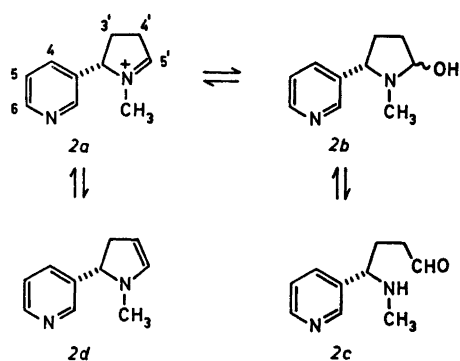# Synthesis, Structure and Stability of Nicotine $\Delta^{1'(5')}$

Iminium Ion, an Intermediary Metabolite of Nicotine

SVANTE BRANDÄNGE and LARS LINDBLOM

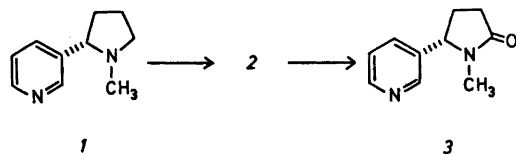Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

The crystalline iminium salt *5* was synthesised from (−)-cotinine (*3*) and its structure and stability in aqueous solutions of varying pH were investigated with the aid of NMR spectroscopy. The iminium form *2a* was the only form observed in freshly prepared, acidic or neutral solutions, and the carbinolamine *2b* was the only form observed in strongly alkaline solutions; no time-dependence was seen in the latter case. In weakly alkaline solutions ($7.8 \lesssim pD_c \lesssim 8.9$), *2a* was observed together with *2b*. After some time, the dimer *6* appeared in all except the strongly acidic and the strongly alkaline solutions. In neutral and weakly alkaline solutions, *6* was later accompanied by other new compounds.
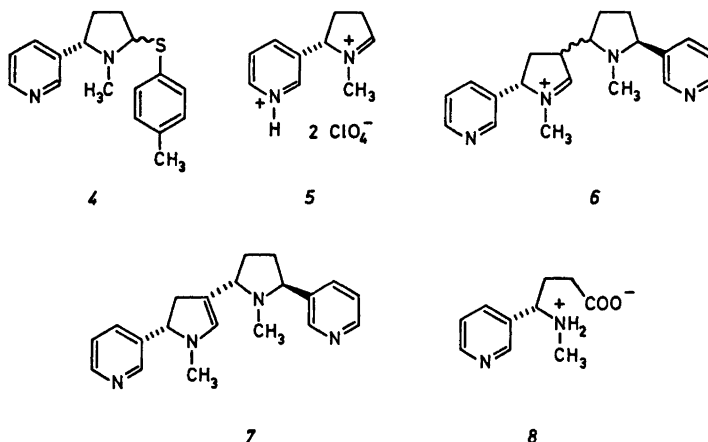

The major pathway for the metabolism of (−)-nicotine (*1*) in man involves the formation of (−)-cotinine (*3*).[1,2] There is good evidence that the conversion of *1* into *3* proceeds via an inter-

mediate (*2*); this has been formulated as an iminium ion (*2a*),[3] as a carbinolamine (*2b*),[4] or as an amino aldehyde (*2c*).[5] According to classical theory, these forms are assumed to be in equilibrium in aqueous solutions.[6] The presence of the forms *2a* and *2c* has been demonstrated by derivatisation,[3,4] but no quantitative measurements have been performed. Previous attempts to isolate *2* in its base form

have failed because of the instability of the compound.[4,7,8]

To elucidate the structure of *2* in aqueous solutions, and also for metabolism studies, we synthesised the iminium salt *5*, starting from *3*. Reduction of *3* with sodium dihydro bis(2-methoxyethoxy)aluminate, followed by the addition of *p*-methylbenzenethiol and subsequent purification on alumina gave the *N,S*-acetal *4*, free from *1* and *3*. A ¹H NMR spectrum showed that the two stereoisomers of *4* were formed in approximately equal amounts. Treatment of more or less purified *4* with anhydrous perchloric acid in dichloromethane afforded the crystalline diperchlorate *5* which could be recrystallised from methanol. This new quenching technique involving *p*-methylbenzenethiol was applied since the direct quenching with perchloric acid proved unsatisfactory. Reduction of *5* with sodium borohydride in methanol gave only nicotine (TLC, GLC), the optical rotation of which showed that *5* was optically pure.

4   *5*   *6*

7   *8*

STRUCTURE IN AQUEOUS SOLUTIONS

Since there seemed to be nothing published about the effect of pH on the ring — chain tautomeric, and related, equilibria of 3,4-dihydro-2*H*-pyrrolium salts, such a study of *5* was undertaken.

