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Circular Dichroic Determination of the Preferred Conformation of Nicotine and Related Chiral Alkaloids in Aqueous Solution

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SUMMARY

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A negative Cotton effect for the pyridine ^1L_b band of aqueous solutions of S-(−)-nicotine, S-(−)-nornicotine, S-(−)-anabasine, S-(−)-methylanabasine in the neutral and monoprotonated (*N*-alicyclic protonated) forms, and S-(−)-cotinine in its neutral form was measured by circular dichroism. Theoretical and experimental arguments suggest that Snatzke's sector rule for the ^1L_b band of monosubstituted benzene derivatives can be applied to the pyridine derivatives investigated. Two regions of the torsion angle τ[τ = 120–140 degrees (+ *anti*clinal) and 300–320 degrees (− *syn*clinal)] were found by the use of molecular models to predict a negative Cotton effect for these alkaloids; the results are in good agreement with data obtained by quantum calculations and X-ray crystallography by other workers.

INTRODUCTION

Besides the effect of changes in chemical structure on the affinity, intrinsic activity, and ability to desensitize the receptors of nicotine and related compounds at the ganglia and neuromuscular junction (1), one must also consider the state of protonation of the basic centers, the absolute configuration at the elements of chirality, and the conformational problems involved.

Taylor (2) suggested that nicotine was active as the univalent cation (protonation of the alicyclic nitrogen), a fact substantiated by the relationship between pH and activity shown by Barlow and Hamilton (3). The activity of compounds related to nicotine but of different basicity will therefore be a function of the extent of ionization at physiological pH; the presence of a lipid-soluble, un-ionized form of the compound to reach the receptor site is necessary, however.

The stereoselective activity of the nicotine enantiomers (4, 5) is presumably due to the spatial characteristics of S-(−)-nicotine fitting more closely to the acetylcholine receptor (6).

Many recent studies of conformational aspects of drug molecules have focused on the preferred conformation, which is readily determined by physical methods (e.g., X-ray crystallography, NMR spectroscopy); little is known, however, of the pharmacophoric conformation of such molecules (7). Drug

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molecules are supposed to act in a preferred or in a less favorable conformation, depending on the steric requirements of the receptor. Recent studies, however, have shown different molecules acting at the same receptor to display similar spatial disposition of chemical groups when in their preferred conformation: for example, various molecules acting on *alpha*-adrenergic receptor sites (8), histamine and analogues (9, 10), and choline derivatives (11). Thus a knowledge of the preferred conformation of drug molecules may give an insight into structural features required by drug receptors.

The preferred conformation of nicotine has been studied by various methods. Theoretical calculations have been made by Kier (12), using the extended Hückel theory, and by Pullman and co-workers (13), using the method of perturbative configuration interaction with localized molecular orbitals (PCILO). These calculations are made for molecules in a vacuum, and the necessary computing simplifications led to models whose deviation from reality affords results needing experimental confirmation.

The conformation of nicotine dihydroiodide has been determined by X-ray crystallography (14). However, the packing restriction of the crystalline state may influence the conformational energy barrier (7), thus allowing only cautious extrapolation of these results to the molecule in solution.

To study the conformation of nicotine in solution, Simpson and co-workers (15) used NMR and dipole moments; their results cannot be critically compared with other data, because of the broad range of possible torsional angles (see later) determined between the rings.

METHODS

Materials. S-(−)-Nicotine was supplied by British Drug Houses, and S-(−)-anabasine by Fluka AG (Buchs, Switzerland). S-(−)-Methylanabasine was a gift from the Imperial Tobacco Company (Bristol, England), and S-(−)-nornicotine was donated by A. H. Sheikh, Chelsea College, who freshly prepared it by demethylation of S-(−)-nicotine. S-(−)-Cotinine was synthesized according to the method of Bowman and McKennis (16).

Spectroscopic techniques. The ultraviolet spectra were obtained with a Unicam SP 800 spectrophotometer. CD[3] measurements were made using a Roussel-Jouan Dichrograph and 0.2- or 0.5-cm cells. ORD spectra were obtained with a Bellingham Stanley/Bendix-Ericson Polarmatic 62 (0.5- and 1.0-cm cells). All spectra were determined at room temperature.

The following molecular rotations (ϕ) and molecular ellipticities (θ) were obtained for the above alkaloids. Concentrations (c) are in grams per 100 ml.

