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ORIGINAL RESEARCH

Specificity of ^{15}N NMR chemical shifts to the nature of substituents and tautomerism in substituted pyridine *N*-oxides

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Abstract ^{1}H, ^{13}C, and ^{15}N NMR chemical shifts have been measured for 2-aminopyridine *N*-oxide (**1**), its eleven derivatives (**2–10**, **13**, **14**), and 3-Cl and 3-Br substituted 4-nitropyridine *N*-oxides (**11**, **12**). $\delta(^{15}N)$ of pyridine ring nitrogen in 2-acetylaminopyridine *N*-oxides are 5.9–11.5 ppm deshielded from those in 2-aminopyridine *N*-oxides. When amino and acetylamino substituents are in 4-position, $\delta(^{15}N)$ of ring nitrogen is 21.3 ppm deshielded in the acetylated derivative. The strong resonance interaction between 2-amino and 5-nitro groups reflects in the decrease of amino nitrogen shielding about 5.3–17.9 ppm. Also, ^{1}H and ^{13}C NMR spectral data are in agreement with ^{15}N NMR results reflected as deshielded amino protons and carbons C-2 and C-5. The pyridine nitrogen chemical shift in all amino- and acetylamino derivatives vary between −101.2 and −126.7 ppm, which has been connected with the tautomeric balance in our earlier studies.

Keywords ^{1}H · ^{13}C · ^{15}N · NMR · 2-Aminopyridine *N*-oxide · 2-Amino-5-nitropyridine *N*-oxide · 2-Acetylaminopyridine *N*-oxide · 4-Aminopyridine *N*-oxide · 4-Acetylaminopyridine *N*-oxide

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Introduction

^{15}N isotope is the most sensitive NMR nucleus to the effect of a substituent introduced to pyridine *N*-oxide (2- and 4-amino, 2- and 4-acetylamino, methyl group in different positions together with 2-amino and/or 2-acetylamino, 5-nitro with 2-amino, 4-nitro together with 3-chloro and 3-bromo e.g.). Pyridine *N*-oxides possess an N–O-moiety, a dual resonance functionality, that can act as both a π-electron donor and a π-electron acceptor [1]. Acceptors in the ring decrease and donors increase the shielding of ^{15}N. A linear relationship has been observed between the substituent chemical shifts of ^{15}N and ^{13}C for the related substituted benzenes.

Only few research reports on ^{15}N and ^{14}N NMR studies of aminopyridine *N*-oxides are found in the literature [2–5]. In this paper, we present our studies regarding the possible tautomerism in amino and acetylaminopyridine *N*-oxides because this problem has not yet been unambiguously solved. Another interest lies in the 5-nitro-substituted compounds (**10**, **13**, **14**), where the electron lone pair of amino moiety is involved in the π-electron conjugation with the aromatic ring the nitro group acting as an electron acceptor.

Recently, 2-amino-5-nitropyridine derivatives have been shown to be very interesting owing to their promising non-linear optical properties as these molecules possess high hyperpolarizability and highly delocalised π-electron system for reason that acceptor and donor group are situated in *para*-position to each other [6].

Experimental

2-Aminopyridine *N*-oxide and its 3- and 5-methyl derivatives were obtained by protecting the primary amino group

by acetylation during the oxidation of ring nitrogen. Otherwise, oxidation would have transformed aminopyridines into nitropyridines [7–15]. In hydrolysis, acetylamino derivatives of pyridine *N*-oxide gave the corresponding amino compounds [7]. 2-Amino- and 2-acetylaminopyridine *N*-oxides as well as their 3-, 4-, 5-, and 6-methyl derivatives are reported in literature [8, 9], but their synthesis were greatly improved [14] compared to the earlier reported methods [8–13].

Synthesis

The modified synthesis of 2-acetylaminopyridine *N*-oxides (**2**, **4**) and 4-, 5-, and 6-methyl-(2-acetylaminopyridine-*N*-oxides) (**7**, **8**, **9**) [8–10, 13] has been presented previously [14]. By modification of methods by Brown and Adam et al. [9, 10], a remarkable shortening in reaction time (9 to 2 h) was achieved by substituting acetic acid by acetic anhydride in the reaction.

The syntheses of 2-amino- (**1**), 4-amino- (**3**), 2-amino-3-methyl- (**5**), 2-amino-5-methylpyridine *N*-oxide (**6**) have also been reported earlier [16]. These compounds were obtained by hydrolysis of the corresponding acetylaminopyridine *N*-oxides [13]. By Herz's hydrolysis method [13] with 50 % H_2SO_4 instead of 10 % NaOH decreases, the reaction time varied from 5 to 1 h [9, 10].

