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The Effect of *N*-Alkyloxycarbonyl Group on the Anticonvulsant Activities of *N*-Alkyloxycarbonyl-α-amino-*N*-methylsuccinimides

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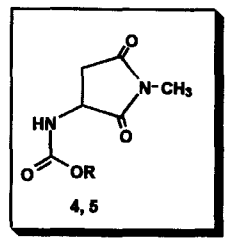
In connection with the development of new anticonvulsant agents with a broad spectrum, we found that *N*-Cbz-α-amino-*N*-alkylsuccinimides showed significant anticonvulsant activities, and the pharmacological activities of these compounds were dependent on their stereochemistry and *N*-substituted alkyl group. These results prompted us to define the effects of other functional group on the anticonvulsant activities of these compounds. Therefore a series of *N*-alkoxycarbonyl-α-amino-N-methylsuccinimide were prepared from *N-Cbz*-aspartic acid and were evaluated with their anticonvulsant activities againt the MES and PTZ tests, in order to define the effect of *N*-substituted alkoxy carbonyl group with the anticonvulsant activities. From these studies, it was found that all the tested *N*-alkoxycarbonyl-α-amino-*N*-methylsuccinimides exhibited significant anticonvulsant activities in the PTZ test and were not active in the MES test. The most active compound in the PTZ test was (S) *N*-ethoxycarbonyl-α-amino-*N*-methyl-succinimide. We found that the pharmacological activities in the PTZ test were dependent on their *N*-alkoxycarbonyl groups. They follow as such; The order of anticonvulsant activities for (R) series as evaluated by ED_{50} was *N*-phenoxycarbonyl=*N*-4-nitrobenzyloxycarbonyl > *N*-ethoxycarbonyl > *N*-allyloxycarbonyl > *N*-tert. butoxycarbonyl compound; For the (S) series *N*-ethoxycarbonyl > *N*-phenoxycarbonyl > *N*-allyloxycarbonyl compound. From the above results, it was conceivable that *N*-substituted alkoxycarbonyl group had certain effects on the anticonvulsant activities of *N*-alkoxycarbonyl-α-amino-*N*-methylsuccinimides.

Key words : α-Amino-*N*-methylsuccinimide, PTZ test, MES test, Anticonvulsant activity

INTRODUCTION

The preceding papers (Park *et al.*, 1996; Lee *et al.*, 1997) reported that *N-Cbz*-α-amino-*N*-alkylsuccinimide exhibited significant anticonvulsant activities in the MES (maximal electroshock seizure) and PTZ (pentylene tetrazole) tests enough to be recomended as new anticonvulsant agents. It was found that *N*-Cbz-α-amino-*N*-methylsuccinimide **1, 2** were most active, and their anticonvulsant activities were dependent on the *N*-substituted alkyl chain. This estimate prompted us to prepare the various analogs of the *N*-substituted alkyl chain to develop more active compounds and to define the effects of the functional group with their anticonvulsant activities. Based on the previous results, we seleceted the (R) and (S) *N*-Cbz-α-amino-*N*-methyl-succinimide **1, 2** as the lead compounds for the further investigation. As a related studies, we prepared *N*-alkyloxycabonyl-α-amino-*N*-methylsuccinimides **4, 5**, in which amino groups were substituted by various alkyloxycarbonyl groups instead of Cbz group, and evaluated their anticonvulsant activities in order to define the effects of substituted alkyloxy carbonyl group on the anticonvulsant activities of these compounds. Herein we wish to report the synthesis and the anticonvulsant activities of the various *N*-alkyloxy carbonyl-α-amino-*N*-methylsuccinimide as shown in Fig 1.

