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THE SYNTHESIS OF 1'-N-METHYL-2'-OXOANABASINE, AN ANALOGUE OF COTININE

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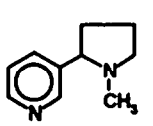
Abstract. The synthesis of 1'-N-methyl-2'-oxoanabasine, a hepatic microsomal metabolite of 1'-N-methylanabasine is described. Treatment of 1'-N-methylanabasine with bromine yielded an intermediate geminal dibrominated derivative, the structure of which has been confirmed by DEPT and COSY spectra. Treatment of the intermediate with zinc yielded the required compound.

The metabolism of nicotine, **1**, and related tobacco alkaloids is a topic of considerable research activity due to the potential insight into the adverse effects of smoking that such studies may provide. A major route of metabolism of nicotine involves cytochrome P450 mediated α-C-oxidation to yield the corresponding iminium ion, which subsequently undergoes oxidation to yield cotinine, **4**. We have previously reported on the metabolism of the minor tobacco alkaloid anabasine, **2**[1,2]. Extension of these studies to 1'-N-methylanabasine, **3**[3,4] necessitated the synthesis of 1'-N-methyl-2'-oxoanabasine, **5**, the lactam corresponding to cotinine, for use as an analytical reference compound. Cotinine, **4**, was first synthesised by Bowman and McKennis[5] by treatment of nicotine with bromine in acetic acid. These workers proposed that the reaction proceeded via the formation of a dibrominated intermediate, 3',5'-dibromocotinine hydrobromide perbromide, **8**. Later, investigators[6] concerned



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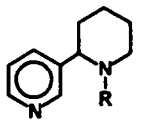
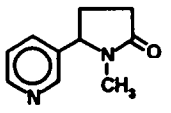


2 R=H
3 R=CH_3



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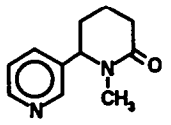

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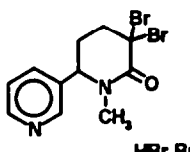
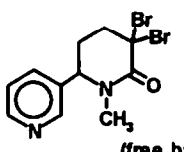
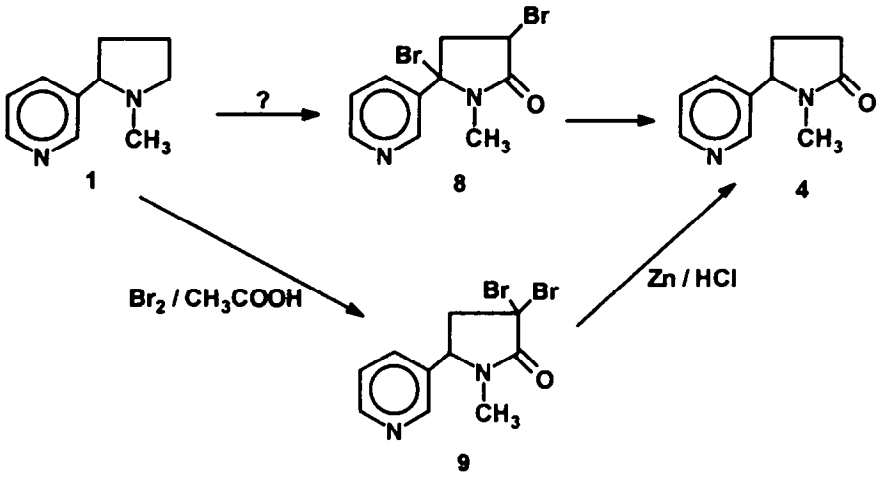


6 HBr Br_2



7 (free base)

with the elucidation of the mass spectral fragmentation pathways of nicotine and related compounds, indicated that the brominated intermediate was the geminal dibromolactam, 9.



Schematic representation of the synthesis of cotinine.

In this communication we report the preparation of 1'-N-methyl-2'-oxoanabasine, **5**, from 1'-N-methylanabasine, **3**, and investigate the above synthetic procedures. Thus treatment of **3** with seven equivalents of bromine in aqueous acetic acid (80% v/v) yielded the required compound **6**. Examination of the electron impact mass spectrum (EI-MS) of the intermediate yellow precipitate **6** yielded a pattern of peaks in the molecular ion region at m/z $(M-H)^+$ 345:347:349 in the ratio 1:2:1, indicative of a dibrominated product. The EI-MS spectrum for the product, **7**, obtained following alkaline extraction of **6** yielded a spectrum with two peaks of mass m/z 268:270 of 1:1, consistent with the molecular ion of a monobrominated compound. Implicating the loss of one atom of bromine during the extraction procedure and/or the mass spectral analysis. In order to elucidate the structure of the synthetic intermediate **6** proton nuclear magnetic resonance (^{1}H-NMR, 300 MHz)[7], ^{13}C-NMR with DEPT (Distortion enhancement by polarisation transfer) and ^{1}H-^{13}C COSY (250 MHz)[7] spectra were determined.