The syntheses of 2-amino-5-nitro- (**10**), 2-amino-5-nitro-3-methyl- (**13**) and 2-amino-5-nitro-6-methylpyridine *N*-oxides (**14**) have been presented previously [16, 17]. These compounds were obtained in rearrangement reaction of the corresponding nitraminopyridine *N*-oxides [16, 17].

3-Chloro- (**11**) and 3-bromo-4-nitropyridine *N*-oxides (**12**) were prepared by oxidation of 3-chloro- and 3-bromopyridine by 30 % H_2O_2 in the presence of acetic anhydride followed by nitration of the crude products after the excess acid was removed [18]. The modification of this synthesis in comparison with earlier applied methods [7] consists an improvement of *N*-oxidation (using acetic anhydride instead of acetic acid) and separation of final product from reaction mixture (using 25 % NH_4OH and NH_4HCO_3 instead of NaOH) giving the pure product due to low temperature during the neutralization process.

NMR spectroscopy

The ^{1}H, ^{13}C and PFG [19] ^{1}H, ^{13}C HMQC [20, 21], and PFG ^{1}H,*X* (*X* = ^{13}C or ^{15}N) HMBC [22] spectra were recorded for 0.5 M DMSO-d_6 solution in a 5-mm sample tube at 30 °C on a Bruker Avance DRX 500 spectrometer working at 500.13 MHz (proton), 125.77 MHz (carbon-13) and 50.70 MHz (nitrogen-15), respectively.

In ^{1}H NMR experiments, the number of data points was 64 K giving a spectral resolution of 0.05 Hz, the number of scans was 8 and the flip angle 30°. An exponential window function of the spectral resolution was used before FT. The ^{1}H NMR chemical shifts are referenced to the signal of residual DMSO-d_5 (δ = 2.50 ppm from TMS).

In ^{13}C experiments, the number of data points was 32 K giving a spectral resolution of 0.5 Hz, the number of scans was 64, and flip angle 30°. A composite pulse decoupling, Waltz-16, was used to remove proton couplings. An exponential window function of the spectral resolution was used before FT. The ^{13}C NMR chemical shifts are referenced to the center peak of the solvent DMSO-d_6 (δ = 39.50 ppm from TMS). The number of data points in PFG ^{1}H,^{13}C HMQC, and HMBC measurements were 1,024 (f_2) × 256 (f_1). This matrix was zero filled to 2,048 × 1,024 and apodized by a shifted sine bell window function along both axes before FT.

In PFG ^{1}H, ^{15}N HMBC experiments, the digital resolution was <0.5 ppm and a 50-ms delay was used for the evolution of long-range couplings. The number of data points were 1,024 (f_2) × 512/450 ppm (f_1 = ^{15}N). This matrix was zero filled to 1,024 × 1,024 and apodized by a shifted sine bell window function along both axes before FT. ^{15}N NMR chemical shifts are referenced to the signal of an external CH_3NO_2 (δ = 0.0 ppm).

Results and discussion

The structures of compounds **1–14** are presented in Fig. 1. The ^{1}H, ^{13}C, and ^{15}N NMR chemical shifts are collected in Tables 1, 2, and 3.

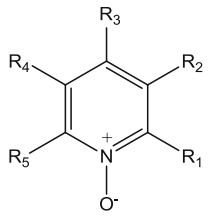
Regarding ^{15}N NMR shifts, we can use our previous papers for comparison [6, 23, 24].

^{1}H NMR shifts

The ^{1}H NMR data reveal the electron acceptor ability of the acetyl group via δ(H-3) (*ortho* to acetylamino). In **2**, δ(H-3) is 1.49 ppm and in **7–9** 1.34–1.38 ppm deshielded in comparison with **1** showing the strong electron withdrawing property of acetylamino group. In 4-acetylamino substituted derivative, δ(H-3/5) are just 1.08 ppm deshielded from that of 4-amino derivative (**3**). In 5-nitro derivatives, the greatest deshieldings, 0.89 and 0.88 ppm for H-6 and H-4, respectively (**10**), 0.82 ppm for both protons (**13**) and 0.79 ppm for H-4 (**14**), are observed. Joint with these effects, one can observe deshieldings of amino protons by 1.31 ppm (**10**), 1.20 ppm (**13**), and 1.16 ppm (**14**) which refer to resonance interaction between amino and nitro group.

