Pergamon

Tetrahedron Vol. 50, No. 39, pp. 11329-11338, 1994

0040-4020/94 $7.00+0.00

0040-4020(94)00697-0

Cis- and *Trans*-Configurations of α,α'-Disubstituted Piperidines and Pyrrolidines by GC-FTIR; Application to Decahydroquinoline Stereochemistry

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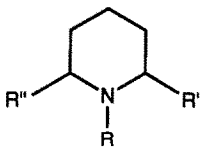
Abstract: GC-FTIR spectroscopy provides a convenient, rapid method to distinguish *cis*- and *trans*-α,α'-disubstituted piperidines and pyrrolidines; the pyrrolidines usually first require N-methylation. *Cis*-isomers exhibit significant Bohlmann bands, *trans*-isomers only very weak bands. The method is also applicable to alkaloids containing a piperidine ring. For example, a 2-propyl-5-methyldecahydroquinoline, named *trans*-**195A**, recently detected in skin extracts of a dendrobatid frog is shown by GC-FTIR to have C(2) and C(8a) in a *cis*-orientation.

Cis- and *trans*-2-alkyl-6-methylpiperidines occur as venom components in ants of the myrmicine genus *Solenopsis* ("fire ants")[1], and various *cis*- and *trans*-2,6-dialkylpiperidines occur in ants of the genus *Monomorium*[2]. Only the *trans*-isomers of 2,5-disubstituted pyrrolidines, however, are detected in venoms of ants of the myrmicine genera *Solenopsis, Monomorium* and *Megalomyrmex*[3]. *Trans*-2-butyl-5-pentylpyrrolidine has been found both in ants[3a] and in skin extracts from dendrobatid frogs (genus *Dendrobates*)[4]. In the frog it has been given the code name *trans*-**197B** and its absolute configuration determined as 2S,5S[5]. *Cis*-2-propyl-6-methylpiperidine and two side-chain oxygenated derivatives have been recently detected as minor alkaloids in extracts of the Mexican bean beetle[6]. 2,6-Disubstituted piperidines also occur in the plant kingdom: *cis*-2-alkyl-6-methylpiperidines have been detected in pine trees (*Pinus*), while spruce trees (*Picea*) contain both *cis*- and *trans*-isomers[7].

While *cis*- and *trans*-configurations of identically α,α'-disubstituted piperidines and pyrrolidines can be unambiguously assigned by the ^{1}H-NMR method of Hill and Chan[8], whereby N-benzylation results in a benzyl methylene singlet from a *cis*-isomer and an AB-quartet from a *trans*-, the method suffers limitations in requiring enough pure material to detect these signals in the δ 3-4 ppm region without interference from other signals and in being applicable only to rings with identical α- and α'-substitutents. ^{13}C-NMR, when sample size and purity permit, can be used to distinguish non-identically substituted *cis*- and *trans*-piperidines by their C-2 and C-6 signals (see ref. 7). Otherwise, the most commonly used method for both piperidines and pyrrolidines involve gc comparisons with both isomers where, usually, the *cis*-isomer has a shorter retention time (see ref. 6).

We report here a simple, more general method for distinguishing *cis*- from *trans*-isomers, which is not limited to rings with identical substitutents and is applicable to microgram quantities present in complex mixtures, such as alkaloid extracts of ants or frog skins, without isolation. We have found, with a series of eight synthetic mixtures[9] of *cis*/*trans*-2,6-disubstituted piperidines (Figure 1, 1, R=H), that

the appearance in FTIR spectra[10] of weak Bohlmann bands[11] at ~2800-2600 cm^{-1} in *cis*- but not *trans*-isomers (Figure 2), permits ready configurational assignments to be made. Bohlmann bands are indicated with arrows in all Figures.



1

R	R'	R''
H	n-C_5H_{11}	H
H	n-C_9H_{19}	CH_3
CH_3	"	"
H	n-$C_{11}H_{23}$	"
H	n-$C_{13}H_{27}$	"
H	$(CH_2)_3CH{=}CH_2$	$(CH_2)_3CH{=}CH_2$
H	$(CH_2)_3CH{=}CH_2$	n-C_5H_{11}
H	n-C_5H_{11}	"
H	$(CH_2)_3CH{=}CH_2$	$(CH_2)_5CH{=}CH_2$
H	n-C_5H_{11}	n-C_7H_{15}
CH_3	"	"
CD_3	"	"

Figure 1: Synthetic *cis/trans* mixtures of 2,6-disubstituted piperidines studied by GC-FTIR.

