

Synthesis and Anticonvulsant Evaluation of (R)- and (S)-3-Carbobenzyloxy-amino-1-oxysuccinimides

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A series of (R)- and (S)-3-carbobenzyloxy-amino-1-oxysuccinimides (**5a-d**) [(S)-3-carbobenzyloxy-amino-1-benzoyloxysuccinimide, (R)-3-carbobenzyloxy-amino-1-acetyloxysuccinimide, (R)-3-carbobenzyloxy-amino-1,4-nitrobenzoyloxysuccinimide, (R)-3-carbobenzyloxy-amino-1,4-fluorobenzoyloxysuccinimide] were synthesized and investigated their anticonvulsant activities in maximal electric shock seizure test and pentylenetetrazole induced seizure test.

Key Words: Amino-1-oxysuccinimides, Maximal electric shock seizure test, Pentylenetetrazole induced seizure test, Anticonvulsant activity.

INTRODUCTION

Epilepsy is a common and diverse set of chronic neurological disorders characterized by seizures recent estimate indicate that 1 % of population is affected some forms of epilepsy and that 20-40 % of epileptic patients failed to experience significant seizure control with the drugs currently available¹⁻⁵. Furthermore, the antiepileptic drug presently used in clinical practices suffer from a broad range of adverse side effects including sedation, tetratogenicity, cognitive dulling and liver toxicity⁶⁻⁹. The clinically epilepsy consists of various forms of seizure, so that there is a need for combination and repeat therapy to control the such complex convulsion. Owing to this multitherapy, there is a danger of toxic and troublesome side effect. Consequently, there is a need for the development of new antiepileptic compound having broader clinical spectrum and lower toxic side effects. Recently, there has been many trials for the development of new typed anticonvulsant compounds including derivatives of various amino acid such as alanine derivatives and N-benzoyl and N-phenyl glycine amide and structural modification of currently used drug such as hydantoin, succinimides and glutarimides and various GABA related compounds¹⁰⁻¹⁴.

But these compounds had some limitation in clinical use that some drug showed anticonvulsant effect only in maximal electric shock seizure test (MES) or pentylenetetrazole induced seizure test (PTZ). So we were interested in the development of new anticonvulsant compounds of broad spectrum. In connection with the studies for the development of anticonvulsants, we tried examining the structural similarities of currently available

anticonvulsant compounds, known to act by different pharmacological mechanisms each other. From the studies of structural similarities of anticonvulsants, we find out the interesting facts that anticonvulsant compounds included the common structural moieties such as NH-CO-C-N and imide in their structures and also some MNDA antagonist, showing anticonvulsive effect, had structural similarity to aspartic acid, known as excitatory amino acid, in view of bioisoster. So we thought that the new anticonvulsants could be possibly developed from the aspartic acid in view of their structures. We designed the following imides such as A, having aforesaid common structures such as NH-CO-C-N, imide and aminocarbonyl of GABA in their structure originated from aspartic acid as shown in Fig. 1.

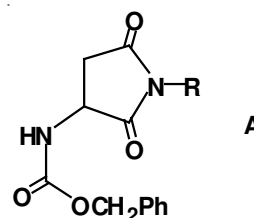
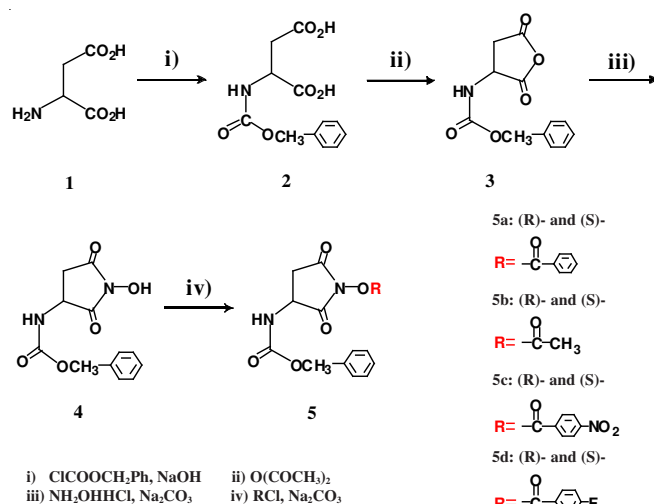


Fig. 1

Usually the stereoisomers exhibited different pharmacological activities, so we tried preparing all the (R)- and (S)-compounds in order to investigate the pharmacological differences between their stereoisomers. The compounds (**1a-d**) could be prepared from the corresponding (R)- or (S)-aspartic acid in moderate yields by known chemical reactions as shown in **Scheme-I**.



