ASPECTS OF PEPTIDE CONFORMATION*†

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ABSTRACT

The paper consists of a critical review of the potential functions and other data that have been proposed for use in the theory of peptide conformation. It is pointed out that, although they all agree broadly in regard to the low-energy regions of the (ϕ, ψ)*-map for an alanyl dipeptide, all of them disagree with the experimentally determined probability distribution (Ref.* 9), *in which the region between* $\psi = 60°$ *and* $\psi = 120°$ *is relatively unpopulated. From this, and other quantum chemical calculations, as also the observed distribution of* ψ*-values in amino acids and amides, a new form is suggested for* $V(\psi)$, *namely* $V(\psi) = 2{\cdot}0\ (1 - \cos 2\psi)$. *Further, the need to have softer potentials for H . . . H and H . . . X interactions is pointed out. These changes lead to much better agreement with the observed conformational parameters for the LL-bend. So also, observed characteristic ratios of random coils of polypeptides having a* C^{β}*-atom attached to* C^{α} *agree better with the theoretical value if such modifications are made in the potential functions. The author feels that the potential functions used at present should be carefully reviewed and revised by comparison with authenticated observational data.*

Comparison between calculated minimum energy locations and orientations, and those observed, of benzene and N-methyl acetamide in their crystal structures indicate that the functions proposed from different laboratories lead to widely different values for the stabilization energy, although they all agree as to the location and orientation of the molecule in the case of benzene. Although there is reasonable agreement between theory and experiment for N-methyl acetamide, it is not considered to be good enough, and the potential functions have to be evaluated more critically before they are applied to more complicated examples.

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The non-planarity of the peptide unit is considered both from quantum chemical calculations and its observed distribution in about thirty examples of accurate crystal structures. Both indicate that changes in ω *from planarity of upto* 10°, *and of* θ_N *(describing the non-planarity of the three bonds meeting at the nitrogen atom) upto* 25°, *are quite feasible and lead only to destabilizing energies less than RT. Similar results are also obtained for formamide, where INDO calculations agree well with microwave data on non-planarity. The non-planar peptide unit requires careful consideration in relation to the solution of crystal structures of globular proteins.*

Key words: Conformation of peptides, Potential energy functions, Planarity of peptide units.

1. INTRODUCTION

In a paper submitted to the Conference on 'Conformation of Biological Molecules and Polymers' [1] held in Jerusalem last year, the author discussed the accuracy of the empirical potential functions adopted in his laboratory. In fact, the form of the potential functions adopted by different groups [2], [3], [4], [5], [6], particularly for the non-bonded interactions, are not very different from one another. However, the values of the constants used differ to some extent. The general impression from the experience in the author's laboratory seems to be that, when a relatively stable minimum-energy conformation exists, it can be detected to a reasonably good accuracy, using potential functions having widely different parameters. An example of this, namely, the orientation of the benzene molecule in its orthorhombic crystal structure at low temperatures, is discussed in one of the succeeding sections. In this case, the calculated Eulerian angles were closely similar when obtained from the use of potential functions which differ as much from one another, as those shown in Fig. 1. On the other hand, the question is not so simple when one considers torsional potential functions, as applied to the conformation of a pair of peptide units. In this case also, the energy contours in the (ϕ, ψ)-plane are very similar for the different types of formulae and constants used by different laboratories [2], [4], [7], [8]. On the other hand, they all differ from the probability distribution of observed conformations in the (ϕ, ψ)-plane as observed in various globular proteins (reported by Pohl [9]). In particular, the region between $\psi = +45°$ and $+135°$ is relatively unocccupied in Pohl's diagram, while a low energy contour extends down to about $\psi = +90°$ from higher values of ψ in the various diagrams calculated by theoretical workers, as mentioned above. This brings to focus the idea that probably the variation of the potential energy $V(\psi)$ with ψ may not have the correct form as currently adopted and that it may have a much higher barrier near about $\psi = 90°$. This is discussed below.

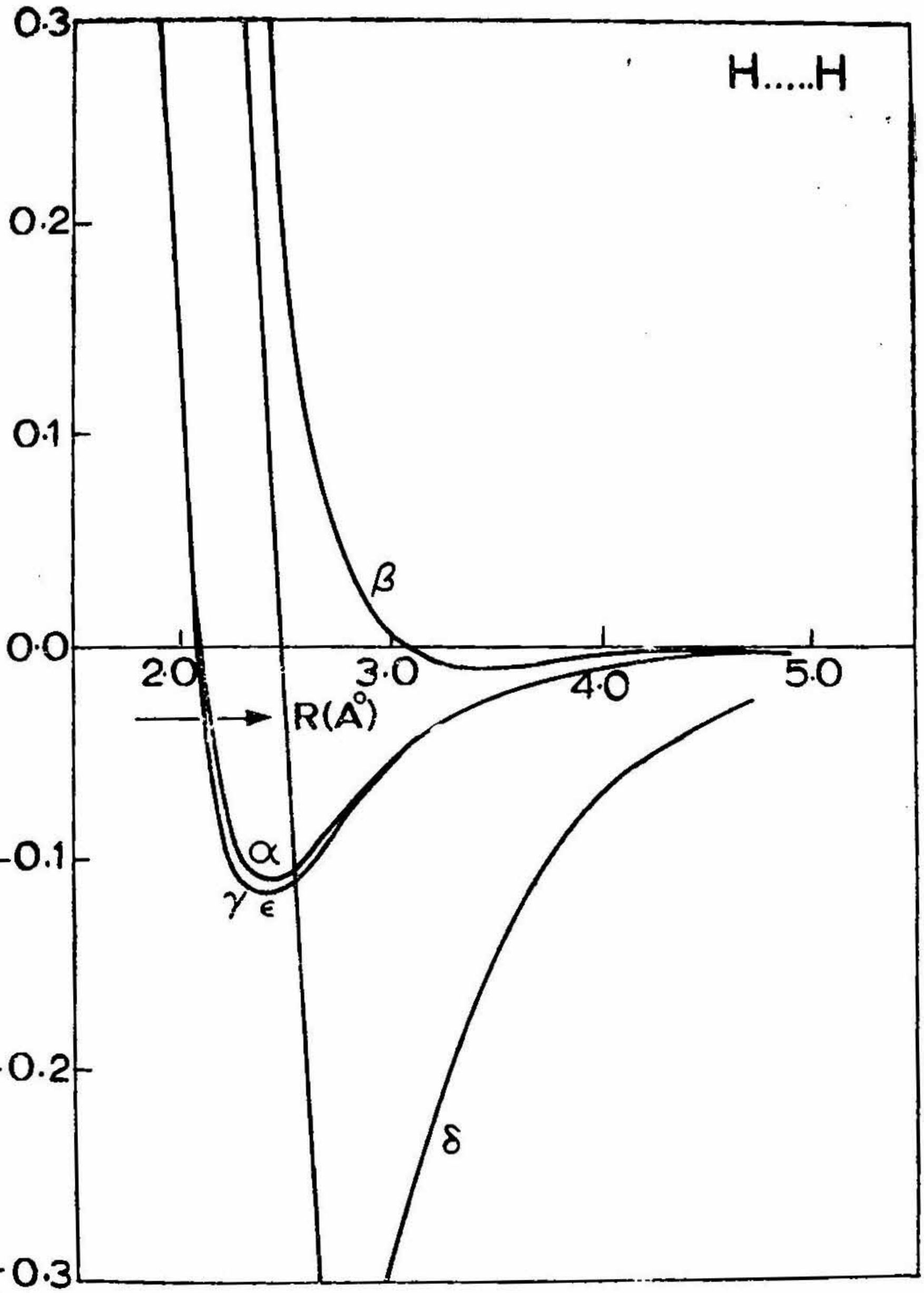


FIG. 1. The variation of the potential functions for the non-bonded interactions H...H, C...H and C...C, as adopted in different laboratories. These are (α) ours [2], (β) Williams [3], (γ) Scheraga [4], [δ] Scheraga (packing) [5] and (ε) Kitaigorodsky [6].

So also, in a recent study reported, about the barrier to rotation around glycosidic C—N linkages in nucleosides and nucleotides [10], the observational

data appear to be best fitted by using a van der Waals radius of 1·0 Å for the hydrogen atom, rather than the normally accepted value of 1·2 Å [2], [11]. It can be readily shown by qualitative considerations that the bridge region, marked IV, in the contact map on the (ϕ, ψ)-plane [*see* for contact map Fig. 12 (*a*) in page 328 of Ref. 2] would become reduced if the normal and the extreme limits are relaxed by 0·2 Å for the H . . . H contact and 0·1 Å for H . . . X contacts, where X may be C, N or O. Correspondingly, the region on either side of the line $\psi = 0°$ in the middle of the left hand side of the (ϕ, ψ) map will also have appreciably lower energies. This region is relatively well populated in Pohl's probability map.

All these indicate that the currently employed potential functions in the biopolymer field have to be carefully checked and revised, by taking into account various observational facts like those mentioned above. In doing so, it would be worthwhile checking whether other observational data may be found, which are better explained by the newer criteria for the parameters put into the potential functions. This paper deals essentially with such problems, with special reference to the observed conformations of peptides and the molecular packing of N-methyl acetamide in its crystal structure.

In addition to these, we shall also consider the energy variation involved in the non-planar distortions of the peptide unit itself. Recent studies made in the author's laboratory [12], [13], [14] indicate that such distortions are quite feasible and must be taken into account if a complete picture is to be obtained of the energies associated with different conformations of a peptide chain. In fact, discussions with workers in the field of protein crystal structures have indicated that a much better fit of the experimental electron density can be obtained if the peptide unit used in building the model is not rigidly planar, but has appreciable flexibility. As will be shown below, even the crystal structure data of simple peptides indicate that ω-distortions of 10° and more can occur. Although quantum chemical calculations were first used to justify the proposal of a non-planar peptide unit [12], we shall present in this paper an outline of experimental energy curves obtained from microwave data and from X-ray crystallography, which closely correspond to the theoretical predictions, indicating that this feature of the peptide unit can be considered to be firmly established from experimental observations.

2. MODIFIED PSI POTENTIAL

Considering the backbone atoms of two linked peptide units C_1^{α}—C_1—N_1—C_2^{α}—C_2—N_2—C_3^{α}, the potential functions associated with the

torsional angles ϕ (C_1—N_1—C_2^{α}—C_2) and ψ (N_1—C_2^{α}—C_2—N_2) have normally been taken to have the following forms:

$$V(\phi) = \tfrac{1}{2} V_{\phi} (1 \pm \cos 3\phi) \qquad (1)$$

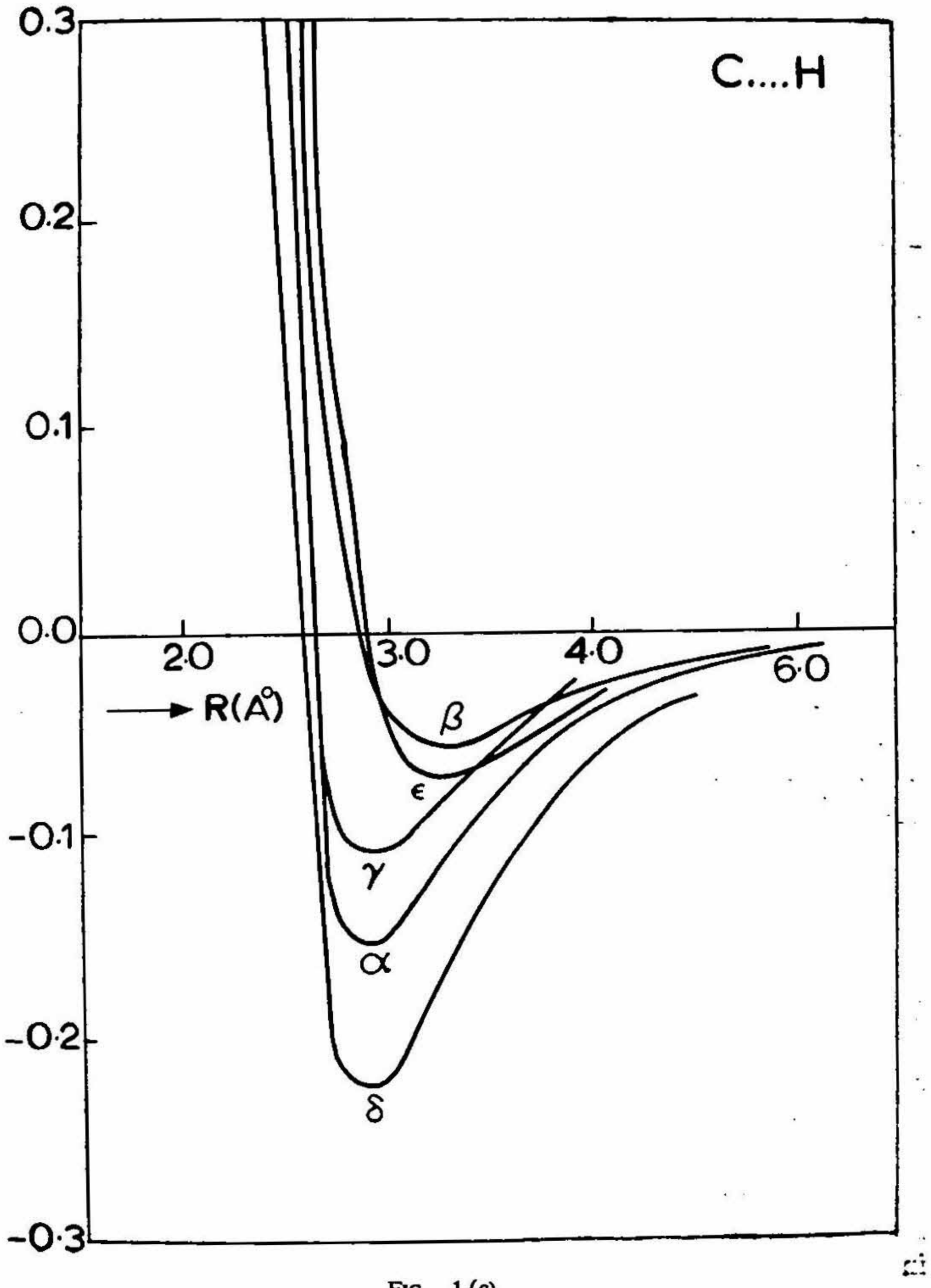


FIG. 1 (*a*)