S-(−)-Nicotine. ORD (c, 0.011, in 2-propanol) $[\phi]_{589}$ −250; $[\phi]_{273}$ −9500 (trough); $[\phi]_{263}$ 0; $[\phi]_{254}$ +6500 (peak); $[\phi]_{245}$ 0; $[\phi]_{222}$ −13,400.

ORD (c, 0.01, in 0.1 N HCl) $[\phi]_{300}$ −850; $[\phi]_{258}$ −2400 (trough); $[\phi]_{253}$ +300 (peak); $[\phi]_{222}$ −1400.

CD (c, 0.008, in sodium phosphate buffer, pH 5.36) $[\theta]_{280}$ 0; $[\theta]_{263}$ −3600; $[\theta]_{255}$ 0; $[\theta]_{240}$ +4000; $[\theta]_{228}$ 0.

CD (c, 0.022, in Na_2CO_3-NaOH buffer, pH 11.5) $[\theta]_{279}$ 0; $[\theta]_{263}$ −5300; $[\theta]_{251}$ 0; $[\theta]_{240}$ +3600; $[\theta]_{227}$ 0.

S-(−)-Nornicotine. ORD (c, 0.022, in 2-propanol) $[\phi]_{589}$ −100; $[\phi]_{273}$ −5100 (trough); $[\phi]_{263}$ 0; $[\phi]_{253}$ +3500 (peak); $[\phi]_{245}$ 0; $[\phi]_{222}$ −6700.

CD (c, 0.022, in sodium phosphate buffer, pH 6.10) $[\theta]_{278}$ 0; $[\theta]_{263}$ −3300; $[\theta]_{252}$ 0; $[\theta]_{242}$ +2300; $[\theta]_{229}$ 0.

CD (c, 0.022, in Na_2CO_3–NaOH buffer, pH 11.5) $[\theta]_{278}$ 0; $[\theta]_{261}$ −4300; $[\theta]_{250}$ 0; $[\theta]_{241}$ +2300; $[\theta]_{228}$ 0.

S-(−)-Methylanabasine. ORD (c, 0.008, in 2-propanol) $[\phi]_{589}$ −200; $[\phi]_{273}$ −3600 (trough); $[\phi]_{267}$ 0; $[\phi]_{254}$ +4600 (peak); $[\phi]_{247}$ 0; $[\phi]_{222}$ −6300.

CD (c, 0.007, in sodium phosphate buffer, pH 5.30) $[\theta]_{279}$ 0; $[\theta]_{266}$ −3300; $[\theta]_{256}$ 0; $[\theta]_{242}$ +3600; $[\theta]_{231}$ 0.

CD (c, 0.022, in Na_2CO_3–NaOH buffer, pH 11.5) $[\theta]_{278}$ 0; $[\theta]_{264}$ −4000; $[\theta]_{254}$ 0; $[\theta]_{241}$ +4000; $[\theta]_{227}$ 0.

S-(−)-Anabasine. ORD (c, 0.019, in 2-propanol) $[\phi]_{589}$ −100; $[\phi]_{272}$ −4100 (trough); $[\phi]_{265}$ 0; $[\phi]_{252}$ +3800 (peak); $[\phi]_{242}$ 0; $[\phi]_{222}$ −2400.

[3] The abbreviations used are: CD, circular dichroism; ORD, optical rotatory dispersion.

CD (*c*, 0.006, in sodium phosphate buffer, pH 5.80) $[\theta]_{280}$ 0; $[\theta]_{266}$ −1700; $[\theta]_{257}$ 0; $[\theta]_{245}$ +2000; $[\theta]_{233}$ 0.

CD (*c*, 0.025, in Na_2CO_3–NaOH buffer, pH 11.5) $[\theta]_{278}$ 0; $[\theta]_{263}$ −3600; $[\theta]_{252}$ 0; $[\theta]_{243}$ +2600; $[\theta]_{230}$ 0.

S-(−)-Cotinine. ORD (*c*, 0.004, in 2-propanol) $[\phi]_{589}$ −100; $[\phi]_{272}$ −7000 (trough); $[\phi]_{263}$ 0; $[\phi]_{253}$ +5900 (peak); $[\phi]_{235}$ +1500 (trough); $[\phi]_{222}$ +9400.

CD (*c*, 0.017, in sodium phosphate buffer, pH 7.4) $[\theta]_{280}$ 0; $[\theta]_{264}$ −5000; $[\theta]_{252}$ 0; $[\theta]_{240}$ +2600.

