Tetrahedron Letters No. 29, pp 2911 - 2914, 1972. Pergamon Press. Printed in Great Britain.

NEW β-LACTAM ANTIBIOTICS: STRUCTURE DETERMINATION OF CEPHAMYCIN A AND B.

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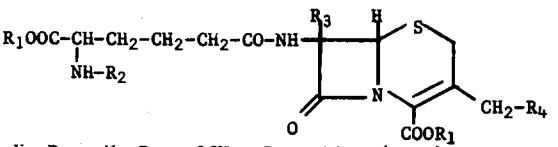
(Received in USA 12 May 1972; received in UK for publication 9 June 1972)

The discoveries and antibiotic properties of a group of new β-lactam antibiotics have recently been reported[1]. In this communication we wish to present evidence for structures 1a and 1b for the cephamycins A and B of *Streptomyces griseus*.



1a $R_1 = H$, $R_2 = H$, $R_3 = OCH_3$, $R_4 = OCO\text{-}C(OCH_3)=CH\text{-}C_6H_4\text{-}OSO_2OH(p)$

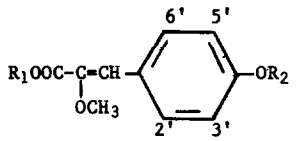
1b $R_1 = H$, $R_2 = H$, $R_3 = OCH_3$, $R_4 = OCO\text{-}C(OCH_3)=CH\text{-}C_6H_4\text{-}OH(p)$

1c $R_1 = H$, $R_2 = H$, $R_3 = OCH_3$, $R_4 = OCONH_2$

2a $R_1 = CH_3$, $R_2 = COCH_3$, $R_3 = OCH_3$, $R_4 = OCOC(OCH_3)=CH\text{-}C_6H_4\text{-}OSO_2OH(p)$

2b $R_1 = CH_3$, $R_2 = COCH_3$, $R_3 = OCH_3$, $R_4 = OCOC(OCH_3)=CH\text{-}C_6H_4\text{-}OCOCH_3$

Cephamycin A (λ_{max} 288 nm, E% 437) and B (λ_{max} 305 nm, E% 524) contain the 7-aminoadipoyl-7-methoxy-cephem skeleton as known for cephamycin C (1c)[2]. The nmr spectra, in addition, show the absorptions of a para-disubstituted benzene moiety, of a single vinylic proton and of a further methoxy group (cephamycin A: 2.19τ, 2H, d, J=8Hz; 2.65τ, 2H, d, J=8Hz; 2.88τ, 1H, s; 6.25τ, 3H, s; cephamycin B: 2.30τ, 2H, d, J=8Hz, 3.10τ, 2H, d, J=8Hz; 3.06τ, 1H, s; 6.23τ, 3H, s; D_2O, int. DSS). These absorptions are caused by α-methoxy-p-hydroxy cinnamic acid moieties, which have been isolated as 5 (λ_{max} 301 nm; nmr, acetone-d_6, TMS : 2.29τ, 2H, d, J=8.5Hz; 3.11τ, 2H, d, J=8.5Hz; 3.03τ, 1H, s; 6.23τ, 3H, s; M^+ 194) and 6 (2.23τ, 2H, d, J=8Hz; 2.66τ, 2H, d, J=8Hz; 3.18τ, 1H, s; 6.30τ, 3H, s; D_2O, int. DSS).



5, R_1 = H, R_2 = H 7, R_1 = CH_3, R_2 = H 9, R_1 = CH_3, R_2 = SO_2OCH_3

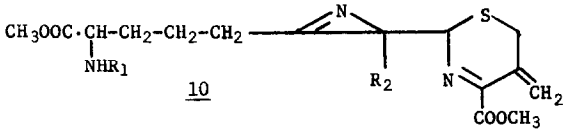
6, R_1 = H, R_2 = SO_2OH 8, R_1 = CH_3, R_2 = SO_2OH

Degradation of cephamycin A to cephamycin B can be accomplished in moist acetone. Further hydrolysis leads to 5. A slow deep-seated degradation of cephamycin A occurs in water at room temperature whereby the methoxy-cephem skeleton is destroyed and the sulfate ester 6 is liberated. 5 and 6 were converted with diazomethane into the monomethyl esters 7 (2.32τ, 2H, d, J=8.5Hz; 3.13τ, 2H, d, J=8.5Hz; 3.10τ, 1H, s; 6.20τ, 3H, s; 6.25τ, 3H, s; acetone-d_6, TMS) and 8 (2.25τ, 2H, d, J=8.5Hz; 2.65τ, 2H, d, J=8.5Hz; 3.06τ, 1H, s; 6.23τ, 3H, s; 6.28τ, 3H, s; acetone-d_6, TMS). The loss of the sulfate group from the phenolic position is reflected in these spectra, as in the cephamycin A and B spectra, by the large upfield shift to normal phenolic positions of the absorptions of the two protons at C-3' and C-5'. The dimethyl ester 9 was analysed by the high-resolution mass spectrometry (M^+ 302.0475, $C_{12}H_{14}O_7S$; m/e 207.0663, $C_{11}H_{11}O_4$; 179.0721, $C_{10}H_{11}O_3$; 243.0316, $C_{10}H_{11}O_5S$; 228.0113, $C_9H_8O_5S$; 133.0294, $C_8H_5O_2$; 105.0344, C_7H_5O). The alternative β-methoxy structure is not compatible with this fragmentation and also unlikely for biogenetic reasons.

