Conformational, Aqueous Solvation, and pK_a Contributions to the Binding and Activity of Cocaine, WIN 32 065-2, and the WIN Vinyl Analog†

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Abstract: Conformational properties of cocaine, WIN 32 065-2, and the WIN vinyl analog were studied via Austin Model 1 (AM1) semiempirical calculations. The conformational space of the molecules, as defined by the dihedral angles representing the orientation of the 2β and 3β side chains, was calculated on a 13 × 13 grid in the gas phase and included full geometry optimization at each grid point. Aqueous solvation energy surfaces were obtained using the AM1–Solvation Model 2 (AM1–SM2). The lowest energy points from the surfaces were fully optimized in both the gas and aqueous phases. Results predict the minimum 2β and 3β side chain conformations to be similar between the gas and aqueous phases and between the three molecules studied. A detailed analysis of the relationship of structural contributions to changes in conformational properties is presented. Population analysis of the minimum energy regions indicates the neutral forms of WIN and the WIN vinyl analog to be more conformationally restricted than cocaine. This conformational restriction is related to steric interactions between the 2β and 3β moieties of the WIN compounds. Cocaine is calculated to be more favorably solvated than the WIN compounds due to the presence of the ester groups in the 2β and 3β moieties. The calculations predict the pK_as of the tropane nitrogen of the WIN compounds to be higher than in cocaine. A model is presented relating the decreased conformational flexibility, decreased aqueous solvation, and increased pK_as of the WIN compounds to their increased binding affinity to the cocaine receptor. Based on the present results, separate models are presented for binding of the neutral and protonated forms of cocaine to the receptor.

I. Introduction

Cocaine has multiple pharmacological effects. It is a local anesthetic,[1] an indirect sympathomimetic,[2] and a psychomotor stimulant.[3] The central stimulant action of cocaine is a result of inhibition of reuptake of the biogenic amines such as norepinephrine (NE), dopamine (DA), and serotonin (5-HT).[4] However, the reinforcing and addictive properties of cocaine are believed to be associated with its inhibition of the DA transporter.[5] Thus, the DA transporter is recognized as the cocaine receptor relevant to the cocaine abuse potential. These receptors are identified by binding of ^{3}H-cocaine, cocaine analogs, or other ^{3}H-labeled DA transporter inhibitors (e.g. ^{3}H-mazindol)[6] and by inhibition of transport of ^{3}H-DA into synaptosomes or cell culture.[7]

During the last five years numerous cocaine analogs and derivatives were synthesized and their affinity for the cocaine receptor and potencies for inhibiting DA transport were determined.[8] These studies identified several cocaine analogs with higher affinity for the receptor and higher potency for inhibiting DA transporter but no cocaine antagonists have yet been discovered. One of these compounds WIN 32 065-2 (WIN) had *in vitro* and *in vivo* effects similar to cocaine but was more potent.[9] Another WIN derivative in which the C2 carbomethoxy group was replaced with a vinyl group[10] also had high affinity for the cocaine receptor. The structures of the three compounds are shown in Figure 1.

Despite extensive experimental studies, it is still not known whether the protonated or neutral form of cocaine binds to the receptor. The pK_a of cocaine is 8.65.[11] In plasma or other aqueous physiological environments approximately 90% of cocaine and most cocaine analogs exist in the positively charged protonated

† Abbreviations: Austin Model 1, AM1; WIN 32 065-2, WIN; Solvation Model 2, SM2; central nervous system, CNS.

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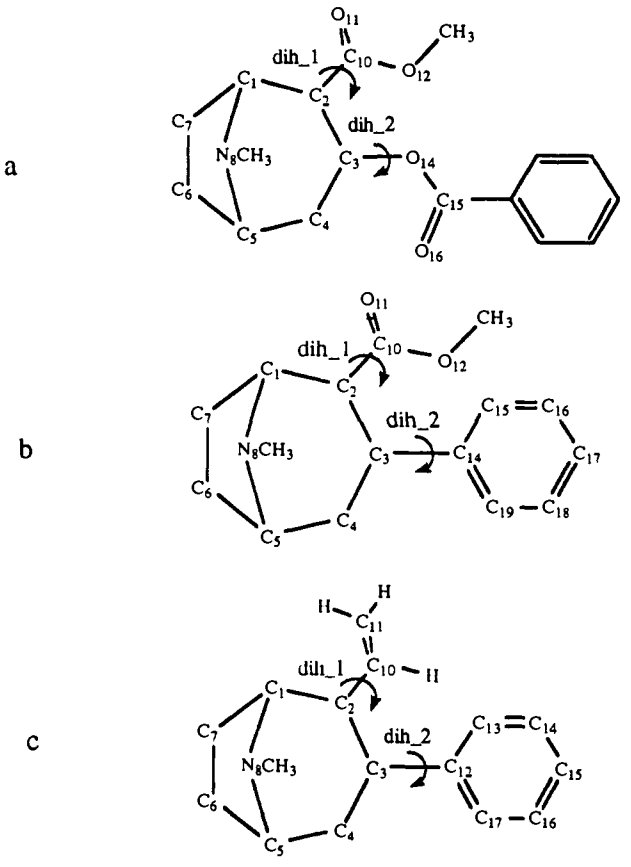

Figure 1. Structures of (a) cocaine, (b) WIN 32 065-2, and (c) the WIN vinyl analog.

form. This is also generally true for sympathomimetic catecholamines. Thus, one would expect the protonated form to be active and the binding to involve an ionic site on the receptor. There is some evidence, however, that suggests that this may not be the case. For example, the methyl quaternary salt analog of cocaine is dramatically less potent than cocaine as evidenced by decreased binding to the dopamine transporter,[7a] although changes in binding due to alteration of the hydrogen bonding properties with respect to a protonated tertiary amine or steric effects due to the additional methyl group cannot be excluded. On the other hand, decreased binding has also been observed in compounds where the *N*-methyl group has been replaced by an electron-withdrawing moiety such as the acetyl group,[12] which are expected to be in the neutral state at physiological pH. Therefore, in order to properly relate the conformational properties of cocaine to its biological function, both the neutral and protonated forms need to be studied.

The lack of success in developing cocaine antagonists emphasizes the importance of better understanding the conformational properties of cocaine with respect to its physiological functions. Greater knowledge of the structure–function relationship of cocaine will aid in the rational design of a cocaine antagonist which may also be useful in the treatment of other dopaminergic system-associated diseases, such as Parkinson's disease.[13] Several computational studies have been presented on cocaine and cocaine analogs in attempts to elucidate the relationship of the structure of cocaine to its biological function.[14] These studies are limited by the calculations being performed in the gas phase and on only one of the two protonation states of cocaine. In the present study we have extended previous work to include the influence of both protonation and solvation on the conformational properties of cocaine, WIN, and the WIN vinyl analog. This was performed via a systematic study of the conformations of these molecules with respect to rotation of the 2β and 3β substituents (see Figure 1).

Presented are gas phase semiempirical calculations using Austin-Model 1 (AM1) and aqueous solvation free energy calculations using the AM1–Solvent Model 2 (AM1-SM2) semiempirical model[15] on both the neutral and protonated forms of cocaine, WIN, and the WIN vinyl analog. Section II presents the computational methods. Following in Section III are the results and discussion on both the gas phase and aqueous phase studies of cocaine, WIN, and the WIN vinyl analog. A summary of the results and the presentation of preliminary models for binding of the studied compounds are given in the conclusion in Section IV.

II. Methods

Calculations were performed with the AMSOL program[16] on a Silicon Graphics IRIS Personal Workstation. Gas phase studies were performed at the AM1 level of theory[17] and calculations in aqueous solution were performed using AM1-SM2.[16b] Energy surfaces were obtained with respect to rotation of the 2β and 3β substituents for both the neutral (free base) and protonated forms of the N8 atom in the tropane ring. The dihedrals used in the energy surfaces are defined as shown in Figure 1a,b,c. Dihedral_1 (dih_1) is defined as O11–C10–C2–C3 for both cocaine and WIN and [ω(C11–C10–C2–C3) + 180] for the WIN vinyl analog (this offset allows for direct comparison of dih_1 between the three compounds); Dihedral_2 (dih_2) is C15–O14–C3–C4 for cocaine, C15–C14–C3–C4 for WIN, and C13–C12–C3–C4 for the WIN vinyl analog. Definitions for the conformers associated with rotation around dih_1 are as follows. In the cis conformer the ester oxygen O12 in cocaine and WIN or C11 in the WIN vinyl analog points away from the tropane nuclei, corresponding to values of 0–60° and 240–360° for dih_1. In the trans conformer the ester oxygen O12 or C11 points toward the tropane nuclei, corresponding to values of 60–240° for dih_1. Potential energy surfaces were obtained on a 13 × 13 grid generated by rotating dih_1 and dih_2 in 30° increments from 0 to 360°. At each point on the grid the structure was fully optimized in the gas phase with dih_1 and dih_2 fixed at their respective values. Each individual grid point was approached from multiple directions and the energies were compared to ensure convergence. The potential energy at each grid point is defined as the heat of formation minus the heat of formation of the lowest energy point on the surface. One-dimensional energy profiles as a function of either dih_1 or dih_2 were extracted from the two-dimensional energy surfaces at the minimum energy value of the fixed dihedral angle. Global minima were obtained from a full geometry optimization of the lowest energy grid point on the potential energy surfaces.