RESULTS AND DISCUSSION

Pyridine chromophore; attribution of Cotton effects bands. The optical rotatory dispersion spectrum of S-(−)-nicotine shows both a negative Cotton effect at 260–270 nm and a positive Cotton effect at approximately 250 nm, in agreement with the common features for such alkaloids shown by Craig and Roy (17). These Cotton effects are better resolved by circular dichroism (18). The two CD bands of opposite sign displayed by the pyridine chromophore could be assigned to the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions by a theoretical treatment (19); the unambiguous attribution of a band to each transition, however, was not possible (19).

The low-energy $\pi \rightarrow \pi^*$ transition of pyridine is at approximately 250 nm, and its $n \rightarrow \pi^*$ transition is at approximately 270 nm (20). The $n \rightarrow \pi^*$ bands of azines are shifted toward the blue region by electron-donating substituents (e.g., pyrrolidine), and toward the red region by electron-accepting substituents; the $\pi \rightarrow \pi^*$ bands undergo bathochromic shifts with both types of substituents (21).

In nicotine the $\pi \rightarrow \pi^*$ transition at approximately 260 nm, which is due to the bathochromic shift, can be assigned by the known hyperchromic effect of proton donors (20). When the ultraviolet spectrum of nicotine in hexane is examined in the presence of increasing concentrations of acetic acid (Fig. 1A), a clear hyperchromic effect is noted in the 260 nm region. The hyperchromic effect on this band is even more pronounced in water when the spectra of nicotine in a buffer solution near neutrality (protonation of alicyclic nitrogen only; see later) and in an acid solution (diprotonated form) are compared (Fig. 1B).

Further evidence for the assignment of the $\pi \rightarrow \pi^*$ band is found in the ORD spectrum of S-(−)-nicotine in acid solution (see METHODS), in which only a weak negative Cotton effect remains. The positive band is canceled by protonation of the aromatic nitrogen. It is concluded that the negative Cotton effect of nicotine corresponds to the $\pi \rightarrow \pi^*$ transition.

Sector rules for pyridine chromophore. For pyridine no empirical rule is known which correlates the atomic environment of the asymmetrically perturbated chromophore with the Cotton effects, in contrast to the classical octant rule of the keto chromophore (e.g., ref. 22). An octant rule based on the

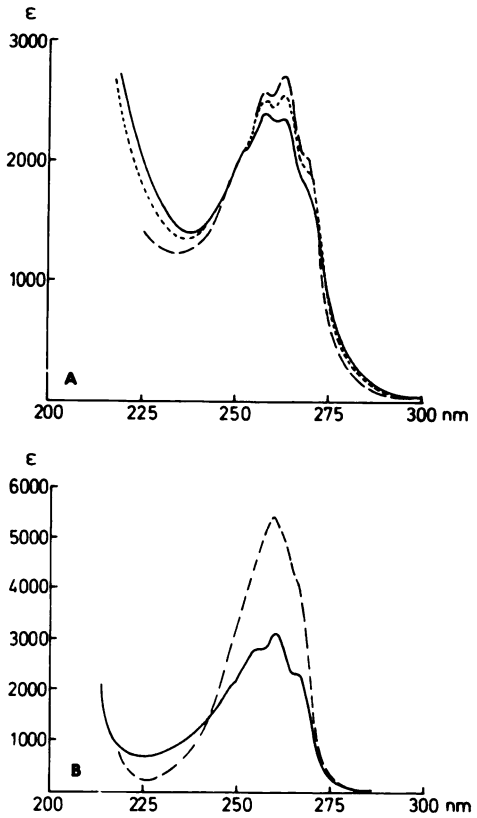


FIG. 1. *Ultraviolet spectra of nicotine*
A. In hexane: ——, as such; -----, with 0.01 N acetic acid; – – –, with 0.1 N acetic acid. B. In water: ——, pH 5.36; – – –, pH 1.

C_{2v} symmetry of pyridine was suggested by Gottarelli and Samori;[4] one plane was the nodal plane of the nitrogen lone pair of electrons.

Consideration of the pyridine π-system, however, reveals a great similarity with the benzene π-system, and it is evident that the deviation of the pyridine π-cloud from 6-fold symmetry, owing to the different electronegativity of the nitrogen atom, is not very important (see refs. 23–32).

