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

Ralf-Siegbert Hauck¹ and Heinz Nau¹,²

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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 (4R,5S)-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 stereochiral configuration and lower than 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 mechanisms 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; Depaken; 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 (4R,5S)-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); (4R,5S)-4-methyl-5-phenyl-2-oxazolidinone, S-4-benzyl-2-oxazolidinone, valeryl chloride, Lindlar catalyst, Lindlar catalyst additive, and S-(−)-phenethylamine (99+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-butylithium, hydrogen, organic substrates, and standard chemicals were obtained from Merck (Darmstadt, Germany); and N-methyl-N-trimethylsilyltrifluorooracetamide (MSTFA) was from Pierce (Rockford; Ill.).

Instrumentation

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

¹ Institute of Toxicology and Embryopharmacology, Free University Berlin, Garsstraße 5, D-1000 Berlin 33, Germany.
² To whom correspondence should be addressed.
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.
relative to the internal standard tetramethylsilane. The gas chromatographic determination of the chemical purities were performed on a Hewlett-Packard 5790A (Böblingen, Germany) using a SE 30 column (1.80 m × 2 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)propane

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

3-Bromo-1-(trimethylsilyl)propane (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)propane and 0.7 ml abs. pyridine in 100 ml of abs. diethyl ether. The reaction mixture was stirred for 3 h at 0°C and was then slowly warmed to room temperature (RT). After 24 h 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 (frac.) distilled to give 55.1 g (288 mmol = 85%) of 3-bromo-1-(trimethylsilyl)propane; bp 49–51°C, 4 mbar [lit. 44–45°C, 2.7 mbar (20)].

S(−)-2-n-Propyl-4-pentyenoic 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 (4R,5S)-4-methyl-5-phenyl-2-oxazolidinone (1) as follows.

**3-Bromo-1-(trimethylsilyl)propane (20)**

4-Phenyl-5-methyl-2-oxazolidinone (1).

A magnetically stirred, cooled (−78°C) solution of 44.3 g (250 mmol) of oxazolidinone 1 in abs. THF (330 ml) was metalted with 156 ml (255 mmol) of n-butyllithium (1.6 M in hexane), until the orange-red color of the dienion just persisted, and acylated immediately with 30.7 ml (255 mmol) of freshly distilled valeryl chloride. The reaction mixture was warmed slowly to RT and stirred for 4 h, before being quenched with saturated ammonium chloride solution (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 a yellow solid. Recrystallization from n-pentane afforded the white crystalline

![Fig. 1. Pathways for the enantioselective synthesis of S(−)-2-propyl-4-pentyenoic acid (7) and R(+)2-n-propyl-4-pentyenoic acid (8).](image)

3. Yield 54.9 g (210 mmol = 84%); mp 47°C [lit. 45.5–47°C (21)]; [α]D22 +46.5 (c = 2.1, CHCl3).

(4R,5S,2’S)-4-Methyl-3-[1-oxo-2-n-propyl-5-(trimethylsilyl)-4-pentylenyl]-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 h at −78°C, the resultant lithium enolate was treated with 15.3 g (80 mmol) of 3-bromo-1-(trimethylsilyl)propane for 2.0 h at −20°C and 4.0 h 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%).

1H-NMR (CDCl3): δ = 0.11 [s, 9H, Si(CH3)3], 0.88–0.96 (m, 6H, 4-CH3, 3'-H), 1.28–1.44 (m, 2H, 2''-H), 1.48–1.63 (m, 1'H, 1''-H), 1.68–1.84 (m, 1H, 1''-H), 2.52–2.57 (m, 2H, 3''-H), 4.01–4.12 (m, 1H, 2'-H), 4.82 (dq, J1 = J2 = 7 Hz, 1H, 4-H), 5.67 (d, J = 7 Hz, 1H, 5-H), 7.29–7.48 (m, 5H, aromatic-H). [α]D21 +27.4 (c = 1.2, CHCl3).

S(−)-2-n-Propyl-4-pentyenoic acid [S(−)-4-yn-VPA] (7).

To a cooled (5°C) solution of 18.6 g (50 mmol) 5 in 700 ml