Structure–Activity Relationships of Unsaturated Analogues of Valproic Acid

Jan Palaty and Frank S. Abbott*

Faculty of Pharmaceutical Sciences, 2146 East Mall, University of British Columbia, Vancouver, Canada V6T 1Z3

Received May 2, 1995[⊗]

The principal metabolite of valproic acid (VPA), 2-ene VPA, appears to share most of VPA's pharmacological and therapeutic properties while lacking its hepatotoxicity and teratogenicity, thus making it a useful lead compound for the development of safer antiepileptic drugs. Analogues of 2-ene VPA were evaluated for anticonvulsant activity in mice using the subcutaneous pentylenetetrazole test. Cyclooctylideneacetic acid exhibited a potency markedly exceeding that of VPA itself with only modest levels of sedation. Potency, as either ED_{50} or brain concentration, was highly correlated ($r > 0.85$) with volume and lipophilicity rather than with one of the shape parameters calculated by molecular modeling techniques, arguing against the existence of a specific receptor site. Instead, a role for the plasma membrane in mediating the anticonvulsant effect is suggested.

Introduction

Valproic acid (2-propylpentanoic acid, VPA, **1**) is an established antiepileptic drug with a simple chemical structure but an unusually broad spectrum of action which includes tonic-clonic, partial complex, and absence seizures.[1] The drug is generally well-tolerated but has two rare but potentially fatal side effects: hepatotoxicity and teratogenicity. These drawbacks are apparently not shared by its equipotent metabolite (*E*)-2-propyl-2-pentenoic acid (2-ene VPA, **7**), which thus holds considerable promise as a successor to VPA.[2] Despite its similarity to the parent, 2-ene VPA shows some different properties in its displacement of GABA from $GABA_A$ binding sites[3] and failure to inhibit high-frequency action potentials in mouse neurons.[4] The lack of reports in the literature on the anticonvulsant properties of 2-ene VPA analogues thus prompted us to evaluate a series of these compounds for their effective potency (ED_{50}) and intrinsic potency (brain concentration following an ED_{50} dose) and to search for correlations with physicochemical parameters such as lipophilicity, volume, and shape. As VPA and 2-ene VPA are somewhat more effective in protecting against absence rather than tonic-clonic seizures,[2] we chose the subcutaneous pentylenetetrazole (scPTZ) test to measure anticonvulsant potency owing to its selectivity for such drugs.

Chemistry

The 2-ene VPA analogues evaluated in this study are shown in Figure 1. The preparation of the (*E*)-2-alkyl-2-pentenoic acids (**5**–**8**) was based on the highly stereoselective addition of propionaldehyde to dibutylboryl enolates derived from racemic *N*-acylisopropyloxazolidinones (Scheme 1).[5] The adducts **22a**–**f** were then isolated and characterized by GC–MS (as their silyl ethers) and ^{1}H-NMR. The spectra were consistent with the presence of a pair of *erythro* enantiomers as supported by the fact that all of the subsequent unsaturated esters **25a**–**e**, where the double bond had been formed by a stereospecific E2 elimination mechanism, were found to exist exclusively as their *E*-isomers. Hydrolysis

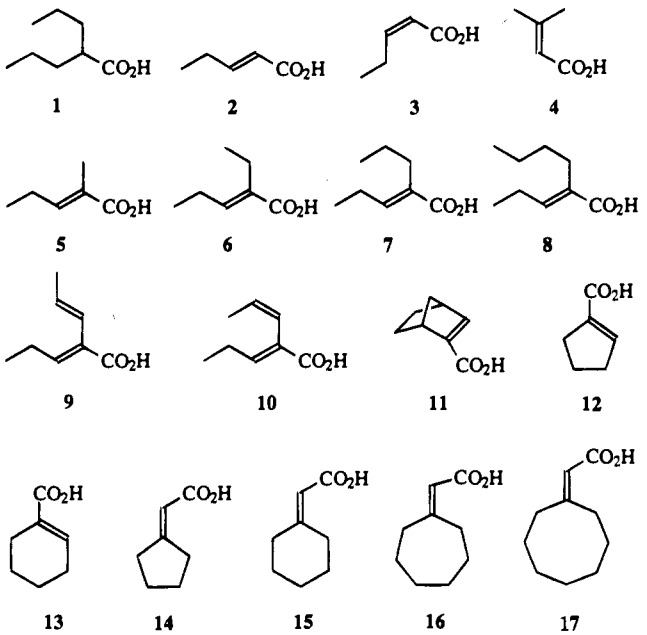


Figure 1. Compounds evaluated for anticonvulsant activity.

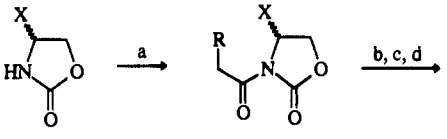
of the adducts **22a**–**d** with lithium hydroperoxide[6] followed by treatment with diazomethane afforded the methyl hydroxyesters **24a**–**d**. However, the branched side-chain adducts **22e** and **22f** were very slowly hydrolyzed (isopropyl, 29%; cyclopentyl, 15%) even when the reaction was conducted at room temperature, presumably due to an excess of steric crowding in the region of the "amide" carbonyl group. Assuming that the *Z* stereochemistry of the dibutylboryl enolate alone would be sufficient to direct the reaction to the *erythro* adduct,[7] we synthesized the isopropyl-substituted adduct **23a** derived from 2-oxazolidone **19** itself. The stereoselectivity of the process had not been compromised, as indicated by ^{1}H-NMR spectroscopy, but the overall conversion of carboximide **21a** to hydroxy ester **24e** proceeded in 70% yield. A similar yield was obtained with the cyclopentyl analogue **21b**.

The subsequent "dehydration" step proved to be surprisingly difficult with the saturated analogues, although many earlier reactions with the aldol adducts themselves as well as precursors of the bis-unsaturated acids **9** and **10** invariably proceeded in high yields using

[⊗] Abstract published in *Advance ACS Abstracts*, July 15, 1995.

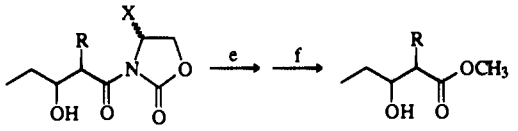
0022-2623/95/1838-3398$09.00/0

Scheme 1



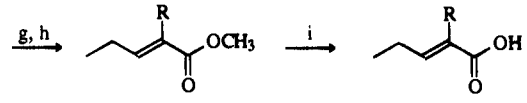
18: X = *i*-Pr
19: X = H

20a: X = *i*-Pr; R = Me
20b: X = *i*-Pr; R = Et
20c: X = *i*-Pr; R = Pr
20d: X = *i*-Pr; R = Bu
20e: X = *i*-Pr; R = *i*-Pr
20f: X = *i*-Pr; R = cyclopentyl
21a: X = H; R = *i*-Pr
21b: X = H; R = cyclopentyl



22a: X = *i*-Pr; R = Me
22b: X = *i*-Pr; R = Et
22c: X = *i*-Pr; R = Pr
22d: X = *i*-Pr; R = Bu
22e: X = *i*-Pr; R = *i*-Pr
22f: X = *i*-Pr; R = cyclopentyl
23a: X = H; R = *i*-Pr
23b: X = H; R = cyclopentyl

24a: R = Me
24b: R = Et
24c: R = Pr
24d: R = Bu
24e: R = *i*-Pr
24f: R = cyclopentyl

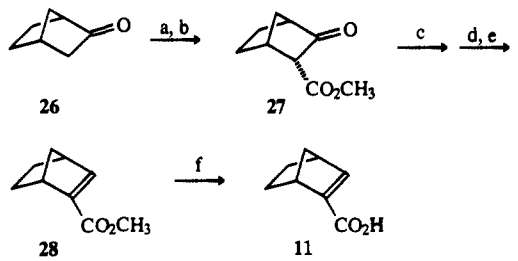


25a: R = Me
25b: R = Et
25c: R = Pr
25d: R = Bu
25e: R = *i*-Pr

5: R = Me
6: R = Et
7: R = Pr
8: R = Bu

[a] Reagent: (a) BuLi, RCH_2COCl; (b) Bu_2BuOTf, Et_3N; (c) CH_3CH_2CHO; (d) H_2O_2, H_2O; (e) LiOOH; (f) CH_2N_2; (g) MsCl, Et_3N; (h) DBU; (i) KOH.

Scheme 2



26 27 28 11

[a] Reagent: (a) LDA, THF; (b) $NCCO_2CH_3$, HMPA; (c) $NaBH_4$; (d) MsCl, Et_3N; (e) DBU; (f) aqueous KOH.

1.1 equiv of all reagents (Et_3N, methanesulfonyl chloride, and DBU) and stirring overnight at room temperature.[8] It was found that using 4 equiv of all reagents and conducting the elimination step under reflux for 1 h[9] afforded reasonable yields of esters **25a–d**, although only trace amounts were obtained for **25e** and none at all for the cyclopentyl-substituted analogue. All products were isolated as single isomers, indicating that the integrity of the E2 elimination was maintained at the elevated temperature. The final acid products were then obtained by basic hydrolysis as single isomers with the exception of compound **5**, the ^{1}H-NMR spectrum of which indicated the presence of two isomers in an 85:15 (*E*:*Z*) ratio.

The norcamphor derivative **11** was prepared by the methoxycarbonylation of (±)-norcamphor[10] followed by reduction with sodium borohydride, dehydration, and hydrolysis. A similar approach was used for compounds **12** and **13** except that the starting materials were the commercially available ethyl 2-oxocyclopentanecarboxylate and ethyl cyclohexenecarboxylate, respectively. Compounds **14–17**, where the double bond is exocyclic to the ring, were prepared by reaction of the corresponding ketone with the ylide of triethyl phosphonoacetate[11] followed by hydrolysis of the resultant ester.

