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Synthesis and pharmacological evaluation of prodrugs of valproic acid

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We report the synthesis of (±)-3,6-Di-*O*-valproil-1,2 : 4,5-di-*O*-isopropylidene-myo-inositol (**5**) as well as (±)-3,6-Di-*O*-valproil-4,5-*O*-isopropylidene-myo-inositol (**6**) and (±)-3,6-Di-*O*-valproil-myo-inositol (**7**), which results from acid hydrolysis of the formers. The anticonvulsant activity of the compound **7** (MES test) expressed as ED_{50} is four times higher than that reported for valproic acid.

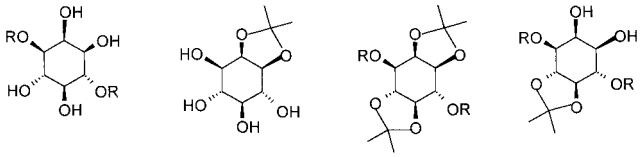
1. Introduction

After the discovery of the anticonvulsant properties of valproic acid (VPA) by Meunier et al. [1], the drug found widespread use as a valuable therapeutic agent for the treatment of several forms of epilepsy. VPA was introduced in the market during the 70's and is now one of the major antiepileptic drugs. It has a broad spectrum of activity against both convulsive and nonconvulsive generalized epilepsies. Unfortunately, several toxic effects associated with its therapy are of importance. Teratogenic effects have been observed [2, 3], and also fatal hepatic necrosis resembling Reye's Syndrome has been reported [4–6]. VPA has also developed teratogenic toxicity in a variety of experimental animals [7–10]. On the other hand, a therapeutic concentration of VPA in the central nervous systems (CNS) high enough to trigger an efficient therapeutic response manifested in seizure control implies a high concentration of VPA in the blood, which generates the unwanted side effects. In order to avoid this, the treatment of brain diseases, such as epilepsy, has to be supported in pharmacologically active agents capable of being delivered to the CNS. This process is, however, severely limited by the selectivity of the protective blood brain barrier (BBB). In this framework, the derivatization of the molecules via prodrug formation is increasing its credibility among drug design strategies. Prodrug strategy, as a mean of improving the delivery of the drugs to the site of therapeutic action, has become a field of practical application. This is particularly true when this strategy involves the derivatization of carboxyl or hydroxyl groups to form esters, which hydrolyse *in vivo*, either chemically or enzymatically, to release the parent drugs.

We report, in this communication, new prodrugs of VPA obtained by esterification with myo-inositol (**1**). Due to its physiological role as a second messenger, the selection of myo-iniositol as a carrier group capable of delivering VPA to its therapeutic site of action can be predicted as successful. Prodrugs that are lipophilic enough to penetrate the BBB allow in this way brain uptake. Further enzymatic conversion leads to therapeutic activity.

2. Investigations, results and discussion

As a natural and physiological compound, myo-inositol is easily metabolized in the body. For the purpose of drug transport, it has the advantage of being capable of attaching several drugs as ester derivatives. The synthetic routes present, however, serious difficulties, because derivatives can only be obtained after selective protection of the positions that are not involved in ester functions. For this reason, we have selectively protected myo-inositol derivatives with di-*O*-isopropylidene functions [11].



1 R=H; **7** R=Pr_2CHCO — **2** — **3** R=H; **4** R=Ac; **5** R=Pr_2CHCO — **6** R=Pr_2CHCO

We prepared the compounds (±)-1,2-*O*-isopropylidene-myo-inositol (**2**) and (±)-1,2 : 4,5-di-*O*-isopropylidene-myo-inositol (**3**) according to Gigg et al. [11]. Compound **3** was purified by silica gel column chromatography and further fractional crystallisation and characterised as the corresponding diacetates. The structure of compound **3** was assigned on the basis of spectral and physical data.

The reaction of **3** with valproic anhydride catalyzed in basic media (triethylamine) gave a mixture of (±)-3,6-Di-*O*-valproil-1,2 : 4,5-di-*O*-isopropylidene-myo-*inositol* (**5**) and other di-valproil-di-*O*-isopropylidene derivatives with structures that have not been yet succesfully determined. When compound **5** was hydrolysed in 75% (v/v) acetic acid solution [12], it gave two products that result from partial and total hydrolysis (**6** and **7**) together with the starting materials.

The structure of compound **5** has been determined by X-Ray diffraction studies. The experimentally determined structure is in agreement with the one that results from a conformational analysis that uses an AM1 model Hamiltonian [12] (MOPAC 7.0 package [13]).