The ^{1}H-NMR spectrum of **6** indicated that the signal due to the protons of the methyl group shifted downfield (2.80ppm) compared to the signal of the methyl protons of **3** ; a broad signal between 9 and 10ppm revealed the presence of a protonated pyridine nitrogen. The integration of the aliphatic region was consistent

with a dibrominated compound, but the resolution was not sufficient to determine the position of the bromine atoms in the molecule.

Examination of the DEPT spectrum of **6** revealed the presence of three quaternary carbon atoms corresponding to the C=O (164ppm), the C3 (140ppm) of the pyridine ring and an additional carbon atom (60ppm). Thus providing evidence that the intermediate product was a geminal substituted dibromolactam. Following examination of the ^{1}H-^{13}C COSY spectrum of **6**, it was deduced that the position of the substitution was possibly the 3'. The relaxation/exchange time, however, for this salt was too short for two dimentional work (^{1}H-^{1}H COSY). Examination of the corresponding proton, carbon and DEPT-NMR spectra of **7** was consistent with the above observations. The ^{1}H-^{1}H COSY spectrum indicated that the 6' proton (5.00ppm) was coupled to the protons at 2.00 and 2.50ppm. The protons at 2.00 and 2.50ppm were coupled to the protons at 2.80 and 2.95ppm. Therefore the protons at 2.00 and 2.50ppm were at the 5' carbon atom. The protons at 2.80 and 2.95ppm were coupled to the 5' protons but not to the 6' and therefore they occupy the 4' position. Hence bromine substitution must occur at the 3' position. In the case of cotinine, **4**, the position of bromine substitution in the synthetic intermediate was elucidated by mass spectral analysis and the structure was concluded to be **9**. To the best of our knowledge the full characterisation of **9** by modern spectroscopic techniques has not been previously reported. Thus the spectroscopic characterisation of **6** confirms for the first time, the nature of the intermediates in this synthetic procedure.

Treatment of **6** in hydrochloric acid suspension with zinc powder yielded the required lactam **5**. This compound has subsequently been identified as a major metabolite of **3** following incubation with hepatic microsomes from various mammalian species[3,4]. The formation of this compound **5** indicates the potential involvement of an iminium ion species in the metabolism of **3**. Additional evidence for the involvement of an iminium ion species in the *in vitro* metabolism of **3**, has been obtained using the radioactive cyanide trapping technique[8]. Further investigations are in progress in an attempt to isolate or trap this reactive intermediate.

Experimental

1'-N-Methylanabasine, **3**, was prepared from anabasine as reported previously[4], all other materials were reagent grade and purchased from British Drug House, Poole, Dorset, UK.

Bromine (8ml, 156mmoles) in aqueous acetic acid (28ml, 80% v/v) was added dropwise to a solution of **3** (4.3g, 26mmoles) in aqueous acetic acid (61ml, 80% v/v) over a 2 hr period. The mixture was left stirring for an additional 1 hr at room temperature, after which it was heated with continuous stirring up to 85°C. The solution was allowed to cool slowly to room temperature and a yellow powder (m.p. 178-182°C)[9] separated (7g, 47% yield based on the formula

$C_{11}H_{12}N_2OBr_2 \cdot HBr \cdot Br_2$, 1'-N-methyl-2'-oxo-3',3'-dibromoanabasine hydrobromide perbromide, **6**) and collected by filtration. Elemental analysis found: C, 22.55; H, 2.23; N, 4.67; Br, 67.73. $C_{11}H_{12}N_2OBr_2 \cdot HBr \cdot Br_2$ requires: C, 22.34; H, 2.54; N, 4.74; Br, 67.68%. Positive result for the presence of ionic bromine was obtained by the addition of silver nitrate to a solution of **6** in water:methanol (50:50 v/v). ^{1}H-NMR (300 MHz, d_6-DMSO) δ in ppm: 9.90 (1H, broad singlet, pyridine N^+-H), 8.90 (1H, d, J=5.56 Hz, H6), 8.82 (1H, s, H2), 8.32 (1H, d, J= 8.12 Hz, H4), 8.12 (1H, m, H5), 5.13 (1H, t, J=5.57 Hz, H6'), 2.87 (2H, m, H4'), 2.73 (3H, s, N-CH_3), 2.46 (1H, m, H5'), 1.96 (1H, m, H5'). ^{13}C-NMR (62.89 MHz, d_6-DMSO) δ in ppm: 164.1 (C=O), 143.9 (C4), 141.3 (C6), 140.4 (C2), 140.1 (C3), 127.8 (C5), 60.0 (C3'), 59.8 (C6'), 42.0 (C4'), 35.2 (CH_3), 29.2 (C5'). ^{13}C-DEPT (CH_3, CH: -ve; CH_2: +ve; C(Q): 0): C4, C6, C2, C2', N-CH_3: -ve; C4', C5': +ve; C=O, C3, C3': 0. ^{1}H-^{13}C COSY (δ in ppm): 8.90-141.3, 8.82-140.4, 8.32-143.9, 8.12-127.8, 5.13-59.8, 2.87-42.0, 2.73-35.2, 1.96-29.2. EI-MS: $(M-H)^+$ 345:347:349, 5.8:10.1:5.2%. FAB-MS: $(M+H)^+$ 347:349:351, 0.9:1.7:1.1%.

