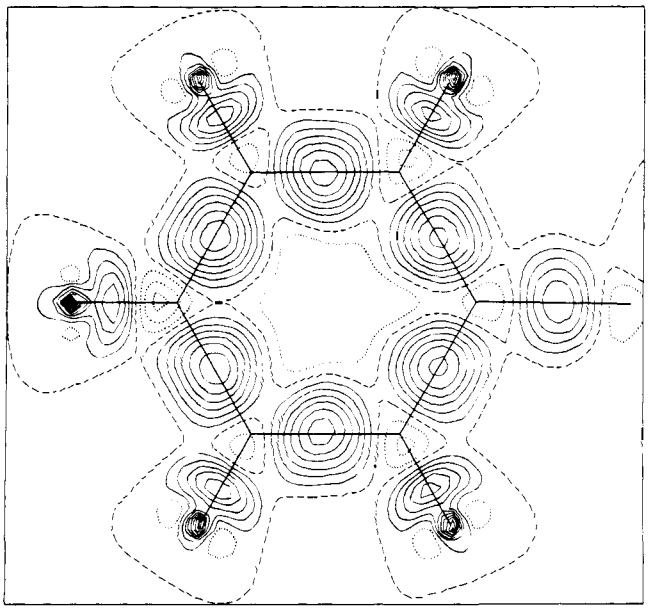

Figure 3. Deformation density section through the plane of the phenyl ring. Contours are as in Figure 1.

These charge distributions are in agreement with classical theory of π electrons in cumulenic systems.

The bonding density distribution in the phenyl plane is shown in Figure 3. The density sections through the aromatic bonds (Figure 2c) show the π character above (and below) the phenyl plane, whereas the electron distribution in the exocyclic C–C bond (Figure 2d) appears to be purely σ.

The net charge[8] in each of the several C–C bonds (Table I) is related to the bond length, varying between 0.1 and 0.3 electrons for bonds between 1.48 and 1.26 Å.

The electron density peak in the inner bond appears to be more circular in shape than that in the outer bond, suggesting a measure of out-of-plane π density in this bond. Moreover, this inner C=C bond is decidedly shorter (1.260 vs. 1.348 Å) and contains more electrons (0.3 vs. 0.22 e) than the outer C=C bond implying some C≡C triple bond character.

Acknowledgments. We thank Professor M. D. Cohen for his support, Professor F. L. Hirshfeld for his assistance, and Dr. M. Lahav for his interest. Support by a MINERVA grant is acknowledged.

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(3) Similar investigations are presently being carried out on the allene system (in the 1:1 complex allenedicarboxylic acid, acetamide), together with Nader. These works are part of a joint study of cumulenes, carried out by us and by F. Nader and H. Irngartinger, Chemistry Department, University of Heidelberg.
(4) The cell constants (100°K) are: a = 10.035 (3), b = 10.458 (4), c = 9.978 (3) Å; α = 105.04 (4), β = 105.27 (3), γ = 92.17 (3)°. Space group $P\bar{1}$, Z = 2.
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(7) The molecule is nonplanar, the phenyl rings A,B,C,D make dihedral angles of 30, 28, 39, and 42°, respectively, with the plane through the butatriene system >C=C=C=C< (see structure in Table I). The butatriene chain is almost linear, the C=C=C angles being 176.2°.
(8) The net charge in each bond was computed by numerical integration.

Ziva Berkovitch-Yellin, Leslie Leiserowitz*
Department of Structural Chemistry
The Weizmann Institute of Science, Rehovot, Israel
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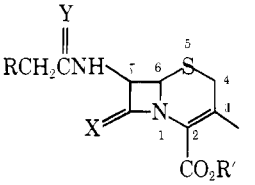
β-Thionolactam Analogs of Cephalosporins and Penicillins

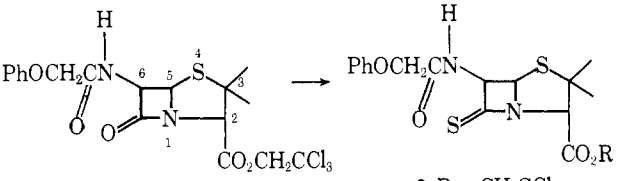
Sir:

