was poured on to cracked ice and extracted into 2 *N* HCl and the acid extract poured slowly on to cracked ice–NH_3 solution: product: yellow crystals; 85%; mp 93.5–95.5°. Dihydroquininone was prepared similarly in 80% yield, mp 94–96° from C_4H_9Cl. The tertiary alcohols from these ketones by addition of MeLi are described in Table III.

Epoxidation Route (for Two-Carbon Side Chains). 2-Phenyl-4-(2-propenyl)quinoline.—Freshly fused $KHSO_4$ (0.28 mol) pulverized with 0.057 mol of α,α-dimethyl-2-phenyl-4-quinolinemethanol (see Table I) was held in an oil bath at 170° for 4 hr with occasional stirring of the mixture. The mixture was cooled, made strongly alkaline, and extracted (Et_2O); the residue [bp 167° (0.13 mm), 14 g, 86%] was a viscous, pale yellow oil; nmr ($CDCl_3$) δ 7.2–8.3 (10, m, aromatic H), 5.1 and 5.4 (2, m, vinyl H), 2.2 (3, d, CH_3); HCl salt, mp 180–182°, needles from *i*-PrOH.

2-(4-Chlorophenyl)-6,8-dimethyl-4-(2-propenyl)quinoline was prepared as above from 0.14 mol of 2-(4-chlorophenyl)-6,8-α,α-tetramethyl-4-quinolinemethanol except that the reaction mixture was held at 180° for 6 hr: product: 40 g; 93%; mp 110–112°; golden crystals from MeOH (and Norit), mp 114–115°; nmr ($CDCl_3$) δ 7.4–8.3 (m, 7, aromatic H), 5.15 and 5.5 (m, 2, vinyl H), 2.87 and 2.48 (s, 3, aromatic CH_3) 2.25 (d, 3, vinyl CH_3).

2-(4-Chlorophenyl)-6,8-dimethyl-4-(2-epoxypropyl)quinoline.—The alkene (0.016 mol) above and 0.0735 mol of freshly prepared monoperoxyphthalic acid solution[14] in 115 ml of Et_2O were held at 25° for 6 days. The phthalic acid was filtered, and the filtrate was washed with four 70-ml portions of 5% aqueous $NaHCO_3$: product: 4.8 g; 91%; mp 135–140°; from MeOH fine, yellow crystals, mp 143–144°; nmr ($CDCl_3$); δ 7.45–8.4 (m, 7, aromatic H), 2.85 and 2.5 (s, 3, aromatic CH_3), 3.1 (q, 2, CH_2), 1.85 (s, 3, CH_3).

Similar treatment of 2-phenyl-4-(2-propenyl)quinoline gave 67% (crude) of 4-(2-epoxypropyl)-2-phenylquinoline 1-oxide, yellow crystals from C_6H_6–C_6H_{14}, mp 140.5–141.5°.

2-(4-Chlorophenyl)-α-(dialkylaminomethyl)-6,8-α-trimethyl-4-quinolinemethanols.—The corresponding epoxide (0.025 mol) and 0.4 mol of dialkylamine were stirred magnetically under N_2 in a simple Parr pressure vessel at 140° for 72 hr (with Me_2NH, 50 ml of DMF was used as the solvent). The mixture was steam distilled to remove excess secondary amine, and the residue extracted into 10% aq HCl which was then basified. If the precipitate was a solid, it was filtered, washed, and recrystallized (MeOH). If an oil, it was dissolved in Et_2O and precipitated as the dihydrochloride with HCl. Results are shown in Table IV.

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Chemical Conversion of Desacetylcephalothin Lactone into Desacetylcephalothin. The Final Link in a Total Synthesis of Cephalosporanic Acid Derivatives

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Desacetylcephalothin lactone was rapidly hydrolyzed to desacetylcephalothin in 10–20% yield in phosphate and borate buffers (pH 6–9, 100°), in borate and carbonate buffers (pH 9–10.8, 25°), and in 0.01 *N* KOH (25°). In addition, other uncharacterized products were formed. The desacetylcephalothin formed was isolated as its cephalothin Me ester after esterification and acetylation. These findings establish the final link in the total synthesis of cephalosporins by the lactone route.

