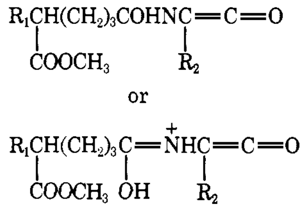
The nmr spectrum of *N*-chloroacetylcephalosporin C dimethyl ester exhibits two one-proton exchangeable doublets at τ 1.18 and 1.38, while *N,N*-phthaloylcephalosporin C dimethyl ester shows a one-proton exchangeable doublet at 1.17. Thus, the protons at τ 1.18 and 1.17 can be assigned to the 7-N*H* 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, 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**.

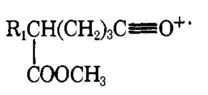


$\underset{COOCH_3}{R_1CH}(CH_2)_3COHN\underset{R_2}{C}{=}C{=}O$

or

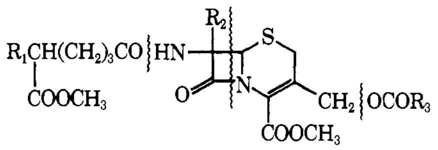
$\underset{COOCH_3}{R_1CH}(CH_2)_3\underset{OH}{C}{=}\overset{+}{N}H\underset{R_2}{C}{=}C{=}O$

g



$\underset{COOCH_3}{R_1CH}(CH_2)_3C{\equiv}O^{+\cdot}$

h

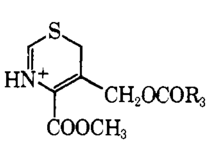


1c, R_1 = $NHCOCH_2Cl$; R_2 = H; R_3 = CH_3

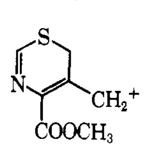
2c, R_1 = $NHCOCH_2Cl$; R_2 = OCH_3; R_3 = CH_3

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

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

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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

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(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).

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(6) D. Hodgkin and E. N. Maslen, *Biochem. J.*, **79**, 393 (1961).

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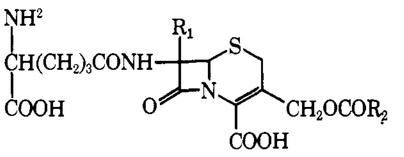
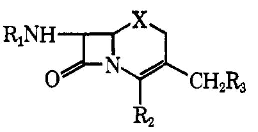
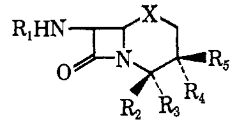
Chart I



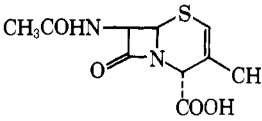
1, $R_1 = H; R_2 = CH_3$
2, $R_1 = OCH_3; R_2 = CH_3$
3, $R_1 = H; R_2 = NH_2$
4, $R_1 = OCH_3; R_2 = NH_2$



5, $R_1 = H; R_2 = COOH; R_3 = OCOCH_3; X = S$
6, $R_1 = COCH_3; R_2 = COOH; R_3 = OCOCH_3; X = S$
7, $R_1 = COCH_3; R_2 = COOCH_3; R_3 = OCOCH_3; X = S$
8, $R_1 = COCH_3; R_2 = COOCH_3; R_3 = OCOCH_3; X = SO_2$
9, $R_1 = COCH_3; R_2 = COOCH_3; R_3 = SCH_3; X = S$
10, $R_1 = C_6H_5OCH_2CO; R_2 = R_3 = H; X = S$



12, $R_1 = COCH_3; R_2 = COOCH_3; R_3 = R_4 = H; R_5 = CH_3; X = SO_2$
13, $R_1 = C_6H_5OCH_2CO; R_2 = R_5 = H; R_3 = COOCH_3; R_4 = CH_3; X = S$
14, $R_1 = C_6H_5OCH_2CO; R_2 = R_5 = H; R_3 = COOCH_3; R_4 = CH_3; X = SO_2$