Aqueous phase potential energy surfaces were obtained using the gas phase geometries with the AM1–SM2 Hamiltonian.[16b] These calculations included electronic relaxation in the presence of the solvation term (AMSOL option: 1SCF). Global minima were obtained by taking the geometries with the lowest free energy from the surfaces and subjecting them to a full geometry optimization at the AM1–SM2 level of theory.

An approximate population analysis was obtained from the energy surfaces by assuming a Boltzmann distribution between all possible conformers, according to:

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$$x_i = \frac{C_i}{\sum_{i=1}^{n} C_i} = \frac{e^{-\Delta E_i/RT}}{\sum_{i=1}^{n} e^{-\Delta E_i/RT}} \quad (1)$$

where x_i is the probability of conformer i at $T = 298$ K, C_i is the concentration of conformer i, ΔE_i is the energy of conformer i, R is the gas constant, and the summation is performed over all points in the surface.[18] The energy ΔE_i represents either the relative gas phase potential energy or the relative free energy in aqueous solution. The thermal enthalpic and entropic vibrational contributions are assumed to be equivalent for all conformations.[19] Summations of the populations in the minimum energy regions were calculated over the minimum energy structure and the eight nearest neighbor structures.

Determination of the change in pK_a between two species may be performed based on knowledge of the difference in the aqueous solvation free energy between the protonated forms of the compounds, $\Delta\Delta G_{AH}$, the difference in the aqueous solvation free energy of the neutral forms of the compounds, $\Delta\Delta G_A$, and the difference in the free energy of protonation in the gas phase, $\Delta\Delta G_{gas}$, and applying the following expression:

$$\Delta pK_a = \frac{\Delta\Delta G_A - \Delta\Delta G_{AH} + \Delta\Delta G_{gas}}{2.3RT} \quad (2)$$

where R is the gas constant and T is the temperature.[20] The various $\Delta\Delta G$ values were obtained directly from the gas phase AM1 and aqueous solvation AM1–SM2 calculations. The free energy of solvation is calculated as the heat of formation for the fully relaxed structure in the presence of the aqueous solvation term minus the heat of formation of the fully relaxed gas phase structure. Free energies of protonation in the gas phase were calculated both by assuming that the vibrational contributions were negligible and including those terms explicitly. The vibrational contributions to the gas phase free energy were included based on the AM1 calculated frequencies and the standard statistical mechanical treatment.[21]

III. Results and Discussion

Cocaine, WIN, and the WIN vinyl analog all contain the tropane nucleus. Experimental and computational studies have shown that the chair conformation of the tropane ring with the *N*-methyl group in the equatorial position is preferred.[14a,22,23] Due to conjugation and as confirmed by X-ray studies,[22] the substituents at the 2β and 3β positions are planar. Therefore, the conformation of the molecules may be described by the rotation of the dihedrals, dih_1 and dih_2, as displayed in Figure 1a,b,c.

1. Gas Phase Calculations. a. Potential Energy Contour Surfaces. The gas phase potential energy contour maps are shown in Figure 2. Several observations may be summarized from analysis of the contour maps. For both protonation states of cocaine and WIN, low energy regions occur between 270 and 360° in the dih_1 dimension and between 120 and 180° with respect to dih_2; high energy areas occur in the region of 180 to 330° in the dimension of dih_2. For the WIN vinyl analog, the low-energy region spans from approximately 270 to 360° in both the dih_1 and dih_2 dimensions. For WIN and its vinyl analog the 0–180° region is identical to the 180–360° region with respect to dih_2 due to the symmetry of the phenyl moiety at the 3β position of the tropane ring.

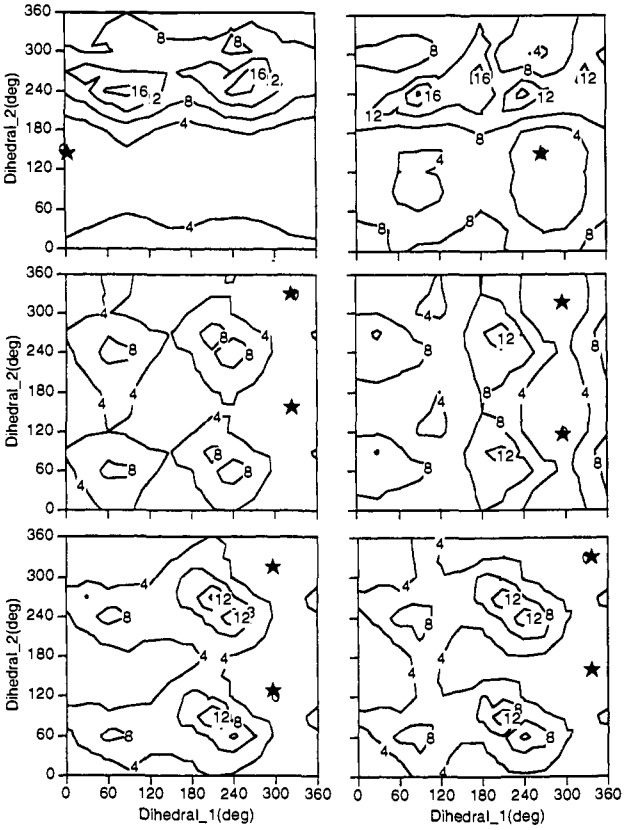

Figure 2. Potential energy surfaces calculated as a function of dih_1 and dih_2 in the gas phase for cocaine (first row), WIN (second row), and the WIN vinyl analog (third row). Left column: the neutral compounds; right column: the protonated compounds. Energy minima are indicated by the ★.

b. Minimum Energy Structures. The gas phase minimum energy structures may be compared with structures obtained from previous computational studies and with experimental structures obtained from X-ray crystallography. Figure 3 displays the low-energy gas phase structures of the neutral and protonated species of the three compounds studied and Table 1 presents the corresponding values for the dihedrals dih_1 and dih_2 along with data with several X-ray crystallographic studies. Computational studies have previously determined structures of the protonated forms of cocaine and a fluorinated WIN analog[14c] and the free base forms of cocaine[14a] and several WIN analogs.[14b] The studies on the protonated compounds were performed via empirical force field calculations using the MM2-87 program and parameter set[24] and the studies of the free base forms of the compounds were done using semiempirical AM1 calculations. The structures obtained in these studies are qualitatively similar to the present structures. Omission of quantitative structural data in all but one of the previous studies precludes more detailed comparisons. Comparison of the present results with experimental X-ray crystallography shows satisfactory agreement. Experimental and calculated values for dih_1 (Table 1) are in excellent agreement, with the trend in the X-ray studies showing a decrease in dih_1 upon protonation of the tropane nitrogen to be reproduced by the calculations. With dih_2 the agreement is not as good, especially in the case of free base cocaine. Analysis of the potential

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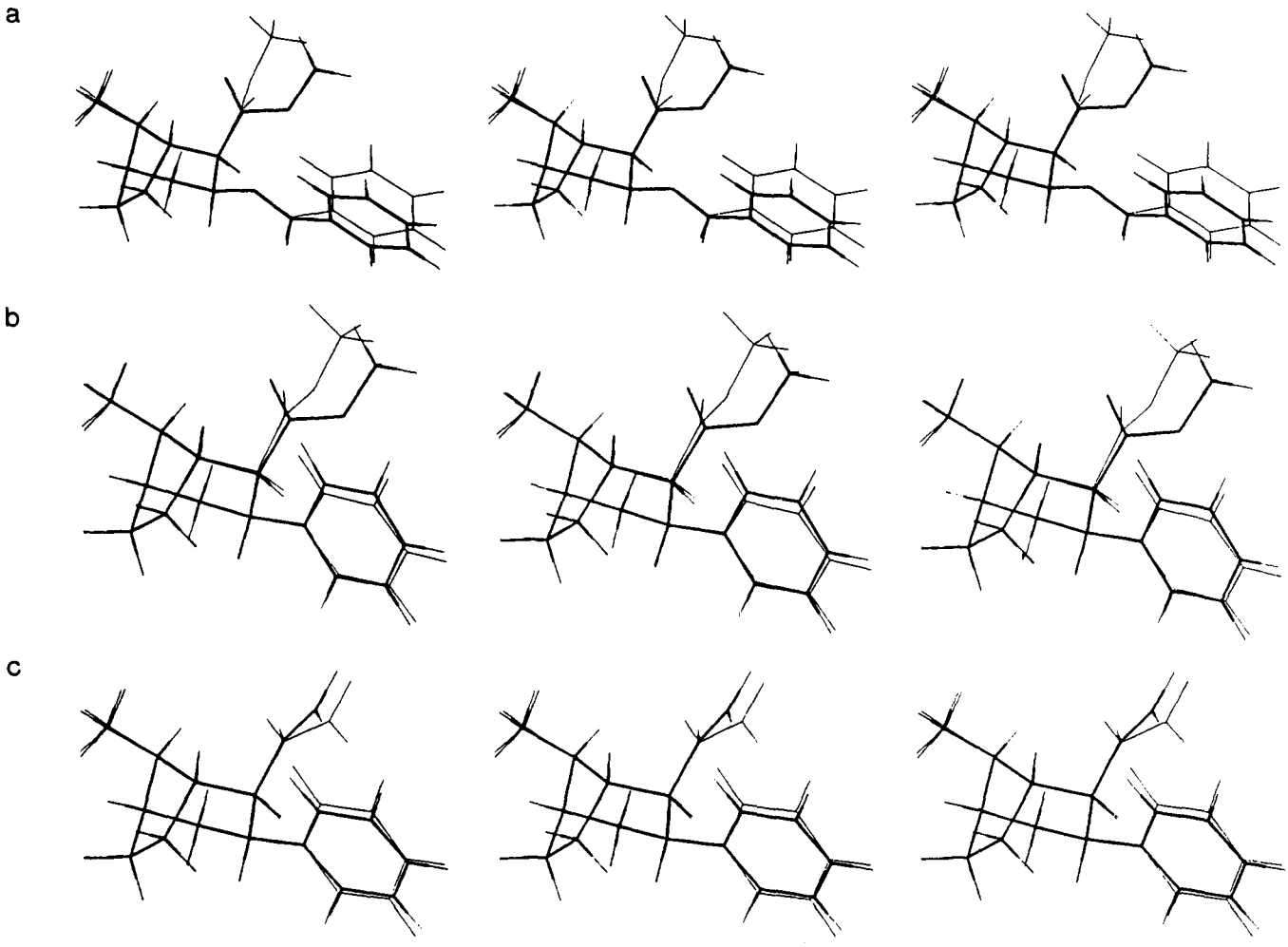

