922. *Cephalosporanic Acids. Part II.* Displacement of the Acetoxy-group by Nucleophiles*

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Cephalosporin C (I), the amine (II), and derived cephalosporanic acids (III; $R^1 = H$) are converted into new derivatives by replacing the acetoxy-group with various sulphur, nitrogen, and carbon nucleophiles.

7-Aminocephalosporanic acid (II) is produced by the action of thiourea on 7-chloroacetamidocephalosporanic acid or spontaneously from 4′-chlorobutanamido- or 5′-chloropentanamidocephalosporanic acids as a result of intramolecular displacement.

Conversion of the antibiotic cephalosporin C (I) into 7-aminocephalosporanic acid (II) and subsequent acylation to give a series of 7-acylaminocephalosporanic acids (III; $R^1 = H$) have been described.[1-3] We have converted such acids into their methyl esters (III; $R^1 = Me$), lactones (IV), and 1-oxides (VIII; Y = OAc).[4] The stereochemistry of the oxides has not been studied. Hale, Newton, and Abraham described [5] the transformation of cephalosporin C (I) into the pyridinium betaine (VI; $X = C_5H_5N^+$) and showed that a range of substituted pyridines formed similar derivatives (C_A compounds) by replacing the acetoxy-group. The formation of a Bunte salt {IX; $Y = S_2O_3Na$ $R = [CH_2]_3{\cdot}CH(NH_2){\cdot}CO_2H$}, when cephalosporin C is treated with sodium thiosulphate was proposed [6] to account for the resulting increase in antibacterial activity. We have studied [7] the substitution of the acetoxy-group in salts of 7-acylaminocephalosporanic acids (III; $R^1 = H$) by azide, *NN*-dimethyldithiocarbamate, thiobenzoate, and

* Part I, G. F. H. Green, J. E. Page, and Susan E. Staniforth, *J.*, 1965, 1595.

[1] E. P. Abraham and G. G. F. Newton, *Biochem. J.*, 1961, **79**, 377; B. Loder, G. G. F. Newton, and E. P. Abraham, *ibid.*, p. 408.

[2] R. B. Morin, B. G. Jackson, E. H. Flynn, and R. W. Roeske, *J. Amer. Chem. Soc.*, 1962, **84**, 3400.

[3] R. R. Chauvette, E. H. Flynn, B. G. Jackson, E. R. Lavagnino, R. B. Morin, R. A. Mueller, R. P. Pioch, R. W. Roeske, C. W. Ryan, J. L. Spencer, and E. Van Heyningen, *J. Amer. Chem. Soc.*, 1962, **84**, 3401.

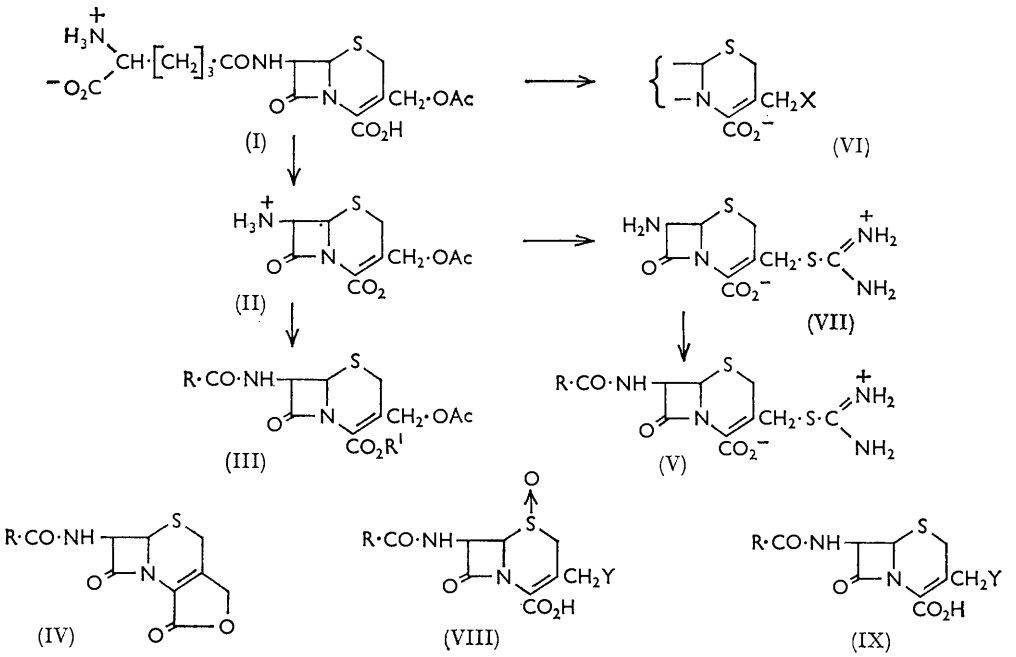
[4] Cf. " The Chemistry of Penicillin," eds. H. T. Clarke, J. R. Johnson, and R. Robinson, University Press, Princeton, 1949, pp. 156, 185; A. W. Chow, N. M. Hall, and J. R. E. Hoover, *J. Org. Chem.*, 1962, **27**, 1381.

[5] C. W. Hale, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, 1961, **79**, 403.

[6] A. L. Demain, J. F. Newkirk, G. E. Davies, and R. E. Harman, *Appl. Microbiol.*, 1963, **11**, 58; A. L. Demain, *Trans. N.Y. Acad. Sci.*, 1963, **25**, 731.

[7] S. Eardley, G. I. Gregory, M. E. Hall, and A. G. Long, Abstracts of Congress Lectures and Scientific Papers, 19th International Congress of Pure and Applied Chemistry, London, 1963, p. 308; E. Van Heyningen and C. N. Bronon, *J. Med. Chem.*, 1965, **8**, 174.

toluene-*p*-sulphinate ions as well as by ethanethiol, pyridine-2-thiol, alkylpyrimidine-2-thiols, and thiourea. The corresponding derivatives (IX) were formed when an aqueous or aqueous-acetone solution of the reactants was warmed at 35° for several days or at 50° for about 24 hr.; many non-aqueous solvents were tried without success. Several derivatives [*e.g.*, compound (V), and the sodium salts of compounds (IX; Y = S·COPh or



$SO_2{\cdot}C_6H_4{\cdot}CH_3$-*p*)] were readily isolated, as they were sparingly soluble. Other derivatives yielded to purification by countercurrent separation [*e.g.*, compound (IX; Y = N_3 or SEt)] or by crystallisation of the free acids or their cyclohexylamine salts [*e.g.*, (IX; Y = N_3 or S·CS·NMe_2)]. Several derivatives were characterised as their methyl esters or their 1-oxides (VIII).

The structure of the thiouronium derivative (V; R = CH_2Ph) was supported by analytical, electrophoretic (no net charge at pH 4·0 or 7·0; migrates as a cation at pH 1·9), and spectroscopic measurements (*e.g.*, $\varepsilon \sim 8200$ at 235 mμ and $\varepsilon_{max.}$ 9800 at 260 mμ [8] in water indicated *S*- rather than *N*-substitution [9] on the thiourea). These indications are in accord with the distinctive nitroprusside test obtained when the thiouronium compound [but not the acetates (III) or thiourea] was treated with bases.[10] Replacing the acetoxy-group in cephalosporin C (I) with thiourea gave the derivative [VI; X = S·C(:$\overset{+}{N}H_2$)NH_2]. The aminoadipoyl side-chain was removed with 2N-hydrochloric acid [1] or with nitrosyl chloride [2] in formic acid to give the amine (VII). Phenylacetylation of this compound under carefully controlled conditions gave the thiouronium derivative (V; R = CH_2Ph). A pure sample of the amine (VII) was obtained as a methanol solvate by direct replacement of the acetoxy-group in 7-aminocephalosporanic acid (II).

The azide was reduced with tin and hydrochloric acid, or by catalytic hydrogenation, to the amino-acid (IX; R = CH_2Ph, Y = NH_2) which was characterised by phenylacetylation to give the amide (IX; R = CH_2Ph, Y = NH·CO·CH_2Ph).

[8] D. M. Green, A. G. Long, P. J. May, and A. F. Turner, *J.*, 1964, 766.
[9] S. F. Mason, *J.*, 1954, 2074.
[10] M. Schenck and H. Kirchhof, *Z. physiol. Chem.*, 1926, **158**, 90.

Replacement of the acetoxy-group in cephalosporin C (I), 7-aminocephalosporanic acid (II), and the 7-acylaminocephalosporanic acids (III; $R^1 = H$) with a wide range of highly polarisable sulphur nucleophiles, with the azide ion, or with the pyridines suggests a common mechanism. We note that: (*a*) these substitutions occur very little with the 1-oxide (VIII; R = CH_2Ph, Y = OAc) (acetate is lost about four times more slowly than from the corresponding sulphide, and the β-lactam ring undergoes fission); (*b*) the methyl esters (III; $R^1 = Me$) and the lactones (IV) do not undergo analogous replacement reactions; and (*c*) oxygen nucleophiles (*e.g.*, carboxylic acids, alcohols, phenols, oximes) do not lead to simple displacement of the acetate group in 7-phenylacetamidocephalosporanic acid (III; R = CH_2Ph, $R^1 = H$) although, under anhydrous conditions with sodium benzyloxide, the β-lactam ring is opened with concurrent loss of the acetoxy-group,[11] and with hydroxylamine the ceph-3-em nucleus, like that in the penicillins,[12] undergoes rapid β-lactam fission at 0°.

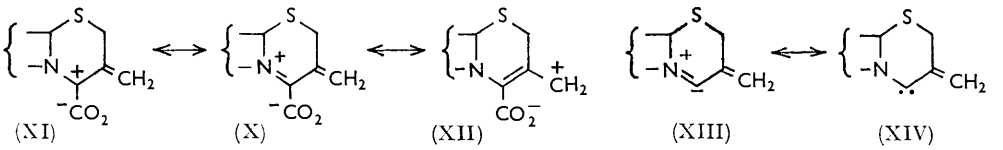
Replacement of the acetoxy-group in the sodium salt of 7-phenylacetamidocephalosporanic acid (III; R = CH_2Ph, $R^1 = H$) by 4,6-dimethylpyrimidine-2-thiol or the azide ion in water at 50° provides the corresponding derivatives (IX; R = CH_2Ph, Y = S·C(:N·C(Me):CH·C(Me):N) and N_3) in yields of *ca.* 80 and 75%, respectively. Carbon dioxide (⩾15%) was evolved over the 24-hour period needed to complete these reactions. Polarimetric studies on the reaction of sodium 7-phenylacetamidocephalosporanate in water alone and with 4,6-dimethylpyrimidine-2-thiol showed that the rate of reaction was independent of the nucleophile and of its concentration and was not significantly affected by changes in pH in the range 5·5—7·5 or by an increase from 1 to 4 in molar equivalents of added sodium chloride. These reactions were apparently first-order in sodium 7-phenylacetamidocephalosporanate. No products from the solvolysis reaction could be identified by chromatography or proton magnetic resonance (p.m.r.). Substitutions by the azide ion as well as solvolysis in water alone were followed by p.m.r., with reference to the integrals due to covalently bound $-O·CO·CH_3$ (7·9 τ) and the acetate anion (8·05 τ); further information was drawn from the signals due to the exocyclic methylene group (see Part I) in the starting material and products. These measurements confirmed the polarimetric results and yielded a secondary isotope factor $k_{H_2O}/k_{D_2O} = 1·4$ for the effect of the solvent, which is fitting for an S_N1 displacement assisted by solvation of the leaving group.[13]

We have found no evidence for the nucleophilic replacement of the hydroxy-groups in the sodium salts of the hydroxy-acids {IX; R = CH_2Ph or $[CH_2]_3·CH(NH_2)·CO_2H$, Y = OH} nor is the thiobenzoate group in compound (IX; R = CH_2Ph, Y = S·CO·Ph) replaced by pyridine unless promoted by electrophilic reagents;[14] similarly phenyl-2,4,6-trimethoxyphenylmethyl acetate undergoes ready oxygen–alkyl fission whereas the corresponding thioacetate is resistant to displacement.[15]

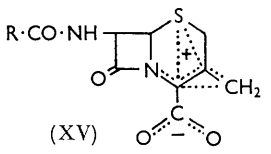
Replacements involving alkyl–oxygen fission of primary allylic esters, such as those described in this Paper, are rare. In several instances [16-18] the departure of the anion may be aided by a nitrogen atom, although this has not always been suggested. Other examples include benzyl esters carrying electron-releasing groups in the *p*-position [19] and vinylogues

[11] S. H. Eggers, T. R. Emerson, V. V. Kane, and G. Lowe, *Proc. Chem. Soc.*, 1963, 248.
[12] J. H. Ford, *Analyt. Chem.*, 1947, **19**, 1004.
[13] C. A. Bunton and V. J. Shriner, *J. Amer. Chem. Soc.*, 1961, **83**, 42, 3207.
[14] Glaxo Laboratories Ltd., Belg. Pat. 650,444.
[15] J. Kenyon and R. F. Mason *J.* 1952, 4964; T. C. Bruice in " Organic Sulfur Compounds," ed. N. Kharasch, Pergamon, Oxford, 1961, p. 430.
[16] E. Galantay, A. Szabo, and J. Fried, *J. Org. Chem.*, 1963, **28**, 98; cf. A. W. Burgstahler, *J. Amer. Chem. Soc.*, 1951, **73**, 3021.
[17] C. C. J. Culvenor, A. T. Dann, and A. T. Dick, *Nature*, 1962, **195**, 570; V. N. Iyer and W. Szybalski, *Science*, 1964, **145**, 55.
[18] T. C. Bruice *et al.*, *J. Amer. Chem. Soc.*, 1961, **83**, 1124; *ibid.*, 1964, **86**, 4109.
[19] A. G. Davies and J. Kenyon, *Quart. Rev.*, 1955, **9**, 203.