with V_ϕ varying between 0·6 and 1·5 kcal/mole; and

$$V(\psi) = \tfrac{1}{2}V_\psi (1 - \cos 3\psi) \tag{2}$$

with V_ψ having values between 0·25 and 1·0 kcal/mole. The shape of the energy contours in the (ϕ, ψ)-plane are not appreciably modified by changing the values of V_ϕ and V_ψ within these ranges. In fact, as mentioned in Ref. 1, the mean value of the proton-proton coupling constant J ($H^\alpha C^\alpha$—NH), averaged over variations of the conformational angles, agrees reasonably well with the observed J-values for a number of different side chains attached to C_2^α, when the potential functions adopted in our laboratory [2] are used. This would indicate, at first sight, that the theoretical energy distribution in the (ϕ, ψ)-plane is reasonably accurate. However, other considerations, like those mentioned in the introduction, namely, the agreement of the theoretical energy contours with the observed distribution of conformations as found in various globular proteins, indicate that the torsional potential $V(\psi)$ must have a different form of variation with ψ. Infrared studies of amides made by Shimanouchi [15] also indicate that the most stable conformations correspond to $\psi = 0°$ or $\psi = 180°$. This is also confirmed by the data on the conformation of the terminal carboxyl group in a large number of amino acids and peptides in all of which $\psi^{(1)}$ and $\psi^{(2)}$ are close to 0° and 180° [16], [17]. In view of these, quantum chemical calculations were made in our laboratory, using the IEHT and an *ab-initio* method on model compounds [18] which indicated that $V(\psi)$ has minima for two values of ψ, namely, $\psi = 0°$ and 180° and a barrier of approximately 4·5 kcal/mole in between these. In fact, Pople and Radom [19] presented some data in the last Jerusalem symposium which also indicated similar results, but pointing to probably larger values of the barrier height.

All the quantum chemical calculations agree as regards the existence of two-fold minima for $V(\psi)$ and the value of a barrier height of about 4·0 kcal/mole or more, which is much larger than the barrier height of 1·0 kcal/mole, which is the largest value used at present. Also, the three-fold minima for $V(\psi)$, as currently used, is contradicted. In view of these, a function of the form

$$V(\psi) = 2{\cdot}0 (1 - \cos 2\psi) \tag{3}$$

was employed to calculate the energy distribution in the (ϕ, ψ)-plane. The result is shown in Fig. 2 (*b*) and is seen to be markedly different from that in Fig. 2 (*a*) which was calculated using the conventional form of the

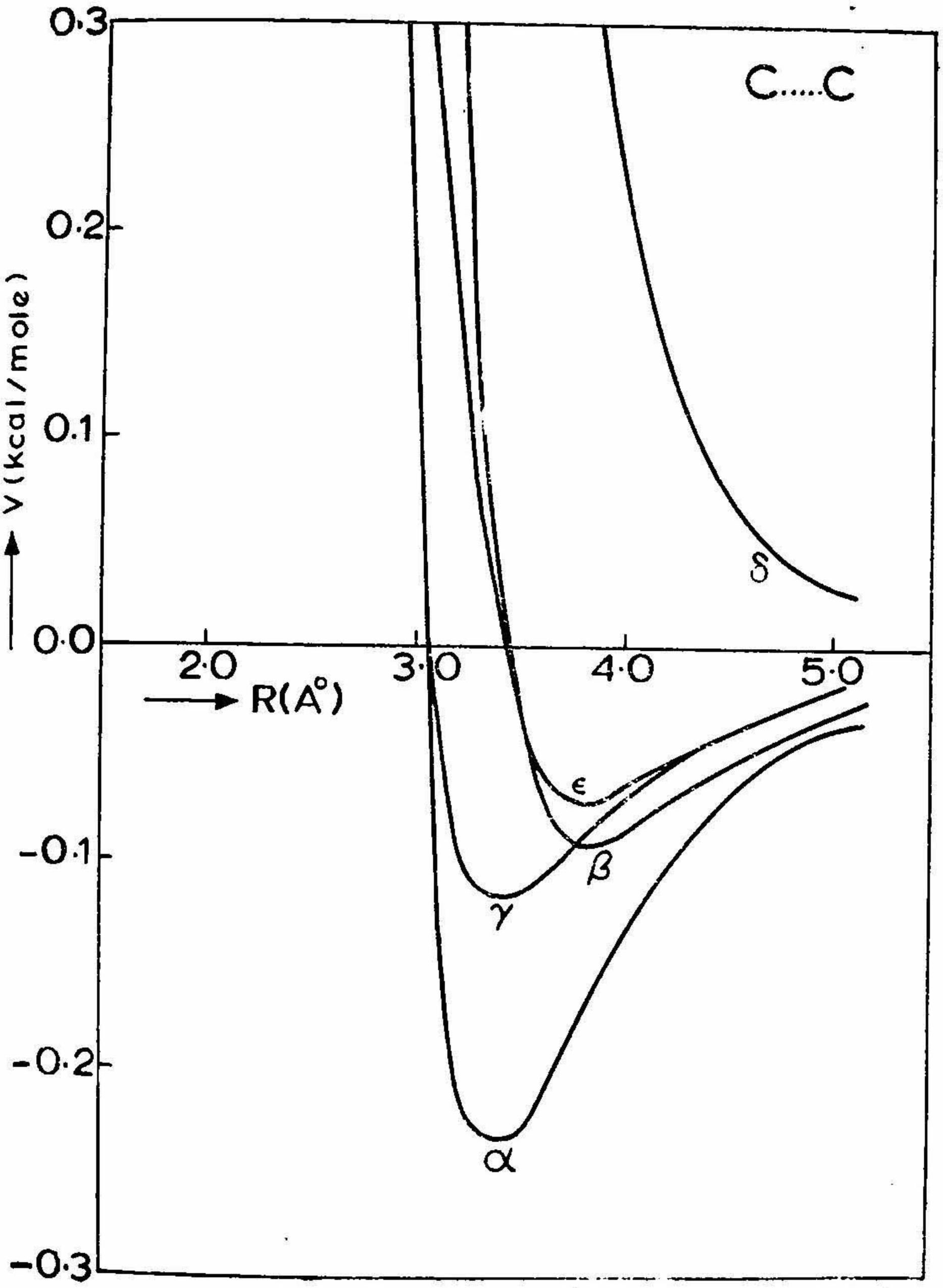


FIG. 1 (*b*)

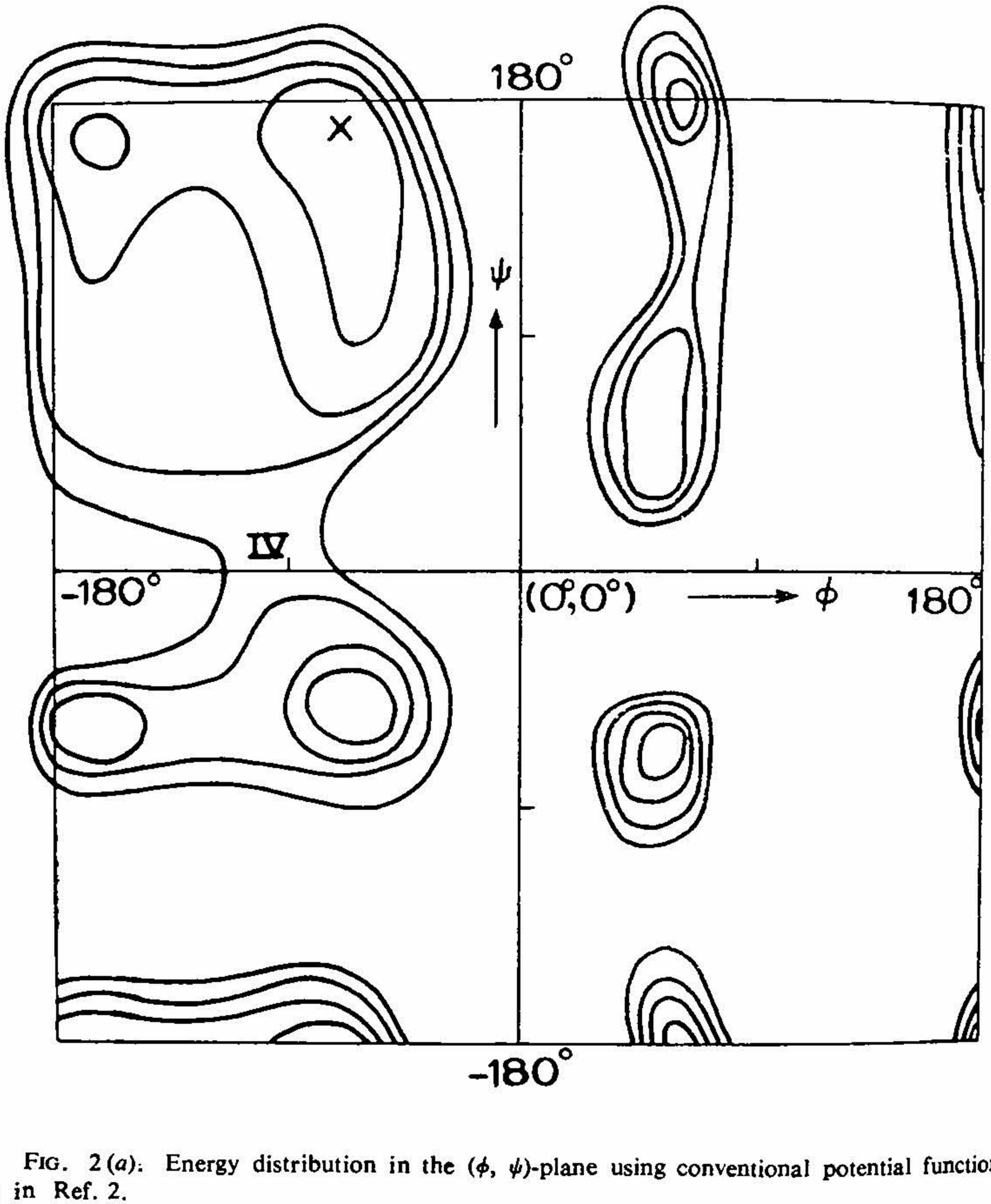


FIG. 2 (*a*). Energy distribution in the (ϕ, ψ)-plane using conventional potential functions as in Ref. 2.

potential functions. The experimental distributions as obtained from globular protein data [9] is shown in Fig. 3. It will be readily observed that Fig. 2 (*b*) is in much better agreement with Fig. 3 than Fig. 2 (*a*). In making this comparison, it should, however, be remembered that the experimental distribution obtained from protein structures will be modified by the existence of regular structures like alpha-helical segments (ϕ, ψ) near ($-60°$, $-60°$)

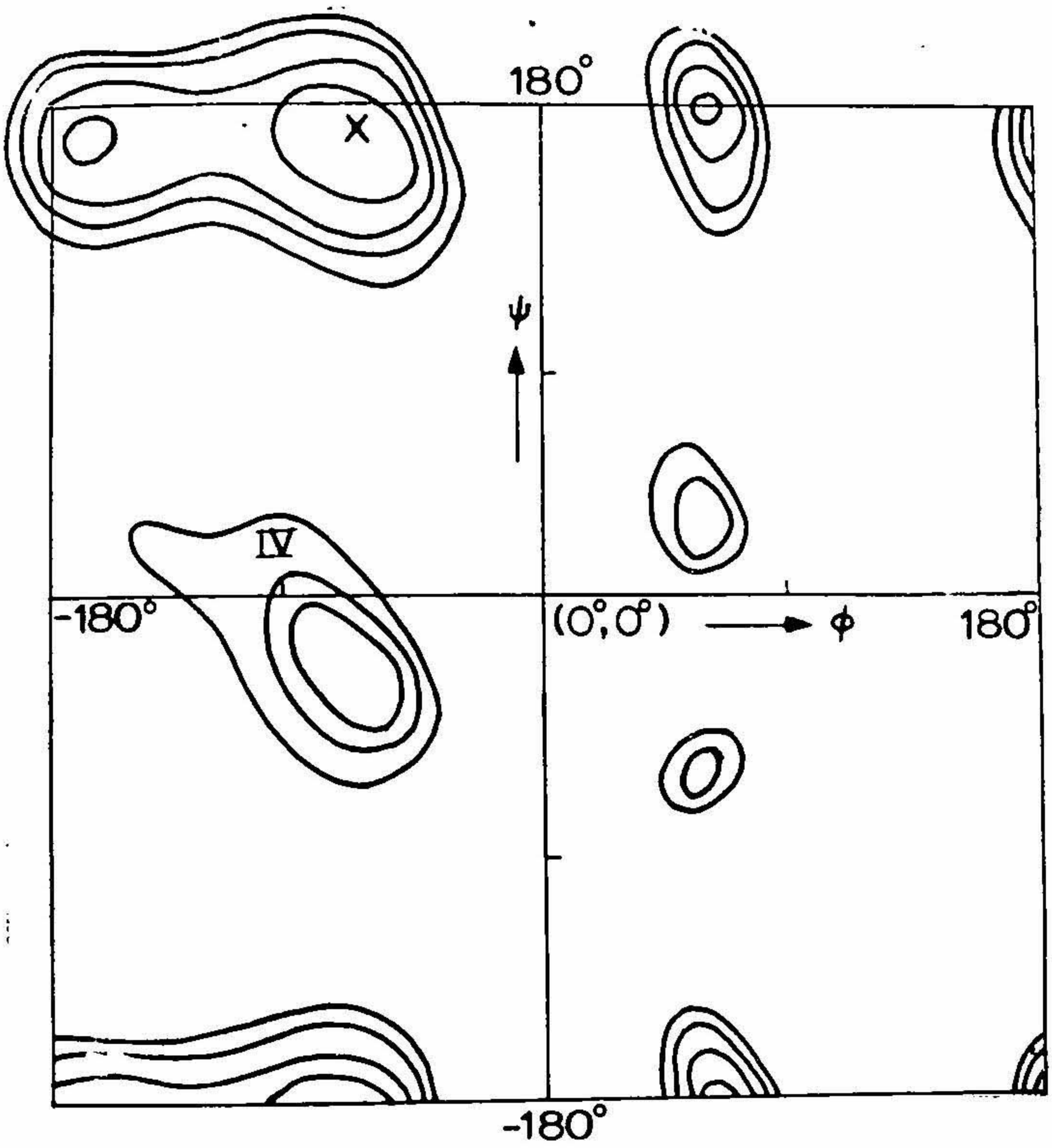


FIG. 2 (*b*). Energy map using the potential function $V(\psi) = 2{\cdot}0\ (1 - \cos 2\psi)$, the other functions being the same as in Ref. 2.

and plated beta-sheets (ϕ, ψ) near ($-$ 120°, + 120°), whose presence is not to be expected to be emphasized in the energy map for the interaction of a pair of peptide units.

