Cephalosporanic Acids. Part VI.[1] Action of Primary and Secondary Aromatic Amines on Cephalosporanic Acids

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Aniline and anisidine displace the acetate group from 7β-phenylacetamidocephalosporanic acid (I; R^1 = $NH{\cdot}CO{\cdot}CH_2Ph$, R^2 = OH, R^3 = OAc); if the pH is not controlled, the products may cyclize to the γ-lactams (II). If the pH is kept at 7·5 this cyclization is prevented; the vinylamines (III), which resemble the penamaldic acids, are by-products. Formation of the lactams is avoided in reactions with *N*-substituted anilines as nucleophiles. We have observed no products of nucleophilic attack by carbon atoms of the aromatic molecules. 7-Phenylacetamidocephalosporanic acid reacts in acetone at 50° with aniline, to give the lactam (V). This change represents initial nucleophilic attack at the 8-position; the dihydrothiazine (V) is protected by the stability of its γ-lactam system. The result is similar to the conversion of penicillanic acids into derivatives of the penicilloic acids.

CEPHALOSPORANIC acids react with pyridine bases to give C_A-compounds, which are betaines formed by replacement of the acetate group.[2] Reactions with certain vinylamines occur with coupling on a carbon atom,[3] but the action of aromatic amines has not been reported.

The pH of an aqueous solution of sodium 7β-phenylacetamidocephalosporanate (I; $R^1 = NH{\cdot}CO{\cdot}CH_2Ph$, $R^2 = O^-Na^+$, R^3 = OAc) became 7·3 when treated with 3 equivalents of aniline. It had dropped to about 5 after 3 days at 35°; solid had separated, and the mixture smelt of hydrogen sulphide. Paper chromatography showed the presence of many compounds, and we were able to purify a dextrorotatory solid, $\lambda_{max.}$ 229—230 and 254—256 nm., $\lambda_{infl.}$ 290 nm., and $\lambda_{min.}$ 244—246 nm.; it fluoresced white on paper chromatograms irradiated with ultraviolet light. The compound, $\nu_{max.}$ 1772 and 1790 $cm.^{-1}$, still contained the azetidinone ring; infrared spectroscopy and ^1H n.m.r. did not indicate the presence of an acetate group, but a band, $\nu_{max.}$ 1688 $cm.^{-1}$, indicated another carbonyl group. The product was neutral, and showed n.m.r. signals for ten protons in phenyl rings and six in three discrete methylene groups. The pattern for the coupled single protons at the 6- and 7-positions confirmed the presence of the azetidinone ring,[4] and we assign the lactam structure (II; R = H) to this

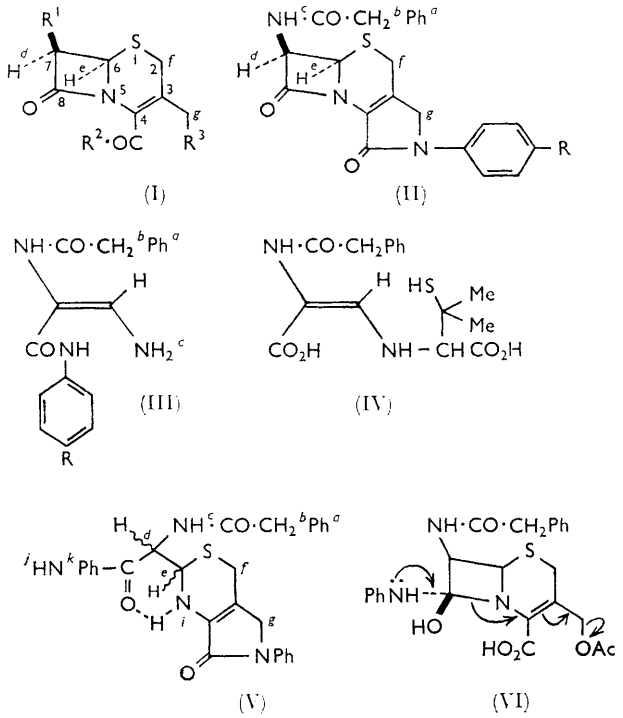
[1] Part V, H. Fazakerley, D. A. Gilbert, G. I. Gregory, J. K. Lazenby, and A. G. Long, *J. Chem. Soc.* (*C*), 1967, 1959.

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

[3] Part II, J. D. Cocker, B. R. Cowley, J. S. G. Cox, S. Eardley, G. I. Gregory, J. K. Lazenby, A. G. Long, J. C. P. Sly, and G. A. Somerfield, *J. Chem. Soc.*, 1965, 5015.

[4] Part I, G. F. H. Green, J. E. Page, and S. E. Staniforth, *J. Chem. Soc.*, 1965, 1595.

product. The ultraviolet absorption[5] of *N*-methylacetanilide, $\lambda_{max.}$ 225 nm., indicates the origin of the band, $\lambda_{max.}$ 229 nm., in the spectrum of this lactam. Formation of the lactam ring and the number of phenyl protons proved that the aniline residue was *N*- rather than *C*-coupled.



The small letters are for reference in the quotation of n.m.r. data

In order to reduce cyclization to the lactam, we treated sodium 7-phenylacetamidocephalosporanate at 80° with aniline in an aqueous solution kept at pH 7·5. A non-polar sulphur-free product separated within 10 min.; it was optically inactive. Its infrared spectrum, $\nu_{max.}$ 1690, 1644, and 1565 cm.$^{-1}$, suggested the presence of two amide groups, and phenyl absorption, $\nu_{max.}$ 702 and 756 cm.$^{-1}$, was evident; the ^{1}H n.m.r. signals corresponded to the presence of two phenyl rings and one discrete methylene group; there were also some low-field resonances due to protons which exchanged in deuterium oxide. An intense ion in the mass spectrum, *m/e* 295·1321, gave the empirical formula $C_{17}H_{17}N_3O_2$ (there were traces of an impurity, *M*, 371·1642, corresponding to the formula $C_{23}H_{21}N_3O_2$); fragmentation peaks indicated loss of CO, NH_3, Ph, and $PhCH_2$.

