# The Enantiomers of the Valproic Acid Analogue 2-*n*-Propyl-4-pentynoic Acid (4-yn-VPA): Asymmetric Synthesis and Highly Stereoselective Teratogenicity in Mice

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The teratogenic activities of *R*(+)- and *S*(−)-2-*n*-propyl-4-pentynoic acid (*R* and *S*-4-yn-VPA), the enantiomers of the highly teratogenic valproic acid (VPA) analogues (±)-4-yn-VPA, were investigated in mice. The enantiomers were prepared via asymmetric synthesis, each in three steps employing the chiral auxiliaries (4*R*,5*S*)-4-methyl-5-phenyl-2-oxazolidinone and *S*-4-benzyl-2-oxazolidinone. The determination of the absolute configurations and the optical purities is described. *R*(+)-4-yn-VPA contained 7%, and *S*(−)-4-yn-VPA 8%, of the respective antipodes. The aqueous solutions of the sodium salts of *R*- and *S*-4-yn-VPA were administered as single i.p. injections during early organogenesis in the mouse (day 8 of gestation) using the induction of exencephaly as the teratological end point. Dose/exencephaly curves indicated that *S*-4-yn-VPA is 7.5 times more teratogenic than its antipode, 1.9 times more teratogenic than (±)-4-yn-VPA and 3.9 times more teratogenic than the parent drug VPA. In contrast, the neurotoxicity (maternal toxicity) of the 4-yn-VPA enantiomers was found to be independent of the stereochemical configuration and lower than achieved after VPA administration. Due to its low neurotoxicity and highly stereoselective neural tube-inducing activity, *S*-4-yn-VPA should prove an important tool for the investigation of molecular mechanism of the teratogenic action in this class of compounds; *R*-4-yn-VPA could act as the negative control in these studies.

KEY WORDS: *R*(+)- and *S*(−)-2-*n*-propyl-4-pentynoic acid; asymmetric synthesis; enantioselective teratogenicity; valproic acid.

## INTRODUCTION

The antiepileptic drug valproic acid (VPA;[3] 2-*n*-propylpentanoic acid; Depekan; Abbott Laboratories) has proven to be particularly useful for the treatment of absence seizures as well as partial and generalized tonic-clonic seizures. However, VPA was found to be teratogenic in the human (1–7) and in various species of experimental animals (8,9). In the human the most striking malformations observed were neural tube defects (spina bifida aperta), which were detected in 1–2% of VPA-exposed conceptuses (10,11). In the mouse, exencephaly can be induced by VPA administration, as the main externally visible neural tube defect (12,13).

It has been shown that the parent drug molecule, and not a metabolite, is responsible for the teratogenic effect (14). This opened the possibility of searching for the fundamental structural elements (pharmacophore) responsible for the teratogenic potency of VPA and related α-branched carboxylic acids. The aim of these investigations is twofold: on the one hand, to search for increasingly potent neural tube defect-inducing agents which can be used for mechanistic studies; on the other hand, to develop alternative antiepileptic agents with low teratogenic potential (15).

Previous studies with VPA and a number of analogous substances demonstrated a high structural specificity of teratogenicity in this class of compounds using the induction of exencephaly in NMRI mice as the teratological end point (9,16). The most potent teratogens were found to have two unbranched alkyl groups with three carbon atoms as well as a free carboxylic group and a hydrogen atom in the α position (16–18). Parallel dose/exencephaly curves indicated that the VPA analogue 2-*n*-propyl-4-pentynoic acid (racemic 4-yn-VPA; (±)-4-yn-VPA) is approximately twice as teratogenic in mice as the parent compound VPA. (±)-4-yn-VPA was found to be less neurotoxic (maternal toxicity) than VPA. (±)-4-yn-VPA therefore was suggested as a useful VPA-related compound to obtain more information on the molecular mechanism of teratogenic action in this class of compounds (18).

The teratogenic potency of a chiral branched-chain carboxylic acid depends on the stereochemical configuration as demonstrated for the 2-*n*-propyl-4-pentenoic acid (4-en-VPA) and 2-ethylhexanoic acid enantiomers (17,19).

We synthesized the enantiomers of 4-yn-VPA via asymmetric synthesis using the chiral auxiliaries (4*R*,5*S*)-4-methyl-5-phenyl-2-oxazolidinone and *S*-4-benzyl-2-oxazolidinone. The teratogenic potency of both enantiomers was determined and shown to be highly stereoselective.