Scheme-I

EXPERIMENTAL

Melting points were determined on a Büchi 510 capillary melting point apparatus and uncorrected. Infrared spectra were recorded on a Perkin-Elmer 683 spectrophotometer. NMR spectra were recorded on a Varian XL-300 or Bruker AC 200 FT-NMR spectrometer in CDCl_3 containing Me_4Si as an internal reference. Mass spectra were obtained by using JEOL JMS DX 303 or HP 5892 mass spectrometer.

(R)-3-Carbobenzyloxy-amino-aspartic acid (2): To a solution of (R)-aspartic acid **1** (5.20 g, 40 mmol) and NaOH (3.52 g, 88 mmol) in H_2O (35 mL) and acetone (35 mL) at 0°C was added solution of carbobenzyloxy chloride (6.82 g, 40 mmol) and NaOH (1.76 g, 44 mmol) in H_2O (17 mL) and acetone (17 mL). The reaction mixture was stirred at room temperature for 7 h. After reaction solution was concentrated *in vacuo*, it was carefully acidified ($\text{pH} = 2$) with conc HCl. Then the white solid was obtained in good yield (8.20 g, 77 %). m.p. $115\text{--}116^\circ\text{C}$; R_f 0.6 (TLC eluent; benzene:THF:formic acid = 15:5:1, v/v/v); IR (neat, ν_{max} , cm^{-1}): 3500, 3350, 3100, 1700, 1680; ^1H NMR (200 MHz, DMSO) δ 2.70 (2H, m), 4.30 (1H, m), 5.16 (2H, s), 7.55 (5H, m), 12.64 (1H, br) (S)-3-carbobenzyloxy-amino-aspartic acid **2**: yield 77 %; m.p. $115\text{--}116^\circ\text{C}$; R_f 0.6 (TLC eluent; benzene:THF:formic acid = 15:5:1, v/v/v); the IR and ^1H NMR spectra of (R)-**2** was identical with the IR and ^1H NMR spectra of (R)-**2**.

(R)-3-Carbobenzyloxy-amino-aspartic anhydride (3): A mixture of synthesized (R)-**2** 10 g, 37 mmol) and acetic anhydride (100 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo*. After concentrating it was added diethyl ether 100 mL. Then the white solid was obtained in good yield (8.4 g, 91 %) m.p. $134\text{--}135^\circ\text{C}$; R_f 0.5 (TLC eluent; benzene:THF:formic acid = 15:5:1, v/v/v); IR (neat, ν_{max} , cm^{-1}): 3370, 3350, 3110, 1710, 1500, 1220; ^1H NMR (200 MHz, DMSO) δ 2.50 (2H, m), 2.99 (1H, m), 4.42 (1H, s), 5.05 (2H, s), 7.57 (5H, m), 10.82 (1H, s).

(S)-3-Carbobenzyloxy-amino-aspartic anhydride (3): m.p. $95\text{--}96^\circ\text{C}$; R_f 0.8 (TLC eluent; ethylacetate); the IR and ^1H NMR spectra of (S)-**3** was identical with the IR and ^1H NMR spectra of (R)-**3**.

(R)-3-Carbobenzyloxy-amino-1-hydroxysuccinimide (4): A mixture of synthesized (R)-3-carbobenzyloxy-amino-

aspartic anhydride **3** (2.49 g, 10 mmol) and hydroxylamine hydrochloride (0.83 g, 12 mmol) in H_2O (2 mL) with Na_2CO_3 (0.64 g) was stirred at 0°C for 1 h. The reaction solution was concentrated *in vacuo*. The white crystal (1.45 g, 55 %) was recrystallized by solution of ethanol and H_2O . Yield 55 % m.p. $^\circ\text{C}$; R_f 0.5 (TLC eluent; benzene:THF:formic acid = 15:5:1, v/v/v); IR (KBr, ν_{max} , cm^{-1}): 3500, 3370, 3110, 1710, 1500, 1220; ^1H NMR (DMSO) δ 2.50 (2H, m), 2.99 (1H, m), 4.42 (1H, s), 5.05 (2H, s), 7.67 (5H, s), 10.82 (1H, s).

(S)-3-Carbobenzyloxy-amino-1-hydroxy succinimide (4): yield 54 % The IR and ^1H NMR spectra of (S)-**4** were identical with the IR and ^1H NMR spectra of (R)-**4**.