In this paper, we focused on the effects of *N*-substituted alkyloxycarbonyl group with their anticonvulsant



4 a: (R), R=C_2H_5 b: (R), R=Allyl c: (R), R=4-nitrobenzyl d: (R), R=phenyl e: (R), R= tert.butyl

5 a: (S), R=C_2H_5 b: (S), R=Allyl c: (S), R=4-nitrobenzyl d: (S), R=phenyl e: (S), R= tert.butyl

Fig. 1. *N*-alkyloxycarbonyl-α-amino-*N*-methylsuccinimides **4** and **5**.

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activities.

MATERIALS AND METHODS

Melting points were determined by a Electrothermal digital melting point apparatus and were incorrected. IR spectra were taken in KBr disk with JASCO FT/IR 200 and were recorded in cm^{-1}. ^{1}H-NMR spectra were recorded in $CDCl_3$ on JNM-EX90A and chemical shifts were reported as δ values in parts per million from TMS as an internal standard. The pharmacological tests were carried out according to the protocol of the Anti-epileptic Drug Development Program of the National Institute of Neurological Disorders and Stroke (Swinyard *et al.*, 1989).

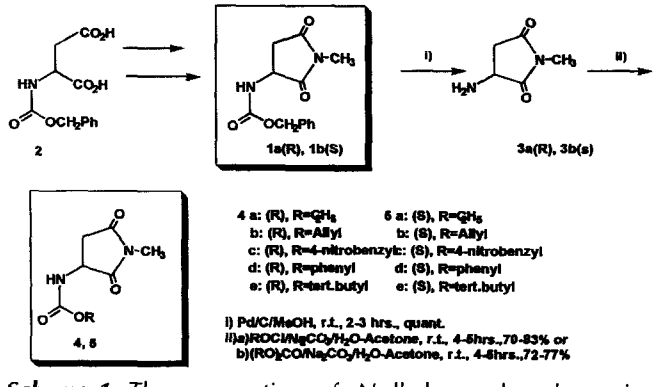
Synthesis

The synthetic methods of (R) and (S) *N-Cbz*-α-amino-*N*-methylsuccinimide were reported in our previous paper and the final compounds were prepared from the corresponding (R) or (S) *N-Cbz*-α-amino-*N*-methyl-succinimide by hydrogenolysis with Pd/C and acylation with various alkoxycarbonyl chlorides or di tert. butyl-dicarbonate. The synthetic procedure is outlined in Scheme 1.

(R) α-Amino-*N*-methylsuccinimide 1a: (R) *N-Cbz*-α-Amino-*N*-methylsuccinimide (524 mg) was subjected to catalytic hydrogenation with 10% palladium on charcoal (52 mg) in methanol (50 mL) at room temperature for 2~3 hrs. The reaction mixture was filtered and the filtrate was evapoated *in vacuo* to give 256 mg of white solid. mp: 73.1°C IR (KBr) cm^{-1}: 1700, 1720, 3300. This compound was used as a synthetic intemediate for the next step without further purification.

(S) *N*-Cbz-α-Amino-*N*-methylsuccinimide 1b: This compund was obtained by the same procedure as described above.

(R) *N*-ethoxycarbonyl-α-amino-*N*-methylsuccinimide 4a: To the sol'n of (R) α-amino-*N*-methylsuccinimide (256 mg) and Na_2CO_3 (254 mg) in acetone (3 mL) and H_2O (3 mL), the sol'n of ethoxycarbonylchloride (259 mg) in acetone (3 mL) was added. Then the reaction mixture was stirred for 4~5 hrs at room temperature. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in EtOAc (200 mL). The EtOAc layer was washed with 10% aqueous $NaHCO_3$ (25 mL x 2), 5% aqueous HCl (25 mL x 2) and H_2O (25 mL x 2) and saturated NaCl solution (25 mL x 2) successively and dried over anhydrous $MgSO_4$. The EtOAc layer was evaporated to give a brown solid. This crude product was purified with a silica gel column chromatography (EtOAc:hexane=2:1) to afford 303 mg of white solid (76%). mp: 129.7°C; $[\alpha]_D^{25°}$: +35.506 (c=1.00%, CH_3OH); IR (KBr) cm^{-1}: 1700, 1720, 3410; ^{1}H-NMR (DMSO-d_6): δ 1.24 (3H, t, *J*=7.2), 2.60~2.95 (2H, m), 3.04 (3H, s), 4.14 (2H, q, *J*=7.2), 4.20~4.50 (1H, m), 5.58 (1H, br).



