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THEORY OF THE NON-PLANAR PEPTIDE UNIT*

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SUMMARY

By means of CNDO/2 calculations on N-methyl acetamide, it is shown that the state of minimum energy of the *trans*-peptide unit is a non-planar conformation, with the NH and $NC_2{}^a$ bonds being significantly out of the plane formed by the atoms $C_1{}^a$, C', O and N.

THEORY OF THE NON-PLANAR PEPTIDE UNIT

In the last few years, many detailed calculations on simple peptides and polypeptides have been carried out using empirical potential functions (see the reviews by Ramachandran and Sasisekharan[1] and by Scheraga[2]) and also using quantum chemical methods[3-5]. In most of these calculations, the standard, completely planar, *trans*-peptide unit of dimensions given by Pauling and Corey[6] as early as 1951 (Fig. 1) has been used without any significant changes. However, the necessity of considering non-planar peptide units was pointed out by Ramachandran[7] in the case of small cyclic peptides. More recently, the non-planar distortions of the *trans*-peptide unit were considered by Levitt and Lifson[8] and by Winkler and Dunitz[9]. Very recently[10], during a study made in the authors' laboratory to correlate the NH–C^{a}H coupling constants (J) and the potential energy calculations in peptides, it was found that, although the data for most peptides were in agreement with theory, the calculated values of J in a few cases with bulky side chains did not agree with the measured values. It appears that the agreement can be improved in these cases when the concept of a strictly planar peptide unit is relaxed and out-of-plane distortions of the N–H bond are permitted. All these pointed to the need for making theoretical calculations on the non-planar peptide unit of the most general type.

Deviations from the *trans*-amide unit, characterised simply by the rotation ω (we follow the IUPAC–IUB conventions[11] regarding the notations) about the C'–N bond leading to the change from a *trans*- to a *cis*-peptide unit has been studied in some detail[12-14] and the deviations can be represented by a potential of the type

$$V(\omega) = \tfrac{1}{2} K_\omega (1 - \cos 2\omega) \tag{1}$$

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and a rounded-off value of 20 kcal/mole has been recommended for K_ω by Ramachandran and Sasisekharan[1] and by Levitt and Lifson[8]. In addition to this simple ω-rotation, Ramachandran and Sasisekharan[1] had indicated other possible types of non-planar distortions of the peptide unit which produce an out-of-plane movement of the carbonyl oxygen and the amide hydrogen. They described these by the symbols v_O and v_H. The existence of such distortions in several medium-ring lactams has been demonstrated by Winkler and Dunitz[9]. These distortions have also been observed in crystal structures of open chain peptides[15,16]. While it is almost certain that large non-planar distortions in lactams like caprylolactam[9] are demanded by cyclization, it is not clear whether the free peptide unit has the distortions or whether they are produced by intermolecular interactions in the other cases cited above. It was therefore considered desirable to calculate the energy of a single peptide unit as a function of the non-planar distortion angles.

NOTATION

As mentioned above, the out-of-plane movement of O and H were described by Ramachandran and Sasisekharan by the angles v_O and v_H. In their study on lactams, Winkler and Dunitz have discussed in great detail the dihedral angles describing the non-planar peptide unit and we shall mostly follow their notation in this paper, but with some minor changes. They define four torsion angles as follows:

$$\omega(C_1 C'N C_2) = \omega_1 = \omega \tag{2a}$$
$$\omega(OC'NH) = \omega_2 \text{ (not used in this study)} \tag{2b}$$
$$\omega(OC'NC_2) = \omega_3 = v_O \tag{2c}$$
$$\omega(C_1 C'NH) = \omega_4 = v_H \tag{2d}$$

Of these, ω_1 is the conventional ω-rotation, which is equal to $0°$ for a planar *cis*-peptide unit and takes the value $180°$ for a planar *trans*-peptide unit. The last two, namely ω_3 and ω_4 are identical with the rotations v_O and v_H, respectively, as defined by Ramachandran and Sasisekharan. The second angle ω_2 is not explicitly used here. In fact it is not an independent variable, but is related to the other three by the equation

$$(\omega_1 + \omega_2) - (\omega_3 + \omega_4) = 0 \ (\text{mod} 2\pi) \tag{3}$$

In addition to these, Winkler and Dunitz have defined two other angles, which indicate the non-coplanarity of the three bonds meeting at C' and N, for which they have used the symbols χ_C and χ_N, respectively. We prefer to use the symbols $\theta_{C'}$ and θ_N for these angles, since the symbol χ has been reserved in the IUPAC–IUB conventions[11] for the side-chain dihedral angles. (Note that C' is used in the subscript rather than C to indicate that the carbonyl carbon C' is involved in this distortion). Following Winkler and Dunitz, we may denote these by the descriptive notations,

$$\theta_{C'} = \theta \ (C_1{}^\alpha, O; C'N) = \omega_1 - \omega_3 + \pi \tag{4a}$$
$$\theta_N = \theta \ (C_2{}^\alpha, H; NC') = -\omega_1 + \omega_4 + \pi \tag{4b}$$

The angle $\theta_{C'} = \theta \ (C_1{}^\alpha, O; C'N)$ is the angle of rotation (positive in the clockwise sense when looking from N towards C') from the plane of $NC'C_1{}^\alpha$ to the plane of $NC'O$, the angle being less than $180°$. Likewise, the angle $\theta_N = \theta \ (C_2{}^\alpha, H; NC')$ is the angle of rotation (positive when clockwise looking from C' towards N) from the plane $C'NC_2{}^\alpha$ to C'NH.

