CBPF-NF-050/01

Crucial Effect of Hydration in a Model for the Antiepileptic Vigabatrin and the Prosthetic Group Interaction*

Mario Giambiagi[a], Myriam Segre de Giambiagi[a] and Marçal de Oliveira Neto[b]

[a]Centro Brasileiro de Pesquisas Físicas, Rua Dr. Xavier Sigaud 150, 22290-180 Rio de Janeiro, RJ, Brasil

E-mail: mar@cbpf.br; rio@cbpf.br

Fax: (55)(21)586.7400

[b]Instituto de Química, Universidade de Brasília, Campus Universitário Asa Norte, 70910-900 Brasília, D.F. Brasil

E-mail: marcal@unb.br

Abstract

The feasibility of a model regarding the first step of the interaction between the antiepileptic VGB (γ-vinyl GABA) and the prosthetic group PLP (pyridoxal phosphate) is studied. In this model, CγH in VGB is oriented towards the center of the PLP ring previous to a Schiff base formation between them. Hydration is mimicked through molecular mechanics first and molecular dynamics afterwards, both with the Amber force field. The first hydration shell of six water molecules is shown to play a crucial role. Hydrogen bonds are formed between them and a) the carboxyl VGB group; b) the phosphate PLP group. The corresponding 3-center MO bond indices have, accordingly, significant values.

*Proofs and correspondence to:

Mario Giambiagi

Centro Brasileiro de Pesquisas Físicas

Rua Dr. Xavier Sigaud 150, 22290-180 Rio de Janeiro, RJ, Brasil

E-mail: mar@cbpf.br

Introduction

Severe adverse effects are verified in epileptic patients treated with usual anticonvulsionants. The need therefore arises for the study of new antiepileptic drugs. The last decade has witnessed the proposal of several new drugs.

The increase in brain concentration of the major inhibitory neurotransmitter, γ-aminobutyric acid (GABA) seems to have an anticonvulsionant effect[1]. The 4-amino-5-hexenoic acid, γ-vinyl GABA, Vigabatrin (VGB) acts by increasing GABA levels in the presynaptic nerve terminal, through inhibition of the GABA transaminase (GABA-T) activity[2]. This irreversible mechanism results in a two-to-three fold GABA elevation[3]; despite its indirect mechanism of action, nuclear magnetic resonance spectroscopy (NMRS) measurements have allowed the setting up of a correlation between the VGB effects and the GABA levels, reaching a maximum of three times the normal one[4].

However, severe micro-vaculation was found in brains of rats treated with VGB[5,6] and recently, advice has been raised against the clinical use of VGB, as disturbances in the visual field are found, possibly due to vacuolations in the optical nerve[7]. To our knowledge, it is not clear whether this adverse effect is related or not to the inactivation process[8,9].

VGB exists as a racemic mixture of R(−) and S(+) isomers, only enantiomer S being associated with its activity[6]. Several different experimental schemes have been proposed in order to explain the inactivation of pig brain GABA-T molecular mechanism, involving interactions between VGB and the prosthetic group pyridoxal phosphate (PLP)[10,11]. It has been shown[11a] that the inactivation mechanism of GABA aminotransferase by VGB functioned by following two separate pathways, one leading to covalent attachment to the protein and the other leading to covalent attachment to the active site PLP. This study was later extended to VGB analogues containing fluorine[11b,c].

We study in this work, from the quantum chemistry viewpoint, the feasibility of a

model where the $C_\gamma H$ bond in VGB is oriented towards the PLP ring previous to a Schiff base formation between them, along the lines of Ref.[12]. Within this approach, we want to explore which the role of hydration and, consequently, of the hydrogen bond formation regarding VGB-PLP interaction is. We give quantitative estimates for the hydrogen bonds, through a multicenter index[13] which has been successfully applied to biological systems[14] and, particularly, to another antiepileptic, lamotrigine[15].

