1-Chloro-5-phenyl-2-pentanone: bp 127–132 °C (7 Torr), solidified and melted at 26–29 °C; n^{30}_D 1.5211; NMR (Me_2SO-d_6) δ 1.84 (q, 2 H, $-COCH_2CH_2CH_2C_6H_5$), 2.41 (m, 4 H, $-COCH_2CH_2CH_2C_6H_5$), 3.88 (s, 2 H, $-COCH_2Cl$), 7.20 (s, 5 H, $-C_6H_5$). Anal. ($C_{11}H_{13}C_{10}$) C, H. 1-Chloro-6-phenyl-2-hexanone: bp 111–116 °C (3 Torr); n^{20}_D 1.5230; NMR (Me_2SO-d_6) δ 1.47 (m, 4 H, $-COCH_2CH_2CH_2CH_2C_6H_5$), 2.40 (m, 4 H, $-COCH_2-CH_2CH_2CH_2C_6H_5$), 3.85 (s, 2 H, $-COCH_2Cl$), 7.13 (s, 5 H, $-C_6H_5$). Anal. ($C_{12}H_{15}ClO$) C, H. 1-Chloro-3-phenyl-2-propanone,[16] 1-chloro-4-phenyl-2-butanone,[16,17] and 1-chloro-3-heptanone[18] physical constants were in agreement with literature values.

7*H*-Pyrimido[4,5-*b*][1,4]oxazines (8-Oxadihydropteridines) (1). The 2-amino-4-hydroxy-6-ω-phenylalkyl derivatives **1g–1j** and the 6-pentyl derivative **1k** were synthesized by the same general procedures. A suspension of 2.06 g (0.01 mol) of 2,5-diamino-4,6-pyrimidinediol[11] hydrochloride sesquihydrate in 500 mL of 1:1 ethanol–water was heated under reflux, and an appropriate α-halo ketone (0.02 mol) dissolved in 25 mL of ethanol was added dropwise. After 10–15 min, 25 mL of aqueous sodium bicarbonate (1.68 g, 0.02 mol) was added, heating was continued for 6 h, and the mixture was cooled overnight. The precipitated 8-oxadihydropteridine was separated by filtration, washed, and dried under reduced pressure. Physical constants and spectral data of all new compounds are summarized in Table II.

The 2,4-diamino derivatives **2** were synthesized from 2,4,5-triamino-6-pyrimidinol using a previously described procedure.[12]

Dihydrofolate Reductase Assay. The enzyme was obtained as previously described,[14] except that centrifugation in an International refrigerated centrifuge Model B-20 at 10000*g* and 2 °C for 0.5–1 h was used to separate precipitated protein instead of filtration through Celite. After a threefold dilution, 50 μL of enzyme solution gave an optical density change of 0.0062 unit/min.

For a typical assay, 50 μL of dihydrofolate reductase solution and 75 (reference cuvette) or 100 μL (sample cuvette) of 0.744 mM TPNH were mixed with sufficient buffer (0.05 M Tris, pH 7.4; 10 mM mercaptoethanol; and 1 mM EDTA) to make a total volume of 3 (reference cuvette) or 2.95 mL (sample cuvette). The enzymatic reaction was initiated by the addition of 50 μL of 0.186 mM dihydrofolic acid solution to the sample cuvette, and the decrease in absorbancy at 340 nm was recorded.[19] Solutions of the analogues were prepared by dissolving them in 25% *N,N*-dimethylformamide or 0.002 N sodium hydroxide and adjusting the pH to 7.4 if necessary. A maximum of 1 mL of inhibitor solution was used in the assay.

Acknowledgment. The authors express appreciation to the Samuel Roberts Noble Foundation for a research fellowship to D.L.D. and to the National Cancer Institute (Grant CA-18610) for partial support of this study.

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Synthesis and Antibacterial Activity of 2-Oxocephalosporins

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Received November 10, 1978

The first reported synthesis of 2-oxocephalosporin derivatives has been achieved via ozonolysis of the corresponding 2-methylenecephalosporins. The new cephalosporin derivatives showed some antibacterial activity against Gram-positive bacteria, but the 2-oxo analogue of cephalothin was much less active than cephalothin itself.

