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# Iridoid glycoside acyl esters from the whole plants of *Linaria canadensis*

**Akihiro Imadzu, Sachiko Sugimoto, Katsuyoshi Matsunami and Hideaki Otsuka**

**Abstract**
From the 1-BuOH-soluble fraction of a MeOH extract of *Linaria canadensis*, collected in the suburbs of Hiroshima City, four new iridoid glycosides (1–4) were isolated, along with eight known compounds. By extensive spectroscopic analyses, the structures of new compounds were elucidated to be 6'-*O*-(*E*)-feruloyl, 6'-O-(Z)-feruloyl, 6-O-(E)-feruloyl and 6-O-(Z)-feruloyl antirrhinoides.

**Keywords:** Antimycotic activity, minimum inhibitory concentration, *Argemone mexicana* L.

## Introduction

*L. canadensis* is a small annual or biennial herb and naturalized plant during 1940's from North America. Extensive phytochemical studies on *L. japonica* have been performed by Kitagawa *et al.* [1, 2] and Otsuka,[3-7] and a related species, *L. canadensis*, called Canada toadflax is also investigated by Mizouchi *et al.* [8] In folk medicine, the leaves are used to treat hemorrhoids. They're also used in a tea as both a diuretic and laxative. Some biological investigation was performed on the European *Linaria* species, *L. vulgaris*. [9] Independent investigation of *L. canadensis*, collected in the suburbs of Hiroshima City, resulted in the isolation of four new iridoid glycoside acyl esters (1–4) along with eight known compounds (5–12). Known compounds were identified as antirrhinoides (5),[10] 6-*O*-(*E*)- (6), [11] and 6-*O*-(*Z*)-*p*-coumaroyl antirrhinoides (7), [10–12] 4,6-di-(7-deoxyiridolactonyl) b-D-glucopyranoside (8), [8] apigenin 7-*O*-rutinoside (9), [13] acacetin 7-O-(6"-O-a-L-rhamnopyranosyl)-$\beta$-D-glucopyranoside (10), [14] benzyl alcohol *O*-(2'-*O*-$\beta$-D-xylopyranose)-$\beta$-D-glucopyranoside (11) [15] and methyl 6,7-dihydrofoliamenthonate 8-*O*-$\beta$-D-glucopyranoside (12)[16] by comparison of spectroscopic data with those reported in the literature. The structures of new compounds were elucidated by extensive spectroscopic analyses.

## Results and Discussion

From the 1-BuOH-soluble fraction of a MeOH extract of *Linaria canadensis,* collected in Hiroshima, four new iridoid glycosides were isolated along with eight known ones by a combination of various kinds of chromatography. The structures of the new compounds were elucidated as follows.

Compound 1, $[\alpha]_D^{25}$ –51.3, was isolated as an amorphous powder and its elemental composition was determined to be $C_{25}H_{30}O_{12}$ by high-resolution (HR)-electrospray ionization (ESI)-mass spectrometry (MS). The IR spectrum exhibited absorptions assignable to hydroxyl groups (3382 cm$^{-1}$), an ester linkage (1701 cm$^{-1}$) and double bonds (1654 cm$^{-1}$). In the $^1$H-NMR spectrum, two acetal protons [$\delta_H$ 5.17 (1H, d, *J*=8.0 Hz, H-1) and 4.71 (1H, d, *J*=7.9 Hz, H-1')], two sets of olefinic protons coupled in *trans* and *cis* geometries [$\delta_H$ 7.62 (1H, d, *J*=15.9 Hz, H-7") and 6.37 (1H, d, *J*=15.9 Hz, H-8"),and 6.35 (1H, d, *J*=6.2 Hz, H-3) and 4.86 (1H, d, *J*=6.2 Hz, H-4), respectively] and three aromatic protons coupled in an ABX system were observed. The presence of two acetal carbon signals and highly deshielded olefinic carbon ($\delta_C$ 142.8) with a hydrogen atom were observed in the $^{13}$C-NMR spectrum (Table 1), along with a carbonyl carbon, further three olefinic carbon and six aromatic carbon signals. The remaining $^{13}$C-NMR signals composed one methyl, two methines with an oxygen atom, two oxygenated tertiary carbons, a methine carbon and a methoxy carbon. From the above evidence, the structure of compound 1 was implied to be an iridoid glycoside with an acyl moiety. Mild alkaline hydrolysis of 1 have antirrhinoides (1a=5) [10] (Table 1) and methyl ferulate (1b),[17]

and acid hydrolysis of 1 ensured the presence of D-glucose. The ester position of the acyl moiety was determined to be at the hydroxyl group on the C-6' by relatively deshielded chemical shifts of $H_2$-6 and HMBC spectrum. Therefore, the structure of 1 was elucidated to be 6'-*O*-(*E*)-feruloyl antirrhinoides, as shown in Fig. 1.