However, even the energy map in Fig. 2 (*b*) does not appear to be sufficient to explain the fact that the accumulation of points in the occupied regions near $\psi = 0°$ is of the same order as for the region near $\psi = 180°$,

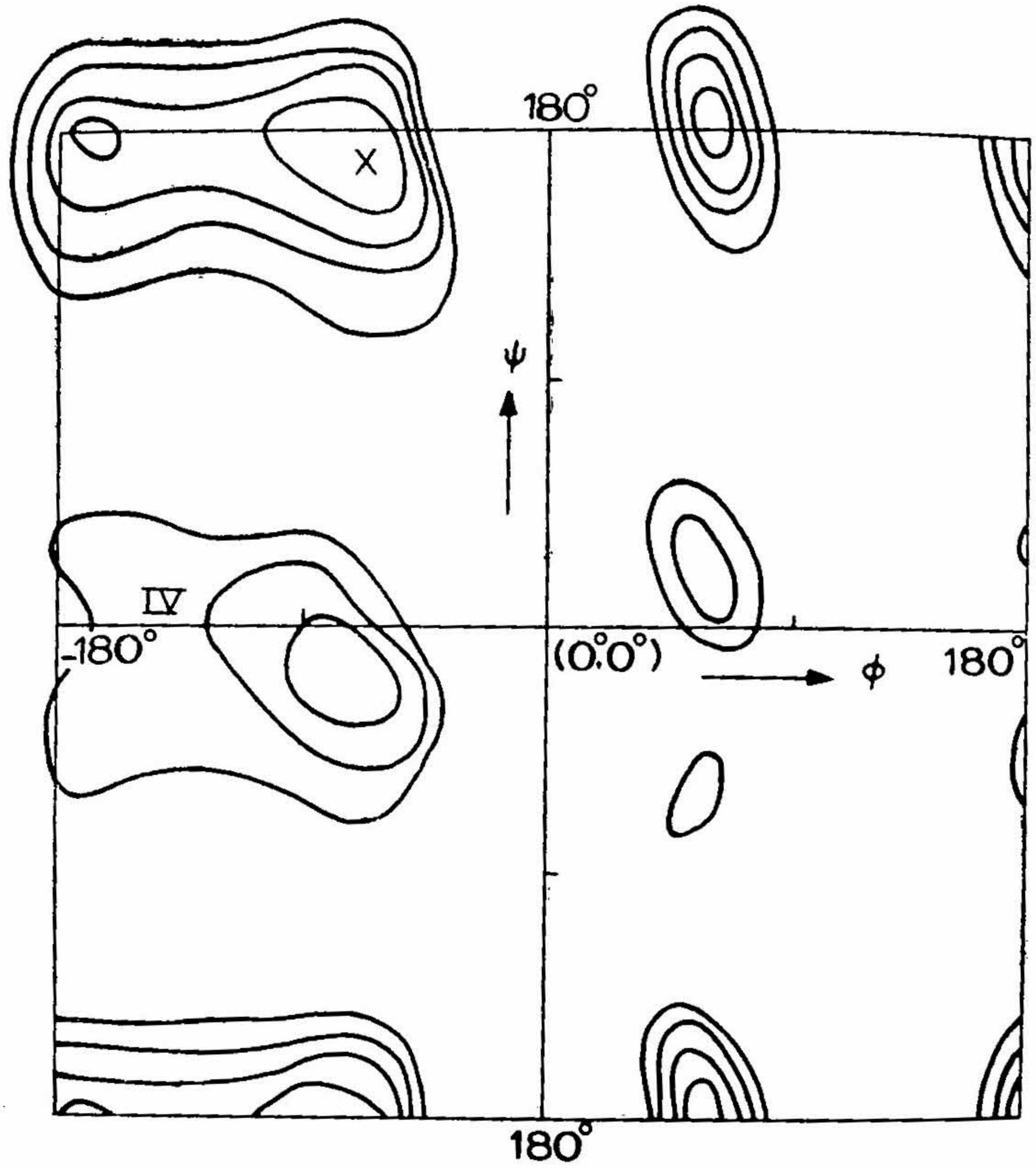


FIG. 2 (*c*). Energy map using $V(\psi) = 2{\cdot}0\ (1 - \cos 2\psi)$ and all interactions involving hydrogen atoms being softened (see text for details).

in Fig. 3. In fact, the region near $\psi = 180°$ is much more occupied in the theoretical plot in Fig. 2 (*b*). This is due to the fact that there are restricting short contacts of the types $N_1 \ldots H_2$ and $H_1 \ldots H_2$ near $\psi = 0°$, if thg contact criteria in Ref. 2 are used. Correspondingly, with standard van der Waals radii, the bridge region IV, will have comparatively high energy values in the energy map. It would appear, therefore, that this region has greater probability of occurring than what is predicted by our theory in Fig. 2 (*b*). One possible method whereby this greater probability, which

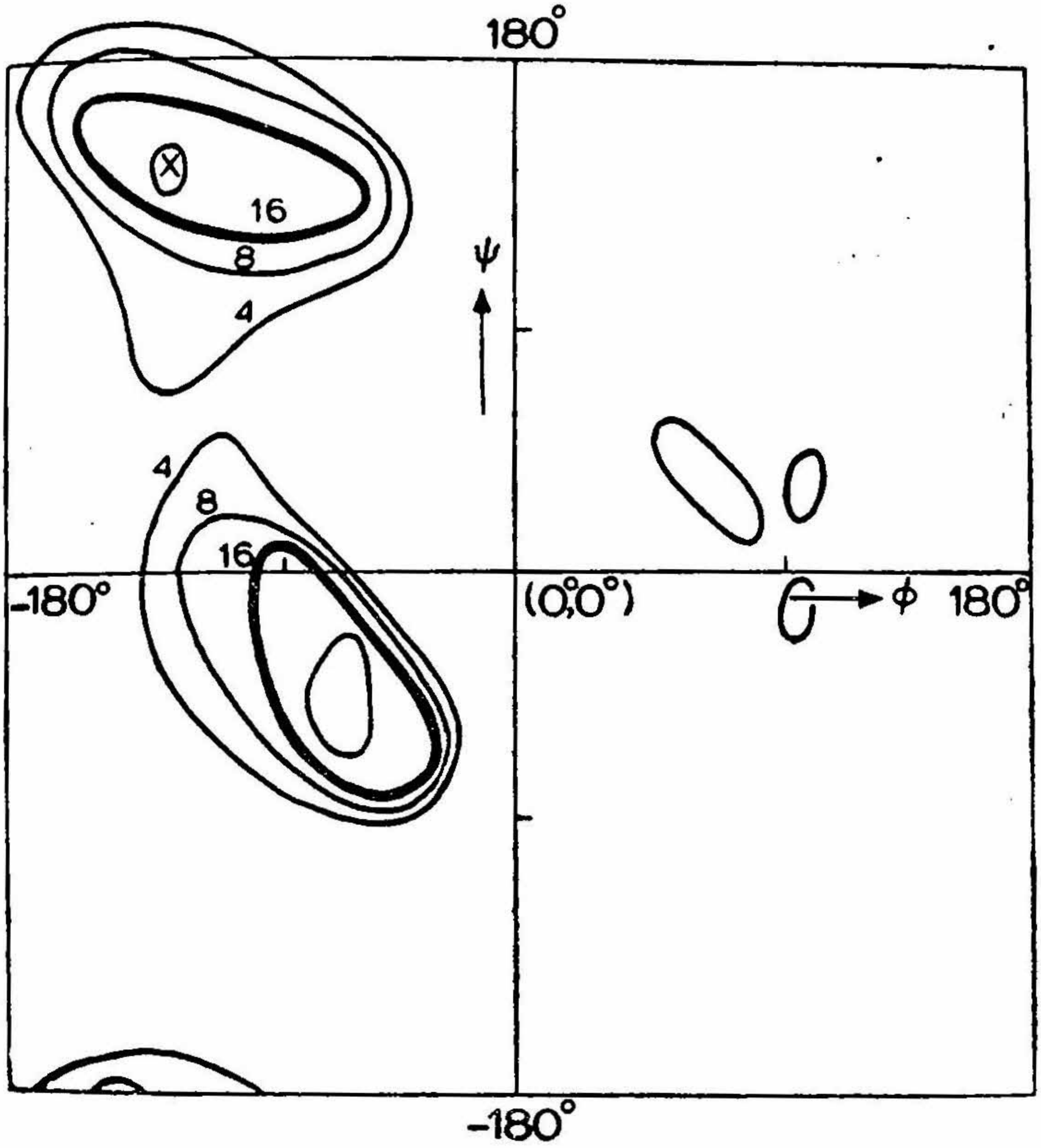


FIG. 3. Probability distributions in the (ϕ, ψ)-plane of the conformations observed in a number of crystal structures of globular proteins (copied from Ref. 9).

corresponds to lowering of energy, can be achieved is by making the interactions between hydrogens and other atoms more soft, that is to have lower energies for smaller values of the contact distances. As was mentioned in the introduction, such a reduction of the van der Waals radius of hydrogen has been found to be reasonable from nucleotide data [10]. (In a personal discussion in 1973, Prof. H. A. Scheraga also indicated to the author that his laboratory was also thinking on similar lines. These ideas are referred

to, but not outlined, in the paper by Ponnuswamy *et al.* [20], from his laboratory.) Therefore, the consequences of introducing both the high barrier, the two-fold $V(\psi)$ potential, and the softer interactions between hydrogen and other atoms, was tried in our laboratory [22] and the result is shown in Fig. 2 (*c*). It is seen that the low energy contours in Fig. 2 (*c*) are in better agreement with the experimental probability contours in Fig. 3, than those in Fig. 2 (*b*), indicating that the softening of the potentials for interactions involving hydrogen atoms is quite reasonable.

