

NMR STUDIES OF PENICILLINS AND
CEPHALOSPORINS. III¹⁾
3-METHYLENE SUBSTITUENT
EFFECT ON STRUCTURE-REACTIVITY
RELATIONSHIP OF CEPHALOSPORINS
STUDIED BY CARBON-13
NMR SPECTROSCOPY

Sir:

In recent years, great interest has been attached to investigating the structure-activity relationship of cephalosporins²⁾. The reactivity of the β -lactam ring of cephalosporins is well known to play a major role in their antibacterial activities²⁾. A number of parameters have been proposed for characterization of the chemical reactivity of the β -lactam ring of cephalosporin, for example, frequency variations in IR carbonyl stretching bands^{3,4)}, C-N and C=O bond length differences⁵⁾, hydrolysis rate constants^{4,6)}, and distributions of CNDO charge density^{7,8)}. In addition to these physical factors, much interest has been shown in the NMR chemical shifts (δ) of C-8 and N-5, which should provide information regarding the electron distribution^{9,10)}. However, the δ (C-8) and the δ (N-5) values have been reported to change only within a relatively narrow range with changes in substituent Y at the C-3 methylene and substituent X at the C-7 acyl amide group⁹⁻¹²⁾. PASCHAL *et al.*¹⁰⁾ suggested a broad correlation between ¹³C chemical shifts and the activity of cephalosporinate ions by the fact that differences between δ (C-3) and δ (C-4), $\Delta\delta$ (4-3), are comparatively large in cephalothin (4) and cephaloridine (9), two clinically useful cephalosporins. However, they also found that cephalixin (X=PhCH(NH₂), Y=Z=H) had a small shift difference¹⁰⁾.

In a previous paper¹⁾, we reported full ¹³C NMR signal assignments for cephalosporin free acids and methyl esters as well as their Na-salts⁹⁻¹²⁾, particularly of the C-3 and C-4 signals, which had previously been confused^{13,14)}. As a result, we suggested¹⁾ that there exists a good linear relationship between the logarithms of the rate constants k_{OH^-} for the OH⁻-catalyzed degradation of cephalosporins and the $\Delta\delta$ (4-3) values of cephalosporinate ions and esters. During our further investigation of various substituent effects of cephalosporins upon ¹³C spectral parameters in the structure-reactivity relationships, we found after observing the ¹³C spectra

of a series of diphenylmethyl esters of 7-(2-thienylacetyl)cephalosporins and some other derivatives, that the $\Delta\delta$ (4-3) values may generally be used as good reactivity indices of cephalosporins having substituent Y on the C-3 methylene group. We report our results here.

The ¹³C spectra of the diphenylmethyl esters of the compounds listed in Table 1 were measured in (CD₃)₂SO and/or CDCl₃. Their ¹³C signals were assigned by various methods reported previously^{1,9,11)}. The ¹³C spectral parameters are listed in Table 1, in which the data on cephalosporinate ions including reported values are also shown for comparison.

INDELICATO *et al.*⁴⁾ earlier suggested a correlation of the pseudo-first-order rates of the cephalosporin β -lactam ring opening, observed at pH 10 and 35°C, with the aliphatic σ_I values of 3-methylene substituents. We found good linear relationships between the inductive σ_I constants¹⁵⁾ and not only δ (C-3) and δ (C-4) but also the $\Delta\delta$ (4-3) values observed (Table 1). This suggests the presence of a good linear relationship between $\Delta\delta$ (4-3) and $\log k_{OH^-}$ ¹⁾.

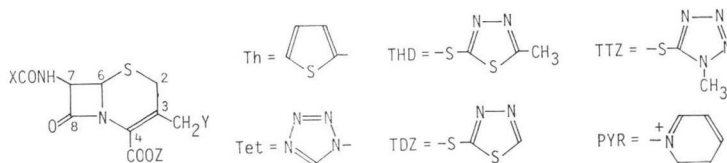
Recently, BOYD *et al.*⁸⁾ calculated the transition state energy, regarded as the theoretical index of reactivity, as the difference in total energies (TSE) between the "transition-state" complex (a model nucleophile, OH⁻, and a 3-cephem model structure with a substituent R at position 3) and the infinitely separated reactants, and suggested a parabolic relationship between -TSE and minimum inhibitory concentrations (MIC's) of 7-(2-thienylacetyl)cephalosporins against five Gram-negative pathogenic microbes. We plotted the TSE-values of BOYD *et al.* against the $\Delta\delta$ (4-3) values listed in Table 1 and found good linear relationships between them both for the diphenylmethyl ester and the Na-salt series (see Fig. 1). A change in the C-7 acyl amide substituent X has been found to affect slightly the $\Delta\delta$ (4-3) values¹⁰⁾. However, data on some compounds of this kind (5, 7, 8, 10 and 17~20) fitted well with the relationship derived for the 7-(2-thienylacetyl)cephalosporins (Table 1).

