Synthesis and Evaluation of Amino Analogues of Valproic Acid

K. R. Scott,[1,3] Sandra Adesioye,[1] Patricia B. Ayuk,[1] Ivan O. Edafiogho,[1] Dolly John,[1] Patrick Kodwin,[1] Thomasena Maxwell-Irving,[1] Jacqueline A. Moore,[1] and Jesse M. Nicholson[2]

Received July 19, 1993; accepted November 12, 1993

Valproic acid, an antiepileptic drug, is extensively metabolized in humans. Two putative metabolites, 2-*n*-propyl-3-aminopentanoic acid (3-aminovalproic acid, 3-amino-VPA; 2a) and 2-*n*-propyl-4-aminopentanoic acid (4-amino-valproic acid, 4-amino-VPA; **4a**), which may result from the transamination of the respective keto acids **1a** and **3a** may explain the unusual extended seizure protection elicited by valproic acid. The title compounds were synthesized as their diasteriomeric ethyl esters **2b** and **4b** and submitted for anticonvulsant evaluation by the Antiepileptic Drug Development Program of the National Institute of Neurological and Communicative Disorders and Stroke. The results verified our hypothesis, as **4b** was active in the subcutaneous pentylenetetrazol (scMet) evaluation at 30 mg/kg. Both compounds were highly toxic at 300 mg/kg.

KEY WORDS: valproic acid; amino acids; metabolism; chirality.

INTRODUCTION

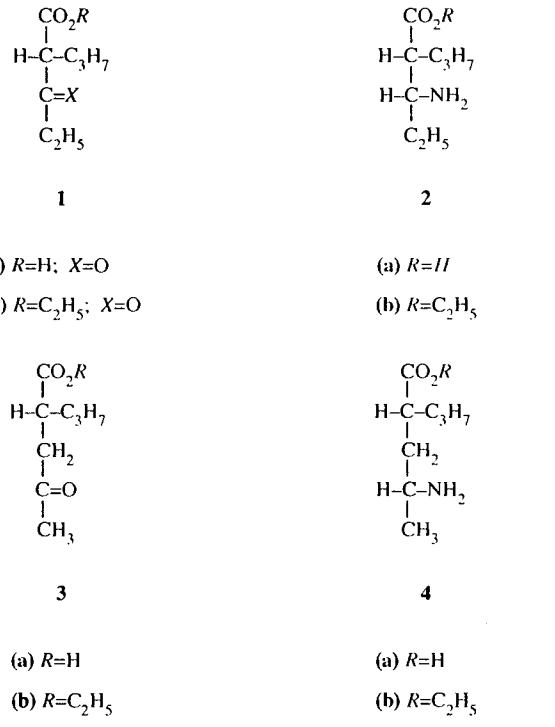
Valproic acid (di-*n*-propylacetic acid) was introduced in the United States in 1978 as an anticonvulsant specifically for the treatment of absence (petit mal) seizures (1). There have, however, been reported cases of fatal hepatotoxicity associated with valproic acid treatment (2), with microvesicular steatosis the most common feature (3). With this side effect in mind, structural analogues of valproic acid were synthesized in our laboratory and evaluated for anticonvulsant activity (4–7). Our laboratory has also become intrigued by the unusual kinetics of valproic acid. It has been shown that the anticonvulsant effect of valproic acid correlates poorly with the steady-state serum valproic acid concentration (8–10). Additionally, there is a distinct difference between the serum valproic acid concentration and the time course of anticonvulsant response, both in patients (11) and in several experimental models (12). These data disclosed that the maximal anticonvulsant response was usually not observed during initial drug therapy until sometime after the steady-state serum valproic acid concentration had been achieved. Following discontinuation of valproic acid administration, seizure control persists long after the parent compound has been cleared from the systemic circulation (12). While investigation of the anticonvulsant profile of the metabolites of valproic acid has been attempted (13), only 2-*n*-propyl-2-pentenoic acid possessed significant anticonvulsant activity (13). 2-*n*-Propyl-3-oxopentanoic acid (3-oxo-VPA; **1a**), while found in an appreciable quantity in the plasma, was not detected in the brain of laboratory animals (13). More recently, **1a** was found in the cerebrospinal fluid and the brain of humans at a low concentration (14). The isomeric 2-*n*-propyl-4-oxopentanoic acid (4-oxo-VPA; **3a**) has also been found in the urine of humans (15). We have postulated that serum transaminases may be converting **1a** and **3a** into their diasteriomeric amino acids, 2-*n*-propyl-3-aminopentanoic acid (3-amino-VPA; **2a**) and 2-*n*-propyl-4-aminopentanoic acid (4-amino-VPA; **4a**), respectively, as shown in Scheme I.

