Allowed Conformations for Protonated and Unprotonated Lidocaine: Three-dimensional Isopotential Energy Surfaces

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The conformational energies of unprotonated and protonated lidocaine were calculated using non-bonded, electrostatic, and torsional potential energy functions. Calculations were performed varying three central torsion angles in the molecules from 0 to 360° at 10° intervals. The results of the energy calculations were represented as three-dimensional isopotential energy surfaces from which the regions of low-energy conformations allowed for each molecule could be selected. Each of these calculations was repeated as a fourth dihedral angle was varied in increments of 20° between 0 and 360°. In this manner, a global minimum was found for unprotonated and for protonated lidocaine. A comparison of the three-dimensional isopotential energy surfaces for unprotonated lidocaine with that of protonated lidocaine shows a striking difference for the global minimum for the N–CO–CH_2–N dihedral angle. The angle is near 70° for unprotonated lidocaine and 220° for protonated lidocaine, resulting in a more extended structure for the protonated molecule. The three-dimensional isopotential surfaces also reveal that the conformational freedom allowed for lidocaine is greatly reduced when it is protonated. The difference in the electrostatic interaction between the two forms of lidocaine is responsible for the large differences in conformational preference. The three-dimensional isopotential energy surfaces suggest minimum-energy pathways by which the molecules may interconvert from one to another low-energy conformational region.

While the molecular mechanism for anaesthesia induced by local anaesthetics remains controversial, the primary site of action of anaesthetics is considered to be the cellular membrane.[1] Local anaesthetics inhibit the conduction of nerve impulses in the cellular membrane by preventing the passage of sodium ions in the membrane's ion channel. There is evidence that local anaesthetics may bind selectively to membranes. For example, stereoisomers of some local anaesthetics differ in their capability for frequency-dependent blocking of sodium channels in nerves, providing evidence for a specific drug–receptor interaction in the sodium ion channel.[2] On the premise that the molecular structure of a local anaesthetic is important to biological activity, we determined, *via* real-time computer graphics–energy calculations, several low-energy conformational regions allowed for lidocaine, both protonated and unprotonated. Lidocaine is a local anaesthetic of the aromatic tertiary amine type and is easily protonated at the amine nitrogen atom, (pK_a 7.7).[3] While both protonated and unprotonated forms of tertiary amine local anaesthetics are present at physiological pH, it is usually assumed that the protonated (charged) species is the biologically active one. We show in this work that the protonated and unprotonated forms of lidocaine have quite different preferred conformations. These different conformations may relate to different biological functions of the anaesthetic in the membrane. The fact that anaesthetic molecules are surrounded by solvent molecules in problems of physiological interest is taken into account in a qualitative manner in the calculations by using a distance-dependent dielectric constant.

Recently, one of us has shown, using a combination of conformational energy calculations and n.m.r. lanthanide induced shifts (L.I.S.s), that the allowed conformations for unprotonated lidocaine in solution[4] are quite different from both the structure of the protonated salt, lidocaine hydrohexafluoroarsenate,[5] and the unprotonated lidocaine crystal,[6] as determined by *X*-ray crystallography. The significant difference was in the value of the N–CO–CH_2–N dihedral angle (angle 3 in Figure 1). This angle is 192.0° (Table 1) in the protonated

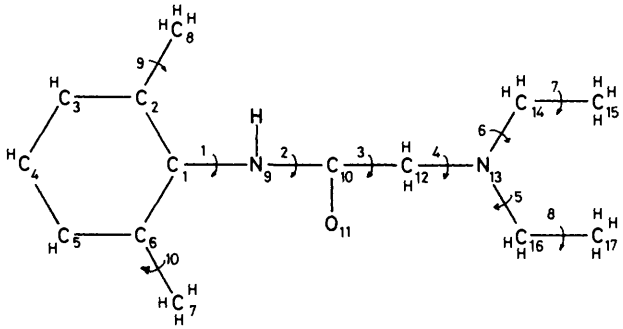


Figure 1. Numbering of the atoms and torsion angles for lidocaine

Table 1. Defining atoms for torsion angles and crystal-structure values for lidocaine hydrohexafluoridoarsenate