The improvement of the predictions from theory, when the new features mentioned above are also included, may be seen from another example in which it is applied, namely internally hydrogen-bonded hairpin bends (also known as beta-bends). There are two types of such bends, the so-called LL bend and the LD bend (Fig. 4). It is to be noted that either an L or D alpha-carbon atom can be substituted by a glycyl C^{α}-atom. Using the earlier potential functions [2] [namely those used for Fig. 2 (*a*)], these have

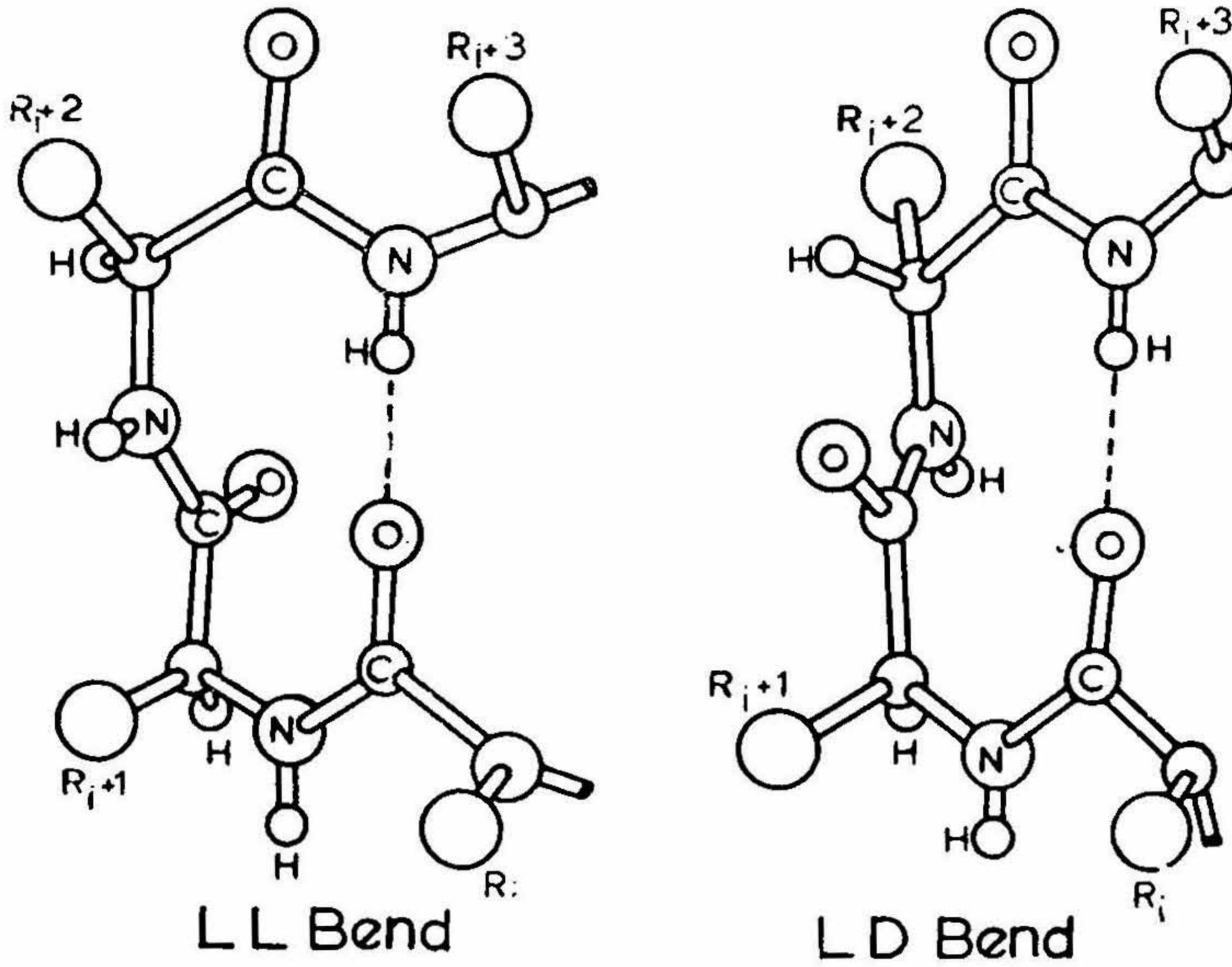


FIG. 4. Diagrams showing typical conformations of the LL and LD bends.

minimum energies for the dihedral angles about bonds meeting at C_2^{α} and C_3^{α}, as given in Table I, in row 1, marked standard potential functions [2] (data from Ref. 21). The mean of the observed conformations, as derived from X-ray structure analyses of small peptides and cyclic hexapeptides as listed in Ref. 21 are also shown in Table I. Similarly some of the protein crystallographic data (from the crystal structures of lysozyme and chymotrypsin), given in Ref. 21, are averaged and included in Table I.

TABLE I

Minimum energy conformation of the LL-bend of a peptide fragment $C_1^{\alpha}—C_1O_1—N_2H_2—C_2^{\alpha}—C_2O_2—N_3H_3—C_3^{\alpha}—C_3O_3—N_4H_4—C_4^{\alpha}$ *having a hydrogen bond of the type* $N_4H_4 \ldots O_1$, *as compared with observational data*

Dihedral angle	ϕ_2	ψ_2	ϕ_3	ψ_3
THEORY (Only for LL)				
Standard potential functions [2], [21]	−50°	−50°	−110°	+40°
Standard functions with new V (ψ) [18]	−60°	−30°	− 90°	20°
Standard functions, with new V (ψ) and softened hydrogen interactions [23]	−60°	−20°	−110°	10°
CRYSTAL STRUCTURE DATA (LL, LG, GL or GG)				
Mean of observed data in lysozyme and chymotrypsin (data from Ref. 21)	−49°	−40°	− 96°	14°
Mean of eight molecules in crystal structures of small peptides (data from Ref. 21)	−68°	−28°	−101°	9°

It will be readily seen from Table I that there is a deviation of ± 20° or more in some of the dihedral angles as predicted by the potential functions in common use [2] and the set of potential functions with the new improvements [18], [22]. Also, it appears that the latter are in better agreement with the observational data. Thus, the results using also the softened potential

(row 3 of Table I) are very close to the mean of eight determinations from accurate crystal structures. In particular, this is so for the angle ψ_3, which is $\approx 10°$ in both of them—quite different from the value 40° given by the conventional potential functions.

3. Test of Potential Functions Using Crystal Packing

As mentioned earlier, there are appreciable differences in the variation of the non-bonded potential with interatomic distance, as adopted in different laboratories (Fig. 1). It would be worthwhile to find out if a discrimination can be made between these by using some criteria for testing them, or whether a better set of functions can be deduced by a combination of theoretical and observational approaches. One way of doing this will be to take the crystal structures of very simple compounds containing the atoms normally found in biopolymers, such as C, H, O, N, etc. This was done in our laboratory in two examples, namely, those of benzene and N-methyl acetamide [24], [25]. In the case of benzene, whose space group is *Pbca* [26], [27], there are four molecules in the unit cell, but they are related by symmetry in such a way that the centres of the molecules occur only at definite locations, namely, the inversion centres at 0, 0, 0; 0, $\frac{1}{2}$, $\frac{1}{2}$; $\frac{1}{2}$, 0, $\frac{1}{2}$; and $\frac{1}{2}$, $\frac{1}{2}$, 0. There is therefore no translational freedom for the benzene molecules. The only freedom available to each molecule is, therefore, a possibility of rotation about three perpendicular axes, with the centre of the molecule fixed at a point. The most convenient way of representing the rotations is by means of Eulerian angles, as shown in Fig. 5. The values observed for these three angles, as calculated from the crystal structure determination, is $\psi = 2{\cdot}3°$, $\theta = 46{\cdot}6°$, and $\phi = 104{\cdot}4°$. An attempt was made, therefore, to determine the interaction energy of one molecule of benzene with its surroundings in the crystal, for the whole range of Eulerian angles, at intervals of 10°. No appreciable charges occur in this molecule, and therefore, the electrostatic interaction was neglected and the total energy was calculated purely as the sum of the non-bonded interactions, V (H, H), V (C, H) and V (C, C). The potential functions first used had the form and values of parameters normally adopted in our laboratory [2]. On examining the calculated data, it was found that the lowest minimum occurred at $\psi = 0°$, $\theta = 50°$ and $\phi = 110°$ (70°), which are very close to those experimentally observed. The two possible values of ϕ correspond to identical structures, and, therefore, only the one with $\phi = 110°$ was explored further. A second minimum was also found in the θ *vs.* ϕ energy map for $\psi = 0°$, at $\theta = 90°$ and $\phi = 50°$ (130°). The calcu-

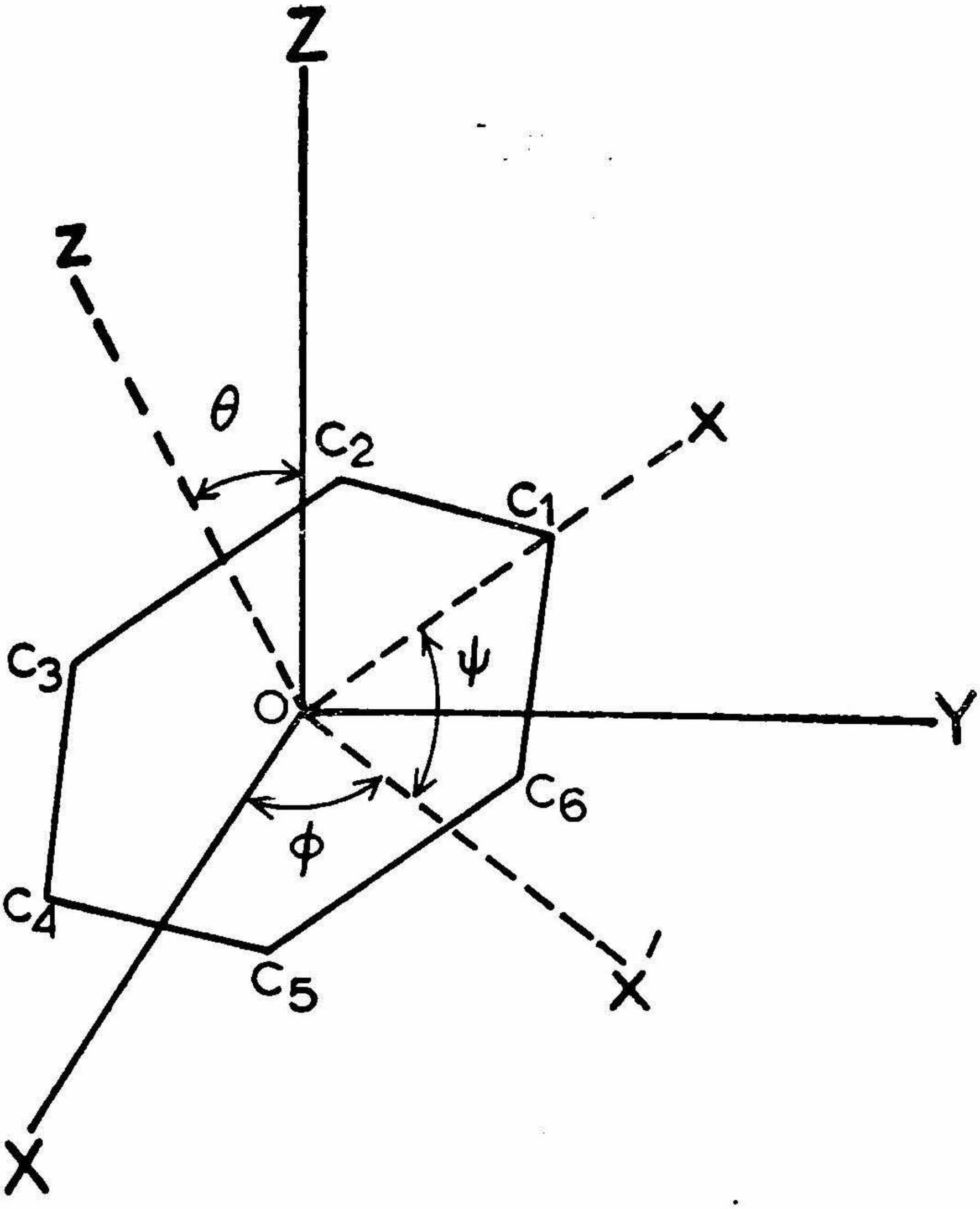


FIG. 5. Diagram showing the Eulerian angles of rotation adopted for the benzene molecule.

lated values for the plane $\psi = 0°$ is shown in Fig. 6. It will be seen from this that the lowest minimum is not appreciably lower than the next higher minimum, the two differing only by about 0·9 kcal/mole and the two being connected together by a low valley of energy values. However it is interesting to note that the lowest energy did in fact occur very close to the observed values. In fact, when the energy calculations were repeated at closer intervals of 1°, it was found that the absolute minimum occurred at $\psi = 2°$,

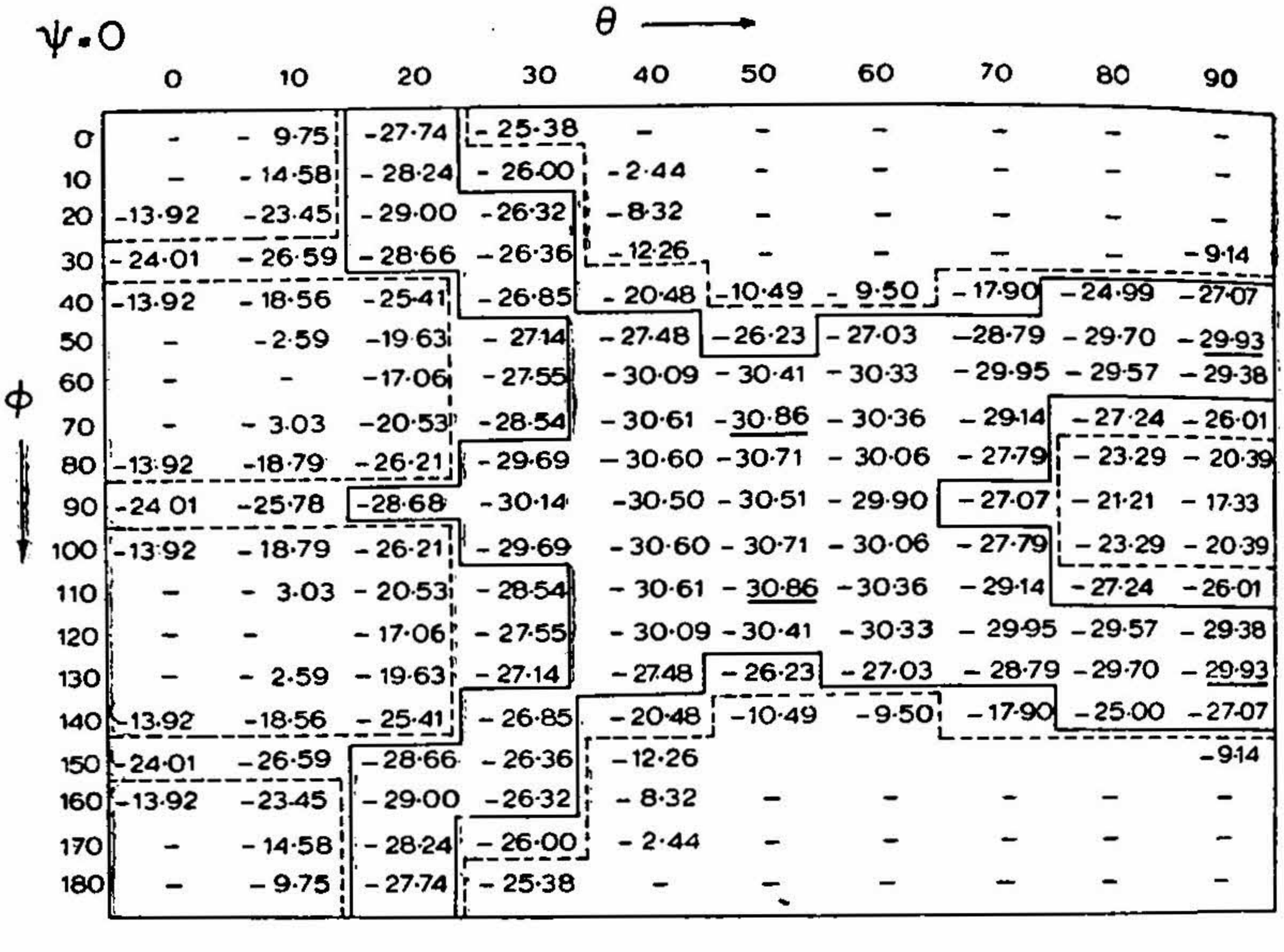


FIG. 6. Packing energy per benzene molecule of the crystal structure, showing the variation with θ and ϕ, for $\psi = 0°$. The lowest and the second lowest minima are underlined. The contact map is also shown, corresponding to normal limits——and extreme limits– – –.

