Theoret. Chim. Acta (Berl.) 36, 317—328 (1975)

Molecular Orbital Studies on the Conformation of GABA (γ-Aminobutyric Acid)

The Isolated Molecule and the Solvent Effect

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Received September 26, 1974

In distinction to Extended Hückel Theory which predicts as the most stable conformation of free zwitterionic GABA a totally extended form, PCILO and SCF *ab initio* studies show that the intrinsically preferred conformation of the isolated molecule is a highly folded one, resulting from strong interactions between the two charged ends. Computations are also carried out for hydrated GABA in the "supermolecule" approach allowing moreover for the flexibility of binding of some of the water molecules of the first hydration shell. They predict the coexistence in solution of a large number of conformations showing different degrees of folding (or extension), a result confirmed by recent NMR studies. This and a number of similar results show that we have to adapt our thinking on the role of conformations in pharmacological activity to this situation, which was frequently obscured by the more abundant results of X-ray crystallography yielding a single conformation.

Key words: γ-aminobutyric acid, conformation of ~

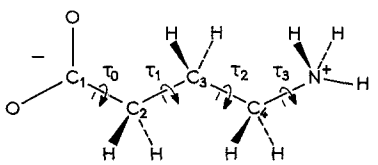
Evidence is accumulating pointing to an important role of GABA in the central nervous system and this raises the question, among other, of the preferred conformation of this molecular species [1–4]. The most precise experimental informations come from X-ray crystal studies, in which this molecules exists generally in the zwitterionic form (Fig. 1) [5–7] with the exception of the crystal of its hydrochloride [8,9] in which it is present as a cation $HOOC-(CH_2)_3-N^+H_3$. In these crystals, the cationic head is always *trans* with respect to the aliphatic chain, while the conformation about the C_2–C_3 bond is *trans* or *gauche*. From the pharmacological point of view it is, however, the conformation in solution which may be important and here the evidence seems contradictory. Measurements of dielectric constants of aqueous solution of α-ω-aminoacids indicate that such molecules exist in water as zwitterions [10]. However, while older studies of dielectric increments [11, 12] suggested that such compounds behave in aqueous solution as freely rotating chains a recent publication [13] questions this conclusion and suggests that they exist in water in a fully extended conformation.

This situation prompts a theoretical investigation of the problem. So far, the only available computation was carried out for the "isolated" molecule by the Extended Hückel Theory [14] and it predicts as the most stable conformation

of the free zwitterion the completely extended one. Because of known limitations of this methodology which lead frequently to erronous results we have re-investigated the problem by more refined quantum mechanical procedures. We have also distinguished explicitly between the study of the free molecule and the molecule in solution.

1. The Method

The zwitterion of GABA posseses four *a priori* important torsion angles, denoted τ_0–τ_3 in Fig. 1, of which, in fact, two, τ_1 and τ_2, are essential. We recall that the torsion angle τ between the bonded atoms A–B–C–D is the angle between the planes ABC and BCD. Viewed from the direction of A, τ is positive for clockwise and negative for counterclockwise rotations. The value $\tau = 0°$ corresponds to the planar-*cis* arrangement of bonds AB and CD.



GABA

Fig. 1. Definition of torsion angles in GABA: $\tau_0 = \tau(O\text{–}C_1\text{–}C_2\text{–}C_3)$, $\tau_1 = \tau(C_1\text{–}C_2\text{–}C_3\text{–}C_4)$, $\tau_2 = \tau(C_2\text{–}C_3\text{–}C_4\text{–}N^+)$, $\tau_3 = \tau(C_3\text{–}C_4\text{–}N^+\text{–}H)$

The computations for the isolated molecule have been carried out by the PCILO (Perturbative Configuration Interaction using Localized Orbitals) method [15, 16] and by the SCF *ab initio* procedure using the program Gaussian 70 [17] with an STO 3 G basis set [18]. This study was made as a function of the two torsion angles τ_1 and τ_2, the cationic head being maintained staggered with respect to the C_3–C_4 bond ($\tau_3 = \pm 60°$ or 180°) and the angle τ_0 being fixed at 0°.

