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NOTE

Two new glycosides from *Viburnum plicatum* Thunb. ex Murray var. *plicatum* f. *plicatum*

Masao Kikuchi · Rie Onoguchi · Yasunori Yaoita · Koichi Machida

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Abstract Two new glycosides, named plicatumoside A (**1**) and (+)-neomedioresinol 4,4′-di-*O*-*β*-D-glucopyranoside (**2**), together with 13 known compounds, were isolated from the leaves of *Viburnum plicatum* Thunb. ex Murray var. *plicatum* f. *plicatum*. Their structures were established on the basis of NMR, MS, and chemical data.

Keywords *Viburnum plicatum* · Caplifoliaceae · Glycoside

Introduction

We have reported the isolation of three new glycosides and seven known ones from the leaves of *Viburnum plicatum* Thunb. var. *tomentosum* Miq. (Capriforiaceae), known as the Hú dié shù in Chinese herbal medicine [1]. It has been used for lymphadenitis, ringworm, and infantile tantrum. In a continuation of our investigation of the chemical constituents from plants of the genus *Viburnum* species, we have now examined the chemical constituents of the leaves of *V. plicatum* Thunb. ex Murray var. *plicatum* f. *plicatum*. As far as we know, there are no reports regarding the chemical constituents of this plant. This paper describes the structural elucidation and identification of 2 new glycosides, named plicatumoside A (**1**) and (+)-neomedioresinol 4,4′-*O*-di-*β*-D-glucopyranoside (**2**), isolated along with 13 known compounds (**3**–**15**) from this plant.

M. Kikuchi (✉) · R. Onoguchi · Y. Yaoita · K. Machida
Tohoku Pharmaceutical University,
4-4-1 Komatsushima, Aoba-ku, Sendai,
Miyagi 981-8558, Japan
e-mail: mkikuchi@tohoku-pharm.ac.jp

Results and discussion

The leaves of *V. plicatum* Thunb. ex Murray var. *plicatum* f. *plicatum* were extracted with MeOH. The MeOH extract was fractionated among $CHCl_3$, AcOEt, *n*-BuOH, and H_2O. The *n*-BuOH- and H_2O-soluble fractions were each subjected to separation by a combination of chromatographic procedures. From the *n*-BuOH-soluble fraction, compounds **1** and **3**–**6** were isolated, while compounds **2** and **7**–**15** were isolated from the H_2O-soluble fraction. The known compounds **3**–**15** were identified as henryoside (**3**) [2], 5-*O*-caffeoyl shikimic acid (**4**) [3], 1-*O*-caffeoyl *β*-D-glucopyranoside (**5**) [4], chlorogenic acid (**6**) [5], (+)-medioresinol 4,4′-*O*-di-*β*-D-glucopyranoside (**7**) [6, 7], (+)-pinoresinol 4,4′-*O*-di-*β*-D-glucopyranoside (**8**) [7], (+)-syringaresinol 4,4′-*O*-di-*β*-D-glucopyranoside (**9**) [7], kaempferol 3-*O*-rutinoside (**10**) [8], kaempferol 3-*O*-robiobioside (**11**) [8], quercetin 3-*O*-rutinoside (**12**) [8], quercetin 3-*O*-robiobioside (**13**) [8], secoxyloganin (**14**) [9], and loganic acid (**15**) [10] by comparison of their spectroscopic data with those previously described in the literature (Fig. 1).

Compound **1** was obtained as a hygroscopic amorphous powder, $[\alpha]_D$ −40.3° (MeOH). The molecular formula of **1** was determined as $C_{26}H_{32}O_{15}$ on the basis of a pseudo molecular ion peak at *m/z* 607.1636 $[M + Na]^+$ in the high-resolution (HR)-FAB-MS. The ^{1}H-NMR spectrum of **1** showed the signals due to one 1,2,6-trisubstituted aromatic ring at δ_H 6.56 (1H, br d, $J = 8.4$ Hz, H-5), 6.68 (1H, br d, $J = 8.1$ Hz, H-3), and 7.25 (1H, dd, $J = 8.4$, 8.1 Hz, H-4), one *ortho*-disubstituted aromatic ring at δ_H 7.40 (2H, br t, $J = 7.7$ Hz, H-4′, H-5′) and 7.51 (2H, dd, $J = 7.7$, 1.5 Hz, H-3′, H-6′), one CH_2 as an AB system at δ_H 5.37 (1H, d, $J = 13.2$ Hz, Ha-7) and 5.39 (1H, d, $J = 13.2$ Hz, Hb-7), and two anomeric protons at δ_H 4.44

