Table I. J(E–H), Hz

	E = ^{15}N[a]	E = ^{31}P[b]
$C_6H_5EH_2$	79–81	205.5[c]
$C_6H_5EHC(CH_3)_3$		205.7 ± 0.5
$C_6H_5EHSi(CH_3)_3$	76.0	200.6 ± 0.5
$C_6H_5EHGe(CH_3)_3$	77.1	194[d]
$C_6H_5EHSn(CH_3)_3$	73.8	187 ± 0.5
$[(CH_3)_2Si]_2EH$	66.5	186[e]

[a] Data taken from ref 1b. [b] Recorded on 20% solutions in benzene using a Varian HA-100D spectrometer. [c] Reference 9. [d] Reference 8. [e] E. Fluck, H. Burger, and V. Goetze, *Z. Naturforsch. B*, **22**, 912 (1967).

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P. G. Harrison, S. E. Ulrich, J. J. Zuckerman*
Department of Chemistry, State University of New York at Albany
Albany, New York 12203
Received October 14, 1970

β-Lactam Antibiotics from *Streptomyces*

Sir:

Several taxonomically unrelated true fungi produce penicillins,[1] and one species of *Streptomyces* has been reported to yield penicillin N.[2] Cephalosporin C (**1**) has been isolated from only one species of *Cephalosporium*.[3] We report here the identification of penicillin N, and isolation and structure elucidation of three new β-lactam antibiotics of the cephalosporin C type from two species of *Streptomyces*. Metabolite **2** was produced by a strain of *Streptomyces lipmanii* NRRL 3584. A new streptomycete species,[4] *Streptomyces clavuligerus* NRRL 3585, afforded antibiotics **3** and **4**.

The antibiotics present in the broth filtrate were concentrated by carbon and anion exchange resin column chromatography. Final purification was achieved by chromatography on cellulose and silica gel to yield the purified antibiotics. The three antibiotics exhibited some common properties. All had a band at about 1770 cm^{-1} in the ir spectra, suggesting the presence of a β-lactam carbonyl group.[5,6] The uv spectra showed absorption maxima at *ca.* 260 nm, characteristic of the 3-cephem chromophore[3,7,8] (Table I). Potentiometric titration revealed the presence of three ionizable groups, and amino acid determination on acid hydrolysates by the Spackman–Stein–Moore method[9] yielded about 2 μmol/mg of α-aminoadipic acid. An acetyl determination with **2** gave a value of 9.0%. Alkoxyl analysis of **2** and **4** afforded 4.5 and 5.8% methoxyl, respectively.

Characteristic features of the nmr spectrum of cephalosporin C (**1**) are the three pairs of AB doublets originating from the vicinally coupled β-lactam protons (J = 4.7 Hz), and the geminally coupled 2-methylene and 3′-methylene groups (J = 18 and 13 Hz),[10] respectively. In addition, the spectrum exhibits the three-proton acetyl singlet and the multiplet due to the seven α-aminoadipyl protons (Table I). The nmr spectrum of **3** shows all the spectral characteristics of **1**, except that the three-proton acetyl singlet is absent. This suggests that the difference between the antibiotic **3** and cephalosporin C is in the functionality on the 3′-methylene group. The nmr spectra of the antibiotics **1** and **2** reveal that in cephalosporin C the H-6 proton occurs as a doublet at τ 4.86, while in **2** there is a one-proton singlet at 4.84, Further, in **2** there is a three-proton singlet at τ 6.47. This indicates that the β-lactam ring has been modified in **2**. The nmr spectrum of **4** shows a one-proton singlet at τ 4.81 and a three-proton singlet at 6.47, and the acetyl singlet is not present. Consequently, in antibiotic **4**, both the functionality on the 3′-methylene group and the β-lactam ring substitution are different from that of cephalosporin C. The presence of a methoxyl group in **2** and **4** is established by alkoxyl analysis and by the three-proton singlets at τ 6.47.[13] Chemical shifts of the one-proton singlets at τ 4.84 and 4.81 in **2** and **4** correspond closely to the chemical shift of the H-6 doublet of cephalosporin C. These data suggest that in **2** and **4** there is a methoxyl group at C-7 of the β-lactam ring.

