

Molecular Mechanics and SCF MO Conformational Analysis of Indol-3-ylacetic Acid Phytohormone (Auxin)

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Conformational analysis of indol-3-ylacetic acid (IAA)—plant growth hormone has been performed using molecular mechanics and the *ab initio* SCF MO theory. The equilibrium geometry of IAA has been determined. Relative energies of alternative conformations, their charge distribution, dipole moment and energy barriers between them have been calculated.

The position of the carboxyl group relative to the indole ring depends on two torsion angles, T1(C2-C3-C8-C9) and T2(C3-C8-C9=O2).

Rotational barriers for these two angles were explored and it emerged that both rotations (about the C3-C8 and C8-C9 bonds) can be accomplished in a reasonable time period at room temperature (the barrier height is about 4.6–10.9 (T1) and 1.7–3.8 (T2) kJ/mol respectively, according to *ab initio* calculations).

Ab initio (GAMESS) and molecular mechanics (DISCOVER (CVFF and cff91), SYBYL (TRIPOS) and MM2(87), calculations revealed qualitatively the same shape of potential energy surface ($E = f(T1, T2)$). However, energy differences between various conformations depend on the basis set (*ab initio* calculations) and force field (molecular mechanics) used.

INTRODUCTION

Indol-3-ylacetic acid (IAA, Figure 1) is a plant growth hormone that regulates physiological functions, such as cell divisions and enlargement, developmental differentiation, and the syntheses of specific proteins.¹ Its crystal structure was determined in 1964² and refined in 1982.³ However, hypotheses on the mechanism of biological activity have been based on the chemical models,^{4,5} solely. Because of biological

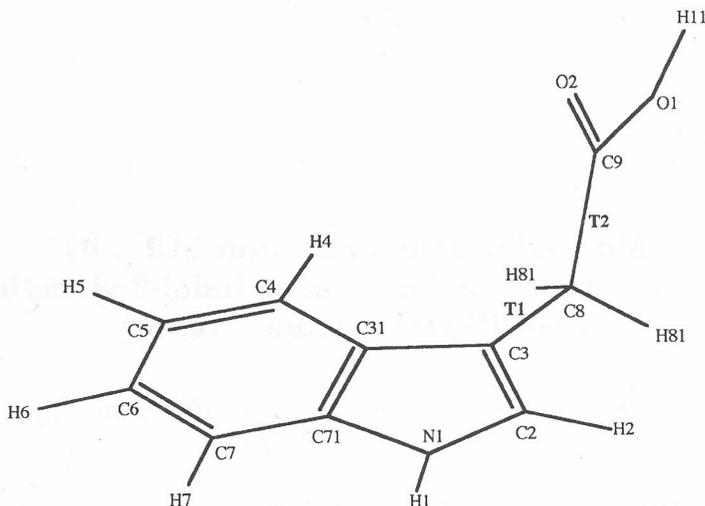


Figure 1. Schematic diagram of indol-3-ylacetic acid (IAA) with atom numbering and torsion angle labels.

relevance, a large number of its conjugates and derivatives has been chemically characterized and the crystal structures of some of them were determined.^{6,7} As a part of this investigation, a conformational search has been performed for IAA using the molecular mechanics programs MM2(87),^{8,9} DISCOVER¹⁰ and SYBYL.¹¹ To assess the quality of these results and to obtain information on the charge distribution of IAA, SCF MO calculations (GAMESS^{12,13}) have been performed.

MOLECULAR MECHANICS RESULTS

Starting from the structure of IAA, determined by X-ray diffraction, energy minimizations were carried out using the programs DISCOVER, MM2(87) and SYBYL. By DISCOVER, calculations were performed with the CVFF^{14,15} and cff91¹⁶ force fields. In the MM2(87) force field, new torsion parameters for N (pyrrol type) were used.¹⁷ By SYBYL, calculations were performed with TRIPOS¹⁸ force field using the atomic charges calculated by the method of Pullman and Berthod.¹⁹ For the full geometry optimizations, the following methods were used: steepest descent, conjugate gradients, Newton-Raphson and quasi-Newton Raphson (BFGS).^{20,21} In geometry optimized conformations, the bond C8-C9 (see Figure 1) remained perpendicular to the indole ring plane, as it was in the crystal structure, but the COOH group changed its orientation and rotation of 90° about the C8-C9 bond occurred (T2 changed from -9.2° to ≈ -90°) (Table I). The COOH plane became almost perpendicular to the C3-C8 bond; (-) periplanar conformation about the C8-C9 bond found in the crystal lattice changed to (-) anticlinal for the molecule in *vacuo*.