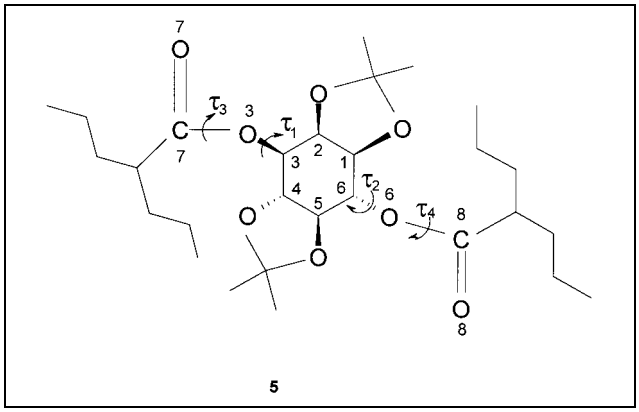


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Fig.: (±)-3,6-Divalproil-1,2,4,5-di-*O*-isopropilliden-myo-inositol

The pharmacological tests were performed according to standard procedures provided by the Antiepileptic Drug Development Program of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) [15, 16]. Maximal electroshock seizure (MES) test was employed to determine the anticonvulsant activity. Rotorod test was used to determine the acute toxicity. The AE activity was expressed as ED_{50} (the dose that is effective in 50% of the animals tested), and estimated, with their 95% confidence limits, by probit analysis [17]. We determined the time when compound **7** had the maximal protection (Table 1) and the ED_{50} value. VPA: $ED_{50} = 1261$ (1155–1371) μmol/kg, time 0.25 h; Compound **7**: ED_{50} 183.6 (133.6–252.5) μmol/kg, time 4 h. This prodrug does not manifest toxicity in the rotorod test. The AE activity of compounds **5** and **6** could not be determined due to solubility problems.

Table 1: Anticonvulsant activity of compound 7 by MES test (mice, intraperitoneally)

Time (h)	Protective[1]	n	Protective[2]	n
1/2	20%	5	50%	8
1	0%	5	37%	8
2	0%	5	0%	8
4	20%	5	62%	8
6	–	–	50%	4

* MES: maximal electroshock test. [1] 0.1 mmol/kg, [2] 0.3 mmol/kg

3. Experimental

3.1 Chemistry

NMR spectra were recorded on a Bruker AC-200 spectrometer: ^1H (200 MHz), and ^{13}C (50.3 MHz). The ^1H NMR spectra were referenced to tetramethylsilane (TMS), ^{13}C NMR spectra were referenced to TMS. IR spectra were recorded on a FT-IR Perkin Elmer 1600 spectrometer. FAB MS were recorded on a Kratos MS80RFA instrument (Kratos Analyticals, Manchester, UK). Xenon beam, (8 keV energy), Center for Drug Discovery, University of Florida. M.p.'s were measured on an Electrothermal apparatus and are uncorrected. Elemental analyses were measured on a Carlo Erba EA 1108. All the results were in an acceptable range. Flash column chromatography was performed using Silica gel 60 (0.063–0.200 mm, Merck). TLC was performed using aluminium sheets Silica gel 60 F254 (Merck). Spots were visualised under 254 nm UV light or with the sulfomolybdic reactive.

Due to the size and the flexibility of compound **5**, a random procedure was used to generate the starting guesses for the geometry optimization procedure. Molecular dynamic calculations T = 600 K, heat time = 0.1 ps, step size = 0.0005 ps, run times = 2, 5, 10, 20 ps, cooling times = 0.5 ps result in only one stable structure characterized by a value of τ_3 and τ_4 (Fig.) close to 180°. Further optimization, using an AM1 model hamiltonian, gives a structure in good agreement with the X-ray derived one (Tables 2 and 3).

3.1.1 (±)-1,2:4,5-Di-O-isopropylidene-myo-inositol (**3**)

Diol **3** was prepared by the method of Gigg et al. [11] from (±)-1,2-*O*-isopropylidene-myo-inositol, 2,2-dimethoxypropane, and *p*-toluenesulfonic acid monohydrate. The crude product was eluted from a column of silica gel with dichloromethane and methanol (100/0 to 10/1 v/v). IR (KBr): ν 3470–3550 cm^{-1} (OH); 1 H NMR ($CDCl_3$): δ 1.29, 1.35, 1.39, 1.45 (4s, 12 H, 2 $C(CH_3)_2$), 2.82 (d, J = 8.1 Hz, 1 H, 3-OH), 3.18–3.34 (m, 2 H, 6-OH, H-5), 3.70–3.85 (m, 2 H, H-4, H-6), 3.90–4.02 (m, 2 H, H-1, H-3), 4.36–4.42 (m, 1 H, H-2).
$C_{12}H_{20}O_6$

