

Nuclear Magnetic Resonance (NMR) Spectroscopic Investigation of Interaction Energies of Ephedrine Stereoisomers in Noncrystalline Solids and Its Correlation with Thermodynamic Data

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Equations relating the interaction energies of each of the binary mixtures of ephedrine from linear combinations of the energies of the individual isomers are presented. The interaction energies in the noncrystalline solid mixtures measured from NMR chemical shift data using cross-polarization magic angle spinning nuclear magnetic resonance ¹³C cross-polarization magic angle spinning nuclear magnetic resonance (¹³CP/MAS NMR) spectroscopy correlate strongly with interaction energy from thermodynamic data. The summation of changes in relative frequencies for structurally equivalent carbons is used as a measure of differences in electron shielding on mixing. The relative direction of polarization of individual stereoisomers is found to affect association in noncrystalline binary mixtures of solids. NMR chemical shift data of solids may be useful in confirming spectroscopically the interactions of stereoisomers observed thermodynamically.

KEY WORDS: interaction energy; ¹³C cross-polarization magic angle spinning nuclear magnetic resonance (¹³C CP/MAS NMR) solids; chemical shift; ephedrine; pseudoephedrine; relative direction of polarization.

INTRODUCTION

The four diastereoisomers (1R:2R)-(-)-pseudoephedrine (-ψ), (1S:2S)-(+)-pseudoephedrine (+ψ), (1R:2S)-(-)-ephedrine (-E), and (1S:2R)-(+)-ephedrine (+E) (Fig. 1) are bronchodilators, which have vasopressor side effects differing significantly among the isomers (1,2). Differences in biological activity between ephedrine and pseudoephedrine isomers in solution have been attributed to methine erythro and threo conformations, respectively, measured from ³J_{HH} coupling constants (3-6). In both ¹H and ¹³C NMR spectra, chemical shift differences between -ψ and -E (and between +ψ and +E) have been attributed to torsional angle differences and steric effects (7-9). These reports concern the properties of individual isomers, and not those of the binary mixtures.

Recent research into the thermodynamics of mixing ephedrine isomers relates mixture solubility with heats of fusion and transition temperatures of the solids (10). This

suggests that structural changes in the solid may occur which correspond to the differences in association energies of the isomeric mixtures in the solid state and are also predictive of their interaction energies in solution. Structural differences for pure isomers have been reported using X-ray crystallography (11,12), but the technique cannot be used for binary mixtures of the isomers because the resulting solids are noncrystalline.

Pirkel and Pochapsky used chemical shift data to calculate diastereoisomeric association constants in solution (13). The advantages of solid-state NMR in drug research have recently been reported (14). Isomeric purity of diastereoisomeric mixtures in solids has been determined using ¹³C cross-polarization magic angle spinning nuclear magnetic resonance (CP/MAS NMR) spectroscopy (15). Studies of interactions of structurally similar compounds using ¹³C CP/MAS NMR in the solid state have also been published (15). This suggests that ¹³C CP/MAS NMR may be useful in investigating the interactions of binary mixtures of ephedrine isomers in the solid state. The difference in interaction energy among binary mixtures of ephedrine isomers (determined thermodynamically) suggests that spectroscopic differences on mixing could also occur. NMR studies on mixing in solids are investigated to avoid potential complicating influences of solvent and concentration effects on chemical shift in solution (16).

MATERIALS AND METHODS

Each binary mixture of ephedrine (Aldrich Chemical Co.) and pseudoephedrine (Sigma Chemical Co.) isomers was melted and allowed to cool three times in a closed glass vial, remelted, transferred (as a liquid) to the NMR rotor, and allowed to solidify in the capped rotor. X-ray diffraction of the solids confirmed the mixtures and the individual components to be noncrystalline. The ¹³C CP/MAS NMR spectra are obtained on a Varian XL-200 NMR spectrometer operating at a field of 4.69 T (¹H = 200 MHz, ¹³C = 50.28 MHz), using a Varian variable-temperature solid probe with cylindrical glass rotors. The spectra were recorded at room temperature over a spectral width of 16,000 Hz, using a 90° pulse of 7.5 μsec. Each spectrum is the result of 1000 transients, with a contact time of 50 msec and a 1.5-sec delay time between scans. A TOSS program at a spinning rate of 4000 Hz is used to suppress spinning side bands. A faster spinning rate more strongly suppresses the spinning side bands but also, for some samples, caused difficulty in maintaining uniform spinning rates. Hexamethylbenzene was used as the external reference standard.

RESULTS AND DISCUSSION

Upon mixing, changes in chemical shift were observed for each binary combination of the four diastereoisomers. These changes could be intramolecular or intermolecular in nature. Since the solids are noncrystalline, no contribution to the interaction energy from crystal lattice or net macroscopic orientational effects would be anticipated. The interactions of the diastereoisomers in the noncrystalline solids are thus reduced to intramolecular interactions, and not to