Methodology

In the first step of our study, the geometries of the Schiff base formed by PLP and an enzymatic lysine residue, and VGB have been separately optimized using the AM1 Hamiltonian of the MOPAC quantum package[16] (see Fig. 1). After this optimization, different calculations were performed so as to obtain the experimentally proposed interaction scheme. The quantum AM1 approximation was tried, as well as molecular mechanics (MM) using the Amber force field. Neither of them yielded any VGB-PLP interaction profile; we were hence led to explore if the medium could play a basic role in the problem. Let us underline that in this work we do not attempt an exhaustive discussion of the features involved in the problem, regarding the solvent effect or the presence of salts in the solution, on the VGB-prosthetic group interaction. The adopted procedure is actually a simple calculation protocol with the purpose of setting a scheme with water molecules taking part in the interaction between both molecular systems.

We have introduced the solvent around the two above mentioned systems by employing periodic boundary conditions in a cubic cell with $9\mathring{A}$ sides. MM calculations were carried out, in order to minimize the interaction energy of the systems lysine residue-PLP and VGB in the presence of 182 water molecules. MM uses only force fields, not involving temperature or time dependence either. Simulations were performed using MM from the Hyperchem package[17]. The force field used was the Amber all-atom force field[18a,b], adapted by introducing the appropriate parameters for the present atomic interactions.

Our energy minimizations employ successively steepest descents, conjugated gradient and quasi Newton-Raphson techniques. The initial set of atomic coordinates was freely minimized until the maximum gradient for all atoms was less than 0.01 kcal.mol^{-1}.$\AA^{-1}$. The final energy value was -3691.80 kcal.mol^{-1}. No significant complexation between the solvent and the PLP-VGB system was observed at the end of this stage.

In the second step we have applied Molecular Dynamics (MD) with the same force fields. The optimized system previously obtained was used as a starting point for the MD simulation. The system was submitted to a run of 20 ps, at a temperature range of $0-300$ K. The temperature step was 30 K, the heating time 0.5 ps and the time step of 0.5 fs was used in the integration algorithm. In this stage a significant interaction between the PLP-VGB system and water was not observed either. The total energy was -3565.80 kcal.mol^{-1}.

In order to obtain a lower energy minimum, the system was cooled down to a run of 10 ps from 300 K to 0 K, in time steps of 1.0 ps and temperature steps of 10 K. The total energy lowers to -3846.20 kcal.mol^{-1}, i. e. more stable by 154 kcal.mol^{-1} relative to that obtained through MM only. In this stage, it was possible to obtain an interaction scheme where the water molecules form a hydrogen-bounded structure with systems lysine-PLP and VGB. Fig. 2b shows only the relevant solvent structure belonging to the first hydration shell, namelly a set of six water molecules. This system is now subjected to a new MOPAC calculation, as explained in the next section.

Results and discussion

The MM minimization of the systems enzymatic lysine residue-PLP and VGB, carried out with the Amber force field prior to hydration, gives the conformation of Fig. 2a. After hydration, the Cγ-H bond of the S(+) isomer of VGB turns out to be oriented perpendicular to the coenzyme ring center (Fig. 2b), while previously it was away off this orientation by 62^o. That is, the allowance of interaction between the mentioned systems

and water as described in the previous section, makes the model orientation possible as in Fig. 2b. Moreover, the corresponding C_γ-ring distance is then $4.4\mathring{A}$, within the range of distances which have been estimated in complexes between heavy atoms (bonded to H) and the center of an acceptor benzene ring[12]. For the bond indices calculation we have used the PM3 MOPAC Hamiltonian, which is known to perform better than other semi-empirical ones for hydrogen bonded systems[19]. The PM3 atomic charges predict a charge transfer of ~ 0.13 electrons from VGB towards PLP and the first hydration shell water molecules; most of this charge (0.10) goes to the hydration shell; we shall return to this subject further on in this section.

Let us remark that, prior to hydration (Fig. 2a), a H bond is found between O_1 in VGB and N_1-H of PLP. Hydration causes a rotation of the carboxylic COO^- group; since the O_1-H distance above mentioned becomes $3.92\mathring{A}$, the previous H bond is no longer possible. Instead, the other COO^- oxygen (O_2) joins a H bond with a water molecule. The $N_\delta-H$ bond in VGB entered a H-bond with $O_{2'}$ of PLP (Fig. 2a); after hydration, it is replaced by another one with the $O_{5'}$ phosphate (N_δ-H-$O_{5'}$) (Fig. 2b), favouring the desired C_γ-H orientation.

