Synthesis and Binding Activity of 4-Azanicotine

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4-Azanicotine (**1**) has been synthesized by the cyclopropylimine rearrangement. A study of several Lewis acids as possible catalysts for the NMF-mediated cyclopropyl imine rearrangement of cyclopropyl-2-pyrazinylmethanone (**4**) indicated that both aluminum chloride and magnesium chloride are suitable catalysts, lithium bromide shows only a trace of activity and zinc chloride, titanium tetrachloride, and titanium tetraisopropoxide show no activity. Compound **1**, in which the aromatic ring is less basic than in nicotine, and 6-aminonicotine (**5**), in which the aromatic ring is more basic, have been compared with the binding properties of nicotine in the P2 rat brain preparation. Compound **1** binds with an affinity very similar to that of nicotine at two of the receptor sites, **5** binds at these sites but with a lower affinity. Unlike nicotine, **1** and **5** do not bind at the very high affinity 'upregulatory' site.

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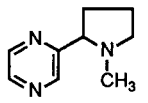
A number of studies have shown the importance of the pyridine ring to the reactivity of nicotine [1-9]. However, the effect of the basicity of the pyridine ring has not been directly assessed. One approach to a nicotine analog with a less basic aromatic ring is to replace the pyridine ring with the less basic pyrazine ring. The p*K*a's of the conjugate acids of these two rings are respectively 5.23 and 0.51. Replacement of the pyrazine ring is also a good choice because of the similarity in size between the CH and the N which replaced it. In this report we describe the synthesis and binding activity of 4-azanicotine (2-(1'-methyl-2'-pyrrolidinyl)pyrazine) (**1**), a compound in which the pyridine ring of nicotine has been replaced by a pyrazine ring.



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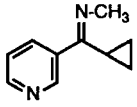

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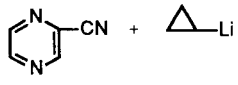

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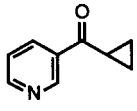

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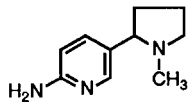

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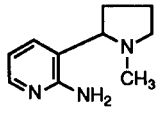

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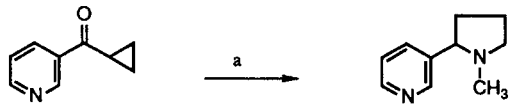
We found what appeared to be a fairly straightforward model synthetic route in the nicotine synthesis devised by Breuer [10]. This synthesis presumably proceeds by a cyclopropyl imine rearrangement. It is presumed that it is actually the imine **2** which undergoes the rearrangement. The cyclopropyl imine rearrangement has been used widely in a variety of syntheses [10-19] and has been studied extensively by R. V. Stevens. Generally, the reaction involves a separate step of imine synthesis, and the imine then rearranges to a pyrroline. The mechanism suggested by Stevens accounts for the fact that an acid catalyst is required for the rearrangement and that it is important that the catalyst possesses a nucleophilic counterion. Breuer's synthesis of nicotine is a slight variation of the usual cyclopropyl imine rearrangement. Normally, the cyclopropyl imine rearranges to the unsaturated pyrroline. However, in Breuer's synthesis, the end result of the one-pot rearrangement is the saturated pyrrolidine. An *in situ* reduction occurs in which formic acid is the reducing agent. Presumably a trace of water in the *N*-methylformamide causes some hydrolysis to occur. The formic acid would then be available for reduction and methylamine would be available for imine formation.

Scheme I



a. HCONHMe, $MgCl_2$, 180°, 24 hours

Cyanopyrazine (**3**), prepared by dehydration of pyrazine carboxamide, was the starting point for our synthesis of 4-azanicotine. The addition of cyclopropyl lithium to this nitrile was complicated by the poor solubility of cyanopyrazine in ether as compared to the solubility of 3-cyanopyridine. Substituting THF for ether did improve the solubility, but none of the desired product was obtained. It was eventually found possible to run the reaction in ether by cooling the solution to only -32° (rather than the -78° used by Breuer).

