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SAR Study of the Anticonvulsant Activity of Amides and Esters of the Valproic Acid

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SUMMARY. The conformational and electronic characteristics of valproic acid (vpa), valpromide (vpd), ethyl valproate (evp), propyl valproate (pvp), butyl valproate (bvp), isobutyl valproate (ibvp) and valproyl valproate (vvp), are analyzed at a semiempirical level, in comparison with their lipophilic properties. The goal is to understand the origin of the activity enhancement of vpa by amidation, and the suppression of its activity by esterification. The feasibility of considering the amide and the esters as prodrugs of vpa is discussed in the light of different hypothesis that include, among others: (a) effective or non effective biotransformation of the esters to the acid and (b) anionic or neutral form of the acid in the site of action. Among the conformational, electronic and lipophilic parameters, the conformational ones appear as determinant for the anticonvulsant activity, and point to the O–C–C–H portion, which might define a pharmacophore or is, at least, more closely related to the activity.

RESUMEN. "Estudio SAR de la actividad anticonvulsivante de amidas y ésteres del ácido valproico". Las características conformacionales y electrónicas del acido valproico (vpa), valpramida (vpd), valproato de etilo (evp), valproato de propilo (pvp), valproato de butilo (bvp), valproato de isobutilo (ibvp) y valproato de valproilo (vvp), fueron analizadas por medio de la aplicación de métodos semiempíricos y evaluadas en comparación con las propiedades lipofílicas. El objetivo es comprender el origen del incremento de la actividad del vpa por amidación y la supresión de ésta por esterificación. Se discute la factibilidad de considerar a las amidas y ésteres como prodrogas del vpa, a partir de diferentes hipotesis que incluyen, entre otras: a) efectiva o no efectiva biotransformación de los ésteres a ácido y b) forma aniónica o neutra del vpa en el momento de interacción en el sitio de unión. La evaluación de los descriptores conformacionales, electrónicos y lipofílicos, muestran los requerimientos conformacionales como determinantes de la actividad anticonvulsivante, asociando la porción O–C–C–H a los requerimientos estructurales del farmacóforo.

KEYWORDS: Molecular Orbital calculations, Pharmacophore, Anticonvulsant Activity, Prodrugs, Valproic Acid Derivatives
PALABRAS CLAVE: Cálculo de Orbitales Moleculares, Actividad anticonvulsivante, Derivados del Ácido Valproico, Farmacóforo, Prodrogas

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INTRODUCTION

Epilepsy is one of the most common disorders of the central nervous system, which affects about 1% of the global population [1-3]. Since epileptic seizures must be viewed as multifactorial, antiepileptic drugs (AED) with several mechanisms of action, such as valproic acid (vpa) [4, 5] have advantages in terms of antiepileptic efficacy compared to drugs with a selective effect. Vpa enhances γ-aminobutiric acid (GABA)-mediated inhibition and decreases excitatory amino-acidergic transmission, being also involved in the decrease of Na^+ conductance, and inhibiting, thence, repetitive neuronal firing [3]. It is a non-sedative drug, which is effective and sufficiently non-toxic for chronic use, but several seizures are resistant to its therapy. This resistance to the drugs, which define as intractable several kinds of epilepsy *(i.e.,* Lennox-Gastant syndrome, severe myoclonic epilepsies in infancy), explains the need for the development of new antiepileptic molecules [6].

There are, at least, three strategies that are currently used for the development of new AED: (a) random screening of newly synthesized chemical compounds of diverse structural categories for anticonvulsant activity, (b) structural variation of known antiepileptic drugs, and (c) rational design, based on the knowledge of the events responsible for epilepsy. An alternate strategy to the traditional structural variation of known drugs is the development of transport forms (prodrugs) to help drug distribution to the specific site of action [3]. Prodrugs are biologically inert molecules [7] which enhance drug delivery to a particular organ or site, and require several steps (chemical or enzymatic reactions) to release the active drug [8]. By preferential delivery of the drug to the site of action, the overall toxicity is significantly reduced while maintaining its therapeutic benefits. In the case of vpa, prodrugs can minimize side effects such as teratogenicity [9,10], hepatotoxicity, and even gastric irritation. The slow release of the active drug also assists with the pharmacokinetical drawback of having the shortest half-life of all the existing antiepileptics [11,12], a fact that forces a high frequency dosing regime. The rate of biotransformation of the prodrug is used, thence, to obtain sustained levels of vpa in plasma.

