NICOTINE PHARMACOLOGY AS INFLUENCED BY STRUCTURAL MODIFICATION: BIOLOGICAL EFFECTS ELUCIDATED BY CHEMICAL REACTION MODELING

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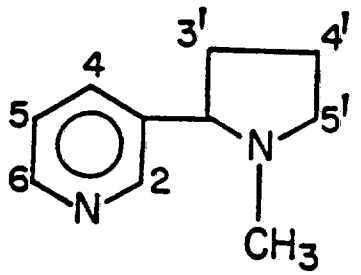
The observation that a small structural change in a biologically active molecule can greatly alter its activity is not uncommon; however, the structural correlates with such a change are rarely understood. For instance, Haglid reported that 4-methylnicotine (4) "lacked pharmacological effects" and suggested that the pyridine methyl group interfered with the conformations required by the nicotinic receptor.[5-7] An examination of a series of methylated nicotine analogues shows the inapplicability of Haglid's postulate. We propose that steric hindrance at the nicotinoid's pyrrolidine nitrogen is a crucial factor. A correlation is found between pyrrolidine nitrogen chemical reactivity (alkylation with iodomethane[22]) and pharmacological activity. In the case of 4-methylnicotine and 2-methylnicotine, chemical reaction modeling reveals an unprecedented steric effect in these conformationally mobile systems. This steric effect would not have been predicted based on molecular models alone. The value of explaining biological activities using chemical reaction modeling is emphasized.

Experimentation on the pharmacologic, psychopharmacologic, and physiologic effects of nicotine (1) continues at a high level. Among the major findings reported recently include: a stereospecific cholinergic binding site for nicotine in rat brain membranes[1]; a noncholinergic receptor for nicotine binding to a second brain membrane preparation[2]; and stereospecific behavioral responses for nicotine.[3] Despite this interest in nicotine, few structure-activity relationships (SAR) have been proposed and no SAR that encompass both central and peripheral nicotinic systems has been advanced. Efforts to establish such relationships would be facilitated by the availability of additional analogues[4] possessing the nicotine ring geometry.

Some time ago, Haglid reported that 4-methylnicotine (4) was devoid of nicotinic cholinergic activity, and he suggested that the pyridine methyl group of 4, which is ortho to its N-methylpyrrolidine ring, interferes with the ring-ring conformation required at the nicotinic receptor.[5-7] We now report pharmacologic testing results which suggest that methyl substitution on the nicotine molecule acts not by limiting the availability of important conformations at the nicotinic receptor (Haglid's postulate[5-7]) but by reducing accessibility to the pyrrolidine nitrogen. By employing chemical reactions as models for biological action, we have observed novel steric effects in these conformationally mobile systems which can be correlated with the pharmacologic activities observed. The nicotinoids included in this series demonstrate a wide range of activity for the tests examined and can serve as the basis for more detailed SAR in the future.



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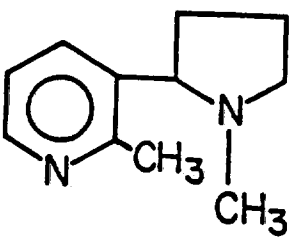

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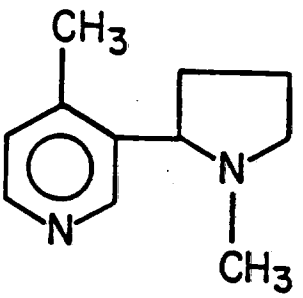

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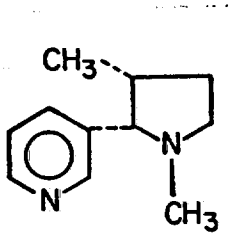
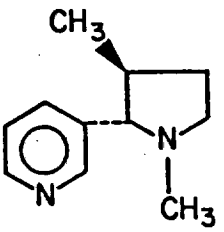
The results of pharmacologic tests using nicotinoids designed[9] to pinpoint specific critical structural features are shown in Table I.[10] Placement of a single methyl group on the nicotine nucleus can clearly have a substantial effect, 6-methylnicotine (6) being 3-7 times more active in the LD_{50} and guinea pig ileum tests and 2-methylnicotine (2) being 100-800 times less active than nicotine in these same two tests.