It has also been demonstrated that transport systems exist for amino acids and other essential hydrophilic materials so that they may enter the brain (16). Thus, the low concentration of **1a** reported in the brain may not be due to its hydrophilicity, but to the conversion into 3-amino-VPA, **2a**. The recent finding (17) that 3-alkyl glutamic acid analogues, branched-chained amino acids similar to **2a**, displayed glutamic acid decarboxylase activation activity and anticonvulsant activity reinforced the need for evaluation of these agents as potential anticonvulsants. We herein report the results of our studies on the synthesis and evaluation of **2a** and 4-amino-VPA, **4a**.

MATERIALS AND METHODS

Chemistry

Melting points were determined on a Thomas–Hoover capillary melting point apparatus and are uncorrected. Observed boiling points were also uncorrected. IR spectra were



Scheme I

[1] Department of Medicinal Chemistry, College of Pharmacy and Pharmacal Sciences,
[2] Department of Chemistry, Graduate School of Arts and Sciences, Howard University, Washington, D.C. 20059;
[3] To whom correspondence should be addressed.

0724-8741/94/0400-0571$07.00/0

recorded on samples of Nujol, as diluted chloroform solution in matched sodium chloride cells, or neat with a Perkin–Elmer 1330 spectrophotometer. ^{1}H NMR spectra were recorded on a General Electric QE 300-MHz spectrometer in deuterated solvents using tetramethylsilane as an internal reference. Coupling patterns are described as follows: s, singlet; bs, broad singlet; dq, doublet of quartets; t, triplet; m, multiplet; and 1H, 2H, 3H, etc., the number of hydrogens integrated within a given coupling pattern. Elemental analyses (C, H, N, and halogen) were performed by Schwarzkopf Microanalytical Laboratory (Woodside, NY). Ethyl 2-propyl-3-oxopentanoate (3-keto-VPA; **1b**) (18) was modified as indicated, and 2-propyl-4-oxopentanoic acid (4-keto-VPA; **3a**) (14) was prepared as reported.

