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A Computer Program for the Simulation of the NOE Effect in NMR Spectroscopy, and Its Implementation into the CICADA Conformational Search Program[#]

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Abstract

Motivation. Three–dimensional structure plays increasing role in the design of molecules with predefined features. Therefore, further development of methods that give 3–D information is necessary. Many molecules of interest are conformationally flexible and difficulties with interpretation of experimental (for example, Nuclear Magnetic Resonance) data may occur. Computational methods are promising tools that can assist to eliminate the above problems. To begin with, however, computational methods and computer programs must be developed that are able to reproduce data for conformationally rigid molecules.

Method. The previously developed computer program CICADA was used to describe conformational Potential Energy Surfaces (PESs) of selected molecules. The conformational flexibility of these molecules was deduced from the PESs. Equations describing the NMR technique termed Nuclear Overhauser Enhancement (NOE) were used to characterize NOE for different conformations.

Results. The computer program NOESIM for NOE enhancement calculation has been developed. This program is implemented as part of the CICADA computer program package. Five different models have been implemented, tested for relaxation rate calculation of methyl groups, and their results compared.

Conclusions. It is shown that NOESIM adequately predicts the experimental NOE effects for rigid molecules. The effort in the future will be improve its performance also for use with flexible systems, for example, Boltzmann distribution of conformers or flexibility of conformers.

Availability. The program is available upon request by E–mail from zdenek@chemi.muni.cz.

Keywords. Nuclear Overhauser Enhancement; computer simulation; molecular mechanics; single coordinate driving method.

Abbreviations and notations

MM, Molecular Mechanics	QM, Quantum Mechanics
NMR, Nuclear Magnetic Resonance	SCD, Single Coordinate Driving
NOE, Nuclear Overhauser Enhancement	

[#] Dedicated to Professor Haruo Hosoya on the occasion of the 65th birthday.

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1 INTRODUCTION

Molecular structure in solution may be elucidated by a variety of spectroscopic methods (UV–VIS, IR, NMR), and the solid state molecular structure can be investigated by the X–ray crystallography. X–ray crystallographic analysis only gives information about the "static" 3–D structures of a molecule in the crystal. On the other hand, NMR measurements may be used to explore the static as well as the dynamic properties of the molecule, which are of interest for many purposes, *e.g*. the understanding of the interactions of molecules with biological receptors, leading to drug design. However, studying the dynamic properties of molecules using NMR techniques is a time consuming process, which in many cases is difficult to perform without molecular modeling. Therefore, there has been an increasing effort in developing computer models simulating these processes. Molecular structures may be studied by computational methods using a variety of procedures, based either on quantum mechanics (QM) or force field methods in molecular mechanics (MM) programs. For studying large molecules the programs using the MM methods for energy calculations basically represent the only practically applicable methods. Comparison of experimental values with those obtained by computational methods often proves the latter to give surprisingly accurate information about the structure of the molecules.

The Nuclear Overhauser Enhancement (NOE) effect is an NMR technique that relies on the relaxation phenomena of the magnetic nuclei and which has proven to be an indispensable tool for probing interatomic distances. The magnitude of the NOE effect is directly proportional to r^{-6}, where r is the spatial distance between two nuclei. The method has therefore found broad application for obtaining information on the 3–D structure and the dynamic properties of molecules. The standard formulation of the NOE theory may, however, only be applicable to molecules that are rigid or moderately flexible in solution [1]. A few computer programs for NOE calculations have been reported, such as CALCNOE [2, 3, 4], FIRM [5], CORMA and MARDIGRAS [6] and MORASS [7].