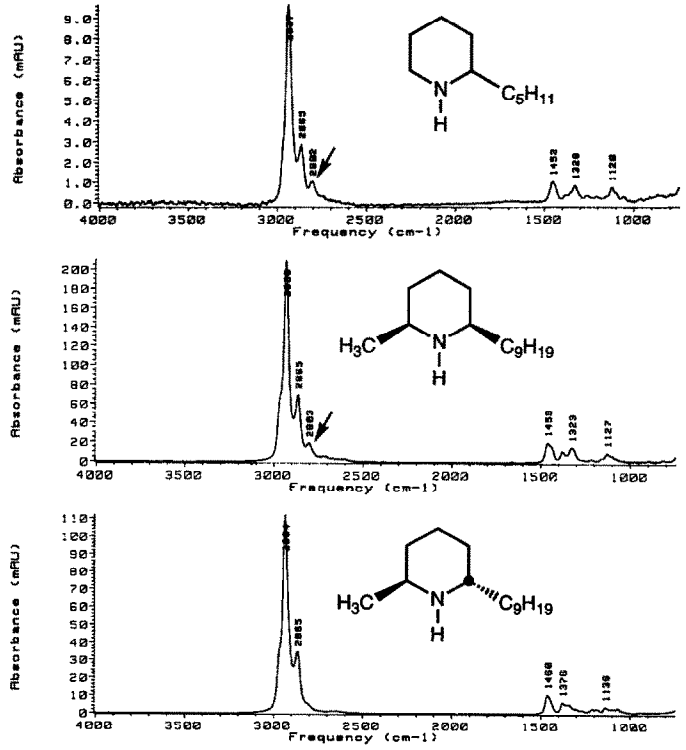


Figure 2: GC-FTIR spectra of 2-pentylpiperidine, and *cis*- and *trans*-2-methyl-6-nonylpiperidine (relative configurations shown).

The Bohlmann band pattern for the *cis*-isomer closely resembles that seen with 2-alkylpiperidines (see 2-pentylpiperidine, Figure 2). We also noted that the *cis*-isomers all had a sharper and more intense infrared absorption in the region of $\nu_{C\text{-}N}$ vibrations at 1330 cm^{-1} than did the *trans*-, although this difference was less pronounced with piperidines containing a 2-methyl substituent. Base-line separations of *cis/trans* isomers were observed using a 25m (0.32mm i.d.) HP-5 (poly 5% diphenylsiloxane-95% dimethylsiloxane) fused silica capillary GC column, where the *cis*-isomer invariably eluted first.

Eschweiler-Clarke methylation [$(CH_2O)_n$, HCO_2H, 60°C, 18 h] on a microscale gave the N-methylated *cis/trans*-piperidines indicated in Figure 1 (**1**, R=CH_3). The cis-isomers had an enhanced Bohlmann band at 2780 cm^{-1} while the *trans*-isomers showed a weaker absorption at 2795 cm^{-1} (Figure 3).