In recent years an increasing number of nuclear modified β-lactam antibiotics have been described, in some instances with retention[1] or enhancement[2] of antibacterial activity. Excepting 7 (6)[3] and 2[4] (2)[5] substituted cephalosporins (penicillins), these modifications are remote from the 1–8 (1–7) bond, the rupture of which is an essential step in the mechanism of action of β-lactam antibiotics. To determine the effect of substitution adjacent to this bond, we set out to prepare the β-thionolactam counterparts of β-lactam antibiotics.

Phosphorus pentasulfide is reported[6] to react preferentially with the side-chain amide of trichloroethyl ester **1**. The product (**2**) of this reaction was converted, via hydrolysis of an intermediate iminothioether, to the free 7β-amine. This conversion establishes the position of sulfur in **2** unambiguously. Boron sulfide is more reactive than phosphorus pentasulfide in carbonyl–thiocarbonyl transformations.[7] This communication describes the utility of boron sulfide for preparation of β-thionolactam analogs of cephalosporins and penicillins.



No.	R	Y	X	R′
1	PhO	O	O	CH_2CCl_3
2	PhO	S	O	CH_2CCl_3
3	PhO	O	S	CH_2CCl_3
4	PhO	S	S	CH_2CCl_3
5	Ph	O	O	CH_2CCl_3
6	Ph	O	S	CH_2CCl_3
9	PhO	O	S	H
10	Ph	O	S	H



7 **8**, R = CH_2CCl_3; **11**, R = H

Reaction of **1** with 2 equiv of boron sulfide in refluxing chloroform overnight gave two major products and unreacted **1**. Chromatography on silica gel gave the β-thionolactams **3** and **4** in 20 and 10% yields, respectively; preparative TLC gave analytically pure **3** (C, H, N, Cl, S)[8] and **4** (C, H, N, Cl, S)[8] as foams. The absence of the characteristic "β-lactam" band at 1780 cm^{-1} and the appearance of a new intense band at 1400 cm^{-1} in their infrared spectra distinguished **3** and **4** from their β-lactam precursor **1**.[9] All of the β-thionolactams prepared exhibited a band in the region of 1370–1420 cm^{-1} attributable to the β-thionolactam moiety. The ultraviolet spectra of **3** and **4** exhibit intense maxima at 313 nm.

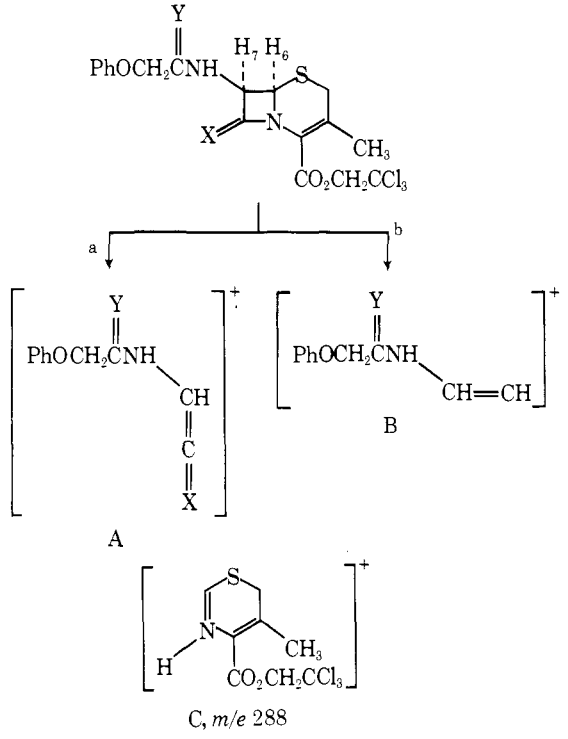
The chemical shift data in Table I are in accord with the assignment of side-chain thionamide in **2** and β-thionolactam in **3**; in particular, the downfield shift of the side-chain methylene protons of **2** and **4** and the opposite shifts of H_6 and H_7 in **3** are noted. A comparison of the mass spectra of **1**–**4** confirms these assignments. Observation of the appro-

