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ORIGINAL ARTICLE

# Phenolic extractives in the trunk of *Toxicodendron vernicifluum*: chemical characteristics, contents and radial distribution

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**Abstract** Phenolic extractives in the trunk of *Toxicodendron vernicifluum* (syn. *Rhus verniciflua*) were investigated. Seventeen compounds, gallic acid, protocatechuic acid, (−)-fisetinidol-4β-ol, (−)-fisetinidol-4α-ol, 2-benzyl-2,6,3′,4′-tetrahydroxycoumaran-3-one, (−)-fustin, 1,2,3,6-Tetra-*O*-galloyl-β-D-glucose, (−)-epifustin, (+)-taxifolin, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose, (−)-garbanzol, (−)-fustin-3-*O*-gallate, (−)-epifustin-3-*O*-gallate, fisetin, sulfuretin, quercetin and butein, were identified from the heartwood extractives. It was found that only (+)-taxifolin which had 5,7-dihydroxy A-ring possessed a 3*R* configuration although other flavonoids which had 7-hydroxy A-ring possessed a 3*S* configuration. Quantitative analysis revealed that the total phenolic contents were much higher in the heartwood (5–7 wt%) than in the sapwood and bark (1–2 wt%), and (−)-fustin was the most abundant extractive in the heartwood (1.4–2.4 wt%). For the radial distribution of phenolic extractives, it was generally found that their content was lowest in the sapwood, increased to the highest in the outer heartwood, and then decreased in the inner heartwood.

**Keywords** Flavonoids · Heartwood · Phenolic extractives · Radial distribution · *Toxicodendron vernicifluum*

## Introduction

The lacquer tree, *Toxicodendron vernicifluum* (syn. *Rhus verniciflua*), is economically and culturally important, particularly for the production of lacquer sap, which is used to manufacture lacquerwares and cultural properties [1]. In Japan, the trees are generally felled after taking the sap and their wood was previously used for tubs and fishing net floats. However, they are now little used and many of them have been incinerated [2].

*T. vernicifluum* has distinguishing yellow-colored heartwood, which contains large amounts of phenolic compounds such as fustin, fisetin and other flavonoids [3, 4]. In Korea, the branch and bark of *T. vernicifluum* are used as a food supplement and traditional herbal medicine, and their phenolic extracts reportedly have various pharmacological properties, such as anti-mutagenic [4], antioxidant [5, 6], anti-microbial [6], anti-viral [7], anti-inflammatory [8] and anti-rheumatoid arthritis properties [9]. Accordingly, the unused wood and bark of *T. vernicifluum* would be good sources of valuable phenolic extracts.

To utilize the unused tree as an ingredient of the phenolic extractives, it is important to clarify the chemical characteristics of the extractives and their contents in the tree, but these fundamental properties have yet to be fully studied especially for the tree after taking the sap. In this study, we targeted the trunk of *T. vernicifluum* after taking the sap and examined the chemical characteristics of phenolic extractives in the heartwood, as well as their contents and radial distribution in the trunk.

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## Materials and methods

### Instrumental analyses

NMR spectra were obtained using a JEOL ALPHA-500 spectrometer at room temperature (RT, ~25 °C). FAB-MS spectra were measured on a JEOL HX-110A spectrometer using glycerol as a matrix. Circular dichroism (CD) analyses were performed with a Jasco J-720W spectrometer at RT under nitrogen atmosphere using methanol as a solvent. Optical rotations were measured on a Jasco P-1020 polarimeter. Analytical and preparative HPLC was carried out with Shimadzu Prominence and LC-VP systems. HPLC with UV and CD detectors (HPLC–UV/CD) was performed using a Jasco Gulliver system equipped with a Jasco CD-1595 detector.

### Authentic samples

Gallic acid and protocatechuic acid were purchased from Wako Pure Chemical Industries Ltd. Other authentic samples were purchased from Extrasynthese S.A., except for 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (Sigma-Aldrich Co. LLC).

### Plant materials

Lacquer sap was collected from *T. vernicifluum* by wounding the trunk in June–September, and then the tree was felled in January of the next year. Three felled trees (A, B, C) were taken from three stands in Ibaraki (Ib), Iwate (Iw) and Niigata (Ni) prefectures in Japan. Disks (2–5 cm thick) were cut off from the trunks of the trees at breast height and then manually separated into heartwood, sapwood and bark. To determine the radial distribution of the extractives, the xylem part of the disk from Ni-A, B and C was further separated into the small blocks containing 2–3 annual rings. All samples were air-dried and ground to under 0.5 mm.

### Extraction and isolation of phenolic compounds from heartwood

Heartwood powder from Ib-A (100 g) was extracted three times with 1 L of 70 vol% aqueous acetone at RT for 6–12 h. The extract solution was then evaporated and freeze-dried to give 7.9 g of 70 vol% acetone extracts. A portion (5.0 g) of the extracts was dispersed in 500 mL of deionized water, and then extracted five times with *n*-hexane (400 mL) and ethyl acetate (400 mL), successively. The yields of *n*-hexane, ethyl acetate and water layer fractions were 0.16, 4.26, and 0.86 g, respectively. The ethyl acetate extracts (1 g) were dissolved in 50 vol% aqueous methanol to isolate compounds by preparative HPLC: column, L-column2 ODS (5 μm, 250 × 20 mm i.d., CERI); column temperature, 40 °C; mobile phase A, 1 vol% acetic acid aqueous solution; mobile phase B, methanol; gradient condition, 35–80 vol% of B (0–40 min, linear); flow rate, 5 mL/min; detection, UV at 280 nm. Seventeen compounds were isolated and identified as gallic acid (**1**, 31.1 mg), protocatechuic acid (**2**, 1.0 mg), (−)-fustin (**6**, 134.5 mg), (+)-taxifolin (**9**, 28.7 mg), 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**10**, 63.1 mg), fisetin (**14**, 28.2 mg), sulfuretin (**15**, 16.4 mg), quercetin (**16**, 3.2 mg), butein (**17**, 4.2 mg) and the other compounds described below.

