*Full Length Research Paper*

# Anticonvulsant activity and neurotoxicity of the enantiomers of DL-HEPP

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**DL-3-hydroxy-3-phenylpentanamide (DL-HEPP) is an anticonvulsant with a broad profile of activity. In order to study if there exists differences in biological activity between its enantiomers, we resolved the racemate from the (-) brucine and (-) 1-phenylethylamine salts of the acids. The optically active acids were then esterified with diazomethane and reacted with ammonia to give (+)-3-hydroxy-3-phenylpentanamide ((+)-HEPP) and (-)-HEPP. The enantiomeric purity of the amides was determined using proton magnetic resonance in the presence of europium tris-[3-(trifluoromethylhydroxymethylene)-(+)camphorate] and chiral high performance liquid chromatography. Optical purity of the amides was greater than 99% enantiomeric excess, impurities were not detected. Pharmacologically, DL-HEPP and its enantiomers have a similar significant anticonvulsant activity at peak drug effect against pentylenetetrazol-induced seizures, but a variation in time between the enantiomers was found with the anticonvulsant activity. In the rotarod ataxia test, the neurotoxicity of the enantiomers of DL-HEPP was also similar. The therapeutic indices of DL-HEPP and its enantiomers against the seizures induced by pentylenetrazol were better than valproate, an antiepileptic widely used in clinics.**

**Key words:** Anticonvulsants, DL-3-hydroxy-3-phenylpentanamide enantiomers, DL-HEPP, resolution, (-) HEPP, (+) HEPP, valproate.

## INTRODUCTION

Epilepsy is a brain disorder that is characterized by recurrent seizures that affects 1% of the population worldwide (McNamara, 1999). Despite the antiepileptic drugs (AEDs) available, at present, 30% of patients with epilepsy continue to have seizures and even among those considered controlled, many unpleasant side effects are still endured (Dichter, 1994). There is clearly a need for more and better AEDs.

The compounds DL-2-hydroxy-2-phenylbutyramide (1, DL-HEPA), DL-3-hydroxy-3-phenylpentanamide (2, DL-HEPP) and DL-4-hydroxy-4-phenylhexanamide (3, DL-HEPB) have a broad profile of anticonvulsant activity (Figure 1). They protect mice against seizures induced by pentylenetetrazol, maximal electroshock, bicuculline, 4-aminopyridine, thiosemicarbazide (Meza-Toledo et al., 1990) and they also protected cats and rats against

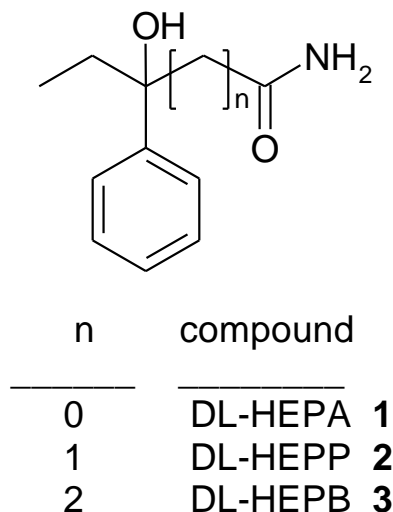*Corresponding author. E-mail: semeza@hotmail.com.

| n | compound |
|---|---|
| 0 | DL-HEPA **1** |
| 1 | DL-HEPP **2** |
| 2 | DL-HEPB **3** |

**Figure 1.** Structures of phenyl alcohol amides 1 to 3.

hippocampal "kindling" (Solís et al., 1979, 1996). From this series, compound 2 possesses the lowest toxicity (Chamorro et al., 1994). Compound 2 also protects against the γ-aminobutyric acid (GABA) withdrawal syndrome, a model of focal epilepsy, which has shown an extraordinary resistance to classic antiepileptics including diazepam, one of the most effective agent for treating status epilepticus (Brailowsky et al., 1992). Additionally, it also produces a significant decrease of focal spike activity in the genetic absence epilepsy rats of the Strasbourg model (GAERS) (Brailowsky et al., 1992).

The profile of anticonvulsant activity of the homologous series of phenyl alcohol amides suggests that they are promising anticonvulsant drugs against epilepsy of the absence type (Carvajal-Sandoval et al., 1998; Gómez-Martínez, 2007; Meza-Toledo et al., 2008) and they are currently undergoing preclinical development. The pharmacokinetic behavior of compound 2 has been tested in animals and healthy volunteers after the oral administration of single and multiple doses (Gómez and Lehmann, 1995a, b; González-Esquivel et al., 1998, 2004; García et al., 2003). These studies showed that compound 2 has a rapid absorption, a long half-life, low protein binding and clinically adverse effects have been minor, so compound 2 shows great promise as a useful antiepileptic in drug therapy. However, further clinical investigation in humans is necessary to determine its use in clinical practice.

In order to continue with the pharmacological eva-luation of DL-HEPP it is necessary to resolve racemate in order to study if there exist differences in biological activity between its enantiomers. In this paper, we report the enantioselective synthesis of 3-hydroxy-3-phenylpentanamide (HEPP) and the anticonvulsant activity and neurotoxicity of its enantiomers.

