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A Quantum Chemical Study of Levcromakalim[a]

Gerhard Ecker[1], **Wilhelm Fleischhacker**[1], **Christian Wolf**[1], **Petra Weiß-Greiler**[2], and **Peter Wolschann**[2,*]

[1] Institut für Pharmazeutische Chemie, Universität Wien, A-1090 Wien, Austria
[2] Institut für Theoretische Chemie und Strahlenchemie, Universität Wien, A-1090 Wien, Austria

Summary. The conformations of the potassium channel opener levcromakalim are analyzed with the aid of quantum chemical calculations in order to determine the energetically most favourable structures. The influence of intramolecular hydrogen bonding on the conformations and on the rotational potential of the molecule is investigated. The structures of different conformationally restricted analogs are compared with the energetically accessible conformational space of levcromakalim with emphasis on similarities in molecular shape.

Keywords. Molecular calculations; *ab initio* Calculations; Conformational analysis; Levcromakalim; Potassium channel opener.

Quantenchemische Untersuchungen an Levcromakalim

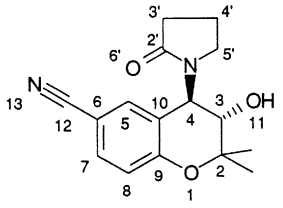
Zusammenfassung. Die Konformationen des Kaliumkanalöffners Levcromakalim werden mit Hilfe quantenchemischer Molekülrechnungen untersucht, um die energetisch günstigsten Strukturen zu bestimmen. Der Einfluß der intramolekularen Wasserstoffbrücke auf die Konformationen und die Dynamik des Moleküls wird analysiert. Die Geometrien verschiedener rigider Analoga werden mit den konformativen Möglichkeiten des Levcromakalims verglichen, um Ähnlichkeiten der Molekülgestalt festzustellen.

Introduction

Potassium channels regulated by intracellular *ATP* (K_{ATP} channels) have been associated with important cellular functions like vasodilation, hormone secretion, cardiac action potential duration, and neurotransmitter release. Thus, potassium channel openers have gained increasing actuality in various therapeutic applications like hypertension, bronchial disorders, or acute myocardial ischemia [1]. One of the lead compounds within this new class of drugs is the benzopyrane levcromakalim (**1**, (3*S*,4*R*)-3-hydroxy-2,2-dimethyl-4-(2-oxo-1-pyrrolidinyl)-6-chromancarbonitril, CAS 94535-50-9) [2]. Although often referred to as lemakalim, levcromakalim is the British approved name for (−)-cromakalim [3].

[a] Dedicated with our best wishes to Prof. Dr. *G. Heinisch* on the occasion of his 60th birthday
* Corresponding author

Structure-activity relationship studies of benzopyrane-type potassium channel openers have shown that the key functional groups necessary for high activity are an electron withdrawing substituent at C-6 and a hydrogen bond accepting functional group at C-4 [4].



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Moreover, the torsional angle between the benzopyrane ring and the cyclic substituent on C-4 seems to be of importance [5]. However, only limited and sometimes contradictory information is given in literature on the conformation of benzopyrane-type potassium channel openers. *Cassidy et al.* have reported [6] that levcromakalim adopts a rigid conformation in solution with the pyrrolidone ring orthogonal to the benzopyrane ring and the pyrrolidone carbonyl group on the same side of the benzopyrane ring as the hydrogen atom located at C-4. This conformation is similar to that observed in the crystalline state by means of an X-ray analysis [7] and to that suggested by *Gadwood et al.* [8] who have synthesized spirocyclic analogs. On the opposite, *Thomas et al.* have postulated two conformational minima with a barrier of rotation around the C-4 – N bond of 48–56 kJ/mol, indicating that rotation is still very fast at ambient temperatures [8]. These two minimum conformations have also been found in force field calculations [9] and by means of semiempirical AM1 investigations [4, 10, 11]. Thus, in continuation of our studies [12] on conformationally restricted analogs of levcromakalim, we used *ab initio* methods for its conformational analysis. In particular, the torsional potential of the C-4 – N bond was studied extensively together with the distortion of the six- and five-membered heterocyclic ring systems.

