Tetrahedron Letters, Vol.22, No.29, pp 2793 - 2796, 1981
Printed in Great Britain

0040-4039/81/292793-04$02.00/0

^{13}C NMR SPECTRA OF CEPHALOSPORINS.[1] SIGNAL ASSIGNMENTS OF FREE ACIDS AND ESTERS

Kazuo Tori,* Junko Nishikawa, and Yoshito Takeuchi†

Shionogi Research Laboratories, Shionogi & Co., Ltd., Fukushima-ku, Osaka, 553 Japan.
†Department of Chemistry, College of General Education, The University of Tokyo, Komaba, Meguro-ku, Tokyo, 153 Japan

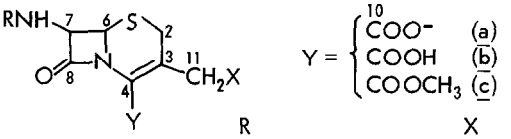
Abstract. ^{13}C NMR signals were assigned for several cephalosporin free acids and esters as well as cephalosporinate ions using T_1 measurements, selective NOE, and pD-dependent chemical shifts to investigate the structure-reactivity relationship.

Much interest has been shown in the chemistry of cephalosporins in relation to their useful biological activities in recent years.[2] ^{13}C and ^{15}N chemical shifts have been regarded as important indices in investigating the structure-activity relationship, and NMR signals were assigned for many cephalosporins, in particular, for cephalosporin sodium salts (a).[2-6] The polarization of the C-8 carbonyl group has frequently been discussed[3] because the chemical reactivity of the β-lactam ring at the C-8–N-5 bond[7] was shown to be closely correlated with the antibiotic activity.[2,3] However, the chemical shifts (δ) of C-8 and N-5 have been reported to be limited within a relatively narrow range.[3-6]

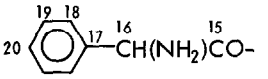
Recently, Paschal et al.[4] suggested the importance of the C-3=C-4 double bond polarization to the activity in cephalosporinate ions (a), observing that the chemical shift differences between C-3 and C-4 [Δδ(4-3)] are large for cephalothin (5a) and cephaloridine (6a) which have been great commercial successes. The discrimination of the C-3 and C-4 signals had been a controversial problem,[5,8,9] which was solved by measuring the dipole-dipole relaxation times (T_1) in 5a in D_2O: δ (T_1) values are 118.8 (1.79 s) and 133.9 (5.13 s) for C-3 and C-4, respectively.[5] We have also confirmed this result for cefazolin sodium salt (3a) and 6a.[10] Therefore, complete ^1H-decoupled ^{13}C spectra of cephalosporinate ions generally display the C-3 signal more intense than the C-4 signal owing to NOE differences arising from the C-2 and C-11 protons;[11,12] this fact is very useful for distinguishing between these two signals.

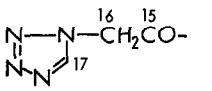
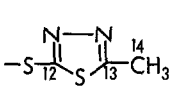
On the other hand, little attention has been paid to the ^{13}C spectra of cephalosporin free acids (b) and esters (c), where the C-3 and C-4 signals have been assigned in analogy to sodium salts (a).[13,14] However, during our studies of NMR spectra of cephalosporins, these signal assignments for (b) and (c) were found to be the reverse of those for (a) in view of their signal intensities. We thus report here the unambiguous signal assignments of 7-aminodeacetoxy- (1) and 7-aminocephalosporanic acids (4), cephalexin (2), 3, 5, and 6 in the three states (a), (b), and (c), and discuss the Δδ(4-3) values in relation to the reactivity of the β-lactam ring.

