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Ring–Chain Tautomerism of Pseudooxynicotine and Some Other Iminium Compounds

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The ring–chain tautomerism in aqueous solution of the nicotine metabolite pseudooxynicotine (*1*) has been studied. Of the four possible forms of *1*, only the chain form *1a* and the iminium form *1c* could be observed by NMR spectroscopy. The chain form *1a* was strongly preponderant at high or low pH. The proportion of *1c* reached a maximum level of 52–53 % in a neutral solution of *1*. These results differ strongly from those published by other workers. Some analogous compounds (*e.g.* *5* and *6*) have also been investigated.

Pseudooxynicotine (*1*) was first synthesized in 1892 [1] and was later found to be a bacterial metabolite of nicotine (*2*).[2] The compound is capable of displaying ring-chain tautomerism [3,4] and, according to classical theory, aqueous solutions of *1* may contain four forms in equilibrium, *1a*–*1d* (Fig. 1). The elemental composition and IR spectrum of the crystalline dihydrochloride indicate that this salt contains the dication derived from the chain form *1a*.[5] Extraction of an alkaline aqueous solution of *1* with chloroform followed by distillation yields the unstable enamine *1d*, also known as *N*-methylmyosmine.[5,6]

A large number of aromatic heterocyclic cations have been studied with respect to their formation of pseudobases by covalent addition of a hydroxyl ion, but much less information is available about the corresponding reactions in the aliphatic series, *e.g.* of iminium ions.[4,7] A structural investigation of *1*, mainly performed by means of ^{1}H NMR spectroscopy, was recently undertaken by Maeda *et al.*[8] and, to the best of our knowledge, their report constituted the first

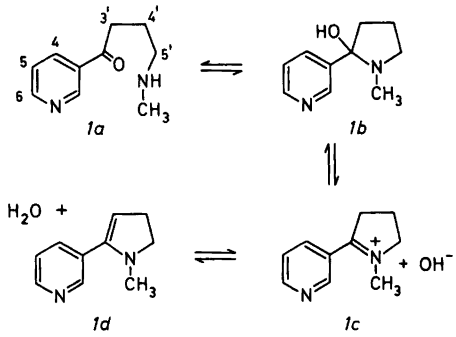

Fig. 1. Conceivable forms (*1a*–*1d*) of pseudooxynicotine in aqueous solution. The numbering of the carbons in nicotine has been kept throughout and is shown for *1a*.

study of the ring-chain tautomerism of a γ-(alkylamino) ketone in aqueous solution as a function of pH. Maeda *et al.* concluded that *1a* was present at pH 1–4, *1c* at pH 2–9.5, and *1d* at pH 4–11. We have now carried out a similar study of *1* and we obtained ^{1}H NMR spectra which were similar to those of Maeda *et al.* However, our interpretations of the spectra and the conclusions reported below differ strongly from those of Maeda *et al.*[8] Knowledge of the structures of nicotine metabolites in aqueous solution is necessary for the detailed mapping of the reaction pathways involved in the metabolism of nicotine. We have previously described a structural study of nicotine $\Delta^{1'(5')}$-iminium ion,[9] which is an isomer of *1c*, while others [10] have studied the 5'-oxo analogue of *1b*.*

* The nicotine numbering, as in *1a*, is used throughout.

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STUDIES OF PSEUDOOXYNICOTINE