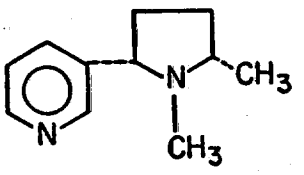
A number of important observations can be made: (1) substitution of a pyridine methyl group ortho to the pyrrolidine ring at either C-2 (e.g., 2) or C-4 (e.g., 4) significantly decreases activity in all tests; (2) cis-3'-methyl- and trans-3'-methylnicotine (3'c and 3't) are nearly equipotent though both are less active than nicotine; (3) substitution close to the pyrrolidine nitrogen (e.g., 5'c)causes loss of activity; (4) substitution close to the pyridine nitrogen increases activity in one case (6) and decrease activity in another (2); and (5) substitution distant from the pyrrolidine ring (e.g., 5 and 6) has only a modest effect on activity, increasing activity in the case of 6-methylnicotine.



3'c



3't



5'c

In order to evaluate Haglid's postulate[5-7] of conformational control of activity as stated above, we included in our study a number of compounds which we considered to have specific conformational features based on inspection of Dreiding models. To quantify the intramolecular interactions arising in these nicotinoids and to substantiate the conformational analyses based on Dreiding models, we performed a series of molecular orbital calculations at the level of intermediate neglect of differential overlap (INDO).[11]

The important structural conclusions from these calculations (see Table II) include: (a) 4-methylnicotine[5-8] (4) and 2-methylnicotine[5-8,15] (2) have a methyl group ortho to the pyrrolidine ring and have similar pyridine-pyrrolidine conformational profiles; (b) cis-3'-methylnicotine (3'c) possesses a $C_{2,4}$-H: C_3'-CH_3 cross ring interaction which is analogous to the C-CH_3:C_3'-H interaction in 2 and 4; (c) the $C_{3'}$-or $C_{5'}$-methyl group of trans-3'-methylnicotine[16,17] (3't) and cis-5'-methylnicotine (5'c) respectively affect pyrrolidine ring interactions without modifying ring-ring energetics; and (d) the pyridine methyl group of 5-methyl- and 6-methylnicotine (5 and 6) have no effect on either ring-ring conformations or pyrrolidine ring conformations.

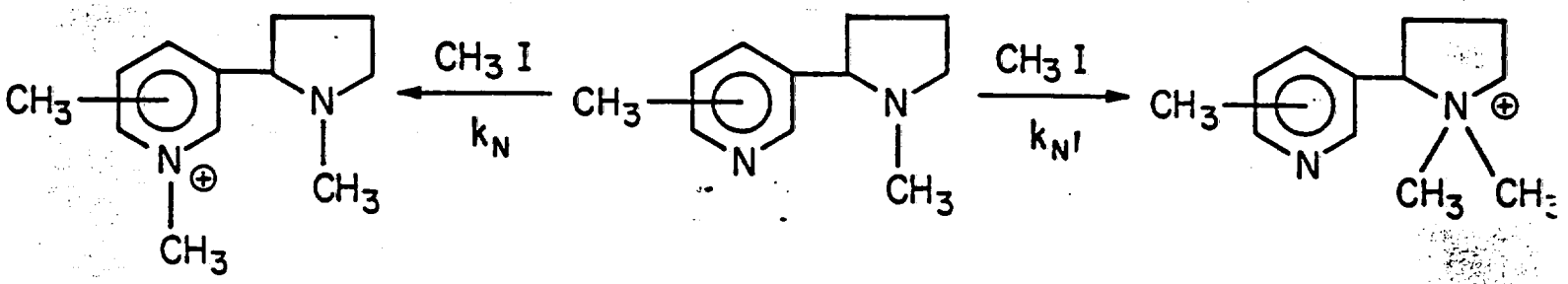
The marked increase in rotational barriers for 2, 3'c and 4 bears out the prediction that these three compounds should have similar cross ring interactions. Consequently, conformations of 2-methyl- and 4-methylnicotine destabilized by the pyridyl methyl group are also destabilized in cis-3'-methylnicotine (3'c) by the $C_{3'}$-methyl substituent. On the other hand, trans-3'-methylnicotine (3't) does not exhibit any increase in rotational barriers compared to nicotine, 6-methyl- or 5-methylnicotine.