## METHODOLOGY

The melting points were determined with a Mettler-Toledo apparatus FP-62 model. Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum GX 2000 FT-IR spectrophotometer with attenuated total reflectance (ATR). The IR absorption frequencies are reported in $cm^{-1}$. The $^1H$ and $^{13}C$ NMR spectra were obtained in a Varian VNMRS-500 spectrometer, at 500 MHz (125.787 MHz for $^{13}C$). The samples were dissolved in $CDCl_3$ using tetramethylsilane (TMS) as internal reference. high-resolution mass spectra (HRMS) data were obtained in a JEOL GCmateTM II spectrometer in electron impact (EI, 70 eV) mode. Optical rotations were measured on a Perkin Elmer 341 polarimeter equipped with a 1 dm cell at 589 nm (sodium-D-line). Analytical chromatography was performed with a modular high-performance liquid chromatograph (HPLC) Beckman System Gold equipped with a 166 variable wavelength detector, a 128 pump and an injector. A Chiracel column OJ (250 × 4.1 mm) packed with cellulose tris(4-methylbenzoate) (10 μm particle diameter) was used. Rotarod tests were performed on a Rotarod (M) 85052-4 Series. Maximal electroshock test was determined using a constant current electroshock unit Ugo Basile model 7801.

## Chemistry

Compounds 5a, 5b, 6a, 6b, 7a and 7b were synthesized according to Figure 2. The DL-(±)-hydroxyester, compound 4, was hydrolyzed to give the hydroxy acid DL-(±)-3-hydroxy-3-phenylpentanoic acid, compound 5, and resolved by means of its (-)-brucine and (-)-1-phenylethylamine salts. The (+) and (-) acids were then esterified with diazomethane to produce the hydroxyesters 6a and 6b which were reacted with ammonia to give the optically active isomers of HEPP (Figure 2). The addition of (-)-brucine to the racemic solution of 3-hydroxy-3-phenyl-pentanoic acid 5 afforded the diasteromeric insoluble salt of 5a that was collected by simple vacuum filtration, this salt was composed of (-)-brucine and the (+) enantiomer. The water soluble salt of 5b was composed of (-)-brucine and the (-) enantiomer. Both complexes were hydrolyzed separately with diluted HCl to produce 5a and 5b. The partially resolved acid 5b was treated with (-)-1-phenylethylamine to give 5b. The optically liberated acids 5a and 5b were reacted with diazomethane to form the corresponding (+) and (-) methyl esters 6a and 6b. Amoniolysis of 6a and 6b formed the beta-hydroxyamides 7a and 7b which exhibited $[\alpha]_D^{20°}$= +35.02° and -34.97° (c = 3, ethanol), respectively.

## DL-(±)-Ethyl 3-hydroxy-3-phenylpentanoate (compound 4)

The compound 4 was synthesized as described previously (Barltrop et al., 1956). IR and NMR spectra were found to be identical with the ones described (Fukuzawa and Hirai, 1993).

## DL-(±) 3-Hydroxy-3-phenylpentanoic acid (compound 5)

A solution of 44.4 g (200 mmol) of compound 4 containing 2 N KOH solution in 130 ml anhydrous methanol was stirred at room temperature for 7 h. After saponification, the methanol was evaporated at reduced pressure. The residue was taken up with 500 ml water, extracted with diethyl ether (100 ml × 3), and the organic phase was discarded. The aqueous phase was cooled and acidified (pH 2.5) with 35 ml 6 N HCl and extracted with diethyl ether (100 ml × 4). The combined ether extracts were washed with $H_2O$ (30 ml × 2), saturated NaCl solution (30 ml × 2), and dried over $Na_2SO_4$ and concentrated *in vacuo*. The precipitate was recrystallized from water to afford 37.5 g (96.6 %) compound 5 as a white solid [melting point: 123 to 124°C (mp 121°C)] (Maroni-Barnaud et al., 1966).
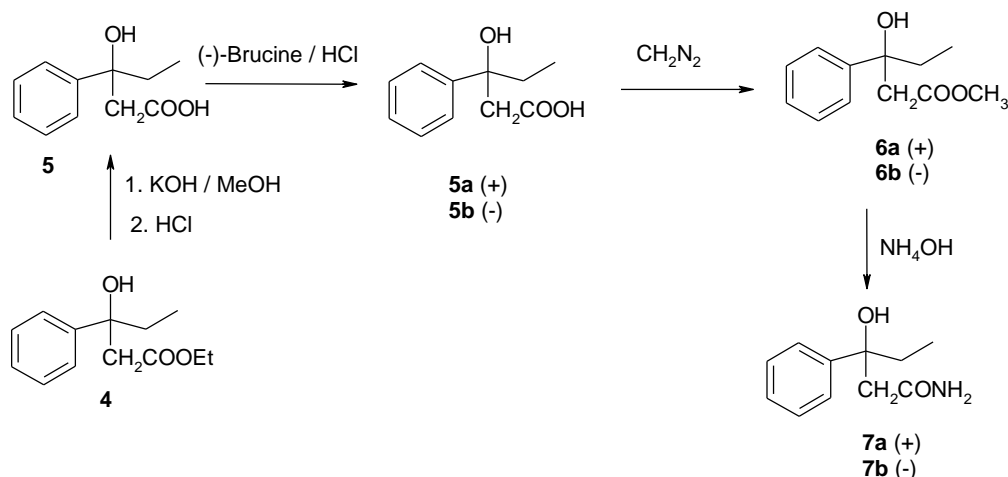
**Figure 2.** Synthesis of the enantiomers of HEPP.