This information indicates the structure (III; R = H), which contains a β-aminoacrylic anilide system, with unknown geometry about the double bond. Such a system might also be generated from 6β-phenylacetamidopenicillanic acid and aniline, but the nearest known analogues are the penamaldic acids (IV),[6] $\lambda_{max.}$ 282 nm. The ultraviolet absorption of our product, $\lambda_{max.}$ 314—316 nm. (ε 26,900), $\lambda_{infl.}$ 290 nm. (ε 17,600), and $\lambda_{min.}$ 250—253 nm. (ε 3050), indicated the bathochromic effect of the anilide in comparison with the carboxy-group. Electrophoresis at pH 1·9 showed that the anilide (III; R = H) was not protonated.

We have insufficient information to single out a pathway for the formation of this anilide. The steps are probably common to other reactions in which cephalosporin C and penicillin N yield cephalosporidine.[1,7]

Sodium 7-phenylacetamidocephalosporanate and aniline, in aqueous solution at 80° and pH 7·5, yield other products, detected by paper chromatography: one, which gave a spot fluorescing white when irradiated with ultraviolet light, was probably the γ-lactam (II; R = H), present in traces too small for isolation. Two other components, both dextrorotatory, behaved as acids; they were isolated at pH 4, and separated by preparative paper chromatography and by chromatography of their sodium salts on polyamide columns.

The less polar acid product, $\lambda_{max.}$ 242 nm. (ε 13,900), $\lambda_{infl.}$ 262 nm. (ε 11,300), gave a dark blue patch on paper irradiated with ultraviolet light. Its infrared absorption and ^{1}H n.m.r. signals indicate the integrity of the 7β-phenylacetamidoceph-3-em-4-carboxylate system. The acetate group in the starting material had been replaced by a phenyl system (five protons); therefore it is joined to the cephem through the nitrogen of the aniline residue, and the compound has structure (I; R^1 = $NH{\cdot}CO{\cdot}CH_2Ph$, R^2 = OH, R^3 = NHPh). The ultraviolet absorption comprises a peak due to the *N*-substituted aniline (*N*-methylaniline, $\lambda_{max.}$ 244 nm.)[8] and an inflexion denoting the contribution from the chromophore in the ceph-3-em system.[9] The more polar acidic product, $\lambda_{max.}$ 252—253 nm., $\lambda_{infl.}$ 265 nm., was probably the bis(ceph-3-em-3-ylmethyl)aniline formed by reaction of the less polar acid with 7-phenylacetamidocephalosporanic acid (cf. *NN*-dimethylaniline,[10] $\lambda_{max.}$ 252 nm., and *N*-methylaniline derivatives described below). The two products behaved as anions on electrophoretograms at pH 7. Ethyl acetate extracts them from aqueous solutions at pH 4.

In acetone at 50° 7-phenylacetamidocephalosporanic acid reacted overnight with aniline, to give a dark red solution smelling of mercaptans. A dextrorotatory solid was isolated, and purified by concentration and crystallisation from *NN*-dimethylformamide and water; it bore no net charge at pH 1·9 or 7·0, and fluoresced white on paper under ultraviolet light. The infrared absorption indicated the presence of amide, $\nu_{max.}$ 1652 and 1538 cm.$^{-1}$, and phenyl groups, $\nu_{max.}$ 754 and 690 cm.$^{-1}$;

[5] P. L. Southwick, D. I. Sapper, and L. A. Pursglove, *J. Amer. Chem. Soc.*, 1950, **72**, 4940; cf. H. E. Ungnade, *ibid.*, 1954, **76**, 5133.

[6] R. B. Woodward, A. Neuberger, and N. R. Trenner, in 'Chemistry of Penicillin,' ed. H. T. Clarke, J. R. Johnson, and R. Robinson, Princeton University Press, 1949, p. 427.

[7] J. D. Jeffery, E. P. Abraham, and G. G. F. Newton, *Biochem. J.*, 1960, **75**, 216.

[8] R. H. Eastman and F. L. Detert, *J. Amer. Chem. Soc.*, 1951, **73**, 4511.

[9] D. M. Green, A. G. Long, P. J. May, and A. F. Turner, *J. Chem. Soc.*, 1964, 766.

[10] H. Walba and G. E. K. Branch, *J. Amer. Chem. Soc.*, 1951, **73**, 3341.

the spectrum lacked absorption due to azetidinone and acetate groups. The ^{1}H n.m.r. signals confirmed and extended these inferences: in particular, three five-proton phenyl systems and three discrete methylene groups were indicated, together with an AB-system of two single protons and three low-field exchangeable protons, probably NH. Analysis by combustion showed four nitrogen atoms to one of sulphur; consequently, we assign to the product structure (V), which contains chromophores due to an *N*-acylanilide, an *N*-acyl-*N*-alkylanilide,[5] and a dihydrothiazine,[9,11] expected at about 242, 250, and 285 nm., respectively. The ultraviolet absorption of the compound includes $\lambda_{max.}$ 248—250 nm. (ε 25,300) and $\lambda_{infl.}$ 290 nm. (ε 6300).

As nucleophilic replacement of the acetate group in cephalosporanic acids requires protic solvents which favour a carbonium-carboxylate intermediate,[3,12] we attribute generation of the lactam (V) in the solvent acetone to prior attack at the 8-position [see (VI)],[9,11] with subsequent addition of aniline to the methylene-dihydrothiazine and final cyclization to a lactam. The stability of the γ-lactam ring preserves the molecule from further reactions due to an anticlockwise electronic flow in the six-membered ring.[1] Solvent would be expected to play a large part in these reactions, since attack at the CH_2·OAc group depends on a dissociation into two charged species, of the type RCH_2·OAc $\longrightarrow$ $RCH_2^+ + OAc^-$; this reaction would therefore be at a disadvantage in solvents of low dielectric constant.[3]

At 80° and pH 7·5, aqueous solutions of sodium 7-phenylacetamidocephalosporanate reacted within 5 minutes with *p*-anisidine, to yield a precipitate which gave a light blue fluorescence on paper illuminated in the ultraviolet. Its properties likened it to the vinylamine, $\lambda_{max.}$ 312—314 and 290 nm., obtained with aniline; consequently we assign structure (III; R = OMe) to it. The *p*-methoxy-substituent causes bathochromic shifts to $\lambda_{max.}$ 323 and 300 nm. More of the product of ring fission was formed in reactions with *p*-anisidine than in those with aniline, and we did not obtain a pure specimen of the simple substitution product (I; R^1 = NH·CO·CH_2Ph, R^2 = OH, R^3 = NH·C_6H_4·OMe-*p*).

