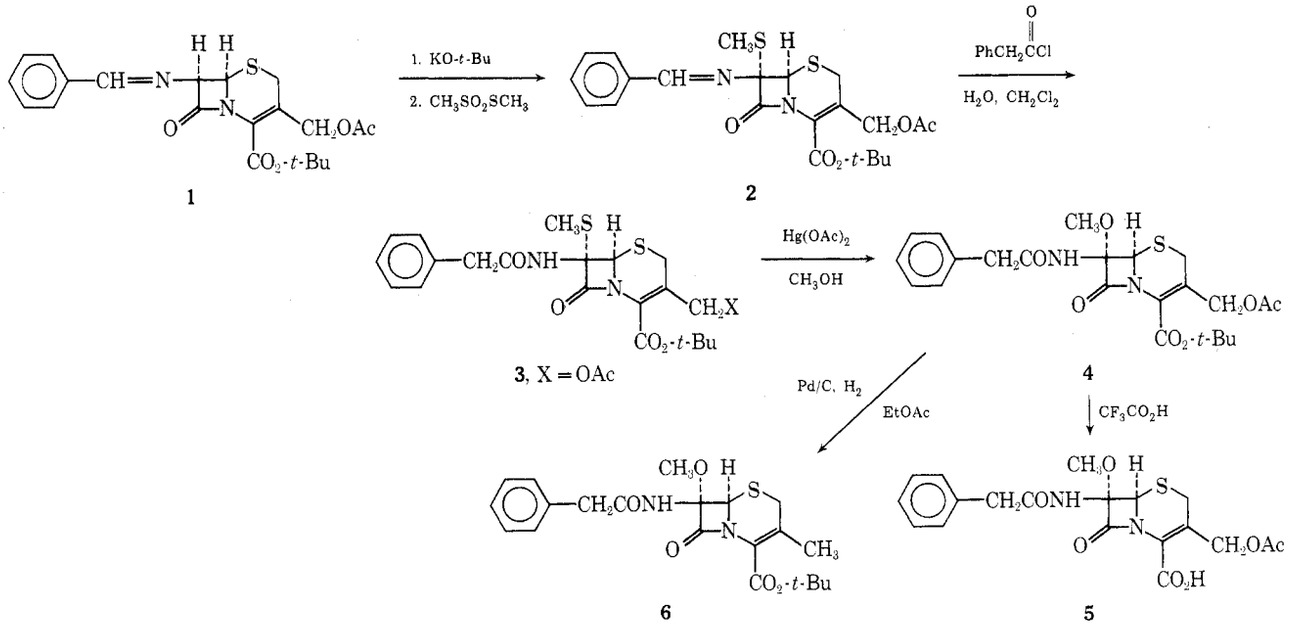
J. Z. Gougoutas

Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455

Received June 6, 1974

We have previously published a novel synthetic route to 7α-substituted cephalosporins and 6α-substituted penicillins,[1] and the volume of literature in this biologically important area is growing rapidly as a result of the work of numerous groups.[2] Our earlier publication reported that a Schiff base (*e.g.*, **1**) could be readily converted to an anion, then alkylated stereoselectively to give a predominance of the α-oriented substituent adjacent to the β-lactam carbonyl.[1a] X-Ray studies corroborated the assignments of structures, and confirmed the usefulness of studies of nuclear Overhauser effects (NOE) to facilitate stereochemical assignment.[1b] We have extended the earlier synthesis[3] of 7-methoxy-7-aminodeacetoxycephalosporins to compounds derived from a 7-aminocephalosporanic acid nucleus itself. The synthesis described here provides an extremely convenient method for synthesizing 7α-methoxycephalosporins.

7α-Methoxy-7-phenylacetamidocephalosporanic acid (**5**) was readily synthesized according to modifications of our previous published procedure, as shown in the sequence below.