A 0.025 M solution of *5* in D_2O showed $pD_c = 2.75$ (pH meter reading $+0.40^9$). The ^1H NMR spectrum of this solution displayed signals at δ 3.46 (*N*-CH$_3$ group) and δ 5.83 (H-2'). These values are higher than those expected from *2b* or *2c* but the former value is close to that of the *N*-CH$_3$ group in 1,5-dimethyl-3,4-dihydro-2*H*-pyrrolium perchlorate in TFA solution (δ 3.56).[10] Five protons are resonating in the low-field region of the spectrum. Four of them are the pyridyl protons and the fifth, which is partially obscured by H-2 and H-6, is ascribed to H-5' in *2a* (δ 8.93). Protons of this type generally fall into the δ 7.5 — 10.0 region.[11] A Partially Relaxed Fourier Transform (PRFT) ^{13}C NMR spectrum of a strongly acidic solution of *5* in D_2O also showed that the iminium ion *2a* is the main species in this solution. The signal at 186.8 ppm was ascribed to C-5'.

The ^1H NMR spectrum of a freshly prepared 0.025 M, neutralised solution of *5* in D_2O ($pD_c = 7.5$) displayed only one signal from the *N*-CH$_3$ group (δ 3.41), and a signal from H-5' at δ 8.93, the latter now being separated from the signals of H-2 and H-6. This spectrum thus showed that the iminium form *2a* is also the main species at pD_c 7.5, and that the content of other species (*2b*, *2c*, *2d*) must be low (< 5 %).

^1H NMR investigation of freshly prepared 0.025 M alkaline solutions revealed that a structural change takes place between approximately pD_c 7.8 and 8.9. In going from neutral solutions to increasingly alkaline ones, a new *N*-CH$_3$ singlet appeared at δ 2.19 when pD_c 7.8 was reached and this new singlet then gradually replaced the iminium methyl singlet at δ 3.41 so that at pD_c 8.9 only that at δ 2.19 was present. The emerging form must be *2b* (see below). The NMR measurements on these weakly alkaline solutions were often complicated by turbidity development and concomitant broadening of the signals. Another complication was decreases in pD_c during the NMR measurements. Use of phosphate buffer, even at low concentration, was unsuitable since it caused a more rapid turbidity development. It seems reasonable to assume that the turbidity was due to the formation of *7* (see below).

A further increase of pD_c up to 13.4 caused only small changes in the spectrum. The ^1H and ^{13}C NMR spectra were compatible both with *2b* and with the hydrate of *2c*, but not with *2d*. Thus, the characteristic signals from the C-5' methine group were found at δ 4.70 in the ^1H NMR spectrum and at 90.2 ppm in the ^{13}C NMR spectrum. The former signal was safely observed as a discrete entity only after an up-field transfer of the HDO signal (to δ 4.36) by heating to 70 °C. Of the two alternatives mentioned, the hydrate of *2c* is less likely for several reasons. In aqueous solutions of aldehydes such as propanal and butanal the equilibrium concentration of free aldehyde
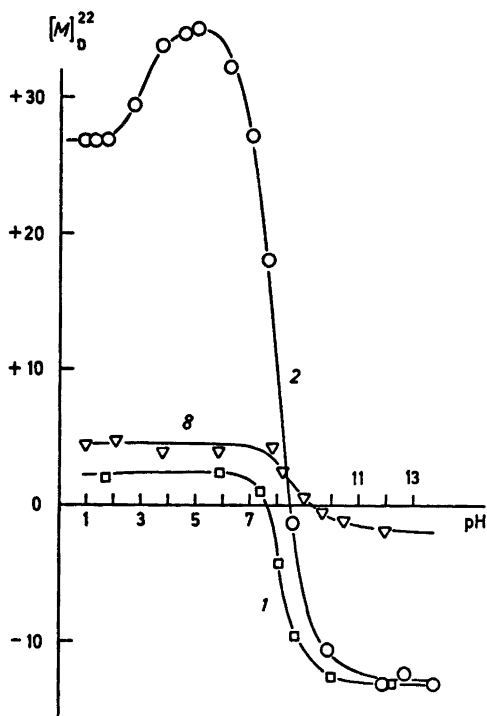
Fig. 1. Molecular rotations of compounds *1*, *2* and *8* versus pH.

is higher than that of the hydrate and signals from both species are seen in the ^{1}H NMR spectra of the solutions.[12,13] No signals from either the hydrogen or the carbon of the aldehyde group in *2c* were observed. Furthermore, owing to the stability of the solutions (see below), no appreciable amount of free aldehyde can be present. Other evidence is the fact that the $[M]_D$ of *2* in the strongly alkaline region is close to that of *1* but clearly different from that of *8* [14] (Fig. 1).