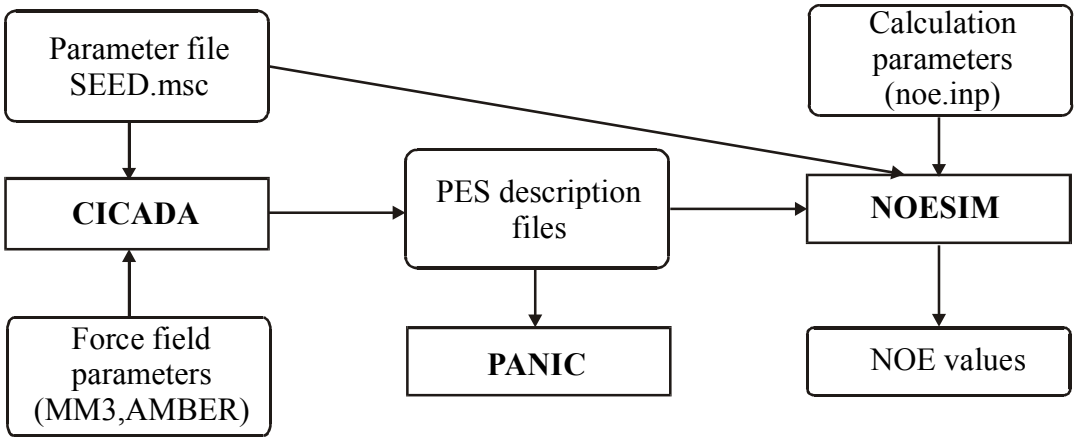
2 METHODS

2.1 Conformational analysis. The program CICADA

CICADA is a computer program for the conformational analysis of organic molecules that has been described elsewhere [8,9]. The CICADA suite consists of a number of programs: input (ROSE [10]), analysis (PANIC [11], COMBINE [12], and conformational clustering VCLU, CCA (unpublished software), FAMILY [13], together with several "in house" visualization and display tools. The program CICADA is based on the Single Coordinate Driving (SCD) method [8,14] for the exploration of the conformational potential energy (hyper) surface (PES), and generates information about the essential critical points only, *i.e*., minima and conformational transition states

of the PES low energy areas. When handling multidimensional conformational spaces, the CICADA procedure has an advantage over most of the traditional strategies such as grid search methods, as these in general generate vast amounts of useless surplus information. Using this PES search procedure reduces the risk of a combinatorial explosion. With the critical points of the PES in hand, all static as well as dynamic properties of a molecule may be fully described. Applying the results from these analyses, for the first time a tool was developed for the quantitative estimation of the flexibilities of molecules. The terms absolute and relative flexibility coefficients, were defined [13,15], respectively, as well as the conformational softness f^0 [16]. The quantitative elucidation of molecular flexibility is important as flexible molecules are much more abundant that rigid ones. The quantitative flexibility analyses may prove valuable and useful in QSAR studies, although reports dealing with this application of flexibility calculations, to the best of our knowledge, have not so far appeared in the literature.

The information about the structural and dynamic properties of the molecule may be used to elucidate and simulate a variety of observed properties, such as NOE effects, which depend on the interatomic distances and the dynamics of the molecule. We wish here to report on the new program, NOESIM, for the simulation of the NOE effect and its implementation into the CICADA suite of programs.



Scheme 1. Cooperation of used programs. The program CICADA is used for conformational analysis. Different force field parameters can be used for description of the molecule's behavior, c.f., MM3, AMBER. Necessary parameters for calculations are stored in the file SEED.msc. The results of conformational analysis are used for flexibility calculations carried out by the program PANIC. The program NOESIM calculates NOE values. Cartesian coordinates of low energy conformers are used as input data and other necessary parameters are included in the noe.inp file.

2.2 Theory of the NOE

The Nuclear Overhauser Effect (NOE) is observed upon decoupling of, *e.g.*, a proton resulting in an enhancement of intensities of the resonances of nuclei in close spatial proximity to the irradiated nucleus. The NOE is the result of dipolar relaxation induced by molecular motions in the solution. The rotation of bonds in tumbling molecules induces fluctuating magnetic fields, arising from the

magnetic moments rotating bonds is a rotating dipole. Provided molecular motions include frequencies in the order of Larmor frequencies of a magnetic nucleus, transitions between spin energy levels are induced by these motions. The magnitude of the NOE effect can be described and modeled in a number of ways.

The NOE value has been defined by Noggle and Schirmer [1] as:

$$R_i \cdot f_i(k) + \sum_{j \neq i} \sigma_{ij} \cdot f_j(k) = \sigma_{ik} \tag{1}$$

where $f_i(k) = NOE$ for proton i upon saturation of proton k, R_i is the total dipolar relaxation rate of proton i as expressed by:

$$R_i = \frac{2\pi}{5} \cdot \gamma^4 \hbar^2 \sum_{j \neq i} [6J(2\omega) + 3J\omega) + J(0)] + R^S \tag{2}$$

and σ_{ij} is the cross relaxation rate between protons i and j defined as:

$$\sigma_{ij} = \frac{2\pi}{5} \cdot \gamma^4 \hbar^2 [6J(2\omega) - J(0)] \tag{3}$$

where γ is the proton gyromagnetic ratio, $\hbar$ is Plank's constant over 2π, $J(\omega)$ is the spectral density function, which for a rigid molecule undergoing isotropic tumbling with a correlation time of t_c gives:

$$J(n\omega) = \frac{\tau_c}{1 + n^2\omega^2\tau_c^2} \cdot \frac{1}{4\pi \cdot r_{ij}^6} \tag{4}$$

where ω is the Larmor frequency of protons and τ_c is the isotropic rotational correlation time and r_{ij} is the distance between protons i and j. The simultaneous equations (1) are solved by Gaussian elimination [17].

When methyl groups are present, the internal rotation of these groups, characterized by rotational correlation time τ_m, must also be taken into account. Five different approaches for calculation of the NOE for molecules containing methyl groups are included in the program.

