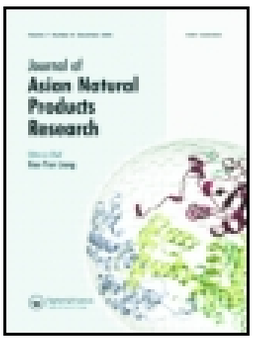

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Ferulic acid esters of glucosylglucose from *Allium macrostemon* Bunge

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ABSTRACT

Three new ferulic acid esters of glucosylglucose, 1-*O*-(*E*)-feruloyl-β-*d*-glucopyranosyl (1-2)-[β- *d*-glucopyranosyl (1-6)]-β-*d*-glucopyranose (allimacronoid A, **1**), 1-*O*-(*E*)-feruloyl-{β-*d*-glucopyranosyl (1-4)-[β-*d*-glucopyranosyl (1-2)]}-[β- *d*-glucopyranosyl (1-6)]-β-*d*-glucopyranose (allimacronoid B, **2**), and 1-*O*-(*E*)-feruloyl-{β-*d*-glucopyranosyl (1-6)-[β-*d*-glucopyranosyl (1-2)]}-[β- *d*-glucopyranosyl (1-6)]-β-*d*-glucopyranose (allimacronoid C, **3**) were isolated together with tuberonoid A (**4**), from the leaves of *Allium macrostemon* Bunge. The chemical structures were elucidated based on the analyses of the spectroscopic and chemical data.

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KEYWORDS
Amaryllidaceae; *Allium macrostemon*; ferulic acid; glucosylglucose; ester

1. Introduction

Allium macrostemon Bunge (Amaryllidaceae), known as wild onion, is widely distributed in East Asian countries. Some steroidal saponins have been isolated from the bulb [1], which showed various biological activities [2–5] such as acute myocardial ischemia, hyperglycemia, hyperlipidemia, and visceral obesity, etc. In Japan, the species is well-known edible weed like other *Allium* species such as onion (*A. cepa* L.), garlic (*A. sativum* L.), and Welsh onion (*A. fistulosum* L.), etc. Nakane *et al.* reported the isolation and HPLC analysis of the flavonol glycoside constituents in the aerial part (leaf) of this plant [6]. In the present chemical research on the phenolic compounds in the leaves of this species, three new ferulic acid esters of glucosylglucose (**1–3**) were isolated, together with a known one tuberonoid A (**4**) [7], and the chemical structures were elucidated.

2. Results and discussion

The known compound **4** was identified as tuberonoid A {1-*O*-(*E*)-feruloyl-β-*d*-glucopyranosyl (1-2)-β-*d*-glucopyranose}, which was isolated from *A. tuberosum*, by comparison of the spectroscopic data with those of reported [7].

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A new compound **1** gave a quasi-molecular ion peak at *m/z* 679.2100 $[M-H]^-$, which corresponded to the molecular formula $C_{28}H_{40}O_{19}$. The ^{1}H-NMR spectrum (Table 1) of compound **1** showed ABX-type (δ 6.81, J = 8.2 Hz, 1H; δ 7.11 J = 2.0, 8.2 Hz, 1H and δ 7.23, J = 2.0 Hz, 1H) aromatic proton signals together with *trans*-olefinic (δ 6.42 and 7.72, J = 15.8 Hz, each 1H) and a methoxyl (δ 3.90, 3H) signals and three sugar anomeric (δ 5.69, J = 7.8 Hz, δ 4.59, J = 7.8 Hz, and δ 4.33, J = 7.8 Hz) proton signals. In the ^{13}C-NMR spectrum of **1** (Table 2), 9 carbon signals of an aglycone (δ 111.9, 114.9, 116.5, 124.5, 127.5, 148.3, 149.4, 151.0, and 167.4), together with a methoxyl signal (δ 56.5), which were attributable to ferulic acid [7], were observed, as well as other sugar signals (18 carbons), which indicated the presence of 3 hexose moieties. The nuclear magnetic resonance (NMR) data of **1** were very similar to those of **4**, with the major difference of the existence of additional sugar unit, showing **1** to be as a ferulic acid ester of triglycoside. By the method of Tanaka *et al.* [8], the hexose components of **1** were identified as *d*-glucose. The positions of the glucoses

Table 1. ^{1}H NMR spectral data (δ) of compounds **1–3** (CD_3OD).

