153. *Studies Related to Cephalosporin C. Part I. 3-Hydroxy- and 3-Amino-furan-2(5H)-ones.*

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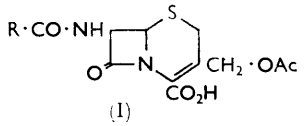
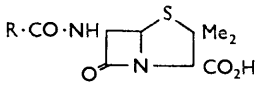
The syntheses of some 3-hydroxy- and 3-amino-furan-2(5*H*)-ones are described. The spectroscopic properties, pK_a values, and potential synthetic usefulness of these compounds are discussed with respect to the antibiotic cephalosporin C.

The elucidation of the structure of cephalosporin C {I; R = D-^-O_2C·CH(NH_3^+)·[CH_2]$_3$·} by chemical [1] and *X*-ray [2] methods revealed a close similarity to the penicillins (II) and in particular to penicillin N * {II; R = D-^-O_2C·CH(NH_3^+)·[CH_2]$_3$·}.[3] This makes plausible the observed antibacterial activity [4] of cephalosporin C and its resistance to, and inhibition of, the enzyme, penicillinase.[5]



(I)



(II)

Although cephalosporin C has relatively low activity against *Staphylococcus aureus* (strain N.C.T.C. 6571, penicillin-sensitive) compared with the penicillins, it was recognised at an early stage that the α-aminoadipic acid side-chain was probably responsible. This was confirmed when it was found that 7-phenylacetamidocephalosporanic acid (I; R = Ph·CH_2) gave a product with about one-hundred times the activity of cephalosporin C against *Staph. aureus*.[6] The elegant method for the preparation of 7-aminocephalosporanic acid from cephalosporin C [7] has made it possible to prepare a range of cephalosporin C analogues.[8] Their observed antibacterial activities indicate that the cephalosporins have activity similar to those of the corresponding penicillins against the Gram-positive bacterium *Staph. aureus* (strain 209P, penicillin-sensitive) but that the cephalosporins are effective also against clinical isolates of penicillin-resistant *Staph. aureus*. The cephalosporins are as good as, or better than, the corresponding penicillins against the Gram-negative bacteria so far tested.[8] Further modifications of the antibacterial

* Also known as cephalosporin N and synnematin B, see ref. 3.

[1] Abraham and Newton, *Biochem. J.*, 1961, **79**, 377.
[2] Hodgkin and Maslen, *Biochem. J.*, 1961, **79**, 393.
[3] Abraham, Newton, and Hale, *Biochem. J.*, 1954, **58**, 94; Newton and Abraham, *Biochem. J.*, 1954, **58**, 103; Abraham, *Pharm. Rev.*, 1962, **14**, 473.
[4] Newton and Abraham, *Biochem. J.*, 1956, **62**, 651.
[5] Abraham and Newton, *Biochem. J.*, 1956, **63**, 628.
[6] Loder, Newton, and Abraham, *Biochem. J.*, 1961, **79**, 408.
[7] Morin, Jackson, Flynn, and Roeske, *J. Amer. Chem. Soc.*, 1962, **84**, 3400.
[8] Chauvette, Flynn, Jackson, Lavagnino, Morin, Mueller, Pioch, Roeske, Ryan, Spencer, and Van Heyningen, *J. Amer. Chem. Soc.*, 1962, **84**, 3401.

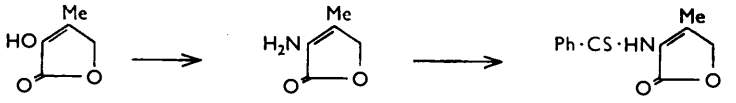
properties of cephalosporin C have been observed by replacing the acetoxy-group by a variety of nucleophiles, such as pyridine.[9] The range of antibiotics already obtained encourages the belief that modifications at other positions on the nucleus, and even of the nucleus itself, could lead to useful antibiotics.

The synthetical problem presented by cephalosporin C is primarily that of obtaining a route to the unknown 3,6-dihydro-2*H*-1,3-thiazine system which will allow suitably substituted intermediates to be prepared. It was expected, however, that synthesis of the 3,6-dihydro-2*H*-1,3-thiazine β-lactam ring system would be more difficult than that of the thiazolidine β-lactam ring system (which has been achieved by Sheehan and his collaborators [10]) because of the weaker nucleophilic character of the amine on which all methods of cyclisation depend.