## MATERIALS AND METHODS

### Chemicals

(±)-2-*n*-Propyl-4-pentynoic acid was synthesized as described previously (18); (4*R*,5*S*)-4-methyl-5-phenyl-2-oxazolidinone, *S*-4-benzyl-2-oxazolidinone, valeroyl chloride, Lindlar catalyst, Lindlar catalyst additive, and *S*(−)-phenethylamine (>99% ee) were supplied by Fluka (Neu-Ulm, Germany); lithium hydroxide, 2-propyn-1-ol, and phosphorus tribromide were purchased from Aldrich (Steinheim, Germany); *n*-butyllithium, hydrogen, organic substances, and standard chemicals were obtained from Merck (Darmstadt, Germany); and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) was from Pierce (Rockford; Ill.).

### Instrumentation

NMR ($^1$H and $^{13}$C) spectra were recorded with a Bruker WH270 spectrometer (Karlsruhe, Germany) at 270 MHz. $^1$H and $^{13}$C chemical shifts are reported as parts per million

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[3] Abbreviations used: VPA, valproic acid (2-*n*-propylpentanoic acid); 4-en-VPA, 2-*n*-propyl-4-pentenoic acid; 4-yn-VPA, 2-*n*-propyl-4-pentynoic acid; fract., fractional; RT, room temperature; THF, tetrahydrofuran; abs., absolute.

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relative to the internal standard tetramethylsilane. The gas chromatographic determination of the chemical purities were performed on a Hewlett–Packard 5700A (Böblingen, Germany) using a SE 30 column (1.80 m × 2 mm i.d.; Applied Science Laboratories, Penn.) and flame ionization detection. The optical purities were determined on a Carlo Erba Fractovap 4160 gas chromatograph (Hofheim, Germany) using a DB-210 capillary column (30 m × 0.25-mm i.d.; 0.5-μm film thickness; J & W Scientific, Ventura, Va.) and nitrogen selective detection. The oven temperature was maintained at 80°C for 2 min, then raised at 20°C/min to 220°C; after 1 min at 220°C, the temperature was raised at 10°C/min to 230°C (to elute the compounds of interest) and was maintained at this final temperature for 4 min for elution of late peaks. The optical rotations were measured at 589 nm using a Dr. Kernchen Gyromat-HP polarimeter (Seelze, Germany).

## Synthetic Methods

### *3-Hydroxy-1-(trimethylsilyl)propyne*

This was synthesized starting with 2-propyn-1-ol according to known procedures (20).

### *3-Bromo-1-(trimethylsilyl)propyne (20)*

Eleven and one-half milliliters (122 mmol) of freshly distilled phosphorus tribromide was added dropwise to a magnetically stirred, cooled (0°C) solution of 50.0 g (340 mmol) 3-hydroxy-1-(trimethylsilyl)propyne and 0.7 ml abs. pyridine in 100 ml of abs. diethyl ether. The reaction mixture was stirred for 3 hr at 0°C and was then slowly warmed to room temperature (RT). After 24 hr the reaction mixture was successively washed with ice water, 1 *M* sodium carbonate solution, and saturated sodium chloride solution, dried over anhydrous sodium sulfate, and fractional (fract.) distilled to give 55.1 g (288 mmol = 85%) of 3-bromo-1-(trimethylsilyl)propyne; bp 49–51°C, 4 mbar [lit. 44–45°C, 2.7 mbar (20)].

### S(−)-2-n-*Propyl-4-pentynoic acid [S(−)-4-yn-VPA] (7)*

The *S*(−)-4-yn-VPA enantiomer was synthesized as outlined in Fig. 1, in a three-step sequence starting from (4*R*,5*S*)-4-methyl-5-phenyl-2-oxazolidinone (1) as follows.

