The Cephalosporin C Group

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1 Introduction

Cephalosporin C (1) was discovered at Oxford during a chemical study of the structure of penicillin N (2), a penicillin with a δ-(D-α-aminoadipoyl) side-chain produced by a species of *Cephalosporium* which had been isolated near a sewage outfall off the coast of Sardinia.[1,2] When the penillic acid from a partly purified preparation of penicillin N was chromatographed on an anion-exchange resin it was followed from the column by a second substance that was detected by its ultraviolet absorption at 260 mμ. This substance, which was given the trivial name Cephalosporin C, was readily isolated as a crystalline sodium salt and assigned the molecular formula $C_{16}H_{21}O_8N_3S$.[3] It was subsequently found to have antibacterial activity,[3] to resist hydrolysis by penicillinase from *B. cereus*,[3] to show an extraordinary lack of toxicity to mice, and to protect mice from infection with a penicillin-resistant strain of *Staph. aureus* which produced penicillinase.[4] It closely resembled the penicillins, and penicillin N in particular, in some of its chemical and biological properties, but differed from them strikingly in others.

Cephalosporin C was formed in too small an amount by the Sardinian *Cephalosporium sp.* to have been detected in culture fluid by its antibacterial activity. Its presence was only revealed after it had been concentrated, with penicillin N, during the purification of the latter. The production of cephalosporin C in sufficient quantity for detailed chemical study was greatly facilitated by the isolation, at the Medical Research Council's former Antibiotics Research Station, of a mutant strain from which much higher yields could be obtained. Culture fluids from this strain were incubated at pH 3, to convert penicillin N into its penillic acid, and the unchanged cephalosporin C then purified by chromatography in volatile buffers on anion-exchange resins. The results of chemical investigations led to the suggestion of structure (1),[5] and the validity of this structure was soon demonstrated by an *X*-ray crystallographic analysis.[6]

The antibacterial activity of cephalosporin C *in vitro* was low. But the apparent relationship of the substance to the penicillin family, coupled with its resistance to penicillinase from *Staph. aureus*, gave it at once a potential clinical interest.

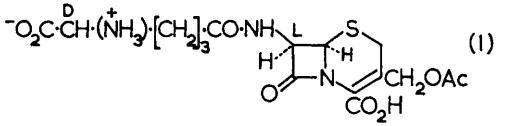
[1] G. Brotzu, *Lav. Ist. Igiene Cagliari*, 1948.
[2] E. P. Abraham and G. G. F. Newton, *Biochem. J.*, 1954, **58**, 266.
[3] G. G. F. Newton and E. P. Abraham, *Biochem. J.*, 1956, **62**, 651.
[4] H. W. Florey, *Ann. Internal Med.*, 1955, **43**, 480.
[5] E. P. Abraham and G. G. F. Newton, *Biochem. J.*, 1961, **79**, 377.
[6] D. Hodgkin and E. N. Maslen, *Biochem. J.*, 1961, **79**, 393.

Before cephalosporin C could be assigned a definitive structure the possibility was envisaged of obtaining more active compounds by exchanging its δ-(D-α-aminoadipoyl) group for other types of side-chain, since benzylpenicillin was known to have a much higher activity than penicillin N against most gram-positive bacteria. This possibility was realised when the nucleus of cephalosporin C (7-aminocephalosporanic acid) was obtained in low yield by mild acid hydrolysis and converted into *N*-acyl derivatives.[7] A further series of active compounds became accessible when it was discovered that the acetoxy-group of cephalosporin C and 7-aminocephalosporanic acid could be displaced by pyridine and other weak heterocyclic bases to yield betaines.[8]

From an early stage work in this field was supported by the National Research Development Corporation, which made agreements with a number of pharmaceutical companies. Efforts by the pharmaceutical industry resulted in the isolation of more highly yielding strains and in the production of cephalosporin C on a large scale. A chemical method for removing the side-chain of cephalosporin C to yield 7-aminocephalosporanic acid in good yield was discovered in the Lilly Research Laboratories.[9] In consequence, two derivatives of cephalosporin C, with approved names cephalothin and cephaloridine, have been introduced into medicine by Eli Lilly and Company and Glaxo Laboratories respectively. The total synthesis of cephalosporin C and of cephalothin has recently been achieved at the Woodward Research Institute in Basel.[10]

2 The Chemistry of Cephalosporin C

A. Nomenclature.—The name cepham has been suggested for the 1-aza-5-thia-6*R*-bicyclo[4,2,0]octan-8-one system by analogy with penam for the bicyclic system (3) in penicillanic acid.[9] The systematic nomenclature of compounds containing the β-lactam–dihydrothiazine ring system of cephalosporin C (1)



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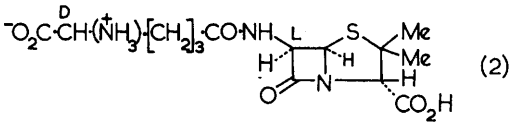

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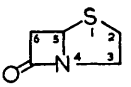

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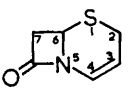


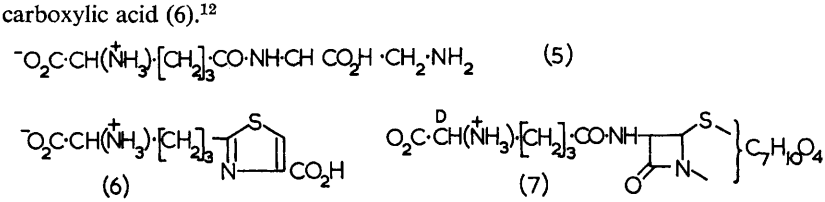
(4)

[7] B. Loder, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, 1961, **79**, 408.
[8] C. W. Hale, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, 1961, **79**, 403.
[9] R. B. Morin, B. G. Jackson, E. H. Flynn, and R. W. Roeske, *J. Amer. Chem. Soc.*, 1962, **84**, 3400.
[10] R. B. Woodward, K. Heusler, J. Gosteli, P. Naegeli, W. Oppolzer, R. Ramage, S. Ranganathan, and H. Vorbrüggen, *J. Amer. Chem. Soc.*, 1966, **88**, 852.

is thus based on the trivial name Δ^3-cephem for the bicyclic system (4) numbered as shown.

B. Structure and Conformation.—Cephalosporin C sodium salt (λ_{max} 260 mμ, log ϵ 3·95; $[\alpha]_D^{20}$ + 103°) gave a positive ninhydrin reaction and behaved as an aminodicarboxylic acid on electrometric titration, showing ionisable groups with pK_a values <2·6, 3·1, and 9·8 respectively.[2,11] Its infrared absorption spectrum showed a band at 5·61 μ, a position at which the penicillins show absorption associated with the stretching vibration of the C = O of the β-lactam ring in the fused β-lactam–thiazolidine ring system. It also showed bands at 5·77 μ and 9·7 μ which could be attributed to an ester grouping.