The fact that 3'c and 3't are essentially equipotent (Table I) indicates that the low activity of 4-methylnicotine (4) and 2-methylnicotine (2) cannot be attributed solely to destabilization of particular ring-ring orientations or conformations required for activity (Haglid's postulate[5-7]). In addition, the fact that introduction of a C_2 methyl group destabilizes N---N' syn conformations[11] in 2 implies that both 2 and 4 are not inactive for conformational reasons alone since 4 would be destabilized in N---N' anti conformations.[11] The low activity of 2-methylnicotine is unlikely to be due to steric congestion at the pyridine nitrogen since significant activity is found for 6-methylnicotine. A particularly striking structural feature of the least active analogues (2 and 4) is that their pyridine methyl groups produce severe steric

hindrance at the pyrrolidine nitrogen for large sectors of their possible conformations. We therefore suggest that pyrrolidine (N') nitrogen accessibility[18] is crucial for biological activity, and that the pyridine methyl groups in 2 and 4 decrease that accessibility.

In order to quantitate pyrrolidine (N') nitrogen congestion, the relative rates of alkylation (Menschutkin Reaction[19-21]) of nicotine and a number of these analogues were determined (c.f. Scheme I[22]). The Menschutkin Reaction was chosen as a model reaction since it has been established that quaternization of tertiary amines and pyridine derivatives are very sensitive to both steric hindrance and nitrogen electron density[19-22].

SCHEME I



N N'

Table II indicates the relative rates of pyridine:pyrrolidine iodomethylation for nicotine and its pyridine monomethylated derivatives. The relative partial rate factors for N and N' alkylation are derived from the ratio of N/N' attack. Note the last column in Table II; $f_{N'}(2)$ and $f_{N'}(4)$ are both significantly less than $f_{N'}(1)$, indicating the important conclusion that a pyridine methyl group ortho to the C_3-C_2, pivot bond decreases the overall relative pyrrolidine nitrogen (N') iodomethylation rate by ca. four-fold relative to nicotine. This large rate retardation is particularly novel in view of the presence of rapid ring-ring rotation about the C_3-C_2, pivot bond which would tend to favor conformations in which there is minimum cross-ring interference. As there is no precedent in the chemical literature[22] for such

a significant steric effect in a conformational mobile molecule like 2 (and 4), it is reasonable that other structural explanations were proposed[5-7] to account for the inactivity of 4.

A comparison of the chemical model for N' accessibility with the pharmacological tests is shown in Figure 1. A similar pattern is found for nicotine and the four pyridine methyl substituted homologues. These observations indicate a relationship between pharmacologic activity and N' accessibility. The low activity of cis-5'-methylnicotine (5'c) (c.f. Table I) cannot be due to conformational factors: substitution at the 5'-position of nicotine is unlikely to effect ring-ring conformational profiles while it will have significant effects on pyrrolidine nitrogen accessibility. The steric accessibility model is also supported by results on other nicotine analogues, including N'-alkylnornicotines[23] and 2',5',5'-trimethylnicotine[24] which also exhibit both decreased activity and increased pyrrolidine nitrogen congestion.[25]

In conclusion, we have demonstrated that the inactivity of 2 and 4 is not due to conformational restrictions as proposed earlier.[5-7] We have also observed a significant correlation between the rate of pyrrolidine nitrogen alkylation and peripheral pharmacolgic activity for these nicotinoids, and have suggested that steric accessibility at nicotine's pyrrolidine nitrogen is an important factor in nicotinic activity.

Chemical reaction modeling of biologic activity is thus valuable for series of compounds, such as those reported here, in which steric (or other) effects would not be predictable based on "intuitive" considerations of structure alone. Our results suggest that ultimately, comprehensive structure-activity relationships for nicotine and other biologically active compounds will be based not only on physical properties and calculated parameters (e.g., electron densities and polarizability) but on chemical reaction modeling as well.

The authors thank Dr. J. F. Whidby, Mr. R. L. Bassfield, and Dr. J. Wooten for obtaining the nmr results and Mr. Robert Maher for invaluable technical assistance. We also thank Drs. T. P. Pitner, J. Lephardt, L. Abood, A. Burger and H. McKennis for critical reading of the manuscript.

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4. For simplicity, we have numbered the nicotine analogues in the text according to the position of the methyl substitutent, e.g., 2-methylnicotine is 2.

