Electrochemical Reduction of Cephalosporanic Acids

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Electrochemical reduction of cephalosporanic acid derivatives (I) bearing various substituents at the 3-position gave the corresponding 3-methylenecepham derivatives (II), a new class of cephalosporins. Reductive opening of the 3-hydroxymethyl-3-cephem-4-carboxylic acid lactone (IV) was effected to give (II) by cathodic reaction. The 3-methylenecepham derivatives (II) were readily isomerized to the 3-methyl-3-cephem derivatives (III) providing a new synthesis of cephalexin [7-(D-2-amino-2-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid] (IIIc). The reaction mechanism is discussed on the basis of deuteriation and polarography of the cephalosporins involved.

In a preliminary communication,[1] we reported that electrochemical reduction of cephalosporanic acid derivatives (I) produced 3-methylenecepham derivatives (II),†‡ a novel class of cephalosporins, which were isomerized to the corresponding 3-methyl-3-cephem derivatives (III), and provided an alternative route to cephalexin (IIIc).[1] Although extensive work[4] has been done on cephalosporin chemistry, to our knowledge no report has appeared on the electrochemistry of cephalosporins.§ The present electrochemical reduction produces a new class of cephalosporins, 3-methylenecephams (II) under mild conditions, and this paper describes further application of this reaction to a wide range of cephalosporins and considers the mechanism.

RESULTS AND DISCUSSION

Preparative Electrolysis.—A typical experimental procedure utilizes a cell with two compartments which are separated from one another by sintered glass. A mercury pool serves as the cathode and a horizontal platinum sheet as the anode. The results of the attempted electroreduction of various cephalosporanic acid derivatives (Ia—f) to the corresponding 3-methylene-

† The 3-methylenecepham derivatives (II) have also been synthesized by Cr^{II} reduction of (I) in our laboratories.[2]

‡ The stereochemistry of (II) at the 4-position has been determined.[3]

§ Polarography of cephalosporin derivatives was studied only from analytical standpoint.[5]

[1] M. Ochiai, O. Aki, A. Morimoto, T. Okada, K. Shinozaki, and Y. Asahi, *Tetrahedron Letters*, 1972, 2341.

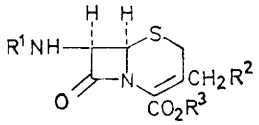
[2] M. Ochiai, O. Aki, A. Morimoto, T. Okada, and H. Shimadzu, (*a*) *J.C.S. Chem. Comm.*, 1972, 800; (*b*) submitted for publication in *Tetrahedron*.

[3] (*a*) M. Ochiai, O. Aki, A. Morimoto, and T. Okada, *Tetrahedron Letters*, 1972, 3241; (*b*) M. Ochiai, E. Mizuta, O. Aki, A. Morimoto, and T. Okada, *ibid.*, p. 3245.

[4] E. H. Flynn, 'Cephalosporins and Penicillins: Chemistry and Biology,' Academic Press, New York, 1972.

[5] (*a*) I. F. Jones and C. T. Rhodes, *J. Pharm. Pharmacol.*, 1968, **20**, 45; (*b*) D. A. Hall, *J. Pharm. Sci.*, 1973, **62**, 980.

cepham derivatives (IIa—f) are shown in Table 1. From Table 1 it is apparent that variation of the group R^1

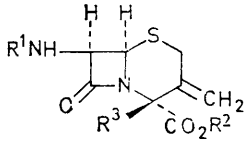


(I)