When the cyclopropyl-2-pyrazinylmethanone (**4**) thus obtained was subjected to the same rearrangement conditions as had been used for cyclopropyl-3-pyridinylmethanone in the synthesis of nicotine, none of the rearranged product was obtained. By decreasing the temperature from reflux (180-190°) to 155° and reaction time from 24 hours to 18 hours an optimum crude yield of 20-25% was obtained. Below 140° the rearrangement did not proceed, and above 170° no identifiable products were recovered.

The yield was disappointing, but not necessarily surprising. Cyclopropylphenylmethanone rearranges in 80% yield [20], whereas, cyclopropyl-3-pyridinylmethanone gives only 30% product [10]. Cyclopropyl-2-pyrazinylmethanone, with two nitrogens in the ring, gave a yield that was not unreasonable, when considering the trend shown by the other two examples mentioned. The nmr spectroscopy of the crude material indicated the presence of *N*-methylformamide and other impurities. The purification process eventually required column chromatography followed by preparative tlc, as described in the Experimental.

In order to obtain a more balanced picture of the importance of basicity of the six-membered ring to binding, it would also be important to have a compound that possessed a more basic aromatic ring. This requirement of greater basicity is satisfied by replacing the pyridine ring of nicotine by 2-aminopyridine (p*K*a of conjugate acid is 6.86). In a cursory examination, 2-aminopyridine might not seem as satisfactory as the aza anaolg from a steric perspective. However, in earlier studies of nicotine analogs [8,21-26] it was seen that substitution of methyl at the 6-position on the pyridine ring of both nicotine and *N*-(3-picolyl)pyrrolidine led to compounds of essentially unaltered biological activity in comparison with parent molecules. If this positional effect is fairly general, the 2-aminopyridine moiety is sterically (at least with regards to binding) not much different from pyridine. It might also be argued that 2-aminopyridine might not be a good replacement for pyridine because binding might take place at the amino group. This possibility can not be ruled out, but by analogy to the results with either protonation or alkylation of aminopyridines, it seems likely that the most probable point of binding would be the ring nitrogen. It has long been known that 2- and 4-aminopyridines are protonated and alkylated at the ring nitrogen. Initially, this result was explained on the basis of the possible existence of tautomeric forms [27-29]. Angyal and Angyal later attributed the results of 2-aminopyridine to enhanced nucleophilicity of the ring nitrogens [30]. Whatever the cause, the result argues for the ring nitrogen being the point of binding, if this is, indeed, a point of attachment in nicotine itself.

In an effort to improve the yield of the NMF-mediated cyclopropyl imine rearrangement of cyclopropyl-2-pyrazinylmethanone a brief study was made of the effect of Lewis acid catalysts on the reaction. Both magnesium chloride and magnesium sulfate have been used as catalysts in the formamide mediated cyclopropyl imine rearrangement [33,34], but only magnesium chloride has been reported in the NMF mediated reaction [20,35]. The original choice of magnesium chloride seems to have been a fortuitous one, rather than being based on any systematic exploration of catalysts. Breuer and Stein [33] had tried to synthesize arylcyclopropylcarbinyl amines from arylcyclopropyl ketones and formamide (Scheme II). When magnesium chloride, a reagent known to catalyze the Leuckhart reaction, was added, the major product was a 1-formyl-2-arylpyrrolidine. Thus magnesium chloride was employed in subsequent uses of this reaction. Since magnesium chloride was the only catalyst reported in NMF mediated cyclopropyl imine rearrangements, we have explored the effect of several Lewis acids catalysts on the rearrangement. The results are summarized in Table I. With zinc chloride as catalyst, unreacted ketone was recovered. With both titanium catalysts, some ketone was recovered, but there were also unidentified products. Lithium bromide promoted the rearrangement, but certainly would not be satisfactory replacement for magnesium chloride. The rearrangement proceeded with improved yields under milder conditions when aluminum chloride was used. The yield and reaction conditions shown for aluminum chloride in Table I are optimized. Results when reaction conditions were varied are shown in Table II. Aluminum chloride did not promote the reaction below 120°. Above 160° there was extensive decomposition of either product or reactant and yields decreased. When the reaction temperature was 140°, all the ketone had disappeared in 4 hours and yields were not improved by extending the reaction time.