STABILITY OF THE SOLUTIONS

As mentioned above, the weakly acidic, neutral, and weakly alkaline solutions were investigated without delay after being prepared, the reason being the low stability of the solutions. Reasonably concentrated aqueous solutions containing the iminium ion *2a* are stable only at pH $\lesssim 1$. In less acidic solutions, the dimeric ion *6* is formed, this being easily detected and quantified by the *N*-CH$_3$ singlet

at δ 2.72. A 0.1 M solution of *5* in D$_2$O (22 °C, pD$_c$ 2.6) contained approximately 10 % of *6* after 25 min, 27 % after 3 h, and 40 % after 5 h. This type of dimerisation (and even trimerisation) has previously been observed in studies of the oxidation of 1-methylpyrrolidine with mercury (II) acetate [15] and the reduction of 2-pyrrolidones with lithium aluminium hydride.[16] The content of *6* seemed to approach a maximum level of approximately 40 %, indicating that the dimerisation reaction is reversible (*cf.* Ref. 15). The same equilibrium mixture could also be obtained by starting from *7* (see below) and adjusting the pD$_c$ to 2.6. In 0.03 M solution the equilibrium content of *6* was approximately 25 %.

In neutral solution (pD$_c$ 7.5), the formation of *6* was faster and after some time new compounds with unknown structures were formed. The ^{1}H NMR spectrum of a solution which had been left at 23 °C for six days contained at least six peaks which could be ascribed to *N*-CH$_3$ groups and the content of *2a* had decreased to approximately 20 % (integration over the δ 8.93 signal).

A striking increase in stability occurs in going to strongly alkaline solutions. No new *N*-CH$_3$ signals appeared over a period of one week at pD$_c$ 13.4 (23 °C). A similar effect of pH on the stability has been noted for 2-carboxy-1-methyl-3,4-dihydro-2*H*-pyrrolium chloride, but the structure of the solute was not studied.[17]

When a solution of *5* was made alkaline (pH 10−11) and then extracted with dichloromethane, *7* was obtained in a reasonably pure state (TLC, NMR) and the IR spectrum (CHCl$_3$) of the extracted material was devoid of carbonyl absorption peaks (*cf.* Ref. 7). Only two intense signals were found in the *N*-CH$_3$ region of the ^{1}H NMR spectrum, indicating that one of the two possible stereoisomers was strongly predominating (> 80 %), probably the *trans* isomer (*7*). Catalytic hydrogenation gave dihydro products which formed two peaks on GLC. The stereostructures of these products have not been further investigated.

CONCLUDING REMARKS

The iminium salt *5* is readily transformed into *3* in high yields by the action of guinea-pig

liver extract (140 000 g supernatant), but only in low yields by using washed microsomes.[18] This shows that the transformation is mediated mainly, if not solely, by a soluble enzyme. The nature of this enzyme is under investigation.

The content of *2b* at the pH of cytoplasm (7.4) is of interest in studying the metabolism of *2* into *3*. From the molecular rotation curve (Fig. 1), a content of approximately 25 % can be calculated if contributions to the rotation from species other than *2a* and *2b* are neglected and if it is assumed that the maximum (+ 35°) and minimum (− 13°) rotations represent the $[M]_D$ values of *2a* and *2b*, respectively. Similar measurements in 0.2 M phosphate buffers indicated only a 10 % content of *2b* at pH 7.4. These figures are probably the best obtainable since ¹H NMR measurements proved to be of little use in this respect (see above). The most informative ¹H NMR spectrum was obtained from a measurement on an unbuffered and dilute (0.025 M) solution which was investigated within 3 min after it had been prepared. During this time the pD_c fell from 7.85 to 7.70 and *2b* was detected as a minor form, but no accurate integration was possible.

