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Cephalosporanic acids: a new look at reactions at the C-3′ position

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Nucleophilic displacement of the acetoxy group of cephalosporanic acids by thiols in aqueous solution at neutral pH provides 3-thiomethyl-substituted compounds with a broad spectrum of antibiotic activity. The aqueous displacement reaction is often destructive of much of the cephalosporanic acid, and products generally require extensive purification. Displacements at a lower pH are complicated by unwanted lactone formation. However, reactions conducted under acid conditions in a variety of anhydrous organic solvents give 3-thiomethyl-substituted compounds in very high yield and quality; no lactone formation is observed. The kinetics of the reaction support an S_N1 mechanism. Protonation of the departing acetoxy group appears therefore critical; the more basic solvents, e.g. dimethylsulphoxide and *N*,*N*-dimethylformamide, significantly retard the rate of reaction.

Introduction

Chemical modification of the naturally occurring cephalosporin C(**1**) (figure 1) at C-7 and at C-3 have provided a number of commercially available cephalosporin antibiotics with enhanced therapeutic value (Flynn 1972; Perlman 1977). Still other compounds are currently being evaluated for eventual introduction into the marketplace.

Since the cephalosporin fermentation is capable of incorporating only the D-α-aminoadipic acid side chain at C-7, early work was directed toward development of methods for producing large quantities of 7-aminocephalosporanic acid (**2**) (7-ACA, figure 1). This was accomplished first by acid hydrolysis in aqueous solution (Abraham *et al.* 1961) and later in a more efficient manner with nitrosyl chloride (Morin *et al.* 1962, 1969) and with phosphorus pentachloride (Fetig *et al.* 1968). The availability of 7-ACA paved the way for the simple preparation of hundreds of compounds with differing acylamido groups at C-7. Cephalothin (**3**) was the first commercially available antibiotic derived from this work, and it quickly gained wide acceptance by the medical community.

Intensified investigation of the chemical and biological properties of the new 7-acylamido compounds and 7-ACA led to the replacement of the C-3′-acetoxy group by a variety of nitrogen and sulphur nucleophiles. This chemistry provided an entry into a new series of cephalosporin antibiotics with an expanded spectrum of antibiotic activity. In this report we review the chemistry that permitted the preparation and clinical evaluation of many new cephalosporin compounds and describe more recent data concerning the nucleophilic displacement of the C-3′-acetoxy group.

Nucleophilic displacement in aqueous solution

The facility with which the acetoxy group is displaced by nucleophiles was first noticed when cephalosporin C(**1**) was allowed to stand in an aqueous pyridine–acetate buffer solution. A small amount of a second, more active compound was produced, which proved to be the

betaine (**4**), generally referred to as cephalosporin C_A (figure 2; Hale *et al.* 1961). Extensive development of the pyridine reaction allowed the manufacture and marketing of a broad spectrum antibiotic under the generic name cephaloridine (**5**; Spencer *et al.* 1967).

Displacement with other nitrogen nucleophiles such as azide ion (Cocker *et al.* 1965), primary aromatic amines and *N*-substituted anilines (Bradshaw *et al.* 1968) provided interesting synthetic derivatives of cephalosporanic acids at the C-3′ position, but no compounds of significant biological importance have emerged.

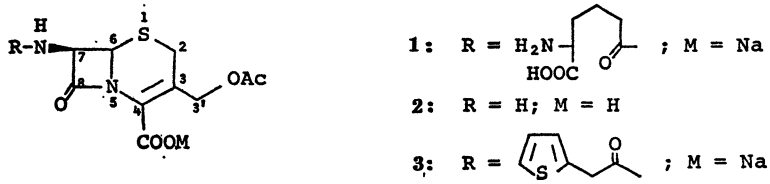


FIGURE 1

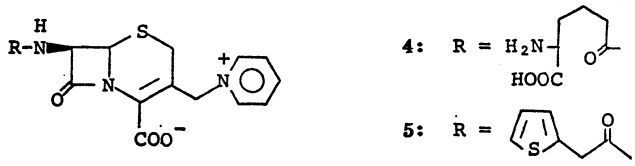
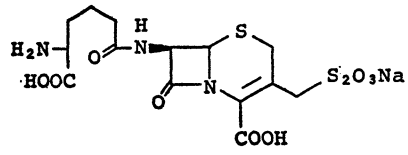


FIGURE 2



6

FIGURE 3

Reactions in which the acetoxy group is replaced with a sulphur nucleophile have been the most extensively investigated, since the compounds derived from these reactions have shown enhanced antibiotic activity (Perlman 1977).