6-Aminonicotine (**5**) was synthesized by the method of Chichibabin [31]. Since the 4-azanicotine synthesized for binding studies was racemic, natural (-)-nicotine was racemized [32] before being converted to 6-aminonicotine. The Chichibabin reaction on nicotine produces both 5-aminonicotine and 2-aminonicotine (**6**). The two isomers are easily separated on the basis of solubility. Although the 6-amino isomer was the desired compound for testing,

Scheme II

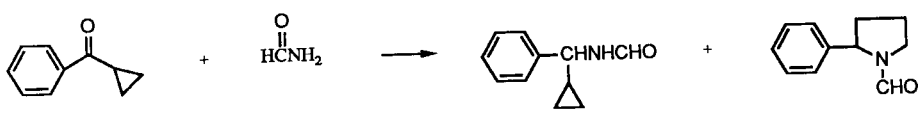


Table I

Effect of Various Lewis Acids on the Yield of the NMF-Mediated Cyclopropyl Imine Rearrangement of Cyclopropyl-2-pyrazinylmethanone [a]

Lewis Acid	% yield [b]
$MgCl_2$ [c]	23-25
$ZnCl_2$	---
$TiCl_4$	---
$Ti(O\text{-}i\text{-}Pr)_4$	---
LiBr	<5
$AlCl_3$ [d]	39

[a] Reaction temperature is 155° and reaction time is 24 hours unless otherwise stated. [b] Yield based on nmr analysis. [c] Reaction time 18 hours. [d] Reaction temperature 140°, reaction time 4 hours.

Table II

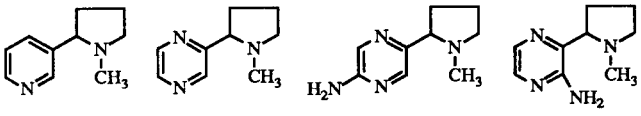
Optimazation or Reaction conditions for Aluminum Chloride Catalyzed Rearrangement of **4** to **1**

Temperature (°C)	Reaction Time (h)	% Yield [a] of **27**
50	48	---
80	24	---
100	24	trace
120	24	21
140	4	39
160	6	10

[a] Based on nmr integration.

Table III

K_D's of Nicotine and Analogues



1.4×10^{-8} 1.1×10^{-6}	2.5×10^{-8} 1.5×10^{-5}	2.1×10^{-6} 1.8×10^{-3}	2.3×10^{-5}

the 2-isomer was also isolated and subjected to binding assays for comparison purposes.

The procedure employed for the binding studies is described elsewhere [36]. Table III gives the K_D's calculated for these compounds and comparable ones for (±)-nicotine [37]. Compound **1** binds with affinity very similar to that of nicotine at two of the receptor sites, the high affinity site and a lower affinity site. However, **1** did not show binding at the very high affinity site (not given in the Table III), but seen with (±)-nicotine ($K_D = 4.9 \times 10^{-13}$) and (-)-nicotine ($K_D = 2.2 \times 10^{-11}$). The failure of the pyrazine analogue **1** to show binding to the very high affinity site may be a result of the concentration range not being wide enough or too few homogenate preparations (three in this case) being used for the study. Nicotine causes enhanced binding at lower concentrations ($< 10^{-8}$ *M*). It is believed [36,38] that this is because nicotine binds to a site that somehow modifies other receptor sites, leading to enhanced binding. These nicotinic receptors are referred to as 'upregulatory'. Compound **1** did not show significant binding to the 'upregulatory' site. Although the inhibition curve for **1** showed a small range of enhanced binding at *ca.* 10^{-11} *M*, statistical analysis indicated this was nonsignificant.