The ultraviolet absorption spectrum ($\lambda_{max.}$ 2600 Å; ε 9000) is also an interesting feature of cephalosporin C. This apparently anomalous spectrum was referred to by Abraham and Newton [1] who compared it with that of α-acetamido-β-methylcrotonic acid ($\lambda_{max.}$ 2230 Å). The explanation offered for the large bathochromic shift was that there are stereochemical reasons for believing that amide resonance is suppressed in a fused β-lactam ring system, thus allowing the nitrogen lone pair of electrons to become part of the chromophoric system. We now report the preparation of some 3-aminofuran-2(5*H*)-ones whose spectra partially support this explanation and which also serve as possible starting points for syntheses.

3-Hydroxy-4-methylfuran-2(5*H*)-one [11] (III) when fused with ammonium acetate at 100° gave the enamine (IV).[12] Its ultraviolet absorption maximum ($\lambda_{max.}$ 2480 Å) was identical with that reported by Abraham and Newton [1] for their degradation product (compound 5) which was assigned this structure. The pK_a of 1·5 which was determined spectroscopically also agrees with that (*ca.* 2) reported by Abraham and Newton, but the difference in extinction coefficient and in melting point suggests that the degradation product was not pure.

Attempts to thioacetylate the enamine (IV) with dithioacetic acid [13] and methyl dithioacetate [14] were unsuccessful, starting material being recovered. Reaction with (thiobenzoylthio)acetic acid,[15] Ph·CS·S·CH_2·CO_2H, however, gave the thioamide (V).



(III) (IV) (V)

It was then felt that if the methyl group in the thioamide (V) were substituted with a suitable leaving group, cyclisation to a 6*H*-1,3-thiazine lactone should occur. The Mannich base hydrochloride (VI) was therefore prepared by the method of Mannich and Bauroth [16] from pyruvic acid, dimethylamine hydrochloride, and formaldehyde. Attempts to prepare the enamine of the Mannich base by fusion with ammonium salts were, however, abortive, at best only traces of suspected enamine being obtained.

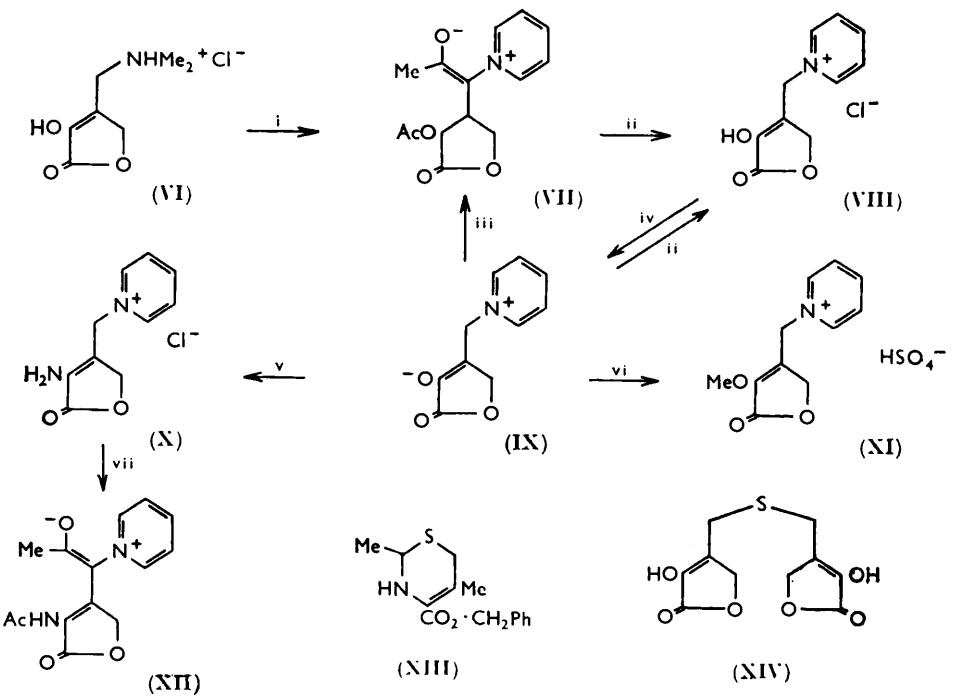
An attempt to prepare the enol acetate of the Mannich base hydrochloride (VI) with acetic anhydride in pyridine led to an orange-yellow crystalline product which had an ultraviolet and visible absorption spectrum characteristic of a pyridinium enol-betaine.[17] Its infrared spectrum revealed that it was also an enol acetate. These facts together with