The total synthesis of compounds related to the antibiotic cephalosporin C (Ib) has been a concern of several laboratories.[1]

Two possible routes to these compounds have been described employing intermediate lactone derivatives with the general structure II in which R is one of several different amino, amido, or imido groups.[1a,d] Compounds of this type have been synthesized from cephalosporanic acid derivatives[2] and are now available by totally synthetic means.[1a,d] However, no chemical method for opening the lactone ring without simultaneous destruction of the β-lactam ring has been available for the conversion of II into III.[3] In addition, the possibility of microbial hydrolysis has been deemed unlikely.[4]

We report, in this paper, a simple chemical procedure for performing this transformation in yields of 10–20% (estimated biologically).

Studies were performed with pure desacetylcephalothin lactone (IIa) prepared from cephalothin[5] (Ia) by a modification of the procedure of Chauvette and Flynn.[6] Hydrolysis of the lactone was accomplished by adding IIa in DMSO to a buffer of appropriate pH at 25–100°, or to dilute KOH at 25° for 10 min or less. The reaction mixture was chromatographed in parallel with standard IIIa,[6] then bioautographed on *Staphylococcus aureus* 209P.[7] Both residual IIa and product IIIa were thus visualized, and yield estimates could be made.

Conversion of IIa into IIIa occurred in phosphate, borate, and carbonate buffer of pH 6–11 maintained at 25–100° for 2–10 min. Except in pH 11.5 KOH solution where 2 min was optimal, at least 10-min incubations were required to obtain product at temperatures below 50°. Buffers prepared from strong nucleophiles such as imidazole, glycine, and mercaptoethanol caused destruction of IIa, with no detectable IIIa formed, under a variety of conditions of temperature and pH. It should be emphasized that at pH 6–8, heating was required to bring about the conversion of IIa into IIIa,

(1) (a) R. Heymes, G. Amiard, and G. Nomine, *C. R. Acad. Sci.* (Paris), **263**, 170 (1966); (b) R. B. Woodward, *Science*, **153**, 487 (1966); (c) E. Galantay, H. Engel, A. Szabo, J. Fried, *J. Org. Chem.*, **29**, 3560 (1964); (d) J. E. Dolfini, J. Schwartz, and F. Weisenborn, *ibid.*, **34**, 1582 (1969).

(2) (a) S. Kukoija, *J. Med. Chem.*, **11**, 1067 (1968); (b) B. Loder, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, **79**, 408 (1961); (d) R. R. Chauvette, E. H. Flynn, B. G. Jackson, E. R. Lavagnino, R. B. Morin, R. A. Mueller, R. P. Pivch, R. W. Roeske, C. W. Ryan, J. L. Spencer, and E. V. Heyningen, *J. Amer. Chem. Soc.*, **84**, 3401 (1962); (d) R. R. Chauvette and E. H. Flynn, *J. Med. Chem.*, **9**, 741 (1966).

(3) K. Heusler in "Topics in Pharmaceutical Sciences," Vol. 1, D. Perlman, Ed., Interscience Publishers, New York, N. Y., 1968, p 33.