Scheme 1. The preparation of *N*-alkyloxycarbonyl-α-aminosuccinimides **4** and **5**.

The following compounds were prepared according to the above procedure.

(S) *N*-Ethoxycarbonyl-α-amino-*N*-methylsuccinimide 5a: 77%; mp: 130.1°C: $[\alpha]_D^{25°}$: -36.094 (c=1.00, CH_3-OH), The IR and ^{1}H NMR spectra were identical with **4a**.

(R) *N*-allyloxycarbonyl-α-amino-*N*-methylsuccinimide 4b: 69%; mp: 104.2°C; $[\alpha]_D^{25°}$: +38.860 (c=1.00, CH_3-OH); IR (KBr) cm^{-1}: 1680, 1720, 1790, 3400; ^{1}H NMR (DMSO-d_6): d 2.60~2.95 (2H, m), 3.02 (3H, s), 4.20~4.50 (1H, m), 4.56 (2H, d, *J*=5.0), 5.10~5.50 (2H, m), 5.70~5.90 (1H, m), 6.00 (1H, br).

(S) *N*-allyloxycarbonyl-α-amino-*N*-methylsuccinimide 5b: 60%; mp: 102.4°C; $[\alpha]_D^{25°}$: -38.152 (c=1.00, CH_3-OH); The IR and ^{1}H NMR spectra were identical with **4b**.

(R) *N*-4-nitrobenzyloxycarbonyl-α-amino-*N*-methyl-succinimide 4c: 73%; mp: 139.3°C; $[\alpha]_D^{25°}$: +40.788 (c=1.00, CH_3OH); IR (KBr) cm^{-1}: 1690, 1720, 1780, 3310; ^{1}H NMR: 2.60~2.90 (2H, m), 3.03 (3H, m), 4. 20~4.50 (1H, m), 5.20 (2H, s), 5.74 (1H, br), 7.49 (2H, d, *J*=8.2), 8.21 (2H, d, *J*=8.2).

(S) *N*-4-nitrobenzyloxycarbonyl-α-amino-*N*-methyl-succinimide 5c: 75%; mp: 138.6°C; $[\alpha]_D^{25°}$: -40.596 (c =1.00, CH_3OH); The IR and ^{1}H NMR spectra were identical with **4c**.

(R) *N*-phenoxycarbonyl-α-amino-N-methylsuccinimide 4d: 63%; mp: 181.4°C; $[\alpha]_D^{25°}$: +37.556 (c=1.00, CH_3-OH); IR (KBr) cm^{-1}: 1700, 1790, 3320; ^{1}H NMR (DMSO-d_6): 2.60~2.90 (2H, m), 3.01 (3H, s), 4.41 (1H, m), 6.80~7.40 (5H, m), 7.70 (1H, br).

(S) *N*-phenoxycarbonyl-α-amino-*N*-methylsuccinimide 5d: 64%; mp: 180.8°C; $[\alpha]_D^{25°}$: -37.800 (c=1.00, CH_3-OH); The IR and ^{1}H NMR spectra were identical with **4d**.