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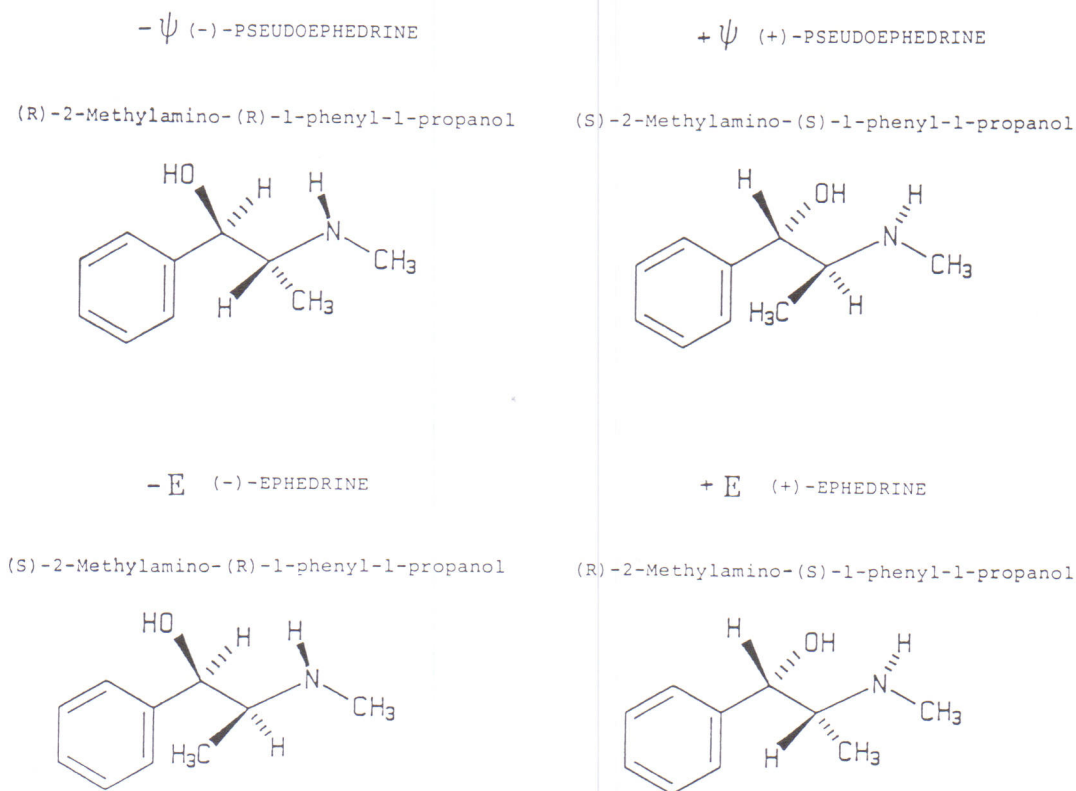


Fig. 1. Structures of ephedrine isomers. Vapor-phase molecular mechanics investigation of ephedrine isomers calculates conformational stabilization at the amine nitrogen from hydrogen bonding between the hydroxyl oxygen and the amine hydrogen. No preferred configuration about the amine nitrogen in solution, however, should be assumed because different solvents may favor either or neither configuration. The nitrogen configurations presented are also consistent with X-ray data of ephedrine isomers as crystals (11,12).

energy differences due to crystal structure. The same remelt procedure was used as in the original paper (10), and no interferences or chemical decomposition by mass spectrometry or NMR, IR, or UV spectroscopy was detected from the remelt procedure.

Classical NMR line shape theory on chemical exchange between two sites demonstrates that lines are sharp when they are magnetically nonequivalent and/or magnetically equivalent. Peak broadening occurs with the partial coalescence between two magnetically nonequivalent peaks (18). Line broadening for corresponding carbon peaks is similar in the each of the equimolar binary mixtures to that of the pure compounds. This suggests that the difference between structurally equivalent carbons in binary equimolar mixtures of ephedrine and pseudoephedrine is primarily a question of whether they are magnetically equivalent or magnetically nonequivalent.

A single set of aliphatic chemical shifts (σ) by ^{13}C CP/MAS for the noncrystalline solids of ephedrine isomers for pure $-\psi$ (or $+\psi$) (Fig. 2A) and for only $-E$ (or $+E$) (Fig. 2C) were observed. The chemical shifts for corresponding carbons of ephedrine and pseudoephedrine were nonequivalent. In binary mixtures of $-E$ with $+\psi$ and $+E$ with $-\psi$, the chemical shifts remain nonequivalent, but both sets were shifted downfield from those of the original isomers. In binary equimolar mixtures of $-E$ with $-\psi$ and of $+E$ with $+\psi$ the corresponding peaks in ephedrine and pseudoephedrine appear magnetically equivalent (Fig. 2D). Thus in binary

equimolar mixtures of the diastereoisomers, two sets of chemical shifts are different from each other and two sets are similar. The sets which appear magnetically equivalent polarize light in the same direction. The sets which are magnetically nonequivalent polarize light in the opposite direction.

Each binary mixture of diastereoisomers has a significant downfield chemical shift compared to that of the corresponding carbons in each of the pure isomers (Table I). A downfield chemical shift corresponds to lower energy. The changes in chemical shift could be due to differences in electron shielding (19) compared to that of the pure compounds.