Hydrolysis of cephalosporin C with hot acid yielded CO_2 and D-α-aminoadipic acid, products which had been obtained earlier on hydrolysis of penicillin N. But cephalosporin C yielded two mol. of ammonia whereas only one was obtained under similar conditions from penicillin N. After hydrogenolysis of cephalosporin C with Raney nickel, hydrolysis gave D-α-aminoadipic acid and L-alanine and partial hydrolysis gave a dipeptide of D-α-aminoadipic acid and $\alpha\beta$-diaminopropionic acid (5). In neutral aqueous solution at 37° cephalosporin C was partially converted into D-2-(4-amino-4-carboxybutyl)thiazole-4-carboxylic acid (6).[12]



(5)

(6)

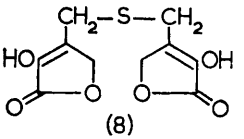
(7)

The formation of these products and the infrared absorption spectrum could be accounted for by the partial structure (7), the thiazole (6) arising from opening of the β-lactam ring, fission between sulphur and the remainder of the molecule and a nucleophilic attack of sulphur on the amide carbon of the side-chain.

An acetoxyl group accounted for two of the unplaced carbon atoms in structure (7). The remaining five atoms, like those of a corresponding fragment of the penicillins, formed the carbon skeleton of valine, since DL-valine and some α-oxoisovaleric acid were among the products formed when cephalosporin C was hydrogenolysed with Raney nickel. Under similar conditions D-valine was obtained from penicillin N by removal of sulphur from the penicillamine fragment of the molecule. However, cephalosporin C, unlike the penicillins, did not yield penicillamine on hydrolysis and its nuclear magnetic resonance spectrum, which did not give a signal at 7·9 p.p.m., indicated that a *gem*-dimethyl group was not present in the molecule. Moreover, cephalosporin C was much more stable at pH 3 than most of the penicillins and did not undergo a penicillin–penillic acid type of rearrangement.

[11] E. P. Abraham and G. G. F. Newton, *Biochem. J.*, 1956, **62**, 658.
[12] J. d'A. Jeffery, E. P. Abraham, and G. G. F. Newton, *Biochem. J.*, 1960, **75**, 216.

Two sulphur-containing lactones were obtained by hydrolysis of cephalosporin C with 1·25N-HCl at 100°. On the basis of their physical and chemical properties one of these compounds appeared to have the α-tetronic acid structure (8) and the other to be a corresponding thiolactone (9). When treated with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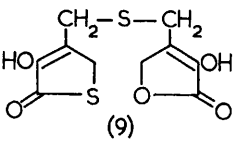
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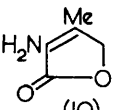
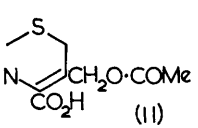

(10)

Raney nickel both compounds gave β-methyl-α-tetronic acid. They were clearly formed by condensation of the five-carbon fragments from two molecules of cephalosporin C. In 0·1N-HCl at room temperature cephalosporin C itself lost an *O*-acetyl group and yielded a lactone which was named cephalosporin C_c (λ_{max} 257 mμ). Treatment of this lactone with Raney nickel gave α-amino-β-methylbutenolide (10), which could be hydrogenated in the presence of Adams catalyst to γ-hydroxyvaline lactone.

The formation of compounds (8), (9), and (10) could be accounted for by the presence of the grouping (11) in cephalosporin C. The position assigned to the double bond was consistent with the isolation of hydroxyacetone 2,4-dinitro-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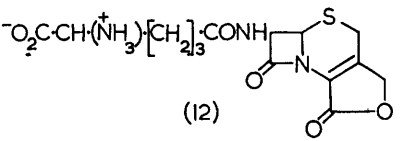
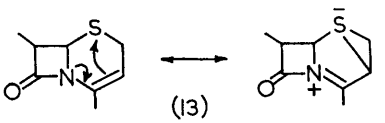

(12)

phenylosazone after ozonolysis of cephalosporin C and treatment of the product with Raney nickel. Hence the structure (1) was suggested, on chemical grounds, for cephalosporin C and (12) for deacetylcephalosporin C lactone (cephalosporin C_c).[5] Oxidation of cephalosporin C under mild conditions with hydrogen peroxide yielded a sulphoxide.

The ultraviolet absorption of cephalosporin C in relation to structure (1) has been the subject of some speculation. It was suggested that the lone pair of electrons of the β-lactam nitrogen atom are involved in the chromophore because amide resonance is suppressed in the fused ring system[5] and also that the sulphur is involved in resonance, as shown, for example, in (13).[13] The carboxyl group at C(4) is not an essential part of the cephalosporin C chromophore since (14) shows λ_{max} 256 mμ.[14]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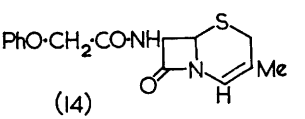
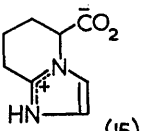

(14)

[13] A. G. Long and A. F. Turner, *Tetrahedron Letters*, 1963, **7**, 421.
[14] R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon, and S. L. Andrews, *J. Amer. Chem. Soc.*, 1963, **85**, 1896.

An *X*-ray crystallographic analysis of cephalosporin C sodium salt has given positions for the atoms which correspond with structure (1) and the correct absolute configuration of the molecule.[6] The region comprising the β-lactam ring and the atoms directly attached to it is closely similar to the corresponding region in benzylpenicillin. But the carboxyl group attached to C(6) of the rather flat dihydrothiazine ring lies much more in the plane of the ring than does the carboxyl group of benzylpenicillin, which is turned at about right-angles to the thiazolidine ring.

An *X*-ray crystallographic analysis of deacetylcephalosporin C lactone gave atomic positions which were defined with greater accuracy than in the earlier analysis of cephalosporin C and corresponded with (12). There was no significant difference in the β-lactam ring of the cephalosporin and that of 6-aminopenicillanic acid, but the angle between the β-lactam ring and the dihydrothiazine ring was less acute than that between the β-lactam and the thiazolidine ring.[15]

C. Other Chemical Reactions.—In boiling aqueous solution cephalosporin C is converted partly into a compound containing a fused imidazole–piperidine-2-carboxylic acid ring system (15) which has been given the trivial name cephalosporidine.[16,17] This compound is derived from the α-aminoadipoyl side-chain and C(6) and C(7) of the β-lactam ring. It is formed from penicillin N as well as from cephalosporin C. Many of the other chemical reactions of cephalosporin C involve changes in its ring system which can also be made with analogues in which the D-δ-(α-aminoadipoyl) side-chain has been replaced by other groups. The infrared and proton magnetic resonance spectra of these analogues and their derivatives have been described in detail.[18]



(15)

