ЖУРНАЛ СТРУКТУРНОЙ ХИМИИ

2012. Том 53, № 6 *Ноябрь – декабрь* *С. 1216 – 1220*

КРАТКИЕ СООБЩЕНИЯ

UDC 541.67:548.737

A MULTINUCLEAR ^{1}H, ^{13}C, AND ^{15}N MAGNETIC RESONANCE STUDY OF TEN 4-NITROPYRIDINE N-OXIDES

A. Puszko[1], K. Laihia[2], E. Kolehmainen[2], Z. Talik[1]

[1]*Department of Bioorganic Chemistry, Faculty of Industry and Economics, University of Economics, Wrocław, Poland,* e-mail: apuszko@gmail.com
[2]*Department of Chemistry, University of Jyväskylä, Finland*

Received August, 30, 2011 *Revised — November, 22, 2011*

The ^{1}H, ^{13}C, and ^{15}N NMR chemical shifts of ten 4-nitropyridine N-oxide derivatives are assigned. The shielding of the pyridine ring nitrogen is sensitive to ring substitution through inductive effects, steric effects by *ortho*-substituents, and the possibility for electron delocalisation (resonance energy). In solution, 3-ethylnitrosoamino-4-nitropyridine N-oxide has two tautomers. The proposed reason is the steric crowding between vicinal 4-nitro and 3-ethylnitrosoamino groups, causing a disturbance to amino nitrogen that can delocalize its lone pair to the oxygen atom of the nitroso group.

Keywords: ^{1}H, ^{13}C, ^{15}N NMR, 4-nitropyridine N-oxide.

It is known that the derivatives of pyridine N-oxide have raised widespread interest due to their exceptional bioactivity as antibiotic, cancerostatic, mutagenic, anticonvulsive, and fungistatic efficacy [1—8]. The presence of a 3-alkylnitrosoamino moiety has been particularly crucial for developing the anticancer efficacy in 4-nitropyridine N-oxide [2, 3]. In order to get a better insight into the structures and electronic characteristics of this class of compounds we have determined ^{1}H, ^{13}C, and ^{15} N NMR chemical shifts for six new 4-nitropyridine N-oxides. The ^{1}H and ^{13}C NMR chemical shifts for four compounds have previously been presented [9—13], but some of those results need to be corrected.

In our earlier multinuclear magnetic resonance study of 4-nitro-2-ethylnitrosoaminopyridine N-oxide derivatives, the rotation of the bulky 2-substituent was so slow in the NMR time scale that the ^{1}H, ^{13}C, and ^{15}N NMR chemical shifts could be measured for a pair of conformers [14, 15]. In 2-ethylnitrosoamino-3-methyl-4-nitropyridine N-oxide, the rotations of both 2-ethylnitrosoamino substituent and its ethyl group were restricted owing to the steric crowding by 3-methyl substituent making amino nitrogen chiral and the neighbouring methylene protons inequivalent [14]. Similar results we obtained later in the ^{15}N NMR study of 3-ethylnitramino-4-nitropyridine N-oxide [15]. Therefore, 3-ethylnitrosoamino-4-nitropyridine N-oxide was synthesised to find out whether the similar chirality exists in its amino group.

Experimental. Compounds. The studied compound were 2-chloro-4-nitropyridine N-oxide (**1**), 2-bromo-4-nitropyridine N-oxide (**2**), 2-iodo-4-nitropyridine N-oxide (**3**), 3-ethylamino-4-nitropyridine N-oxide (**4**), 3-ethylnitrosoamino-4-nitropyridine N-oxide (**5A**, **5B**), 3-iodo-4-nitropyridine N-oxide (**6**), 2-chloro-3-methyl-4-nitropyridine N-oxide (**7**), 2-bromo-3-methyl-4-nitropyridine N-oxide (**8**), 2-chloro-6-methyl-4-nitropyridine N-oxide (**9**), and 2-iodo-6-methyl-4-nitropyridine N-oxide (**10**).

