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Notes

Application of ^{15}N Nuclear Magnetic Resonance Spectroscopy to the Determination of the Stability of Aryl Nitrogen Mustards

Derry E. V. Wilman,*,[†] Brian D. Palmer,[‡] and William A. Denny[‡]

Cancer Research Campaign Centre for Cancer Therapeutics, Institute of Cancer Research, Cotswold Road, Sutton, Surrey SM2 5NG, U.K., and Cancer Research Laboratory, School of Medicine, University of Auckland, Private Bag 92019, Auckland, New Zealand

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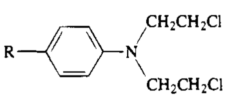
An excellent correlation has been shown to exist between the ^{15}N NMR chemical shifts of a series of aryl nitrogen mustards and the Hammett constant, σ, which is much improved by the use of σ^-. These chemical shifts also correlate well with the hydrolysis rates of the compounds in 50% aqueous acetone at 66 °C and their alkylation of 4-(4′-nitrobenzyl)pyridine under similar conditions. Thus ^{15}N NMR is a straightforward and material-conserving method for estimating the relative stabilities of aryl nitrogen mustards.

The antibody-directed enzyme prodrug therapy (ADEPT) approach to cancer chemotherapy[1] has led to a renewed interest in derivatives of bis(2-chloroethyl)amine (nornitrogen mustard). In particular, aryl nitrogen mustards **1**, derivatives of aniline mustard (*N,N*-bis(2-chloroethyl)aniline), have been considered as both prodrugs and active metabolites.[2] In deciding on likely prodrug–metabolite combinations, it is very important to be able to determine the stability/reactivity differential between them. The stability (reactivity) of nitrogen mustards has traditionally been determined titrimetrically, as the half-life for aqueous hydrolysis under physiological conditions of temperature and pH,[3–5] or by their reaction with 4-(4′-nitrobenzyl)pyridine (NBP).[4,5] However, this approach has inherent difficulties for compounds with either very long or very short half-lives and those of high lipophilicity.



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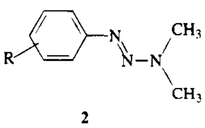

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The chemical stability of the aryl nitrogen mustards is directly related to electron distribution in the molecule as a whole, as determined by the Hammett substituent constant, σ, for substituents in the aryl ring.[6] This electron distribution is responsible for other effects also, including NMR chemical shifts. For instance, in the case of the 3,3-dimethyl-1-(4-substituted phenyl)triazenes **2**, there is a strong correlation between σ, for the 4-substituent, and the chemical shifts in the ^{15}N NMR spectrum[7] (eqs 1–3).

$$\delta_{N1} = -14.97(\pm 0.72)\sigma_p - 26.32 \tag{1}$$

$$n = 12, r = -0.99, s = 0.80$$

$$\delta_{N2} = 5.52(\pm 0.43)\sigma_p + 68.60 \tag{2}$$

$$n = 12, r = 0.97, s = 0.48$$

$$\delta_{N3} = 14.69(\pm 0.99)\sigma_p - 224.37 \tag{3}$$

$$n = 12, r = 0.98, s = 1.09$$

It was therefore reasonable to expect that such a correlation might exist for aryl nitrogen mustards, between the ^{15}N chemical shift of the mustard nitrogen and σ for substituents in the aryl ring, and that this might further correlate with the stability of the compounds. The ^{15}N NMR spectra were therefore determined for a series of 30 *N,N*-bis(2-chloroethyl)anilines, and the existence of any correlation between the chemical shift and σ was investigated. The existence of a correlation between the chemical shift and the stability of the compounds was investigated for the previously determined hydrolysis rates of a subset of these compounds in 50% aqueous acetone,[8] together with their rate of reaction with 4-(4′-nitrobenzyl)pyridine.[8]

Results and Discussion

Table 1 contains the ^{15}N NMR chemical shift data for the series of 30 *N,N*-bis(2-chloroethyl)anilines investigated, together with their σ and σ^- values, where they are known.[9] Equation 4 describes the correlation between the chemical shifts (δ) and σ and equation 5 that with σ^-.

$$\delta = 19.09(\pm 1.91)\sigma - 315.68 \tag{4}$$

$$n = 30, r = 0.88, s = 3.58$$

[†] Institute of Cancer Research.