EXPERIMENTAL

Thin-layer chromatography was run on DC-Fertigplatten (Merck) Kieselgel 60 F_{254} and Aluminiumoxid 60 F-254 (Typ E). Direct inlet mass spectra were recorded on an LKB 2091 spectrometer operated at 70 eV. ¹H NMR spectra were recorded on a Varian XL-100 or a JEOL JNM-FX 100 instrument using sodium 3-(trimethylsilyl)propanesulfonate or TMS as internal references in D_2O or $CDCl_3$, respectively. PRFT ¹³C NMR spectra were obtained on a Varian XL-100-12 WG spectrometer equipped with an S-124 XL FT accessory using the pulse sequence $(180° − t − 30°)_n$. The instrument was operated at 25.16 MHz and controlled *via* a Varian 620 L 16K computer. The samples were in acidic or alkaline aqueous solutions and D_2O in a coaxial capillary was used for the locking. Chemical shifts were calibrated from internal dioxane (67.4 ppm). Neutral and weakly alkaline solutions of *5* in D_2O were prepared at 0 − 5 °C by dissolving *5* in D_2O and then adding $NaOD/D_2O$. The ¹H NMR spectra were then run at 23 °C within 20 min. Measurements of pH were performed using a PHM 62 Standard pH meter (Radiometer, Copenhagen, accuracy 0.01 unit). The 0.010 M solutions needed for the study of $[M]_D$ as a function of pH were prepared by dissolving *5* in H_2O and

then adding conc. sulfuric acid or strong aqueous NaOH. The measurements were carried out on a Perkin-Elmer 241 polarimeter within 5 min. Elemental analyses were carried out at Alfred Bernhardt Mikroanalytisches Laboratorium, Elbach über Engelskirchen, Federal Republic of Germany. In discussing the spectra of *2a − 2c* the numbering given in the formula of *2a* has been kept throughout.

(−)-Cotinine (3) was synthesised from (−)-nicotine according to the literature procedure.[19] It showed $[\alpha]_D^{25}$ − 20.2° (*c* 5.7, methanol); lit.[20] value: $[\alpha]_{546}^{30}$ − 19.85° (*c* 5.59, methanol).

N,S-Acetal 4. A 70 % solution (3.0 g) of sodium dihydrobis(2-methoxyethoxy)aluminate (10 mmol) in benzene was diluted with ether (50 ml) and the resulting solution was added dropwise (30 min) under an atmosphere of nitrogen to a stirred and ice-cooled solution of (−)-cotinine (3.0 g, 17 mmol) in ether (75 ml). The mixture was then allowed to reach room temperature and stirring continued for 18 h. An additional 0.5 g of the reducing agent was added and the mixture stirred for 1 h. After cooling with ice-water, a solution of *p*-methylbenzenethiol (0.3 g, 24 mmol) in ether (30 ml) was added during 10 min. The mixture was allowed to react for 10 min and was then kept at − 18 °C for 1 h. The clear solution was decanted, the solvent evaporated and the remaining material purified on neutral alumina (5 × 30 cm), using light petroleum (40 − 60 °C) − ether − methanol (40:20:1.5) as eluent. The major contaminant showed a lower mobility (R_F 0.29) than did *4* (R_F 0.61) on TLC using aluminium oxide plates and this solvent system. ¹H NMR ($CDCl_3$): δ 8.6 − 8.4 (m, 2H, H-2, and H-6); 7.65 − 6.9 (m, ≈6H); 5.5, 4.06, 3.83, 3.51 (m, each ≈0.5 H, H-2′ and H-5′ in *cis* and *trans* isomers); 2.33, 2.32, 2.23 (s, ≈6 H, N-CH_3 and S-CH_3); 2.7 − 1.8 (m, ≈4 H). MS (direct inlet) at room temperature showed peaks from *p*-methylbenzenethiol and its corresponding disulfide (m/e = 246, 124, 123 and 91) but also fragments from the nitrogen-containing moiety (m/e = 160, 159, 82). On heating to 120 °C, peaks at m/e = 320, 319, 215, 200, 199 and 185 appeared, probably arising from the dimer *7* (see below).

Diperchlorate 5. Anhydrous perchloric acid in methylene chloride was prepared by mixing a 70 % aqueous solution of perchloric acid (6.0 g) with acetic anhydride (10.4 g) and methylene chloride (60 ml) at − 18 °C (20 h). The major part of this solution (60 ml) was added under an atmosphere of nitrogen to the eluate containing *4*, cooled to − 30 °C. After being stirred for 5 min, the solvent was decanted and the precipitate was washed with cold methanol and then with dry ether to give 2.2 g of crude *5*. Another 0.8 g could be obtained from the mother liquor; total yield: 49 % (calc. on *3*). Recrystallisation from methanol containing anhydrous perchloric acid afforded an analytical sample, which on rapid