(−)-Fisetinidol-4β-ol (**3**, 2.9 mg): CD (in methanol) [$\theta$] (nm): +0.28 (300), +4,400 (289), −3,900 (275), −390 (250), −6,500 (238), −3,000 (231), −6,600 (220). FAB-MS *m/z*: 291 $[M+H]^+$. $^1$H-NMR [in acetone-$d_6$/$D_2O$ (95/5, v/v)]: $\delta$ 3.89 (1H, dd, $J$ = 3.5, 9.5 Hz, H-3), 4.56 (1H, d, $J$ = 3.5 Hz, H-4), 4.85 (1H, d, $J$ = 9.5 Hz, H-2), 6.24 (1H, d, $J$ = 2.5 Hz, H-8), 6.40 (1H, dd, $J$ = 2.5, 8.0 Hz, H-6), 6.74 (1H, dd, $J$ = 2.0, 8.0 Hz, H-6′), 6.78 (1H, d, $J$ = 8.0 Hz, H-5′), 6.91 (1H, d, $J$ = 2.0 Hz, H-2′), 7.11 (1H, d, $J$ = 8.0 Hz, H-5). $^{13}$C-NMR [in acetone-$d_6$/$D_2O$ (95/5, v/v)]: $\delta$ 66.6 (C-4), 71.32 (C-3), 77.56 (C-2), 102.87 (C-8), 109.19 (C-6), 115.53, 115.56 (C-2′ and 5′), 115.73 (C-4a), 120.18 (C-6′), 131.38 (C-1′), 132.28 (C-5), 145.56 (C-3′), 145.78 (C-4′), 156.12 (C-8a), 159.37 (C-7).

(−)-Fisetinidol-4α-ol (**4**, 7.7 mg): CD (in methanol) [$\theta$] (nm): −44 (300), +2,300 (289), +140 (262), +34,000 (237), +15,000 (224), +18,000 (220). FAB-MS *m/z*: 291 $[M+H]^+$. $^1$H-NMR [in acetone-$d_6$/$D_2O$ (95/5, v/v)]: $\delta$ 3.73 (1H, dd, $J$ = 8.0, 10.0 Hz, H-3), 4.60 (1H, d, $J$ = 10.0 Hz, H-2), 4.65 (1H, d, $J$ = 8.0 Hz, H-4), 6.19 (1H, d, $J$ = 2.5 Hz, H-8), 6.43 (1H, dd, $J$ = 2.5, 8.0 Hz, H-6), 6.76 (1H, dd, $J$ = 1.5, 8.0 Hz, H-6′), 6.79 (1H, d, $J$ = 8.0 Hz, H-5′), 6.93 (1H, d, $J$ = 1.5 Hz, H-2′), 7.27 (1H, d, $J$ = 8.5 Hz, H-5). $^{13}$C-NMR [in acetone-$d_6$/$D_2O$ (95/5, v/v)]: $\delta$ 72.15 (C-4), 74.19 (C-3), 82.03 (C-2), 102.65 (C-8), 109.31 (C-6), 115.49 (C-5′), 115.72 (C-2′), 117.27 (C-4a), 120.57 (C-6′), 129.33 (C-5), 130.87 (C-1′), 145.52 (C-3′), 145.94 (C-4′), 155.83 (C-8a), 158.53 (C-7).

2-Benzyl-2,6,3′,4′-tetrahydroxycoumaran-3-one (**5**, 4.3 mg): FAB-MS *m/z*: 289 $[M+H]^+$. $^1$H-NMR (in $CD_3OD$): $\delta$ 3.01 (1H, d, $J$ = 14.0 Hz, Ha-8), 3.06 (1H, d, $J$ = 14.0 Hz, Hb-8), 6.24 (1H, d, $J$ = 2.0 Hz, H-7), 6.38 (1H, dd, $J$ = 2.0, 8.5 Hz, H-5), 6.46 (1H, dd, $J$ = 2.0, 8.0 Hz, H-6′), 6.51 (1H, d, $J$ = 8.0 Hz, H-5′), 6.61 (1H, d, $J$ = 2.0, Hz, H-2′), 7.28 (1H, d, $J$ = 8.5 Hz, H-4). $^{13}$C-NMR (in $CD_3OD$): $\delta$ 42.33 (C-8), 98.85 (C-7), 107.56 (C-2), 112.63 (C-5), 113.18 (C-3a), 115.82 (C-5′), 118.71 (C-2′), 123.05 (C-6′), 126.28 (C-1′), 127.01 (C-4), 145.12 (C-4′), 145.65 (C-3′), 169.70 (C-6), 174.76 (C-7a), 199.26 (C-3).

1,2,3,6-Tetra-*O*-galloyl-β-D-glucose (**7**, 6.8 mg): FAB-MS *m/z*: 789 $[M+H]^+$. $^1$H-NMR (in $CD_3OD$): $\delta$ 3.95 (1H, t, $J = 9.5$ Hz, H-4), 4.01 (1H, ddd, $J = 2.0$, 4.5, 9.5 Hz, H-5), 4.51 (1H, dd, $J = 4.5$, 12.0 Hz, Ha-6), 4.60 (1H, dd, $J = 2.0$, 12.0 Hz, Hb-6), 5.43 (1H, dd, $J = 8.5$, 10.0 Hz, H-2), 5.57 (1H, dd, $J = 9.5$, 10.0 Hz, H-3), 6.09 (1H, d, $J = 8.5$ Hz, H-1), 6.92 (2H, s, H-2, 6 of galloyl-*O*-2), 7.02 (2H, s, H-2, 6 of galloyl-*O*-1), 7.03 (2H, s, H-2, 6 of galloyl-*O*-3), 7.11 (2H, s, H-2, 6 of galloyl-*O*-6). $^{13}$C-NMR (in $CD_3OD$): $\delta$ 64.00 (C-6), 69.67 (C-4), 72.39 (C-2), 76.50 (C-3), 76.65 (C-5), 93.93 (C-1), 110.24 (C-2, 6 of galloyl-*O*-6), 110.40, 110.43 (C-2, 6 of galloyl-*O*-2 and 3), 110.61 (C-2, 6 of galloyl-*O*-1), 119.89 (C-1 of galloyl-*O*-3), 120.44 (C-1 of galloyl-*O*-2), 121.02 (C-1 of galloyl-*O*-1), 121.25 (C-1 of galloyl-*O*-6), 139.97, 139.99 (C-4 of galloyl-*O*-3 and 6), 140.21 (C-4 of galloyl-*O*-2), 140.69 (C-4 of galloyl-*O*-1), 146.35, 146.37 (C-3, 5 of galloyl-*O*-1 and 2), 146.52, 146.54 (C-3, 5 of galloyl-*O*-3 and 6), 166.33 (C-7 of galloyl-*O*-1), 167.20 (C-7 of galloyl-*O*-2), 167.74 (C-7 of galloyl-*O*-3), 168.18 (C-7 of galloyl-*O*-6).