No.	Compound **1**	Compound **2**	Compound **3**
Ferulic acid			
2	7.23 (1H, d, 2.0)	7.22 (1H, d, 1.9)	7.23 (1H, d, 1.9)
5	6.81 (1H, d, 8.2)	6.81 (1H, d, 8.2)	6.83(1H, d, 8.2)
6	7.11 (1H, dd, 2.0, 8.2)	7.11 (1H, dd, 1.9, 8.2)	7.14 (1H, dd, 1.9, 8.2)
7	7.72 (1H, d, 15.8)	7.72 (1H, d, 16.1)	7.71 (1H, d, 15.9)
8	6.42 (1H, d, 15.8)	6.42 (1H, d, 16.1)	6.41 (1H, d, 15.9)
OMe	3.90 (3H, s)	3.90 (3H, s)	3.91 (3H, s)
Glucose-1			
1	5.69 (1H, d, 7.8)	5.71 (1H, d, 7.8)	5.71 (1H, d, 8.1)
2	3.61–3.69 (1H, m)	3.62–3.68 (1H, m)	3.79–3.81 (1H, m)
3	3.61–3.69 (1H, m)	3.62–3.68 (1H, m)	3.64–3.69 (1H, m)
4	3.49 (1H, t, 9.5)	3.47–3.54 (1H, m)	3.54 (1H, t, 8.8)
5	3.54–3.60 (1H, m)	3.55–3.62 (1H, m)	3.56–3.62 (1H, m)
6	3.78 (1H, dd, 11.5, 5.1)	3.74–3.79 (1H, m)	3.77–3.82 (1H, m)
	4.17 (1H, dd, 11.5, 1.7)	4.17 (1H, dd, 11.5, 1.7)	4.16 (1H, dd, 11.4, 1.6)
Glucose-2			
1′	4.59 (1H, d, 7.8)	4.61 (1H, d, 7.8)	4.61 (1H, d, 7.8)
2′	3.18 (1H, dd, 7.8, 8.5)	3.22–3.26 (1H, m)	3.16–3.20 (1H, m)
3′	3.36 (1H, t, 8.5)	3.51–3.54 (1H, m)	3.31–3.38 (1H, m)
4′	3.30–3.34 (1H, m)	3.51–3.55 (1H, m)	3.30–3.34 (1H, m)
5′	3.22–3.29 (1H, m)	3.32–3.38 (1H, m)	3.37–3.42 (1H, m)
6′	3.54–3.60 (1H, m)	3.69–3.75 (2H, m)	3.68–3.73 (1H, m)
	3.61–3.67 (1H, m)		4.00 (1H, dd, 11.6, 2.0)
Glucose-3			
1″	4.33 (1H, d, 7.8)	4.31 (1H, d, 8.8)	4.34 (1H, d, 7.8)
2″	3.20 (1H, t, 7.8)	3.20 (1H, t, 8.8)	3.19–3.26 (1H, m)
3″	3.22–3.29 (1H, m)	3.25–3.30 (1H, m)	3.31–3.38 (1H, m)
4″	3.22–3.29 (1H, m)	3.25–3.30 (1H, m)	3.22–3.30 (1H, m)
5″	3.22–3.29 (1H, m)	3.30–3.35 (1H, m)	3.21–3.28 (1H, m)
6″	3.61–3.69 (1H, m)	3.62–3.68 (1H, m)	3.60–3.65 (1H, m)
	3.84 (1H, dd, 11.7, 1.5)	3.84 (1H, br.d, 10.7)	3.81–3.86 (1H, m)
Glucose-4			
1‴		4.35 (1H, d, 8.1)	4.42 (1H, d, 7.8)
2‴		3.17 (1H, t, 8.1)	3.16 (1H, t, 7.8)
3‴		3.25–3.30 (1H, m)	3.31–3.38 (1H, m)
4‴		3.25–3.30 (1H, m)	3.22–3.30 (1H, m)
5‴		3.25–3.30 (1H, m)	3.21–3.28 (1H, m)
6‴		3.57–3.68 (2H, m)	3.64–3.68 (1H, m)
			3.75–3.78 (1H, m)

Table 2. ^{13}C NMR spectral data (δ) of compounds **1–3** (CD_3OD).