11

absorption at 260 nm for 3-cephems has been attributed to the interaction of the nitrogen lone-pair electrons with the π electrons of the double bond.[1] In β,γ-unsaturated sulfides, even though the sulfur and the double bond are insulated by a methylene group, a bathochromic shift occurs.[8,9] Consequently, it was suggested that the sulfur, ring nitrogen, and the double bond may be essential for the 260-nm absorption.[2-4] However, unlike the red shift observed for β,γ-unsaturated sulfides, no similar effect is observed in β,γ-unsaturated sulfones.[10] The CD curves of the sulfide 7 and sulfone 8 do show a blue shift of about 7 nm. Further, in the sulfide 9, there are two sulfur atoms homoallylic to the double bond, and the uv spectra of the sulfides 7 and 9 reveal a bathochromic shift of 5 nm (Figure 2). These data suggest that the sulfur atom of the 3-cephem skeleton contributes a bathochromic shift of about 5 nm for the 260-nm absorption. The uv and CD spectra of 10, and its carboxy derivatives 6 and 7, show that the 4-carboxy function contributes a red shift of about 10 nm, and from the known alkyl substitution effect of olefins, the 3-methyl or methylene-acetoxy function should contribute a bathochromic shift of 5 nm to the 260-nm absorption. Cyclic enamines and enamides have absorption bands at about 240 nm.[11]

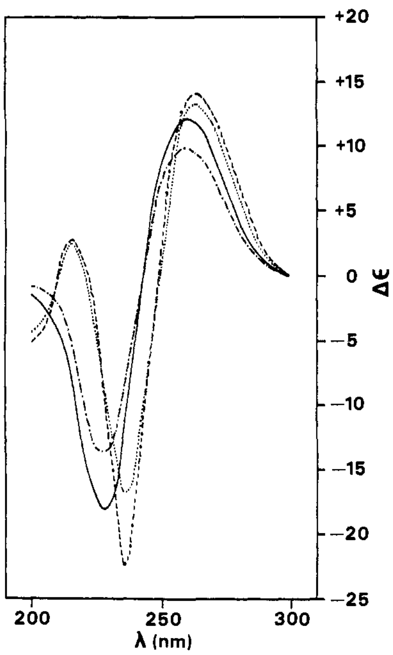


Figure 1. Circular dichroism curves of antibiotics **1** (—), **2** (- - -), **3** (- · -), and **4** (· · ·) in water.

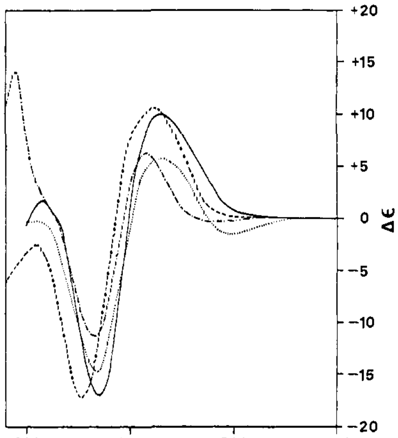


Figure 2. Circular dichroism curves of **5** (- - -) in water, and **7** (—), **8** (- · -), and **9** (· · ·) in methanol.

The second transition of the 3-cephem chromophore at 230 nm could have its origin in (a) the n → σ^* transition of the sulfur,[12] (b) the n → π^* of the β-

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[8] E. A. Fehnel and M. Carmack, *J. Amer. Chem. Soc.*, 71, 84 (1949).
[9] H. P. Koch, *J. Chem. Soc.*, 387 (1949).
[10] E. A. Fehnel and M. Carmack, *J. Amer. Chem. Soc.*, 71, 231 (1949).
[11] A. I. Scott, "Interpretation of Ultraviolet Spectra of Natural Products," McMillan, New York, N. Y., 1964, p 27.

Table II. Solvent Effect on the Uv and CD spectra of *N*-Acetyl-7-aminocephalosporanic Acid (**6**)

	Water		Methanol		Acetonitrile		Dioxane	
CD, maxima ($\Delta\epsilon$)	195	+0.85	206	+3.43	208	+1.39	210	+1.88
	229	−17.7	234.5	−19.4	235.5	−21.7	237	−20.9
	260	+12.9	264.5	+11.1	265	+10.1	265	+13.4
Uv, λ_{max} (ϵ)	260.5	9200	263	8300	263.5	7800	266	7800