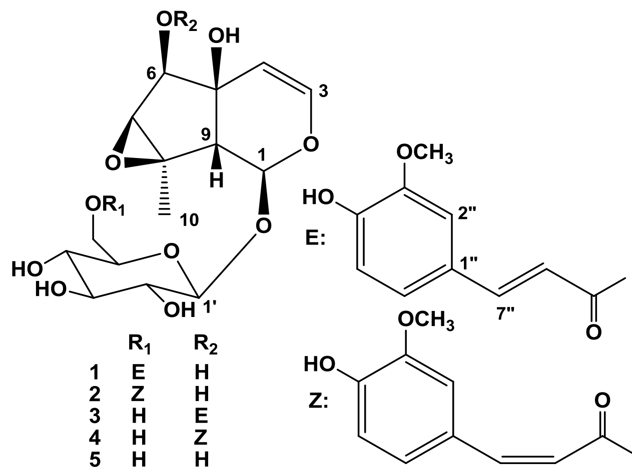


**Fig 1**

Compound 2, $[\alpha]_D^{25}$ –57.9, was isolated as an amorphous powder and its elemental composition was the same as that of 1. Spectroscopic data of 2 showed high similarity to those of 1, except for the coupling constant of the one the double bonds [$\delta_H$ 6.87 (1H, d, $J$=12.9 Hz, H-7") and 5.79 (1H, d, $J$=12.9 Hz, H-8")]. Therefore, the structure of 2 was elucidated to be 6'-*O*-(*Z*)-feruloyl antirrhinoides, as shown in Fig. 1.

**Table 1:** $^{13}$C-NMR spectroscopic data for compounds 1–5 (100 MHz, $CD_3OD$)

| C | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 95.6 | 95.7 | 94.8 | 94.9 | 95.3 |
| 3 | 142.8 | 142.8 | 143.4 | 143.4 | 143.0 |
| 4 | 107.9 | 107.8 | 107.5 | 107.6 | 107.8 |
| 5 | 74.7 | 74.7 | 74.9 | 74.9 | 74.8 |
| 6 | 78.7 | 78.9 | 79.5 | 79.4 | 78.6 |
| 7 | 66.2 | 66.1 | 64.4 | 64.5 | 66.3 |
| 8 | 64.0 | 64.0 | 64.5 | 64.6 | 64.2 |
| 9 | 53.4 | 53.4 | 53.5 | 53.5 | 53.3 |
| 10 | 17.7 | 17.7 | 17.4 | 17.5 | 17.6 |
| 1' | 99.9 | 100.0 | 99.8 | 99.9 | 99.7 |
| 2' | 74.8 | 74.8 | 74.8 | 74.8 | 74.8 |
| 3' | 77.7 | 77.8 | 77.9 | 77.9 | 77.8 |
| 4' | 71.7 | 71.8 | 71.9 | 71.9 | 71.9 |
| 5' | 75.9 | 75.7 | 78.5 | 78.6 | 78.4 |
| 6' | 64.0 | 63.9 | 63.0 | 63.1 | 63.1 |
| 1" | 127.6 | 128.1 | 128.6 | 128.2 | |
| 2" | 111.9 | 115.5 | 112.0 | 115.3 | |
| 3" | 149.5 | 149.8 | 150.8 | 148.4 | |
| 4" | 150.8 | 149.8 | 151.9 | 150.3 | |
| 5" | 116.6 | 115.9 | 116.6 | 115.8 | |
| 6" | 124.2 | 127.0 | 124.2 | 126.9 | |
| 7" | 147.1 | 145.9 | 147.6 | 146.3 | |
| 8" | 115.3 | 116.2 | 115.0 | 116.1 | |
| 9" | 168.9 | 167.9 | 168.6 | 167.6 | |
| -OCH3 | 56.6 | 56.6 | 56.5 | 56.5 | |