Displacement by a sulphur nucleophile was first observed during a media evaluation in the fermentation of cephalosporin C. Addition of sodium thiosulphate resulted in an increase in potency which was traced to the formation of the more active Bunte salt **6** (figure 3; Demain *et al.* 1963). Since then, a large number of sulphur-derived compounds have been prepared. These include products derived from reactions with alkyl and aryl mercaptans, heterocyclic thiols, sulphinates, thioureas, xanthates and dithiocarbamates (Cocker *et al.* 1965; Van Heyningen & Brown 1965; Fazakerley *et al.* 1967). Heterocyclic thiols gave highly active cephalosporin antibiotics with a range of activity against both Gram-positive and Gram-negative organisms. Numerous heterocyclic thiol-derived compounds have been clinically evaluated, or are currently undergoing clinical testing.

Kinetic data compiled on a number of sulphur and nitrogen nucleophiles (Cocker *et al.* 1965; Taylor 1965) demonstrated that the reaction in aqueous solution at near neutral pH proceeds by an S_N1 mechanism. Solvent-assisted ionization of acetate ($k_{H_2O}/k_{D_2O} = 1.4$) to give the resonance stabilized, allylic cation **7** (figure 4) proved to be the rate-determining step.

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Very little, if any, ionic recombination occurs as evidenced by the absence of a common ion effect. Rate constants for pyridine, azide, thiosulphate and thiopicolinate did not deviate significantly from a value of 2.0×10^{-5} s^{-1} at 46 °C and pH 7.0.

That the cation **7** is produced in these reactions was demonstrated by its ability to undergo aromatic electrophilic substitution when generated in the presence of resorcinol or *N*-methylindole to give **8** and **9**, respectively (figure 5; Cocker *et al.* 1965).

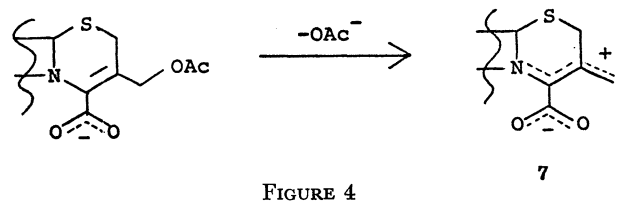
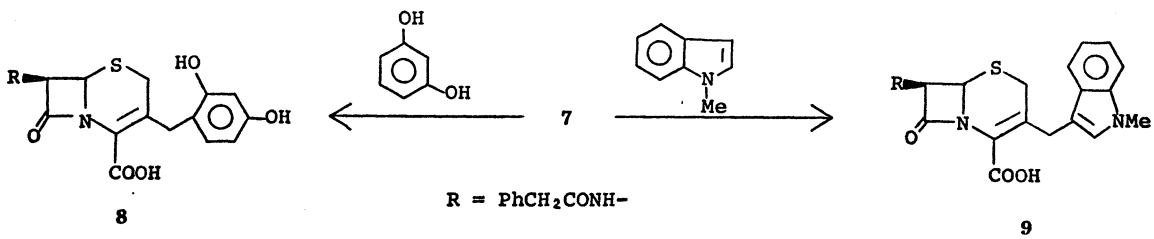
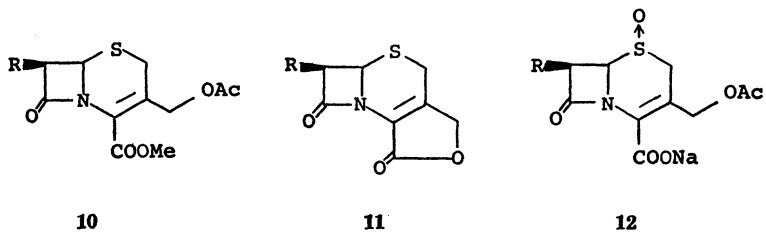