Angle	Defining atoms[a]	Crystal structure[b] (°)
1	C(2)–C(1)–N(9)–C(10)	289.9
2	C(1)–N(9)–C(10)–C(12)	186.7
3	N(9)–C(10)–C(12)–N(13)	192.0
4	C(10)–C(12)–N(13)–C(16)	86.6
5	C(12)–N(13)–C(16)–C(17)	180.9
6	C(12)–N(13)–C(14)–C(15)	292.6

[a] Torsion angle 4 is defined as C(10)–C(12)–N(13)–C(14) in ref. 4. To convert a value of angle 4 in this work into a value from ref. 4 one must add 120°. Also, torsion angles 5 and 6 are interchanged from the earlier work. [b] Torsion angles computed from ref. 5. Molecule is in the protonated form.

lidocaine salt,[5] near 0° in the unprotonated lidocaine crystal,[6] and ±60° in unprotonated lidocaine in solution.[4] While the *X*-ray structure represents one static conformation allowed for the molecule in the crystal, the potential energy calculations reported in this work allow the determination of the most energetically favourable conformations for the protonated and unprotonated forms of the lidocaine molecule. Low activation energy pathways through which the protonated to unprotonated interconversion may occur in the cell membrane may be followed along the isopotential energy surfaces.

In most graphical representations of molecular conformational energy calculations to date, only two torsional angles are varied, while the other torsional angles are held fixed, resulting in two-dimensional energy plots. This technique has been generalized to three dimensions by Pattabiraman and Langridge[7] who showed that conformational energy calculations could be carried out by varying three torsional angles and representing the energies as three-dimensional isopotential surfaces.[7] Real-time colour computer graphics are used to study such isopotential energy surfaces.

Calculations

Figure 1 shows the structure of the unprotonated lidocaine molecule. There are ten single-bond rotations for this molecule. The defining atoms for these rotations, as well as the values of these dihedral angles in the crystal lidocaine hydrohexafluoroarsenate as determined by *X*-ray diffraction,[5] are given in Table 1. Owing to the bulky methyl groups attached to the ring, the plane of the aromatic ring is perpendicular to the plane of the amide bond.[4] For this reason, torsion angle 1 was restricted to ±90°. Torsion angle 2 has been shown to be in the *trans* conformation[8] (the C=O bond is *trans* to the NH bond), thus torsion angle 2 was set at 180°. The torsion angles defining the orientation of methyl groups (*i.e.* angles 7—10) were held fixed for staggered conformations. Thus, the torsion angles 3—6 are the only variables which must be considered in determining the allowed conformations for either the protonated or unprotonated form of lidocaine. The starting co-ordinates were generated from the *X*-ray crystal structure of lidocaine hydrohexafluoroarsenate.[5]

Conformational energy calculations were carried out using non-bonded, electrostatic, and torsional potential functions[9] for the protonated and unprotonated lidocaine molecules. Only the amide proton was included explicitly in the calculations, while a united atom representation was followed for carbons with methyl, methylene, or aromatic hydrogens. The 6—12 potential function parameters in the united atom representation for the non-bonded atom pairs were taken from Weiner *et al.*[10] and the charges were obtained from CNDO/2 calculations.[11]

The question naturally arises as to whether the minimum-energy conformations generated here are the same as those which would occur in physiological media. The theoretical and computational difficulties in the calculation of solvation energies for solutes in polar solvents such as water have been discussed in detail by Warshel.[12] The effective dielectric constant for short-range interactions in water increases with the distance between ions.[12] We have therefore used a dielectric constant (unitless) of $\varepsilon = r_{ij}$ (in Å), where r_{ij} is the distance between non-bonded atoms.[11] The contribution of the resulting electrostatic energy to the total potential energy function is thus $V_{ij} = q_i q_j / r_{ij}\varepsilon$, where q_i is the partial charge of the solute atoms. This method of accounting for the effect of the solvation energy upon the electrostatic energy by using a distance-dependent dielectric constant has also been used by others[11,13,14] to take into account the effect of aqueous media upon biological molecules.