$\theta = 47°$ and $\phi = 106°$, which are extremely close to those observed, as mentioned above.

It would appear from this that these calculations may be taken to be a very good verification of the correctness of the potential functions adopted. On the other hand, when the calculations were repeated with other sets of potential functions .(those used for instance, in other laboratories [3], [4], [5], [6]), the results in all the calculations were not far different. In every case, the calculations at intervals of 10° gave the absolute minimum at exactly the same place as the one obtained using our functions, namely, $\psi = 0°$, $\theta = 50°$ and $\phi = 110°$ (70°), and the second lowest minimum also occurs for the same values of (ψ, θ, ϕ), namely, $\psi = 0°$, $\theta = 90°$, $\phi = 50°$ (130°). However, the interesting observation was that the relative difference between the absolute minimum and the second lowest minimum of energy was appreciably different for the different functions [28]. The data are given in Table II. On looking at this, it will be seen that the differ-

TABLE II

Energy values of the benzene molecule in its orthorhombic crystal structure, calculated using the different potential functions shown in Fig. 1

Potential functions used	Energy, in kcal/mole of		
	Lowest minimum	Next lowest minimum	Difference
Our laboratory [2]	−30·86	−29·93	0·93
Kitaigorodsky [6]	−13·46	−13·03	0·43
Scheraga [4]	−20·84	−20·28	0·56
Williams [3]	−17·98	−15·60	2·38
Scheraga (packing) [5]	−18·28	− 7·75	10·53
Our usual potential functions [2], adding quadrupole-quadrupole interactions [28]	−31·91	−29·27	2·64

ence varies from 0·5 kcal/mole to 10 kcal/mole. It may be mentioned that we have neglected the stabilizing energy from quadrupole-quadrupole interactions of the benzene molecules in these calculations. The contribution from this is appreciably larger (negative) for the lowest minimum and less (positive) for the second lowest minimum. This adds about 1·7 kcal/mole to the difference between the two minima, and the energy difference is more than 2 kcal/mole for all the sets of potential functions. The consequence of all these in relation to the stability of the observed crystal structure is very significant. This problem obviously requires further study. In fact, neutron diffraction studies, made at different temperatures [27], indicate that the pattern of the crystal packing, as given by its space group, as well as the parameters defining the exact location of the molecules, do not change appreciably with temperature from − 135° C to − 3° C. However, at a higher pressure and temperature (25 kilobars and 21° C), benzene undergoes a phase transition [29], leading to a crystal having a space group $P2_1/c$. We have not yet examined the stability of this structure, and how well this structure is explained by theory.

The fact, that different potential functions having their energy minima at somewhat different values of the interatomic distances (*see* for instance Fig. 1) yield exactly the same position for the benzene structure with the lowest energy, requires some special examination, because this is a feature that has been observed also in the map for a dipeptide using the different functions. As mentioned in the last section, (ϕ, ψ)-maps calculated using the different sets of potential functions currently in use have generally the same features, and also yield the same regions of low energy (say, less than 3·0 kcal/mole above the minimum value) in all the cases. What is more interesting is the fact that these regions of low energy could, in fact, have been predicted by merely examining the interatomic contact distances, as is done in the so-called contact map. The allowed regions predicted by the contact map very well represent the low energy regions calculated using any one of the set of potential functions. This situation has both its advantage and its demerits. For instance, this means that, to a first approximation, one could predict allowed protein or peptide structures reasonably well without going too deeply into the exact values of the parameters used in the potential functions. However, it only gives what might be called "allowed conformations", and this approach cannot be expected to yield the "minimum-energy conformation" with great accuracy in any one case. In this connection, we might mention the fact pointed out in the previous section on the ψ-potential function, namely, that the conformation of lowest energy for the hairpin bend turns out to be some 30° away for some of the dihedral angles defining the bend, if the correct potential function for V (ψ) is not used, and if proper attention is not paid to the softening of the interatomic potentials between hydrogen and other atoms. In fact, we believe that the good agreement obtained with observational data, using the softened potential functions and the high barrier ψ-potential functions, is a good justification for the use of these functions in all future calculations of protein and peptide energies. However, the precise way in which the softening is to be made has to be investigated further.

Thus, in judging the agreement between experiment and theory as regards potential functions, it is necessary to take the information from a large number of observational data, especially those derived from different types of experiments. When the end-to-end distance of a random coil of polypeptides was calculated from theory for this purpose, it was found that the value obtained agreed reasonably well with observation when the potential functions used earlier in our laboratory [2] were adopted. However, as mentioned in the last section, the energy map in the (ϕ, ψ)-plane obtained

with this function does not agree well with the distribution of conformation in this plane. When changes were made only in the ψ-potential function to make it have two-fold minima and a high barrier, the agreement with the distribution became reasonably good, but the calculated root-mean-square end-to-end distance became far too large [30]. The reason becomes obvious on examining Fig. 2 (*b*), in which the region near $\psi = 180°$ is much more occupied than the region near $x = 0°$, relative to the observed distribution. The value of the helical unit height (h) is of the order of 3 Å for the former region, and is small, of the order of 1 Å, for the latter region, in which the chain tightly coils without moving far away from the initial unit. Since the second region is relatively less important in this map, the average end-to-end distance becomes high. On the other hand, if softening of the particular interatomic functions involving hydrogen is also included and the map shown in Fig. 2 (*c*) is obtained, we notice that not only does the agreement with the observed distribution become better, but the root-mean-square end-to-end distance also is in better agreement with the data [31] (Table III). Also, an increase of the backbone bond angle at C^{α} further improves the fit with observational data (Table III, row 4). It is surmised that the improve-

TABLE III

Calculated values of the characteristic ratio, using different types of potential functions

Nature of Potential Function	Characteristic ratio
THEORY	
1. Using usual potential functions [2], [30]	10
2. With 2-fold ψ-potential ($\tau = 110°$) [31]	18
3. With 2-fold ψ-potential and softening ($\tau = 110°$) [31]	14
4. Same as row 3, but $\tau = 112°$ [31]	11
5. Same as row 4, but with barrier for $V(\psi) = 2$ kcal/mole [31]	8
EXPERIMENTAL DATA	
6. Range from several reported studies	7·5—9·5

ment comes not so much from the alteration in the geometrical features (namely increase of $\tau(C^a)$ from 110° to 112°, *per se*), as from the resultant greater density of conformations in the bridge region IV, near $\psi = 0°$. In fact, if the barrier of the two-fold potential $V(\psi)$ is also brought down to 2 kcal/mole (rather than the value of 4 kcal/mole adopted in rows 2 to 4), while retaining the softening of the H . . . X interactions, the agreement with observation becomes quite good indeed (Table III, row 5). Obviously, there is a great need to examine further the fit with this type of information.

The validity of our potential functions has also been tested in another example of crystal packing, namely that of N-methyl acetamide [25]. This crystal has the symmetry of the space group *Pnma* and also is a suitable crystal to study, because the number of parameters defining its degrees of freedom are only three, as shown in Fig. 7. These are the two parameters x, z ($y = 0{\cdot}25$ for this molecule) and a third one, θ, to indicate the angle which the C—N bond direction makes with the x-axis (Fig. 7). The details of the calculations are not given here as they are described in Ref. 25, except to state that the non-bonded potential functions as adopted in our laboratory,

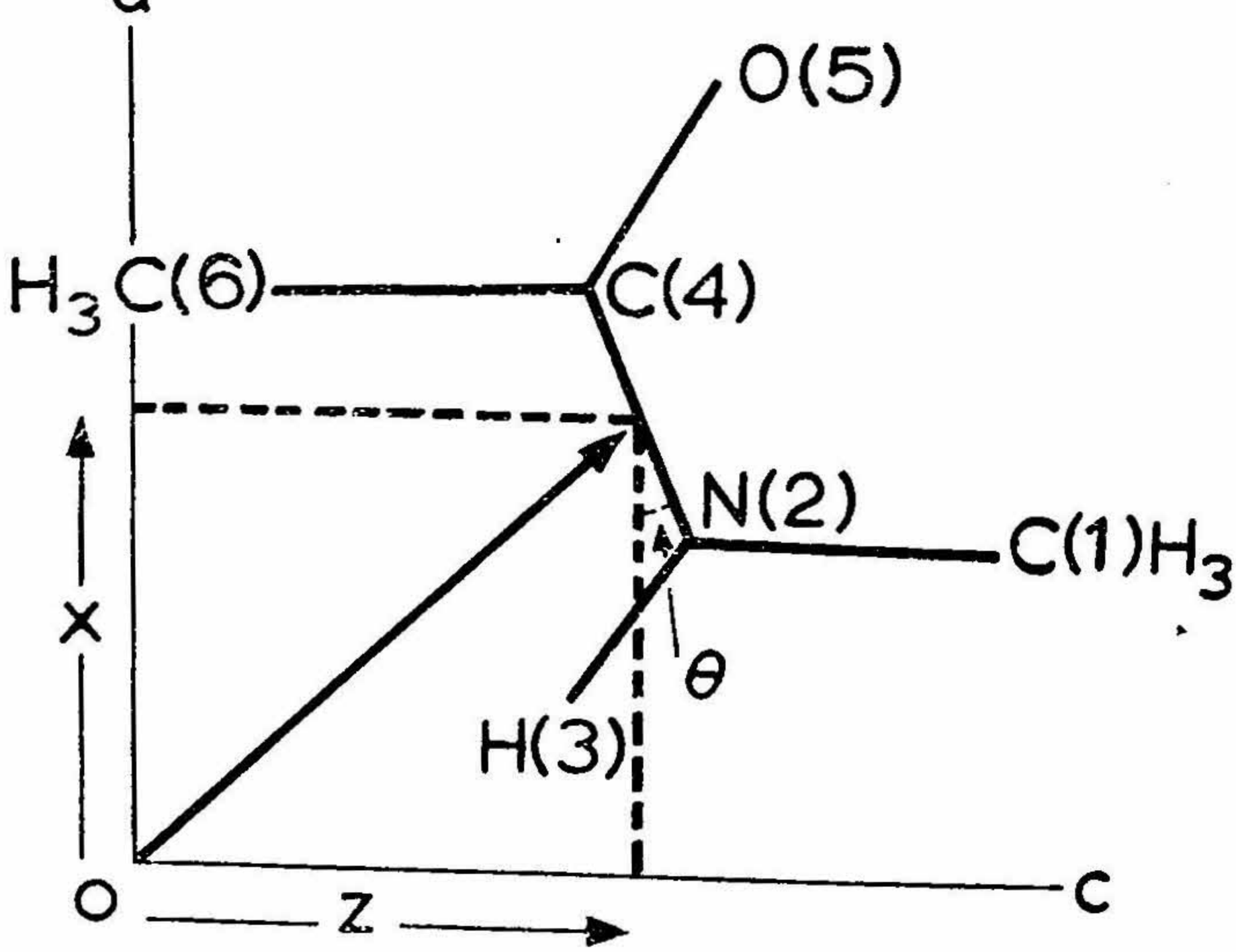


FIG. 7. Diagram showing the definitions of the variable parameters x, z and θ, for the location and orientation of the molecule of N-methyl acetamide in its crystal structure.

were used, in addition to electrostatic potentials with the partial atomic charges present in the molecule [2]. Since a hydrogen bond occurs between N—H and C = O, the potential function describing the variation of energy with hydrogen bond length and bond angle, as proposed from our laboratory [32], was used for this purpose. There are other functions also reported in the literature, such as those in references [4], [33], [34]. In fact, De Santis *et al.* have studied the same crystal structure, using the non-bonded potential functions and hydrogen bond potential functions normally in use in their laboratory [33]. Reasonably good agreement between the crystallographically determined location of the molecules and the theoretically calculated one was obtained in our studies [25] and the positions of none of the atoms in the unit were different from the actual locations by more than 0·5 Å in our calculations. De Santis *et al.* found that the agreement was not good when they used their usual hydrogen bond potential function [33], but, when they used modified potential functions (taking into account the possible non-linearity of the hydrogen bond, as given by the potential functions adopted in our laboratory [32]), they got very good agreement. Although our theoretical structure differed from the observed one in the crystal by only a small amount (none of the atoms being more than 0·5 Å away from the true positions), we believe that this amount of agreement is not good enough for such a simple structure. It is not immediately obvious what additional refinements should be made. In fact, four different possibilities of the orientations of the methyl hydrogens, in relation to the backbone atoms, were tried and only one of them gave good agreement in our calculations, although the absolute energy as calculated from theory was lower by about 1·5 kcal/mole in another example. There is obviously much more to be investigated in detail about the precise nature of the interatomic forces and the exact values of the parameters.