Frequency can be related to electron shielding effects ($1 - \sigma$) on mixing by the formula (20)

$$\nu = \frac{\gamma}{2\pi} B_0 (1 - \sigma) \quad (1)$$

in which ν is the resonance frequency, γ is the gyromagnetic ratio for carbons, σ is the shielding constant, and B_0 is the strength of the magnetic field. A difference in frequency in a strong magnetic field for two structurally equivalent carbons corresponds to a difference in electron shielding between the atoms:

$$\Delta\nu = \frac{\gamma}{2\pi} B_0 \Delta(1 - \sigma) \quad (2)$$

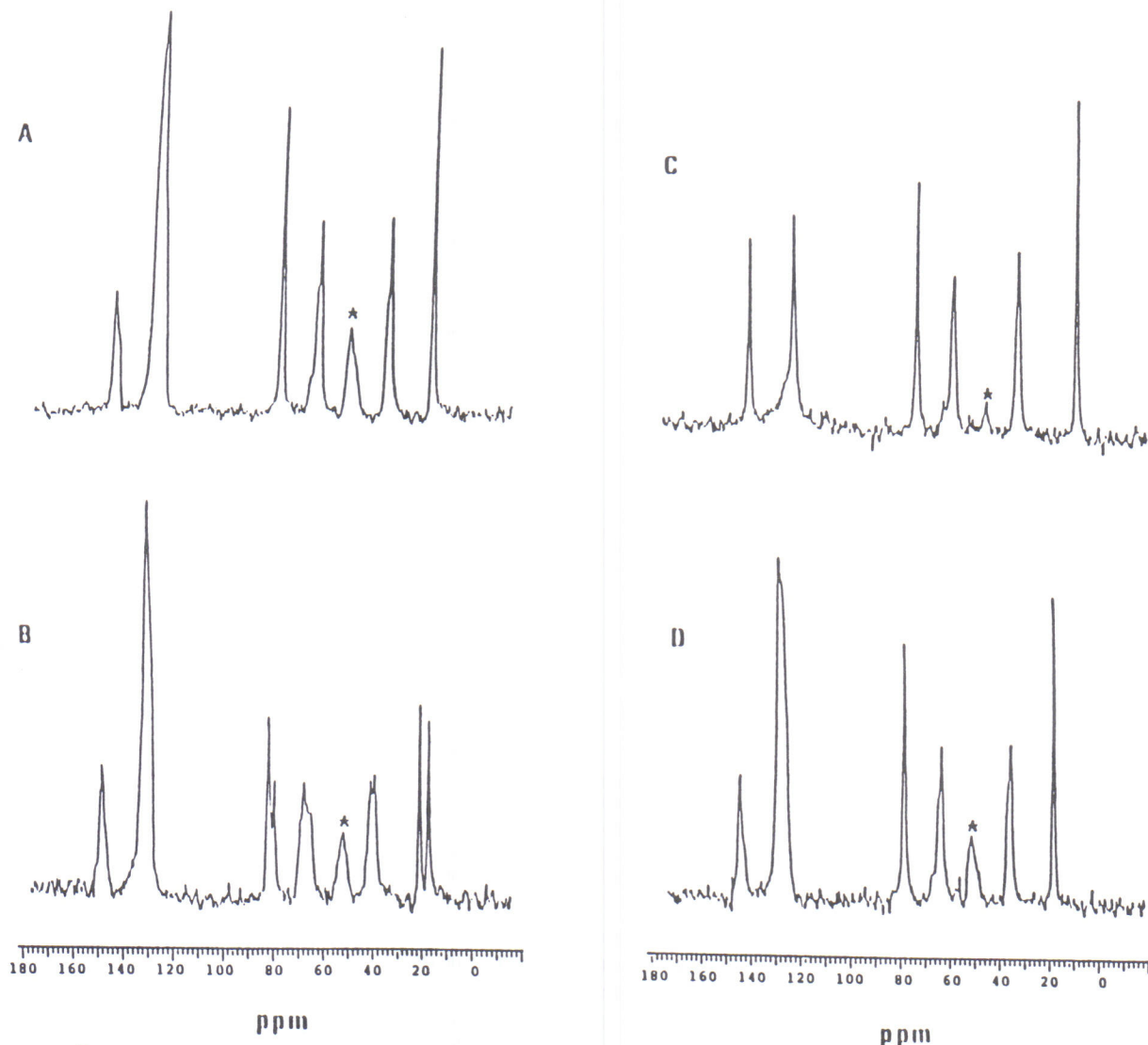


Fig. 2. ^{13}C CP/MAS spectra of noncrystalline solids of ephedrine isomers: (A) pure (-)-pseudoephedrine ($-\psi$); (B) binary equimolar mixture with opposite directions of polarization ($-\psi/+E$) and similar to ($+\psi/-E$); (C) pure (+)-ephedrine ($+E$); (D) binary equimolar mixture with similar directions of polarization ($-\psi/-E$) and similar to ($+\psi/+E$). The asterisk denotes spinning side bands. Conditions: 50.28-MHz ^{13}C -NMR spectrometer; spectral width, 16,000 Hz; contact time, 50 msec; delay time, 1.5 sec; and spinning rate, 4000 Hz.

Relative chemical shift δ equals $10^6(1 - \sigma)$, which converts shielding into parts per million (ppm) units (20). Therefore with $\Delta\delta$ in units of 10^{-6} ,

$$\Delta\nu = \frac{\gamma}{2\pi} B_0 \Delta\delta \quad (3)$$

The absence of a change in the NMR chemical shift on mixing would indicate no change in electron shielding and no change in energy between the different isomers for a particular structural feature in the mixture. Because some change in chemical shift is observed on mixing for nearly all carbons in each binary mixture of isomers, and because some changes are positive and others are negative, the summation of chemical shifts ($\Sigma\Delta\delta_A$) is used as a measure of net change in electron shielding over the entire molecule. The summation of chemical shifts for two isomers ($\Sigma\Delta\delta_{AB}$) is used as a

measure of the changes in energy from electron shielding effects in the total mixture (Table II). Chemical shifts for corresponding carbons are each calculated relative to those of (-)-ephedrine.