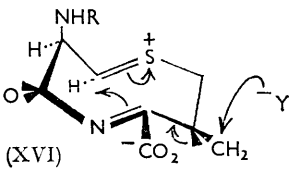
of the carbonium–immonium cation (cf. intermediates in the Mannich reaction [20]), which may be stabilised by resonance in the forms (X), (XI), and (XII). The ion (X) involves much strain [21] and might be expected to lose carbon dioxide readily [22] to give ylides (XIII) capable of resonance with carbene forms (XIV). Slow evolution of carbon dioxide during replacement of the acetoxy-group has been mentioned above; this may arise from the 4- or 8-positions, or from both sources, and represents at most only 15% of the ceph-3-em



(XI) (X) (XII) (XIII) (XIV)



(XV)



(XVI)

derivative. Evidence already presented implicates the 1-sulphur atom in the mechanism of the displacement reaction; to accommodate this factor we may invoke the summarising structure (XV) [in a succeeding Paper [23] we show that the ceph-2-em derivative (XVII) is not an intermediate in these displacements]. Another way of accommodating these factors is in an ionic valence-bond tautomer (XVI), in which stereospecific re-formation of the ceph-3-em systems depends on the asymmetry at its 7-position and possible rigidity in the 8-membered ring. The failure of esters and lactones to undergo the substitution reaction suggests that in these molecules the polarisation of the double bond countervails the displacement discussed above.

The absence of ready γ-lactonisation is surprising, but is in keeping with our failure to demonstrate simple substitution of the acetate group by oxygen nucleophiles, presumably hindered by solvation of the anions.[24] The acid group at the 4-position may be involved in a seven-membered ring with the acetate group [25] or in an α-lactone [25a] or its corresponding zwitter-ion (XI). Further, the geometry of the ion (XVI) precludes ready lactone formation.

We studied the reaction between the sodium salt of 7-phenylacetamidocephalosporanic acid (III; R = CH_2Ph, R^1 = H) and a variety of carbon nucleophiles which take part in the Mannich reaction.[26] With resorcinol at 50° in aqueous solution a new and less polar compound was detected by paper chromatography; isolation by fractional extraction gave 3-(2,4-dihydroxybenzyl)-7-phenylacetamidoceph-3-emoic acid (XVIII; R = H). The infrared and p.m.r. spectra confirmed the absence of an acetate group and suggested the presence of a 1,2,4-trisubstituted benzene ring (as in 2,4-dihydroxyethylbenzene). Methylation with diazomethane gave the dimethoxy-methyl ester (XVIII; R = Me).

[20] (*a*) H. Hellmann and G. Opitz, " α-Aminoalkylierung," Verlag Chemie, Weinheim, 1960, p. 216. (*b*) H. Hellmann in " Neuere Methoden der Präparativen Organischen Chemie," vol. II, Verlag Chemie, Weinheim, 1960, pp. 160 *et seq.*; (*c*) H. Böhme, H. J. Bohn, E. Köhler, and J. Roehr, *Annalen*, 1963, **664**, 130.

[21] F. S. Fawcett, *Chem. Rev.*, 1950, **47**, 219; cf. R. D. Kimbrough, *J. Org. Chem.*, 1963, **28**, 3577; I. Heming and J. Harley-Mason, *J.*, 1964, 2165.

[22] P. Haake and J. Mantecón, *J. Amer. Chem. Soc.*, 1964, **86**, 5230.

[23] J. D. Cocker, S. Eardley, G. I. Gregory, M. E. Hall, and A. G. Long, unpublished work.

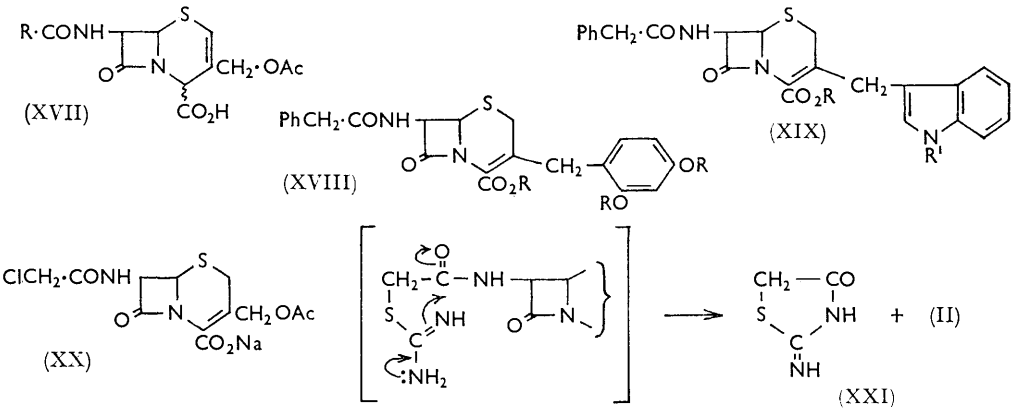
[24] C. A. Bunton, " Nucleophilic Substitution at a Saturated Carbon Atom," Elsevier, Amsterdam, 1963, pp. 75, 131.

[25] B. Capon, *Tetrahedron Letters*, 1963, 911.

[25a] E. Grunwald and S. Winstein, *J. Amer. Chem. Soc.*, 1948, **70**, 841; C. K. Ingold, " Structure and Mechanism in Organic Chemistry," Bell, London, 1953, p. 383; H. Zahn and W. Pätzold; *Chem. Ber,*. 1963, **96**, 2566.

[26] Ref. 20 (*a*), pp. 84 *et seq.*

With indole and *N*-methylindole in aqueous acetone, we obtained the derivatives (XIX; R = R^1 = H and R = H, R^1 = Me). Substitution at the 3-position in the indole nucleus is considered most likely by analogy with the Mannich reaction [26] and other electrophilic substitutions.[27] Further evidence (paper chromatography) was obtained for the formation of a similar derivative with 2-methylindole but not with 3-methylindole.



When the sodium salt of 7-chloroacetamidocephalosporanic acid (III; R = CH_2Cl, R^1 = H) was treated with 2-mercaptomethylthiophen, *p*-chlorothiophenol, or toluene-ω-thiol in aqueous acetone the corresponding 7-substituted thioacetamidocephalosporanic acids (III; R^1 = H) were rapidly formed. With thiourea in water the chloroacetamido-derivative (XX) was converted into 7-aminocephalosporanic acid (II); paper chromatography indicated the presence of the iminothiazolidone (XXI) in the mother-liquors. We attribute this reaction to an intramolecular displacement of the acyl group by thiourea, similar to that reported for the cleavage of 2-chloro-*NN*-dipropylacetamide.[28,29] Formation of an intermediate imino-oxiran [30] is less likely since no formation of the amine (II) was detected when simple thiols were used. Spontaneous formation of the amine (II) from 7-ω-chloroacylaminocephalosporanic acids (III; R = $CH_2{\cdot}[CH_2]_2{\cdot}Cl$ or $CH_2{\cdot}[CH_2]_3{\cdot}Cl$, R^1 = H) was detected by paper chromatography. Evidence has been adduced [31] for internal participation of this kind in *N*-substituted-γ-chloroalkanamides, giving the halide ion and γ-butyrolactones.

Experimental

Melting points (uncorrected) were determined on a Kofler hot-stage microscope except for those designated " cap.," which were measured in open capillaries. Unless otherwise stated, optical rotations are for solutions at room temperature in dioxan (at 1% ±0·2%); ultraviolet and infrared spectra [32] are for solutions in 0·1M-phosphate buffer at pH 6·0 and for Nujol mulls, respectively. P.m.r. spectra [32] were measured at 38° with a Varian Associates A60 spectrometer and were calibrated against either tetramethylsilane (Me_4Si = 10·0 τ) used as an internal standard or, for D_2O solutions, against t-butyl alcohol used as an internal standard and the results referred to tetramethylsilane.

[27] A. R. Katritzky and J. M. Lagowski, " Heterocyclic Chemistry," Methuen, London, 1960, pp. 159 *et seq.*

[28] A. J. Speziale and P. C. Hamm, *J. Amer. Chem. Soc.*, 1956, **78**, 5580; A. J. Speziale, *J. Org. Chem.*, 1958, **23**, 1231.

[29] Cf. E. D. Sverdlov, V. L. Vasilevski, V. M. Fedoseev, and A. B. Silaev, *Zhur. obshchei Khim.*, 1963, **33**, 3373.

[30] H. E. Baumgarten, J. F. Fuerholzer, R. D. Clark, and R. D. Thompson, *J. Amer. Chem. Soc*, 1963, **85**, 3303.

[31] B. Capon, *Quart. Rev.*, 1964, **18**, 71; P. de Mayo, " Molecular Rearrangements," Pt. 2, Interscience, New York, 1964, p. 1004.

[32] Part I, *J.*, 1965, 1595.

Paper chromatography on equilibrated Whatman No. 1 paper involved the following solvents: (A) descending, at 37° on papers buffered at pH 5, with the upper phase from ethyl acetate–butan-1-ol–0·1M-sodium acetate buffer at pH 5 (8 : 1 : 8 v/v), lower phase in the tank; (B) ascending, at room temperature using propan-1-ol–water (7 : 3 v/v); and (C) butan-1-ol–ethanol–water (4 : 1 : 5 v/v), descending, at room temperature using the upper layer as mobile phase, lower layer in the tank. Paper electrophoresis was performed on Whatman No. 3 MM paper at 10—30 v/cm. or on Whatman No. 1 paper at 70 v/cm. under light petroleum (b. p. 40—60°) [33] using either (*a*) pH 1·9 buffer consisting of formic acid (16·7 ml.; 98%), acetic acid (84 ml.), acetone (105 ml.), and water (495 ml.) [33] or (*b*) a pH 7·0 buffer made by adding phosphoric acid to 0·2M-disodium hydrogen phosphate. Spots were located by visual examination with a Hanovia " chromatolite " ultraviolet lamp. 7-Phenylacetamidocephalosporanic acid, (III; R = CH_2Ph, R^1 = H), R_p 1·00, was used as standard.

Hydroxylamine assays were determined as previously described [12] and, after correction for molecular-weight differences, are referred to the sodium salt of 7-phenylacetamidocephalosporanic acid as standard (100%).

Magnesium sulphate or sodium sulphate was used for drying solutions in organic solvents.

Equivalent weights were determined by electrometric titration of acids with 0·1N-sodium hydroxide.

7-Acylaminocephalosporanic Acids (III; R^1 = H).—*Method A.* A solution of 7-aminocephalosporanic acid [2] (2·72 g., 10 mmoles) (II) in water (40 ml.) containing sodium hydrogen carbonate (2·1 g., 25 mmoles) and acetone (30 ml.) was cooled to 0—5°, stirred and treated with a solution of an acid chloride (10 mmoles) in acetone. After 15 min. a further portion of the acid chloride (2·5 mmoles) in acetone was added and the mixture was stirred a further 15 min. The acetone was removed *in vacuo*, the aqueous solution was washed with ethyl acetate, acidified to pH 2 with 2N-hydrochloric acid, and extracted with ethyl acetate. The combined extracts were washed with water and dried. Evaporation and crystallisation from acetone–water furnished the required cephalosporanic acids (Table). In some cases the product was characterised by preparing the sodium salt as follows. The crude acid (10 mmoles) in ethyl acetate (100 ml.) was treated with a 10% solution of sodium 2-ethylhexanoate in butan-1-ol (25 ml.). The precipitated sodium salt was crystallised by suspending it in boiling acetone and adding water until solution was almost complete; the hot solution was filtered and cooled.

Method B. 7-Aminocephalosporanic acid (II) (20 mmoles) was suspended in boiling ethyl acetate (250 ml.) and treated with the appropriate acid chloride (40 mmoles). Refluxing was continued for 20—30 min. The cooled and filtered mixture was treated with aniline (60 mmoles) and, after 60 min., extracted several times with 3% aqueous sodium hydrogen carbonate solution. The combined aqueous extracts were washed with ethyl acetate acidified to pH 2 with 2N-hydrochloric acid and extracted three times with ethyl acetate. The combined extracts were washed with water, dried, and evaporated to give the cephalosporanic acid, which was purified by trituration with ethyl acetate or recrystallised from aqueous acetone.