3 Biosynthesis

The cephalosporin C structure can be formally dissected into residues of D-α-aminoadipic acid, L-cysteine, αβ-dehydro-γ-hydroxyvaline, and acetic acid, whereas a dissection of the penicillins produced by *P. chrysogenum* gives a monosubstituted acetic acid, L-cysteine, and D-valine. When [2-^{14}C]DL-α-aminoadipic acid, [3-^{14}C]DL-mesocystine and [1-^{14}C]DL-valine were added to fermentations with a *Cephalosporium sp.*, the carbon-14 was incorporated mainly into the side-chain, the carbon of the β-lactam ring, and the valine-yielding fragment of cephalosporin C respectively.[19] [1-^{14}C]Acetate labelled the *O*-acetyl

[15] R. D. Diamond, D.Phil. Thesis, Oxford, 1963.
[16] E. P. Abraham and P. W. Trown, *Biochem. J.*, 1963, **86**, 271.
[17] E. O. Bishop and R. E. Richards, *Biochem. J.*, 1963, **86**, 277.
[18] G. F. H. Green, J. E. Page, and S. E. Staniforth, *J. Chem. Soc.*, 1965, 1595.
[19] P. W. Trown, B. Smith, and E. P. Abraham, *Biochem. J.*, 1963, **86**, 284.

group and the D-α-aminoadipoyl side-chain. The distribution of carbon-14 in the side-chain of cephalosporin C labelled from [1-^{14}C]acetate and from [5-^{14}C]-α-oxoglutarate indicated that the formation of the D-α-aminoadipic acid involved the condensation of acetyl coenzyme A and α-oxoglutarate by reactions analogous to those by which α-oxoglutarate is formed from acetyl coenzyme A and oxaloacetate in the citric acid cycle.[20,21] This is also the mechanism by which L-α-aminoadipic acid is formed in yeast.[22]

L-α-Aminoadipic acid is used by the *Cephalosporium sp.* for the synthesis of lysine, saccharopine [ε-*N*-(2-glutaryl)lysine] being a probable intermediate.[23] Despite the presence of a D-α-aminoadipic acid residue in the side-chains of cephalosporin C and penicillin N, free α-aminoadipic acid extracted from the mycelium has almost entirely (>99%) the L-configuration. In suspensions of washed mycelium L-α-aminoadipic acid is a more efficient precursor of the side-chain than the D-isomer.[24] Thus the stage at which the D-configuration arises remains to be determined.

Carbon-14 from both L- and D-[^{14}C]valine is incorporated into the 5-carbon fragment of cephalosporin C by mycelial suspensions, but D-valine is converted into L-valine in the mycelium.[24,25] No evidence has been obtained that free γ-hydroxyvaline is an intermediate and the oxidation of a valine methyl group may occur at a later stage.

A variety of penicillins with different side-chains can be obtained by the addition of appropriate side-chain precursors to fermentations with *Penicillium chrysogenum*, but no analogue of penicillin N and cephalosporin C with side-chains other than D-α-aminoadipoyl has been obtained from the *Cephalosporium sp.* However, isopenicillin N, with an L-α-aminoadipoyl side-chain,[26,27] and a peptide which appears to be δ-(α-aminoadipoyl)cysteinylvaline[28] are present in *P. chrysogenum*. It may be that L-α-aminoadipic acid is involved in the synthesis of all penicillins by *P. chrysogenum*, but that no mechanism exists for the replacement of the D-α-aminoadipic acid residue by other groups in the synthetic pathways which occur in the *Cephalosporium sp.*

4 Derivatives and Analogues of Cephalosporin C

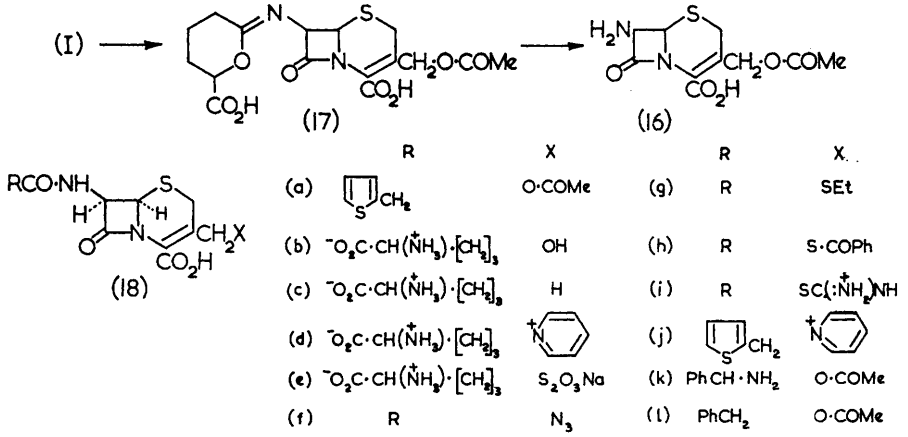
Both the α-aminoadipoyl and *O*-acetyl group of cephalosporin can be removed selectively and replaced by other groups.

[20] P. W. Trown, E. P. Abraham, G. G. F. Newton, C. W. Hale, and G. A. Miller, *Biochem. J.*, 1962, **84**, 157.
[21] P. W. Trown, M. Sharp, and E. P. Abraham, *Biochem. J.*, 1963, **86**, 280.
[22] M. Strassman, L. W. Ceci, and B. E. Silverman, *Biochem. Biophys. Res. Comm.*, 1964, **14**, 268.
[23] E. P. Abraham, G. G. F. Newton, and S. C. Warren, *I.A.M. Smyp. Appl. Microbiol. (Tokyo)*, 1964, **6**, 79.
[24] S. C. Warren, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, 1967, **103**, 891.
[25] A. L. Demain, *Biochem. Biophys. Res. Comm.*, 1963, **10**, 45.
[26] E. H. Flynn, M. H. McCormick, M. C. Stamper, H. DeValeria, and C. W. Godzeski, *J. Amer. Chem. Soc.*, 1962, **84**, 4594.
[27] M. Cole and F. R. Batchelor, *Nature*, 1963, **198**, 383.
[28] H. R. V. Arnstein, M. Artman, D. Morris, and E. J. Toms, *Biochem. J.*, 1960, **76**, 353, 357.

A. 7-Aminocephalosporanic Acid.—Hydrolysis of cephalosporin C with aqueous acid under mild conditions resulted in the removal of the α-aminoadipoyl side-chain and the production, in very small yield, of a compound in which the remainder of the molecule was unchanged.[7] This compound [3-acetoxymethyl-7-aminoceph-3-em-4-oic acid (16)] was given the trivial name 7-aminocephalosporanic acid, by analogy with 6-aminopenicillanic acid in the penicillin series. It readily yielded *N*-acyl derivatives with acid chlorides in aqueous acetone containing bicarbonate. But competing reactions, such as the removal of the *O*-acetyl group and subsequent lactonisation, prevented this hydrolytic method from being a practical one for the preparation of 7-aminocephalosporanic acid in quantity.