The procedure of the synthesis of 3-ethylnitrosoamino-4-nitropyridine N-oxide (**5A**, **5B**) is as follows: a 2.0 g (11.0 mmol) portion of 3-ethylamino-4-nitropyridine N-oxide (**4**) (its synthesis was described previously [15]) was dissolved in10 ml of diluted hydrochloric acid (1:4;1 volume of HCl and 3 volumes of H_2O) and then 2.0 ml of a saturated solution of $NaNO_2$ was added dropwise maintaining

the temperature at 0 °C for 20 minutes. Then the reaction product was separated by filtering, dried in air, and recrystallized from methanol. The synthesis of halogenated derivatives 2-Cl— (**1**), 2-Br— (**2**), 2-I— (**3**), and 3-I—4-NO_2-pyridine N-oxide (**6**) have been described before [14] as well as of some of their methyl derivatives, e.g. 2-Cl—3-CH_3— (**7**), 2-Br—3-CH_3— (**8**), 2-Cl—6-CH_3— (**9**), and 2-I—6-CH_3—4-NO_2-pyridine N-oxide (**10**) [10].

NMR-spectroscopy. The ^{1}H, ^{13}C and PFG [16] ^{1}H,^{13}C HMQC [17, 18] and PFG ^{1}H, X (X = ^{13}C or ^{15}N) HMBC [19] spectra were recorded for a 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 the ^{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 was 30°. An exponential window function of the spectral resolution was used prior to FT. The ^{1}H NMR chemical shifts are referenced to the signal of residual DMSO-d_5 (δ = 2.50 ppm from int. TMS).

In the ^{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 the flip angle was 30°. A composite pulse decoupling (Waltz-16) was used to remove proton couplings. An exponential window function of the spectral resolution was used prior to FT. The ^{13}C NMR chemical shifts are referenced to the central peak of the DMSO-d_6 solvent (δ = 39.50 ppm from int. TMS). The number of data points in PFG ^{1}H, ^{13}C HMQC, and HMBC measurements were 1024(f_2)×256(f_1). This matrix was zero filled to 2048×512 and apodized with a shifted sine bell window function along both axes prior to FT.

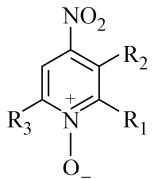
In the PFG ^{1}H, ^{15}N HMBC experiments, the digital resolution was <0.5 ppm and a 50 ms delay was used for evolution of long-range couplings. The number of data points were 1024(f_2)× ×512/450 ppm (f_1 = ^{15}N). This matrix was zero filled to 1024×1024 and apodized with a shifted sine bell window function along both axes prior to FT. The ^{15}N NMR chemical shifts are referenced to the signal of external CH_3NO_2 (δ = 0.0 ppm).

Results and discussion. The structure of compounds **1—10** are presented in Fig. 1 and the ^{1} H, ^{13}C, and ^{15}N NMR chemical shifts are presented in Tables 1—3.

Although the ^{1}H and ^{13}C NMR chemical shifts of 4-nitromethylpyridine N-oxides, which have halogen substituents, have been studied earlier [9—13], however, there is no published data for their ^{15}N NMR chemical shifts. Moreover, in 4-nitropyridine N-oxide derivatives, the interaction between 4-nitro and N-oxide groups may be determined by monitoring the shieldings of the N-ring and the N-nitro group when a new substituent is introduced. The highest contribution of the quinoid resonance structure among the studied compounds is characteristic of 4-nitropyridine N-oxide and its derivatives, which apart from the 4-nitro group, also contain electron-acceptor substituents. Effects of steric inhibition of conjugation between pyridine nitrogen and the 4-nitro group were reported previously based on the ^{13}C NMR spectra for methyl derivatives of 4-nitropyridine N-oxide [20].

It is important that the precise determination of the influence of the methyl group at various positions of the pyridine ring on the spectra of 2-halo-4-nitropyridines can be performed by comparing the NMR spectra of 2-halogen-4-nitropyridine N-oxides (**1**—**3**, **6**) and derivatives with the added methyl substituent in the ring (**7**—**10**).