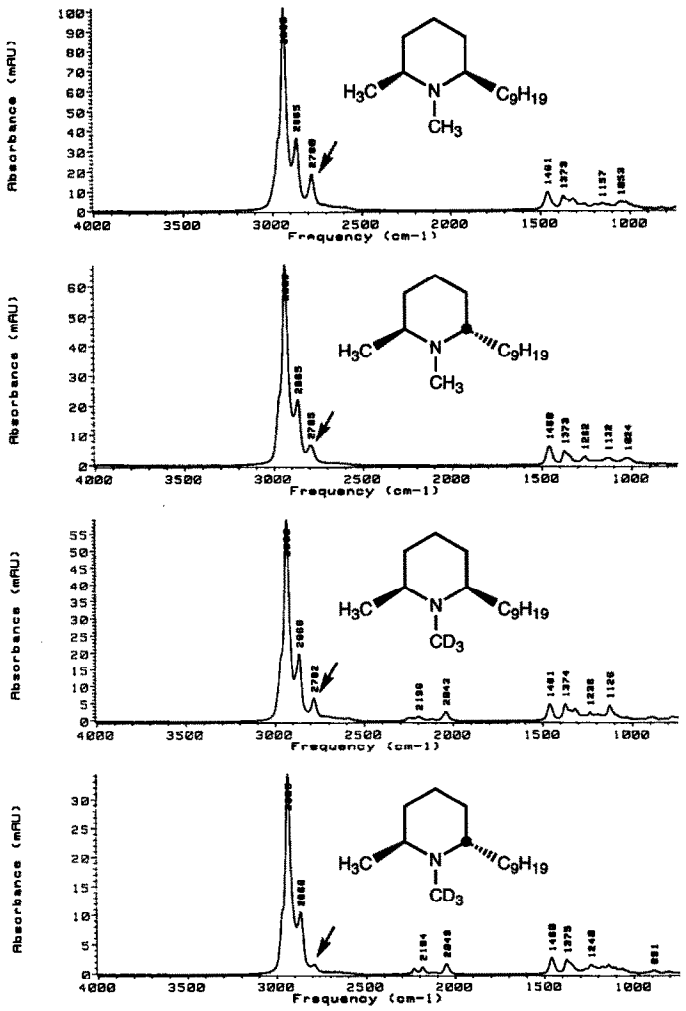


Figure 3: GC-FTIR spectra of N-CH_3 and N-CD_3 derivatives of *cis*- and *trans*-2-methyl-6-nonylpiperidine (relative configurations shown).

Methylation of *cis/trans*-2-methyl-6-nonylpiperidine with $(CD_2O)_n/DCO_2D$[12] provided the N-CD_3 derivatives (Figure 1, **1**, R=CD_3); the *cis*-isomer showed a Bohlmann band at 2782 cm^{-1}, roughly one-half the intensity of the N-CH_3 derivative, while the *trans*-N-CD_3 derivative exhibited only a very weak absorption at ~2795 cm^{-1} (Figure 3). A sharp small band at 1126 cm^{-1} was seen with the *cis*- but not the *trans*-N-CD_3 derivative (Figure 3). N-methylation, while unnecessary in view of the significant difference between unmethylated *cis*- and *trans*-piperidines, does appear to provide useful confirmatory data.

The dihedral angle (ϕ) between the N-lone-pair and the α C-H bond, as it approaches the optimal value of 180° should be reflected in the shift of the Bohlmann band to lower frequencies, reaching a maximum shift at 180°. A contribution to the lowering of this frequency may also be provided by the inductive effect of the methyl group (see discussion in ref. 11) (Figure 4). Bohlmann band *intensities*, on the other hand, should depend on the number of α C-H bonds adopting the optimal *trans* antiparallel (TAP) orientation. Computer molecular modelling (Figure 4) of *cis*-α,α'-disubstituted piperidines indicate $\phi \approx 175°$ with an observed Bohlmann band at 2800 cm^{-1}; N-methylation results in $\phi \approx 180°$ with a more intense Bohlmann band at 2780 cm^{-1} in accordance with the above prediction. The intensity increase stems from a third TAP hydrogen provided by the N-methyl group. Note (Figures 2 and 3) that the N-CD_3 *cis*-derivative shows the same absorption frequency as the N-CH_3 derivative yet a smaller increase in intensity of the band over the unmethylated piperidine. Athough the number (two) of TAP α C-H bonds is the same as the unmethylated piperidine we assume that the N-lone-pair may be more axially-oriented due to the replacement of NH by an N-CH_3 or N-CD_3 group, which preempts the equatorial orientation[13]. This increases the proportion of α hydrogens TAP to the lone-pair and consequently increases the intensity of the absorption.

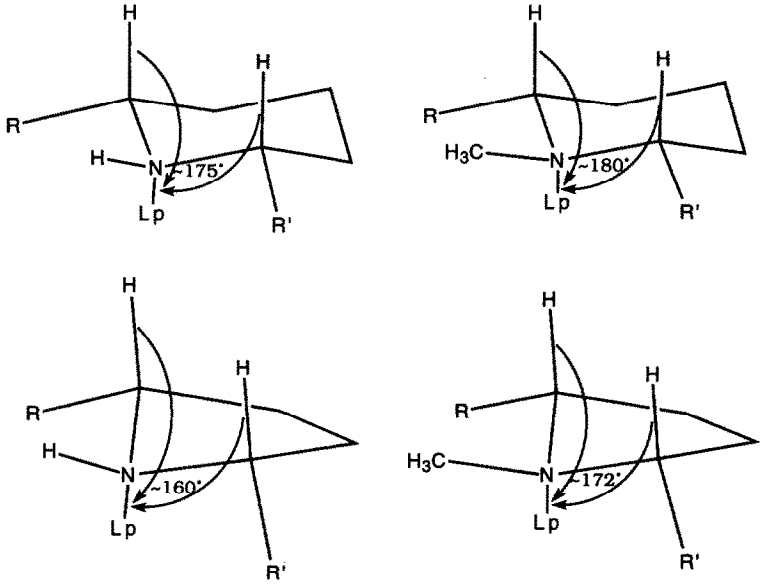
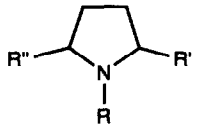


Figure 4: Dihedral angles between the N-lone-pair (Lp) and TAP α C-H bonds in α,α'-disubstituted piperidines and pyrrolidines, before and after N-methylation.