Results

Table 1 reveals that effective anticonvulsant potency, as measured by the ED_{50} dose for protection against scPTZ-induced seizures, increased with the size of the molecule. Although this trend was less evident with the most potent drugs, the highest molecular weight drug (compound **17**) was also the most potent. The sedative potencies of the drugs also followed size, but the effect was much more noticeable for the acyclic drugs. With the exception of compound **17**, all cyclic compounds showed no sedative effects at the ED_{50} dose. This property was exploited in compounds **16** and **17**, which preserved or even exceeded the potency of 2-ene VPA while remaining free of substantial sedation.

Having both the ED_{50} values as well as the resultant brain concentrations C allows for an estimate of the relative ability of the drugs to penetrate into the brain at their therapeutic dose. This parameter *R* can be considered as a distribution ratio between the brain and overall tissue compartments under non-steady-state conditions.

$$R = \frac{C\ (\mu\text{mol/g of wet brain tissue})}{ED_{50}\ (\mu\text{mol/g of body weight})} \quad (1)$$

The link between biological and physicochemical parameters was then investigated by analyzing for all possible correlations as shown in Table 2. Lipophilicity was found to be significantly correlated with molecular volume *V* and most shape descriptors (Figure 2). Consequently, it is not possible to unambiguously distinguish the roles of shape and lipophilicity in drug potency. The best correlations of $\log(1/ED_{50})$ with these physicochemical properties for all analogues except VPA are shown below, along with the relevant statistical parameters: *n* is the number of analogues employed, *s* is the standard error of estimate, *r* is the correlation coefficient, *F* is the calculated *F* test value. In each equation, the numbers in parentheses are the standard errors associated with the coefficients. The introduction of a shape parameter (Y^+ or Y^-, chosen on the basis of their low correlation with volume and log *P*) failed to significantly improve the correlation.

$$\log(1/ED_{50}) = 0.0138(\pm 0.0011)V + 0.96(\pm 0.15) \quad (2)$$

$$n = 16;\ s = 0.105;\ r = 0.955;\ F = 146;\ F_{0.01(1),1,14} = 8.9$$

$$\log(1/ED_{50}) = 0.432(\pm 0.049)\log P + 1.84(\pm 0.10) \quad (3)$$

$$n = 16;\ s = 0.138;\ r = 0.921;\ F = 79;\ F_{0.01(1),1,14} = 8.9$$

Since the ED_{50} values in Table 1 are a measure of all the rate constants involved between the injection of drug and its interaction at the final site, they are a poor measure of the actual pharmacodynamic properties of

Table 1. Anticonvulsant and Sedative Properties and Brain Concentrations of VPA and Its Analogues

compound	ED_{50} (mmol/kg) [95% CI]	C^a (nmol/g brain)	sedation	R^b	volume (Å^3) [no. conf.]^d	log P^c
VPA (1)	0.83 [0.48–1.1]	620 ± 240	+	0.74	151.6 [43]	2.72
(*E*)-2-pentenoic acid (**2**)	6.0 [5.2–6.5]	2220 ± 940	−	0.37	94.0 [4]	1.22
(*Z*)-2-pentenoic acid (**3**)	7.0 [5.6–9.8]	4460 ± 840	−	0.64	93.9 [5]	1.22
Dimethylacrylic acid (**4**)	5.2 [3.5–6.1]	2710 ± 590[e]	−	0.52	94.2 [2]	1.09
(*E*)-2-methyl-2–pentenoic acid (**5**)	4.4 [2.4–17]	1520 ± 470	−	0.35	110.0 [3]	1.53
(*E*)-2-ethyl-2–pentenoic acid (**6**)	1.6 [0.68–1.7]	660 ± 40	−	0.41	127.7 [2]	2.06
2-ene VPA (7)	0.84 [0.44–1.1]	260 ± 70	+	0.31	144.7 [6]	2.59
(*E*)-2-butyl-2–pentenoic acid (8)	0.87 [0.59–1.0]	140 ± 50	++	0.17	161.9 [26]	3.11
(*E,E*)-2-(1′-propenyl)-2-pentenoic acid (**9**)	1.4 [0.46–1.6]	680 ± 90	f	0.49	138.0 [10]	2.22
(*E,Z*)-2-(1′-propenyl)-2-pentenoic acid (**10**)	0.78 [0.26–1.7]	350 ± 40[e]	−	0.45	139.1 [9]	2.22
(±)-Bicyclo[2.2.1]hept-2–ene-2-carboxylic acid (11)	2.9 [1.2–3.8]	2150 ± 330[e]	−	0.75	123.1 [2]	1.42
1-Cyclopentenyl-1-carboxylic acid (**12**)	4.7 [2.3–5.5]	500 ± 80[e]	−	0.11	99.8 [2]	1.10
1-Cyclohexenyl-1-carboxylic acid (**13**)	2.2 [1.6–2.5]	1210 ± 240	−	0.55	117.1 [2]	1.66
Cyclopentylideneacetic acid (**14**)	2.3 [1.3–3.7]	450 ± 60[e]	−	0.20	117.4 [2]	1.72
Cyclohexylideneacetic acid (**15**)	1.4 [0.77–1.9]	320 ± 100	−	0.23	133.3 [2]	2.28
Cycloheptylideneacetic acid (**16**)	0.96 [0.73–1.3]	159 ± 31[e]	−	0.17	150.5 [13]	2.84
Cyclooctylideneacetic acid (17)	0.73 [0.65–0.80]	94 ± 37	+	0.13	166.6 [14]	3.40

[a] Drug levels ± SD at 15 min postdose in whole brain homogenates from $n = 5$ mice given an ED_{50} dose and sampled in triplicate. [b] Calculated by eq 1. [c] Calculated 1-octanol/water partition coefficient. [d] Number of conformers within 10 kJ of global minimum for carboxylate in aqueous medium. [e] As in *a*, but $n = 4$ only. [f] Animals exhibited severe ataxia and rigidity rather than sedation.

Table 2. Correlation Matrix of Biological and Physicochemical Properties of VPA Analogues

	log(1/ED_{50})	log(1/*C*)	log P	V^a	$X^{+\,b}$	ΔY^b	$Y^{-\,b}$	$Y^{+\,b}$	ΔZ^b	$Z^{-\,b}$	$Z^{+\,b}$
log(1/ED_{50})	1.00										
log(1/*C*)	0.87	1.00									
log *P*	0.92	0.89	1.00								
V	0.96	0.87	0.97	1.00							
X^+	0.64	0.58	0.57	0.60	1.00						
ΔY	0.68	0.55	0.69	0.69	0.15	1.00					
Y^-	0.19	0.29	0.35	0.22	−0.05	0.25	1.00				
Y^+	0.45	0.26	0.33	0.43	0.18	0.68	−0.54	1.00			
ΔZ	0.84	0.70	0.82	0.88	0.44	0.68	0.01	0.58	1.00		
Z^-	0.73	0.51	0.63	0.72	0.36	0.56	−0.07	0.53	0.91	1.00	
Z^+	0.75	0.67	0.77	0.82	0.41	0.70	−0.04	0.79	0.92	0.74	1.00

[a] Volume. [b] Shape parameter (Figure 2).

the drug. Hence, the initial absorption steps were eliminated by replacing ED_{50} in the previous equations with the brain concentrations of the drug following an ED_{50} dose and measured at the time of seizure onset in the anticonvulsant test.

$$\log(1/C) = 0.0184(\pm 0.0027)V + 0.89(\pm 0.35) \quad (4)$$

$n = 16$; $s = 0.251$; $r = 0.874$; $F = 45$; $F_{0.01(1),1,14} = 8.9$

Discussion

The evaluation of 2-ene VPA and its analogues *in vivo* supported earlier findings for VPA analogues[12,13] showing that increasing the size of the aliphatic group attached to a carboxylic acid fragment also increased anticonvulsant potency. This was shown especially clearly in the cases of the (*E*)-2-pentenoic acids (compounds **2** and **5**–**8**) and the cycloalkylideneacetic acids **14**–**17**. Quantitative support for this phenonomenon is provided by eq 2, depicted graphically in Figure 3, which shows a high degree of correlation between log(ED_{50}) and volume. This relationship largely persists when ED_{50} is replaced by the brain concentration (eq 4, illustrated in Figure 4) although the correlation parameters are clearly inferior. This finding is significant, as much of the work to date has focused strictly on ED_{50} itself as the biological indicator of potency, which suffers from the drawback of incorporating pharmacokinetic as well as pharmacodynamic terms. Thus, the intrinsic potency of the drugs is governed to a lesser degree by shape-independent properties such as lipophilicity than the overall effective potency, where the drug's passage from the periphery into the brain must be considered. Although this evidence is less than robust, it supports the involvement of a receptor site

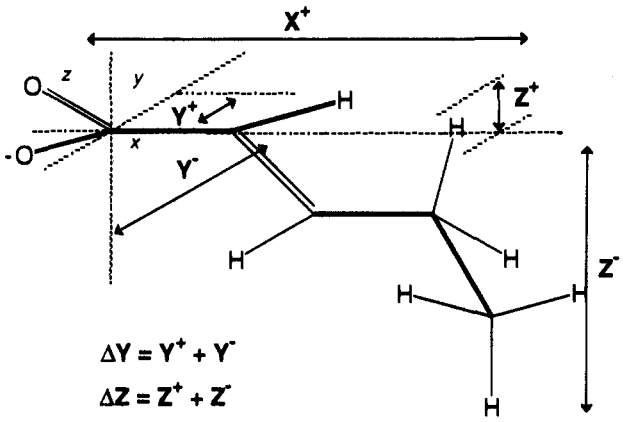


Figure 2. Illustration of width parameters for (*E*)-2-pentenoate.