FIGURE 4



R = $PhCH_2CONH$-

FIGURE 5



R = $PhCH_2CONH$-

FIGURE 6

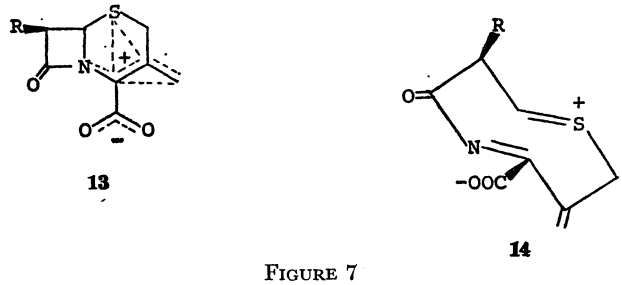


FIGURE 7

The failure of ester **10** and lactone **11** (figure 6) to undergo the displacement reaction emphasizes the importance of the carboxylate group. The picture was somewhat complicated by the observation that displacement reactions on the sulphoxide **12** occurred at one-fifth of the rate of the corresponding sulphide. In an attempt to implicate sulphur in the mechanism, the structures **13** and **14** were proposed (figure 7; Cocker *et al.* 1965). While structure **13** may more completely describe the nature of the cation **7**, product formation via the ionic valence-bond tautomer **14** is less likely since it would involve stereospecific re-formation of the β-lactam, and this in turn would require unusual rigidity in the 8-membered ring.

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For the most part, displacement of the acetoxy group of cephalosporin carboxylates was conducted only in water or mixtures of water with polar organic solvents, such as formamide, acetone or dimethylsulphoxide at near neutral pH at 46–60 °C, or in anhydrous organic solvents such as dimethyl sulphoxide and *N*,*N*-dimethylformamide at 60 °C (Sugimoto *et al.* 1975). Except where product crystallization occurs during the course of the reaction (Cocker *et al.* 1965), processes conducted in aqueous medium (pH 7) result in substantial hydrolysis of the acetate ester and are generally destructive of the sensitive β-lactam ring, or complicated by unwanted lactone formation at lower pH values. Lactone **11** precipitated in approximately 30 % yield during an attempted displacement on the corresponding carboxylic acid at pH 2 in aqueous dioxan (Taylor 1965). Therefore, products prepared in aqueous solution often require extensive purification.

Our efforts in C-3′-acetoxy displacement reactions involved the development of methods that decreased hydrolytic reactions of the acetate ester and were less destructive of the β-lactam ring.

Acid-catalysed displacement in non-aqueous solvents

An acid-catalysed displacement reaction in anhydrous organic solvents appeared to be the answer to circumventing problems associated with acetate and β-lactam hydrolysis and the requirement for highly polar solvents necessary to assist acetate ionization (Ingold 1967). The mechanism of such a reaction is compared with acetate ionization (figure 8). Protonation of the acetoxy carbonyl function and subsequent loss of acetic acid (path *b*) would give the equivalent neutral zwitterion **7** that is produced by solvent-assisted acetate ionization from cephalosporin carboxylates in polar solvents (path *a*). Furthermore, it was assumed that formation of **7** would be essentially irreversible as in more polar solvents (Cocker *et al.* 1965; Taylor 1965).

The major problem in acid-catalysed reactions was lactonization, a serious problem with cephalosporin carboxylic acids in aqueous solution. The mechanism of lactone formation was investigated in the following manner (figure 9): refluxing anhydrous 7-(2-thienylacetamido)-cephalosporanic acid (**15**) in dry acetonitrile resulted in loss of acetic acid and substantial decomposition, but no lactone was produced. When the experiment was repeated with approximately 5 % aqueous acetonitrile, both desacetyl compound **16** and lactone **17** were formed, the latter being produced at the expense of the former. This is in agreement with the rapid acid-catalysed formation of cephalosporin C lactone from the corresponding desacetyl derivative (Abraham & Newton 1961). Furthermore, it has been shown that the intermediate **7** does not react with oxygen nucleophiles, water and alcohols, except by destruction of the β-lactam ring (Cocker *et al.* 1965). Lactone formation must therefore occur by initial hydrolysis to the desacetyl compound **16** followed by acid-catalysed cyclization, and once **7** is formed, it will not produce lactone **17** either by direct cyclization or by reaction with water to give **16**.