Method C. 7-Chloroacetamidocephalosporanic acid (III; R = CH_2Cl, R^1 = H) (3·48 g., 10 mmoles) suspended in acetone (60 ml.) was treated with a solution of sodium hydrogen carbonate (1·68 g., 20 mmoles) and immediately with a thiol (10—30 mmoles). The reaction mixture was stirred at room temperature for a period (0·5—4 hr.) determined by following the course of the reaction by paper chromatography (solvent A). The acetone was then removed by evaporation, the aqueous solution was washed with ethyl acetate (100 ml.), separated, and acidified (pH *ca.* 2) with 2N-hydrochloric acid. The product was extracted into ethyl acetate (2 × 100 ml.) and purified as described above.

Methyl 7-Phenylacetamidocephalosporanate (III; R = CH_2Ph, R^1 = Me) (with Dr. J. KENNEDY).—7-Phenylacetamidocephalosporanic acid (III; R = CH_2Ph, R^1 = H) (3 g.) in dry dioxan (10 ml.) was treated with an ethereal solution of diazomethane until a yellow colour persisted. After 30 min. a precipitate had formed and this was collected and crystallised from ethyl acetate to give the *ester* (1·8 g., 60%), m. p. 178—180°, $[\alpha]_D^{18·5}$ +67° (*c* 1·0, Me_2CO), $\lambda_{max.}$ (EtOH) 260 mμ (ε 7800), $\nu_{max.}$ (Nujol) 3270 (NH), 1762 (β-lactam), 1748 and 1240 (OAc), 1726 (CO_2Me), 1652 and 1535 $cm.^{-1}$ (CONH), $\nu_{max.}$ ($CHBr_3$) 3430 (NH), 1788 (β-lactam), 1740—1730 and 1230 (CO_2Me and OAc), 1680 and 1506 $cm.^{-1}$ (CONH) (Found: C, 56·5; H, 5·0; N, 7·4; S, 8·0. $C_{19}H_{20}N_2O_6S$ requires C, 56·4; H, 5·0; N, 6·9; S, 7·9%).

[33] H. Michl in " Chromatographic Reviews," Elsevier, Amsterdam, 1959, Vol. 1, p. 11.

The following methyl esters were prepared similarly: *methyl* 7-(2-*thienylacetamido*)*cephalosporanate* (III; R = 2-thienylmethyl, R^1 = Me), m. p. 178—180°, $[\alpha]_D^{20}$ +60° (*c* 1·0, Me_2CO), $\lambda_{max.}$ (EtOH) 237 mμ (ε 12,900), inflection at 260 mμ (ε 7800), $\nu_{max.}$ 1782 (β-lactam), 1730 and 1226 (CO_2R), 1712 and 1268 (CO_2R), 1654 and 1530 cm.$^{-1}$ (CONH) (Found: C, 50·9, 50·9; H, 4·4; N, 6·8; S, 15·5. $C_{17}H_{18}N_2O_6S_2$ requires C, 49·7; H, 4·4; N, 6·8; S, 15·6%; the duplicated high carbon analysis is inexplicable); *methyl* 7-*pentanamidocephalosporanate* (III; R = Bu^n, R^1 = Me), m. p. 154—157°, $\lambda_{max.}$ (EtOH) 261—263 mμ (ε 7800), $\nu_{max.}$ 1776 (β-lactam), 1745 and 1235 (CO_2R), 1725 and 1235 (CO_2R), 1648 and 1540 cm.$^{-1}$ (CONH) (Found: C, 51·7; H, 6·0; N, 7·7; S, 8·7. $C_{16}H_{22}N_2O_6S$ requires C, 51·9; H, 6·0; N, 7·6; S, 8·7%).

1-*Oxides* (VIII; Y = OAc) *of* 7-*Acylaminocephalosporanic Acids*.—7-Phenylacetamidoceph-

Cephalosporanic acids (III; R^1 = H)

No.	R	Salt	Method	$[\alpha]_D$	$\lambda_{max.}$ (mμ)	ε
1	CH_2Ph	—	A	+91°	259	9300
2	CH_2·C:CH·CH:CH·S	Na	B	+133 *	237	14,800
3	CH_2Cl	—	B	+78	260	9320
4	CH_2·SMe	—	A	+91	258·5	9000
5	CH_2·SEt	Na	A	+118*	259	9050
6	CH_2·S·CH_2Ph ‡	Na	A, C	+100	259	9600
7	Bu^n †	—	B	+97	260	9300
8	CH_2·$[CH_2]_2$·Cl	—	B	+81	261	8950
9	CH_2·$[CH_2]_3$·Cl	—	B	+88	260·5	8730
10	CH_2·S·CH_2·C:CH·CH:CH·S	Na	C	+101 *	238	16,200
11	*p*-CH_2·S·C_6H_4·Cl	—	C	+125	255·5	15,600

	Found (%)					Molecular	Requires (%)				
No.	C	H	N	S	Cl	formula	C	H	N	S	Cl
1	55·5	4·7	7·5	7·9	—	$C_{18}H_{18}N_2O_6S$	55·4	4·65	7·2	8·2	—
2	46·2	3·6	6·3	15·2	—	$C_{16}H_{15}N_2NaO_6S_2$	45·9	3·6	6·7	15·3	—
3	41·3	3·7	7·9	8·7	10·0	$C_{12}H_{13}ClN_2O_6S$	41·4	3·8	9·0	9·2	10·2
4	43·7	4·6	8·2	17·5	—	$C_{13}H_{16}N_2O_6S_2$	43·3	4·5	7·8	17·8	—
5	42·3	4·4	6·9	16·4	—	$C_{14}H_{17}N_2NaO_6S_2$	42·4	4·3	7·1	16·2	—
6	50·1	4·2	6·25	13·9	—	$C_{19}H_{19}N_2NaO_6S_2$	49·8	4·2	6·1	14·0	—
7	50·7	5·7	8·0	9·4	—	$C_{15}H_{20}N_2O_6S$	51·0	5·7	7·8	9·0	—
8	44·5	4·5	7·3	8·6	9·3	$C_{14}H_{17}ClN_2O_6S$	44·6	4·55	7·4	8·5	9·4
9	46·4	4·9	7·3	8·2	9·2	$C_{15}H_{19}ClN_2O_6S$	46·1	4·9	7·2	8·2	9·1
10	44·0	3·7	6·2	20·5	—	$C_{17}H_{17}N_2NaO_6S_3$	44·0	3·7	5·9	20·7	—
11	47·0	3·7	5·9	14·5	7·7	$C_{18}H_{17}ClN_2O_6S_2$	47·3	3·75	6·1	14·0	7·5

* $[\alpha]_D$ for aqueous solutions. † Hydroxylamine assay 98%. ‡ *Cyclohexylamine salt*, $\lambda_{max.}$ 258 mμ (ε 9550) (Found: C, 56·2; H, 6·4; N, 8·1; S, 12·2. $C_{25}H_{33}N_3O_6S_2$ requires C, 56·1; H, 6·2; N, 7·8; S, 12·0).

alosporanic acid (4·86 g.) was dissolved in water (110 ml.) containing sodium hydrogen carbonate (1·05 g.) and mixed with a solution of sodium metaperiodate (2·94 g.) in water (50 ml.). After 16 hr. at room temperature (subsequent experiments showed that equally satisfactory results were obtained after 1 hr.) the reaction mixture was washed with ethyl acetate (100 ml.). The aqueous layer was acidified to pH 1—2 with concentrated hydrochloric acid, the white solid was collected, washed with water, and dried. The product was refluxed with ethanol or acetone, cooled, filtered (2·9 g., 57%), and finally crystallised from dimethylformamide, to give the *sulphoxide* (VIII; R = CH_2Ph, Y = OAc) $[\alpha]_D$ +143° (*c* 0·74 Me_2SO), $\lambda_{max.}$ 258 mμ (ε 11,050), $\nu_{max.}$ 3300 (NH), 1778 (β-lactam), 1747 and 1235 (OAc), 2578 and 1728 (CO_2H), 1663 and 1535 cm.$^{-1}$ (CONH) and 992 cm.$^{-1}$ (S ⟶ O); hydroxylamine assay 85% (Found: C, 53·5; H, 4·6; N, 6·5; S, 7·7%; Equiv., 409. $C_{18}H_{18}N_2O_7S$ requires C, 53·2; H, 4·5; N, 6·9; S, 7·9%; Equiv., 406·4). The *sodium salt* was prepared by neutralising a suspension of the sulphoxide (0·17 g.) in water (10 ml.), lyophilising the solution, and drying the white solid over phosphorus pentoxide *in vacuo* for 24 hr., $\lambda_{max.}$ 258 mμ (ε 10,900), $\nu_{max.}$ 1756 (β-lactam), 1734 and 1240 (OAc), 1642 and 1538 (CONH), 1605 (CO_2^-) and 1032 cm.$^{-1}$ (S ⟶ O) (Found: C, 46·2; H, 4·4; N, 5·8; S, 6·7. $C_{18}H_{17}N_2NaO_7S,2H_2O$ requires C, 46·5; H, 4·6; N, 6·05; S, 6·9%). In a similar way we obtained 7-*pentanamidocephalosporanic acid* 1-*oxide* (VIII; R = Bu^n, Y = OAc) (53%), $[\alpha]_D$ +152° (*c* 0·85, Me_2SO), $\lambda_{max.}$ 257—258 mμ (ε 10,800), $\nu_{max.}$ 3300 (NH), 1780 (β-lactam), 1750 and 1220 (OAc), 2535 and 1722 (CO_2H), 1650 and 1528 (CONH) and 992 cm.$^{-1}$ (S ⟶ O)

(Found: C, 48·3; H, 5·55; N, 7·6; S, 8·6. $C_{15}H_{20}N_2O_7S$ requires C, 48·4; H, 5·4; N, 7·5; S, 8·6%).

Lactones (IV) *from 7-Acylaminocephalosporanic Acids* (III; R^1 = H) (with Dr. J. KENNEDY). —7-Phenylacetamidocephalosporanic acid (5 g.) in aqueous acetone (1 : 1 v/v; 100 ml.) was treated dropwise with concentrated hydrochloric acid (15 ml.). The mixture was left at room temperature, under nitrogen, for 16 hr. The resulting solid (2·4 g.) was collected by filtration. The filtrate was extracted with methylene chloride (2 × 100 ml.) and the combined extracts were dried and reduced to a small volume, yielding a solid. The combined solids were crystallised from ethanol to give *3-hydroxymethyl-7-phenylacetamidoceph-3-em-4-oic acid lactone* (IV; R = CH_2Ph) (2·09 g., 50%), m. p. 210° (from acetic acid), $[\alpha]_D$ +170° (*c* 1·0, Me_2CO), $\lambda_{max.}$ ($CHCl_3$) 257—258 mμ (ε 7500), $\nu_{max.}$ 1782 (γ-lactone), 1755 (β-lactam), 1650 and 1520 $cm.^{-1}$ (CONH); hydroxylamine assay 82% (Found: C, 58·3; H, 4·3; N, 8·4; S, 9·8. $C_{16}H_{14}N_2O_4S$ requires C, 58·2; H, 4·3; N, 8·5; S, 9·7%). Similarly prepared was *7-benzylthioacetamido-3-hydroxymethylceph-3-em-4-oic acid lactone* (IV; R = $CH_2{\cdot}S{\cdot}CH_2Ph$), m. p. 165—166°, $\lambda_{max.}$ (EtOH) 254 mμ (ε 8000), $\nu_{max.}$ 1784 (γ-lactone), 1756 (β-lactam), 1660 and 1552 $cm.^{-1}$ (CONH), R_p 3·05 (solvent A) (Found: C, 54·3; H, 4·2; N, 7·45; S, 16·9. $C_{17}H_{16}N_2O_4S_2$ requires C, 54·2; H, 4·3; N, 7·4; S, 17·0%).