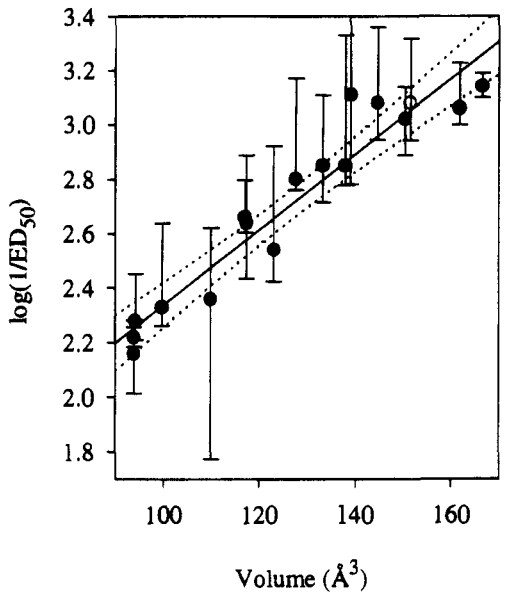


Figure 3. log(1/ED_{50}) *vs* volume for 2-ene VPA analogues. Error bars indicate 95% confidence intervals. VPA (not used in equation) is shown as an empty circle.

with some minor shape requirements which mediates the anticonvulsant effect.

Comparing cyclic and acyclic compounds with equal numbers of carbon atoms indicates that the introduction of a ring closure caused a reduction in activity most readily explained by parallel decreases in both volume and lipophilicity as revealed by Table 1. Thus, structure is apparently less important than the simple bulk property of volume or lipophilicity. This relationship is demonstrated clearly by the C_8 acids 2-ene VPA, **15** and **11**.

As has been noted earlier,[14] compound **9** ((*E,E*)-diene VPA) induced a profound neurotoxicity characterized primarily by rigidity. This was in marked contrast to the ataxia and decreased muscle tone observed as the sedative effects of the other drugs, including the closely-related compound **10** ((*E,Z*)-diene VPA).

Considering the chemical similarity of the compounds tested, it is relevant to ask whether or not a QSAR study is justifiable in this case. The only physicochemical terms involved here are highly correlated parameters describing volume, shape, and lipophilicity, since all compounds are identical in the electrostatic or inductive properties of their substituents on the basic α,β-unsaturated carboxylic acid unit, with the minor exception of compounds **9** and **10**. Thus, is there enough diversity in the set to obtained a balanced picture of the cause of the anticonvulsant effect? We believe the answer to be in the affirmative if the ranges of lipophilicities and potencies are considered. For example, the compounds vary from those which are nearly water-miscible in the undissociated form (compounds **2** and **3**) to one which is barely water-soluble even as its sodium salt (compound **17**). Similarly, ED_{50} potencies vary from 0.7 to 7.0 mmol/kg, demonstrating that evaluated acids represent a diverse group of physical and biological, if not chemical, properties.

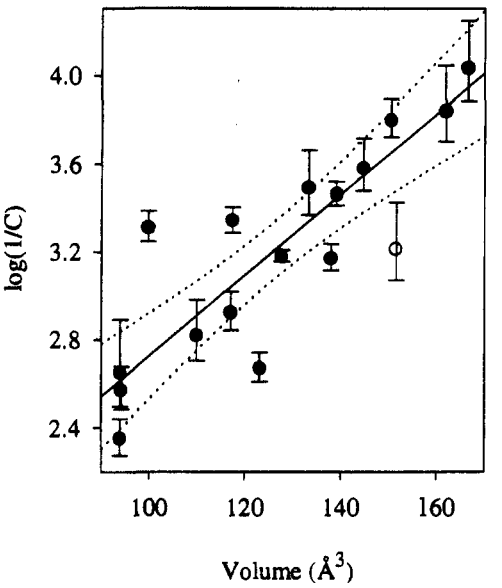


Figure 4. log(1/C) *vs* volume for 2-ene VPA analogues. Error bars indicate SD. VPA (not used in equation) is shown as an empty circle.

More importantly, our primary objective here was to study the interaction of the drugs with the effector leading to seizure protection. For this assumption of a common mechanism to hold, it is evident that the tested analogues must not stray far from the fundamental structure of 2-ene VPA. The simplicity of this molecule clearly restricted allowed substituents on the α,β-unsaturated carboxylic acid moiety to those of an aliphatic nonpolar nature, although some liberties were taken by the use of cyclic, branched, or unsaturated substituents. Introduction of strong electrostatic charges or strongly electron-withdrawing species, for example, would profoundly alter the properties of the molecule which could lead to altered pharmacokinetics (notably transfer across the blood–brain barrier) and quite possibly different pharmacodynamics as well. When combined with the fact that the scPTZ anticonvulsant test is fairly nonspecific (for example, the mechanistically and therapeutically unrelated drugs ethosuximide and clonazepam are both effective here[15]), this could clearly lead to considerable ambiguity about how a drug is exerting its anticonvulsant effect. Consequently, correlation equations with ED_{50} or brain concentration for a group of pharmacologically diverse compounds would no longer carry implications about the mechanism through which seizure protection was being achieved.

Whereas VPA remained as one of the most effective overall anticonvulsant drugs as measured by its ED_{50} value, its intrinsic potency was remarkably low in

comparison with a large number of other analogues, including 2-ene VPA. This was shown by the compact group of analogues with ED_{50} less than 1 mmol/kg which afforded anticonvulsant protection equal to that of VPA at essentially the same dose but at substantially lower effective brain concentrations. The therapeutic advantage of VPA must therefore be derived mainly from its ability to be efficiently transported into the brain rather than from an exceptionally effective interaction with its final neurochemical target. A truly significant reduction in ED_{50} can thus be achieved by dealing primarily with the issue of blood–brain barrier transport. Specifically, a rational step to further improve the effectiveness of compound **17** would not be to increase size, for example by increasing the size of the ring or adding further substituents, but rather to incorporate the molecule into a prodrug such as a diacylglyceryl ester,[16] which has been shown to be an efficient carrier of VPA into the brain. The need to consider primarily pharmacokinetics rather than intrinsic potency was also illustrated by the difficulties encountered in preparing an aqueous solution of **17** as its sodium salt for an ED_{50} dose, which demonstrated that lipophilicity cannot be increased significantly beyond this point if sufficient aqueous solubility is to be retained.

Dividing the brain concentration by the ED_{50} dose was taken as an index of the drug's ability to penetrate into the brain from the initial site of injection at its therapeutic dose (R), or a drug's instantaneous distribution ratio between the two compartments. Although the results appear randomly distributed at first, some distinct trends do emerge. First, VPA clearly is very efficient in reaching the brain, judging by its high value of R. This is likely due to the lack of a double bond conjugated with the carboxyl moiety, although it is less clear how this feature is expressed biochemically. It may represent a lower degree of plasma protein binding due to altered charge densities on the carboxyl group or reduced dissociation leading to a greater free concentration and thus a potentially larger gradient with the CSF compartment. This notion is undermined by the fact that the dienes **9** and **10** have above-average values of R despite being the most acidic compounds in the table (pK_a VPA = 4.95; pK_a 2-ene VPA = 4.36; pK_a **9**, **10** = 4.02[13]). Also, the unsaturated analogues **3** and **11** have values of R comparable to that of VPA itself.

There is an appreciable correlation between sedation and a molecule's floppiness, as indicated by the number of conformers found by the conformational search. This is shown clearly when one compares the 2-substituted 2-pentenoic acids (**2** and **5**–**8**), which exhibit rapidly increasing sedation with size, with the cyclic compounds, which show noticeable sedation only at the level of the conformationally labile cyclooctylidene ring. The literature of VPA analogues generally supports this trend[12,17–20] but not without exception. It has been reported that the large rigid molecule spiro[4.6]undecane-2-carboxylic acid gave an scPTZ ED_{50} value of 0.42 mmol/kg while its neurotoxicity was about twice that of VPA.[21] This suggests that sedation may simply be a function of volume rather than conformational lability, which would account for the seemingly contradictory observation that while compounds **16** and **17** have an equally high number of conformations, only the latter shows sedative effects. However, the acid **16** has a greater volume/lipophilicity than 2-ene VPA, and yet only the latter is markedly sedating. A possible explanation is that it is the spatial range of the conformational interconversions which must be considered rather than simply the volume of the molecule in a given conformation. Specifically, the coordinates of given carbon atoms in compound **16**, unlike those in 2-ene VPA, do not show appreciable variation between conformations and result in a relatively small time-averaged volume. Therefore, the decreased sedation of compound **16** may in fact stem from the corresponding reduction in volume as noted previously.

Nevertheless, it is clear that although both anticonvulsant potency and sedation increase with volume regardless of the structure, the presence of a ring system in the molecule can selectively diminish the dependence of sedation. Thus, a branched C_{10} acid would be profoundly sedating unless it was a cyclic molecule such as compound **17**. As shown in Figures 3 and 4, the absence of outliers representing different structural classes indicates that there is no such relationship with anticonvulsant potency.

Having shown that both anticonvulsant activity and sedation increase with volume and lipophilicity, it is pertinent to ask what the actual effector site might be. The repeatedly demonstrated effects of VPA on membrane fluidity (*in vitro* and *in vivo*)[22,23] and sodium channel kinetics[4,24,25] justify proposing a role for membrane disordering in the pharmacodynamics of VPA, analogous to the one for volatile anesthetics.[26] The existence of such a target site is further supported by recent work[27] showing that neither VPA nor 2-ene VPA readily crosses the plasma membrane, which greatly limits their access to intracellular enzymes.

In conclusion, we have demonstrated that the intrinsic anticonvulsant potency of 2-ene VPA analogues has a strong dependence on lipophilicity and molecular size with no significant contributions from any specific shape descriptor. This suggests that the drug's receptor site may be the neuronal plasma membrane. Finally, it was shown that some 2-ene VPA analogues, especially compound **17**, exhibit a much greater anticonvulsant potency than VPA when compared on the basis of their respective brain concentrations.