a; R^1 = H, R^2 = OAc, R^3 = H
b; R^1 = $HO_2C(H_2N)\cdot CH\cdot[CH_2]_3\cdot CO$, R^2 = OAc, R^3 = H
c; R^1 = D-$Ph(NH_2)CH\cdot CO$, R^2 = OAc, R^3 = H
d; R^1 = $MeSO_2\cdot CH_2\cdot CH_2\cdot O\cdot CO$, R^2 = OAc, R^3 = H
e; R^1 = $PhCH_2\cdot CO$, R^2 = OAc, R^3 = H
f; R^1 = $PhO\cdot CH_2\cdot CO$, R^2 = OAc, R^3 = H
g; R^1 = 2-Thienylacetyl, R^2 = OAc, R^3 = Na (H)
h; R^1 = 2-Thienylacetyl, R^2 = SAc, R^3 = Na
i; R^1 = 2-Thienylacetyl, R^2 = S·(1-oxo-2-pyridyl), R^3 = Na
j; R^1 = 2-Thienylacetyl, R^2 = $S\cdot SO_2\cdot O\cdot Na$, R^3 = Na
k; R^1 = 2-Thienylacetyl, R^2 = $\overset{+}{N}C_5H_5$, R^3 = anion
l; R^1 = 2-Thienylacetyl, R^2 = OAc, R^3 = Me

does not affect the reaction, and that the reaction only occurs at R^2.

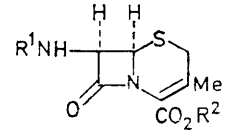
The applicability of the reaction to other cephalosporin derivatives was next examined, and the cephalosporin derivatives (Ig—l) with various substituents at



(II)

a; R^1 = H, R^2 = H, R^3 = H
b; R^1 = $HO_2C(H_2N)\cdot CH\cdot[CH_2]_3\cdot CO$, R^2 = H, R^3 = H
c; R^1 = D-$Ph(NH_2)CH\cdot CO$, R^2 = H, R^3 = H
d; R^1 = $MeSO_2\cdot CH_2\cdot CH_2\cdot O\cdot CO$, R^2 = H, R^3 = H
e; R^1 = $PhCH_2\cdot CO$, R^2 = H, R^3 = H
f; R^1 = $PhO\cdot CH_2\cdot CO$, R^2 = H, R^3 = H
g; R^1 = 2-Thienylacetyl, R^2 = Na (H), R^3 = H
l; R^1 = 2-Thienylacetyl, R^2 = Me, R^3 = H
m; R^1 = 2-Thienylacetyl, R^2 = H, R^3 = D
n; R^1 = 2-Thienylacetyl, R^2 = Me, R^3 = D

the 3-position were also converted into the corresponding 3-methylenecepham derivatives (IIg) and (III) in

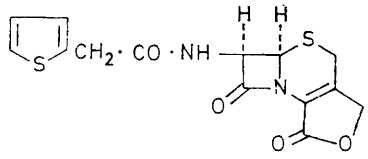


(III)

a; R^1 = H, R^2 = H
c; R^1 = D-$Ph(NH_2)CH\cdot CO$, R^2 = H
g; R^1 = 2-Thienylacetyl, R^2 = H
l; R^1 = 2-Thienylacetyl, R^2 = Me

reasonable yields under similar conditions. The results are summarized in Table 2. These results imply that the cathodic reduction takes place generally in the cephalosporin bearing a CH_2R^2 group (R^2 = O, N, or S substituents) at the 3-position.

It is worthy of note that the methyl ester (Il) also underwent the reaction because this suggests the possibility of opening the lactone ring of 3-deacetylcephalosporanic acid lactone (IV).



(IV)

The reactions of (Ig—k) were all conducted in an aqueous phase (buffer solution), but water–tetrahydrofuran was employed to effect the reaction of the methyl ester (Il) since (Il) is insoluble in water.

Reductive Opening of the Lactone (IV).—Electrochemical reduction of 3-hydroxymethyl-7-(2-thienylacetamido)-3-cephem-4-carboxylic acid lactone [7] (IV) in a mixture of acetonitrile and sodium phosphate buffer was carried out at room temperature at 15 V under stirring for 14 h. The usual work-up afforded (IIg), which was converted into the methyl ester (III) with diazomethane. The methyl ester (III) was isomerized [2] quantitatively by treatment with base to the 3-methyl-3-cepham derivative [8] (IIIl).