2.2.1 Static model

In this case the distance between protons of the methyl group and other protons is calculated as the average distance over methyl protons [7]:

$$\frac{1}{m} \sum_{j=1}^{m} \frac{1}{\left\langle r_{ij}^3 \right\rangle^2} \tag{5}$$

For a methyl/non–methyl proton pair, $m = 3$, and for an inter–methyl proton pair, $m = 9$. Rotation correlation time is then replaced by the effective correlation τ_r which is given by the expression:

$$\frac{1}{\tau_r} = \frac{1}{\tau_c} + \frac{1}{\tau_m} \tag{6}$$

2.2.2 Pseudo–atom model

Methyl protons are substituted by one pseudo atom situated in the center of the plane defined by the three methyl protons. Rotation correlation time is then replaced by the effective correlation τ_r according to Eq. (6).

2.2.3 *N*–site jump model

The simplification used in this model is to treat methyl protons as "jumping" between equivalent sites [5,18]. For methyl protons undergoing rapid jumps between equivalent positions, the spectral density function is given by:

$$\begin{aligned} J(n\omega) &= \frac{1}{5}\left[\frac{\tau_c}{1+n^2\omega^2\tau_c^2}\cdot A + \frac{\tau_r}{1+n^2\omega^2\tau_r^2}\cdot B\right] \\ A &= \sum_{m=-2}^{2}\left|\sum_{i=1}^{M}\sum_{j=1}^{N}\frac{Y_{2m}(\Theta_{ij},\Phi_{ij})}{r_{ij}^3}\right|^2 \\ B &= \sum_{m=-2}^{2}\left[\frac{1}{NM}\sum_{i=1}^{M}\sum_{j=1}^{N}\left|\frac{Y_{2m}(\Theta_{ij},\Phi_{ij})}{r_{ij}^3}\right|^2 - A\right] \end{aligned} \tag{7}$$

where N is number of equivalent proton sites of a methyl group, $M = 1$ for interactions between a single proton and methyl group and $M = N$ for interactions between two methyl groups. Y_{2m} are normalized spherical harmonics [5,18] and Θ and Φ are polar angles of the inter–nuclear vector in the molecular coordinate frame. The spherical harmonics functions are given by:

$$\begin{aligned} Y_{20} &= \sqrt{\frac{5}{4\pi}}\cdot\left(\frac{3}{2}\cos^2\Theta - \frac{1}{2}\right) \\ Y_{21} &= \sqrt{\frac{15}{8\pi}}\cdot\sin\Theta\cdot\cos\Theta e^{i\Phi} \\ Y_{22} &= \sqrt{\frac{15}{2\pi}}\cdot\sin^2\Theta e^{2i\Phi} \\ Y_{2-m} &= (-1)^m\cdot(Y_{2m}) \end{aligned} \tag{8}$$

Usually $N = 3$ for methyl groups, but N approaches infinity for freely rotating methyl groups. In the simplest case, where $\tau_r << \tau_c$, the second term of Eq. (7) is negligible. In the N–site jump model, the spectral density functions for interactions between protons of the same methyl group are [19]:

$$J(n\omega) = \frac{1}{4\pi\cdot r_h^6}\cdot\left[\frac{1}{4}\cdot\frac{\tau_c}{1+n^2\omega^2\tau_c^2} + \frac{3}{4}\cdot\frac{\tau_r}{1+n^2\omega^2\tau_r^2}\right] \tag{9}$$

2.2.4 The Rowan model

Rowan *et. al*. [19–22] have developed expressions for the case where one nucleus of a methyl group containing three equilibrium positions and a proton outside the methyl group are positioned with equal distances between two of the methyl protons. In this case the quotient τ_c / r_{ij}^6 in the spectral density function (4) was replaced by the expression:

$$A \cdot \tau_c + B \cdot \tau_r$$

where coefficients *A* and *B* are given by:

$$A = \frac{2}{9}\left\{\left(\frac{1}{r_f^6} + \frac{1}{2r_n^6}\right) + \left[\frac{(1 - 6l_f^2 + 6l_f^4)}{r_f^6} + \frac{3(m_n m_f + n_n n_f)^2 - 1}{r_n^3 r_f^3}\right]\right\} \tag{10}$$

$$B = \frac{2}{9}\left\{\left(\frac{2}{r_f^6} + \frac{1}{2r_n^6}\right) - \left[\frac{(1 - 6l_f^2 + 6l_f^4)}{r_f^6} + \frac{3(m_n m_f + n_n n_f)^2 - 1}{r_n^3 r_f^3}\right]\right\} \tag{11}$$

where r_f and r_n are the distances between a non–methyl proton and most distant and the nearest proton of a methyl group, respectively. Details are described in [21]. The coefficients l_f, l_n, m_f, m_n, n_f, n_n are directional cosines of r_n and r_f.