Displacement of the Acetoxy-group in Cephalosporin C (I) *with Thiourea.*—A mixture of the sodium salt of cephalosporin C (I) (5·0 g.) and thiourea (8·0 g.) in water (200 ml.) was set aside at 37°. After 5 days, paper chromatography (solvent B) indicated that only traces of cephalosporin C remained. Acetone (1 l.) was added and the mixture cooled to 0°. The product obtained by centrifuging was triturated with acetone to give a brown solid (3·7 g.). A solution of this product in water (500 ml.) was passed through a column (26·0 × 2·5 cm.) of Dowex 1 × 8 (acetate cycle). Paper chromatography of the eluates showed that the thiouronium derivative [VI; X = $S{\cdot}C(:NH_2^+)NH_2$] was eluted rapidly and was free from thiourea and cephalosporin C. Selected fractions (25 ml.) were freeze-dried to give a cream solid (2·58 g.). Trituration with a small volume of absolute methanol gave a fluffy solid (2·18 g.). Crystallisation from methanol–water gave a white solid, m. p. >180° (cap.), $[\alpha]_D$ +62° (*c* 0·2, H_2O). An analytical specimen of *S-[7-D-(5-amino-5-carboxypentanamido)-4-carboxyceph-3-em-3-ylmethyl]-isothiourea* [VI; X = $S{\cdot}C(:NH_2^+)NH_2$] was obtained by passing an aqueous solution through a column of equal volumes of charcoal and Celite, freeze-drying the eluates, and crystallising from aqueous methanol to give colourless blades, m. p. 197° (cap., decomp.), $[\alpha]_D$ +66° (*c* 4·0, H_2O), $\lambda_{max.}$ 259—262 mμ (ε 8050), $\nu_{max.}$ (Infracord, uncorr. frequencies) at 3500, 3300 (NH), 1760 (β-lactam), 1676 (CONH), and 1610 (CO_2^-) $cm.^{-1}$ (Found: C, 39·5; H, 5·8; N, 12·5; S, 12·8; OMe, 5·4. $C_{15}H_{21}N_5O_6S_2,CH_3OH,H_2O$ requires C, 39·9; H, 5·65; N, 14·5; S, 13·3; OMe, 6·44%). This compound showed no net charge when subjected to electophoresis at pH 7·0 or 4·0. R_F (solvent B) 0·11 compared with cephalosporin C, R_F 0·22. Thiourea and α-aminoadipic acid were detected by paper chromatography after acid hydrolysis of the thiouronium compound.

A similar experiment using 7-D-(5-amino-5-carboxypentanamido)-4-carboxy-3-hydroxymethylceph-3-em-4-oic acid [34] {IX; R = $[CH_2]_3{\cdot}CH(NH_2){\cdot}CO_2H$, Y = OH} as its sodium salt did not yield the thiouronium derivative.

S-[7-Amino-4-carboxyceph-3-em-3-ylmethyl]isothiourea (VII).—(*a*) *Acid hydrolysis of S-[7-D-(5-amino-5-carboxypentanamido)-4-carboxyceph-3-em-3-ylmethyl]isothiourea* [VI; X = $S{\cdot}C(:\overset{+}{N}H_2)NH_2$]. The cephalosporin C derivative (2·0 g.) in 2N-hydrochloric acid (80 ml.) was kept at room temperature for 3 days. The reaction mixture was adjusted to pH 4·1 by adding 5% ammonium hydroxide solution. The resulting solution was passed down a column (40 cm. × 3·5 cm.) of Dowex 50 × 8 (200—400 mesh, NH_4^+ form); [35] the column was washed with 0·2M-ammonium formate buffer, pH 4·1, until a consistently low optical density at 260 mμ was obtained on successive fractions. These washings (2 l.) contained unhydrolysed material. The column was developed with 0·5M-ammonium acetate buffer, pH 6·8; 25-ml. fractions were collected and their optical densities measured at 260 mμ. On this basis fractions 27—47 were combined and lyophilised three times to leave a residue (0·23 g.), $\lambda_{max.}$ 234—235 ($E_{1\,cm.}^{1\%}$ 246) and 260 mμ ($E_{1\,cm.}^{1\%}$ 172). Part (0·185 g.) of this residue was dissolved in water (1·8 ml.), and acetone (25 ml.) was added. After centrifuging for 20 min. at 2000 r.p.m., the supernatant

[34] J. D'A. Jeffery, E. P. Abraham, and G. G. F. Newton, *Biochem. J.*, 1961, **81**, 591.
[35] C. H. W. Hirs, S. Moore, and W. H. Stein, *J. Biol. Chem.*, 1952, **195**, 669.

liquid was decanted and the gummy residue was washed with 95% acetone–water. The residue was dissolved in water and freeze-dried to give compound (VII) (0·135 g., 15·5%), $\lambda_{max.}$ 265 mμ ($E^{1\%}_{1\,cm.}$ 205). Paper chromatography (solvent B) showed a single spot R_F 0·36 in good agreement with the purer sample described below (*c*).

(*b*) *Cleavage of* S-[7-D-(5-*amino*-5-*carboxypentaramido*)-4-*carboxyceph*-3-*em*-3-*ylmethyl*]*isothiourea* [VI; X = S·C(:$\overset{+}{N}H_2$)NH_2] *with nitrosyl chloride.* The cephalosporin derivative (2·0 g.) in formic acid (98%; 7·5 ml.) at 0° was treated for 30 sec. with a solution of nitrosyl chloride (0·728 g.) in formic acid (98%; 6·64 ml.). The solution was stirred for a total of 15 min. when half of the solution was evaporated *in vacuo* as rapidly as possible on a rotary evaporator. The gum was dissolved in water, adjusted to pH 6·8 by adding De-Acidite G(SRA 101), and freeze-dried to give a solid (0·217 g.). Preparative paper chromatography (solvent B) on Whatman No. 17 paper gave starting material (88 mg.), $\lambda_{max.}$ 262 mμ ($E^{1\%}_{1\,cm.}$ 175), and compound (VII) (93 mg.), $\lambda_{max.}$ 267 mμ ($E^{1\%}_{1\,cm.}$ 188). The remaining half of the reaction mixture was stirred for a further 15 min. and worked up as above to give the two components [VI; X = S·C(:NH_2^+)NH_2] (44 mg.), $\lambda_{max.}$ 263 mμ ($E^{1\%}_{1\,cm.}$ 179), and compound (VII) (47 mg.), $\lambda_{max.}$ 267 mμ ($E^{1\%}_{1\,cm.}$ 201). Paper chromatography and electrophoresis confirmed the nature and homogeneity of these samples.

(*c*) *Displacement of the acetoxy-group in* 7-*aminocephalosporanic acid* (II) *with thiourea.** A suspension of 7-aminocephalosporanic acid (5·44 g., 20 mmoles) in water (500 ml.) was titrated to pH 7 with 2N-ammonium hydroxide solution, and thiourea (1·52 g., 20 mmoles) was added. After 70 hr. at room temperature the reaction mixture was passed through a column (3·6 cm. × 20 cm.) of Dowex 1 × 8 (100—200 mesh, acetate form). The column was washed with water (500 ml.) and the combined eluates were adjusted to pH 3·0 by adding Dowex 50 (H^+ form). The resin was filtered off, washed with water, and resuspended in water (100 ml.). 2N-Ammonium hydroxide was added to adjust the pH to 7, the resin was filtered off, and the filtrate was freeze-dried to give S-(7-*amino*-4-*carboxyceph*-3-*em*-3-*ylmethyl*)*isothiourea* (VII) (0·86 g., 16·5%), $\lambda_{max.}$ 260 mμ (ε 6500), $\nu_{max.}$ 1752 (β-lactam) and 1600 $cm.^{-1}$ (CO_2^-), R_F (solvent B) 0·38, which migrated towards the cathode as a single spot on electrophoresis at pH 1·9 and was detected by the formation of a characteristic yellow colour with ninhydrin. An analytical sample (32 mg.) was obtained by dissolving a portion (0·2 g.) of the product in water (5 ml.), adding methanol (5 ml.), and precipitating with acetone (50 ml.) (Found: C, 38·0; H, 5·3; N, 16·1; S, 18·2. $C_9H_{12}N_4O_3S_2,2CH_3OH$ requires C, 37·6; H, 5·7; N, 15·9; S, 18·2%).

When this displacement reaction was carried out on a more concentrated reaction mixture (40 mmoles 7-aminocephalosporanic acid in 120 ml., water) a by-product, precipitated at pH 3·0, was obtained. A similar product was obtained when thiourea was omitted, suggesting that it was formed by self-condensation of 7-aminocephalosporanic acid. This material has not been obtained in a pure state and characterisation was incomplete.

S-(4-*Carboxy*-7-*phenylacetamidoceph*-3-*em*-3-*ylmethyl*)*isothiourea* (V; R = CH_2Ph).—(*a*) *Phenylacetylation of* S-(7-*amino*-4-*carboxyceph*-3-*em*-3-*ylmethyl*)*isothiourea* (VII). A crude sample of the isothiouronium derivative (VII) (0·50 g., $E^{1\%}_{1\,cm.}$ 187 at 260 mμ) was dissolved in water (45 ml.), diluted with acetone (45 ml.), and cooled to 0°. A portion (1·65 ml., 1 equiv.) of a solution of phenylacetyl chloride (3·43 ml.) in acetone (25 ml.) was added; the pH dropped to 1·6 and a small quantity of a precipitated gum was removed by centrifuging. The pH was raised to *ca.* 4·5 by adding De-Acidite G(SRA 101, hydroxide form). A further portion (6·6 ml., 4 equiv.) of the phenylacetyl chloride solution was added and the procedure was repeated several times until a total of 10 equiv. phenylacetyl chloride had been added during 88 min. The resin was removed by filtration and washed with water. The filtrate and washings were combined and the acetone was removed *in vacuo* at 0—5°. The pH was adjusted to 3·0 with 0·2N-hydrochloric acid and the mixture was extracted successively with benzene (3 × 50 ml.), ethyl acetate (2 × 50 ml.), and light petroleum (b. p. 40—60°; 50 ml.). The aqueous phase was flushed with nitrogen for 25 min., adjusted to pH 6·5 with De-Acidite G (SRA 101, hydroxide form), filtered, and rotary-evaporated at ⩾25° to a small volume. The precipitate was collected by filtration, washed with water (2·0 ml.), and dried, giving the *isothiourea* (V; R = CH_2Ph) (0·157 g., 22·4%), $\lambda_{max.}$ (H_2O) 258—263 mμ (ε 8600), infrared spectrum similar to that described below (Found: C, 45·8; H, 4·85; N, 12·1, 12·7; S, 13·8, 14·2. $C_{17}H_{18}N_4O_4S_2,2H_2O$ requires C, 46·1; H, 5·0; N, 12·7; S, 14·5%).

* With the assistance of Dr. M. E. Hall.

(*b*) *From 7-phenylacetamidocephalosporanic acid* (III; R = CH_2Ph, R^1 = H). The acid (40·0 g.) was dissolved in a solution (284 ml.) of sodium hydrogen carbonate (8·77 g., 1·02 equiv.) whilst a stream of nitrogen was passed through the solution to remove dissolved carbon dioxide. The resulting solution was filtered, the pH of the filtrate being 6·8. A filtered solution of thiourea (11·7 g., 1·5 equiv.) in water (200 ml.) was added, the total volume was adjusted to 1500 ml., and the mixture was agitated for 65 hr. at 37°. The precipitated solid was collected by filtration and washed free from thiourea by suspending in water (800 ml.) and stirring vigorously for 40 min. The washing procedure was repeated. The solid was dried *in vacuo* over phosphorus pentoxide to give the sparingly soluble (*ca.* 0·2% w/v in water at 25°) isothiouronium derivative (V; R = CH_2Ph) (22·0 g., 52·7%), $[\alpha]_D$ +151° (*c* 0·7 in Me_2SO), $\lambda_{max.}$ (H_2O) 260 mμ (ε 8800), $\nu_{max.}$ 1764 (β-lactam), 1660 and 1538 (CONH), and 1590 cm.$^{-1}$ (CO_2^-); hydroxylamine assay 64%; R_F 0·69 (solvent C), 0·1 (solvent A) (Found: C, 47·3; H, 5·0; N, 12·6; S, 15·3. $C_{17}H_{18}N_4O_4S_2,1{\cdot}5H_2O$ requires C, 47·1; H, 4·9; N, 12·9; S, 14·8%). This compound showed no net charge on electrophoresis at pH 7·0 or 4·0; at pH 1·9 it migrated as a single spot towards the cathode (detected with Grote's reagent [36]).

We failed to prepare the isothiouronium derivative by replacing the hydroxy-group in sodium 3-hydroxymethyl-7-phenylacetamidoceph-3-em-4-oate [3] with thiourea under the conditions described above. The following isothiouronium compounds (V) were prepared from the corresponding 7-acylaminocephalosporanic acids (III; R^1 = H) by method (*b*): S-(4-*carboxy*-7-*ethylthioacetamidoceph*-3-*em*-3-*ylmethyl*)*isothiourea* (V; R = CH_2·SEt) *monohydrate*, $\lambda_{max.}$ 261—262 mμ (ε 9300), $\nu_{max.}$ 1780 (β-lactam), 1668 and 1536 (CONH), and 1600 cm.$^{-1}$ (CO_2^-) (Found: C, 38·25; H, 4·7; N, 13·55; S, 23·1. $C_{13}H_{18}N_4O_4S_3,H_2O$ requires C, 38·2; H, 4·9; N, 13·7; S, 23·55%); crystallisation from water gave a different form, $\lambda_{max.}$ 262 mμ (ε 9950), $\nu_{max.}$ 1762 (β-lactam), 1644 and 1530 (CONH), and 1592 cm.$^{-1}$ (CO_2^-); the infrared spectra of both forms in dimethyl sulphoxide were similar, $\nu_{max.}$ (Me_2SO) 1770 (β-lactam), 1674 and 1550 (CONH), and 1610 cm.$^{-1}$ (CO_2^-); S-(7-*benzylthioacetamido*-4-*carboxyceph*-3-*em*-3-*ylmethyl*)*isothiourea* (V; R = CH_2·S·CH_2Ph), $\lambda_{max.}$ 260 mμ (ε 9450), $\nu_{max.}$ 1756 (β-lactam), 1654 and 1533 (CONH), and 1580 cm.$^{-1}$ (CO_2^-) (Found: C, 45·45; H, 4·7; N, 12·2; S, 20·9. $C_{18}H_{20}N_4O_4S_3,H_2O$ requires C, 45·9; H, 4·7; N, 11·9; S, 20·4%); S-(4-*carboxy*-7-*n-pentanamidoceph*-3-*em*-3-*ylmethyl*)*isothiourea* (V; R = $[CH_2]_3$·CH_3), $\lambda_{max.}$ 262 mμ (ε 9350), $\nu_{max.}$ 1768 (β-lactam), 1657 and 1638 (CONH), and 1590 cm.$^{-1}$ (CO_2^-) (Found: C, 43·6; H, 5·45; N, 14·4; S, 16·8. $C_{14}H_{20}N_4O_4S_2,H_2O$ requires C, 43·1; H, 5·7; N, 14·35; S, 16·4%).