The product **5** was found to be highly active against both gram-positive and gram-negative bacteria. Minimum inhibitory concentrations against several susceptible gram-positive and gram-negative organisms ranged from 0.1 to 10 μg/ml. Nuclear Overhauser effects were observed for various adjacent groups, but the close proximity of the absorption peaks prevented quantitation. Hydrogenolysis of the ester **4**, however, provided a corresponding deacetoxy analog (**6**) that was identical with the major 7-methoxy epimer (75% yield), which we had obtained by mercuric acetate–methanol solvolysis of the deacetoxy analog of **3** (X = H), but was assigned the *7β-methoxy orientation* on the basis of NOE studies.[3] To resolve this ambiguity, we resorted to an X-ray crystallographic determination of the major 7-methoxy epimer resulting from treatment of the deacetoxy analog of **3** with mercuric acetate–methanol.

The molecular geometry indicated clearly that the methoxy group is α, or cis, to the hydrogen at the 6 position.

The 7α-methoxy free acid derived from **6** was inactive at 100 μg/ml against the gram-negative and gram-positive microorganisms tested. The NOE values previously reported for compound **6** were OCH_3-6-H, 5%, and NH-6-H, 10%. These values have been confirmed by a repeat study on the original sample. A solution of a freshly prepared and purified sample, however, gave opposite values: OCH_3-6-H, 16%, and NH-6-H, 5%. The reason for the anomalous results observed with the original sample is not yet known.

Although there has been no question of the correctness of assignments of other 7α-methoxy structures previously reported,[2b,c] this study represents the first confirmation of the absolute configuration of a 7-methoxycephalosporin with marked microbiological activity.

Experimental Section

The pmr spectra were obtained on Varian nuclear magnetic resonance spectrometers (Models T-60 and XL-100-15), and chemical shifts are reported on the τ scale, with tetramethylsilane used as an internal standard. Perkin-Elmer spectrometers (Models 257 and 621) were used to measure infrared spectra, and mass spectra were obtained from an AEI-MS-902 mass spectrometer. Melting points are uncorrected.

7α-Methylthio-7-benzaliminocephalosporanic Acid *tert*-Butyl Ester (2). The 7α-methylthio Schiff base **2** was prepared in a facile manner by modification of the previously reported procedure, which provided **2** in 21% yield and required extensive chromatography. The improved procedure affords **2** as a crystalline