To investigate the COOH group ability to change its orientation, rotations about the C8-C9 bond (Figure 1) were performed. Rotations were also accomplished about the bond C3-C8, to see whether the conformation with the side chain coplanar to the plane of the indole ring (T1 = C2-C3-C8-C9 ≈ 0°) represents a local minimum

TABLE I

Comparison of some bond lengths (Å) and torsion angles (°) about two relevant bonds obtained by X-ray structure analysis, molecular mechanics and *ab initio* calculations

BOND	X-RAY	MM287	CVFF	eff91	TRIPOS	GAMESS 9STO-3G)
C2-C3	1.36	1.37	1.42	1.38	1.34	1.35
C31-C4	1.40	1.42	1.43	1.41	1.51	1.41
C4-C5	1.38	1.38	1.39	1.39	1.41	1.37
C5-C6	1.39	1.42	1.41	1.42	1.49	1.41
C6-C7	1.37	1.38	1.39	1.39	1.41	1.37
C7-C71	1.39	1.42	1.42	1.40	1.51	1.40
C71-C31	1.39	1.40	1.42	1.41	1.33	1.40
C71-N1	1.38	1.38	1.38	1.39	1.35	1.40
N1-N2	1.36	1.38	1.41	1.38	1.35	1.40
C3-C8	1.48	1.50	1.56	1.49	1.51	1.52
C8-C9	1.51	1.52	1.52	1.50	1.51	1.54
TORSION ANGLE						
C2-C3-C8-C9	95.5	92.0	96.3	91.0	101.8	108.4
C3-C8-C9=O2	-9.2	-108.6	-94.8	-91.2	-88.5	109.7

for IAA. Such conformation was found in the crystal structures of some *n*-alkylindole acetic acids.⁷

By MM2(87), rotations about the C3-C8 bond were performed in steps of 15°, from 0° to 360°, followed by energy minimization with respect to all the other internal coordinates.

During these reoptimizations, T2 always remained in its local minimum (*i.e.* T2 $\approx \pm 90^\circ$). The results show that, in *vacuo*, the conformation with an approximately coplanar side chain (T1 $\approx 0^\circ$) is a point of inflection with a conformational energy of 2.9 kJ/mol (MM2(87)) above that of the minimum, *e.g.* the conformation with a

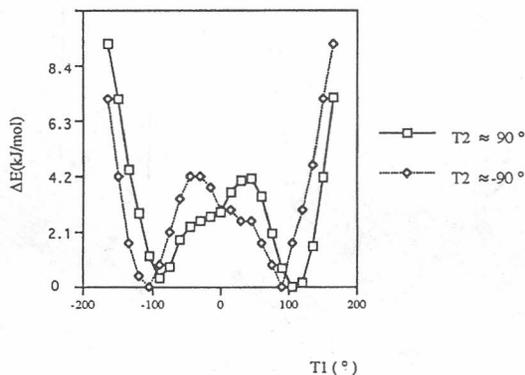


Figure 2. The relative conformational energy as a function of the torsion angle T1 for T2 $\approx \pm 90^\circ$; values calculated by MM2(87).