Sodium Salt of 3-*Ethylthiomethyl*-7-*phenylacetamidoceph*-3-*em*-4-*oic Acid* (IX; R = CH_2Ph, Y = SEt).—A mixture of sodium 7-phenylacetamidocephalosporanate (4·125 g., 10 mmoles) and ethanethiol (3·7 ml., 50 mmoles) in acetone–water (1 : 1, 100 ml.) was heated at 37° in a sealed tube for 137 hr. The acetone and excess of ethanethiol were removed *in vacuo*. The aqueous solution was acidified to pH 2·0 with 2N-hydrochloric acid and extracted with ethyl acetate (250 ml., and 2 × 100 ml.). The combined, dried extracts were evaporated to a pale yellow foam (3·98 g.). This material was dissolved in ethyl acetate (40 ml.) that had been equilibrated with 0·1M-sodium dihydrogen phosphate (adjusted to pH 4·5). The solution was subjected to counter-current distribution using ethyl acetate (40 ml.)–0·1M-sodium dihydrogen phosphate in a 50-tube Craig apparatus. After 142 transfers 93 fractions were collected and selected fractions were assessed by paper chromatography (solvent A). Fractions 1—7 were combined, separated from the lower phase, dried, and evaporated to give a yellow foam (2·1 g.). This product in ethyl acetate (25 ml.) was treated with a solution of sodium 2-ethylhexanoate in butan-1-ol (10% w/v, 15 ml.). The white precipitate was collected, washed with ethyl acetate, and dried to give the *sodium salt of* 3-*ethylthiomethyl*-7-*phenylacetamidoceph*-3-*em*-4-*oic acid* (IX; R = CH_2Ph, Y = SEt) (0·74 g.), $[\alpha]_D$ +79° (*c* 1·07, H_2O), $\lambda_{max.}$ 264 mμ (ε 10,350), $\nu_{max.}$ 1756 (β-lactam), 1666 and 1540 (CONH), and 1608 cm.$^{-1}$ (CO_2^-) (Found: C, 52·0; H, 4·8; N, 6·6; S, 15·1. $C_{18}H_{19}N_2NaO_4S_2$ requires C, 52·2; H, 4·6; N, 6·8; S, 15·5%). Fractions 9—46, treated as above, led to the recovery of sodium 7-phenylacetamidocephalosporanate (0·91 g.).

S-(4-*Carboxy*-7-*phenylacetamidoceph*-3-*em*-3-*ylmethyl*)-NN-*dimethyldithiocarbamate* (IX; R = CH_2Ph, Y = S·CS·NMe_2) *Salts*.—A solution of the sodium salt of 7-phenylacetamidocephalosporanic acid (4·12 g., 10 mmoles) in water (60 ml.) containing sodium dimethyldithiocarbamate (2·86 g., 16 mmoles) was heated to 50° for 24 hr. The cooled solution was covered

[36] I. W. Grote, *J. Biol. Chem.*, 1931, **93**, 25.

with ethyl acetate (200 ml.) and slowly acidified to pH 2·0 with 2N-hydrochloric acid. The aqueous layer was extracted with ethyl acetate (2 × 100 ml.) and the combined extracts were washed with water, filtered, and evaporated to dryness. The product was dissolved in aqueous acetone (1 : 1, 30 ml.) and neutralised to pH 7 with N-sodium hydroxide solution. The acetone was removed *in vacuo*, the aqueous solution was extracted with ethyl acetate (50 ml.) and treated with cyclohexylamine hydrochloride (1·81 g., 13·3 mmoles) to give S-(4-*carboxy*-7-*phenylacetamidoceph*-3-*em*-3-*ylmethyl*)-NN-*dimethyldithiocarbamate cyclohexylamine salt* (2·06 g., 37·4%) as pale-cream crystals, $\lambda_{max.}$ 268 mμ (ε 23,550), $\nu_{max.}$ 1786 (β-lactam), 1666 and 1536 (CONH), and 1570 cm.$^{-1}$ (CO_2^-) (Found: C, 54·5; H, 6·3; N, 10·0; S, 17·1. $C_{25}H_{34}N_4O_4S_3$ requires C, 54·5; H, 6·2; N, 10·2; S, 17·5%). This salt (0·549 g., 1 mmole) was suspended in water (30 ml.), covered with ethyl acetate (75 ml.), acidified with 2N-hydrochloric acid (0·6 ml.), and shaken until complete solution was achieved. The ethyl acetate layer was dried and evaporated. The resulting foam was dissolved in aqueous acetone (1 : 1, 25 ml.), neutralised with N-sodium hydroxide solution, the acetone evaporated and the aqueous solution freeze-dried to give the sodium salt (0·43 g.), $[\alpha]_D$ +4·3° (*c* 0·82, H_2O), $\lambda_{max.}$ 268·5 mμ (ε 21,150), $\nu_{max.}$ 1758 (β-lactam), 1662 and 1530 (CONH), and 1600 cm.$^{-1}$ (CO_2^-). Methylation of the cyclohexylamine salt in methanol–acetone (1 : 1) with an excess of ethereal diazomethane gave S-(4-*methoxycarbonyl*-7-*phenylacetamidoceph*-3-*em*-3-*ylmethyl*)-NN-*dimethyldithiocarbamate*, m. p. 198—200° (cap.), $[\alpha]_D$ −200° (*c* 0·8), $\lambda_{max.}$ (EtOH) 274—275 mμ (ε 18,400), $\nu_{max.}$ ($CHBr_3$) 3410 (NH), 1784 (β-lactam), 1728 and 1250 (CO_2R), 1682 and 1532 cm.$^{-1}$ (CONH) (Found: C, 51·9; H, 5·1; N, 9·3; S, 20·4. $C_{20}H_{23}N_3O_4S_3$ requires C, 51·6; H, 5·0; N, 9·0; S, 20·7%).

Similarly prepared were: the *cyclohexylamine salt of* S-(4-*carboxy*-7,2′-*thienylacetamidoceph*-3-*em*-3-*ylmethyl*)-NN-*dimethyldithiocarbamate* (IX; 2-thienylmethyl, Y = S·CS·NMe_2) m. p. 190° (cap., decomp.), $[\alpha]_D$ −53° (*c* 1·0, Me_2SO), $\lambda_{max.}$ 267—271 mμ (ε 24,100), $\nu_{max.}$ 1758 (β-lactam), 1650 and 1520 (CONH), and 1580 cm.$^{-1}$ (CO_2^-) (Found: C, 49·9; H, 6·1; N, 9·9; S, 22·65. $C_{23}H_{32}N_4O_4S_4$ requires C, 49·6; H, 5·8; N, 10·1; S, 23·0%), and the corresponding sodium salt, m. p. 250—260° (cap., decomp.), $\lambda_{max.}$ 268 mμ (ε 22,500), $\nu_{max.}$ 3300 (NH), 1750 (β-lactam), 1660 and 1542 (CONH), and 1606 cm.$^{-1}$ (CO_2^-) (with Dr. V. Arkley); S-(7-*benzylthioacetamido*-4-*carboxyceph*-3-*em*-3-*ylmethyl*)-NN-*dimethyldithiocarbamate* (IX; R = CH_2·S·CH_2Ph, Y = S·CS·NMe_2) *cyclohexylamine salt*, $\lambda_{max.}$ 269—270 mμ (ε 22,900), $\nu_{max.}$ 1766 (β-lactam), 1663 and 1526 (CONH), and 1583 cm.$^{-1}$ (CO_2^-) (Found: C, 52·2; H, 5·9; N, 9·45; S, 21·4. $C_{26}H_{36}N_4O_4S_4$ requires C, 52·3; H, 6·1; N, 9·4; S, 21·5%); S-(4-*carboxy*-7-*n-pentanamidoceph*-3-*em*-3-*ylmethyl*)-NN-*dimethyldithiocarbamate* (IX; R = Bu^n, Y = S·CS·NMe_2) *cyclohexylamine salt*, $\lambda_{max.}$ 269 mμ (ε 24,550), $\nu_{max.}$ 1764 (β-lactam), 1658 and 1526 (CONH), and 1584 cm.$^{-1}$ (CO_2^-) (Found: C, 50·6; H, 6·8; N, 10·1, 10·4; S, 18·5. $C_{22}H_{36}N_4O_4S_3$ requires C, 51·1; H, 7·0; N, 10·8; S, 18·6%) and the corresponding sodium salt, $[\alpha]_D$ −22° (*c* 1·0, H_2O), $\lambda_{max.}$ 269 mμ (ε 23,300), $\nu_{max.}$ 1758 (β-lactam), 1660 and 1530 (CONH) and 1600 cm.$^{-1}$ (CO_2^-).

3-*Benzoylthiomethyl*-7-*phenylacetamidoceph*-3-*em*-4-*oic Acid* (IX; R = CH_2Ph, Y = S·COPh). —Thiobenzoic acid (4·14 g., 30 mmoles) was added to a solution of sodium hydrogen carbonate (2·52 g., 30 mmoles) in water (50 ml.) and warmed for a short time in a stream of nitrogen. A solution of 7-phenylacetamidocephalosporanic acid sodium salt (4·125 g., 10 mmoles) in water (50 ml.) was added, and the mixture was filtered through kieselguhr. The filtrate (pH 7·1) was heated at 50° for 17·5 hr.; a white solid began to precipitate within 2 hr. The cooled mixture was filtered, the solid was washed with water, dried (2·35 g., 48%), and crystallised from water–acetone–ethanol (1 : 1 : 1, 210 ml.) to give *sodium* 3-*benzoylthiomethyl*-7-*phenylacetamidoceph*-3-*em*-4-*oate*, $[\alpha]_D$ −29° (*c* 1·120, Me_2SO), $\lambda_{max.}$ (EtOH) 234 (ε 21,150) and 273 mμ (ε 16,100), $\nu_{max.}$ 1758 and 1744 (β-lactam), 1658 and 1524 (CONH), 1644 (COPh), and 1622 cm.$^{-1}$ (CO_2^-), R_P 2·54 (solvent A) (Found: C, 56·1; H, 4·2; N, 5·85; S, 13·4. $C_{23}H_{19}N_2NaO_5S_2$ requires C, 56·3; H, 3·9; N, 5·7; S, 13·1%). This salt, suspended in acetone–water (1 : 1, 60 ml.), was covered with ethyl acetate and acidified to pH 2 with 2N-hydrochloric acid. The mixture was shaken until solution was achieved; the aqueous layer was washed with ethyl acetate (2 × 25 ml.). The combined extracts were dried and evaporated to give a white crystalline solid (0·95 g.) which was triturated with cold acetone–water (3 : 1, 25 ml.), collected by filtration and dried over phosphorus pentoxide *in vacuo* to give 3-*benzoylthiomethyl*-7-*phenylacetamidoceph*-3-*em*-4-*oic acid* (0·85 g., 91%) $[\alpha]_D^{26}$ −132° (*c* 0·876), $\lambda_{max.}$ 242 (ε 17,100) and 272 mμ (ε 21,250), $\nu_{max.}$ 3300 (NH), 1770 (β-lactam), 1722 and 1700 (CO_2H), 1682 (COPh), 1640 and 1530 (CONH) and 732 and 682 cm.$^{-1}$ (Ph); hydroxylamine assay 100% (Found: C, 59·0; H, 4·4; N, 6·3; S, 13·2; Equiv., 468; $C_{23}H_{20}N_2O_5S_2$ requires C, 59·0; H, 4·3; N, 6·0; S, 13·7%; Equiv.,

468·5). An attempt to replace the hydroxy-group in the sodium salt of 3-hydroxymethyl-7-phenylacetamidoceph-3-em-4-oic acid [3] (IX; R = CH_2Ph, Y = OH) with sodium thiobenzoate was unsuccessful.