(R) *N*-tert. butoxy-α-amino-*N*-methylsuccinimide 4e: To the sol'n of (R) α-amino-*N*-methylsuccinimide (256

mg) and Na_2CO_3 (259 mg) in acetone (3 mL) and H_2O (3 mL), the sol'n of di tert. butyl-dicarbonate (466 mg) in acetone (4 mL) was added. Then the reaction mixture was stirred for 4~5 hrs at room temperature. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in EtOAc (200 mL). The EtOAc layer was washed with 10% aqueous $NaHCO_3$ (25 mL x 2), 5% aqueous HCl (25 mL x 2) and H_2O (25 mL x 2) and saturated NaCl solution (25 mL x 2) successively and dried over anhydrous $MgSO_4$. The EtOAc layer was evaporated to give a brown solid. This crude product was purified with a silica gel column chromatography (EtOAc:hexane=2:1) to afford 322 mg of white solid (71%). mp: 104.3°C; $[\alpha]_D^{25°}$: +23.532 (c=1.00, CH_3OH); IR (KBr) cm^{-1}: 1700, 1720, 1740, 3300; ^{1}H-NMR (DMSO-d_6): 1.53 (9H, s), 2.60~2.90 (2H, m), 3.02 (3H, s), 4.25 (1H, m), 5.59 (1H, br).

(S) *N*-tert. butoxy-α-amino-*N*-methylsuccinimide 5e: 68%; mp: 104.3°C; $[\alpha]_D^{25°}$: -23.164 (c=1.00); The IR and ^{1}H NMR spectra were identical with **4e**.

Pharmacology

The anticonvulsant activities for *N*-alkyloxycarbonyl-α-amino-*N*-methylglutarimides **4, 5** in the maximal electric shock seizure (MES) and the pentylenetetrazole induced seizure (PTZ) tests were carried out according to the protocol of the Antiepileptic Drug Developement Program of the National Institute of Neurological Disorders and Stroke (Swinyard *et al.*, 1988). They follow as such : All tested compounds were dissolved in polyethylene glycol 400 and administered ip to ICR male mice at doses of 25, 50, 75 and 100 mg/kg. The anticonvulsant tests were performed 30 min after administration in groups of 4 mice, also we determined the lowest dose that all the tested animals could be induced seizures at the stage of preliminary screening. Seizure was then artificially induced by either electric shock or pentylenetetrazole. The maximal elelectric shock seizure (MES) tests were elicited with a 60-cycle a.c. of 50 mA intensity delivered for 0.2 s via corneal electrods with a ECT unit (UGO Basline, Itlay). A drop of 0.9% saline was instilled in the eye prior to application of electrods. Protection in this test was defined as the abolition of hind limb tonic extension component of seizure. The pentylenetetrazole seizure (PTZ) test entailed the administration of 80 mg/kg of pentylenetetrazole as a 0.5% solution subcutaneously in the posterior midline of the mice, and observation lsated for 30 min. Protection was defined as the failure to observe even a threshold seizure, a single episode of chronic spasms that persist for at least 5 sec. duration, and the ED_{50} acts as a quantitative anticonvulsant evaluations was estimated from the dose-response data.The effects of the compounds on the forced and spontaneous motor activities were evaluated in mice by the rotorod test with a Rotorod treadmill for mice (UGO Baseline, Itlay). They follow as such: The previously trained animal was placed on an 1 inch diameter knurled plastic rod rotating at 6 rpm after the administration of the tested compounds. Normal mice can remain on a rod at this speed indefinitely. Neurological toxicity was defined as the failure of the animal to remain on the rod for 2 min. Finally, the median neurotoxic dose (TD_{50}) was estimated from the dose-response data.

RESULTS AND DISCUSSION

As seen in Scheme 1, all tested compounds were prepared from the corresponding (R) or (S) *N*-alkoxycarbonyl-α-amino-*N*-methylsuccinimide from the *N*-*Cbz*-α-amino-*N*-methylsuccinimide **1** via hydrogenolysis with Pd/C, and acylation by usual methods in moderate yields. And all the compounds gave the satisfactory spectral data. We investigated the anticonvulsant activities for those compounds in both the MES and PTZ test. The results of preliminary anticonvulsant activities are summarized in Table I and II.