Most ^{13}C signals of the compound examined were easily assigned by using ^1H single-frequency and noise off-resonance decouplings, ^1H non-decoupling with NOE in the gated mode,[5] and comparison of the chemical shifts with those of related compounds[3-6,8,9] (see the TABLE). However, some ^{13}C signal assignments were not straightforward, particularly for C-3 and C-4 in (b) and (c). For example, the C-3 signal (δ 122.8) is more intense than the C-4 signal (127.4) for

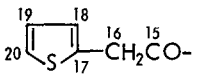


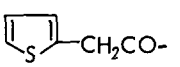
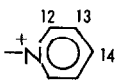
7-Aminodeacetoxy-cephalosporanic acid (1) — R: H; X: H

Cephalexin (2) — R:  ; X: H

Cefazolin (3) — R:  ; X: 

7-Aminocephalosporanic acid (4) — R: H; X: OCOCH3

Cephalothin (5) — R:  ; X: OCOCH3

Cephaloridine (6) — R:  ; X: 

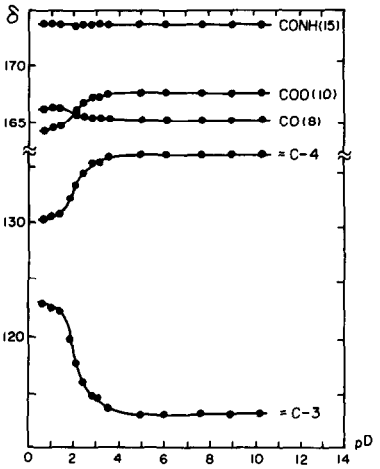


Fig. 1. pD Dependence of chemical shifts in cephaloridine (6).

2a in D_2O, but comparison of the C-3 with the C-4 signal intensity for 2c in $CDCl_3$ shows that the signal at the lower field (δ 131.6) is more intense than that at the higher field (122.5).

Thus, we first measured the T_1 values and NOE factors for 2c and 4c by the usual inversion-recovery method and the gated-decoupling method, respectively.[11] As expected, the lower-field signals between C-3 and C-4 had shorter T_1 values and larger NOE factors, and hence were assigned to C-3 (see the TABLE). Next, we attempted to follow the pD dependence of ^{13}C chemical shifts of the cephalosporins in D_2O to confirm that some signals, particularly the C-3 and the C-4 signal, mutually exchange their positions on going from an alkaline to an acidic solution, but 1-5 were only soluble in D_2O in limited pD ranges. Therefore, we measured the pD dependence of the spectra of a betaine type of 6. Figure 1 shows plots of δ against pD for five carbons. As the pD value[15] decreases to less than about 2, the C-3 and C-4 signals rapidly approach each other. The strong pD dependence of these signals for 6 reasonably suggests that these signal positions may be reversed from state (a) to (b) of the usual cephalosporins.

Confirmatory evidence for the assignments of the C-3 and C-4 signals in (b) was provided by the selective NOE measurement[16] for 1b in D_2O-DCl; we observed the NOE enhancements of the C-3 (δ 144.0) and C-4 (122.6) signals by irradiating the CH_3 protons at C-3 selectively with a weak coherent-wave rf field[16] using the gated-decoupling methods. Obviously, the C-3 signal was enhanced by 1.2, while the C-4 signal was not.

The data obtained are listed in the TABLE; the C-3 and C-4 signals for the other cases were assigned from the signal intensities. Discrimination of the C-8, C-10, and C-15 signals was frequently possible also from the signal intensities (see their T_1 values).

As shown in the TABLE also, the Δδ(4-3) values for (b) and (c) are negative in almost all cases, whereas those for (a) are positive. However, the values apparently increase algebraically on going from 1 to 6 in each type, though small solvent effects or pD effects were seen. The difference in Δδ(4-3) between acid-form (b) and ester-form (c) was seen for 2.[17]

Good linear relationships were found between the logarithms of the rate constants k_{OH} re-

TABLE. ^{13}C Chemical Shifts (δ), T_1 (s), and NOE Factors[a]