The influence of water on the conformation of the zwitterion was studied by the "microscopic supermolecular" approach, which consists of fixing water molecules at the most favorable hydration sites and calculating the conformational map of the new "supermolecule". The most favorable hydration sites are determined by *ab initio* studies on model compounds, following the procedure indicated in Refs. [19–22] and recently reviewed in [23]. The conformational map of the new supermolecule, representing hydrated GABA, was computed by the PCILO method alone, as the hydrated compound is too large for computations *ab initio*. The success of this mode of approach has been strikingly illustrated in recent studies on histamine [24], indolealkylamines [25], and phenethylamines [26]. Moreover, in this case, we have taken into account partially the flexibility of the hydration shell.

2. Results and Discussion

2.1. The Isolated Molecule

The PCILO conformational energy map of zwitterionic GABA, as a function of τ_1 and τ_2 is represented in Fig. 2. It shows a very acute global energy minimum at $\tau_1 = \pm 60°$, $\tau_2 = 0°$, corresponding to a highly folded form, followed by a secondary energy minimum at $\tau_1 = \pm 60°$, $\tau_2 = \mp 90°$ representing also a folded form. The totally extended conformation ($\tau_1 = \tau_2 = 180°$) is situated 54 kcal/mole above the global minimum, which indicates a very strong preference of the isolated molecule for a highly folded structure, favored by the intramolecular attraction of the two ionic ends. We have verified that this result is independent of the orientation ($\tau_0 = 0°$ or 90°) of the COO^- group with respect to the chain. (In the crystal structures this orientation corresponds to $\tau_0 = 0°$ with the exception of the Cu-GABA complex [7] where τ_0 is close to 90°.) Figure 2 indicates also the crystallographic conformations of GABA with respect to τ_1 and τ_2. It is obvious that the observed conformations are high energy ones, which must be due to the effect of strong crystal packing forces.

A similar PCILO conformational energy map was also constructed for the cationic form. The global energy minimum is found for the same folded conformation as in the case of the zwitterion. The essential and appreciable difference between the two maps concerns the energy differences of the other conformations with respect to the most stable one, which are much smaller in

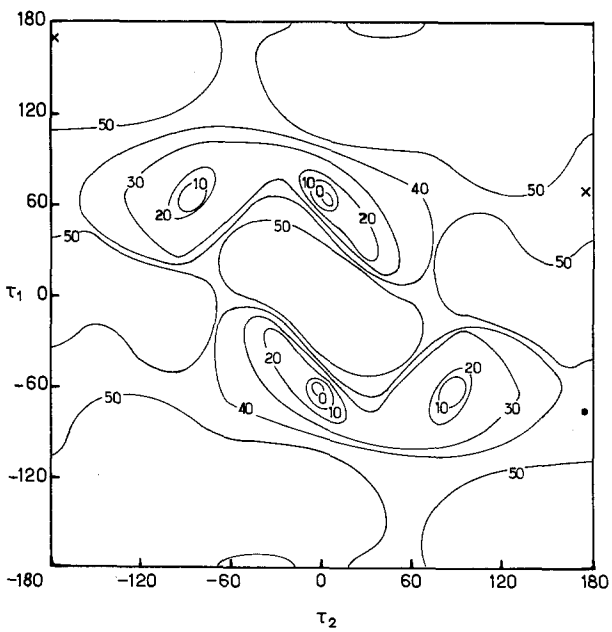


Fig. 2. PCILO conformational energy map for zwitterionic GABA. Isoenergy curves in kcal/mole, with respect to the most stable conformation taken as energy zero. ● crystallographic conformation [1, 2], × crystallographic conformation in Cu–GABA complex [7]