Despite extensive efforts,[9] no method has so far been developed for the hydrolytic opening of the cephalosporin lactone ring except one insufficient procedure.[10]

TABLE 1

Electrochemical reduction of cephalosporins

Substrate	System [a]	Time (h)	Product (%)
(Ia)	A	34·0	(IIa) (64)
(Ib)	A	5·5	(IIb) (81)
(Ic)	B	2·0	(IIc) (12)
(Id) [b]	A	4·0	(IId) [c] (9)
(Ie)	B	3·0	(IIe) [c,d]
(If)	B	3·0	(IIf) [c,d]

[a] A, 0·1M-Na_2HPO_4–HCl (pH 7·0); B, 0·1M-$MeCO_2Na$–$MeCO_2H$ (pH 4·1). [b] Takeda, Jap.P. 47–39,088. [c] Ref. 2. [d] Microscale experiment.

TABLE 2

Electrochemical reduction of cephalosporins

Substrate	System [a]	Time (h)	Product (%)
(Ig)	A	2·5	(IIg) (50)
(Ig)	B	12·0	(IIg) (47)
(Ih)	B	5·0	(IIg) (36)
(Ii) [b]	C	22·0	(IIg) (54)
(Ij) [c]	A	5·0	(IIg) (53)
(Ik)	A	3·5	(IIg) (7)
(Il)	D	3·5	(IIl) (73)

[a] A, 0·1M-Na_2HPO_4–HCl (pH 7·0); B, 0·1M-$MeCO_2Na$–$MeCO_2H$ (pH 4·1); C, B + LiBr,H_2O; D, C + tetrahydrofuran. [b] Ref. 6. [c] Prepared in our laboratories (see Experimental section).

[6] M. Ochiai, O. Aki, A. Morimoto, T. Okada, and T. Kaneko, *Tetrahedron Letters*, 1972, 2345.

[7] R. R. Chauvette and E. H. Flynn, *J. Medicin. Chem.*, 1966, **9**, 741.

[8] R. J. Stedman, K. Swered, and J. R. E. Hoover, *J. Medicin. Chem.*, 1964, **7**, 117.

[9] K. Heusler in ' Topics in Pharmaceutical Sciences,' vol. 1, ed. D. Perlman, Interscience, New York, 1968, p. 33.

[10] S. L. Neidleman, S. C. Pan, J. A. Last, and J. E. Dolfini, *J. Medicin. Chem.*, 1970, **13**, 386.

The present electrochemical opening of the lactone (IV) and the subsequent isomerization of the 3-methylenecepham derivative (III) to the 3-methyl-3-cephem derivative (IIII) thus shed light on a new approach to an alternative total synthesis of cephalosporanic acid *via* the lactone, in view of the fact that the transformation of desacetoxycephalosporin to cephalosporin has already been accomplished.[11]

Synthesis of Cephalexin.—Principally, two methods have been available for the synthesis of 7-(D-2-amino-2-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid, cephalexin (CEX), which is an orally active cephalosporin antibiotic. One involves catalytic hydrogenolysis [8,12] of the corresponding cephalosporanic acid and the other a multi-step synthesis *via* the ring expansion of penicillin derivatives.[13]

We have devised a new synthesis of CEX by the use of 3-methylenecepham derivatives (II) as mentioned below. Compound (IIa) was treated with phenylglycyl chloride to give the corresponding 3-methylenecepham derivative (IIc), which was isomerized quantitatively to the corresponding 3-deacetoxycephalosporanic acid derivative (IIIc), CEX, on treatment with pyridine and chlorotrimethylsilane. The 3-methylenecepham derivative (IIc) was alternatively obtained by cathodic reduction of cephaloglycine (Ic) as indicated in Table 1.