Table I. NMR[a] and MS[b] of Thionocephems

H_6	H_7	$OCH_2C{=}Y$	No.	X	Y	*m/e* A	*m/e* B
5.07	5.90	4.59	1	O	O	191	176
5.18	6.18	4.94	2	O	S	207	192
5.48	5.60	4.60	3	S	O	207	176
5.64	5.75	4.96	4	S	S	223	192

[a] Chemical shifts are quoted in ppm relative to internal TMS in $CDCl_3$ solution. [b] Mass spectra were obtained at 70 eV (<150° source temperature) on an A.E.I. MS 902 instrument.

Scheme I



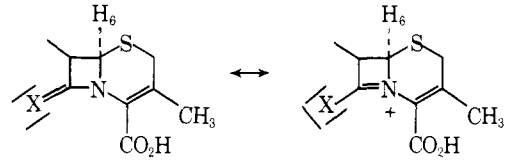
priate ion derived from cleavage via path a (Scheme I) dictates the placement of the additional sulfur (s) in fragment A. Similarly, the occurrence of path b allows the differentiation of side-chain and β-lactam substitution. An intense ion at *m/e* 288 in the spectra of **1–4,** attributable to ion C, confirms the integrity of the ester function and the dihydrothiazine ring.

Reaction of lactam **5** with boron sulfide gave the crystalline β-thionolactam **6** (mp 150–151°; C, H, N, Cl, S)[8] in 4% yield. Similar treatment of penicillin **7** gave a 1% yield of **8** (amorphous; *m/e* M^+ = 495.9848; calcd for $C_{18}H_{15}Cl_3N_2O_4S_2$, 495.9851) after extensive chromatography. Attempts to improve these yields were unsuccessful.

Treatment of **3, 6,** and **8** with 10 equiv of zinc dust in 90% aqueous acetic acid (**3** and **6** at room temperature, **8** at 0°) for 2–3 hr effected facile deesterification, as judged by TLC. Dilution with ethyl acetate and extraction into water at pH 8 and 0°, followed by acidification to pH 2 and extraction with ethyl acetate, gave the acids **9** (70%; trimethylsilyl ester had *m/e* M^+ = 436.0947; calcd for $C_{19}H_{24}N_2O_4S_2Si$, 436.0929), **10** (17%; mp 176–176.5°; C, H, N, S as hemihydrate[8]), and **11** (25%; trimethylsilyl ester had *m/e* M^+ = 438.1103; calcd for $C_{19}H_{26}N_2O_4S_2Si$, 438.1120). Paper electrophoresis of **9–11** indicated purities of 93, 98, and 100%, respectively (analysis by ultraviolet spectrodensitometry at 263 nm). Bioautography of each of these electropherograms against *S. aureus* 209 P revealed the presence of only one microbiologically active component; the zone of inhibition corresponded exactly with the position of the β-thionolactam on the paper. Thin layer chromatography on silica gel (1-butanol–acetic acid–water (3:1:1) development) separated completely thionolactams **9–11** from their β-lactam counterparts. In each case, the absence of the β-lactam counterpart was noted as was the presence of several minor impurities (ultraviolet assay). Bioautography of these TLC plates against *S. aureus* 209 P again revealed the presence of only one active component in each case, the zone of inhibition being coincident with the position of the β-thionolactam.