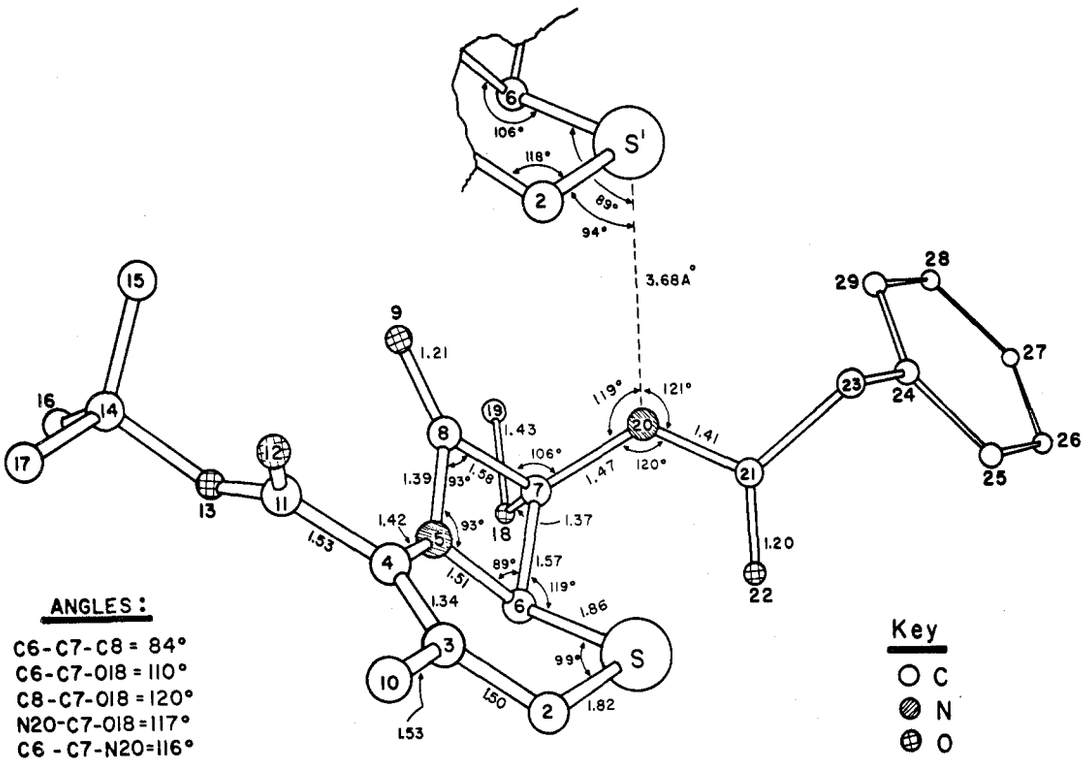


Figure 1.

product in 50% yield (85% yield as determined from pmr integrations of crude material).

To a stirred solution of Schiff base **1** (23.8 g, 57 mmol) in dimethoxyethane (530 ml, freshly distilled from $LiAlH_4$) at −50 to −60° under N_2 was added sublimed potassium *tert*-butoxide (6.13 g, 57 mmol). The dark-red solution was stirred for 3 min. Methyl methanethiolsulfonate (7.03 g, 57 mmol) in dimethoxyethane (10 ml) was added, and the mixture was stirred for 50 min at −50°. The dark mixture was poured into ice-cold 0.2 *M* pH 6.6 phosphate buffer (1500 ml) and extracted with $CHCl_3$ (3 × 700 ml). The $CHCl_3$ extract was washed with saturated NaCl solution, dried (Na_2SO_4), and evaporated *in vacuo* to a residue, which crystallized readily from CH_3OH to give 13.2 g (50% yield) of 7α-methylthio Schiff base **2** having mp 125–126° and spectral properties as previously described. Further quantities of **2** could be obtained by chromatography of the mother liquor on silica gel, using $CHCl_3$–hexane (9:1) as solvent.

7α-Methylthio-7-phenylacetamidocephalosporanic Acid *tert*-Butyl Ester (3, X = OAc). **3** (X = OAc) was prepared in gram quantities, as previously described.

7α-Methoxy-7-phenylacetamidocephalosporanic Acid *tert*-Butyl Ester (4). To 7α-methylthio *tert*-butyl ester **3** (449 mg, 0.91 mmol) in dry CH_3OH (4 ml) under N_2 was added mercuric acetate (291 mg, 0.91 mmol). The mixture was stirred at room temperature for 40 min and evacuated *in vacuo* to a residue. The residue was washed repeatedly with $CHCl_3$, and the $CHCl_3$ extract was washed with water (4 × 50 ml), dried (Na_2SO_4), and evaporated to a residue (410 mg). Further purification was effected by tlc chromatography on silica gel (three PQIF plates, 20 cm × 40 cm × 1 mm) in the system $CHCl_3$–hexane (9:1), which provided **4** as a colorless residue (202 mg, 47% yield): pmr ($DCCl_3$) τ 8.48 (9 H, s, *tert*-butyl) 7.93 (3 H, s, *O*-acetyl), 6.83, 6.43 (2 H, AB q, J = 19 Hz, C-2), 6.55 (3 H, s, OCH_3), 6.30 [2 H, s, $PhCH_2(C{=}O)N$], 5.22, 4.92 (2 H, AB q, J = 14 Hz, C-3 methylene), 4.93 (1 H, s, C-6), 3.27 (1 H, broad s, NH), and 2.6 (5 H, s, aromatics); ir ($CHCl_3$) 1782 (β-lactam C=O), 1730 (broad, ester C=O's), and 1695 cm^{-1} (amide C=O); mass spectrum, weak molecular ion at *m/e* 476.