No.	Compound **1**	Compound **2**	Compound **3**
Ferulic acid			
1	127.5	127.5	127.5
2	111.9	111.9	112.1
3	149.4	149.4	149.5
4	151.0	151.0	151.1
5	116.5	116.6	116.6
6	124.5	124.5	124.5
7	148.3	148.4	148.3
8	114.9	114.9	115.0
9	167.4	167.3	167.4
OMe	56.5	56.5	56.6
Glucose-1			
1	94.4	94.3	94.5
2	82.7	83.2	81.5
3	77.5*	77.4	77.6
4	70.6	70.6	70.6
5	78.0*	77.6	77.4
6	69.5	69.4	69.5
Glucose-2			
1′	105.6	105.6	104.8
2′	75.9	75.7	75.7
3′	77.6*	76.0	77.7*
4′	71.0	80.3	71.4
5′	78.0*	76.5	77.3
6′	62.2	61.5	69.5
Glucose-3			
1″	104.5	104.5*	104.6
2″	75.1	75.1	75.0
3″	77.7*	77.8**	77.8*
4″	71.5	71.5	71.5
5″	78.0*	77.9**	77.8*
6″	62.7	62.7	62.6
Glucose-4			
1‴		104.6*	104.5
2‴		74.9	75.0
3‴		77.9**	77.9*
4‴		71.3	71.5
5‴		78.0**	78.0*
6‴		62.4	62.7

*, ** assignments bearing the same superscript may be interchangeable in the same column.

were confirmed by NMR (COSY, HSQC, and HMBC) spectral data of **1**. In particular, the HMBC spectrum showed the correlations originated from the carbon linkages between glucose C-1 – ferulic acid C-9, glucose C-1′ – glucose C-2, and glucose C-1″ – glucose C-6 (Figure 1). The large *J* values of three anomeric proton signals in the ^{1}H-NMR spectrum of **1** (Table 1) also confirmed the β configurations. From the data mentioned, **1** was concluded to be as 1-*O*-(*E*)-feruloyl-β-*d*-glucopyranosyl (1–2)-[β- *d*-glucopyranosyl (1–6)]-β-*d*-glucopyranose and named as allimacronoid A (**1**).

Compound **2** showed a quasi-molecular ion peak at *m/z* 841.2620 $[M-H]^-$ (corresponded to the molecular formula $C_{34}H_{50}O_{24}$). The ^{1}H- and ^{13}C-NMR spectral data of **2** (Tables 1 and 2) whose assignment was also certified by COSY, HSQC, and HMBC spectra, were similar to those of **1** with the difference of the signals coming from an additional hexose moiety, suggesting **2** to be as a ferulic acid ester of tetraglycoside. The sugar components of