[9] Hale, Newton, and Abraham, *Biochem. J.*, 1961, **79**, 403.
[10] Sheehan and Henery-Logan, *J. Amer. Chem. Soc.*, 1962, **84**, 2983, and references there cited.
[11] Schinz and Hinder, *Helv. Chim. Acta*, 1947, **30**, 1349.
[12] Green, Long, May, and Turner, preceding paper.
[13] McOmie, *Ann. Reports*, 1948, **45**, 207.
[14] Houben and Schultze, *Ber.*, 1910, **43**, 2482.
[15] Crawhall and Elliott, *J.*, 1951, 2071; Kurzer, *Chem. and Ind.*, 1961, 1333.
[16] Mannich and Bauroth, *Ber.*, 1924, **57**, 1108.
[17] Frangatos and Taurins, *Canad. J. Chem.*, 1961, **39**, 410, and references there cited.

its nuclear magnetic resonance spectrum and the reactions outlined below led to its formulation as the betaine (VII).

The betaine (VII) was insoluble in water, but dissolved rapidly in 6N-hydrochloric acid. Evaporation of the resulting solution gave colourless crystals which gave a wine-red colour with ferric chloride solution and had ultraviolet absorption maxima at 2320 Å in aqueous solution and 2570, 2620, and 2680 (shoulder) Å in aqueous alkali. These values suggest the presence of a 3-hydroxyfuran-2(5*H*)-one and pyridinium group, hence the compound must be the pyridinium chloride (VIII). The corresponding pyridinium betaine (IX) was precipitated when an aqueous solution of the pyridinium chloride was carefully adjusted to pH 9. The structural relation between the betaines (VII) and (IX) was demonstrated by ready conversion of the latter into the former with acetic anhydride at room temperature. The structures assigned to the betaines (VII) and (IX) were further confirmed by their nuclear magnetic resonance spectra, the latter as its methyl ether (XI). Although aqueous acid normally converts a pyridinium enol betaine into its keto-pyridinium salt,[17] the conversion of the betaine (VII) into the pyridinium chloride (VIII) is more analogous to the acidic hydrolysis of a β-triketone to a β-diketone.[18]

Fusion of the betaine (IX) with ammonium acetate at 140° gave the enamine which was precipitated as the pyridinium chloride (X) with hydrogen chloride from the solution of the fusion product in chloroform. The enamine was treated with aqueous ammonia solution and then with acetic anhydride, yielding thus the brick-red betaine (XII), which



Reagents: (i) Ac_2O–C_5H_5N. (ii) Aq. HCl. (iii) Ac_2O. (iv) NaOH. (v) NH_4·OAc,HCl. (vi) Me_2SO_4. (vii) Aq. NH_3,Ac_2O.

proves the structural similarity to the enol (VIII). Attempts to thioacylate the pyridinium enamine (X) with dithioacetic acid, methyl dithioacetate, (thiobenzoylthio)acetic acid, thioacetamide, or thiobenzoyl chloride failed. It appears that the pyridinium group has removed the last vestige of nucleophilic character from the enamine, and the possibility of forming a 2*H*-1,3-thiazine by this route seems remote.

[18] Birch and Elliott, *Austral. J. Chem.*, 1956, **9**, 238.