Compound 3, $[\alpha]_D^{25}$ –90.7, was isolated as an amorphous powder and its elemental composition was the same as that of aforementioned compounds. Spectroscopic data were similar to those of 1 and obvious down field and up field shifts were observed for H-6 ($d_H$ 3.89 → 5.08 and $H_2$-6' 4.53 and 4.44 → 3.93 and 3.65) in the $^1$H-NMR spectra, respectively. HMBC correlation between H-6 and C-9" ($d_C$ 168.6) confirmed the structure of 3 to be 6-*O*-(*E*)-feruloyl antirrhinoides, as shown in Fig. 1.

Compound 4, $[a]_D^{26}$ –64.0, was isolated as an amorphous powder and its elemental composition was also the same as that of aforementioned compounds. From the same logical consequence between compounds 1 and 2, the structure of 4 was determined to be 6-*O*-(*Z*)-feruloyl antirrhinoides, as shown in Fig. 1.

## Experimental

General experimental procedure Optical rotations were measured on a JASCO P-1030 digital polarimeter. IR and UV spectra were measured on Horiba FT-710 and JASCO V-520 UV/Vis spectrophotometers, respectively. $^1$H- and $^{13}$C-NMR spectra were taken on a JEOL JNM α-400 at 400 MHz and 100 MHz with tetramethylsilane as an internal standard. Positive-ion HR-ESI-MS was performed with an Applied Biosystems QSTAR XL NanoSprayTM System.

A highly-porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel column chromatography (CC) was performed on silica gel 60 (E. Merck, Darmstadt, Germany), and ODS open CC on Cosmosil 75$C_{18}$-OPN (Nacalai Tesque, Kyoto) [$\Phi$ = 50 mm, $L$ = 20 cm, linear gradient: MeOH-$H_2O$ (1:9, 1 L) → (1:1, 1 L) → (9:1, 1 L), fractions of 10 g being collected]. The DCCC (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns ($\Phi$ = 2 mm, $L$ = 40 cm), the lower and upper layers of a solvent mixture of $CHCl_3$-MeOH-$H_2O$-1-PrOH (9:12:8:2) being used as the stationary and mobile phases, respectively. Five-gram fractions were collected and numbered according to their order of elution with the mobile phase. HPLC was performed on an ODS column (Inertsil; GL Science, Tokyo, Japan; $\Phi$ = 6 mm, $L$ = 25 cm, 1.6 mL/ min), and the eluate was monitored with UV (254 nm) and refractive index monitors.

Plant material Whole plants of *L. canadensis* were collected in the suburbs of Hiroshima City and a voucher specimen was deposited in the Herbarium of Faculty of Pharmaceutical Sciences, Graduate School of Biomedical and Health Sciences, Hiroshima University (02-LC-Hiroshima-0515).

## Extraction and isolation

Whole plants of *L. canadiens* (5.62 kg) were extracted three times with MeOH (30 L × 3) at room temperature for one week and then concentrated to 3 L *in vacuo*. The concentrated extract was washed with *n*-hexane (3 L, 61.4 g), and then the MeOH layer was concentrated to a gummy mass. The latter was suspended in water (3 L) and then extracted with EtOAc (3 L) to give 54.3 g of an EtOAc-soluble fraction. The aqueous layer was extracted with 1-BuOH (3 L) to give a 1-BuOH-soluble fraction (134 g), and the remaining water-layer was concentrated to furnish 734 g of a water-soluble fraction. The 1-BuOH-soluble fraction (130 g) was subjected to Diaion HP-20 CC ($\Phi$ =60 mm, $L$ = 45 cm), using $H_2O$-MeOH (4:1, 6 L), (3:2, 6 L), (2:3, 6 L), and (1:4, 6 L), and MeOH (6 L), 1 L fractions being collected. The residue (10.2 g) in fractions 9–13 was subjected to silica gel ($\Phi$ = 35 mm, $L$ = 45 cm) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (3 L), and $CHCl_3$-MeOH (49:1, 1.5 L), (24:1, 1.5 L), (9:1, 1.5 L), (17:3, 1.5 L), (4:1, 1.5 L), (3:1, 1.5 L), and (7:3, 1.5 L)], 250 mL fractions being collected. The residue (3.27 g) in fractions 40–48 was separated ODS open CC and the residue (221 mg) in fractions 50–57 was subjected to DCCC, followed by the