At 50° and an initial pH of 7·1, sulphanilamide converted sodium 7-phenylacetamidocephalosporanate in 3 days into several products, and the highly insoluble γ-lactam (II; R = SO_2·NH_2), $\lambda_{max.}$ 246—250 and 264—267 nm., separated out. It gave a white fluorescence on papers illuminated in the ultraviolet. *p*-Acetylsulphamoylaniline gave the γ-lactam (II; R = SO_2·NHAc) likewise; this was crystallised from *NN*-dimethylformamide, and was too sparingly soluble for accurate measurements of optical density ($\lambda_{max.}$ 270 nm.). In 1 hour at 85° and at constant pH 7·5, sulphanilamide gave (after chromatography of the product on a polyamide) the dextrorotatory sodium salt (I; R^1 = NH·CO·CH_2Ph, R^2 = O^-Na^+, R^3 = NH·C_6H_4·SO_2·NH_2-*p*), $\lambda_{max.}$ 273 nm.

[11] G. C. Barrett, S. H. Eggers, T. R. Emerson, and G. Lowe, *J. Chem. Soc.*, 1964, 788.

The complications in reactions with primary aromatic amines arise from formation of lactams and from attack on the 8-carbonyl group of ceph-3-em systems. Seeking to avoid these difficulties, we treated sodium 7β-phenylacetamidocephalosporanate for 1 hr. with *N*-methylaniline in aqueous solution at 87° and at (constant) pH 7·5, and obtained a mixture simpler than that from the reactions with aniline. After removal of the base and neutral products, dextrorotatory material was extracted into an organic solvent from the aqueous solution at pH 4. We obtained the sodium salt of the derivative (I; R^1 = NH·CO·CH_2Ph, R^2 = O^-Na^+, R^3 = NMePh) (36%) by addition of sodium 2-ethylhexanoate. Its ultraviolet absorption, $\lambda_{max.}$ 251—252 nm. (ε 18,500), represents the predominance of the dialkylaniline chromophore (cf. *NN*-dimethylaniline,[10] $\lambda_{max.}$ 252 nm.). During electrophoresis at pH 7·0 the compound hardly moves as an acid, and at pH 1·9 it moves very slightly to the cathode; therefore the pK_a of the 4-carboxylic acid group must be <1·9, and the pK_a of the conjugate acid of the amine is >7. (The pK_a of the *NN*-dimethylanilinium ion [13] is 5·06. Bulkier substituents increase the value by hampering the base-weakening aromatic mesomerism; the cephem-4-carboxylate ion will exert a base-strengthening $+I$ effect and provide a stabilizing negative charge.)

In similar reactions we used *N*-methylaniline to replace the acetate group in cephalothin (I; R^1 = 2-thienylacetamido-, R^2 = OH, R^3 = OAc), 7β-benzylthioacetamidocephalosporanic acid (I; R^1 = NH·CO·CH_2·S·CH_2Ph, R^2 = OH, R^3 = OAc), and in 7β-aminocephalosporanic acid (I; R^1 = NH_3^+, R^2 = O^-, R^3 = OAc); and with *N*-ethylaniline, *N*-benzylaniline, and ethyl anilinoacetate we made the corresponding derivatives of 7β-phenylacetamidocephalosporanic acid.

The *N*-methylaniline derivative of 7-aminocephalosporanic acid crystallizes from aqueous solution at about pH 6, presumably as the aminobetaine (I; R^1 = NH_2, R^2 = O^-, R^3 = $\overset{+}{N}$HMePh). We plump for protonation on the anilino-nitrogen atom, as it is likely (for reasons already advanced in this paper) to be more strongly basic than the 7β-amino-group (cf. 7β-aminocephalosporanic acid,[14] pK_a 4·63 and 1·75); accordingly, it migrated twice as far as 7-aminocephalosporanic acid towards the cathode on electrophoresis at pH 1·9 and travelled more slowly to the anode at pH 7·0, which indicates that at the latter pH the anilino-group still existed in appreciable amount as the conjugate acid. (At pH 1·9, 7-aminocephalosporanic acid behaves as if the zwitterionic form, with no net charge, is contributing appreciably to the equilibrium. Comparisons of pK_a values and electrophoretic mobilities must take account of the solvents: pK_a values are for aqueous solutions,

[12] Part III, A. B. Taylor, *J. Chem. Soc.*, 1965, 7020.

[13] A. Albert and E. P. Serjeant, 'Ionisation Constants of Acids and Bases,' Methuen, London, 1962, p. 139.

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

whereas electrophoresis at pH 1·9 is carried out on solutions containing other solvents).

Note Added in Proof: By recent spectroscopic measurements, Miss A. Gale has found pK_a *ca.* 4·7 for the *N*-methylanilinium group in our compounds, as solutions in pure water. The isoelectric point for the derivative (I; $R^1 = NH{\cdot}CO{\cdot}CH_2Ph$, $R^2 = O^-$, $R^3 = \overset{+}{N}HMePh$) is 3·3, and the p$K_a$ of the 4-CO_2H group is $<1{\cdot}9$ (by deduction); therefore, the two basic groups in the diamino-carboxylate anion (I; $R^1 = NH_2$, $R^2 = O^-$, $R^3 = NMePh$) are of nearly the same strength, and deductions from electrophoretic behaviour are shown to be tricky. As most of the amino-acids in this series bear weakly basic amino-groups, the uncharged forms are probably important in non-polar and aprotic solvents, but the dipolar forms predominate in water (see E. J. Cohn and J. T. Edsall, 'Proteins, Amino Acids and Peptides as Ions and Dipolar Ions,' Reinhold, New York, 1943, pp. 90 *et seq.*).