*(4*R,5S*-4-Methyl-3-(1-oxopentyl)-5-phenyl-oxazolidinone (3) (21).* A magnetically stirred, cooled (−78°C) solution of 44.3 g (250 mmol) of oxazolidinone 1 in abs. THF (330 ml) was metalated with 156 ml (255 mmol) of *n*-butyllithium (1.6 *M* in hexane), until the orange-red color of the dianion just persisted, and acylated immediately with 30.7 ml (255 mmol) of freshly distilled valeroyl chloride. The reaction mixture was slowly warmed to RT and stirred for 4 hr, before being quenched with saturated ammonium chloride solution (200 ml). The volatile substances were removed by rotary evaporation, and the resulting slurry was extracted with methylene chloride (3 × 200 ml). The combined organic extracts were successively washed with water and saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to give a yellow solid. Recrystallization from *n*-pentane afforded the white crystalline

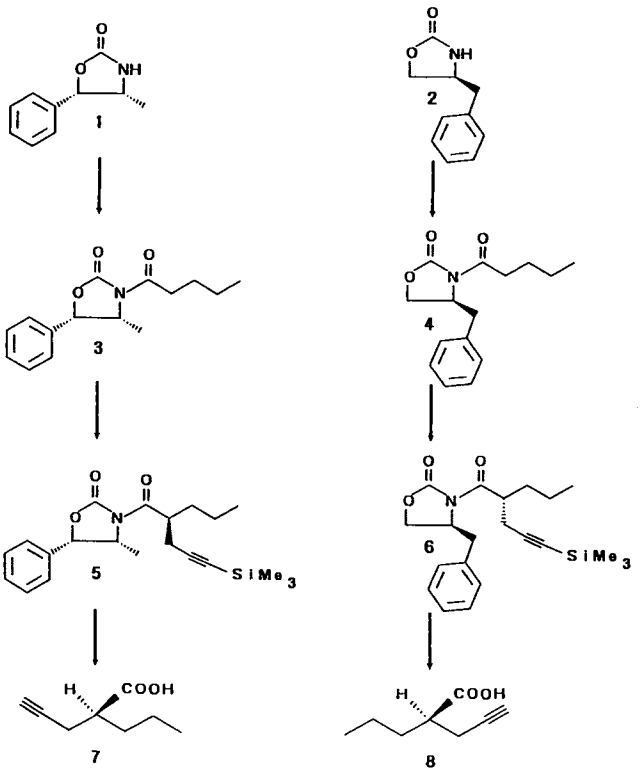


Fig. 1. Pathways for the enantioselective synthesis of *S*(−)-2-*n*-propyl-4-pentynoic acid (7) and *R*(+)-2-*n*-propyl-4-pentynoic acid (8).

3. Yield 54.9 g (210 mmol = 84%); mp 47°C [lit. 45.5–47°C (21)]; $[\alpha]_D^{22} = +46.5$ ($c$ = 2.1, $CHCl_3$).

*(4*R,5S,2′S*-4-Methyl-3-[1-oxo-2-n-propyl-5-(trimethylsilyl)-4-pentynyl]-5-phenyl-2-oxazolidinone (5).* A magnetically stirred, cooled (−78°C) solution of lithium diisopropylamide [prepared from 70 mmol of diisopropylamine in 85 ml of THF and 70 mmol of *n*-butyllithium (1.6 *M* in *n*-hexane)] was used to enolize 17.0 g (65 mmol) of 3 in 25 ml of THF. After stirring for 0.5 hr at −78°C, the resultant lithium enolate was treated with 15.3 g (80 mmol) of 3-bromo-1-(trimethylsilyl)propyne for 2.0 hr at −20°C and 4.0 hr at 10°C. The reaction was quenched by addition of half-saturated aqueous ammonium chloride (200 ml). The volatile substances were removed by rotary evaporation, and the acidified (1 *M* HCl) resulting slurry was extracted with methylene chloride (3 × 200 ml). The combined organic extracts were successively washed with water and saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated *in vacuo* to give the yellow oily 5, which was purified by flash chromatography on silica gel using a mobile phase of ethyl acetate/*n*-hexane (5:95, v/v). Yield 20.5 g (55.3 mmol = 85%). $^1$H-NMR ($CDCl_3$): δ = 0.11 [s, 9H, $Si(CH_3)_3$], 0.88–0.96 (m, 6H, 4-$CH_3$, 3″-H), 1.28–1.44 (m, 2H, 2″-H), 1.48–1.63 (m, 1H, 1″-$H_b$), 1.68–1.84 (m, 1H, 1″-$H_a$), 2.52–2.57 (m, 2H, 3′-H), 4.01–4.12 (m, 1H, 2′-H), 4.82 (dq, $J_1 = J_2$ = 7 Hz, 1H, 4-H), 5.67 (d, $J$ = 7 Hz, 1H, 5-H), 7.29–7.48 (m, 5H, aromatic-H). $[\alpha]_D^{21} = +27.4$ ($c$ = 1.2, $CHCl_3$).