Reaction of **2**, **3**, and **4** with chloroacetyl chloride and *N*-carbethoxyphthalimide afforded the corresponding *N*-chloroacetyl (**2a**, **3a**, and **4a**) and *N*,*N*-phthaloyl (**2b**, **3b**, and **4b**) derivatives. Potentiometric titration of these *N*-acyl derivatives shows they all contain two ionizable groups. A comparison of the dissociation constants of **1**, **2**, **3**, and **4** and their *N*-acyl derivatives reveals that the antibiotics **2**, **3**, and **4** contain the amino and the two carboxyl groups present in cephalosporin C.

Reaction of the *N*-chloroacetyl (**2a**, **3a**, **4b**) and *N*,*N*-phthaloyl (**2b**, **3b**, **4b**) derivatives of **2**, **3**, and **4** with diazomethane gave the corresponding *N*-acyl dimethyl esters **2c**, **3c**, and **4c**, and **2d**, **3d**, and **4d**, respectively. The chemical shifts of the 3′-methylene protons in the nmr spectra of the antibiotics **1**, **2**, **3**, and **4** and their *N*-acyl dimethyl esters are similar (Table II). Consequently, the groups deshielding the 3′-methylene groups should be structurally similar. The *N*-chloroacetyl derivative of **1** reveals a three-proton acetyl singlet at τ 8.0, while the corresponding derivative of **3** has a two-proton exchangeable singlet at 3.41. Whereas the *N*-chloroacetylcephalosporin C dimethyl

(1) A. G. Sanders, "Antibiotics," H. W. Florey, *et al.*, Ed., Vol. 2, Oxford University Press, London, 1949, p 672.
(2) I. M. Miller, E. O. Stapley, and L. Chaiet, *Bact. Proc.*, 32 (1962).
(3) E. P. Abraham and G. G. F. Newton, *Biochem. J.*, **79**, 377 (1961).
(4) C. E. Higgens and R. E. Kastner, *Int. J. Syst. Bacteriol.*, in press.
(5) R. B. Woodward, "The Chemistry of Penicillin," H. T. Clarke, J. R. Johnson, and R. Robinson, Ed., Princeton University Press, Princeton, N. J., 1949, p 444.
(6) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," Methuen & Co., London, England, 1964, p 214.
(7) D. M. Green, A. G. Long, P. J. May, and A. F. Turner, *J. Chem. Soc.*, 766 (1964).
(8) R. Nagarajan and D. O. Spry, *J. Amer. Chem. Soc.*, **93**, 2310 (1971).
(9) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).
(10) Though both the 2-methylene and 3′-methylene groups are adjacent to the double bond, in the flat, rigid dihydrothiazine ring[11] the angle between the 2-methylene protons and the π orbital is 30–35°, causing the greater negative geminal coupling.[12] This large negative coupling is diagnostic of the rigid dihydrothiazine ring geometry in 3-cephems.
(11) D. Hodgkin and E. N. Maslen, *Biochem. J.*, **79**, 393 (1961).
(12) M. Barfield and D. M. Grant, *J. Amer. Chem. Soc.*, **85**, 1899 (1963).
(13) L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, New York, N. Y., 1969, p 180.

Table I. Physical-Chemical Properties of Cephalosporin Antibiotics

	1	2	3	4
Ir (mull), cm^{-1}	1780 (β-lactam); 1730, 1230 (*O*-acetyl)	1770 (β-lactam); 1730, 1230 (*O*-acetyl)	1770 (β-lactam); 1700 (carbamate)	1770 (β-lactam); 1710 (carbamate)
Uv (H_2O) (λ_{max} in nm)	260 ($E_{1\ cm}^{1\%}$ 188)	265 ($E_{1\ cm}^{1\%}$ 155); 242 ($E_{1\ cm}^{1\%}$ 130)	261 ($E_{1\ cm}^{1\%}$ 161)	264 ($E_{1\ cm}^{1\%}$ 149); 242 ($E_{1\ cm}^{1\%}$ 124)
Amino acid (μmol/mg of α-aminoadipic acid)	2.46	2.39	2.09	1.97
Potentiometric titration (66% DMF)	pK_a's 3.9, 5.3, 10.5 mol wt 440	pK_a's 3.9, 5.3, 10.5 mol wt 480	pK_a's 4.0, 5.3, 10.5 mol wt 460	pK_a's 4.2, 5.6, 10.4 mol wt 450
Nmr (D_2O)	4.34 (H-7, d, 4.7); 4.85 (H-6, d, 4.7); 5.05, 5.25 (3-CH_2, dd, 12.5); 6.15–6.3 (NH_2CH-COOH, m); 6.28, 6.60 (2-CH_2, dd, 18); 7.40–7.70 CH_2CO, m); 7.87 (CH_3, s); 7.85–8.50 (CH_2CH_2, m)	4.84 (1 H, s); 5.14 (1 H, d, 12.5); 5.32 (1 H, d, 12.5); 6.1–6.3 (1 H, m); 6.33 (1 H, d, 18); 6.47 (3 H, s); 6.71 (1 H, d, 18); 7.4–7.7 (2 H, m); 7.90 (3 H, s); 7.9–8.4 (4 H, m)	4.33 (1 H, d, 5); 4.85 (1 H, d, 5); 5.0 (1 H, d, 13); 5.32 (1 H, d, 13); 6.1–6.3 (1 H, m); 6.31 (1 H, d, 18); 6.61 (1 H, d, 18); 7.4–7.7 (2 H, m); 7.9–8.5 (4 H, m)	4.81 (1 H, s); 5.06 (1 H, d, 13); 5.26 (1 H, d, 13); 6.0–6.2 (1 H, m); 6.32 (1 H, d, 18); 6.47 (3 H, s); 6.68 (1 H, d, 18); 7.4–7.6 (2 H, m); 7.9–8.4 (4 H, m)
Functional groups	One acetyl, one primary amino, and two carboxyl	One acetyl, one methoxyl, one primary amino, and two carboxyl	One primary amino and two carboxyl	One methoxyl, one primary amino, and two carboxyl