Antiepileptic (AE) prodrugs should contain specifically designed brain target [8,13]. However, in general, the design of an AE prodrug has been based on an increase of the lipophilicity, so that the drug is distributed in the whole body, including the brain, as well as on the consideration of the easy release of the drug from its biologically inactive precursor by enzymatic hydrolysis. The first attempts, in relation to vpa therapy, were centered on the esters of vpa, because of the increase of the lipophilicity by esterification. However, controversial results on the subject have been published during the last decade: Pharmacokinetic analysis of monoalkyl esters of vpa has shown that, although they underwent rapid biotransformation to vpa in dogs, they were not active against convulsion in mice [2, 11, 12] This result is not self-supporting, because rapid and complete conversion to vpa should imply, at least, the same anticonvulsant activity.

Valpramide (vpd), a primary amide of vpa, has been found to be a prodrug in humans, where it is rapidly and almost completely metabolized to vpa [2, 14-18] The anticonvulsant activity screened in mice has shown that vpd is 2-5 times more potent than vpa [2, 14]. Moreover, it has also been reported [19-23] that vpd possesses specific properties of its own (unrelated to vpa): it induces an elevation in the

plasma level of carbamazepine-10,11-epoxide, the active metabolite of carbamazepine. Thence, its consideration as a prodrug of vpa in mice, is still under discussion.

Looking for an explanation of these experimental facts, we have decided to analyze the structural, electronic and lipophilic properties of vpd and 5 monoalkyl esters of vpa, judging the reliability of their definition as prodrugs of vpa. A recent QSAR (Quantitative Structure Activity Relationship) study of a series of vpa derivatives generated by substitution on the alkyl moiety [24], has shown that lipophilic and electronic effects have to be considered together to quantify the anticonvulsant activity. Moreover, the statistical analysis has demonstrated that the CO group of the carboxylic function seems to be significantly associated with the actual reaction site for the series under study. Although no QSAR study can be performed for vpa, vpd and the monoalkyl esters, because of the lack of activity of the last set of compounds, we present, in this article, a thorough analysis of the lipophilic, conformational and electronic characteristics of valproic acid (vpa), Valpramide (vpd), ethyl valproate (evp), propyl valproate (pvp), butyl valproate (bvp), isobutyl valproate (ibvp) and valproyl valproate (vvp), with the aim of understanding, by means of a SAR (Structure Activity Relationship) analysis, the effects associated with the activity enhancement of vpa by amidation and its decrease by esterification.

METHODS

Calculation procedure

The analysis presented in this article is based on the evaluation of lipophilic, conformational, and electronic parameters of vpa, vpd, evp, pvp, bvp, ibvp and vvp.

The quantitative expression of lipophilicity as the partition coefficient (log P, Table 2) is the result of calculations that use the atomic parameters derived by Ghose and coworkers [25] within the Chem-Plus extension of Hyperchem [26]. The same package has been used to calculate the surface area and the volume, with the method described by Bodor *et al.* [27].