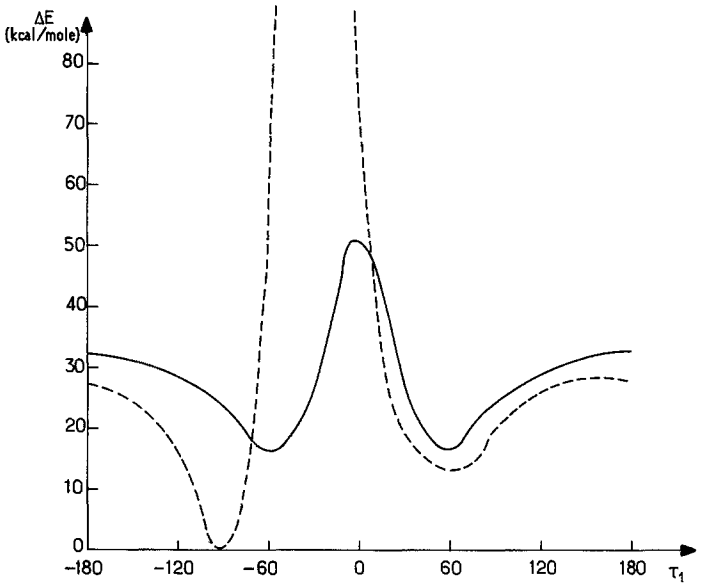


Fig. 3. *Ab initio* (STO 3 G) conformational energy of zwitterionic GABA as a function of τ_1, the lowest energy being taken as energy zero (kcal/mole). ——— results for $\tau_2 = 60°$; ——— results for $\tau_2 = 180°$

Table 1. Relative energies of conformations of GABA: comparison of PCILO vs *ab initio* results

Conformation	τ_1	τ_2	ΔE (kcal/mole)	
			PCILO	*Ab initio*
Global minimum of PCILO	± 60°	0°	0	0
gg	± 60°	∓ 90°	9	14
gt	± 60°	180°	41	30
tt	180°	180°	54	44

the cation. In particular the fully extended form of the cation is only 5 kcal/mole above the global minimum.

The *ab initio* computations, much more expensive, have been limited to the evaluation of the energy variation as a function of τ_1 for two preselected values of τ_2 equal, respectively, to 180° (*trans*) and 60° (*gauche*). The results shown in Fig. 3 indicate that in both cases the minima of the curves occur for values of τ_1 corresponding to *gauche* conformations. The energy minimum occurs at $\tau_1 = -90°$, $\tau_2 = +60°$, 30 kcal/mole below the value corresponding to the *trans-trans* form. Moreover, an *ab initio* calculation performed for the conformation corresponding to the global energy minimum of the PCILO computation ($\tau_1 = 60°$, $\tau_2 = 0°$) confirms the previous results by indicating an energy lowering of 44 kcal/mole with respect to the totally extended conformation.

These results are summed up in Table 1, for the different *gauche* (*g*) and *trans* (*t*) torsions about τ_1 and τ_2, with respect to the most stable conformation found by PCILO. It is evident that the evolution of the energy differences in the *ab initio* computations parallels the PCILO results and confirms the predominant stability of the folded form for the isolated zwitterion. These refined computations contradict thus the earlier EHT results. This situation also means that the conformations observed in the crystals are quite different from the intrinsically preferred one. This is in no way astonishing when one considers the possibilities of strong intermolecular interactions in the solid.

2.2. *The Molecule in Water*

The question now arises, which has a more direct pharmacodynamic significance, of what becomes of this conformational preference of the free zwitterionic GABA in solution. This section represents an attempt to answer it.