The ultraviolet absorption spectra of the 3-aminofuran-2(5*H*)-ones obtained [(IV) $\lambda_{max.}$ 2480 Å; (X) $\lambda_{max.}$ 2530 Å] indicate that the availability of the lone pair on the nitrogen atom in the β-lactam of cephalosporin C is insufficient fully to account for the spectrum shown by cephalosporin C, although it makes an important contribution. Fehnel and Carmack [19] have, however, shown that a sulphide group which is separated by only one methylene group from a chromophore has a considerable bathochromic effect. In the following paper the dihydrothiazine (XIII) is found to have an absorption maximum at 2850 Å and comparison of the absorption spectra of the enolate ions of (III), $\lambda_{max.}$ 2650 Å, and (XIV),[1,20,21] $\lambda_{max.}$ 2800 Å, reveals a similar bathochromic shift due to sulphur. It appears likely, therefore, that in cephalosporin C ($\lambda_{max.}$ 2600 Å) the lone pair of electrons on the nitrogen of the β-lactam is not completely free, and the sulphide group should be regarded as an integral part of the chromophore. The nature of the electronic transition giving rise to the absorption band in cephalosporin C has already been a subject for speculation.[12,21]

Experimental

Ultraviolet (Cary model 14M double-beam recording spectrometer) and infrared (Perkin-Elmer 21) absorption spectra were recorded for ethanol and chloroform solutions, respectively, except where otherwise stated. M. p.s were determined on a Kofler block. Nuclear magnetic resonance spectra were measured on an A.E.I. RS2 spectrometer operating at 60 Mc./sec. for dimethyl sulphoxide solutions with tetramethylsilane as internal reference. pK_a values were determined spectroscopically.

3-Hydroxy-4-methylfuran-2(5H)-one (III).—This compound was prepared by the method of Schinz and Hinder [11] and had m. p. 88—90° (lit., 90—92°); τ (p.p.m.) 8·00 (triplet, J = 1 c./sec., CH_3), 5·27 (quartet, J = 1 c./sec., CH_2), and 0·47 (singlet, OH) (cf. ref. 20).

3-Amino-4-methylfuran-2(5H)-one (IV).—A mixture of 3-hydroxy-4-methylfuran-2(5*H*)-one (0·7 g.) and ammonium acetate (2·1 g.) was fused at 100° for 5 min. The residue was dissolved in water (25 ml.) and continuously extracted with ether for 24 hr. Evaporation of the dried ($MgSO_4$) ethereal extract gave 3-amino-4-methylfuran-2(5*H*)-one [12,21] (0·66 g.), m. p. 146°, which crystallised as needles (from ether), m. p. 147—148°, pK_a *ca.* 1·5 (Found: C, 53·3; H, 6·3; N, 12·2. Calc. for $C_5H_7NO_2$: C, 53·1; H, 6·2; N, 12·4%); $\lambda_{max.}$ (in H_2O) 2480 Å (ε 8700), $\lambda_{max.}$ (in EtOH) 2550 Å (ε 8400), $\nu_{max.}$ 3400 and 3320 (NH_2), 1750 (lactone), 1690 (enamine), 1350, 975, and 910 cm.$^{-1}$; τ (p.p.m.) 8·22 (triplet, J = 1 c./sec., CH_3), 5·37 (quartet, J = 1 c./sec., CH_2).

4-Methyl-3-thiobenzamidofuran-2(5H)-one (V).—A solution of (thiobenzoylthio)-acetic acid [15] (0·57 g.) in 0·1N-sodium hydroxide (30 ml.) was added to a solution of 3-amino-4-methylfuran-2(5*H*)-one (0·30 g.) in ethanol (6 ml.). The solution was brought to pH 7 with 2N-hydrochloric acid and kept at 20°. After 4 days, pale yellow needles (0·12 g.) had separated and after 3 weeks a second crop (0·41 g.) was collected. The two crops were combined and crystallised from ethanol, to give the *thioamide* as yellow needles, m. p. 217—218° (Found: C, 61·6; H, 4·9; N, 5·8; S, 13·3. $C_{12}H_{11}NO_2S$ requires C, 61·7; H, 4·7; N, 6·0; S, 13·7%); $\lambda_{max.}$ 2540 (ε 13,800), $\lambda_{infl.}$ 3050 Å (ε 6150), $\nu_{max.}$ (in Nujol) 3230 (NH) 1748 (lactone), 1690 (enamine), and 1600 cm.$^{-1}$; τ (p.p.m.) 7·99 (singlet, CH_3), 4·84 (singlet, CH_2), 1·70 and 1·10 (multiplets, C_6H_5).