**(+)-3-Hydroxy-3-phenylpentanoic acid (compound 5a)**

To a stirred solution of 24.7 g (127.3 mmol) of compound 5, 300 ml ethyl acetate at 60°C was added 52.57 g (119.6 mmol) of (-)-brucine. The solution was heated under reflux for 10 min, cooled at -20°C for 24 h and concentrated. The residue was treated with 50 ml hexane and the solid was filtered off and crystallized from 90 ml ethyl acetate. The brucine salt of compound 5a was filtered off, and the mother liquors were evaporated to dryness to obtain 26.9 g the brucine salt of compound 5b which was treated with 31 ml 5% HCl in 350 ml diethyl ether. The ethereal layer was separated and compound 5b was extracted with acetone (150 ml × 2). The combined ethereal and acetone extracts were concentrated and the solid was crystallized from water to give 8.3 g (42.8 mmol) of partially resolved compound 5b, $[\alpha]_D^{20°}$ = -15° (c = 3.0, ethanol). The crystalline brucine salt of compound 5a was heated with 80 ml ethyl acetate, cooled and filtered off several times to give the brucine salt of compound 5a (18 g), mp 118 to 119°C, which was treated with 21 ml 5% HCl in 230 ml diethyl ether, the ethereal layer was separated and compound 5a was extracted with diethyl ether (100 ml × 2). The combined ether extracts were concentrated to give compound 5a as a white solid. The solid was crystallized from water to give 8 g (32.4%) of compound 5a [(Mp 92 to 93°C; $[\alpha]_D^{20°}$ = +21.7° (c = 3.0, ethanol) (Mp 98°C; $[\alpha]_D^{24°}$ = +22° (ethanol)] (Mitsui and Kudo, 1965).

**(-)-3-Hydroxy-3-phenylpentanoic acid (compound 5b)**

5.84 g (30.1 mmol) of compound 5b partially resolved was mixed with 30 ml ethanol, 3.64 g (30.1 mmol) of (-)-1-phenylethylamine and heated at 40°C for 5 min. The mixture was concentrated to dryness and the solid was crystallized several times from ethanol to give the 1-phenylethylamine salt of compound 5b (6 g) as white crystals, mp 174 to 175°C which was treated with 5 ml 40% NaOH, 24 ml water and 48 ml benzene. The aqueous layer was washed with benzene (22 ml × 4), cooled and the pH of the solution was adjusted to 1 with 3.7 ml 37% HCl. The precipitate was filtered off and the solid was crystallized from water to give 2.0 g (34.2%) of compound 5b as a white solid [mp 92 to 93°C; $[\alpha]_D^{20°}$ = -21.3° (c = 3.0, ethanol) ($[\alpha]_D$= -17.0° (c = 10.0, benzene)] (Mitsui and Kudo, 1967); IR (ATR): ν = 1683, 3504 cm$^{-1}$; $^1$H-NMR (500 MHz, CDCl$_3$): δ = 0.77 (t, 3 H, J = 7 Hz), 1.77 (dq, 1 H, J = 7 Hz, J = 14 Hz), 1.82 (dq, 1 H, J = 7 and 14 Hz), 2.83 (d, 1 H, J = 16 Hz), 2.99 (d, 1 H, J = 16 Hz), 3.53 (s, 1 H), 4.40 (br, 1 H), 7.23 (d,d, 1 H, J = 5 Hz, J = 10 Hz); 7.31 (d,d, 2 H, J = 10 Hz), 7.35 (d, 2 H, J = 5 Hz) ppm; $^{13}$C-NMR (125 MHz, CDCl$_3$): δ = 7.72, 35.81, 44.52, 75.37, 125.44, 126.77, 128.22, 144.22, 177.38. HRMS EI: *m/z* calculated for $C_{11}H_{14}O_3$ (M$^+$) 194.0943, found 194.0935 (M$^+$).

**(+)-Methyl 3-hydroxy-3-phenylpentanoate (compound 6a)**

To a stirred solution of 4.5 g (23.2 mmol) of compound 5a in 15 ml diethyl ether was added a solution of 1 g (23.8 mmol) of diazometane in 90 ml diethyl ether. The reaction mixture was stirred for 10 min liberating nitrogen. Then, it was concentrated at reduced pressure to obtain 4.8 g (99.5%) of compound 6a ($[\alpha]_D^{20°}$ = +2.2° (c = 3, ethanol) ($[\alpha]_D^{14°}$ = +1.64° (c = 0.85, ethanol)] (Mitsui and Kudo, 1965). IR and NMR spectra were found to be identical with the ones described (Denmark et al., 2005).

**(-)-Methyl 3-hydroxy-3-phenylpentanoate (compound 6b)**

This compound was obtained from compound 5b following a similar procedure to the preparation of compound compound 6a. Yield 98.02%, $[\alpha]_D^{20°}$ = -2.2° (c = 3, ethanol) ($[\alpha]_D^{21°}$ = -0.97° (c = 1.03, ethanol)] (Denmark et al., 2005). NMR spectra were found to be identical with the ones described (Adachi and Harada, 2008).