6-Aminonicotine (**5**) binds to two sites, but with lower affinity than **1**. Although **5** seemed to induce enhanced binding over a wide range, statistical analysis indicated that this enhancement is nonsignificant. Compound **5**, whose six-membered ring is more basic than the pyridine ring of nicotine, binds to rat brain nicotinic receptors with lower affinity than nicotine. On the other hand, **1** seems to have a binding affinity very similar to that of nicotine, although it apparently does not bind to the very high affinity site. One interpretation is that the change from the pyridine ring of nicotine to the less basic pyrazine ring of **1** does not appreciably affect the binding at the two sites, but that the more basic pyridine ring is necessary for binding at the very high affinity site. From our results, it would appear that there may be an optimum basicity range for binding and that **5** falls outside that range. With regard to assessing the steric importance of the 6-amino group, our results with 2-aminonicotine (**6**) do confirm previous observations that substitution at the 6-position of the pyridine of nicotine has less effect on inherent nicotine activity than does substitution at the 2-position.

EXPERIMENTAL

Melting points (mp) were recorded on a Fisher-Johns melting point apparatus and are uncorrected. Infrared spectra (ir) were recorded on Beckman IR20A-X, Beckman Acculab 1, Perkin-Elmer 1420 ir, or Perkin-Elmer 1320 Spectrophotometers as KBr pellets, solutions in carbon tetrachloride or deuteriochloroform or as neat liquids. Mass spectra (ms) were recorded on a Hitachi Perkin-Elmer RMU-6E mass spectrometer or VG Instruments ZAB-2F Micro Mass spectrometer by Mass Spectrometry Center, Department of Chemistry, University of Kentucky. Carbon 13-nmr spectra were recorded on a Varian XL-200 spectrometer at 49.5 MHz in the Nuclear Magnetic Resonance Center, Department of Chemistry, University of Kentucky. Proton nmr spectra were recorded on a Varian EM-390 90 MHz or Varian XL-200 200 MHz spectrometers. The chemical shifts are given on the δ-scale (ppm) and were referenced to internal tetramethylsilane (TMS). Thin layer chromatography (tlc) was conducted on Analtech silica gel GF plates (0.25 mm) or Machery-Nagel polygram silica gel G/UV plates (0.25 mm). Preparative plates were made from Machery-Nagel silica gel F254. Column chromatography was performed on Machery-Nagel silica gel 60. Elemental

analyses were performed by Atlantic Microlab, Inc., Atlanta, Georgia. Starting materials and solvents used in this work were reagent grade in 98% or higher purity and used without further purification or purified by standard literature procedures. Optical rotation was determined in an O. C. Rudolph and Sons, Inc., model 63 polarimeter with a sodium lamp. Hydrogenations were performed in a Parr apparatus.

Cyclopropyl-2-pyrazinylmethanone (**4**).

To 1.35 g (0.195 mole) of lithium wire in small pieces (*ca.* 3 mm x 1 cm) in 50 ml anhydrous ether under argon was added 11.5 g (0.0950 mole) cyclopropylbromide in 15 ml anhydrous ether. The addition rate was such as to maintain a gentle reflux throughout the addition. The resulting orange/brown cyclopropyllithium mixture was stirred an additional 4 hours at room temperature before being used.