^{1}H NMR spectra of neutral aqueous solutions of pseudooxynicotine (*1*) showed that two forms of *1* are present in approximately equal amounts and the structures *1a* and *1c* could be ascribed to these forms. Thus, the signals at δ 2.82 (48 %) and 3.71 (52 %) were ascribed to the *N*–CH_3 of *1a* and *1c*, respectively. The signals ascribed to the *N*-methylpyrrolinium moiety of *1c* agree well with those obtained from 3,4-dihydro-1-methyl-5-phenyl-2*H*-pyrrolium perchlorate in trifluoroacetic acid solution.[11] The assignments are further supported by the effects of pH on the NMR spectra. Changes in pH did not affect the positions of the signals ascribed to the protons of the five-membered ring of *1c*; on the other hand, the corresponding signals ascribed to *1a* varied with pH in the manner expected for an amine (see below). The ^{13}C NMR spectrum of a neutral solution of *1* in H_2O is in accord with the ^{1}H NMR spectrum; the chemical shifts obtained for *1a* and *1c* are given in Table 1. In the downfield region two weak signals were observed at 201.7 and 184.7 ppm, respectively. The former has practically the same shift as the signal from the carbonyl carbon of *3* and is therefore assigned to C-2′ of *1a*; the 184.7 ppm resonance is assigned to C-2′ of *1c*.

With the assignment of the NMR signals from *1a* and *1c* at hand, it was readily seen that either acidification or alkalization of the neutral solution leads to an increased content of *1a* and a corresponding decrease in *1c* (Fig. 2). Only these two forms could be observed by NMR measurements on aqueous solutions of *1*; we observed no signals which could be assigned to the carbinola-

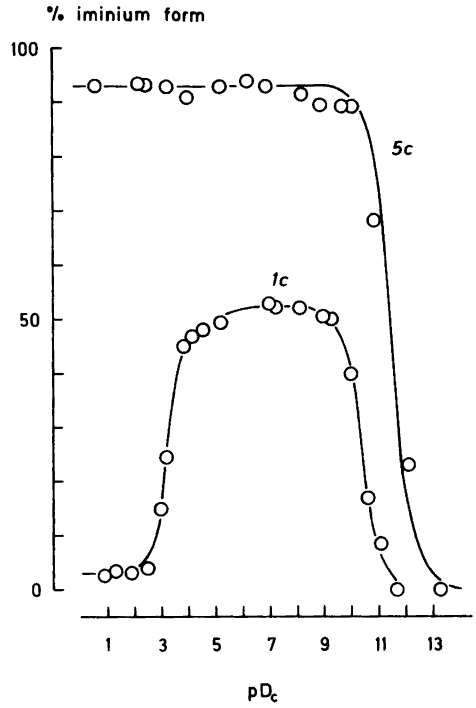


Fig. 2. Equilibrium contents of iminium forms *1c* and *5c* as a function of pD_c. For both compounds, the remaining part up to 100 %, as seen by ^{1}H NMR spectroscopy, is the amino ketone form, *i.e. 1a* and *5a*, respectively.

Table 1. ^{13}C NMR chemical shifts obtained for the pseudooxynicotine forms *1a*, *1c* and *1d*, for nicotine (*2*) and for the reference compound *3*.

Carbon No.	*1a* [a] pH 1	*1a* [a] pH 7	*1a* [a] pH 13	*1c* [a] pH 7	*1d* [b]	*2* [c] $pD_c>11$	*3* [d] $pD_c\approx13$
2	145.2	149.2	148.3	149.1	149.6*	149.0*	149.2
3	135.5	132.7	136.2*	124.6	148.6*	138.8*	133.0
4	142.4	137.6	136.9*	138.7	134.7*	137.3*	137.2
5	128.8	125.4	125.2	125.4	124.2*	125.5	125.3
6	146.5	153.7	151.1	154.3	152.0*	149.3*	153.7
2′	197.6	201.7	n.o.	184.7	130.4*	69.6	201.2
3′	36.8	36.4	39.9*	40.8	105.5	34.9	n.o.
4′	20.3	20.5	22.1	19.1	29.7	22.9	21.8
5′	49.1	49.4	51.9	64.7	57.0	57.4	58.7
N-CH_3	33.9	34.0	34.4*	39.9	40.5	40.4	45.1

[a] in H_2O; [b] in $(CD_3)_2SO$ with dioxane (67.40 ppm) as internal reference; [c] in D_2O ($pD_c>11$); shifts are relative to external TMS; [d] in D_2O ($pD_c\approx13$)+$(CD_3)_2SO$(≈1:2); *: tentative assignment; n.o.: not observed.