Table II. Nmr Spectral Data of *N*-Acylcephalosporin Dimethyl Esters[a]

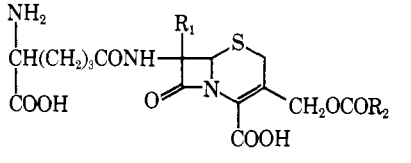
Compd	2-CH_2	3-CH_2	H-6	R_2 = H or OCH_3	R_3 = CH_3 or NH_2[b]	7-NH[b]	R_1 = $NHCOCH_2Cl$[b]
1c	6.29, 6.52 (18)	5.03, 5.31 (13)	4.86 (4.7)	4.27 (8.5, 4.7)	7.97 (s)	1.16 (8.5)	1.39 (7.5)
1d	6.34, 6.59 (18)	5.06, 5.33 (13)	4.91 (4.5)	4.33 (8.5, 4.5)	7.97 (s)	1.17 (8.5)	
2c	6.34, 6.65 (18)	5.08, 5.33 (13)	4.81 (s)	6.60 (s)	7.97 (s)	0.79 (s)	1.37 (7.5)
2d	6.37, 6.72 (18)	5.08, 5.33 (13)	4.84 (s)	6.62 (s)	7.97 (s)	0.83 (s)	
3c	6.33, 6.54 (18)	5.11, 5.39 (13)	4.85 (5.0)	4.31 (8.0, 5.0)	3.41 (s)	1.15 (8.0)	1.38 (8.0)
3d	6.38, 6.63 (18)	5.11, 5.40 (13)	4.88 (4.5)	4.34 (8.5, 4.5)	3.38 (s)	1.17 (8.5)	
4c	6.37, 6.69 (18)	5.16, 5.42 (13)	4.81 (s)	6.60 (s)	3.41 (s)	0.81 (s)	1.37 (7.5)
4d	6.41, 6.76 (18)	5.16, 5.42 (13)	4.84 (s)	6.62 (s)	3.39 (s)	0.84 (s)	

[a] The nmr spectra of all these compounds were determined in DMSO-d_6, with TMS as internal standard. Chemical shifts are expressed in τ values, and the figures in parentheses are coupling constants in hertz; s = singlet. The chemical shifts of the α-aminoadipyl, phthalimido, methylene of the *N*-chloroacetyl, and the carbomethoxy protons are not included in the table. [b] The signal due to these protons disappeared on shaking with D_2O.