The previously mentioned study of model compounds (alkylammonium and formate ions, Refs. [20, 21]) indicates that the principal hydration sites of GABA will be its two charged ends. A preferential strong fixation of six water molecules is predictable, three at each end, following the scheme of Fig. 4 (with $N^+ \ldots O_{water}$ distance of 2.6 Å and $O^- \ldots O_{water}$ distance of 2.65 Å). Evaluated with an STO 3 G basis set in groups of three, the energy of binding is 28 kcal/mole for a molecule of water at the cationic head and 21 kcal/mole for a molecule of water at the anionic end. However, while in the study of the formate ion the angle $\theta = \overline{C\text{–}O \ldots HO}$, between the C–O and the O–O axis was found to be preferentially equal to 110°, it is obvious that the steric hindrance due to the methylene groups will tend to modify (enlarge) this angle in GABA. We have therefore investigated the effect of the flexibility of the value of θ for the two corresponding water molecules on the conformational possibilities. During this search τ_3 was maintained at the value of 180° representing a staggered arrangement of the cationic head with respect to the C_3–C_4 bond. In a further improvement, once the optimum value of θ determined, the effect of variation of τ_3 and τ_0 was also explored.

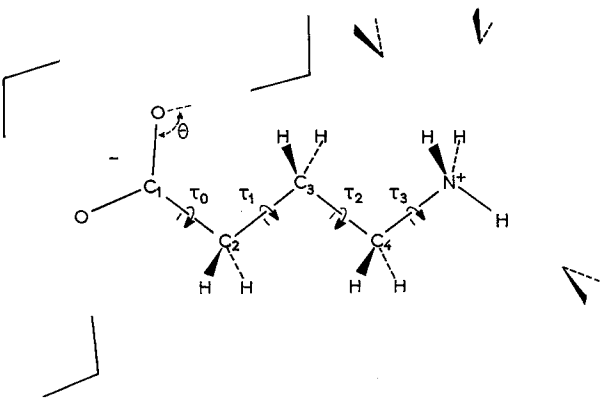


Fig. 4. The first hydration shell of GABA

2.2.1. The Effect of Variation of θ

For $\theta = 110°$ or $120°$ the conformational energy map of hydrated GABA is extremely restricted, steric hindrance preventing rotation about τ_1 which is constrained to the value of 180°. The rotation about τ_2 presents more freedom, the most stable conformation corresponding to $\tau_1 = 180°$, $\tau_2 = \pm 30°$ with a secondary energy minimum for the totally extended form at $\tau_1 = \tau_2 = 180°$, 4 kcal/mole above the global one for $\theta = 110°$ and 2 kcal/mole above the global one for $\theta = 120°$. The situation is illustrated for $\theta = 120°$ in Fig. 5. As can be seen also from Table 2 the release of the steric hindrance by changing θ from 110° to 120° is associated with a stabilization of the preferred conformation by 41 kcal/mole.

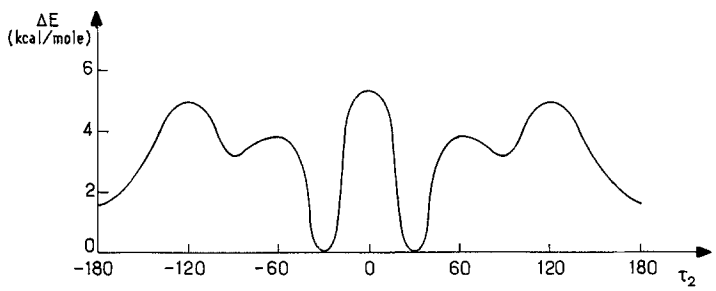


Fig. 5. PCILO conformational energy curve (kcal/mole) of hydrated GABA as a function of τ_2 for $\tau_1 = 180°$, $\theta = 120°$ with respect to the most stable conformation taken as energy zero