METHOD

The total molecular energy of the model compound *N*-methyl acetamide was calculated as a function of the angles ω, θ_N and θ_C' by employing quantum chemical methods. The CNDO/2 method devised by Pople and Segal [17] and found to be successful in predicting molecular geometries[18] was used in our studies. The Fortran IV computer program, QCPE 141, was obtained for this purpose from the Quantum Chemistry Program Exchange, Indiana University, Bloomington, Ind. The geometry shown in Fig. 1 was adopted for the molecule and the two atoms $C_1{}^a$ and $C_2{}^a$ were taken to be methyl groups with C–H bonds of length 1.1 Å and having tetrahedral symmetry.
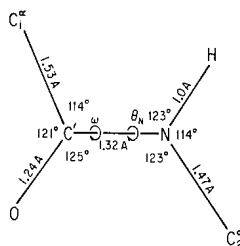

Fig. 1. Bond lengths and bond angles of the peptide unit, used in the calculations. The relevant dihedral angles are $\omega = \omega(C_1{}^a C' N C_2{}^a)$ and $\theta_N = \theta(C_2{}^a H, N C')$.

The methyl groups were kept in the staggered conformation about the C'–C or the N–C bond. *N*-Methyl acetamide has been used as a model compound for the peptide unit by several workers and its use here for calculations of non-planarity also appears to be reasonable.

RESULTS

Two sets of calculations were carried out, by introducing out-of-plane distortions at the atom N, and at the atom C', separately. In terms of the dihedral angles defined above, this would correspond to the following variations: (A) $\theta_C' = 0$; vary ω (by $\Delta\omega$ from the planar conformation with $\omega = 180°$), and, for each ω, vary θ_N. (B) $\theta_N = 0$; vary ω by $\Delta\omega$, and, for each ω, vary θ_C'.

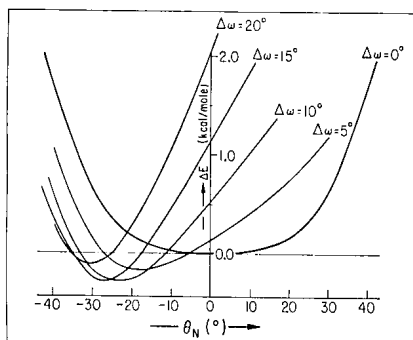Fig. 2. Variation of ΔE, the change in energy from the planar conformation, with ω and θ_N. Note that the minimum energy corresponds to a non-planar peptide unit, characterised by $\Delta\omega$ and θ_N different from 0°.

The variation of the total energy with non-planar distortions at N (Set A) is shown graphically in Fig. 2, for $\Delta\omega = 0°, 5°, 10°, 15°$ and $20°$ and θ_N varying from about $-40°$ to about $+40°$. The energy values ΔE are given relative to the energy for the completely planar *trans*-conformation, namely, $\Delta\omega = \theta_N = \theta_C' = 0°$ ($\omega = 180°$). Thus,

$$\Delta E (\omega,\theta_N) = E (\omega,\theta_N,\theta_C' = 0) - E (180°, 0°, 0°) \tag{5}$$

It can be seen from the energy variation that when $\Delta\omega = 0°$, ($\omega = 180°$) the energy increases for all θ_N; in other words, when the atoms $C_1{}^\alpha$, C', N and $C_2{}^\alpha$ are coplanar, moving the amide hydrogen atom H out of plane only increases the energy. But this is not so when ω is different from $180°$. For instance, for $\Delta\omega = 5°$, the energy of the distorted peptide is clearly less than that of the planar unit for θ_N lying between about $-5°$ and $-25°$. Our calculations seem to show that the energy decrease is largest when $\Delta\omega$ is between $10°$ and $15°$ (say $12.5°$) and θ_N between $-22°$ and $-28°$, (say $-25°$) and has a value of -0.26 kcal/mole. For $\Delta\omega$ greater than $20°$, *i.e.* $\omega > -160°$, the curve for ΔE does not go below the horizontal axis ($\Delta E = 0$).

Similar calculations made by varying ω and θ_C', (Set B, keeping $\theta_N = 0°$) did not yield any range of these distortion angles for which ΔE was negative. Also, the energy differences for a given change in θ_C' were much larger for this distortion than for corresponding values of θ_N.