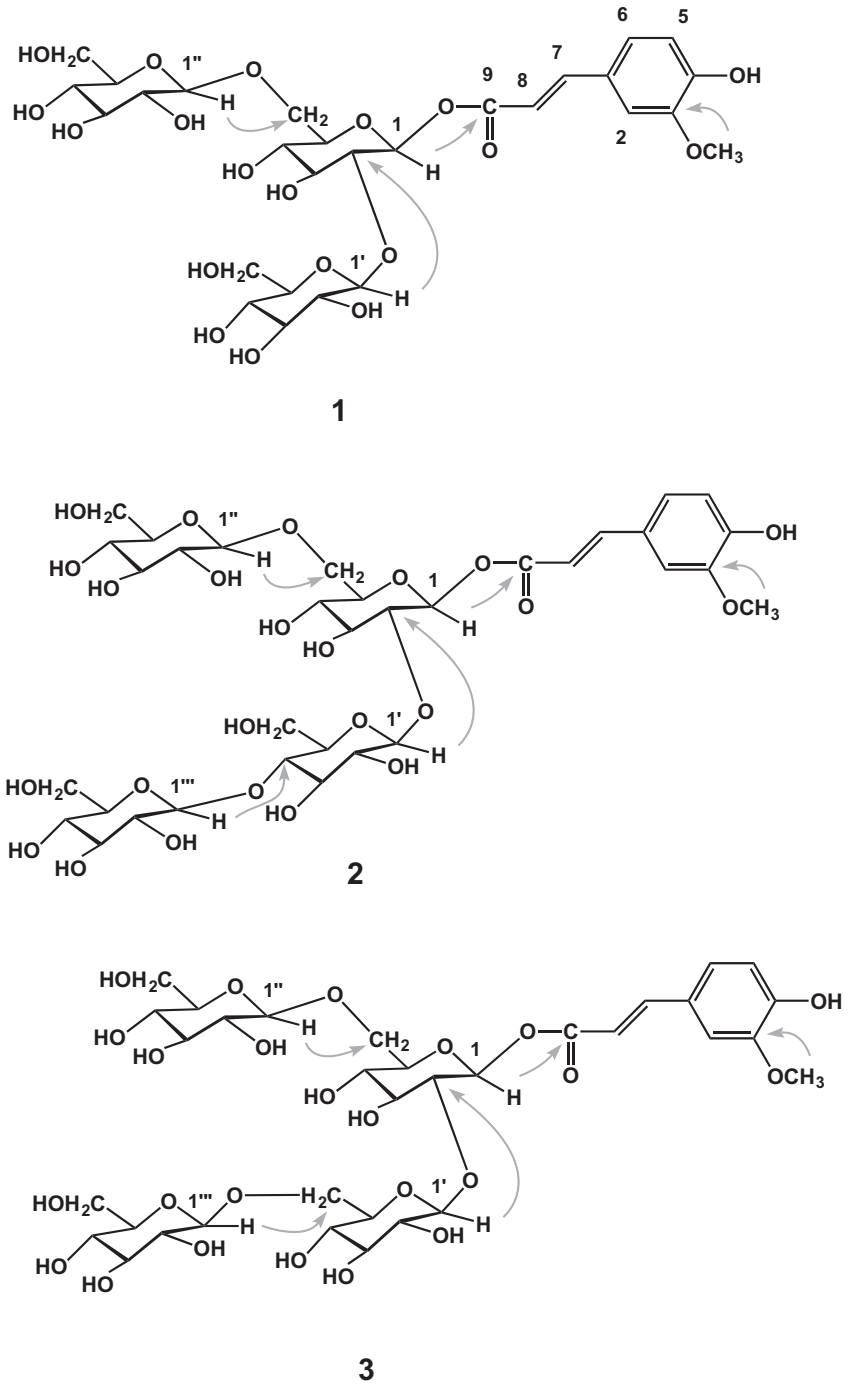

Figure 1. Chemical structures and the selected HMBC correlations (H→C) of **1–3**.

2 were identified as *d*-glucose by HPLC analysis [8]. The HMBC spectrum of **2** also showed the correlations arising from the carbon linkages between glucose C-1 – ferulic acid C-9, glucose C-1′ – glucose C-2, glucose C-1″ – glucose C-6 and glucose C-1‴ – glucose C-4′

(Figure 1). The *J* values of the four anomeric proton signals in the ^{1}H-NMR spectrum of **2** (Table 1) indicated *β* configurations of the anomeric carbons. From the data described above, **2** was concluded to be 1-*O*-(*E*)-feruloyl-{β-*d*-glucopyranosyl (1-4)-[β-*d*-glucopyranosyl (1-2)]}-[β- *d*-glucopyranosyl (1-6)]-β-*d*-glucopyranose and named as allimacronoid B (**2**).

Compound **3** showed a quasi-molecular ion peak at *m/z* 841.2624 $[M-H]^-$ (corresponded to the molecular formula $C_{34}H_{50}O_{24}$). The ^{1}H- and ^{13}C-NMR spectral data of **3** (Tables 1 and 2, assignment was certified by COSY, HSQC, and HMBC spectra) were almost identical with those of **2**, suggesting **3** to be as a ferulic acid ester of tetraglycoside. The sugar components of **3** were identified as *d*-glucose [8]. The HMBC spectrum of **3** certified the carbon linkages between glucose C-1 – ferulic acid C-9, glucose C-1′ – glucose C-2, glucose C-1″ – glucose C-6, and glucose C-1‴ – glucose C-6′ (Figure 1). The large *J* values of the four anomeric proton signals in the ^{1}H-NMR spectrum (Table 1) indicated β configurations of the anomeric carbons, and **3** was elucidated to be as 1-*O*-(*E*)-feruloyl-{β-*d*-glucopyranosyl (1-6)-[β-*d*-glucopyranosyl (1-2)]}-[β- *d*-glucopyranosyl (1-6)]-β-*d*-glucopyranose and named as allimacronoid C (**3**).

