Probing Intermolecular Interactions in Chiral Chromatography with NMR Spectroscopy

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Abstract

NMR spectroscopy was used to probe the intermolecular interactions in chiral chromatography. Three chiral sypathiometric analytes were studied; ephedrine, norephedrine, and pseudoephedrine along with a chiral surfactant; N-dodecocycarbonylvaline (DDCV). One dimensional diffusion experiments were performed to determine the complex with the greatest binding constant. Two dimensional ROESY experiments were collected to determine the intermolecular and intramolecular interactions of the analyte:surfactant mixtures and to generate binding maps. Experimental results determined that ephedrine exhibited stronger binding to the micelles than the pseudoephedrine. The norephedrine:DDCV association constants spanned a wider range than ephedrine or pseudoephedrine. The two-dimensional ROESY data suggested that one strong H-bond between the analyte and the micelle produced a lower energy complex than two weak H-bond and/or two competing H-bonds between the –OH and the –NH donor sites on the analyte. The two-dimensional ROESY data also suggested that once the analyte is H-bonded to the micelle, the analyte remained near the micelle surface within the chiral pocket, with the aromatic ring pointing towards the hydrocarbon chain, rather than interacting with the hydrophobic core of the micelle.

Introduction

In this study NMR spectroscopy was utilized to probe the intermolecular interactions in chiral chromatography. The mixtures studied contained three different sypathiometric drugs. Sympathomimetric drugs mimic the effects of the sympathetic nervous system, and are used as decongestents, bronchiodilators for asthma, and vasopressors. These drugs work by occupying the andrenergic receptor sites and act as an agonist, or increase the release of the neurotransmitter norepinephrine[3]. The three drugs investigated were pseudoephedrine, ephedrine, and norephedrine. Each of the three drugs studied contained two chiral centers. For pseudoephedrine and norephedrine, both enantiomers were studied, while only the 1R,2S enantiomer of ephedrine was studied. The structures of the drugs are shown below

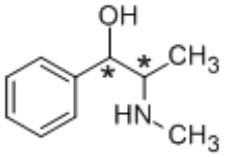

Figure I: Ephedrine (1R,2S & 1S,2R) and Pseudoephedrine (1S,2S & 1R,2R)
**denotes a chiral center*

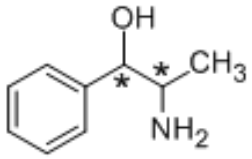

Figure II: Norephedrine **(**1R,2S (-) & 1S, 2R (+)**)**
**denotes a chiral center*

The surfactant used in this study contains one chiral center, which allows the surfactant to form chiral micelles in solution. The structure for the surfactant N-dodecocycarbonylvaline (DDCV) is shown below:

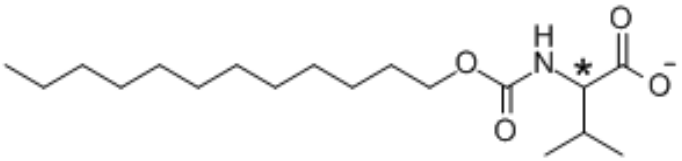

Figure III: DDCV
**denotes a chiral center*

All possible combinations of analyte:surfactant enantiomers were studied. For example the norephedrine study contained the mixtures: R-DDCV:(+)-norephedrine, R-DDCV:(-)-norephedrine, S-DDCV:(+)-norephedrine, and S-DDCV:(-)-norephedrine.

Although the enantiomers have the same chemical formula, the configuration of the chiral center can change the way binding occurs between the analyte and the surfactant. This difference in binding can cause different physiological properties of enantiomers of the drugs within the body. The importance of understanding the different properties of the enantiomers is seen in an example of a drug called thalidomide. Thalidomide was prescribed to pregnant women in the early 1960's to help alleviate the symptoms of morning sickness. The drug was given as a racemic mixture, but was found to be a teratogen. Subsequent research showed that the R enantiomer was the form that caused the desired effects of alleviating the symptoms of morning sickness while the S enantiomer caused abnormal prenatal development[1].

Characterization of the interactions between the micelle and the analyte with NMR spectroscopy gives greater understanding into the intermolecular interactions responsible for the separation of chiral drugs. Chiral chromatography is the process used to separate chiral compounds into their R and S enantiomers. Peterson, et. al. have used chiral chromatography to separate enantiomers ephedrine, pseudoephedrine, and norephedrine[4]. In this study enantiomers were separated based upon their differential interactions with the chiral DDCV micelles. The results of this study showed the following.

- In mixtures containing (S)-DDCV and pseudoephedrine enantiomers, SS-pseudoephedrine eluted before RR-pseudoephedrine suggesting that SS-pseudoephedrine bound more strongly to the micelles.
- The elution order of the chiral analytes was reversed when the surfactant DDCV was switched from the S-form to the R-form, when all other conditions were kept constant[1].

In this research, we set out to measure the association constants and free energies of binding of the analytes to the micelles to determine if these thermodynamic variables governed elution order. We also hoped to use 2D-NMR to obtain a detailed picture of the structures of the analyte:DDCV complexes. The association equilibrium investigated for all drug:micelle mixtures is shown below.

Drug + Surfactant $\rightleftharpoons$ Drug:Surfactant Complex

Therefore, the binding constant for the drug:surfactant interaction is as follows.

$$=[\qquad\qquad]\qquad[\qquad\qquad\qquad]$$

Binding constants of the drugs to the surfactant were determined by performing one dimensional NMR diffusion experiments. Details of this experiment have been reported previously[4]. The intensities of peaks were recorded as a function of increasing magnetic field gradient strength, G. Plots were then prepared with the quantity ((26753*G*0.004)^2)*(0.25-(0.004/3)) on the x-axis and Ln of the peak intensity on the y-axis. The slope of these graphs was –D, where D is the diffusion coefficient. The equations used to calculate the K value from the D value are given in the experimental section. The equation relating ΔG to K is given below.

$$\Delta G = -R \bullet T \bullet \ln(K)$$

*T= 298 K, R= 8.314 J/K•mol

Large K values and large negative ΔG values denote strong intermolecular interactions between the drug and the micelles. The stronger the interaction between the drug and micelle, the lower in energy the complex.

The difference between the ΔG values between two enantiomers can be used to calculate a Δ(ΔG) value. This value allows for a quantitative comparison of the free energy differences between two sets of enantiomers. The equation for Δ(ΔG) is shown below.

$$\Delta(\Delta G) = [\Delta G(\text{enantiomer \#1}) - \Delta G(\text{enantiomer \#2})]$$

Two dimensional NMR ROESY experiments show which hydrogens in the analyte:surfactant complex and are within 5 Å of one another. This data can be used to perform molecular modeling which in turn shows probable structures of the analyte, the surfactant, and the complex. ROESY spectra were collected for each mixture and the 2D spectra were used to calculate sum projections to create binding maps between the analyte and the micelle. The binding maps assign the closest analyte hydrogen to a specific surfactant hydrogen at 100% and the relative distances of the rest of the analyte hydrogens to the micelle can be determined based off the closest analyte hydrogen.

Experimental Procedures

A buffer was needed to ensure that no epimerization occured in solution for the samples. Numerous buffers were made, and the suitable buffer was determined to be D_2O at pH 10. All ephedrine, pseudoephedrine, and norephedrine solutions were prepared in a solution of D_2O adjusted to pH 10. To adjust the D_2O to the correct pH, diluted NaOD was slowly added until the pH reached 10.