heating (4 °/min) melted at 228 − 232 °C. (dec., sealed tube), $[\alpha]_D^{25}$ + 77° (c 1.3, water). Found: C 33.10; H 3.77; N 7.73. Calc. for $C_{10}H_{14}Cl_2N_2O_8$: C 33.26; H 3.91; N 7.76. IR (KBr): 1680 cm^{-1}. ^{1}H NMR (10 % DCl in D_2O): δ 9.2 − 8.8 (m, 3 H), 8.8 − 8.6 (m, 1 H), 8.4 − 8.1 (m, 1 H), 5.86 (broad t, 1 H), 3.8 − 2.2 (7 H, including a 3 H singlet at 3.52). ^{13}C NMR (10 % HCl in H_2O): 186.8 (C-5'), 147.3 (C-4), 143.6 (C-6), 142.1 (C-2), 136.0 (C-3), 129.6 (C-5), 73.4 (C-2'), 40.6 (N-CH$_3$), 36.5 (C-4'), 29.8 (C-3') ppm.

NMR spectra of 2b. ^{1}H NMR (D_2O, pD$_c$ 13.4, 70 °C): δ 8.56 − 8.40 (H-2 and H-6) 7.97 − 7.80 (H-4), 7.57 − 7.38 (H-5), 4.70 (s, H-5'), 3.74 (t, H-2'), 2.5 − 1.7 (5 H, including a 3 H singlet at 2.21). ^{13}C NMR (H_2O, pH 12.5): 149.3, 148.7, 139.0 (C-3), 137.6, 125.2, 90.4 (C-5'), 65.1 (C-2'), 34.9, 32.6 (C-4', disappears in D_2O-NaOD), 31.4 ppm.

Preparation of 5 from less purified 4. The decanted clear solution obtained after quenching with *p*-methylbenzenethiol in the preparation of *4* was rapidly filtered through a column of neutral alumina (40 g), rinsing with ether. The solution (60 ml) of perchloric acid described above was added at − 30 °C to the eluate, the precipitate was allowed to settle and the solvent was then decanted. Methanol (50 ml) containing a little of the perchloric acid solution was added to the precipitate, the crystals were filtered off and washed first with cold methanol − perchloric acid and finally with dry ether. After a second crop of 0.4 g had been obtained from the mother liquor, the total yield was 3.5 g (67 %).

Reduction of 5 to nicotine. A suspension of *5* (200 mg) in methanol (5 ml) was treated with sodium borohydride (50 mg). After being stirred for 1 h (22 °C), the reaction mixture was concentrated, and an aqueous solution of sodium carbonate was added. The nicotine was extracted with ether and purified by preparative GLC on a 3 % JXR column (0.3 × 180 cm, 110 °C). $[\alpha]_D^{22}$ − 165° (c 0.39, acetone); authentic (−)-nicotine showed $[\alpha]_D^{22}$ − 169° (c 0.44, acetone).

Preparation of 7. Cotinine was reduced with sodium dihydrobis(2-methoxyethoxy)aluminate as described above and the reaction mixture was poured into 1 M hydrochloric acid. The mixture was made alkaline (pH 10 − 11) and extracted with dichloromethane, and the extract was then dried (Na_2SO_4). TLC on silica gel with methanol − ethyl acetate (5:4) showed a major spot at R_F 0.73. ^{1}H NMR (CDCl$_3$): δ 8.7 − 8.35 (m, 4 H), 7.85 − 7.5 (m, 2 H), 7.35 − 7.05 (m, 2 H), 5.91 (m, 1 H), 3.94 (d of d, 1 H), 3.5 − 1.4 (14 H, including 3 H singlets at 2.43 and 2.12). MS (*m/e*, relative intensity): M$^+$= 320(40), 319(50), 215(30), 200(46), 199(46), 185(47), 161(75), 160(51), 159(36), 82(67), 42(100).

Reduction of 7. The product obtained on catalytic hydrogenation (Pt/C) of *7* (113 mg) in isooctane − dioxane showed two spots on TLC (R_F = 0.55 and 0.49) using methanol − ethyl acetate (5:4) as solvent and two partially resolved peaks on GLC (3 % JXR, 0.2 × 180 cm, 225 °C). ^{1}H NMR (CDCl$_3$): δ 8.7 − 8.3 (m, 4 H), 7.8 − 7.5 (m, 2 H), 7.4 − 7.1 (m, 2 H), 3.5 − 1.4 (18 H, including N-CH$_3$ singlets at 2.15 and 2.13). MS (direct inlet): M$^+$= 322(5), 291(7), 217(24), 187(22), 186(26), 162(34), 161(100), 130(80). On GLC-MS, similar spectra were obtained for both peaks.

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