Experimental Section

General Procedures. Acids **3**, **9**, and **10** were prepared as described by Lee *et al.*[8] Acids **2** and **4** were obtained commercially (Aldrich). Flash chromatography was performed with silica gel 60 (Merck 9285, 230–400 mesh). Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on Bruker 200 or 300 MHz spectrometers with chemical shifts reported in ppm relative to tetramethylsilane. IR spectra were recorded on a Bomem MB-100 instrument. Qualitative GC–MS analyses were performed on a Hewlett-Packard HP5700A gas chromatograph (packing, 3% Dexsil 300; oven, 50 °C initial to 260 °C at 16 °C/min or 32 °C/min) interfaced to a Varian MAT-111 mass spectrometer. Mass spectral peak intensities (in parentheses) are reported relative to the base peak. Where noted, alcohols and acids were silylated with *N*-(trimethylsilyl)-*N*-methyltrifluoroacetamide or *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (Pierce) in EtOAc prior to analysis. Elemental analyses were performed by Mr. Peter Borda at the Department of Chemistry, UBC. Male CD-1 mice were obtained from University of British Columbia Animal Services. Linear regression calculations were performed using QSAR-PC (BIOSOFT, Cambridge, MA).

(±)-**4-(1-Methylethyl)-2-oxazolidinone (18).** (±)-Valinol (25 g, 240 mmol) was dissolved in diethyl carbonate (170 mL). Potassium *tert*-butoxide (7.0 g, 62 mmol) was added and the mixture brought to reflux. Distillate was collected until the stillhead temperature rose to 126 °C. The solution was allowed to cool and then diluted with ether and washed with saturated ammonium chloride and brine. The organic extract was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The solid residue was decanted with petroleum spirit (2 × 30 mL) and 1:9 and 1:4 ether/petroleum spirit solutions (v/v, 30 mL each). The resultant suspension was refluxed briefly with a 2:3 solution (30 mL), cooled to −10 °C, filtered, and washed with 1:4 ether/petroleum spirit (2 × 15 mL) to afford the oxazolidinone (21.2 g, 68%) as a white powder: mp 69−72 °C; ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.90 (6H, m, CH_3), 1.68 (1H, m, C*H*(CH_3)$_2$), 3.62 (1H, m, *CH*NH), 4.08 (1H, dd, J = 9, 8 Hz, CH_2O), 4.40 (1H, dd, J = 9, 9 Hz, CH_2O), 6.90 (1H, br s, NH); IR ($CHCl_3$, cm^{-1}) 3244, 2950, 1749; GC−MS m/z 129 (3, M$^+$), 86 (100), 42 (98).

General Procedure for the Synthesis of (±)-3-(1-Oxoalkyl)-4-(1-methylethyl)-2-oxazolidinones (20a−f) and (±)-3-(1-Oxoalkyl)-2-oxazolidinones (21a,b). Cyclopentylacetyl chloride and pentanoyl chloride were prepared from their respective acids by the procedure described by Furniss *et al.*[28] The remaining acid chlorides were obtained commercially (Aldrich). The corresponding carboximides **20a−f** and **21a,b** were then prepared as described by Evans *et al.*[29]

(±)-**3-(1-Oxopropyl)-4-(1-methylethyl)-2-oxazolidinone (20a):** 7.35 g (85%, bp 82−87 °C/0.1 mmHg); ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.85 (3H, d, J = 7.0 Hz, CH(C*H*$_3$)$_2$), 0.91 (3H, d, J = 7 Hz, CH(C*H*$_3$)$_2$), 1.08 (3H, t, J = 7.5 Hz, H(3)), 2.20−2.43 (1H, m, C*H*(CH_3)$_2$), 2.70−3.05 (2H, m, CH_2CO), 4.20−4.50 (3H, m, $CHCH_2O$); IR (neat, cm^{-1}) 2956, 1778, 1704; GC−MS (m/z): 185 (3, M$^+$), 142 (12), 85 (8), 68 (5), 57 (100).

(±)-**3-(1-Oxobutyl)-4-(1-methylethyl)-2-oxazolidinone (20b):** 7.62 g (82%, bp 83 °C/0.05 mmHg); ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.80−1.00 (9H, m, 3 × CH_3), 1.65 (2H, m, CH_2-CH_2CO), 2.31 (1H, septet of d, J = 5, 4 Hz, C*H*(CH_3)$_2$), 2.67−3.02 (2H, m, CH_2CO), 4.26−4.52 (3H, m, $CHCH_2O$); IR (neat, cm^{-1}): 2953, 1777, 1701; GC−MS (m/z): 199 (28, M$^+$), 184 (3), 171 (22), 156 (60), 130 (27), 85 (55), 71 (100).

(±)-**3-(1-Oxopentyl)-4-(1-methylethyl)-2-oxazolidinone (20c):** 8.77 g (89%, 97−105 °C/0.05 mmHg); ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.80−1.00 (9H, m, 3 × CH_3), 1.28−1.48 (2H, m, CH_3C*H*$_2$), 1.48−1.70 (2H, m, C*H*$_2$CH$_2$CO), 2.20−2.44 (1H, m, C*H*(CH_3)$_2$), 2.70−3.09 (2H, m, CH_2CO), 4.28−4.56 (3H, m, $CHCH_2O$); IR (neat, cm^{-1}): 2948, 1779, 1708; GC−MS (m/z): 213 (3, M$^+$), 198 (1), 184 (10), 171 (25), 130 (10), 85 (100).

(±)-**3-(1-Oxohexyl)-4-(1-methylethyl)-2-oxazolidinone (20d):** 7.49 g (64%, bp 104−6 °C/0.05 mmHg); ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.68−0.80 (9H, m, 3 × CH_3), 1.09−1.23 (4H, m, CH_3C*H*$_2$C*H*$_2$), 1.38−1.60 (2H, m, C*H*$_2$CH$_2$CO), 2.02−2.29 (1H, m, C*H*(CH_3)$_2$), 2.51−2.88 (2H, m, CH_2CO), 4.03−4.39 (3H, m, $CHCH_2O$); IR (neat, cm^{-1}): 2944, 1772, 1702; GC−MS (m/z): 228 (2, M$^+$), 184 (10), 171 (13), 130 (8), 99 (100).

(±)-**3-(1-Oxo-3-methylbutyl)-4-(1-methylethyl)-2-oxazolidinone (20e):** 6.78 g (91%, bp 94−98 °C/0.05 mmHg) at 3/4 scale of above; ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.80−1.00 (12H, m, CH(C*H*$_3$)$_2$), 2.00−2.12, 2.12−2.41 (1H, 1H, m, m, 2 × C*H*(CH_3)$_2$), 2.60 (2H, dd, J = 7.0, 15.0 Hz, CH_2CO), 4.22−4.55 (3H, m, $CHCH_2O$); IR (neat, cm^{-1}): 2954, 1778, 1700; GC−MS (m/z): 213 (2, M$^+$), 198 (3), 171 (10), 130 (8), 85 (100).

(±)-**3-(1-Oxo-2-cyclopentylethyl)-4-(1-methylethyl)-2-oxazolidinone (20f):** 9.13 g (82%, bp 127−130 °C/0.2 mmHg); mp 33−37 °C; ^{1}H-NMR (300 MHz, acetone-d_6) δ: 0.88, 0.95 (3H, 3H, d, d, J = 7 Hz each, CH(C*H*$_3$)$_2$), 1.10−1.32 (2H, m, cyclopentyl), 1.46−1.73 (4H, m, cyclopentyl), 1.73−1.92 (2H, m, cyclopentyl), 2.21−2.40 (2H, m, C*H*(CH_3)$_2$, H(3)), 2.77 (1H, dd, J = 7, 16 Hz, CH_2CO), 3.04 (1H, dd, J = 7, 16 Hz, H(2)), 4.28−4.52 (3H, m, $CHCH_2O$); IR ($CHCl_3$, cm^{-1}): 2957, 1777, 1699; GC−MS (m/z): 239 (2, M$^+$), 196 (5), 171 (30), 130 (27), 111 (100).

(±)-**3-(1-Oxo-3-methylbutyl)-2-oxazolidinone (21a):** 10.44 g (66%, bp 89−92 °C/0.05 mmHg); ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.95 (6H, d, J = 7 Hz, CH(C*H*$_3$)$_2$), 2.0−2.2 (1H, C*H*(CH_3)$_2$), 2.75 (2H, d, J = 7 Hz, CH_2CO), 4.00 (2H, t, J = 8 Hz, NCH_2CH_2O), 4.44 (2H, dt, J = 1, 8 Hz, NCH_2CH_2O); IR ($CHCl_3$, cm^{-1}): 2964, 1781, 1699; GC−MS (m/z): 156 (23, M$^+$ − 15), 129 (68), 114 (10), 101 (23).

(±)-**3-(1-Oxo-2-cyclopentylethyl)-2-oxazolidinone (21b):** 1.91 g (58%, bp 118 °C/0.05 mmHg); mp 44−46 °C; ^{1}H-NMR (200 MHz, acetone-d_6) δ: 1.05−1.29 (2H, m, cyclopentyl), 1.44−1.72 (4H, m, cyclopentyl), 1.72−1.94 (2H, m, cyclopentyl), 2.27 (1H, br septet, J = 7 Hz, H(3)), 2.88 (2H, d, J = 7 Hz, CH_2CO), 4.00 (2H, br t, J = 8 Hz, NCH_2CH_2O), 4.45 (2H, br t, J = 8 Hz, NCH_2CH_2O); IR ($CHCl_3$, cm^{-1}): 2960, 1781, 1702; GC−MS (m/z): 197 (2, M$^+$), 154 (5), 129 (93), 111 (28), 101 (22), 88 (100).

(±)-***erythro*-3-(3-Hydroxy-2-alkyl-1-oxopentyl)-4-(1-methylethyl)-2-oxazolidinones (22a−f) and (±)-*erythro*-3-(3-Hydroxy-2-alkyl-1-oxopentyl)-2-oxazolidinones (23a,b).** The aldol adducts **22a−f** and **23a,b** were prepared from their corresponding carboximides as described by the method of Evans *et al.*[6] Samples of the adducts were further purified by flash chromatography.

