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CARBOHYDRATE ESTERS OF CINNAMIC ACID FROM FRUITS OF *PHYSALIS PERUVIANA*, *PSIDIUM GUAJAVA* AND *VACCINIUM VITIS-IDAEA*

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Key Word Index—*Physalis peruviana*; Solanaceae; cape gooseberry; *Psidium guajava*; Myrtaceae; guava; *Vaccinium vitis-idaea*; Ericaceae; cowberry; cinnamic acid carbohydrate ester; 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose; 1-*O*-*trans*-cinnamoyl-β-D-gentiobiose; 1-*O*-*trans*-cinnamoyl-α-L-arabinofuranosyl-(1 → 6)-β-D-glucopyranose.

Abstract—1-*O*-*trans*-Cinnamoyl-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranose was isolated from fruits of *Physalis peruviana* and 1-*O*-*trans*-cinnamoyl-α-L-arabinofuranosyl-(1 → 6)-β-D-glucopyranose was obtained from fruits of *Psidium guajava*. Fruits of *Vaccinium vitis-idaea* and *P. guajava* were found to be rich sources of 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Solid phase extracts of fruits of *Physalis peruviana* (cape gooseberry), *Psidium guajava* (guava) and *Vaccinium vitis-idaea* (cowberry) were analysed as part of our investigations on natural progenitors of cinnamic acid-derived volatiles in fruits [1]. HPLC separation and diode array detection indicated the presence of cinnamic acid 1-*O*-glucopyranose ester (**1**) and the existence of two novel cinnamic acid derivatives. The structural elucidation of the latter is reported herein.

RESULTS AND DISCUSSION

1-*O*-*trans*-Cinnamoyl-β-D-glucopyranose (**1**) was obtained from solid phase extracts of homogenized fruits of *P. guajava* and *V. vitis-idaea* (Table 1) by semi-preparative HPLC. The ^{1}H and ^{13}C NMR data, including the ^{1}H COSY and ^{1}H–^{13}C spectra, were consistent with the proposed structure and with data reported previously for the structure of 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose from *Fragaria ananassa* [1]. The IR spectrum of compound **1** corresponded exactly with data detailed in refs [2–4]. Acetylation of **1** afforded the tetraacetate (**1a**), whose particle beam EI-mass spectrum exhibited the $[M]^+$ at *m/z* 478 and the $[M - \text{cinnamic acid}]^+$ ion at *m/z* 331. The natural occurrence of compound **1** has only been reported in *Spiraea thunbergii* [4], *Bauhinia manca* [5] and *Salix sacchalinensis* [6], whereas much evidence is given for its generation after addition of exogenous *trans*-cinnamic acid to plant cell cultures or tissue slices [7–10].

1-*O*-*trans*-Cinnamoyl-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranose (**2**) was isolated from homogenized fruits of *P. peruviana*. The ^{13}C/DEPT spectrum (Table 2) revealed the presence of 21 carbon atoms (two × CH_2, 17 × CH, two × C). By comparison with ^{13}C/DEPT data of **1**, the presence of a disaccharide moiety in the upfield range (12 resonances) and a downfield cinnamoyl group was suggested. The ^{1}H NMR spectrum clearly confirmed the presence of *trans*-cinnamic acid. Proton resonances at δ 6.44 and 7.70 (1H each,

Table 1. Content of compounds **1–3** in fruits

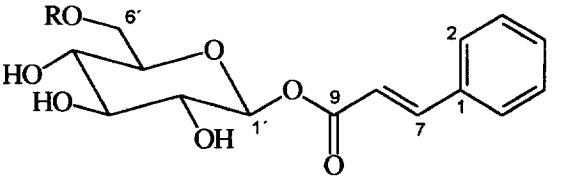
Compound	Source	Content (mg kg^{-1})
1-*O*-*trans*-Cinnamoyl-β-D-glucopyranose (**1**)	Guava (*Psidium guajava*)	7.3
	Strawberry (*Fragaria* × *ananassa*)	67.6*
	Cowberry (*Vaccinium vitis-idaea*)	84.2
1-*O*-*trans*-Cinnamoyl-β-D-gentiobiose (**2**)	Cape gooseberry (*Physalis peruviana*)	47.8
1-*O*-*trans*-Cinnamoyl 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranose (**3**)	Guava (*Psidium guajava*)	3.2

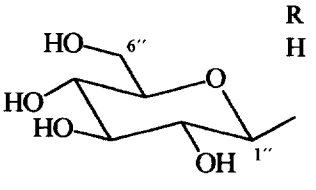
*Data published in ref. [1].