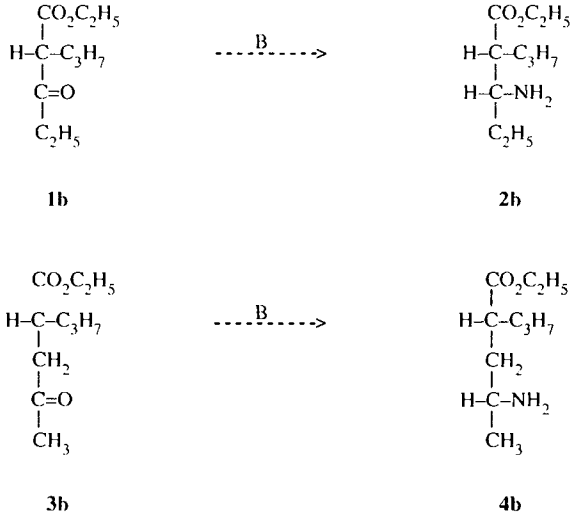
*Ethyl 2-*n*-Propyl-3-oxopentanoate (1b).* *n*-Butyl lithium (15 g, 230 mmol, 92 mL of a 2.5 *M* solution) was introduced into a 500-mL three-neck flask equipped with an inlet tube for nitrogen, a mechanical stirrer, and a condenser. The system was cooled in a 1-L DeWar flask to 0°C. With a stream of nitrogen flowing, 23.3 g (230 mmol) of diisopropyl amine was added over 30 min. The resulting paste was diluted with 100 mL of dry tetrahydrofuran (THF; previously cooled to 0°C), which allowed uniform stirring. Following a period of 30 min at −20°C, the mixture was cooled to −78°C and ethyl valerate (14.97 g, 120 mmol) was added over 30 min. After an additional 30 min, propionyl chloride (10.64 g, 120 mmol) was rapidly added via a syringe and the reaction was allowed to proceed for a further 2 hr before being quenched with 20% HCl. After warming to room temperature, the organic layer was separated. The aqueous layer was extracted with ether (3 × 100 mL) and the combined extracts were washed with saturated $KHCO_3$ (3 × 50 mL) and dried (Na_2SO_4). Evaporation of the solvents and distillation produced four fractions: fraction 1, bp 62–70°C (0.5 mm); fraction 2, bp 70–77°C (0.5 mm); fraction 3, bp 77–103°C (0.5 mm); and fraction 4, bp 103–130°C (0.5 mm). ^{1}H NMR ($CDCl_3$) analysis of fraction 3: δ 0.93 (3H, t, J = 7.27 Hz, CH_3), 1.07 (3H, t, J = 7.29 Hz, CH_3), 1.27 (3H, t, J = 7.06 Hz, CH_3), 1.00–2.75 (6H, m, 3 × CH_2), 3.45 (1H, t, J = 7.36 Hz, CH), 4.18 (2H, dq, J = 7.08 Hz, OCH_2-) proved to be authentic **1b** [lit. (18), bp 64–65°C (0.4 mm)]; yield, 5.6 g (25.1%). An additional 1.9 g was obtained on redistillation of fractions 1 and 2; total yield, 33.6%.

*(±)-Ethyl 2-*n*-Propyl-3-aminopentanoate (2b).* In a 300-mL flask 11.11 g (60 mmol) **1b** was dissolved in 160 mL dry methanol, ammonium acetate (45.93 g, 590 mmol) was added, and after solution, sodium cyanoborohydride (5.65 g, 90 mmol) was added. The mixture was connected to a drying tube and stirred at room temperature for 48 hr. The solution was acidified to pH <2 with concentrated hydrochloric acid and evaporated under reduced pressure to form a white paste. The mass was dissolved in 150 mL of water and extracted with ether (3 × 50 mL). The aqueous solution was treated with cold saturated KOH solution (8 *M*) to pH ~12 and then with sodium chloride until saturated. The suspension was placed in a liquid–liquid extraction apparatus and extracted with ether for 24 hr. The ether was dried and evaporated, and the residue distilled, producing three fractions: fraction 1, bp 54–59°C (0.4 mm); fraction 2, 59–64°C (0.4 mm); and fraction 3, 64–69°C (0.4 mm). Redistillation of fraction 3 produced three fractions: fraction 1, 63–69°C (0.6 mm); fraction 2, 70–86°C (0.6 mm); and fraction 3, 80–124°C (0.6 mm). In contrast to the first three colorless fractions, fraction 3 was yellow and was found to be impure. Fraction 2, which contained the largest quantity (8.2 g, 73%) was analytically pure. ^{1}H NMR ($CDCl_3$): δ 0.93 (3H, t, J = 7 Hz, CH_3), 0.97 (3H, t, J = 7.27 Hz, CH_3), 1.27 (3H, t, J = 7.06 Hz, CH_3), 1.10–1.75 (6H, m, 3 × CH_2), 2.35 (1H, m, CH), 2.81 (1 H, m, CH), 4.15 (2H, dq, J = 7.08 Hz, OCH_2-). The amino group was unobserved, due, probably, to solvent exchange. *Anal.* Calc. for $C_{10}H_{21}NO_2$: C, 64.11; H, 11.32; N, 7.48. Found: C, 64.16; H, 11.22; N, 7.79.