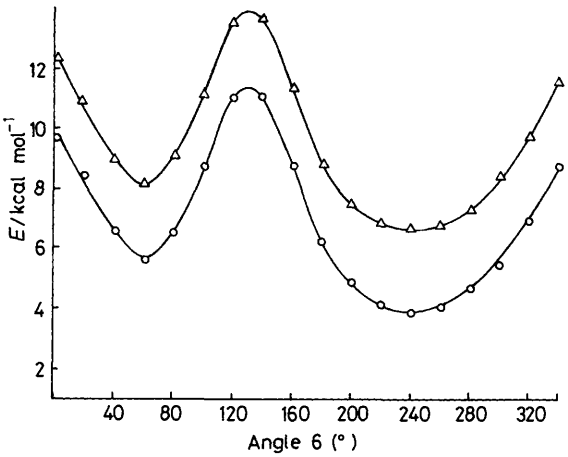


Figure 2. The global minimum energy (kcal mol^{-1}) of lidocaine *versus* torsion angle 6. The ○, unprotonated; △, protonated lidocaine

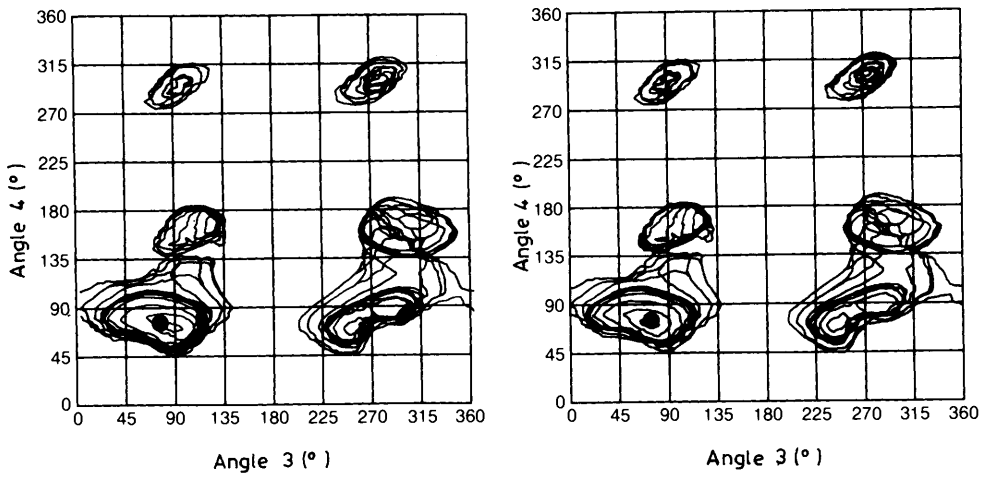
For a given value of the torsion angle 5, potential energies were calculated by varying the torsion angles 3 and 4 from 0 to 360° in intervals of 10°. These calculations were repeated for each value of the torsion angle 5 from 0 to 360° at 10° intervals. The planes formed by angles 3 and 4 were stacked for a given value of the torsion angle 5 such that the angle 3 axis is along the *x*-axis, the angle 4 axis is along the *y*-axis, and the angle 5 axis is along the *z*-axis in an orthogonal co-ordinate system. The minimum energy in each plane was found, as well as the global minimum in three dimensions. As described earlier,[7] isopotential energy contours at a given interval above the global minimum were drawn on each plane thereby producing three-dimensional isopotential energy surfaces. The isopotential surfaces thus obtained were displayed by computer programs MIDAS and BILD[15] on an Evans & Sutherland calligraphics Color Picture System 2.

The effect of torsion angle 6 on the conformations was investigated by generating the entire three-dimensional isopotential energy surface (of the type just described) for each value of angle 6 from 0 to 360° at 20° intervals.