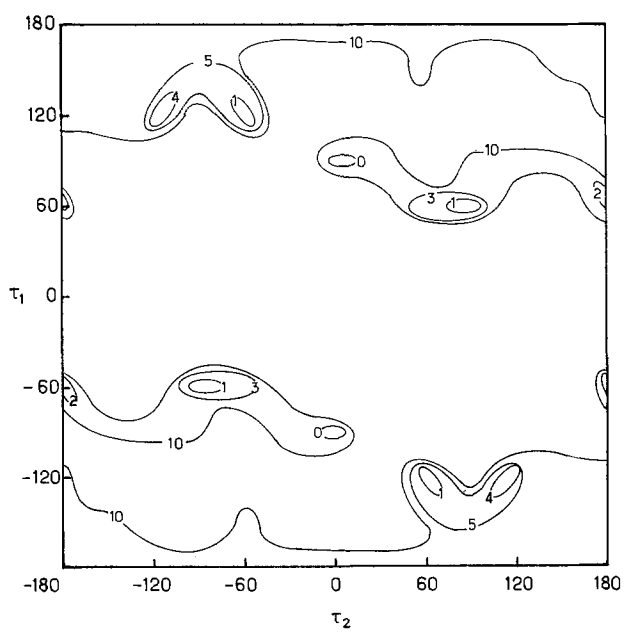


Fig. 6. PCILO conformational energy map of hydrated GABA, for $\theta = 140°$, $\tau_3 = 180°$. Isoenergy curves in kcal/mole with respect to the most stable conformation taken as energy zero

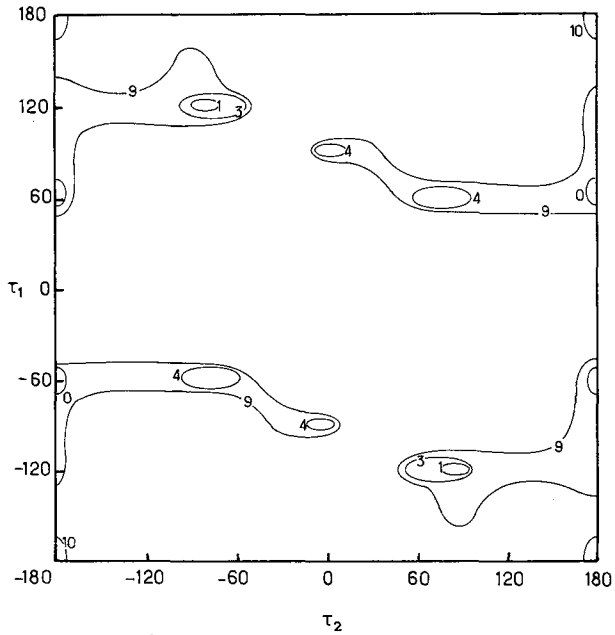


Fig. 7. PCILO conformational energy map of hydrated GABA, for $\theta = 150°$, $\tau_3 = 180°$. Isoenergy curves in kcal/mole with respect to the most stable conformation taken as energy zero

Table 2. Lowest energies of the conformations of hydrated GABA with respect to the global energy minimum taken as energy zero

θ	τ_0	τ_1	τ_2	τ_3	ΔE (kcal/mole)
110	0	180	± 30	180	56
120	0	180	± 30	180	15
130	0	± 90	0	180	7.6
140	0	± 90	0	180	4.3
150	0	± 60	180	180	0.9
150	0	90	−120	195	0
150	0	90	− 60	210	0.4
150	0	− 60	180	225	3.2
150	0	90	−150	240	4.3
150	90	± 60	∓120	180	0.1

The further increase in the value of θ increases appreciably the conformational possibilities of the hydrated species and leads to further stabilization of the preferred conformations. Figures 6 and 7 represent the conformational energy maps of hydrated GABA corresponding to $\theta = 140°$ and 150° respectively. New energy minima appear, global and local, corresponding to a number of energetically nearly equivalent conformations. For $\theta = 140°$, the global energy minimum is at $\tau_1 = \pm 90°$, $\tau_2 = 0°$, with a secondary minimum at $\tau_1 = \pm 120°$, $\tau_2 = \mp 60°$, 0.7 kcal/mole above the global one. For $\theta = 150°$ the global minimum

is at $\tau_1 = \pm 60°$, $\tau_2 = 180°$, with a close secondary minimum at $\tau_1 = \pm 120°$, $\tau_2 = \mp 90°$. As indicated in Table 2 the increase in θ is associated with a continuous stabilization of the global energy minimum, which seems however to be near its limit for $\theta = 150°$.