As seen in Table I and Table II, none of the tested

Table I. Anticonvulsant activities of (R)-*N*-alkyloxycarbonyl-α-aminosuccinimides (**4**) in mice

Compound	Config.	R	Dose[a]	MES[b]	PTZ[c]
4a	R	C_2H_5	25		
			50		4/4(3/4)[d]
			75		2/4
			100	4/4	1/4(0/4)[e]
4b	R	Allyl	25		
			50		4/4
			75		3/4
			100	4/4	2/4(0/4)[f]
4c	R	4-nitrobenzyl	25		4/4
			50		3/4
			75		2/4
			100	4/4	1/4(0/4)[g]
4d	R	phenyl	25		
			50		4/4
			75		3/4(2/4)[g]
			100	4/4(3/4)[e]	1/4(0/4)[g]
4e	R	tert.butyl	75		4/4
			100	4/4(0/4)[e]	3/4(0/4)[f]

[a]All compounds were dissolved in polyethyleneglycol400 and administerd i.p to ICR male mice. Dose was denoted in mg/kg.
[b]The MES test : 50 mA, 60 Hz, ac, 0.2 sec., via corneal eletrods, 30 min post administration of test compound. And the results were denoted as non-protected animals/tested animals.
[c]The PTZ test: Subcutaneous pentylenetetrazol (80 mg/kg) 30 min post administration of test compound. And the results were denoted as non-protected animals/tested animals.
[d] at a dose of 60 mg/kg. [e] at a dose of 200 mg/kg. [f] at a dose of 150 mg/kg. [g] at a dose of 125 mg/kg.

compounds exhibited anticonvulsant activities against the MES test at a dose of 100 mg/kg, and *N*-4-nitrobenzyloxycarbonyl-α-amino-*N*-methylsuccinimide **4c, 5c**, *N*-phenoxycarbonyl-α-amino-*N*-methylsuccinimide **4d, 5d** and *N*-tert. butoxy-α-amino-*N*-methyl-α-amino-*N*-methylsuccinimide **4e, 5e** showed some anticonvulsant activities at a dose of 200 mg/kg. But in PTZ test, all tested compounds were found to be active at the lower dose than 100 mg/kg. According to the protocol for the development of new anticonvulsant, the compounds, showing the antionvulsant activity at dose of 100 mg/kg in mice, were recommended to further investigation of quantification. So only PTZ tests for the tested compounds were carried out for the quantitative anticonvulsant evaluation and rotorod test were carried out to evaluate the neurotoxicity. The results of quantitative anticonvulsant activities and rotorod test are summarized in Table III.

Table II. Anticonvulsant activities of (S)-*N*-alkyloxycarbonyl-α-aminosuccinimides (**5**) in Mice

Compound	Config.	R	Dose[a]	MES[b]	PTZ[c]
5a	S	C_2H_5	25		3/4(4/4)[d]
			50		2/4
			75		1/4
			100	4/4	0/4
5b	S	Allyl	25		4/4
			50		3/4
			75		2/4
			100	4/4	1/4(0/4)[e]
5c	S	4-nitrobenzyl	100	4/4(3/4)[f]	4/4(0/4)[f]
5d	S	phenyl	25		4/4
			50		3/4
			75		2/4
			100	4/4(2/4)[f]	1/4(0/4)[g]
5e	S	tert.butyl	100	4/4	4/4

[a]All compounds were dissolved in polyethyleneglycol400 and administerd i.p to ICR male mice. Dose was denoted in mg/kg.
[b]The MES test: 50 mA, 60 Hz, ac, 0.2 sec., via corneal eletrods, 30 min post administration of test compound. And the results were denoted as non-protected animals/tested animals.
[c]The PTZ test: Subcutaneous pentylenetetrazol (80 mg/kg) 30 min post administration of test compound. And the results were denoted as non-protected animals/tested animals.
[d] at a dose of 15 mg/kg. [e] at adose of 150 mg/kg. [f] at a dose of 200 mg/kg. [g] at a dose of 125 mg/kg.