Structures of **1**—**10**



Compounds	R_1	R_2	R_3
1	Cl	H	H
2	Br	H	H
3	I	H	H
4	H	NHC_2H_5	H
5	H	$N(NO)C_2H_5$	H
6	H	I	H
7	Cl	CH_3	H
8	Br	CH_3	H
9	Cl	H	CH_3
10	I	H	CH_3

Table 1

^{1}H NMR *chemical shifts* (ppm *from int.* TMS) *of* 4-*nitropyridine* N-*oxides* **1**—**10** *in* 0.5 M DMSO-d_6 *solution at* 30 °C

Compound	δ(^{1}H), ppm						
	2	**3**	**5**	**6**	CH_2	CH_3	NH/NH_2
4-NO_2-pyr NO[a]	8.39	8.19	8.19	8.39	—	—	—
1	—	8.71	8.19	8.64	—	—	—
2	—	8.75	8.20	8.62	—	—	—
3	—	8.77	8.19	8.57	—	—	—
4	8.14	—	8.01	7.47	3.38	1.19	8.10
5A[b]	8.93	—	8.29	8.53	4.00	1.06	—
5B[b]	8.67	—	8.25	8.50	4.69	1.42	—
6	8.89	—	8.09	8.35	—	—	—
7	—	—	8.04	8.53	—	2.58	—
8	—	—	8.05	8.54	—	2.61	—
9	—	8.58	8.43	—	—	2.07	—
10	—	8.65	8.41	—	—	2.50	—

[a] From ref. [9].
[b] Compounds **5A** and **5B** are a couple of tautomers.

^{1}H NMR shifts. The introduction of a halogen atom in position 2 (**1**—**3**) of 4-nitropyridine N-oxide results in a deshielding effect on H-3 (0.52—0.58 ppm, Table 1), whereas the chemical shifts of H-5 remain unchanged. This fact may be explained by a negative inductive effect of halogen. It can be expected that the back donation in 4-nitropyridine N-oxide is increased under halogen substitution in C-2 and a part of the quinoid form is also increased in resonance structures. The quality of halogen has not much influence on the H-3 chemical shifts, which vary from 8.71 ppm to 8.77 ppm. A comparison of the ^{1}H NMR chemical shifts of 2-halogen-4-nitropyridine N-oxides (**1**, **2**) and their 3-methyl analogues (**7**, **8**) does not show a great difference in the H-5 chemical shifts and even less in the H-6 chemical shifts. The deshielding effect on H-5 is 0.15 ppm for both pairs of compounds and the shielding effect for H-6 is just 0.11 ppm and 0.08 ppm). The methyl group in H-6 (**9**, **10**) increases the shielding in H-3 (0.12 ppm and 0.13 ppm) and deshielding in H-5 (0.24 ppm and 0.22 ppm) respectively. When an iodine atom is brought to position 3 (**6**), the effect is the greatest on the H-2 chemical shift where the deshielding effect is 0.50 ppm.

Compound **5** exists in one conformer that, however, possesses two tautomeric forms. The proximity of the nitro group and its electronegative oxygen help to push the sterically close amino nitrogen lone pair to nitroso nitrogen and forward to oxygen. The CH_2 protons are equivalent in the ethyl group.

^{13}C NMR shifts. The assignment of the ^{13}C NMR chemical shift is based on the literature data and substituent-induced chemical shifts [9—15, 21—25] (Table 2). In the ^{13}C NMR spectra, the effect of halogen on the chemical shifts of 4-nitropyridine N-oxide is stronger than its effect on the ^{1}H NMR chemical shifts and the quality of halogen is also obvious. The heavy atom effects are seen in C-2 (**1**—**3**, **7**—**8,** and **9**—**10**) [26]. It is also obvious in C-3 of compound **6**. Only Cl has deshielding effects; other halogens are shielding for C-2. The heavy atom effect caused by iodine in C-2 is 27.39 ppm in **3** and **6**, and in C-3 it is 32.41 ppm. The effect and quality of halogen can also be observed in the chemical shift of methyl groups situated in positions 3 and 6 in the pyridine ring. These effects oscillate from 16.86 ppm to 19.70 ppm. This shielding effect of the large iodine substituent on C-3 (*ipso* carbon in **6)** is 32.41 ppm [23].