lactam carbonyl,[13] (c) the red-shifted n → π* transition of β-lactam carbonyl caused by the overlap of the sulfur lone-pair electrons with the β-lactam carbonyl, or (d) the overlap of the π system of the double bond with the p and π orbitals of the β-lactam carbonyl group. The CD curve of **10** proves that the removal of the 4-carboxyl has a minor effect on the 230-nm transition. The CD spectra of the sulfone **8**, and the sulfides **7** and **13**, show that the 230-nm transition does not arise from the n → σ* transition of the sulfur. A comparison of the CD spectra of the sulfones **8**, **12**, and **14** with the spectra of the corresponding sulfides **7** and **13** shows that the 230-nm transition is not due to the red-shifted n → π* transition caused by the overlap of the sulfur lone-pair electron with the β-lactam carbonyl group. The n → π* transition of six- and five-membered lactams shows amplitudes[14] of the same order as the n → π* transitions of ketones, and the amplitude of the four-membered lactam carbonyl transition would be expected to be similar in magnitude. The 230-nm transition of the 3-cephem chromophore is associated with high extinction and ellipticity. The saturated sulfones **12** and **14** do not show the 230-nm negative Cotton effect and, consequently, the latter transition cannot be assigned to the n → π* transition of the β-lactam carbonyl group.

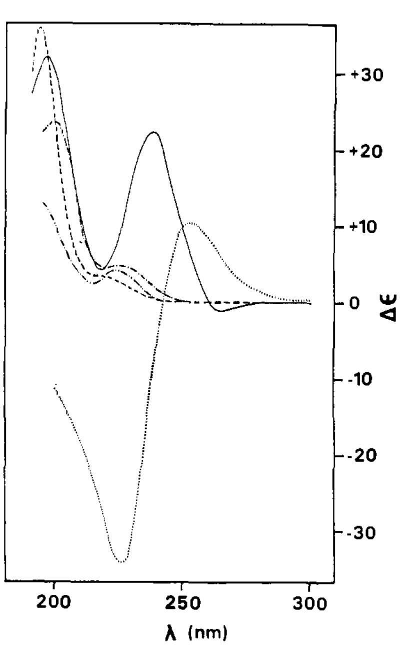

Figure 3. Circular dichroism curves of **10** (· · ·), **11** (—), **12** (- - -), **13** (- · -), and **14** (– · · ·) in methanol.

The double bond and β-lactam carbonyl can be considered as a homoconjugated π system constituting an inherently dissymmetric chromophore.[15,16] Such chromophores are associated with high amplitudes, and indeed the ellipticity of the 230-nm transition is high, $\Delta\epsilon = -10$ to -35. Dreiding models of the 3-cephem chromophore show that there is overlap between the π system of the double bond and the p and π orbitals of the β-lactam carbonyl group (Figure 4). The generalized octant rule[17] or the quadrant rule[18] predicts a negative sign, and the 230-nm transition shows a negative Cotton effect. The effect of solvent on the 230-nm transition of the acid **6** supports the assignment that the 230-nm absorption is due to a n → π* transition (Table II). The 2-cephem derivative **11** is devoid of the 230-nm negative Cotton effect, and the 237-nm positive Cotton effect is due to the electron-transfer transition of the α,β-unsaturated sulfide. The red shift and the intensification observed for the 230-nm transition in the antibiotics **2** and **4** may be due to the influence of the methoxyl group being adjacent to the β-lactam carbonyl group.

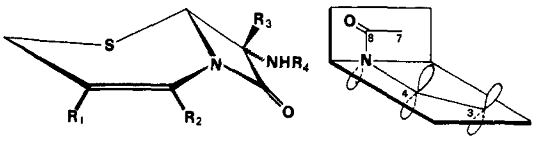

Figure 4. Conformation of the 3-cephem chromophore.

Acknowledgments. We gratefully acknowledge the technical assistance of F. W. Beasley, H. E. Martlage, R. D. Miller, S. M. Nash, and E. A. Wible.

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(12) M. Goodman, K. C. Su, and G. C. C. Niu, *J. Amer. Chem. Soc.*, **92**, 5220 (1970).

(13) L. Neelakantan and D. W. Urry, Abstracts of the 158th National Meeting of the American Chemical Society, Sept 1969, No. 176. These authors report that the n → π* transition of the peptide chromophore (β-lactam carbonyl) is observed near 210 nm for penicillins.

(14) H. Wolf, "Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry," G. Snatzke, Ed., Heyden & Son, London, England, 1967, p 361.

(15) K. Mislow, "Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry," G. Snatzke, Ed., Heyden & Son, London, England, 1967, p 162. Also see p 221 and references cited in these two sections.

(16) The 3-cephem chromophore exhibits both enamine and enamide character,[6,7] and consequently the sulfur, double bond, nitrogen lone pair, and β-lactam carbonyl could be considered as a conjugated chromophore; however, the extended conjugation is partially disrupted due to the deviation from planarity of the β-lactam nitrogen and carbonyl groups.

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