Figure 3. Stereo diagrams of the gas phase neutral and protonated low-energy structures of (a) cocaine, (b) WIN, and (c) the WIN vinyl analog. Thin lines represent the neutral species and thick lines the protonated species. The non-hydrogen atoms in the tropane ring of the neutral and protonated forms of the molecules were subjected to a least squares fit prior to viewing. The left and center pictures comprise the cross-eyed stereo pair and the center and right pictures comprise the wall-eyed stereo pair. In all structures the hydrogen atoms are presented as thin lines.

Table 1. Dihedral Angles of the Global Energy Minima Calculated in the Gas and Aqueous Phases and Experimental Values from X-ray Crystallography[a]

	calculated						
	gas phase		aqueous phase			experimental	
	dih_1	dih_2	dih_1	dih_2		dih_1	dih_2
neutral Species							
cocaine	356	150	330	150	free base cocaine	358	96
WIN	329	178	330	180			
WIN vinyl analog	308	130	120	120			
protonated Species							
cocaine	279	150	270	90	cocaine hydrochloride	314	157
WIN	289	127	300	120	norcocaine hydrochloride	291	82
WIN vinyl analog	330	141	150	149			

[a] All angles in degrees. Experimental data for free base cocaine is from ref 22b, for cocaine hydrochloride is from ref 22a, and for norcocaine hydrochloride is from 22c.

energy surfaces for both the neutral and protonated forms of cocaine (Figure 2, row 1) show the energy minima to be located in broad wells with respect to the dih_2 dimension. These wells extend between dih_2 values of 60 to 150°, which encompasses the X-ray crystallographic values, suggesting that the calculated structures do not contradict those from experiment. More details of the conformational surfaces will be presented below. Thus, the present calculations qualitatively reproduce previous computational work as well as yield good agreement with experiment suggesting the present approach to yield physically relevant results and, therefore, be of value for the interpretation of structure–activity relationships in cocaine, WIN, and the WIN vinyl analog.

Comparison of the minimum energy structures may be performed with respect to protonation of the tropane nitrogen (Figure 3) and with respect to the three compounds studied (Figure 4). In all cases the presented compounds have been overlapped based on the tropane ring non-hydrogen atoms. Analysis of Figure 3 allows for a qualitative picture of the influence of protonation on the gas phase minimum energy structures to be obtained. In all three compounds the largest change occurs in the conformation of the 2β substituent while the 3β substituent is virtually unchanged. Changes in the 2β moiety are due primarily to interactions with the tropane nitrogen atom, as will be discussed below.

The overlapped minimum energy structures of cocaine, WIN, and WIN vinyl analog are shown in Figure 4, parts a and b, for the neutral and protonated species, respectively. The minimum energy structures are qualitatively similar for the three compounds.

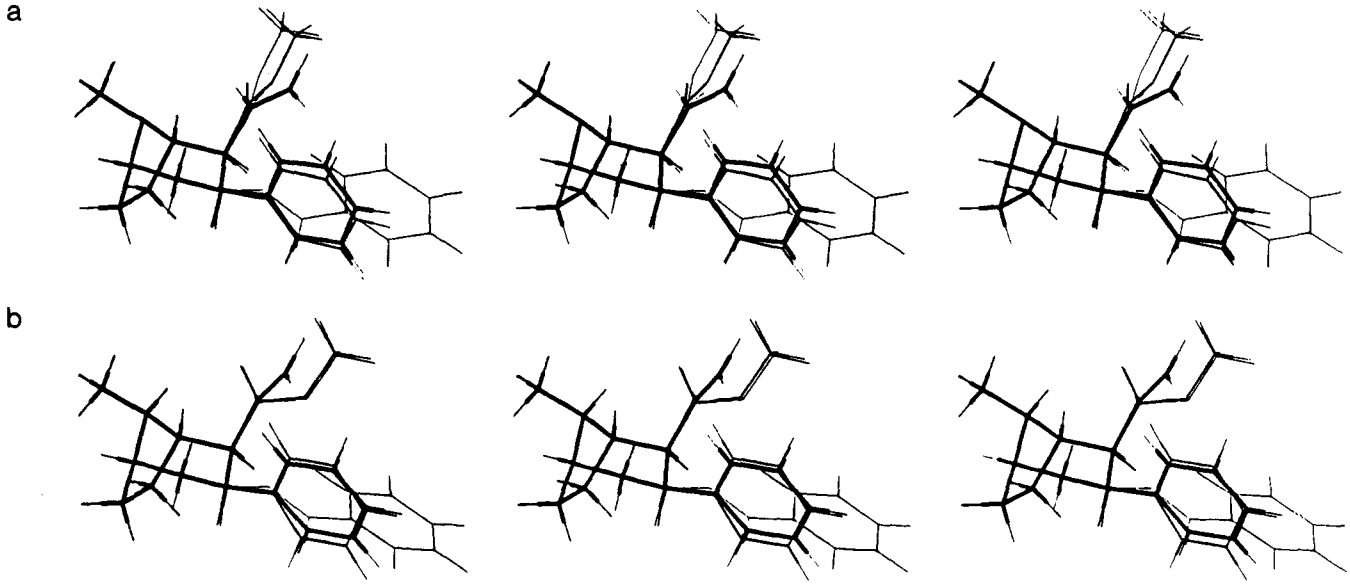

Figure 4. Stereo diagrams of the gas phase (a) neutral and (b) protonated low-energy structures of cocaine, WIN, and the WIN vinyl analog. Thin lines represent cocaine, medium lines represent WIN, and thick lines represent the WIN vinyl analog. The non-hydrogen atoms in the tropane rings of the three molecules were subjected to a least squares fit prior to viewing. The left and center pictures comprise the cross-eyed stereo pair and the center and right pictures comprise the wall-eyed stereo pair. In all structures the hydrogen atoms are presented as thin lines.

In the neutral species the orientation of the 2β substituent for all three compounds is cis. This places the carbonyl oxygen of cocaine and WIN in an orientation where it lies between the tropane ring bridge nitrogen and the 3β substituent. Protonation of the tropane nitrogen allows for the formation of a hydrogen bond between the carbonyl oxygen and the nitrogen leading to a decrease in dih_1 upon protonation of both cocaine and WIN (see Table 1). Omission of the hydrogen bond acceptor in the WIN vinyl analog leads to a smaller change in the conformation of the 2β substituent upon protonation; however, a shift does occur. For the 3β substituent the minimum orientation has the O14—C15 or C14—C15 bond anti-staggered to the C3—C4 bond of the tropane ring. The largest deviations in the 3β substituent upon protonation occur in cocaine (see Figure 3a), which is associated with its additional flexibility as compared to the WIN compounds (see below).

Despite the overall structural similarity of the minimum energy geometries, there exists consistent differences in dih_1 upon going from the neutral to the protonated state. The calculated differences in dih_1 between the protonated and neutral states are −77, −40 and 22°, for cocaine, WIN, and the WIN vinyl analog, respectively. The predicted conformational differences between the protonated and neutral compounds are in agreement with both X-ray crystallographic and ^{13}C-NMR studies[22,23] on cocaine. Note that in neutral cocaine the energy surface in the region of minima is relatively flat (see Figure 5). This allows neutral cocaine to sample regions of dih_1 down to 270°, which corresponds to the minimum energy region of the protonated species. Thus, the change in conformation between the neutral and protonated species may be considered to be negligible within the context of the conformational space accessible to the neutral species at room temperature.

c. Dih_1 and Dih_2 One-Dimensional Dihedral Surfaces. To analyze the potential energy surfaces in more detail, one-dimensional dih_1 and dih_2 potential energy maps were extracted from the two-dimensional surfaces (Figure 2) at the second dimension energy minima. The resulting surfaces are presented in Figure 5 for both protonation states of the three compounds studied. It should be noted that direct extraction of a single dimension from the two-dimensional surfaces may overestimate the energy barrier heights. The energy barriers heights would decrease upon full relaxation with only the dihedral of interest restrained.