The ephedrine and pseudoephedrine: surfactant mixtures were prepared as follows. The amount of 0.0033 g (20mM) ephedrine or pseudoephedrine and 0.0082 g (25mM) S-dodecocycarbonylvaline (DDCV) were added to a sample vial. Then, one milliliter of the prepared D_2O was added to the sample vial with an automatic pipette. The solution was mixed on the vortex machine, and placed into the sonicator until the solution was completely dissolved. The solution was then pipetted into a labeled NMR tube and capped. The norephedrine:surfactant mixture was prepared in the same manner, except 0.0030 (20mM) of norephedrine was added.

An NMR diffusion experiment was run on each sample prepared. Analyte and polymer peak intensities were analyzed. The following % gradients were used in the diffusion experiment: 5, 10, 12, 14, 16, 18, 20, 22, 24, 26, 30, 34, 38, 40, 45, 50, 55, and 60. Two peak intensities from ephedrine/pseudoephedrine and two polymer peaks were read for the diffusion experiment which consisted of three separate trials of 18 spectra each (a total of 54 spectra). The measured peak intensities were put into an EXCEL spreadsheet and graphs plotting ((26753*G*0.004)^2)*(0.25-(0.004/3)) on the x-axis and Ln of the peak intensity on the y-axis were generated for the analyte and polymer in the sample. As indicated in the introduction, the slope of the lines on these graphs was –D, where D is the diffusion coefficient. The D_b and D_{obs} values were determined from the EXCEL graphs and were used to calculate the f_{bound} and K values. The equations used to calculate the K values are shown below:

$$f_{bound} = \frac{D_{(Analyte)} - D_{free\ (Analyte)}}{D_b - D_{free\ (Analyte)}} \qquad K = \frac{f_{bound\ (DDCV)}}{(1-f_{bound\ (DDCV)}) \bullet [\ DDCV]}$$

Conductivity experiments were done previously by Erin Zimmerman to determine the surfactant's critical micelle concentration. The results showed that the CMC was below 2.9 ± 0.33 mM. From this information, it was determined that a diffusion experiment could be run at a concentration of 1.5 mM of DDCV to determine the surfactant D_{free} value of the DDCV. The D_{free} value of the DDCV surfactant was measured by preparing a 1.5 mM solution of DDCV in D_2O at pH 10. The solution was created by adding 0.0005 g of DDCV to a small sample vial. Then, one milliliter of the prepared D_2O was added to the sample vial with an automatic pipette. The solution was mixed on the vortex machine, and placed into the sonicator until the solution was completely dissolved. The solution was then pipetted into a labeled NMR tube and capped. The following % gradients were used in the diffusion experiment: 2, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23. The experiment consisted of three separate trials with 11 scans each (totaling 33 spectra). The measured peak intensities were put into EXCEL and the same graphs as described above were created. The K values found from these graphs were used as the D_{free} of the DDCV which were used to calculate the f_{bound} values of DDCV.

An NMR diffusion experiment was also run on one enantiomer of each analyte in free solution. The following % gradients were used in the diffusion experiment for the analyte: 3, 5, 7, 9, 11, 12, 14, 16, 18, 20, 22 and 24. Two peak intensities from each analyte were read for the diffusion experiment which consisted of three separate trials of 12 spectra each (a total of 36 spectra). The diffusion experiments were done on the analyte to find the D_{free} of the analyte to use in determining the diffusion constant of the analyte:polymer mixture.

Two-dimensional NMR ROESY experiments were performed on each analyte:surfactant sample. The ROESY experiment consisted of a total of 80 scans. The resulting ROESY spectrum was assigned and spreadsheets showing the hydrogens that are located $\leq$ 5 Å from each other were created.

Binding maps were generated from the two-dimensional NMR ROESY experiments performed on each analyte:surfactant sample. Sum projections for the alpha hydrogen (δ = 3.90) and hydrocarbon chain (δ = 1.20) of the surfactant were calculated. The hydrogens on the analyte were integrated, the largest integration was set at 100% and the remaining hydrogens "relative closeness" were calculated based on the closest hydrogen.

Results

The proton assignments for the three analytes studied are shown below. See the structures for the proton labels used in the tables.

Table I. Ephedrine assignments

Assignment	Chemical Shift
H_a	5.01 ppm
H_b	3.27 ppm
H_c	0.92 ppm
H_d	2.61 ppm
H_e	7.28 ppm

Table II. Pseudophedrine assignments

Assignment	Chemical Shift
H_a	4.40 ppm
H_b	2.77 ppm
H_c	0.88 ppm
H_d	2.25 ppm
H_e	7.28 ppm

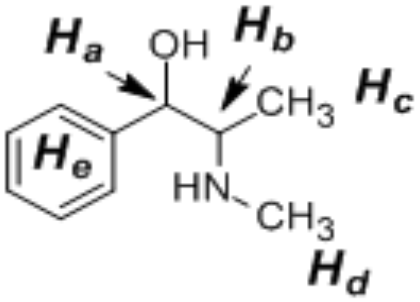

Figure IV. Ephedrine/Pseudoephedrine

Table III. Norephedrine proton assignments

Assignment	Chemical Shift
H_a	4.82
H_b	3.40
H_c	0.97
H_e	7.28

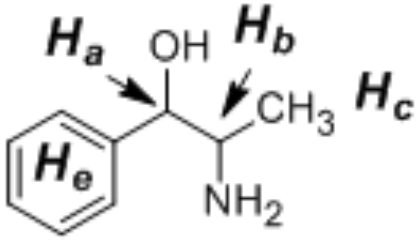

Figure V. Norephedrine

Table IV. DDCV proton assignments

Assignment	Chemical Shift
α	4.05 ppm
β	2.03 ppm
γ	0.78 ppm
1	3.75 ppm
2	1.50 ppm
3	1.15 ppm

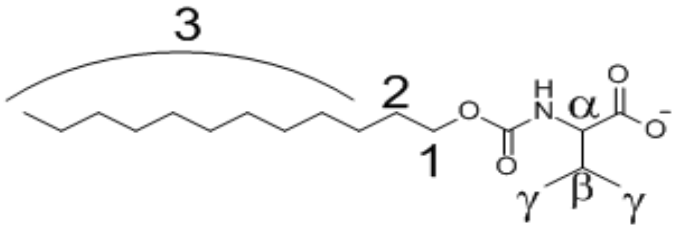

Figure VI. DDCV

Figure VII shows a stack plot of pseudoephedrine/ephedrine mixtures. These spectra were used to identify the best buffer conditions for subsequent experiments.