Eight different synthetic mixtures[9] of *cis/trans*-2,5-disubstituted pyrrolidines (Figure 5, **2**, R=H), when examined by GC-FTIR, showed only very minor spectral differences between isomers, in the fingerprint region[14]. Computer molecular modelling predicts a dihedral angle of *ca.* 160° between the N-lone-pair and TAP α C-H bonds (Figure 4). In the case of the *cis*-isomer, the Bohlmann band is a scarcely

observable shoulder on the lower frequency side of the methylene C-H stretching absorption, while the *trans*-isomer has no detectable Bohlmann band (Figure 6). Eschweiler-Clarke methylation provided the N-methylated *cis/trans*-pyrrolidines indicated in Figure 5 (2, R=CH_3). Molecular modelling of these N-methyl derivatives indicated $\phi \approx 172°$ with, in every case, a prominent Bohlmann band at 2784 cm^{-1} observed for the *cis*-isomer, while the *trans*-isomer had a weaker absorption at 2792 cm^{-1} but still more intense than with unmethylated pyrrolidines. Thus, the same results obtain as with the piperidines. N-methylation produces a more optimal ϕ resulting in a shift to lower frequency and the intensity increases as another TAP hydrogen is provided by the N-methyl group.



2

R	R'	R''
H	*n*-C_6H_{13}	C_2H_5
CH_3	"	"
Ac	"	"
H	*n*-C_7H_{15}	$(CH_2)_2CH{=}CH_2$
Ac	"	"
H	$(CH_2)_3CH{=}CH_2$	*n*-C_4H_9
Ac	"	"
H	"	"
H	*n*-C_7H_{15}	"
CH_3	"	"
CH_2D	"	"
CHD_2	"	"
CD_3	"	"
H	$(CH_2)_7CH{=}CH_2$	"
Ac	"	"
H	*n*-C_7H_{15}	$(CH_2)_4CH{=}CH_2$
CH_3	"	*n*-C_6H_{13}
H	*n*-C_9H_{19}	"
CH_3	"	"

Figure 5: Synthetic *cis/trans*-mixtures of 2,5-disubstituted pyrrolidines studied by GC-FTIR.

In addition, a weak absorption at 1122 cm^{-1} is seen in *cis*-isomers, but is scarcely detectable in the *trans*-(Figure 6). Unmethylated *cis/trans*-2,6-dialkyl-pyrrolidines are barely separable on a 25 meter HP-5 column, with the *cis*-isomer eluting just ahead of the *trans*-isomer; however, the N-methyl derivatives are well-separated (50-60 sec.) with the *cis*-isomer eluting first, as was the case with the two N-methyl piperidines examined.

CD_3-methylation of a *cis/trans*-2-butyl-5-heptylpyrrolidine mixture afforded a *cis*-derivative (Figure 5, 2, R=CD_3) with a pronounced Bohlmann band at 2795 cm^{-1}; the *trans*-derivative had only a weak shoulder at approximately 2810 cm^{-1} (Figure 7, H, I, K, L). Here the effect of the N-CD_3 group in enhancing Bohlmann bands must be a conformational one as in the piperidines since no additional TAP hydrogens can be involved. The frequency shift from the unmethylated pyrrolidine is consistent with a more optimal dihedral angle and the increased intensity may stem from the lone pair being forced into a more axial orientation by the equatorial N-substituent.

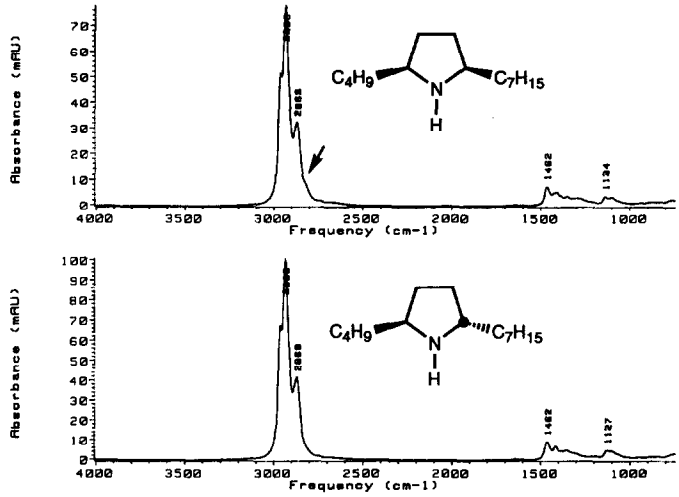


Figure 6: GC-FTIR spectra of *cis*- and *trans*-2-butyl-5-heptylpyrrolidine (relative configurations shown).