Paper electrophoresis was performed with 25 μg of substance spotted on Whatman 1 paper utilizing a 0.1 *N* triethylammonium acetate buffer. A voltage gradient of 14 V/cm is applied for 90 min after which the sheets are air dried. Bioautography of these paper strips was done on *S. aureus* 209 P plates (phosphate buffered agar plates at pH 7.0 incubated for 16 hr at 37°C). It should be noted that this technique does not separate the β-thionolactams from their β-lactam counterparts. For this purpose, thin layer chromatography was performed on glass silica gel plates (Merck 5765) using the solvent system 1-butanol–acetic acid–water (3:1:1). R_f values for the β-thionolactams **9–11** and their β-lactam counterparts (in parentheses) under these conditions are 0.60 (0.55), 0.64 (0.57), and 0.72 (0.66), respectively. Bioautography was performed as before with a moistened paper sheet between the glass plate and the agar. Each pair of thionolactam–lactam was chromatographed on the same plate (25 μg of material being spotted in each case). Complete separation on 20-cm plates was achieved. Bioautography showed the β-lactam's zone of inhibition to be much larger than that of the β-thionolactam.

The deshielding of H_6 (H_5) in the NMR spectra of these compounds and the appearance of the 313-nm band in the ultraviolet spectra of cephems may be explained by increased contribution of resonance form **12′.** The larger 1–8



12 X = O, S **12′**

(1–7) bond order that results should be reflected in a decrease in reactivity at this bond and reduced antimicrobial activity. Indeed, antimicrobial activities of **9, 10,** and **11** were substantially less than those of their β-lactam counterparts (tube-dilution assay). Minimal inhibitory concentrations (μg/ml) against *S. pyogenes* were 10.9 (0.5), 18.7 (0.09), and 1.8 (0.006) for **9, 10,** and **11,** respectively (numbers in parentheses refer to the parent β-lactam).

In summary, β-thionolactams have been prepared in the cephalosporin and penicillin series by amide–thioamide transformation with boron sulfide. These compounds show substantially reduced antimicrobial activity in vitro.

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(10) Squibb Postdoctoral Fellow.

P. W. Wojtkowski,*[10] J. E. Dolfini
Octavian Kocy, Christopher M. Cimarusti
The Squibb Institute for Medical Research
Princeton, New Jersey 08540
Received May 12, 1975

Conformational Analysis of Amino Acids and Peptides Using Specific Isotope Substitution. II. Conformation of Serine, Tyrosine, Phenylalanine, Aspartic Acid, Asparagine, and Aspartic Acid β-Methyl Ester in Various Ionization States

Sir:

As pointed out by Roberts and Jardetzky,[1] an uncertainty in the assignment of the two protons attached to the β-carbon has been a major problem in the conformational study by NMR of amino acids in solution. Very recently Feeney et al.[2] showed that the vicinal coupling constant between the carbonyl carbon and the β-proton might be useful as a conformational index of amino acids, if one could analyze the proton NMR spectrum independently. Due to the lack of any reliable empirical relation between the vicinal carbon–proton coupling constant and the dihedral angle, this method is particularly unsatisfactory if the two β-protons are incidentally overlapping. In practice, the chemical shift difference between these two protons frequently is very small. Stereoselective deuteration, therefore, must be exploited.[3]

In the present communication, we describe a conformational study of Ser, Phe, Tyr, and Asp and its derivatives, using stereoselective deuteration. A major object of our study is to understand qualitatively the factors responsible for the conformational changes in various ionization states. We therefore used the most convenient method of analysis, developed by Pachler[4] (Figure 1).

The proton NMR spectrum of (2*S*)-Asp at pD 5.8 shows a typical ABX pattern (Figure 2a).[5] A straightforward analysis of this spectrum gives us three chemical shifts, δ_α, δ_{β_1}, and δ_{β_2} and three coupling constants, $J_{\alpha\beta_1}$, $J_{\alpha\beta_2}$, and $J_{\beta_1\beta_2}$. The unambiguous assignment of β_1 and β_2 can be made using selectively deuterated amino acid[6] which gives an AB pattern at any pD (Figure 2b). An analysis of this