(−)-Epifustin (**8**, 8.8 mg): FAB-MS *m/z*: 289 $[M+H]^+$. $^1$H-NMR (in $CD_3OD$): $\delta$ 4.27 (1H, d, $J = 3.5$ Hz, H-3), 5.34 (1H, d, $J = 3.5$ Hz, H-2), 6.38 (1H, d, $J = 2.5$ Hz, H-8), 6.50 (1H, dd, $J = 2.5$, 8.5 Hz, H-6), 6.73 (1H, d, $J = 8.5$ Hz, H-5′), 6.81 (1H, dd, $J = 2.0$, 8.5 Hz, H-6′), 6.98 (1H, d, $J = 2.0$ Hz, H-2′), 7.71 (1H, d, $J = 8.5$ Hz, H-5). $^{13}$C-NMR (in $CD_3OD$): $\delta$ 73.83 (C-3), 83.28 (C-2), 103.85 (C-8), 112.00 (C-6), 113.51 (C-4a), 115.91 (C-2′ and 5′), 120.16 (C-6′), 128.99 (C-1′), 130.22 (C-5), 146.08 (C-3′), 146.48 (C-4′), 164.89 (C-8a), 166.84 (C-7), 192.82 (C-4).

(−)-Garbanzol (**11**, 4.4 mg): FAB-MS *m/z*: 273 $[M+H]^+$. $^1$H-NMR (in $CD_3OD$): $\delta$ 4.51 (1H, d, $J = 12.0$ Hz, H-3), 4.99 (1H, d, $J = 12.0$ Hz, H-2), 6.32 (1H, d, $J = 2.0$ Hz, H-8), 6.53 (1H, dd, $J = 2.0$, 8.5 Hz, H-6), 6.83 (2H, d, $J = 8.5$ Hz, H-3′ and 5′), 7.36 (2H, d, $J = 8.5$ Hz, H-2′ and 6′), 7.72 (1H, d, $J = 8.5$ Hz, H-5). $^{13}$C-NMR (in $CD_3OD$): $\delta$ 74.55 (C-3), 85.50 (C-2), 103.71 (C-8), 112.12 (C-6), 113.48 (C-4a), 116.13 (C-3′ and 5′), 129.52 (C-1′), 130.10 (C-5), 130.38 (C-2′ and 6′), 159.18 (C-4′), 165.14 (C-8a), 166.85 (C-7), 194.55 (C-4).

(−)-Fustin-3-*O*-gallate (**12**, 9.4 mg): FAB-MS *m/z*: 441 $[M+H]^+$. $^1$H-NMR (in $CD_3OD$): δ 5.40 (1H, d, $J = 12.0$ Hz, H-2), 5.84 (1H, d, $J = 12.0$ Hz, H-3), 6.40 (1H, d, $J = 2.0$ Hz, H-8), 6.57 (1H, dd, $J = 2.0$, 8.5 Hz, H-6), 6.74 (1H, d, $J = 8.0$ Hz, H-5′), 6.84 (1H, dd, $J = 2.0$, 8.0 Hz, H-6′), 6.95 (2H, s, H-2, 6 of galloyl-*O*-3), 6.99 (1H, d, $J = 2.0$ Hz, H-2′), 7.73 (1H, d, $J = 8.5$ Hz, H-5). $^{13}$C-NMR (in $CD_3OD$): $\delta$ 75.28 (C-3), 83.33 (C-2), 103.88 (C-8), 110.34 (C-2, 6 of galloyl-*O*-3), 112.47 (C-6), 113.73 (C-4a), 115.46 (C-2′), 116.16 (C-5′), 120.56 (C-1 of galloyl-*O*-3), 120.75 (C-6′), 128.90 (C-1′), 130.29 (C-5), 140.12 (C-4 of galloyl-*O*-3), 146.40 (C-3, 5 of galloyl-*O*-3), 146.42 (C-3′), 147.32 (C-4′), 164.96 (C-8a), 166.77 (C-7 of galloyl-*O*-3), 167.18 (C-7), 189.47 (C-4).

(−)-Epifustin-3-*O*-gallate (**13**, 3.9 mg): FAB-MS *m/z*: 441 $[M+H]^+$. $^1$H-NMR (in $CD_3OD$): $\delta$ 5.61 (1H, d, $J = 3.5$ Hz, H-2), 5.80 (1H, d, $J = 3.5$ Hz, H-3), 6.44 (1H, d, $J = 2.5$ Hz, H-8), 6.55 (1H, dd, $J = 2.5$, 8.5 Hz, H-6), 6.68 (1H, d, $J = 8.5$ Hz, H-5′), 6.80 (1H, dd, $J = 2.0$, 8.0 Hz, H-6′), 6.91 (1H, d, $J = 2.0$ Hz, H-2′), 6.96 (2H, s, H-2, 6 of galloyl-*O*-3), 7.74 (1H, d, $J = 8.5$ Hz, H-5). $^{13}$C-NMR (in $CD_3OD$): $\delta$ 73.37 (C-3), 81.96 (C-2), 103.98 (C-8), 110.40 (C-2, 6 of galloyl-*O*-3), 112.40 (C-6), 113.95 (C-4a), 115.49 (C-2′), 116.14 (C-5′), 120.03 (C-6′), 120.42 (C-1 of galloyl-*O*-3), 128.17 (C-1′), 130.39 (C-5), 140.12 (C-4 of galloyl-*O*-3), 146.22 (C-3′), 146.47 (C-3, 5 of galloyl-*O*-3), 146.73 (C-4′), 164.83 (C-8a), 166.39 (C-7 of galloyl-*O*-3), 167.29 (C-7), 188.17 (C-4).