S(−)-2-n-*Propyl-4-pentynoic acid [S(−)-4-yn-VPA] (7).* To a cooled (5°C) solution of 18.6 g (50 mmol) 5 in 700 ml

THF/$H_2O$ (3:1) were added subsequently 38.7 ml (340 mmol) hydrogen peroxide (30% in water) and 4.2 g (100 mmol) lithium hydroxide (LiOH × $H_2O$) in 65 ml water. After stirring for 6.0 hr the reaction was quenched by addition of 47.3 g (375 mmol) sodium sulfide in 200 ml water. The volatile substances were removed by rotary evaporation, the resulting slurry was treated with 5 *M* NaOH (pH >10), and the oxazolidinone **1** was recovered by methylene chloride extraction. The aqueous layers were acidified to pH <2 by addition of 1 *M* HCl. The desired carboxylic acid was extracted with diethyl ether (3×). The combined organic layers were dried over anhydrous sodium sulfate. Concentration and fract. distillation afforded *S*(−)-2-*n*-propyl-4-pentynoic acid. Yield 6.5 g (46 mmol = 91%); bp 72–74°C, 0.3–0.4 mbar. $^1$H-NMR ($CDCl_3$): δ = 0.94 (t, *J* = 8 Hz, 3H, 3′-H), 1.28–1.50 (m, 2H, 2′-H), 1.57–1.81 (m, 2H, 1′-H), 2.02 (t, *J* = 2.5 Hz, 5-H), 2.35–2.53 (m, 2H, 3-H), 2.54–2.70 (m, 1H, 2-H), 12.00 (s, 1H, COOH). $^{13}$C-NMR ($CDCl_3$): δ = 13.71, 19.95, 20.65, 33.02, 44.05, 69.92, 81.06, 181.10. $[\alpha]_D^{22}$ = −8.2 (*c* = 2.2, $CHCl_3$).

*R(+)-2-*n*-Propyl-4-pentynoic acid [R(+)-4-yn-VPA] (8)*

The *R*(+)-4-yn-VPA enantiomer was synthesized as outlined in Fig. 1, in a three-step sequence starting from *S*-4-benzyl-2-oxazolidinone (**2**) according to the synthetic procedure described for *S*(−)-2-*n*-propyl-4-pentynoic acid.

*S-4-Benzyl-3-(1-oxopentyl)-2-oxazolidinone (4).* Yield 96%. $^1$H-NMR ($CDCl_3$): δ = 0.95 (t, *J* = 6.5 Hz, 3H, 5′-H), 1.42 (dt, $J_1$ = 8 Hz, $J_2$ = 6.5 Hz, 2H, 4′-H), 1.60–1.75 (m, 2H, 3′-H), 2.77 (dd, $J_1$ = 13.5 Hz, $J_2$ = 9 Hz, 1H, $PhCH_b$), 2.83–3.05 (m, 2H, 2′-H), 3.30 (dd, $J_1$ = 13.5 Hz, $J_2$ = 3 Hz, 1H, $PhCH_a$), 4.12–4.24 (m, 2H, 5-H), 4.63–4.73 (m, 1H, 4-H), 7.18–7.37 (m, 5H, aromatic-H). $[\alpha]_D^{22}$ = +53.2 (*c* = 2.4, $CHCl_3$).

*(4S,2′R)-4-Benzyl-3-[1-oxo-2-*n*-propyl-5-(trimethylsilyl)-4-pentynyl]-2-oxazolidinone (6).* Yield 75%. $^1$H-NMR ($CDCl_3$): δ = 0.20–0.26 [m, 9H, $Si(CH_3)_3$], 0.96–1.06 (m, 3H, 3″-H), 1.35–1.52 (m, 2H, 2″-H), 1.58–1.72 (m, 1H, 1″-$H_b$), 1.80–1.95 (m, 1H, 1″-$H_a$), 2.69 (d, *J* = 7 Hz, 2H, 3′-H), 2.85 (dd, $J_1$ = 13.5 Hz, $J_2$ = 9.5 Hz, 1H, $PhCH_b$), 3.44 (dd, $J_1$ = 13.5 Hz, $J_2$ = 3 Hz, 1H, $PhCH_a$), 4.05–4.16 (m, 1H, 2′-H), 4.23–4.34 (m, 2H, 5-H), 4.76–4.86 (m, 1H, 4-H), 7.28–7.49 (m, 5H, aromatic-H). $[\alpha]_D^{22}$ = +49.4 (*c* = 1.5, $CHCl_3$).