Oxidation of the sodium salt of the derivative (IX; R = CH_2Ph, Y = S·COPh) (0·49 g.) in aqueous acetone (1 : 1; 30 ml.) with sodium periodate (0·225 g.) for 3 hr. at room temperature gave, after acidification and crystallisation from acetone, the *sulphoxide* (VIII; R = CH_2Ph, Y = S·COPh), $\lambda_{max.}$ 268 mμ (ε 21,600), $\nu_{max.}$ 3300 (NH), 1772 (β-lactam), 1720 and 2550 (CO_2H), 1670 and 1534 (CONH), 1658 (COPh), and 998 cm.$^{-1}$ (S ⟶ O) (Found: C, 56·8; H, 4·3; N, 5·8; S, 12·95. $C_{23}H_{20}N_2O_6S_2$ requires C, 57·0; H, 4·2; N, 5·8; S, 13·2%). Similarly prepared were: 7-D-(5-*amino*-5-*carboxypentanamido*)-3-*benzoylthiomethylceph*-3-*em*-4-*oic acid* (IX; Y = S·COPh R = $[CH_2]_3$·$CH(NH_2)$·CO_2H), *monopotassium salt* $[\alpha]_D^{20}$ −30·3° (*c* 1·0, H_2O), $\lambda_{max.}$ 243—244 (ε 15,800) and 273—274 mμ (ε 20,700), $\nu_{max.}$ 1772 (β-lactam), 1660 and 1540 (CONH), and 1600 cm.$^{-1}$ (CO_2^-) (Found: C, 44·0; H, 4·9; N, 7·2; S, 11·45. $C_{21}H_{22}KN_3O_7S_2,2H_2O$ requires C, 44·4; H, 4·6; N, 7·4; S, 11·3%) (with Dr. B. M. Bain); 3-*benzoylthiomethyl*-7,2′-*thienylacetamidoceph*-3-*em*-4-*oic acid* (IX; R = 2-thienylmethyl, Y = S·COPh), $[\alpha]_D^{26}$ −138° (*c* 0·95), $\lambda_{max.}$ 238—239 (ε 23,450) and 273—274 mμ (ε 21,050), $\nu_{max.}$ 1772 (β-lactam), 1722 and 1700 (CO_2H), 1680 and 1536 (CONH), and 1640 cm.$^{-1}$ (S·COPh), R_P 4·45 (solvent A) (Found: C, 53·4; H, 4·1; N, 6·1; S, 20·0. $C_{21}H_{18}N_2O_5S_3$ requires C, 53·1; H, 3·8; N, 5·9; S, 20·3%); the corresponding 1-*oxide* (VIII; R = 2-thienylmethyl, Y = S·COPh), $[\alpha]_D$ −46° (*c* 0·4), $\lambda_{max.}$ 239—240 (ε 21,750) and 271—272 mμ (ε 21,350), $\nu_{max.}$ 1778 (β-lactam), 1734 (CO_2H), 1668 and 1534 (CONH), and 995 cm.$^{-1}$ (S ⟶ O) (Found: C, 51·1; H, 4·0; N, 5·8; S, 19·4. $C_{21}H_{18}N_2O_6S_3$ requires C, 51·4; H, 3·7; N, 5·7; S, 19·6%); *methyl* 3-*benzoylthiomethyl*-7,2′-*thienylacetamidoceph*-3-*em*-4-*oate*, m. p. 219—221° (cap.), $[\alpha]_D$ −120° (*c* 0·92), $\lambda_{max.}$ (EtOH) 238 (ε 22,600) and 275 mμ (ε 17,500), $\lambda_{max.}$ ($CHBr_3$) 1780 (β-lactam), 1722 and 1200 (CO_2R), 1672 and 1504 (CONH), and 1665 cm.$^{-1}$ (S·COPh) (Found: C, 54·3; H, 4·2; N, 5·6; S, 19·2. $C_{22}H_{20}N_2O_5S_3$ requires C, 54·1; H, 4·1; N, 5·7; S, 19·7%); 3-*benzoylthiomethyl*-7-*methylthioacetamidoceph*-3-*em*-4-*oic acid* (IX; R = CH_2·S·Me, Y = S·COPh) *sodium salt*, $[\alpha]_D^{26}$ −66° (*c* 1·05, Me_2SO), $\lambda_{max.}$ 244 (ε 17,250) and 274 mμ (ε 22,000), $\nu_{max.}$ 1745 (β-lactam), 1660 and 1530 (CONH), 1648 (COPh) and 1608 cm.$^{-1}$ (CO_2^-), R_P 2·2 (solvent A) (Found: C, 46·35; H, 4·1; N, 6·3; S, 20·4. $C_{18}H_{17}N_2NaO_5S_3,0·5H_2O$ requires C, 46·0; H, 3·9; N, 6·0; S, 20·5%).

Sodium Salt of 7-*Benzoylthioacetamido*-3-*benzoylthiomethylceph*-3-*em*-4-*oic Acid* (IX; R = CH_2·S·COPh, Y = S·COPh).—This compound was prepared from 7-chloroacetamidocephalosporanic acid (III; R = CH_2Cl, R^1 = H) by replacing both the chlorine and acetoxy-groups under the conditions described above. A crystallised sample had $[\alpha]_D^{26}$ −17° (*c* 1·0, Me_2SO), $\lambda_{max.}$ (EtOH) 238 (ε 28,200) and 271 mμ (ε 24,200), $\nu_{max.}$ 1750 (β-lactam), 1662 and 1538 (CONH), 1648 (COPh), and 1605 cm.$^{-1}$ (CO_2^-), R_P *ca.* 3·0 (solvent A) (Found: C, 49·9; H, 3·6; N, 4·6; S, 16·8. $C_{24}H_{19}N_2NaO_6S_3,1·5H_2O$ requires C, 49·9; H, 3·8; N, 4·85; S, 16·65%).

7-*Phenylacetamido*-3-*toluene*-p-*sulphonylmethylceph*-3-*em*-4-*oic Acid* (IX; R = CH_2Ph, Y = SO_2Ph).—The sodium salt of 7-phenylacetamidocephalosporanic acid (5·0 g., 12·1 mmoles) in water (15 ml.) was filtered and added to a filtered solution of sodium toluene-*p*-sulphinate (10·8 g., 60·5 mmoles) in water (40 ml.) and the mixture was warmed at 47° for 26 hr. A white solid began to precipitate after a few minutes. The reaction mixture was chilled at 0° overnight, when the solid was collected by filtration, slurried twice with ice–water (20 and 10 ml., respectively), and dried to give the sodium salt of the title compound as an off-white powder (3·3 g.). This product (0·2 g.) was dissolved in dimethyl sulphoxide (2·0 ml.) and precipitated with acetic acid (1·0 ml.); reprecipitation gave the *title compound*, (0·14 g.), $[\alpha]_D^{25}$ +91° (*c* 0·7, Me_2SO), $\lambda_{max.}$ (H_2O) 227 (ε 15,700) and 266 mμ (ε 9,500), $\nu_{max.}$ 1760 (β-lactam), 1654 and 1530 (CONH), and 1610 cm.$^{-1}$ (CO_2^-) (Found: C, 56·6; H, 4·5; N, 5·4; S, 13·2. $C_{23}H_{22}N_2O_6S_2$ requires C, 56·8; H, 4·6; N, 5·8; S, 13·2%).

2-(4-*Carboxy*-7-*phenylacetamidoceph*-3-*em*-3-*ylmethylthio*)*pyridine* (IX; R = CH_2Ph, Y = 2-pyridylthio).—The sodium salt of 7-phenylacetamidocephalosporanic acid (10·0 g., 24·2 mmoles) was dissolved in water (100 ml.) containing pyridine-2-thiol (8·2 g., 73·5 mmoles) and the pH was adjusted to 7·0. The mixture was warmed at 47° for 43 hr.; the pH was adjusted with 2N-sodium hydroxide to 7·0 twice during this period and again at the end of the reaction. The reaction mixture was extracted several times with methylene chloride (2 × 100 ml., 4 × 50 ml.). The pH of the aqueous layer was reduced to 4·5 by adding Dowex 50 W (H^+ form); a pale cream precipitate began to separate at pH 5·5. The resin and precipitate were collected by filtration and the precipitated material was extracted into hot acetone. Evaporation

gave a pale yellow solid which was triturated with a little acetone to give a white solid (0·93 g.). Further material (0·22 g.) was obtained from the mother-liquors and yet more (0·26 g.) by suspending the resin in water, adjusting the pH to 7·0 with 2N-sodium hydroxide, filtering, and acidifying the filtrate to pH 3·5, followed by crystallisation from acetone. The combined product (1·41 g., 13%) was crystallised from methanol to give translucent needles of the *pyridine*, $[\alpha]_D^{25}$ −146° (*c* 0·7, DMF), $\lambda_{max.}$ 249 mμ (ε 13,450), shoulders at *ca.* 266 and 289 mμ, $\nu_{max.}$ 1772 (β-lactam), 1705 (CO_2H), and 1650 and 1528 cm.$^{-1}$ (CONH) (Found: C, 57·35; H, 4·6; N, 9·4; S, 14·4. $C_{21}H_{19}N_3O_4S_2$ requires C, 57·1; H, 4·3; N, 9·5; S, 14·5%). The acidic mother-liquors from the above preparation contained a second component, R_F 0·18 (solvent A), 0·76 (solvent B) which behaves as a zwitterion on paper electrophoresis at pH 7. The formation of this material is reflected by a change in the ultraviolet absorption of the major product on acidification to pH *ca.* 2·0 to give $\lambda_{max.}$ 251 (ε 8890) and 325 mμ (ε 4580), $\lambda_{min.}$ 289 mμ (ε 1870).

2-(4-*Carboxy*-7-*phenylacetamidoceph*-3-*em*-3-*ylmethylthio*)-4-*methylpyrimidine* (IX; R = CH_2Ph, Y = 4-methylpyrimidin-2-ylthio).—The sodium salt of 7-phenylacetamidocephalosporanic acid (2·5 g.) in water (5·0 ml.) was mixed with a solution of 4-methylpyrimidine-2-thiol hydrochloride (0·98 g., 1 equiv.) in water–acetone (2 : 1; 45 ml.) which had been adjusted to pH 7·0 with 2N-sodium hydroxide solution. Water was added to bring the final volume to 75 ml. and the mixture warmed at 47° for 48 hr.; the pH was adjusted to 7·0 after 24 hr. by adding 2N-sodium hydroxide solution. The reaction mixture was cooled and filtered, and the acetone was removed by evaporation under reduced pressure. Acidification with N-hydrochloric acid to pH 1·5 precipitated a yellow solid which was collected, washed, and dried (2·16 g., 74%). Crystallisation from acetone and decolorisation of a solution in dimethylformamide with subsequent precipitation by adding water gave the *pyrimidine*, $[\alpha]_D$ −15·7° (*c* 1·4, 1% aq. $NaHCO_3$), $\lambda_{max.}$ 258 mμ (ε 17,700), $\nu_{max.}$ 1780 (β-lactam), 1710 (CO_2H), and 1660 and 1545 cm.$^{-1}$ (CONH) (Found: C, 54·9; H, 4·5; N, 11·9; S, 13·9. $C_{21}H_{20}N_4O_4S_2$ requires C, 55·2; H, 4·4; N, 12·3; S, 14·0%).

A similar preparation gave 2-[4-*carboxy*-7-(2-*thienylacetamido*)*ceph*-3-*em*-3-*ylmethylthio*]-4-*methylpyrimidine* (IX; R = 2-thienylmethyl, Y = 4-methylpyrimidin-2-ylthio), $[\alpha]_D$ −89° (*c* 0·5), $\lambda_{max.}$ 242 mμ (ε 20,800) and shoulder at 260 mμ (ε 17,600), $\nu_{max.}$ 1786 (β-lactam), 1708 (CO_2H), and 1664 and 1554 cm.$^{-1}$ (CONH) (Found: C, 49·6; H, 4·3; N, 11·9; S, 20·7. $C_{19}H_{18}N_4O_4S_3$ requires C, 49·35; H, 3·9; N, 12·1; S, 20·8%). Similarly sodium 7-phenylacetamidocephalosporanate and 4,6-dimethylpyrimidine-2-thiol hydrochloride gave 2-(4-*carboxy*-7-*phenylacetamidoceph*-3-*em*-3-*ylmethylthio*)-4,6-*dimethylpyrimidine* [IX; R = CH_2Ph, Y = 4,6-dimethylpyrimidin-2-ylthio], $[\alpha]_D$ −77·5° (*c* 0·4), $\lambda_{max.}$ 264 mμ (ε 18,500), $\nu_{max.}$ 1790 (β-lactam), 1702 and 1682 (CO_2H), and 1657 and 1536 cm.$^{-1}$ (CONH) (Found: C, 56·0; H, 4·9; N, 12·0; S, 13·4. $C_{22}H_{22}N_4O_4S_2$ requires C, 56·1; H, 4·7; N, 11·9; S, 13·6%).