Table 2: Torsional angles (°) for ester unions in compound 5

Angles	Experimental values*	Calculated values**
τ_1 = C2–C3–O3–C7	−83.3 (7)	−83.03
τ_2 = C5–C6–O8–C8	112.1 (6)	118.67
τ_3 = O7–C7–O3–C3	−1.1 (13)	−0.38
τ_4 = O8–C8–O6–C6	1.0 (11)	4.66

Standard deviations in parentheses
* Data obtained from X-ray
** Data calculated by the AM1 conformational analysis method

Table 3: Torsional angles (°) in the inositol ring in compound 5

Angles	Experimental values*	Calculated values**
C2–C3–C4–C5	54.4 (7)	−58.33
C3–C4–C5–C6	−74.4 (6)	78.93
C4–C5–C6–C1	67.5(6)	−60.25
C5–C6–C1–C2	−47.7(6)	30.69
C6–C1–C2–C3	33.6(8)	−16.69
C4–C3–C2–C1	−35.4(8)	29.76

Standard deviations in parentheses
* Data obtained from X-ray
** Data calculated by the AM1 conformational analysis method

3.1.2. (±)-3,6-Di-O-acetyl-1,2:4,5-di-O-isopropylidene-myo-inositol (**4**)

Acetylation of **3** gave compound **4**, m.p. 227–228.5 °C (from acetone); lit. 230–232 °C [10].

3.1.3. (±)-3,6-Di-O-valproil-1,2:4,5-di-O-isopropylidene-myo-inositol (**5**)

Compound **3** (9.50 g, 36.54 mmol) was mixed with dry triethylamine (43 ml, 308 mmol) and 4-pyrrolidinopyridine (1.90 g, 12.76 mmol). Valproic anhydride (24.70 g, 91.50 mmol) was added to the suspension at room temperature. After 28 h the solvent was evaporated under reduced pressure. The solid phase was isolated by centrifugation. This crude product was crystallised from MeOH (33 ml) to give **5** as a white crystals (6.62 g, 12.93 mmol, 35.4%) melting at 143.5–145 °C; Rf = 0.52 (dichloromethane) 1 (dichloromethane-acetone 10:1).
IR (KBr): ν 1731 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 0.86, 0.89, 0.90, 0.93 (4s, 12 H, $(CH_3)_4$), 1.23–1.72 (m, 16 H, CH_2), 1.27, 1.38, 1.43, 1.56 (4s, 12 H, 2 $C(CH_3)_2$), 2.35–2.55 (m, 2 H, CH), 3.45 (dd, J = 11.1, J = 9.4 Hz, 1 H, H-5), 4.13 (m, 2 H, H-1, H-4), 4.58 (t, J = 4.5 Hz, 1 H, H-2), 5.12 (dd, J = 10.6, J = 4.2 Hz, 1 H, H-3), 5.29 (dd, J = 11.1, J = 6.9 Hz, 1 H, H-6); ^{13}C NMR ($CDCl_3$): δ13.9 (valproil 4 × CH_3), 20.1, 20.2, 20.3, 20.4 (4 × CH_2CH_3), 25.7, 26.7, 26.8, 27.6 (4 × CH_3), 34.1, 34.4, 34.5 (4 × $CH_2CH_2CH_3$, 1 overlapping), 44.9, 45.2 (2 × CH), 70.2, 73.5, 74.8, 75.1, 76.5, 79.3 (inositol 6 × CH), 110.2, 112.5(2 × $C(CH_3)_2$), 175.2, 176.1 (2 × COO); MS (FAB): observed accurate mass 535.6 $[M^+Na]^+$, $C_{28}H_{48}O_8$ requires 535.673.
$C_{28}H_{48}O_8$

3.1.4. (±)-3,6-Di-O-valproil-4,5-O-isopropylidene-myo-inositol (**6**) *and (±)-3,6-Di-O-valproil-myo-inositol* (**7**)