*R(+)-2-*n*-Propyl-4-pentynoic acid [R(+)-4-yn-VPA] (8).* Yield 93%; bp 72–74°C, 0.3–0.4 mbar. $^1$H- and $^{13}$C-NMR ($CDCl_3$) spectra are identical with those of 7. $[\alpha]_D^{22}$ = +8.4 (*c* = 2.1, $CHCl_3$).

*Determination of the Absolute Configuration*

Three hundred twenty-four milligram (2.31 mmol) of (+)-2-*n*-propyl-4-pentynoic acid in 10 ml of *n*-hexane was reduced via catalytic hydrogenation using 104 mg of Lindlar catalyst (Pd on calcium carbonate poisoned with lead) and 52 mg of Lindlar catalyst additive in 10 ml of *n*-hexane and a hydrogen pressure of 2 atm (20°C, 3 hr) to give 279 mg (1.96 mmol = 85%) of *R*(+)-2-*n*-propyl-4-pentenoic acid (17,22). $^1$H-NMR ($CDCl_3$): δ = 0.91 (t, *J* = 8 Hz, 3H, 3′-H), 1.30–1.73 (m, 4H, 1′-H, 2′-H), 2.20–2.54 (m, 3H, 2-H, 3-H), 5.02–5.15 (m, 2H, 5-H), 5.80 (mc, 1H, 4-H), 12.05 (s, 1H, COOH). $^{13}$C-NMR ($CDCl_3$): δ = 13.84, 20.36, 33.63, 36.07, 45.02, 116.84, 135.18, 182.51. $[\alpha]_D^{24}$ = +5.5 (*c* = 4.4, $CHCl_3$).

*Determination of the Optical Purity*

The enantiomers of 4-yn-VPA were separated by gas chromatography as their diastereomeric amides formed with *S*(−)-1-phenethylamine. The racemate served as standard: *R*(+)-4-yn-VPA, 86% ee [=93%(*R*) + 7%(*S*)]; *S*(−)-4-yn-VPA, 84% ee [=92%(*S*) + 8%(*R*)].

### Animal Experiments

Female mice (Han: NMRI 29–36 g) were mated between 6:00 and 9:00 AM. The animals with vaginal plugs were separated, and the first 24 hr after conception was designated gestational day 0. The mice were fed an Altromin 1324 diet, given access to water ad libitum, and kept in an artificial light/dark cycle (10:00 AM to 10:00 PM, lights on). Controlled conditions were maintained (RT, 21 ± 1°C; air moisture, 50 ± 5%). The substances were injected i.p. as their sodium salts during the active phase on day 8 between 7:00 and 9:00 AM (23). The injected solutions in the applied dosages were prepared as aqueous solutions of the sodium salts (10 ml/kg body wt). On day 18 of gestation the implantations as well as the resorptions and dead fetuses (embryolethality) were counted and every living fetus was weighed individually and examined for exencephaly.

## RESULTS

### Synthesis

Synthetic pathways are shown in Fig. 1. The enantiomers of 4-yn-VPA (**7** and **8**) were synthesized according to general procedures described by Evans *et al.* (24,25).

The N-acylation of the two chiral oxazolidinones **1** and **2**, via their lithium conjugates, with valeroylchloride yielded the carboximides **3** and **4**. Enolization of carboximides **3** and **4** with lithium diisopropylamide and subsequent treatment with 3-bromo-1-(trimethylsilyl)propyne yielded the desired carboximides **5** and **6**, respectively. After hydrolytic cleavage of **5** and **6** using lithium hydroperoxide solution (25), the terminal silyl ether linkage was quantitatively hydrolyzed using alkaline conditions (pH >10) during the workup procedure to obtain the enantiomeric acids **7** and **8** and to recover the chiral auxiliaries **1** and **2**. *R*-4-yn-VPA contained 7%, and *S*-4-yn-VPA 8%, of its antipode.

