Synthesis and Pharmacological Activity of O-(5-Isoxazolyl)-l-serine

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A novel isoxazole derivative, O-(5-isoxazolyl)-l-serine (OIS, 1), was synthesized by a Mitsunobu reaction of isoxazolin-5-one (4) with N-Boc-l-serine tert-buty1 ester (5) and subsequent deprotection of the coupling product. Its structure was elucidated by spectroscopic analyses. The pharmacological activity of 1 was also examined with cloned glutamate receptors and transporters using a Xenopus oocyte-expressing system showing substrate activity on an excitatory amino acid carrier 1 (EAAC 1) as a glutamate transporter.

Key words synthesis; O-(5-isoxazolyl)-l-serine; pharmacological activity; excitatory amino acid carrier 1; Xenopus oocyte

O-(5-Isoxazolyl)-l-serine (OIS, 1) is thought to be a structural isomer of naturally occurring isoxazolinone derivatives, β-(isoxazolin-5-on-2-yl)-l-alanine (BIA, 2) and β-(isoxazolin-5-on-4-yl)-l-alanine (TAN, 3), but it has not yet been found in nature. BIA (2) was identified in the leguminous genus Lathyrus, Lens and Pisum, was confirmed to be the biosynthetic precursor of the neurotoxin 3-N-oxaly-L-2,3-diaminopropionic acid (β-ODAP) in grass pea (Lathyrus sativus L.),1–5 and was also found to have antimycotic activity.6 BIA was synthesized from N-Boc-l-serine (9) by Baldwin et al.7 TAN (3) was isolated from Streptomyces platensis as an antifungal antibiotic,8 and was also synthesized by Tsubotani et al.9 However, OIS (1) has not yet been synthesized, in spite of its stable structure.

During the pharmacological study of isoxazolinone derivatives and such related compounds as neurotoxins, which cause crippling human neuropathy, we reported that TAN was a potential agonist for glutamate receptors (Glu R) and also a causative agent of neuropathy, which is caused by eating the grass pea seeds, whereas BIA had almost no activity.10–12 Therefore, we have focused on the synthesis of an isomer, OIS (1), to clarify the structure–activity relationship of isoxazolinone compounds. We now report the synthesis and pharmacological activity of 1 in comparison with its two isomers and other related compounds.

Results and Discussion

Synthesis of OIS (1) OIS (1) was synthesized by a Mitsunobu reaction13 of isoxazolin-5-one (4) with N-Boc-l-serine tert-buty1 ester (5) and subsequent deprotection of the coupling product (6), as shown in Chart 1. Its structure was elucidated by spectroscopic analyses. The IR spectrum of 1 showed an absorption band for carbonyl functionality (1689 cm⁻¹) of an amino acid. The 1H-NMR spectrum revealed two doublet signals of aromatic protons of the isoxazole ring at δ 5.46 (d, 1H, J=2.2 Hz, C₂H₃) and 8.17 (d, 1H, J=2.2 Hz, C₄H), and two multiplet signals of one methine group at the α-position of an amino acid at δ 4.50 (m, 1H) and the adjacent oxymethylene group at δ 4.29 (m, 2H). The 13C-NMR spectrum also showed three signals (δ 79.3, 154.8, 173.2) due to an isoxazole skeleton, together with three signals (δ 54.5, 71.2, 171.7) of serine moiety. A downfield shifted carbon signal at δ 71.2, which does not appear in the spectrum of BIA (2) or TAN (3), clearly indicated that this carbon is bonded to the isoxazole ring through an oxygen atom (Fig. 1). Information concerning the coupling position was obtained from the heteronuclear multiple bond correlation (HMBC) spectrum of protected OIS (6), in which a cross-peak was observed between the oxymethylene proton (δ 4.54) and quaternary carbon (δ 172.9) of the isoxazole nucleus, indicating that a serine moiety is attached to the C-5 position of the isoxazole ring. This structure was reinforced by comparison of the previously observed NMR data for 2 and 3.13 Finally, the structure of newly synthesized 1 was concluded to be a positional isomer of 2 and 3, and is represented by the structure formula in Chart 1.