7α-Methoxy-7-phenylacetamidocephalosporanic Acid (5). To 7α-methoxy *tert*-butyl ester **4** (128 mg, 0.27 mmol) in a stoppered flask at 0° was added trifluoroacetic acid (5 ml). The flask was removed from the ice bath and allowed to warm to room temperature over the course of 15 min, during which time the stopper was loosened to release pressure. The trifluoroacetic acid was removed *in vacuo*, and the residue was taken up in $CHCl_3$–H_2O. The pH was adjusted to 7.5 with aqueous $NaHCO_3$ and, after shaking, the $CHCl_3$ layer was removed. Fresh $CHCl_3$ was added to the aqueous layer, and the pH was adjusted to 2.0 with 1 *N* HCl. Solid NaCl was added, and the acid layer was extracted repeatedly with $CHCl_3$. The combined $CHCl_3$ extracts were dried (Na_2SO_4) and evaporated to give 72 mg (63% yield) of crude acid **5**: pmr ($DCCl_3$–CD_3OD) τ 7.92 (3 H, s, *O*-acetyl), 6.80, 6.43 (2 H, AB q, J = 19 Hz, C-2), 6.53 (3 H, s, OCH_3), 6.30 [2 H, s, $PhCH_2(C{=}O)N$], 5.12, 4.82 (2 H, AB q, J = 14 Hz, C-3 methylene), 4.92 (1 H, s, C-6), and 2.6 (5 H, s, aromatics); ir ($CHCl_3$) 1780 (β-lactam C=O), 1735 (broad, acid and ester C=O's), and 1690 cm^{-1} (amide C=O); mass spectrum, no molecular ion but peaks at *m/e* 360 (M − CH_3COOH) and 205 [$C_6H_5CH_2C(=O)NHC(OCH_3)=C=O$]; mass spectrum molecular ion of trimethylsilyl ester at *m/e* 492.

Recrystallization from acetone–hexane provided crystals, mp 161–162°.

Anal. Calcd for $C_{19}H_{20}N_2O_7S$: C, 54.28; H, 4.80; N, 6.66; S, 7.62. Found: C, 53.74; H, 5.13; N, 6.29; S, 7.85.

Hydrogenolysis of 7α-Methoxy-7-phenylacetamidocephalosporanic Acid *tert*-Butyl Ester (4) to 7α-Methoxy-7-phenylacetamidodeacetoxycephalosporanic Acid *tert*-Butyl Ester (6). Prior to hydrogenolysis, 7α-methoxy *tert*-butyl ester **4** was dissolved in EtOAc and filtered through charcoal. The ester **4** (132 mg) and 10% palladium on charcoal (530 mg) in 10 ml of EtOAc was shaken with hydrogen at 35 psi for 3 days at room temperature. The catalyst was removed by filtration, and the EtOAc was evaporated to a residue. Silica gel tlc in the system hexane–$CHCl_3$ (1:1), followed by pmr analysis, indicated a mixture of esters **4** and **6**. Preparative silica gel tlc in the system hexane–$CHCl_3$ (1:1) provided two major components, the less polar of which yielded 22 mg of **6**, mp 168–170°, on crystallization from CH_3OH. This sample and the major epimer from the mercuric acetate methanolysis of the deacetoxy analog of **3** were found to be identical in comparisons of pmr, ir (KBr), mixture melting point, and silica gel tlc [EtOAc–hexane (1:1)].