The phosphate group plays an essential role in the hydration process. Each of its three oxygen atoms ($O_{4'}$, $O_{5'}$, $O_{6'}$) are involved respectively in three, two and one H bonds. The energy balance and charge density distributions for the interactions between water and the oxygen atoms in the phosphate and dimethyl phosphate anions have been extensively analyzed in the literature[20–22]. The predominating attraction suffered by the six water molecules of the first hydration shell is, as seen in Fig. 2b, the one towards the phosphate oxygens and the above mentioned O_2 of VGB; outside from the corresponding H bonds, we find only one bond between two water molecules (W_5 and W_6). This agrees with the conclusion of Langlet et al., pointing out that the water molecules in the first hydration shell are bound to DMP^- rather than between themselves[21].

Multicenter bond indices have been shown to give valuable information[23]. In the

three-center case, it is particularly suitable as a measure of hydrogen bonds[13]. Let us give briefly the necessary formulation. In non-orthogonal bases, the idempotency of the Π matrix (2Π is the first-order density matrix) leads to the definition of a bond index I_{AB} for the bond between atoms A and B in the closed-shells case[24,25]:

$$I_{AB} = 4 \sum_{a \in A, b \in B} \Pi_a^b \Pi_b^a \tag{1}$$

which is the generalization of the Wiberg bond index[26] to non-orthogonal bases. As the idempotency of Π holds for any power, it is possible to write for three-center bonds (see Refs.[13,23] for multicenter bonds in general)

$$I_{ABC} = 8 \sum_{a \in A, b \in B, c \in C} \Pi_a^b \Pi_b^c \Pi_c^a \tag{2}$$

We have shown that the PM3 approximation[16] gives quite meaningful results for I_{XHY} in systems of biological interest[14,15].

Table 1 shows the I_{ABC} values, together with I_{BC} and the corresponding distances and angles, for the H bonds of Fig. 2b. The first row reports the values for the only VGB-water bridge, while the last one corresponds to the only water-water bridge. The first one is the weakest, for it is the farthest from linearity, almost a bent bond; this is consistent with a similar case we have met with[14].

To our knowledge, there are very few I_{ABC} values for hydrogen bonds in the literature; we have shown its appropriateness, since the three-center index makes a clear distinction between strong and normal H bonds[13]. The values for systems involved in biological problems are even fewer[13–15]. These quantities, we believe, could play a leading role in the description of hydrated clusters in biomolecules; the increasing growth of this new link between chemistry and biophysics has been surveyed in a very recent review[27].

All our H-bonds I_{XHY} values are negative, both for strong and normal H bonds. We have shown that no *a priori* distinction between positive and negative values is to be expected in this case[28]. The quantities reported in Table 1 are not enough to allow a

sound conclusion about the relative influence of H bond distances and angles on I_{XHY} indices. Nevertheless, at first glance, it would seem that the clearly highest value for the ($O(W_3)$-H-$O_{5'}$) bond is due mostly to the short distance. Leaving aside the first row, the deviation from linearity falls within a range of 8^o; the distance range is more appreciable instead. All I_{XHY} values ($0.01-0.03$) save one characterize normal H bonds. There is only one NHO bond, that correspond to the longest XY distance, linking VGB to PLP; the average I_{OHO} value is -0.021.It is also seen in the table that the I_{HY} indices match well the behaviour of the I_{XHY} indices.

Let us look for cooperative effects[29] on the I_{XHY} indices. The H bond "chain" [($O_{4'}$-H-$O(W_5)$)($O(W_5)$-H-$O(W_6)$)($O(W_6)$-H-O_2)] does not manifest mutual reinforcement when compared with the only bond not involved neither in a chain, nor in a sharing scheme, ($O_{6'}$-H-$O(W_2)$); on the contrary, they decrease. We would therefore not conclude in favour of cooperativity.

The six water molecules of course reflect on their I_{OH} values (not reported in the tables) the effect of H bonding. The five OH bonds not involved in H bonds keep their I_{OH} values within the narrow range (0.95-0.97). I_{OH} in W_3 falls to 0.82, while the six other indices of the OH bonds taking part in a bridge decrease to the range (0.88-0.93).