A one-pot synthesis of CEX (IIIc) from (IIa) can be achieved more conveniently. Thus (IIa) was acylated with phenylglycyl chloride hydrochloride in dichloromethane in the presence of triethylamine, *NN*-dimethylaniline, and chlorotrimethylsilane. The (IIc) thus formed was isomerized without isolation to CEX (IIIc) by the addition of dry pyridine.

Reaction in Deuteriated Solvent.—Electrolysis of (Ig) was conducted in a deuteriated solvent system (0·1M-$MeCO_2Na$–$MeCO_2D$ in D_2O) under the conditions comparable to those described in Table 2. The usual work-up followed by esterification with diazomethane afforded (IIn) (16%). Examination of the product (IIn) by n.m.r. and mass spectrometry revealed that the deuterium derived from the solvent was incorporated exclusively at the 4β-position. Compound (IIn) was identical with the specimen obtained by the Cr^{II} reduction of (Ig) in deuteriated solvent followed by subsequent esterification.[2]

Polarography.—Polarographic measurements were made in order to obtain mechanistic insights of the cathodic reaction and to know the reduction potentials of the related compounds. The results are summarized in Table 3. Cephalothin (Ig; $R^3 = H$) and its methyl ester (II) showed reduction potentials of $-1{\cdot}2 \sim -1{\cdot}8$ V in aqueous or acetonitrile solutions at pH 4—7. The wave heights are proportional to the concentrations of the substrates, and the diffusion current constant (k_D) is 2·7 at pH 4·0, which is considered to correspond to a two electron reduction wave.[5b,14] However, the reduction wave of (Ig) did not divide into two steps even in acetonitrile solution,[15] which suggests the instability

TABLE 3

Polarography of cephalosporins

Substrate	MeCN (%) [a]	Electrolyte [b]	pH	$E_{\frac{1}{2}}$ [c]	k_D
(Ig) ($R^3 = H$)	20	E	4·0	−1·28	2·7
(II)	100	B		−1·70	4·5
	50	P	7·7	−1·55	1·6
	20	A	6·4	−1·38	2·3
	20	E	5·6	−1·29	2·2
(IIg) ($R^3 = H$)	0	E	6·5		
(III)	100	B			
(IV) (lactone)	100	B			
	50	P	7·7		
(IIIg)	0	E	6·5		
(IIII)	100	B			
(Ia)	0	A	4·1	(−1·50)	(260) [d]
	0	P	6·4	(−1·63)	(84) [d]
	0	P	7·0	(−1·65)	(10) [d]
	0	P	8·0	(−1·63)	(3) [d]
(Ic)	0	M		(−1·50)	(1·8) [d]
	0	P	7·0	(−1·70)	(8·0) [d]
(IIIc) (CEX)	0	M		(−1·60)	(0·9) [d]
	0	P	7·0	(−1·70)	(10) [d]

[a] Percent present in water. [b] E, 0·2M-Et_4NI; A, 0·2M-$MeCO_2Na$–$MeCO_2H$ buffer; B, $Bu^n_4NClO_4$; M, 0·2M-Me_4NBr; P, 0·1M-KH_2PO_4–Na_2HPO_4 buffer. [c] Corrected for the anode potential *vs.* a normal calomel electrode. [d] Catalytic hydrogen wave.

of the intermediate radical anion (V). Both the 3-methylenecepham (IIg; $R^3 = H$) and the 3-methyl-3-cephem (IIIg; $R^3 = H$) did not show any appreciable reduction wave in the range from 0 to −2·8 V, indicating that further electrochemical reaction of the products is unlikely, and suggesting that the presence of the 3-CH_2R^2 (R^2 = O, N, or S substituents) substituted 3-cephem system is the essential requirement for the electrochemical reduction.