Although the α,α'-disubstituted piperidines and pyrrolidines of Figure 7 have differing α and α'-substituents, a comparison of their Bohlmann bands is informative. The Bohlmann bands of the unmethylated *cis*-piperidines have a lower frequency than the bands of the corresponding *cis*-pyrrolidines because of a more optimal φ, but the same intensity because of the same number of TAP α C-H hydrogens (Figure 7, A, G). The same comparison holds for the N-CH_3 and N-CD_3 derivatives, although small differences can be seen due to differing α- and α'-substituents (Figure 7, B, C, H, I). Although the unmethylated *cis*-piperidine and the N-CD_3-*cis*-pyrrolidine have roughly the same φ and number of TAP hydrogens, the latter has a more intense Bohlmann band due to the lone-pair being more in an axial orientation (Figure 7, A, I).

Methylation of a *cis/trans*-2-butyl-5-heptylpyrrolidine mixture with $(CD_2O)_n/HCO_2H$ or $(CH_2O)_n/DCO_2D$ gave N-CHD_2 or N-CH_2D derivatives (Figure 5, **2**, R=CHD_2 and R=CH_2D) respectively, which permitted a rough estimate of the contributions of *trans*-antiparallel N-methyl hydrogens and the above conformational effect in enhancing Bohlmann bands. In every case, the *cis*-isomer had a more pronounced Bohlmann band pattern than the *trans*-, with the intensity of the *cis*-isomer's Bohlmann band at 2795-2802 cm^{-1} (relative to the intensity (100) of ν_{CH} at 2935 cm^{-1}) decreasing in the order: N-CH_2D (27) > N-CH_3 (24) > N-CHD_2 (20) > N-CD_3 (14) (Figure 7, H, I, K, L, and Figure 8, A, B, C, D). Since the dihedral angle is the same in every case, the difference between the isotopically labelled pyrrolidines should reflect only the probability of another α C-H of the proper TAP orientation. Surprisingly, the N-CH_2D-substituted pyrrolidine gave a more intense Bohlmann band than the N-CH_3-substituted pyrrolidine. Otherwise the effect of replacing one and two hydrogens in N-CD_3 is roughly additive, consistent with a doubling of the probability of one α C-H being in the correct TAP orientation. As seen in Figures 7 and 8, C-D bands can be detected in these compounds in the range 2000-2200 cm^{-1} and are related to the fundamental C-H stretching frequencies by a factor of ~1.38[15]. The most prominent absorption in every case, the lower frequency vibration, corresponding to the symmetrical C-D streching mode, increases in intensity and also is shifted to lower frequencies, regularly in the series CH_2D, CHD_2, CD_3.

Acetylation (Ac_2O/Py, 18 h) of four different pairs of *cis/trans*-pyrrolidines (Figure 5, **2**, R=Ac) reversed the order of elution of isomers from the HP-5 column with the *trans*-isomer now eluting first. Small IR spectral differences between *cis*- and *trans*-N-acetyl isomers could be detected with the *cis*- showing a pair of sharp absorptions at 1228 and 1170 cm^{-1}, while the *trans*-isomers had a broad absorption at 1196 cm^{-1}; otherwise their spectra including the $\nu_{C=O}$ absorption (1672 cm^{-1}) were identical.

While such differences could be useful confirmatory data, N-methylation would appear to distinguish more easily between *cis*- and *trans*-pyrrolidines.