A 500-ml round-bottomed, two-necked flask was equipped with a stirrer bar, low temperature thermometer and a septum for argon inlet and addition of reactants. The flask was cooled to -30°, and 100 ml of anhydrous ether was added. Cyanopyrazine [35] (5.00 g, 47.6 mmoles) in 40 ml of anhydrous ether was added, and the contents of the flask were allowed to come to -32°. The cyclopropyllithium prepared above was transferred, *via* syringe, to the cooled mixture at a rate to maintain the reaction temperature below -27°. The mixture was then stirred for several hours at -32°, allowed to warm overnight to room temperature, and stirred for about 2 hours at room temperature. After cooling to 5°, the reaction was quenched with 40 ml of a 30% ammonium chloride solution, followed by the addition of 75 ml of 6 *M* hydrochloric acid. The aqueous layer was removed, and the ether was washed with 2 x 25 ml of 10% hydrochloric acid. The combined aqueous phases were allowed to stand 1 hour at room temperature, then were cooled to 5°. After being made basic with a 50% sodium hydroxide solution, the water layer was extracted with 4 x 60 ml of ether. The ether was dried (magnesium sulfate) and removed to give 3.47 g of a dark brown liquid. The original ether layer was concentrated to half volume and extracted with 2 x 25 ml 20% hydrochloric acid. The aqueous extracts were allowed to stand at room temperature for 1 hour, were made basic and were extracted with 3 x 30 ml of ether. The ether was dried (magnesium sulfate) and removed to give an additional 0.76 g of crude ketone (total crude yield, 60%). ^{1}H nmr analysis revealed the purity of the ketone to be >90%, and the ketone was used without further purification. Pure ketone could be obtained by column chromatography with hexane/ethyl acetate (60/40); ir (deuteriochloroform): 1682 (C=O) cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 1.06-1.40 (4 H, m, cyclopropyl C2H), 3.39 (1 H, tt, J = 8 Hz, J = 3 Hz, cyclopropyl C1H), 8.70 (1 H, d, J = 1.5 Hz, C5H), 8.78 (1 H, d, J = 1.5 Hz, C6H), 9.20 (1 H, s, C3H); ms: m/e 148 (M^+), 69 (M^+-$C_4H_3N_2$), 41 ($C_3H_5^+$).

Anal. Calcd. for $C_8H_8N_2O$: C, 64.85; H, 5.44; N, 18.91. Found: C, 64.92; H, 5.44; N, 18.96.

4-Azanicotine (**1**).

Cyclopropylpyrazinylmethanone (17.63 g, 0.119 mole) was combined with 2.32 g (24.3 mmoles) of anhydrous magnesium chloride and 42 ml (0.718 mole) of *N*-methylformamide (NMF) in a 50-ml round-bottomed flask and heated under argon at 160° for 18 hours. After the reaction mixture cooled it was extracted with 2 x 25 ml ether and the ether in turn extracted with 4 x 10 ml of 20% sodium hydroxide. The ether was dried (sodium hydroxide) and removed to give 7.97 g of a dark brown liquid, which nmr showed to contain a large amount of NMF. The crude liquid was shaken with 15 ml of ether and allowed to stand. Two layers formed, and the bottom layer (NMF) was removed. The ether was shaken with 3 x 5 ml of 20% sodium hydroxide, dried (magnesium sulfate) and removed to give 2.53 g of a dark brown liquid containing starting ketone, NMF and product. An initial purification was accomplished by running the crude product through a column (8-10 cm diameter) with *ca.* 3" of silica. The column was packed and initially eluted with neat ethyl acetate. Solvent polarity was increased with increments of methanol up to 10%. Product (1.10 g), still containing NMF, was recovered. This crude was divided on 4 preparative tlc plates, and each was eluted 4 times in neat ethyl acetate. The desired product had an R_f of *ca.* 0.2, and 210 mg (1.1%) was recovered; ir (neat): 3040 (C-H arom), 2960 (C-H aliph) cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 1.80-2.10 (3 H, m, C3a'H, C4'H), 2.21-2.50 (2 H, m, C3b'H, C5a'H), 2.26 (3 H, s, methyl), 3.22-3.46 (2 H, m, C2'H, C5b'H), 8.50 (1 H, d, J = 2 Hz, C5H), 8.56 (1 H, dd, J = 1 Hz, J = 2 Hz, C6H), 8.71 (1 H, d, J = 1 Hz, C3H); ms: m/e 163 (M^+), 84 (M^+-$C_4H_3N_2$); exact mass Calcd for $C_9H_{13}N_3$: 163.1111. Found: 163.1106.

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