4. Possible Non-planar Distortions of the Peptide Unit

We have so far been considering the interactions between atoms which are not covalently bonded to one another. On the other hand, in the case of the peptide unit, which may be represented by Fig. 8 (*a*), it is normally believed that its most stable conformation is the planar one, in which all the six atoms shown in Fig. 8 (*a*) lie on a plane, and that the deviations of the atoms from this plane are relatively small. However, non-planar structures for the peptide unit had not been ruled out earlier, although the idea of a partial double bonded character for the bond C—N would indicate a

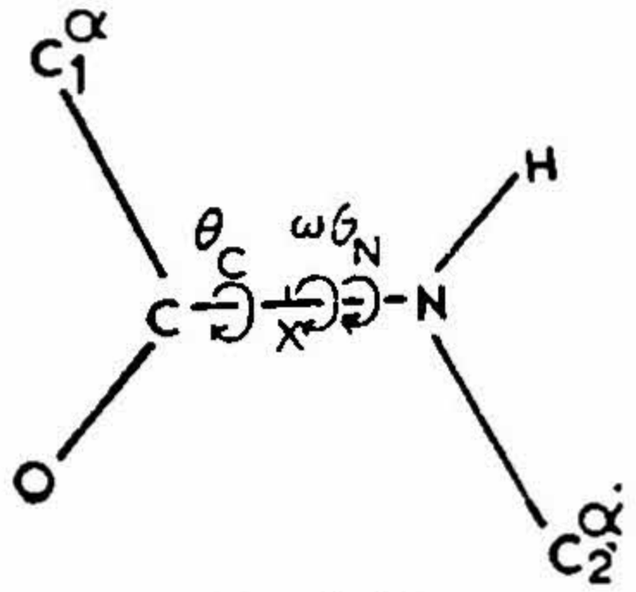


FIG. 8 (*a*)

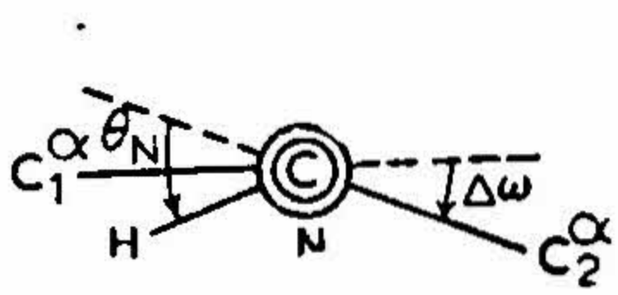


FIG. 8 (*b*)

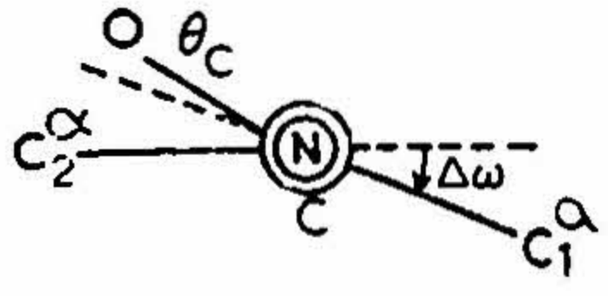


FIG. 8 (*c*)

FIG. 8 (*a*). Diagram showing the nomenclature of the atoms in a peptide unit and the dihedral angles ω, θ_N and θ_C.

FIG. 8 (*b*). Definition of the angles $\Delta\omega$ and θ_N, in the Newman projection down the bond direction CN.

FIG. 8 (*c*) Definition of the angles $\Delta\omega$ and θ_C in the Newman projection down the bond NC.

relatively high planar rigidity to the six atoms in the systems as a whole. For example, even in the review published by the author and Sasisekharan as early as 1968 [2], notations were adopted for describing such non-planar distortions of the peptide unit. More recently, Winkler and Dunitz [35] have analysed the existence of such distortions in various examples, and have indicated that they are not unimportant. However, the precise nature of the non-planar distortions that occur, and the relative ease of their occurrence, do not seem to have been investigated, either theoretically, or by a careful analysis of the observed data, until very recently. We have carefully studied this subject during the last two years and it is perhaps worthwhile to summarize our results here, because they appear to be very important for the construction of the peptide and protein chains that are of biological importance [12], [13], [14]. In fact, the possibility that the three bonds meeting at the nitrogen atom may not all be coplanar was pointed out forcibly to the author by Prof. R. S. Mulliken during a discussion which the author had with him in 1971. It was pointed out in that discussion that the pyramidal character of the three bonds meeting at the nitrogen, which is so characteristic of this atom (*e.g.*, in ammonia molecule), could not be completely obliterated by the N—C bond having a partial double bond character. Therefore, calculations were made using the CNDO/2 formulation of quantum chemistry and the energy of the peptide unit was calculated for various conformations in which the three bonds meeting at the nitrogen, or at the carbonyl carbon atom, are non-planar. In order to satisfactorily describe the results, it is necessary to introduce a suitable definition of non-planarity. In fact, the non-planarity introduced by an ω-rotation about the C—N bond on the backbone of the

peptide unit is fairly well recognised. If the ω-rotation were the only distortion to exist, then the peptide unit could be described as consisting of two segments joined together, say at the point X, which is the mid-point of the C—N bond [Fig. 8 (*a*)]. The atoms C_1^{α}, O and the bond CX would be coplanar with C, while the atoms C_2^{α}, H and the bond NX would be coplanar with N, the two planes being relatively twisted by the angle ω about C—N.

The ω-distortion has been studied in great detail in various laboratories, including the author's previous laboratory at Madras—*e.g.*, theoretically using quantum chemistry [36], [37], by NMR [38], and by IR studies [39]. However, it was only recently recognized that, if the three bonds meeting at nitrogen were pyramidal, then it could be described by a dihedral angle between the two planes passing through the atoms C, N, C_2^{α} and C, N, H. Since dihedral angles, obtained by a rotation about the various bonds, have become the standard technique of representing the conformation of biomolecules, this dihedral angle was taken to be the extra degree of freedom required to specify the non-planarity of the three bonds meeting at the nitrogen atom. We have used the symbol θ_N for this angle, and it is also a rotational angle about the bond C—N, as indicated in Fig. 8 (*a*). The two dihedral angles $\triangle\omega$ and θ_N are also particularly clearly described in the diagram shown in Fig. 8 (*b*). The corresponding diagram giving the definition of the angle θ_C, indicating the possible non-planar arrangement of the atoms attached to the carbonyl C, is shown in Fig. 8 (*c*). It is readily verified that these three parameters $\triangle\omega$, θ_N, and θ_C are sufficient to describe all the non-planar distortions that are possible for the peptide unit.

When the calculations with the CNDO/2 method were made using a peptide unit with standard bond lengths and bond angles, for the planar structure and for different values of $\triangle\omega$ and θ_N, the results shown in Fig. 9 (*a*) were obtained (redrawn from the data in ref. 12). As will be seen from this figure, the lowest energy occurs at about $\triangle\omega = 10°$ and $\theta_N = -25°$ (The possible minimum at $\triangle\omega = -10°$, $\theta_N = +25°$, which is symmetrically related to this, is disregarded for the present). It is also clear from Fig. 9 (*a*) that the low energy conformations lie in a narrow valley lying on either side of the line $\theta_N = -2\triangle\omega$. This brings out the interesting fact, namely that the change in the angle $\triangle\omega$ alone is not sufficient to represent the non-planar distortion of the peptide unit. Actually, the hydrogen atom also moves to the same side of the plane containing the atoms C_1^{α}, C and N, as

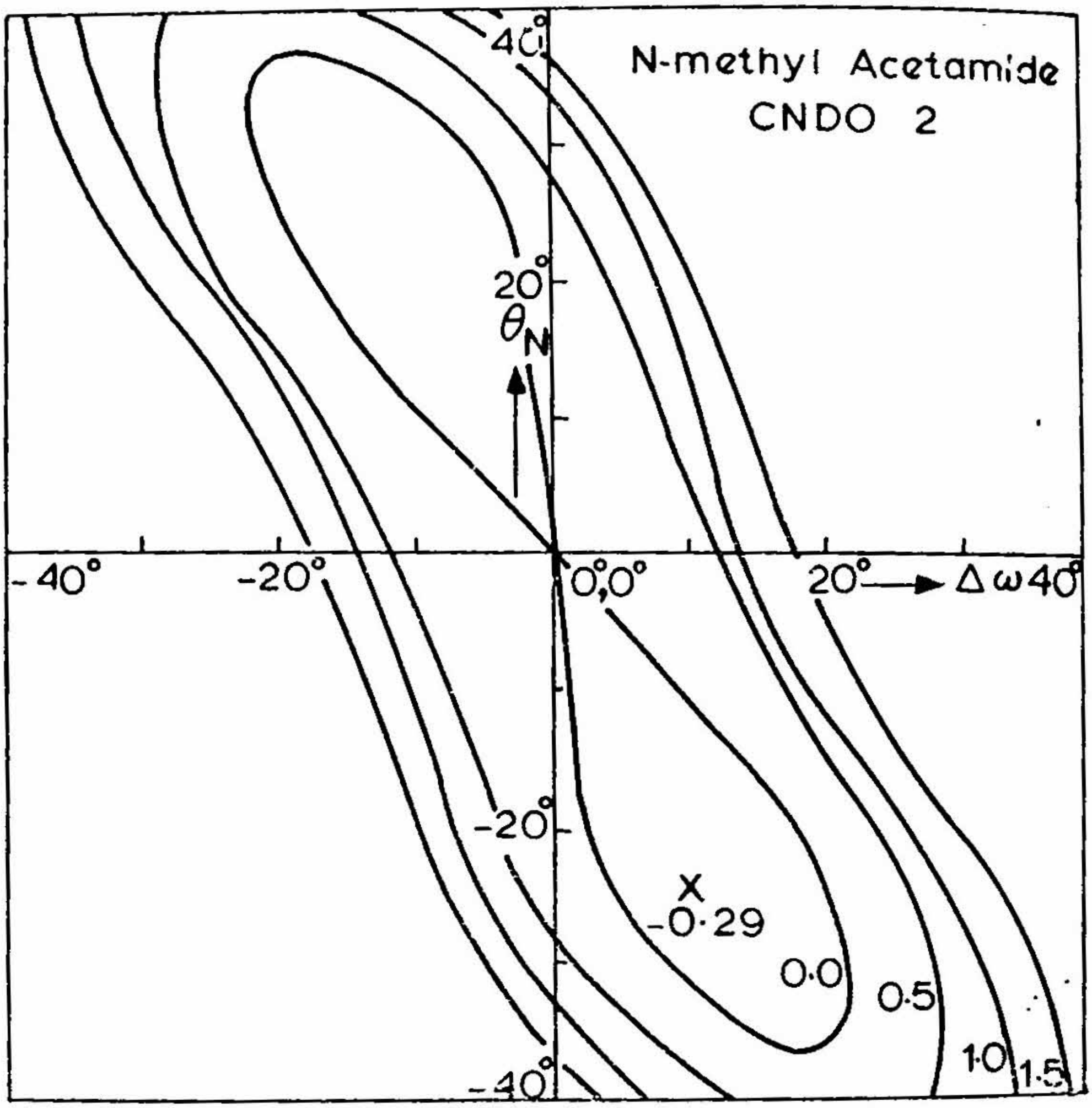


FIG. 9 (*a*). Energy contours in the plane with $\Delta\omega$ along the x-axis and θ_N along the y-axis drawn from the computational data, using the CNDO/2 method.

the atom C_2^{α}. In fact, this is the situation shown in Fig. 8 (*b*), where it will be noticed that the magnitude of θ_N is approximately double the value of $\Delta\omega$ and its sense is opposite to that of $\Delta\omega$. In other words, *the greater the value of* $\Delta\omega$ *the greater is the pyramidal character of the bonds meeting at the nitrogen atom.* We consider this to be the most significant result that has been obtained out of these calculations from quantum chemistry.