purification of the residue (87.1 mg) in fractions 32–45 was purified by HPLC (H2O-MeOH, 4:1) to give 3.2 mg of 11 from the peak at 32 min. The residue (79.1 mg) in fractions 141–202 obtained on ODS open CC was purified by DCCC to give 4.9 mg of in 12 in fractions 131–144.

The residue (18.7 g) in fractions 14–18 obtained on Diaion HP-20 CC was subjected to silica gel ($\Phi$ = 55 mm, $L$ = 40 cm) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (6 L), and $CHCl_3$-MeOH (49:1, 3 L), (24:1, 3 L), (9:1, 3 L), (17:3, 3 L), (4:1, 3 L), (3:1, 3 L), and (7:3, 3 L)], 500 mL fractions being collected. The residue (1.08 g out of 1.37 g) in fractions 31–37 was separated by ODS open CC and the residue (160 mg) was subjected to DCCC. The residue (39.0 mg) in fractions was purified by HPLC ($H_2O$-MeOH, 3:2) to give 11.4 mg of 7 and 7.7 mg of 6 from the peaks at 14 min and 21 min, respectively. The residue (48.4 mg) in fractions 120–135 was purified by HPLC ($H_2O$-MeOH, 3:2; flow rate: 1 mL/min) to yield 20.3 mg of 1 and 4.4 mg of 2 from the peaks at 20 min and 25 min, respectively. The residue (301 mg) in fractions 160–169 was subjected to DCCC and the residue (68.2 m) in fractions 141–179 was purified by HPLC ($H_2O$-MeOH, 13:7; flow rate: 1 mL/min) to afford 33.6 mg of 8 from the peaks at 63 min and 68 min. The residue (213 mg) in fractions 170–182 was subjected to DCCC, and the residue (10.1 mg) in fractions 47-53 was purified by HPLC ($H_2O$-MeOH, 3:2) to give 0.7 mg of 4 and 2.1 mg of 3 from the peaks at 17 min and 24 min, respectively, and the residue (39.0 mg) in fractions 94–111 was purified by HPLC ($H_2O$-MeOH, 3:2) to afford 11.4 mg of 7 and 7.7 mg of 6 from the peaks at 14 min and 21 min, respectively. The residue (1.42 g) in fractions 55–61 obtained on silica gel CC was subjected to ODS open CC. The first residue (43.8 mg) in 14–23 was purified by HPLC ($H_2O$-MeOH, 9:1; flow rate: 1 mL/min) to give 4.3 mg of 5 from the peak at 13 min. The second residue (373 mg) was subjected to DCCC and the residue (81.5 mg) in fractions was purified by HPLC ($H_2O$-MeOH, 13:7) to give 17.1 mg of 9 from the peak at 62 min. The third residue (334 mg) in fractions 138–230 was purified by DCCC to give 5.3 mg of 10 in fractions 91–110.

Compound 1 Amorphous powder, $[\alpha]_D^{25}$ –51.3 ($c$=1.28, MeOH); IR $\nu_{max}$ (film) $cm^{-1}$: 3382, 2931, 1701, 1654, 1515, 1273, 1162, 1014; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 322 (4.01), 237 (3.87), 217 (3.84); $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$: 7.62 (1H, d, $J$=15.9 Hz, H-7"), 7.18 (1H, d, $J$=1.9 Hz, H-2"), 7.07 (1H, dd, $J$=8.2, 1.9 Hz, H-6"), 6.82 (1H, d, $J$=8.2 Hz, H-5"), 6.37 (1H, d, $J$=15.9 Hz, H-8"), 6.35 (1H, d, $J$=6.2 Hz, H-3), 5.17 (1H, d, $J$=8.0 Hz, H-1), 4.86 (1H, d, $J$=6.2 Hz, H-4), 4.71 (1H, d, $J$=7.9 Hz, H-1'), 4.53 (1H, dd, $J$=12.0, 2.5 Hz, H-6'a), 4.44 (1H, dd, $J$=12.0, 5.8 Hz, H-6'b), 3.89 (3H, s, $CH_3O$-), 3.89 (1H, m, H-6), 3.54 (1H, m, H-5'), 3.40 (2H, m, H-3' and 4'), 3.35 (1H, br s, H-7), 3.27 (1H, dd, $J$ = 9.0, 7.9 Hz, H-2'), 2.36 (1H,d, $J$=8.0 Hz, H-9), 1.43 (3H, s, $H_3$-10); $^{13}$C-NMR ($CD_3OD$, 100 MHz): Table 1; HR-ESI-MS (positive-ion mode) $m/z$: 561.1584 $[M+Na]^+$ ($C_{25}H_{30}O_{12}Na$: 561.1579).