![Chart 1. Synthetic Routes of OIS (1)](image)

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Pharmacological Activity of OIS (1) 

The pharmacological activity of OIS (1) was examined for its activity on Glu R and Glu T by using a Xenopus oocyte-expressing system in comparison with its two isomers, BIA and TAN, and the results are summarized in Table 1. Macrosopic currents were recorded using a two-electrode voltage-clamp method as described previously.11,12) In the voltage-clamp experiments using cloned rat Glu R and Glu T expressed in Xenopus oocytes, 1 at 0.1 mM showed weak substrate activity on an excitatory amino acid carrier 1 (EAAC 1), a neuron-type Glu T, while 2 had no activity. OIS (1) also had slight agonistic activity on the glycine site of EAAC 1-type Glu T (Table 1). In summary, 1 had moderate activity toward the glycine site of the NMDA receptor as well as toward the glycine site of a neuron-type Glu T, while 2 showed no activity toward the EAAC 1-type Glu T in sharp contrast to its isoergic activity toward the glycine site of the NMDA receptor as well as toward the glycine site of the AMP A-subtype of Glu R composed of 1A and 2B subunits. However, 3 had a potential activity against all Glu R and Glu T examined, and β-ODAP also showed agonistic activity on the AMPA receptor, while 2 showed no activity toward Glu R or Glu T (Table 1). In conclusion, a novel isoxazole compound 1 was synthesized from ethyl propiolate (via ethyl malonialdehyde oxime (8), 400.5 mg, 3.07 mmol) as colorless prisms (305.5 mg, 69.2%) in accordance with the method of Sarlo.15) A mixed solution of N-Boc-serine (9, 1,003 g, 4.89 mmol) and N,N-dimethylformamide di-tert-butylicarbonate (DFBA, 6,21 ml, d=0.848, 25.9 mmol) in dry benzene (8.0 ml) was refluxed for 19 h under Ar. To the mixture, 5% aq. NaHCO3 was added and stirred for 30 min, then an adequate amount of CH2OH was added to give one layer. After extraction with AcOEt, its fraction was rinsed 3 times with H2O and once with sat. NaCl aq., which was dried over MgSO4 and filtered. The AcOEt solution was concentrated in vacuo to give a yellow oil (1.427 g), which was purified by CC [SiO2, Kieselgel Art. 7734, 60 (230—400 mesh) (Merck)]. 1 was synthesized from ethyl propiolate (via ethyl malonialdehyde oxime (8), 400.5 mg, 3.07 mmol) as colorless prisms (305.5 mg, 69.2%) in accordance with the method of Sarlo.15) After extraction with AcOEt, its fraction was rinsed 3 times with H2O and once with sat. NaCl aq., which was dried over MgSO4 and filtered. The AcOEt solution was concentrated in vacuo to give a yellow oil (1.427 g), which was purified by CC [SiO2, Kieselgel Art. 7734, 60 (230—400 mesh) (Merck)].

**Experimental**

Melting points were measured on a Yanagimoto apparatus and are uncorrected. IR spectra were recorded on a JASCO FT/IR-300E spectrometer by the diffuse reflection measurement method. NMR spectra were measured on a JEOL GSX-500er (500 MHz for 1H and 125 MHz for 13C) in CDCl3 solution and on a JEOL GSX-400er (400 MHz for 1H and 100 MHz for 13C) in D2O solution, and chemical shifts were reported in δ (ppm) from tetramethylsilane (TMS) as an internal standard. FAB-MS were recorded on a JEOL JMS-HX110A spectrometer in a nitrobenzylalcohol (NBA) matrix in the positive ion mode. The electrospray ionization-mass spectra (ESI-MS) were obtained on a JEOL JMS-700T spectrometer in H2O:CH3CN:CH2OH:AcOH=33:33:6:1. TLC: Kieselgel 60 F254, 0.25 mm (Merck). Column chromatography (CC): Kieselgel 60 (230—400 mesh) (Merck).

BIA (2) was obtained from plants as described previously.11) TAN (3) and β-ODAP were purchased from Takeda Chemical Ind., Ltd., and from Tocris Cookson, Ltd., respectively. All chemicals used in the pharmacological study were dissolved in glass-distilled water.