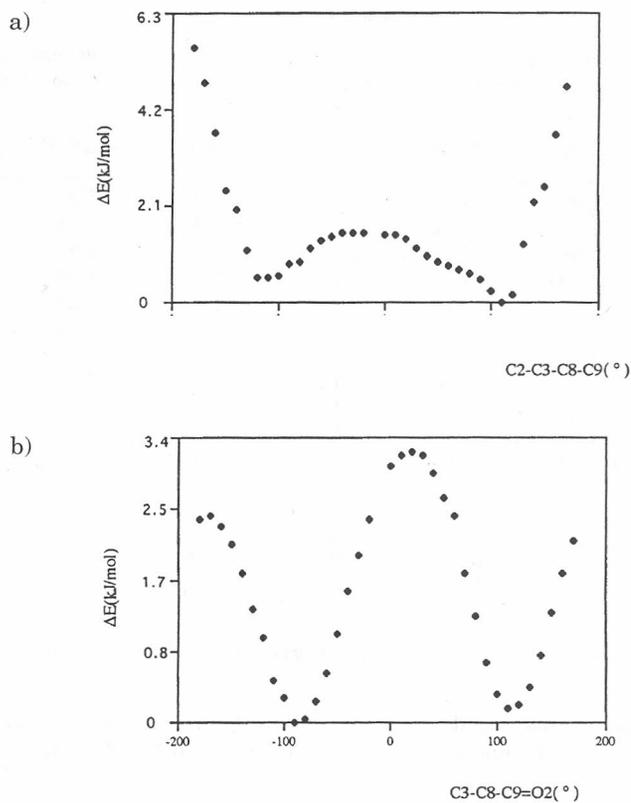


Figure 3. The conformational energy as a function of torsions: a) T1 ($T2 \approx -90^\circ$) and b) T2 ($T1 \approx 90^\circ$) calculated by SYBYL (TRIPOS force field).

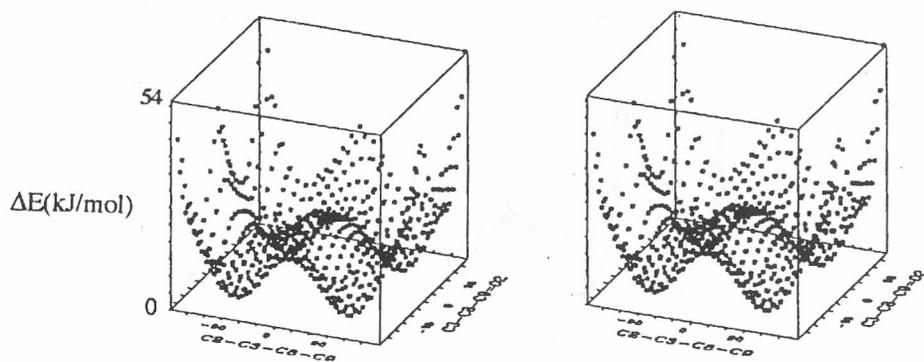


Figure 4. The conformational energy as a function of both relevant torsions T1 and T2 values determined in CVFF (DISCOVER).

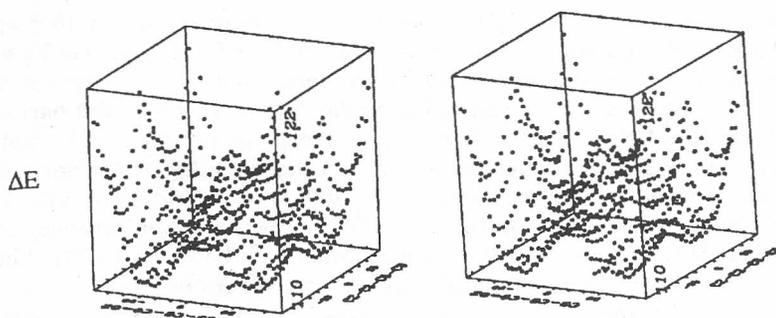


Figure 5. The conformational energy as a function of both relevant torsions T1 and T2 values determined in cff91 (DISCOVER). Energy is in kcal/mol (1kcal/mol = 4.1868 kJ/mol).