4-Dimethylaminomethyl-3-hydroxyfuran-2(5H)-one Hydrochloride (VI).—This material was prepared as described by Mannich and Bauroth.[16] After recrystallisation from concentrated hydrochloric acid it had m. p. 168° (decomp.), pK_a *ca.* 5·5 (enol), $\lambda_{max.}$ (in aq. alkali) 2670 Å (ε 8750) and $\lambda_{max.}$ (in aq. acid) 2340 Å (ε 7250).

1-[1-(4-Acetoxy-2,5-dihydro-5-oxo-3-furyl)-2-hydroxypropenyl]pyridinium Betaine (VII).—Acetic anhydride (30 ml.) was added to a suspension of 4-dimethylaminomethyl-3-hydroxyfuran-2(5*H*)-one hydrochloride (10 g.) in pyridine (30 ml.); the solid dissolved. The dark solution deposited crystals (2·5 g.) after 4 hr., which were washed with acetic anhydride and chloroform and after recrystallisation from chloroform gave orange needles of the *betaine*, m. p. 190—191·5° (Found: C, 60·6; H, 4·7; N, 5·4. $C_{14}H_{13}NO_5$ requires C, 61·1; H, 4·8;

[19] Fehnel and Carmack, *J. Amer. Chem. Soc.*, 1949, **71**, 84.
[20] Galantay, Szabo, and Fried, *Tetrahedron Letters*, 1963, 415.
[21] Long and Turner, *Tetrahedron Letters*, 1963, 421.

N, 5·1%); $\lambda_{max.}$ 2160 (ε 4700) and 4020 Å (ε 44,600); $\nu_{max.}$ 1780sh (enol acetate), 1752 (lactone), 1710, 1655, and 1610 cm.$^{-1}$; τ (p.p.m.) 6·17 (singlet, ·CH_2·O·CO), 5·03 [singlet, CH_3·C(O$^-$)=C], 2·70 and 1·46 (multiplets, $C_5H_5N^+$); the solvent would obscure the enol acetate signal.

1-(2,5-*Dihydro*-4-*hydroxy*-5-*oxo*-3-*furylmethyl*)*pyridinium Chloride* (VIII).—The betaine (VII) (4·04 g.) was dissolved in 6N-hydrochloric acid at 20°, and after 5 min. the pale yellow solution was evaporated to dryness. The residue (3·28 g.), m. p. 200° (decomp.), was recrystallised from 10% aqueous ethanol by the gradual addition of ether, to give prisms, m. p. 204—206° (decomp.), of the *enol* (Found: C, 53·1; H, 4·6; Cl, 15·5; N, 6·1. $C_{10}H_{10}ClNO_3$ requires C, 52·8; H, 4·4; Cl, 15·6; N, 6·1%); $\lambda_{max.}$ (aq. acid) 2310 Å (ε 32,100), $\lambda_{max.}$ (aq. alkali) 2570 (ε 27,500), 2620 (ε 27,500), and 2680 Å (ε 27,500); $\nu_{max.}$ (Nujol) 1770 (lactone), 1670 (enol), 1630, and 1600 cm.$^{-1}$.

1-(2,5-*Dihydro*-4-*hydroxy*-5-*oxo*-3-*furylmethyl*)*pyridinium Betaine* (IX).—2N-Sodium hydroxide solution was added to a solution of the enol (VIII) (0·454 g.) in water (5 ml.) to bring the pH to 9. The *betaine* (0·330 g.) was precipitated; recrystallisation from ethanol gave prisms, m. p. 188—191° (Found: C, 62·7; H, 4·8; N, 7·4. $C_{10}H_9NO_3$ requires C, 62·8; H, 4·7; N, 7·3%); $\nu_{max.}$ 1752 (lactone), 1700 (enolate), 1600, and 1120 cm.$^{-1}$. The ultraviolet absorption spectra were identical with those of the pyridinium chloride in acid and alkaline media. Evaporation of a solution of the enol betaine in hydrochloric acid gave material identical with the pyridinium chloride.

1-[1-(4-*Acetoxy*-2,5-*dihydro*-5-*oxo*-3-*furyl*)-2-*hydroxypropenyl*]*pyridinium Betaine* (VII) *from* 1-(2,5-*Dihydro*-4-*hydroxy*-5-*oxo*-3-*furylmethyl*)*pyridinium Betaine* (IX).—The enol betaine (0·5 g.) was suspended in acetic anhydride at 20°. After 20 min. the enol acetate (0·6 g.) was obtained as orange needles, m. p. and mixed m. p. 190—191°.