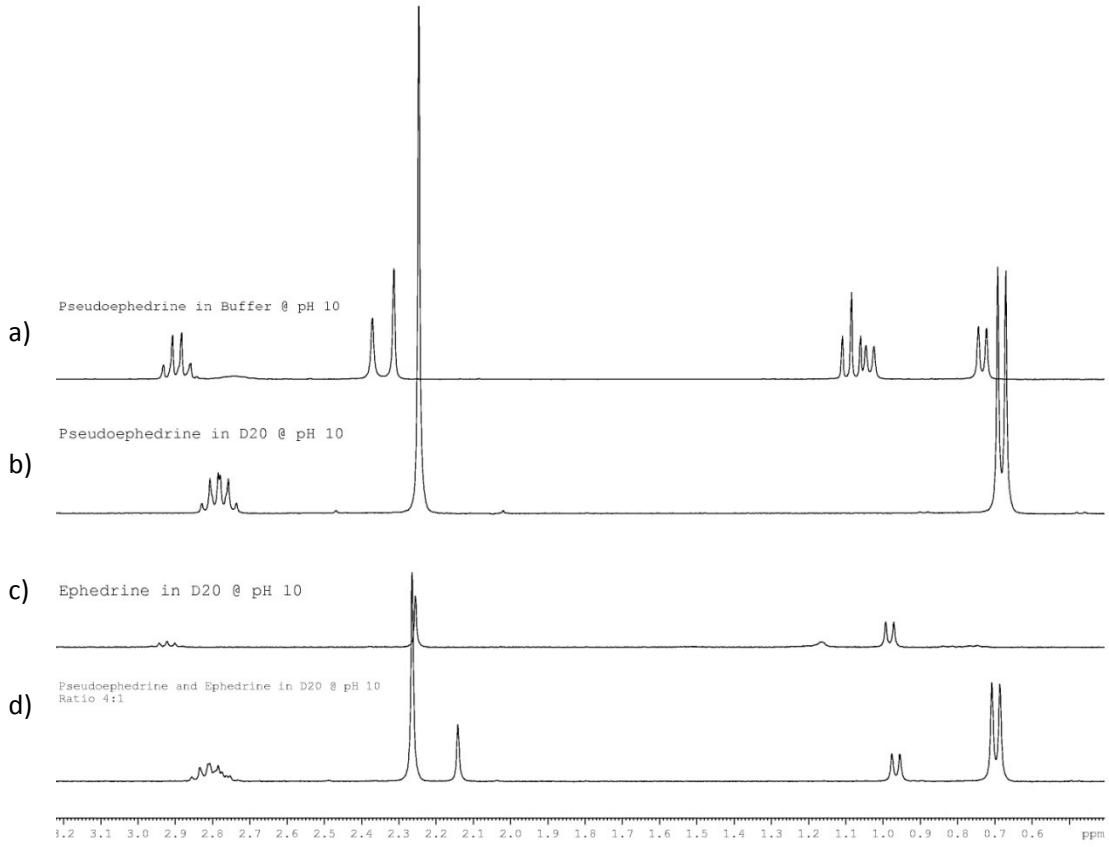

Figure tack plot of NMR spectrum
seudoephedrine in buffer at pH 10
seudoephedrine in D_2O at pH 10
c) Ephedrine in D_2O at pH 10
d) Pseudoephedrine and Ephedrine in D_2O at pH 10 (Ratio 4:1)

The following tables show the average D values, the D_{free} values, and the F_{bound} values for the micelle and ephedrine, as well as the K values and ΔG values for the complexes.

Table V. Ephedrine:DDCV mixtures

S-Micelle & R,S-ephedrine					
	D of S-Micelle x 10^{-6} (cm^2 s^{-1})	D of R,S- Analyte x 10^{-6} (cm^2 s^{-1})	Analyte-D_{Free} x 10^{-6}	DDCV- D_{Free} x 10^{-6}	D_{bound} value x 10^{-6}
	0.617	2.32	6.42	3.86	0.613 ± 0.002
	0.613	2.26	6.33	3.88	
	0.623	2.31	6.42	3.82	
Average	0.618 ± 0.005	2.30 ± 0.03	6.39 ± 0.05	3.85 ± 0.03	
F_{bound} DDCV	[DDCV] (mol/L)	F_{bound} Peptide	K (Equil Const)	ΔG (kJ/mol)	
0.999 ± 0.008	0.0250 ± 0.0002	0.708 ± 0.012	97.1 ± 2.5	-11.3 ± 0.3	
R-Micelle & R,S-ephedrine					
	D of R-Micelle x 10^{-6} (cm^2 s^{-1})	D of R,S- Analyte x 10^{-6} (cm^2 s^{-1})	Analyte D_{Free} x 10^{-6}	DDCV- D_{Free} x 10^{-6}	D_{bound} value x 10^{-6}
	0.703	2.74	6.42	3.86	0.613 ± 0.002
	0.700	2.74	6.32	3.88	
	0.702	2.74	6.42	3.82	
Average	0.702 ± 0.002	2.74 ± 0.00	6.39 ± 0.05	3.85 ± 0.03	
F_{bound} DDCV	[DDCV]	F_{bound} Peptide	K (Equil Const)	ΔG (kJ/mol)	
0.973 ± 0.007	0.0243 ± 0.0002	0.631 ± 0.011	70.5 ± 1.8	-10.5 ± 0.3	

The following tables show the average D values, the D_{free} values, and the F_{bound} values for the micelle and pseudoephedrine, as well as the K values and ΔG values for the complexes.

Table VI. Pseudoephedrine:DDCV mixtures

S-Micelle & R,R-pseudoephedrine					
	D of S-Micelle x 10^{-6} (cm^2 s^{-1})	D of R,R- Analyte x 10^{-6} (cm^2 s^{-1})	Analyte-D_{Free} x 10^{-6}	DDCV- D_{Free} x 10^{-6}	D_{bound} value x 10^{-6}
	0.668	2.52	5.28	3.86	0.613 ± 0.002
	0.670	2.53	5.24	3.88	
	0.670	2.53	5.25	3.82	
Average	0.670 ± 0.001	2.53 ± 0.01	5.26 ± 0.02	3.85 ± 0.03	
F_{bound} DDCV	[DDCV] (mol/L)	F_{bound} Peptide	K Value	ΔG (kJ/mol)	
0.983 ± 0.007	(2.46 ± 0.02)E-02	0.588 ± 0.005	58.1 ± 0.8	-10.1 ± 0.1	
S-Micelle & S,S-pseudoephedrine					
	D of S-Micelle x 10^{-6} (cm^2 s^{-1})	D of S,S-Analyte x 10^{-6} (cm^2 s^{-1})	Analyte D_{Free} x 10^{-6}	DDCV- D_{Free} x 10^{-6}	D_{bound} value x 10^{-6}
	0.654	2.44	5.28	3.86	0.613 ± 0.002
	0.653	2.42	5.24	3.88	
	0.653	2.43	5.25	3.82	
Average	0.653 ± 0.001	2.43 ± 0.01	5.26 ± 0.02	3.85 ± 0.03	
F_{bound} DDCV	[DDCV]	F_{bound} Peptide	K Value	ΔG (kJ/mol)	
0.988 ± 0.007	0.0247 ± 0.000171	0.608 ± 0.005	62.9 ± 0.9	-10.3 ± 0.1	
R-Micelle & R,R pseudoephedrine					
	D of R-Micelle x 10^{-6} (cm^2 s^{-1})	D of R,R- Analyte x 10^{-6} (cm^2 s^{-1})	Analyte D_{Free} x 10^{-6}	DDCV- D_{Free} x 10^{-6}	D_{bound} value x 10^{-6}
	0.670	2.76	5.28	3.86	0.613 ± 0.002
	0.670	2.75	5.24	3.88	
	0.670	2.76	5.25	3.82	
Average	0.670 ± 0.001	2.76 ± 0.01	5.26 ± 0.02	3.85 ± 0.03	
F_{bound} DDCV	[DDCV]	F_{bound} Peptide	K (Equil Const)	ΔG (kJ/mol)	
0.983 ± 0.007	0.0246 ± 0.0002	0.538 ± 0.005	47.5 ± 0.7	-9.56 ± 0.14	
R-Micelle & S,S-Pseudoephedrine					
	D of R-Micelle x 10^{-6} (cm^2 s^{-1})	D of S,S-Analyte x 10^{-6} (cm^2 s^{-1})	Analyte D_{Free} x 10^{-6}	DDCV- D_{Free} x 10^{-6}	D_{bound} value x 10^{-6}
	0.614	2.32	5.28	3.86	0.613 ± 0.002
	0.613	2.27	5.24	3.88	
	0.625	2.32	5.25	3.82	
Average	0.618 ± 0.007	2.30 ± 0.03	5.26 ± 0.02	3.85 ± 0.03	
F_{bound} DDCV	[DDCV]	F_{bound} Peptide	K (Equil Const)	ΔG (kJ/mol)	
0.999 ± 0.008	0.0250 ± 0.0002	0.636 ± 0.008	69.9 ± 1.4	-10.5 ± 0.2	

The following tables show the average D values, the D_{free} values, and the F_{bound} values for the micelle and norephedrine, as well as the K values and ΔG values for the complexes.