Table III. High-Resolution Mass Spectral Data of *N*-Acylcephalosporin Dimethyl Esters

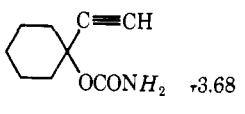
	1c		2c		3d		4d	
	Mass (Intensity)	Composition	Mass (Intensity)	Composition	Mass (Intensity)	Composition	Mass (Intensity)	Composition
e	230.048 (98)	$C_9H_{12}O_4NS$	230.053 (50)	$C_9H_{12}O_4NS$	231.047 (0.5)	$C_8H_{11}O_4N_2S$	231.050 (4)	$C_8H_{11}O_4N_2S$
f	170.027 (100)	$C_7H_8O_2NS$	170.028 (100)	$C_7H_8O_2NS$	170.028 (33)	$C_7H_8O_2NS$	170.024 (4)	$C_7H_8O_2NS$
g	290.070 (15)	$C_{11}H_{15}O_5N_2Cl$	320.081 (4)	$C_{12}H_{17}O_6N_2Cl$	345.106 (0.5)	$C_{17}H_{17}O_6N_2$		
h	234.057 (25)	$C_9H_{13}O_4NCl$	234.057 (20)	$C_9H_{13}O_4NCl$	288.087 (1)	$C_{15}H_{14}O_3N$	288.088 (8)	$C_{15}H_{14}O_3N$

ester is a $C_{20}H_{26}O_9N_3SCl$ compound, the corresponding derivative of **3** is a $C_{19}H_{25}O_9N_4SCl$ diester. Consequently, instead of a CH_3 group in the cephalosporin C derivative, the corresponding derivative of **3** contains an NH_2 group. The NH_2 protons of carbamates resonate at τ 3.3–3.7 in DMSO-d_6.[14] The structure of the antibiotic, **3**, from *Streptomyces clavuligerus* is thus 7-(5-amino-5-carboxyvaleramido)-3-carbamoyloxymethyl-3-cephem-4-carboxylic acid.[15]

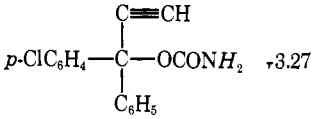


1, R_1 = H; R_2 = CH_3
2, R_1 = OCH_3; R_2 = CH_3
3, R_1 = H; R_2 = NH_2
4, R_1 = OCH_3; R_2 = NH_2

(14) The chemical shifts of the NH_2 protons in DMSO-d_6 of two authentic carbamates are



τ3.68



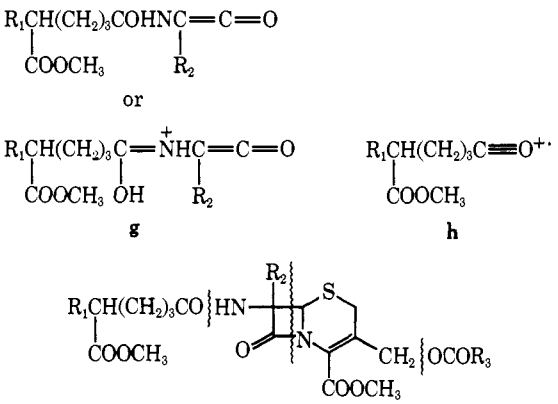
τ3.27

The carbamate protons resonate at τ 5.0–5.2 in $CDCl_3$.

(15) Cephalosporin C has been converted by a series of reactions to **3d**, thus establishing the relationship between **1** and **3**; unpublished work of C. F. Murphy and R. E. Koehler.

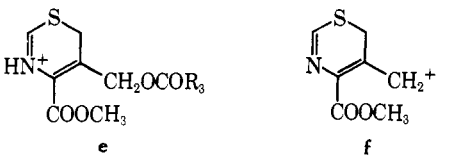
The nmr spectrum of *N*-chloroacetylcephalosporin C dimethyl ester exhibits two one-proton exchangeable doublets at τ 1.18 and 1.38, while *N*,*N*-phthaloylcephalosporin C dimethyl ester shows a one-proton exchangeable doublet at 1.17. Thus, the protons at τ 1.18 and 1.17 can be assigned to the 7-N*H* protons. The *N*-chloroacetyl dimethyl esters of **2** and **4** have two exchangeable protons at τ 0.79 and 1.37, and 0.81 and 1.37, respectively. Clearly, the exchangeable one-proton singlets at about τ 0.8 in the *N*-acyl dimethyl esters can be assigned to the 7-N*H* proton, and its singlet nature confirms the placement of a methoxyl group at the 7 position in **2** and **4**. Finally, the *N*-acyl dimethyl ester derivatives of **2** have a three-proton singlet at τ 7.97, and the *N*-acyl dimethyl esters of **4** show a two-proton exchangeable singlet at *ca.* τ 3.4. These data establish the structure of the antibiotic **2** of *Streptomyces lipmanii* as 7-(5-amino-5-carboxyvaleramido)-7-methoxycephalosporanic acid, and that of the metabolite, **4**, from *Streptomyces clavuligerus* as 7-(5-amino-5-carboxyvaleramido)-7-methoxy-3-carbamoyloxymethyl-3-cephem-4-carboxylic acid.