The absolute configuration of the carboxylic acids **7** and **8** was determined via catalytic hydrogenation of (+)-2-*n*-propyl-4-pentynoic acid into the known *R*(+)-2-*n*-propyl-4-pentenoic acid (17,22). Therefore the absolute configuration of (+)-2-*n*-propyl-4-pentynoic acid is *R*.

### Teratogenicity in Mice

The results are summarized in Table 1. The exencephaly rate induced by *S*-**4**-yn-VPA increased from 3% (0.40 mmol/kg body wt) to 65% (1.05 mmol/kg body wt). *R*-4-yn-VPA was a much less potent neural tube defect-inducing agent;

Table I. Teratogenicity of the Enantiomers of 4-yn-VPA in Mice

| Substance | Dose (mmol/kg)[a] | Litter (*n*) | Live fetuses (*n*) | Fetal weight (g)[b] | Embryolethality (%)[c] | Exencephaly (%)[d] |
|---|---|---|---|---|---|---|
| *S*(−)-4-yn-VPA[e] | 0.40 | 12 | 138 | 1.13 ± 0.06 | 10 | 3 |
| | 0.60 | 10 | 107 | 1.12 ± 0.06 | 8 | 23 |
| | 0.73 | 12 | 125 | 1.12 ± 0.05 | 17 | 38 |
| | 0.86 | 14 | 113 | 1.05 ± 0.08* | 38 | 51 |
| | 1.05 | 17 | 96 | 1.04 ± 0.10* | 51 | 65 |
| *R*(+)-4-yn-VPA[f] | 3.00 | 12 | 139 | 1.12 ± 0.11 | 10 | 1 |
| | 4.50 | 9 | 88 | 1.11 ± 0.11 | 16 | 26 |
| | 6.00 | 10 | 66 | 1.00 ± 0.10* | 45 | 38 |
| Control[g] | — | 10 | 126 | 1.14 ± 0.05 | 6 | 0 |

[a] Single i.p. dose of the sodium salts per kilogram body weight on the morning of day 8 of gestation.
[b] Mean ± SD.
[c] Resorptions and dead fetuses as percentage of total implants.
[d] Percentage of live fetuses.
[e] Containing 8% *R*(+)-4-yn-VPA.
[f] Containing 7% *S*(−)-4-yn-VPA.
[g] 3.0 mmol aqueous NaCl/kg body weight.
* Significantly different from the control ($P < 0.01$, Student's *t* test).

the exencephaly rate induced by *R*-4-yn-VPA increased from 1% (3.00 mmol/kg body wt) to 38% (6.00 mmol/kg body wt). The fetal weight retardation became significantly different from the control weight at dose levels higher than 0.73 mmol/kg body wt for *S*-4-yn-VPA and at 6.00 mmol/kg body wt for *R*-4-yn-VPA.

Dose/exencephaly curves of *R*-, *S*-, (±)-4-yn-VPA, and VPA are shown in Fig. 2. The $ED_{50}$ dose values demonstrate that the *S*-enantiomer was 7.5 times as potent as the *R*-enantiomer (Table II).

A comparable degree of neurotoxicity (maternal toxicity) was observed following i.p. administration of 1.5 mmol/kg body wt, *R*-, *S*-, and (±)-4-yn-VPA, respectively, in nonpregnant mice using a Rotorod apparatus described in Ref. (26).

## DISCUSSION

(±)-4-yn-VPA was the first racemic VPA analogue with a neural tube-inducing potency exceeding that of VPA in mice. (±)-4-yn-VPA combined a high teratogenic potency with a low maternal toxicity (neurotoxicity) and was therefore suggested as a useful compound to investigate the molecular reasons of teratogenic action of VPA-related substances (18). (±)-4-yn-VPA is an equivalent mixture of two substances (enantiomers) with different three-dimensional structures.

We found a highly enantioselective teratogenicity for the 4-yn-VPA enantiomers (27). *S*-4-yn-VPA was significantly more teratogenic than the *R*-enantiomer (Table 1, Fig. 2). In contrast, the neurotoxicity (maternal toxicity) was found to be independent of the stereochemical configuration. Because of the low neurotoxicity of 4-yn-VPA compared to VPA (18), it was possible to determine the dose/exencephaly curve for the less teratogenic *R*-4-yn-VPA enantiomer at high dose levels (Table 1, Fig. 2).