Results and Discussion

Geometry optimization of levcromakalim

The optimized geometries of levcromakalim as obtained at HF/3–21 G, HF/6–31 G**, and B3LYP/6–31 G** levels are given in Table 1 in comparison with the crystal structure data [4]. The sum of the absolute values of the differences between calculated and experimental bond lengths divided by the number of considered bonds is lowest for the HF/3–21 G basis set (0.012), whereas the HF/6–31 G** basis set results in a slightly higher value (0.013). The density functional method (B3LYP/6–31 G**) surprisingly leads to higher deviations (0.015). The differences for the bond angles (Table 1) are 1.24 (HF/3–21 G), 0.91 (HF/6–31 G**), and 1.06 (B3LYP/6–31 G**). The calculated bond lengths are on the average larger for all methods (the sum of the differences between calculated and experimental bond lengths divided by the number of shown bond lengths amounts to +0.081 for HF/3–21 G, +0.0187 for HF/6–31 G**, and +0.139 for B3LYP/6–31 G**). The

Table 1. Selected conformational parameters of levcromakalim estimated by *ab initio* methods and by X-ray analysis [4]; results of fully geometrical optimization are given for the HF/3–21 G, HF/6–31G**, and B3LYP/6–31 G** density functional levels; distances are given in Å, angles in degree

Basis set	HF/3–21 G	HF/6–31 G**	B3LYP/6–31 G**	Experimental
O1-C2	1.466	1.434	1.462	1.459
C2-C3	1.529	1.536	1.546	1.529
C3-C4	1.538	1.530	1.538	1.520
C4-C10	1.515	1.517	1.516	1.510
C10-C5	1.384	1.387	1.395	1.379
C5-C6	1.384	1.385	1.402	1.394
C6-C7	1.395	1.395	1.408	1.392
C7-C8	1.371	1.373	1.384	1.373
C8-C9	1.391	1.395	1.405	1.390
C9-C10	1.386	1.392	1.408	1.390
C6-C12	1.426	1.442	1.431	1.422
C12-N13	1.141	1.137	1.164	1.152
C3-O11	1.432	1.396	1.415	1.420
C4-N1′	1.470	1.454	1.466	1.452
N1′-C2′	1.356	1.357	1.371	1.340
N1′-C5′	1.475	1.459	1.467	1.460
C2′-O	1.225	1.204	1.230	1.239
C2′-C3′	1.520	1.513	1.523	1.496
C3′-C4′	1.542	1.531	1.538	1.499
C4′-C5′	1.550	1.534	1.543	1.518
O1-C9-C10	122.5	122.9	123.3	123.7
C9-C10-C4	120.4	119.9	119.9	119.4
C10-C4-C3	112.3	110.8	111.6	109.3
C4-C3-O11	111.5	111.8	112.0	110.8
C2-O1-C9	119.0	119.9	118.6	120.7
C10-C4-N1′	111.2	112.9	112.8	112.5
C4-N1′-C2′	120.3	120.0	119.8	122.7
N1′-C2′-O	124.1	125.0	124.7	124.4
N1′-C2′-C3′	108.6	108.4	108.2	108.9
C4′-C3′-C2′	103.9	104.1	104.3	104.3
O1-C9-C10-C5	178.3	179.4	179.0	182.5
C2-O1-C9-C10	338.9	341.0	341.8	353.7
C4-C10-C5-C6	180.0	179.4	180.4	180.0
C3-C4-C10-C5	171.4	166.7	167.2	154.0
O-C3-C4-C10	158.8	168.7	167.0	172.1
N1′-C4-C10-C5	47.2	40.7	40.6	29.8
C2′-N1′-C4-C10	206.4	213.7	210.2	231.2
O6′-C2′-N1′-C4	−0.04	5.7	3.7	9.8
C3′-C2′-N1′-C4	183.0	187.5	186.1	191.0
C4′-C3′-C2′-N1′	349.7	350.2	351.0	344.7

corresponding data for the angle differences are −0.306 for HF/3–21 G, −0.112 for HF/6–31 G**, and −0.159 for B3LYP/6–31 G**. The calculated bond angles are therefore generally smaller than the values determined by the X-ray investigation. Nevertheless, the deviations between calculated and experimental values are small