Attempts to find an enzyme which will catalyse the removal of the α-aminoadipoyl side-chain from cephalosporin C have hitherto been unsuccessful. In contrast, enzymes which will remove the phenylacetyl and other non-polar side-chains from both the penicillins and analogues of cephalosporin C are widely distributed in micro-organisms. The specificity of these acylases is associated with the nature of the *N*-acyl side-chain rather than with the structure to which the latter is attached.[29,30] However, chemical methods have been devised by which 7-aminocephalosporanic acid can be obtained from cephalosporin C in good yield.

Treatment of cephalosporin C with nitrosyl chloride in anhydrous formic acid gave an intermediate iminolactone (17) which was hydrolysed to 7-aminocephalosporanic acid and α-hydroxyadipic acid when dissolved in water.[9] Cephalosporins which have found general clinical use have so far been prepared from 7-aminocephalosporanic acid obtained in this way[31] and have the general

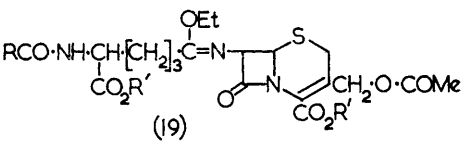


[29] M. Cole, *Nature*, 1964, **203**, 519.
[30] W. Kaufmann and K. Bauer, *Nature*, 1964, **203**, 520.
[31] R. R. Chauvette, E. H. Flynn, B. G. Jackson, E. R. Lavagnino, R. B. Morin, R. A. Mueller, R. P. Pioch, R. W. Roeske, C. W. Ryan, J. L. Spencer, and E. van Heyningen, Antimicrobial Agents and Chemotherapy, *Amer. Soc. Microbiol.*, Ann Arbor, Michigan, 1962, p. 687.

structure (18). For example, cephalothin (18a) is the sodium salt of 7-(thiophen-2-acetamido)cephalosporanic acid.

In another procedure for the preparation of 7-aminocephalosporanic acid, cephalosporin C is first converted into a diester by protection of its free NH_2 with a benzylcarbonyl or other suitable group, esterification, and removal of the protecting group. An ester of 7-aminocephalosporanic acid is obtained in good yield, together with the ester of 6-oxopiperidine-2-carboxylic acid, when the diester of cephalosporin C is kept for several days in methylene dichloride containing acetic acid at room temperature.[32] In a third procedure the diester of an *N*-acyl derivative of cephalosporin C is converted into an iminochloride by treatment with phosphorus oxychloride and then to an amino-ether (19). The latter yields 7-aminocephalosporanic acid ester on hydrolysis in dioxan–aqueous phosphoric acid.[33a] The use of benzyl and diphenylmethyl esters, from which the corresponding acids can be obtained by hydrogenolysis and by hydrolysis with trifluoroacetic acid in anisole respectively, enables free 7-aminocephalosporanic acid to be prepared by these procedures. Hydrolysis of the t-butyl ester of 7-aminocephalosporanic acid is also readily achieved with trifluoroacetic acid.[33b]

7-Chloroacetamidocephalosporanic acid reacts with thiourea in water to give 7-aminocephalosporanic acid and an iminothiazolidone, presumably by the intramolecular displacement shown in (20).[34]



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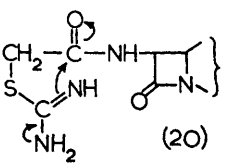


(20)

B. Deacetyl and Deacetoxy-cephalosporins.—Treatment of cephalosporin C with an acetyl esterase yielded deacetylcephalosporin C (18b), which readily lactonised in acid solution.[35] Hydrolysis of the *O*-acetyl group of other *N*-acyl derivatives of 7-aminocephalosporanic acids has been carried out similarly and the resulting deacetyl compounds [(18), X = OH] have been assigned the trivial name cephalosporadesic acids. *O*-Aroyl derivatives of cephalosporadesic acids have been prepared by a Schotten–Baumann reaction with aroyl chlorides and sodium hydroxide in aqueous acetone.[36]

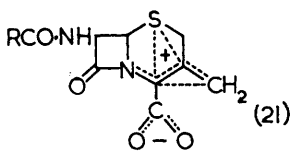
Hydrogenation of cephalosporin C in the presence of a large amount of palladium catalyst results in hydrogenolysis of the allylic acetoxy-group and the

[32] Ciba, Belg. P., 645,157/1964.
[33] (*a*) Ciba, Belg. P., 643,899/1964; (*b*) R. J. Stedman, *J. Med. Chem.*, 1966, **9**, 444.
[34] 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; H. Fazakerley, D. A. Gilbert, G. I. Gregory, J. K. Lazenby, and A. G. Long, Autumn Meeting of the Chemical Society, Nottingham, Sept. 1965.
[35] J. d'A. Jeffery, E. P. Abraham, and G. G. F. Newton, *Biochem. J.*, 1961, **81**, 591.
[36] E. van Hayningen, *J. Med. Chem.*, 1965, **8**, 22.

formation of deacetoxycephalosporin C (18c).[14,37] Removal of the acetoxy-group from 7-aminocephalosporanic acid has been reported to occur under similar conditions.

C. Displacement of the Acetoxy-group by Nucleophiles.—During the purification of cephalosporin C in pyridine acetate buffer a second active compound was encountered which showed no net charge at pH 7 and was given the trivial name cephalosporin C_A (pyridine) (18d). This compound was shown to be a pyridinium betaine formed by displacement of the acetoxy-group.[38] Similar displacements were found to occur with surprising facility with a variety of other heterocyclic weak bases and with 7-aminocephalosporanic acid as well as with cephalosporin C itself. With sodium thiosulphate cephalosporin C yielded a Bunte salt (18e).[39,40]

The displacement of the acetoxy-group in a variety of 7-acylaminocephalosporanic acids has been studied in detail. Substitution occurred with azide (18f), ethanethiol (18g), thiobenzoate (18h), thiourea (18i), dithiocarbamates, and xanthates, as well as with pyridine and other heterocyclic bases.[34,41] The reaction proceeds by an S_N1 mechanism.[42] With certain bidentate nucleophiles, such as pyrid-2-thione, displacement by the sulphur is followed by an internal Michael addition by the nitrogen atom to the double bond of the thiazine ring and the formation of spirocyclic compounds.[34] However, the methyl esters, hydroxy-acids, and lactone derived from (18) did not undergo analogous replacement reactions and little substitution occurred with a 1-oxide. It has been suggested that the 1-sulphur atom is implicated in the displacement reaction and that alkyl–oxygen fission of the acetyl ester is facilitated by resonance forms



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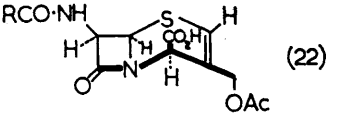


(22)

indicated in (21).[34] Cephaloridine (18j), which is used clinically, is the pyridinium betaine derived from cephalothin [7-(2-thienyl)acetamido-3-(1-pyridylmethyl)-3-cephem-4-carboxylate betaine].[43]