Electronic parameters have been calculated by means of quantum chemical procedures. They have shown to be useful in several opportunities, not only as activity quantifiers, but also as descriptors of the relative importance of the different atomic centers in the determination of the activity of a molecule. They allow, in this way, preliminary inferences on the reaction mechanism responsible for drug action. Atomic charges have been widely used for this purpose [28]. They are not, however, quantum mechanical observables, and their definition, together with the population analysis itself from which they derive, are arbitrary concepts. They have to be used with caution, and special care has to be taken in choosing the best method, or the best theoretical model, for their calculation, mainly when dealing with medium-size molecules like vpa derivatives, for which the use of high-quality *ab-initio* calculations is precluded. Semiempirical calculations are often the choice for these systems, as they can be used with confidence to analyze trends in a given series. However, different semiempirical methodologies may lead to different results, not only at a quantitative, but also at a qualitative level, and, thence, to different conclusions. We have chosen AM1 as the calculation proce-

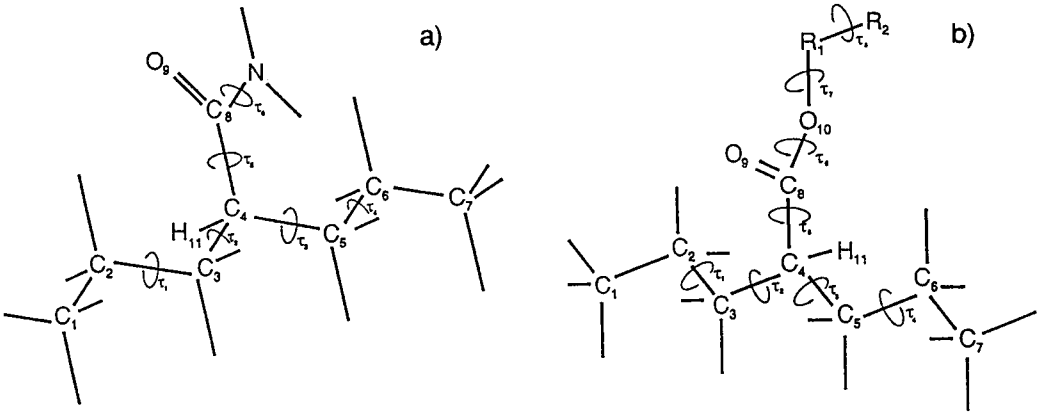

Figure 1. Most stable conformations of vpd (a) and vpa and its esters (b), derived from AM1 calculations. τ_i = torsional angles varied in the conformational study.

	vpa	evp	pvp	Bvp	Ibvp	vvp	vpd
τ_1	-179.54	177.96	175.78	174.40	176.62	178.52	177.83
τ_2	68.65	-71.39	-70.80	-66.28	-70.78	-73.85	-61.90
τ_3	- 69.83	71.23	71.69	70.48	68.03	68.13	64.75
τ_4	176.36	-173.56	-179.52	-175.92	-175.76	-78.15	-178.67
τ_5	-58.51	-50.61	-55.73	-53.62	-61.62	-78.15	119.47
τ_6	-0.08	-0.23	-177.43	-175.64	-1.24	-2.98	0.25
τ_1	-	-77.41	81.65	82.29	-83.10	78.96	-
τ_1	-	-	179.32	-179.54	172.99	53.93	-

Table 1. Torsional angles of the most stable conformers of each of the compounds analyzed. Only the most relevant for the definition of the conformation are reported.
τ_1: C_1 -C_2 - C_3 - C_4, τ_2 : C_2 -C_3 - C_4 - C_8, τ_3: C_6 -C_5 -C_4 -C_8, τ_4: C_7 -C_6 - C_5 - C_4, τ_5: C_3 -C_4 - C_8 - C_9, τ_6: C_9 -C_8 - C_{10} - R_1, τ_7: C_8 -C_{10} - R_1 - R_2.