mine *1b* or the enamine *1d*. There was no spectral change near pD_c 4 in the ^1H and ^{13}C NMR spectra which Maeda *et al.*[8] claimed to occur in this pH region and which should be associated with a change from *1a* to *1d*. There is a gradual change in the NMR spectra between $pD_c \approx 2$ and 5 which is most pronounced between pD_c 3 and 4 but this must be attributed to the protonation of the pyridine ring; the protonation effect on the ^{13}C NMR spectrum of *1a* is evident from the values given in Table 1 (*cf.* the shifts for *1a* at pH 1 and 7).

Also at pH values higher than 7, the only forms of *1* observed by NMR spectroscopy were *1a* and *1c* (Fig. 2). The recording of the ^1H and, in particular, of the ^{13}C NMR spectra was, however, complicated by the instability of *1* in alkaline solutions. In the ^1H spectra, the signal ascribed to *N*-CH_3 in *1a* was at $\delta \approx 2.82$ up to $pD_c \approx 8.7$ (pD_c=pH meter reading+0.40 [12]). A further increase in pD_c led to upfield shifts; δ 2.18 being reached between pD_c 12 and 13. The magnitudes of pertinent chemical shifts indicate that the only form of *1* observed in strongly alkaline solution is *1a* rather than *1d* as claimed [8] by Maeda *et al.* Thus, in D_2O at pD_c 13 the two H-4′ hydrogens show a triplet at δ 1.89 but in distilled *1d*, dissolved in DMSO-d_6, they give a signal at δ 2.55. This difference in chemical shift seems too large to be due to a solvent effect on a C–H hydrogen but can be accounted for by assuming that the H-4′ hydrogens are allylic, as in *1d*, in DMSO-d_6 but not in D_2O solution, as in *1a*. Similar but smaller differences were also observed for the *N*-CH_3 and H-5′ hydrogens ($\Delta\delta$=0.27 and 0.45 ppm, respectively). The ^{13}C NMR spectra of *1a* in strongly alkaline aqueous solutions differed clearly from that of *1d* in DMSO-d_6 (Table 1). No signal from the carbonyl C-2′ in *1a* was detected under the actual recording conditions but this was considered less significant since *3*, which is more stable, behaved similarly and gave only a weak signal for C-2′ at pD_c 13.

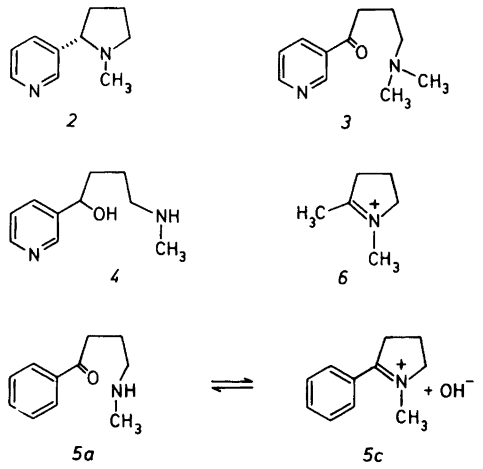
As seen in Table 1, the NMR signals obtained from the aromatic carbons of *1a* at pH 7 are close to those of *3* at $pD_c \approx 13$. This is reasonable since the pyridine ring in *1a* is unprotonated in neutral solution. However, a strongly alkaline solution of *1* gives a partly altered set of signals and this observation raises the question of whether the reactions *1a* ⇄ *1b* are rapid on the NMR time scale. Under the assumption that they are rapid, and if the equilibrium mol fraction of *1b* is large enough, one may expect alterations in the observed chemical shifts. To settle this matter we recorded the UV spectra of *1*, nicotine and 3-acetylpyridine in alkaline aqueous solutions (Fig. 3). The 230 nm band, which is common to *1* and 3-acetylpyridine only, seems to undergo a slight decrease in intensity with increasing pH but, since the two compounds behave similarly, this decrease could not be due to the formation of carbinolamine. If nicotine is accepted as a UV model compound for the carbinolamine *1b*, these spectra clearly show that the major form of pseudooxynicotine at pH 12 is the amino ketone *1a*. Similarly, the 3-indolyl analogue of *1c* gives an amino ketone on treatment with aqueous sodium hydroxide and extraction with ether.[13]