2.2.5 The Heatley model

This model agrees with the Rowan *et. al*. approach, but is extended to the general case where the three possible *i–j* distances of methyl protons *i* and non–methyl proton *j* are all different [23]. These distances are denoted $r_{ij}^{(1)}, r_{ij}^{(2)}, r_{ij}^{(3)}$, and $\beta_{ij}^{(ab)}$ represents the angle between vectors $r_{ij}^{(a)}$ and $r_{ij}^{(b)}$. The coefficients τ_c / r_{ij}^6 in the spectral density functions $J(n\omega)$ are replaced by expression (12):

$$\begin{aligned} &\frac{1}{9}\left[(A + 2B) \cdot \tau_c + 2(A + B)\tau_r\right] \\ &A = \sum_{a=1}^{3} (r_{ij}^{(a)})^{-6} \\ &B = \sum_{a=1}^{2} \sum_{b>a} \frac{1}{2} \cdot (3\cos^2 \beta_{ij}^{(ab)} - 1) \cdot (r_{ij}^{(a)} r_{ij}^{(b)})^{-3} \end{aligned} \tag{12}$$

The relaxation rate for protons of the same methyl group is as defined by Eq. (9).

NOE values are dependent not only on the spectrometer frequency, but also on the correlation times. The correlation time τ_c describes the tumbling behavior of a molecule in a solvent and τ_m the free rotation of the methyl groups. Because we are concerned with isotropic molecular tumbling, τ_c is therefore related to the time it takes the molecule to rotate by 1 radian about any axis. τ_c can be approximated to the rotational relaxation time given by Debye [24]:

$$\tau_c = \frac{4 \cdot \pi \cdot \eta \cdot a^3}{3 \cdot k \cdot T} \tag{13}$$

where η is the viscosity of the solvent and *a* is the radius of molecule. Using this approximation, and using typical values for the viscosity for organic solvents, one can write a *very approximate* estimate of τ_c as:

$$\tau_c \approx M_W \cdot 10^{-12} \tag{14}$$

where M_W is the molecular mass.

Because of the small rotation barrier of the methyl group the rotation is extremely rapid and correlation time is usually much smaller than τ_c. For the τ_m values the following approximation has been used [23]:

$$\tau_m \approx \frac{h}{kT} \cdot e^{E_a / RT} \tag{15}$$

where E_a is the energy barrier of the rotation, *h* is Planck's constant, *T* is the absolute temperature, *R* the gas constant and *k* is the Boltzmann constant.

3 RESULTS AND DISCUSSION

The ability of the program NOESIM has been first tested on a series of rigid molecules. The test molecules were: 5–(2,4–dinitroanilino)penta–2,4–dienal, *cis*– and *trans*–crotonaldehyde, and Apparicine. It will be shown that the computed NOE values for the protons of the rigid part of the molecules are in good agreement with experimental values.

Conformational searches were performed by the program CICADA version 2002 [9] using the MM3 molecular mechanics force field [25–28]. The dielectric constant for MM calculations was set to 1.5 to mimic a nonpolar environment. All low energy minima with relative energies of less than 50 kcal/mol were taken into the conformational search. Rotation correlation times τ_c were calculated from molecular weights using Eq. (14). Internal correlation times for methyl groups were arranged from rotational barriers using Eq. (15).

3.1 5–(2,4–dinitroanilino)penta–2,4–dienal

The molecule, with proton numbering, is shown in Figure 1.

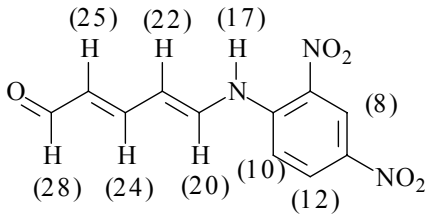


Figure 1. Proton numbering in the 5–(2,4–dinitroanilino)penta–2,4–dienal molecule.

The CICADA conformational search shows three different conformations with Boltzmann population 40.5 %, 12.0 %, and 6.8 %. Calculated Boltzmann weight–averaged interproton distances are summarized in Table 1. The molecular weight of the structure is 260.02 $g \cdot mol^{-1}$, which corresponds with the correlation time τ_c = 0.26 ns. Because this structure has no methyl group, results of all included methods for relaxation rate calculation and NOE values are the same. Calculated and experimental NOE values published in [29] are summarized in Table 2.

Table 1. Calculated inter–proton distances (in Å) for 5–(2,4–dinitroanilino)penta–2,4–dienal. For atom numbering see Figure 1.

Proton #	**8**	**10**	**12**	**20**	**22**	**24**	**25**	**28**
8	0.00	4.84	4.21	5.63	6.91	8.00	9.08	10.28
10	4.84	0.00	2.40	2.53	4.58	4.71	6.31	6.97
12	4.21	2.40	0.00	4.53	6.82	6.86	8.63	9.15
20	5.63	2.53	4.53	0.00	3.07	2.50	4.42	4.85
22	6.91	4.58	6.82	3.07	0.00	3.01	2.47	4.49
24	8.00	4.71	6.86	2.50	3.01	0.00	3.07	2.46
25	9.08	6.31	8.63	4.42	2.47	3.07	0.00	2.96
28	10.28	6.97	9.15	4.85	4.49	2.46	2.96	0.00

Table 2. Calculated and experimental NOE values (in %) for 5–(2,4–dinitroanilino)penta–2,4–dienal. For proton numbering see Figure 1.