## HPLC–UV/CD analysis

The 70 vol% acetone extracts of the heartwood from each tree were dissolved in 50 vol% aqueous methanol for HPLC–UV/CD analysis under the following conditions: column, L-column2 ODS (3 μm, 150 × 4.6 mm i.d., CERI); column temperature, 40 °C; mobile phase A, 10 mM $H_3PO_4$ aqueous solution; mobile phase B, methanol; gradient condition, 10–62 vol% of B (0–60 min, linear); flow rate, 0.5 mL/min; detection, UV at 330 nm and CD at 330 nm. The enantiomeric purity of fustin was estimated from the *G* value [10, 11] using isolated and recrystallized (−)-fustin as an enantiopure standard, according to the following equations:

$$G = (\text{Peak area of fustin at CD 330 nm})/(\text{Peak area of fustin at UV 330 nm}).$$

$$\text{Enantiomeric purity of fustin [\% of (}-\text{)-fustin]} = 100-\{[G\,\text{of (}-\text{)-fustin}]-[G\,\text{of sample}]\}/\{[G\,\text{of (}-\text{)-fustin}]\times 2\}\times 100.$$

## Quantitative analysis of phenolic compounds

The total phenolic content of the 70 vol% acetone extract solution was measured by the Folin–Ciocalteu method [12] using gallic acid as a standard reference. Quantitative HPLC analysis for the 70 vol% acetone extract solution was performed under the same condition used for HPLC–UV/CD analysis except for the gradient conditions [10–62 vol% of B (0–30 min, linear)] and flow rate (1 mL/min). UV detection was monitored at 280 nm for compounds **1** and **10**, 320 nm for compounds **5**, **6**, **8**, **9**, and **12**, 370 nm for compounds **14** and **17**, and 400 nm for compound **15**. The contents of the phenolic compounds were calculated from the peak areas using a calibration

**Table 1** Basic information on the trunk of *T. vernicifluum*

| Sample | Age | Diameter (cm) | Content (wt%) | | |
|---|---|---|---|---|---|
| | | | Bark | Sapwood | Heartwood |
| Ib | | | | | |
| A | 10 | 23 | 7.3 | 11.7 | 81.0 |
| B | 10 | 21 | 7.7 | 10.6 | 82.5 |
| C | 10 | 22 | 9.1 | 13.1 | 79.0 |
| Average | | | 8.0 | 11.8 | 80.8 |
| Iw | | | | | |
| A | 17 | 16 | 12.8 | 18.3 | 68.9 |
| B | 17 | 16 | 13.6 | 22.7 | 66.7 |
| C | 17 | 15 | 11.7 | 17.5 | 72.9 |
| Average | | | 12.7 | 19.5 | 69.5 |
| Ni | | | | | |
| A | 25 | 23 | 10.7 | 8.6 | 80.6 |
| B | 25 | 23 | 13.5 | 11.9 | 76.0 |
| C | 25 | 24 | 10.4 | 10.2 | 80.6 |
| Average | | | 11.6 | 11.1 | 79.1 |

The diameter was measured at breast height and the contents were calculated based on the air-dried weight. Individual samples (A, B, C) were obtained from the stands in Ibaraki (Ib), Iwate (Iw) and Niigata (Ni) prefectures


**Fig. 1** Cross-section of the trunk of *T. vernicifluum* (Ni-A)

curve prepared with each standard or isolated compound. These quantifications were performed in triplicate for each sample.

## Results and discussion

### Basic information on the trunk of *T. vernicifluum*

Basic information on the samples and cross-section of the trunk are shown in Table 1 and Fig. 1, respectively. The ratio of the heartwood, with a characteristic yellow color, was remarkably high compared to other parts. This indicates that heartwood is the most quantitatively important part for the utilization of the trunk.

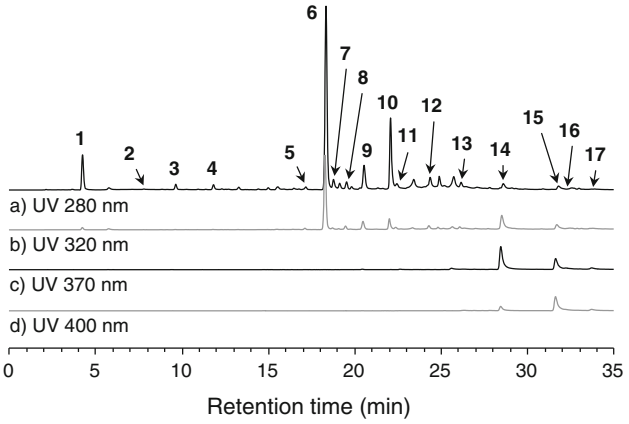


**Fig. 2** HPLC chromatograms of the ethyl acetate extracts from the heartwood of *T. vernicifluum* (Ib-A). Chromatograms were detected at UV 280 nm (**a**), 320 nm (**b**), 370 nm (**c**) and 400 nm (**d**)

### Chemical characteristics of phenolic extractives

Figure 2 shows the HPLC chromatograms of the ethyl acetate extracts prepared from the 70 vol% acetone extracts from the heartwood of Ib-A. Among the peaks detected on the chromatograms, seventeen compounds (**1–17**) could be isolated by preparative HPLC. By comparing the NMR and FAB-MS data and the HPLC retention time with those of authentic samples, gallic acid (**1**), protocatechuic acid (**2**), fisetin (**14**), sulfuretin (**15**), quercetin (**16**) and butein (**17**) were identified (Fig. 3). Relative structures of compound **6**, **9** and **10** were also determined in a similar manner, and their absolute configurations were established by comparing the optical rotations with literature values (Table 2) to identify them as (−)-fustin, (+)-taxifolin and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose, respectively [15, 17, 18].

