Performance of Methods Used For the Methyl Orange Structure Modelling

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Abstract: Methyl orange (MO) structure obtained by experimental X-ray crystallography data was previously compared by statistical analysis to the conformations of minimal energy obtained by molecular mechanics calculations using the MM2 force field and by the AM1, PM3 and DFT quantum chemical methods. Good performance was obtained by molecular mechanics, as well as by the DFT approach. This paper presents a comparison of MO structure obtained by additional molecular mechanics methods, which use the MMFF94s, MMFF and AMBER force fields, with the previously studied ones. The conformations of minimal energy thus obtained were then compared by superposition on the heavy atoms by a least-square procedure with the experimental X-ray crystallography data and, also, with the conformations obtained by the previously studied methods. Comparison of these structures was performed by the RMS value thus obtained and by the torsion angle between the phenyl rings of the MO structure. Better RMS value was noticed by the superposition of X-ray structure with the conformation obtained by DFT. The conformations derived from the AM1 semiempirical approach and from the molecular mechanics methods employing the MM, MMFF and MMFF94s force fields gave still reasonable accurate superposition results, as compared to the X-ray structure.

Keywords: methyl orange, Omega, Hyperchem, MMFF94s, MMFF, Amber

1. Introduction

Methyl orange (MO) (Fig.1) is a pH indicator, frequently used in titrating most mineral acids, strong bases and estimating alkalinity of waters [1]. It is also employed as reagent for detection of bromine [2].

The MO protein-binding properties have been of interest for nearly as long [3]. It is, also, used as reagent to form ion pairs with, and thereby isolate, certain compounds from biological material.

Methyl orange has given no evidence of carcinogenic potential in limited oral and injection studies in the rat [http://www.bibra-information.co.uk/profile-55.html]. It was mutagenic in Ames bacterial tests following metabolic activation, but has mainly given negative results in genotoxic assays in mammalian cells in culture and did not induce chromosomal effects in mice treated by intraperitoneal injection. A high acute oral toxicity was demonstrated in rats. There was some evidence of skin sensitization in man.

Experimental crystallographic X-ray data [3] of methyl orange indicated that the two phenyl rings of the azobenzene nucleus are inclined to each other at 10°.

In previous studies, bond lengths and angles of minimum energy structures were then compared to the experimental X-ray crystallographic data by linear regression analysis and, also the RMS values were derived from the experimental vs. optimized structures resulting from atom by atom superposition.

Thus, the structure of MO was energy minimized by molecular mechanics calculations using the MM2 force field, by AM1 [4] and PM3 [5] semiempirical quantum chemical methods and compared to the experimental X-ray one. Methyl orange structure obtained by the molecular mechanics calculations was found to be in good agreement with the crystallographic data.

DFT calculations were performed and compared to the previously minimized structures obtained by semiempirical calculations [6]. Dependence of calculated bond lengths and angles versus the experimental ones was evaluated by multiple linear regression. The statistical results indicated that the DFT approach was found to better model the MO structure, in comparison to molecular mechanics and the AM1 and PM3 semiempirical methods.

This paper presents a comparison of MO structure obtained by additional molecular mechanics methods which use the MMFF94s, MMFF and AMBER force fields with the previously studied ones.
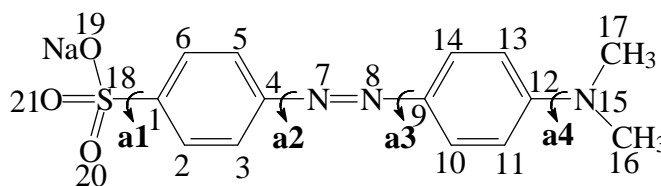

Figure 1. Methyl orange structure

2. Methods

2.1. Methyl orange structure simulation by OMEGA software

The neutral molecular structure of the diarylazo phosphate was modelled by the conformational search ability of the Omega v.2.3.2 [7] program. Omega employs a rule-based algorithm [8] in combination with variants of the Merck force field 94 [9]. MO SMILES structure was used as program input.

For the preparation of MO conformers, following parameters were used: a maximum of 200 conformers per compound, an energy cutoff of 10 kcal/mol relative to global minimum identified from the search, the default dielectric constant of 1 and two types of force fields: the MMFF94s force field with coulomb interactions and attractive part of the van der Waals interactions and the MMFF force field, without coulomb interactions and attractive part of the van der Waals interactions. In order to avoid redundant conformers the RMSD fit value of 0.4 Å was used.

2.2. Methyl orange structure simulation by HYPERCHEM

Methyl orange structure was built by the Hyperchem software [10] and conformational analysis was performed by molecular mechanics calculations, using the AMBER force fields, with the RMS gradient value of 0.01 kcal/Å·mol, as criterion to choose an optimized conformation and Polak-Ribiere as conjugate gradient, an acceptance energy cutoff of 10 kcal/mol above the minimum energy, a maximum number of conformers to be generated was set to 5000 and 100 conformers over the minimum energy conformer were kept. Four torsion angles

were considered in the conformational search: a1 (O19-S18-C1-C2); a2 (C3-C4-N7-N8); a3 (N7-N8-C9-C10); a4 (C11-C12-N15-C17), in Fig. 1.