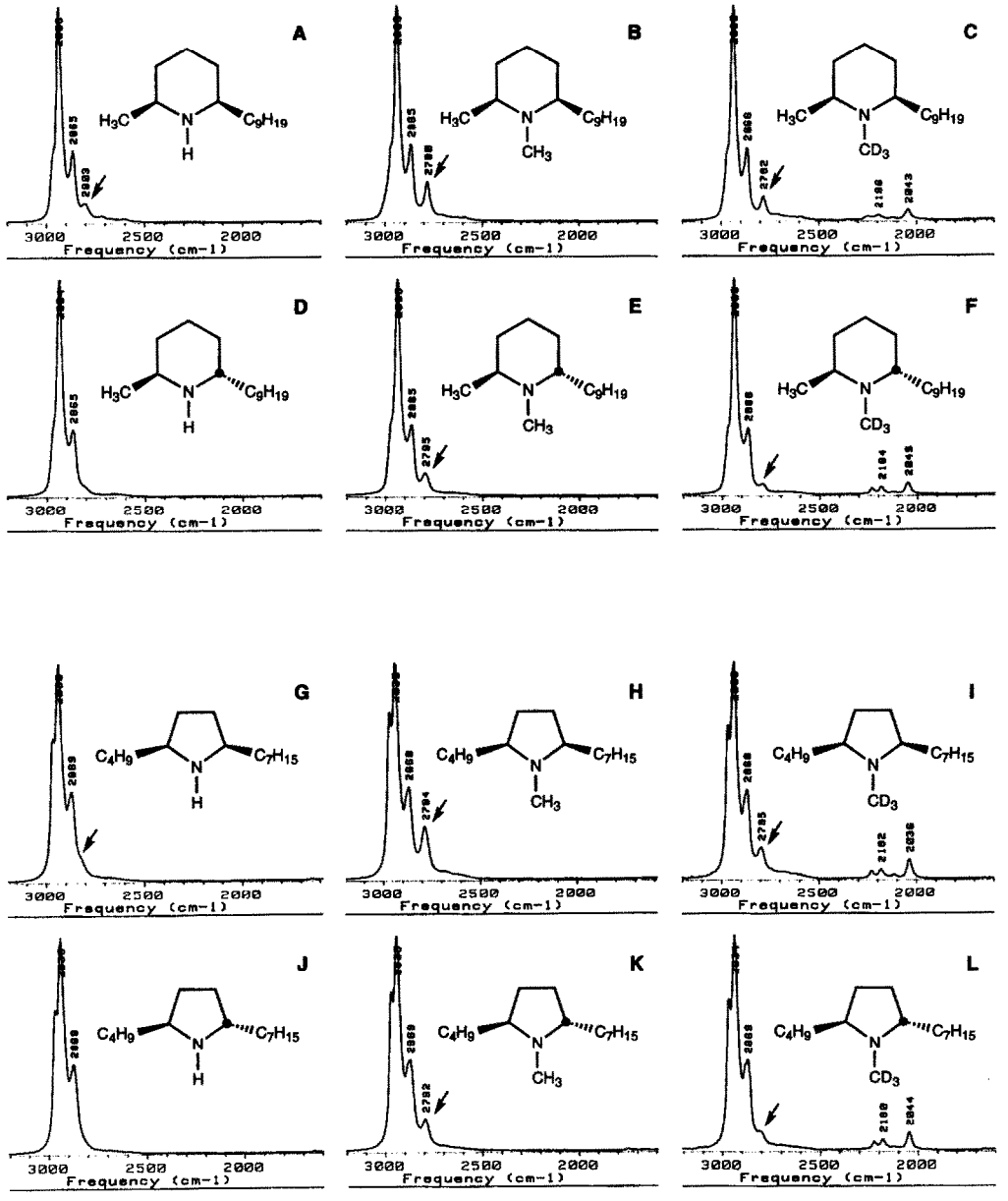


Figure 7: GC-FTIR spectra (1600 to 3200 cm^{-1}) of *cis*- and *trans*-2-methyl-6-nonyl-piperidine (A and D), the corresponding N-CH_3 and N-CD_3 derivatives (B, C, E, F), *cis*- and *trans*-2-butyl-5-heptylpyrrolidine (G and J) and the corresponding N-CH_3 and N-CD_3 derivatives (H, I, K, L) (relative configurations shown).

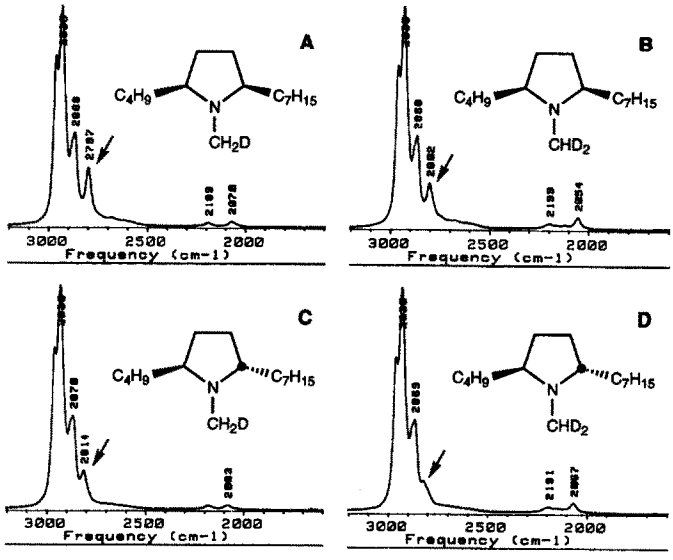
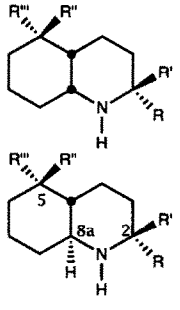