It was decided to further purify **6**: this salt (1.1g) was added to water (~2ml) , the pH of the suspension was adjusted to pH 9 using ammonium hydroxide and subsequently extracted with chloroform to afford 400mg of the free base, **7**[10] (m.p. 91-94°C). ^{1}H-NMR (250 MHz, d_6-DMSO) δ in ppm: 8.58 (1H, dd, J=1.61, 3.07 Hz, H6), 8.45 (1H, d, J=2.00 Hz, H2), 7.58 (1H, dd, J= 1.98, 4.01 Hz, H4), 7.50 (1H, m, H5), 4.97 (1H, t, J=5.77 Hz, H6'), 2.88 (2H, m, H4'), 2.74 (3H, s, N-CH_3), 2.42 (1H, m, H5'), 1.95 (1H, m, H5'). ^{13}C-NMR (62.89 MHz, d_6-DMSO) δ in ppm: 163.9 (C=O), 148.9 (C6), 147.7 (C2), 135.8 (C3), 134.0 (C4), 124.0 (C5), 60.6 (C3'), 60.5 (C6'), 42.4 (C4'), 34.9 (N-CH_3), 29.7 (C5'). ^{13}C-DEPT (CH_3, CH: +ve; CH_2: -ve; C(Q): 0): C6, C2, C4, C5, C2', N-CH_3 : +ve, C4', C5': -ve, C=O, C3, C3': 0. ^{1}H-^{13}C COSY (δ: in ppm): 8.58-148.9, 8.45-147.7, 7.58-134.0, 7.50-124.0, 4.97-60.5, 2.93-42.4, 2.83-42.4, 2.74-34.9, 2.42-29.7, 1.95-29.7. EI-MS: highest observed mass m/z 268:270, 0.6:0.8%. FAB-MS: $(M+H)^+$ 347:349:351, 13.9:41.8:7.8%. The ^{1}H-^{1}H COSY spectrum is presented in Figure 1.

1'-N-Methyl-2'-oxo-3',3'-dibromoanabasine hydrobromide perbromide, **6**, (4.6g) was subsequently reduced with zinc (3.5g) and concentrated hydrochloric acid (3ml), over 10 minutes at 0°C and vigorous stirring. Excess zinc was removed by filtration, water was added to the filtrate and the pH adjusted to 9 with ammonium hydroxide. Extraction with dichloromethane (4 x 60 ml), followed by rotary film evaporation yielded 1.2 g of **5**. Ultraviolet (UV): maximum wavelength 260nm, infrared (IR): C=O 1625, N-CH_3 (lactam) 1575 cm^{-1} and elemental analysis found: C, 66.09; H, 7.41; N, 13.87. $C_{11}H_{14}N_2O \cdot 1/2H_2O$ requires: C, 66.30; H, 7.54; N, 14.07%. ^{1}H-NMR (250 MHz, d_6-DMSO) δ in ppm: 8.52 (1H, dd, J=1.57, 3.09 Hz, H6), 8.45 (1H, d, J=2.13 Hz, H2), 7.61 (1H, dd, J=2.00, 3.79 Hz, H4), 7.42 (1H, m, H5), 4.69 (1H, t,