Carbon No.	1a[b] D_2O[d] δ[h]	1b[b] D_2O[e] δ	1c $CDCl_3$ δ	1c DMSO δ	2a[b] D_2O[d] δ[h]	2b D_2O δ	2b D_2O[e] δ	2c $CDCl_3$ δ	2c DMSO δ	2c CD_2Cl_2 δ	2c CD_2Cl_2 T_1 (NOE)	3a D_2O[g] δ[h]	3a DMSO[g] δ	3b[c] DMSO δ
C-2	28.9	31.3	29.9	28.4	29.1	29.0	30.8	30.1	29.0	30.3	0.27(1.47)	27.7	26.6	27.0
3	122.7	144.0	130.6	129.4	122.8	122.7	138.9	131.6	131.0	132.0	6.7 (1.19)	119.8	115.3	125.9
4	127.4	122.6	122.7	121.6	127.4	127.1	122.2	122.5	121.6	122.7	12.7 (0.96)	131.9	133.8	125.9
6	58.6	55.5	58.7	58.5	57.8	57.7	58.3	57.3	57.2	57.6	0.34(1.21)	58.1	57.0	57.2
7	62.5	58.5	63.6	63.4	59.3	59.3	59.5	59.0	58.6	59.2	0.38(1.47)	59.8	58.4	58.9
8	170.0	161.7	168.7	169.5	164.8	164.3	165.5	164.6	164.4	165.0	9.0 (0.98)	165.1	163.0	163.9
10	171.1	165.6	163.0	162.7	170.7	170.7	165.8	162.6	162.4	163.0	11.2 (0.93)	168.4	164.7	162.7
11	19.4	20.5	20.0	19.2	19.3	19.2	20.5	19.9	19.3	20.0	0.94(1.23)	38.8	36.5	35.8
12	---	---	---	---	---	---	---	---	---	---	---	167.0	165.5	163.9
13	---	---	---	---	---	---	---	---	---	---	---	170.9	165.9	166.2
14	---	---	---	---	---	---	---	---	---	---	---	15.7	15.1	15.2
15	---	---	---	---	175.3	170.2	169.9	173.7	174.1	174.0	5.3 (0.94)	168.1	165.7	165.6
16	---	---	---	---	58.6	57.4	57.4	59.7	58.2	59.8	0.41(1.58)	50.5	49.1	49.0
17[j]	---	---	---	---	138.2	132.4	132.3	140.4	142.0	141.0	6.6 (1.05)	146.1	145.1	145.1
18[j]	---	---	---	---	128.1	129.1	129.1	127.0	126.6	127.4	0.71(1.31)	---	---	---
19[j]	---	---	---	---	130.0	130.5	130.5	128.9	127.9	129.1	0.65(1.26)	---	---	---
20	---	---	---	---	130.0	131.4	131.4	128.1	126.9	128.3	0.50(1.33)	---	---	---
OCH_3	---	---	52.3	51.8	---	---	---	52.3	51.9	52.5	0.90	---	---	---
Δδ(4-3)	4.7	-21.4	-7.9	-7.8	4.6	4.4	-16.7	-9.1	-9.4	-9.3	---	12.1	17.5	0.0