Initial experiments showed that **15** reacted with 1-methyltetrazole-5-thiol in dry acetonitrile containing a catalytic amount of anhydrous methanesulphonic acid at 25 °C to give the expected product **19**, but substantial decomposition also occurred. Markedly better results were obtained by refluxing a mixture of **15** and 1-methyltetrazole-5-thiol in acetonitrile without an external acid catalyst. The reaction progressed smoothly; little or no decomposition occurred; no lactone was observed; and the product **19** was isolated as the dicyclohexylamine salt in 69 % yield. Replacing acetonitrile with 1,2-dichloroethane and refluxing for 6 h resulted in direct crystallization of **19** in 72 % yield (figure 10). These data show that cephalosporanic

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acids themselves are sufficiently acidic to effect the acid-catalysed conversion. The method proved to be quite general and table 1 summarizes data obtained from the reaction of 7β-(2-thienylacetamido)cephalosporanic acid (**15**) with thiols in a variety of organic solvents. Numerous other compounds have been prepared in a similar manner. In most cases, the proper choice of cephalosporanic acid, thiol and solvent resulted in direct crystallization of product from the reaction mixture.

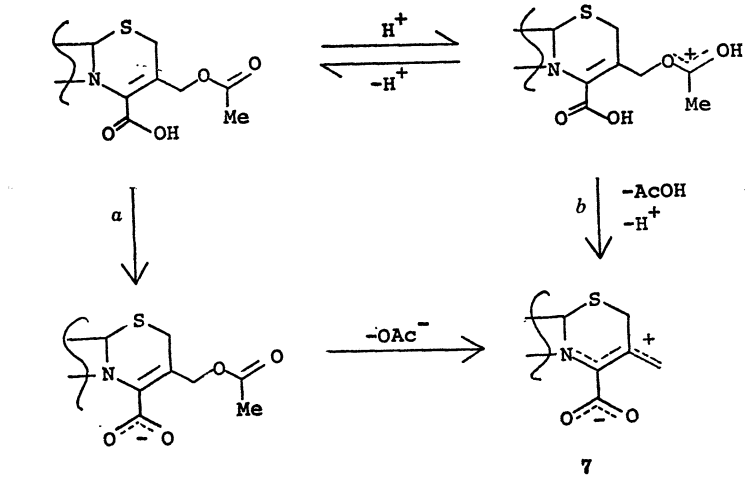

FIGURE 8

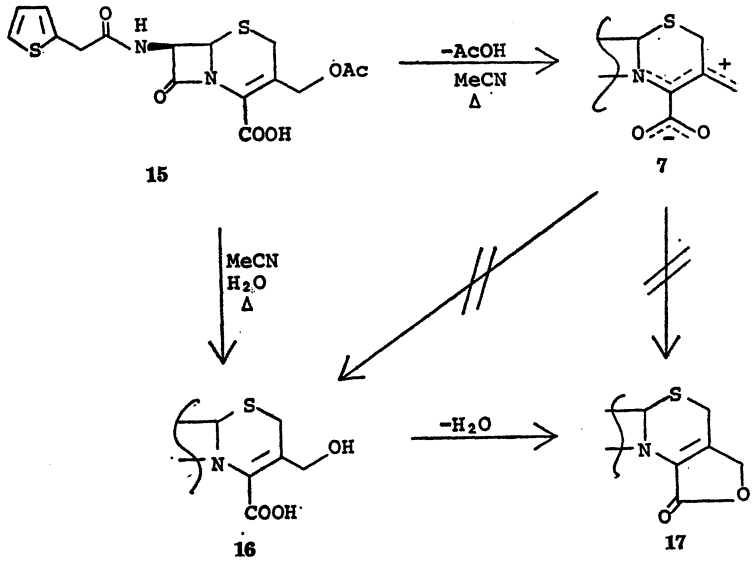

FIGURE 9

The acid-catalysed reaction shows a dramatic similarity to acetate ionization in polar solvents. First-order kinetics were observed ($k = 1.6 \times 10^{-5}$ s^{-1} in acetonitrile at 75 °C), and no reaction occurred with ester **10** or lactone **11**. Reactions