TableVII. Norephedrine:DDCV mixtures

S-Micelle & R,S (-) norephedrine					
	D of S-Micelle x 10^{-6} (cm^2 s^{-1})	D of (-) Analyte x 10^{-6} (cm^2 s^{-1})	Analyte-D_{Free} x 10^{-6}	DDCV- D_{Free} x 10^{-6}	D_{bound} value x 10^{-6}
	0.717	2.54	7.25	3.86	0.613 ± 0.002
	0.713	2.51	7.25	3.88	
	0.713	2.52	7.35	3.82	
Average	0.714 ± 0.002	2.52 ± 0.02	7.28 ± 0.06	3.85 ± 0.03	
F_{bound} DDCV	[DDCV] (mol/L)	F_{bound} Peptide	K (Equil Const)	ΔG (kJ/mol)	
0.969 ± 0.007	0.0242 ± 0.0002	0.714 ± 0.011	103.0 ± 2.3	-11.5 ± 0.3	
S-Micelle & S,R (+) norephedrine					
	D of S-Micelle x 10^{-6} x 10^{-6} (cm^2 s^{-1})	D of (+) Analyte x 10^{-6} (cm^2 s^{-1})	Analyte D_{Free} x 10^{-6}	DDCV- D_{Free} x 10^{-6}	D_{bound} value x 10^{-6}
	0.667	3.05	7.25	3.86	0.613 ± 0.002
	0.674	3.07	7.25	3.88	
	0.678	2.98	7.35	3.82	
Average	0.673 ± 0.006	3.03 ± 0.05	7.28 ± 0.06	3.85 ± 0.03	
F_{bound} DDCV	[DDCV] (mol/L)	F_{bound} Peptide	K (Equil Const)	ΔG (kJ/mol)	
0.982 ± 0.008	0.0245 ± 0.0002	0.637 ± 0.012	71.5 ± 2.1	-10.6 ± 0.3	
R-Micelle & R,S (-) norephedrine					
	D of R-Micelle x 10^{-6} (cm^2 s^{-1})	D of (-) Analyte x 10^{-6} (cm^2 s^{-1})	Analyte D_{Free} x 10^{-6}	DDCV- D_{Free} x 10^{-6}	D_{bound} value x 10^{-6}
	0.786	4.13	7.25	3.86	0.613 ± 0.002
	0.784	4.14	7.25	3.88	
	0.784	4.13	7.35	3.82	
Average	0.785 ± 0.001	4.13 ± 0.01	7.28 ± 0.06	3.85 ± 0.03	
F_{bound} DDCV	[DDCV] (mol/L)	F_{bound} Peptide	K (Equil Const)	ΔG (kJ/mol)	
0.947 ± 0.006	0.0237 ± 0.0001	0.472 ± 0.009	37.8 ± 1.1	-9.00 ± 0.26	
R-Micelle & S,R (+) norephedrine					
	D of R-Micelle x 10^{-6} (cm^2 s^{-1})	D of (+) Analyte x 10^{-6} (cm^2 s^{-1})	Analyte D_{Free} x 10^{-6}	DDCV- D_{Free} x 10^{-6}	D_{bound} value x 10^{-6}
	0.692	2.66	7.25	3.86	0.613 ± 0.002
	0.693	2.66	7.25	3.88	
	0.699	2.67	7.35	3.82	
Average	0.695 ± 0.004	2.66 ± 0.01	7.28 ± 0.06	3.85 ± 0.03	
F_{bound} DDCV	[DDCV] (mol/L)	F_{bound} Peptide	K (Equil Const)	ΔG (kJ/mol)	
0.975 ± 0.007	0.0243 ± 0.0002	0.693 ± 0.010	92.5 ± 2.1	-11.2 ± 0.3	

Table VIII. Δ(ΔG) values

Combination	**Δ(ΔG) kJ/mol**
R-RR/R-RS R micelle with analytes	0.97 ± 0.05
S-RR/R-RR RR pseudoephedrine with surfactant	0.50 ± 0.01
S-RS/R-RS RS ephedrine with surfactant	0.79 ± 0.03
S+/S- S micelle with norepehdrine analytes	0.91 ± 0.03
R+/R- R micelle with norephedrine analytes	2.22 ± 0.08
***All values were T-tested to be statistically different at the 95% confidence level**	

The binding maps shown in Table VIII below were generated from ROESY cross peak data. The ROESY assignments for each two-dimensional spectrum collected are tabulated in Appendix A.

Table IX. Binding maps for all mixtures studied

Binding Maps												
Hydrocarbon Chain							Alpha Hydrogen					
	Ha	**Hb**	**Hc**	**Hd**	**He**			**Ha**	**Hb**	**Hc**	**Hd**	**He**
<u>Pseudoephedrine Complex</u>							**<u>Pseudoephedrine Complex</u>**					
R, RR	15	31	100	7	56		R, RR	91	100	20	9	22
R, SS	22	57	56	7	100		R, SS	100	76	47	8	18
S, RR	42	81	25	13	100		S, RR	100	85	84	85	73
S, SS	27	49	87	16	100		S, SS	100	48	27	48	24
<u>Ephedrine Complex</u>							**<u>Ephedrine Complex</u>**					
R, RS	N/A	62	37	8	100		R, RS	49	100	50	55	24
S, RS	13	54	3	9	100		S, RS	41	87	41	100	N/A
<u>Norephedrine Complex</u>							**<u>Norephedrine Complex</u>**					
R, +	36	30	23	x	100		R, +	100	62	94	x	32
R, -	30	40	5	x	100		R, -	100	N/A	40	x	11
S, +	27	30	8	x	100		S, +	79	100	100	x	42
S, -	27	40	16	X	100		S, -	8	1	100	X	66

Discussion

Epimerization in Buffer Solution

A suitable solvent was needed to use throughout the study, however when an NMR spectrum was collected of pseudoephedrine in the buffer used in the chromatography study, major and minor species were detected for three different hydrogen peaks, and sometimes a fourth. It was determined that they were a major and minor species because the ratio of integrations of the major and minor peaks were done for each and found to be consistent for each hydrogen. The ratio of the major and minor species changed when placed in different buffers. The buffer used in the chromatography study contained triethylamine (TEA). A buffer containing boric acid was also used. The pH was adjusted between 7.8 and 11. An interesting occurrence happened when the boric acid buffer was adjusted to a pH of 7.8, the major and minor species switched, where the ratio of the major to the minor species became less than one. Interestingly, when the pseudoephedrine was dissolved in only deuterium oxide, a clean spectrum was obtained with only one major species peak. The pseudoephedrine was also dissolved in deuterium oxide and the pH was adjusted to 10, and the spectrum only showed one major species. This result indicated that the epimerization (switching from one diasteriomer to another) was not dependent upon pH, but rather on the buffer itself. A diffusion experiment was run on the pseudoephedrine in the buffer solution, and the diffusion coefficients were measured for both the major and minor species were found to be equal. This result indicated that the major and minor species are diasteriomers of one another. To determine whether the pseudoephedrine was indeed the major species, a solution was made with pseudoephedrine and ephedrine in a ratio of 4:1, and a spectrum was obtained. When the spectrum was compared to the pseudoephedrine spectrum collected in the buffer, it was determined that the major form in the buffer was ephedrine rather than pseudoephedrine. The spectra used to reach these conclusions can be seen in Figure VII of the results section.