NOE enhancement	Experimental value	Calculated value
28 → 24	14.0	10.5
24 → 28	23.0	21.8
22 → 25	19.0	17.6
25 → 22	–	18.2
24 → 20	–	11.8
20 → 24	14.0	10.5
20 → 10	23.0	18.2
10 → 20	20.0	12.2
10 → 12	26.0	24.2

The reason for the largest deviation 20 → 10 and 10 → 20 is that the exchangeable proton number 17 (NH group) was not included into the calculation. Protons of NH groups must be specially parameterized for NOE calculation. This is a problem which will be addressed in a later version of the program.

3.2 Crotonaldehyde

Two isomers of crotonaldehyde were chosen as a test example for the model of calculation of the relaxation rate of the methyl group. Experimental data were published in [21]. Both isomers were minimized using the quantum mechanics approach. Structure optimization was carried out using the program Gaussian 98 [29], at the Hartree–Fock level of theory with the 6–31G* basis set. The proton numbering is shown in Figures 2A and 2B. Calculated interproton distances are summarized in Table 3.

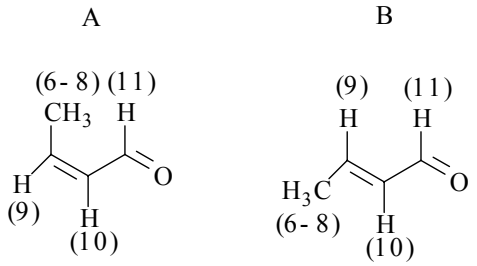


Figure 2. Proton numbering in *cis*–crotonaldehyde (A) and *trans*–crotonaldehyde (B).

Table 3. Calculated inter–proton distances (in Å) for *cis*–crotonaldehyde and for *trans*–crotonaldehyde.

	cis–crotonaldehyde						*trans*–crotonaldehyde					
Proton	**6**	**7**	**8**	**9**	**10**	**11**	**6**	**7**	**8**	**9**	**10**	**11**
6	0.00	1.79	1.79	3.17	3.87	2.13	0.00	1.80	1.80	3.17	2.41	4.76
7	1.79	0.00	1.80	2.58	4.15	3.59	1.80	0.00	1.81	2.60	3.50	4.77
8	1.79	1.80	0.00	2.58	4.15	3.59	1.80	1.81	0.00	2.60	3.50	4.77
9	3.17	2.58	2.58	0.00	2.38	3.93	3.17	2.60	2.60	0.00	3.11	2.50
10	3.87	4.15	4.15	2.38	0.00	3.20	2.41	3.50	3.50	3.11	0.00	3.20
11	2.13	3.59	3.59	3.93	3.20	0.00	4.76	4.77	4.77	2.50	3.20	0.00

Molecular weight of the crotonaldehyde is 70.09 g·mol^{-1}, which corresponds to a τ_c value of 0.070 ns. The calculated rotation barriers for the methyl group are 0.6 kcal·mol^{-1} and the corresponding correlation time for this group calculated by equation (15) is 0.476 ps. Calculated NOE values using different relaxation rate calculation methods are summarized in Tables 4 for *cis*–crotonaldehyde and 5 for *trans*–crotonaldehyde, respectively.

Table 4. Calculated NOE values (in %) for *cis*–crotonaldehyde using different methods for relaxation rate calculation.[a] For details see the chapter 2.2.

	Irradiated proton 11			Irradiated proton 10		
Method	**6**	**9**	**10**	**6**	**9**	**11**
1	0.1	1.7	4.8	0.0	42.2	35.5
2	3.8	0.8	5.5	0.2	25.3	5.9
3	3.2	1.6	4.8	–0.9	45.4	41.9
4(3)[b]	0.2	1.7	4.8	0.0	42.2	35.9
4(6)	0.1	1.7	4.8	0.0	42.1	36.4
4(9)	0.2	1.6	4.8	0.0	41.9	35.5
4(12)	0.1	1.7	4.8	0.0	42.1	36.4
5	3.9	1.0	5.3	0.4	30.6	11.8
experiment	4.7	0.8	4.4	1.5	23.3	1.5
	Irradiated proton 9			Irradiated proton 6		
	6	**10**	**11**	**9**	**10**	**11**
1	0.1	27.8	9.9	15.3	0.0	1.8
2	2.0	34.9	1.6	15.3	3.3	36.4
3	–6.1	25.8	11.6	31.9	1.1	45.5
4(3)	0.1	27.7	10.1	0.3	0.0	2.4
4(6)	0.1	27.4	10.2	0.3	0.0	1.8
4(9)	0.2	27.8	9.9	0.6	0.0	2.9
4(12)	0.1	27.4	10.2	0.3	0.0	1.8
5	3.1	35.1	3.2	11.1	2.1	32.0
experiment	1.5	32.8	1.3	18.6	3.8	32.6