Cephamycin A and B were acetylated in dimethyl formamide-acetic anhydride. Cephamycin A gave a monoacetyl dimethyl ester 2a (2.26τ, 2H, d, J=8Hz; 2.70τ, 2H, d, J=8Hz; 3.03τ, 1H, s; 4.89τ, 1H, s; 6.14τ, 3H, s; 6.27τ, 3H, s; 6.32τ, 3H, s; 6.49τ, 3H, s; 8.04τ, 3H, s; 4.78 and 5.10τ, 1H each, 2d, J=13Hz; 6.27τ, 1H, d, J=18Hz; 7.64τ, 2H, t; 8.23τ, 4H, m; high field doublet of SCH_2 hidden; CD_3OD, TMS) and cephamycin B the N,O-diacetyl dimethyl ester 2b (2.30τ, 2H, d, J=8Hz; 2.96τ, 2H, d, J=8Hz; 3.11τ, 1H, s; 4.95τ, 1H, s; 6.16τ, 3H, s; 6.29τ, 3H, s; 6.33τ, 3H, s; 6.51τ, 3H, s; 7.74τ, 3H, s; 8.02τ, 3H, s; 4.80 and 5.09τ, 1H each, d, J=13Hz; 6.72τ, 1H, d, J=18Hz; 7.65τ, 2H, m; 8.23τ, 4H, m; low field doublet of SCH_2 hidden,

$CDCl_3$, TMS. $C_{31}H_{37}N_3O_{13}S$: fd. 53.33, H 5.43, N 6.03, S 3.70%; calc. C 53.83, H 5.39, N 6.07, S 4.64%).

Elimination of acetic acid during vaporization is noticeable in mass spectra of 7-phenylacetamido- and 7-phenoxyacetamido cephalosporanic esters[3a] and a major factor to be considered in the spectra of aminoadipoyl-cephalosporanic acid derivatives. Subsequent loss of carbondioxide leads to abundant ions of the tentative azirine structure 10 (e.g. N-carbomethoxy cephalosporin C dimethylester: m/e 397.1300; N-carbomethoxy cephamycin C dimethylester: m/e 427.1409). Spectra of the di-acetyl derivative 2b show the expected peak at m/e 411.1450 ($C_{18}H_{25}N_3O_6S$, calc. 411.1464). In addition, an intense peak at m/e 405.0899 ($C_{19}H_{17}NO_6S$, calc. 405.0882)



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is observed which corresponds to the most characteristic fragmentation of penicillins[3b] and cephalosporins[3a] through the β-lactam ring. (The prominent appearance of this ion in spectra of cephamycin B but hardly of cephamycin C derivatives may have been caused by some $\Delta^{2,3}$-isomer, the presence of which is indicated by nmr signals at 3.4 (s; -S-CH=), 6.22τ (s; C_4-$COOCH_3$) and 6.52τ (s; C_7-OCH_3). We have since observed the higher thermal stability of iso-cephamycins and iso-cephalosporins in many instances.) Mass spectra of 2b therefore contain the evidence for the central dihydrothiazine structure connected to both the amino-adipoyl and the hydroxyphenylpyruvic moieties.

The configuration at the cinnamic acid double bond is being determined by nmr comparison with both synthetic partial structures. Reasonable assumptions concerning the configuration at C7 of the cephamycins have been confirmed by synthesis[4].

Acknowledgement

We thank Mr. R. Boos and his staff for microanalyses, Drs. N.R. Trenner and J.L. Beck for their technical assistance and Dr. J.D. White, Department of Chemistry, Harvard University for his critical discussions.

References

1(a) R. Nagarajan, L.D. Boeck, M. Gorman, R.L. Hamill, C.E. Higgens, M.M. Hoehn, W.M. Stark, J.G. Whiney; J. Amer. Chem. Soc. 93 2308 (1971);

(b) E.O. Stapley, D. Hendlin, S. Hernandez, M. Jackson, J.M. Mata, A.K. Miller, H.B. Woodruff, T.W. Miller, G. Albers-Schönberg, B.H. Arison, J.L. Smith; Abstracts XIth Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlantic City, 1971, p. 8.

(c) T.W. Miller, R.T. Goegelman, R.G. Weston, I. Putter, F.J. Wolf; Antimicrobial Agents & Chemotherapy, in press.

2 G. Albers-Schönberg, B.H. Arison, J.L. Smith and F.J. Wolf, unpublished results. The structure of cephamycin C has after completion of our analysis been reported for a metabolite of *Streptomyces clavuligerus*[1a].

3(a) W. Richter and K. Biemann, Monatschefte 96 494 (1965);

(b) W. Richter and K. Biemann, Monatschefte 95 766 (1964).

4 R.W. Ratcliffe and B.G. Christensen, Tetrahedron Letters, submitted for publication.