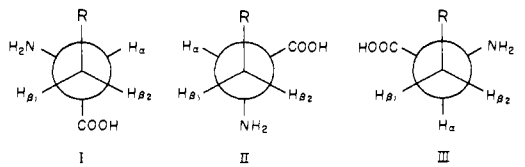

Figure 1. Newman projections about the C_α–C_β bond for the three rotamers of L-amino acids. The configurational notation for the C-2 of L-amino acids is usually *S*, except for L-Cys which is 2*R*. The configurational notation of H_{β_1} and H_{β_2} are pro-*S* and pro-*R* for L-(2*S*)-Asp, Asn, AspOMe, and Ser, but pro-*R* and pro-*S* for L-(2*S*)-Phe and Tyr. The fractional population P_I, P_{II}, and P_{III} for each conformer can be given by the following equations: $P_I = (J_{\alpha\beta_1} - J_g)/(J_t - J_g)$, $P_{II} = (J_{\alpha\beta_2} - J_g)/(J_t - J_g)$, $P_{III} = 1 - (P_I + P_{II})$, in which $J_g = 2.60$ Hz and $J_t = 13.56$ Hz (ref 4).

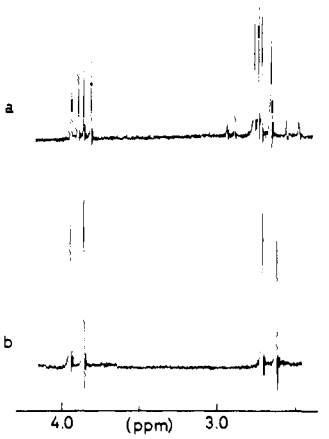

Figure 2. The 100-MHz ^{1}H NMR spectra of (a) L-(2*S*)-aspartic acid at pD 5.8; (b) L-(2*S*,3*R*)-[3-^{2}H]aspartic acid at pD 6.6 with a deuterium decoupling. Chemical shifts refer to internal DSS.

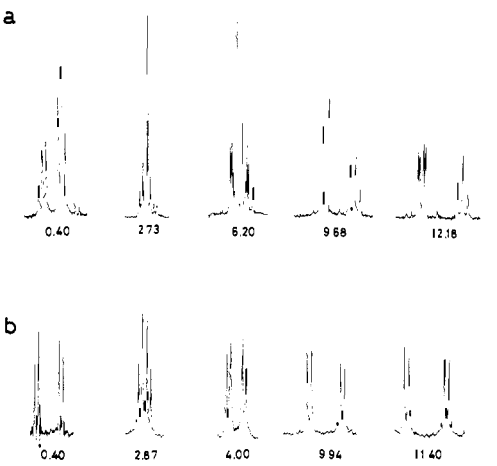

Figure 3. The 100-MHz ^{1}H NMR spectra of (a) L-(2*S*)-serine; and (b) DL-{(2*S*,3*S*);(2*R*,3*R*)}-[3-^{2}H]serine with a deuterium irradiation. Numbers below each spectrum denote the pD's.

spectrum affords $J_{\alpha\beta_1}$, δ_α, and δ_{β_1} because in this case the β_2 proton was replaced by a deuterium. There was no observable iostope effect in the vicinal coupling constants, which indicates the population of the rotational isomers is not affected by deuteration.

The proton spectra of (2*S*)-Ser and a racemic mixture of (2*S*,3*S*)- and (2*R*,3*R*)-[3-^{2}H]Ser[7] at various pD's are shown in Figure 3. As was demonstrated by Ogura et al.[8] the spectra of (2*S*)-Ser can only be solved by an ABC treatment. At around pD 2, the chemical shifts of the three protons became very close, which makes the spectral analysis difficult. A racemic mixture of [^{2}H]Ser, instead, gives a simple AB spectrum at any pD, except pD 2.3, where the chemical shifts of α and β_2 become identical. The NMR parameters obtained by the analysis of [^{2}H]Ser therefore gives very useful information for analysis of the complex ABC spectra, and of course absolute assignment of each proton.

Proton NMR spectra of all the other amino acids we examined were analyzed in a similar way.[9] The calculated fractional populations are summarized in Table I.

The dominant form of Asp, Asn, and AspOMe at low pD, where these compounds exist in the cationic forms, is III. As this conformer seems to be the least favorable in terms of steric hindrance, there might be a compensation due to the water structure, that is a smaller free energy of the total system can be obtained by putting all large substit-