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8. E. Leete and S. A. S. Leete, J. Org. Chem., 43, 2122-2125 (1978). Note, in particular, the pharmacology discussion in the experimental section of this paper.

9. Full details of the synthesis of the new compounds reported herein will be described elsewhere. Satisfactory elemental and spectroscopic analyses were obtained for all compounds. Unless otherwise indicated, all compounds are racemic mixtures.

10. The various pharmacologic tests reported herein represent the traditional ones used for screening nicotinic cholinergic agents. It is recognized that many different sites of action as well as mechanisms of action are clearly involved. We are particularly interested in the activity trends observed rather than the operative detailed pharmacologic mechanisms. All pharmacologic tests reported herein were conducted under contract outside of Philip Morris.

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11. As a baseline geometry for the calculations, a partially optimized nicotine coordinate system was used. The nicotine coordinates evolved from fully optimizing pyridine and N-methylpyrrolidine structures separately, and subsequently using these geometries together for the full nicotine calculations. The nicotine structural parameters varied for energy minimization included the inter-ring distance, the side-to-side and up-and-down torsions of the pyridine ring, the methyl out-of-plane and side-to-side motions, and the H(2) and H(4) in-plane angles. At fixed values of τ, the inter-ring torsion angle, the above mentioned structural parameters were varied independently until the energy minimum was found. This procedure was repeated at 10° increments of τ. These nicotine geometries were used for the INDO studies of the methylnicotine conformations by substituting $-CH_3$ coordinates in place of the appropriate -H coordinates. For each compound, the methyl rotational angles were chosen to minimize the energy. For comparison, an INDO calculation[12] was also run on the reported x-ray structure[13] of nicotine using standard hydrogen

parameters; the molecular energy we calculated from these x-ray data greatly exceeded our results on the partially optimized nicotine geometry at all values of τ. Table II lists the calculated energy minima and maxima for each compound as well as the rotational barriers relative to nicotine. The calculated barriers are large. Further calculations in which additional structural parameters are relaxed would undoubtedly reduce these barriers but probably would not change the trend seen for this molecular series. The actual barrier heights are not reported as we place more emphasis on the relative order observed than on the actual values calculated, and it is important to note that unrealistically high barriers are often the results of such procedures.[14] Full details of these calculations will be reported elsewhere by one of us (R. W. Dwyer). N---N' refers to the relative orientation of the pyridine and pyrrolidine nitrogen atoms.

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25. The lower activity of 3'c and 3't may also be related to decreased pyrrolidine accessibility demonstrated in preliminary iodomethylation studies on these compounds (J. I. Seeman and H. V. Secor, unpublished results). Torsional motion about the $C_2,-C_3,$ and $C_3,-C_4,$ bonds can be a major source of relief of steric strain for these compounds.

Caption for Figure 1

Figure 1. A comparison of the chemical model for pyrrolidine nitrogen (N') accessibility with pharmacological tests of five nicotinoids. Note that the number in parenthesis adjacent to the top of the off-scale values represents the respective activity and the number above each bar represents the compound identity as indicated in the text.