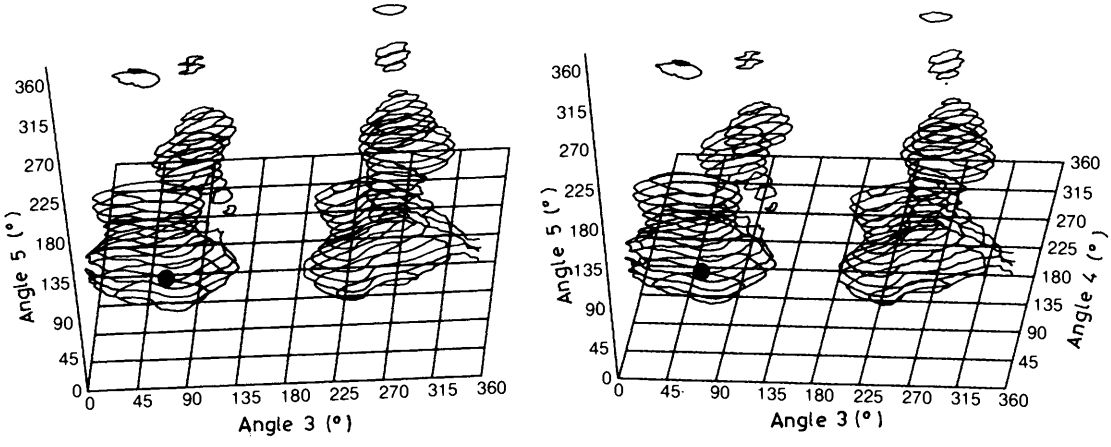
Results

The global minimum energy obtained for each of the three-dimensional isopotential surfaces *versus* angle 6 is shown in Figure 2. The lower line is for the unprotonated molecule and the upper line is for the protonated form. For both molecules, there are two minima for torsion angle 6, one at 60° and the other at 240°. Only the 240° minimum will be considered in this paper since it is a broader, lower energy minimum.

Figure 3(a) shows a stereoview of the isopotential energy surface for the unprotonated form looking down the angle 5 axis. In Figure 3(a) the closed circle is at the global minimum for this isopotential energy surface. The global minimum is at 70, 70, and 70° for torsion angles 3—5 respectively. All contour lines are drawn at 2 kcal mol^{-1} above the global minimum. Figure 3(b) shows another stereoview of the isopotential surfaces. These isopotential surfaces show the energetically favourable regions for the three torsional angles. Table 2 lists the ranges for the three torsion angles 3—5 for the six energetically favourable regions. The minimum-energy regions found here for unprotonated lidocaine agree with those found earlier for unprotonated lidocaine in CCl_4 solution by a combination of n.m.r. L.I.S. and conformational energy calculations.[4] From Table 2 and Figure 3(a), it is clear that there is a pseudo-two-fold symmetry about torsion angle 3 for the



(a)



(b)

Figure 3. Stereoviews (a) and (b) of the isopotential energy surfaces for the unprotonated form of lidocaine. All contour lines are drawn at 2 kcal mol^{-1} above the global minimum (represented by a closed circle)

Table 2. Ranges (°) torsion angles 3—5 for the energetically favourable regions of lidocaine[a]

Angle 3	Angle 4	Angle 5
Unprotonated form		
0—135[b]	45—135[b]	50—170[b]
200—360	45—170	50—170
60—135	135—185	100—150
260—330	135—190	100—170
50—120	270—315	50—110
230—295	270—315	50—110
Protonated form		
55—290[c]	45—140[c]	40—80[c]
140—260	45—110	90—160
90—165	140—180	90—160

[a] Angle 6 is 240° (see text and Figure 1). [b] The global minimum occurs in this region (Figure 3). [c] The global minimum occurs in this region (Figure 4).

unprotonated form. Another interesting result which these surfaces illustrate is that it is possible to follow the minimum-energy pathway by which the molecule may move from one local minimum to another. As an example, the minimum-energy pathway between the region in which the global minimum occurs for unprotonated lidocaine (Figure 3) and the region defined by the three torsion angles in row 2 of Table 2 is through a small barrier (< 2 kcal mol^{-1}) at angle 3 equal to 360° (Figure 3).

Figure 4(a) shows a stereoview of the isopotential energy surface of protonated lidocaine looking down the torsion angle 5 axis. In Figure 4(a) the closed circle is at the global minimum for this isopotential energy surface. The global minimum is at 220, 70, and 70° for torsion angles 3—5 respectively. All contour lines were drawn at 2 kcal mol^{-1} above the global minimum. Figure 4(b) shows another stereoview of the isopotential surfaces. The ranges for the three torsion angles 3—5 for the three energetically favourable regions of protonated lidocaine are given in Table 2. Figures 4(a) and (b) show that the three regions are interconnected.