Table 2 shows the I_{AB} values for effective and secondary (i.e. formal) bonds for the $-O-PO_3$ group in PLP and for the CO_2^- group in VGB, before and after joining the VGB-PLP-water system. It is well known[13,25] that oxygen usually takes part in secondary bonds; i.e. OO' "bonds" are particularly significant in most cases. We have thus shown in the table not only the $I_{OO'}$ values for the PO_3 moiety, but also the values involving $O_{2'}$. After joining the complex, the bond charges (the bond indices are the electronic charges along the bonds, see Refs.[24,25]) become altered and those corresponding to the formal bonds more than those of effective bonds. As there is a great reorganization, and $O_{4'}$ and $O_{5'}$ take part in three and two H bonds respectively, it is not possible to follow its details. The three $I_{OO'}$ values in PO_3 decrease, $I_{4'5'}$ most strikingly. Of the I_{PO} values,

two of them decrease, one increases and $I_{3'5'}$ increases slightly. The relative variations of the formal bonds are much greater. We have mentioned that there is an overall charge transfer from PLP to water; to our knowledge, it has not been remarked before that charge transfer may be in some cases mostly ascribed to the bond charges.

As to the (CO_2^-) group in VGB, the OO' index remains almost unchanged. The I_{CO} values, instead, become more similar under interaction, i.e. they depict a more conjugated system.

In order to compare other multicenter bond indices (the parenthesis indicating the values previous to the interaction), let us report:

$$I_{COO'}(VGB) = -0.2046(-0.2037); \quad I_{POO'O''}(PLP) = -0.0149(-0.0600);$$

$$I_{ring}(PLP) = 0.0260(0.0288)$$

The three-center CO_2^- index of VGB is maintained; the four-center PO_3 index decreases, in agreement with the above discussion. As to the ring in PLP, it indicates a moderate, non-negligible conjugation[30]; much more significant, for instance, than the one for the $(S_3N_3)^-$ system[23].

Two separate pathways have been proposed for the inactivation mechanism of GABA aminotransferase by VGB[11a]. The present study, which intends to be an approximation to a much more complicated problem involving features not considered here, concerns only one of them: namely, the one related to the covalent attachment to the active site PLP[11a,b]. The proposed fluorinated analogues of VGB[11b] are likely to have an influence on the obtained hydration scheme; we propose to approach this problem in the near future.

We hope that the appealing results obtained with our model shall prompt other theoretical and experimental work about the VGB interactions mechanisms.

Conclusion

Hydration of the enzymatic lysine residue-PLP and VGB systems gives rise to hydro-

gen bonds between the first hydration shell of six water molecules as well as, on one side, the carboxyl group of VGB and on the other side, most heavily, the phosphate group of PLP. The proposed model, with the C_γ-H of VGB perpendicular to the PLP ring, becomes thus feasible.

Acknowledgement

The authors wish to acknowledge Prof. José Maria Pires for his preliminary calculations and Prof. Ricardo Bicca de Alencastro for the generous discussions carried out.

Figure Captions

Fig. 1. Labelling of a) vigabatrin (VGB) and of b) the Schiff base formed by pyridoxal-5'-phosphate (PLP) with the ε-amino group of a specific lysine residue at the active site; in the calculations that follow, we replace the enzyme by a hydrogen atom.

Fig. 2a). The systems lysine residue-PLP and VGB prior to hydration. The two H-bonds formed before hydration are shown. The C_γ-H bond of VGB is not oriented towards the PLP ring. The enzymatic fragment attached to the PLP ring is replaced by a hydrogen atom.

Fig. 2b). The system lysine residue PLP-VGB after hydration, together with the six water molecules W of the first hydration shell. The phosphate $O_{5'}$ has replaced $O_{2'}$ in the H-bond formed by N_δ-H. The N_1-H-O_1 bond, which prevented the orientation of the C_δ-H bond towards the PLP ring, has disappeared. It is thus possible to obtain the required orientation. All the H-bonds formed by the water molecules are shown in the Figure.