Although ferulic acid is commonly distributed in *Allium* species [9], the ester derivatives with oligo-glucose are rarely found. Han *et al.* reported tuberonoid A (**4**) from *A. tuberosum* [7] and some flavonol sophorosides (glucosyl C_1 – C_2 glucose) were isolated from the leaf epidermis of *A. cepa* [10]. It is very noteworthy that *A. macrostemon* Bunge contains various types of ferulic acid esters with oligo-glucose.

3. Experimental

3.1. General experimental procedures

Optical rotation was measured with JASCO DIP-1000 Digital Polarimeter (JASCO Ltd., Tokyo, Japan). Ultra–violet spectra were measured in MeOH using JASCO V-530 UV/VIS spectrophotometer (JASCO Ltd., Tokyo, Japan). Infrared spectra were measured using JASCO FT/IR-400 Spectrophotometer (JASCO Ltd., Tokyo, Japan). NMR spectra were recorded in CD_3OD using a JEOL JNM-A500 spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) (JEOL Ltd., Tokyo, Japan). Negative-ion HRESI-TOF-MS was recorded on an Agilent Technologies 6540 Accurate-Mass Q-TOF LC/MS spectrometer (Agilent Technologies Japan Ltd., Tokyo, Japan). For column chromatography, DIAION HP20SS (Mitsubishi Chemical Corporation), Sephadex LH20 (Pharmacia Corporation) and Cosmosil 140C_{18}-OPN (Nacalai Tesque Inc.) were used. Preparative HPLC was performed on a JASCO preparative HPLC system (Pump: PU-2086 Plus, Detector: UV-2075 Plus, detected at 254 nm) equipped with a ZORBAX SB-C18 (9.4 mm i.d. × 250 mm, Agilent) column, with 30% MeOH (3 ml/min) as the eluent.

3.2. Plant material

The leaves of *Allium macrostemon* Bunge, grown in the campus of Saga University, Japan, were collected in February 2015. The plant material was identified by one of the authors (K. Ohshima, professor of the laboratory of Plant Virology, Saga University). A voucher sample is deposited in the laboratory of Analysis of Plant Metabolism at the Faculty of Agriculture, Saga University.

3.3. Extraction and isolation

The fresh leaves (600 g) of *A. macrostemon* Bunge were extracted with 60% aqueous EtOH (2.5 L) for 2 days. The solvent was concentrated *in vacuo* and subjected to a column of Cosmosil 140C_{18}-OPN, which was eluted with H_2O containing increasing amount of MeOH to afford three fractions of Fr. 1 (15 g), Fr. 2 (8 g), and Fr. 3 (8 g). Fr. 1 was purified with Sephadex LH20 (60% aqueous EtOH) and DIAION HP20SS (stepwise elution with H_2O and MeOH) column chromatographies to afford two fractions (Fr. 1-1 and Fr. 1-2). Fr. 1-1 (1 g) was applied to Cosmosil 140C_{18}-OPN (stepwise elution with H_2O and MeOH) and DIAION HP20SS (stepwise elution with H_2O and MeOH) column chromatographies to afford two fractions (Fr. 1-1-1 and Fr.1-1-2). Fr. 1-1-1 (0.1 g) was purified by preparative HPLC separation to give **3** (6 mg, Rt = 6.5 min). Fr. 1-1-2 (0.3 g) was purified by preparative HPLC separation to give **1** (16 mg, Rt = 9.5 min) and **2** (10 mg, Rt = 7.3 min). Fr. 1-2 (3 g) was applied to Cosmosil 140C_{18}-OPN (stepwise elution with H_2O and MeOH) column chromatography, followed by preparative HPLC separation to give **4** (36 mg, Rt = 13.5 min).