The MIC values reported by BOYD *et al.*⁸⁾ plotted against the $\Delta\delta$ (4-3) values gave parabolic relationships similar to that between MIC and -TSE, as shown in Fig. 2.

In conclusion, the $\Delta\delta$ (4-3) values in ¹³C NMR spectra were confirmed to be good indices for predicting changes in the β -lactam ring reactivity

Table 1. Carbon-13 NMR spectral data on cephalosporins.^{a, b}

Compound No.	Substituents			δ					$\Delta\delta(4-3)$ (ppm)	Reference
	X	Y	Z	C-3	C-4	C-8	CONH	4-COO		
1	ThCH ₂	H	Na	123.2	127.5	165.0	174.6	170.6	+ 4.3	10)
2	ThCH ₂	OH	Na	122.1	130.3	165.5	174.9	169.8	+ 8.2	10)
3	ThCH ₂	SCH ₃	Na	120.9	130.4	165.1	174.4	169.5	+ 9.5	10)
4	ThCH ₂	OCOCH ₃	Na	117.3	132.4	165.5	174.3	169.0	+15.1	1)
				116.9	132.3	165.4	174.8	169.1	+15.4	10)
5	{ThCH ₂ {7 α -OCH ₃	CONH ₂	Na	118.4	132.0	161.2	175.1	168.6	+13.6	This work
6	ThCH ₂	THD	Na	119.1	131.9	165.3	174.3	168.4	+12.8	10)
7	TetCH ₂	TDZ	Na	119.6	132.1	165.1	168.0	168.4	+12.5	This work
8	PhCH ₂	TTZ	Na	118.9	131.8	165.4	176.0	168.5	+12.9	This work
9	ThCH ₂	PYR	—	113.0	136.1	165.2	173.9	167.7	+23.1	1)
				113.1	135.9	165.4	174.5	167.9	+22.8	10)
10	PhCH SO ₃ Na	PYR- (4-CONH ₂)	—	113.2	136.2	165.3	169.6	167.7	+23.0	This work
11	ThCH ₂	H	CHPh ₂	134.9	122.6	164.8	170.1	161.1	-12.3	This work
				(133.6)	(121.6)	(164.5)	(169.7)	(160.9)	(-12.0)	
12	PhCH ₂	H	CHPh ₂	134.2	122.6	164.8	171.3	161.2	-11.6	This work
				(133.6)	(121.6)	(164.8)	(171.0)	(161.0)	(-12.0)	
13 ^c	TetCH ₂	H	CHPh ₂	(134.2)	(121.6)	(164.1)	(165.6)	(160.9)	(-12.6)	This work
14 ^{c, d}	ThCH ₂	OH	CHPh ₂	(134.6)	(121.9)	(165.0)	(169.9)	(160.8)	(-12.7)	This work
15	ThCH ₂	SCH ₃	CH ₃	130.1	122.4	164.5	170.2	162.1	- 7.7	This work
				(129.5)	(121.6)	(164.4)	(169.9)	(162.0)	(- 7.9)	
16	ThCH ₂	OCOCH ₃	CHPh ₂	128.1	125.1	165.0	170.2	160.5	- 3.0	This work
				(126.3)	(124.9)	(164.9)	(169.9)	(160.5)	(- 1.4)	
17	{ThCH ₂ {7 α -OCH ₃	CONH ₂	CHPh ₂	132.6	125.2	161.0	171.1	160.3	- 7.4	This work
				(130.4)	(124.3)	(160.7)	(170.3)	(160.1)	(- 6.1)	
18 ^c	TetCH ₂	THD	CHPh ₂	(128.9)	(124.7)	(164.5)	(165.6)	(160.4)	(- 4.2)	This work
19 ^c	TetCH ₂	TDZ	CHPh ₂	(128.8)	(124.9)	(164.5)	(165.6)	(160.5)	(- 3.9)	This work
20	PhCH ₂	TTZ	CHPh ₂	130.2	125.2	164.9	171.2	161.0	- 5.0	This work
				(128.6)	(124.6)	(165.1)	(170.9)	(160.5)	(- 4.0)	