Because each of the four isomers changes differently in the presence of each of the three other isomers, 12 potentially different individual interactions can be postulated. Two separate terms are necessary to describe changes in chemical shift in the binary mixtures. For example, the terms $-\psi/-E$ and $-E/-\psi$ are used to distinguish between the chemical shift of $-\psi$ in the presence of $-E$ and the considerably different chemical shift of $-E$ in the presence of $-\psi$. The changes in chemical shift on mixing in each combination are calculated (Table II).

Differences in chemical shift changes on mixing may be related to differences in interaction energy between the di-

Table Ia. Chemical Shifts (ppm) and Assignments in Individual and Equimolar Mixtures of Solids^a

Isomer ^b	C-CH ₃	N-CH ₃	N-C-H	O-C-H	Ar-C	Ar-C' - 1
Mixture (1/1)						
-ψ/-E	17.7	35.8	65.0	80.7	132.4	149.1
-E/-ψ	17.7	35.8	65.0	80.7	131.2	149.1
+ψ/+E	17.6	35.7	64.9	80.7	132.7	149.5
+E/+ψ	17.6	35.7	64.9	80.7	131.9	149.5
-ψ/+E	17.6	38.4	66.1	80.5	131.0	149.7
+E/-ψ	13.8	36.6	65.1	78.4	131.0	149.7
+ψ/-E	17.6	38.3	66.0	80.6	131.2	149.8
-E/+ψ	13.8	36.6	65.1	78.3	131.2	149.8
-ψ/+ψ	16.4	37.4	64.6	77.8	128.7	147.2
+ψ/-ψ	16.4	37.4	64.6	77.8	128.7	147.2
+E/-E	16.5	36.2	61.8	75.9	129.0	143.7
-E/+E	16.5	36.2	61.8	75.9	129.0	143.7
Single						
-ψ	15.2	34.0	63.4	78.3	128.8	145.8
+ψ	15.4	34.2	63.6	78.5	129.0	147.2
-E	11.4	35.5	63.1	77.9	128.9	146.7
+E	12.4	38.4	65.9	79.5	130.9	146.8

^a The term -ψ/-E denotes -ψ in the presence of equimolar -E, whereas -E/-ψ denotes -E in the presence of equimolar -ψ.

^b Ar-C are aromatic carbons and Ar-C' - 1 is the C' - 1 aromatic carbon. Hexamethylbenzene is used as an external reference standard.

astereoisomers. Interaction energy changes on mixing of ephedrine isomers have been reported from thermodynamic data (10). However, NMR interaction energies are measured in terms of frequency units, and not free energy units. Multiplying the thermodynamic energy units by a constant converts them to frequency units (Appendix A). Since each of the interaction energies of the mixture has been determined, the energy changes in the solids as obtained from both the thermodynamic and the electron shielding data can be compared.

Energy diagrams (Figs. 3 and 4) from the thermodynamic data simplify explanations of the diastereoisomeric

interactions. Each of the 12 interaction energies can be generated quantitatively from only the frequencies of the four individual isomers (Appendix A). Because the difference in the energy levels of -ψ to -E is larger than the difference in energy levels +ψ to +E, the energy of mixed energy levels -ψ/-E is also greater than for the mixed energy levels +ψ/+E. The assignment of the energy states to individual isomers is unambiguous from the thermodynamic data.

The energy diagram from summation of chemical shift data is constructed from calculations in Table II. The $\Sigma\Delta\delta_{AB}$ for -E/+ψ is the same vertical length as the vertical distance between +E/-E and +ψ/-ψ. The $\Sigma\Delta\delta_{AB}$ for -E/-ψ

Table Ib. Downfield Chemical Shifts (ppm) in Individual and Equimolar Mixtures of Solids Relative to (-)-Ephedrine^a

Mixture (1/1) ^b	C-CH ₃	N-CH ₃	N-C-H	O-C-H	Ar-C	Ar-C' 1	$\Sigma\Delta\delta_A$
-ψ/-E	6.3	0.3	1.9	2.8	3.5	2.4	17.2
-E/-ψ	6.3	0.3	1.9	2.8	2.3	2.4	16.0
+ψ/+E	6.2	0.2	1.8	2.8	3.8	2.8	17.6
+E/+ψ	6.2	0.2	1.8	2.8	3.0	2.8	16.8
-ψ/+E	6.2	2.9	3.0	2.6	2.1	3.0	19.8
+E/-ψ	2.4	1.1	2.0	0.5	2.1	3.0	11.1
+ψ/-E	6.2	2.8	2.9	2.7	2.3	3.1	20.0
-E/+ψ	2.4	1.1	2.0	0.4	2.3	3.1	11.3
-ψ/+ψ	5.0	1.9	1.5	-0.1	-0.2	0.5	8.6
+ψ/-ψ	5.0	1.9	1.5	-0.1	-0.2	0.5	8.6
-E/+E	5.1	0.7	-1.3	-2.0	0.1	-3.0	-0.4
+E/-E	5.1	0.7	-1.3	-2.0	0.1	-3.0	-0.4
-ψ	3.8	-1.5	0.3	0.4	-0.1	-0.9	2.0
+ψ	4.0	-1.3	0.5	0.6	0.1	0.5	4.4
-E	0.0	0.0	0.0	0.0	0.0	0.0	0.0
+E	1.0	2.9	2.8	1.6	2.0	0.1	10.4

^a The term -ψ/-E denotes -ψ in the presence of equimolar -E, whereas -E/-ψ denotes -E in the presence of equimolar -ψ.