**(+)-3-Hydroxy-3-phenylpentanamide (compound 7a)**

A mixture containing 4.8 g (23 mmol) of compound 6a, 15 ml ethanol and 15 ml 28% aqueous ammonia was cooled at 0°C and saturated with ammonia gas. The flask was closed with a rubber stopper and held at room temperature for 19 days. Then, the mixture was cooled, and it was extracted with diethyl ether (15 ml × 4), dried over Na$_2$SO$_4$, filtered and concentrated *in vacuo*. The residue was treated with 10 ml benzene, the precipitate was filtered off and the solid was crystallized from benzene to give 3 g (67.3 %) of compound 7a as a white solid (mp 51 to 52°C; $[\alpha]_D^{20°}$ = +35.02° (c = 3, ethanol); IR (ATR): ν = 1656, 3186, 3334 cm$^{-1}$; $^1$H-NMR (500 MHz, CDCl$_3$): δ = 0.73 (t, 3 H), 1.76 (dq, 1 H, J = 6.5 Hz, J = 13 Hz), 1.82 (dq, 1 H, J = 6.5 Hz, J = 13 Hz), 2.65 (d, 1 H, J = 15 Hz), 2.70 (d, 1 H, J = 15 Hz), 5.11 (br, 1 H), 5.70 (br, 1 H), 5.80 (br, 1 H), 7.20 (tt, 1 H, J = 9 Hz, J = 15 Hz), 7.23 (tt, 2 H, J = 9 Hz, J = 15 Hz), 7.34

(dt, 2H, J = 9 Hz, J = 11 Hz) ppm; $^{13}$C-NMR (125 MHz, CDCl$_3$): δ = 7.98, 36.07, 46.06, 75.82, 125.4, 126.93, 128.4, 145.35, 175.29 ppm. HRMS EI: *m/z* calculated for $C_{11}H_{13}NO$ (M-H$_2$O) 175.0997, found 175.0995 (M-H$_2$O).

**(-)-3-Hydroxy-3-phenylpentanamide (compound 7b)**

Compound 7b was obtained from compound 6b, following a similar procedure to the preparation of compound 7a. Yield 56.4%, Mp 51 to 52°C; $[α]_D^{20°}$ = -34.97° (c = 2.98, ethanol). IR, $^1$H-NMR and $^{13}$C-NMR spectra and HRMS data were identical to compound 7a.

**DL-(±)-3-hydroxy-3-phenylpentanamide (compound 2)**

Compound 2 was synthesized as described previously (Meza-Toledo et al., 1990) [mp 101 to 102°C (mp 101 to 102°C (Meza-Toledo et al., 1990)].

**Chromatographic determination of the enantiomeric purity**

The enantiomeric purity of compounds 7a and 7b was determined by chiral HPLC using a Chiracel OJ column (4.1 × 250 mm), eluting with n-hexane/2-propanol (85:15) at a flow rate of 0.9 ml/min; detection was at 221 nm. 20 µl of each enantiomer dissolved in n-hexane:2-propanol (85:15) (400 ng/ml) was injected into the column and the enantiomeric excess was determined.

**Determination of the enantiomeric purity using Europium tris-[3-(trifluoromethylhydroxymethylene)-(+)camphorate] [Eu[TFH-cam-d]]**

To establish the enantiomeric purity by $^1$H-NMR (500 MHz), Eu[TFH-cam-d] (0.017 g, 0.02 mmol) was added either to the racemic compound 2 (0.019 g, 0.01 mmol) or their enantiomers 7a and 7b (0.019 g, 0.01 mmol) dissolved in 0.5 ml CDCl$_3$. $^1$H-NMR spectra were performed. Chemical shift differences (Δδ) were calculated by subtracting the low field signal to the high field signal of both enantiomers resolved in the spectrum.

**Pharmacology**

*Animals and treatment*

Male albino Swiss Webster mice (Birmex, Mexico City) weighing 25 to 30 g were housed in groups of 5, at room temperature (20 to 24°C), with tap water and food (pellet type Lab Rodent Diet 5008; PMI Nutrition International, Brentwood, MO, USA) *ad libitum*, with a 12-h light-dark cycle (light on: 6.00 a.m). Mice were used in the mouse anticonvulsant and rotarod tests. The experiments were carried out according to the National Institutes of Health animal care and use guidelines, and were approved by our scientific research committee. Each treatment group and vehicle control group consisted of 7 to 10 animals.

*Anticonvulsant activity*

The anticonvulsant activity (Meza-Toledo et al., 1990) of DL-HEPP and its enantiomers 7a and 7b was evaluated using pentylenetetrazol (PTZ) and maximal electroshock (MES) models. Compounds DL-HEPP, 7a and 7b were dissolved in a 10% polyethyleneglycol-400 solution; sodium valproate and PTZ were dissolved in water. All the compounds were administered intraperitoneally (i.p). The convulsant dose of PTZ inducing

seizures and death in 100% of mice was determined and used in the pharmacological test. The time of peak drug effect (TPE) was evaluated for each anticonvulsant before determining the dose-response curves. PTZ was administered i.p at 80 mg/kg, to four groups of 7 to 10 mice, and suppression of clonic seizures and death was considered the end point. In the MES test, seizures were induced by application of an electrical current across the brain via earclip electrodes. Shocks were delivered at constant current of 20 mA with a frequency of 100 Hz, a pulse width of 0.4 ms and a duration of 0.2 s. Compounds DL-HEPP, 7a, 7b and sodium valproate were tested at TPE. The dose at which the hind limb tonic seizure was blocked in 50% of the animals (ED$_{50}$ value) was determined by probit analysis. ED$_{50}$, TD$_{50}$ and 95% confidence intervals were calculated by the method described previously (Litchfield and Wilcoxon, 1949).