A comparison of Figures 3 and 4 reveals that the flexibility of the protonated molecule is restricted as compared with that of the unprotonated molecule. Protonation of lidocaine removes the energy barrier (at a torsion angle 3 of *ca.* 180°) between the low-energy regions. Instead, a wide barrier now exists (at a torsion angle 3 of *ca.* 0 ± 60°) for the protonated form. The

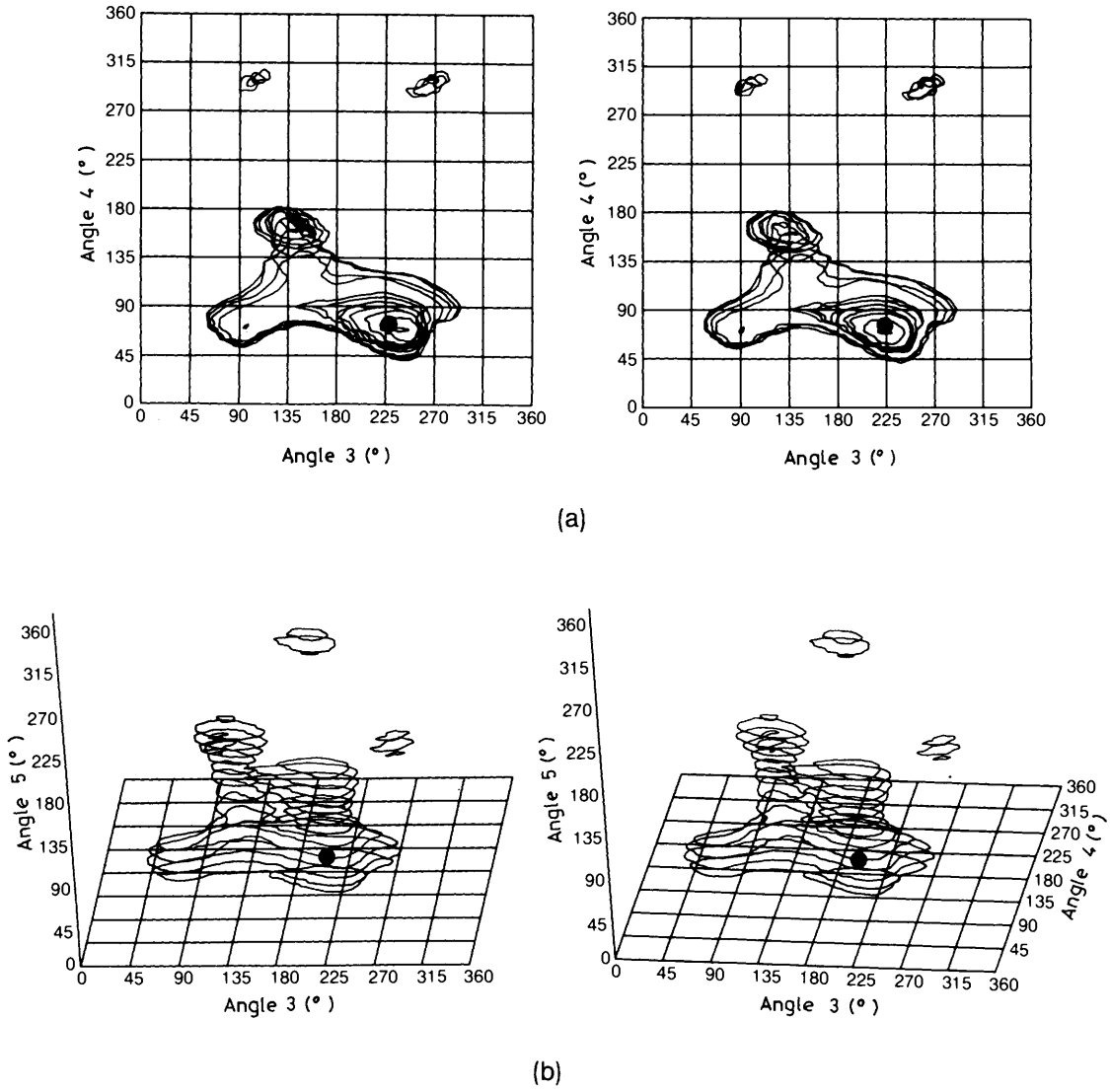


Figure 4. Stereoviews (a) and (b) of the isopotential energy surfaces for the protonated form of lidocaine. All contour lines are drawn at 2 kcal mol^{-1} above the global minimum (represented by a closed circle)