The enamine structure *1d* has been assigned to a tobacco alkaloid [14,16] and a metabolite [15] of nicotine or its *N*′-oxide. Our results show that if a single structure is to be assigned to this alkaloid (metabolite), the iminium form *1c* should be preferred as this form predominates at physiological pH.

Two chemical reactions with *1* in aqueous solution were carried out. When sodium borohydride was added to a weakly alkaline solution (pH 9.0) of *1* in water, nicotine was formed in large excess over the alcohol *4* (^1H NMR). At this pH, *1a* and *1c* are present in comparable amounts but *1c* is evidently reduced much faster than *1a*. When the reduction was started at pH 11.7, nicotine and *4* were formed in approximately equal amounts, demonstrating that the concentration of *1c* was much lower at this pH. These findings also show that the reaction *1a* → *1c* is fast enough to provide more *1c* as the reduction proceeds. Benzoylation of *1* with benzoyl chloride in strongly alkaline solution gave the *N*-benzoyl derivative of *1a* in an 87 % yield.

STUDIES OF ANALOGOUS COMPOUNDS

Some compounds analogous to *1* have also been studied with respect to ring-chain tautomerism. The crystalline perchlorates of *5c* [11] and *6* [21] were investigated by ^1H NMR spectroscopy as described above. The iminium ion *6* was the only form observed by ^1H NMR spectroscopy up to $pD_c \approx 12$. Compound *5*, like its pyridyl analogue



1, existed mainly as its amino ketone form (*5a*) and iminium form (*5c*); the percentage of *5c* is given as a function of pD_c in Fig. 2.

An investigation of neutral aqueous solutions of *5* and five analogues *p*- or *m*-substituted in the benzene ring shows that the equilibrium content of the iminium form increases when the substituent is electron-donating and decreases when it is electron-attracting. Moreover, some preliminary measurements indicate that the contents of iminium ion in acidic solutions are the same as in neutral solutions. Pseudooxynicotine (*1*) behaves differently and shows no constant level of iminium ion (*1c*) in the acidic region (Fig. 2). Two plateau levels can, however, be discerned, one at 2–3 % and the other at 52–53 %. These should correspond to the species in which the pyridine ring is protonated and unprotonated, respectively, and the mole fractions of these species are then in agreement with the substituent effects discussed above for *5* and its analogues.