2-[4-*Carboxy*-7-(2-*thienylacetamido*)*ceph*-3-*em*-3-*ylmethylthio*]*benzothiazole* (IX; R = 2-thienylmethyl, Y = 2-benzothiazolyl).—The sodium salt of 7-(2-thienylacetamido)cephalosporanic acid (2·09 g., 5 mmoles) was added to a refluxing solution of benzothiazole-2-thiol (0·835 g., 5 mmoles) in aqueous acetone (1 : 1; 30 ml.) under nitrogen. After 45 min. the reaction mixture was cooled to deposit a solid. Paper chromatography showed the presence of unreacted starting material. The reaction mixture was evaporated to incipient precipitation (10 min.) and the mixture was refluxed for a further 20 min. Complete removal of the acetone gave a yellow solid which was collected by filtration. The mother liquors had pH 5·5; further acidification did not provide more of the required compound. The solid was suspended in water, stirred, and neutralised with 2N-sodium hydroxide solution. A gel formed and was collected by centrifuging and filtration. This product was washed with a mixture of ethyl acetate–acetone to leave the derivative as a white gel (0·405 g.). The filtrate and washings were freed from organic solvents and filtered, and the filtrate was acidified to give a second crop (0·44 g.), total yield 0·845 g., 32%. Further purification was effected by treating a solution of the derivative (0·25 g.) in ethanol (25 ml.) with water (75 ml.) to give the *benzothiazole*, $[\alpha]_D^{25}$ −184° (*c* 0·52), $\lambda_{max.}$ 225 (ε 29,800), 278 (ε 17,500), 289 (shoulder) (ε 16,400), and 300 mμ (14,600), $\nu_{max.}$ 1780 (β-lactam), 1710 (CO_2H), and 1656 and 1532 cm.$^{-1}$ (CONH) (Found: C, 50·6; H, 3·8; N, 8·5; S, 24·2. $C_{21}H_{17}N_3O_4S_4$,0·5EtOH requires C, 50·3; H, 3·8; N, 8·0; S, 24·4%).

3-*Azidomethyl*-7-*phenylacetamidoceph*-3-*em*-4-*oic Acid* (IX; R = CH_2Ph, Y = N_3).—7-Phenylacetamidocephalosporanic acid (20·0 g.) and sodium azide (7·0 g.) were dissolved in

water (120 ml.) and N-sodium hydrogen carbonate (45 ml.), and the solution was warmed at 50° for 16 hr. The cooled solution was covered with ethyl acetate (600 ml.), stirred, and acidified. Addition of sodium chloride helped the layers to separate. The aqueous layer was extracted with ethyl acetate (3 × 200 ml.). The combined extracts were dried and evaporated to give a brown froth (19·7 g.). This material was purified by countercurrent distribution in a 50-tube Craig apparatus (40 ml. phases) using the solvent system ethyl acetate–0·2M-sodium acetate–acetic acid buffer (pH 4·4) (1 : 1 v/v). A solution of the froth in the upper phase (120 ml.) was distributed equally between the first three tubes and subjected to 147 transfers to give 100 fractions obtained automatically by a coupled fraction collector. Paper chromatography (solvent A) of every third fraction showed the azide to be present in fractions 10—100. Suitable fractions were combined, dissolved in acetone, and converted into the *sodium salt* of the *acid* (IX; R = CH_2Ph, Y = N_3) (10·5 g., 51%) with an excess of sodium 2-ethylhexanoate in butan-1-ol. Crystallisation from acetone–methanol gave needles, $[\alpha]_D$ +150° (*c* 1·0, H_2O), $\lambda_{max.}$ 261 mμ (ε 9150), $\nu_{max.}$ 3300 (NH), 2112 (N_3), 1758 (β-lactam), and 1636 and 1542 cm.$^{-1}$ (CONH); hydroxylamine assay 108% (Found: C, 47·8; H, 3·8; N, 17·2; S, 7·3. $C_{16}H_{14}N_5NaO_4S,0·5H_2O$ requires C, 47·5; H, 3·7; N, 17·3; S, 7·9%). This was converted in the usual way into the *methyl ester*, m. p. 161—163° (cap.), $[\alpha]_D$ +88° (*c* 0·7), $\lambda_{max.}$ (EtOH) 264 mμ (ε 8860), $\nu_{max.}$ ($CHBr_3$) 3410 (NH), 2120 (N_3), 1786 (β-lactam), 1728 and 1250 (CO_2R), and 1680 and 1506 cm.$^{-1}$ (CONH) (Found: C, 52·6; H, 4·7; N, 17·95; S, 8·25. $C_{17}H_{17}N_5O_4S$ requires C, 52·7; H, 4·4; N, 18·1; S, 8·3%) and the 1-*oxide* (VIII; R = CH_2Ph, Y = N_3), m. p. 195—196° (cap.), $\lambda_{max.}$ 258 mμ (ε 10,800), $\nu_{max.}$ 3300 (NH), 2120 (N_3), 1778 (β-lactam), 2550 and 1726 (CO_2H), 1660 and 1540 (CONH), and 995 cm.$^{-1}$ (S ⟶ O) (Found: C, 49·2; H, 4·1; N, 16·8; S, 8·0. $C_{16}H_{15}N_5O_5S$ requires C, 49·3; H, 3·9; N, 18·0; S, 8·2%).

Other azides made in a similar way were: 3-*azidomethyl*-7-*benzylthioacetamidoceph*-3-*em*-4-*oic acid* (IX; R = CH_2·S·CH_2Ph, Y = N_3), m. p. 116—118° (cap., decomp.), $[\alpha]_D^{24}$ +106° (*c* 0·8), $\lambda_{max.}$ 260 mμ (ε 9800), $\nu_{max.}$ 2110 (N_3), 1784 (β-lactam), 1720 and 1690 (CO_2H), and 1656 and 1544 cm.$^{-1}$ (CONH) (Found: C, 47·2; H, 4·2; N, 16·6; S, 15·2. $C_{17}H_{17}N_5O_4S_2,0·5H_2O$ requires C, 47·7; H, 4·2; N, 16·3; S, 15·0%); the corresponding *sodium salt* was characterised by $[\alpha]_D^{26}$ +104° (*c* 1·09, H_2O), $\lambda_{max.}$ 260—262 mμ (ε 9500), $\nu_{max.}$ 2110 (N_3), 1748 (β-lactam), 1650 and 1536 (CONH), and 1594 cm.$^{-1}$ (CO_2^-); hydroxylamine assay 125% (Found: C, 44·5; H, 4·25; N, 15·2; S, 13·8. $C_{17}H_{16}N_5NaO_4S_2,H_2O$ requires C, 44·4; H, 3·95; N, 15·2; S, 14·0%); isolation and purification of the above acid was achieved through its *cyclohexylamine salt*, m. p. 149—151° (with previous sintering, cap., decomp.), $\lambda_{max.}$ 260 mμ (ε 10,200), $\nu_{max.}$ 2110 (N_3), 1790 (β-lactam), 1666 and 1524 (CONH), and 1572 cm.$^{-1}$ (CO_2^-) (Found: C, 53·4; H, 6·2; N, 15·8; S, 12·0. $C_{23}H_{30}N_6O_4S_2$ requires C, 53·3; H, 5·8; N, 16·2; S, 12·4%); 3-*azidomethyl*-7-*pentanamidoceph*-3-*em*-4-*oic acid* (IX; R = $[CH_2]_3$·CH_3, Y = N_3) *sodium salt*, $[\alpha]_D^{27}$ +124° (*c* 1·03, H_2O), $\lambda_{max.}$ 261 mμ (ε 8750), $\nu_{max.}$ 2120 (N_3), 1748 (β-lactam), 1654 and 1535 (CONH), and 1610 cm.$^{-1}$ (CO_2^-), R_P 1·33 (solvent A) (Found: C, 43·5; H, 4·6; N, 20·5, 20·8; S, 8·6. $C_{13}H_{16}N_5NaO_4S$ requires C, 43·2; H, 4·5; N, 19·4; S, 8·9%).

3-*Aminomethyl*-7-*phenylacetamidoceph*-3-*em*-4-*oic Acid* (IX; R = CH_2Ph, Y = NH_2) (with Dr. J. KENNEDY).—The sodium salt of 7-phenylacetamidocephalosporanic acid (40 g., 0·097 mole) was converted into the crude azide (IX; R = CH_2Ph, Y = N_3) as described above. A solution of this product in ethanol (350 ml.) was mixed with tin powder (20 g., 0·17 mole) and concentrated hydrochloric acid was added dropwise. The mixture was stirred for 20 min., the excess of tin removed by filtration, and the filtrate diluted with water (300 ml.) giving a solid which was removed by filtration. The filtrate was saturated with hydrogen sulphide and the precipitate removed by filtration through Kieselguhr. The filtrate was concentrated to half-volume at 40°, the pH was adjusted to 4·5 with ammonia (*d* 0·88) and the solution was added to a column (7·5 cm. × 11 cm.) of Dowex 1 × 8 (acetate form). The column was washed with water until an optically active eluate was obtained, when further elution was with 5% v/v aqueous acetic acid. The combined optically active eluates were freeze-dried and the resulting solid was redissolved in water (250 ml.), adjusted to pH 4·5, and passed through a column of Dowex 1 as described above. The freeze-dried solid was triturated with ethanol to give the *amine* (7·1 g., 21%), $[\alpha]_D^{23}$ +168° (*c* 1·0, H_2O), $\lambda_{max.}$ 257 mμ (ε 7500), $\nu_{max.}$ 1765 (β-lactam), 1664 and 1552 (CONH), and 1598 cm.$^{-1}$ (CO_2^-), R_F 0·11 (solvent A), 0·52 (solvent B) (Found: C, 55·4; H, 5·3; N, 11·9; S, 8·8. $C_{16}H_{17}N_3O_4S$ requires C, 55·4; H, 4·9; N, 12·1; S, 9·2%). Electrophoresis at pH 1·9 shows a spot moving as a cation, no migration at pH 7·0; detection was by forming an orange colour with ninhydrin.

A crude sample of the amine was obtained by hydrogenating an ethanolic solution of the azide (IX; R = CH_2Ph, Y = N_3) over platinum oxide at 4—5 atm. in the presence of perchloric acid.

7-Phenylacetamido-3-phenylacetamidomethylceph-3-em-4-oic Acid (IX; R = CH_2Ph, Y = $NH{\cdot}CO{\cdot}CH_2Ph$).—Phenylacetyl chloride (0·18 ml.), in acetone (5 ml.) was slowly added to a stirred solution of the crude amine (IX; R = CH_2Ph, Y = NH_2) (0·40 g.) in acetone–water (1 : 1; 40 ml.) and N-sodium hydrogen carbonate solution (1·5 ml.) at 0°. Subsequently the mixture was stirred at 0° for 30 min. and at room temperature for a further 30 min. During this period a small volume (0·2 ml.) of N-sodium hydrogen carbonate was added to maintain the pH at 7·0. The acetone was removed *in vacuo*, and the aqueous solution was acidified and extracted with ethyl acetate. The washed and dried extracts were evaporated to a brown froth (0·53 g.). A solution of this product in acetone (5 ml.) was allowed to evaporate, depositing colourless prisms of the *amide* (0·15 g.), m. p. 199—200° decomp. from 180° (cap. uncorr.), $\lambda_{max.}$ 258—262 mμ (ε 10,200). A sample was recrystallised from aqueous dimethylformamide as colourless prisms (Found: C, 60·3; H, 5·2; N, 9·1; S, 6·6. $C_{24}H_{23}N_3O_5S,0{\cdot}5H_2O$ requires C, 60·7; H, 5·1; N, 8·9; S, 6·8%); R_P 1·5 (solvent A) and 1·2 (solvent C); paper electrophoresis (pH 4 and 7) showed one spot with mobility 0·9 that of 7-phenylacetamidocephalosporanic acid.