[a] Method number 1 – static model, 2 – pure Rowan *et. al*. model, 3 – pseudoatom model, 4 – *N*–site jump model, 5 – Heatley *et. al*. model (for details see chapter 2.2).
[b] Numbers in parentheses are step numbers in the *N*–site jump calculation method

Table 5. Calculated NOE values (in %) for *trans*–crotonaldehyde using different methods for relaxation rate calculation.[a] For details see chapter 2.2.

	Irradiated proton 11			Irradiated proton 10		
Method	**6**	**9**	**10**	**6**	**9**	**11**
1	0.0	28.3	17.6	0.5	6.7	6.9
2	0.1	18.6	7.3	2.2	3.6	7.6
3	0.0	29.7	21.1	1.8	7.2	6.7
4(3)[b]	0.0	29.4	20.6	0.1	7.4	6.7
4(6)	0.0	29.3	20.6	0.1	7.4	6.7
4(9)	0.0	29.4	20.0	0.2	7.3	6.7
4(12)	0.0	29.3	20.6	0.1	7.4	6.7
5	0.3	21.9	11.3	2.2	4.5	7.3
experiment	–2.1	17.4	2.4	3.8	–2.0	2.3
	Irradiated proton 9			Irradiated proton 6		
	6	**10**	**11**	**9**	**10**	**11**
1	0.7	21.0	33.3	2.1	5.3	0.1
2	2.2	6.3	32.1	14.2	29.4	1.6
3	0.3	25.0	32.0	23.0	45.7	1.3
4(3)	0.1	24.6	32.2	0.2	0.8	0.0
4(6)	0.1	24.6	32.2	0.2	0.7	0.0
4(9)	0.2	23.9	32.4	0.4	1.9	0.0
4(12)	0.1	24.6	32.1	0.2	0.7	0.0
5	2.2	8.0	31.6	9.9	20.7	1.6
experiment	0.6	4.0	25.7	11.6	24.2	0.1

[a] Method number 1 – static model, 2 – pure Rowan *et. al*. model, 3 – pseudoatom model, 4 – *N* site jump model, 5 – Heatley *et. al*. model (for details see chapter 2.2)
[b] Numbers in parentheses are step numbers in the *N*–site jump calculation method

3.3 Apparicine

Apparicine has been chosen as the next representative of a relatively rigid molecule with a conformationally constrained methyl group. The structure of Apparicine with proton numbering is shown in Figure 3.

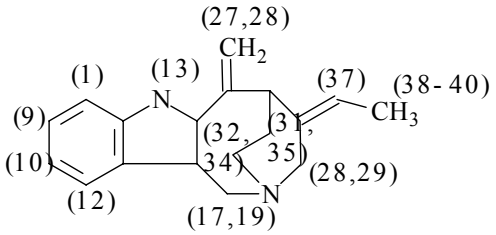


Figure 3. Structure and proton numbering of Apparicine. (The numbering is same as used in the program CICADA.)

The conformational search was performed for all torsions in the tricyclic part of the molecule. Two low energy conformations with Boltzmann populations of 97.2 % and 2.8 % were found. The calculated energy barriers for interconversion between the conformations were between 2.6 and 7.9 kcal·mol^{-1}. The superimposition of both low energy conformations is shown in Figure 4. Boltzmann averaged interproton distances are summarized in Table 6.

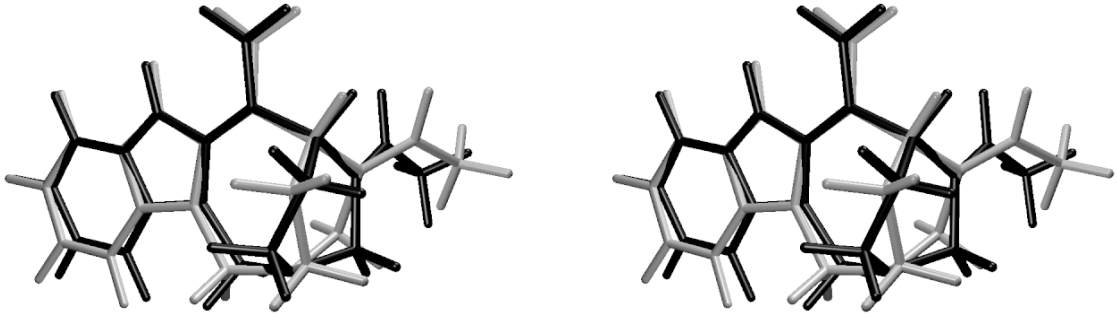


Figure 4. Superimposition of the low energy conformations of Apparicine found by the program CICADA. The global minimum is colored black.