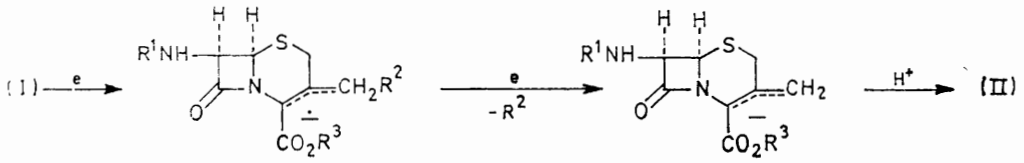
The lactone (IV) was reduced to give (IIg; $R^3 = H$) in 14% yield at 15 V in a preparative experiment, although (IV) did not show any apparent reduction wave in the range from 0 to −2·0 V in polarographic measurements.

Polarography of the derivatives (Ia), (Ic), and (IIIc) having an amino-group was examined. These compounds showed the reduction wave at $E_{\frac{1}{2}} - 1{\cdot}5 \sim -1{\cdot}7$ V. The wave heights became extraordinarily large in more acidic solutions, but comparably small in non-buffer solutions, and this may be due to a catalytic hydrogen wave. In these compounds accurate reduction potentials could not be measured owing to overlap of the catalytic hydrogen wave.

The Reaction Mechanism.—The observations described above may indicate the operation of the mechanism depicted in the Scheme. The substrate (I) is electrochemically reduced in a one-electron step to afford the

[11] J. A. Webber, E. M. Van Heyningen, and R. T. Vasileff, *J. Amer. Chem. Soc.*, 1969, **91**, 5674.
[12] C. W. Ryan, R. L. Simon, and E. M. Van Heyningen, *J. Medicin. Chem.*, 1969, **12**, 310.
[13] R. R. Chauvette, P. A. Pennington, C. W. Ryan, R. D. G. Cooper, F. L. Jose, I. G. Wright, E. M. Van Heyningen, and G. W. Huffman, *J. Org. Chem.*, 1971, **36**, 1259.
[14] O. H. Müller in ' Technique of Organic Chemistry, Physical Methods of Organic Chemistry,' 3rd edn., vol. 1, Part IV, ed. A. Weissberger, Interscience, New York, 1960, p. 3178.
[15] A. H. Maki and D. H. Geske, *J. Amer. Chem. Soc.*, 1961, **83**, 1852.

very short-lived anion radical (V), which is subsequently reduced to the anion (VI) by the second one-electron step, although neither of these steps was confirmed directly. The anion (VI) is then protonated to give the product (II). The preferential formation of 3-methylenecepham derivative (II), not of 3-methyl-3-cephem derivative (III), may be explained in terms of a kinetically controlled protonation [16] of the anion (VI) at the 4-position. In addition, the configuration at the 4-position may be determined by the steric requirement of the whole molecule.



(V) (VI)

SCHEME

EXPERIMENTAL

The i.r. spectra were obtained on a Hitachi 215 spectrophotometer in KBr discs. The n.m.r. spectra were taken with a Varian HA-100 spectrometer and chemical shifts are reported in δ values relative to tetramethylsilane as an internal standard in $CDCl_3$ or CF_3CO_2D solution, or external standard in D_2O solution. The u.v. spectra were recorded in water on a Hitachi EPS-3T spectrophotometer. The mass spectra were obtained with a Hitachi RMU-6D machine.

General Procedure for Electrochemical Reductions.—(*a*) Macroscale electroreductions were conducted in a cell made from a 1 l three-necked flask which contained a mercury pool cathode (*ca.* 34 cm^2) and a horizontal platinum sheet anode (5 cm × 11 cm), separated from one another by a sintered glass partition of medium porosity, at room temperature under stirring. The applied voltage was maintained constant by means of a Yanagimoto Controlled Electrolyser VE-3. The progress of the reaction was monitored using u.v. spectra of aliquot portions. The characteristic absorption of the 3-cephem chromophore at 258 nm gradually decreased as the reaction proceeded.

(*b*) Microscale electroreductions were conducted with a solution of the substrate (5—10 mg) in a buffer (250 ml) in a similar cell as described above. The progress of the reaction was monitored by u.v. analysis of undiluted aliquot portions.