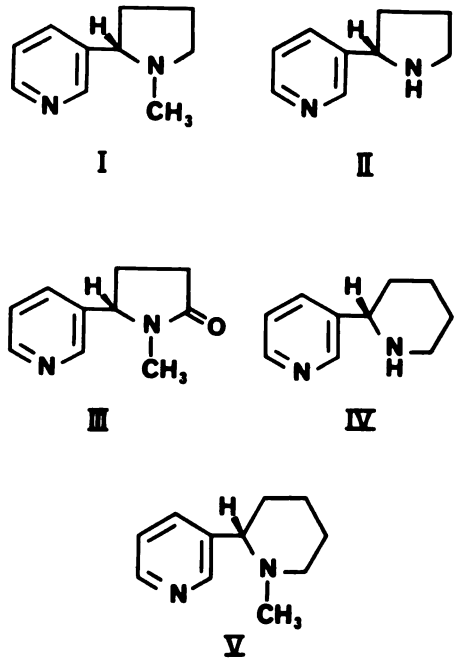
It may therefore be tentatively suggested that as a first approximation the asymmetrically perturbated $\pi \rightarrow \pi^*$ transition of both the phenyl and pyridyl chromophores, which are responsible for the ^1L_b CD band, do not differ significantly with regard to Cotton effects.

The sector rules developed for the benzene chromophore must be applied to the CD spectra of chiral pyridine derivatives in order to check this hypothesis and to see whether the perturbation of the nitrogen atom on the π-cloud symmetry can be neglected at this stage.

Thus we have considered the 260–270 nm $\pi \rightarrow \pi^*$ transition [^1L_b band (33–35)], using both the sector rules developed by Snatzke (36–38) for the benzene ^1L_b band (second and third chiral spheres) and the pyridine octant rule suggested by Gottarelli and Samori.[4]

Successful prediction of the sign of the ^1L_b Cotton effect of some optically active pyridine derivatives [various substituted tetralines and indane analogues[5] and pyridylmethylcarbinol (19)] was obtained by using the benzene sector rules, whereas application of the octant rule was only partially successful. These results suggest that the perturbative effect of the nitrogen atom on the Cotton effects of the $\pi \rightarrow \pi^*$ transition can indeed be neglected in a first approximation. We therefore feel that until a better rule is found Snatzke's benzene rules can also be applied to the ^1L_b band of the pyridine chromophore.

CD studies of investigated alkaloids. The CD spectra of S-(−)-nicotine (I), S-(−)-nornicotine (II), S-(−)-anabasine (IV), and S-(−)-methylanabasine (V) were measured in buffered solutions for the monoprotonated (alicyclic nitrogen protonated) and nonprotonated forms of these alkaloids, since these two forms are present at physiological pH in a ratio dependent upon pK_a (see Table 1). S-(−)-cotinine (III), a weakly basic cyclic amide, exists only as the neutral form at physiological pH. Because the two pK_a values are too close to allow sufficient separation of the mono- and diprotonated forms, we determined the CD spectrum of only the neutral form. The pK_a values at 25° were taken from Yamamoto (40). Since no data were available for methylanabasine, its pK_a values were calculated by subtracting the difference between the pK_a values of nicotine and nornicotine from those of anabasine.



To obtain the monoprotonated forms of the alkaloids, a pH region giving a good balance between maximum and minimum protonation of the alicyclic and aromatic nitrogens, respectively, was calculated from the pK_a values (Table 1). The ionic strength of the solutions (0.1) was included in the calculations.

Cotinine contains an additional chromo-

[4] G. Gottarelli and B. Samori, personal communication, 1971.

[5] G. Gottarelli and B. Samori, personal communication, 1972.

TABLE 1
Degrees of Protonation for Nicotine and Related Chiral Alkaloids

Compound	pK_a values at 25°		Protonation						
	pK_{a1} (alicyclic N)	pK_{a2} (aromatic N)	At 37° and pH 7.4[a]			At 25°		At 25° and pH 11.5[b]	
			Alicyclic N	Aromatic N	pH[b]	Alicyclic N	Aromatic N	Alicyclic N	Aromatic N
			%	%		%	%	%	%
Nicotine	7.9	3.1	70.0	0.01	5.36	99.6	1.3	0.03	~0
Nornicotine	9.0	3.3	96.2	0.02	6.10	99.9	0.4	0.4	~0
Anabasine	8.7	3.1	92.6	0.01	5.80	99.9	0.5	0.2	~0
Methylanabasine	(7.6)[c]	(2.9)[c]	50.0	0.01	5.30	99.6	1.0	0.02	~0
Cotinine	4.5	2.7	0.1	~0	7.40	0.2	~0		

[a] The temperature corrections for the pK_a values were made according to Perrin (39). An ionic strength of 0.1 was assumed.