X-Ray Determination of the Structure of 6. Crystals of **6** from methanol were found to be monoclinic with a = 23.14, b = 5.796, c = 17.69 Å, β = 116.0°, d_{meas} = 1.33 g/cm^3, and space group $C2$ with Z = 4. All of the nonhydrogen atoms were located by Patterson and Fourier methods based on 1070 symmetry-independent intensities measured on a Syntex $P2_1$ automatic diffractometer (Cu Kα, λ = 1.542 Å). Least-squares refinements of all coordinates (except y for S) and individual isotropic temperature parameters reduced the conventional R factor to its present value of 0.09 for

the 865 observed intensities.[4] The fractional atomic coordinates relative to a twofold axis through the origin are given in Table I;[4] Figure 1 presents bond distances and agles in the solid-state molecular conformation.

Although no attempt was made to locate the hydrogen atoms, the positions of all but the methyl hydrogens may be defined within small limits. The molecular geometry clearly indicates that the methoxy group is α, or cis, to the hydrogen attached to C-6. The sulfur atom is displaced by 0.76 Å from the least-squares plane (P1) of the five other atoms of the dihydrothiazine ring (rms displacement of these five atoms 0.06 Å). The dihedral angle between P1 and the least-squares plane (P2) through the four atoms of the β-lactam ring (rms displacement 0.00 Å) is 30°. The least-squares plane (P3) of the exocyclic amide and benzylic carbon atoms, N-20, O-22, C-21, C-23, defines dihedral angles of 53 and 108° with P2 and the plane of the phenyl ring, respectively. The peptide-like hydrogen bonding between the exocyclic amide groups of neighboring molecules commonly found in crystal structures of cephalosporin derivatives[5] is not present in this crystal structure, nor is any other type of (N-20)–H···O hydrogen bonding evident. Instead, the (N-20)–H bond points directly toward the sulfur atom, S^1, of a (*b*) translationally related molecule (see Figure 1 for the intermolecular geometry of this approach), with S^1 displaced by only 0.67 Å from the exocyclic amide plane, P3.

Acknowledgment. We thank Dr. P. Funke for obtaining mass spectra, Mr. J. Alicino for combustion analyses, and Mr. H. Basch for microbiological data.

Registry No.—**1**, 36954-82-2; **2**, 37787-02-3; **3** (X = OAc), 37787-03-4; **4**, 51932-72-0; **5**, 51932-73-1; **6**, 37786-97-3.

Supplementary Material Available. Tables of fractional coordinates and isotropic temperature factors will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JOC-74-2794.

References and Notes

(1) (a) E. H. W. Bohme, H. E. Applegate, B. Toeplitz, J. E. Dolfini, and J. Z. Gougoutas, *J. Amer. Chem. Soc.*, **93**, 4324 (1971). (b) E. H. W. Bohme, H. E. Applegate, J. B. Ewing, P. T. Funke, M. S. Puar, and J. E. Dolfini, *J. Org. Chem.*, **38**, 230 (1973).
(2) *Vide, inter alia*, (a) R. A. Firestone, N. Schelechow, D. B. R. Johnston, and B. G. Christensen, *Tetrahedron Lett.*, 375 (1972); (b) L. D. Cama, W. J. Leanza, T. R. Beattie, and B. G. Christensen, *J. Amer. Chem. Soc.*, **94**, 1408 (1972); (c) J. E. Baldwin, F. J. Urban, R. D. G. Cooper, and F. L. Jose, *ibid.*, **95**, 2401 (1973); G. A. Koppel and R. E. Koehler, *ibid.*, **95**, 2403 (1973).
(3) W. A. Slusarchyk, H. E. Applegate, P. T. Funke, W. Koster, M. S. Puar, M. Young, and J. E. Dolfini, *J. Org. Chem.*, **38**, 943 (1973). *Cf.* T. Jen, F. Frazee, and J. R. E. Hoover, *ibid.*, **38**, 2857 (1973).
(4) See paragraph at end of paper regarding supplementary material.
(5) R. M. Sweet in "Cephalosporins and Penicillins Chemistry and Biology," E. H. Flynn, Ed., Academic Press, New York, N. Y., 1972, pp 300–301.

