ON THE CONFORMATIONAL STRUCTURE OF NICOTINAMIDE AND 1-METHYL-1,4-DIHYDRONICOTINAMIDE*

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Received September 4th, 1978

The conformation of nicotinamide (*I*) and 1-methyl-1,4-dihydronicotinamide (*II*) was examined using the NDDO method. The influence of solvent on the molecular structure of the title compounds was estimated by means of a continuum model. Analysis of the NDDO wave functions contributes to the knowledge about the mechanism of the NADH reduction.

The knowledge of conformation of the NAD^+-type coenzymes and their constituents should be an important prerequisite to gain more insight into the mechanism of their action. Nicotinamide(*I*) and 1-methyl-1,4-dihydronicotinamide(*II*) represent suitable models for the pyridine components of these coenzymes. Numerous attempts have been undertaken to predict the conformation of both compounds by means of quantum chemical methods[1-7]. Relative to the preferred conformations, the predictions of semiempirical methods as EHT (ref.[1,2,7]) PCILO (ref.[3]), and HMO (ref.[4]) including non-bonding interactions are in qualitatively good agreement with X-ray data for the solid state of *I* and the 1-benzyl analogue of *II*. However, the PCILO results are strongly dependent on the manner in which the π-bonds are localized in the pyridine ring[8]. SCF type methods, CNDO/2 and MINDO/2, provide no correct results in the conformational analysis of the title compounds[6], in accordance with calculations on other conjugated systems[9,10]. In previous papers, it had been shown that within the semiempirical all valence electron SCF-MO formalism the NDDO method tends to be well suitable to investigate the molecular structure of conjugated compounds[10-13]. Considering the importance of the NAD^+-type coenzymes in biochemical processes[14,15], conformational analysis of the title compounds by means of this method seems to be recommendable. The examination of solvent effects on both structures, may be useful as well. Up to now, only little is known about conformations of *I* and *II* in solution[7,16,17].

* Part XV in the series Quantum Chemical Calculations for the Determination of the Molecular Structure of Conjugated Compounds; Part XIV: J. Mol. Struct., in press, and Part X in the series on Calculations of Biologically Important Compounds: Part IX: This Journal *43*, 3064 (1978).

CALCULATIONS

The parametrization of the NDDO method is given in detail in ref.[18]. To estimate the solvent effect the continuum approximation has been employed. The change of the molecular energy under the influence of solvent is written as the sum of the electrostatic energy, the cavity energy, and the dispersion energy. The formalism for the calculation of the single terms is desribed in refs[19,20]. The geometry was taken from X-ray studies on *I*, ref.[21] and on the 1-benzyl analogue of *II*, ref.[22].

RESULTS AND DISCUSSION

The most important conformational problem with *I* and *II* is the orientation of the amide group with respect to the ring. Fig. 1 shows the NDDO potential energy curve for the rotation of the amide group in nicotinamide (*I*). There are two minima at values of $\Theta = 30°$ and 150°, respectively. The absolute minimum at $\Theta = 150°$ corresponds wel to the value of $\Theta = 156°$ determined[20] experimentally. For comparison, the NDDO potential curve of benzamide ($\Theta^{NDDO} = 20°$ (ref.[12]), $\Theta_{exp} = 26°$ (ref.[23])) is also given in Fig. 1. The similarity between the conformations of both compounds is obvious. Due to the presence of the ring nitrogen atom, there are not excessive changes of the potential curve. The NDDO energy difference between the minimum energy conformations of *I* amounts to 3·2 kJ mol^{-1}. The rotational barrier is 7·05 kJ mol^{-1}. These results suggest a conformational equilibrium with a certain predominance of the twisted conformation *Ib*. The equation

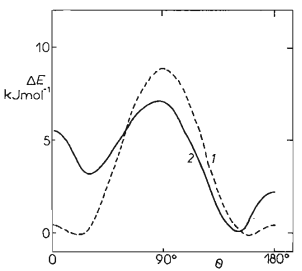


FIG. 1

NDDO Potential Energy Curves for Nicotinamide (2) and Benzamide (1)

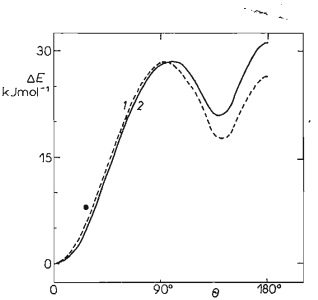


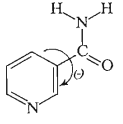
FIG. 2

NDDO Potential Energy Curve for 1-Methyl-1,4-dihydronicotinamide without (2) and with (1) Inclusion of the Solvent (tetrachloromethane)

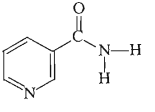
$$\mu^2_{exp} = x_1\mu^2_{Ia} + x_2\mu^2_{Ib} \qquad (1)$$

enables an estimation of the relative population of the conformers. In this equation, μ_{Ia} and μ_{Ib} are the dipole moments of the minimum conformations, x_1 and x_2 represent the weight fractions. Using CNDO/2 dipole moments, contributions of 65% and 35% have been estimated for *Ib* and *Ia*, respectively[17]. Calculations based on the NDDO dipole moments resulted in the same values. The NDDO potential curve for 1-methyl-1,4-dihydronicotinamide (*II*) is given in Fig. 2. The global minimum exists at $\Theta = 0°C$. A local minimum at $\Theta = 140°$ belongs to a conformation which is less stable by about 20·9 kJ mol^{-1}. This results is again in good accordance with the experimental value of $\Theta = 4°$ determined for the 1-benzyl analogue of *II* in the solid state[21]. The rotational barrier between the minimum conformations amounts to 28·1 kJ mol^{-1}. By performing an analogous analysis of the relative population of both conformers as in the case of *I*, based on the dipole moment values measured[6] experimentally ($\mu = 4·00$ D) and calculated by the NDDO method for the minimum conformations, a value of 75% for *IIa* is obtained.