*(±)-Ethyl 2-*n*-Propyl-4-aminopentanoate (4b).* To 4-oxo-VPA, **3a** (15), 2.42 g (15.3 mmol), was added absolute ethanol (5 mL) and sulfuric acid (0.3 g), and the mixture refluxed for 12 hr. The reaction mixture was extracted with ether (2 × 15 mL). The organic layer was washed successively with saturated $KHCO_3$ (2 × 15 mL) and saturated NaCl (2 × 15 mL) and dried (Na_2SO_4). Evaporation of the solvent and distillation provided ethyl 4-oxo-VPA, **3b** [2.82 g, 98.6%, bp 52–56°C (0.145 mm)]. IR (neat): ν, 1722 and 2874–2960 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.91 (3H, t, J = 7.35 Hz, CH_3), 1.26 (3H, t, J = 7.35 Hz, CH_3), 1.30–1.66 (3H, m, CH_2 + CH), 2.20–2.96 (4H, m, 2 × CH_2), 4.13 (2H, q, J = 7.35 Hz, OCH_2-).

To a solution of ethyl 4-oxo-VPA, **3b** (2.7 g, 14.5 mmol), in 40 mL dry methanol and ammonium acetate (11.5 g, 149.4 mmol) was added sodium cyanoborohydride (1.41 g, 22.5 mmol) and the procedure for **2b** followed. The title compound **4b** was isolated in a 43% yield [bp 83–84°C (0.05 mm)]. ^{1}H NMR ($CDCl_3$): δ 0.93 (3H, t, J = 7.35 Hz, CH_3), 1.22 (6H, t, J = 6.62 Hz, 2 × CH_3), 1.26–1.48 (1H, m, CH), 1.74–2.04 (4H, m, 2 × CH_2), 2.36–2.54 (3H, m, CH + CH_2), 3.61–3.83 (2H, m, $CH_2C{=}O$), 6.91 (2H, bs, NH_2).

Attempted Resolution of 3b. L-Menthol (50 g, 0.3 mol) was converted into ethyl-l-menthyl carbonate [60 g, bp 89–90°C (0.50 mm), 88%; lit. (19), bp 121°C (9 mm), 163°C]. ^{1}H NMR ($CDCl_3$): δ 0.80–0.86 (9H, m, 3 × CH_3), 1.30 (3H, t, J = 7 Hz, OCH_2CH_3), 0.74–2.12 (9H, m, cyclohexane ring),



B = $NaCNBH_3$; NH_4OAc.

Scheme II

Table I. Phase I Intraperitoneal Anticonvulsant Testing in the Mouse

Compound	Dose (mg/kg)	MES[a] 0.5 hr	MES[a] 4 hr	scMet[a] 0.5 hr	scMet[a] 4 hr	Tox[b] 0.5 hr	Tox[b] 4 hr
2b	30	0/1	0/1	0/1	0/1	0/4	0/2
	100	—[c]	—	—	—	8/8[d]	—
	300	—	—	—	—	4/4[d]	—
4b	30	0/1	0/1	2/5	0/1	0/4	0/2
	100	0/3	0/3	0/1	0/1	0/8	0/4
	300	—	—	—	—	4/4[d]	—

[a] Number of animals protected/number of animals tested.
[b] Number of animals exhibiting toxicity/number of animals tested.
[c] Not tested.
[d] All animals died.

4.18 (2H, q, J = 7 Hz, OCH_2CH_3), 4.45 (1H, m, CH of isopropyl group).

Ethyl-l-menthyl carbonate (60 g, 0.26 mol) was converted into l-menthyldrazide [54.1 g, 97%, mp 96–97°C (from ligroine), bp 70–90°C; lit. (19), mp 101.5–102°C]. IR (neat): ν 3362, 3331, 3222, 1682, 1377, 1277, and 1184 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.67–2.12 (18H, m, 3 × CH_3 + cyclohexane ring), 3.78 (2H, bs, NH_2), 4.60 (1H, m, CH of isopropyl group).

Resolution of **3b** followed the method of Woodward and coworkers (19) for hindered ketones. A solution of **3b** (2.75 g, 15 mmol), sodium acetate (30 mg), acetic acid (15 mg) in 15 mL of ethanol was refluxed for 9 days. During this period, 0.5 mL of the reaction mixture was withdrawn and chromatographed. Distillation of the reaction provided the starting material.