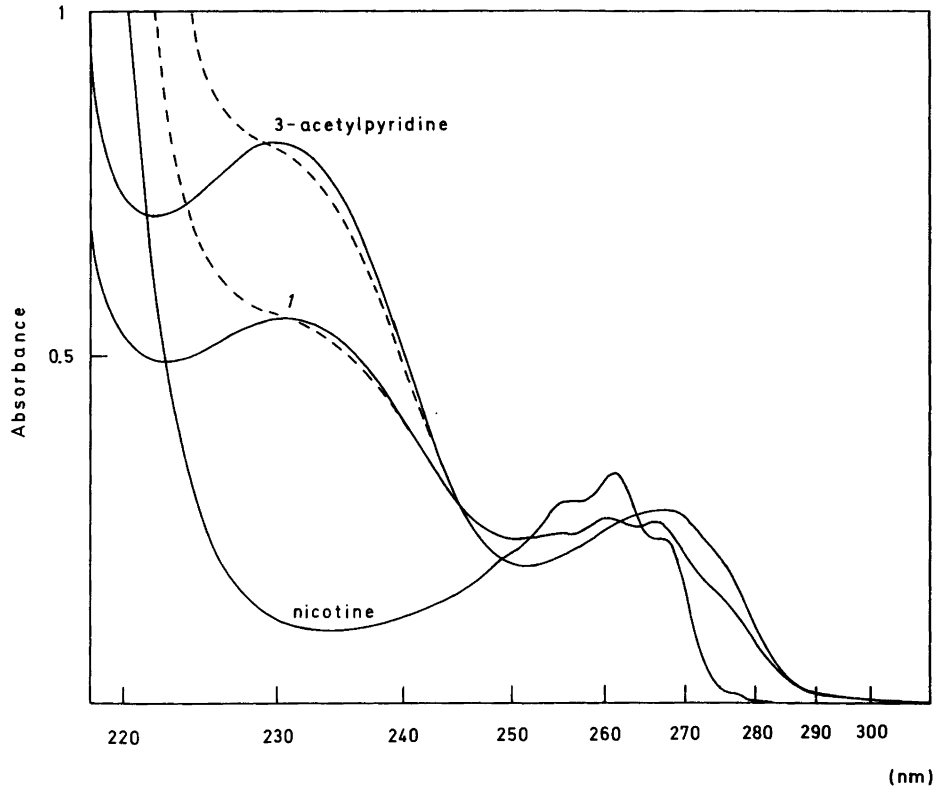


Fig. 3. UV spectra of nicotine (97 μM), pseudooxynicotine (*1*, 80 μM) and 3-acetylpyridine (83 μM) in H_2O at pH 11.9–12.0 (unbroken curves) and 12.9 (broken curves).

EXPERIMENTAL

^1H NMR spectra. In order to obtain simple spectra, isotope exchange of the H-3′ hydrogens was effected by treating the dihydrochloride twice with refluxing 20 % DCl in D_2O overnight. Solutions of *1* (0.04 M) were prepared by dissolving the resulting salt in D_2O and adjusting the pD_c with NaOD or DCl in D_2O. The ratios *1a*:*1c* are averages of the three ratios obtained from integration of the N-CH_3, H-4′ and H-4 signals. Spectra were recorded at 23–25 °C on a Varian XL-100 or JEOL FX-100 instrument using sodium 2,2-dimethyl-2-silapentane-5-sulfonate as internal reference. ^1H NMR shifts measured in DMSO-d_6 or $CDCl_3$ are given relative to internal TMS.

^{13}C NMR spectra were recorded at 25 MHz using the same instruments. When the spectra of *1* were taken in strongly alkaline solutions (pH >10.5), the salt content was minimized by dissolving distilled *1d* in D_2O and then adding $NaOD/D_2O$. For the samples run in H_2O, a coaxial capillary containing D_2O was used for the locking and chemical shifts were calibrated from internal dioxane (67.40 ppm). The references used for the samples in D_2O and $(CD_3)_2SO$ were the same as those for the ^1H spectra. Single frequency off-resonance (SFOR) spectra were obtained by irradiating at 400 Hz upfield from TMS in the proton spectrum. Assignments of the signals were based on multiplicities obtained in SFOR spectra, chemical shift considerations,[17] results after exchanges of the H-3′ hydrogens in D_2O, comparison between *1* and *3*, and comparison of spectra run at pH 1 and pH 7.

Measurements of pD_c or pH were performed on a PHM 62 standard pH meter (Radiometer, Copenhagen, accuracy 0.01 unit); UV spectra were recorded on a Beckman DK-2 spectrometer. Melting points are corrected. Nicotine and 3-acetylpyridine were purchased from Merck and distilled before use.

Pseudooxynicotine dihydrochloride (*1a* dihydrochloride) was prepared from ethyl nicotinate and *N*-methylpyrrolidone as previously described,[18] but using sodium hydride in toluene (reflux, 3 h) as base instead of sodium ethoxide. After two recrystallizations from ethanol–light petroleum (1:1), the compound melted at 200–202 °C (sealed tube); lit.[5] m.p. 196–198 °C; IR (KBr): 1692 cm^{-1}, broad ammonium bands around 2400 cm^{-1}.