$J = 16.0$ Hz) were attributable to *trans*-olefinic protons. Doublets at δ 4.38 and 5.58 (1H each, $J = 7.5$ Hz) indicated two β-configurated anomeric protons (vicinal H-1′/2′ and H-1″/2″ in *trans* position). The partial similarities of NMR spectra suggested that compound **1** was enlarged by a second glucopyranose moiety to result in **2**. The signal due to C-6′ in **1** (δ 63.1) was shifted downfield by 7.8 ppm and that of C-5′ was shifted upfield by 1.4 ppm. This indicated location of the terminal glucopyranose in **2** at C-6′, corroborated by a downfield shift of the H-6′a and H-6′b signals [11, 12]. The (1→6) linkage was confirmed by ^{1}H-^{13}C NMR data. From the cross-peak correlations, the two carbon signals C-6′ (δ 70.9) and C-1″ (δ 105.3) were associated with their respective protons resonating at δ 3.78/4.10 (H-6′b/a, $J = 12.0$ Hz each) and δ 4.38 (H-1″). The cross-peak between the anomeric proton H-1′ (δ 5.58) and the carbon peak at δ 96.9 (C-1′) proved the β-glycosidic linkage with cinnamic acid. The signals of the remaining gentiobiose protons appeared in a very congested area of the ^{1}H NMR spectrum (δ 3.15–3.76), preventing their complete assignment. Definitive evidence for the proposed structure therefore necessitated the synthesis of compound **2** [3]. ^{1}H NMR and ^{13}C/APT spectra were fully superimposable on those of natural **2**. Remaining assignments of ^{13}C NMR gentiobiose signals were achieved by analyzing published data of phenolic gentiobiosides [11, 12]. Compound **2** shared several UV (λ_{max} 287 nm, HPLC-DAD) and IR properties (see Experimental) with **1**. Glycoconjugation caused a bathochrome shift of 10 nm of the UV spectrum compared to cinnamic acid (λ_{max} 277 nm, HPLC-DAD). On acetylation, **2** yielded the heptaacetate (**2a**). The particle beam EI-mass spectrum showed the $[M]^+$ at m/z 766, the $[M -$ cinnamic acid$]^+$ ion at m/z 619 and the $[M -$ cinnamic acid $-$ tri-*O*-acetylglucose$]^+$ fragment (terminal tetra-*O*-acetylglucose) at m/z 331. The chromatographic properties of compound **2** were also studied. Cochromatography of natural and synthetic **2** by reverse-phase HPLC showed no differences in retention time. Alkaline hydrolysis of **2**, just as highly specific cleavage by β-glucosidase, afforded cinnamic acid and gentiobiose. The disaccharide was analyzed by HPLC-ED (electrochemical detection). To our knowledge, 1-*O*-*trans*-cinnamoyl-β-D-gentiobiose (**2**) is described as a naturally occurring compound for the first time.