(±)-***erythro*-3-(3-Hydroxy-2-methyl-1-oxopentyl)-4-(1-methylethyl)-2-oxazolidinone (22a):** ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.80−0.95 (9H, m, 3 × CH_3), 1.20 (3H, d, J = 7 Hz, C*H*$_3$CHCO), 1.31−1.50 (2H, m, CH_3C*H*$_2$), 2.30 (1H, septet of d, J = 4, 7 Hz, C*H*(CH_3)$_2$), 3.61 (1H, d, J = 5 Hz, OH), 3.62−3.85 (2H, m, CHCHCO), 4.25−4.55 (3H, m, $CHCH_2O$); IR ($CHCl_3$, cm^{-1}): 3538, 2964, 1779, 1684; GC−MS (m/z, TMS ether) 300 (2, M$^+$ − 15), 230 (20), 170 (10), 158 (13), 143 (40).

(±)-***erythro*-3-(3-Hydroxy-2-ethyl-1-oxopentyl)-4-(1-methylethyl)-2-oxazolidinone (22b):** ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.79−1.05 (12H, m, 4 × CH_3), 1.35−1.90 (4H, m, 2 × CH_3C*H*$_2$), 2.20−2.43 (1H, m, C*H*(CH_3)$_2$), 3.67 (1H, s, OH), 3.78−3.88 (1H, m, *CH*CHCO), 3.90−4.05 (1H, m, CHCO), 4.30−4.59 (3H, m, $CHCH_2O$); IR (neat, cm^{-1}): 3436, 2933, 1764, 1693; GC−MS (m/z, TMS ether) 314 (43, M$^+$ − 15), 270 (7), 244 (50), 184 (17), 143 (100).

(±)-***erythro*-3-(3-Hydroxy-2-propyl-1-oxopentyl)-4-(1-methylethyl)-2-oxazolidinone (22c):** ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.85−0.98 (12H, m, 4 × CH_3), 1.22−1.55 (4H, m, 2 × CH_3C*H*$_2$), 1.63−1.89 (2H, m, CH_3CH_2C*H*$_2$), 2.31 (1H, septet of d, J = 7, 4 Hz, C*H*(CH_3)$_2$), 3.56−3.68 (1H, m, C*H*CHCO), 3.72 (1H, d, J = 6 Hz, OH), 4.00−4.12 (1H, m, CHCO), 4.30−4.60 (3H, $CHCH_2O$); IR (neat, cm^{-1}): 3445, 2923, 1771, 1690; GC−MS (m/z, TMS ether) 328 (7, M$^+$ − 15), 284 (7), 258 (53), 198 (13), 186 (17), 158 (25), 143 (88), 125 (40).

(±)-***erythro*-3-(3-Hydroxy-2-butyl-1-oxopentyl)-4-(1-methylethyl)-2-oxazolidinone (22d):** ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.83−0.98 (12H, m, 4 × CH_3), 1.21−1.54 (6H, m, CH_3C*H*$_2$C*H*$_2$C*H*$_2$), 1.72−1.86 (2H, m, CH_3C*H*$_2$CH), 2.34 (1H, septet of d, J = 7, 4 Hz, C*H*(CH_3)$_2$), 3.55−3.70 (1H, m, C*H*CHCO), 3.73 (1H, d, J = 6 Hz, OH), 3.99−4.10 (1H, m, CHCO), 4.28−4.60 (3H, m, $CHCH_2O$); IR (neat, cm^{-1}): 3474, 2921, 1770, 1690; GC−MS (m/z, TMS ether) 342 (8, M$^+$ − 15), 298 (10), 272 (53), 212 (13), 200 (17), 158 (25), 143 (46).

(±)-***erythro*-3-[3-Hydroxy-2-(1-methylethyl)-1-oxopentyl]-4-(1-methylethyl)-2-oxazolidinone (22e):** mp 88−93 °C; ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.85−1.04 (15H, m, 5 × CH_3), 1.20−1.60 (2H, m, CH_3C*H*$_2$), 2.14−2.43 (2H, m, 2 × C*H*(CH_3)$_2$), 3.71−3.87 (2H, m, C*HOH*), 4.12 (1H, dd, J = 6, 8 Hz, CHCO), 4.28−4.58 (3H, m, $CHCH_2O$); IR ($CHCl_3$, cm^{-1}): 3414, 2967, 1768, 1678; GC−MS (m/z, *t*-BDMS ether) 328 (77, M$^+$ − 57), 284 (48), 242 (22), 198 (48), 125 (90).

(±)-***erythro*-3-(3-Hydroxy-2-cyclopentyl-1-oxopentyl)-4-(1-methylethyl)-2-oxazolidinone (22f):** ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.88−1.05 (9H, m, 3 × CH_3), 1.1−2.4 (12H, m, cyclopentyl, C*H*(CH_3)$_2$, CH_3C*H*$_2$), 3.69−3.85 (2H, m, OH, CHCO), 4.19 (1H, dd, J = 6, 10 Hz, $CHCH_2O$), 4.29−4.57 (3H, m, H(3), $CHCH_2O$); IR ($CHCl_3$, cm^{-1}): 3511, 2961, 1766, 1692; GC−MS (m/z, TMS ether) 354 (2, M$^+$ − 15), 340 (5), 311 (10), 211 (53).

(±)-***erythro*-3-[3-Hydroxy-2-(1-methylethyl)-1-oxopentyl]-2-oxazolidinone (23a):** ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.84−1.00 (9H, m, CH_3), 1.2−1.6 (2H, m, CH_3C*H*$_2$), 2.23

(1H, septet of d, J = 7 Hz, C*H*(CH_3)$_2$), 3.69 (1H, d, J = 6 Hz, OH, disappears with D_2O), 3.73–3.88 (1H, m, C*H*CHCO), 4.00–4.15 (3H, m, $CHCH_2O$, CHCO), 4.39–4.50 (2H, dd, J = 9, 9 Hz, $CHCH_2O$); IR ($CHCl_3$, cm^{-1}): 3525, 2968, 1776, 1684; GC–MS (m/z, TMS ether) 286 (28, $M^+ - 15$), 258 (20), 242 (32), 162 (27), 144 (15), 125 (55).

(±)-*erythro*-3-(3-Hydroxy-2-cyclopentyl-1-oxopentyl)-2-oxazolidinone (23b): mp 91–94 °C; ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.92 (3H, t, J = 8 Hz, H(5)), 1.2–2.0 (10H, m, cyclopentyl, CH_3CH_2), 2.13–2.38 (1H, m, $(CH_2)_2CHCH$), 3.50–3.85 (2H, m, OH, CHCO), 4.00–4.20 (3H, m, C*H*CHCO, CH_2CH_2O), 4.38–4.52 (2H, m, CH_2CH_2O); IR ($CHCl_3$, cm^{-1}): 3535, 2954, 1772, 1694; GC–MS (m/z, TMS ether) 312 (23, $M^+ - 15$), 284 (25), 268 (38), 162 (40), 151 (53), 123 (28).

General Procedure for the Synthesis of Methyl (±)-(*E*)-2-Alkylpentenoates (25a–e) and Methyl (±)-*erythro*-2-Cyclopentyl-3-hydroxypentenoate (24f).[6] The crude adduct, **22a–f** or **23a,b**, was dissolved in THF/water (400 mL, 3:1 v/v) and cooled to 0 °C. 3,5-Dibutyl-4-hydroxytoluene (80 mg, 0.4 mmol), hydrogen peroxide (30%, 16.4 mL, 145 mmol, 4 equiv), and lithium hydroxide monohydrate (3.06 g (73 mmol, 2 equiv) in 5 mL water) were then added, and the solution was stirred for 3 h at 0 °C. Aqueous sodium sulfite (1.5 M, 106 mL, 160 mmol) and saturated aqueous $NaHCO_3$ (50 mL) were then added and the THF evaporated *in vacuo*. The aqueous residue was extracted with $CHCl_3$ (200 mL), acidified with 1 M HCl, and extracted with EtOAc (2 × 200 mL). The EtOAc extract was washed with brine, dried over $MgSO_4$, filtered, and evaporated *in vacuo* to afford the 3-hydroxy acid. The hydroxy acid was then dissolved in anhydrous ether and treated with diazomethane. The ethereal solution was evaporated and the ester redissolved in dry dichloromethane (0.14 M) at 0 °C. Et_3N (4 equiv) and methanesulfonyl chloride (4 equiv) were added, and the solution was stirred for 1 h and then evaporated *in vacuo* and filtered with an equal volume of THF. DBU (4 equiv) was then added and the solution refluxed for 1 h. The mixture was diluted with an equal volume of petroleum spirit and washed with 1 M HCl, saturated aqueous $NaHCO_3$, and brine. The extract was dried over $MgSO_4$, filtered, and evaporated *in vacuo* to afford a clear oil which was then purified by fractional distillation.

Methyl (*E*)-2-methyl-2-pentenoate (25a): 2.18 g (55% from carboximide **20a**, bp 133–136 °C, lit. 51 °C/11 mmHg[30]); ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.90 (3H, t, J = 8 Hz, C*H*$_3$CH$_2$), 1.70 (3H, m, C*H*$_3$CHCO), 2.08 (2H, m, CH_3CH_2), 3.56 (3H, s, OCH_3), 6.60 (1H, t, J = 8 Hz, CH=C); IR (neat, cm^{-1}): 2950, 1720; GC–MS (m/z): 128 (23, M^+), 113 (8), 97 (24).

Methyl (*E*)-2-ethyl-2-pentenoate (25b): 1.21 g (31% from carboximide **20b**, bp 112–117 °C/20 mmHg); ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.90–1.10 (6H, m, 2 × CH_3CH_2), 2.10–2.30 (4H, m, 2 × CH_3CH_2), 3.68 (3H, s, OCH_3), 6.68 (1H, t, J = 15 Hz, CH=C); IR (neat, cm^{-1}): 2961, 2876, 1714, 1647; GC–MS (m/z): 142 (62, M^+), 127 (45), 113 (43), 111 (37), 95 (37), 83 (38), 67 (43).