3.3.1. Allimacronoid A (1)

Off-white amorphous powder; $[\alpha]_D^{21}$ −36.0 (*c* 0.13, MeOH). UV λ_{max}^{MeOH} nm (log ε): 329 (4.25). IR ν_{max} cm^{-1}: 3379, 2924, 1712, 1631, 1596, 1516, 1276, 1165, 1089, 897, 579. ^{1}H-NMR spectral data, see Table 1. ^{13}C-NMR spectral data, see Table 2. HRESI-TOF-MS (negative-ion mode) *m/z*: 679.2100 $[M-H]^-$ (calcd for $C_{28}H_{39}O_{19}$, 679.2085).

3.3.2. Allimacronoid B (2)

Off-white amorphous powder, $[\alpha]_D^{18}$ −13.9 (*c* 0.09, MeOH). UV λ_{max}^{MeOH} nm (log ε): 330 (4.00). IR ν_{max} cm^{-1}: 3439, 2923, 1712, 1630, 1595, 1516, 1430, 1373, 1275, 1164, 1092, 635. ^{1}H-NMR spectral data, see Table 1. ^{13}C-NMR spectral data, see Table 2. HRESI-TOF-MS (negative-ion mode) *m/z*: 841.2620 $[M-H]^-$ (calcd for $C_{34}H_{49}O_{24}$, 841.2613).

3.3.3. Allimacronoid C (3)

Off-white amorphous powder, $[\alpha]_D^{18}$ −21.7 (*c* 0.13, MeOH). UV λ_{max}^{MeOH} nm (log ε): 329 (4.14). IR ν_{max} cm^{-1}: 3415, 2921, 1711, 1630, 1594, 1515, 1271, 1065, 1029. ^{1}H-NMR spectral data, see Table 1. ^{13}C-NMR spectral data, see Table 2. HRESI-TOF-MS (negative-ion mode) *m/z*: 841.2624 $[M-H]^-$ (calcd for $C_{34}H_{49}O_{24}$, 841.2613).

3.4. HPLC-QTOF-MS analysis of 1–3

Negative-ion HRESI-TOF-MS of **1–3** was obtained in the HPLC-QTOF-MS analysis as following conditions. HPLC system (Agilent 1100 series); column: Agilent Eclipse Plus C18 (2.1 mm i.d. × 100 mm, 1.8 μm), mobile phase: 0.1% formic acid with 2.5 mM $AcONH_4$-CH_3CN [90:10 (0 min)→10:90 (20 min)], flow rate: 0.2 ml/min, column temperature: 40 °C, detection: 280 nm (UV), retention times (min): **3** (2.0), **2** (2.4) and **1** (3.0). TOFMS system (Agilent G1969A); ionization: ESI (negative), drying gas: N_2, 350 °C, 10 L/min, nebulizer gas: N_2, 50 psig, capillary: 4000 V, fragmentor: 150 V, mass range: 50–1700 *m/z*.

3.5. Identification of sugar moieties of 1–3

Identification of the sugar moieties of **1–3** was done by the following analytical method of Tanaka *et al.* [8]. A solution of **1–3** (0.5 mg) in 0.5 M HCl (0.1 ml) was heated at 95 °C in a screw-capped vial for 2 h. The mixture was neutralized by addition of Amberlite IRA400 (OH^- form) and filtered. The filtrate was dried *in vacuo*, dissolved in 0.1 ml of pyridine containing L-cysteine methyl ester (5 mg/ml) and reacted at 60 °C for 1 h. To the mixture, a solution (0.1 ml) of *o*-toryl isothiocyanate in pyridine (5 mg/ml) was added and it was heated at 60 °C for 1 h. The final mixture was directly analyzed by HPLC [Cosmosil $5C_{18}$ AR II (250 × 4.6 mm i.d., Nacalai Tesque Inc.); 25% CH_3CN in 50 mM H_3PO_4; flow rate, 0.8 ml/min; column temperature, 35 °C; detection, 250 nm]. The t_Rs of the peak at 15.84 min for **1**, 15.83 for **2**, and 15.89 min for **3** coincided with that of *d*-glucose (15.82 min).

Disclosure statement

No potential conflict of interest was reported by the authors.

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