1 - *O* - *trans* - Cinnamoyl - α - L - arabinofuranosyl - (1→6)-β-D-glucopyranose (3) was obtained from fruits of *P. guajava*. ^{13}C/DEPT data (Table 2) revealed the presence of 20 carbon atoms (two × CH_2, 16 × CH, two × C). Like compound **2**, partial similarities of the ^{13}C NMR spectra of **1–3** suggested that compound **1** was enlarged by an arabinofuranosyl moiety to result in **3**. ^{13}C/DEPT shift values of five carbon signals were consistent with data on L-α-arabinose [13–15]. The interglycosidic (1→6) linkage between the terminal arabinose and the inner glucose unit was deduced from a downfield shift of C-6′ (63.1 in **1**) by 6.1 ppm and C-5′ (79.5 in **1**) by 1.4 ppm, whereas the other glucose carbon signals were nearly unchanged. The anomeric configuration of L-arabinofuranose was concluded to be α, because its H-1″ proton signal appeared as a singlet at δ 4.88 [13]. Remaining ^{1}H and ^{13}C NMR data of **3** were essentially the same as those for the cinnamoyl-glucose moiety of compound **2**. The IR and UV (HPLC-DAD) data were in accordance with those of compounds **1** and **2**. Acetylation of **3** afforded the hexaacetate (**3a**), whose particle beam EI-mass spectrum exhibited the $[M]^+$ at m/z 694 and the $[M -$ cinnamic acid$]^+$ ion at m/z 547. In accordance with its substrate specificity, highly purified β-glucosidase was able to cleave **1** directly and **2** sequentially, but, as expected, no cleavage was observed for **3**. Further confirmation of the L-α-arabinofuranosyl moiety was achieved by HPLC-ED after acid hydrolysis of **3**. Compared to commercial L(+)-arabinose, there were no differences in retention times. To our knowledge, the occurrence of **3** in a natural source has not been reported before.

Comparable to CoA-dependent activation of acyl moieties, 1-*O*-glucose esters of (hydroxy)cinnamic acids are believed to be activated conjugates. They can be used by relevant transferases to give new esters [7]. However, non-enzymatic transesterification is also feasible under alkaline conditions. Spontaneous formation of methyl cinnamate could be observed in a 20 mM methanolic solution (MeOH–H_2O, 3:2) of purified 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose (**1**) at pH 7.8 and elevated temperature (40°). This result corresponds well with earlier studies on the volatile composition of fruits of *P. peruviana* [16]: methanolic and ethanolic aroma extracts of cape gooseberry (neutralized with excess $NaHCO_3$) exhibited increased concentrations of methyl and ethyl cinnamate, respectively. Thus, the presence of activated cinnamoyl moieties was concluded [16]. Regarding the chemical reactivity of the





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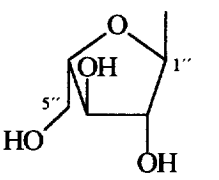

3

Table 2. ^{13}C NMR data of compounds **1–3** (300 MHz, D_2O)

		1	2	3
Cinnamic acid	1	136.4	136.4	136.4
	2, 6	131.3	131.3	131.3
	3, 5	131.8	131.9	131.8
	4	134.0	134.0	133.9
	7	150.7	150.8	150.7
	8	118.7	118.7	118.7
	9	170.1	169.9	170.0
Glucose	1′	96.9	96.9	96.8
	2′	74.8	75.8	74.7
	3′	78.2	78.4[a]	78.4
	4′	71.9	72.3	72.0
	5′	79.5	78.1	78.1
	6′	63.1	70.9	69.2
Other sugar	1″	—	Glucose 105.3	Arabinose 110.8
	2″	—	74.7	83.7
	3″	—	78.5[a]	79.3
	4″	—	71.7	86.6
	5″	—	78.6	63.8
	6″	—	63.5	—

[a]Assignments may be reversed.

ester bond, there certainly are no significant differences between the 1-*O*-gentiobiose ester (**2**) isolated from *P. peruviana* and compound **1**.

EXPERIMENTAL

General. ^1H and ^{13}C NMR (DEPT, APT): 300 MHz (D_2O). Chemical shifts are quoted in δ units relative to TMS. ^1H COSY and ^{13}C-^1H correlation: standard pulse sequences employed. Particle beam LC/EIMS at 70 eV was performed in columnless mode. Operation details: *n*-hexane as mobile phase, flow rate 0.2 ml min^{-1}, desolvation chamber temp 70°, He-pressure at nebulizer 4.8×10^5 Pa. The peracetylation reaction mixts were directly injected (5 μl). IR: KBr.

Plant material. Fruits of Colombian *Physalis peruviana* L., Brazilian *Psidium guajava* L. and German *Vaccinium vitis-idaea* L. were purchased from the local market and stored at −70° until used for analysis.