Cocaine and WIN both have two local energy minima in the

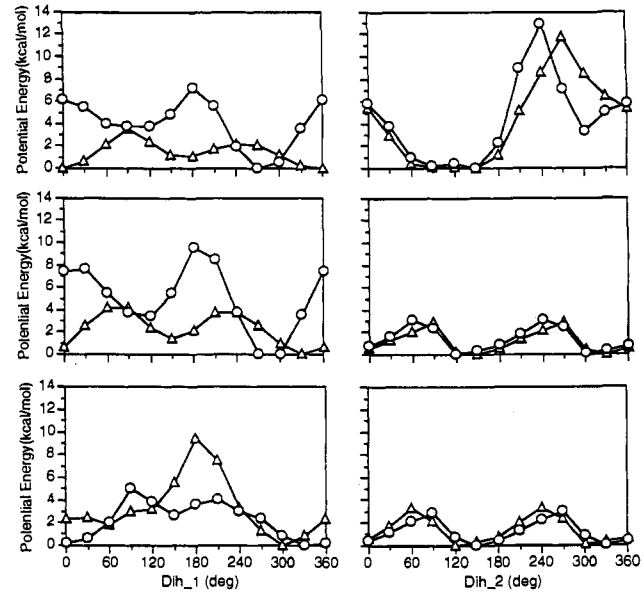

Figure 5. Potential energy surfaces as a function of dih_1 (left column) and dih_2 (right column) calculated in the gas phase with dih_2 or dih_1, respectively, at its minimum energy value. First row: cocaine; second row: WIN; third row: the WIN vinyl analog. Legend: (△) the neutral compounds and (○) the protonated compounds.

vicinity of 120–180° and 270–360° in the dih_1 surface, namely the trans and cis conformers, respectively. To better understand the intramolecular interactions responsible for the dih_1 potential energy surfaces in the cocaine and WIN neutral species, distances between the 2β and 3β substituents were analyzed. In the neutral minimum energy structure of cocaine the 2β ester oxygens are approximately equidistinct to the tropane nitrogen. This orientation suggests that the 2β ester oxygen to tropane nitrogen lone pair repulsion dictates the location of the cis and trans minima. In the trans structure the methoxy O12 to 3β O14 distance is 2.49 Å as compared to 2.69 Å for the carbonyl O11 to O14 distance in the cis structure. These repulsive interactions, however, may be counterbalanced by dipole–dipole interaction between the 2β and 3β ester groups due to those groups being oriented approximately perpendicular to each other (Figure 3a). It is suggested that in the cis structure the ester dipole–dipole interactions are more favorable than in the trans structure, leading

to a lower energy. Additional analysis of the 2β to 3β distances shows the methoxy O12 to the nearest phenyl C distance to be 3.56 Å in the trans structure and the carbonyl O11 to phenyl C distance to be 3.77 Å in the cis structure. These interactions may also contribute to the cis structure being of a lower energy.

Analysis of the WIN neutral minimum energy structures indicates the 2β to 3β substituent interactions to have a significant influence on both the location and relative energies of the minimum energy structures. In contrast to cocaine, the O11 versus O12 to N8 distances for the minimum energy structures differ significantly. Furthermore, the 2β to phenyl ring carbon distances are significantly shorter in the WIN minimum than occur with cocaine. It is suggested that the 2β carbonyl oxygen to 3β phenyl C interaction leads to the shift in the dih_1 minima of WIN as compared to cocaine (Table 1). Concerning the ordering of the minima, interactions between the 2β substituent and both the tropane nitrogen and 3β phenyl ring were analyzed. The trans-methoxy O12 to N8 distance of 3.09 Å and the cis carbonyl O11 to N8 distance of 3.19 Å are similar. The ester oxygens to 3β phenyl C minimum distances are also similar, with the trans methoxy O12 to C distance being 2.97 Å and the cis carbonyl O11 to C distance being 2.92 Å. The similarity of the cis and trans distances makes it difficult to discern the cause of the neutral WIN minima energy difference based on analysis of the calculated structures. Attempts to clarify the relative contributions of these interactions by model compound calculations were not successful (not shown).

To dissect the structural contributions to the energy barriers to rotation about dih_1 in neutral cocaine and WIN, detailed inspection was made of the structures at the energy barriers. In both the cocaine and WIN 90° structures and the WIN 240° structure the shortest distances are the 2β ester oxygen to tropane N8 distances, indicating that interaction to be responsible for the barrier to rotation. The larger barrier for both cocaine and WIN at 90° is associated with shorter methoxy O12 to N8 distances as compared to the carbonyl O11 to N8 distance in the WIN structure at 240°. The shortest distance in the neutral cocaine 240° structure is the 2β methoxy to 3β methoxy distance of 2.65 Å, while the next short interaction involves a carbonyl O11 to tropane N8 distance of 3.01 Å. It is suggested that these steric interactions lead to the 240° barrier in neutral cocaine, however, the 2β to 3β esters dipole–dipole interactions may counterbalance these steric interactions, leading to the relatively low barrier at 240°.

In WIN, the larger energy barriers and the similarity of the 90 and 240° barriers as compared to cocaine are due to the interactions between the 2β ester oxygens and the 3β phenyl ring. This is evidenced by shorter ester oxygen O11/O12 to the phenyl C minimum distances as compared to cocaine. The 2β ester oxygens interact with the center of the 3β phenyl ring, such that oxygen to aromatic π-cloud interactions may dominate. This differs from the minimum energy structures where the 2β to 3β interactions involve the ester oxygens and phenyl ring hydrogens. *Ab inito* calculations showing the interaction of cations with the benzene π cloud to be favorable support the present results.[25] Thus, the closer spatial orientation of the 2β ester group and the 3β phenyl group in WIN, due to the removal of the 3β ester group, appears to contribute to the larger barriers to rotation of the 2β substituent as compared to cocaine.

In the protonated forms of the drugs the minima associated with dih_1 shift by −77 and −40° as compared to the neutral forms of cocaine and WIN, respectively. This shift aligns the 2β O11 and tropane N8 atoms in the cis structures allowing for the formation of a hydrogen bond (see Figure 3a,b). The trans protonated species also has a hydrogen bond due to the interaction of the ester methoxy oxygen (O12) with the tropane N8 atom. In the cis conformers the O11 to N8 distances are 2.84 and 2.86 Å for cocaine and WIN, respectively, and in the trans conformer the O12 to N8 distances are 2.98 and 2.85 Å for cocaine and WIN, respectively. The lower energy of the cis conformer is due to the carbonyl oxygen being a better hydrogen bond acceptor than the methoxy oxygen. *Ab initio* calculations on the interaction between water and methyl acetate with both the carbonyl and ester oxygens support this assertion.[26] These hydrogen bonds also lead to a lowering of the energy minima relative to the rotation barriers, such that the barrier heights in the protonated species are larger than in the neutral compounds.

Overall, in cocaine and WIN the conformation of the 2β substituent is influenced by interactions with both the N8 atom of the tropane ring and the 3β substituent. In the neutral species the 2β ester O to tropane N repulsion influences the position of the energy minima and maxima whereas the ester O to tropane N hydrogen bond dictates the minimum energy structures upon protonation of the tropane nitrogen. This alteration is most obvious in cocaine where the values of dih_1 for the energy minima and maxima are reversed upon going from the neutral to the protonated species, emphasizing the dominate role of the 2β ester to tropane nitrogen atom in that compound. The relative energies of the cis versus trans minima, however, are associated with interactions between the 2β and 3β substituents. Repulsion of the 2β ester oxygens with the tropane nitrogen contributes to the barriers to rotation in the neutral compounds, with additional contributions from repulsive interactions between the 2β and 3β substituents leading to the larger barriers in WIN. The greater role of interactions between the 2β and 3β substituents in WIN is emphasized by the change in the position of the minima and maxima by approximately −30 to −60° upon protonation as compared to the approximately −90° shift in cocaine. In the charged species the barriers to rotation are dominated by breaking of the 2β ester to tropane N8 hydrogen bonds; however, shorter distances between the 2β methoxy O and the 3β substituent lead to the 180° barrier being larger than the 0° barrier for both protonated cocaine and protonated WIN (not shown).