*Effects of time*

Groups of 10 mice were dosed i.p with DL-HEPP, 7a and 7b, 100 mg/kg, and protection against convulsions and death produced by pentylenetetrazol, 80 mg/kg, i.p, was evaluated at different times.

*Neurotoxic effects*

Separate groups of mice were trained to stay on a rotarod that rotated at 10 rpm. The drum diameter was 2.54 cm. Four groups of 7 to 10 trained mice were dosed with the test compound or drug vehicle (10% polyethyleneglycol-400 solution) and were tested at TPE to measure the effect of the drug on motor performance (Meza-Toledo et al., 1990). Animals which fell off before 120 s were considered ataxic. The dose at which 50% of the animals fell off the rotarod (TD$_{50}$) was determined by probit analysis (Litchfield and Wilcoxon, 1949).

*Protective index*

It was calculated by dividing the TD$_{50}$ value by the respective ED$_{50}$ values as determined in either PTZ or MES tests. The protective index is considered to be an index representing the margin of safety and tolerability between ED$_{50}$ and TD$_{50}$ values (Löscher and Nolting, 1991).

**RESULTS**

**Chemistry**

Melting point of compound 5, 123 to 124°C, and those of their enantiomers 5a and 5b, 92 to 93°C, were different. Similarly, the melting point of DL-HEPP, 101 to 102°C, decreased to 51 to 52°C in the enantiomers 7a and 7b. Examination of the $^1$H and $^{13}$C-NMR spectra of DL-HEPP and their enantiomers 7a and 7b in a CDCl$_3$ solution showed identical chemical shifts. This agrees with literature (Nógrádi, 1981), where racemates because of their different crystal structure have melting points which may be different from those of the pure enantiomers. In order to study the enantiomeric purity of compounds 7a and 7b they were analyzed by using a chiral HPLC column to resolve the racemate. Figure 3A showed the chromatographic resolution of DL-HEPP exhibiting a
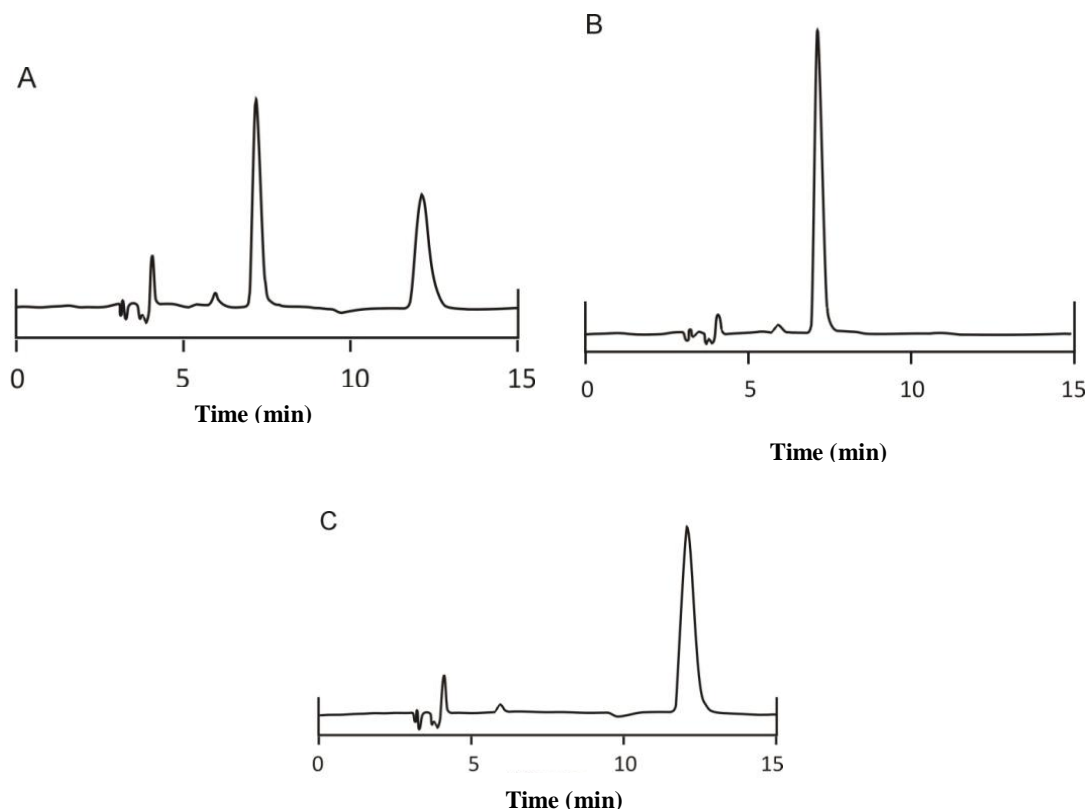
**Figure 3**. Chromatographic separation of the enantiomers of HEPP in a Chiracel OJ column. A) DL-HEPP; B) (-) HEPP; C) (+) HEPP.

retention time for 7a (12.08 min) and 7b (7.16 min). Figure 3b and c showed the chromatogram of pure enantiomers 7b and 7a, impurities were not detected and they have at least 99% ee.