Δ (ΔG) Values

The fact that with both R and S surfactant ΔG value changes when the chirality of Carbon B of the analyte changes from R to S suggests that the N-H group is involved in hydrogen bonding because the position of the N-H group affects the ΔG value. In general the S surfactant binds both analytes more strongly than the R for ephedrine and both enantiomers of pseudoephedrine. Ephedrine interacts more strongly with both the R and the S DDCV than the pseudoephedrine.

Δ(ΔG) is bigger when the stereochemistry of the analyte changes compared to the stereochemistry of the surfactant. For example, the Δ(ΔG) for S-DDCV:RR-pseudoephedrine compared to the S-DDCV:RS-ephedrine is 0.79 kJ/mol and the Δ(ΔG) for R-DDCV:RR-pseudoephedrine compared to the R-DDCV:RS-ephedrine is 0.97 kJ/mol. This result is consistent with the presence of a strong hydrogen bonding interaction between the analytes and the surfactant. The analyte chiral carbon is attached directly to the hydrogen bond donor/acceptor atoms whereas the DDCV Hα is adjacent to the donor/acceptor atoms on the surfactant. Changing the chirality of the analyte would be expected have a greater effect on the ability of the analyte to form intermolecular hydrogen bonds.

There is a larger Δ(ΔG) value for the R micelle than the S- micelles in both the pseudoephedrine and norephedrine samples. This result would suggest that the R-micelle would be favorable in a chiral chromatography column for both of these analytes because it would cause a greater separation of the enantiomers.

Comparison of Ephedrine to Pseudoephedrine

From the association constant measurements, it was determined that overall ephedrine bound more strongly to the micelle than pseudoephedrine. For example, the weakest interaction of the ephedrine:micelle complex (R-DDCV:RS-ephedrine complex) had a binding coefficient of 70.5 ± 1.8 while the strongest interaction of the pseudoephedrine:micelle complex (R-DDCV:SS-pseudoephedrine complex) had a binding coefficient of 69.9 ± 1.4. The strongest interaction for ephedrine was with the S micelle, while the strongest interaction with pseudoephedrine was between the R-micelle and SS-pseudoephedrine. The weakest interaction was between the R-micelle and the RR-pseudoephedrine. The SS-pseudoephedrine enantiomer had a stronger interaction with both micelles over the RR-pseudoephdrine. This result is inconsistent with previous chromatography data which states that the elution order of the analytes switches when the chirality of the micelle changes. A reasonable explanation of this difference is likely the phenomenon that was observed when the pseudoephedrine formed major and minor species in a buffer solution. The chiral chromatography is carried out in a TEA buffer solution, so the chromatogram could possibly be labeled incorrectly if the major and minor species were not taken into account by the previous researchers.

Ephedrine

The binding map data for ephedrine suggests is favors a H-bond to the surfactant through the –NH rather than the –OH. In the binding map to the alpha hydrogen of the DDCV there is a higher percentage for the ephedrine Hb and Hd protons, and a lower percentage to the Ha proton. These results are significant because the analyte Ha is attached to the carbon containing the –OH and both Hb and Hd are attached to the carbon containing the –NH. This is seen with the S-RS complex which has binding percentages of 41, 87, and 100 for Ha, Hb, and Hd respectively. This result suggests that a single, strong H-bond leads to higher K values. This conclusion is also supported further by additional results discussed below.

Pseudoephedrine with S-DDCV

The SS enantiomer of pseudoephedrine binds more strongly to the micelle than the RR enantiomer. This result is consistent with the chromatography results in which the enantiomer RR eluted before SS[4]. Because the RR enantiomer eluted first, it would suggest that the binding to the surfactant would be lower or weaker than the binding of SS which eluted last. This result was confirmed by the association constant measurements.

From the binding maps, the S-SS complex seems to have predominantly one H-bond through the analyte –OH. This conclusion is a result of the proximity of the DDCV alpha hydrogen of the surfactant to the hydrogen Ha of the analyte. The S-RR complex binding map seems to indicate that both the analyte –OH and –NH are involved in the hydrogen bonding because comparable percentages of 100% and 85% were detected for Ha and Hd respectively in the binding map to the DDCV Hα. This result could mean that the complex can form two hydrogen bonds or that there is an equilibrium between the complexes bound through the –OH or –NH of the analyte. Again the binding maps suggest that a single, strong hydrogen bond through the analyte –OH may lead to a greater binding affinity than either two weak hydrogen bonds involving both –OH and –NH hydrogen bonds through the analyte, or an equilibrium between the two hydrogen bond sites.

The binding maps to the hydrocarbon chain of the surfactant show that in all analyte complexes the closest hydrogen of the analyte is from aromatic ring. Nine out of the ten complexes studied have 100% binding at the aromatic ring of the analyte. This result suggests that upon hydrogen bonding to the headgroup, the ring is directed towards the hydrocarbon chain of the surfactant for all complexes. This data is consistent for ephedrine, pseudoephedrine, and norephedrine.

The binding maps to the hydrocarbon chain of the surfactant also show a relatively small % for many of the other protons of the pseudoephedrine. This result suggests that the analyte spends most of its time near the surface of the micelle with the ring pointed towards the chain. There is likely not an interaction where the analyte is deep within the hydrophobic core of the micelle, because if this were the case, we would expect a comparable % for all of the pseudoephedrine hydrogens. This same conclusion can be made with the ephedrine and norephedrine within the DDCV complexes.

Norephedrine

According to the K values, the complex with the largest biding constant is the S-micelle with the (-)-norephedrine at 103.0 ± 2.3. When examining (+)-norephedrine, it is seen that the R-micelle complex binds more strongly than the S-micelle complex. Furthermore, the binding maps suggest that the R-DDCV:(+)-norephedrine complex hydrogen bond is primarily through the –OH because the percentages for the norephedrine Ha (100%) is much greater than the percentage of Hb (62%). The S-DDCV:(+)-norephedrine binding map shows that the Hb (100%) percent is higher than the Ha (79%), but the two values are comparable. This data once again suggests that a single, strong hydrogen bond may lead to stronger interactions and a lower energy complex than either a competing –OH/-NH hydrogen bonds and/or a complex with two weak hydrogen bonds to both the –NH and –OH.

The norephedrine binding constants show that the S-micelle interacts more strongly with the (-)-norephedrine enantiomer while the R-micelle interacts more strongly with the (+)-norephedrine. This data is consistent with previous research that shows the elution order of the chiral chromatography column switches when the chirality of the micelle is switched. The K values obtained would suggest that in chiral chromatography the R-micelle would elute the enantiomers in the order (-) then (+) while the S-micelle would elute the enantiomers in the order (+) then (-).

Binding for Analyte:Micelle Complex

The following figures (Figure VIII and Figure IX) show a general positioning of the analyte to the surfactant determined by the two-dimensional ROESY data.