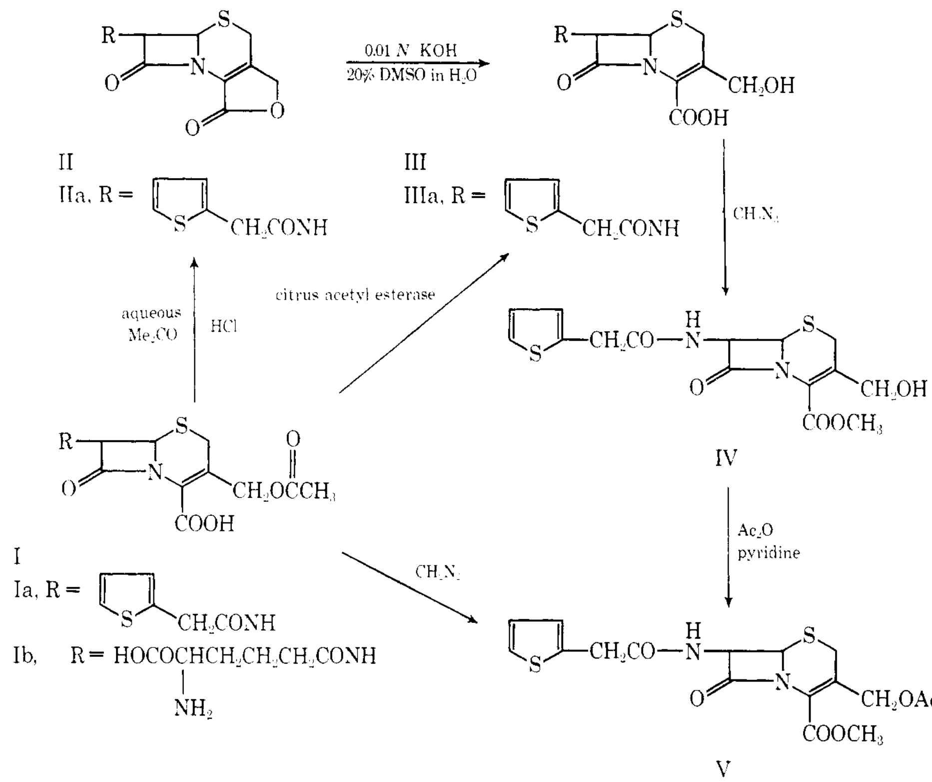
(4) E. P. Abraham, ref 3, p 1.

(5) Cephalothin is the generic name given to 7-[2-(2-thienyl)-acetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid.

(6) (a) J. D'A. Jeffery, E. P. Abraham, and G. G. F. Newton, *Biochem. J.*, **81**, 591 (1961); (b) E. Van Heyningen, *J. Med. Chem.*, **8**, 22 (1965); (c) We thank Dr. R. C. Erickson, of this Institute, for preparing IIIa from Ia with citrus acetyl esterase.

(7) E. Meyers and R. C. Erickson, *J. Chromatogr.*, **26**, 531 (1967).

SCHEME I



while at pH 9–10.8, and in 0.01 N KOH, the conversion proceeded at 25°.

As the pH was increased, the reaction time for optimum yield decreased, indicating a dependence of the reaction on the concentration of OH^- ion. Probably the saponification of the lactone is a second-order reaction and not due to a first-order solvolysis mechanism. Cocker and coworkers have shown more rigorously that cephalosporin lactones do not solvolytically react with nucleophiles in contrast with a variety of cephalosporanic acid derivatives with substituted Me groups that do.[8]

It should be pointed out that chromatograms of all reaction mixtures showed other uv-absorbing products in addition to IIIa. These were all inactive against *S. aureus* 209P. The major inactive product, which gave a positive starch–I_2 reaction without KOH treatment,[9] is possibly IIa with its β-lactam ring cleaved. As judged from the intensity of its uv absorption, it was estimated to represent the degradation of over 50% of IIa. This derivative was not studied further.

Compound IIIa, prepared from either IIa by the procedure of this paper or Ia with citrus acetyl esterase, suffered extensive decomposition during tlc on silica gel or cellulose, as well as upon elution from these chromatograms. Accordingly, to prove that the product formed from IIa by our method, in fact, was IIIa, a stable derivative had to be prepared. This was accomplished as shown in Scheme I.

Compound IIIa was converted into its Me ester IV with CH_2N_2.[10] Compound IV, however, readily reverted to IIa upon elution from cellulose tlc, a reflection of the known instability of such compounds.[11] If IV was treated with Ac_2O in pyridine, cephalothin Me ester V was obtained. Compound V was sufficiently stable to be recovered in satisfactory yield from cellulose tlc.

It is worthy of note that some isomerization of the double bond to the Δ^2 compound has been observed during the pyridine–Ac_2O esterification of the CH_2OH group of desacetylcephalosporins. That the compound isolated in our work was indeed the authentic Δ^3 isomer was indicated by direct comparison with cephalothin Me ester prepared by reaction of cephalothin with CH_2N_2. The compounds showed identical chromatographic mobilities, ir and uv spectra, and melting points (with undepressed mixture melting point).