The $^1$H-NMR spectrum of DL-HEPP showed an AB coupled system for diasterotopic protons H-2 centered at 2.77 ppm; H-4 was observed as an overlapped system $ABX_3$ at 1.82 ppm (Figure 4a). The addition of Europium tris-[3-(trifluoromethylhydroxymethylene)-(+)camphorate] (Eu[TFH-cam-d]) to racemic 7a/7b split the methylene H-2 signals of isomer 7a at 3.20 ppm and for the isomer 7b at 3.08 ppm (Figure 4b). Protons H-4 for (-) isomer were shifted at 2.18 ppm, same protons for (+) isomer were located at 2.10 ppm.

Figure 4c showed the proton NMR spectrum of isomer (+)-7a pure. The addition of 0.01 mmol of Eu[TFH-cam-d] to these compounds shifted protons H-2 to 3.08 ppm; methylene H-4 displayed two different signals, one at 2.15 ppm and other at 2.0 ppm for each diastereomeric proton. Due pseudo-contact, interaction between Eu[TFH-cam-d] and enantiomers (-) and (+) are different, isomer (-)-**7b** pure in Figure 4d displayed an AB coupled system for protons H-2 at 2.95 and 2.82 ppm, respectively, nevertheless, the diastereotopic methylene H-4 remained as a multiplet centered at 1.92 ppm.

**Pharmacology**

The anticonvulsant activity and neurotoxicity of DL-HEPP, 7a, 7b and sodium valproate after intraperitoneal administration is shown in Table 1. The compound DL-HEPP has been previously shown to be endowed with anticonvulsant activity in several animal seizure tests but individual evaluation of each of these enantiomers 7a and 7b was still lacking. From the data of Table 1, it can be seen that not only DL-HEPP but also each of its enantiomers exhibit interesting anticonvulsant protections in these seizure tests that are as potent as valproate, a reference antiepileptic drug widely used in human clinics. With respect to the PTZ test, compounds DL-HEPP, 7a and 7b showed a similar significant anticonvulsant activity ($ED_{50}$: 55, 61 and 50 mg/kg, respectively) and sodium valproate was the least potent ($ED_{50}$: 120 mg/kg) (Table 1). Sodium valproate exhibited a 50% protection by the MES test at a dose of 237 mg/kg whereas the anticonvulsant activity of DL-HEPP and its enantiomers (+) 7a and (-) 7b was something different ($ED_{50}$: 138, 168 and 108 mg/kg, respectively).

The anticonvulsant activity of (+) HEPP declined rapidly after the first two hours exhibiting 10% protection while (-) HEPP showed the highest activity (90% protection) and
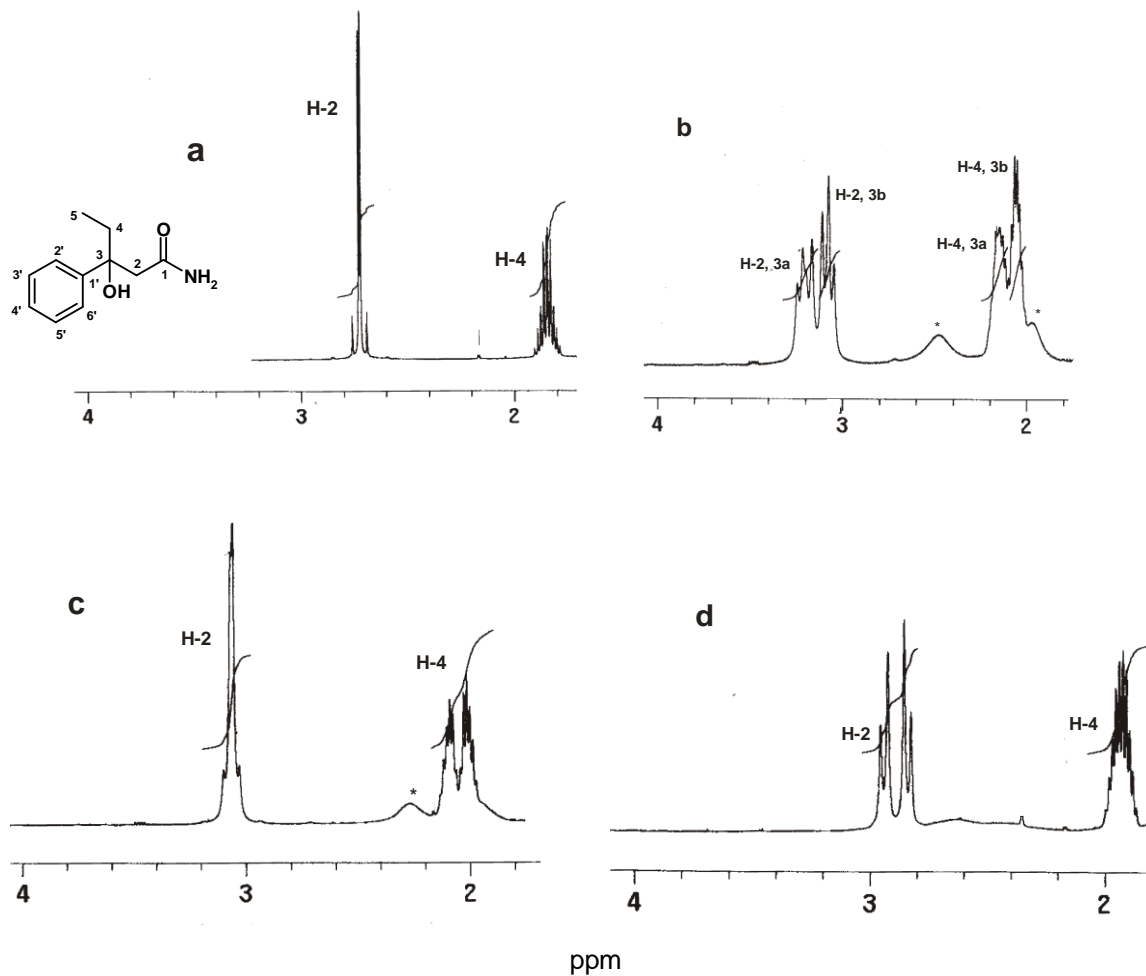
**Figure 4.** ^{1}H-NMR spectra (500 MHz): a) DL-HEPP; b) DL-HEPP added with 0.02 mmol Eu[TFH-cam-d]; c) (+) HEPP added with 0.01 mmol Eu[TFH-cam-d]; d) (-) HEPP added with 0.01 mmol of Eu[TFH-cam-d].
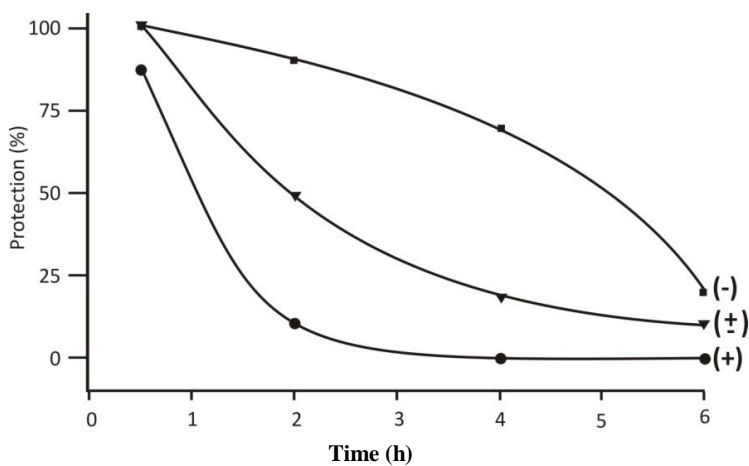