High-resolution mass spectral analysis of *N*-acyl dimethyl esters **1c**, **2c**, **3d**, and **4d** confirm the above structural assignments.[16] The differences in the composition of the dihydrothiazine fragments **e** and **f** derived from **2c**, **3d**, and **4d** show the nature of the substituent,



g **h**

1c, $R_1 = NHCOCH_2Cl$; $R_2 = H$; $R_3 = CH_3$
2c, $R_1 = NHCOCH_2Cl$; $R_2 = OCH_3$; $R_3 = CH_3$
3d, $R_1 = N$, *N*-phthaloyl; $R_2 = H$; $R_3 = NH_2$
4d, $R_1 = N$, *N*-phthaloyl; $R_2 = OCH_3$; $R_3 = NH_2$



e **f**

$R_2 = CH_3$ in **2**, and $R_2 = NH_2$ in **3** and **4** (Table III). The substituent $R_1 = OCH_3$ in **2** is also supported by the analysis of side-chain fragments **g** and **h** derived from **2c**.

The antibiotics **2** and **4** exhibited greater activity than cephalosporin C against gram-negative organisms, while **3** exhibited activity comparable to cephalosporin C.

(16) W. Richter and K. Biemann, *Monatsh. Chem.*, **96**, 484 (1965).

R. Nagarajan,* L. D. Boeck, M. Gorman, R. L. Hamill
C. E. Higgens, M. M. Hoehn, W. M. Stark, J. G. Whitney
The Lilly Research Laboratories, Eli Lilly and Company
Indianapolis, Indiana 46206
Received February 24, 1971

The 3-Cephem Chromophore

Sir:

The spectral characteristics of the 3-cephem chromophore, the basic skeleton of the cephalosporin antibiotics, have evoked considerable comment.[1-4] The 3-cephem chromophore shows an absorption maximum at 260 nm. During the structure elucidation of the antibiotics **2**, **3**, and **4**, from *Streptomyces*[5] (Chart I), we noticed that **2** and **4** showed two absorption maxima at about 265 and 240 nm (Table I). This observation prompted a detailed investigation of the 3-cephem chromophore.

Table I. Uv Spectra of Cephalosporins

Compd	Solvent[a]	λ_{max}, nm	ϵ
1	W	260	8900
2	W	265	7100
		242	6000
3	W	261	7000
4	W	264	6900
		242	5700
5	W	262	8000
7	M	262.5	8100
8	M	264	9500
9	M	267	9100
10	M	257	9100
11	M	232	5600

[a] W = water; M = methanol.

The CD curves of the antibiotics **2** and **4** show two Cotton effects, a positive maximum at 263 and a negative maximum at 236 nm. Similarly, the CD spectra of the antibiotics **1** and **3** show two Cotton effects, a positive at 259 and negative at 228 nm (Figure 1). Apparently, cephalosporins have two transitions, but due to the low intensity of the lower transitions in **1** and **3**, they are not discernible in their uv spectra. The uv and CD spectra of a number of model 3-cephems confirm the conclusion that the 3-cephem chromophore has two transitions (Table I and Figure 2).

Bond length data obtained by X-ray analysis of cephalosporins[6,7] afford good evidence that in the 3-cephem moiety the lone-pair electrons of the nonplanar nitrogen are involved to some degree in amide, as well as enamine, resonance.[7] The long-wavelength

(1) E. P. Abraham and G. G. F. Newton, *Biochem. J.*, **79**, 377 (1961).
(2) A. G. Long and A. F. Turner, *Tetrahedron Lett.*, 421 (1963).
(3) D. M. Green, A. G. Long, P. J. May, and A. F. Turner, *J. Chem. Soc.*, 766 (1964).
(4) G. C. Barrett, V. V. Kane, and G. Lowe, *ibid.*, 783 (1964).
(5) R. Nagarajan, L. D. Boeck, M. Gorman, R. L. Hamill, C. E. Higgens, M. M. Hoehn, W. M. Stark, and J. G. Whitney, *J. Amer. Chem. Soc.*, **93**, 2308 (1971).
(6) D. Hodgkin and E. N. Maslen, *Biochem. J.*, **79**, 393 (1961).
(7) R. M. Sweet and L. F. Dahl, *J. Amer. Chem. Soc.*, **92**, 5489 (1970).