Compound	Log P	Surface Area A2	Volumen A3	DE_{50} (mg/Kg)	
				MES	scMet
vpa	2.61	361.35	551.32	200(a,b)	146(a,b)
evp	2.98	416.91	659.01	NA(a)	NA(a)
pvp	3.45	447.60	710.46	NA(b)	NA(b)
bvp	3.85	483.04	764.64	NA(b)	NA(b)
ibvp	3.85	463.45	754.27	NA(b)	NA(b)
vvp	5.44	540.31	922.04	NA(a)	NA(a)
vpd	1.91	368.36	563.71	56(a,b)	55(a,b)
vp	0.17	356.45	542.00	200(a)	146(a)

Table 2. Calculated hydrophobic (log P), steric descriptors and AE (ED50) activity measured in mice (a,b) using the MES and scMet tests.MES: maximal electroshock, scMet: subcutaneous pentylentetrazol a) ref. 11; b) ref. 12. NA, non active compound.
More details in the text.

formation is more stable for vpd, as it minimizes steric interactions of the more voluminous amine group with the adjacent substituents. We have calculated the energy barriers involved in the interconversion between both conformations, for τ_5 the reaction coordinate. The calculated energy barriers, which are close to three kcal/mol, demonstrate that the rotation around the C_4-C_8 bond occurs at no energy cost. However, in the physiological media, the influence of the solvent (water [37]) cannot be neglected. Hydrogen-bond coordination of water molecules to the Oxygen atoms, aminic Hydrogens and Hydrogen 11 increases the rotational barriers in 10 kcal/mol, allowing one to clearly differentiate syn and antiperiplanar conformations. Recent X-ray studies of N-4-carboxyphenylvalpramide has shown that a tridimensional structure is stabilized through intermolecular H-bonds, involving the same atoms that we have associated with water molecules [38].

Moreover, the solvent has been modeled by the immediate surrounding water molecules, without simulating the solvent effect as a dielectric media [39,40]. Its consideration might further increases the rotational barriers.

Lipophilic and electronic descriptors. Comparative analysis of the results

Lipophilic (log P) and steric parameters are given in Table 2, together with the anticonvulsant activity, expressed as ED_{50}.

Tables 3-6 show the electronic parameters calculated by the different methodologies. The local charges on the atomic centers belong to a Mulliken population analysis, which gives, at the AM1 level, a fairly accurate description of the electronic distribution in acetamide [33]. We assume that it will also render the most trustworthy description of the vpa-related molecules.

Only the local charges in the most representative part of the molecule are reported. There is no important separation of the charge in the alkyl chain, where all the Carbon atoms share almost the same negative charge of 0.1 a.u. This part of the molecule does not contribute to the total dipole moment either, because of the mutual cancellation of the local dipoles on each of the propyl chains, oriented in opposite directions. Variations of the charges, which are reflected in the dipole moment, are mainly centered on the amide/carboxylic functions, where the modifications have been made. It has to be mentioned, however, that, although being irrelevant for the electronic description of the molecule, the propyl substituents define a structural requirement for the AE activity (*i.e.,* acetic acid is not an AED), which is probably related to lipophilic needs.

Data obtained with different methodologies show that the most serious discrepancies are related to the electronic description of the amide function, for which even AM1 and PM3 give different results (Tables 3 and 4). After the results obtained for acetamide, we have based our further discussion on the results of AM1 calculations.

According to the AM1 calculations (Table 3), a negative density charge characterizes the carbonyl Oxygen (O_9) as well as the hydroxyl and ester Oxygen (O_{10}) and amide Nitrogen atoms. Whereas the charge on the first (atom 9) is larger than on atom 10 (Fig. 1 b) on both the esters and vpa, it is smaller than the negative charge on the N-atom of the amide function, in coincidence with the *ab-initio* results obtained for acetamide [33]. However, the –OR, –OH, and $–NH_2$, as entire