In a search for unique and potent cephalosporin antibiotics, we have prepared a series of 2-oxocephalosporins. By total synthsis, Woodward and co-workers prepared a 2-oxocepham[1] which lacks the crucial Δ^3 double bond.[2] We anticipated that the 2-keto group in conjugation with the Δ^3 double bond would modify the reactivity of the β-lactam carbonyl and thereby enhance the antibacterial activity of this class of cephalosporin derivatives.[3]

Chemistry. The 2-oxocephalosporins were prepared by a brief treatment of 2-methylenecephalosporins[4] with ozone at low temperatures as outlined in Scheme I. Prolonged reaction times resulted in ozonolysis of the Δ^3 double bond. Cleavage of the phenoxyacetyl group of compound **2a** was accomplished by the procedure of Peter and Bikel[5] to afford the key intermediate **4**, from which new 2-oxocephem derivatives could be synthesized as exemplified by the preparation of compound **6**. 2-Oxocephalosporin derivatives were found to be quite stable under acidic conditions. For example, the treatment of the 2-oxocephalosporin diphenylmethyl ester **2a** with trifluoroacetic acid and anisole produced the free acid **3a** in high yield. However, 2-oxocephalosporins are quite labile under basic

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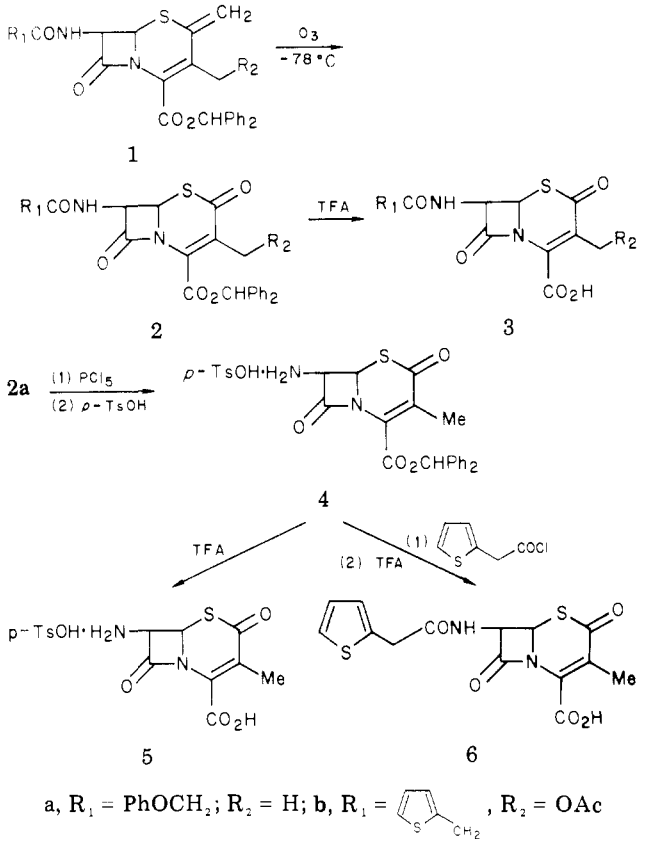
Scheme I



a, R_1 = $PhOCH_2$; R_2 = H; b, R_1 = , R_2 = OAc

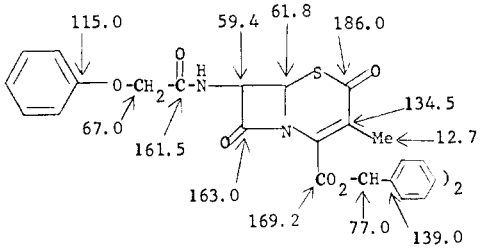


Figure 1. ^{13}C NMR (ppm) in $CDCl_3$ of compound 2a.

conditions and attempts to prepare the sodium salt of **3a** were not successful under a variety of conditions. The half-life of compound **3a** in 5% sodium bicarbonate in D_2O at room temperature was estimated to be approximately 2 min by following the decomposition with NMR spectroscopy.