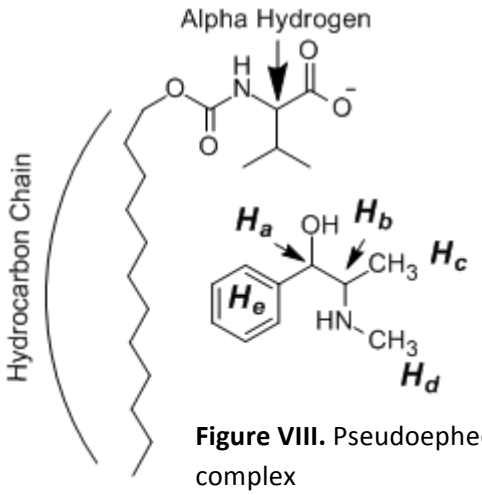

Figure VIII. Pseudoephedrine/Ephedrine:DDCV complex

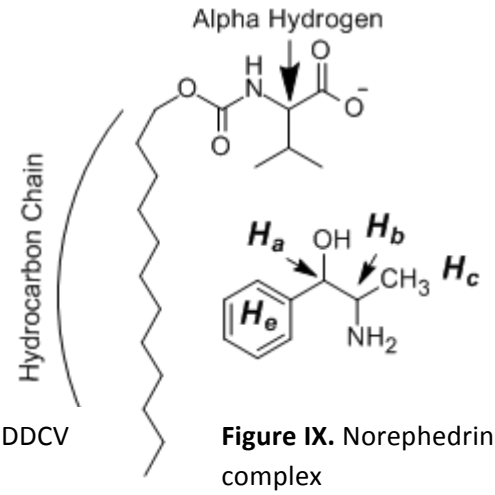

Figure IX. Norephedrine:DDCV complex

Conclusions

- The suitable solvent for the analyte:surfactant mixture was determined to be deuterium oxide adjusted to pH 10
- When comparing the binding constants of the analytes pseudoephedrine and ephedrine, ephedrine complexes were determined to have stronger binding than the pseudoephedrine complexes.
- Norephedrine:DDCV association constants spanned a wider range than ephedrine and pseudoephedrine.
- When comparing experimental results that were obtained from this experiment to previous research by Peterson et. al
    - The SS-pseudoephedrine enantiomer bound more strongly with both micelles, which suggests in chiral chromatography, the elution order would not switch when the chirality of the surfactant DDCV switched. This is contradictory to the previous research, and a reasonable explanation can be found when observing the NMR spectrum of pseudoephedrine in the buffer used in the experiment that contains major and minor species.
    - Norephedrine association constants showed that micelles containing the R- and S-DDCV had a stronger binding to different norephedrine enantiomers, which is consistent with the previous research.
- Two-dimensional ROESY data suggests that one strong H-bond between the analyte and the micelle produces a lower energy complex than two weak H-bonds and/or two competing H-bonds between the –OH and the –NH donor site on the analyte.
- Two-dimensional ROESY data suggests that once the analyte is H-bonded to the micelle, the analyte remains near the surface, with the aromatic ring pointing towards the hydrocarbon chain, rather than interacting with the hydrophobic center of the micelle.

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Appendix A

ROESY cross peak and assignments for all analyte:micelle mixtures

- S,SS Complex
  - Table I-A: Intramolecular DDCV Peaks
  - Table II-A: Intramolecular Pseudoephedrine Peaks
  - Table III-A: Intermolecular Peaks
- S,RR Complex
  - Table IV-A: Intramolecular DDCV Peaks
  - Table V-A: Intramolecular Pseudoephedrine Peaks
  - Table VI-A: Intermolecular Peaks
- R,SS Complex
  - Table VII-A: Intramolecular DDCV Peaks
  - Table VIII-A: Intramolecular Pseudoephedrine Peaks
  - Table IX-A: Intermolecular Peaks
- R,RR Complex
  - Table X-A: Intramolecular DDCV Peaks
  - Table XI-A: Intramolecular Pseudoephedrine Peaks
  - Table XII-A: Intermolecular Peaks
- S,RS Complex
  - Table XIII-A: Intramolecular DDCV Peaks
  - Table XIV-A: Intramolecular Ephedrine Peaks
  - Table XV-A: Intermolecular Peaks
- R,RS Complex
  - Table XVI-A: Intramolecular DDCV Peaks
  - Table XVII-A: Intramolecular Ephedrine Peaks
  - Table XVIII-A: Intermolecular Peaks
- S,(+) Complex
  - Table XIX-A: Intramolecular DDCV Peaks
  - Table XX-A: Intramolecular Norephedrine Peaks
  - Table XXI-A: Intermolecular Peaks
- S,(-) Complex
  - Table XXII-A: Intramolecular DDCV Peaks
  - Table XXIII-A: Intramolecular Norephedrine Peaks
  - Table XXIV-A: Intermolecular Peaks
- R,(+) Complex
  - Table XXV-A: Intramolecular DDCV Peaks
  - Table XXVI-A: Intramolecular Norephedrine Peaks
  - Table XXVII-A: Intermolecular Peaks
- R,(-) Complex
  - Table XXVIII-A: Intramolecular DDCV Peaks
  - Table XXIX-A: Intramolecular Norephedrine Peaks
  - Table XXX-A: Intermolecular Peaks

S, SS Complex ROESY Data

Table I-A: Intramolecular NOE's of DDCV for S,SS Complex

Intramolecular NOE's DDCV (S,SS complex)	
Cross Peak	Assignment
3.93, 1.90	α --> β
3.92, 0.65	α --> γ
3.92, 1.36	α --> C2
3.93, 1.14	α --> C3
1.90, 0.69	β --> γ
1.917, 3.71	β --> C1
1.92, 1.14	β --> C3
0.636, 3.72	γ --> C1
0.68, 1.19	γ --> C3
3.714, 1.17	C1 --> C3

Table II-A: Intramolecular NOE's of Pseudoephedrine for S,SS Complex

Intramolecular NOE's Pseudoephedrine (S,SS complex)	
Cross Peak	Assignment
4.51, 0.861	Ha --> Hc
4.51, 2.58	Ha --> Hd
4.51, 7.28	Ha --> He
3.26, 2.59	Hb --> Hd
3.27, 7.28	Hb --> He
0.89, 2.59	Hc --> Hd
0.871, 7.28	Hc --> He
2.61, 7.28	Hd --> He

Table III-A: Intermolecular NOE's of S,SS Complex

Intermolecular peaks (S, SS complex)	
Cross Peak	Assignment
3.92, 0.83	α --> Hc
3.91, 7.28	α --> He
1.91, 0.866	β --> Hc
1.92, 2.58	β --> Hd
1.90, 7.28	β --> He
0.608, 4.52	γ --> Ha
0.65, 3.24	γ --> Hb
0.65, 0.83	γ --> Hc
0.66, 2.58	γ --> Hd
0.60, 7.28	γ --> He
3.71, 4.52	C1 --> Ha
3.70, 3.26	C1 --> Hb
3.713, 0.86	C1 --> Hc
3.71, 2.58	C1 --> Hd
3.70, 7.28	C1 --> He

S,RR Complex ROESY Data

Table IV-A; Intramolecular NOE's of DDCV for S, RR Complex

Intramolecular NOE's DDCV (S,RR complex)	
Cross Peak	Assignment
3.93, 1.90	α --> β
3.92, 0.65	α --> γ
3.92, 1.36	α --> C2
3.93, 1.14	α --> C3
1.90, 0.69	β --> γ
1.917, 3.71	β --> C1
1.92, 1.14	β --> C3
0.636, 3.72	γ --> C1
0.68, 1.19	γ --> C3
3.714, 1.17	C1 --> C3