Methyl (*E*)-2-propyl-2-pentenoate (25c): 4.02 g (54% from carboximide **20c**, purified by flash chromagraphy using 1:19 ether/petroleum spirit, v/v); ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.88 (3H, t, J = 8 Hz, C*H*$_3$CH$_2$CH$_2$), 1.04 (3H, t, J = 8 Hz, C*H*$_3$CH$_2$CH=), 1.25–1.51 (2H, m, $CH_3CH_2CH_2$), 1.75–1.84 (2H, m, $CH_3CH_2CH_2$), 2.17–2.34 (2H, m, CH_3CH_2CH=), 3.69 (3H, s, OCH_3), 6.71 (1H, t, J = 8 Hz, CH=C); IR (neat, cm^{-1}): 2951, 2874, 1715, 1646; GC–MS (m/z): 156 (82, M^+), 127 (100), 113 (20), 95 (100), 81 (23).

Methyl (*E*)-2-butyl-2-pentenoate (25d): 4.12 g (64% from carboximide **20d**, bp 64–90 °C/8 mm); ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.80–1.00 (3H, m, C*H*$_3$(CH_2)$_3$), 1.04 (3H, t, J = 8 Hz, C*H*$_3$CH$_2$CH=), 1.20–1.40 (4H, m, $CH_3CH_2CH_2CH_2$), 2.15–2.40 (4H, m, C*H*$_2$CH=C(C*H*$_2$)CO), 3.68 (3H, s, OCH_3), 6.72 (1H, t, J = 15 Hz, CH=C); IR (neat, cm^{-1}): 2946, 2869, 1716, 1646; GC–MS (m/z): 170 (22, M^+), 155 (2), 141 (87), 127 (63), 109 (43), 95 (100), 81 (37), 69 (93).

Methyl (*E*)-2-(1-Methylethyl)-2-pentenoate (25e). Using 1.6 g of the hydroxy ester **24e** (obtained in 4.47 g (70%) yield from carboximide **21a**) gave only 100 mg of the methyl ester **25e** using the above procedure: ^{1}H-NMR (200 MHz, acetone-d_6) δ: 1.02 (3H, t, J = 8 Hz, C*H*$_3$CH$_2$), 1.2–1.4 (6H, m, CH(C*H*$_3$)$_2$), 2.20 (2H, q, J = 8 Hz, CH_3CH_2), 2.2–2.3 (1H, m, C*H*(CH_3)$_2$), 3.65 (3H, s, OCH_3), 6.68 (1H, t, J = 8 Hz, CH=C); IR ($CHCl_3$, cm^{-1}): 2960, 1708; GC–MS (m/z): 156 (37, M^+), 141 (30), 127 (60), 109 (65), 95 (70), 81 (67).

(±)-*erythro*-Methyl 2-cyclopentyl-3-hydroxypentanoate (24f): 0.94 g (62% from carboximide **21b**); ^{1}H-NMR (200 MHz, acetone-d_6) δ: 1.0–1.9 (13H, m, cyclopentyl, CH_3CH_2), 2.12–2.28 (1H, m, CHC*H*(CH_2)$_2$), 3.50–3.64 (5H, m, OCH_3, CHCO, OH), 3.78–3.82 (1H, m, C*H*CHCO); IR ($CHCl_3$, cm^{-1}): 3450, 2954, 1725, 1443; GC–MS (m/z): 142 (2, $M^+ - 58$), 111 (18), 99 (15), 83 (18), 74 (100).

General Procedure for the Synthesis of (*E*)-2-Alkyl-2-pentenoic Acids (5–8). The ester **25a–d** was dissolved in methanol (8 mL/mmol ester). Aqueous potassium hydroxide (2 M, 8 mL/mmol ester) was added and the solution refluxed for 2 h before the methanol was removed *in vacuo*. The solution was acidified with 6 M HCl and extracted with ether. The organic extract was washed with brine, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by distillation or flash chromatography on silica using ether/petroleum spirit as an eluent to afford the pure acid.

(*E*)-2-Methyl-2-pentenoic acid (5): 1.17 g of an oily crystalline solid (60%; bp 79–88 °C/0.7 mmHg; lit. bp 106.5 °C[31]); ^{1}H-NMR (200 MHz, acetone-d_6) δ: 1.03 (3H, t, J = 8 Hz, C*H*$_3$CH$_2$ (*E*)), 1.17 (3H, t, J = 7 Hz, C*H*$_3$CH$_2$ (*Z*)), 1.64 (3H, d, J = 5 Hz, CH=CC*H*$_3$ (*Z*)), 1.78 (3H, d, J = 1 Hz, CH=CCH3 (*Z*)), 2.20 (2H, q of d, J = 8, 8 Hz, CH_3CH_2 (*E*)), 2.46 (2H, m, CH_3CH_2 (*Z*)), 5.55 (1H, m, CH=C (*Z*)), 6.75 (1H, t of d, J = 1, 8 Hz, CH=C (*E*)), *E*:*Z* = 85:15; IR (neat, cm^{-1}): 3200–3040, 2849, 1693, 1645; GC–MS (m/z, TMS ester) 186 (5, M^+), 171 (100), 157 (33), 127 (17), 97 (40). Anal. ($C_6H_{10}O_2$) C, H.

(*E*)-2-Ethyl-2-pentenoic acid (6): 1.01 g of a clear oil (62%: bp 88 °C/0.5 mmHg); ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.89–1.08 (6H, m, 2 × CH_3), 2.10–2.38 (4H, m, 2 × CH_3CH_2), 6.70 (1H, t, J = 9 Hz, CH=C); IR (neat, cm^{-1}): 3200–3040, 2964, 1688, 1642; GC–MS (m/z, *t*-BDMS ester) 185 (100, $M^+ - 57$), 141 (5), 111 (32), 99 (13). Anal. ($C_7H_{12}O_2$) C, H.

(*E*)-2-Propyl-2-pentenoic acid (7, 2-ene VPA): 1.78 g of a crystalline solid (49%: purified by flash chromatography); mp 33–4 °C; ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.90 (3H, t, J = 8 Hz, C*H*$_3$CH$_2$CH$_2$), 1.04 (3H, t, J = 8 Hz, C*H*$_3$CH$_2$CH=), 1.3–1.4 (2H, m, $CH_3CH_2CH_2$), 2.10–2.26 (4H, m, C*H*$_2$CH=C-(C*H*$_2$)CO), 6.75 (1H, t, J = 9 Hz, CH=C); IR (neat, cm^{-1}): 3200–3040, 2932, 1674, 1632; GC–MS (m/z, TMS ester) 214 (13, M^+), 199 (47), 185 (10), 169 (13), 124 (42). Anal. ($C_8H_{14}O_2$) C, H.

(*E*)-2-Butyl-2-pentenoic acid (8): 2.74 g of an oily crystalline solid (76%; bp 135–139 °C/4 mmHg); ^{1}H-NMR (200 MHz, acetone-d_6) δ: 0.90 (3H, t, J = 7 Hz, C*H*$_3$(CH_2)$_3$), 1.05 (3H, t, J = 8 Hz, C*H*$_3$CH$_2$CH=), 1.20–1.47 (4H, m, $CH_3CH_2CH_2$-CH_2), 2.14–2.36 (4H, m, C*H*$_2$CH=C(C*H*$_2$)CO), 6.75 (1H, t, J = 7.6 Hz, CH=C); IR (neat, cm^{-1}): 3200–3040, 2898, 1692, 1639; GC–MS (m/z, *t*-BDMS ester): 213 (100, $M^+ - 57$), 179 (18), 139 (20), 105 (10). Anal. ($C_9H_{16}O_2$) C, H.

1-Cyclopentene-1-carboxylic Acid (12). To a solution of ethyl 2-oxocyclopentanecarboxylate (6.0 g, 38 mmol) in methanol (100 mL) at 0 °C was added sodium borohydride (1.6 g, 42 mmol). The solution was stirred for 1 h at 0 °C, and then water (30 mL) was added, followed by acidification with 6 M HCl. The solution was condensed *in vacuo* and extracted with ether (2 × 100 mL), and the extract was washed with brine, dried over $MgSO_4$, filtered, and evaporated *in vacuo* to afford an orange oil, which was diluted with ether (80 mL) and washed with saturated aqueous $NaHCO_3$ and water before being dried over $MgSO_4$, filtered and evaporated to afford a yellow oil (4.94 g). Owing to apparent decomposition during distillation in a previous experiment, the crude material was used directly in the next (dehydration) step. Dry dichloromethane (100 mL) was added, followed by Et_3N (3.48 g, 34.3 mmol) and methanesulfonyl chloride (3.94 g, 34.3 mmol), each as dichloromethane solutions (5 mL). The solution was stirred 1 h at room temperature, then evaporated *in vacuo*, and filtered with THF (50 mL). DBU (5.22 g, 34.3 mmol) in THF (10 mL) was added and the mixture stirred 1 d at room temperature before

being diluted with ether (100 mL), washed with 1 M HCl and brine, dried over $MgSO_4$, filtered, and evaporated *in vacuo*. Distillation afforded ethyl cyclopentenecarboxylate (bp 82–105 °C/10 mmHg; 3.02 g, 56% overall) as a clear liquid: IR ($CHCl_3$, cm^{-1}): 2941, 1716, 1448; GC–MS (*m/z*): 140 (30, M^+), 112 (22), 95 (100), 67 (98). The ester was then hydrolyzed as described above to produce cyclopentenecarboxylic acid as a crystalline white solid (0.80 g, 96%): mp 114–117 °C (lit. mp 123–124 °C[32]); ^{1}H-NMR (200 MHz, acetone-d_6) δ: 1.82–2.00 (2H, m, $CH_2CH_2CH_2$), 2.40–2.56 (4H, m, $CH_2CH_2CH_2$), 6.69–6.77 (1H, m, CH=C); IR ($CHCl_3$, cm^{-1}): 3040, 2950, 1691, 1621; GC–MS (*m/z*, *t*-BDMS ester) 169 (100, $M^+ - 57$), 125 (42), 95 (28). Anal. ($C_6H_8O_2$) C, H.