Polarography.—Polarographic reduction potentials were measured on a Yanagimoto Polarograph PA-2. A dropping mercury electrode (k_c = 1·54 $mg^{\frac{2}{3}}$ $sec^{-\frac{1}{2}}$) was used as cathode and mercury pool electrode as anode. The reduction potentials were corrected for the anode potential *vs.* a normal calomel electrode. When necessary, aqueous solutions of samples were neutralized with tetra-n-butylammonium hydroxide containing 0·2M-tetraethylammonium iodide in order to prevent the undesirable effect of Na^+ or H^+ ions. Samples were thoroughly deaerated and kept under nitrogen prior to measurements. Measurements were made at 25°. Tetramethylammonium bromide and tetraethylammonium iodide as supporting electrolytes were commercially available. Tetra-n-butylammonium perchlorate was prepared by neutralizing 10% aqueous tetra-n-butylammonium hydroxide with perchloric acid. After two recrystallizations from 50% aqueous ethanol no contaminant wave was observed in polarography. Acetonitrile was purified as described in the literature.[17]

7-Amino-3-methylenecepham-4-carboxylic Acid (IIa).—A solution of 7-aminocephalosporanic acid (Ia) (2·0 g, 7·3 mmol) and sodium hydrogen carbonate (610 mg, 7·3 mmol) in a sodium phosphate buffer (400 ml; pH 6·9) was electrolysed at 15 V for 34 h under stirring. After completion of the electrolysis, insoluble material was filtered off and the mixture was then chromatographed on a charcoal * column (60 g). Elution with water and lyophilization afforded the *carboxylic acid* (IIa) (1·03 g, 63%), m.p. 212—214° (decomp.) (Found: C, 43·75; H, 4·75; N, 12·4; S, 14·45. $C_8H_{10}N_2O_3S$,0·25H_2O requires C, 43·9; H, 4·85; N, 12·8; S, 14·65%), $\nu_{max.}$ 1770 cm^{-1} (β-lactam C=O), δ (CF_3CO_2D) 3·61 (2H, ABq, 2-CH_2), 5·18 (1H, d, 6-H), 5·36 (1H, s, 4-H), 5·48br (2H, s, 3 =CH_2), and 5·68 (1H, d, 7-CH).

7-(D-5-Amino-5-carboxypentanamido)-3-methylenecepham-4-carboxylic Acid Monosodium Salt (IIb).—A solution of cephalosporin C monosodium salt dihydrate (Ib) (2·0 g, 3·76 mmol; 88·9% purity) in a sodium phosphate buffer (400 ml; pH 7·0) was electrolysed at 15 V for 5·5 h. Purification on a charcoal column followed by lyophilization afforded the *monosodium salt* (IIb) (1·16 g, 81%), m.p. 205—208° (decomp.) Found: C, 40·3; H, 5·65; N, 10·35. $C_{14}H_{18}N_3NaO_6S$,2H_2O requires C, 40·5; H, 5·35; N, 10·1%), $\nu_{max.}$ 1760 (β-lactam C=O) and 920 cm^{-1} (C=CH_2), δ (D_2O) 1·80—3·40 (4H, m, CH_2CH_2), 2·61 (2H, t, CH_2CO), 3·69 (2H, ABq, 2-CH_2), 3·93 (1H, t, N–CH), 5·15 (1H s, 4-H), 5·44 (1H, d, 7-CH), and 5·59 (2H, s, 3 =CH_2).