As expected, all compounds showed a shift in UV absorption maxima to longer wavelengths (314–318 nm) than normal cephalosporins (around 260 nm)[6] due to the conjugation of the 2-carbonyl with the Δ^3 double bond. Similarly, the absorption of the β-lactam carbonyl of the 2-oxocephems in the infrared is shifted to higher wavenumbers (e.g., 1795 cm^{-1} for **3b** vs. 1775 cm^{-1} for cephalothin).[4] The characteristic change in the chemical shift of the C-6 proton in the NMR spectra[7] supported the assigned structure. The peak assignments for the ^{13}C NMR spectrum of compound **2a** are shown in Figure 1.

Biological Results. The new cephalosporins **3a**, **3b**, **5**, and **6** were tested in vitro against several strains of Gram-positive and Gram-negative bacteria, and the results are shown in Table I. Neither derivative having a 3-methyl substituent (**3a** and **6**) displayed significant antibacterial activity under the test conditions. The incorporation of a 3-acetoxymethyl led to enhanced activity relative to the corresponding 3-methyl (**3b** vs. **6**). The 2-oxo analogue of cephalothin, **3b**, was substantially less active than cephalothin itself. Since 2-oxocephems seem to be quite unstable under basic conditions, it is not known whether the rather poor antibacterial activity observed is due to instability, inappropriate 3- and/or 7-substituted side chains, or is inherent in the nucleus itself.

Table I. Antibacterial Activities of 2-Oxocephalosporin Derivatives[a]

organism	strain no.	Cephalothin	3a	3b	5	6
Str. pneumoniae	1985	0.06	32	1	63	63
Str. pyogenes	9604	0.13	32	1	125	125
Staph. aureus	9537	0.13	8	4	32	8
Staph. aureus & 50% serum	9537	8	>63	63	>63	>63
Staph. aureus (Pen-Res)	9606	0.5	125	16	>125	>125
Staph. aureus (Meth-Res)	15097	32	>125	>125	125	>125
Str. faecalis	20688	32	63	125	32	63
Pr. mirabilis	9900	1	125	32	>125	63
Pr. mirabilis	9716	2	125	63	125	63
Pr. rettgeri	21205	63	>125	>125	125	>125

[a] The in vitro antibacterial activities are reported as minimum inhibitory concentrations (MIC) in μg/mL. The MIC were determined by a twofold serial dilution technique in nutrient broth. The MIC for all compounds was $\geq$125 for the following organisms: *K. pneumoniae* 20468; *Pr. morganii* 15153; *Ser. marcescens* 20019; *Ent. cloacae* 9659, 9656; *Ps. aeruginosa* 9843. For the following organisms, the MIC of cephalothin were 16–32 and the MIC of 3a, 3b, 5, and 6 were >125: *E. coli* 15119, 20341; *K. pneumoniae* 15130.

Experimental Section

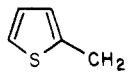
Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The IR spectra were recorded on a Beckman 5240 spectrophotometer using KBr pellets; NMR spectra were obtained on a Varian HA-100 spectrometer using Me_4Si as an internal standard. The IR and NMR data on all compounds were consistent with the assigned structure. The ^{13}C NMR spectrum of compound **2a** was obtained on a Varian XL-100A in $CDCl_3$. The ozone was generated by a Welsbach Model T-23 ozonizer with an output of 1.1 mmol of O_3/min. No attempt was made to monitor the uptake of ozone. Analyses are indicated only by symbols of the elements and were within ±0.4% of the calculated figures.