TABLE II

Ab initio and empirical energies and dipole moments of a few optimized conformations

	1*	2*	3	4	5	6	7	8
T1(C2-C3-C8-C9) (°)	108.4	106.9	180.0 ^a	0.0	-15.0	104.9	106.2	0.0
T2(C3-C8-C9=O2) (°)	109.7	-90.7	-99.7	-111.7	-111.7	0.0	180.0	180.0
Energy(STO-3G) (kJ/mol)	0.0	0.8	15.9	4.6	4.2	1.7	3.8	15.1
Energy(4-31G) (kJ/mol)	0.0	1.3	-	9.6	7.5	3.4	-	-
Energy(6-31G ^s) (kJ/mol)	0.0	1.7	-	10.9	-	3.8	-	-
Dipole Moment(D) (STO-3G)	2.6	1.7	2.4	1.8	2.0	1.9	-	2.2
Dipole Moment(D) (4-31G)	3.5	2.2	-	2.3	-	2.4	-	-
Dipole Moment(D) (6-31G ^s)	3.6	2.2	-	2.5	-	2.4	-	-
Energy(kJ/mol) (DISCOVER-CVFF) ^b	0.4	0.0	13.0	10.0	9.6	8.4	11.7	23.4
Energy(kJ/mol) (DISCOVER-cff91) ^b	0.8	0.0	5.0	4.6	5.0	21.8	28.1	44.8
Dipole Moment(D) (CVFF-DISCOVER)	1.4	1.6	1.5	1.5	1.5	1.8	1.3	1.2
Dipole Moment(D) (cff91-DISCOVER)	3.3	1.8	2.6	2.6	2.8	1.2	3.2	3.4

1* Optimized X-ray structure.

2* In the conformation 1*, the COOH group was rotated by 180°; modified conformer was optimized.

^a One of torsion angles was kept fixed during minimization; its value is given in bold.

^b Energy of the nearest conformation obtained by simultaneous rotations about the bonds C3-C8 and C8-C9.

perpendicular side chain, $T1 \approx \pm 100^\circ$ (see Figure 2). Barriers are at 180° approximately 9.2 kJ/mol high and at -45° approximately 4.2 kJ/mol high (for $T2 \approx -90^\circ$). Similar rotations (with the step of 10°) were performed by the program SYBYL (TRIPOS) and the results are given in Figure 3a). There is a broad flat barrier from $T1 \approx -50^\circ$ to $T1 \approx 10^\circ$, 1.7 kJ/mol high, and a sharp one at 180° , 5.4 kJ/mol above the minima ($T1 \approx \pm 100^\circ$). Rotations around the bond C8-C9, (see Figure 3b), revealed minima about $\approx \pm 110^\circ$ and barriers at -160° , and 15° (for $T1 \approx 100^\circ$), 2.5 and 3.3 kJ/mol high, respectively. By the DISCOVER program, simultaneous rotations about the bonds C3-C8 and C8-C9 were performed with CVFF and cff91 (Figures 4 and 5). In both force fields, four local minima were determined with (\pm) anticlinal conformation about these bonds (conformations $(100^\circ, 100^\circ)$ and $(-100^\circ, -100^\circ)$ are mirror images of each other, and likewise for $(100^\circ, -100^\circ)$, and $(-100^\circ, 100^\circ)$). From Figure 4 it is obvious that for any $T1$ value there are two preferred orientations of the carboxyl group, with $T2 \approx \pm 100^\circ$. The energy barrier for rotation about the bond C8-C9 ($T2 \approx 0^\circ$) is about 8 kJ/mol for $T1 \approx 90^\circ$, and about 21 (25) kJ/mol for $T1 \approx 0^\circ$ (180°), in CVFF; in cff91 they are about 21 and 29 (46) kJ/mol, respectively (Figures 4 and 5 and Table II). For the rotation about the bond C3-C8, the barriers are at 0° , and 180° , 10.0 and 13.0 kJ/mol high, respectively (CVFF). In cff91, barriers of approximately equal height (4.6 kJ/mol) are at -160° , and 30° (for $T2 \approx 90^\circ$).