EXPERIMENTAL

Our general procedures have been described.[3,4] In this work the infrared absorption was measured for Nujol mulls; the hydrated crystals gave broad bands at about 3420 cm.$^{-1}$. The ultraviolet spectra were measured for the sodium salts in aqueous phosphate buffer at pH 6, and for the neutral compounds in ethanol. We thank A.E.I. Ltd., Manchester, for the mass spectrum, measured on an MS 9 spectrometer. The polyamide powder used for column chromatography was Merck item no. 7435.

Proton Magnetic Resonance Spectra.—τ Values are given as in refs. 1 and 4. (I; $R^1 = NH_2$, $R^2 = O^-$, $R^3 = NMePh$): D_2O (containing $NaHCO_3$); 4·58 (J = 4·5 c./sec.; *d*), 5·02 (J = 4·5 c./sec.; *e*), 6·80 (2; *f*), and 5·78 (2; *g*).

(I; $R^1 = NH{\cdot}CO{\cdot}CH_2Ph$, $R^2 = O^-$, $R^3 = NHPh$): D_2O; 2·78 (10; *a*), 6·46 (2; *b*), 4·48 (J = 4·5 c./sec.; *d*), 5·21 (J = 4·5 c./sec.; *e*), 6·83 (2; *f*), 6·0 (2; *g*). ($R^3 = NMePh$): D_2O; 2·65 (10; *a*), 6·48 (2; *b*), 4·47 (J = 4·5 c./sec.; *d*), 5·20 (J = 4·5 c./sec.; *e*), 7·12 and 5·86 (2 × 2; *f* and *g*). ($R^3 = NPh{\cdot}CH_2{\cdot}CO_2Et$): D_2O; 2·83 (10; *a*), 6·00, 6·92, and 5·80 (3 × 2; *b*, *f*, and *g*), 4·43 (J = 4·5 c./sec.; *d*), and 5·13 (J = 4·5 c./sec.; *e*). ($R^3 = NH{\cdot}C_6H_4{\cdot}SO_2{\cdot}NH_2$-*p*): D_2O; 2·68 (9; *a*), 6·39 (2; *b*), 4·45 (J = 4·5 c./sec.; *d*), 5·13 (J = 4·5 c./sec.; *e*), *ca.* 6·73 (2; *f*), and *ca.* 5·93 (2; *g*).

(I; R^1 = 2-thienylacetamido, $R^2 = O^-$, $R^3 = NMePh$): D_2O; 6·23 (2; *b*), 4·55 (J = 4·5 c./sec.; *d*), 5·17 (J = 4·5 c./sec.; *e*), *ca.* 7·04 (2; *f*), and *ca.* 5·83 (2; *g*).

(II; R = H): DMSO;* 2·67 (10; *a*), 6·12 (2; *b*),* 0·87 (J = 8 c./sec.; *c*), 4·15 (J = 5 and 8 c./sec.; *d*), 4·96 (J = 5 c./sec.; *e*), 5·70 (2; *f*),* and 5·35 (2; *g*).* ($R = SO_2{\cdot}NH_2$): DMSO;* 2·67 (9; *a*), 0·83 (J = 8 c./sec.; *c*), 4·14 (J = 5 and 8 c./sec.; *d*), 4·85 (J = 5 c./sec.; *e*), and 5·28 (2; *g*)*: pyridine; 6·13 (2; *b*), 3·64 (J = 5 and 8 c./sec.; *d*), 4·27 (J = 5 c./sec.; *e*), 6·13 and 6·45 (J = 19 c./sec.; *f*), and 5·64 and 6·03 (J = 18 c./sec.; *g*).

(III; R = H): [$^2H_6$]DMSO; 2·60 (5; *a*), 6·22 (2; *b*), and *ca.* 4·20 (2; *c*): $CF_3{\cdot}CO_2H$; 2·52 (5; *a*), 5·98 (2; *b*).

(V): DMSO; 2·66 (15; *a*), −0·36 (*i*): pyridine; 6·13 (2; *b*), 0·80 (*c*), 4·20 and 4·60 (complex; *d* and *e*), 6·13 (2; *f*), 6·28 and 6·63 (J = 17 c./sec., *g*), and −1·35 (*i*).

3-Anilinomethyl-7β-phenylacetamidoceph-3-em-4-carboxylic Acid, γ-Lactam (II; R = H).—A solution of sodium 7β-phenylacetamidocephalosporanate (I; $R^1 = NH{\cdot}CO{\cdot}CH_2Ph$, $R^2 = O^-Na^+$, R^3 = OAc) (5·0 g.) and redistilled aniline (3·3 ml., 3·0 equiv.) in water (100 ml.), initially at pH 7·3, was left at 37° for 3 days. It turned yellow and smelt of hydrogen sulphide, and the pH changed to about 5. A yellow solid (3·43 g.) precipitated which gave five spots on a thin-layer chromatogram; it was stirred for 1 hr. with glacial acetic acid. The mixture was filtered and the residue washed with acetone, and dried (0·30 g.). Part (0·27 g.) of this solid was dissolved in *NN*-dimethylformamide (10 ml.). Water (50 ml.) was added slowly, with stirring, to precipitate a white solid (0·19 g.), $[\alpha]_D$ +164° [*c* 1·01 in dimethylformamide (DMF)]; part (0·151 g.) of this material was stirred with ethyl acetate (10 ml.) containing DMF (1%), and the insoluble part was filtered off, washed with ethyl acetate, and dried to give the *lactam* (II; R = H) (0·144 g.), $\lambda_{max.}$ 229—230 (ε 16,500) and 254—256 nm. (ε 12,800), $\lambda_{infl.}$ 290 nm. (ε 5600), $\lambda_{min.}$ 244—246 nm. (ε 11,600), $\nu_{max.}$ 3270 (NH), 1790 and 1772 (azetidinone), 1688 (αβ-unsaturated γ-lactam), and 1668 and 1540 (CONH) cm.$^{-1}$ (Found: C, 64·7, 64·25; H, 4·8, 4·5; N, 10·2; S, 7·6. $C_{22}H_{19}N_3O_3S,0{\cdot}25H_2O$ requires C, 64·4; H, 4·8; N, 10·2; S, 7·8%). This compound streaked on paper chromatograms; it fluoresced white when the papers were held under ultraviolet light. (The bands at 1790 and 1772 cm.$^{-1}$ probably represent hydrated and non-hydrated forms.)