^{13}C NMR shifts

The assignments of ^{13}C NMR chemical shifts (Table 2) are based on literature data and substituent induced chemical



Compound	R_1	R_2	R_3	R_4	R_5
1.	NH_2	H	H	H	H
2.	$NHCOCH_3$	H	H	H	H
3.	H	H	NH_2	H	H
4.	H	H	$NHCOCH_3$	H	H
5.	NH_2	CH_3	H	H	H
6.	NH_2	H	H	CH_3	H
7.	$NHCOCH_3$	H	CH_3	H	H
8.	$NHCOCH_3$	H	H	CH_3	H
9.	$NHCOCH_3$	H	H	H	CH_3
10.	NH_2	H	H	NO_2	H
11.	H	Cl	NO_2	H	H
12.	H	Br	NO_2	H	H
13.	NH_2	CH_3	H	NO_2	H
14.	NH_2	H	H	NO_2	CH_3

Fig. 1 Structures of **1–14**

shifts [6, 23–25] as well as homo- and heteronuclear 2D chemical shift correlation measurements. When C-2 in pyridine *N*-oxide is substituted with an amino group the *ipso* carbon chemical shift changes from 138.2 ppm [25] to 150.69 ppm (**1**). In its methylated derivatives (**5**) and (**6**), the 2-*ipso* carbon is 1.11 (**5**) and 2.18 ppm (**6**) shielded from that of **1**. The substitution of hydrogen in amino group by acetyl group (**2**) increases the shielding in *ipso* carbon C-2 ppm by 6.97 ppm and in the methylated derivatives by ca. 4–9 ppm (**7**, **8**, and **9**). When the methyl group locates at C-5 (**8**), the deshielding of C-5 is 17.0 ppm due to hyperconjugation with the methyl group. When a 5-nitro group is introduced into 2-amino-pyridine *N*-oxide (**10**) and its 3- (**13**) and 6-methyl (**14**) derivatives, the increase in δ(C-2) is 3.0–4.4 ppm and in δ(C-5) 21–23 ppm. Again these changes are due to resonance interaction between the 2-amino and 5-nitro group.

^{15}N NMR shifts

Our earlier studies on pyridine *N*-oxides show that in nitraminopyridine and *N*-alkylamino-4-nitro derivatives possessing a tertiary amino group at C-2, $\delta(^{15}N)$ of the ring nitrogen varies from −92.7 to −93.0 ppm. It means that these compounds exist mainly as amino tautomers [6, 23, 24]. In neat pyridine *N*-oxide, the corresponding $\delta(^{15}N)$ is −86.8 ppm [25]. Also, in a compound with a secondary amino substituent at C-3 $\delta(^{15}N)$ is −86.9 ppm revealing its tautomeric preference as an amino form. In 3-methyl-2-nitraminopyridine *N*-oxide, the corresponding value of $\delta(^{15}N)$ is −94.4 ppm. The predominance of the amino tautomer is in agreement with quantum chemical ab initio HF/6-311G** structure optimization and energy calculations [6]. The greater stability of the amino tautomer can be explained that while the N–NO_2 group is twisted to one side of the plane of the aromatic ring the spatial vicinity of two substituents inhibit the conjugation between the

Table 1 ^{1}H NMR chemical shifts of **1–14** (ppm from int. TMS) in 0.5 M DMSO-d_6 at 30 °C

Compound	$\delta(^1H)$								
	H-2	H-3	H-4	H-5	H-6	CH_3	$COCH_3$	NH_2	$NHCOCH_3$
1	–	6.78	7.09	6.56	8.02	–	–	6.82	–
2	–	8.27	7.38	7.11	8.35	–	2.26	–	10.49
3	7.77	6.52	–	6.52	7.77	–	–	6.30	–
4	8.10	7.60	–	7.60	8.10	–	2.07	–	10.99
5	–	–	6.99	6.52	7.91	2.15	–	6.65	–
6	–	6.72	6.95	–	7.88	2.10	–	6.53	–
7	–	8.12	–	6.94	8.21	2.30	2.25	–	10.41
8	–	8.15	7.22	–	8.25	2.23	2.21	–	10.39
9	–	8.16	7.28	7.16	–	2.42	2.25	–	10.52
10	–	6.87	7.97	–	8.91	–	–	8.13	–
11	8.80	–	–	8.21	8.37	–	–	–	–
12	8.86	–	–	8.18	8.39	–	–	–	–
13	–	–	7.91	–	8.84	2.25	–	8.02	–
14	–	6.79	7.88	–	–	2.74	–	7.98	–

Table 2 ^{13}C NMR chemical shifts of **1–14** (ppm from int. TMS) in 0.5 M DMSO-d_6 at 30 °C

Compound	$\delta(^{13}C)$							
	C-2	C-3	C-4	C-5	C-6	CH_3	$COCH_3$	CO
1	150.69	109.00	126.83	112.05	136.96	–	–	–
2	143.72	114.58	126.86	119.12	137.37	–	24.24	169.57
3	138.55	110.12	147.91	110.12	138.55	–	–	–
4	138.84	115.72	150.27	115.72	138.84	–	23.96	169.14
5	149.58	118.08	127.18	111.30	134.42	16.65	–	–
6	148.51	108.76	128.05	121.43	136.23	16.67	–	–
7	142.88	114.67	137.85	119.81	136.47	20.29	24.27	169.48
8	141.41	114.11	127.79	129.01	136.83	17.02	24.15	169.30
9	146.53	111.77	125.63	119.19	143.55	17.54	24.32	169.41
10	155.04	106.30	123.01	133.89	133.79	–	–	–
11	140.57	126.22	141.19	122.86	139.04	–	–	–
12	142.66	113.93	143.11	122.71	139.16	–	–	–
13	154.22	116.34	122.72	133.11	131.53	16.71	–	–
14	153.66	103.56	123.66	135.33	145.56	14.92	–	–