In CVFF, the barrier for rotation around the bond C3-C8 is at 0° , as determined by *ab initio* calculations (see *Ab initio* RESULTS); in all the other force fields, it is shifted about 30° from zero. The results obtained by various force fields and *ab initio* calculations, together with experimental findings,^{6,7} suggest that rotations around the relevant bonds can be easily accomplished at room temperature. According to previous analyses, it seems that the barriers for rotation about the C8-C9 bond in cff91 are overestimated.

The bond lengths of the optimized conformations were compared with the bond lengths in the crystal structure^{2,3} (see Table I) and it was found that MM2(87) and cff91 force fields reproduce them reasonably well (especially the shortening of C6-C7

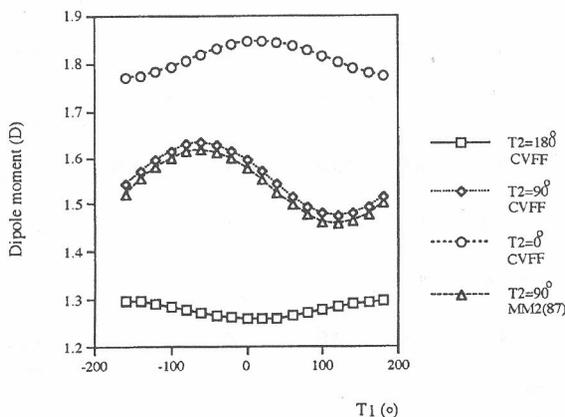


Figure 6. Dipole moment as a function of torsion angles $T1$ and $T2$, calculated by DISCOVER(CVFF) and MM2(87).

and C4-C5 bonds, which were observed in many of indole-3-acetic acid derivatives⁷) while it is not true for CVFF.

Dipole moments of various conformations were determined in the CVFF, cff91 and MM2(87) force field (Table II and Figure 6).

Ab initio RESULTS

In order to verify the results obtained by molecular mechanics, and to determine the molecular charge distribution, *ab initio* SCF MO calculations at the RHF level, using the computer program GAMESS,^{12,13} have been performed in the following basis sets: STO-3G, 4-31G and 6-31G*.

The X-ray structure ($T1 = 95.5^\circ$ and $T2 = -9.2^\circ$) was used as the initial structure and the indole ring geometry was kept fixed. The optimization of the other parameters in the minimal (STO-3G) basis set produced rotation of the COOH group about 90° to $T2 = 109.7^\circ$, the rest of the side chain remained in its initial position. The dihedral around the C3-C8 bond remained (+) anticlinal ($T1$ about 90°), as it is in the crystal, while that around the C8-C9 bond changed from (-) periplanar ($T2 = -9.2^\circ$) to (+) anticlinal ($T2 = 109.7^\circ$). Partial optimization with GAMESS was performed for seven other ($T1$, $T2$) conformations chosen on the basis of the molecular mechanics results, keeping either $T1$ or $T2$ fixed. The final energies and values of the dipole moments are given in Table II (including the results of molecular mechanics calculations performed with CVFF and cff91).

The results obtained agree with those of the molecular mechanics calculations: the preferred conformers are those with a perpendicular side chain and anticlinal conformations (+ and -) around the C8-C9 bond. In STO-3G²⁰ basis set, the energies of these conformations (conformations 1 and 2 in Table II) are about 4 kJ/mol lower than the energy of those with the same orientation of the carboxyl group but with the side chain coplanar to the IAA ring plane (conformations 4 and 5 in Table II). In the 4-31G and 6-31G* basis sets, these differences are about 8 kJ/mol (Table II). The conformations with the perpendicular side chain but with the opposite orientations of the COOH group, (+) anticlinal *vs.* (-) anticlinal, differ a little in energy. The energy of the latter is about 1.7, 1.3 and 0.8 kJ/mol higher (than the former) in the 6-31G*, 4-31G and STO-3G basis sets, respectively. There are two barriers for COOH rotation: at $T2 \approx 0^\circ$ and 180° . The height of the lower (conformation 6 in Table II) is 3.8, 3.4 and 1.7 kJ/mol in the 6-31G*, 4-31G and STO-3G basis sets, respectively.