7β-Phenylacetamido-3(p-sulphamoylanilinomethyl)ceph-3-em-4-carboxylic Acid, γ-Lactam (II; $R = SO_2{\cdot}NH_2$).—In a similar experiment sodium 7-phenylacetamidocephalosporanate (2·0 g.) and sulphanilamide (1·08 g., 3·0 equiv.) gave a solid (1·14 g.), $[\alpha]_D$ +85° (*c* 1·0 in DMF), R_P 1·32 (white fluorescence); part (0·30 g.) was crystallized from acetone to give the *γ-lactam* (II; $R = SO_2{\cdot}NH_2$) (0·101 g.), $\lambda_{max.}$ 246—250 and 264—267 nm. (optical densities could not be measured, owing to low solubility), $\nu_{max.}$ 3260 (NH), 1776 (azetidinone), 1700 (αβ-unsaturated γ-lactam), and 1668 and 1545 (CONH) cm.$^{-1}$ (Found: C, 54·5; H, 4·0; N, 11·0; S, 12·7. $C_{22}H_{20}N_4O_5S_2$ requires C, 54·5; H, 4·2; N, 11·6; S, 13·2%).

3(p-Acetylsulphamoylanilinomethyl)-7β-phenylacetamidoceph-3-em-4-carboxylic Acid, γ-Lactam (II; $R = SO_2{\cdot}NHAc$).—In an experiment like the last, but with *p*-acetylsulphamoylaniline as nucleophile we obtained the *compound* (II; $R = SO_2{\cdot}NHAc$) (0·90 g.). Crystallization from DMF yielded pure material (51 mg.), $[\alpha]_D$ +117° (*c* 1·06 in DMF), $\lambda_{max.}$ 270 nm. (too insoluble for optical density measurements), $\nu_{max.}$ 1792 (azetidinone), 1738 (Ac), 1700 (αβ-unsaturated γ-lactam), and 1665 and 1548 (CONH) cm.$^{-1}$ (Found: C, 53·9; H, 4·3; N, 10·8; S, 11·5. $C_{24}H_{22}N_4O_6S_2{\cdot}0{\cdot}5H_2O$ requires C, 53·8; H, 4·3; N, 10·5; S, 11·8%).

α-(Aminomethylene)-α-(phenylacetamido)acetanilide (II; R = H).—A solution of sodium 7β-phenylacetamidocephalosporanate (I; $R^1 = NH{\cdot}CO{\cdot}CH_2Ph$, $R^2 = O^-Na^+$, R^3 = OAc) (2·0 g.) and redistilled aniline (0·53 ml., 1·2 equiv.) in water (40 ml.) was kept at 80° for 1 hr. at pH 7·5, maintained by additions of N-sodium hydroxide. After 10 min. a white solid began to separate from the yellow reaction mixture. This was collected, washed with water, and dried (0·25 g.); part (0·20 g.) was stirred with acetone (15 ml.) for 30 min., and the resulting product was filtered off and dried to give the *anilide* (III; R = H) (0·17 g.), m. p. 230°, $\lambda_{max.}$ 314—316 nm. (ε 26,900), $\lambda_{infl.}$ 290 nm. (ε 17,600), $\lambda_{min.}$ 250—253 nm. (ε 3050), $\nu_{max.}$ 1690 and 1565

* Dimethyl sulphoxide. † Assignment uncertain.

(CONH), 1644 (CONH), 756 (Ph), and 702 (Ph) cm.$^{-1}$ (Found: C, 70·5, 70·5; H, 5·6, 5·8; N, 14·0, 14·2; S, 0·2. $C_{17}H_{17}N_3O_2$ requires C, 69·15; H, 5·8; N, 14·2%, *M*, 295). Mass spectrum (*m/e*): 371·1642 ($C_{23}H_{21}N_3O_2$ impurity; weak), 295·1321 ($C_{17}H_{17}N_3O_2$), 278·1061 ($C_{17}H_{14}N_2O_2$), 91 (benzyl), 77 (phenyl), and 28 (CO). In a similar experiment, part (0·80 g.) of the white solid (0·94 g.), m. p. 225°, from the reaction was crystallized from water (5 ml.) and DMF (12 ml.); the product (0·73 g.), recrystallized similarly, had properties similar to those of the foregoing specimen (Found: C, 68·75; H, 5·6; N, 13·8; S, 0·0%) and showed no net charge on electrophoresis at pH 1·9 or 7·0.

In a similar experiment, but with *p*-anisidine (0·7 g., 1·2 equiv.) in place of aniline, we obtained α-(*aminomethylene*)-α-(*phenylacetamido*)-p-*methoxyacetanilide* (III; R = OMe) (0·24 g.), m. p. 214—216°, R_P 1·87 (light-blue fluorescence), $\lambda_{max.}$ 300 (ε 23,800) and 323 (ε 21,600) nm., $\lambda_{min.}$ 314 (ε 21,200) and 256 (ε 140) nm., $\nu_{max.}$ 3460, 3300, and 3240 (NH_2 and NH), 1685 and 1568, and 1642 and 1522 (CONH) cm.$^{-1}$ (Found: C, 66·3; H, 6·1; N, 12·5; S, 0·4. $C_{18}H_{19}N_3O_3$ requires C, 66·4; H, 5·9; N, 12·9%).