2.2.2. The Effect of Variation of τ_3

Maintaining θ at 150° we then proceed to vary simultaneously τ_1, τ_2, and τ_3, this last angle from 180° (staggered) to 240° (eclipsed) in 15° rotations. Figures 8–11 reproduce the four most stable conformational energy maps obtained. As can be seen from Table 2, the rotation of τ_3 to 195° and 210° produces a stabilization of the global energy minimum with respect to $\tau_3 = 180°$ by about 0.5–1 kcal/mole; a further rotation has an inverse effect so that in the eclipsed arrangement of the cationic head, the global energy minimum is about 3.4 kcal/mole above that of the staggered form. What is, however, most striking when we look at Figs. 7–9, which represent the most stable situations, is the multiplicity of conformations of nearly equal energy which may be associated with hydrated GABA. Thus in Fig. 8 alone there are three degenerate global energy minima at $\tau_1 = 90°$, $\tau_2 = -120°$, $\tau_1 = -120°$, $\tau_2 = 90°$, and $\tau_1 = -60°$, $\tau_2 = -30°$. To these may be added the global minima of Figs. 9 ($\tau_1 = 90°$, $\tau_2 = -60°$) and 7 ($\tau_1 = -60°$, $\tau_2 = 180°$) which are nearly degenerate with the three previous ones.

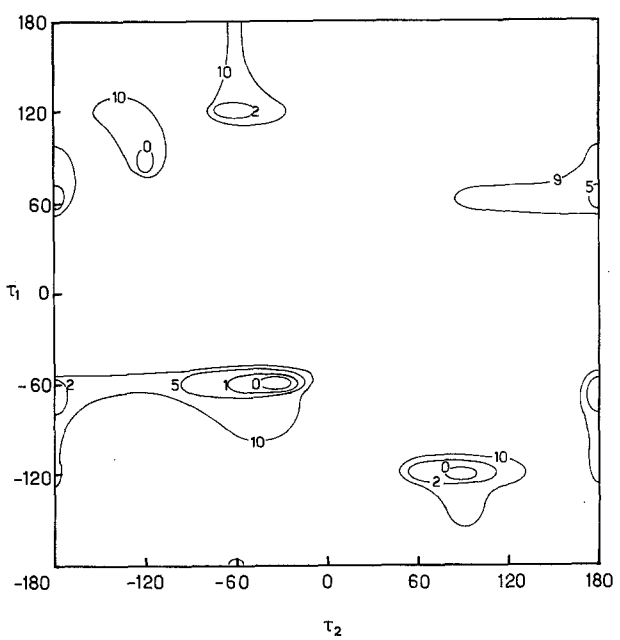


Fig. 8. PCILO conformational energy map of hydrated GABA for $\theta = 150°$, $\tau_3 = 195°$. Isoenergy curves in kcal/mole with respect to the most stable conformation taken as energy zero