On the other hand, when similar calculations were made for non-planar distortions at the carbonyl carbon atom, defined by the angles $\Delta\omega$ and θ_C, very large positive changes in energy were obtained even for small values

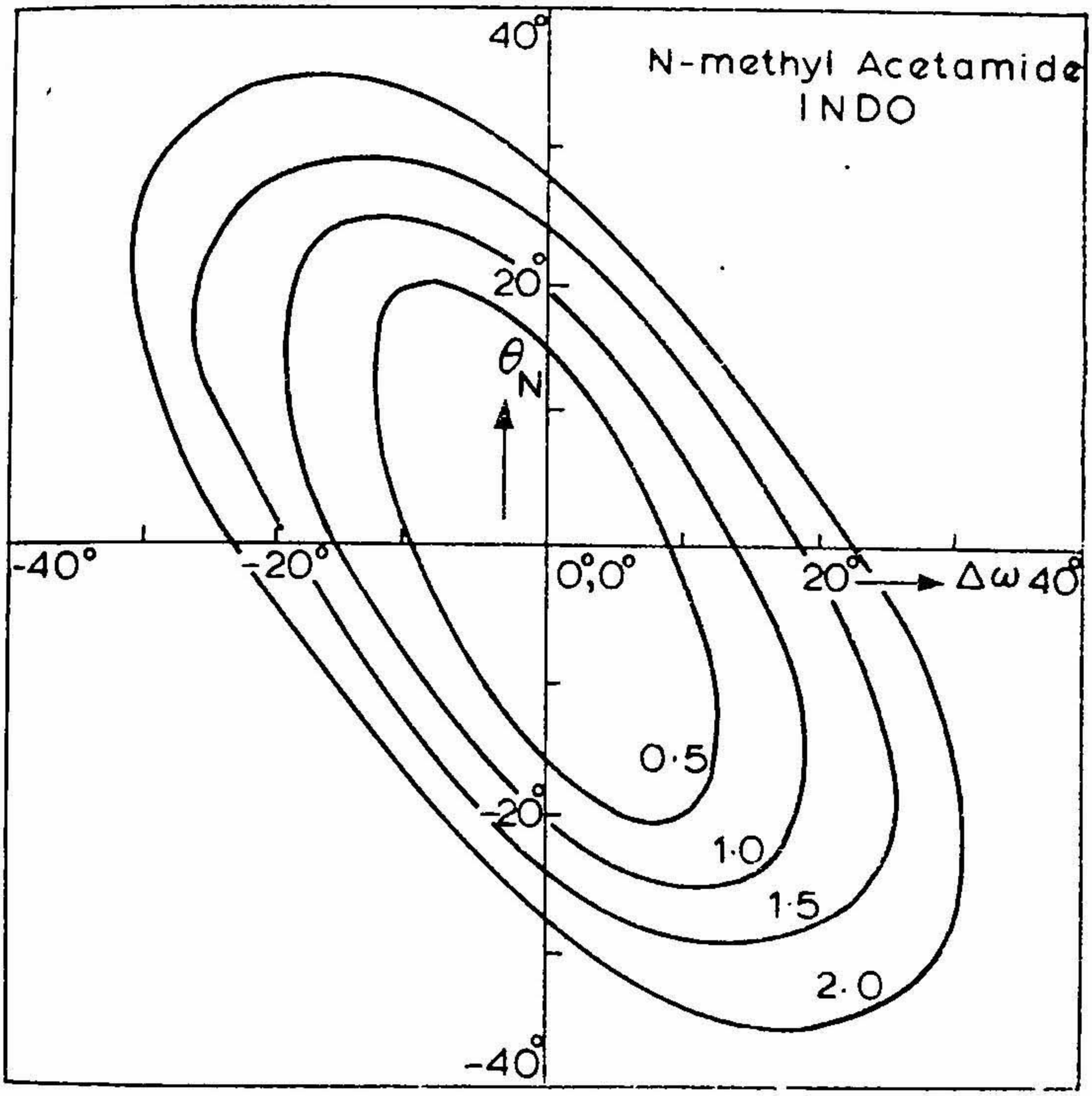


FIG. 9(*b*). Similar to (*a*), but obtained using the INDO method.

of θ_C, showing that very little non-planarity of the three bonds meeting at C can actually occur. Thus, one can say that the essential modifications of the structure that occur in a non-planar peptide unit are that the dihedral angle $\triangle\omega$ can undergo a small change ($|\triangle\omega| < 15°$) and, in addition, the three bonds meeting at the nitrogen atom can be non-planar, which is denoted by the rotational angle θ_N, which may have values as high as 25°. However, a correlated variation exists between $\triangle\omega$ and θ_N, given by the relation $\theta_N = -2\triangle\omega$, leading to increased pyramidal nature of the three bonds meeting at N, with increasing $\triangle\omega$. In fact, Fig. 9 (*b*), which gives the results obtained from using the INDO method, also shows an extended region of

low energies along the line $\theta_N = -2\triangle\omega$. In this case, the minimum energy occurs at $\triangle\omega = 0°$ and $\theta_N = 0°$. However, the change in energy for small variations of $\triangle\omega$, upto 10°, are quite small, (less than 0·5 kcal/mole) along the line $\theta_N = -2\triangle\omega$, and such deviations are therefore very likely to be observed in actual structures of peptides.

This prediction from theory, of the correlated variation between θ_N and $\triangle\omega$ in the model compound N-methyl acetamide, has been verified from an examination of the observed crystal structures of small peptides. The observations in about thirty examples are plotted in Fig. 10 (*a*), and it may

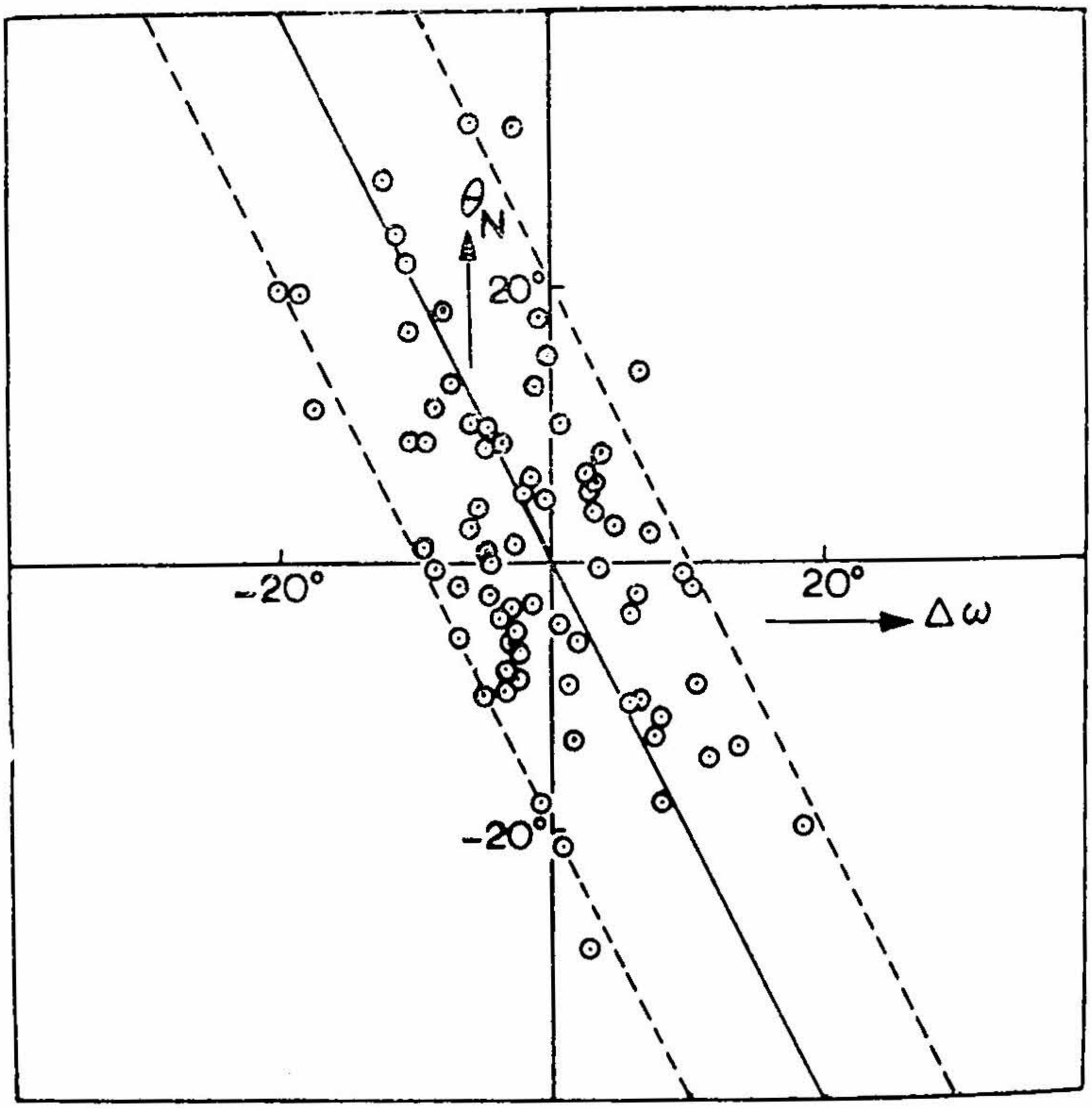


FIG. 10 (*a*). The observational data from a number of crystal structures of simple peptides and related compounds, shown by circles (data taken from Ref. 14), in the ($\triangle\omega$, θ_N)-plane. Note that all the data lie close to, and on either side of, the line $\theta_N = -2\triangle\omega$,

be seen from this that the observed data all lie in the low energy regions of these energy maps in Fig. 9. From the distribution of the observed data, it is also possible to deduce the variation of energy with $\triangle\omega$ (by using the standard Boltzmann distribution law) and this is shown in Fig. 11, along with the theoretical variation of the energy, according to the INDO calculations, along the line $\theta_N = -2\triangle\omega$. The very close correspondence between the two is extremely striking and forms, perhaps, the best proof for the essential correctness of the theoretical deductions obtained from quantum chemistry. On the other hand, it is seen from the distribution of θ_C *vs.* $\triangle\omega$, shown in Fig. 10 (*b*), that the observational points in the ($\triangle\omega$, θ_C)-plane lie very close to the horizontal $\triangle\omega$-axis. This means that there is practically no correlated variation between θ_C and $\triangle\omega$. In fact, according to Fig. 10 (*b*), θ_C is randomly distributed over a very small range of about 5° on either side of the line $\theta_C = 0°$, over the whole range of observed values of $\triangle\omega$. Here again, we see that the theoretical prediction that there is very little non-planarity of the three bonds meeting at the carbonyl C atom, is substantiated by experiment.

In view of the success of the INDO prediction in N-methyl acetamide, it will be worthwhile commenting here about what happens in the related

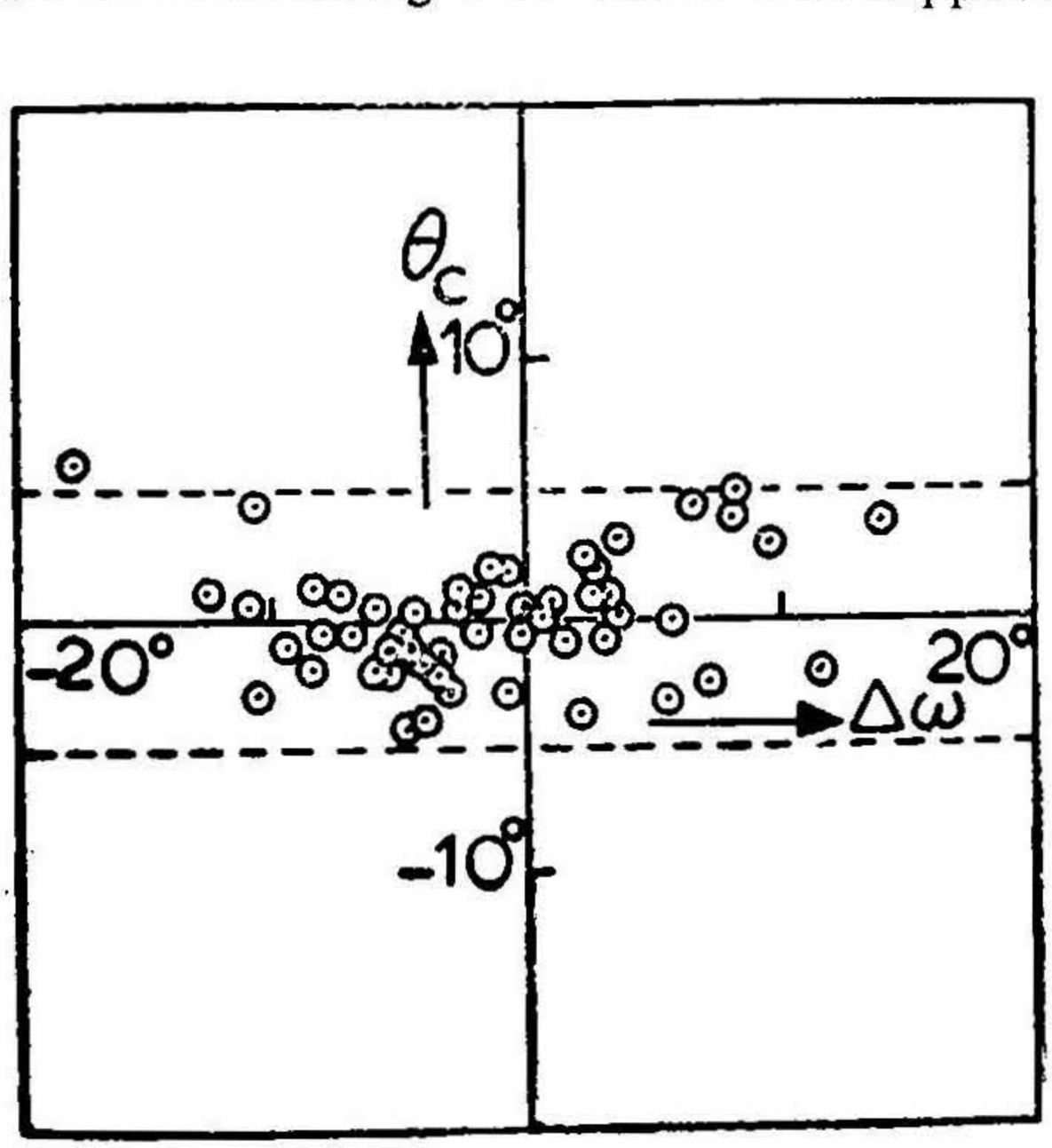


FIG. 10(*b*). Distribution of observed data in the ($\triangle\omega$, θ_N)-plane. Note that all of them lie close to the horizontal $\triangle\omega$-axis, and are much less spread out than in Fig. 10(*a*).