Sodium 7β-*Phenylacetamido*-3-*anilinomethylceph*-3-*em*-4-*carboxylate* (I; R^1 = NH·CO·CH_2Ph, R^2 = O^-Na^+, R^3 = NHPh).—A solution of sodium 7β-phenylacetamidocephalosporanate (I; R^1 = NH·CO·CH_2Ph, R^2 = O^-Na^+, R^3 = OAc) (5·0 g.) and redistilled aniline (1·33 ml., 1·1 equiv.) in water (100 ml.) was kept for 45 min. at 85°; the pH was raised from 7·1 to 7·5 and kept at the higher figure by additions of N-sodium hydroxide (12·4 ml.). The mixture was cooled, and the precipitate [mainly (III; R = H)] (0·67 g.) was filtered off. The orange filtrate was extracted with ethyl acetate (3 × 50 ml.) and with methyl isobutyl ketone (3 × 50 ml.); organic solvents were distilled out of the remaining aqueous phase, which was freeze-dried. A solution of the residue (4·94 g.) in water (50 ml.), pH 7·6, was covered with ethyl acetate (25 ml.), and 2N-hydrochloric acid was added (to pH 4·0). The organic layer and extracts of the aqueous phase were combined, dried, concentrated, and treated with sodium 2-ethylhexanoate in acetone (10%; 10 ml.). The solution was concentrated again until separation (0·19 g.) began. The yellow filtrate was added, with stirring, to ether (100 ml.), and part (1·0 g.) of the precipitate (1·46 g.), R_P 0·72, 1·32, and 1·52 (white fluorescence) was dissolved in water (4 ml.); the solution was filtered and applied to Whatman No. 17 papers, which were irrigated downwards overnight with solvent C.* Two bands of material were traced by their ultraviolet absorption; they were cut out and eluted with water, the solutions were freeze-dried, and the residues were triturated with ether.

Part (0·39 g.) of the less polar fraction (0·42 g.), R_P 1·41 and 1·78 (faint), was stirred with water (8 ml.) and filtered, and the filtrate was run on to a column of polyamide (30 ml.), which was washed with water. Eluates were examined by paper chromatography, and the richest fractions were freeze-dried. The residue was rubbed with ether to give the *aniline derivative* (I; R^1 = NH·CO·CH_2Ph, R^2 = O^-Na^+, R^3 = NHPh) (0·19 g.), $[\alpha]_D$ +85° (*c* 1·07 in water), R_P 1·32 and 1·47 (weak) (white), $\lambda_{max.}$ 242 nm. (ε 13,900), $\lambda_{infl.}$ 262 nm. (ε 11,300), $\nu_{max.}$ 1750 (azetidinone), 1658 and 1505 (CONH), and 1600 (CO_2^-) cm.$^{-1}$ (Found: C, 53·1, 52·6; H, 4·9, 4·8; N, 8·9; S, 6·4. $C_{22}H_{20}NaN_3O_4S,3H_2O$ requires C, 52·9; H, 5·25; N, 8·4; S, 6·4%). The compound migrated 2 cm. as an anion on electrophoresis at pH 7·0.

The more polar fraction was worked up similarly to give an antibacterially active substance (93 mg.), $[\alpha]_D$ +93° (*c* 1·03 in water), R_P 0·62, $\lambda_{max.}$ 252—253 nm. ($E^{1\%}_{1cm.}$ 240), $\lambda_{infl.}$ 265 nm. ($E^{1\%}_{1cm.}$ 214). It could not be obtained pure; its probable structure is discussed on p. 802. It ran 1·7 cm. towards the anode on electrophoresis at pH 7·0.

Sodium 7β-*Phenylacetamido*-3(p-*sulphamoylanilinomethylceph*-3-*em*-4-*carboxylate* (I; R^1 = NH·CO·CH_2Ph, R^2 = O^-Na^+, R^3 = NH·C_6H_4·SO_2·NH_2-*p*).—In an experiment similar to the last, except that sulphanilamide (2·49 g., 1·2 equiv.) was used as the nucleophile, we obtained the *sulphanilamide derivative* (0·19 g.), $[\alpha]_D$ +82° (*c* 1·01 in water), R_P 0·8, $\lambda_{max.}$ 273 nm. (ε 23,600), $\lambda_{min.}$ 233—234 nm. (ε 7,300), $\nu_{max.}$ 1752 (azetidinone), 1662 and 1522 (CONH), and 1600 (CO_2^-) cm.$^{-1}$ (Found: C, 49·0; H, 4·4; N, 10·0; S, 11·6. $C_{22}H_{21}N_4NaO_6S_2,H_2O$ requires C, 48·7; H, 4·3; N, 10·3; S, 11·8%).

α-(5-*Anilinomethyl*-4-*carboxy*-3,6-*dihydro*-2H-1,3-*thiazin*-2-*yl*)-α-(*phenylacetamido*)*acetanilide*, γ-*Lactam* (V).—A solution of 7β-phenylacetamidocephalosporanic acid (I; R^1 = NH·CO·CH_2Ph, R^2 = OH, R^3 = OAc) (5·0 g.) and freshly distilled aniline (3·3 ml., 3·3 equiv.) in acetone (100 ml.) was kept at 50° for 16 hr. The solution turned from yellow to deep red. It was concentrated, and an orange-brown solid (1·20 g.) separated, part (0·555 g.) of which was crystallized in two crops from DMF (5 ml.) and water (2 ml.). Recrystallization likewise gave needles (0·256 g.) of the *lactam* (V), m. p. 214°, $[\alpha]_D$ +33·5° (*c* 1·0 in DMF), $\lambda_{max.}$ 248—250 nm. (ε 25,300), $\lambda_{infl.}$ 290 nm. (ε 6300), $\nu_{max.}$ 1698 (αβ-unsaturated γ-lactam), 1652 and 1538 (CONH), and 754 and 690 (phenyls) cm.$^{-1}$ (Found: C, 67·4; H, 5·4; N, 11·3; S, 5·85. $C_{28}H_{26}N_4O_3S$ requires C, 67·45; H, 5·3; N, 11·2; S, 6·4%). This material bore no net charge on electrophoresis at pH 1·9 or 7·0.

The same product was obtained from a similar reaction mixture left for 1 week at 37°.