Diphenylmethyl 3-Methyl-2-oxo-7-(phenoxyacetamido)-ceph-3-em-4-carboxylate (2a). Into a cooled (–78 °C) solution of 3.0 g (5.7 mol) of diphenylmethyl 3-methyl-2-methylene-7-(phenoxyacetamido)ceph-3-em-4-carboxylate (**1a**)[4] in 40 mL of CH_2Cl_2–MeOH (4:1) was bubbled ozone with a flow rate of 1.1 mmol/min for 18 min. Thin-layer chromatography (silica gel, 4:1 CH_2Cl_2–EtOAc) indicated no starting material was left. The reaction was purged with nitrogen for 5 min, then the cooling bath was removed, and the reaction was allowed to warm to room temperature over 15 min and kept 30 min. Evaporation of solvents under reduced pressure and chromatography of the residue over silica gel using a CH_2Cl_2–EtOAc gradient elution solvent system gave 1.67 g (56%) of **2a** as colorless amorphous powder: IR 1795 (β-lactam), 1725 (ester), 1680 (amide), 1645 cm^{-1} (thiolactone); NMR ($CDCl_3$) δ 2.10 (s, 3 H, methyl), 4.65 (s, 2 H, $PhOCH_2$), 5.85 (d, J = 4.5 Hz, 1 H, 6-H), 6.05 (q, J = 4.5 and 9.0 Hz, 1 H, 7-H), 6.9–7.7 (m, 17 H); UV λ_{max} (EtOH) 318 nm (ϵ 7450). Anal. ($C_{29}H_{24}N_2O_6S$) C, H, N.

3-Methyl-2-oxo-7-(phenoxyacetamido)ceph-3-em-4-carboxylic Acid (3a). The 2-oxocephem ester **2a** (220 mg, 0.42 mmol) was treated with 3 mL of trifluoroacetic acid and 0.5 mL of anisole for 3 min at 5 °C. All volatile materials were evaporated under high vacuum and the residual oil was triturated with Et_2O–*n*-pentane to give 140 mg (92%) of **3a** as a colorless amorphous powder: NMR ($CDCl_3$) δ 2.05 (s, 3 H, methyl), 4.60 (s, 2 H, OCH_2), 6.10 (q, J = 4.5 and 11.5 Hz, 1 H, H-7), 5.75 (d,

J = 4.5 Hz, 1 H, H-6), 6.8–7.4 (m, 5 H); IR 1790 (β-lactam), 1725 (ester), 1680 (amide), and 1640 cm^{-1} (thiolactone); UV λ_{max} (EtOH) 318 nm (ε 6870). Anal. ($C_{16}H_{14}N_2O_6S$) C, H, N.

3-(Acetoxymethyl)-2-oxo-7-(2-thienylacetamido)ceph-3-em-4-carboxylic Acid (3b). The preparation was carried out in the same way as described for **3a**, starting from diphenylmethyl 3-(acetoxymethyl)-2-methylene-7-(2-thienylacetamido)ceph-3-em-4-carboxylate (**1b**). The overall yield from compound **1b** to compound **3b** was 42%: NMR ($CDCl_3$) δ 2.00 (s, 3 H, OAc), 3.78 (s, 2 H, i), 4.72 (d, J = 14.5 Hz, 1 H), 4.95 (d, J = 14.5 Hz, 1 H),



i

5.70 (1 H, q, J = 4.5 and 9.0 Hz, H-7), 6.05 (d, J = 4.5 Hz, H-6), 6.9–7.4 (m, 3 H); IR 1795 (β-lactam), 1735 (ester), 1680 (amide), 1640 cm^{-1} (thiolactone); UV λ_{max} (EtOH) 316 nm (ε 5950). Anal. ($C_{16}H_{14}N_2O_7S$) C, H, N.

Diphenylmethyl 7-Amino-3-methyl-2-oxoceph-3-em-4-carboxylate *p*-Toluenesulfonic Acid Salt (4).[5] To a solution of 1.06 g (2 mmol) of the 2-oxocephem **2a** in 30 mL of CH_2Cl_2 was added 1.9 mL (24 mmol) of pyridine. The solution was cooled to −20 °C, then 1.24 g (6 mmol) of PCl_5 was added in one portion, and the mixture was stirred for 30 min at −10 °C and then 30 min at 0 °C under a nitrogen atmosphere. The reaction was then cooled to −78 °C and a solution of 10 mL of MeOH in 15 mL of CH_2Cl_2 was added dropwise, keeping the reaction temperature below −50 °C. After 5 min of stirring below −50 °C, the reaction was stirred at −30 °C for 60 min and then allowed to warm to 0 °C over 15 min. Aqueous NaH_2PO_4 (0.5 M, 20 mL) was then added, and the reaction mixture was vigorously stirred for 30 min at 0 °C. The reaction was extracted with 30 mL of CH_2Cl_2, and the organic layer was dried ($MgSO_4$). Evaporation of the solvent afforded a yellow viscous oil, which was dissolved in 5 mL of acetone and treated with 300 mg (1.5 mmol) of *p*-toluenesulfonic acid monohydrate. Dilution with 70 mL of ether afforded 610 mg (54%) of compound 4 as a colorless amorphous solid: IR 1785 (β-lactam), 1725 (ester), 1640 cm^{-1} (thiolactone); NMR δ 2.05 (s, 3 H, methyl), 2.35 (s, 3 H, methyl), 4.5–5.5 (br, 4 H), 5.75 (d, 1 H, J = 4.5 Hz, 6-H), 7.05 (s, 1 H, $CHCPh_2$), 7.2–7.8 (m, 14 H); UV λ_{max} (EtOH) 316 nm (ε 5660). Anal. ($C_{28}H_{26}H_2O_7S_2$) C, H, N.