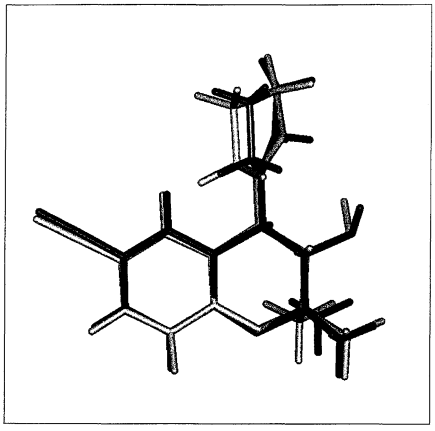


Fig. 1. Superposition of the global minimum of levcromakalim as obtained by *ab initio* calculations (HF/3–21 G basis set, grey structure) and the geometry found by X-ray analysis [4] (dark structure)

for all methods, and the results given above should not be overemphasized, especially because the error limits of the X-ray analysis are not known accurately enough.

The dihedral angles are much more sensitive to the environment, and so the differences between molecular calculations, which consider gas phase geometries, and X-ray analysis, where the influence of intermolecular interactions are rather strong, are quite large. The rotation of the pyrrolidone ring is monitored by the dihedral angle φ_1 (C-2′ – N-1′ – C-4 – C-10). Different values are obtained with the various *ab initio* methods. Deviations of 7.5 degrees indicate that the torsional potential is rather soft in the neighbourhood of the minimum, but the fact that the experimental value is 20 degrees higher than the calculated ones shows that the conformation of the compound in the crystal is different from the geometries as obtained by the calculations. Figure 1 gives a superposition of the calculated geometry and the X-ray structure [4].

The experimentally determined angles which characterize the distortion in the five- and the six-membered heterocyclic rings are also different from the calculated ones. The origin of these discrepancies may be due to the slightly different conformational behaviour with respect to the dihedral angle φ_1. In addition, there exists an *intramolecular* hydrogen bond in the conformation found as the global minimum by the calculations, whereas only *intermolecular* hydrogen bonds are observed in the crystal. The latter aspect will be considered in more detail in the following.

Conformational analysis of levcromakalim

Levcromakalim possesses one rotatable single bond: the connection between the ring system (C-4) and the pyrrolidone ring. Additionally, the saturated six-membered ring as well as the pyrrolidone ring may exist in different conformations. First, the torsional potential of the C-4 – N single bond of levcromakalim was calculated choosing the dihedral angle φ_1 (C-2′ – N-1′ – C-4 – C-10) as parameter. The results are shown in Fig. 2.