**Figure 5.** Anticonvulsant activity in time of DL-HEPP and its enantiomers against pentylenetetrazol induced seizures in mice.

DL-HEPP showed an intermediate activity (50% protection) (Figure 5). At 4 h after administration, the anticonvulsant activities of DL-HEPP and (-) 7b decreased to 20 and 70% protection, respectively, at this

**Table 1.** Anticonvulsant activity and neurotoxicity of DL-HEPP and its enantiomers.

| Compound | Pharmacological test[a] | | | | |
|---|---|---|---|---|---|
| | $ED_{50}$ (mg/kg) PTZ[c] | $ED_{50}$ (mg/kg) MES[d] | $TD_{50}$ (mg/kg) Rotarod ataxia | Protective index[b] | |
| | | | | PTZ | MES |
| DL-HEPP | 55 (51-60)[e] | 138 (135-142) | 212 (209-216) | 3.85 | 1.54 |
| (+) HEPP | 61 (55-68) | 168 (164-172) | 223 (212-234) | 3.66 | 1.32 |
| (-) HEPP | 50 (43-58) | 108 (101-115) | 207 (203-210) | 4.14 | 1.91 |
| Valproate[f] | 120 (110-132) | 237 (221-253) | 380 (357-404) | 3.16 | 1.60 |

[a]Time of test: 30 min post-dosing to peak drug effect. [b]$TD_{50}/ED_{50}$. Seizures induced by: [c]pentylenetetrazol and [d]maximal electroshock. [e]95% confidence interval. [f]Time of test: 30 ($TD50$) and 45 (PTZ and MES) min post-dosing.

time compound (+) HEPP had no protection. At 6 h after injection the anticonvulsant activity of DL-HEPP and (-) 7b fell to 10 and 20% protection, respectively (Figure 5). There was no difference in neurotoxicity between DL-HEPP and its enantiomers (+) 7a and (-) 7b ($TD_{50}$: 212, 223 and 207 mg/kg, respectively). In this test, sodium valproate was the least toxic ($TD_{50}$: 380 mg/kg) (Table 1).

Compounds DL-HEPP, (+) 7a and (-) 7b showed protective indices (PI: 3.85, 3.66 and 4.14, respectively) that are better than sodium valproate (PI = 3.16) against PTZ induced seizures in mice (Table 1). However, in the MES test sodium valproate (PI = 1.6) had similar protective indices as those of DL-HEPP, (+) 7a and (-) 7b (PI: 1.54, 1.32 and 1.91, respectively).

## DISCUSSION

Synthesis of optically pure 5a has been reported previously (Mitsui and Kudo, 1965) and the absolute configuration was determined to be S $[\alpha]_D^{24°}= +22°$ (ethanol). Since none of the reactions to produce 7a from 5a affected directly the chiral carbon atom and there is no acidic protons to promote racemization, the absolute configuration of 6a and 7a was deducted to be S by comparing the optical rotation obtained for 5a $[\alpha]_D^{20°}= +21.7°$ (c = 3.0, ethanol). Denmark et al. (2005) reported a specific rotation of +16° for the corresponding carboxylic acid 5a produced from the hydrolysis of 6a. Compounds 6a and 6b has been synthesized from catalytic enantioselective aldol reaction of propiophenone (Denmark et al., 2005; Adachi and Harada, 2008; Oisaki et al., 2006).