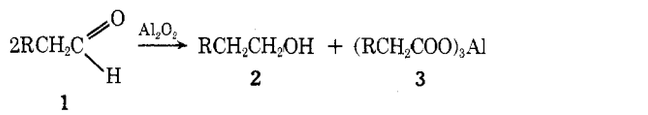
Oxidation–Reduction of 9-(*p*-Methoxyphenyl)-9-fluorenylacetaldehyde on Activated Alumina

Frederick A. Lamb, Philip N. Cote, Bruce Slutsky, and Bruno M. Vittimberga*[1]

Department of Chemistry, University of Rhode Island, Kingston, Rhode Island 02881

Received July 17, 1973

During a study of the alumina-catalyzed condensation reactions of aldehydes, we found that when 9-(*p*-methoxyphenyl)-9-fluorenylacetaldehyde (1) is chromatogrammed in the usual manner through a column prepared with activated alumina (Woelm neutral, activity grade I, pH 7.5), it undergoes an oxidation and a reduction reaction, reminiscent of the Cannizzaro reaction, yielding, in approximately equal amounts, the alcohol **2**, mp 107.5–109°, and a salt tentatively assigned the aluminum salt **3**.[2] The overall yield for the reaction was 90% based on converted aldehyde.



1 **2** **3**

R = [9-(*p*-methoxyphenyl)-9-fluorenyl]

Structure **2** was readily assigned on the basis of infrared and nmr analysis. The infrared spectrum of **3** is typical of a carboxylate salt. For identification purposes **3** was converted to the parent acid (**4**), mp 193–195°, by treatment with 5% sulfuric acid and extraction with ether. The parent acid was then identified by the usual spectral methods. Final confirmation of structure was obtained by synthesis. Reduction of **1** with sodium borohydride yields **2** (94%) while oxidation with silver oxide yields **4** (89%). The identity of these compounds with those obtained from the column was established by comparison of ir and nmr spectra and by mixture melting point determination.

The results were found to be reproducible. The length of the column and the amount of alumina used did not affect the course of the reaction.[3] Elution was carried out with mixtures of hexane, benzene, chloroform, ether, and methanol in increasing polarity and finally with 5% acetic acid–methanol solution. Unreacted aldehyde was eluted in early chloroform fractions followed by **2**. The compound **3** was isolated from the column with 5% acetic acid–methanol elution. Apparently the alumina surface serves a unique role in this conversion, since the base-catalyzed reaction of **1** under the usual Cannizzaro reaction conditions gave no reaction.[4]

The alumina oxidation of alcohols to carboxylic acids at elevated temperatures[5,6] and the reduction of benzaldehydes to benzyl alcohols[7] have previously been reported in the literature. Very recently, Kuiper, *et al.*,[8] reported a spectral study of benzaldehyde adsorbed on alumina. They observed bands in the infrared and Raman spectra which they attributed to benzoate and benzyl alcoholate bonds formed on the alumina surface. Products were not isolated, though benzyl alcohol was detected by gas chromatography.

The present study reports the first instance in which both oxidation and reduction products have been isolated from a column reaction.

It would be rather premature to attempt to describe a precise mechanism at this time without additional information. However, a general mechanism can be postulated using Peri's description of the γ-alumina catalyst.[9] According to this description, the alumina surface is composed of exposed aluminum ions, oxide groups, and hydroxide groups of various basicity.

If one aldehyde group reacts with an oxide group while a second molecule reacts at a neighboring exposed aluminum ion site, hydride transfer would occur as shown in Scheme I.[10,11] Upon elution, the catalyst surface undergoes an exchange reaction, releasing **2** and **3**.

It is interesting to note that 9-carbazolylacetaldehyde, which is substituted with a less bulky group at the α carbon, undergoes an alumina-catalyzed condensation reaction instead.[12] The condensation may require enol formation, which is more favorable in the carbazole compound than in **1**.[13]

The mechanism of this reaction is under investigation and results will be reported at a later time.