Figure 8: GC-FTIR spectra (1600 to 3200 cm^{-1}) of N-CH_2D and N-CHD_2 derivatives of *cis*- and *trans*-2-butyl-5-heptylpyrrolidine (relative configurations shown).

We are currently applying the piperidine Bohlmann band correlations to the study of the stereochemistry of decahydroquinolines, an alkaloid class commonly found in dendrobatid frogs[4]. Using as references, *cis*-**195A** ("pumiliotoxin C")(**3**) and three synthetic epimers (**4**-**6**), we have assigned the indicated C(2)- and C(8a)-relative configurations to *trans*-**195A** (**7**), a new diastereomer detected as a major alkaloid in skin extracts of the Peruvian frog, *Epipedobates bassleri* (Figure 9). The mass spectrum (ion trap) is virtually identical with that of **3** [m/z, 196 (M+1), 152 (100), 109 (11), 107 (6)]. The *trans*-ring junction is indicated by sharp IR absorptions at 1334 and 1132 cm^{-1} [18], while the C(2) configuration, relative to that at C(8a), is indicated as *cis*- by the observation of a Bohlmann band at 2798 cm^{-1} in the FTIR spectrum of **7**, typical of a *cis*-2,6-disubstituted piperidine. The relative stereochemistry at C(5) of **7** remains unknown (Figure 10). The same arguments apply to the FTIR spectra presented in ref. 18 for several dendrobatid decahydroquinolines (cis-**219A**, trans-**219A**, cis-**243A**, trans-**243A** and 5-epi-trans-**243A**), confirming that the relative configuration of three of the four stereocenters of the decahydroquinoline ring can be assigned by FTIR.



3	R=*n*-C_3H_7	R'=R'''=H	R''=CH_3
4	R=R'''=H	R'=*n*-C_3H_7	R''=CH_3 [16,19]
5	R=*n*-C_3H_7	R'=R''=H	R'''=CH_3 [17]
6	R=*n*-C_3H_7	R'=R'''=H	R''=CH_3 [17]
7	R=H	R'=*n*-C_3H_7	R''=CH_3, R'''=H or R''=H, R'''=CH_3

Figure 9: *Cis*- and *trans*-fused decahydroquinolines (relative configurations shown).

It would appear likely that the C(2)-C(7a) configurations of perhydroindoles, could after methylation, be assigned in a similar manner by comparisons with *cis*- and *trans*-2,5-disubstituted pyrrolidines. However, perhydroindoles have not as yet been reported as natural products.

In conclusion, we have presented a simple GC-FTIR method to determine the relative configurations of *cis*- and *trans*-α,α'-differently disubstituted piperidines and pyrrolidines in mixtures and in very small amounts. The method should also be applicable to alkaloids with piperidine or pyrrolidine moieties. We have proposed a rationalization of our results using a simple model which stresses i) the dihedral angle between the N-lone-pair and α C-H bonds and ii) the number of TAP α-hydrogens.

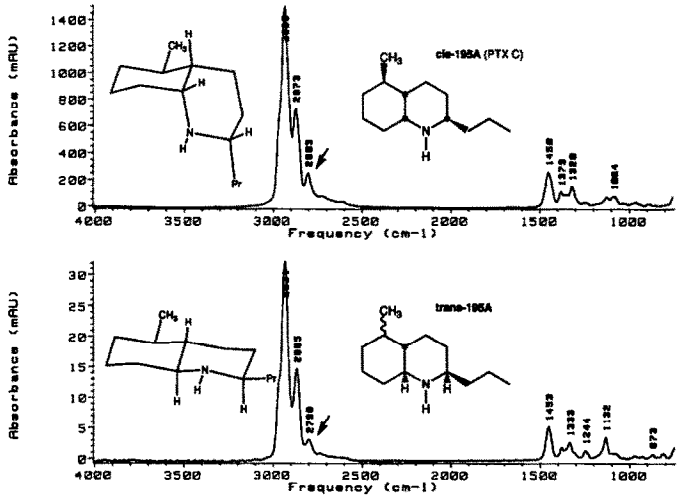


Figure 10: GC-FTIR spectra of cis-**195A** (PTX C) and trans-**195A**.