Oizaki et al. (2006) reported a specific rotation of +1.64° (77% enantiomeric excess) for the (+) hydroxyester 6a. Adachi et al. (2008) reported a specific rotation of -0.97° for the (-) hydroxyester 6b. For compounds 6a and 6b we reported specific rotations of +2.2° and -2.2°, respectively. Compounds 7a and 7b have not been reported previously. From the higher optical rotation, values for compounds 6a and 6b respect those published previously, and considering that compounds

7a and 7b have optical purities greater than 99% enantiomeric excess, it is assumed that compounds 6a and 6b reported in this paper have at least 99% enantiomeric excess. In line with previously published methods for the preparation of (+) and (-) hydroxyesters (Denmark et al., 2005; Adachi and Harada, 2008; Oisaki et al., 2006), the advantages in the reported method were the higher optical purities obtained.

While the anticonvulsant activities of DL-HEPP and its enantiomers were similar in the PTZ assay at peak drug effect, in the MES model were different. PTZ interacts with $GABA_A$ receptor (Ramanjaneyulu and Ticku, 1984; Squires et al., 1984) and sodium channel blockers like diphenylhydantoin are effective in the MES model of epilepsy (Willow and Catterall, 1982; McNamara, 2011). It is reported that hydroxyphenylamides such as DL-2-(3-chlorophenyl)-2-hydroxynonanamide and its (-) and (+) enantiomers blocked sodium channels with inhibitory concentration 50 values of: 1.81, 1.88 and 2.61 mM, respectively (Davis et al., 2012). As DL-HEPP and its enantiomers are hydroxyphenylamides it may be possible that they also block sodium channels. This could explain their effect in the MES test.

The rapid onset of the anticonvulsant effect suggests that DL-HEPP and its enantiomers readily penetrate the blood-brain barrier. This finding agrees well with the low serum protein binding of HEPP as previously published (Gómez et al., 1995a). The strong direct relationship between the concentrations of HEPP in plasma and/or brain and the anticonvulsant effect demonstrated that the parent compound is responsible for the anticonvulsant action (Gómez et al., 1995a). When DL-HEPP was administered with diphenylhydantoin to rabbits, plasma HEPP levels decreased. This result suggested a pharmacokinetic interaction between diphenylhydantoin and HEPP, probably on the drug-metabolizing enzyme system in the liver (Medina et al., 1998). As phenytoin acts as an enzyme inducer of microsomal P450, it is probable that DL-HEPP and its enantiomers might be metabolized by cytochrome P450, perhaps of the same genetic subfamily on which phenytoin acts as enzyme inducer. However, it will be necessary to perform HEPP

metabolism studies with the racemate and its enantiomers in order to determine the mechanisms involved in the biotransformation of this drug. The variation in the anticonvulsant activity over time between the enantiomers (+) 7a and (-) 7b could be due to differences in their metabolism or distribution. Further studies to explore it are warranted.

The rotarod ataxia test was used to evaluate the neurotoxicity. In this test, the neurotoxicity of DL-HEPP, (+) 7a and (-) 7b was similar. The mechanism underlying the anticonvulsant activity of DL-HEPP and its homologues DL-HEPA and DL-HEPB is not known. They protect against seizures induced by bicuculline, a GABA$_A$ receptor antagonist (Pérez de la Mora and Tapia, 1973; Tapia et al., 1979; Meza-Toledo et al., 1990). DL-HEPP also reversed GABA mediated inhibition of electrically and potassium chloride evoked exogenous [$^3$H]-GABA release from rat substantia nigra slices without having any effect on evoked release in the absence of GABA. DL-HEPP also counteracted the inhibition in electrically evoked release of [$^3$H]-GABA produced by the GABA$_A$ receptor antagonists picrotoxinin and bicuculline. DL-HEPP might be acting as a modulator at the GABA$_A$ receptor complex (Meza-Toledo and Bowery, 2008).

In support of this idea, it has been reported that DL-HEPP displaces [$^3$H]-flunitrazepam and [$^{35}$S]-tert-butylbicyclophosphorothionate from benzodiazepine and picrotoxin sites on GABA$_A$ receptor complex in rat brain crude synaptic membranes (Chávez and Martínez, 1996). It is published that the hydroxybencenamide DL-3,3,3-trifluoro-2-hydroxy-2-phenyl-propionamide (Choudhury-Mukherjee et al., 2003) enhanced GABA$_A$ current evoked by GABA (10 µM) in rat hippocampal neurons (Choudhury-Mukherjee et al., 2003). It is probable that DL-HEPP and its enantiomers may also modulate GABA$_A$ current evoked by GABA in neurons. The enantio-selective synthesis of HEPP will help us to elucidate the mechanism of action underlying the anticonvulsant action of HEPP.

## Conclusion

At the time of peak drug effect (30 min) there was no differences either in the anticonvulsant activity against pentylenetetrazol induced seizures or in neurotoxicity between DL-HEPP and its enantiomers, which suggests that the chiral separation of DL-HEPP and its homo-logues is not necessary for further preclinical studies.

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