Our results represent the first reported conversion of a cephalosporin lactone II into an intact hydroxy acid III. Since a method for the transformation of desacetylcephalosporanic acids to cephalosporins has been published previously,[12] our findings supply the final link in the total synthesis of cephalosporins by the lactone route.

Experimental Section

Desacetylcephalothin Lactone[2d] (IIa).—To a solution of 550 mg of the Na salt of Ia in 6 ml of H_2O were added 5 ml of Me_2CO and 2 ml of concentrated HCl. The reaction mixture was stirred at room temperature. The lactone IIa continuously precipitated from solution, and, after 2 hr, 194 mg of IIa was collected by filtration; after an additional 10 hr, another 214 mg of IIa was obtained. The total yield of IIa was 408 mg (77%). This material was further purified to remove residual traces of Ia. To the crude product IIa in 3 l. of Me_2CO–EtOAc (1:2) was added 10 ml of 5% aq $NaHCO_3$, followed by 500 ml of H_2O. The mixture was shaken vigorously, then the phases were allowed

(8) J. D. Cocker, B. R. Crowley, J. S. O. Cox, S. Earsdley, G. I. Gregory, J. K. Lazenby, A. G. Long, J. L. P. Sly, and G. A. Somerfield, *J. Chem. Soc.*, 5015 (1965).

(9) R. Thomas, *Nature*, **191**, 116 (1961).

(10) J. W. Chamberlin, and J. B. Campbell, *J. Med. Chem.*, **10**, 966 (1967).

(11) E. Van Heyningen, *ibid.*, **8**, 27 (1965).

(12) Glaxo Labs Ltd., British Patent 52,288 (1964).

to separate. The upper layer, containing IIa completely free of residual unreacted Ia, was concentrated *in vacuo* on a rotary evaporator at room temperature. Crystalline IIa separated, mp 228–230° dec (lit. mp 230–232°[2d]). *Anal.* ($C_{14}H_{12}N_2O_4S_2$) C, 49.94; H, 3.57; N, 8.32. Found: C, 49.74; H, 3.72; N, 8.23.

Hydrolysis of Desacetylcepthalothin Lactone.—IIa (1 mg) was dissolved in 0.2 ml of DMSO and added to 0.8 ml of 0.25 *M* KH_2PO_4–K_2HPO_4 buffer, pH 8.0. The reaction mixture was heated to 100° for 5 min, then rapidly cooled to 25°. An identical mixture, kept at 25°, was also prepared. The reaction mixtures, authentic IIIa made from Ia by the action of citrus acetyl esterase, and a control of unreacted IIa were chromatographed on Whatman No. 1 paper with EtOAc–*n*-BuOH–H_2O (2:5:1). The chromatogram was air dried and bioautographed against *S. aureus* 209P. A zone of growth inhibition that was detected in the heated reaction mixture corresponded to authentic IIIa (R_f = 0.03), with no residual IIa. The major uv-absorbing product (R_f = 0.1) gave no growth inhibition. In the unheated reaction mixture, only unreacted IIa was observed (R_f = 0.86).

The yield of IIIa in our conversion was estimated by comparison of the diameter of zones of growth inhibition obtained with the heated sample and various amounts of authentic IIIa. The results indicated a 10–20% transformation. A similar yield was obtained fom a 2-min incubation of a DMSO solution of IIa in 0.01 *N* KOH at 25°.

These same procedures were followed for the experiments indicated in Table I. The data in Table I were obtained by chromatographing aliquots of the reaction mixtures after 0.5, 1, 2, 5, and 30 min, along with known concentrations of authentic IIIa.

TABLE I

OPTIMUM REACTION TIME FOR CONVERSION OF IIA into IIIA[a]

Buffer[b] or KOH	pH	Temp (°C)	Time (min)
KH_2PO_4–K_2HPO_4	7.0	100	5
KH_2PO_4–K_2HPO_4	7.5	100	2
KH_2PO_4–K_2HPO_4	8.0	100	1
0.01 *N* KOH	11.5	25	0.5

[a] Visualized by bioautography with *S. aureus* 209P. [b] Buffers at 0.25 *M*.