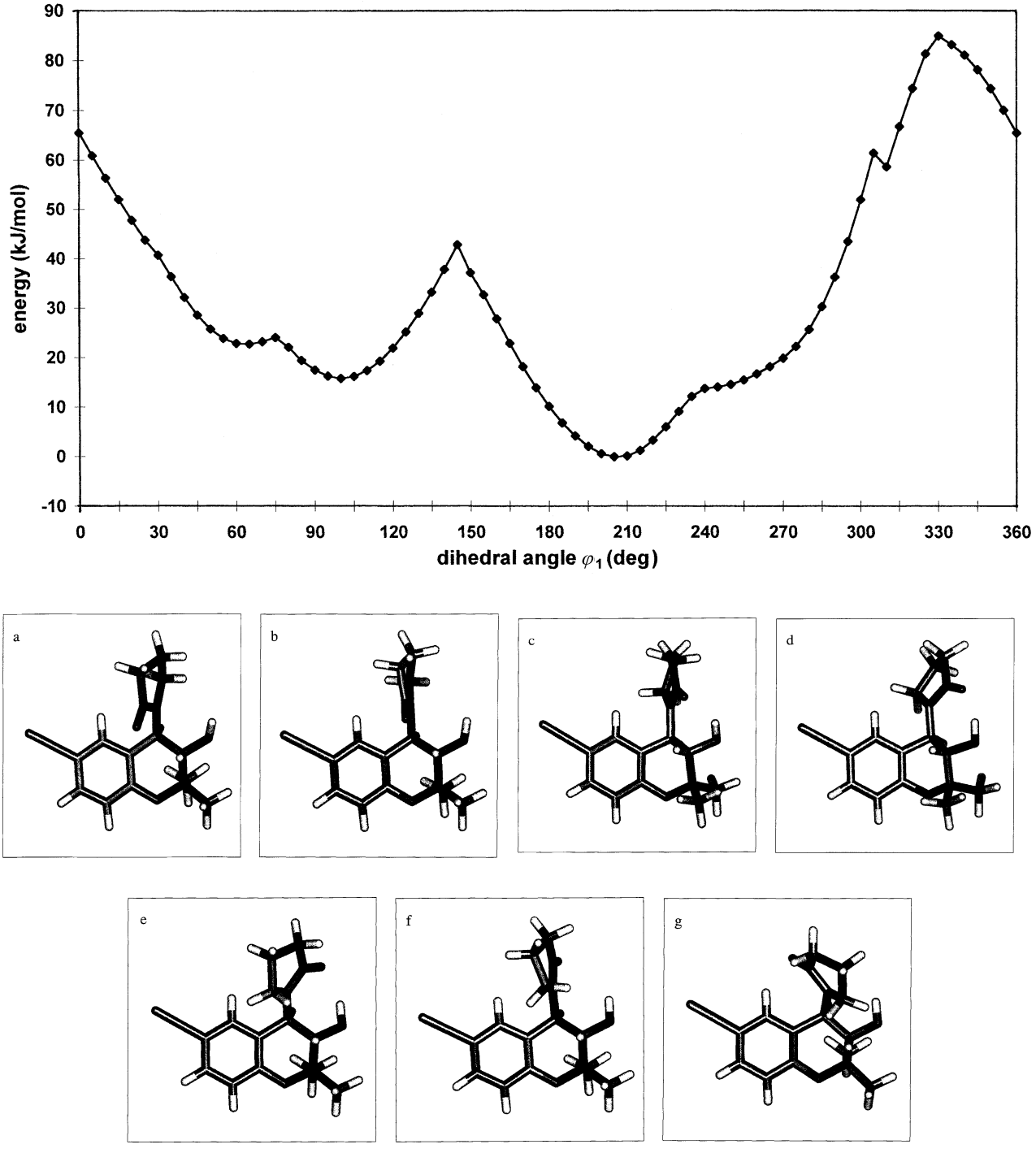


Fig. 2. Torsional potential of the dihedral angle φ_1; selected conformations along the potential curve are shown; **a**: 35°, **b**: 60°, **c**: 101°, **d**: 150°, **e**: 206°, **f**: 245°, **g**: 310°

Three conformational minima are observed, with the absolute energy minimum corresponding to a dihedral angle of $\varphi_1 = 206$ degrees. The related conformation is shown in Fig. 2 as conformation **e**. The pyrrolidone ring adopts a perpendicular position with respect to the ring system. Steric interactions between the methylene group of the five-membered ring and the other part of the molecule are small. A hydrogen bond between the hydroxyl group and the carbonyl group is formed. A second pronounced local minimum appears at $\varphi_1 = 100$ degrees, 15 kJ/mol higher than the absolute one (Fig. 2, conformation **c**). The hydrogen bond still exists (the distance between the oxygen atoms within the hydrogen bond is 2.45 Å), but the position of the pyrrolidone ring is different, with the carbonyl group located on the other side of the plane of the aromatic ring. Minimized steric energy as well as the hydrogen bond are responsible for the geometry of the molecule. The third minimum along the torsional potential close to the second minimum can be observed at a dihedral angle of $\varphi = 62$ degrees. In this conformation the hydrogen bond is broken (conformation **b**, Fig. 2), and the pyrrolidone ring is perpendicular to the plane of the aromatic ring. A shoulder near the minimum structure to higher values of the dihedral angle also corresponds to a structure with a broken hydrogen bond (conformation **f**, Fig. 2). Energetically unfavourable geometries along the torsional potential are given by conformations **a**, **d**, and **g**. Especially conformation **d** shows a transition state geometry, still with a hydrogen bond but with enhanced steric interactions.