Table V: Intramolecular NOE's of Pseudoephedrine for S,RR Complex

Intramolecular peaks Pseudoephedrine (S,RR complex)	
Cross Peak	Assignment
4.51, 0.882	Ha --> Hc
4.51, 2.59	Ha --> Hd
3.26, 0.882	Hb --> Hc
3.26, 2.602	Hb --> Hd
3.26, 7.26	Hb --> He
0.90, 2.62	Hc --> Hd
0.88, 7.27	Hc --> He
2.59, 7.26	Hd --> He

Table IV: Intermolecular NOE's of S,RR Complex

Intermolecular peaks (S,RR complex)	
3.90, 0.885	α --> Hc
3.88, 7.26	α --> He
1.91, 0.86	β --> Hc
0.619, 4.511	γ --> Ha
0.63, 3.26	γ --> Hb
0.65, 2.62	γ --> Hd
0.606, 7.263	γ --> He
3.72, 4.50	C1 --> Ha
3.71, 0.88	C1 --> Hc
3.71, 2.60	C1 --> Hd
3.72, 7.26	C1 --> He
1.35, 7.26	C2 --> He
1.18, 4.50	C3 --> Ha
1.14, 3.26	C3 --> Hb
1.18, 0.81	C3 --> Hc
1.16, 7.27	C3 --> He

R,SS Complex ROESY Data

Table VII-A: Intramolecular NOE's of DDCV

Intramolecular NOE's- DDCV (R,SS complex)	
Cross Peak	Assignment
3.89, 1.362	α --> C2
3.901, 1.145	α --> C3
1.919, 0.71	β --> γ
1.918, 3.739	β --> C1
1.912, 1.175	β --> C3
0.70, 3.74	γ --> C1
0.746, 1.37	γ --> C2
3.738, 1.171	C1 --> C3

Table VIII-A: Intramolecular NOE's of Pseudoephedrine

Intramolecular NOE's Pseudoephedrine (R,SS complex)	
Cross Peak	Assignment
4.51, 0.861	Ha --> Hc
4.51, 2.58	Ha --> Hd
4.51, 7.28	Ha --> He
3.26, 2.59	Hb --> Hd
3.27, 7.28	Hb --> He
0.89, 2.59	Hc --> Hd
0.871, 7.28	Hc --> He
2.61, 7.28	Hd --> He

Table IX-A: Intermolecular NOE's of R,SS Complex

Intermolecular peaks (R,SS complex)	
Cross Peak	Assignment
3.936, 4.48	α --> Ha
3.90, 7.23	α--> He
1.909, 0.866	β --> Hc
2.62, 1.92	β --> Hd
0.618, 4.69	γ --> Ha
0.647, 0.873	γ --> Hc
0.624, 2.615	γ --> Hd
0.672, 7.264	γ --> He
3.73, 4.48	C1 --> Ha
3.74, 0.889	C1 --> Hc
3.741, 2.62	C1 --> Hd
3.738, 7.268	C1 --> He
1.41, 2.612	C2 --> Hd
1.368, 7.2671	C2 --> He
1.16, 4.52	C3 --> Ha
1.22, 0.90	C3 --> Hc
1.17, 2.62	C3 --> Hd
1.176, 7.265	C3 --> He

R,RR Complex ROESY Data

Table X-A: Intramolecular NOE's of DDCV

Intramolecular NOE's – DDCV (R,RR complex)	
Cross Peak	Assignment
3.91, 0.60	α --> γ
3.91, 1.35	α --> C2
3.91, 1.15	α --> C3
1.90, 0.664	β --> γ
1.90, 3.71	β --> C1
1.90, 1.16	β --> C3
0.69, 3.71	γ --> C1
0.68, 1.36	γ --> C2
0.82, 1.17	γ --> C3
3.71, 1.17	C1 --> C3

Table XI-A: Intramolecular NOE's of Pseudoephedrine

Intramolecular NOE's Pseudoephedrine (R,RR complex)	
Cross Peak	Assignment
4.51, 3.24	Ha --> Hb
4.506, 0.858	Ha --> Hc
4.50, 2.55	Ha --> Hd
4.48, 7.28	Ha --> He
3.23, 0.86	Hb --> Hc
3.23, 2.56	Hb --> Hd
3.22, 7.27	Hb --> He
0.86, 2.56	Hc --> Hd
0.854, 7.2823	Hc --> He
2.562, 7.28	Hd --> He

Table XII-A: Intermolecular NOE's of R,RR Complex

Intermolecular peaks (R,RR complex)	
Cross Peak	Assignment
3.93, 4.48	α --> Ha
3.91, 0.83	α --> Hc
3.92, 7.28	α --> He
1.90, 0.83	β --> Hc
1.91, 2.56	β --> Hd
4.506, 0.606	γ --> Ha
0.62, 3.23	γ --> Hb
0.66, 0.86	γ --> Hc
0.61, 2.56	γ --> Hd
7.27, 0.593	γ --> He
3.70, 4.50	C1 --> Ha
3.71, 0.86	C1 --> Hc
3.71. 2.548	C1 --> Hd
3.70, 7.28	C1--> He
1.35, 3.23	C2 --> Hb
1.36, 0.82	C2 --> Hc
1.32, 2.56	C2 --> Hd
1.35, 7.27	C2 --> He
1.17, 4.50	C3 --> Ha
1.16, 3.23	C3 --> Hb
1.17, 0.82	C3 --> Hc
1.18, 2.56	C3 --> Hd
1.16, 7.268	C3 --> He

S,RS Complex ROESY Data

Table XIII-A: Intramolecular NOE's of DDCV

Intramolecular NOE's – DDCV (S,RS complex)	
Cross Peak	Assignment
3.88, 0.65	α --> γ
3.88, 1.32	α --> C2
3.89, 1.17	α --> C3
1.90, 0.67	β --> γ
1.92, 3.70	β --> C1
1.92, 1.35	β --> C2
1.91, 1.17	β --> C3
0.67, 3.69	γ --> C1
0.64, 1.36	γ --> C2
0.64, 1.17	γ --> C3
3.70, 1.15	C1 --> C3

Table XIVI-A: Intramolecular NOE's of Ephedrine

Intramolecular NOE's – Ephedrine (S,RS complex)	
Cross Peak	Assignment
4.69, 3.22	Ha --> Hb
4.69, 0.93	Ha --> Hc
4.69, 2.58	Ha --> Hd
3.23, 0.90	Hb --> Hc
3.22, 2.57	Hb --> Hd
3.23, 7.26	Hb --> He
0.92, 2.58	Hc --> Hd
0.93, 7.27	Hc --> He
2.57, 7.27	Hd --> He

Table XV-A: Intermolecular NOE's of S,RS Complex

Intermolecular peaks (S,RS complex)	
Cross Peak	Assignment
3.86, 4.69	α --> Ha
3.88, 0.93	α --> Hc
3.88, 2.57	α --> Hd
3.88, 7.26	α --> He
1.92, 4.71	β --> Ha
1.92, 3.23	β --> Hb
1.91, 0.92	β --> Hc
1.91, 2.55	β --> Hd
1.92, 7.27	β --> He
0.62, 4.96	γ --> Ha
0.62, 3.23	γ --> Hb
0.64, 2.57	γ --> Hd
0.627, 7.27	γ --> He
3.69, 4.69	C1 --> Ha
3.70, 3.22	C1 --> Hb
3.71, 0.93	C1 --> Hc
3.70, 2.57	C1 --> Hd
3.69, 7.27	C1 --> He
1.35, 0.92	C2 --> Hc
1.34, 7.27	C2 --> He
1.19, 4.69	C3 --> Ha
1.17, 3.23	C3 --> Hb
1.17, 7.27	C3 --> He