Attempted Resolution of 2b. The method of Carpino (20) was employed. To a solution of **2b** (2.5 g, 13.3 mmol) in 15 mL of dichloromethane, containing 1.1 g (13.8 mmol) of pyridine, was added (−)-methyl chloroformate (3.0 g, 13.7 mmol) in 5 mL of dichloromethane through a pressure-equilibrating funnel over 30 min. After 4 days at room temperature, a precipitate formed. The mixture was washed with water (3 × 15 mL) and dried (Na_2SO_4). Evaporation of the solvent provided the amide **5**. After two recrystallizations from ethanol–water, a constant melting point was obtained (69°C); yield, 2.3 g (53%).

RESULTS AND DISCUSSION

Chemistry

The synthesis of 3-amino-VPA and 4-amino-VPA followed Scheme II. Due to the instability of 3-oxo-VPA [**1a** (18)], ethyl 3-oxo-VPA (**1b**) was synthesized by modification of the published procedure (18), while 4-oxo-VPA was synthesized as published (15). Attempted resolution of these ketones with l-menthyldrazide (19) failed, due apparently to the low energy difference between the enantiomeric forms of the two keto esters. Initially, our synthetic scheme to **2a** involved the conversion of **1b** into its oxime **1** (R = C_2H_5; X = NOH), followed by catalytic reduction (21); however, due to the rapid enolization of the ester in base (22), this method was abandoned. Reductive amination of the keto esters with sodium cyanoborohydride (23) produced the diasteriomeric amino esters, **2b** and **4b**. Resolution of the amino ester **2b** with (−)-menthyl chloroformate (20a) produced an analytically pure amide.

Pharmacology

Preliminary pharmacological testing of the ethyl esters **2b** and **3b** has been provided by the Antiepileptic Drug Development Program, Epilepsy Branch, Neurological Disorders Program, National Institutes of Neurological and Communicative Disorders and Stroke (NINCDS), by testing procedures that have been described (24). Phase I results are shown in Table I. The three tests were maximal electroshock seizure (MES), subcutaneous pentylenetetrazol (scMet), and neurologic (rotorod) toxicity (Tox). For comparative purposes, intraperitoneal (ip) ED_{50} in the MES evaluation of valproate and phenytoin are 271.1 and 9.50 mg/kg, respectively, while in the scMet evaluation valproate provides an ip ED_{50} of 148.6 mg/kg (25). Phenytoin is ineffective in the latter evaluation (25). As expected, both compounds displayed no activity against seizures induced by MES. While **2b** did not protect the animals against pentylenetetrazol-induced seizures, **4b** was active at an ip dose of 30 mg/kg (2/5 mice protected at 30 min) but not at 100 mg/kg (0/1 mice protected at 30 min). The lack of protection at the higher dosage was probably due to the small sample size. A separate evaluation in our laboratory will be undertaken with additional animals to verify this initial finding. It is to be noted that at a high dosage, both of these analogues were lethal (**2b**, 4/4 animals dead at 100 mg/kg ip; **4b**, 4/4 animals dead at 300 mg/kg ip). The latter finding was not unexpected, as branched-chain amino acids such as aspartic acid are known toxic convulsants (26). Thus, the hypothesis that anticonvulsant activity of valproic acid may also be due to potential amino acid metabolites was validated. Further work is currently in progress to resolve the esters and hydrolyze each enantiomer into its amino acids.

ACKNOWLEDGMENTS

The authors wish to acknowledge the generous financial support of the Minority Biomedical Research Support Program (GM08244-06) and the Graduate School of Arts and Sciences for the support of the High Field NMR spectrometer. The authors express appreciation to the Council for

International Exchange of Scholars (CIES) for the Fulbright Senior Research Award to Dr. Ivan O. Edafiogho. Special thanks are extended to Dr. Frank S. Abbott, University of British Columbia, and Dr. Penelope L. Codding, University of Calgary, for their encouragement and for providing a forum at their universities for discussion of this project with their faculties during a visit by the principal author, and to Dr. Harvey J. Kupferberg and Mr. James P. Stables for providing pharmacological data through the Antiepileptic Drug Development Program, National Institutes of Health.

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