Replacement of the 2β methyl ester side chain with a vinyl group distinguishes the WIN vinyl analog from cocaine and WIN. As can be seen in Figure 5, rotation around dih_1 in the WIN vinyl analog exhibits an energy minima in the vicinity of 330° for both the neutral and protonated form. Less favorable minimum energy regions are found at 60 and 150° for the neutral and protonated species, respectively. The dih_1 surface in the WIN vinyl analog is dominated by steric overlap between the vinyl moiety and both the tropane nitrogen atom and the 3β phenyl ring. Repulsion between the vinyl and phenyl moieties leads to the energy barrier in both the neutral and protonated compounds at 180°. For the protonated WIN vinyl analog a significantly smaller energy barrier occurs at 180° and a broader energy minimum occurs in the vicinity of 330°. In the protonated WIN vinyl analog the tropane N8 nitrogen eclipses the π orbitals of the vinyl double bond in the regions of both 180 and 330°. At 0° the N8 to C10 and C11 distances are 2.92 and 3.61 Å, respectively, and at 330° those distances are 2.95 and 3.92 Å, both in the range where favorable interactions occur. Thus, it is predicted that favorable interactions between the tropane N8 proton and the vinyl moiety double bond leads to a lowering of the energy barrier at 180° and a shift of the energy minimum to 330° in the protonated species. The repulsive nature of the neutral tropane nitrogen to double bond interaction contributes to the energy barrier at 180° and leads to the small energy barrier at 0° in the neutral compound.

Gas phase potential energy surfaces associated with the rotation of dih_2 are presented in the second column of Figure 5. Comparison of the neutral versus protonated species for all three compounds show the energy surfaces to be similar. The largest difference occurs with cocaine, where a second minimum at 300°

(25) Kearney, P. C.; Mizoue, L. S.; Kumpf, R. A.; Forman, J. E.; McCurdy, A.; Dougherty, D. A. *J. Am. Chem. Soc.* **1993**, *115*, 9907–9919.

(26) Briggs, J. M., Nguyen, T. B.; Jorgensen, W. L. *J. Phys. Chem.* **1991**, *95*, 3315.

is predicted for the protonated species. Cocaine has a broad minimum in the region of 60 to 180° versus two narrower wells for WIN and the WIN vinyl analog in the vicinity of 120–180° and 300–360°. Analysis of the barriers to rotation show significantly higher energies in cocaine. In cocaine, the larger barriers at 210 and 240° in the protonated and neutral species, respectively, are due to a combination of repulsion between the 2β and 3β ester groups and steric overlap when the 3β carbonyl oxygen (O16) eclipses the tropane C4 atom. In the neutral cocaine high-energy structure at 240° O16 is 2.46 and 2.87 Å away from O11 and C2, respectively, leading to significant repulsion. While the presence of the ester on the 3β substituent of cocaine leads to the larger barrier to rotation, by positioning the phenyl ring further from the tropane nucleus, it is also responsible for the broad energy minimum in the 60 to 180° region of dih_2. This is caused by negligible interactions between the phenyl ring and both the 2β substituent and the bridging nitrogen atom of the tropane ring. The local minimum at 300° in protonated cocaine is due to the movement of the 2β carbonyl oxygen away from the 3β substituent upon hydrogen bond formation, as evidenced by the decrease in dih_1 by 90° upon going from the neutral to protonated cocaine minima (Figure 5). This allows for a low-energy region between the barriers at 240 and 360°. The 240° barrier is due to repulsion of the 2β and 3β esters and at 360° steric repulsion when the 3β carbonyl oxygen (O16) eclipses the tropane C4 atom leads to the barrier. In WIN and the WIN vinyl analog omission of the ester group and direct attachment of the phenyl ring to the tropane nucleus at the 3β position lowers the repulsion between the 2β and the 3β substituents as compared to cocaine, leading to a lower barrier to rotation. The barrier in these compounds is due to overlap of the 3β phenyl ring with the 2β substituent. In addition, repulsion in cocaine associated with the eclipsing of the tropane C4 atom by the carbonyl oxygen (O16) which contributes to the barrier in the region of 0° is avoided. The energy minima in the WIN compounds are narrower due to the symmetry of the 3β phenyl ring leading to the second barrier. For cocaine, WIN, and the WIN vinyl analog the −30° shift and slight energy increase in the dih_2 barriers upon protonation are associated with increased unfavorable interactions between the 2β and 3β substituents due to the slight rotation of the 2β substituent.

2. Aqueous Phase Calculations. Full geometry optimization using the AM1–SM2 solvation term is CPU intensive. Test cases comparing the frozen geometry versus full geometry optimization showed the difference between two calculatiosn not to exceed 0.6 kcal/mol for the minima of the three species studied. We conclude that a single SCF calculation using the AM1–SM2 Hamiltonian and the gas phase optimized geometry is a reasonable approximation to full geometry optimization for the purpose of studying the aqueous potential energy surfaces. Therefore, the aqueous solvation surfaces were obtained via electronic relaxation using AM1–SM2 Hamiltonian (AMSOL option: 1SCF) on the gas phase optimized geometries. Only the global minima were fully relaxed in the aqueous phase.

a. Aqueous Potential Energy Contour Surfaces. The aqueous potential energy surfaces generated using the gas phase geometries are displayed in Figure 6. Comparison with the gas phase potential energy surfaces (Figure 2) show there to be no significant changes in the overall topologies. The only significant changes in the location of the minima occur in cocaine. In neutral cocaine the position of the minimum shifts with respect to dih_1 while a shift in dih_2 occurs in protonated cocaine. In both protonation states of WIN and the WIN vinyl analog changes in the minima are all less than 30°. Due to the high-energy regions of the map being associated with steric overlap only minor changes in the positions and magnitude of the maxima are calculated.

b. Minimum Energy Structures. The structures of the global energy minima as described by dih_1 and dih_2 are included in Table 1. As noted above, several changes have occurred with respect to the gas phase for cocaine. In neutral cocaine the

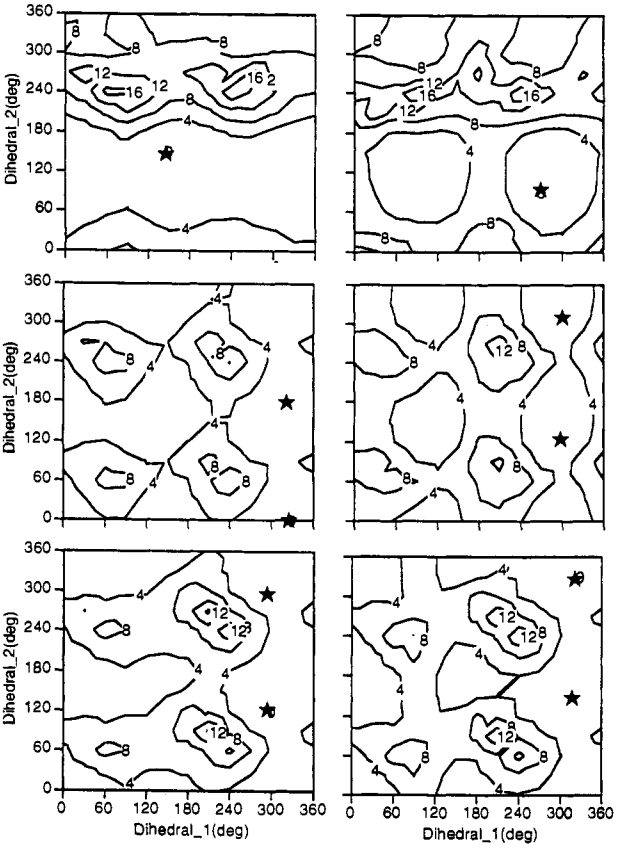

Figure 6. Aqueous solvation energy surfaces calculated as a function of dih_1 and dih_2 in the aqueous phase for cocaine (first row), WIN (second row), and the WIN vinyl analog (third row). Left column: the neutral compounds; right column: the protonated compounds. Energy minima are indicated by the ★.

minimum is now the trans conformer with respect to dih_1; however, the energy difference between the cis and trans geometries is only 0.06 kcal/mol. This difference may be considered insignificant with respect to the accuracy of the applied level of theory. The protonated cocaine minimum has shifted from 150° in the gas phase to 90° in aqueous solution with respect to dih_2. In accord with the broad energy well in the dih_2 dimension only a small energetic difference is predicted to be present between the 90 and 150° conformers (see below).

c. Dih_1 and Dih_2 One-Dimensional Dihedral Surfaces. A comparison of the dih_1 rotation profiles in the gas phase and in aqueous solution are shown in Figure 7. For the neutral species, where solvation effects are expected to be smaller, the surfaces from the two phases are similar. In neutral cocaine there is a lowering of the energies in the region of 90 to 180° indicating the trans conformer to be stabilized with respect to the cis. This stabilization is associated with increased exposure of the 2β carbonyl oxygen (O11) in the trans conformer. Presented in Table 2 are the solvent accessibilities and the free energies of solvation associated with the 2β ester oxygens for cocaine and WIN. As may be seen, the solvent accessibilities of the carbonyl and methoxy oxygens in the cis conformer of neutral cocaine are approximately equal, corresponding to the carbonyl oxygen free energy of solvation being about 2 kcal/mol more favorable than that of the methoxy oxygen. This is due to the carbonyl oxygen being a better hydrogen bond acceptor than the methoxy oxygen, as discussed above. Upon going to the trans conformer, the carbonyl oxygen accessibility increases while that of the methoxy oxygen decreases leading to free energies of solvation of −7.0 and −0.8 kcal/mol for the carbonyl and methoxy oxygens, respectively. In neutral WIN a slight lowering of the energy in the 0 to 180° region of dih_1 occurs. That change is smaller than in neutral cocaine in accord with the smaller energy difference between the cis and trans free energies of solvation of the 2β ester oxygens