Isolation and purification. Whole frozen fruits (250 g) were blended with 250 ml of MeOH–0.1 M $HClO_4$ (2:5) for 5 min at 14 000 r.p.m. The homogenate was centrifuged for 45 min at 1° and 2900 *g*. In a batch process, the supernatant was solid phase-extracted with 30 g of Lewatit OC 1064 [17] (preconditioned) and the resin rinsed with H_2O (3 × 200 ml). The Me_2CO eluate (5 × 100 ml) was concd *in vacuo* (30°) to 5 ml. Semi-prep. HPLC was carried out on ODS-Hypersil 5 μm (column 250 × 16 mm i.d.) at a flow rate of 10 ml min^{-1}. Linear gradient: 100% H_2O to 100% MeCN–H_2O (2:3) within 100 min, UV-detection at 280 nm. Frs between 440 and 460 ml contained **2**, compound **3** eluted between 460 and 480 ml, and **1** between 480 and 500 ml. Complete purification was achieved by subjecting the combined and concd frs to a second semi-prep. HPLC as described above. Fruits of *V. vitis-idaea* L. (500 g) yielded 17 mg of **1**, *P. peruviana* L. (1 kg) yielded 35 mg of **2** and *P. guajava* L. (4 kg) afforded 23 mg of **1** and 10 mg of compound **3**.

Peracetylation. Compounds **1–3** (0.5 mg each) were dissolved in 0.2 ml pyridine–Ac_2O (1:1) and kept 24 hr at ambient temp.

1-O-trans-*Cinnamoyl*-β-D-*glucopyranose* (**1**). Amorphous powder. ^{13}C NMR: Table 2. ^1H NMR (300 MHz, D_2O): δ 3.24–4.00 (6H, obsc, H-2′ to 6′), 5.54 (1H, *d*, J = 7.5 Hz, H-1′), 6.42 (1H, *d*, J = 16.0 Hz, H-8), 7.32 (3H, *m*, H-3, 4, 5), 7.50 (2H, *m*, H-2, 6), 7.68 (1H, *d*, J = 16.0 Hz, H-7). IR ν_{max}^{KBr} cm^{-1}: 3200–3600 (OH), 2925 (C–H), 1717 (>C=O, ester), 1635 (C=C), 1508 (Ar), 1497, 1450, 1310, 950–1270 (C–O and *trans*-C=C), 769 (monosubst. Ar).

Tetraacetate of **1** (**1a**). EIMS (particle beam) 70 eV, scan range *m/z* 105–1000, *m/z* (rel. int.): 478 $[M]^+$ (<1), 436 $[M - CH_2CO]^+$ (<1), 418 $[M - HOAc]^+$ (<1), 376 $[M - HOAc - CH_2CO]^+$ (<1), 358 $[M - HOAc - HOAc]^+$ (<1), 331 [M − cinnamic acid]$^+$ (5), 271 [M − cinnamic acid − HOAc]$^+$ (2), 242 (4), 200 (2), 169 (24), 131 (100).

1-O-trans-*Cinnamoyl*-β-D-*glucopyranosyl*-(1→6)-β-D-*glucopyranose* (**2**). Amorphous powder. ^{13}C NMR: Table 2. ^1H NMR (300 MHz, D_2O): δ 3.15–3.76 (10H, obsc, H-2′ to 5′ and H-2″ to 6″), 3.78 (1H, *d*, J = 12.0 Hz, H-6′b), 4.10 (1H, *d*, J = 12.0 Hz, H-6′a), 4.38 (1H, *d*, J = 7.5 Hz, H-1″), 5.58 (1H, *d*, J = 7.5 Hz, H-1′), 6.44 (1H, *d*, J = 16.0 Hz, H-8), 7.38 (3H, *m*, H-3, 4, 5), 7.52 (2H, *m*, H-2, 6), 7.70 (1H, *d*, J = 16.0 Hz, H-7). IR ν_{max}^{KBr} cm^{-1}: 3200–3600 (OH), 2923 (C–H), 1719 (>C=O, ester), 1635 (C=C), 1511 (Ar), 1497, 1450, 1311, 950–1270 (C–O and *trans*-C=C), 770 (monosubst. Ar).