By structural elucidation based on the results from a series of NMR ($^{1}$H, $^{13}$C, $^{13}$C-DEPT, $^{1}$H-$^{1}$H COSY, HSQC and HMBC) and FAB-MS experiments, the relative structures of the other isolated compounds were determined. Compound **5** was identified as 2-benzyl-2,6,3′,4′-tetrahydroxycoumaran-3-one, which is a kind of auronol and reported to be a major compound contained in the heartwood of *T. succedaneum* (hazenoki) [19]. Because optical activity was not observed on the optical rotation and CD analyses, compound **5** was found to be a racemic form, similar to the auronol analog of alphitonin [20, 21]. Compound **7** was identified as 1,2,3,6-Tetra-*O*-galloyl-β-D-glucose, the absolute configuration of which was confirmed by the optical rotation data (Table 2) [16]. The relative structure of **4** was identified as fisetinidol-4α-ol, which has large $^{1}$H-$^{1}$H vicinal coupling constants of $J_{2,3} = 10.0$ Hz and $J_{3,4} = 8.0$ Hz, while that of **3** was identified as fisetinidol-4β-ol, which has the large $J_{2,3}$ (9.5 Hz) and small $J_{3,4}$ (3.5 Hz). The relative structures of **8** and **11** were

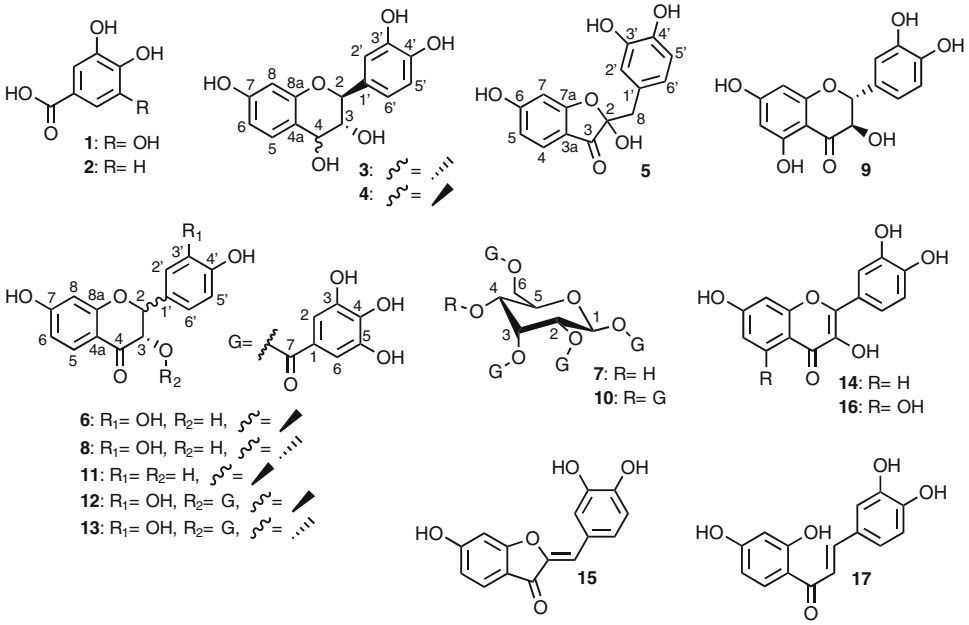


**Fig. 3** Chemical structures of the isolated compounds

**Table 2** Optical rotations of the isolated compounds

| Compound | Solvent | Optical rotation, $[\alpha]_D$ | | |
|---|---|---|---|---|
| | | Observed (25 °C) | Literature | |
| | | | (+) | (−) |
| **3** | Acetone/$H_2O$ | −54.8° ($c = 0.24$) | | |
| **4** | Acetone/$H_2O$ | −28.5° ($c = 0.47$) | +27.3° (20 °C, $c = 0.8$) [13] | −26.2° (20 °C, $c = 0.7$) [14] |
| **5** | Acetone/$H_2O$ | –0.1° ($c = 0.44$) | | |
| **6** | Acetone/$H_2O$ | –25.7° ($c = 0.79$) | +28.3° (25 °C, $c = 0.9$) [15] | –26° [15] |
| **7** | Acetone | +42.4° ($c = 0.71$) | +31.8° (20 °C, $c = 0.38$) [16] | |
| **8** | Acetone/$H_2O$ | –42.1° ($c = 0.46$) | | |
| **9** | Acetone | +19.9° ($c = 0.72$) | +17.3° (20 °C, $c = 0.57$) [17] | –20.6° (20 °C, $c = 0.32$) [17] |
| **10** | Acetone | +19.7° ($c = 0.78$) | +18.3° (20 °C, $c = 0.38$) [18] | |
| **11** | Acetone/$H_2O$ | –31.7° ($c = 0.37$) | | |
| **12** | Acetone/$H_2O$ | –87.0° ($c = 0.47$) | | |
| **13** | Acetone/$H_2O$ | –204.0° ($c = 0.33$) | | |

Acetone/$H_2O$ = 1/1 (v/v)

identified as epifustin, 2,3-cis-isomer ($J_{2,3} = 3.5$ Hz) of 2,3-trans-fustin (**6**, $J_{2,3} = 12.0$ Hz), and garbanzol, the 3′-dehydroxy analog of fustin, respectively. Compounds **12** and **13** were revealed as galloylated fustin and epifustin. The galloylated position was determined by the HMBC correlation between H-3 and the carbonyl C-7 of the galloyl group, hence the relative structures of **12** and **13** were identified as fustin-3-*O*-gallate and epifustin-3-*O*-gallate, respectively.

The CD data of both **3** and **4** showed positive Cotton effect (CE) at around 289 nm (see spectral data in "Materials and methods"), which is related to the $^1L_b$ band of the $\pi \rightarrow \pi^*$ transition of the aromatic chromophore. Accordingly, the absolute configurations at C-2 were confirmed as 2*S* according to the helicity rule for flavan-3,4-diols [22]. From these data, the absolute structures of **3** and **4** were identified as (−)-(2*S*,3*R*,4*R*)-fisetinidol-4β-ol and (−)-(2*S*,3*R*,4*S*)-fisetinidol-4α-ol, respectively. The stereochemistry of **4** was also confirmed by the optical rotation data (Table 2) [13, 14]. These flavan-3,4-diols have been reported in the synthetic study of profisetinidins although some spectral data differ from those in this study [23]. The CD spectra of 2,3-dihydroflavonols (**6**, **8**, **9**, **11–13**) were shown in Fig. 4. All compounds showed clear CE around