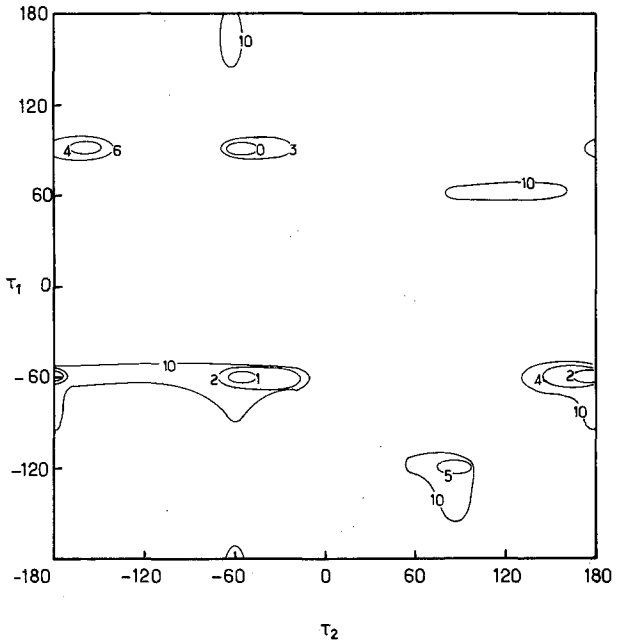


Fig. 9. PCILO conformational energy map of hydrated GABA, for $\theta = 150°$, $\tau_3 = 210°$. Isoenergy curves in kcal/mole with respect to the most stable conformation taken as energy zero

2.2.3. The Effect of Variation of τ_0

Finally, as indicated by the bottom line of Table 2, the effect of variation of τ_0 seems relatively unimportant. An energy minimum comparable to the previously underlined energy minima (which were computed for $\tau_0 = 0°$) is found for $\tau_0 = 90°$ at $\tau_1 = \pm 60°$, $\tau_2 = \mp 120°$ (with θ fixed at 150° and τ_3 at 180°).

3. Conclusion

One of the most striking results of this investigation is certainly given by the large number of stable or possible conformations which may be predicted for GABA in aqueous solution. The principal such conformations are shown in Fig. 12. They offer a wide variety of arrangements, both *gauche* and *trans*, with respect to the principal torsion angles involved. Although we do not expect that the entire solution behavior of GABA can be explained by a treatment reduced to the consideration of the first hydration shell, even if we allow, as it is done here, a certain degree of flexibility in the arrangement of some of the water molecules around the hydration sites, it seems reasonable to consider, in particular in view of the previous successes of the "supermolecule" approach [24–26], that the produce gives an acceptable indication on the direction and magnitude of changes in conformational preferences of the isolated molecule when it enters aqueous solution. From that point of view an essential conclusion of this study is that a

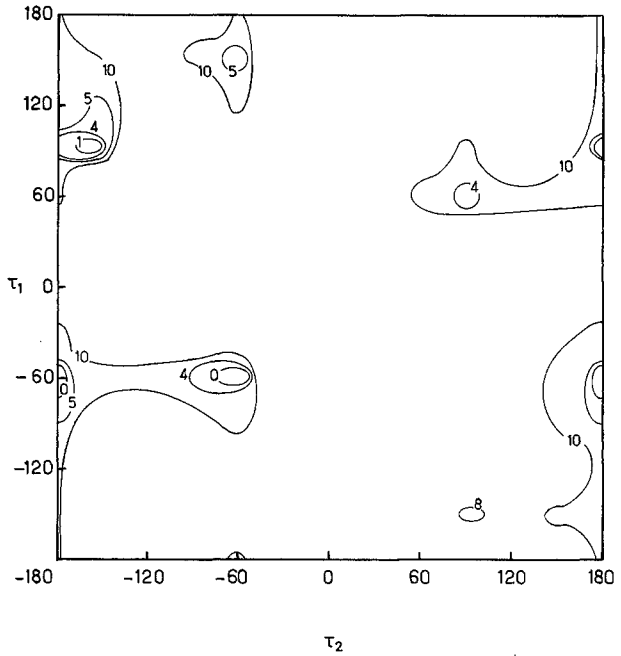


Fig. 10. PCILO conformational energy map of hydrated GABA, for $\theta = 150°$, $\tau_3 = 225°$. Isoenergy curves in kcal/mole with respect to the most stable conformation taken as energy zero