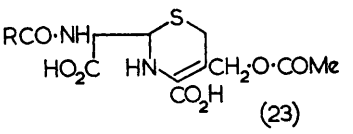
D. Isomerisation to 7-Acylaminoceph-2-em-4-carboxylic Acids.—In the presence of pyridine and acetic anhydride, 7-acylaminocephalosporanic acids undergo an isomerisation involving a shift of the double bond to the 2,3 position. These compounds isomerise slowly in the presence of pyridine alone, but their esters

[37] R. J. Stedman, K. Swered, and J. R. E. Hoover, *J. Med. Chem.*, 1964, **7**, 177.
[38] C. W. Hale, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, 1961, **79**, 403.
[39] A. L. Demain, J. F. Newkirk, G. E. Davies, and E. R. Harman, *Appl. Microbiol.*, 1963, **11**, 58.
[40] A. L. Demain, *Trans. N.Y. Acad. Sci.*, 1963, **25**, 731.
[41] E. van Heyningen and C. N. Brown, *J. Med. Chem.*, 1965, **8**, 174.
[42] A. B. Taylor, *J. Chem. Soc.*, 1965, 7020.
[43] P. W. Muggleton, C. H. O'Callaghan, and W. K. Stevens, *Brit. Med. J.*, 1964, **2**, 1234.

do so more readily. An equilibrium is reached when the proportion of $\Delta^2 : \Delta^3$ is about 7:3. The Δ^2 compounds absorb strongly between 220 and 300 mμ and their esters show λ_{max}, or an inflexion, between 245 and 250 mμ.[44]

The acetate group in the ceph-2-em-4-carboxylic acids is replaced by pyridine, thiourea, and thiobenzoate, but the reactions are slower than the corresponding ones with the Δ^3 compounds. Hence the Δ^2 compounds are presumably not involved in the displacements with 7-acylaminocephalosporanic acids. Conformations such as (22), with the 4-carboxy-group pseudo-axial to the six-membered ring, have been suggested for the Δ^2 compounds.[44]

E. Base- and Enzyme-catalysed Hydrolysis of Derivatives of 7-Aminocephalosporanic Acid.—When the β-lactam ring of the penicillins is opened by mild alkaline hydrolysis, or with enzyme penicillinase, the immediate products are penicilloates which are well defined and relatively stable compounds. Early attempts to characterise the products of a similar hydrolysis of cephalosporin C indicated that the corresponding compound (23) had no more than a transitory



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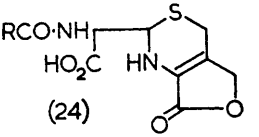
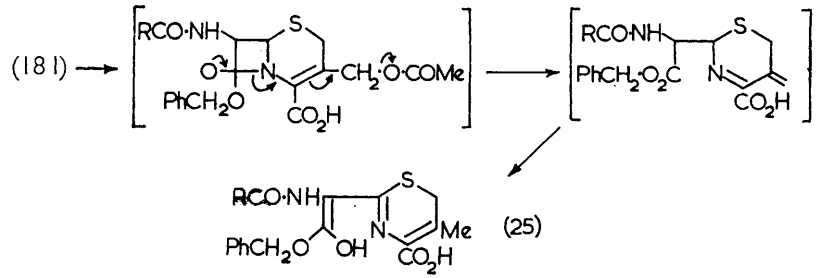


(24)

existence and was rapidly fragmented in aqueous solution.[15] Opening of the β-lactam ring of cephalosporin C and cephalothin by a β-lactamase from *Pseudomonas pyocyanea* at pH 7 is accompanied by the spontaneous expulsion of acetate and a similar hydrolysis of cephaloridine by the expulsion of pyridine.[45] Deacetylcephalosporin C also undergoes further fragmentation when its β-lactam ring is opened, but the corresponding lactone yields a relatively stable product (λ_{max} 265 mμ) which presumably has the structure (24).

Expulsion of the acetoxy-group, probably as part of a concerted reaction involving a prototropic rearrangement, occurs on fission of the β-lactam ring of 7-phenylacetamidocephalosporanic acid [cephaloram (18l)] with sodium benzyloxide in benzyl alcohol, but in this case a major product of the reaction



(25)

Scheme 1

[44] J. D. Cocker, S. Eardley, G. I. Gregory, M. E. Hall, and A. G. Long, *J. Chem. Soc.* (*C*), 1966, 1142.
[45] L. D. Sabath, M. Jago, and E. P. Abraham, *Biochem. J.*, 1965, **96**, 739.

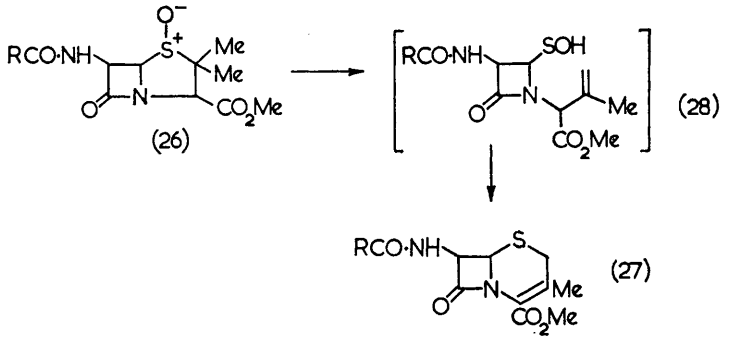
has been isolated and shown to be the 6*H*-1,3-thiazine (25) or a tautomer. The latter may[46] be formed as shown in Scheme 1.

The β-lactam rings of the ceph-2-ems are much more stable to alkali than the corresponding Δ^3-compounds.[44]

5 Chemical Routes from the Penicillin to the Cephalosporin Series

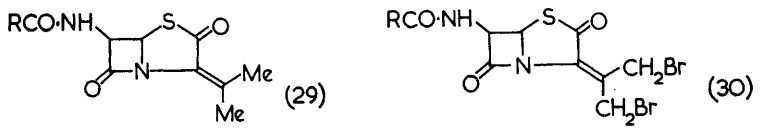
Two chemical methods have been reported for the conversion of the fused β-lactam-thiazolidine ring system into β-lactam–dihydrothiazines.

The sulphoxide of phenoxymethyl penicillin ester [(26), R = $C_6H_5OCH_2$] gives a compound containing the 3-methylceph-3-em-4-carboxylic acid ring system (27) in 15% yield on refluxing in xylene in the presence of traces of toluenesulphonic acid.[14] The transformation (Scheme 2) is presumed to involve the intermediate (28). Activation of the carboxyl group of a penicillin, followed



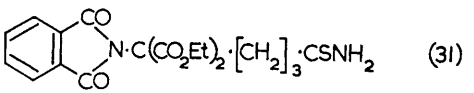
Scheme 2

by refluxing in the presence of a base, yields an anhydropenicillin (29). It has been stated that anhydropenicillins can be converted into compounds of the cephalosporin C series, either by allylic bromination with *N*-bromosuccinimide to give (30) and treatment of the latter with base, or by hydroxylation with microbial enzymes,[47] but no details of these processes have been given.