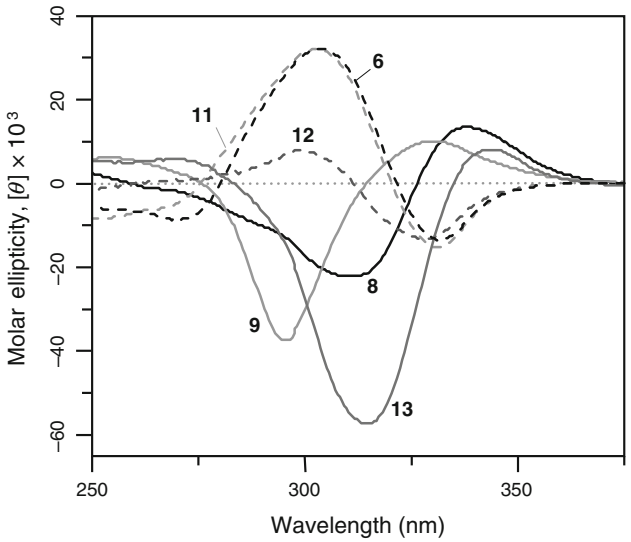


**Fig. 4** CD spectra of 2,3-dihydroflavonols (**6**, **8**, **9**, **11–13**)

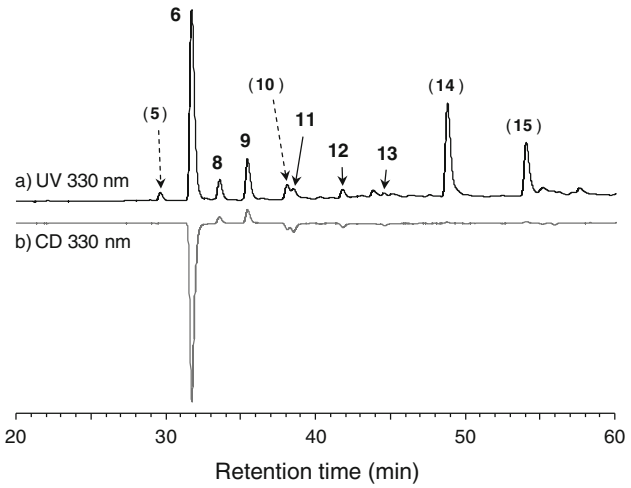


**Fig. 5** HPLC–UV/CD chromatograms of the 70 vol% acetone extracts from the heartwood of *T. vernicifluum* (Ib-A). Chromatograms were detected at UV 330 nm (**a**) and CD 330 nm (**b**)

330–340 nm, which have been assigned to the n → π* transition of the conjugated carbonyl chromophore of acetophenone moiety. In relation to 2,3-dihydroflavonols, it has been reported that the positive n → π* CE indicates a 2*R* and the negative CE indicates a 2*S* configuration [22]. Accordingly, compounds **8**, **9** and **13** showing positive CE were revealed to have a 2*R* whereas **6**, **11** and **12** showing negative CE were shown to have a 2*S* configuration. As these results, compounds **8**, **11**, **12** and **13** were identified as (−)-(2*R*,3*S*)-epifustin, (−)-(2*S*,3*S*)-garbanzol, (−)-(2*S*,3*S*)-fustin-3-*O*-gallate and (−)-(2*R*,3*S*)-epifustin-3-*O*-gallate, respectively. Confirmation of the C-2 configuration by CD experiment also supported the absolute structures of (−)-(2*S*,3*S*)-fustin (**6**) and (+)-(2*R*,3*R*)-taxifolin (**9**) established by the optical rotations.

Although the phenolic compounds contained in the wood and bark of *T. vernicifluum* have been reported, their absolute configurations have not been investigated in many studies [5–8]. We determined the absolute structures of the isolated compounds, and found that the 2,3-dihydroflavonols (**6**, **8**, **11–13**) which had 7-hydroxy (also known as 5-deoxy) A-ring possessed a 3*S* configuration, while (+)-taxifolin (**9**) which had 5,7-dihydroxy A-ring possessed a 3*R* configuration. However, some studies reported that the heartwood of *T. vernicifluum* contained (+)-mollisacasidin (the same as fisetinidol-4α-ol), (+)-fustin and (+)-garbanzol [4, 9], which are the enantiomers of **4**, **6** and **11**, not identified in this study. To discuss the enantiomers of optically active flavonoids, direct analysis of the heartwood extracts was conducted using HPLC–UV/CD. Figure 5 shows the HPLC–UV/CD chromatograms of the 70 vol% acetone extracts from the heartwood of Ib-A. The isolated 2,3-dihydroflavonols (**6**, **8**, **9**, **11–13**) were detected at UV 330 nm (a), and the positive or negative peaks assigned to the respective compounds were observed on the CD detection at 330 nm (b). Because the CD peaks showed the same direction as observed in the CD spectra of corresponding isolates (Fig. 4), it was indicated that these compounds mainly consisted the same isolated isomer. Similar results were obtained for all heartwood extracts from other trees (data not shown). The enantiomeric purity of fustin (**6**), the most abundant compound isolated from the heartwood, was estimated from the *G* value [10, 11]. The measured purities were close to 100 % of (−)-fustin (>97 %) for all samples (Table 3). Therefore, all the heartwood samples were revealed to contain (−)-fustin as an almost single enantiomer. It has been reported that (−)-fustin is also contained in the wood of *Cotinus coggygria* (syn. *Rhus cotinus*) [24], whereas the heartwood of *Acacia* spp. contains (+)-fustin [15, 25]. In addition, the heartwood of *R. javanica* L. var. *roxburghii* was reported to contain a racemic mixture of (+)- and (−)-fustin [26].

## Contents and radial distribution of phenolic extractives in the wood and bark

The major phenolic compounds in both the sapwood and bark extracts were the same as the identified compounds in the heartwood extracts (data not shown). The total phenolic content (TP) and the contents of 10 major compounds were determined and their averages were calculated for each stand (Table 4). Regardless of the stands, TP in the heartwood (∼5–7 wt%) was much higher than those in the sapwood and bark (∼1–2 wt%), and the contents of the respective compounds and their total amount (Total) were also high in the heartwood. Therefore, the heartwood was revealed as the richest part in phenolic extractives. In addition, (−)-fustin (Fus) was

**Table 3** Enantiomeric purities of fustin contained in heartwood extracts

| | Enantiomeric purity of fustin [% of (−)-fustin] | | |
|---|---|---|---|
| | A | B | C |
| Ib | 99.6 | 99.2 | 98.9 |
| Iw | 99.4 | 99.8 | 98.9 |
| Ni | 98.2 | 98.0 | 97.2 |

The enantiomeric purity of fustin was estimated from the *G* value obtained by HPLC–UV/CD analysis of the heartwood extracts

found to be the most abundant phenolic compound accumulated in the heartwood (1.4–2.4 wt%).