Compound 2 Amorphous powder, $[\alpha]_D^{25}$ –57.9 ($c$=0.29, MeOH); IR $\nu_{max}$ (film) $cm^{-1}$: 3374, 2930, 1713, 1654, 1514, 1279, 1171, 1014; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 325 (3.95), 235 (3.79), 212 (3.93); $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$: 7.18 (1H, d, $J$=1.9 Hz, H-2"), 7.18 (1H, dd, $J$=8.3, 1.9 Hz, H-6"), 6.87 (1H, d, $J$=12.9 Hz, H-7"), 6.78 (1H, d, $J$=8.3 Hz, H-5"), 6.35 (1H, d, $J$=6.2 Hz, H-3), 5.79 (1H, d, $J$=12.9 Hz, H-8"), 5.10 (1H, d, $J$=8.0 Hz, H-1), 4.88 (1H, d, $J$=6.2 Hz, H-4). 4.68 (1H, d, $J$=7.9 Hz, H-1'), 4.50 (1H, dd, $J$=12.0, 2.4 Hz, H-6'a), 4.38 (1H, dd, $J$=12.0, 6.0 Hz, H-6'b), 3.89 (3H, s, $CH_3O$-), 3.86 (1H, d, $J$=1.3 Hz, H-6), 3.50 (1H, m, H-5'), 3.40 (2H, m, H-3' and 4'), 3.34 (1H, br s, H-7), 3.23 (1H, m, H-2'), 2.32 (1H,d, $J$=8.0 Hz, H-9), 1.37 (3H, s, $H_3$-10); $^{13}$C-NMR ($CD_3OD$, 100 MHz): Table 1; HR-ESI-MS (positive-ion mode) $m/z$: 561.1586 $[M+Na]^+$ ($C_{25}H_{30}O_{12}Na$: 561.1579).

Compound 3 Amorphous powder, $[\alpha]_D^{25}$ –90.7 ($c$=0.14, MeOH); IR $\nu_{max}$ (film) $cm^{-1}$: 3388, 2925, 1700, 1653, 1514, 1264, 1158, 1017; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 320 (4.02), 233 (3.86), 217 (3.82); $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$: 7.72 (1H, d, $J$=15.8 Hz, H-7"), 7.22 (1H, d, $J$=1.9 Hz, H-2"), 7.11 (1H, dd, $J$=8.2, 1.9 Hz, H-6"), 6.83 (1H, d, $J$=8.2 Hz, H-5"), 6.45 (1H, d, $J$=15.8 Hz, H-8"), 6.43 (1H, d, $J$=6.4 Hz, H-3), 5.55 (1H, d, $J$=6.4 Hz, H-1), 5.08 (1H, d, $J$=1.9 Hz, H-6), 4.97 (1H, d, $J$=6.4 Hz, H-4), 4.70 (1H, d, $J$=7.9 Hz, H-1'), 3.93 (1H, dd, $J$=12.0, 2.0 Hz, H-6'a), 3.90 (3H, s, $CH_3O$-), 3.65 (1H, dd, $J$=12.0, 6.5 Hz, H-6'b), 3.57 (1H, d, $J$=1.9 Hz, H-7), 3.40 (1H, m, H-3'), 3.33 (1H, m, H-5'), 3.24 (1H, m, H-2'), 3.21 (1H, m, H-4'), 2.51 (1H, d, $J$=6.4 Hz, H-9), 1.52 (3H, s, $H_3$-10); $^{13}$C-NMR ($CD_3OD$, 100 MHz): Table 1; HR-ESI-MS (positive-ion mode) $m/z$: 561.1577 $[M+Na]^+$ ($C_{25}H_{30}O_{12}Na$: 561.1579).