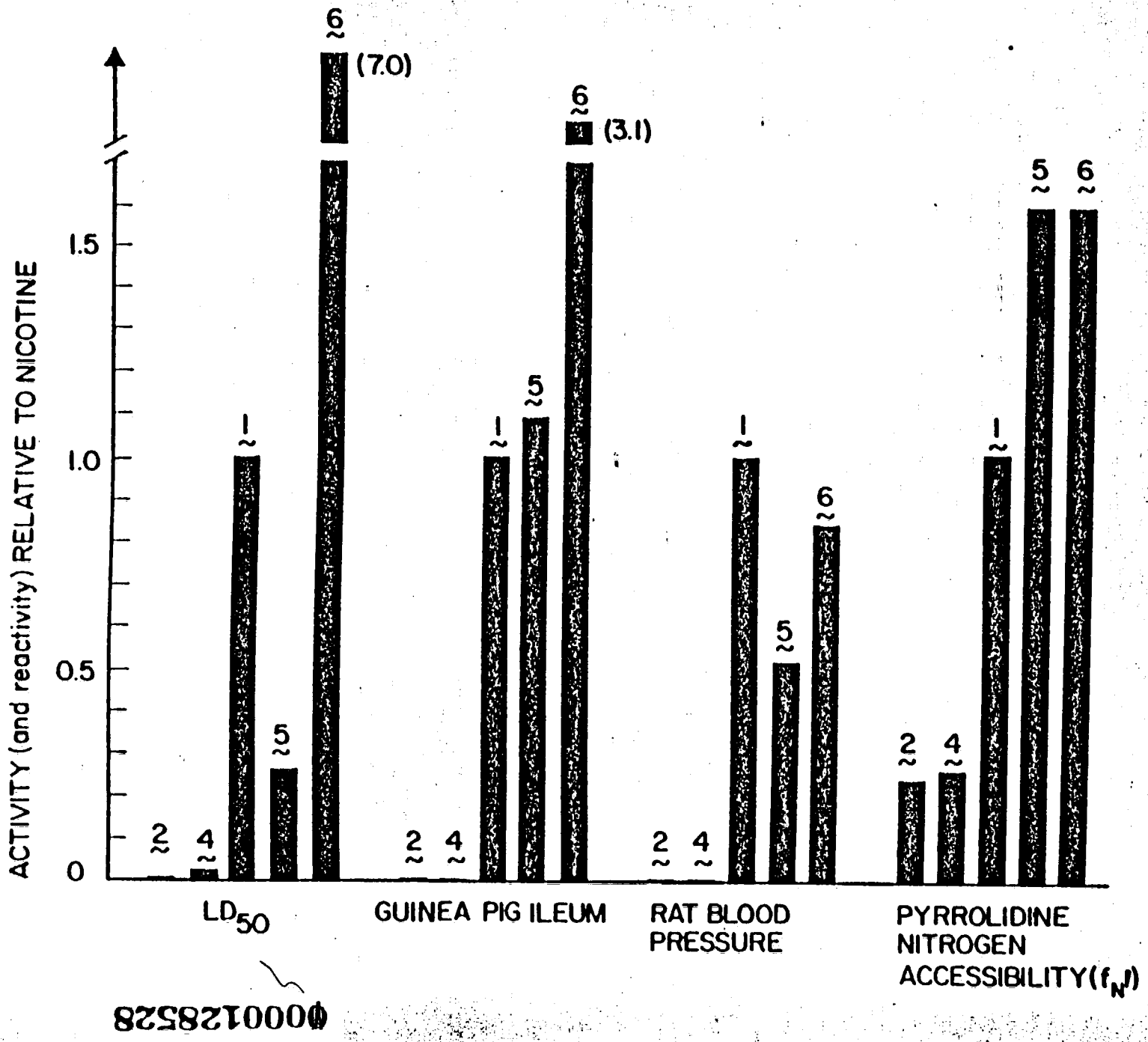

Figure 1

Table I. Activities of Nicotine Analogues[a]

Compound		LD_{50}[b] (mg/kg)[n][c]	GUINEA PIG ILEUM Relative Activity Without Antagonist[d][n][e]	% Reduction of Contraction [n][e] Pretreatment With Atropine[f]	Pretreatment With Hexamethonium[g]	Rat Blood Pressure (mg/kg)[h][n][c]
(S)-Nicotine[i]		0.42±0.04[58][c]	1.3±0.3[4]	29[3][e]	82[6][e]	.041±.010[10]
(R,S)-Nicotine	(1)	0.90±0.09[60]	0.54±.18[4]	55[4]	58[6]	.058±.012[14]
(R,S)-2-Methylnicotine	(2)	91±5[40]	$7.0\pm3.0\times10^{-4}$[3]	45[4]	37[8]	j
(R,S)-cis-3'-Methylnicotine	(3'c)	23.7±1.4[50]	$1.5\pm0.6\times10^{-2}$[3]	52[3]	73[6]	6.3±3.1[11]
(R,S)-trans-3'-Methynicotine	(3't)	16.2±0.7[60]	$1.7\pm0.7\times10^{-2}$[3]	65[3]	74[6]	2.81±.06[8]
(R,S)-4-Methylnicotine	(4)	32±3[40]	$10\pm2\times10^{-3}$[2]	81[3]	5[4]	310±260[13]
(R,S)-5-Methylnicotine	(5)	3.5±0.7[70]	0.6±0.4[3]	46[3]	73[4]	0.27±.12[15]
(R,S)-6-Methylnicotine	(6)	0.13±0.02[40]	1.7±.25[3]	50[3]	84[6]	0.0449±.0016[5][l]
(S)-cis-5'-Methylnicotine	(5'c)	61.9±3.6[50]	2.1×10^{-3}[2][k]	30[4]	8[6]	51±46[9]