with oxygen and nitrogen nucleophiles resulted in extensive decomposition of the β-lactam ring. Further, the sulphoxide **12** or Δ^2-cephalosporanic acids undergo displacement at a substantially slower rate than the corresponding Δ^3-compounds. However, acid-catalysed conversions were much less effective in basic

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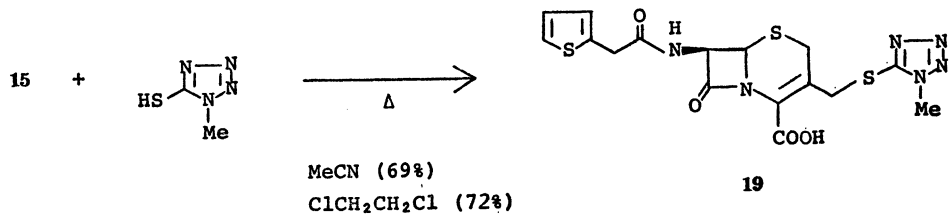
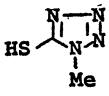
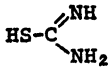
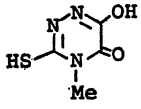
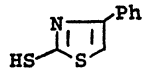
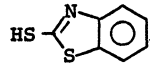
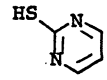
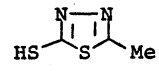


FIGURE 10

TABLE 1. ACID CATALYSED DISPLACEMENT OF THE ACETOXY GROUP IN **15** BY THIOLS IN NON-AQUEOUS SOLVENTS

thiol	solvent	product yield (%)†
	thiophene	80
	fluorobenzene	93‡
	isopropyl acetate	71§
	acetonitrile	76
HS-Ph	1,2-dichloroethane	74
	acetonitrile	83
	acetonitrile	86
	1,2-dichloroethane	88
	acetonitrile	83
	acetic acid	70
	1,2-dichloroethane	70

† Reactions were conducted at the reflux temperature of the indicated solvent, except acetic acid where temperature was kept at 84–86 °C. Each product crystallized directly from the reaction mixture and gave satisfactory analyses.

‡ Heterogeneous reaction that required refluxing for 72 h.

§ Partly heterogeneous reaction that required refluxing for 36 h.

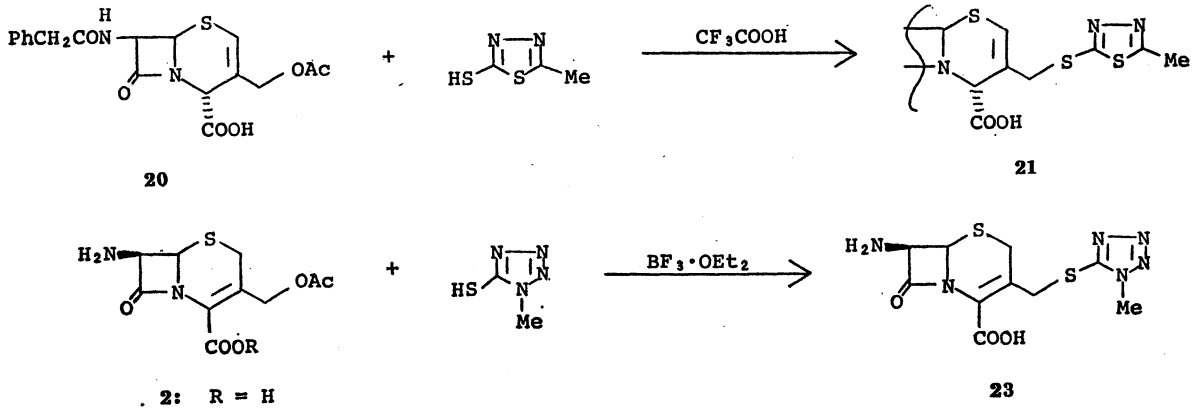


FIGURE 11

solvents of high dielectric constant, e.g. dimethylsulphoxide, *N*,*N*-dimethylformamide, formamide and alcohols, probably owing to ineffective proton transfer to the more weakly basic acetoxy group.