Heptaacetate of **2** (**2a**). EIMS (particle beam) 70 eV, scan range *m/z* 105–1000, *m/z* (rel. int.): 766 $[M]^+$ (<1), 706 $[M - HOAc]^+$ (<1), 646 $[M - HOAc \times 2]^+$

(<1), 619 [M − cinnamic acid]$^+$ (<1), 586 [M − HOAc × 3]$^+$ (<1), 559 [M − cinnamic acid − HOAc]$^+$ (1), 547 (2), 456 (1), 419 (3), 331 [M − cinnamic acid − Glc(OAc)$_3$]$^+$ (4), 259 [M − cinnamic acid − AcOH × 6]$^+$ (70), 169 (88), 131 (100).

1 - O - trans - *Cinnamoyl* - α - L - *arabinofuranosyl* - (1→6)-β-D-*glucopyranose* (**3**). Amorphous powder. ^{13}C NMR: Table 2. ^{1}H NMR (300 MHz, D_2O): δ 3.30–3.95 (11H, obsc, H-2′ to 6′ and H-2″ to 5″), 4.88 (1H, *s*, H-1″), 5.55 (1H, *d*, *J* = 7.5 Hz, H-1′), 6.42 (1H, *d*, *J* = 16.0 Hz, H-8), 7.30 (3H, *m*, H-3, 4, 5), 7.46 (2H, *m*, H-2, 6), 7.68 (1H, *d*, *J* = 16.0 Hz, H-7); IR ν_{max}^{KBr} cm^{-1}: 3200–3600 (OH), 2926 (C–H), 1716 (>C=O, ester), 1617 (C=C), 1521 (Ar), 1384, 950–1270 (C–O and *trans*-C=C), 769 (monosubst. Ar).

Hexaacetate of **3** (**3a**). EIMS (particle beam) 70 eV, scan range *m/z* 105–1000, *m/z* (rel. int): 694 [M]$^+$ (<1), 635 [M − HOAc]$^+$ (<1), 592 [M − HOAc − CH_2CO]$^+$ (<1), 547 [M − cinnamic acid]$^+$ (<1), 487 [M − cinnamic acid − HOAc]$^+$ (<1), 472 [M − CH_2CO − HOAc × 3]$^+$ (<1), 420 (1), 346 (1), 317 (10), 259 (50), 169 (24), 131 (100).

Synthesis of **1** *and* **2**. The 8-hydroxyquinolinyl-ester of *trans*-cinnamic acid was prepared [3]. To a stirred soln of excess β-D(+)-Glc (1.15 g, 6.38 mmol) (β-gentiobiose (2.18 g, 6.38 mmol) for **2**, respectively) in dry pyridine (100 ml) at 0°, the above cinnamic acid ester (0.58 g, 2.12 mmol) and NaH (60% dispersion, 60 mg, 1.5 mmol) were added. The reaction mixt. was kept 4 days in the cold, pyridine was evapd, the residue dissolved in aq. buffer (pH 4, 20 ml) and the soln extracted with $CHCl_3$ (2 × 20 ml). The aq. phase was concd and submitted to semi-prep. HPLC as described above. Purified **1** was obtained as a solid (yield: 14 mg, 2%). ^{13}C NMR (300 MHz, D_2O-CD_3OD, 8:2): δ 61.5 (C-6′), 77.9 (C-5′), 70.2 (C-4′), 76.6 (C-3′), 73.1 (C-2′), 95.2 (C-1′), 117.0 (C-8), 129.5 (2C, C-2, 6), 130.0 (2C, C-3, 5), 132.2 (C-4), 134.7 (C-1), 148.9 (C-7), 168.2 (C-9). ^{1}H NMR (300 MHz, D_2O): δ 3.16–3.83 (6H, obsc, H-2′ to 6′), 5.52 (1H, d, J = 7.5 Hz, H-1′), 6.44 (1H, *d*, *J* = 16.0 Hz, H-8), 7.30 (3H, *m*, H-3, 4, 5), 7.52 (2H, *m*, H-2, 6), 7.68 (1H, *d*, *J* = 16.0 Hz, H-7). Compound **2** yielded 39 mg (4%). ^{13}C NMR (300 MHz, D_2O): 63.5 (C-6″), 71.0 (C-6′), 71.8 (C-4″), 72.4 (C-4′), 74.8 (C-2″), 75.9 (C-2′), 78.1 (C-5′), 78.4 (C-3′), 78.6 (C-3″), 78.7 (C-5″), 96.9 (C-1′), 105.3 (C-1″), 118.8 (C-8), 131.4 (2C, C-2, 6), 131.9 (2C, C-3, 5), 134.1 (C-4), 136.5 (C-1), 150.9 (C-7), 170.0 (C-9). ^{1}H NMR (300 MHz, D_2O): δ 3.08–3.70 (10H, obsc, H-2′ to 5′ and H-2″ to 6″), 3.72 (1H, *d*, *J* = 12.0 Hz, H-6′b), 4.05 (1H, *d*, *J* = 12.0 Hz, H-6′a), 4.32 (1H, *d*, *J* = 7.5 Hz, H-1″), 5.50 (1H, *d*, *J* = 7.5 Hz, H-1′), 6.40 (1H, *d*, *J* = 16.0 Hz, H-8), 7.31 (3H, *m*, H-3, 4, 5), 7.47 (2H, *m*, H-2, 6), 7.68 (1H, *d*, *J* = 16.0 Hz, H-7).