Table 3 ^{15}N NMR shifts (ppm from ext. CH_3NO_2) of **1–14** in 0.5 M DMSO-d_6 at 30 °C

Compound	$\delta(^{15}N)$			
	Ring	NH_2	NO_2	$NHCOCH_3$
1	126.1	315.8	–	–
2	114.6	–	–	254.5
3	122.5	310.8	–	–
4	101.2	–	–	246.7
5	126.7	317.1	–	–
6	126.0	318.9	–	–
7	120.1	–	–	254.4
8	114.6	–	–	255.2
9	116.1	–	–	254.2
10	126.5	298.1	16.7	–
11	74.7	–	19.2	–
13	–	297.9	16.9	–
14	122.9	310.5	–	–

2-nitramino and pyridine ring increasing the energy of the amino form, which, however, is slightly more stable than the imino form. When the molar ratio of the imino form in the tautomer balance is increasing, the $\delta(^{15}N)$ of ring nitrogen becomes more shielded varying from −124.1 to −168.7. In 4-nitro derivatives with secondary 2-amino substituent, $\delta(^{15}N)$ of ring varies from −112.8 to −120.9 ppm [22] and in 5-nitro derivatives with secondary 2-amino substituent from −128.4 to −129.5 [24]. $\delta(^{15}N)$ of ring in pyridine *N*-oxide and 2-aminopyridine *N*-oxide are −86.6 ppm [6] and −126.1 ppm, respectively. In all other derivatives, the aromatic nitrogen chemical shifts from 114.6 to 126.7 ppm mean that the molar ratio of the imino form in the tautomer balance is increasing.

In 2-aminopyridine *N*-oxide (**1**), $\delta(^{15}N)$ of amino is −315.8 ppm. Introduction of methyl group to 2-aminopyridine to C-3 (**5**) or to C-5 (**6**) has a minimal effect on the amino shift differing from that of 5-nitro group (**10**), i.e., 17.7 ppm. The *p*-nitro group together with 3- and 6-methyl substituents (**13**, **14**) decreases the amino nitrogen shift 17.9 and 5.3 ppm, respectively.

In conclusion, taking into account the large chemical shift range and sensitivity to substituents of ^{15}N, we can state that there are characteristic $\delta(^{15}N)$ ranges for pyridine-*N* oxides possessing NH_2, $NHCOCH_3$, and NO_2 substituents. $\delta(^{15}N)$ of ring vary from −74.7 to −126.7 ppm, $\delta(^{15}N)$ of primary amino from −297.9 to −318.9 ppm, $\delta(^{15}N)$ of amino in acetyl derivatives from −246.7 to −255.2 ppm, and $\delta(^{15}N)$ of nitro group from −16.7 to −19.2 ppm. $\delta(^{15}N)$ of ring is somewhat sensitive to the nature of substituent at C-2. When the NH_2 is substituted by $NHCOCH_3$, $\delta(^{15}N)$ of ring becomes slightly deshielded, while the deshielding in amino nitrogen is larger being 61–69 ppm. As a whole, the ^{15}N NMR shifts manifest a strong resonance interaction between 2-amino and 5-nitro groups by 17.7 ppm (**10**), 19.9 ppm (**13**), and 5.3 ppm (**14**) deshielding in $\delta(^{15}N)$ of the amino group.

Conclusions

Among NMR parameters for fifteen substituted pyridine *N*-oxides reported in this study, ^{15}N NMR chemical shifts

are the most sensitive to the effects of substituent and tautomerism.

$\delta(^{15}N)$ of the pyridine ring nitrogen in 2-acetylaminopyridine *N*-oxides are 5.9–11.5 ppm deshielded from those in 2-aminopyridine *N*-oxides. When amino and acetylamino substituents are in 4-position, $\delta(^{15}N)$ of ring nitrogen is 21.3 ppm deshielded in the acetylated derivative. The strong resonance interaction between 2-amino and 5-nitro groups reflects in the decrease of amino nitrogen shielding about 5.3–17.9 ppm. The pyridine nitrogen chemical shift in all amino- and acetylamino derivatives vary between −101.2 and −126.7 ppm, which has been connected with the tautomeric balance in our earlier studies. Also, ^{1}H and ^{13}C NMR spectral data are in agreement with ^{15}N NMR results.

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