The radial distributions of phenolic extractives in the xylem part of Ni-A, B and C were shown in Fig. 6. TP and Total were found to have the lowest value in sapwood, increase drastically to the highest value at the outer heartwood, and then decrease gradually toward the pith. A similar trend was reported for the general distribution pattern of heartwood extractives in various wood species [27–33], and the low content of extractives in inner heartwood was explained by the result of increasing in accumulation of extractives with aging and/or their structural changes (degradation) over time [27, 30, 31]. In our results, TP in the heartwood of Iw and Ni (age 17 and 25) were higher than that of Ib (age 10) (Table 4). Accordingly, the contents of phenolic extractives in the heartwood would be mainly related to tree age, but further studies are needed to discuss the influencing factors because the number and variety of samples in this study were quite limited. A similar distribution trend was observed in the contents of (−)-fustin, (+)-taxifolin (Tax), (−)-epifustin (Efus) and particularly sulfuretin (Sul).

In contrast to these compounds, fisetin (Fis) and 2-benzyl-2,6,3′,4′-tetrahydroxycoumaran-3-one (BTC) showed a different radial distribution trend. The contents of fisetin and the benzylcoumaranone in the heartwood were almost constant and/or increased somewhat toward the pith (Fig. 6). It has been reported that some compounds would be formed in the heartwood by a non-enzymatic reaction during aging [27, 30, 31]. As reported for the reactions of fustin [19] and taxifolin [20, 21], (−)-fustin would undergo an oxidative reaction to form fisetin and a rearrangement reaction to form the benzylcoumaranone, respectively. Accordingly, the non-enzymatic reactions of (−)-fustin during aging were suggested as a potential pathway for the formation of fisetin and 2-benzyl-2,6,3′,4′-tetrahydroxycoumaran-3-one in heartwood, although further studies on these synthetic pathways are required. In addition to these reactions, an equilibrium epimerization at the C-2 position is also expected for 2,3-dihydroflavonols [20, 21]. In this study, reversible epimerization between (−)-fustin and

**Table 4** Contents of phenolic extractives in the trunk of *T. vernicifluum*

| | Average content (standard deviation in parentheses), wt% based on oven-dried weight | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | TP | GA | BTC | Fus | Efus | Tax | PGG | FusG | Fis | Sul | But | Total |
| Heartwood | | | | | | | | | | | | |
| Ib | 5.14 (0.35) | 0.22 (0.06) | 0.02 (0.01) | 1.41 (0.11) | 0.10 (0.02) | 0.25 (0.04) | 0.53 (0.06) | 0.08 (0.005) | 0.22 (0.01) | 0.11 (0.01) | 0.02 (0.002) | 2.95 (0.16) |
| Iw | 7.08 (0.20) | 0.25 (0.05) | 0.02 (0.01) | 2.41 (0.18) | 0.13 (0.02) | 0.38 (0.005) | 0.46 (0.06) | 0.08 (0.002) | 0.21 (0.006) | 0.28 (0.08) | 0.06 (0.01) | 4.28 (0.08) |
| Ni | 7.25 (0.34) | 0.36 (0.05) | 0.14 (0.03) | 2.40 (0.20) | 0.17 (0.01) | 0.33 (0.02) | 0.58 (0.08) | 0.13 (0.02) | 0.35 (0.05) | 0.32 (0.13) | 0.04 (0.01) | 4.84 (0.43) |
| Sapwood | | | | | | | | | | | | |
| Ib | 0.60 (0.06) | 0.01 (0.005) | ND | 0.05 (0.01) | <0.01 | <0.01 | 0.06 (0.02) | ND | <0.01 | <0.01 | <0.01 | 0.12 (0.04) |
| Iw | 2.36 (0.54) | 0.05 (0.008) | ND | 0.20 (0.06) | 0.03 (0.01) | 0.02 (0.01) | 0.31 (0.03) | ND | 0.05 (0.02) | 0.04 (0.01) | 0.01 (0.005) | 0.71 (0.12) |
| Ni | 1.53 (0.30) | 0.05 (0.02) | ND | 0.08 (0.01) | <0.01 | 0.03 (0.01) | 0.32 (0.03) | ND | <0.01 | 0.01 (0.008) | <0.01 | 0.50 (0.07) |
| Bark | | | | | | | | | | | | |
| Ib | 1.37 (0.40) | 0.08 (0.04) | ND | 0.04 (0.006) | <0.01 | <0.01 | 0.13 (0.01) | <0.01 | 0.04 (0.006) | 0.03 (0.005) | 0.02 (0.007) | 0.34 (0.05) |
| Iw | 1.63 (0.24) | 0.09 (0.05) | ND | 0.07 (0.01) | <0.01 | <0.01 | 0.24 (0.10) | <0.01 | 0.05 (0.009) | 0.03 (0.005) | 0.03 (0.005) | 0.51 (0.16) |
| Ni | 1.48 (0.39) | 0.07 (0.03) | ND | 0.08 (0.02) | <0.01 | <0.01 | 0.30 (0.06) | <0.01 | 0.05 (0.02) | 0.04 (0.005) | 0.02 (0.01) | 0.55 (0.11) |

The total phenolic content (TP) was measured by the Folin–Ciocalteu method. Gallic acid (GA, **1**), 2-benzyl-2,6,3′,4′-tetrahydroxycoumaran-3-one (BTC, **5**), (−)-fustin (Fus, **6**), (−)-epifustin (Efus, **8**), (+)-taxifolin (Tax, **9**), 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (PGG, **10**), (−)-fustin-3-*O*-gallate (FusG, **12**), fisetin (Fis, **14**), sulfuretin (Sul, **15**) and butein (But, **17**) were quantified by HPLC analysis, and their total amount (Total) was determined