Table 6. Calculated averaged interproton distances in (Å) for the Apparicine molecule. Proton numbering is same as in the program CICADA.

Proton #	**1**	**9**	**10**	**12**	**17**	**19**	**23**	**24**	**27**	**28**	**29**	**31**	**32**	**34**	**35**	**37**	**38**	**39**	**40**
1	0.0	2.5	4.2	4.9	5.9	6.0	7.8	8.5	6.1	4.4	7.1	6.9	6.2	7.9	8.2	7.2	8.7	9.3	8.0
9	2.5	0.0	2.4	4.2	6.0	6.3	8.4	9.4	8.3	6.7	8.8	8.4	7.1	8.7	9.5	8.8	9.5	10.4	8.9
10	4.2	2.4	0.0	2.4	4.6	5.0	7.3	8.4	8.7	8.2	6.4	7.7	8.9	8.6	8.6	9.8	8.3	4.9	4.3
12	4.9	4.2	2.4	0.0	2.2.	2.7	5.0	6.0	7.4	6.6	6.9	6.4	4.4	5.5	6.8	6.9	7.8	6.5	5.9
17	5.9	6.0	4.6	2.2	0.0	1.8	3.4	4.1	6.3	6.1	5.4	4.7	2.6	3.4	4.8	5.8	5.4	6.8	5.9
19	6.0	6.3	5.0	2.7	1.8	0.0	2.3	3.5	6.0	5.9	4.9	5.1	3.6	4.1	4.9	4.7	3.8	5.3	4.2
23	7.8	8.4	7.3	5.0	3.4	2.3	0.0.	1.8	5.9	6.4	4.2	5.0	4.2	3.8	3.9	3.8	2.1	3.7	3.4
24	8.5	9.4	8.4	6.0	4.1	3.5	1.8	0.0	5.5	6.4	3.5	3.9	3.7	2.8	2.5	4.0	3.1	4.1	4.5
27	6.1	8.3	8.7	7.4	6.3	6.0	5.9	5.5	0.0	1.8	2.1	3.3	4.9	5.6	4.0	3.4	6.2	5.9	5.9
28	4.4	6.7	7.5	6.6	6.1	5.9	6.4	6.4	1.8	0.0	3.5	4.1	5.0	6.2	5.2	4.3	6.9	6.8	6.3
29	7.1	8.8	8.7	6.9	5.4	4.9	4.2	3.5	2.1	3.5	0.0	2.5	4.0	4.2	2.4	2.4	4.6	4.5	4.8
31	6.9	8.4	8.2	6.4	4.7	5.1	5.0	3.9	3.3	4.1	2.5	0.0	2.4	2.7	1.8	4.8	6.2	7.1	6.4
32	6.2	7.1	6.4	4.4	2.6	3.6	4.2	3.7	4.9	5.0	4.0	2.4	0.0	1.8	3.0	5.5	6.0	7.0	6.6
34	7.9	8.7	7.7	5.5	3.4	4.1	3.8	2.8	5.6	6.2	4.2	2.7	1.8	0.0	2.3	5.8	5.7	6.7	6.7
35	8.2	9.5	8.9	6.8	4.8	4.9	3.9	2.5	4.0	5.2	2.4	1.8	3.0	2.3	0.0	4.3	5.1	5.5	6.0
37	7.2	8.8	8.6	6.9	5.8	4.7	3.8	4.0	3.5	4.3	2.5	4.8	5.5	5.8	4.3	0.0	3.1	2.6	2.6
38	8.7	9.5	8.6	6.5	5.4	3.8	2.1	3.1	6.2	6.9	4.6	6.2	6.0	5.7	5.1	3.1	0.0	1.8	9.3
39	10.4	9.8	7.8	6.8	5.3	3.7	4.1	5.9	6.8	4.5	6.6	7.1	7.0	6.7	5.5	2.6	1.8	0.0	1.8
40	8.0	8.9	4.3	5.9	5.9	4.2	3.4	4.5	5.9	6.3	4.8	6.4	6.6	6.7	6.0	2.6	9.3	1.8	0.0

Table 7. Comparison of calculated and experimental NOE values for Apparicine. Proton numbering is same as in Figure 3. The numbers of conformers are the same as used in the CICADA program. Heatley *et. al*. and the *N*–site jump model ($N = 9$, values in parenthesis) models were used for NOE calculation of methyl protons.