[‡] University of Auckland.

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Table 1. ^{15}N Chemical Shifts and Correlation Coefficients[a] for a Series of *N,N*-Bis(2-chloroethyl)arylamines

	R	δ_N	σ	σ^-
1	4-NO	−289.02	0.91	1.60
2	4-NO_2	−296.76	0.78	1.24
3	4-CN	−303.20	0.66	1.00
4	4-N=NPh	−304.08	0.39	0.69
5	4-SO_2CH_3	−304.17	0.72	0.98
6	4-N=NPh, 3-CH_3	−304.75	0.32	0.62
7	4-COOH	−305.81	0.45	0.64
8	4-CF_3	−308.13	0.54	0.65
9	3-NO_2	−309.95	0.71	
10	4-CON$(CH_3)_2$	−310.99	0.36	0.63
11	4-CH=NOH	−311.07	0.10	
12	4-$CH_2CH_2NH_2$	−311.61	−0.06	
13	3-COOH	−313.99	0.37	0.37
14	3-SCH_3	−313.99	0.15	0.19
15	3-CON$(CH_3)_2$	−314.10	0.28	
16	4-Cl	−314.47	0.23	0.27
17	3-OCH_3	−314.76	0.12	0.12
18	4-SCH_3	−315.34	0.00	0.06
19	H	−315.45	0.00	0.00
20	3-CH_3	−315.98	−0.07	−0.07
21	4-CH_2COOH	−316.69	−0.07	
22	4-CH_2CH_2COOH	−317.08	−0.07	
23	4-$CH_2CH_2CH_2COOH$	−317.37	−0.07	
24	4-CH_3	−318.03	−0.17	−0.15
25	4-$NHOCCH_3$	−318.12	0.00	
26	4-F	−318.51	0.06	0.05
27	4-OH	−318.52	−0.37	−0.16
28	4-OCH_2CH_2COOH	−321.31	−0.33	−0.16
29	4-$OCH_2C_6H_5$	−321.51	−0.23	
30	4-OCH_3	−321.77	−0.27	−0.16

[a] Values of σ and σ^- are taken from ref 9.

$$\delta = 16.05(\pm 0.91)\sigma^- - 317.33 \qquad (5)$$

$$n = 21, r = 0.97, s = 2.06$$

There is a fair correlation between the ^{15}N chemical shift and σ, which is considerably enhanced when σ^- is used, albeit that it is a smaller data set. This is not unexpected, as the values of σ^- used are derived from aniline-based systems, rather than the benzoic acid systems which is generally the case for σ.

The reactivity of a selection of these compounds was determined by two methods, both of which involved a 50% aqueous acetone solvent system at 66 °C,[8] after the method of Ross.[10] One involved measurement of the hydrolysis products by HPLC, i.e., disappearance of the compound, and the other the direct alkylation of 4-(4′-nitrobenzyl)pyridine followed by spectrophotometric determination of the resulting blue product,[8] and these results are displayed in Table 2.

Comparison of these data with that of Table 1 led to the relationships of eqs 6−10. Throughout the data there is a spread of values without clustering at either end, even in the small sets, which gives some validity to the correlations.

$$\delta_N = 16.78(\pm 1.56)\sigma - 316.52 \qquad (6)$$

$$n = 6, r = 0.98, s = 1.29$$

$$\log k_W = -2.16(\pm 0.48)\sigma - 3.51 \qquad (7)$$

$$n = 6, r = 0.91, s = 0.40$$

$$\log k_{NBP} = -3.11(\pm 0.40)\sigma - 2.90 \qquad (8)$$

$$n = 6, r = 0.97, s = 0.33$$

Table 2. Kinetic Data Obtained for a Series of 4-(Substituted aryl) Nitrogen Mustards[a]