	vpa	evp	pvp	bvp	lbvp	Vvp	vpd	vp
q_1	-0.0951	-0.0970	-0.0970	-0.0770	-0.0974	-0.0970	-0.1266	-0.1789
q_8	0.3095	0.3064	0.3061	0.3084	0.3057	0.3068	0.3050	0.3308
q_9	-0.3626	-0.3552	-0.3545	-0.3748	-0.3554	-0.3541	-0.3799	-0.5825
q_{10}	-0.3198	-0.2815	-0.2813	-0.2710	-0.2790	-0.2795	-0.4358	-0.5831
q_{R1}	0.2401	-0.0120	-0.0125	0.0700	-0.0125	0.0940	0.2280	-
q_8 -q_{10}	0.6300	0.5900	0.5870	0.5800	0.5840	0.5860	0.7400	0.9130
bo 4-8	0.9156	0.9110	0.9119	0.9210	0.9116	1.5087	1.5250	0.8227
bo 8-9	1.7972	1.8033	1.8042	1.8302	1.8036	1.2321	1.2485	1.5006
bo 8-10	1.0462	1.0238	1.0224	1.0132	1.0233	1.3685	1.3723	1.5006
bo 10-R1	0.9102	0.9363	0.9384	0.9544	0.9398	1.4315	0.9018	-
dm	1.8050	1.4990	1.5060	1.5760	1.5120	1.4810	3.699	7.9760
E_H	-11.2581	-10.9268	-10.9073	-10.9167	-10.8650	-10.8229	-10.4569	-4.1390
E_L	1.1208	1.2780	1.2669	1.0995	1.2739	1.1014	1.6250	7.0500

Table 3. Electronic descriptors derived from AM1 calculation. $\mathbf{q_n}$: local charges on the atomic centers (a.u.), **bo**: bond orders, **dm**: dipole moment (D), $\mathbf{E_H}$:energy of the HOMO (eV), $\mathbf{E_L}$: energy of the LUMO (ev). We recall that vp instead of vpa descriptors have to be considered in the comparative analysis.

	vpa	evp	pvp	bvp	lbvp	vvp	vpd
q_1	-0.0600	-0.0790	-0.0799	-0.0770	-0.0789	-0.0786	-0.0946
q_8	0.3804	0.3752	0.3741	0.3734	0.3731	0.3729	0.2241
q_9	-0.3942	-0.3754	-0.3746	-0.3748	-0.3755	-0.3748	-0.3999
q_{10}	0.9163	-0.2738	-0.2713	-0.2710	-0.2680	-0.2698	-0.0085
q_{R1}	0.2246	0.0780	0.0750	0.0700	0.0690	0.0738	0.0286
bo 4-8	0.9255	0.9214	0.9214	0.9210	0.9209	0.9204	0.9192
bo 8-9	1.8077	1.8299	1.8303	1.8302	1.8286	1.8300	1.7444
bo 8-10	1.0492	1.0138	1.0134	1.0132	1.0152	1.0127	1.1630
bo 10-R1	0.9162	0.9506	0.9536	0.9544	0.9545	0.9547	0.9605
dm	1.7330	1.6100	1.5930	1.5760	1.5820	1.5840	3.4910
E_H	-11.2182	-10.9124	-10.9095	-10.9168	-10.8946	-10.8229	-9.6854
E_L	1.0161	1.1120	1.1005	1.0995	1.1046	1.1014	1.3516

Table 4. The same as Table 3 for PM3 calculations.

groups, bear a charge (qR_1) that is positive and smaller than that on the carbonyl O-atom. The carbonyl O appears, thence, as the center for electrophilic attack, whereas a nucleophilic reactant would be oriented toward the Carboxylic C atom. This description of the charge distribution is in agreement with the localization of the HOMO in the Carbonyl Oxygen and the LUMO in the C atom bonded to it (Fig. 2 a,b), which is a common feature of all the compounds analyzed.