NOE	Conformer number		Average	Experimental
enhancement	1 (population 2.8%)	3 (population 97.2%)	structure	value
17→32	2.1 (1.7)	3.6 (0.9)	3.1 (1.0)	4.0
17→12	2.3 (2.4)	2.9 (6.6)	2.8 (6.5)	4.0
19→12	1.6 (6.0)	10.3 (8.9)	8.2 (8.9)	4.0
19→24	4.1 (1.3)	3.2 (2.0)	3.4 (2.0)	3.0
12→17	2.1 (6.2)	2.9 (6.2)	2.7 (6.2)	4.0
12→19	0.6 (2.0)	2.2 (2.2)	1.6 (2.2)	4.0
31→34	3.6 (1.3)	3.4 (1.7)	3.5 (1.6)	3.5
31→29	6.8 (8.2)	5.2 (8.1)	5.8 (8.2)	5.0
31→24	2.4 (1.0)	3.5 (1.6)	3.2 (1.5)	4.0
35→32	3.5 (3.0)	3.4 (2.5)	3.4 (2.5)	3.0
35→34	4.6 (1.7)	5.3 (1.7)	5.1 (1.7)	4.0
29→31	3.1 (0.7)	3.8 (1.1)	3.7 (1.1)	4.0
29→35	2.4 (2.0)	2.3 (1.4)	2.3 (1.4)	2.0
29→27	0.4 (0.1)	0.4 (0.3)	0.4 (0.3)	–1.4
29→28	4.8 (3.8)	5.2 (3.7)	5.1 (3.7)	5.0
37→24	3.5 (2.9)	4.5 (2.0)	4.4 (2.0)	4.0
23→35	1.2 (1.0)	2.0 (1.9)	1.9 (1.8)	2.0

The molecular weight of Apparicine is 264.37 g·mol^{-1} which corresponds to the rotation correlation time τ_c = 0.264 ns. Computed energy barriers for the rotation of the methyl group is 0.7 kcal·mol^{-1}. Using the approximate formula (15), we obtained a value for the methyl group rotation correlation time τ_m =0.6 ps. NOE values were calculated for both low energy conformers and for Boltzmann distribution weight–average structure. The calculated results and experimental values published in [22,30] are summarized in Table 7.

It is seen that the NOE values calculated from Boltzmann weighted–average structure correspond well with experimental data. The large differences between calculated and experimental values for protons 12, 17, 19 may be explained by the "flipping" of the molecule on this part of the molecule which is seen in Figure 4. It indicates that intramolecular motion must be included in the modification of the τ_c values.

3.4 Summary

The accuracy of calculated data was tested using calculation of absolute and relative variations. The results of the tests are summarized in Table 8.

Table 8. Accuracy of calculated data.

	Method [a]							
	1	2	3	4(3)	4(6)	4(9)	4(12)	5
Molecule 1 (7 NOE values)								
Absolute variation	–24.0	–	–	–	–	–	–	–
Relative variation	–3.43	–	–	–	–	–	–	–
Molecule 2A (12 NOE values)								
Absolute variation	12.4	8.2	79.0	–1.4	–1.9	–1.4	–1.9	12.8
Relative variation	1.03	0.68	6.58	–0.12	–0.16	–0.12	–0.16	1.07
Molecule 2B (12 NOE values)								
Absolute variation	34.5	37.2	105.8	34.1	33.9	34.4	33.9	23.5
Relative variation	2.88	3.10	8.82	2.84	2.83	2.87	2.82	1.96
Molecules 2A + 2B (24 NOE values)								
Absolute variation	46.9	45.4	184.8	32.7	32.0	33.0	32.0	36.3
Relative variation	1.95	3.78	7.70	1.36	1.33	1.37	1.33	1.51
Molecule 3 (17 NOE values)								
Absolute variation	–	–	–	–	–	–	–10.9	2.5
Relative variation	–	–	–	–	–	–	–0.64	0.15

[a] Models used for methyl proton interactions: 1 – static model, 2 – pure Rowan *et. al*. model, 3 – static model, 4 – *N*–site jump model (number in parenthesis are steps in calculations), 5 – Heatley *et. al*. model.

The positive values of variations in Table 7 indicate that the calculated values are larger than the experimental ones, and *vice versa*. It is seen that the best methods for calculation of the NOE enhancement are models by Heatley *et. al*. and the *N*–site jump model. The analysis also shows that in majority of cases the values calculated by all methods are larger than the experimental values, especially in the case of values near 0.0.

4 CONCLUSIONS

A new computer program, NOESIM, for the calculation of Nuclear Overhauser Enhancement in NMR spectroscopic studies of organic compounds has been developed. The program is implemented as a part of the CICADA computer program package. Five different models for relaxation rate calculation of methyl protons have been implemented and tested. We may conclude from the results presented above that the best models for methyl proton relaxation rate calculation are the Heatley *et. al.* and the *N*–site jump models. It has been shown that NOESIM adequately predicts the experimental NOE effects for rigid molecules, but fails to predict the effect for nuclei positioned in the flexible parts of molecules. It will be one effort in the near future to eliminate this disadvantage. Another direction we will follow in the near future is to calculate Boltzmann probability using free energy instead of potential energy, which will make the simulation more realistic. The last direction will be to include the flexibility of the fragments of molecule in the NOE calculations.

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