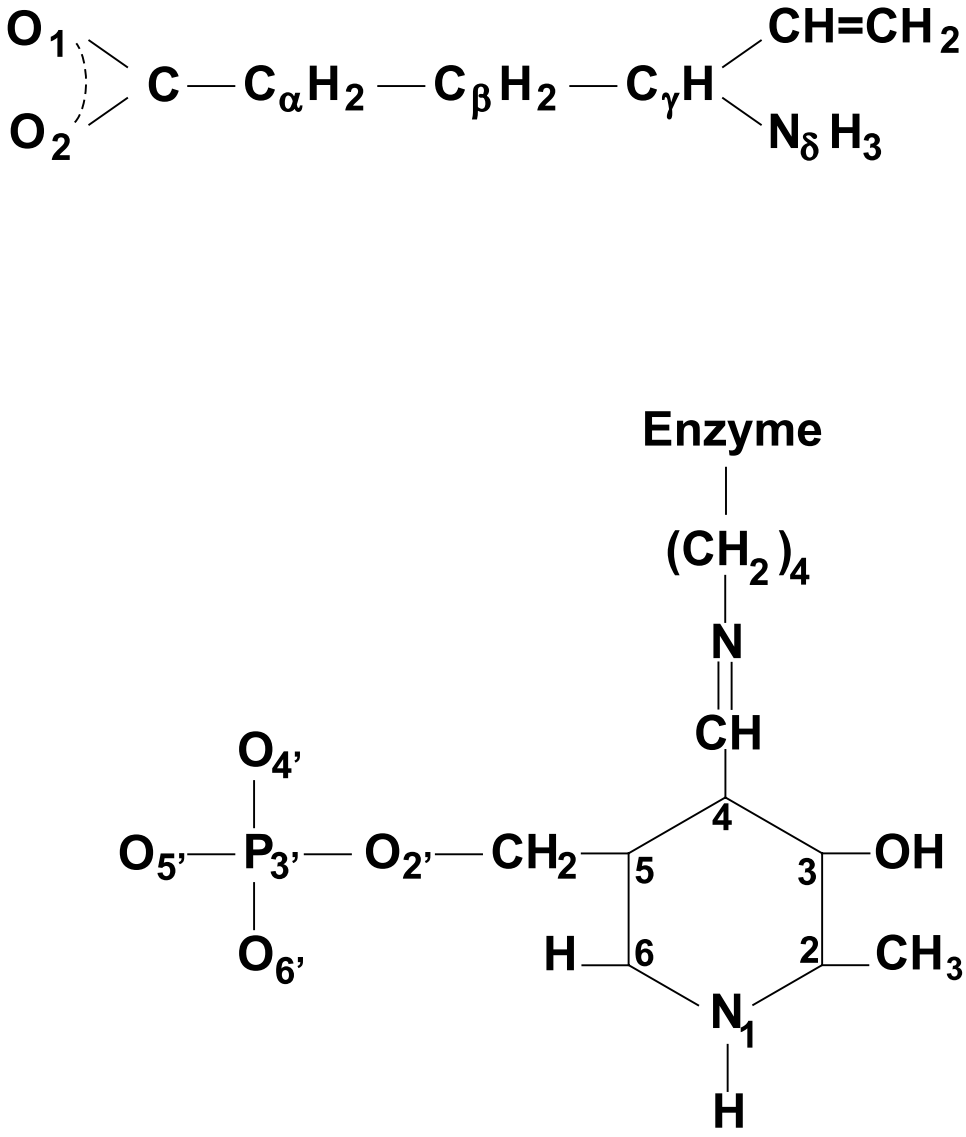
O1
O2
C
Cα H2
Cβ H2
Cγ H
CH=CH2
Nδ H3
Enzyme
(CH2)4
N
CH
O4'
O5'
P3'
O2'
CH2
4
5
3
OH
H
6
2
CH3
O6'
N1
H