At this point it has to be mentioned that, according to its pKa value (4.56), vpa is in its dissociated form at physiological pH (7.4) [41], i.e., vp instead of vpa has to be considered for the analysis of the charges (Table 3). The comparison of the results of Tables 2 and 3 shows that the C_8-O_{10} charge separation ($\Delta_{q8\text{-}q10}$), which is reflected in the value of the dipole moment, is larger for the active com-

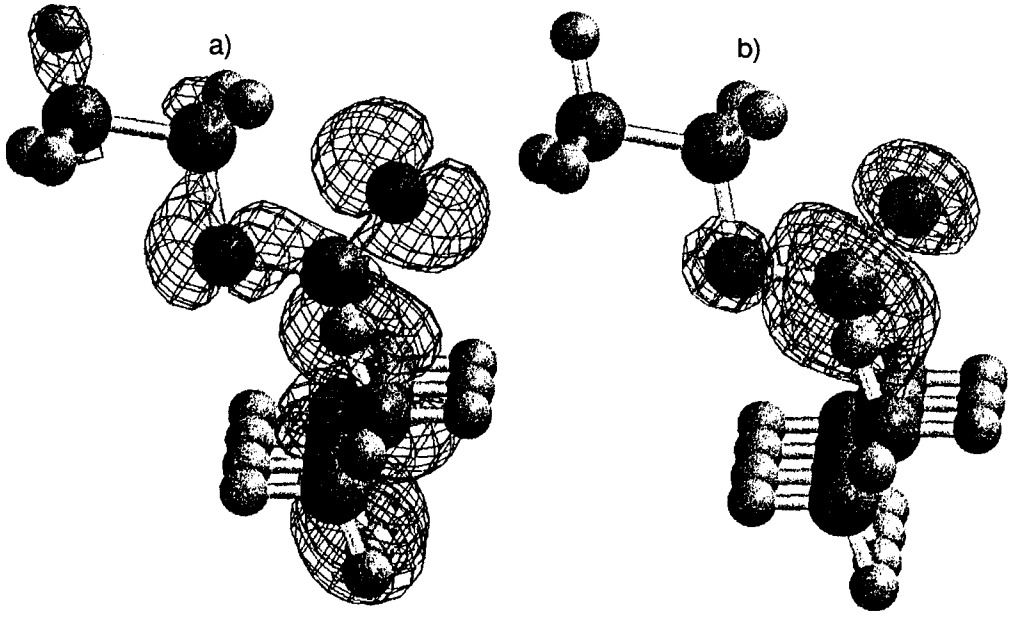

Figure 2. Frontier molecular orbitals: (a) HOMO = Highest Occupied Molecular Orbital, localized on the carbonyl group, (b) LUMO = Lowest Unoccupied Molecular Orbital, localized on the Carbon atom bonded to it. They are exemplified with vpe.

vpa	evp	pvp	bvp	ibvp	vvp	vpd	
q_1	-0.0130	-0.0150	-0.0150	-0.1510	-0.0150	-0.0151	-0.0452
q_8	0.3412	0.3647	0.3650	0.3648	0.3651	0.3660	0.3558
q_9	-0.3668	-0.3602	-0.3600	-0.3596	-0.3589	-0.3591	-0.3698
q_{10}	-0.3034	-0.3403	-0.3412	-0.3413	-0.3415	-0.3433	-0.4169
q_{R1}	0.2150	0.1780	0.1900	0.1899	0.2010	0.1976	0.1900
bo 1-8	0.9073	0.9041	0.9046	0.9042	0.9043	0.9038	0.9021
bo 8-9	1.8243	1.8330	1.8332	1.8338	1.8347	1.8347	1.8064
bo 8-10	1.0336	1.0003	0.9999	0.9995	0.9980	0.9981	1.0590
bo 10-R1	0.9285	0.9242	0.9249	0.9249	0.9265	0.9246	0.9231
dm	1.6400	1.6180	1.6070	1.5910	1.6060	1.6010	3.2713
E_H	-11.4097	-11.2473	-11.2478	-11.2443	-11.2504	-11.2661	-10.5659
E_L	0.9517	1.0453	1.0445	1.0452	1.0342	1.0365	1.4308

Table 5. The same as Table 3 for MNDO calculations.

pounds, vpd and vp, mainly due to changes in q_{10}. The value for vpa is very close to that of the esters. However, when vpd and vp are compared, it is not easy to understand that large values q_{10}, $\Delta_{q8\text{-}q10}$, and of the dipole moment are associated with a higher activity. Different transport mechanisms in the distributions of charged vp and uncharged vpd species [41,42], a fact that invalidates any correlation. Moreover, the minor changes of the electronic descriptors in the uncharged structures, in addition to the lack of activity of the esters precludes any conclusion related to the activity, derived from their correlation.