1-(2,5-*Dihydro*-4-*methoxy*-5-*oxo*-3-*furylmethyl*)*pyridinium Hydrogen Sulphate* (XI).—The enol betaine (3·96 g.) was dissolved in dimethyl sulphate (10 ml.) and, after 5 min., the solution was extracted with water and the aqueous extract washed with ether. Evaporation of the aqueous solution and crystallisation of the residue from ethanol and then aqueous ethanol gave the *enol methyl ether* (5·5 g.) as prisms, m. p. 136—138° (Found: C, 43·5; H, 4·2; N, 4·7; S, 10·5. $C_{11}H_{13}NO_7S$ requires C, 43·6; H, 4·5; N, 4·6; S, 10·6%); $\lambda_{max.}$ (H_2O) 2180 (ε 2720), 2560 Å (ε 2060); $\nu_{max.}$ (Nujol) 1770 (lactone), 1700 (enol ether), and 1652 cm.$^{-1}$; τ (p.p.m.) 5·88 (singlet, ·CH_2·NC_5H_5), 5·58 (singlet, OMe), 5·25 (singlet, ·CH_2·O·CO), 1·95 and 1·04 (two multiplets, $C_5H_5N^+$), (cf. ref. 20).

1-(4-*Amino*-2,5-*dihydro*-5-*oxo*-3-*furylmethyl*)*pyridinium Chloride* (X).—The betaine (IX) (2·16 g.) was fused with ammonium acetate at 140° for 5 min. The cooled residue was dissolved in chloroform (20 ml.), and the solution saturated with dry hydrogen chloride. The precipitated *enamine* (1·64 g.) crystallised from ethanol in buff-coloured prisms, m. p. 190—195° (decomp.), and sublimed (160—170°/0·4 mm.) to colourless prisms, m. p. 195—199° (Found: C, 53·0; H, 4·8; N, 12·3. $C_{10}H_{11}ClN_2O_2$ requires C, 53·0; H, 4·9; N, 12·4%); $\lambda_{max.}$ (H_2O) 2530 Å (ε 2200); $\lambda_{max.}$ (aq. acid) 2160 (ε 1440), 2520 Å (ε 2050); $\lambda_{max.}$ (aq. alkali) 2500 (ε 1200), 2550 (ε 1200), and $\lambda_{infl.}$ 2620 Å (ε 1100), $\nu_{max.}$ 3280 and 3230 (NH_2) 1735 (lactone), 1675 (enamine), 1622, and 1600 cm.$^{-1}$.

3-*Amino*-4-*pyridinomethylfuran*-2(5H)-*one Methylide*.—The enamine (X) (0·40 g.) was suspended in 2N-ammonium hydroxide solution (30 ml.) and extracted with chloroform. Evaporation of the dried ($MgSO_4$) solution gave the *methylide* (0·26 g.) which crystallised from chloroform–ether as prisms, m. p. 90·5—92° (Found: C, 62·5; H, 5·3; N, 14·9. $C_{10}H_{10}N_2O_2$ requires C, 63·1; H, 5·3; N, 14·7%); $\nu_{max.}$ 3510 and 3400 (NH_2), 1770 (lactone), 1700 (enamine), and 1610 cm.$^{-1}$.

1-[1-(4-*Acetamido*-2,5-*dihydro*-5-*oxo*-3-*furyl*)-2-*hydroxypropenyl*]*pyridinium Betaine* (XII).—A solution of the methylide (0·55 g.) in acetic anhydride (2 ml.) deposited brick-red crystals of the *betaine* (0·49 g.), which crystallised as brick-red prisms, m. p. 193—195°, from chloroform (Found: C, 60·5; H, 4·9; N, 10·2. $C_{14}H_{14}N_2O_4$ requires C, 61·3; H, 5·1; N, 10·2%); $\lambda_{max.}$ 2180 (ε 7400) and 4120 Å (ε 30,500); $\nu_{max.}$ 3300 (NH), 1730 (lactone), 1700 (enamine), 1650, 1600, 1365, and 1325 cm.$^{-1}$.

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