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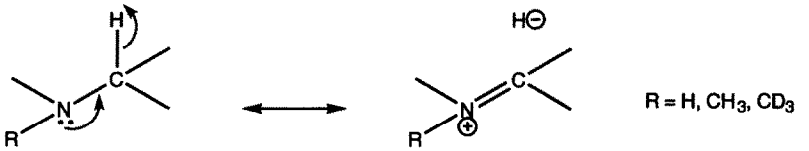
9. These synthetic mixtures were prepared by one of us (THJ) during studies on ant venoms.
10. A Hewlett-Packard model 5890 gas chromatograph fitted with an HP-5 fused silica capillary column (polymer of 5% diphenylsiloxane and 95% dimethylsiloxane, 25 m x 0.32 mm), interfaced with a Hewlett-Packard model 5965B FTIR detector (narrow band, 4000-750 cm^{-1}, resolution 8 cm^{-1}) with a 59970 IRD ChemStation was used to record all FTIR spectra. The GC program was 100° (1 min) to 280° (10 min) at 10°/min.

11. Bohlmann bands were defined as ν_{CH} bands at lower frequencies (~2800-2600 cm^{-1}) than normal aliphatic C-H stretching bands. An intuitive model based on the concept of hyperconjugation provides a convenient rationalization of Bohlmann's empirical observations (Bohlmann, F.; *Chem. Ber.*, **1958**, *91*, 2157-2167) that two or more hydrogens α to a nitrogen lone-pair and *trans*-antiparallel (TAP) are sufficient to observe ν_{CH} bands displaced to lower frequencies (implying a decreased Hooke's law force constant). In our model, it is assumed that the N-lone-pair non-bonding electrons enhance the contribution of the double bond between N and C(α) in the resonance hybrid, concommitantly weakening the α C-H bond.



The following results are explained: i) The Bohlmann band effect is maximum when the dihedral angle between the N-lone-pair sp^3 orbital and a TAP α CH bond is 180°. In this case the mesomeric double bond structure is more significant and has maximum overlap with the σ bond of the TAP α-hydrogen. ii) Electron-releasing substituents, *e.g.* the CH_3 or CD_3 groups, which stabilize hyperconjugation resonance forms, would be expected to shift Bohlmann bands to lower frequencies, even though in the case of CD_3, no additional TAP hydrogens are present. Supporting this interpretation is the observation of a 20 cm^{-1} shift to lower frequency of TAP α-hydrogens in *cis*-N-CD_3-2,6-disubstituted piperidines, ν_{CH}=2780 cm^{-1}, *vs* ν_{CH}=2800 cm^{-1} for the unmethylated *cis*-piperidines (see Figures 2 and 3) that parallels the 35 cm^{-1} decrease in $\nu_{C=O}$ frequency in going from N-methylacetamide to N,N-dimethylacetamide[19], an effect generally attributed to hyperconjugation.

12. D_2-paraformaldehyde ($(CD_2O)_n$) and D_2-formic acid (DCO_2D) are available from Cambridge Isotope Labs., Woburn, MA., 01801.
13. Lambert, J.B.; Keske, R.G.; Carhart, R.E.; Jovanovich, A.P. *J. Am. Chem. Soc.* **1967**, *89*, 3761-3767. The effect of N-methylation in enhancing the Bohlmann bands of piperidine was ascribed by these authors to the shift of the normally equatorially directed N-lone-pair to an axial orientation.
14. Very subtle but reproducible differences can be detected between *cis*- and *trans*-isomers in three different ir regions: *Cis*-pyrrolidines generally have a doublet of absorptions at *ca.* 1130 cm^{-1}, while only one absorption is observed in this region for *trans*-isomers. In a second region, *ca.* 1300 cm^{-1} (ν_{C-N}), *cis*-isomers exhibit a plateau, while *trans*-isomers have a broad absorption with a slope declining toward lower frequencies. Lastly, *cis*-isomers have a slightly more intense plateau at 2600-2800 cm^{-1} than the *trans*- (see Figure 6).
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(*Received in USA* 13 *April* 1994; *accepted* 1 *August* 1994)