Sodium 3-(N-*Methylanilinomethyl*)-7β-*phenylacetamidoceph*-3-*em*-4-*carboxylate* (I; R^1 = NH·CO·CH_2Ph, R^2 = O^-Na^+, R^3 = NMePh).—A solution of sodium 7β-phenylacetamidocephalosporanate (I; R^1 = NH·CO·CH_2Ph, R^2 = O^-Na^+, R^3 = OAc) (5·0 g.) and purified *N*-methylaniline (1·76 ml., 1·2 equiv.) in water (100 ml.) was kept at 87° and pH 7·5 for 1 hr. The pH was maintained by additions of N-sodium hydroxide (11·7 ml.); none was needed after the first 30 min. The yellow solution was extracted with ethyl acetate (3 × 50 ml.) and with methyl isobutyl ketone (2 × 50 ml.). The aqueous phase was then covered with ethyl acetate (50 ml.), and the pH was brought, with stirring, to 4·0 by additions of 2N-hydrochloric acid. The aqueous layer was again extracted with ethyl acetate, and the combined organic phases were concentrated. Sodium 2-ethylhexanoate in acetone (10%; 5 ml.) was added; a solid (1·56 g.), R_P 1·38 and 1·57 (yellow fluorescence), separated and was spun off; addition of ether to the supernantant liquor precipitated a second crop (0·47 g.). A solution of the first crop was acidified to pH 4 and re-extracted with ethyl acetate; the product was converted as before into the sodium salt (0·97 g.), $[\alpha]_D$ +125° (*c* 1·05 in water), part (0·50 g.) of which was stirred with acetone to give the pure *sodium salt* (0·466 g.), $[\alpha]_D$ +134° (*c* 1·01 in water), $\lambda_{max.}$ 251—252 nm. (ε 18,500), $\nu_{max.}$ 3280 (NH), 1756 (azetidinone), 1666 and 1532 (CONH), and 1605 (CO_2^-) cm.$^{-1}$ (Found: C, 59·7; H, 5·3; N, 8·75; S, 6·8. $C_{23}H_{22}N_3NaO_4S,0·25H_2O$ requires C, 59·5; H, 4·9; N, 9·1; S, 6·9%). The compound moved 0·7 cm. towards the anode

on electrophoresis at pH 7·0, and 0·1 cm. towards the cathode at pH 1·9

Sodium 3-(N-*Methylanilinomethyl*)-7β-(2-*thienylacetamido*)*ceph*-3-*em*-4-*carboxylate* (I; R^1 = 2-thienylacetamido, $R^2 = O^-Na^+$, R^3 = NMePh).—In an experiment similar to the last, but with sodium 7β-(2-thienylacetamido)cephalosporanate (I; R^1 = 2-thienylacetamido, $R^2 = O^-Na^+$, R^3 = OAc) (5·0 g.), we obtained the pure *sodium salt* (1·10 g.), $[\alpha]_D$ +127° (*c* 1·0 in water), $\lambda_{max.}$ 246—247 nm. (ε 22,400), $\nu_{max.}$ 1756 (azetidinone), 1660 and 1530 (CONH), and 1605 (CO_2^-) cm.$^{-1}$ (Found: C, 53·7; H, 4·5; N, 9·0; S, 13·45. $C_{21}H_{20}N_3NaO_4S_2,0·25H_2O$ requires C, 53·7; H, 4·4; N, 8·9; S, 13·6%).

Sodium 7β-*Benzylthioacetamido*-3-(N-*methylanilinomethyl*)*ceph*-3-*em*-4-*carboxylate* (I; R^1 = $NH{\cdot}CO{\cdot}CH_2{\cdot}S{\cdot}CH_2Ph$, $R^2 = O^-Na^+$, R^3 = NMePh].—In an experiment similar to the last two, but with sodium benzylthioacetamidocephalosporanate (I; R^1 = $NH{\cdot}CO{\cdot}CH_2{\cdot}S{\cdot}CH_2Ph$, $R^2 = O^-Na^+$, R^3 = OAc) (5·0 g.)[3] and after chromatography of aqueous solutions of the crude sodium salts of the products on columns of polyamide, we obtained the *sodium salt* (0·54 g.), $[\alpha]_D$ +86° (*c* 1·06 in water), $\lambda_{max.}$ 251—252 nm. (ε 17,600), $\lambda_{min.}$ 228—229 nm. (ε 11,200), $\nu_{max.}$ 1752 (azetidinone), 1665 and 1510 (CONH), and 1602 (CO_2^-) cm.$^{-1}$ (Found: C, 53·4; H, 5·2; N, 8·1; S, 12·2. $C_{24}H_{24}N_3NaO_4S_2,2H_2O$ requires C, 53·2; H, 5·3; N, 7·9; S, 12·05%). The product was traced through the separation and purification by its R_F value, which was 1·8 times that of the starting material.

Sodium 3-(N-*Ethylanilinomethyl*)-7β-*phenylacetamidoceph*-3-*em*-4-*carboxylate* (I; $R^1 = NH{\cdot}CO{\cdot}CH_2Ph$, $R^2 = O^-Na^+$, R^3 = NEtPh).—A solution of sodium 7β-phenylacetamidocephalosporanate (5·0 g.) and redistilled *N*-ethylaniline (1·52 ml., 1·0 equiv.) in water (100 ml.) was kept at 85° for 1 hr.; the pH was maintained at 7·5 by additions of N-sodium hydroxide (12·0 ml.). The product was isolated as a sodium salt (2·16 g.), R_P 0·87, 1·10, and 1·44, precipitated with ether, as described for reactions with *N*-methylaniline. A solution of this solid in water (10 ml.) was chromatographed on polyamide, and fractions rich in a component, R_P 1·40, were chromatographed again to obtain, after freeze-drying of the eluates and trituration of the residue with ether, the pure *sodium salt* (0·37 g.), $[\alpha]_D$ +107° (*c* 1·0 in water), $\lambda_{max.}$ 256 nm. (ε 20,000), $\lambda_{min.}$ 227—229 nm. (ε 9600), $\nu_{max.}$ 1756 (azetidinone), 1668 and 1512 (CONH), and 1604 (CO_2^-) cm.$^{-1}$ (Found: C, 59·65; H, 5·3; N, 8·5; S, 6·75. $C_{24}H_{24}N_3NaO_4S,0·5H_2O$ requires C, 59·7; H, 5·2; N, 8·7; S, 6·5%). Further crops from the columns yielded more pure material (0·56 g.).