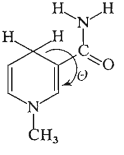
The most striking difference between the conformations of *I* and *II* predicted by the NDDO method is the strong predominance of the conformer *IIa* over *IIb*, whereas the conformer *Ib* is predominant in nicotinamide (*I*). The results of the dipole moment analysis suggest also an identical situation at least in apolar solvents. Biochemical investigations show that the amide group is of importance for the fixation of the pyridine coenzymes at the enzyme[15]. It may be interesting therefore to determine the complete potential curves for *I* and *II* taking into account the solvent effect. Starting from *Ib* to *Ia*, there is a dipole moment change of $\mu = 1·46 - 5·11$ D.



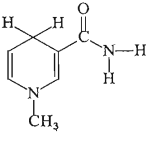
Ia ($\Theta = 0°$)



Ib ($\Theta = 180°$)



IIa



IIb

Hence, a strong solvent dependance of the conformational structure might be expected favouring the conformations with the larger dipole moments for more polar solvents. The potential curve obtained for *I* in tetrachloromethane is given in Fig. 3. The influence of the solvent leads to a stronger stabilization of the conformation *Ia*. Based on our calculations, this structure is even slightly more stable than *Ib*. This tendency is greater with solvents more polar than tetrachloromethane. Comparing this result with values arising from the dipole moment analysis, the stability of the conformation *Ia* seems to be somewhat overestimated. This may be caused by underestimation of the NDDO energy difference between the minimum conformations of *I* taken as reference for the vapour state or by deficiencies in the estimation of the solvation energy contributions. Thus, it is not always sufficient to restrict the calculation of the electrostatic part of the solvation energy solely to the dipole term. The results on 3-pyridinealdehyde indicate possibility that the energy change arising from the dipole term may at least partially be compensated by the quadrupole energy contributions[24]. The change of the dipole moment for *II* ($\mu = 3{\cdot}41 - 5{\cdot}39$ D starting from *IIa* to *IIb*) suggests a stronger stabilization of the conformation *IIb* in solution. Our results illustrated in Fig. 2 confirm this expectation. However, the conformation *IIa* should also dominate in apolar solvents due to the smaller change of the dipole moment in comparison to the situation in nicotinamide (*I*). This finding corresponds to the results of the dipole moment analysis.

There are some indications that the oxidized and reduced part of the pyridine coenzyme are located in a strongly apolar enzyme region. The results taking into account the tetrachloromethane solvation of the compound *II* provide a reasonable basis for the discussion of the molecular structure of the reduced form of the pyridine coenzymes in biochemical processes. The results obtained for nicotinamide (*I*) cannot be used in this way because of the quaternary structure of the oxidized form. Both EHT (ref.[25]) and the first estimations by means of the NDDO method[26] sug-

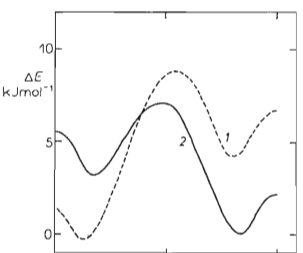


FIG. 3

NDDO Potential Energy Curve for Nicotinamide without 2 and with 1 Inclusion of the Solvent (tetrachloromethane)

gest a molecular arrangement corresponding to the structure *Ia* as the most stable conformation of oxidized nicotinamide. Consequently, the same orientation of the amide group should be preferred in the reduced and oxidized part of the pyridine coenzymes in contrast to the conformation in free nicotinamide (*I*).

Based on NDDO wave functions, the same conclusions can be reached as in the case of the enzymatic redox process as already shown using CNDO/2 and EHT results, respectively[6,7]. NDDO conclusions can therefore be shortly summarized in the following way: *a*) The net charge of the hydrogen atoms ($\Delta Q = -0{\cdot}0360$) in the methylene group of *IIa* is the most negative one of all hydrogen atoms implying a stronger capability of these atoms to act as reducing centre. *b*) The CH bonds of the methylene group in *IIa* possess the lowest value of Wiberg's bond index ($W_{CH} = 0{\cdot}947$) suggesting a preferred cleavage of these bonds in redox processes. *c*) Analysis of the HOMO seems to be useful considering the participation of the methylene group of *IIa* in the enzymatic reduction and assuming the importance of the most reactive electrons. This analysis proves that the HOMO represents a pseudo-π-orbital characterized by the coefficients of the ring atoms including in considerable amount methylene hydrogen atoms. The numerical values of the coefficients are almost identical with the CNDO/2 values already given[6] indicating a hyperconjugative effect of the methylene group.

We wish to express our gratitude to Dr V. Skála, Prague Institute of Chemical Technology, and Dr W. Schöpp, Sektion Biowissenschaften, Karl-Marx-Universität, Leipzig, for the helpful discussions and Dr P. Birner, Sektion Chemie, Karl-Marx-Universität, Leipzig, for the possibility to use his NDDO program.

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