Table 2

^{13}C NMR *chemical shifts* (ppm *from int.* TMS) *of* 4-*nitropyridine* N-*oxides* **1**—**10** *in* 0.5 M DMSO-d_6 *solution at* 30 °C

Compound	δ(^{13}C), ppm						
	C-2	C-3	C-4	C-5	C-6	CH_2	CH_3
4-NO_2-pyr NO[a]	140.20	121.24	142.03	121.24	140.20	—	—
1	141.74	121.89	141.63	119.09	141.03	—	—
2	132.96	125.22	141.49	119.62	140.75	—	—
3	112.81	130.88	140.98	120.25	139.10	—	—
4	125.79	141.99	125.95	122.27	127.13	37.13	13.75
5A[b]	137.82	132.95	138.61	122.97	139.98	41.76	10.77
5B[b]	138.09	129.11	139.59	122.34	140.73	48.73	13.28
6	147.80	88.83	146.42	121.99	139.16	—	—
7	143.32	130.80	143.75	119.30	138.14	—	16.86
8	137.77	133.01	143.60	119.83	138.05	—	19.70
9	141.46	119.15	140.44	118.73	151.25	—	18.00
10	112.59	128.33	140.31	119.90	149.27	—	18.75

[a] From ref. [9].
[b] Compounds **5A** and **5B** are a couple of tautomers.

The 3-nitrosoamino group (**5**) exerts on *ipso* carbon 7.87 ppm and 11.71 ppm deshielding effects depending on the tautomer structure. The steric proximity of the nitro group to amino nitrogen can make the lone pair of amino nitrogen move to nitroso nitrogen and further to nitroso oxygen.

^{15}N NMR shifts. The ^{15}N NMR chemical shifts for compounds **1—10** are presented in Table 3. In the studied compounds there occur four different nitrogen atoms, namely aromatic pyridine-N*→O, CN*O_2 (nitro), N*C_2H_5 NO (amino), and NN*O (nitroso). They have different ranges of ^{15}N NMR chemical shifts and thus their assignments were straightforward.

In 4-nitropyridine N-oxide derivatives, the interaction between 4-nitro and N-oxide groups can be estimated by monitoring the shieldings of N_{nitro} and N_{ring}. Among the derivatives with halogen substituent at C-2, the most significant effect on the ^{15}N shift of pyridine ring nitrogen is caused by Cl, and the effect is shielding (Table 3). The presence of a methyl group in the *ortho*-position to the 4-nitro group (**7**, **8**) forces the nitro group out of the plane of the pyridine ring, and the deshielding effect is increased. On passing from 2-chloro- and 2-bromo-4-nitropyridine (**1**, **2**) to their 3-methyl derivatives (**7**, **8**), the shielding on the nitro group decreases from –18.1 ppm and –18.4 ppm to –13.7 ppm and –13.3 ppm respectively. If the methyl group is introduced to C-6 in 2-chloro- and 2-bromo-4-nitropyridines (**9**, **10**) the shift of the nitro group changes from –18.1 ppm to –17.2 ppm due to lack of the *ortho*-effect and inductive effect of the methyl group. In 2-chloro-, 2-bromo-, 2-iodo-4-nitropyridine N-oxide, the ^{15}N shifts of pyridine nitrogen are –80.1 ppm, –78.2 ppm, –73.9 ppm and the ^{15}N chemical shifts are not much dependent on the nature of the halogen. The proximity of the iodine substituent to the 4-nitro group in 3-iodo-4-nitropyridine N-oxide results in a decreasing chemical shift of the nitro group to –15.3 ppm.

Conclusions. The complete ^{1}H, ^{13}C, and ^{15} N NMR chemical shift assignments for ten 4-nitropyridine N-oxides were reported in this paper. The NMR spectral characterization of these compounds is valuable concerning 3-ethylnitrosoamino-4-nitropyridine because this compound possesses special biological activity. In contrast with our findings on 2-ethylnitrosoamino-3-methyl-4-nitropyridine and 3-ethylnitramino-4-nitropyridine, 3-ethylnitrosoamino-4-nitropyridine N-oxide has no chiral amino nitrogen. However, its ethylnitrosoamino group shows prototropic tautomerism.

Table 3

^{15}N NMR *shifts* (ppm *from ext.* CH_3NO_2) *of* 4-*nitropyridine* N-*oxides* **1**—**10** in 0.5 M DMSO-d_6 *solution at* 30 °C

Compound	–δ (^{15}N), ppm				
	Pyr **N**O	Ar**N**O_2	**N**HR	NR**N**O	*J*(N,H)
4-NO_2-pyr NO[a]	73.4	16.6	—	—	—
1	80.1	18.1	—	—	—
2	78.2	18.4	—	—	—
3	73.9	18.3	—	—	—
4	72.0	14.0	293.0	—	92.3
5A[b]	74.9	18.0	—	128.5	—
5B[b]	72.0	18.0	—	137.6	—
6	75.2	15.3	—	—	—
7	83.4	13.7	—	—	—
8	81.4	13.3	—	—	—
9	82.6	17.2	—	—	—
10	76.7	17.4	—	—	—

[a] From ref. [9].
[b] Compounds **5A** and **5B** are a couple of tautomers.