3-(2,4-Dihydroxybenzyl)-7-phenylacetamidoceph-3-em-4-oic Acid (XVIII; R = H).—The sodium salt of 7-phenylacetamidocephalosporanic acid (4·12 g., 10 mmoles), resorcinol (11·0 g., 100 mmoles), and water (100 ml.) were heated at 50° for 40 hr. when paper chromatography indicated that only a trace of the starting material remained. The reaction mixture was extracted with ether (2 × 75 ml.), acidified to pH 2·0 with 2N-hydrochloric acid, and extracted with ethyl acetate (3 × 50 ml.). These extracts were washed with water, dried, and evaporated to *ca.* 50 ml. This solution was shaken with 0·5M-disodium hydrogen phosphate in water (100 ml.) and the aqueous layer was adjusted to pH 7·0 with phosphoric acid. The lower layer was separated, washed with ethyl acetate (50 ml.), and the pH adjusted to 6·0 with phosphoric acid. Ethyl acetate (2 × 50 ml.) was used to extract the resulting solution. This process was repeated at pH values of 5·5, 5·0, 4·5, 4·29, 4·05, and 2·0. The combined ethyl acetate extracts made at each pH value were dried, evaporated, and examined by paper chromatography. The fraction (0·58 g.), $\lambda_{max.}$ 264—267 mμ ($E^{1\%}_{1cm.}$ 167) isolated at pH 4·5 gave a single spot on paper chromatography, R_P 2·11. Crystallisation from acetone–water (1 : 4) gave *3-(2,4-dihydroxybenzyl)-7-phenylacetamidoceph-3-em-4-oic acid* (XVIII; R = H), m. p. 136—140° (Kofler), $[\alpha]_D^{28{\cdot}5}$ −64° (*c* 0·932), $\lambda_{max.}$ 265·5 mμ (ε 10,200), $\nu_{max.}$ 1752 (β-lactam), 1700 (CO_2H), and 1660 and 1540 cm.$^{-1}$ (CONH) (Found: C, 56·4; H, 4·6; N, 6·1; S, 6·6%; Equiv., 460·0. $C_{22}H_{20}N_2O_6S,1{\cdot}5H_2O$ requires C, 56·5; H, 5·0; N, 6·0; S, 6·9%; Equiv., 467·5). A solution of this derivative (0·23 g.) in methanol (20 ml.) was treated with an excess of ethereal diazomethane at room temperature for 68 hr. when colourless crystals of *methyl 3-(2,4-dimethoxybenzyl)-7-phenylacetamidoceph-3-em-4-oate* (XVIII; R = Me) separated, m. p. 204—208° (from methanol–acetone), $\lambda_{max.}$ (EtOH) 276 mμ (ε 10,250), $\nu_{max.}$ ($CHBr_3$) 1780 (β-lactam), 1728 (CO_2Me), and 1682 and 1510 cm.$^{-1}$ (CONH), (Nujol) 1770 (β-lactam), 1728 (CO_2Me), and 1666 and 1536 cm.$^{-1}$ (CONH) (Found: C, 62·0; H, 5·4; N, 5·4; S, 6·4; OMe, 18·8. $C_{25}H_{26}N_2O_6S$ requires C, 62·2; H, 5·4; N, 5·8; S, 6·6; OMe, 19·3%).

3-(N-Methylindol-3-ylmethyl)-7-phenylacetamidoceph-3-em-4-oic Acid (XIX; R = H, R^1 = Me).—A solution of *N*-methylindole (6·55 g., 50 mmoles) in acetone (50 ml.) was added to a solution of the sodium salt of 7-phenylacetamidocephalosporanic acid (4·125 g., 10 mmoles) in water (50 ml.) at 52°. The mixture was kept at this temperature for 44·5 hr. The acetone was removed and the product was extracted into chloroform. Evaporation of the dried chloroform solution gave an olive liquid (8·2 g.) which was triturated twice with ether (250 ml., and 25 ml.) to give a yellow solid (2·19 g.). Purification was effected by crystallisation from benzene (50 ml.) to give a yellow solid (0·95 g.), $\lambda_{max.}$ (EtOH) 273 mμ ($E^{1\%}_{1cm.}$ 231), and by chromatography on silica gel (50—100 mesh, 90 g.). Fractions eluted with chloroform and chloroform containing 1% v/v methanol were combined and triturated with ether to give the *indole* (0·16 g.), $[\alpha]_D^{26{\cdot}5}$ −101° (*c* 0·87, $CHCl_3$), $\lambda_{max.}$ (EtOH) 271—272 mμ (ε 11,000), $\nu_{max.}$ 1780 (β-lactam), 1702 (CO_2H), and 1662 and 1534 (CONH) (Found: C, 64·8; H, 5·0; N, 8·6, 9·3; S, 6·9. $C_{25}H_{23}N_3O_4S$ requires C, 65·0; H, 5·0; N, 9·1; S, 6·9%). Paper chromatography (solvent A) showed a faint yellow fluorescence on the starting line with the main spot R_P 3·43. Electrophoresis at pH 7·0 showed one spot moving toward the anode.

Methylation of this derivative (46 mg.) in methanol solution with ethereal diazomethane

resulted in the separation of colourless needles of *methyl* 3-(N-*methylindol*-3-*ylmethyl*)-7-*phenylacetamidoceph*-3-*em*-4-*oate* (XIX; R = R^1 = Me) (11 mg.), m. p. 191—192°, $\lambda_{max.}$ (EtOH) 274 mμ (ε 12,200), $\nu_{max.}$ 1762 (β-lactam), 1722 and 1180 (CO_2Me), and 1660 and 1540 cm.$^{-1}$ (CONH) (Found: C, 65·95; H, 5·2. $C_{26}H_{25}N_3O_4S$ requires C, 65·7; H, 5·3%).

In a similar way 3-(*indol*-3-*ylmethyl*)-7-*phenylacetamidoceph*-3-*em*-4-*oic acid* (XIX; R = R^1 = H) was obtained, $[\alpha]_D^{27}$ −81° (*c* 0·91, $CHCl_3$), $\lambda_{max.}$ (EtOH) 271 mμ (ε 12,400), $\nu_{max.}$ 1765 (β-lactam), 1715 (CO_2H), and 1665 and 1530 cm.$^{-1}$ (CONH) (Found, dried at 40°/1·0 mm. for 16 hr.: C, 64·4; H, 5·3; N, 9·0; S, 6·4. $C_{24}H_{21}N_3O_4S,0{\cdot}5C_2H_5OC_2H_5$ requires C, 64·4; H, 5·4; N, 8·7; S, 6·6%). The presence of ether was confirmed by the p.m.r. spectrum.

Evidence (chiefly from paper chromatography and electrophoresis) was obtained for the formation of new derivatives when sodium 7-phenylacetamidocephalosporanate was warmed with the following in water or water–acetone: 4-ethyl-1,2-dihydroxybenzene, pyrogallol, β-naphthol, 2-methylindole (but not 3-methylindole), 5-bromoindole, 5-benzyloxyindole, pyrrole and *N*-methylpyrrole, cyclohexane-1,3-dione and 5,5-dimethylcyclohexanone-1,3-dione, malonic acid, pyruvic acid, dimethylaniline, α-picoline *N*-oxide, and 2-hydroxy-1,4-naphthaquinone.

Reaction between Thiourea and Sodium 7-Chloroacetamidocephalosporanate (III; R = CH_2Cl, R^1 = Na).—7-Chloroacetamidocephalosporanic acid (1·04 g., 3 mmoles) was suspended in water (20 ml.) and the pH was brought to 7·0 with N-sodium hydroxide. Thiourea (0·34 g., 4·5 mmoles) was added and the solution was kept at 31° for 65 hr. The precipitated solid was collected, washed with water (10 ml.), and dried to give 7-aminocephalosporanic acid (II) (0·45 g.), $\lambda_{max.}$ 262—264 mμ ($E_{1\,cm.}^{1\%}$ 288), $\nu_{max.}$ 1800 (β-lactam), 1734 and 1236 (OAc), and 1620 cm.$^{-1}$ (CO_2^-); comparative electrophoresis (pH 1·9) and paper chromatography confirmed the identity of this material with a standard sample. The filtrate was evaporated to dryness; electrophoresis (pH 1·9) showed two positively charged components, the faster-running of which was thiourea. Paper chromatography showed these components as having R_P values of 0·8 and 1·3, respectively. The slower-moving component was tentatively identified as the iminothiazolidone (XXI) by its having electrophoretic, chromatographic, and colour-reaction properties identical with those of a sample prepared as follows:[37] chloroacetic acid (94 mg., 1 mmole), thiourea (114 mg., 1·5 mmoles) in water (7·5 ml.) were heated at 35° for 65 hr. Electrophoresis and paper chromatography showed the presence of thiourea and the iminothiazolidone (R_P 0·8). Further characterisation was not attempted.

Paper-chromatographic Evidence for the Formation of 7-Aminocephalosporanic Acid (II) *from the ω-Chloroalkylamidocephalosporanic Acids* (III; R = $CH_2{\cdot}[CH_2]_2{\cdot}Cl$ *and* $CH_2{\cdot}[CH_2]_3{\cdot}Cl$, R^1 = H).—The title acids gave well-defined spots on paper chromatography (Whatman No. 2 paper treated with a pH 6 phosphate buffer; developed with the upper phase of a butan-1-ol : water : ethyl acetate (1 : 8 : 8) mixture; ultraviolet detection). The paper was sprayed with a ninhydrin reagent (0·5% w/v in EtOH) slowly giving the colour sequence, yellow ⟶ brown ⟶ purple, previously observed with 7-aminocephalosporanic acid (II) but not with the acyl derivatives (III).

Kinetic Methods.—(*a*) *Polarimetric.* The change of optical rotation with time was measured in a 1-dm. cell for a 0·12M-solution of the sodium salt of 7-phenylacetamidocephalosporanic acid (III; R = CH_2Ph, R′ = H) in a 0·1M-phosphate buffer adjusted to pH 6·0 and kept at 47°. A plot of optical rotation with time gave α_∞ from which a collinear plot of log ($\alpha_t - \alpha_\infty$) against time was obtained. The first-order rate constant, k_{H_2O} 4·0 × 10^{-5} sec.$^{-1}$, was unchanged by reducing the initial concentration to 0·081M. The pH fell to *ca.* 5·0 during these measurements. Similar experiments in the presence of 4,6-dimethylpyrimidine-2-thiol hydrochloride at 50° gave the following rate constants (pH adjusted continuously during the reaction):

pH	7·5	6·5	6·5	5·5
Sodium chloride (M)	0·12	0·12	0·5	0·12
4,6-Dimethylpyrimidine-2-thiol (M)	0·12	0·12	0·5	0·12
10^5k (sec.$^{-1}$)	5·4	5·0	4·5	4·3

(*b*) *Proton magnetic resonance measurements.* The generation of free acetate ions during the solvolysis or nucleophilic displacement in 0·24M-solutions of sodium 7-phenylacetamidocephalosporanate in water or deuterium oxide at 52° was measured by recording the p.m.r. spectrum (at *ca.* 40°) at suitable intervals. Analysis of the integrals for the covalent acetate

[37] R. Andreasch, *Monatsh.*, 1887, **8**, 407.

(7·9 τ) and for free acetate ions (8·05 τ) led to collinear plots of log (covalent acetate integral/total acetate integral) against time, from which the following first-order rates were obtained:

Sodium azide (M)	Solvent	10^5k (sec.$^{-1}$)
0·24	D_2O	2·9
0·24	H_2O	4·1
1·20	D_2O	2·9

During these displacement reactions in deuterium oxide, measurements of the integral for the singlet at 5·88 τ due to the protons $-CH_2N_3$ in the product (IX; R = CH_2Ph, Y = N_3) showed that it was formed in *ca.* 75% yield. In a similar experiment by Dr. A. B. Taylor the 1-oxide (VIII; R = 2-thienylmethyl, Y = OAc) released acetate ions (8·05 τ) at only *ca.* one-fourth the rate of that for the parent cephalosporanic acid; in contrast, no evidence (spectroscopic or paper-chromatographic) was obtained for the formation of the azide (VIII; R = CH_2Ph, Y = N_3). Similarly, qualitative experiments with thiourea and sodium thiobenzoate failed to provide the corresponding sulphoxides (VIII; R = CH_2Ph, Y = S·C:(NH_2^+)·NH_2 or S·CO·Ph).

Measurement of Carbon Dioxide Evolved During Displacement Reactions of 7-Phenylacetamidocephalosporanic Acid (III; R = CH_2Ph, R^1 = H).—Sodium 7-phenylacetamidocephalosporanate (2·0 g.) was dissolved in water (40 ml. boiled under reduced pressure to remove dissolved gasses) containing the nucleophile (1—5 moles). The solution was heated at 50° whilst passing a stream of nitrogen previously bubbled through a saturated solution of barium hydroxide. The effluent gases were passed successively through two traps containing saturated barium hydroxide. After suitable intervals the barium carbonate was collected and weighed. Correction was made for material precipitated in blank runs where the cephalosporanic acid salt was omitted from the reaction mixture. A typical result with sodium azide (1·05 moles) was precipitation of barium carbonate corresponding to carbon dioxide evolution of 3·8% and 13·6% after 7 and 24 hr., respectively.

The 7-amino- and 7-acylamino-derivatives in this Paper are assumed to be 7β-compounds (*i.e.*, with the substituent projecting on the same side of the molecule as the sulphur atom); the p.m.r. spectrum [32] indicates a *cis*-disposition of the hydrogen atoms at the 6- and 7-positions, as in cephalosporin C.[38] Similar reservations pertain to the 6-position in derivatives of 6-aminopenicillanic acid.

Biological Results.—Cup-plate bioassays [39] of the derivatives described above were determined against *S. aureus* and lay in the range 0·1—6·0 compared with the standard for 7-phenylacetamidocephalosporanic acid. Exceptions were the 1-oxides (<0·1) and the methyl esters, which were inactive.

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