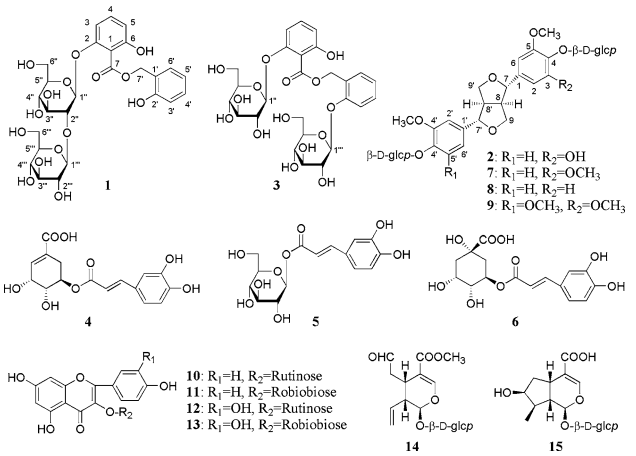

Fig. 1 Structures of 1–15

Table 1 ^{13}C-NMR data for compounds **1** and **3** (150 MHz, CD_3OD)

Position	1	3	Position	1	3
1	110.2	110.5	1′	129.5	126.8
2	157.2	157.2	2′	137.3	157.0
3	106.3	106.7	3′	129.7	117.1
4	133.9	134.2	4′	129.6	130.0
5	111.1	111.7	5′	129.6	123.8
6	160.0	160.1	6′	129.7	130.8
7	169.8	170.1	7′	68.2	63.7
1″	99.7	102.9	1‴	104.5	102.6
2″	83.4	74.8	2‴	76.0	74.9
3″	77.4	78.3	3‴	77.8	78.3
4″	70.7	71.2	4‴	70.8	71.3
5″	77.6	78.2	5‴	77.5	78.1
6″	61.4	62.6	6‴	62.4	62.6

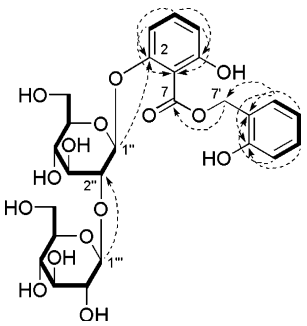

Fig. 2 The main HMBCs of **1**. *Heavy lines* indicate partial structures inferred from ^{1}H-^{1}H COSY

(1H, d, $J = 7.7$ Hz, H-1‴) and 5.28 (1H, d, $J = 7.3$ Hz, H-1″). Acid hydrolysis of **1** yielded only D-glucose, which was identified by its retention time and optical rotation by means of chiral detection in HPLC analysis. The coupling constants of the two anomeric protons indicated that the glycosyl linkages are of β-configuration. These spectral features, including the molecular formula, were very similar to those of henryoside (**3**), except for the ^{13}C-NMR chemical shifts due to the *ortho*-disubstituted aromatic ring and the CH_2 as an AB system (Table 1). The chemical shift variations noted in both **1** and **3** could be explained by the absence of a β-D-glucopyranosyl unit at C-2′ in **1**. This β-D-glucopyranosyl unit was determined to be involved in a glycosyl linkage at C-2″ of the other β-D-glucopyranosyl group bonded to C-2, because the signal due to C-2″ was markedly downfield shifted at δ_C 83.4 (+8.6 ppm), when comparing the ^{13}C-NMR spectrum of **1** with that of **3**. This finding was supported by the HMBC experiment between H-1‴ and C-2″ (Fig. 2). From the above data, the structure of **1** was established as 2-hydroxybenzyl 2-(2-*O*-β-D-glucopyranosyl-β-D-glucopyranosyloxy)-6-hydroxybenzoate, and named plicatumoside A.