HPLC-ED analysis of carbohydrate moiety. To aq. solns of **1** and **2** (4 mg ml^{-1}) were added the same vols of 0.2 M NaOH (alkaline hydrolysis). HPLC conditions: RCX-10 anion-exchange column, (250 × 4.1 mm i.d.), 60 mM NaOH as eluent at a flow rate of 1 ml min^{-1}, measuring electrode: gold, pulsed amperometric detection (PAD, pot 1: +0.30 V, 0.73 sec, pot 2: +0.75 V, 0.12 s, pot 3: −0.60 V, 0.48 sec, integr. interval: 0.55–0.73 s). R_t-values: 6.34 min (carbohydrate released by **1**), 6.35 min (β-D(+)-Glc), 11.00 min (carbohydrate liberated by **2**), 11.07 min (β-gentiobiose). Chromatographic separation of L(+)-arabinose from β-D(+)-Glc was achieved by lowering the flow rate to 0.5 ml min^{-1} and cooling the column (2°). Compound **3** (1 mg) was treated with 6 M HCl (60 μl, acid hydrolysis). R_t-values under changed cond.: 11.28 and 11.68 min (carbohydrates released by **3**), 11.23 min (L(+)-arabinose) and 11.50 min (β-D(+)-Glc).

Quantitative HPLC. Prior to HPLC analysis, the initial concd Lewatit extract (1 ml diluted back to 10 ml aq. soln) was passed through a micro-PA column (preconditioned) and 0.2 M NaOH (10 ml) was added (alkaline hydrolysis). Calculation was based on liberated cinnamic acid (ext. standard curve). HPLC was carried out on Nucleosil 120-5-C_{18} (column 250 × 4 mm i.d. rigged out with 11 × 4 mm i.d. guard column) at a flow rate of 1 ml min^{-1}. Linear gradient: 100% A: 0.2 M Na_2HPO_4–citric acid (pH 4) buffer–MeOH (8:2) to 100% B: MeCN–A (25:75) within 30 min, then increased to 100% MeCN within 10 min. UV-detection at 277 nm. R_t-values: 26.8 min (cinnamic acid) (before hydrolysis: 14.0 min (**2**), 16.6 min (**3**) and 17.7 min (**1**)). Transesterification of (**1**): A 0.02 M MeOH–H_2O (3:2) soln of (**1**) was adjusted to pH 7.8 at 40°. Me cinnamate showed R_t 37.75 min, confirmed by addition of authentic compound.

β-*Glucosidase assay*. Compounds **1**–**3** (1 mg each) were dissolved in 0.1 M Na_2HPO_4–citric acid (pH 5) buffer (50 ml). Almond β-glucosidase (1 mg, 36 units) was dissolved in the same buffer (1 ml) and added to 1 ml of sample soln (blank: (pH 5) buffer instead). Incubation: 15 min at 30°, analytical HPLC as described above.

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