R,RS Complex ROESY Data

Table XVI-A: Intramolecular NOE's of DDCV

Intramolecular NOE's – DDCV (R,RS complex)	
Cross Peak	Assignment
3.87, 1.34	α --> C1
3.87, 1.13	α --> C3
3.70, 1.89	β --> C1
1.91, 0.68	β --> γ
1.91, 1.17	β --> C3
3.70, 0.70	C1 --> γ
1.20, 0.66	C3 --> γ
3.70, 1.17	C1 --> C3

Table XVII-A: Intramolecular NOE's of Ephedrine

Intramolecular NOE's – Ephedrine (R,RS complex)	
Cross Peak	Assignment
4.69. 0.926	Ha --> Hc
4.69, 2.61	Ha --> Hd
3.26, 0.92	Hb --> Hc
3.28, 2.59	Hb --> Hd
0.95, 2.58	Hc --> Hd
0.922, 7.27	Hc --> He
2.62, 7.28	Hd --> He

Table XVIII-A: Intermolecular NOE's of R,RS Complex

Intermolecular peaks(R,RS complex)	
Cross Peak	Assignment
3.86. 4.65	α --> Ha
3.86, 7.28	α --> He
1.91, 0.92	β --> Hc
1.96, 2.61	β --> Hd
0.90, 3.26	γ --> Hb
0.93, 0.68	γ --> Hc
0.92, 2.61	γ --> Hd
3.71, 3.32	C1 --> Hb
3.70, 0.914	C1 --> Hc
3.70, 2.59	C1 --> Hd
3.69, 7.28	C1 --> He
1.18, 7.27	C3 --> He
1.20, 0.82	C3 --> Hc
1.19, 2.61	C3 --> Hd

S, (+) Complex ROESY Data

Table XIX-A: Intramolecular NOE's of DDCV

Intramolecular NOE's - DDCV (S,+ complex)	
Cross Peak	Assignment
3.90, 1.36	α --> C2
3.90, 1.18	α --> C3
1.93, 0.72	β --> γ
1.93, 3.72	β --> C1
1.93, 1.22	β --> C3
0.71, 3.72	γ --> C1
3.72, 1.20	C1 --> C3

Table XX: Intramolecular NOE's of Norephedrine

Intramolecular NOE's – Norephedrine (S,+ complex)	
Cross Peak	Assignment
4.85, 3.43	Ha --> Hb
4.85, 0.982	Ha --> Hc
4.86, 7.29	Ha --> He
3.43, 0.98	Hb --> Hc
3.43, 7.29	Hb --> He
0.98, 7.29	Hc --> He

Table XXI-A: Intermolecular NOE's of S, + Complex

Intermolecular peaks (S,+ complex)	
Cross Peak	Assignment
3.91, 7.29	α --> He
1.93, 0.97	β --> Hc
0.65, 4.85	γ --> Ha
3.72, 4.85	C1 --> Ha
3.72, 0.97	C1 --> Hc
3.72, 7.29	C1 --> He
1.36, 0.97	C2 --> Hc
1.36, 7.29	C2 --> He
1.21, 4.85	C3 --> Ha
1.22, 3.43	C3 --> Hb
1.21, 7.29	C3 --> He

S,(-) Complex ROESY Data

Table XXII-A: Intramolecular NOE's of DDCV

Intramolecular NOE's - DDCV (S, - complex)	
Cross Peak	Assignment
3.90, 0.69	α --> γ
3.91, 1.36	α --> C2
3.91, 1.15	α --> C3
1.93, 0.69	β --> γ
1.94, 3.73	β --> C1
1.95, 1.21	β --> C3
0.69, 3.73	γ --> C1
0.67, 1.37	γ --> C2
0.66, 1.21	γ --> C3
3.73, 1.18	C1 --> C3

Table XXIII-A: Intramolecular NOE's of Norephedrine

Intramolecular NOE's – Norephedrine (S,- complex)	
Cross Peak	Assignment
5.08, 0.95	Ha --> Hb
5.08, 3.34	Ha --> Hc
5.08, 7.30	Ha --> He
3.34, 0.95	Hb --> Hc
3.34, 7.30	Hb --> He
0.95, 7.30	Hc --> He

Table XXIV-A: Intermolecular NOE's of S, - Complex

Intermolecular peaks (S,- complex)	
Cross Peak	Assignment
3.91, 7.30	α --> He
1.94, 0.94	β --> Hc
1.93, 7.30	β --> He
0.66, 5.08	γ --> Ha
0.67, 7.29	γ --> He
3.73, 5.08	C1 --> Ha
3.72. 3.35	C1 --> Hb
3.73, 7.30	C1 --> He
1.36, 7.30	C2 --> He
1.17, 3.36	C3 --> Hb
1.20, 7.29	C3 --> He

R,(+) Complex ROESY Data

Table XXV-A: Intramolecular NOE's of DDCV

Intramolecular NOE's – DDCV (R,+ complex)	
Cross Peak	Assignment
3.91, 1.36	α --> C2
3.91, 1.22	α --> C3

1.93, 0.68	β --> γ
1.93, 3.73	β --> C1
0.69, 3.72	γ --> C1
0.68, 1.22	γ --> C3
3.73, 1.19	C1 --> C3

Table XXVI-A: Intramolecular NOE's of Norephedrine

Intramolecular NOE's – Norephedrine (R,+ complex)	
Cross Peak	Assignment
4.93, 3.45	Ha --> Hb
4.92, 0.97	Ha --> Hc
4.92, 7.28	Ha --> He
3.45, 0.97	Hb --> Hc
3.42, 7.29	Hb --> He
0.971, 7.28	Hc --> He

Table XXVII-A: Intermolecular NOE's- DDCV:Norephedrine complex

Intermolecular peaks(R,+ complex)	
Cross Peak	Assignment
3.92, 0.97	α --> Hc
1.92, 4.92	β --> Ha
0.65, 4.92	γ --> Ha
0.68, 0.97	γ --> Hc
0.653, 7.28	γ --> He
3.72, 4.93	C1 --> Ha
3.72, 0.97	C1 --> Hc
3.72, 7.29	C1 --> He
1.36, 4.92	C2 --> Ha
1.36, 7.29	C2 --> He
1.20, 4.92	C3 --> Ha
1.21, 3.45	C3 --> Hb
1.21, 7.29	C3 --> He

R, (-) Complex ROESY Data

Table XXVIII-A: Intramolecular NOE's of DDCV

Intramolecular NOE's – DDCV (R,- complex)	
Cross Peak	Assignment
3.89, 1.36	α --> C2

3.89, 1.15	α --> C3
1.93, 0.68	β --> γ
1.92, 3.73	β --> C1
0.68, 3.72	γ --> C1
0.67, 1.22	γ --> C3
3.72, 1.18	C1 --> C3

Table XXIX: Intramolecular NOE's of Norephedrine

Intramolecular NOE's – Norephedrine (R,- complex)	
Cross Peak	Assignment
4.87, 3.13	Ha --> Hb
4.88, 0.97	Ha --> Hc
4.87, 7.30	Ha --> He
3.13, 0.96	Hb --> Hc
3.13, 7.30	Hb --> He
0.96, 7.31	Hc --> He

Table XXX: Intermolecular NOE's of R, - Complex

Intermolecular peaks (R,- complex)	
Cross Peak	Assignment
1.92, 7.30	β --> He
0.64, 4.87	γ --> Ha
0.67, 0.96	γ --> Hc
0.63, 7.32	γ --> He
3.71, 4.87	C1 --> Ha
1.37, 7.30	C2 --> He
1.18, 4.86	C3 --> Ha
1.21, 7.30	C3 --> He