Fig. 1

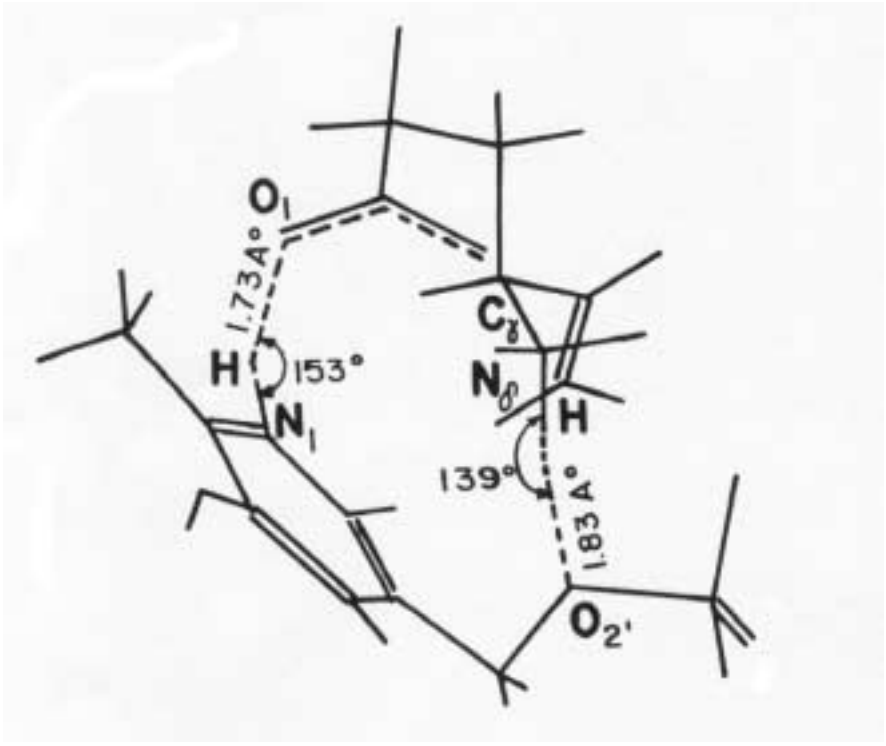

Fig. 2a

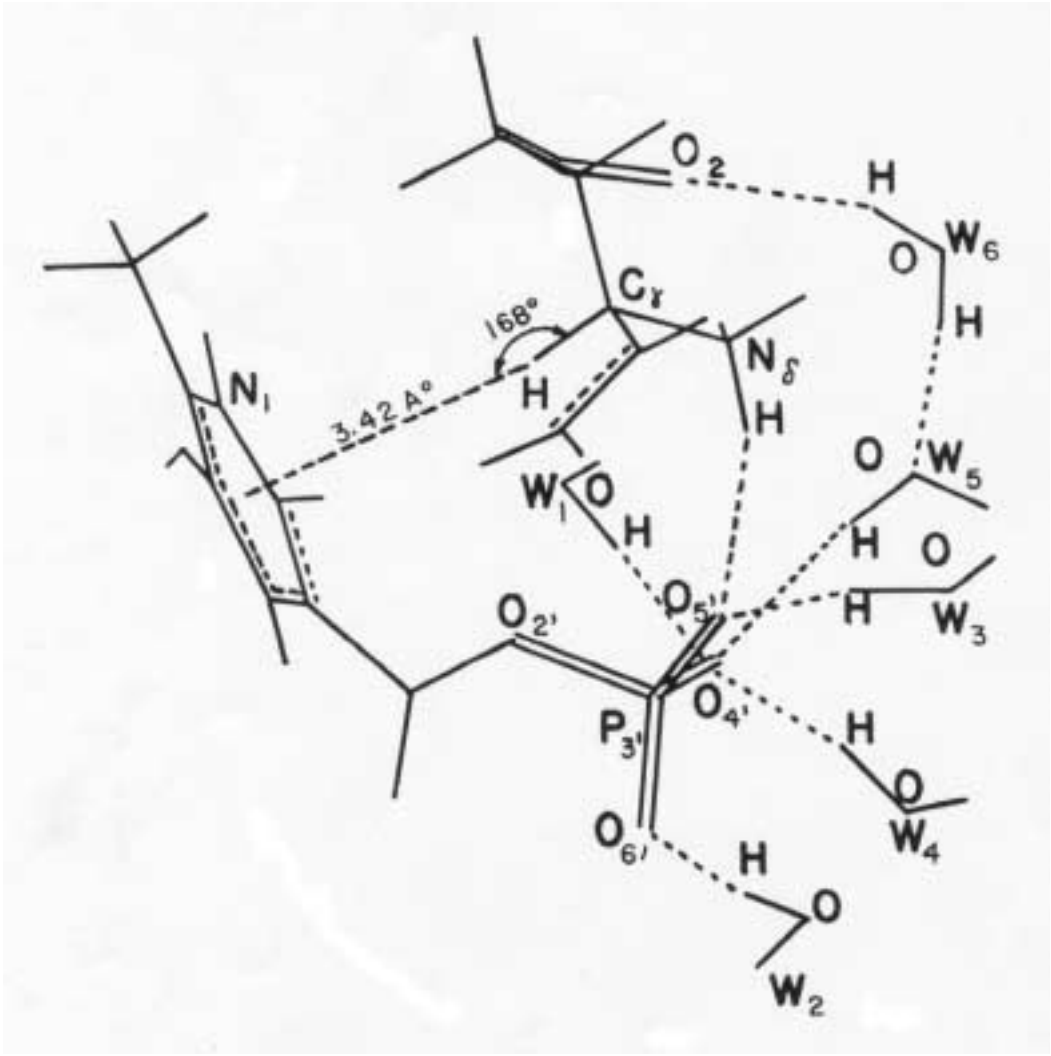
O2
H
W6
O
H
Cγ
168°
Nδ
H
N1
3.42 A°
H
O
W5
W1
O
H
H
O
O5'
H
W3
O2'
O4'
P3'
H
O
W4
O6'
H
O
W2