[b] Buffers used: for pH 5–7.4 sodium phosphate buffers of ionic strength 0.1; for pH 11.5, Na_2CO_3–NaOH buffer of ionic strength 0.1.

[c] Estimated pK_a (see the text).

phore compared to the other alkaloids, namely, an amide group. However, the $n \rightarrow \pi^*$ transition of this group is known to occur at shorter wavelengths than the considered pyridine transitions [e.g., 205 nm for formamide and acetamide (41) and 231 nm for the β-lactam ring (42)].

The CD ^1L_b bands measured were negative for both the neutral and monoprotonated solutes. It has been shown for the phenyl chromophore that protonation of a nitrogen does not change the sign of the Cotton effect (43). The maximum ellipticity values (θ) depended on compound and conditions, but this quantitative aspect will not be considered further, since the numerous factors involved defied our interpretation. Only the qualitative aspect of these results will be considered in the conformational determination.

Conformational interpretation of ^1L_b Cotton effects. The torsion angle τ is a measure of the mutual arrangement of the pyridine and pyrrolidine (or piperidine) rings. We use τ as defined by Pullman *et al.* (13), namely, the planar arrangement of the drawing (Fig. 2) corresponding to the value $\tau = 0$. Viewed from the pyridine ring, τ is positive for a clockwise rotation (Fig. 2, direction of arrow).

Using framework molecular models and Dreiding molecular models, τ has been

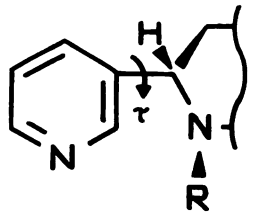


FIG. 2 *Definition of the torsional angle τ.*

rotated from 0 to 360 degrees. The pyrrolidine ring (nicotine, nornicotine, and cotinine) was assumed to be planar, which is likely to be its average conformation (12). The *N*-methyl group attached to the pyrrolidine ring was assumed to be *trans* to the pyridine ring, this arrangement being energetically preferred over the *cis* form (13). The piperidine ring (anabasine and methylanabasine) was taken in its chair conformation, with the pyridyl and *N*-methyl substituents being equatorial.

From the location of the atoms in the various sectors [nitrogen strongly predominating over carbon (43)], the expected Cotton effects of the ^1L_b band have been predicted using Snatzke's benzene sector rules for the third chiral sphere. The angular regions predicted to show a negative Cotton effect are summarized in Table 2. Each compound has two regions where the ^1L_b Cotton effects are expected to be negative.

TABLE 2

Angular regions of expected negative ^1L_b Cotton effect

Compound	τ	τ
	degrees	*degrees*
(−)-Nicotine	120–170	300–350
(−)-Nornicotine	100–170	280–350
(−)-Anabasine	100–170	280–350
(−)-Methylanabasine	100–170	280–350
(−)-Cotinine	70–140	250–320

If the assumption is made that the five compounds in their preferred conformation have torsion angles of comparable amplitude, a common angular region can be found from the results given in Table 2.

This assumption is reasonable, since the chemical structures of the five compounds do not vary greatly in the environment of the rotating bond. It is supported by recent conformational studies in analogous series in which the bond adjacent to the aromatic nucleus was shown to undergo only small changes in rotation for a series of histamine analogues (9), phenothiazines (44), and nucleosides (45).

It is therefore concluded that the CD spectra of the five investigated alkaloids, as monoprotonated and neutral forms in aqueous solution, suggest two preferred conformations having torsion angles of 120–140 degrees [+ *anti*clinal (46)] and 300–320 degrees [− *syn*clinal], respectively.

These results are in good agreement with the values obtained by molecular orbital calculations by Kier (12) (τ = 120 and 300 degrees) and by Pullman *et al.* (13) (τ = 320 degrees, and a less preferred conformation with τ = 120 degrees), or by X-ray diffraction (14) (τ = 118 degrees). However, our CD interpretation does not discriminate between the two preferred conformations or reveal their proportions in the solution.

The biological implications of these results as compared to the preferred conformation of acetylcholine have already been discussed (12–14).

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