Mulliken population analysis and distributed multipole analysis DMA^{23,24} have been performed on all conformations optimized. The charge distribution remains fairly constant in the conformations analyzed; changes in atomic charges are ≤ 0.01 e in the STO-3G and ≤ 0.02 e in the 4-31G basis set. However, differences between the atomic charges in different basis sets are significant: the maximum difference, found in conformation 1 (Table II), between the charges in STO-3G and 4-31G(6-31G*) basis set is 0.38 e (0.43 e) [C9] and 0.66 e (0.55 e) [N1] for DMA and Mulliken population charges, respectively. In Figure 7a) the Mulliken and b) DMA charge distributions of conformation 1 ($T1 = 108.4^\circ$, $T2 = 109.7^\circ$) are given as obtained in the STO-3G and 6-31G* basis sets. The root mean square difference (rms) between the Mulliken population and DMA charges is 0.16, 0.15 and 0.14 in STO-3G and 6-31G* basis set, respectively.

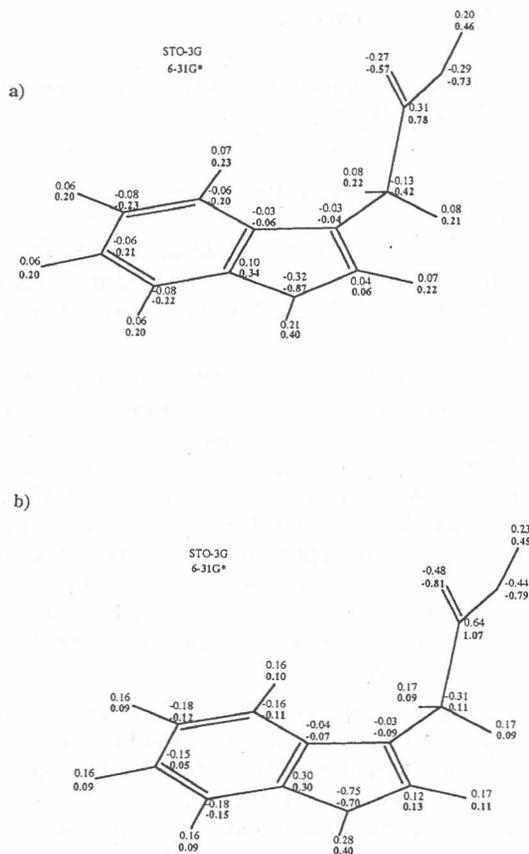


Figure 7. The charge distribution on the optimized IAA conformation (1) determined by *ab initio* SCF MO method in STO-3G and 6-31G^{*} basis sets: a) Mulliken population analysis b) DMA distribution.

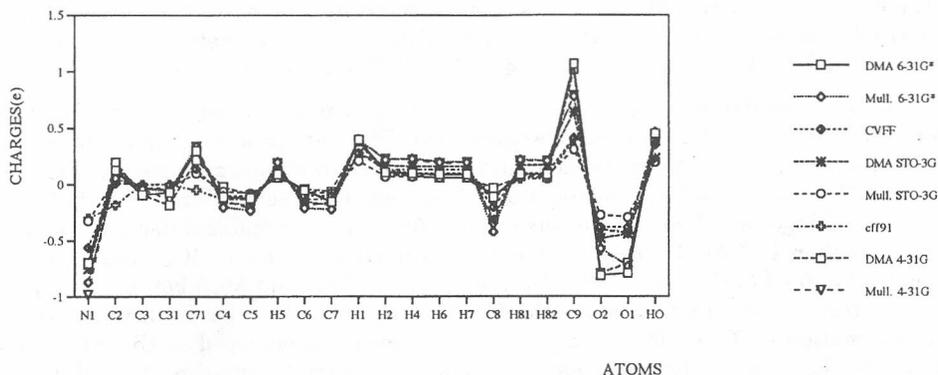


Figure 8. Atomic charges from CVFF and cff91 force fields and charges calculated by the *ab initio* SCF MO method.