Fig. 2b

Table 1. Hydrogen bonds of Fig. 3b. Three-center bond indices I_{XHY}, I_{AB} for the HY bond and geometrical features.

Labelling	XH$\cdots$Y	$-I_{XHY}$	I_{HY}	$r_{HY}(\mathring{A})$	$r_{XY}(\mathring{A})$	$< XHY(^o)$
$O(W_6)HO_2$	OH$\cdots$O	0.0073	0.0127	2.02	2.85	144.3
$O(W_5)HO_{4'}$	OH$\cdots$O	0.0132	0.0182	2.06	2.99	164.2
$O(W_4)HO_{4'}$	OH$\cdots$O	0.0283	0.0533	1.69	2.62	162.4
$O(W_1)HO_{4'}$	OH$\cdots$O	0.0198	0.0283	1.89	2.83	167.0
$N_\delta HO_{5'}$	NH$\cdots$O	0.0125	0.0153	2.12	3.09	161.8
$O(W_3)HO_{5'}$	OH$\cdots$O	0.0339	0.1136	1.45	2.39	167.3
$O(W_2)HO_{6'}$	OH$\cdots$O	0.0283	0.0607	1.64	2.59	169.8
$O(W_6)HO(W_5)$	OH$\cdots$O	0.0208	0.0373	1.71	2.66	169.8

Table 2. I_{AB} values, for effective and secondary bonds, before (I_{AB}) and after (I'_{AB}) joining the VGB-PLP-water system. The values refer to the O-PO_3 fragment in PLP and to the CO_2 group in VGB.

A	B	I_{AB}	I'_{AB}
$P_{3'}$	$O_{4'}$	0.8785	0.9238
$P_{3'}$	$O_{5'}$	0.8376	0.8491
$P_{3'}$	$O_{6'}$	1.0400	0.9782
$P_{3'}$	$O_{2'}$	0.6441	0.5907
$O_{2'}$	$O_{4'}$	0.0437	0.0545
$O_{2'}$	$O_{5'}$	0.0662	0.0353
$O_{2'}$	$O_{6'}$	0.0808	0.0683
$O_{4'}$	$O_{5'}$	0.2133	0.0495
$O_{4'}$	$O_{6'}$	0.3829	0.2023
$O_{5'}$	$O_{6'}$	0.3280	0.1331
C	O_2	1.3615	1.4089
C	O_1	1.6081	1.5548
O_1	O_2	0.1699	0.1678