Hydrogen bonding along the torsional potential is monitored by the oxygen-oxygen distance. The dependence of this parameter on the dihedral angle is given in Fig. 3 (empty circles indicate the oxygen-oxygen distances, empty triangles show the oxygen-hydrogen distance of the hydrogen bond).

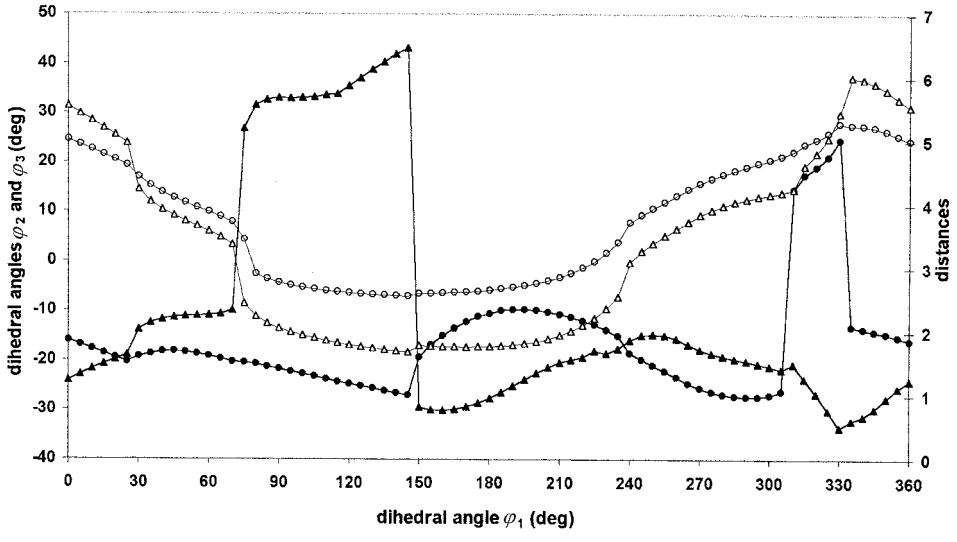


Fig. 3. Dihedral angles φ_2 (filled triangles) and φ_3 (filled circles) in dependence of the dihedral angle φ_1; O–O distance (empty circles) and O-HO distance (empty triangles) depending on the dihedral angle φ_1

The hydrogen bond exists for a rather large range of φ_1; its disruption caused by steric reasons leads to changes of the dihedral angles in the five- and six-membered rings.

The rotation around the C-4-N bond is strongly related to hydrogen bonding to the oxygen of the six-membered ring as well as to the motions of the six-membered ring and of the pyrrolidone ring. In Fig. 3, the distortion of both the six-membered and the five-membered ring is given. The conformational changes within the six-membered ring are monitored by the dihedral angle φ_2 (C-2–O-1–C-9–C-10; filled triangles in Fig. 3), the flipping of the pyrrolidone ring is characterized by the dihedral angle φ_3 (C-3′–C-2′–N-1′–C-4; solid circles in Fig. 3). There are some positions in the energy dependence of the dihedral angle where changes of the slope and edges can be seen. At $\varphi_1 = 75$ degrees, the transition state between two local minima corresponds to the formation of the hydrogen bond and a synchronous flipping of the six-membered ring. A comparable flipping occurs at $\varphi_1 = 145$ degrees, the transition state between the two lowest conformational minima.

For a better understanding of these coupled conformational changes, the energy hypersurface given by the energy dependence of the dihedral angle φ_1 and the angle φ_2 inside the six-membered ring was calculated for both regions around the energy minima; the results are presented in Fig. 4.