Typical experimental procedure for synthesis of (R)-3-carbobenzyloxy-amino-1-benzoyloxysuccinimide: A mixture of synthesized (R)-carbobenzyloxy-amino-1-hydroxysuccinimide (**4**) (0.56 g, 2 mmol) benzoylchloride (0.34 g, 2 mmol) and Na_2CO_3 (0.25 g) with acetone 8 mL H_2O 5 mL were stirred at room temperature for 5 h. After EtOAc (200 mL) was added, the reaction mixture was added saturated NaHCO_3 solution (50 mL), 5 % HCl (50 mL) and H_2O (50 mL) then the confined organic layers were concentrated *in vacuo*. The obtained (R)-3-carbobenzyloxy-amino-1-benzoyloxysuccinimide (**5a**) (0.35 g, 46 %) was recrystallized with EtOAc and *n*-hexane. m.p. $141\text{--}142^\circ\text{C}$; R_f 0.7 (TLC eluent; EtOAc: *n*-hexane = 1:1, %); IR (KBr, ν_{max} , cm^{-1}): 3410, 3100, 1950, 1730, 1690, 1510; ^1H NMR (DMSO) δ 2.85 (2H, m), 4.77 (1H, s), 5.07 (2H, s), 7.77 (10H, m). (S)-3-Carbobenzyloxy-amino-1-benzoyloxysuccinimide (**5a**): yield 0.37 g (49 %). The IR and ^1H NMR spectra of (S)-**5a** were identical with the IR and ^1H NMR spectra of (R)-**5a**.

(R)-3-Carbobenzyloxy-amino-1-acetyloxysuccinimide (5b): yield 56 % m.p. $103\text{--}104^\circ\text{C}$; R_f 0.6 (TLC eluent; EtOAc:*n*-hexane = 1:1, %) ; IR (KBr, ν_{max} , cm^{-1}): 3380, 3370, 3100, 3000, 1720, 1700, 1510, 1210; ^1H NMR (DMSO) δ 2.33 (3H, s), 2.72 (2H, m), 4.64 (1H, s), 5.02 (2H, s), 7.71 (5H, m).

(R)-3-Carbobenzyloxy-amino-1-4-nitrobenzoyloxy-succinimide (5c): yield 48 %; m.p. $175\text{--}176^\circ\text{C}$; R_f 0.5 (TLC eluent; EtOAc:*n*-hexane = 2:1, v/v); IR (KBr, ν_{max} , cm^{-1}): 3400, 3100, 3100, 3000, 1850, 1700, 1320, 1280; ^1H NMR (DMSO) δ 2.86 (2H, m), 2.72 (2H, m), 4.77 (1H, s), 5.08 (2H, s), 8.15 (10H, m).

(R)-3-Carbobenzyloxy-amino-1-4-fluorobenzoyloxy-succinimide (5d): yield 74 %; m.p. $176\text{--}177^\circ\text{C}$; R_f 0.4 (TLC eluent; EtOAc:*n*-hexane = 2:1, v/v); IR (KBr, ν_{max} , cm^{-1}): 3400, 3100, 3100, 3000, 1730, 1690, 1550, 1290, 1190; ^1H NMR (DMSO) δ 2.87 (2H, m), 4.77 (1H, s), 5.08 (2H, s), 7.80 (10H, m). The IR and ^1H NMR spectra of (S)-**5b** (60 %), **5c** (49 %), **5d** (67 %) were identical with the IR and ^1H NMR spectra of (R)-**5b**, **5c** and **5d**.

RESULTS AND DISCUSSION

All products gave satisfactory spectral data. So the compounds **5a-d** were submitted to the following anticonvulsant tests. It was reported that MES test was correlated to generalized tonic clonic seizure and PTZ test to generalized absence seizure. So these two kinds of seizure test are very meaningful for the clinical prediction of anticonvulsant drug candidates. Therefore we investigated the anticonvulsant activity for those compounds((R)-**5a-d** and (S)-**5a-d**) in maximal electric shock

seizure test (MES test) and pentylenetetrazole induced seizure test (PTZ test). The results of anticonvulsant activity are summarized in Table-1.

TABLE-1
SELECTED ANTICONVULSANT EVALUATION OF
(R)-, (S)-CARBOBENZYLOXY-AMINO-1-3-
HYDROXYLSUCCINIMIDES IN MICE

Compound	Config	ED ₅₀ (mg/Kg) ^a	
		MES (PI) ^b	PTZ ^c
5a	R	97.2	103.2
5b	R	172.5	137.5
5c	R	98.3	81.3
5d	R	125.2	100.0
5a	S	121.3	106.3
5b	S	92.3	89.7
5c	S	90.6	112.3
5d	S	102.2	97.2
Phenobarbital ^d	—	21.8(3.1)	13.1(5.3)

^aAll compounds were administered ip to ICR male mice and all anticonvulsant tests were performed in groups of 4 mice 30 min after test compound administration. ^bMaximal electric shock seizure test ; 50 mA, 60 Hz, ac, 02 s. ^cSubcutaneous pentylenetetrazoyl (90 mg/Kg) induced seizure test. ^dRef 14.

It is found that the (R)-isomer was more active than (S)-isomer about the anticonvulsant activity. Especially, (R)- and (S)-**5a-d** were reported to be active in only PTZ. We continue to synthesize their analogs and evaluate their anticonvulsant activities in order to develop more active anticonvulsant compounds and define the structure-activity relationship more distinctly.

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