(a) See footnote 4. (b) Route of administration was i.v. (free base) in male albino mice (SPF). Values calculated using the method of D. J. Finney, "Probit Analysis," Cambridge, 1952. (c) [n] refers to number of animals. (d) Molar ratio of standard (l-nicotine dihydrogen tartrate) to test compound which causes an equivalent response. Each experiment used a freshly prepared ileum strip. The mean (±standard deviation) dose of the standard which caused an 8 mm contraction involved a 50 mL solution of 1.00±0.33 g/mL l-nicotine dihydrogen tartrate. The procedure used is based on R. Magnus, Pfluegers Archiv., 102, 123 (1904). (e) [n] refers to the number of replicates. (f) Atropine sulfate, 2.15×10^{-8} g/ml. (g) Hexamethonium iodide, 2.15×10^{-5} g/ml. (h) Dose necessary to cause a 25% increase in blood pressure by infusion into the cannulated jugular vein. (i) For some recent results on the pharmacology of (S)-nicotine, see M. D. Aceto, et. al., J. Med. Chem., 22, 174 (1979). (j) No blood pressure increase up to 31 mg/kg for 2. (k) A very large error associated with this value, two determinations made. (l) Data obtained on (S)-6-methylnicotine.

Table II. INDO Calculated Parameters and Relative Aklylation Rates of Nicotine and Nicotine Analogues

Compound		Calculated Conformational Parameters: τ Degrees[a,b] Minima	Maxima	Relative Rotational Barriers[c]	Pyridine (N) and Pyrrolidine (N') Alkylations: $k_{N'}/k_N$[d,e]	Relative Partial Rate Factor[f]: f_N	$f_{N'}$
Nicotine	(1)	280/100	340/160	1.0/1.0	2.7±.36	0.37	1
2-Methylnicotine	(2)	100/240	360/160	2.5/1.7	2.3±.12	0.10[g,i]	0.23[f]
4-Methylnicotine	(4)	280/100	180/340	2.9/1.9	0.33±.03	0.76[g]	0.25[f]
5-Methylnicotine	(5)	280/100	160/340	1.0/1.0	2.2±.17	0.64[g]	1.4[f]
6-Methylnicotine	(6)	280/100	340/160	1.0/1.0	8.0±.33	0.18[f]	1.4[h]
cis-3'-Methylnicotine	(3'c)	100/280	180/360	4.5/4.4	---	---	---
trans-3'-Methylnicotine	(3't)	280/100	340/160	1.0/1.0	---	---	---

(a) $\tau = (C_4\text{-}C_3\text{-}C_{2'}\text{-}C_{3'})$ dihedral angle. Clockwise rotation of the pyrrolidine ring relative to the pyridine ring is in the "positive" sense. (b) For each pair, the lower energy conformation is listed first. (c) The order corresponds to lower energy minimum to lower energy maximum, followed by higher minimum to higher maximum. See text and footnote 11 for a discussion. (d) $k_{N'}$ is the Winstein-Holness rate constant and reflects the total rate of pyrrolidine alkylation [c.f. J. I. Seeman and W. A. Farone, J. Org. Chem., 43, 1954 (1978); J. I. Seeman, E. B. Sanders, and W. A. Farone, Tetrahedron, 36, 1173 (1980)]. (e) Determined by nmr analysis of the total reaction product of the alkaloid and iodomethane in acetonitrile at 25°. (f) Calculated from $k_{N'}/k_N = \underline{f}_{N'}/\underline{f}_N$ for each analogue, where $\underline{f}$ refers to partial rate factor. (g) Derived from the relative rates of pyridine and substituted pyridine iodomethylations in acetonitrile at 25°; pyridine:2-methylpyridine:3-methylpyridine:4-methylpyridine:2,3-dimethylpyridine = 1:0.43:1.7:2.1:0.43. (h) Assumed that $f_{N'}(6) = f_{N'}(5)$. (i) Buttressing effects taken into consideration.