In the first picture, the calculated energies are plotted with φ_1 (range from $\varphi_1 = 30$ degrees to $\varphi_1 = 140$ degrees) and φ_2 (-40 to 70 degrees) as variables. The two energy minima can be observed, but not for the same conformation of the six-membered ring. Synchronous change of φ_2 with φ_1 has to take place at the saddle point of the energy minimum reaction path.

The region of the second (the absolute) energy minimum geometry is shown in the second graph. The calculated energy is again plotted against φ_1 and φ_2 (φ_1 between 170 and 280 degrees, φ_2 between -50 and 20 degrees). The energy potentials are rather soft; the molecule is therefore flexible within some limits.

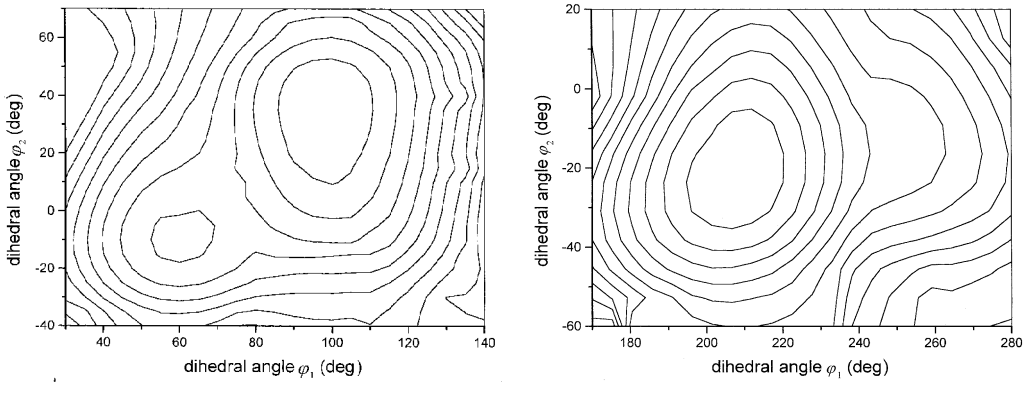
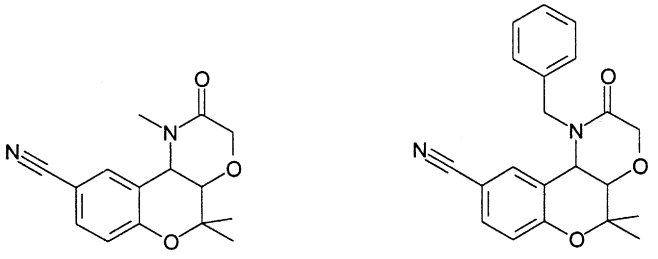


Fig. 4. Energy hypersurfaces; contour lines of the energy dependence on the dihedral angles φ_1 and φ_2; lowest contour line at an energy of 1 kJ/mol; step width of the energy: 1 kJ/mol

Comparison of synthetic compounds with different conformations of levcromakalim

Structure-activity relationship investigations on benzopyrane derivatives showed that the position of the amide carbonyl group of the substituent in position 4 relative to the benzopyrane ring system seems to be of great importance for the biological activity of this group of compounds [5, 15]. Therefore, a series of conformationally restricted 1-benzopyrano[3,4-*b*][1,4]oxazines have been synthesized.



2 **3**

Two of these compounds (**2** and **3**) were used for a molecular comparison with different conformations of levcromakalim. In comparison to levcromakalim, both compounds show decreased vasorelaxant activity by a factor of two [13]. The geometries of these substances were minimized by *ab initio* calculations (HF/3–21 G) and compared with different conformations of levcromakalim. In Fig. 5 the minimized structures of **2** (Fig. 5a) and **3** (Fig. 5b) are superimposed with the best fitting conformations of levcromakalim.