Compound 4 Amorphous powder, $[\alpha]_D^{26}$ –64.0 ($c$=0.05, MeOH); IR $\nu_{max}$ (film) $cm^{-1}$: 3347, 2927, 1714, 1653, 1513, 1263, 1169, 1022; UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 313 (4.03), 218.(4.06); $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$: 7.77 (1H, d, $J$=1.9 Hz, H-2"), 7.16 (1H, dd, $J$=8.3, 1.9 Hz, H-6"), 6.91 (1H, d, $J$=12.8 Hz, H-7"), 6.77 (1H, d, $J$=8.3 Hz, H-5"), 6.42 (1H, d, $J$=6.2 Hz, H-3), 5.92 (1H, d, $J$=12.8 Hz, H-8"), 5.54 (1H, d, $J$=6.3 Hz, H-1), 5.04 (1H, d, $J$=2.0 Hz, H-6), 4.96 (1H, d, $J$=6.2 Hz, H-4), 4.69 (1H, d, $J$=7.9 Hz, H-1'), 3.93 (1H, dd, $J$=11.8, 2.0 Hz, H-6'a), 3.87 (3H, s, $CH_3O$-), 3.66 (1H, dd, $J$=11.8, 6.7 Hz, H-6'b), 3.56 (1H, d, $J$=2.0 Hz, H-7), 3.39 (1H, m, H-3'), 3.33 (1H, m, H-5'), 3.24 (1H, m, H-2'), 3.22 (1H, m, H-4'), 2.48 (1H, d, $J$=6.3 Hz, H-9), 1.51 (3H, s, $H_3$-10); $^{13}$C-NMR ($CD_3OD$, 100 MHz): Table 1; HR-ESI-MS (positive-ion mode) $m/z$: 561.1576 $[M+Na]^+$ ($C_{25}H_{30}O_{12}Na$: 561.1579).

Sugar analysis Compound 1 (500 mg) was hydrolyzed with 1M HCl (0.1 mL) at 90 ºC for 2 h. The reaction mixtures were partitioned with an equal amount of EtOAc (0.1 mL), and the water layers were analyzed with a chiral detector (JASCO OR-2090*plus*) on an amino column [Asahipak $NH_2$P-50 4E, $CH_3CN$-$H_2O$ (4:1), 1 mL/min]. Their hydrolyzate gave a peak for D-glucose at 19.4 min with a positive optical rotation sign. The peak was identified by co-chromatography with the authentic sample.

Mild alkaline hydrolysis of 1 Compound 1 (9.1 mg) was hydrolyzed in 2 mL of 0.1 M NaOH in MeOH at 25 ºC for 1 h. The reaction mixture was neutralized by the addition of Amberlite IR-120B ($H^+$) and then the filtrate was evaporated. The residue was purified by prep. TLC ($CHCl_3$-MeOH-$H_2O$, 15:6:1) to give 1.7 mg of 1a and 0.6 mg of 1b from the spots at $R_f$ 0.82 and 0.27, respectively. Antirrhinoside (1a): $[\alpha]_D^{22}$ –54.5 ($c$=0.11, dioxane); HR-ESI-MS (positive-ion mode) $m/z$: 385.1108 $[M+Na]^+$ ($C_{15}H_{10}O_{12}Na$: 385.1105).[10] Methyl ferulate (1b): $^1$H-NMR ($CD_3OD$, 400 MHz) $\delta$: 7.61 (1H, d, $J$=15.9 Hz, H-7), 7.17 (1H, d, $J$=2.0 Hz, H-2),7.06 (1H, dd, $J$=8.1, 2.0 Hz, H-6), 6.80 (1H, d, $J$=8.1 Hz, H-5), 6.34 (1H, d, $J$=15.9 Hz, H-8), 3.89 (3H, s, $CH_3O$-), 3.76 (3H, s, $CH_3OCO$-);[17] HR-ESI-MS (positive-ion mode) $m/z$: 231.0628 $[M+Na]^+$ ($C_{11}H_{12}O_4Na$: 231.0628).

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