Bond orders (Tables 3-6) have been analyzed in relation to the lability of vpd and the esters, as a measurement of their possible hydrolysis to vpa. The lability of a C_8-N, C_8–O_{10} bonds can be associated to a prodrug mechanism. However, no

		vpa	evp	pvp	bvp	ibvp	vvp	vpd
q_1	CIS	0.0320	0.0290	0.0290	0.0800	0.0290	0.0290	0.0270
	CISD	0.0320	0.0290	0.0290	0.0290	0.0290	0.0290	0.0270
q_8	CIS	0.4450	0.4460	0.4460	0.4470	0.4470	0.4450	0.4010
	CISD	0.4130	0.4230	0.4270	0.4230	0.4200	0.4300	0.3640
q_9	CIS	-0.5160	-0.5110	-0.5110	-0.5130	-0.5130	-0.5100	-0.5030
	CISD	-0.4890	-0.4910	-0.4970	-0.4910	-0.4890	-0.5000	-0.4680
q_{10}	CIS	-0.2270	-0.1830	-0.1850	-0.1854	-0.1840	-0.1840	-0.2630
	CISD	-0.2200	-0.1830	-0.1800	-0.1820	-0.1800	-0.1790	-0.2600
q_{R1}	CIS	0.2000	0.1080	0.1070	0.1010	0.1050	0.1020	0.1410
	CISD	0.2000	0.1080	0.1060	0.1010	0.1050	0.1020	0.1410
bo_{1-8}	CIS	0.9925	0.9925	0.9796	0.9921	0.9921	0.9928	0.9916
bo_{8-9}	CIS	1.7086	1.6998	1.7005	1.6980	1.6986	1.7009	1.7133
bo_{8-10}	CIS	1.1316	1.1359	1.1353	1.1358	1.1362	1.1361	1.1394
bo_{10-R1}	CIS	0.9488	0.9688	0.9680	0.9677	0.9681	0.9670	0.9625
dm	CIS	2.3342	2.4490	2.4430	2.4560	2.5111	2.4620	4.589
	CISD	2.1845	2.4440	2.3630	2.3210	2.3890	2.4620	4.394
E_H	CIS	-10.003	-9.8805	-9.8696	-9.8506	-9.8560	-9.8560	-9.5159
E_H	CIS	0.0711	1.9837	1.9783	1.9946	1.9973	1.9756	1.8123

Table 6. The same as Table 3 for ZINDO/S-MRCI calculations. The results for single (CIS) and doubles (CISD) excitations are reported.

significant difference was found, for this electronic parameter, between vpd and the esters.

The calculated lipophilicity (log P, Table 2), is not able to explain, either, the AE activity of the compounds under study. A high lipophilicity, which increases as the alcoholic hydrocarbon chain becomes longer, characterizes the non-active esters. The activity of vpd, on the other hand, seems to be high enough to overrate the effect of a lower penetration of the blood brain barrier (log P_c =1.91, Table 2). The value of vp corrected for dissociation [43,44], log Pc (= 0.17), is smaller than the vpa and vpd's value (Table 2).In the case of vpd, according to its pKa value, the correction is negligible [41,42].

The lipophilic effects are justifying, thence, the higher anticonvulsant potency of vpd in comparison with vpa, which is actually vp in the site of reaction, but this justification can not be extended to the esters.