^b Ar-C are aromatic carbons and Ar-C' - 1 is the C' - 1 aromatic carbon. Hexamethylbenzene is used as an external reference standard.

Table II. Comparison of NMR Chemical Shift Data with Thermodynamic Interaction Energies^a

Mixture (I/I)	S_A (e.u.)	ν_{exp} (THz)	ν_{calc} (THz)	$\Delta\nu_{AB}$ (THz)	$\Sigma\Delta\delta_A$ (ppm)	$\Sigma\Delta\delta_{AB}$ (ppm)	$\Delta\delta_{\text{ref}}$ (ppm)	$\Sigma\Delta\delta_{AB} - \Delta\delta_{\text{ref}}$ (ppm)
$-\psi/-E$	15.4	48.4	48.4	14.8	17.2	33.2	0.0	33.2
$-E/-\psi$	10.9	33.6	33.5		16.0			
$+\psi/+E$	15.1	47.2	47.2	10.4	17.6	34.4	10.0	24.4
$+E/+ \psi$	11.7	36.8	36.6		16.8			
$-\psi/+E$	15.0	47.5	47.6	11.0	19.8	30.9	5.6	25.3
$+E/-\psi$	11.6	36.5	36.6		11.1			
$+\psi/-E$	14.9	46.9	46.8	13.5	20.0	31.3	0.0	31.3
$-E/+ \psi$	10.8	33.3	33.5		11.3			
$-\psi/+ \psi$	18.9	59.4	59.4	0.0	—	8.6	8.6	—
$+ \psi/-\psi$	18.9	59.4	59.4		—			
$-E/+E$	12.0	33.4	33.5	0.0	-4.8	0.8	0.4	0.4
$+E/-E$	12.0	33.4	33.5		5.6			
$-\psi$	19.1	60.1	60.0	1.3	2.0	6.4	3.2	3.2
$+\psi$	18.7	58.8	58.9		4.4			
$-E$	10.2	32.1	32.0	-2.8	0.0	-10.4	-5.2	-5.2
$+E$	11.1	34.9	35.1		10.4			

^a The term $-\psi/-E$ denotes $-\psi$ in the presence of equimolar $-E$, whereas $-E/-\psi$ denotes $-E$ in the presence of equimolar $-\psi$.

increases relative to the $\Sigma\Delta\delta_{AB} - E/+ \psi$ by the amount due to $-\psi$ (1.9–2.0 ppm). The $\Sigma\Delta\delta_{AB}$ for $+E/-\psi$ differs from the $\Sigma\Delta\delta_{AB}$ for $-E/+ \psi$ by the vertical distance from $+E$ to $+E/-E$ (5.6 ppm). The $\Sigma\Delta\delta_{AB}$ for $+E/+ \psi$ differs from $\Sigma\Delta\delta_{AB}$ for $-E/+ \psi$ by the vertical distance due to $+E$ (5.2–

5.3 ppm). The uncorrected vertical distance $\Sigma\Delta\delta_{AB}$ for $+E/+ \psi$ is 34.4 ppm, which is also the distance between $+E/+ \psi$ and $-\psi$. The vertical length of the line can also be obtained from 34.4 ppm less the length due to $+E$ (5.6 ppm) minus the length due to $+\psi$ (4.4 ppm). This difference in

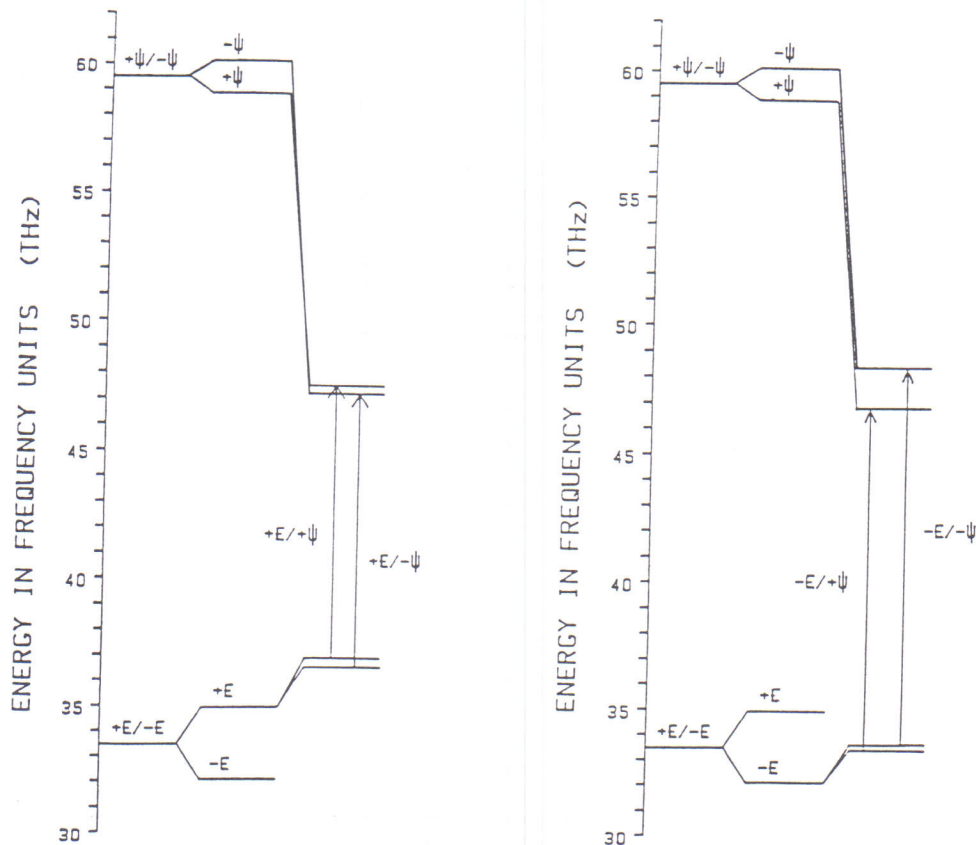


Fig. 3. Energy diagram from thermodynamic data. Data from Table II, columns 1 and 3.