1-Cyclohexene-1-carboxylic acid (13) was prepared by hydrolysis of commercial methyl 1-cyclohexene-1-carboxylate as described above. The crude product was purified by flash chromatography using ether/petroleum spirit (1:2, v/v) to afford the acid 1.98 g (87%) as a white crystalline solid: mp 36–38 °C (lit. mp 38 °C[33]); ^{1}H-NMR (200 MHz, acetone-d_6) δ: 1.5–1.7 (4H, m, $CH_2CH_2CH_2CH_2$), 2.1–2.3 (4H, m, CH_2C=), 6.9–7.0 (1H, m, CH=), 10.1 (1H, br s, CO_2H); IR ($CHCl_3$, cm^{-1}): 3200–3000, 2936, 1686, 1644; GC–MS (*m/z*, *t*-BDMS ester) 183 (100, $M^+ - 57$), 139 (23), 109 (25). Anal. ($C_7H_{10}O_2$) C, H.

(±)-Methyl 3-oxobicyclo[2.2.1]heptane-2-carboxylate (27) was prepared from (±)-norcamphor as described elsewhere[10] to afford the product (5.39 g, 89%) as a yellowish oil: ^{1}H-NMR (200 MHz, acetone-d_6) δ: 1.4–1.9 (6H, m, 3 × CH_2), 2.1–2.3, 2.4–2.6 (1H, 1H, m, m, $CH(CH_2)CH$), 2.9 (1H, m, CHCO), 3.75 (3H, s, OCH_3); IR ($CHCl_3$, cm^{-1}): 3035, 2961, 1764, 1731; GC–MS broad peak indicative of decomposition.

(±)-Bicyclo[2.2.1]hept-2-ene-2-carboxylic Acid (11). The methyl ester **28** was prepared from **27** as described for ethyl cyclopentenecarboxylate. Distillation afforded a crude product (1.14 g, 36%, bp 93–123 °C/10 mmHg) which was hydrolyzed in refluxing potassium hydroxide as described above to the acid (0.40 g, 14% from **27**, bp 132–135 °C/0.4 mmHg) as a clear oil which crystallized upon standing: mp 30–32 °C (lit. mp 21.5–22.5 °C[34]); ^{1}H-NMR (200 MHz, acetone-d_6) δ: 1.0–1.9 (6H, m, 3 × CH_2), 3.00 (1H, m, C=CHCH), 3.20 (1H, m, (CH)-(CO)C=CH), 6.92 (d, 1H, J = 3 Hz, C=CH); IR ($CHCl_3$, cm^{-1}): 3038, 2948, 1691; GC–MS (*m/z*, *t*-BDMS ester) 237 (2, $M^+ - 15$), 195 (100), 167 (75), 123 (28). Anal. ($C_8H_{12}O_2$) C, H.

Cyclohexylideneacetic Acid (15). Ethyl cyclohexylideneacetate was prepared from cyclohexanone as described elsewhere[11] and the crude product then hydrolyzed in refluxing potassium hydroxide as above to afford the crystalline white acid (1.28 g, 46%) after decanting with petroleum spirit: mp 86–88 °C (lit. mp 89 °C[35]); ^{1}H-NMR (200 MHz, acetone-d_6) δ: 1.52–1.74 (6H, m, $CH_2CH_2CH_2CH_2CH_2$), 2.2–2.3 (2H, m, CH_2C=), 2.8–2.9 (2H, m, CH_2C=), 5.63 (1H, t, J = 1 Hz, C=CH), 10.3 (1H, br s, CO_2H); IR ($CHCl_3$, cm^{-1}): 3200–3000, 2935, 2859, 1688, 1643; GC–MS (*m/z*, *t*-BDMS ester) 197 (100, $M^+ - 57$), 153 (22), 123 (20). Anal. ($C_8H_{12}O_2$) C, H.

Cyclopentylideneacetic acid (14) was prepared from cyclopentanone using the method described for cyclohexylideneacetic acid. The final product was purified by flash chromatography using 1:2 ether/petroleum spirit (v/v) to afford the acid (1.60 g, 84%) as a clear oil which crystallized upon standing: mp 58–60 °C (lit. mp 60–61 °C[36]); ^{1}H-NMR (200 MHz, acetone-d_6) δ: 1.5–1.7 (4H, m, $CH_2CH_2CH_2CH_2$), 2.12–2.30 (4H, m, CH_2C=), 6.95–7.05 (1H, m, C=CH), 10.4 (1H, br s, CO_2H); IR ($CHCl_3$, cm^{-1}): 3200–3000, 2911, 1688, 1644; GC–MS (*m/z*, *t*-BDMS ester) 183 (100, $M^+ - 57$), 139 (25), 109 (22). Anal. ($C_7H_{10}O_2$) C, H.

Cycloheptylideneacetic acid (16) was prepared from cycloheptanone using the method described for cyclohexylideneacetic acid. The final acid product was purified by flash chromatography using 1:2 ether/petroleum spirit (v/v) to afford the acid as a clear oil which crystallized upon standing: mp 50–2 °C (lit. mp 54–55 °C[35]); ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.5–1.8 (8H, m, $CH_2(CH_2)_4CH_2$), 2.40 (2H, br t, J = 7 Hz, CH_2C=), 2.85 (2H, br t, J = 7 Hz, CH_2C=), 5.66 (1H, t, J = 1 Hz, C=CH); IR ($CHCl_3$, cm^{-1}): 3200–3000, 2930, 1685, 1628; GC–MS (*m/z*, *t*-BDMS ester) 211 (100, $M^+ - 57$), 167 (15), 137 (23). Anal. ($C_9H_{14}O_2$) C, H.

Cyclooctylideneacetic acid (17) was prepared from cyclooctanone using the method described for cyclohexylideneacetic acid. The final product was purified by flash chromatography using 1:2 ether/petroleum spirit (v/v) to afford the acid as a clear oil which crystallized upon standing: mp 81–3 °C (lit. mp 89–89.5 °C[35]); ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.4–1.9 (10H, m, $CH_2(CH_2)_5CH_2$), 2.38 (2H, t, J = 7 Hz, CH_2C=), 2.78 (2H, t, J = 7 Hz, CH_2C=), 5.78 (1H, s, C=CH); IR ($CHCl_3$, cm^{-1}): 3200–3000, 2913, 1687, 1626; GC–MS (*m/z*, *t*-BDMS ester) 225 (100, $M^+ - 57$), 181 (10), 151 (12). Anal. ($C_{10}H_{16}O_2$) C, H.

Drug Assays. Anticonvulsant potency was determined for each drug using five intraperitoneal doses (4 mL/kg) of its sodium salt and eight 6-week-old mice per dose.[15] Pentylenetetrazole (85 mg/kg, 10 mL/kg) was injected subcutaneously at 10 min, and the animals were observed for a further 30 min. An animal was considered to be unprotected if it showed a 5 s clonus with loss of balance. Administration of PTZ alone produced seizures in 87% of a group of 48 mice, with a latency mean of 7.2 ± 4.3 min and a median of 6 min. ED_{50} was determined from a graph of percentage protection *vs* log(dose).[37] Sedation was evaluated by the degree of activity and muscle tone present during handling when the mice were administered PTZ. Sedation termed "+" in Table 1 implies a noticeable reduction in muscle tone whereas "++" describes a state where the animal lies limp and essentially motionless.[15]

Brain homogenates were prepared from mice administered an ED_{50} dose of each drug and sacrificed at 15 min. The brains were removed, homogenized in 0.32 M sucrose, and frozen at −78 °C until analysis. The samples were thawed, and 500 μL aliquots were mixed with sodium octanoate solution (500 μL, 22.4 nmol) and 1.5 M HCl (1000 μL) and 380 μL aliquots combined with EtOAc (2000 μL). The mixtures were vortexed thoroughly and then subjected to gentle rotation for 30 min. The vials were centrifuged (2060*g*, 10 min), and a 1000 μL portion of the organic layer was removed and dried over sodium sulfate for 10 min. A fraction (800 μL) of the supernatant was removed, evaporated to approximately 100 μL under a flow of dry nitrogen in a 40 °C water bath, and finally derivatized with 500 μL of an EtOAc solution containing 7% MTBSTFA and 0.07% *t*-BDMSCl (v/v) for 1 h at 65 °C. Upon cooling, the solutions were analyzed by GC–MS. The acids in the above assays were analyzed by GC–MS using a Hewlett-Packard HP 5890 gas chromatograph interfaced to a HP 5989A mass spectrometer. GC: 34.5 kPa helium head pressure, Hewlett-Packard HP-1 capillary column (12 m × 0.2 mm i.d. × 0.33 μm film). Oven: 50 °C initial, increasing to 260 °C at 10 °C/min with final hold time 5 min. Injection: 1 μL. Equilibration time: 0.5 min. MS: electron impact ionization potential 70 eV, single ion monitoring at *m/z* ($M^+ - 57$) for *tert*-butyldimethylsilyl esters of drug and internal standard, dwell time 75 ms, source 275 °C, quad 100 °C. A standard autotuning sequence was used.

Physicochemical Parameters. Molecular modeling was performed using MacroModel v. 3.5 on a Silicon Graphics IRIS workstation. The input structure was minimized using the MM2* force field[38] in an aqueous medium and the volume calculated at the resultant local minimum. A Monte Carlo conformational search was then conducted for 500 conformations using a 10 kJ/mol energy cutoff. The atomic Cartesian coordinates for each conformer were then transformed with a custom PASCAL program so as to place the carbonyl atom at the origin, C(2) on the *x*-axis and the C(2) substituent in the *xy* plane. The population-weighted mean internuclear dimensions, expressed as the maximal *x*, *y*, or *z* values, were calculated based on a Boltzmann distribution at 37 °C. These parameters correspond to the STERIMOL scheme proposed[39] as a means of quantifying shape as well as size in a linear free energy relationship.