**Isoxazolin-5-one (4)** 

was synthesized from ethyl propiolate (via ethyl malonialdehyde oxime (8), 400.5 mg, 3.07 mmol) as colorless prisms (305.5 mg, 69.2%) in accordance with the method of Sarlo.15) After extraction with AcOEt, its fraction was rinsed 3 times with H2O and once with sat. NaCl aq., which was dried over MgSO4 and filtered. The AcOEt solution was concentrated in vacuo to give a yellow oil (1.427 g), which was purified by CC [SiO2, Kieselgel Art. 7734, n-hexane:AcOEt=2:1 (v/v)] to give its formyl ester (5) as a by-product.

**N-Boc-serine tert-Butyl Ester (5)** 

Colorless prisms (837.2 mg, 65.8%): mp 79.0—83.5 °C. IR (CHCl3) νnm cm−1: 3432, 1709. 1H-NMR (500 MHz): δ 1.38 (s, 9H, tert-Bu). 1.41 (s, 9H, tert-Bu), 2.62 (s, 1H, OH), 3.82 (d, 2H, J=3.4 Hz, CH2), 4.18 (br s, 1H, CH), 5.41 (d, 1H, J=1.0 Hz, NH). 13C-NMR (125 MHz): δ 27.9 (CH), 28.3 (3CH3), 56.3 (CH3), 63.9 (CH), 80.1 (O-C), 82.6 (O-C), 150.6 (O-C), 170.0 (O-C). FAB-MS m/z: 262 (MH+), 206 (MH+—tert-Bu), 150 (M+—2-tert-Bu).

**N-Boc-formyl-tert-serine tert-Butyl Ester (5)**: Colorless prisms (23.0 mg): IR (CHCl3) νnm cm−1: 3438, 1730. 1H-NMR (500 MHz): δ 1.38 (s, 9H, tert-Bu), 1.40 (s, 9H, tert-Bu), 4.35 (dd, 1H, J=3.0, 11.0 Hz, CH), 4.40 (m, 1H, CH), 4.46 (dd, 1H, J=3.0, 11.0 Hz, CH), 5.23 (d, 1H, J=7.1 Hz, CH), 7.98 (s, 1H, CHO). 13C-NMR (125 MHz): δ 27.9 (3CH3), 28.3 (3CH3), 53.2 (CH), 64.1 (CH2), 80.2 (O-C), 83.0 (O-C), 155.1 (C=O), 160.3 (HC=O), 168.4 (C=O). FAB-MS m/z: 290 (MH+), 234 (MH+—tert-Bu), 178 (MH+—2-tert-Bu).
al., then rinsed 2—3 times with ether after removing the solvent. The resulting yellow oil was applied to CC [SiO₂, Kieselgel, ethanol : H₂O = 4 : 1 (v/v)], and 1 was obtained as a colorless powder (7.5 mg, quant.): mp 109.5—112.0 °C. IR (nujol) νmax cm⁻¹: 3377, 1689, 1596, 1459. ¹H-NMR (400 MHz): δ 4.10 (m, 1H, CH), 4.56 (m, 2H, CH₂), 5.46 (d, 1H, J=2.2 Hz, C₄H), 8.17 (d, 1H, J=2.2 Hz, C₃H). ¹³C-NMR (100 MHz): δ 54.5 (C₈H), 71.2 (CH₂), 79.3 (C₄H), 154.8 (C₃H), 171.7 (C₉=O), 173.2 (C₅-O). ESI-MS m/z: 173 (MH⁺).

Pharmacological Activity Assay  This assay of newly synthesized 1 and related compounds was performed using a Xenopus oocyte-expressing system as previously described.¹¹,¹²)

Amino Acid Analysis  Selected Lathyrus and Pisum seeds were germinated in the dark at 25—26 °C. After 6 or 7 d, the seedlings were collected and extracted in 75% EtOH. Detection of 1 in the seedlings and seeds was attempted using an automatic amino acid analyzer (Hitachi 835-10) equipped with a UV detector (265 nm) under standard operating conditions as described previously:¹⁴) 1 was eluted at about 31 min from the column, and 2 and 3 were eluted at about 23 and 37 min, respectively, at a flow rate of 0.275 ml per min.

References