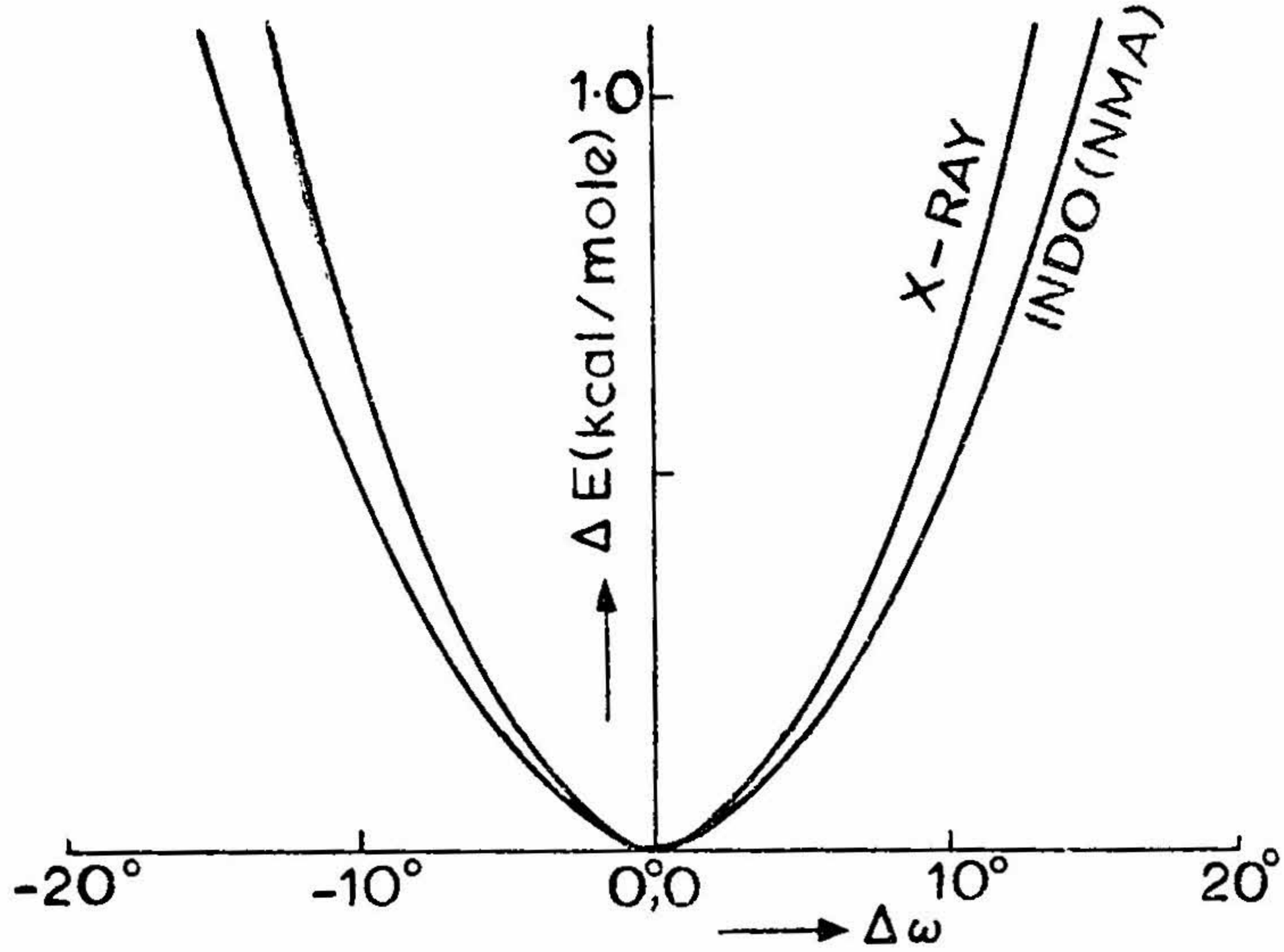
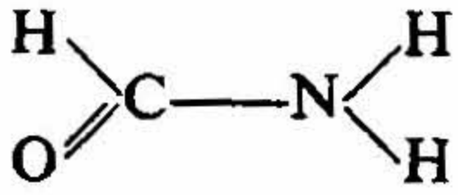


FIG. 11. Variation of energy with $\Delta\omega$ along the line $\theta_N = -2\Delta\omega$, as calculated using the INDO method for N-methyl acetamide. The experimental curve, deduced from observed data in crystal structures, is also shown for comparison.

compound, formamide, which has a much simpler structure than N-methyl acetamide namely,  . We shall not go into the details as these are being prepared for publication elsewhere [14]. We shall only reproduce the variation of energy with $\Delta\omega$ for the correlated variation along the line $\theta_N = -2\Delta\omega$, as predicted by the INDO calculations, and as observed by the microwave studies made by Hirota *et al.* [40], which is shown in Fig. 12. Here again, the variation of ΔE with $\Delta\omega$, as given by theory, and that given by experiment, are very close to each other, showing that the nitrogen atom can have an appreciable amount of non-planarity, approaching towards the pyramidal structure as observed in ammonia.

In the next section, we shall consider the consequences of the results described in this section. Here, we shall merely mention that it is no longer possible to ignore the non-planar distortions of the peptide unit and assume

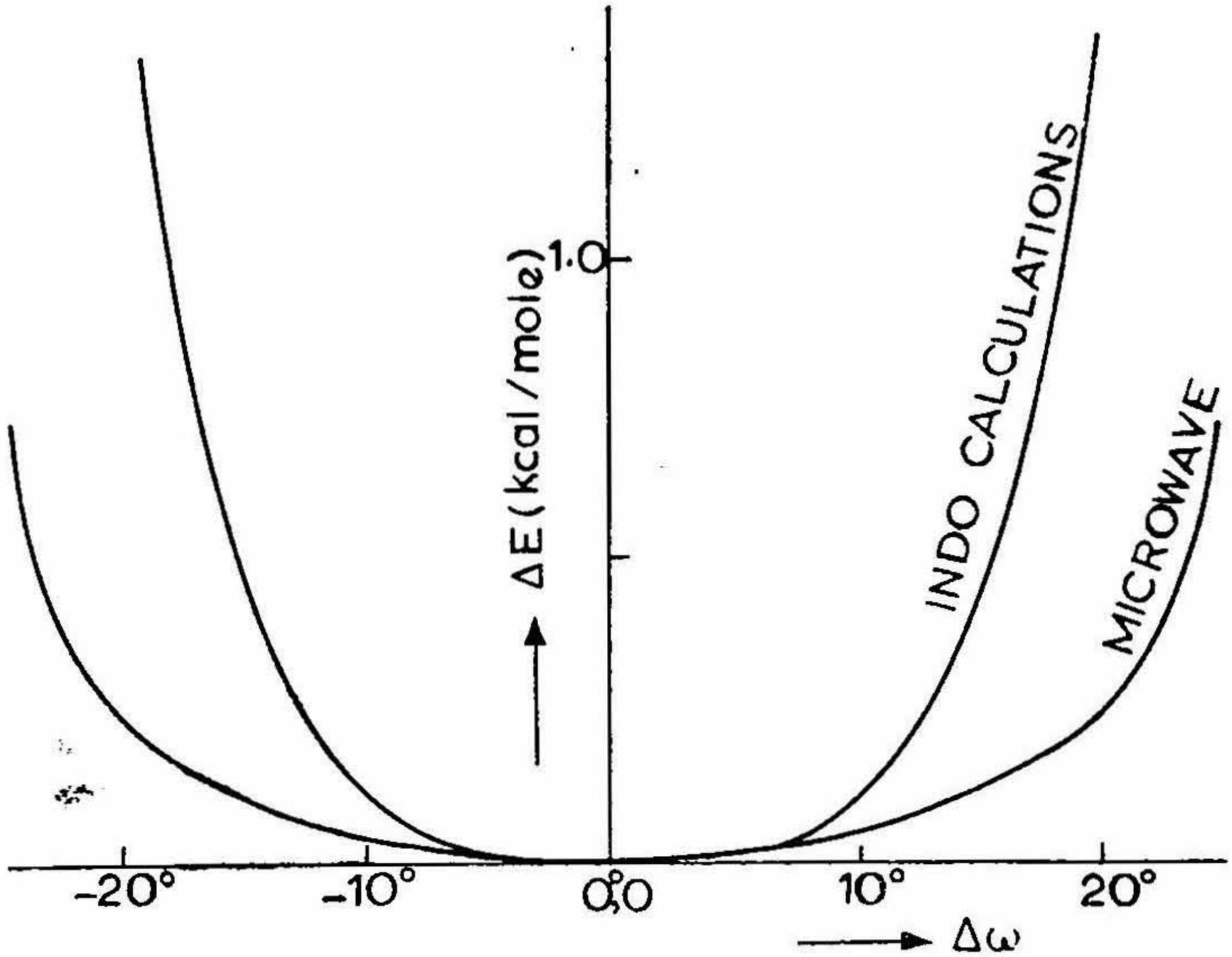


FIG. 12. Similar to Fig. 11, but for formamide. The two curves are respectively from theory (INDO), and from experiment (microwave studies).

that it is planar, to a first approximation, for all practical purposes, in making models of the peptide chains or protein chains, for comparison with the electron density maps obtained from X-ray diffraction studies. In the discussions which the author had with some protein crystallographers, it was strongly brought out that the crystal structure analysis would be able to get much better agreement between the electron density map and the positions of the atoms obtained from model building, if the peptide unit is not as rigid as it is normally assumed, but had a reasonable amount of flexibility. This is exactly what is obtained by allowing the ω-distortion in the backbone of the peptide chain. Also, the fact that, when there is a defined ω-distortion, the amino hydrogen atom also moves in the same direction normal to the mean plane as the C_2^{α} atom, will play an important part in checking for hydrogen bonds. The consequences of the non-planarity have not yet been investigated in detail. Presumably this may play an important part in the stability of the alpha-helix in which the hydrogen bond is not straight in the structure as determined by fibre crystallograph [41].

5. DISCUSSION

The main theme of the present paper is that, even today, after nearly a decade of vigorous studies in various laboratories on peptide conformation, there are several factors, connected with the essential ideas involved in the theoretical analysis, which have yet to be carefully checked, verified and probably revised. For example, the new form of V (ψ), for the variation of energy with the dihedral angle ψ, produces change in the calculated energy by as much as two or three kcal/mole in certain parts of the (ϕ, ψ)-plane. Therefore, if this factor is not taken into account and an energy minimisation is carried out, with even three or four peptide units, there is no guarantee that the final minimum so obtained will be very close to the true one. This is brought out, for instance, by the structure of three units linked together and having a $4 \rightarrow 1$ hydrogen bond. As mentioned in an earlier section. some of the dihedral angles, as predicted from theory, differ by as much as 30°, according as the earlier form of V (ψ), or the newly proposed form V (ψ), is adopted. While this may involve only a change by about 1·0 Å in the positions of some of the atoms in the three-peptide unit structure, if a larger number of units are involved, the positions of the latter may be deviated quite appreciably. This does not, however, mean that we should look with suspicion on all energy minimisations that have been done, using the earlier form of V (ψ). It is quite possible that the non-bonded interactions play such an important part in the production of forbidden regions in the conformational space, that the allowed, or good, structures could be predicted with reasonable accuracy. However, we feel that it is not reasonable to put complete faith in the exact energy-minimised structures that are obtained using the potential functions that are available at present. For instance, we feel that it is unreasonable to suppose that energy minimisation can ever give a structure which is more accurate than the X-ray determined structure, as has sometimes been claimed [20]. We hope that the opportunity of mutual discussion that will be provided by this symposium will bring out errors in, or improvements of, the currently used potential functions. If this paper has stimulated arguments on these lines, the author and his group would feel highly satisfied.

The case of the non-planar peptide unit is also very similar. Normally, when one works out structures from theory, the planar peptide unit is employed, as being a good enough approximation. Our calculations have shown that this need not be the case. Even in simple crystal structures, the peptide unit sometimes has as large a deviation as $\triangle\omega = -12°$ and

$\theta_N = 25°$, as observed for instance, in that of Gly-L-Leu [42]. In this structure, at first sight, one does not see any special reason why so much of non-planarity should exist. However, it is interesting to note that the exigencies of the situation, demanded by packing considerations, have compelled the planar peptide unit to take up such a large amount of non-planar distortion in this case. Therefore, it is quite likely that, in protein and enzyme structures also, some of the peptide units may have appreciably distorted configurations in special cases, where this would help in stabilizing the structure. In other words, we feel that, in calculating the energy-minimised structure, the minimisation should be done not only over the range of the parameters ϕ_i and ψ_i, but also over the two new parameters namely, $\triangle\omega_i$ and θ_{Ni}. The processing of such calculations does not involve any great difficulty in devising the program for the computations, although the time required for the calculations might be appreciably increased. However, it is worthwhile to include the new parameters and find out how these modifications change the possible structures of various peptides, such as cyclic peptides, or small lengths of peptide chains linked by disulphide bridges. It is also necessary to think of having a simple device in the atomic models, whereby the non-planar distortion, having the correlated variation of $\triangle\omega$ and θ_N, can be incorporated. It appears to be not too difficult to think of such a device using the Kendrew models. Their introduction in the space filling models will probably be somewhat more difficult.

In attempting to test the validity of the potential functions that are adopted, simple crystal structures would surely play an important part, because the variables involved can be reduced and also because the structures concerned are well specified, and accurately determined. Therefore, more studies like what we have described earlier, regarding N-methyl acetamide should be carried out. The results coming out of the comparison between theory and from experiment in a large number of examples would help us in obtaining better refinements of the potential functions now in use.

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