Recently, two other reports appeared which describe an acid-catalysed displacement reaction of the acetoxy group under somewhat different conditions (figure 11). Undiluted trifluoroacetic acid proved effective in the conversion of the Δ^2-cephalosporanic acid **20** to the corresponding 3-thiomethyl-substituted compound **21** (Peter *et al.* 1977). Boron trifluoride complexes with ethers, phenol, and acetic acid promoted the conversion of 7-ACA (**2**) or the acid-labile ester **22** to the modified cephalosporin nucleus **23** (Saikawa *et al.* 1978). This reaction worked with a variety of alkyl, aryl and heterocyclic mercaptans in a number of organic solvents over a temperature range of 0–60 °C.

These newer methods thus provide a more efficient synthesis of 3-thiomethyl-substituted cephalosporin compounds, another step in the long history of development of therapeutically effective penicillin and cephalosporin antibiotics.

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References (Hatfield *et al.*)

Abraham, E. P., Newton, G. G. F. & Loder, B. 1961 *Biochem. J.* **79**, 408–416.
Abraham, E. P. & Newton, G. G. F. 1961 *Biochem. J.* **79**, 377–393.
Bradshaw, J., Eardley, S. & Long, A. G. 1968 *J. chem. Soc.* C, pp. 801–806.
Cocker, J. D., Cowley, B. R., Cox, J. S. G., Eardley, S., Gregory, G. I., Lazenby, J. K., Long, A. G., Sly, J. C. P. & Somerfield, G. A. 1965 *J. chem. Soc.*, pp. 5015–5031.
Demain, A. L., Newkirk, J. F., Davis, G. E. & Harman, R. E. 1963 *Appl. Microbiol.* **11**, 58–61.
Fazakerley, H., Gilbert, D. A., Gregory, G. I., Lazenby, J. K. & Long, A. G. 1967 *J. chem. Soc.*, pp. 1959–1963.
Fetig, B., Peter, H., Bickel, H. & Vischer, E. 1968 *Helv. chim. Acta* **51**, 1108–1119.
Flynn, E. H. (ed.) 1972 *Cephalosporins and penicillins: chemistry and biology*, ch. 12, pp. 532–582. New York: Academic Press.
Hale, C. W., Newton, G. G. F. & Abraham, E. P. 1961 *Biochem. J.* **79**, 403–407.
Ingold, C. K. 1967 *Structure and mechanism in organic chemistry*, 2nd edn, ch. 7, pp. 457–473. Cornell University Press.
Morin, R. B., Jackson, B. G., Flynn, E. H., Roescke, W. & Andrews, S. L. 1962 *J. Am. chem. Soc.* **84**, 3400–3401.
Morin, R. B. *et al.* 1969 *J. Am. chem. Soc.* **91**, 1396–1400.
Perlman, D. (ed.) 1977 *Structure activity relationships among the semisynthetic antibiotics*, pp. 87–237. New York: Academic Press.
Peter, H., Muller, B. & Bickel, H. 1977 U.S. Patent 4,014,874 (Ciba-Geigy).
Saikawa, I., Takano, S., Momonoi, K., Takakura, I., Kuroda, S., Tanaka, K., Hoyashi, K., Nagahashi, B. & Kutani, C. 1978 German Offenlegungsschrift 2,804,896 (Toyama).
Spencer, J. L., Siu, F. Y., Jackson, B. G., Higgins, H. M. & Flynn, E. H. 1967 *J. org. Chem.* **32**, 500–501.
Sugimoto, K., Kobayashi, K., Nishiyimi, K. & Morimoto, S. 1975 U.S. Patent 3,872,115 (Takeda).
Taylor, A. B. 1965 *J. chem. Soc.*, pp. 7020–7029.
Van Heyningen, E. & Brown, C. N. 1965 *J. mednl Chem.* **8**, 174–181.

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