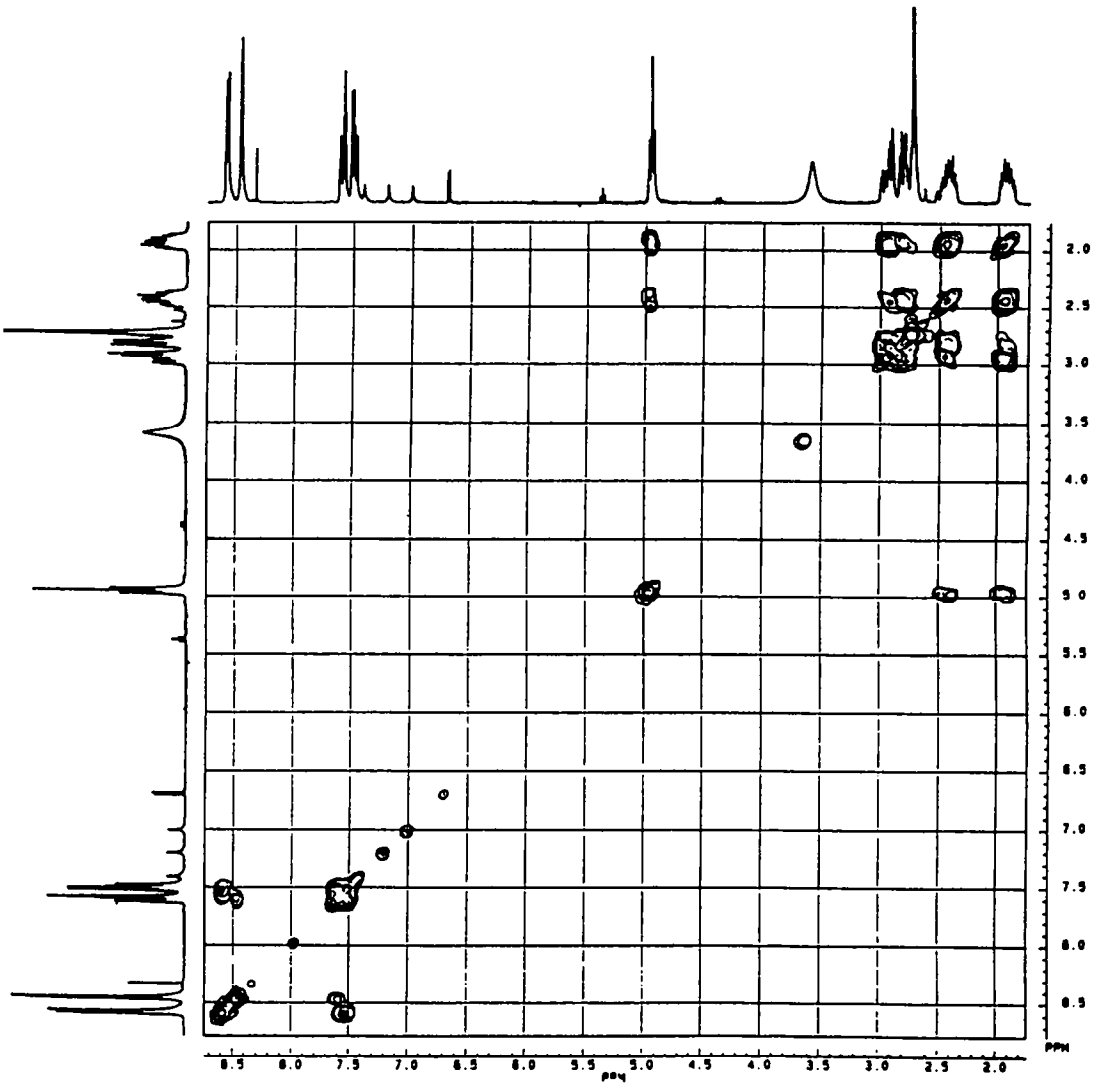


Figure 1. Proton-proton coorelation spectrum of 1'-N-methyl-2'-oxo-3',3'-dibromoanabasine.

J=5.29 Hz, H6'), 2.64 (3H, s, N-CH_3), 2.36 (2H, m, H3'), 2.16 (2H, m, H4'), 1.61 (2H, m, H5'). EI-MS: $(M)^+$ 190, 96.6% (35eV), 190, 48.8% (70eV). The perchlorate salt (m.p. 213-217°C) of **5** was prepared by adding dropwise a solution of perchloric acid in dichloromethane to a solution of **5** in dichloromethane, which was kept at -28°

C. Elemental analysis found: C, 45.29; H, 5.16; N, 9.50; Cl, 12.12. $C_{11}H_{14}N_2O \cdot HClO_4$ requires: C, 45.45; H, 5.20; N, 9.64; Cl, 12.20%.

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9 Melting points were determined by a Gallenkamp melting point apparatus and are reported uncorrected.

10 CD of 7 in methanol and acetonitrile confirmed that 7 has the (S)- configuration.

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