Compound **2** was obtained as colorless needles, $[\alpha]_D$ +82.0° (MeOH). The molecular formula of **2** was determined as $C_{32}H_{42}O_{17}$ on the basis of a pseudo molecular ion peak at *m/z* 699.2481 $[M + H]^+$ in the HR-FAB-MS. In the ^{1}H-NMR spectrum of **2**, two methoxyl groups [δ_H 3.84 (3H, s, OMe-5), 3.87 (3H, s, OMe-3′)], two anomeric proton signals [δ_H 4.65 (1H, d, $J = 7.6$ Hz, H-1″), 4.87 (1H, d, $J = 7.6$ Hz, H-1‴)], a 1,3,4-trisubstituted benzene ring [δ_H 6.91 (1H, dd, $J = 8.2$, 2.0 Hz, H-6′), 7.02 (1H, d, $J = 2.0$ Hz, H-2′), 7.14 (1H, d, $J = 8.2$ Hz, H-5′)], and a set of *meta*-coupled doublets of an asymmetrically 1,3,4,5-tetrasubstituted benzene ring [δ_H 6.54 (1H, d, $J = 2.0$ Hz, H-2), 6.57 (1H, d, $J = 2.0$ Hz, H-6)] were observed. Acid hydrolysis of **2** in the above described manner gave only D-glucose. The ^{1}H-NMR data was closely related to those of (+)-medioresinol 4,4′-*O*-di-β-D-glucopyranoside (**7**), except for the disappearance of an OMe group. Furthermore, compound **2** possesses a set of *meta*-coupled doublets (H-2 and H-6) instead of a chemically equivalent singlet of 1,3,4,5-tetrasubstituted benzene ring in **7**. The molecular formula of **2** represented a loss of a CH_2 unit compared to that of **7**. These findings indicated that an OMe group of 1,3,4,5-tetrasubstituted benzene ring in **7** was replaced by an OH group in **2**. This deduction was supported by the HMBC and the NOE difference (NOED) experiments as follows (Fig. 3): the anomeric proton at H-1″ [δ_H 4.65 (1H, d, $J = 7.6$ Hz)] showed HMBC cross-peak with δ_C 134.5, which is also correlated to the *meta*-coupled doublet proton signal [δ_H 6.57 (1H, d, $J = 2.0$ Hz)]. In the NOED experiment, irradiation of the OMe proton [δ_H 3.84 (3H, s, 5-OMe)] showed an NOE on the signal at δ_H 6.57. Thus, the signals at δ_C 134.5 and δ_H 6.57 were assigned to C-4 and H-6, respectively. Furthermore, **2** was treated with (trimethylsilyl) diazomethane to

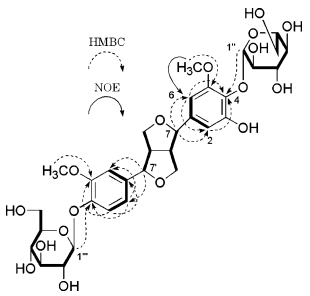

Fig. 3 The main HMBCs and NOE correlation of **2**. *Heavy lines* indicate partial structures inferred from ^{1}H-^{1}H COSY

give **7**, which was identified by comparison of the spectral data including the optical rotation, suggesting that the absolute configuration of **2** was determined to be the same as **7** [6, 7]. From these data, the structure of **2** was elucidated as shown and named (+)-neomedioresinol 4,4′-*O*-di-*β*-D-glucopyranoside.

Experimental

General

Optical rotations were measured with a JASCO DIP-360 digital polarimeter. UV spectra were recorded with a Beckman DU-64 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL JNM-LA 600 (600, 150 MHz, respectively) spectrometers. Chemical shifts are given on a δ (ppm) scale, with tetramethylsilane (TMS) as internal standard. FAB-MS were recorded on a JEOL JMS-DX 303 mass spectrometer. Column chromatography was carried out on Kieselgel 60 (230–400 mesh, Merck, Darmstadt, Germany), Sephadex LH-20 (Pharmacia Fine Chemicals, Sweden), and Diaion HP-20 (Mitsubishi Chemical). HPLC was carried out on a Tosoh HPLC system [pump, CCPS; detector, UV-8020; column, TSK gel ODS 120T (7.8 mm i.d. × 30 cm, Tosoh, Tokyo, Japan)], TSK gel Amide-80 (7.8 mm i.d. × 30 cm, Tosoh), and Cosmosil 5SL (10 mm i.d. × 25 cm, Nacalai, Tokyo, Japan)].