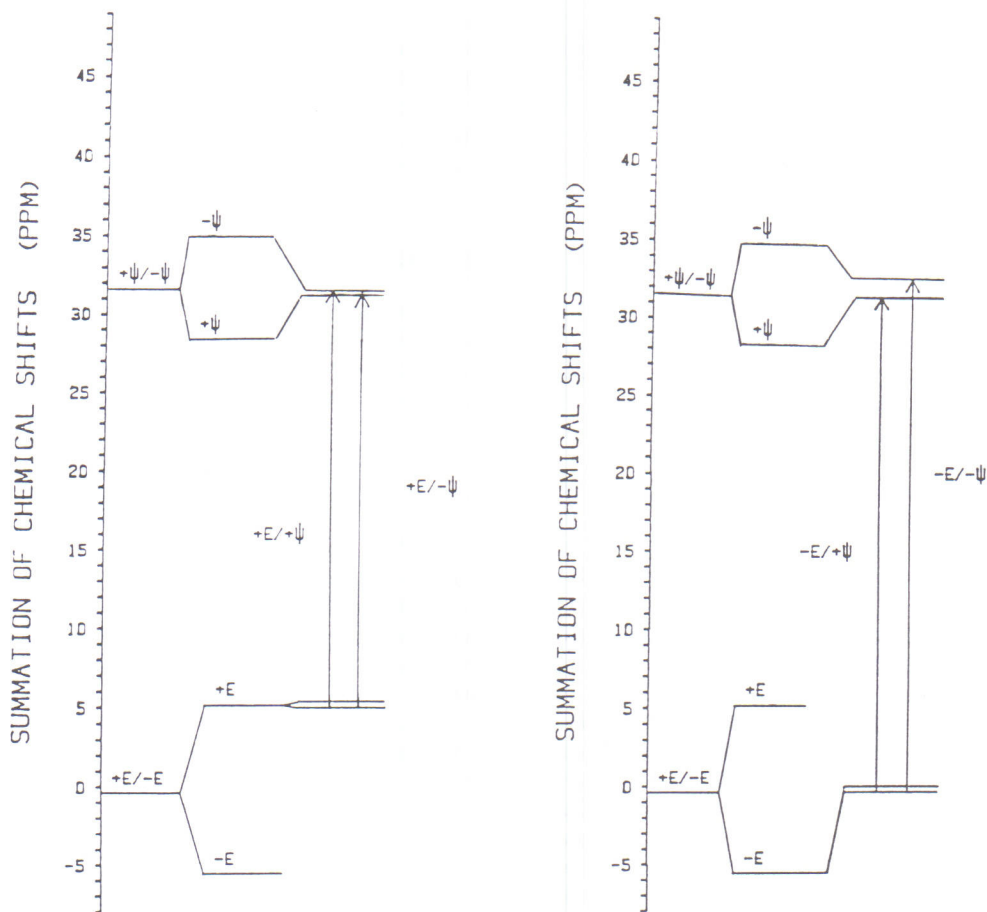


Fig. 4. Energy diagram from NMR summation of chemical shift data. Data from Table II, columns 1 and 9.

chemical shift (10.0 ppm) nearly equals the chemical shift difference between $-E$ and $+E$ (10.4 ppm). The change for $+ψ/+E$ is also consistent with both $+E$ and $+ψ$ changing predicted from linear combinations of the energies of the individual isomers for the mixture (Appendix B).

Experimentally the summation of chemical shifts for the enantiomeric mixture $-ψ/+ψ$ does not (as would be predicted) add to zero. The $\sum\Delta\delta_{AB}$ for $-E/+E$ nearly equals zero. Zero relative to the $-E/+E$ (i.e., when polarization is expected to be zero) would require the addition of 0.4 to each of the summations. This means that the average chemical shift effect for polarization left in $-E$ equals the average chemical shift effect for polarization right in $+E$ and that this value is close to the net zero polarization in $+E/-E$.

Extensive ^1H and ^{13}C NMR studies of the same isomers and binary mixtures of the same isomers at differing concentrations conducted prior to the CP MAS experiments found that identical chemical shifts and relaxation times occur in dilute solutions of $-E$ and of $+E$ free base but that small differences increasing with concentration invariably appeared in the more concentrated solutions independent of the deuterated solvent used. Solids were investigated to eliminate solvent-dependent explanations of the chemical shift data.

Schipper demonstrated that a chiral compound can polarize an achiral compound at molecular distances, i.e., chi-

rality imparts an electromagnetic force on structures (21). Thus forces between two different chiral components could also involve polarization but the forces would be different based on their relative direction of polarization. In a non-crystalline solid in which no crystal lattice forces exist, each molecule is still in the electromagnetic field of the remaining isomers. In a strong magnetic field the direction of the electromagnetic force will be in an opposite direction if the direction of polarization of light is the opposite direction (magnetic circular dichroism). This difference in energy within a strong electromagnetic field means that chemical shifts for $-E$ and $+E$ are not necessarily equivalent in the solid. Relative direction of polarization is used to explain why $-E/-ψ$ differs from $-E/+ψ$. Precisely the same mechanism explains the chemical shift data of $-E$ and $+E$. They are magnetically equivalent in solution because then the chiral molecules are too distant to affect each other's field significantly.