Sodium 7-(D-2-Amino-2-phenylacetamido)-3-methylenecepham-4-carboxylate (IIc).—To a solution of (IIa) (214 mg, 0·9 mmol) and triethylamine (202 mg, 2·0 mmol) in dichloromethane (10 ml) was added *NN*-dimethylaniline (200 mg, 1·65 mmol) and chlorotrimethylsilane (270 mg, 2·5 mmol) in one portion. The mixture was stirred for 4 h at room temperature. To the above solution was added phenylglycyl chloride hydrochloride (205 mg, 1·1 mmol) in four portions at 10 min intervals. The mixture was stirred for 3 h at room temperature and then extracted with water (5 ml). The aqueous extract was neutralized with 1N-sodium hydroxide, and then lyophilized to afford a powder. Chromatographic purification on an Amberlite XAD-2 column followed by lyophilization gave the *sodium salt* (IIc) (80 mg, 24%), m.p. 174—178° (decomp.) (Found: C, 51·75; H, 4·75; N, 10·8. $C_{16}H_{16}N_3NaO_4S$,0·25H_2O requires

* 'Shirasagi K-1,' manufactured by Takeda Chemical Industries, Ltd.

[16] H. O. House, 'Modern Synthetic Reactions,' 2nd edn., W. A. Benjamin, Menlo Park, California, 1972, p. 193.
[17] C. K. Mann, *Electroanalyt. Chem.*, 1969, **3**, 57.

C, 51·4; H, 4·45; N, 11·25%), $\nu_{max.}$ 1750 (β-lactam C=O) and 917 cm^{-1} (C=CH_2), δ (D_2O) 3·50 (2H, ABq, 2-CH_2), 5·07 (1H, s, 4-H), 5·27 (1H, s, PhC*H*), 5·33br (2H, s, =CH_2), 5·49 (1H, d, 6-H), 5·65 (1H, d, 7-H), and 7·68br (5H, s, phenyl).

Isomerization of (IIc) *into* (IIIc).—A solution of (IIc) (56·2 mg, 0·15 mmol) in [$^2H_5$]pyridine (1·0 ml) and chlorotrimethylsilane (0·4 ml) was stirred for 2 days at room temperature in an n.m.r. sample tube, and the progress of the reaction was monitored by n.m.r. The usual work-up and chromatographic purification on an Amberlite XAD-2 column followed by lyophilization afforded (IIIc), identical with an authentic specimen.

Sodium 7-(D-2-*Amino*-2-*phenylacetamido*)-3-*methylene*-*cepham*-4-*carboxylate* (IIc) *by Electrolysis*.—A solution of cephaloglycine [7-(D-2-amino-2-phenylacetamido)cephalosporanic acid] sodium salt (Ic) (1·06 g, 2·48 mmol) in an acetate buffer (250 ml; pH 6·7) was electrolysed at 15 V for 2 h. The mixture was concentrated to *ca.* 10 ml and chromatographed on an Amberlite XAD-2 column. Elution with water and lyophilization afforded (IIc) (56 mg, 12%).

One-pot Synthesis of 7-(D-2-*Amino*-2-*phenylacetamido*)-3-*methyl*-3-*cephem*-4-*carboxylic Acid* (CEX) (IIIc).—To a solution of (IIa) (214 mg, 0·9 mmol) and triethylamine (202 mg, 2·0 mmol) in dichloromethane (15 ml) were added *NN*-dimethylaniline (200 mg, 1·65 mmol) and chlorotrimethylsilane (250 mg, 2·3 mmol) in one portion. The mixture was stirred for 4 h at room temperature, and then phenylglycyl chloride hydrochloride (205 mg, 1·1 mmol) was added in four portions under ice cooling, and the mixture was stirred for 2 h at 0° and for an additional 2 h at room temperature. The solvent was removed under reduced pressure. To the residue was added dry pyridine (6·0 ml) and the mixture was stirred for 12 h at room temperature. Pyridine was evaporated off under reduced pressure to dryness and the residue was taken up in 1N-sodium hydroxide, which was then lyophilized to afford a powder (651 mg). Chromatographic purification on an Amberlite XAD-2 column and lyophilization gave 7-(D-2-amino-2-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid sodium salt (IIIc) (154 mg, 43%), which was identical with an authentic specimen.