Lipophilicity was determined for the undissociated species using the CLOGP program (Daylight Chemical Information Systems, Irvine, CA, v. 3.54). The program was not able to distinguish *cis* and *trans* isomers.

Acknowledgment. We are grateful to Dr. Eric P Bigham of Burroughs Wellcome Co. (Research Triangle

Park, NC) for lipophilicity calculations. We thank Dr. Wei Tang and Mr. Roland Burton (Faculty of Pharmacy, UBC) for the ED_{50} data for VPA and assistance with the GC–MS analyses, respectively. This work was supported from a Medical Research Council of Canada program grant.

References

(1) Rall, T. W.; Schleifer, L. S. Drugs Effective in the Therapy of the Epilepsies. In *The Pharmacological Basis of Therapeutics*, 8th ed.; Gilman, A. G., Rall, T. W., Nies, A. S., Taylor, P., Eds.; Pergamon Press: New York, 1990; pp 436–462.

(2) Loscher, W. Pharmacological, toxicological and neurochemical effects of $\Delta^{2(E)}$-valproate in animals. *Pharm. Weekblad. Sci.* **1992**, *14*, 139–143.

(3) Nau, H.; Loscher, W. Valproic acid and metabolites: pharmacological and toxicological studies. *Epilepsia* **1984**, *25* (Supp. 1), S14–S22.

(4) McLean, M. J.; Macdonald, R. L. Sodium valproate, but not ethosuximide, produces use- and voltage-dependent limitation of high frequency repetitive firing of action potentials of mouse central neurons in cell culture. *J. Pharmacol. Exp. Ther.* **1986**, *237*, 1001–1011.

(5) Evans, D. A.; Sjogren, E. B.; Bartroli, J.; Dow, R. L. Aldol addition reactions of chiral crotonate imides. *Tetrahedron Lett.* **1986**, *27*, 4957–4960.

(6) Evans, D. A.; Britton, T. C.; Ellman, J. A. Contrasteric carboximide hydrolysis with lithiumhydroperoxide. *Tetrahedron Lett.* **1987**, *28*, 6141–6144.

(7) Evans, D. A.; Nelson, J. V.; Taber, T. R. Stereoselective aldol condensations. In *Topics in Stereochemistry*; Allinger, N. L., Eliel, E. L., Wilen, S. H., Eds.; Wiley: New York, 1982; Vol. 13, pp 1–116.

(8) Lee, R. D.; Kassahun, K.; Abbott, F. S. Stereoselective synthesis of the diunsaturated metabolites of valproic acid. *J. Pharm. Sci.* **1989**, *78*, 667–671.

(9) Williams, R. M.; Maruyama, L. K. Synthesis of functionalized bicyclic dioxopiperazines viaintramolecular epoxide opening. *J. Org. Chem.* **1987**, *52*, 4044–4047.

(10) Mander, L. N.; Sethi, S. P. Regioselective synthesis of β-ketoesters from lithium enolates and methyl cyanoformate. *Tetrahedron Lett.* **1983**, *24*, 5425–5428.

(11) Wadsworth, W. S.; Emmons, W. D. Ethyl cyclohexylideneacetate. In *Organic Syntheses*; Baumgarten, H. E., Ed.; Wiley: New York, 1973; Collect. Vol. V, pp 547–549.

(12) Loscher, W.; Nau, H. Pharmacological evaluation of various metabolites and analogues of valproic acid. Anticonvulsant and toxic potencies in mice. *Neuropharmacol.* **1985**, *24*, 427–435.

(13) Abbott, F. S.; Acheampong, A. A. Quantitative structure-anticonvulsant activity relationships of valproic acid, related carboxylic acids and tetrazoles. *Neuropharmacology* **1988**, *27*, 287–294.

(14) Lee, R.; Orr, J.; Abbott, F. S. Tissue distribution, elimination characteristics and anticonvulsant efficacy of *(E,E)*-2,3′-diene VPA compared to *(E)*-2-ene VPA and to valproic acid (VPA) in rats. Submitted to *Epilepsy Res.*

(15) Swinyard, E. A.; Woodhead, J. H.; White, H. S.; Franklin, M. R. Experimental selection, quantification and evaluation of anticonvulsants. In *Antiepileptic Drugs*, 3rd ed.; Levy, R. H., Dreifuss, F. E., Mattson, R. H., Meldrum, B. S., Penry, J. K., Eds.; Raven Press: New York, 1989; pp 85–102.

(16) Mergen, F.; Lambert, D. M.; Saraiva, J. C.; Poupaert, J. H.; Dumont, P. Antiepileptic activity of 1,3-dihexadecanoylamino-2-valproyl-propan-2-ol, a prodrug of valproic acid endowed with tropism for the central nervous system. *J. Pharm. Pharmacol.* **1991**, *43*, 815–816.

(17) Carraz, G. Theory of the activity of the di-*n*-propylacetic acid structure. *Agressologie* **1967**, *8*, 13–20.

(18) Taillandier, G.; Benoit-Guyod, J. C.; Boucherle, A.; Broll, M.; Eymard, P. Dipropylacetic series. XII. Anticonvulsant branched aliphatic acids and alcohols. *Eur. J. Med. Chem.-Chim. Ther.* **1975** *10*, 453–462.

(19) Keane, P. E.; Simiand, J.; Mendes, A.; Santucci, K.; Morre, M. The effects of analogues of valproic acid on seizures induced by pentylenetetrazole and GABA content in brain of mice. *Neuropharmacology* **1983**, *22*, 875–879.

(20) Elmazar, M. M.; Hauck, R.-S.; Nau, H. Anticonvulsant and neurotoxic activities of twelve analogues of valproic acid. *J. Pharm. Sci.* **1993**, *82*, 1255–1258.

(21) Liu, M.; Scott, K. R.; Pollack, G. M. Pharmacokinetics and pharmacodynamics of valproate analogues in rats. I. Spiro[4.6]-undecane-2-carboxylic acid. *Epilepsia* **1994**, *31*, 465–473.

(22) Perlman, B. J.; Goldstein, D. B. Membrane-disordering potency and anticonvulsant action of valproic acid and other short-chain fatty acids. *Mol. Pharmacol.* **1984**, *26*, 83–89.

(23) Tangorra, A.; Curatola, G.; Bertoli, E. Evaluation of antiepileptic drug effect on membrane fluidity. *Exp. Mol. Pathol.* **1991**, *55*, 180–189.

(24) Van Dongen, A. M.; van Erp, M. G.; Voskuyl, R. A. Valproate reduces excitability by blockage of sodium and potassium conductance. *Epilepsia* **1986**, *27*, 177–182.

(25) Zona, C.; Avoli, M. Effects induced by the antiepileptic drug valproic acid upon the ionic currents recorded in rat neocortical neurons in cell culture. *Exp. Brain Res.* **1990**, *81*, 313–317.

(26) Moody, E. J.; Harris, B. D.; Skolnick, P. The potential for safer anaesthesia using stereoselective anaesthetics. *Trends Pharmacol. Sci.* **1994**, *15*, 387–391.

(27) Lucke, A.; Mayer, T.; Altrup, U.; Lehmenkuhler, A.; Dusing, R.; Speckman, E.-J. Simultaneous and continuous measurement of free concentration of valproate in blood and extracellular space of rat cerebral cortex. *Epilepsia* **1994**, *35*, 922–926.

(28) Furniss, B. S.; Hannaford, A. J.; Rogers, V.; Smith, P. W.; Tatchell, A. R. *Vogel's Textbook of Practical Organic Chemistry*; Wiley: New York, 1987; p 498.

(29) Evans, D. A.; Bartroli, J.; Shih, T. L. Enantioselective aldol condensations. 2. *Erythro* selective chiral aldol condensations *via* boron enolates. *J. Am. Chem. Soc.* **1981**, *103*, 2127–2129.

(30) Lide, D. R., Ed. *CRC Handbook of Chemistry and Physics*, 75th ed.; CRC Press: Boca Raton, 1994; p 3249.

(31) Lucas, H. J.; Prater, A. N. The isomeric 2-pentenes. *J. Am. Chem. Soc.* **1937**, *59*, 1682–1686.

(32) Philp, R. P.; Robertson, A. V. Cyclic hydroxycarboxylic acids. II. Lactonization reactions on 2-hydroxycyclopentanecarboxylic acids and 3-hydroxyprolines. *Aust. J. Chem.* **1978**, *30*, 123–130.

(33) Boorman, E. J.; Linstead, R. P. Investigations of the olefinic acids. Part XVI. Additive reactions and tautomeric changes of cyclic unsaturated acids and analogous observations on α-methylpentenoic acids. *J. Chem. Soc.* **1935**, 258–267.

(34) Finnegan, R. A.; McNees, R. S. Organometallic chemistry. VII. The reactions of norbornene, *endo*-5-hydroxymethylnorbornene, nortricyclene and norbornadiene. *J. Org. Chem.* **1964**, *29*, 3234–3241.

(35) Wolinsky, J.; Erickson, K. L. Bromomethylenecycloalkanes. *J. Org. Chem.* **1965**, *30*, 2208–2211.

(36) Weiland, J. H.; Arens, J. F. Chemistry of acetylenic ethers. XVII. The reactions of some Ethoxyethynylcarbinols with mercaptans and thio acids. *Recl. Trav. Chim. Pays-Bas* **1956**, *75*, 1358–1368.

(37) Litchfield, J. T.; Wilcoxon, F. A simplified method of dose-effect experiments. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99–113.

(38) Mohamadi, F.; Richards, N. G.; Guida, W. C.; Liskamp, R.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. MacroModel - an integrated software system for modeling organic and bioorganic molecules using molecular mechanics. *J. Comput. Chem.* **1990**, *11*, 440–467.

(39) Verloop, A.; Hoogenstraaten, W.; Tipker, J. Development and application of new steric substituent parameters in drug design. In *Drug Design*; Ariens, E. J., Ed.; Academic Press: New York, 1976; Vol. 7, pp 165–208.

JM9503207