Diaz *et al.*, in investigating DL-, L-, and D-methionine in crystalline solids by CP MAS, found that both the D- and the L-forms had two different conformations, α and β (22). The relative populations of the α and β forms were quite different between the D- and the L-isomer. Unequal populations of the α and β forms between the two enantiomers means that experimentally the energy levels of the L- and D-isomer in a strong magnetic field are not necessarily equal. The reason

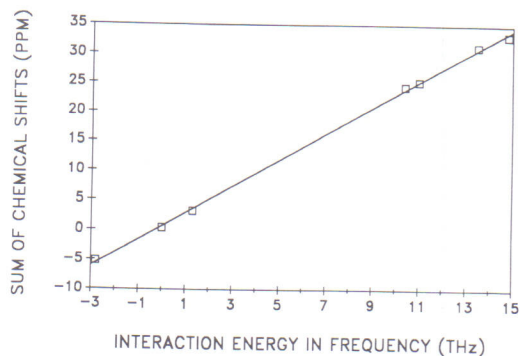


Fig. 5. Correlation line of interaction energy versus summation of chemical shift.

for the inequality could also be due to differences in the relative direction of polarization.

The energies of interaction in the solids of each of the ephedrine isomers from the thermodynamic data are strongly correlated with the summation of chemical shift data. The relative summation of chemical shift compared to the differences in interaction energies from the thermodynamic data (Fig. 5) results in the equation $Y = 2.27 \times 10^{-18} X + 0.6$, in which Y is the summation of chemical shifts and X is the interaction energy. The R^2 correlation coefficient for the line is 0.9998. The units of the slope are in 1/Hz. Assuming that the correct zero in the $-E/+E$ mixture for $\Sigma\Delta\delta_{AB}$ differs by 0.4, the constant increases to 1.0 and the equation becomes $Y = 2.27 \times 10^{-18} X + 1.0$. Solving the equation for X when the $\Sigma\Delta\delta$ shift is zero, $X = -4.4 \times 10^{17}$ Hz, which is in the short-UV wavelength spectral region, i.e., in the spectral region in which optical rotary dispersion of ephedrine isomers has been measured (23).

CONCLUSIONS

The use of ^{13}C CP/MAS NMR spectroscopy in determining the interaction energies in mixtures of diastereoisomers of ephedrine has been demonstrated. Based on these experiments the summation of chemical shifts for structurally equivalent carbon atoms correlates with the changes in energy from the thermodynamic properties of the isomeric mixtures. This approach offers an independent spectroscopic procedure for determination or verification of energies of interaction. Crystalline solids may require an energy correction for crystal lattice effects. The relative direction of electron polarization (measured in optical rotation) explains the association of diastereoisomeric mixtures of ephedrine and pseudoephedrine in the solids. The energy of each binary equimolar mixture may be mathematically calculated from only linear combinations of energies of the four individual isomers. Both the spectroscopic and the thermodynamic techniques may prove useful in correctly characterizing the physical properties of other structurally labile diastereoisomers from their interactions as binary mixtures in non-crystalline solids.

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APPENDIX A

The energy E in frequency units of the individual and mixtures of stereoisomers of ephedrine at room temperature can be converted directly from published entropy data (10) by the formula

$$E = 4.184 * \frac{S_A * T}{n * h} \quad (5)$$

in which S_A is the entropy in entropy units, absolute temperature T is a constant (300 K), n is the Avogadro constant, and h is the Planck constant (24). The factor of 4.184 converts calories to joules. The energy diagram (Fig. 2) summarizes the thermodynamic data of the binary mixtures in frequency units.

The energy of the two racemic mixtures ($\pm E$ and $\pm\psi$) is simply the average of the energies of the component isomers (+ and - isomers).

$$\nu_{\pm E} = \frac{(\nu_{+E} + \nu_{-E})}{2} \quad (6)$$

$$\nu_{\pm\psi} = \frac{(\nu_{+\psi} + \nu_{-\psi})}{2} \quad (7)$$

And therefore,

$$\psi_{+E} = \psi_{\pm E} + \frac{(\Delta\nu_E)}{2} \quad (8)$$

where $\Delta\nu_E = (\nu_{+E} - \nu_{-E})$. The remaining three energy levels of the binary mixtures of enantiomers equal either linear additions or linear subtractions from the energy of the racemic mixtures.