Carbon No.	3c[f] DMSO δ	4a[b] D_2O[d] δ[h]	4b[c] DMSO δ	5a D_2O[g] δ[h]	5a DMSO[g] δ	5b[c] DMSO δ	5c $CDCl_3$ δ	5c DMSO δ	5c $CDCl_3$-CD_2Cl_2(1:1) δ	5c $CDCl_3$-CD_2Cl_2(1:1) T_1 (NOE)	6a D_2O δ[h]	6a DMSO δ	6b D_2O[e] δ
C-2	27.1	26.0	25.0	26.4	25.1	25.5	26.4	25.6	26.7	0.40(2.01)	25.9	24.3	26.8
3	126.7	116.8	122.3	117.3	113.7	123.4	125.7	124.7	126.0	9.3 (1.83)	113.0	108.8	122.8
4	124.7	132.4	126.6	132.4	134.0	126.3	125.5	124.9	125.6	21.2 (1.16)	136.1	138.1	130.3
6	57.3	59.0	58.8	58.2	57.2	57.3	57.4	57.4	57.7	0.69(2.09)	58.3	57.3	58.4
7	59.0	63.0[i]	63.5	60.0	58.5	59.0	59.3	59.2	59.7	0.52(2.04)	60.4	59.0	60.4
8	164.2	170.3[i]	169.8	165.5	163.4	164.6	164.6	164.9	165.0	15.8 (1.35)	165.2	162.9	166.3
10	161.8	169.6[i]	163.1	169.0	164.6	162.7	161.8	161.8	162.2	22.9 (1.30)	167.7	162.9	164.3
11	35.5	65.1	62.8	65.0	64.2	62.6	63.0	62.4	63.2	0.67(2.11)	62.6	61.4	61.8
12	163.5	174.9	170.1	174.8	170.5	170.1	170.5	170.1	170.7	23.7 (1.48)	145.1	145.0	145.1
13	166.2	21.2	20.5	21.2	20.6	20.4	20.7	20.4	20.9	2.26(1.67)	129.2	127.9	129.4
14	15.2	---	---	---	---	---	---	---	---	---	147.1	149.3	147.5
15	165.5	---	---	174.3	169.9	169.9	170.1	169.9	170.4	10.3 (1.74)	173.9	169.8	173.8
16	49.0	---	---	37.0	35.7	35.7	37.1	35.7	37.2	0.88(2.03)	36.8	35.6	36.9
17[j]	145.0	---	---	136.6	136.9	136.8	134.9	136.7	135.6	18.8 (1.22)	136.7	136.7	136.7
18[j]	---	---	---	128.3	126.5	126.5	127.8	126.5	127.8	2.24(1.99)	128.2	126.5	128.3
19[j]	---	---	---	128.3	126.2	126.3	127.5	126.2	127.5	2.13(1.91)	128.2	126.1	128.3
20	---	---	---	126.5	124.8	124.9	125.9	124.9	125.9	2.14(1.86)	126.5	124.7	126.6
OCH_3	52.5	---	---	---	---	---	52.9	52.6	53.1	1.78(1.53)	---	---	---
Δδ(4-3)	-2.0	15.6	4.3	15.1	20.3	2.9	-0.2	0.2	-0.4	---	23.1	29.3	7.5

a: ^{13}C FT NMR spectra were recorded on a Varian NV-14 (at 15.087 MHz) and/or a JEOL FX-90Q (at 22.50 MHz) NMR spectrometer at ca. 30°C in 8- and/or 10-mm spinning tubes, respectively, in organic solvents with internal TMS reference (δ 0) and in D_2O with internal dioxane reference (δ 67.4). Accuracies of δ, T_1, and NOE factor are about ±0.1 ppm, ±10%, and ±10%, respectively. b: Not soluble in D_2O at pD 3-6.5 and DMSO. c: Not soluble or decomposed in D_2O at pD<3. d: Dissolved by adding an equimolar amount of $NaHCO_3$ to the free acids (pD 7.5-8.5). e: Dissolved by adding conc. DCl (pD<1). f: Not soluble in $CDCl_3$. g: Sodium salts were dissolved in D_2O or DMSO. h: These δ valus agree well with those reported.[3-6,9] i: Assignments may be reversed in each column. j: Assignments given here were based on the lanthanide-induced shifts ($\Delta\delta_{18}>\Delta\delta_{19}$) in Yb(fod)$_3$-assisted spectra of 2c and 5c in $CDCl_3$ and the assumption that C-18 has a longer T_1.[11]

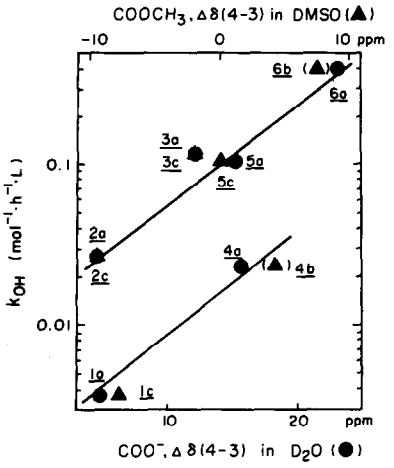


Fig. 2. Relationships of k_{OH} reported and Δδ(4-3) indices.

ported by Yamana and Tsuji[7] for the OH^--catalyzed degradation of cephalosporins 1-6 and the Δδ(4-3) values of (a) for the R = H and R = R_1R_2CHCO series (see Fig. 2). This is also the case with acids (b) and esters (c). Thus, the Δδ(4-3) index should have a considerable significance in predicting the reactivity even at an intermediate ester stage during cephalosporin synthesis, at least for usual C-11-substituted cephalosporins. Recently, Boyd et al.[18] reported a parabolic relationship between antibacterial activity expressed in terms of minimum inhibitory concentration measured for 7(2-thienylacetyl)cephalosporins against five Gram-negative pathogenic microbes and the theoretical index of reactivity called the transition state energy (TSE) calculated for a model nucleophile, i.e., OH^- and a 3-cephem model structure with a substituent X at C-3. We also successfully attempted to correlate Δδ(4-3) with -TSE almost linearly for each state (a), (b), and (c). This correlation might be extended to that with biological activities in limited cases, but not for general cases, as pointed out frequently.[4,18]