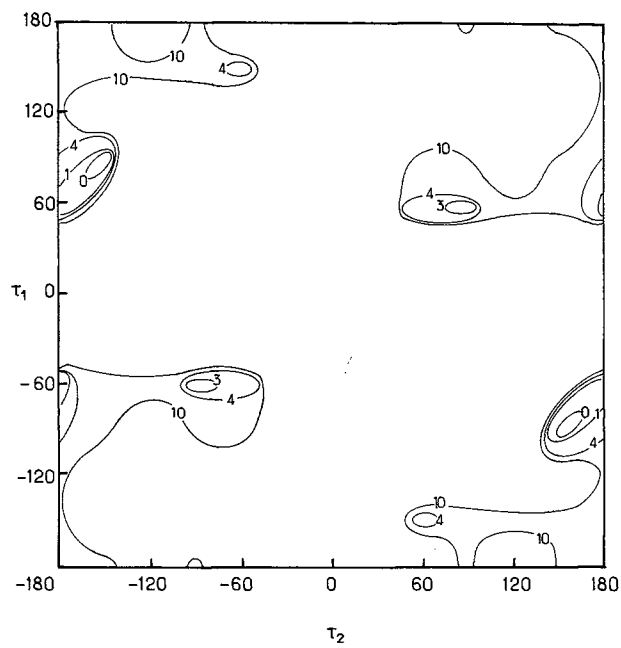


Fig. 11. PCILO conformational energy map of hydrated GABA, for $\theta = 150°$, $\tau_3 = 240°$. Isoenergy curves in kcal/mole with respect to the most stable conformation taken as energy zero

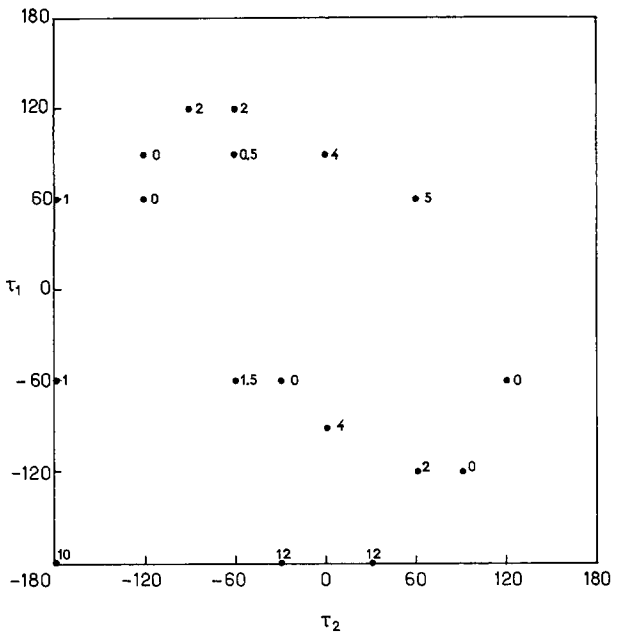


Fig. 12. Conformations susceptible to occur for GABA in solution. Energies (kcal/mole) with respect to the most stable conformations indicated by 0

number of conformations seem possible in solution with an energy difference between them relatively small. This conclusion rejoins a similar one reached in connection with other types of drugs [27]. We have thus to adapt our thinking on the role of conformation in pharmacological activity to this state of affairs which was frequently obscured by the more abundant results of X-ray crystallography of these compounds, yielding a single conformation.

In the particular case of GABA, a very recent NMR study on the conformational properties of its aqueous solution by Ham [28] brings forward a strong support for our proposals. Their author distinguishes at least five different conformations present in various proportions in solution. The most abundant conformation is a *gauche-trans* one with respect to τ_1 and τ_2 obviously corresponding to the stable conformation which we find at $\tau_1 = 60°$, $\tau_2 = 180°$ for $\theta = 150°$. The next most common conformations are a *trans-gauche* one which is close to the most stable conformation which we find for $\theta = 120°$ (which is, however, 14 kcal/mole above the previous one), and the *trans-trans* one which is accessible at about 10 kcal/mole above the most stable one. Finally there are also present in solution a number of *gauche-gauche* conformations more or less reminiscent of the computed ones.

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