The eight energy levels of diastereoisomeric mixtures can also be calculated directly from linear combinations of the frequencies of the two racemic compounds and the two interaction energies. The diastereoisomeric mixtures with a common direction of polarization are functions of the square root of the binary product of three variables differing only in sign. The subscript in the term $\nu_{-\psi/-E}$, for example, denotes the energy of (-)-pseudoephedrine in the presence of equimolar (-)-ephedrine and the term $\nu_{-E/-\psi}$ denotes the energy of (-)-ephedrine in the presence of equimolar (-)-pseudoephedrine.

$$\nu_{-\psi/-E} = \left[\left(\nu_{\pm\psi} + \nu_{\pm E} - \frac{\Delta\nu_\psi}{2} \right) * \left(\nu_{\pm\psi} - \nu_{\pm E} - \frac{\Delta\nu_\psi}{2} \right) \right]^{1/2} \quad (9)$$

$$\nu_{+\psi/+E} = \left[\left(\nu_{\pm\psi} + \nu_{\pm E} - \frac{\Delta\nu_E}{2} \right) * \left(\nu_{\pm\psi} - \nu_{\pm E} - \frac{\Delta\nu_E}{2} \right) \right]^{1/2} \quad (10)$$

$$\nu_{-E/-\psi} = \left[\left(\nu_{\pm E} + \frac{\Delta\nu_E}{2} - \frac{\Delta\nu_\psi}{2} \right) * \left(\nu_{\pm E} - \frac{\Delta\nu_E}{2} + \frac{\Delta\nu_\psi}{2} \right) \right]^{1/2} \quad (11)$$

$$\nu_{+E/+ \psi} = \left[\left(\nu_{\pm E} + \frac{\Delta\nu_E}{2} + \frac{\Delta\nu_\psi}{2} \right) * \left(\nu_{\pm E} + \frac{\Delta\nu_E}{2} + \frac{\Delta\nu_\psi}{2} \right) \right]^{1/2} \quad (12)$$

For the diastereoisomeric pair with an opposite direction of polarization, the square root of the product of the same four (or only three) variables in each set differing only in their signs calculates the individual energy levels.

$$\nu_{-\psi/+E} = \left[\left(\nu_{\pm\psi} + \nu_{\pm E} - \frac{\Delta\nu_\psi}{2} - \frac{\Delta\nu_E}{2} \right) * \left(\nu_{\pm\psi} - \nu_{\pm E} - \frac{\Delta\nu_\psi}{2} + \frac{\Delta\nu_E}{2} \right) \right]^{1/2} \quad (13)$$

$$\nu_{+\psi/-E} = \left[\left(\nu_{\pm\psi} + \nu_{\pm E} - \frac{\Delta\nu_\psi}{2} + \frac{\Delta\nu_E}{2} \right) * \left(\nu_{\pm\psi} - \nu_{\pm E} - \frac{\Delta\nu_\psi}{2} - \frac{\Delta\nu_E}{2} \right) \right]^{1/2} \quad (14)$$

$$\nu_{-E/+ \psi} = \left[\left(\nu_{\pm E} + \frac{\Delta\nu_\psi}{2} + \frac{\Delta\nu_E}{2} \right) * \left(\nu_{\pm E} - \frac{\Delta\nu_\psi}{2} - \frac{\Delta\nu_E}{2} \right) \right]^{1/2} \quad (15)$$

$$\nu_{+E/-\psi} = \left[(\nu_{\pm E} + \Delta\nu_\psi + \Delta\nu_E) * (\nu_{\pm E} + \Delta\nu_\psi - \Delta\nu_E) \right]^{1/2} \quad (16)$$

The 12 interaction energies (Table II) calculated from those of the four single isomers are compared to those previously determined. The R^2 coefficient is 0.99987, with the slope of 0.999 and the intercept 0.04 not significantly different from zero.

APPENDIX B

The effect of the strong NMR magnetic field on the transition states is linear on the relative energy levels which contain two unequal frequency components (already split into a higher and a lower level). In the specific case $+E/+ \psi$, however, the two frequency components are symmetrical. The energy level for $(\nu_{+E/+ \psi})$ in Eq. (12) contains two identical frequency components.

$$\nu_{+E/+ \psi} = \left[\left(\nu_{\pm E} + \frac{\Delta\nu_E}{2} + \frac{\Delta\nu_E}{2} \right) * \left(\nu_{\pm E} + \frac{\Delta\nu_E}{2} + \frac{\Delta\nu_E}{2} \right) \right]^{1/2} \quad (17)$$

The two frequency components in a strong magnetic field become unequal, i.e., one decreases by $(\frac{1}{2} \Delta\delta_E)$ and the other increases by $(\frac{1}{2} \Delta\nu_E)$. The resulting two energy levels are $(\nu_{\pm E} + \frac{1}{2} \Delta\nu_E \pm \frac{1}{2} \Delta\nu_E)$, which again differs by $(\Delta\nu_E)$. Changes in the transitions in $+E/+ \psi$ also change transitions in $+ \psi/+E$. The frequency components $(\nu_{\pm\psi} + \psi_{\pm E} - \frac{1}{2} \Delta\nu_E)$ and $(\nu_{\pm\psi} - \psi_{\pm E} - \frac{1}{2} \Delta\nu_E)$ in Eq. (10),

$$\nu_{+ \psi/+E} = \left[\left(\nu_{\pm\psi} + \psi_{\pm E} - \frac{\Delta\nu_E}{2} \right) * \left(\nu_{\pm\psi} - \psi_{\pm E} - \frac{\Delta\nu_E}{2} \right) \right]^{1/2} \quad (18)$$

split into two different levels, $(\nu_{\pm\psi} + \psi_{\pm E} - \frac{1}{2} \Delta\nu_E \pm \frac{1}{2} \Delta\nu_E)$ and $(\nu_{\pm\psi} + \psi_{\pm E} - \frac{1}{2} \Delta\nu_E \pm \frac{1}{2} \Delta\nu_E)$. The loss in energy

of the higher energy state on mixing $(\frac{1}{2} \Delta\nu_E)$ must equal the gain in energy in the lower energy state $(\frac{1}{2} \Delta\nu_E)$. The net change in energy on mixing is thus $(\Delta\nu_E)$.

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