Cephalothin Methyl Ester (V).—To a solution of 150 mg of IIa in 30 ml of DMSO was added 120 ml of ice-chilled 0.01 *N* KOH. After 2 min at 25°, the reaction mixture was adjusted to pH 5 with 7.5 ml of 0.2 *M* AcOH to stop the reaction. Paper chromatography (as above) of the resulting solution showed IIIa, as visualized by its uv-absorption and antibacterial activity.

This solution was extracted three times with 300-ml portions of $CHCl_3$ to remove most of the DMSO. To the resultant aqueous phase was added 80 g of $(NH_4)_2SO_4$; this solution was extracted twice with 180-ml portions of a mixture consisting of EtOAc–Me_2CO–H_2O (5:5:1). Paper chromatography showed that IIIa was in the solvent phase. The pooled solvent phase was evaporated *in vacuo* to a syrup (mainly residual DMSO). Evaporation was repeated twice at 60°, after the addition of 25 ml of abs EtOH to remove any H_2O. The volume of syrup was 9 ml. It was dissolved in 40 ml of dry EtOAc (Na_2SO_4). To this solution was added an excess of CH_2N_2 in Et_2O. After 2 min at 25°, excess CH_2N_2 was decomposed by the addition of 0.5 ml of glacial AcOH. The resulting solution was washed twice with 25-ml portions of H_2O to remove DMSO. Partition chromatography, performed as follows on Whatman No. 1 paper, showed that IV was in the EtOAc layer. The procedure was: (1) 1 vol of the lower (aqueous) phase of a mixture of C_6H_6–AcOH–H_2O (4:1:4) was diluted with 3 vol of Me_2CO; (2) paper strips spotted with the sample were drawn through this solution, then air dried for 2.5 min; (3) the strips were then developed by descending chromatography with the upper phase of the solvent mixture (*vide supra*) in a chamber saturated with lower phase. Compound IV, detected by its uv absorption and antibacterial activity, moved with R_f = 0.55; authentic IV, prepared by the action of CH_2N_2 on IIIa, obtained by the reaction of Ia with citrus acetyl esterase, had the same R_f. Compound IIa moved on the same chromatogram to an R_f of 0.42, well separated from IV.

The EtOAc solution was brought to dryness *in vacuo*. The residue was taken up in 14 ml of pyridine, and 7 ml of Ac_2O was added; after allowing the mixture to stand at 25° overnight, 5 ml of MeOH and then 25 ml of $CHCl_3$ were added. The resulting solution was washed with 50 ml of 3 *N* HCl and then twice with 25-ml portions of H_2O. Tlc, run in the following manner, showed V to be in the $CHCl_3$ phase. A precoated MN-300 cellulose GF plate (Analtech) was irrigated with a mixture of propylene glycol–MeOH (3:7), as if developing an ascending chromatogram. The plate was then air dried for 15–30 min to remove the MeOH prior to spotting with sample. The plate was developed in an ascending fashion with propylene glycol saturated PhMe. Compound V, detected by its uv absorption and antibacterial activity, moved with R_f = 0.60; authentic V prepared by treating IV with CH_2N_2 had the same R_f.

The $CHCl_3$ solution was concentrated *in vacuo* and chromatographed on 12 MN-300 plates (20 × 20 cm) in the manner described above. Compound V, visualized as an uv-absorbing band, was eluted with MeOH–$CHCl_3$ (1:1).[13] The eluate was partitioned between equal vol of $CHCl_3$ and 50% (v/v) aq MeOH. The $CHCl_3$ phase was washed twice with equal vol of 25% aq MeOH and then dried (Na_2SO_4). On evaporating the $CHCl_3$ solution to dryness *in vacuo*, 9 mg of crude crystalline V was obtained. Recrystallization from MeOH gave V, which exhibited an ir spectrum in $CHCl_3$ that was identical with that of authentic V prepared from Ia.

(13) The probable, but unproved, presence of Δ^2 isomer was indicated by the observation of a slightly slower moving band (R_f = 0.53).