The relative potency (Table II) calculated from the dose/exencephaly curves (Fig. 2) indicated that *S*-4-yn-VPA was

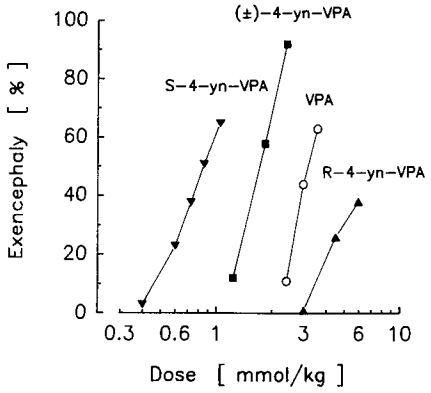


Fig. 2. Dose/exencephaly curves for *R*-, *S*-, and (±)-2-*n*-propyl-4-pentynoic acid and VPA after single i.p. administration of the sodium salt on day 8 of gestation in mice. Values for (±)-4-yn-VPA from Ref. 18; those for VPA from Ref. 31 (3.6 and 2.4 mmol/kg) and Ref. 32 (3.0 mmol/kg)

Table II. Teratogenic Activity (Induction of Exencephaly) of *R*-, *S*-, and (±)-4-yn-VPA and VPA in Mice[a]

| Substance | Slope[b] | $ED_{50}$ (mmol/kg)[c] | Relative potency |
|---|---|---|---|
| *S*(−)-4-yn-VPA | 1.51 (1.41–1.62) | 0.83 (0.79–0.88) | 7.5 |
| *R*(+)-4-yn-VPA | 1.40 (1.27–1.54) | 6.19 (5.74–6.67) | 1.0 |
| (±)-4-yn-VPA | 1.38 (1.19–1.61) | 1.58 (1.47–1.70) | 3.9 |
| VPA | 1.33 (1.17–1.50) | 3.19 (2.98–3.41) | 1.9 |

[a] Results are calculated from the dose/exencephaly curves (Fig. 2) using the method described in Ref. 30.
[b] Slope of the regression line.
[c] Median teratogenic dose.

7.5 times more potent than its antipode, 1.9 more teratogenic than (±)-4-yn-VPA, and 3.9 times more teratogenic than the parent compound VPA. The enantioselectivity of the teratogenic response investigated was more pronounced for the 4-yn-VPA enantiomers than for the 4-en-VPA enantiomers (17). This accords with Pfeiffer's rule (28): the more potent the racemic mixture is (4-yn-VPA > 4-en-VPA), the greater the enantioselectivity of the teratogenic activity becomes.

Because of the high potency of *S*-4-yn-VPA compared to its antipode, it is possible that the exencephaly rates induced by *R*-4-yn-VPA is partly the consequence of the *S*-impurity it contains (7%). However, dose–response calculations show that the "impurity" of *S*-4-yn-VPA in the *R*-4-yn-VPA preparation does not fully account for the teratogenic potency of the *R*-enantiomer preparation. Thus, the *R*-4-yn-VPA is not totally inactive and probably has some low potency itself. This question can be answered fully when a more purified preparation of the *R*-enantiomer becomes available.

Enantioselective teratogenicity could be the result of differing transplacental pharmacokinetics of the two enantiomers or of different intrinsic activities. Pharmacokinetic studies showed that there are no significant enantioselective differences in the transplacental pharmacokinetics of *R*- and *S*-4-yn-VPA following i.p. administration of 300 mg/kg body wt (1.85 mmol/kg body wt) (±)-4-yn-VPA in mice (29). This may indicate that the enantioselective teratogenicity is due to intrinsic activities of the enantiomers and not to pharmacokinetic differences.

Thus the highly specific teratogenic stereoisomer *S*-4-yn-VPA and its "negative control" *R*-4-yn-VPA should be important tools to approach the molecular mechanism of teratogenic action of this class of compounds. Furthermore, stereochemical consideration could be decisive for the development of alternative antiepileptic agents with low teratogenic potential, because the teratogenic action, but not the anticonvulsant activity (15) or neurotoxicity, is stereoselective.

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