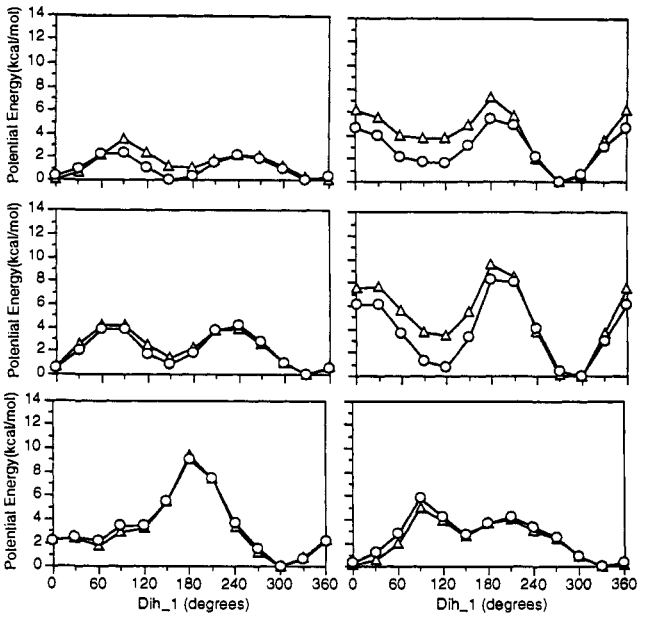

Figure 7. Energy surfaces as a function of dih_1 calculated in the gas phase (△) and aqueous phase (○). Left column: the neutral compounds; right column: the protonated compounds. First row: cocaine; second row: WIN; third row: the WIN vinyl analog.

Table 2. Solvent Accesibilities and Free Energies of Solvation of the 2β Ester Oxygens in the Dihedral_1 Cis and Trans Geometries of Cocaine and WIN[a]

	neutral		protonated	
	cis	trans	cis	trans
	Cocaine			
ester oxygen (O11)				
SA	28.1	51.6	36.1	37.7
FE	-4.0	-7.0	10.9	6.4
methoxy oxygen (O12)				
SA	27.3	5.8	21.2	12.1
FE	-2.1	-0.8	6.1	9.3
nitrogen (N8)				
SA	5.1	4.9	4.5	4.6
FE	-1.9	-1.5	3.9	3.9
	WIN			
ester oxygen (O11)				
SA	27.2	52.7	29.5	48.3
FE	-3.5	-6.3	11.3	7.1
methoxy oxygen (O12)				
SA	28.3	4.4	25.4	8.0
FE	-1.7	-0.5	6.1	9.5
nitrogen (N8)				
SA	5.0	4.8	4.4	4.6
FE	-1.6	-1.3	3.9	3.9

[a] Solvent accessibilities (SA) are in Å² and total solvation free energies (FE) are in kcal/mol.

(Table 2). The hydrophobic character of the vinyl 2β substituent in the WIN vinyl analog leads to negligible differences between the gas and aqueous phase surfaces.

Solvation effects are significantly larger on the dih_1 surfaces in the protonated species due to their positive charge. In addition to the influence of the 2β ester oxygen that occurs in the neutral species, the free energy of solvation of the tropane nitrogen is significant. As shown in Table 3, in the trans conformers of cocaine and WIN the tropane nitrogen is more favorably solvated as compared to the cis conformer. This difference is associated with the less favorable hydrogen bond in the trans conformer (*vide infra*) and acts to counterbalance the favorable carbonyl oxygen (O11) to tropane nitrogen (N8) hydrogen bond which dominates the gas phase dih_1 surfaces for the protonated species. In the WIN vinyl analog the protonated form is only slightly affected by the inclusion of aqueous solvation due to the hydrophobic character of the 2β vinyl moiety.

Table 3. Solvent Accessibilities and Free Energies of Solvation of the Tropane Nitrogen in the Dihedral_1 Cis and Trans Geometries[a]

		neutral		protonated[b]	
		cis	trans	cis	trans
cocaine	SA	5.1	4.9	4.5	4.6
	FE	-1.9	-1.5	-9.0	-9.8
WIN	SA	5.0	4.8	4.4	4.6
	FE	-1.6	-1.3	-8.8	-9.7
WIN vinyl analog	SA	5.0	10.3	7.2	10.4
	FE	-1.0	-1.2	-9.2	-9.7

[a] Solvent accessibilities (SA) are in Å² and total solvation free energies (FE) are in kcal/mol. [b] In the protonated species the results are the sum of the tropane nitrogen and the covalently bound proton.

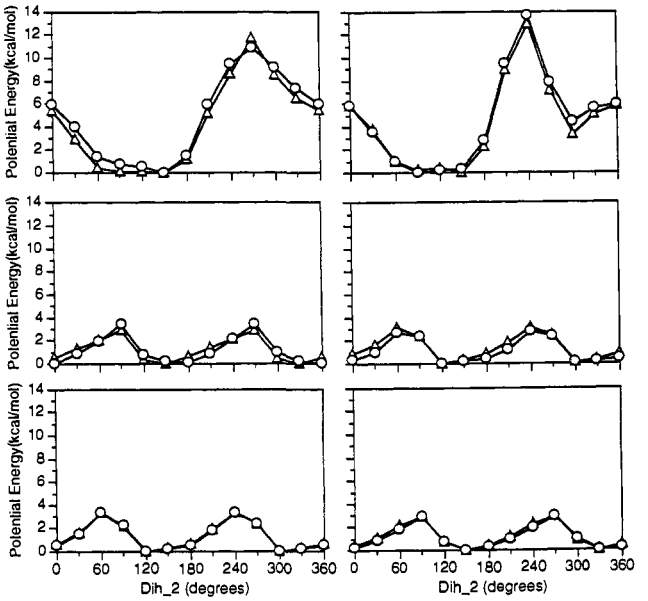

Figure 8. Energy surfaces as a function of dih_2 calculated in the gas phase (△) and aqueous phase (○). Left column: the neutral compounds; right column: the protonated compounds. First row: cocaine; second row: WIN; third row: the WIN vinyl analog.

Aqueous potential energy one-dimensional surfaces for dih_2 are compared with the gas phase surfaces in Figure 8. For all molecules, the gas and aqueous surfaces are similar. This similarity indicates that steric repulsion between the 2β and 3β substituents, rather than electrostatic interactions, dominates the dih_2 surfaces.

3. Conformer Populations in the Gas Phase and in Aqueous Solution. To quantify the conformational freedom of the molecules, a population analysis was performed based on a Boltzmann distribution. Calculations were performed using eq 1. Presented in Figures 9 and 10 are the resulting two-dimensional populations maps calculated in the gas and aqueous phases, respectively, for both the neutral and protonated species. Regions of the energy contour maps which are less than 2 kcal/mol above the minima make a significant contribution to the probability distributions at room temperature. Thus, only limited regions of the dih_1 versus dih_2 surfaces are predicted to be populated at room temperature. Comparison of the neutral versus protonated species for cocaine and WIN in both phases show a decrease in accessible conformational space upon protonation. A trend also occurs where the accessible regions of conformational space increase upon going from the gas to the aqueous phase in cocaine and WIN. This includes an increase in the dih_1 trans conformer population for both forms of cocaine and WIN in the gas phase. In accord with the small changes in the WIN vinyl compound upon protonation discussed above, only minor differences occur between the gas and aqueous phases and between the neutral and protonated species. Overall, the present calculations predict significant populations of all the species studied to occur in similar regions of conformational space. Large populations occur in the

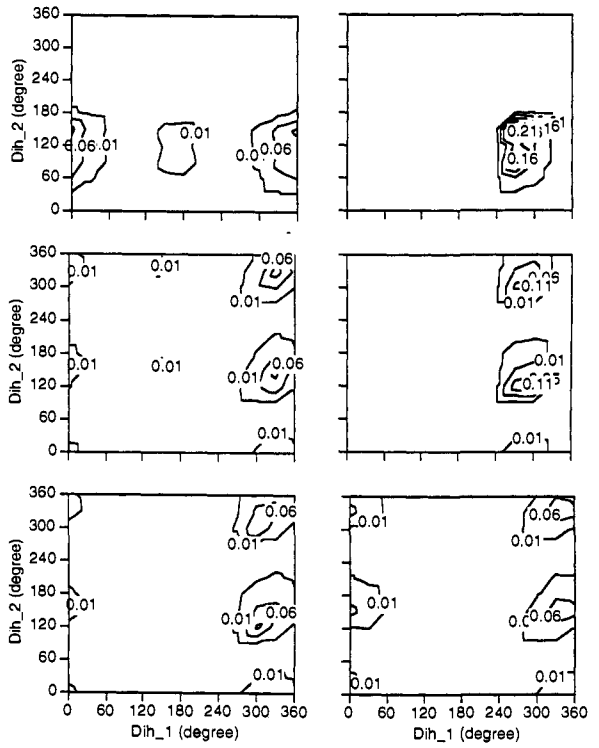

Figure 9. Probability as a function of dih_1 and dih_2 calculated for the neutral compounds (left column) and protonated compounds (right column) in the gas phase. First row: cocaine; second row: WIN; third row: the WIN vinyl analog.