Plant material

Leaves of *V. plicatum* Thunb. ex Murray var. *plicatum* f. *plicatum* were collected in May 2007 in Sendai, Miyagi prefecture, Japan, and identified by one of the authors (M.K.). A voucher specimen (2007-5-KM3) is held in the laboratory of M. Kikuchi.

Extraction and isolation

Fresh leaves of *V. plicatum* Thunb. ex Murray var. *plicatum* f. *plicatum* (860 g) were extracted with MeOH at room temperature for 3 weeks. The MeOH extract was concentrated under reduced pressure, and the residue (74.5 g) was suspended in water. This suspension was successively extracted with $CHCl_3$, AcOEt, *n*-BuOH, and H_2O. The *n*-BuOH-soluble fraction was concentrated under reduced pressure to produce a residue (14.4 g). The extract was chromatographed on a silica gel column using $CHCl_3$-MeOH-H_2O (40:10:1), and the eluate was separated into 20 fractions (frs. 1–20). Fraction 7 was chromatographed on a Sephadex LH-20 column using 50% MeOH, and the eluate was separated into ten fractions (frs. 7-1 to 7-10). Fraction 7-3 was subjected to preparative HPLC [column, TSK gel ODS 120T; mobile phase, MeOH-H_2O (1:2); UV detector, 205 nm; flow rate, 1.0 mL/min; column temperature, 40°C] to give **1** (20.5 mg) and **3** (12.5 mg). Fraction 13 was chromatographed on a Sephadex LH-20 column using 50% MeOH, and the eluate was separated into six fractions (frs. 13-1 to 13-6). Fraction 13-2 was subjected to preparative HPLC [column, TSK gel ODS 120T; mobile phase, MeOH-H_2O (1:4); UV detector, 205 nm; flow rate, 1.5 mL/min; column temperature, 40°C] to give **4** (15.8 mg) and **5** (22.5 mg). Fraction 17 was subjected to preparative HPLC [column, TSK gel ODS 120T; mobile phase, MeOH-H_2O (1:5); UV detector, 205 nm; flow rate, 1.5 mL/min; column temperature, 40°C] to give **6** (8.5 mg). The H_2O layer was passed through a Mitsubishi Diaion HP-20 column, and the adsorbed material was eluted with H_2O and MeOH. The MeOH eluate-fraction from the Diaion HP-20 column was concentrated. The residue (2.4 g) was chromatographed on a silica gel column using $CHCl_3$-MeOH-H_2O (30:10:1), and the eluate was separated into 10 fractions (frs. 1–10). Fraction 5 was subjected to preparative HPLC [column, TSK gel ODS 120T; mobile phase, MeOH-H_2O (1:2); UV detector, 205 nm; flow rate, 1.5 mL/min; column temperature, 40°C] to give **2** (18.0 mg), **7** (15.0 mg), **8** (16.5 mg), **9** (25.5 mg), **10** (23.0 mg), **11** (16.0 mg), **12** (20.0 mg), and **13** (12.0 mg). Fraction 7 was subjected to preparative HPLC [column, TSK gel ODS 120T; mobile phase, MeOH-H_2O (1:2); UV detector, 205 nm; flow rate, 1.5 mL/min; column temperature, 40°C] to give **14** (15.0 mg) and **15** (11.5 mg).

*Plicatumoside A (**1**)*

An amorphous powder; $[\alpha]_D^{25}$ −40.3° (c = 0.31, MeOH); UV λ_{max} (MeOH) nm (log ε): 205 (4.6), 247 (3.9); FAB-MS *m/z*: 607 $[M + Na]^+$; HR-FAB-MS *m/z*: 607.1636 $[M + Na]^+$ (calc'd for $C_{26}H_{32}O_{15}Na$: 607.1639); ^{1}H-NMR (600 MHz, CD_3OD) δ: 3.08 (1H, ddd, J = 9.8, 5.8, 2.6 Hz, H-5‴), 3.15 (1H, dd, J = 9.2, 7.7 Hz, H-2‴), 3.17 (1H, dd, J = 12.0, 5.8 Hz, Ha-6‴), 3.28–3.41 (4H, m, H-4″, H-5″, H-3‴, H-4‴), 3.47 (1H, dd, J = 12.0, 2.6 Hz, Hb-6‴), 3.50 (1H, dd, J = 8.5, 7.3 Hz, H-2″), 3.63 (1H, t, J = 9.2 Hz,