6 Synthesis of Degradation Products

A. Compounds (6) and (15).—The DL form of the thiazole (6) was synthesised by condensation of the thioamide (31) with methyl bromopyruvate and subse-

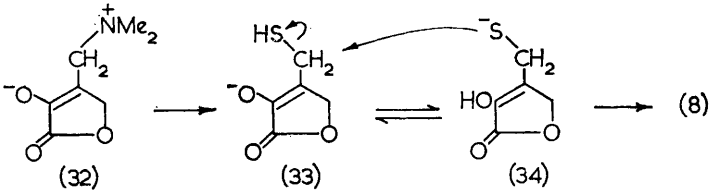


[46] S. H. Eggers, V. V. Kane, and G. Lowe, *J. Chem. Soc.*, 1965, 1262.
[47] S. Wolfe, J. C Godfrey, C. T. Holdrege, and Y. G. Perron, *J. Amer. Chem. Soc.*, 1963, **85**, 643; Belg. P. 621, 452/1963.

quent treatment with 6N-HCl at 110°. It was identical with the compound obtained by racemisation of the thiazole from cephalosporin C with acetic anhydride.[12]

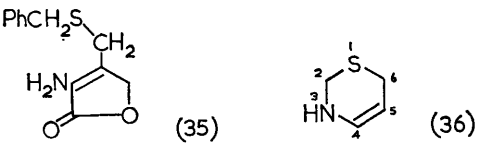
The DL form of cephalosporidine (15) was synthesised by heating DL-δ-(α-aminoadipoyl)aminoacetaldehyde diethylacetal in aqueous acetic acid at 100°. It was identical with the compound formed by racemisation of cephalosporidine at 190°.[16] Cephalosporidine is presumably formed from the D-δ-(α-aminoadipoyl) aminoacetaldehyde fragment of penicillin N and cephalosporin C by intramolecular condensation.

B. Compounds (8) and (10).—Synthetic routes to the sulphur-containing lactone (8) and aminobutenolide (10) start from β-dimethylaminomethyl-α-tetronic acid hydrochloride, first obtained in 1924 from pyruvic acid, dimethylamine hydrochloride, and formaldehyde.[48] The free base (32) reacted with the sulphydryl anion in dimethylformamide to yield (33). It appeared that the enolate salt (33), formed initially, equilibrated with the S-anion (34) and that this was followed by a nucleophilic attack of the latter on the former.[49a,50]



(32) (33) (34)

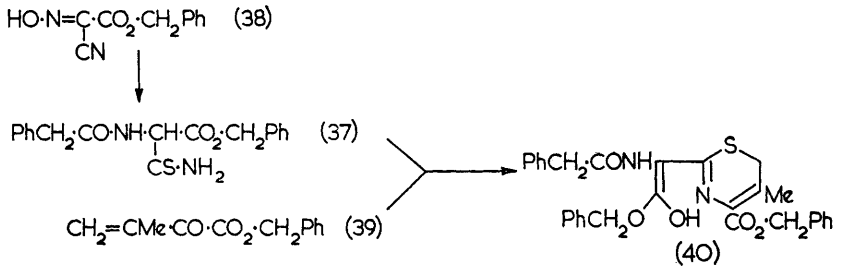
Reaction of (32) with toluene-*w*-thiol gives β-benzylthiolmethyl-α-tetronic acid, from which the enamine (35) is obtained by fusion with ammonium acetate. Treatment of (35) with Raney nickel gives (10).[51] The latter has also been obtained by fusion of β-methyl-α-tetronic acid with ammonium acetate.



(35) (36)

C. Compound (25).—Synthetic routes which have been found to the Δ^4-dihydro-6*H*-1,3-thiazine system (36) and to some 6*H*-1,3-thiazines depend on the addition of a thioamide to an αβ-unsaturated ketone.[52] The thioamide (37) was prepared by reduction of the hydroximino-nitrile (38), acylation of the amine with phenylacetyl chloride, and treatment of the product with hydrogen sulphide.

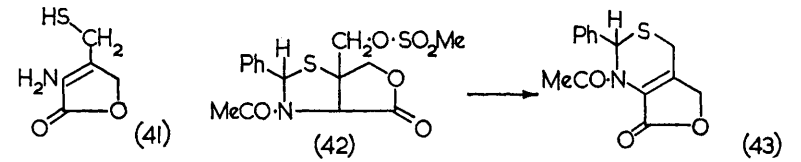
[48] C. Mannich and M. Bauroth, *Ber.*, 1924, **27**, 1108.
[49] (*a*) E. Galantay H.Engel, A. Szabo, and J. Fried, *J. Amer. Chem. Soc.*, 1964, **29**, 3560; (*b*) J. C. Sheehan and J. A. Schneider, *J. Org. Chem.*, 1966, **31**, 1635. (*c*) R. Heymès, G. Amiard, and G. Nominé, *Compt. Rend.*, 1966, **263**, 170.
[50] A. G. Long and A.F. Turner, *Tetrahedron Letters*, 1963, 421.
[51] D. M. Green, A G. Long, P. J. May, and A. F. Turner, *J. Chem. Soc.*, 1964, 766.
[52] G. C. Barrett, S.H. Eggers, T. R. Emerson, and G. Lowe, *J. Chem. Soc.*, 1964, 788.



When (37) reacted with the vinyl keto-ester (39) in dioxan saturated with hydrochloric acid, dehydration as well as addition occurred and the product (λ_{max} 342 and 285 mμ) was the 6*H*-1,3-thiazine (40) or a tautomer. Hydrolysis with aqueous ethanolic sodium carbonate converted (40) into a half-ester, identical with (25) obtained from 7-phenylacetamidocephalosporanic acid.[46]

7. The Total Synthesis of Cephalosporins

Several possible synthetic routes were envisaged which might lead to the 3,6-dihydro(2*H*)-1,3-thiazine–β-lactam ring system of the 7-acylaminocephalosporanic acids. One, which resembled a route followed successfully in the penicillin field and has been used as a synthetic approach to the cephams from homocysteine,[49b] would have involved the condensation of a phthalimidomalonaldehydate ester with the butenolide (41), followed by subsequent closure of the



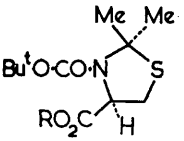
β-lactam ring and re-opening of the lactone ring.[49a] Another involved the addition of a thioamide to a vinyl keto-ester for the construction of the dihydrothiazine ring.[52] A third envisaged ring expansion of a thiazolidine to give the appropriate dihydrothiazine and was analogous, in some respects, to the route from the sulphoxide of phenoxymethyl penicillin ester (26) to (27). In a model experiment the methanesulphonate (42) appeared to be converted in high yield into (43) when refluxed in dioxan with anhydrous sodium acetate.[53] None of these suggested procedures has yet been brought to a successful conclusion. However, a synthesis of D,L-deacetylcephalothin lactone has recently been reported which involves condensation of the unstable β-thiolmethyl-α-tetronic acid (33) with the enamine of phthalimidomalonaldehydate *t*-butyl ester, the subsequent stages being similar to those in the penicillin series.[49c]