7-Amino-3-methyl-2-oxoceph-3-em-4-carboxylic Acid *p*-Toluenesulfonic Acid Salt (5). To a cooled solution of 3 mL of trifluoroacetic acid and 0.5 mL of anisole was added 200 mg (0.35 mmol) of compound 4 in one portion, and the reaction mixture was stirred for 3 min. All solvents were evaporated under reduced pressure, affording a yellow oil which was triturated with 7 mL of Et_2O to obtain 135 mg (93%) of compound **5** as a colorless amorphous powder: IR 1785 (β-lactam), 1710 (ester), 1640 cm^{-1} (thiolactone); NMR (Me_2SO-d_6–D_2O) δ 2.00 (s, 3 H, methyl), 2.30 (s, 3 H, H_3CPh), 5.30 (d, J = 5.0 Hz, 1 H, H-7), 6.05 (d, J = 5.0 Hz, 1 H, H-6), 7.18 (d, J = 10.0 Hz, 2 H, phenyl), 7.52 (d, J = 10.0 Hz, 2 H, phenyl); UV λ_{max} (EtOH) 316 nm (ε 5500). Anal. ($C_{15}H_{16}N_2O_7S_2$) C, H, N.

3-Methyl-2-oxo-7-(2-thienylacetamido)ceph-3-em-4-carboxylic Acid (6). To a solution of 250 mg (0.45 mmol) of compound **4** in 5 mL of CH_2Cl_2 was added at 0 °C 60 mg (0.6 mmol) of triethylamine and 60 mg of dimethylaniline, followed by 80 mg (0.5 mmol) of 2-thienylacetyl chloride in 0.5 mL of CH_2Cl_2, and the mixture was stirred for 60 min at 0 °C under nitrogen. The reaction was diluted with 30 mL of CH_2Cl_2 and washed with ice-cold 10% H_3PO_4 and saturated sodium bicarbonate. The organic layer was dried ($MgSO_4$) and then the solvent was removed under reduced pressure to give a yellow oil. This was purified by chromatography on a column of 20 g of silica gel using a CH_2Cl_2–EtOAc gradient elution solvent system to give 157 mg of a colorless oil. Generation of acid was achieved by treatment of this oil with trifluoroacetic acid (3 mL) and anisole (0.5 mL) for 3 min at 0 °C. Evaporation of all volatile materials under high vacuum and trituration of the residual oil with ether produced 82 mg (52%) of **6** as a colorless amorphous powder: IR 1792 (β-lactam), 1725 (ester), 1660 (amide), 1640 cm^{-1} (thiolactone); NMR ($CDCl_3$) δ 2.05 (s, 3 H, methyl), 3.91 (s, 2 H, i), 5.92 (q, J = 5.0, 8.5 Hz, 1 H, H-7), 6.10 (d, J = 5.0 Hz, 1 H, H-6), 6.9–7.4 (m, 3 H); UV λ_{max} (EtOH) 314 nm (ε 5700). Anal. ($C_{14}H_{12}N_2O_5S_2$) C, H, N.

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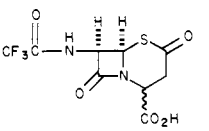

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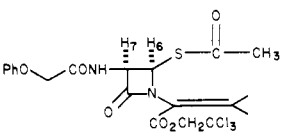

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