A higher AE potency of vp would be expected according to the electrostatic descriptors (larger md and q_{10} values). The fact that its activity is lower than that of vpd is justified by their different transport properties (less efficient transportation of the anionic structure to the site of action).

Neither the lipophilicity, nor the electronic characteristics have been able to justify the lack of AE of the esters. We recall, thence, the conformational analysis previously described. It has shown that only in the vpd structure the eclipsed O_9–to $-H_{11}$ conformation is stabilized (Fig. 1), defining a O_9–C_8–C_1–H_{11} structural portion that is also present in vp, due to the equivalence of the Oxygen atoms. The O–C–C–H molecular portion, with a partial negative charge on the Oxygen, and

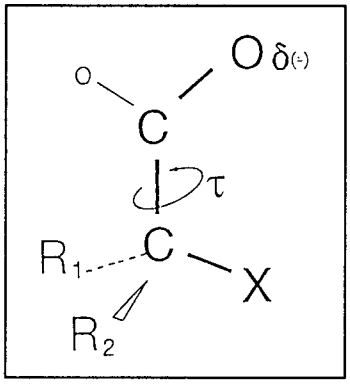

Figure 3. Geometric characteristics of the molecular portion that define the structural requirement associated with the AE activity: boldface lines and symbols. X refers to H. o refers to Oxygen or Nitrogen.

the H atom oriented in the same direction (Fig. 3), appears as a structural requirement for the AE activity, as it is only present in the active formulae, vpd and vp. The free acid (vpa) and its esters are more stable in the opposite O_9– to $-H_{11}$ conformation. We consider this the first step toward the definition of a pharmacophore [45] (Fig. 3) that should contain this portion with the carbonyl Oxygen atom in a well defined orientation relative to the Hydrogen.

The definition of a pharmacophore should be considered, at this point, no more than an inference, as it is only based on the structural characteristics of two active compounds. Looking for a confirmation of this inference, several amides have been synthesized in our lab, the preliminary results of their conformational analysis and AE activity supporting the definition previously given.

CONCLUSIONS

We have presented, in this article, a conformational and electronic study of vpd, vpa and several vpa esters, whose results were analyzed on the basis of the simultaneous consideration of their lipophilic properties.

Neither the lipophilic, nor the electronic properties, are capable of justifying the lack of activity of the esters.

A conformational study, including vpd, vp, vpa and 5 esters of vpa, has allowed us to infer that the justification of the AE activity of vpa and vpd *vs.* the lack of activity of the esters may relay on a structural requirement. The relative value of the AE potency of vpa and vpd is finally justified on the basis of different transport mechanisms for vpa and vp. These conclusions are based on the assumption that the esters are not efficiently biotransformed to vpa, and that the acid is in the dissociated form in the site of action.

The results of the conformational analysis appear, thence, as the unique justification of the AE activity of vp and vpd, and the lack of activity of the esters.

With the elements at hand, nothing can be said about the possibility of considering vpd as a prodrug of vpa. According to its better transport, it can be easily distributed to the brain and hydrolyzed to vpa, which, in the anionic form, renders a higher AE activity. Although this mechanism agrees with the definition of a prodrug, it is not the only one that may be associated with the AE activity of vpd. Based on its conformational characteristics, an AE activity of vpd *per se* can also be inferred.

The esters, at least those analyzed in this article, do not seem to be efficiently biotransformed to vpa in the brain.

The results presented in this article give some light on the different factors that should be considered in the design of vpa derivatives with increased AE potency. Accepting the postulated structural requirement, new derivatives have been design on the basis of a previous conformational analysis. In this way, three amides of vpa with presumed higher activity (N-butyl, N-cyclohexyl, N-(4-carboxy-

phenyl)-valpramide , eclipsed conformation), and one with presumed lower activity (N-morpholin-vpd, opposite conformation) have been synthesized. Their biological tests have confirmed the structural requirement for the AE activity. Details on the results, where the electronic and conformational characteristics of the new derivatives are discussed together, are the subject of the forthcoming article.

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