3-*Sulphothiomethyl*-7-(2-*thienylacetamido*)-3-*cephem*-4-*carboxylic Acid Disodium Salt* (Ij).—A solution of Keflin * (Ig) (2·09 g, 5 mmol) and sodium thiosulphate (790 mg, 5 mmol) in water (50 ml) was stirred for 24 h at 50°. The insoluble material was filtered off and the mixture was chromatographed on an Amberlite XAD-2 column to afford the *disodium salt* (Ij) (706 mg, 27%), m.p. 196—198° (decomp.) (Found: C, 32·2; H, 3·0; N, 5·0. $C_{14}H_{12}N_2Na_2O_7S_4,1·5H_2O$ requires C, 32·25; H, 2·9; N, 5·35%), δ (D_2O) 3·83 (2H, ABq, 2-CH_2), 4·09 (2H, s, CH_2CO), 4·21 (2H, s, 3-CH_2), 5·26 (1H, d, 6-H), 5·80 (1H, d, 7-H), and 7·05—7·60 (3H, m, thiophen).

Sodium 3-*Methylene*-7-(2-*thienylacetamido*)*cepham*-4-*carboxylate* (IIg).—A solution of Keflin (Ig) (1·00 g, 2·4 mmol) in a sodium phosphate buffer (200 ml; pH 7·0) was electrolysed at 15 V for 2·5 h. The solution was then acidified to pH 2·0 by the addition of 1N-hydrochloric acid and extracted with ethyl acetate. The combined organic layers were washed, dried ($MgSO_4$), and concentrated to *ca.* 10 ml, and then treated with 2N-sodium 2-ethylhexanoate in isopropyl alcohol. The resulting precipitate was washed three times with ether and dried *in vacuo* over phosphorus pentoxide to afford the sodium salt (IIg) [2] (432 mg, 50%).

The results listed in Table 2 were obtained by similar procedures to those described above.

Methyl 3-*Methylene*-7-(2-*thienylacetamido*)*cepham*-4-*carboxylate* (III).—Electrochemical reduction of 3-hydroxymethyl-7-(2-thienylacetamido)-3-cephem-4-carboxylic acid lactone [7] (IV) (510 mg, 1·5 mmol) in a mixture of acetonitrile (125 ml) and a sodium phosphate buffer (125 ml) was carried out at 15 V under stirring for 14 h. Addition of lithium bromide monohydrate (2·0 g) was necessary to effect the reaction. The mixture was acidified to pH 2·0 by the addition of 1N-hydrochloric acid and extracted with ethyl acetate (saturated with sodium chloride). The combined organic layers were extracted with 1% aqueous sodium hydrogen carbonate, which was washed with ethyl acetate. The aqueous solution obtained was again acidified to pH 2·0 with 1N-hydrochloric acid and extracted with ethyl acetate. The organic extracts were washed, dried ($MgSO_4$), and concentrated to *ca.* 10 ml, and then treated with an excess of diazomethane in ether. The usual work-up followed by chromatographic purification on silica afforded the methyl ester [2] (III) (76 mg, 14%), which was identical with an authentic specimen.

Methyl [4β-^{2}H]-3-*Methylene*-7-(2-*thienylacetamido*)*cepham*-4α-*carboxylate* (IIn).—A solution of Keflin (Ig) (2·0 g, 4·8 mmol) in a buffer (0·1M-$MeCO_2Na$–$MeCO_2D$ in D_2O; 25 ml) was electrolysed at 15 V for 5 h. After completion of the reduction, the solution was acidified to pH 2·0 with 1N-hydrochloric acid and extracted with ethyl acetate. The combined extracts were washed, dried ($MgSO_4$), and concentrated *in vacuo*. Acetonitrile was added to the jelly-like residue to give crystalline material (IIm) (320 mg). This was esterified with an excess of diazomethane in ether, and chromatographic purification on silica afforded (IIn) [2] (278 mg, 16%).

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* Cephalothin is the generic name for 7-(2-thienylacetamido)-cephalosporanic acid; Keflin is the registered trade name for cephalothin sodium salt.