Incidentally, a change in the C-7 amide group affected Δδ(4-3) only slightly[3-6] except for a phthalimido group [Δδ(4-3) = -21.9 ppm],[19] which might largely interact with the double bond. A change in the C-4 ester group affects Δδ(4-3) slightly. Therefore, the ester group should be fixed when this index is used. Detailed substituent effects will be reported elsewhere.

REFERENCES

(1) NMR Studies of penicillins and cephalosporins. II. For Part I, see K.Tori, T.Tsushima, Y.Tamura, H.Shigemoto, T.Tsuji, H.Ishitobi, and H.Tanida, Tetrahedron Lett. 3307 (1975).
(2) E.H.Flynn, "Cephalosporins and Penicillins: Chemitry and Biology," Academic Press (1972); F.A.Jung, W.R.Pilgrim, J.P.Poyser, and P.J.Siret, Top. Antibiotic Chem. 4, 1 (1980).
(3) R.Mondelli and P.Ventura, J.C.S. Perkin II 1749 (1977), and references therein.
(4) J.W.Paschal, D.E.Dorman, P.R.Srinivasan, and R.L.Lichter, J. Org. Chem. 43, 2013 (1978).
(5) J.-M.Dereppe, A.Schanck, B.Coene, C.Moreau, and M.Van Meerssche, Org. Magn. Resonance 11, 638 (1978).
(6) A.Schanck, B.Coene, M.Van Meerssche, and J.-M.Dereppe, Ibid. 12, 337 (1979).
(7) T.Yamana and A.Tsuji, J. Pharm. Sci. 65, 1563 (1976).
(8) N.Neuss, C.H.Nash, P.A.Lemke, and J.B.Grutzner, J. Am. Chem. Soc. 93, 2337 (1971).
(9) Idem., Proc. Roy. Soc. (London) B179, 335 (1971).
(10) Y.Takeuchi, the 43th Annual Meeting of the Chem. Soc. Jpn., Abstracts, II-1031 (1981).
(11) F.W.Wehrli, Top. C-13 NMR Spectrosc. 2, 343 (1976), and references therein.
(12) Y.Terui, K.Tori, S.Maeda, Y.K.Sawa, Tetrahedron Lett. 2853 (1975).
(13) S.Kukolja, N.D.Jones, M.O.Chaney, T.K.Elzey, M.R.Gleissner, J.W.Paschal, and D.E.Dorman, J. Org. Chem. 40, 2388 (1975).
(14) E.M.Gordon, H.W.Chang, C.M.Cimarusti, B.Toeplitz, and J.Z.Gougoutas, J. Am. Chem. Soc. 102, 1690 (1980).
(15) R.G.Bate, "Determination of pH," 2nd. ed., p. 375, Wiley & Sons (1973).
(16) J.Uzawa and S.Takeuchi, Org. Magn. Resonance 11, 502 (1978).
(17) However, the Δδ(4-3) value for 1b in D_2O-DCl is larger (-21.4 ppm) owing to NH_3^+ at C-7, which affects all δ for the skeletal carbons. The Δδ(4-3) value of -6.4 ppm for 4b in D_2O-DCl was observed, although 4b decomposes rapidly under this condition.
(18) D.B.Boyd, D.K.Herron, W.H.W.Lunn, and W.A.Spitzer, J. Am. Chem. Soc. 102, 1812 (1980).
(19) The assignment originally reported[13] was reversed (also, the C-3 and C-4 signals in a cephalosporin where R = $PhOCH_2CO$, X = H, and Y = $CO_2CH_2C_6H_4$-p-NO_2, were misassigned).[13]

(Received in Japan 17 April 1981)