Sodium 3-(N-*Benzylanilinomethyl*)-7β-*phenylacetamidoceph*-3-*em*-4-*carboxylate* (I; $R^1 = NH{\cdot}CO{\cdot}CH_2Ph$; $R^2 = O^-Na^+$, R^3 = NPh·CH_2Ph).—Sodium 7β-phenylacetamidocephalosporanate (5·0 g.) was added to redistilled *N*-benzylaniline (2·22 g.; 1·0 equiv.) in water (50 ml.) and ethanol (50 ml.); the solution was kept at 85° for 1 hr., and the pH was maintained at 7·5 by additions of N-sodium hydroxide (8·8 ml.). Extraction of neutral materials with ethyl acetate and methyl isobutyl ketone and preparation of the sodium salt was performed as for the *N*-methylaniline derivatives. The crude sodium salt (2·2 g.) was purified by repeated chromatography of aqueous solutions [pH adjusted to 7 with Dowex-50 (H^+)] on polyamide columns, with paper chromatography as a guide. In this way the *sodium salt* was obtained as an off-white solid (0·48 g.), $[\alpha]_D$ +50° (*c* 1·03 in water), R_P 1·53, $\lambda_{max.}$ 251—252 nm. (ε 17,000), $\lambda_{min.}$ 228—229 nm. (ε 10,100), $\nu_{max.}$ 1750 (azetidinone), 1664 and 1510 (CONH), and 1602 (CO_2^-) cm.$^{-1}$ (Found: C, 59·9; H, 5·6; N, 7·5; S, 5·5. $C_{29}H_{27}N_3NaO_4S,2·5H_2O$ requires C, 59·9; H, 5·5; N, 7·2; S, 5·5%).

Sodium 3-(N-*Ethoxycarbonylmethylanilinomethyl*)-7β-*phenylacetamidocephalosporanate* (I; $R^1 = NH{\cdot}CO{\cdot}CH_2Ph$, $R^2 = O^-Na^+$, R^3 = NPh·$CH_2{\cdot}CO_2$Et).—A solution of sodium 7β-phenylacetamidocephalosporanate (5·0 g.) and ethyl α-anilinoacetate (2·16 g., 1·0 equiv.) in water (100 ml.) was kept at 86° for 1 hr.; the pH was maintained at 7·5 by additions of N-sodium hydroxide (14·5 ml.). The cloudy orange solution was extracted with ethyl acetate (3 × 50 ml.), the aqueous phase was covered with more ethyl acetate (50 ml.), and the pH was brought to 4·0 by additions of phosphoric acid. The required material was extracted with ethyl acetate and converted with sodium 2-ethylhexanoate into the sodium salt, which was precipitated from acetone with ether. Part (1·70 g.) of this product (1·85 g.), R_P 0·92 and 1·63, was suspended in water (10 ml.) and dissolved by adjusting the pH to 7·0 with sodium hydrogen carbonate. The product was then extracted, at pH 4·5, into ethyl acetate, and reprecipitated as the sodium salt (1·38 g.). This material was purified by passing its aqueous solutions [at pH 7·0 (saturated sodium hydrogen carbonate)] through polyamide columns, with paper chromatography of the fractions as a guide. Freeze-drying of fractions rich in material, R_P 1·70, gave solids that were triturated with ether to give the *sodium salt* (0·32 g.), $[\alpha]_D$ +76° (*c* 1·06 in water), R_P 1·77, $\lambda_{max.}$ 248 nm. (ε 19,700), $\lambda_{min.}$ 223—225 nm. (ε 10,300), $\nu_{max.}$ 1750 (azetidinone and ester), 1670 and 1535 (CONH), 1602 (CO_2^-), and 1192 (ester) cm.$^{-1}$ (Found: C, 57·7; H, 5·45; N, 7·5; S, 5·9. $C_{26}H_{26}N_3NaO_6S,0·5H_2O$ requires C, 57·0; H, 5·0; N, 7·8; S, 5·9%). Further crops (0·18 g.) of similar material were obtained.

7β-*Amino*-3-(N-*methylanilinomethyl*)*ceph*-3-*em*-4-*carboxylic Acid* (I; $R^1 = NH_2$, $R^2 = O^-$, $R^3 = \overset{+}{N}HMePh$) (with Miss A. Gale).—7β-Aminocephalosporanic acid (I; $R^1 = NH_3^+$, $R^2 = O^-$, R^3 = OAc) (10·9 g.) was added in portions to redistilled *N*-methylaniline (6·52 ml., 1·5 equiv.) in water (320 ml.); the mixture was stirred and kept at 82° for 90 min. Suspended solid was dissolved by raising the pH to 8·5 and then letting it fall to 7·0, at which value it was maintained with N-sodium hydroxide (72 ml.). The cooled solution was washed with methylene dichloride (3 × 200 ml.), and concentrated at 40°. It was decolourised by filtration at pH 8·7 through a column (12 cm. × 4 cm.) of grade I alumina (water-washings of which attain pH 5·2). Optically active fractions were collected (pH 7·9), and acidified with acetic acid to pH 5·5. The *amino-compound* (7·91 g., 62%) precipitated, $[\alpha]_D$ −12° (*c* 0·97 in DMSO). Pure material was obtained by repetition of the treatment with alumina; addition of acetic acid precipitated a white solid, $[\alpha]_D$ −14° (*c* 1·01 in DMSO), R_P 0·30, $\lambda_{max.}$ 251—252 nm. (ε 17,550), $\nu_{max.}$ 1806 (azetidinone), 1540 (CO_2^-), and 750 and 692 (Ph) cm.$^{-1}$ (Found: C, 56·2; H, 5·3; N, 13·05; S, 10·2. $C_{15}H_{17}N_3O_3S$ requires C, 56·4; H, 5·4; N, 13·15; S, 10·0%).

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