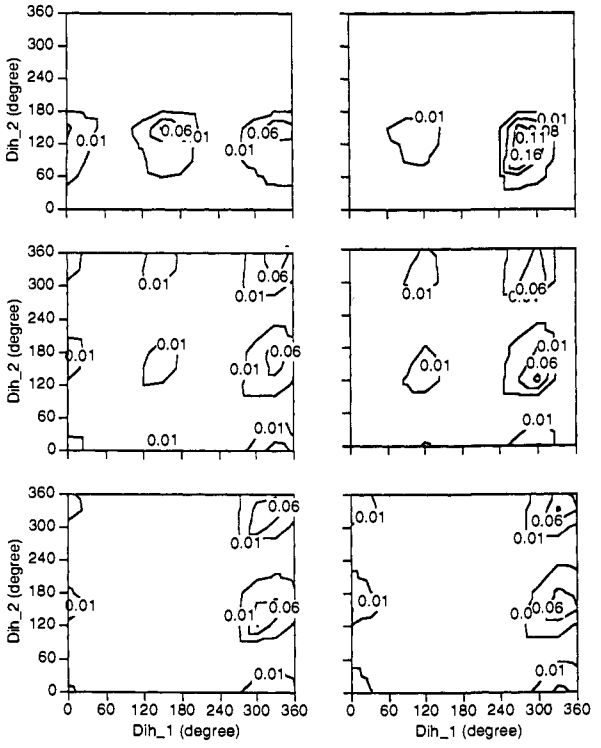

Figure 10. Probability as a function of dih_1 and dih_2 calculated for the neutral compounds (left column) and protonated compounds (right column) in the aqueous phase. First row: cocaine; second row: WIN; third row: the WIN vinyl analog.

lower right quadrant of the plots for all three compounds, in the vicinity of dih_1 values of 300° and dih_2 values of 120°.

Additional analysis of the conformational flexibility was performed by obtaining the population in the region of the minimum energy structures. The results are presented in Table 4. The populations of WIN and the WIN vinyl analog for the cis minimum energy regions are larger than that of cocaine in all

Table 4. Populations of the Cis and Trans Minimum Energy Regions in the Gas and Aqueous Phases[a]

	gas phase		aqueous phase	
	cis	trans	cis	trans
neutral				
cocaine	0.55	0.07	0.30	0.45
WIN	0.84	0.12	0.80	0.04
WIN vinyl analog	0.63	0.03	0.64	0.02
protonated				
cocaine	0.61	0.07	0.57	0.00
WIN	0.86	0.12	0.60	0.00
WIN vinyl analog	0.63	0.01	0.66	0.10

[a] Due to the symmetry of WIN and the WIN vinyl analog, the population is the sum of the equivalent values of dih_2. The presented values are the summations of the populations of the 9 grid points centered about the cis and trans energy minima.

Table 5. Free Energy and pK_a Differences between Cocain and WIN, and Cocaine and the WIN Vinyl Analog[a]

free energy difference	$\Delta\Delta G_{gas}$	$\Delta\Delta G_{solv,A}$	$\Delta\Delta G_{solv,AH^+}$	ΔpK_a
WIN–cocaine	−1.28	3.02	0.50	0.91
WIN vinyl analog–cocaine	0.76	5.07	1.30	3.34

[a] Free energies in kcal/mol.

instances. In cocaine the population of the minimum energy region increases upon protonation, especially in the aqueous phase where the cis and trans conformers of neutral cocaine are similarly populated. In WIN and the WIN vinyl analog the influence of either protonation or aqueous solvation is minimal. The largest difference occurs in the protonated form of WIN in aqueous solution. This is due to the combined influence of aqueous solvation of the 2β ester oxygens and the tropane nitrogen in trans conformer, as shown in Figure 7 and Tables 2 and 3. Thus, the present calculations predict the WIN and the WIN analog compounds to have larger populations in the cis minimum energy conformations as compared to cocaine.

4. Prediction of pK_a Differences between Cocaine, WIN, and the WIN Vinyl Analog. Ambiguities concerning the protonation state of the form of cocaine that interacts with the dopamine transporter and the potential contribution of the neutral form of the molecule to transport across membranes make an analysis of the pK_as of cocaine, WIN, and the WIN vinyl analog relevant. Taking advantage of the availability of the changes in energy upon protonation of the compounds in the gas phase and the free energies of aqueous solvation of the neutral and protonated species (see Table 5) the change in pK_a, ΔpK_a = $pK_{a,\text{WIN (or the WIN vinyl analog)}} - pK_{a,\text{cocaine}}$, was calculated using eq 2. This approach avoids problems associated with the theoretical determination of the absolute pK_a due to inaccuracies in the experimental absolute free energy of solvation of the proton and limitations in the accuracy of the calculated absolute free energies of solvation of the individual species. The absolute free energy of solvation of a proton is not required for determination of a pK_a change and systematic inaccuracies in the heats of formation and free energies of solvation of cocaine, WIN, and the WIN vinyl analog associated with the AM1 and AM1–SM2 levels of theory may be assumed to cancel.[27] Applying the data presented in Table 5 yields pK_a differences of 0.9 and 3.3 for WIN and the WIN vinyl analog with respect to cocaine, respectively. Thus, both WIN and the WIN vinyl analog are predicted to have pK_as larger than that of cocaine, with the WIN vinyl analog being significantly larger. Such an increase in pK_a would lead to an increased population of the protonated forms of the drugs at physiological pH as compared to cocaine. The present predictions should be readily verifiable by experiments.

In the above calculation of the difference in the pK_as, the heats of formation in the gas phase were used directly. In eq 2 it may be seen that the free energy of protonation in the gas phase is

(27) Cramer, C. J. *J. Org. Chem.* **1992**, *57*, 7034–7043.

required for the compounds studied. Since semiempirical calculations are parameterized to yield results that correspond to room temperature it was assumed that the vibrational contributions to the free energy are included implicitly in the heats of formation. To check that this assumption was reasonable the vibrational contributions to the free energies of protonation were obtained from frequency calculations on the fully optimized structures. Using the resulting free energies, pK_a changes of 0.6 and 2.5 are calculated for WIN and the WIN vinyl analog, respectively. The similarity of these values with those in Table 5 indicate that assumptions on the vibrational contributions to the gas phase protonation free energies have a negligible influence on the present results.

Analysis of the results in Table 5 for the three free energy terms included in eq 2 show all three terms to make contributions; however, the change in the free energy of solvation of the neutral species dominates the calculated pK_a differences. The source of the difference is the loss of the ester groups in WIN and the WIN vinyl analog leading to a less favorable free energy of solvation for the WIN compounds. In the protonated species the charged tropane nitrogen dominates the solvation of the compounds such that the loss of the ester groups leads to a smaller change in the free energies of solvation as compared to the neutral WIN compounds. Since the neutral species of cocaine is significantly more favorably solvated than the WIN compounds, it is energetically more favorable for cocaine to lose its proton, leading to a lower pK_a. In other words, the decreased solvation of the neutral forms of WIN and the WIN vinyl compound makes the deprotonation of those compounds more unfavorable than for cocaine, elevating their pK_as. As expected, the loss of two esters in the WIN vinyl analog yields a larger change in the pK_a as compared to WIN.

The relative solvation of cocaine versus WIN and the WIN vinyl analog will contribute to the relative binding of the compounds to the receptor. Assuming that both compounds are desolvated upon receptor binding, the lowered free energy of solvation of WIN and the WIN vinyl analog will lead to tighter binding as compared to cocaine. This is due to a lower free energy requirement for the desolvation of the molecules, allowing them to bind more readily to the receptor. Utilizing a thermodynamic cycle similar to that often used in free energy perturbation calculations,[28] the following equivalence is valid:

$$\frac{K_{eq,cocaine}}{K_{eq,WIN}} = \frac{K_{aqueous}}{K_{receptor}} \quad (3)$$

In equation 3, $K_{eq,cocaine}$ and $K_{eq,WIN}$ are the experimentally determined equilibrium constants for the binding of cocaine and WIN to the receptor, respectively, $K_{aqueous}$ is the relative aqueous solvation of cocaine with respect to WIN and $K_{receptor}$ is the relative binding strength of cocaine versus WIN directly to the receptor. Note that $K_{aqueous}$ and $K_{receptor}$ cannot be measured experimentally and represent alchemical equilibria useful in the present study for the direct understanding of the contribution of changes in aqueous solvation to the relative binding of the compounds. Such an approach allows the contributions of aqueous solvation and receptor interactions to be treated independently in contrast to the traditional analysis of the ratios of the equilibrium constants, where both aqueous solvation and receptor interactions contribute. If it is assumed that the direct binding of cocaine and WIN to the receptor is identical, such that $K_{receptor}$ equals 1, then eq 3 simplifies to the following:

$$\frac{K_{eq,cocaine}}{K_{eq,WIN}} = K_{aqueous} \quad (4)$$

allowing for the change in the binding of the compounds associated with their relative aqueous solvation to be determined. $K_{aqueous}$ may readily be determined from the differences in the AM1–SM2 calculated free energies of solvation via the van't Hoff equation ($\Delta G = -RT \ln K$). Applying the differences in the free energies of solvation for cocaine and WIN reported in Table 5 yields binding ratios of 6.0×10^{-3} and 0.43 between the neutral and protonated forms of cocaine and WIN, respectively, and 1.9×10^{-4} and 0.11 between cocaine and the WIN vinyl analog. The reciprocal of these values (e.g. $K_{eq,WIN}/K_{eq,cocaine}$) predict WIN to bind 167 and 2.3 times more tightly than cocaine in the neutral and protonated states, respectively. For the WIN vinyl analog binding ratios of 5236 and 9.1 are obtained for the neutral and protonated forms, respectively. Comparison of WIN and the WIN vinyl analog predict the neutral and protonated forms of the vinyl analog to bind 316 and 4.0 fold more tightly than the WIN compound, respectively. Thus, based solely on solvation effects it is predicted that the WIN vinyl analog will have the greatest binding affinity followed by WIN and cocaine. Experimental data on partition coefficients of the studied compounds would aid in the verification of the predicted results.