A major difficulty confronted attempts to synthesise cephalosporin C by a route similar to that which was successful with the penicillins and involving closure of the β-lactam ring as the final step. The immediate product of cleavage

[53] G. Stork and H. T. Cheung, *J. Amer. Chem. Soc.*, 1965, **87**, 3783.

of the β-lactam ring of a 7-acylaminocephalosporanic acid, unlike the penicilloates, was highly unstable. The first total synthesis of cephalosporin C and cephalothin has now been achieved by a new and ingenious approach to the problem, and the discovery of a remarkable series of reactions.[10]

L-Cysteine was converted into L-*N*-t-butyloxycarbonyl-2,2-dimethylthiazolidine-4-carboxylic acid [(44), R = H]. The methyl ester [(44), R = CH_3] reacted with an excess of dimethyl azodicarboxylate at 105° to give the hydrazodiester (45).



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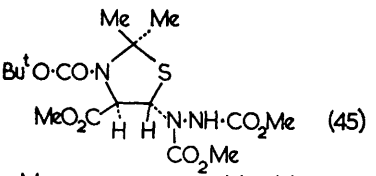
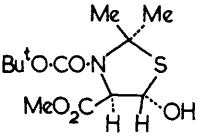


(45)



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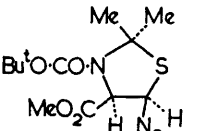

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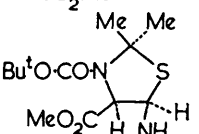
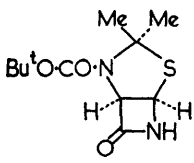


(48)

When (45) was oxidised with lead tetra-acetate in boiling benzene and the reaction mixture treated with sodium acetate, it was converted into the *trans*-hydroxy-ester (46). Treatment of (46) with excess of di-isopropylethylamine and methanesulphonyl chloride led to an intermediate from which the methanesulphonate group could be displaced by azide ion, with normal inversion, to yield the *cis*-azido-ester (47). The latter was reduced with aluminium amalgam to the *cis*-amino-ester (48). The structures of the surprisingly stable *trans*-hydroxy-ester and the *cis*-amino-ester were rigidly established by *X*-ray crystallographic analysis at Harvard. Thus, by these novel reactions a properly oriented nitrogen atom was introduced into the β-position of a cysteine residue to produce an arrangement of atoms corresponding to one moiety of cephalosporin C.

The *cis*-amino-ester (48) yielded the β-lactam (49) on treatment with triisobutylaluminium in toluene.

For reaction with the β-lactam a novel dialdehyde was synthesised, in which a



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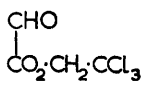

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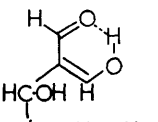

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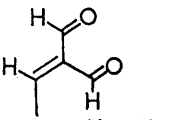
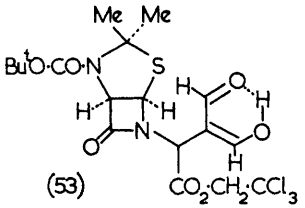


(52)

carboxyl function was covered by a new protective group that could be removed by reduction. Di-βββ-trichlorethyl D-tartrate was oxidised with sodium metaperiodate to βββ-trichloroethyl glyoxylate (50). Condensation of the latter in aqueous solution with the sodium salt of malondialdehyde gave the aldol (51)

which lost water on heating in n-octane with the formation of the dialdehyde (52).

When (52) was heated with the β-lactam (49) in n-octane at 80° for 16 hours the adduct (53) was formed. This yielded the aminoaldehyde (54) in trifluoroacetic acid at room temperature, the reaction involving the formation of a six-



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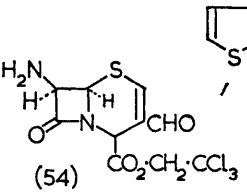

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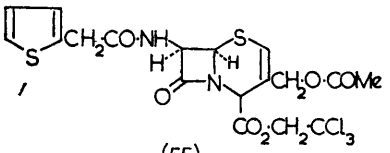

(55)

membered ring by attack of the electrophilic carbon atom of a protonated carbonyl group on the nucleophilic sulphur atom and the removal of the *N*-t-butyloxycarbonyl group.

Acylation of the aminoaldehyde (54) with thiophen-2-acetyl chloride and reduction of the aldehyde group with diborane in tetrahydrofuran yielded an alcohol which gave the Δ^2 isomer of cephalothin ββ*β*-trichloroethyl ester (55) on acetylation with acetic anhydride and pyridine. In anhydrous pyridine, (55) equilibrated with the ββ*β*-trichloroethyl ester of cephalothin and the two isomers were separated by chromatography on silica gel. Reductive removal of the ββ*β*-trichloroethyl group with zinc dust in 90% acetic acid yielded cephalothin itself (18a).

The aminoaldehyde (54) was also acylated with *N*-ββ*β*-trichloroethyloxycarbonyl-D-α-aminoadipic acid and the *N*-acyl derivative esterified with ββ*β*-trichloroethanol in the presence of dicyclohexylcarbodi-imide and pyridine. Reduction of one of the products, followed by acetylation, isomerisation, and reductive removal of the trichloroethyl groups from the Δ^3 isomer, yielded cephalosporin C (1).

8 Structure–Activity Relationships

A. Antibacterial Activity *in Vitro*.—Early studies with cephalosporin C revealed that this antibiotic had a broad antibacterial spectrum, but that its activity was relatively low. It showed only about 0·1% of the activity of benzylpenicillin *in vitro* against a number of gram-positive bacteria. The activity of penicillin N against these organisms was about 1% of that of benzylpenicillin.[3,54] However, cephalosporin C, unlike benzylpenicillin and penicillin N, was as active against penicillinase-producing staphylococci as against non-penicillinase producers.[54] Its activity against the penicillinase-producing organisms could be correlated with its resistance to hydrolysis by staphylococcal penicillinase. This resistance to the enzyme was associated with the ring system of the molecule and not with the nature of the *N*-acyl side-chain, since penicillin N, which also contained a δ-(D-α-aminoadipoyl) side-chain, was rapidly hydrolysed. Comparison of the

[54] H. W. Florey, *Giorn. Microbiol.*, 1956, **2**, 361.

properties of cephalosporin C, penicillin N, and benzylpenicillin therefore suggested that replacement of the *N* acyl side-chain of cephalosporin C by phenylacetyl, or a related group, would lead to compounds with a much higher activity against gram-positive bacteria which retained a resistance to penicillinase. This was shown to be so when 7-phenylacetamidocephalosporanic acid was first prepared.[7] The activity of this compound against the Oxford strain of *Staph. aureus* was several hundred times that of cephalosporin C and of the same order as, though less than, that of benzylpenicillin. Deacetylcephalosporin C shows about 25% of the activity of cephalosporin C, deacetoxycephalosporin C appears to be less active than the deacetyl derivative, and the activity of 7-aminocephalosporanic acid itself is extremely low. In contrast, the betaine obtained on replacement of the acetoxy-group in cephalosporin C by a pyridinium group was about ten times as active against the staphylococcus as cephalosporin C itself.