A solution of compound **5** (3.89 g, 7.61 mmol) in acetic acid 75% (acetic acid/water, 48 ml) was stirred at room temperature. After 160 h the reaction mixture was concentrated in vacuo with dry benzene. Column chromatography (dichloromethane: acetone 100/0 to 100/10) gave **5**, **6** and **7** as a white powder (20.2, 30.0 and 26.9%).
Compound **6**: m.p. = 90–93 °C; Rf = 0.60 (dichloromethane-acetone 10:1).
IR (KBr): ν 3200–3550 (OH) 1720 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 0.87, 0.90, 0.94 (3s, 12 H, $(CH_3)_4$, 1 overlapping), 1.21–1.85 (m, 16 H, CH_2), 1.28, 1.53 (2s, 6 H, $C(CH_3)_2$), 1.95 (br, s, 1 H, OH), 2.38–2.58 (m, 2 H, CH), 2.99 (br, s, 1 H, OH), 3.41 (t, J = 9.8 Hz, 1 H, H-5), 4.01 (t, J = 9.7 Hz, 1 H, H-1), 4.11 (dd, J = 7.7, J = 4.8 Hz, 1 H, H-4) , 4.45 (t, J = 4.4 Hz, 1 H, H-2), 5.00 (dd, J = 10.1, J = 4.1 Hz, 1 H, H-3), 5.09 (dd, J = 10.4, J = 7.7 Hz, 1 H, H-6); ^{13}C NMR ($CDCl_3$): δ 13.9 (valproil 4 × CH_3), 20.3, 20.4, 20.5 (4 × CH_2CH_3, 1 overlapping), 25.8, 27.7 (2 × CH_3), 34.5, 34.7 (4 × $CH_2CH_2CH_3$, 2 overlapping), 45.3, 45.4 (2 × CH), 70.9, 72.6, 73.6, 75.1, (inositol 6 × CH, 2 overlapping), 110.3 ($C(CH_3)_2$), 176.4, 177.1 (2 × COO); MS (FAB): observed accurate mass 495.4 $(M^+Na)^+$.
$C_{25}H_{44}O_8$
Compound **7**: m.p. = at 140–143 °C; Rf = 0.35 (dichloromethane-acetone 10:1).
IR (KBr): ν 3480 (OH) 1717 cm^{-1} (C=O); ^1H NMR ($CDCl_3$): δ 0.87, 0.90, 0.94 (3s, 12 H, $(CH_3)_4$, 1 overlapping], 1.23–1.74 (m, 16 H, CH_2), 2.39–2.52 (m, 2 H, CH), 3.15, 3.19 (2s, 4 H, OH), 3.48(m, 1 H, H-5), 3.64 (m, 1 H, H-1), 4.02 (m, 1 H, H-4), 4.15 (d, J = 2.6 Hz, 1 H, H-2), 4.80 (dd, J = 10.2, J = 2.6 Hz, 1 H, H-3), 5.16 (t, J = 9.7 Hz, 1 H, H-6); ^{13}C NMR ($CDCl_3$): δ 14.0 (valproil 4 × CH_3), 20.42, 20.47, 20.53 (4 × CH_2CH_3, 1 overlapping), 34.5 (4 × $CH_2CH_2CH_3$, 3 overlapping), 45.3, 45.5 (2 × CH), 70.8, 71.0, 72.6, 73.1, 74.9 (inositol 6 × CH, 1 overlapping), 176.4, 178.4 (2 × COO); MS (FAB): observed accurate mass 455.4 $[M^+Na]^+$.
$C_{22}H_{40}O_8$

3.2 Pharmacological studies

3.2.1. Maximal electroshock seizure test (MES)

Adult Swiss albino mice (25 to 31 g) were used as experimental animals in the maximal electroshock seizure (MES) test. At the previously determined time of peak effect of the test substance, a drop of electrolyte solution (0.9% sodium chloride solution) was applied to the ears, the electrodes are applied, and the electrical stimulus (50 mA; 60 Hz) was delivered for 0.2 s.
The animals are restrained by hand and released immediately following stimulation in order to permit observation of the seizure throughout its entire course. The tonic component is considered abolished if the hindleg tonic extension does not exceed a 90° angle with the plane on the body; absence of this component indicates that the test substance has the ability to prevent seizure spread.

3.2.2. Rotorod test

When a normal mouse is placed on a rod that rotates at a speed of 6 rpm, the mouse can maintain its equilibrium for long periods of time. A neurological deficit is indicated by the inability of the animal to maintain its equilibrium for 1 min on this rotating rod at 6 rpm in each of three trials.

Acknowledgements: This work was supported in through grants from Universidad Nacional de La Plata, and Laboratorios Bagó. The authors wish to thank Dra. Guillermina Estiú for comments and Ms Elvie Guy for preparing the manuscript.

This research paper was presented during the 2nd Conference on Retrometabolism based Drug Design and Targeting, May 11–14, 1999, Amelia Island, Florida, USA

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