Compound **2** fits best with the conformation of levcromakalim with an angle of $\varphi_1 = 185°$, compound **3** shows best fitting with the conformation with a dihedral angle of $\varphi_1 = 130°$. Both compounds show largest similarity with structures far from the global minimum geometry of levcromakalim, which might explain

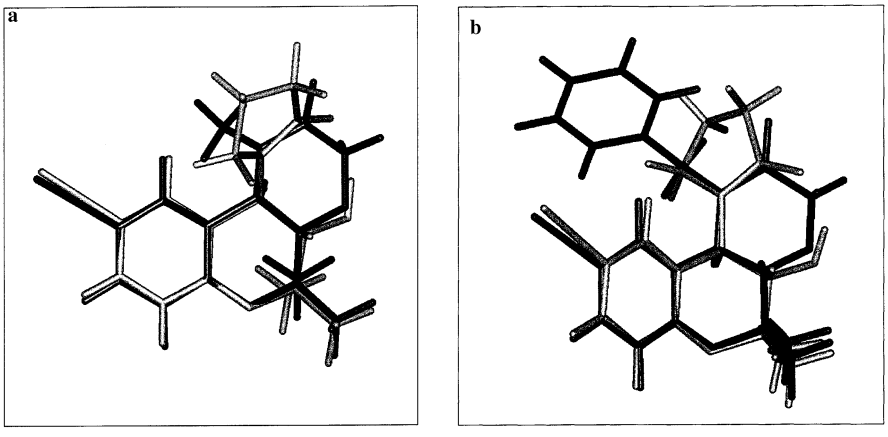


Fig. 5. Superposition of the global minimum geometry of **2** with the conformation of levcromakalim with a dihedral angle of 190° (Picture a) and superposition of the global minimum geometry of **3** with the conformation of levcromakalim with a dihedral angle of 130° (Picture b); the following atoms of levcromakalim are used for the fitting procedure: C1, C7, C8, C9, C10, N1′, C2′

their reduced biological activity. It also seems to be evident that the molecular shape has some range of possible conformations which may associate with the receptor site.

Conclusions

The conformation of the potassium channel opener levcromakalim is mainly influenced by the rotation of the connected pyrrolidone ring. The rotation around the carbon-nitrogen bond (C-4–N1′) shows at least three conformational minima and a shoulder which corresponds to a structure close to the global energy minimum. The energy difference between both pronounced minima amounts to 15 kJ/mol, the energy barrier between both minima was found to be 43 kJ/mol. The rotation around the carbon-nitrogen bond is strongly coupled with the conformational distortions within the five- and six-membered heterocyclic rings. An intramolecular hydrogen bond was found to determine the calculated structure. The studied compound can be therefore assumed to exist predominantly in one conformation, with a dihedral angle between the pyrrolidone ring and the benzopyrane ring system of about 210 degrees, which is rather close to the experimentally found value of about 231 degrees. In the solid state, only intermolecular hydrogen bonding could be detected [4]. From the energy barriers of the rotation it can be concluded that the geometry of levcromakalim is sufficiently flexible, so that conformational changes to fit a receptor site are possible without drastic changes of the internal energy of the ligand. Comparisons with synthetic molecules of similar but more restricted geometry show that the biological activity of these substances seems to be connected with a proper position of the pyrrolidone ring. For a more detailed answer on which conformation is responsible for the biological activity, more molecules with slightly different molecular shapes in the corresponding part of the molecule have to be synthesized.

Materials and Methods

Ab initio molecular orbital calculations on levcromakalim were carried out with the GAUSSIAN 94 series of programs [14]. Initial coordinates were obtained from the molecule building program ALCHEMY [15] and converted to the GAUSSIAN input by the program BABEL [16]. Geometry optimization was performed at the *Hartree-Fock* level using the 3–21 G basis set. This basis set is known to give proper results for intramolecular hydrogen bonding and steric interactions. Torsional potential curves were calculated using the SCAN option of GAUSSIAN with restricted geometry optimization. The 3D energy surfaces were computed at a grid of 22×22 points with 5 degrees intervals. A larger basis set (HF/6–31 G**) and a density functional method (B3LYP/6–31 G**) were used for the geometry optimization of the conformational minimum of levcromakalim only.

Acknowledgements

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