ND means not detected and <0.01 means trace amount

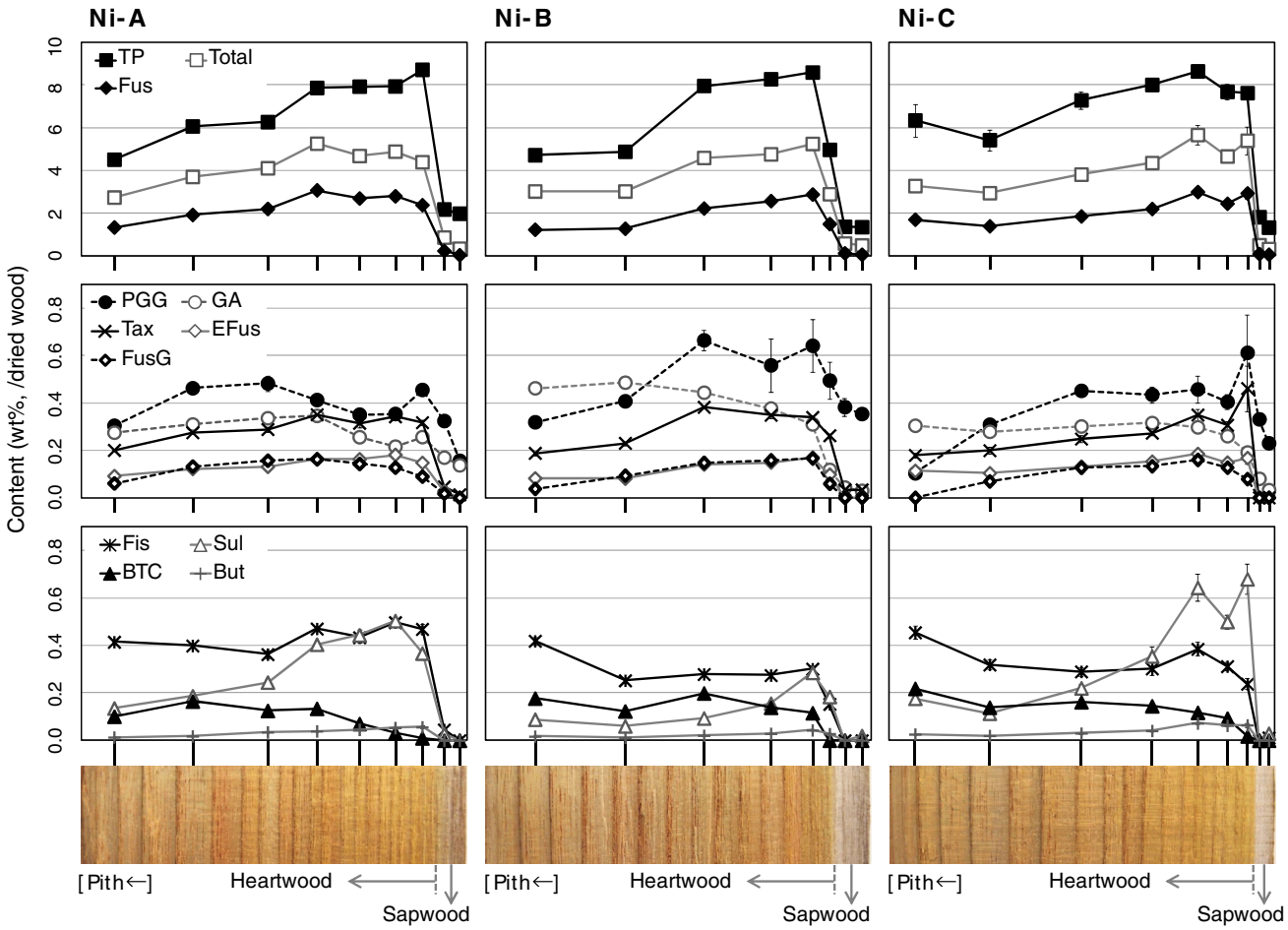


**Fig. 6** Radial distribution of phenolic extractives in the xylem of *T. vernicifluum* (Ni-A, B, C). Total phenolic content (TP) was measured by the Folin-Ciocalteu method. Gallic acid (GA, **1**), 2-benzyl-2,6,3′,4′-tetrahydroxycoumaran-3-one (BTC, **5**), (−)-fustin (Fus, **6**), (−)-epifustin (Efus, **8**), (+)-taxifolin (Tax, **9**), 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (PGG, **10**), (−)-fustin-3-*O*-gallate (FusG, **12**), fisetin (Fis, **14**), sulfuretin (Sul, **15**) and butein (But, **17**) were quantified by HPLC analysis, and their total amount (Total) was determined. The *error bar* indicates ±standard deviation

(−)-epifustin was observed (data not shown) as well as the presence of (−)-epifustin at a constant rate of 5–7 % of (−)-fustin (Table 4; Fig. 6). It was thus suggested that (−)-epifustin contained in the heartwood extracts might be formed by the epimerzation of (−)-fustin.

With respect to the compounds in sapwood sections (Fig. 6), a certain amount of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (PGG), a kind of hydrolyzable gallotannin, was contained, while the contents of the flavonoids were very low. A similar trend was observed in the data of Table 4, particularly for Iw and Ni. It has been reported that the sapwoods of *T. succedaneum* and *Quercus crispula* contain hydrolyzable tannins as the main phenolic components, which would act as defensive compounds [34, 35]. Therefore, it was suggested that the sapwood of *T. vernicifluum* might also contain hydrolyzable tannins including pentagalloylglucose as defensive compounds.

## Conclusion

The phenolic compounds contained in the heartwood of *T. vernicifluum* were identified as mainly flavonoids and hydrolyzable tannins. It was revealed that the 7-hydroxy A-ring type flavonoids, such as (−)-fustin (**6**), had a 3*S* configuration, while the 5,7-dihydroxy A-ring type (+)-taxifolin (**9**) had a 3*R* configuration. Quantitative analysis revealed that the heartwood was the richest part in phenolic extractives and (−)-fustin was the most abundant phenolic compound. For the radial distribution of phenolic extractives it was generally observed that their contents were the lowest in sapwood, increased to the highest in the outer heartwood, and then decreased in the inner heartwood.

In this study, we revealed a number of fundamental characteristics of phenolic extractives in the trunk of *T. vernicifluum*, although several uncertainties remain, such as the difference in content and the factors influencing it.

Further studies will not only facilitate the utilization of phenolic extractives from unused trunks but also the understanding of the biochemistry of phenolic extractives.

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