4-Dimethylamino-1-(3′-pyridinyl)-1-butanone (*3*) was prepared from ethyl nicotinate and ethyl 4-(dimethylamino)butanoate according to method B described for the diethylamino analogue.[19] ^1H NMR ($CDCl_3$): δ 9.2–7.1 (4 H), 2.95 (t, 2 H), 2.5–1.5 (10 H, including an N-CH_3 singlet at 2.11); ^{13}C NMR: See Table 1.

3,4-Dihydro-1-methyl-5-phenyl-2H-pyrrolium (5c) perchlorate and *3,4-dihydro-1,5-dimethyl-2H-pyrrolium (6) perchlorate* were prepared by reactions between the corresponding cyclic imines [20] and methyl iodide and subsequent ion exchange with silver perchlorate. The former iminium salt [11] showed m.p. 116–117 °C and the latter 241–243 °C; lit.[21] m.p. 239–241 °C.

5c: ^1H NMR (D_2O, pD_c 6.9): δ 7.7–7.6 (aromatic hydrogens), 4.36 (t, H-5′), 3.60 (s, N-CH_3), 3.24 (t, H-3′), 2.56–2.20 (m, H-4′).

5a: ^1H NMR (D_2O, pD_c 13.3): δ 8.0–7.4 (aromatic hydrogens), 2.58 (t, H-5′), 2.26 (s, N-CH_3), 1.84 (t, H-4′).

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REFERENCES

1. Pinner, A. and Wolffenstein, R. *Ber. Dtsch. Chem. Ges. 25* (1892) 1428.
2. Wada, E. and Yamasaki, K. *J. Am. Chem. Soc. 76* (1954) 155.
3. Beke, D. *Adv. Heterocycl. Chem. 1* (1963) 167.
4. Bunting, J. W. *Adv. Heterocycl. Chem. 25* (1979) 1.
5. Haines, P. G. and Eisner, A. *J. Am. Chem. Soc. 72* (1950) 1719 and references therein.
6. Maeda, S., Matsushita, H., Mikami, Y. and Kisaki, T. *Agric. Biol. Chem. 42* (1978) 2177.
7. Bunting, J. W. *Heterocycles 14* (1980) 2015.
8. Maeda, S., Matsushita, H., Mikami, Y. and Kisaki, T. *Agric. Biol. Chem. 44* (1980) 1643.
9. Brandänge, S. and Lindblom, L. *Acta Chem. Scand. B 33* (1979) 187.
10. Nguyen, T.-L., Dagne, E., Gruenke, L., Bhargava, H. and Castagnoli, N., Jr. *J. Org. Chem. 46* (1981) 758.
11. Cervinka, O., Katritzky, A. R. and Swinbourne, F. J. *Collect. Czech. Chem. Commun. 30* (1965) 1736.
12. Glasoe, P. K. and Long, F. A. *J. Phys. Chem. 64* (1960) 188.
13. Harris, M. and Joule, J. A. *J. Chem. Res. (S)* (1978) 25.
14. Gorrod, J. W. and Jenner, P. *Essays Toxicol. 6* (1975) 35.

15. Maeda, S., Uchida, S. and Kisaki, T. *Agric. Biol. Chem. 42* (1978) 1455.
16. Hecht, S. S., Chen, C.-H. B. and Hoffmann, D. *Acc. Chem. Res. 12* (1979) 92.
17. Long, K. R., Long, R. C. and Goldstein, J. H. *J. Magn. Reson. 8* (1972) 207.
18. Späth, E. and Bretschneider, H. *Ber. Dtsch. Chem. Ges. 61* (1928) 327.
19. Bloom, M. S., Breslow, D. S. and Hauser, C. R. *J. Am. Chem. Soc. 67* (1945) 2206.
20. Bielawski, J., Brandänge, S. and Lindblom, L. *J. Heterocycl. Chem. 15* (1978) 97.
21. Gitel, S. N. and Gutsche, C. D. *J. Am. Chem. Soc. 97* (1975) 3900.

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