REFERENCES

1. *Albini A., Pietra S.* Heterocyclic N-oxides. – CRC: Press, 1991.
2. *Kalatzis E., Kiriazis E.* // Chim. Chron., New Series. – 1992. – **21**. – P. 41.
3. *Kulkarni C.V., Ray R.* // J. Mol. Struct. – 1981. – **71**. – P. 253.
4. *Nantermet P.G., Burgey C.S., Robinson K.A., Pellicore J.M., Newton C.L., Deng J.Z., Selnick H.G., Lewis S.D., Lucas B.J., Krueger J.A., Miller-Stein C., White R.B., Wong B., McMasters D.R., Wallace A.A., Lynch J.J., Yan Y.W., Chen Z.G., Kuo L., Gardell S.J., Shafer J.A., Vacca J.P., Lyle T.A.* // Bioorg. Med. Chem. Lett. – 2005. – **15**. – P. 2771.
5. *Pool J.A., Scott B.L., Kiplinger J.L.* // Chem. Commun. – 2005. – **20**. – P. 2591.
6. *Pool J.A., Scott B.L., Kiplinger B.L.* // J. Amer. Chem. Soc. – 2005. – **127**. – P. 1338.
7. *Balzarini J., Stevens M., DeClercg E., Schols D., Pannecouque C.J.* // J. Antimicrob. Chemother. – 2005. – **5**. – P. 135.
8. *Balzarini J., Keyaerts E., Vijgen L., Vandermeer F., Stevens M., De Clercg E., Egherink H., Van Ranst M.* // J. Antimicrob. Chemother. – 2006. – **57**. – P. 472.
9. *Talik T., Talik Z.* // Rocz. Chem. – 1962. – **36**. – P. 539.
10. *Talik Z., Puszko A.* // Rocz. Chem. – 1976. – **50**. – P. 2209.
11. *Puszko A.* // Polish J. Chem. – 1992. – **66**. – P. 1979.
12. *Puszko A.* // Magn. Reson. Chem. – 1992. – **30**. – P. 271.
13. *Laihia K., Puszko A., Linnanto J., Kolehmainen E.* // J. Mol. Struct. – 2006. – **783**. – P. 73.
14. *Laihia K., Puszko A., Kolehmainen E., Lorenc J.* // J. Mol. Struct. – 2007. – **831**. – P. 203.
15. *Laihia K., Puszko A., Kolehmainen E., Lorenc J.* // J. Mol. Struct. – 2008. – **889**. – P. 371.
16. *Hurd R.E., John B.K.* // J. Magn. Reson. – 1991. – **91**. – P. 648.
17. *Bax A., Griffey R.H., Hawkins B.L.* // J. Magn. Reson. – 1983. – **55**. – P. 301.
18. *Bax A., Subramanian S.* // J. Magn. Reson. – 1986. – **67**. – P. 565.
19. *Bax A., Summers M.F.* // J. Amer. Chem. Soc. – 1986. – **108**. – P. 2093.
20. *Puszko A., Wasylina L.* // Chem. Paper. – 1995. – **49**. – P. 176.
21. *Breitmeier E., Voelter W.* Carbon-13 NMR Spectroscopy, 3rd Ed. – New York: VCH, 1987.
22. *Laihia K., Kolehmainen E., Kauppinen R., Lorenc J., Puszko A.* // Spectrochim. Acta. – 2002. – **A58**. – P. 1425.
23. *Laihia K., Kolehmainen E., Virtanen E., Nissinen M., Puszko A., Talik Z.* // Magn. Reson. Chem. – 2003. – **41**. – P. 721.
24. *Anet F.A.L., Yavari I.* // J. Org. Chem. – 1976. – **41**. – P. 3589.
25. *Sojka S.A., Dinan F.J., Kolarczyk R.* // J. Org. Chem. – 1979. – **44**. – P. 307.
26. *Neto A.C., Ducati L.C., Rittner R., Tormena C.F., Contreras R.H., Frenking G.* // J. Chem. Theory Comput. – 2009. – **5**. – P. 222.