3. Results and Discussion

Two types of stereoisomers were obtained by the MMFF and MMFF94s force fields: Z and E, with respect to the azo group. 12 Z and 192 E stereoisomers were derived by the MMFF force field and 6 Z and 96 E stereoisomers by MMFF94s. Most stable was the E stereoisomer type in both cases (Table 1). The minimum energy conformer obtained by MMFF force field is presented in Fig. 2.

Only the E stereoisomer type (Fig. 3) was obtained by the Amber force field inside Hyperchem software. The torsion angles of the minimum energy conformer are presented in Table 1 for each case.

The two phenyl rings are placed in the same plane with the azo group in case of all E stereoisomers. The amino group is in the same plane with the rest of the molecule when the MMFF and MMFF94s force fields were ulitized. Same amino nitrogen atom had a pyramidal non-planar form in case of the Amber force field.

The minimum energy structures thus obtained were compared to the experimental X-ray structure of MO. The RMS values obtained by atom per atom superposition of all heavy atoms, except the oxygen atoms of stereoisomers obtained by the Amber, MMFF and MMFF94s force fields are presented in Table 2, together with RMS values obtained for the conformers previously minimized by the AM1 and PM3 hamiltonians, by the DFT approach and by the MM force field. The oxygen atoms were not considered in the superposition procedure for uniformity of results (the experimental data contained the ionized form of the sulfonic acid group, which could not be modeled accurately by some of the presented approaches).

TABLE 1. Torsion angles of minimum energy conformers derived by several approaches

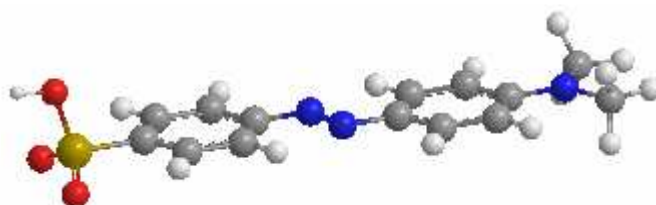| Stereoisomer	Amber energy (kcal/mol)	MMFF energy (kcal/mol)	MMFF94s energy (kcal/mol)	a1 (degree)	a2 (degree)	a3 (degree)	a4 (degree)
E conformer	38.17			88.71	0.32	179.97	36.18
Z conformer		73.58		89.99	90.01	-90.03	-179.9
E conformer		25.77		-90.01	179.86	-0.001	179.35
Z conformer			73.60	-90.01	-90.13	90.002	179.35
E conformer			22.81	90.00	0.002	179.96	-179.92

Figure 2. Methyl orange structure optimized by the MMFF force field
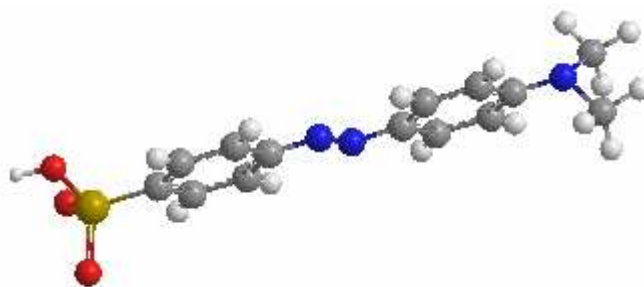
Figure 3. Methyl orange structure optimized by the Amber force field

TABLE 2. RMS values obtained by minimum energy conformer superposition of methyl orange with X-ray experimental structure and the torsion angle between the phenyl rings of the minimum energy structure

Conformational search method	RMS (Å)	Torsion angle (degrees)
Amber	0.41	0.4856
MMFF	0.19	0.174
MMFF94s	0.19	0.174
MM*	0.19	6.71
AM1*	0.21	10.64
PM3*	0.29	22.31
DFT*	0.14	0.081

* previously studied [4-6]

The RMS values obtained by a least-square procedure indicated that accurate results were obtained by molecular mechanics calculations using the MM, MMFF and MMFF94s force fields. Better RMS value was noticed by the supersosition of X-ray structure with the conformation obtained by DFT. The conformations derived from semiempirical approaches and from the Amber force field calculations gave poorer, but still reasonable superposition results, as compared to the X-ray structure.

Closer torsion angle between the phenyl rings of the minimized structures to the experimental value (of 10.02^{o}) was obtained by the AM1 semiempirical hamiltonian and by the MM force field approach. This angle was measured by the Vega ZZ software [11].

Molecular mechanics employing the MM, MMFF and MMFF94s force fields and AM1 and DFT quantum chemical methods modeled accurately the methyl orange structure.

4. Conclusion

Methyl orange structure modelled by several molecular mechanics and quantum chemical methods was compared to the experimental X-ray structure. In addition to previously studies, in which the MM2 force field, the semiempirical AM1 and PM3 hamiltonians and the DFT approach were used, in the present paper the MMFF94s, MMFF and AMBER force fields were used for the conformational analysis. The MO structure of minimum energy thus obtained was compared to the experimental one, by the RMS value and the torsion angle between the phenyl rings. Better RMS value was noticed by the supersosition of X-ray structure with the conformation obtained by DFT. The conformations derived from semiempirical approaches and by molecular mechanics methods (except the Amber force field approach) gave higher RMS value, but still reasonable superposition results. Comparing the torsion angle between the phenyl nuclei of the MO structure, it was observed that AM1 hamiltonian and the MM force field gave a closer value to the X-ray data. Molecular mechanics, as well as quantum chemical approches can model with good results the MO structure.

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