IV. Conclusion

Conformational properties were calculated for both the neutral and protonated species of cocaine, WIN, and the WIN vinyl analog. Energy surfaces were obtained as a function of the dihedrals dih_1 and dih_2 in both the gas phase and in aqueous solution (Figures 2 and 6). The overall topologies of the two-dimensional surfaces were similar in both phases for the compounds studied. However, differences associated with structure, protonation, and aqueous solvation of the compounds are evident.

Comparison of the neutral and protonated species predicts a change in the location of energy minima of the 2β substituent. The largest difference calculated is −77° in cocaine (Figure 3, Table 1). The minima for the protonated species are generally deeper and narrower and those for the WIN compounds are narrower than those of cocaine. Interestingly, for the WIN vinyl analog, the energy barrier to rotation of dih_1 is larger and minimum energy well is narrower for the neutral compound as compared to the protonated compound (Figures 5 and 7). This is due to an interaction between the tropane nitrogen or hydrogen and the vinyl double bond. With respect to the rotation of dih_1, the relative conformational flexibility decreases from cocaine to WIN to the WIN vinyl compound for the neutral species and for the protonated species it decreases from cocaine to the WIN vinyl analog to WIN.

Analysis of the conformation of the 3β substituent via the dih_2 surfaces (Figures 5 and 8) show the location of the energy minima to be similar for all three molecules and protonation states and the presence of a relatively large barrier to rotation in cocaine. The extended 3β chain is responsible for the minimum in the cocaine dih_2 surface being broader than that of the WIN compounds. Thus, it is predicted that removal of the 3β ester group in the WIN compounds leads to a decrease in the conformational flexibility of the molecules, while having only a limited influence on the low-energy conformations as compared to cocaine.

The conformational flexibility of cocaine, WIN, and the WIN vinyl analog was quantitated by calculating the probability distributions based on a Boltzmann distribution. Results show only limited regions of the surfaces to be accessible at room temperature (Figures 9 and 10). The lower right quadrant of the two-dimensional maps are highly populated for all the compounds. Inspection of the populations of the minimum energy regions reveals the WIN and the WIN vinyl analog minima to be populated to a greater extent than cocaine, with the differential being largest for the neutral species in aqueous solution (Table 4).

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analog are known to be similar.[9,10,29] In combination with the similarity of the minimum energy structures of the three molecules (Figures 3, 4 and Table 1) this suggests that the biological conformations are similar to the minimum energy conformations calculated in the present study. Additional support for this assertion is the agreement between the calculated energy minima of cocaine and experimental structures from X-ray crystallography (Table 1).

It is generally thought that the protonated species bind to the cocaine receptor because they are the dominant forms at physiological pH. Several lines of evidence, however, suggest that the neutral form may bind. The quaternary salt of cocaine is not active.[7a] The only well-correlated quantitative structure–activity study of WIN analogs was performed on the neutal species.[14b] Pseudocaine, where the protonated nitrogen is more accessible as compared to cocaine, has a lower binding affinity.[9b] Cloning and expression of cocaine-sensitive dopamine transporter complementary DNA revealed a 12 hydrophobic residue segment which was suggested to possibly be involved in the binding of drugs.[6c] The present work predicts the conformations of the protonated and neutral species of the studied compounds to be similar (Figure 3, 4 and Table 1). Thus, protonation of the tropane nitrogen is predicted not to be required for the studied molecules to assume the active conformation. In the case of cocaine, however, protonation is predicted to increase the population in the region of the assumed active conformation.

Calculation of the change in the pK_a of the tropane nitrogen in WIN and the WIN vinyl analog versus cocaine predicts both compounds to have higher pK_as (Table 5). Such changes would increase the population of the protonated species of the compounds at physiological pH. This difference will contribute to the binding of the WIN compounds assuming the protonated species to interact with the receptor. However, the possibility that changes in environment (e.g. upon moving from aqueous solution to a hydrophobic binding site) may significantly decrease the pK_a of the tropane nitrogen cannot be excluded. Previous theoretical studies on proteins have predicted changes in pK_as of 2–3 pH units associated with alterations in environment.[30] Such changes in pK_a may lead to deprotonation at physiological pH and interaction of the neutral species with the cocaine binding site. Deprotonation of the tropane nitrogen due to changes in the pK_a associated with environment may also be of significance for the movement of cocaine and its analogs across cell membranes.

Aqueous free energies of solvation of the drugs are expected to have a significant role in both receptor binding and bioavailability. A recent theoretical linear solvation energy relationship study supports this assertion.[31] Changes in aqueous solvation (Table 5) are predicted to increase the binding of the protonated forms of WIN and the WIN vinyl analog by 2.3 and 9.1 fold as compared to cocaine, respectively. From the magnitude of these ratios it is evident that solvation does indeed play a significant role in the relative binding of the drugs. Experiments show the binding of WIN to be approximately 5-fold greater than that of cocaine[9] with an additional increase in binding for the WIN vinyl analog based on the relative binding of the WIN and WIN vinyl analogs 3β *p*-chloro analogs.[10] Assuming the protonated forms of the molecules to bind to the receptor, aqueous solvation is predicted to make a 2-fold contribution to the 5-fold increase in binding observed experimentally with WIN. Upon going from WIN to the WIN vinyl analog, the 4-fold predicted aqueous solvation differences is larger than the increase in binding observed experimentally. The larger magnitude of the aqueous solvation contribution as compared to the overall change in binding suggests the possibility that the presence of the vinyl moiety at the 2β position leads to direct interactions with the receptor being less favorable for the vinyl group than those occurring with the methyl ester. It should be emphasized, however, that the present study does not directly address the contribution of structural alterations with respect to interactions between the three molecules and the cocaine receptor.

If the neutral species are involved in the interaction of the drugs with the receptor, the calculated changes in binding due to aqueous solvation of 167 and 5263 fold for WIN and the WIN vinyl analog are significantly larger than the experimentally determined increases. The large contribution of aqueous solvation to binding upon going from neutral cocaine to the neutral WIN compounds lends support to the possibility that the neutral species of the compounds interact with the receptor. These results emphasize the importance of including solvation effects into account when analyzing structure–activity relationships.

The differential in dopamine transporter binding of WIN and the WIN vinyl analog with respect to cocaine may preliminary be modeled as follows: Assuming the protonated species to bind to the receptors, WIN and the WIN vinyl analog bind more tightly due to a combination of decreased conformational flexibility, decreased aqueous solvation, and increased pK_as. The decreased conformational flexibility will lead to a greater population of the active conformers in solution, although the differential between cocaine and the WIN compounds for the protonated species is relatively small. The decreased aqueous solvation of the WIN compounds favors binding due to a lower free energy requirement for desolvation of those molecules as compared to cocaine. Increased pK_as of the WIN compounds will increase the concentration of the protonated species at physiological pH, thereby favoring binding.

If the assumption is made that the neutral form of the compounds interact with the cocaine receptor, the contributions of the predicted changes between the molecules to binding is altered. The favorable contribution of the decreased conformational flexibility of the WIN compounds is increased due to the similar populations of the cis and trans conformers of neutral cocaine in solution. The favorable contribution of aqueous solvation to the binding of the WIN compounds is significantly larger and is predicted to dominate the relative binding of the compounds. Detrimental to binding of the neutral WIN compounds is the predicted increase in pK_as, making the deprotonation of the compounds more difficult if the neutral species does indeed interact with the receptor. In a neutral binding scenario the predominance of the protonated form of the drugs in solution, especially with cocaine, would be predicted to stabilize the compounds in their active conformations. Initial interactions between the drugs and the receptor may occur with the compounds still in their protonated forms. This initial interaction may then place the molecules in a more hydrophobic environment. Once in the hydrophobic site the energetics associated with protonation of the tropane ring nitrogen may be altered, such that the protonated state would be less favored leading to a decrease in the pK_a of the tropane nitrogen, deprotonation of the nitrogen, and binding of the neutral compounds to the receptor.

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Supplementary Material Available: Heats of formation and cartesian coordinates of all global energy minima are included (12 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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