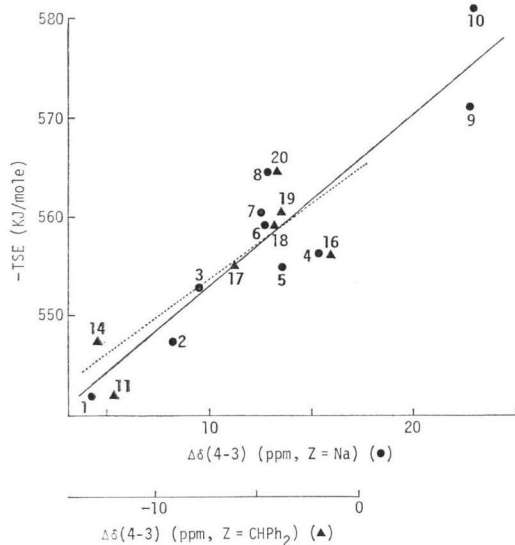
^a Detailed spectral data with full signal assignments will be reported in our full paper.

^b ¹³C NMR spectra were recorded on a Varian NV-14 FT NMR spectrometer at 15.087 MHz in D₂O for Na-salts (internal dioxan reference, δ 67.4) and in CDCl₃ and/or (CD₃)₂SO (in parentheses) for esters (internal TMS reference, δ 0) at ordinary probe temperature (30°C) using about 0.1 mmole/ml solutions in 8-mm spinning tubes. Typical FT NMR measurement parameters are: spectral width, 3923 Hz; pulse width, 13 μ s (flipping angle, 19°); acquisition time, 0.6 s; number of data points, 4820.

^c Sparingly soluble in CDCl₃.

^d The δ (C-3) and the δ (C-4) values were respectively shifted to a lower and a higher field compared to what was found for the acetylated compound (16). This is due to the effect of hydrogen-bonding of (CD₃)₂SO to the allylic OH group. In the same way, the double bond C-2 and C-1 signals in geraniol, (CH₃)₂C=CH(CH₂)₂(CH₃)C(1)=C(2)HCH₂OH, were found to be respectively shifted to a lower (+1.3 ppm) and a higher (-3.5 ppm) field in (CD₃)₂SO than in CDCl₃, whereas these signals were essentially unchanged in both solvents in its acetate.

Fig. 1. Relationships between TSE reported⁹⁾ and $\Delta\delta(4-3)$ values.



and biological activities of cephalosporins due to different C-3 methylene substituents. For such studies, the use of esters rather than sodium salts of cephalosporins may be recommended, because δ_C values of the latter in D_2O sometimes change greatly depending on the pH of the solution¹⁾.

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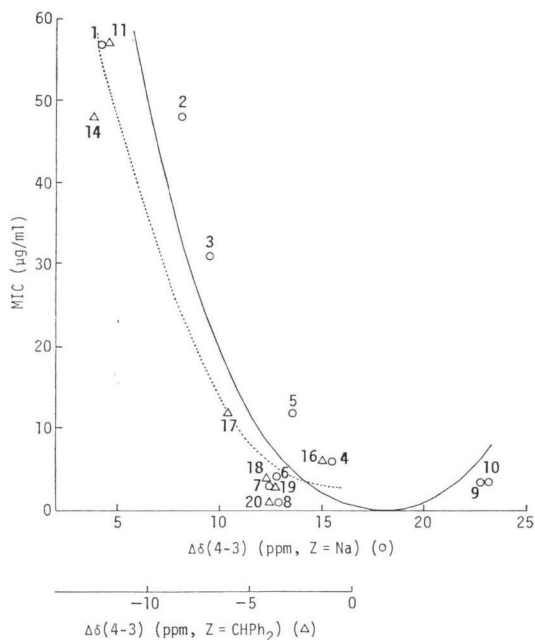
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Fig. 2. Average *in vitro* Gram-negative inhibitory concentration ($\mu\text{g/ml}$) (MIC)⁹⁾ vs. $\Delta\delta(4-3)$ values (see text).



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