References

1 K. Gale, *Epilepsia*, 1989, **30 (Suppl.3)** S1.

2 M.J. Jung, B. Lippert, B.W. Metcalf, P. Böhlen and P.J. Schechter, *J. Neurochem.*, 1977, **29**, 797.

3 P.J. Riekkinen, A. Pitkanen, A. Ylinen, J. Sivenius and T. Halonen, *Epilepsia*, 1989, **30 (Suppl. 3)**, S18.

4 R.H. Mattson, O.A.C. Petroff, D. Rothman and K. Behar, *Acta Neurol. Scand.* , 1995, **Suppl. 162**, 27.

5 R.A. John, E.M.Rimmer, J. Williams, G. Cole, L.J. Fowler and A. Richens, *Biochem. Pharmac.* , 1987, **36**, 1467.

6 E. Bem-Menachem, *Epilepsia*, 1995, **36 (Suppl. 2)**, S95.

7 A. Thomson, private communication.

8 D. De Biase, D. Barra, F. Bossa, P. Pucci and R. A. John, *J. Biol. Chem.*, 1991, **266**, 20056.

9 A.Richens, *Antiepileptic Drugs*, 3rd. Ed.; R. Levy, R. Mattson, B. Meldrum, J.K. Penry and F. E. Dreyfuss eds., Raven Press, N. York, 1989.

10 M. D. Toney, S. Pascarella and D. De Biase, *Protein Sci.*, 1995, **4**, 2366.

11 (a) S. M. Nanavati and R. B. Silverman, *J. Am. Chem. Soc.*, 1991, **113**, 9341; (b) R.B. Silverman, K.A. Bichler and A.J. Leon, *J. Am. Chem. Soc.* 1996, **118**, 1241; (c) R.B. Silverman, K.A. Bichler and A.J. Leon, *J. Am. Chem. Soc.*, 1996, **118**, 1253.

12 B.V. Cheney, M.W. Schulz, J. Cheney and W.G. Richards, *J. Am. Chem. Soc.*, 1988, **110**, 4195.

13 M. Giambiagi, M.S. de Giambiagi and K.C. Mundim, *Struct. Chem.*, 1990, **1**, 423.

14 M. de Oliveira Neto, M.S. de Giambiagi and M. Giambiagi, *Chem Phys. Lett.*, 1998, **290**, 205.

15 M. de Oliveira Neto, J.M. Pires, M. Giambiagi, M.S. de Giambiagi and F. A. Alvarez, *Struct. Chem.*, 1998, **9**, 339.

16 J.J.P. Stewart, J. *Comp.-Aided Mol. Design*, 1990, **4 (special issue)**, 1.

17 Hypercube Inc., 419 Phillip St., Waterloo, Ontario, Canada N2L3X2 (1994).

18 a) S.J. Weiner, P.A. Kollman, D.A. Case, U. Chandra Singh, C. Ghio, G. Alagona, S. Profeta Jr. and P. Weiner, *J. Am. Chem. Soc.*, 1984, **106**, 765. b) S.J. Weiner, P.A. Kollman, D.T. Nguyen and D.A.Case, *J. Comp. Chem.*, 1986, **7**, 230.

19 P.Novak, S. Sekušak, D. Vikic-Topic and Z.Popovic, *J. Chem. Soc., Faraday Trans.*, 1998, **94**, 1051.

20 A. Pullman, B. Pullman and H. Berthod, *Theor. Chim. Acta*, 1978, **47**, 75.

21 J. Langlet, P. Claverie, B. Pullman and D. Piazzola, *Int. J. Quantum Chem., Quantum Biol. Symp.*, 1979, **6**, 404 .

22 G. Alagona, C. Ghio and P. Kollman, *J. Am. Chem. Soc.* , 1983, **105**, 5226.

23 M. Giambiagi and M.S. de Giambiagi, *Trends Chem. Phys.*, 1996, **4**, 111.

24 M. Giambiagi, M.S. de Giambiagi, D.R. Grempel and C.D. Heymann, J. *Chim. Phys.*, 1975, **72**, 15.

25 M.S. de Giambiagi, M. Giambiagi, and F.E. Jorge, *Z. Naturforsch.*, 1984, **39 a**, 1259.

26 K. Wiberg, Tetrahedron, 1968, **24**, 1083.

27 E.G. Robertson and J.P. Simons, *Phys. Chem. Chem. Phys.*, 2001, **3**, 1.

28 K.C. Mundim, M. Giambiagi and M.S. de Giambiagi, *J. Phys. Chem.*, 1994, **18**, 6118.

29 See for instance O. M, M. Yez and J. Elguero, *J. Mol. Struct. (Theochem)*, 1994, *314*, 73.

30 M. Giambiagi, M.S. de Giambiagi, C.D. dos Santos Silva and A. Paiva de Figueiredo, *Phys. Chem. Chem. Phys.*, 2000, **2**, 3381.