	4-substituent	k_W (min^{-1})[b]	log k_W	k_{NBP} (min^{-1})[c]	log k_{NBP}
30	CH_3O	4.09×10^{-3}	−2.388	9.88×10^{-3}	−2.005
24	CH_3	4.71×10^{-4}	−3.327	1.11×10^{-2}	−1.955
18	CH_3S	1.24×10^{-4}	−3.907	9.97×10^{-4}	−3.001
25	CH_3CONH	3.08×10^{-4}	−3.511	4.22×10^{-4}	−3.375
10	$(CH_3)_2NCO$	2.85×10^{-5}	−4.545	9.76×10^{-5}	−4.011
5	CH_3SO_2	1.64×10^{-5}	−4.785	9.58×10^{-6}	−5.019

[a] Data from ref 8. [b] Pseudo-first-order rate constant for hydrolysis in 50% aqueous acetone at 66 °C. [c] Pseudo-first-order rate constant for alkylation of NBP in 50% aqueous acetone at 66 °C with 50 μM drug and 25 mM NBP.

$$\log k_W = -0.13(\pm 0.03)\delta_N - 44.48 \qquad (9)$$

$$n = 6, r = 0.93, s = 0.36$$

$$\log k_{NBP} = -0.17(\pm 0.04)\delta_N - 57.79 \qquad (10)$$

$$n = 6, r = 0.92, s = 0.52$$

While there is a reasonable correlation (r = 0.98) between the ^{15}N chemical shift (δ_N) and the Hammett constant (σ) for this series (eq 6), on this occasion it is not improved by the use of σ^- (r = 0.97) which, while at variance with the results of Panthananickal and coworkers,[6] is in agreement with Hansch's reappraisel of Ross' data.[11] The latter has shown a similar lack of variation between the use of σ or σ^- in the analysis of data obtained from aryl nitrogen mustards. However, σ^- values are not available for the CH_3CONH and $(CH_3)_2NC$ substituents, leaving too small a data set for reliability.

The relationships between σ and the kinetic parameters and σ and δ_N are marginally less good (eqs 7−10). However, the correlation between the δ_N and the rate constant k_W for the aqueous hydrolysis reaction (eq 9) is adequate to demonstrate the utility of this approach.

The correlation observed between the hydrolysis rate and the ^{15}N NMR chemical shifts of substituted aryl nitrogen mustards confirms that this is a suitable and straightforward technique for the determination of their stability. The more sensitive instrumentation currently available means that this may be achieved with substantially less material than was possible in this study. In addition, as NMR is a nondestructive technique, the compound may always be recovered.

Experimental Section

Aryl Nitrogen Mustards. The nitrogen mustards used were in the main available from earlier work, the remainder were prepared as previously described.[10,12]

NMR. Natural abundance ^{15}N NMR spectra were obtained at 25.35 MHz on a Bruker AC250 spectrometer using a 10 mm multinuclear probe. Samples consisted of 1 mmol of compound dissolved in 1.8 mL of DMSO containing 30% (w/w) DMSO-d_6, for the lock, and 20 mg of chromium acetylacetonate, to reduce the relaxation time. Spectra were measured at 305 K with neat nitromethane as external standard. The microprogram INVGATE.AU (standard Bruker software) was used to obtain nuclear Overhauser effect (NOE)-suppressed proton-decoupled ^{15}N spectra by inverse-gated heteronuclear decoupling.

The parameters used were relaxation delay, 2 s; pulse width, 45°; decoupler power, 18 H. The spectral width was 20 000 Hz, which together with 16 K data points gave an acqusition time of 0.41 s and a digital resolution of 2.44 Hz/point. A total of 15 000 transients/spectrum required 10 h to obtain the fid, which was exponentially multiplied (line broadening, 5 Hz) prior to Fourier transformation.

Kinetic Studies. Reaction rate constants for the hydrolysis of a selection of the nitrogen mustards in 50% acetone:water at 66 °C and for alkylation of 4-(4′-nitrobenzyl)pyridine by them, under the same conditions, have been reported previously.[8] These data are displayed in Table 2 for the compounds in question.

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