Although molecular mechanics failed to reproduce *ab initio* dipole moments (Table II), (this is mostly due to the simplified method used for dipole moment calculations), partial charges used are in good agreement with the *ab initio* charges. DMA monopoles and Mulliken population charges determined in STO-3G, 4-31G and 6-31G* basis set, as well as CVFF and cf91 charges, are given in Figure 8. There is very good agreement between CVFF and DMA (Mulliken) charges in the minimal basis set; rms between these charges is 0.10 (0.08). However, to design the binding of IAA and its derivatives to a particular receptor (due to the importance of electrostatics in binding), atomic charges should be derived from the *ab initio* SCF molecular electrostatic potential^{25,26} (determined in the 6-31G* or 6-311G** basis set).

DMA and Mulliken analyses were also done on the free indole ring and on formic acid after optimization in the STO-3G basis set. The molecular charge distribution of indole acetic acid is consistent with the charge distributions of these two compounds. The difference between the atomic charges on the corresponding atoms is significant on the C3 and C8 atoms only. The dipole moment of the various indole acetic acid conformations can be well reproduced by a vector addition of the dipole moments of the free indole ring ($\mu = 1.8$ D) and formic acid ($\mu = 0.6$ D). For example, the dipole moment of conformation 8 (Table II) is 2.2 D, and calculated from fragments, it is 2.0 D.

DISCUSSION AND CONCLUSION

The comparative analysis performed suggest that molecular mechanics methods (force fields) described here can be used in conformational analyses of indole-3-acetic acid derivatives with reasonable reliability.

The *ab initio* and molecular mechanics methods show agreement on the results of conformational analysis of indol-3-ylacetic acid, which may be summarized as follows.

Conformations of IAA with a perpendicular side chain ($T1 \approx \pm 100^\circ$) and anticlinal conformation about the C3-C8 bond are energetically preferred. However, the potential energy of the one determined in crystal lattice, with (-) periplanar conformation around the C8-C9 bond ($T1 = 95.5^\circ$ and $T2 = -9.2^\circ$) is not significantly higher (< 4 kJ/mol). Conformers with (+) anticlinal and (-) anticlinal conformations about the C8-C9 bond ($T2 \approx \pm 100^\circ$) (MM results) are minima for any conformation of T1, but only for $T1 \approx 90.0^\circ$ the rotation of the COOH group can easily occur at room temperature (according to calculations in 6-31G* basis set $\Delta E \approx 1.5$ kT). Conformers with the side chain coplanar with the indole ring ($T1 = 0.0^\circ$ and 180°) correspond to barriers on the potential-energy surface ($\Delta E = 4.6$ and 15.9 kJ/mol in STO-3G, respectively). The interconversion from conformations with positive T1 to those with negative T1 is expected to occur with ease at room temperature. This implies that entropy differences will contribute to hindering of the T1 positive to negative conversion.

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SAŽETAK

Konformacijska analiza indol-3-octene kiseline

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Konformacijska analiza biljnog hormona, indol-3-octene kiseline (IAA) provedena je primjenom molekulske mehanike i *ab initio* SCF MO teorije. Nađene su ravnotežne i prijelazne konformacije IAA i izračunane njihove relativne energije i dipolni momenti te raspodjela naboja. Položaj karboksilne skupine na indolskom prstenu definiran je torzijskim kutovima T1(C2-C3-C8-C9) i T2(C3-C8-C9-O2). Proučavanjem njihovih rotacijskih barijera utvrđeno je da su promjene oba kuta na sobnoj temperaturi moguće u relativno kratkom vremenu: visina barijera kJ mol^{-1} iznosi 4.6 – 10.9 (T1), odnosno 1.7 – 3.8 (T2) (*ab initio* rezultati). *Ab initio* (GAMESS) i molekulsko-mehanički računi (DISCOVER CVFF and cff91), SYBYL (TRIPOS(87)) daju kvalitativno jedan oblik plohe potencijalne energije, $E = f(T1, T2)$. Razlike energija pojedinih konformacija ovise o temeljnom skupu (*ab initio*) odnosno polju sila (molekulska mehanika) s kojim je račun propveden.