DISCUSSION

The results described above clearly indicate that there is a stable conformation of the peptide unit which is non-planar, whose energy is lower than that of a planar peptide unit. This conformation of least energy may be taken to have approximately the dihedral angles $\Delta\omega = +12.5°$ ($\omega = -167.5$), $\theta_N = -25°$ (or $\omega_1 = -167.5°$, $\omega_4 = 180 - 12.5° = 167.5°$). This non-planarity is shown schematically in Fig. 3a.
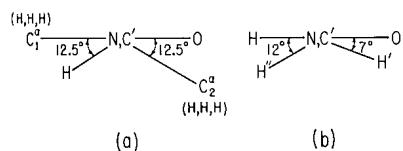

(a)   (b)

Fig. 3. Projection of the peptide unit down the bond NC'— (a) corresponding to the minimum energy conformation obtained from Fig. 2 for *N*-methyl acetamide; (b) as reported for formamide by Costain and Dowling[19].

This may be compared with the non-planar nature of the stable conformation of formamide as deduced by Costain and Dowling[19] from its microwave spectrum, shown in Fig. 3b.

It may be mentioned here that the distortion at N could also have the atoms H and $C_2{}^\alpha$ above the plane formed by the four atoms $C_1{}^\alpha$, C', O and N, instead of being below this plane, as shown in Fig. 3a. Such a stable conformation would correspond to $\Delta\omega = -12.5°$ and $\theta_N = +25°$. As a matter of fact, we have not shown any of the energy curves for negative values of $\Delta\omega$ in Fig. 2 because these are obviously related by a mirror symmetry about $\Delta\omega = 0°$ to those drawn there. However, these two symmetrically related conformations will have the same energy only in com-

pounds like N-methyl acetamide which are not optically active. They may be different in peptide units having amino acid side chains which may have the L- or the D-configuration at C^α and thus be asymmetric.

Thus, the CNDO/2 calculations reported here indicate that the lowest energy for a peptide unit occurs for a non-planar conformation, in which there is a rotation about the N–C′ bond as well as a nonplanar arrangement of the three bonds at N. They also show that it is much more facile to displace H from the plane of the atoms C′, N and $C_2{}^\alpha$ than to displace O from the plane of C′, N and $C_1{}^\alpha$. However, the difference in energy $| \Delta E |$ (=0.26 kcal/mole) between the planar conformation and non-planar conformation of lowest energy is much smaller than the value of kT, which is 0.6 kcal/mole at room temperature. Hence, the molecule may be expected to have a range of conformations in solution near the planar one.

It may be pointed out that the CNDO/2 method is not considered to be accurate in predicting the values for the energy associated with variations of torsional angles. However, our calculations give the correct order of variations of ΔE_ω with ω. For small values of $\Delta\omega$, it has the form

$$\Delta E_\omega = K_\omega (\Delta\omega)^2 \tag{6}$$

where K_ω has a value of 17.5 kcal/mole, close to the accepted value of 20 kcal/mole[1,8]. This gives one some confidence in the theoretical results reported above. Quite apart from this, the fact that the conformation of minimum energy has the same type of non-planarity at N as has been observed for formamide, is in further support of the qualitative prediction of the theory, which should be explored further by using experimental techniques. As in formamide, it is quite likely that the conformation at N may be undergoing rapid inversions, so that the values calculated from the mean planar conformation may be accurate enough for many physical quantities, such as the NMR J-values. However, if, in some compounds, one of the non-planar conformations is hindered by steric repulsion, it would be necessary to include specifically the non-planar nature of the peptide unit in making theoretical calculations from such compounds. We feel that the consideration of such effects would probably resolve the discrepancies noted for the J-values in some dipeptides with bulky side groups. This is being investigated.

It may also be mentioned that the same theory and programme as was used in the present investigation were used to study the non-planarity of the amide group in acetamide and formamide. Both of them were found to have the minimum energy when the three bonds at the nitrogen atom were non-planar, the depression of energy below the planar conformation being about -1.0 to -1.5 kcal/mole in both cases (much larger than the value mentioned above for N-methyl acetamide). The details of these calculations will be reported elsewhere. However, it may be mentioned that the value -1.3 kcal/mole for formamide agrees fairly well with the value -0.9 kcal/mole as calculated from the spectroscopic data of Costain and Dowling.[19]

ADDENDUM (Received November 15th, 1972)

After this paper was sent to the journal, it has been found from an analysis of published crystallographic structures of peptides containing the atoms of a full peptide unit, that the deviation from planarity of the three bonds at the carbonyl

C'-atom is very small, while it is larger in the case of the three bonds attached to the nitrogen atom. Also, the nonplanarity that occurs at the nitrogen atom is of the type deduced in this paper, namely ω and θ_N having opposite signs and θ_N having roughly twice the value of ω.

Further, calculations made with the formulation INDO of quantum chemistry, also yield results which are closely similar to the results arrived at in this paper. These will be published in detail in due course.

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