change in the global minimum for the unprotonated *versus* protonated form is due to electrostatic interaction. For the unprotonated molecule, there is a favourable electrostatic interaction between the amide hydrogen atom and N(13) as shown in Figure 5 (upper). [The amide proton–N(13) distance is 2.9 Å]. Protonation of the atom N(13) causes a favourable electrostatic interaction between the tertiary amine proton and the carbonyl oxygen atom as shown in Figure 5 (lower). [The amine proton–O(11) distance is 2.4 Å.] The co-ordinates of the atoms for Figure 5 were generated from the global minimum-energy conformations found in Figures 3 and 4. The minimum-energy conformation of the unprotonated and protonated forms is seen to be primarily determined by the value of torsion angle 3. The protonated form of lidocaine, which has a value of torsion angle 3 near 220°, is somewhat more extended in space (Figure 5) than the unprotonated lidocaine molecule with torsion angle 3 of *ca.* 70°. On comparing interatomic distances in unprotonated and protonated lidocaine, the following distances differ (CP = centre of phenyl ring): CP–N(13), N(13)–O(11), and N(13)–N(9). They are 5.7, 3.3, and 3.1 Å for the unprotonated and 6.0, 2.8, and 3.6 Å for the protonated molecules, respectively.

Discussion

Conformational energy calculations were carried out varying four torsion angles for the protonated and unprotonated form of the lidocaine molecule. Energetically favourable regions for the molecules were determined using three-dimensional isopotential energy surfaces. The addition of a proton causes large differences between the conformations allowed for protonated *versus* unprotonated lidocaine, due mainly to a reorientation around torsion angle 3 upon protonation caused by a change in electrostatic interactions.

The minimum-energy conformations which we have calculated here should quite adequately represent the conformations present in aqueous solution. When the lidocaine molecule is unprotonated, as it is expected to be if dissolved in the hydrophobic interior of a biological membrane, the conformation should resemble that of Figure 5 (upper), or one of the lower-energy conformations whose dihedral angles are given in Table 2. When the lidocaine molecule becomes protonated, as it is when present in an aqueous medium at physiological pH, the conformation changes considerably [Figure 5 (lower)]. The N–H • • • N bond of the unprotonated state is broken, the N–CO–CH_2–N dihedral angle changes from

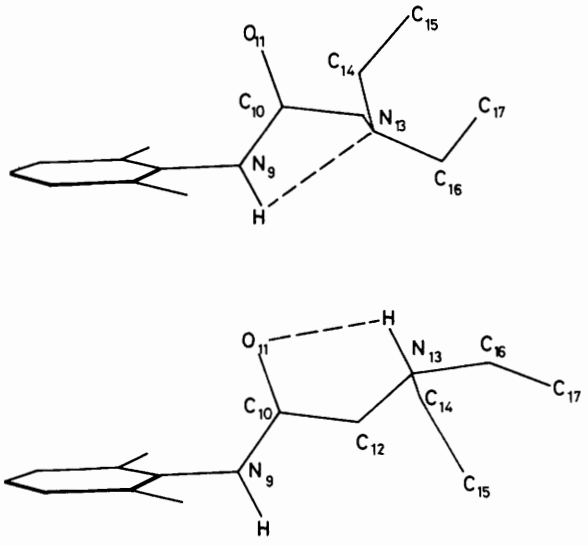


Figure 5. Stereoviews of the minimum-energy conformations for (upper) the unprotonated and (lower) the protonated form of lidocaine. The dashed line represents favourable electrostatic interactions

ca. 70° (range 0 ± 135°) to *ca.* 220° (range 55—290°) and a new electrostatic bond is able to form (Figure 5, lower). Thus the range of conformationally allowed N–CO–CH_2–N bond angles, theoretically determined in this work, includes the published experimental values mentioned earlier, *i.e.* ± 60° for unprotonated lidocaine in solution,[4] *ca.* 0° in the unprotonated lidocaine crystal,[6] and 192° for the protonated lidocaine salt.[5]

The low-energy conformations of each molecule, as presented in Table 2, may aid in the understanding of the biological activity of tertiary amine anaesthetics. They can also serve as starting conformations for future molecular dynamics calculations on these molecules. The use of three-dimensional isopotential energy surfaces combined with a computer graphics display as described in this work is a convenient tool for the conformational analysis of small drug molecules.

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