The discovery of a procedure for the production of 7-aminocephalosporanic acid in quantity has resulted in the production by pharmaceutical companies of many hundreds of cephalosporins with the general structure (18). The preparation of these derivatives has followed that of a series of new penicillins obtained in a similar manner from 6-aminopenicillanic acid.[55] In general, the relative changes in activity against a non-penicillinase producing strain of *Staph. aureus* which occur when one *N*-acyl side-chain is replaced by another are similar in the cephalosporin and penicillin series of compounds. As with cephalosporin C, other *N*-acyl derivatives of 7-aminocephalosporanic acid yield compounds with different activities when changes are made in group X (18).[31,56] It is not easy to predict the effect of changes in R and X together from the effects observed on changing each group separately. But cephalothin (18a) and cephaloridine (18j) have high activities against many gram-positive bacteria and useful activities against a number of gram-negative bacteria.[57] Both compounds have been found to have clinical value for the treatment of a variety of infections.

Important differences between the antibacterial activities of the cephalosporins and penicillins respectively are associated with differences in the behaviours of the two groups of compounds to β-lactamases. It is now clear that β-lactamases from different bacteria may differ greatly in their behaviour to a given member of the cephalosporin or penicillin family, and, in particular, that staphylococcal penicillinase differs from the β-lactamases produced by gram-negative organisms. Compounds with the β-lactam–dihydrothiazine ring system are resistant to hydrolysis by the staphylococcal enzyme even when they have a high affinity for the enzyme.[58] Thus cephalosporins with *N*-acyl side-chains such as phenylacetyl and thiophen-2-acetyl, which confer a high intrinsic activity on the molecule

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[57] M. Barber and P. M. Waterworth, *Brit. Med. J.*, 1964, **2**, 344.
[58] B. Crompton, M. Jago, K. Crawford, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, 1962, **84**, 52.

against most gram-positive bacteria, are also highly active against the penicillinase-producing staphylococcus. In the penicillin series resistance to staphylococcal penicillinase depends on the side-chain and not on the ring system. Penicillins with certain side-chains, such as 2,6-dimethoxybenzoyl, have a very low affinity for the enzyme[59] but a considerable intrinsic activity against the staphylococcus.

In contrast to the staphylococcal enzyme, β-lactamases from some gram-negative bacteria may hydrolyse cephalosporin C, cephalothin, and cephaloridine at a higher maximum rate than benzylpenicillin, or than ampicillin which has a D-phenylglycyl side-chain. β-Lactamases from other gram-negative bacteria appear to show penicillinase activity but little cephalosporinase activity.[60] Penicillins and cephalosporins with a 2,6-dimethoxybenzoyl side-chain show a very high affinity for some of these β-lactamases (in contrast to their low affinities for the staphylococcal enzyme) and are resistant to enzymic hydrolysis, but they have only a very low activity against the organism by which the enzymes are produced.[45,61] It appears that cephalothin and cephaloridine are more active than ampicillin against some gram-negative bacteria but not against others. The relative importance of the rôles played by β-lactamase production and by the inherent resistance of bacterial cells in this context has still to be evaluated. However, ampicillin is normally the penicillin of choice for the treatment of infections by gram-negative bacteria. The question therefore arises whether the respective advantages of ampicillin and of a cephalosporin can be combined in a compound having the side-chain of the former and the ring system of the latter. This compound, named cephaloglycin [7-(D-α-aminophenylacetamido)cephalosporanic acid (18k)], has shown interesting biological properties in a preliminary study.[62]

The available evidence strongly supports the view that the mode of action of the cephalosporins is essentially the same as that of the penicillins.[63,64,65] The latter inhibit the cross-linking, by a transpeptidation reaction, of mucopeptide formed in the synthesis of the bacterial cell-wall. It has been pointed out that there are resemblances between a face of the penicillin molecule and certain faces of *N*-acetylmuramic acid,[66] D-alanyl-D-alanine,[67] and L-alanine-D-glutamic acid[68] respectively, which are components of the mucopeptide network. Hypotheses about the mode of action of penicillin have invoked each of these components in turn as one with which penicillin competes for attachment to an enzyme surface by hydrogen and ionic bonds. This primary combination of

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penicillin with the enzyme may be followed by opening of the β-lactam ring and formation of a covalent bond.

X-Ray crystallographic analyses have shown that the relative positions of the atoms in the β-lactam ring of cephalosporin C and the atoms immediately attached to the ring are similar to, though not identical with, those in benzylpenicillin and 6-aminopenicillanic acid. But the orientation of the carboxyl group attached to C(4) in (18), which lies in the plane of the six-membered ring, is somewhat different from that of the corresponding carboxyl group in the penicillins. Moreover, deacetylcephalosporin lactones (23) may have high antibacterial activity.[31] It seems, therefore, that the presence of a carboxylate ion is not essential for antibacterial activity, but that if this group is present its orientation may be varied within certain limits. When more extensive changes in configuration occur as in synthetic enantiomorphs of the natural penicillins,[69] or when structural changes are made which result in a substantial decrease in the sensitivity of the β-lactam ring to attack by nucleophiles, activity may be lost. Either, or both, of these factors could be responsible for the inactivity of the Δ^2 isomers of cephalosporins in a conformation such as that shown in (22).

B. Absorption and Hypersensitivity.—Two other biological properties appear to be associated specifically with the β-lactam–dihydrothiazine ring system. One is the failure of many cephalosporins to be absorbed as efficiently from the gastro-intestinal tract as are penicillins with the same *N*-acyl side-chains. The precise features of the two ring systems which are responsible for this difference have not been established. A second property is the failure of cephalosporins, in most cases, to produce a reaction in patients reported to be hypersensitive to penicillin. Hypersensitivity to the penicillins is a complex phenomenon and the nature of the antigen responsible for its most serious manifestation, anaphylactic shock, remains to be established.[70] But it is a reasonable assumption that this antigen, like those responsible for milder reactions, is associated with the opening of the penicillin β-lactam ring and linkage of a transformation or degradation product of the molecule to tissue protein. The opening of the β-lactam ring of the cephalosporins in aqueous solution is associated with an extensive fragmentation of the molecule. This could well result in the formation of antigens from the cephalosporins which would not react with antibodies to conjugated proteins derived from the penicillins.

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[70] B. B. Levine, *Fed. Proc.*, 1965, **24**, 45.