As seen in Table III, (S) *N*-ethoxycarbonyl-α-amino-*N*-methylsuccinimide **5a** was the most active among them and the anticonvulsant activity in the PTZ test was 5-fold more active than valpoic acid as evaluated from ED_{50} value. The anticonvulsant activities of other compounds were also comparable to the other anticonvulsant drugs. It was found that the anticonvulsant activities in the PTZ test were dependent on their *N*-alkyloxycarbonyl group. They follow as such; The order of anticonvulsant activities for (R) series as evaluated by ED_{50} was *N*-phenoxycarbonyl=*N*-4-nitrobenzyloxycarbonyl > *N*-ethoxycarbonyl > *N*-allyloxycarbonyl > *N*-tert. butoxycarbonyl compound; For the (S) series *N*-ethoxycarbonyl > *N*-phenoxycarbonyl > *N*-allyloxycarbonyl compound. From the above results, it was conceivable that *N*-substituted alkoxycarbonyl

Table III. The selected anticonvulsant evaluation of *N*-alklcarbonyl-α-aminosuccinimides (**4** and **5**) in mice

Compound	Config.	R	TD_{50}[b] (mg/kg)	ED_{50} (mg/kg)[a]	
				MES (PI)[c]	PTZ (PI)[d]
4a	R	C_2H_5	163.1		80.6 (2.0)
4b	R	Allyl	150.0		100.0 (1.5)
4c	R	4-nitrobenzyl	100.0		75.0 (1.3)
4d	R	phenyl	125.0		75.0 (1.7)
4e	R	tert.butyl	125.0		119.4 (1.1)
5a	S	C_2H_5	150.0		51.9 (2.9)
5b	S	Allyl	125.0		78.1 (1.6)
5c	S	4-nitrobenzyl	150.0		150.6 (1.0)
5d	S	phenyl	150.0		75.0 (2.0)
	Diphenylhydantoin[e]		65.4	9.5 (6.9)	f
	Phenobarbital[e]		69.0	21.8 (3.1)	13.1 (5.3)
	Ethosuximide[e]		440.8	f	130.4 (3.4)
	Methosuximide[e]		130.1	42.6 (3.1)	34.5 (3.7)
	Valproic aid[e]		425.8	271.1 (1.6)	148.6 (2.9)
	Trimethadione[e]		1070.0	704.2 (1.5)	250.5 (4.3)

[a]All 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.
[b]Rotarod test for neurotoxicity in groups of 5 mice. c maximal electric shock seizure test: 50 mA, 60 Hz, ac, 0.2 s. and PI is protective index (TD_{50} ED_{50}).
[d]Subutaneous pentylenetetrazole (80 mg/kg) induced seizure test.
[e]Witak *et al.*, 1972. [f]not effect.

group had certain effects on the anticonvulsant activities of *N*-alkoxycarbonyl-α-amino-*N*-methylsuccinimides.

CONCLUSION

In conclusion, a series of (R) and (S) *N*-alkoxycarbonyl-α-amino-*N*-methylsuccinimides were prepared from the corresponding *N-Cbz*-aspartic acid, their antconvulsant activities in the MES and PTZ tests. They include their neuritoxicities, in order to define the effects of *N*-alkoxycarbonyl group with their anticonvulsant activities. From these studies, it was found that all tested compounds did not show significant activities in the MES test at a dose of 100 mg/kg. However, all tested compounds showed significant anticonvulsant activities in the PTZ test. And (S) *N*-ethoxycarbonyl-α-amino-*N*-methylsuccinimide **5a** was the most active in PTZ test. As evaluated from ED_{50} value and PI index, this compound was thought to be active enough to be recommended for a new anticonvulsant drug candidate. Also we found that the anticonvulsant activities of these compounds were dependent on the *N*-substituted alkoxycarbonyl groups. From these results, even though we could not explain the reason exactly, it was conceived that the *N*-substituted alkyloxycarbonyl group of these compounds played an important role for their anticonvulsant activities and their spectrum of anticonvulsant activities.

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