Table 2 ^{13}C-NMR data for compounds **2** and **7** (150 MHz, CD_3OD)

Position	2	7	Position	2	7
1	140.2	135.7	1′	137.5	137.5
2	108.0	105.4	2′	111.7	111.7
3	152.0	154.5	3′	151.0	151.1
4	134.5	139.6	4′	147.6	147.6
5	154.4	154.5	5′	118.1	118.1
6	102.7	105.4	6′	119.8	119.8
7	87.1	87.2	7′	87.1	87.1
8	55.7	55.7	8′	55.5	55.5
9	70.9	71.4	9′	72.9	72.9
OCH_3	56.79	57.6, 57.6	OCH_3	56.77	56.8
1″	106.9	104.9	1‴	102.9	102.9
2″	75.4	75.8	2‴	75.0	75.0
3″	77.7	77.9	3‴	78.3	78.3
4″	73.0	73.0	4‴	71.4	71.4
5″	78.4	78.4	5‴	77.9	77.9
6″	62.1	62.6	6‴	62.5	62.6

H-3″), 3.66 (1H, dd, J = 12.1, 5.1 Hz, Ha-6″), 3.83 (1H, dd, J = 12.1, 1.8 Hz, Hb-6″), 4.44 (1H, d, J = 7.7 Hz, H-1‴), 5.28 (1H, d, J = 7.3 Hz, H-1″), 5.37 (1H, d, J = 13.2 Hz, Ha-7′), 5.39 (1H, d, J = 13.2 Hz, Hb-7′), 6.56 (1H, br d, J = 8.4 Hz, H-5), 6.68 (1H, br d, J = 8.1 Hz, H-3), 7.25 (1H, dd, J = 8.4, 8.1 Hz, H-4), 7.40 (2H, br t, J = 7.7 Hz, H-4′, H-5′), 7.51 (2H, dd, J = 7.7, 1.5 Hz, H-3′, H-6′); ^{13}C-NMR (CD_3OD): Table 1.

*(+)-Neomedioresinol 4,4′-O-di-β-D-glucopyranoside (**2**)*

Colorless needles, mp 175–176°C; $[\alpha]_D^{25}$ +82.0° (c = 0.12, MeOH); UV λ_{max} (MeOH) nm (log ε): 203 (5.1), 221 (4.5), 273 (3.7); CD ($c = 1.8 \times 10^{-5}$ M, MeOH) Δε (nm): +4.8 (210), −5.9 (230); FAB-MS *m/z*: 699 $[M + H]^+$, 721 $[M + Na]^+$; HR-FAB-MS *m/z*: 699.2481 $[M + H]^+$ (calc'd for $C_{32}H_{43}O_{17}$: 699.2500); ^{1}H-NMR (600 MHz, CD_3OD) δ: 3.10 (2H, m, H-8, H-8′), 3.22–3.49 (8H, m, H-2″, H-3″, H-4″, H-5″, H-2‴, H-3‴, H-4‴, H-5‴), 3.84 (3H, s, CH_3O-5), 3.74 (2H, m, Ha-6″, Ha-6‴), 3.86 (2H, m, Hb-6″, Hb-6‴), 3.87 (3H, s, CH_3O-3′), 3.88 (2H, dd, J = 12.0, 2.7 Hz, Hα-9, Hα-9′), 4.26 (2H, dd, J = 12.0, 6.8 Hz, Hβ-9, Hβ-9′), 4.65 (1H, d, J = 7.6 Hz, H-1″), 4.71 (1H, d, J = 3.9 Hz, H-7), 4.74 (1H, d, J = 4.1 Hz, H-7′), 4.87 (1H, d, J = 7.6 Hz, H-1‴), 6.54 (1H, d, J = 2.0 Hz, H-2), 6.57 (1H, d, J = 2.0 Hz, H-6), 6.91 (1H, dd, J = 8.2, 2.0 Hz, H-6′), 7.02 (1H, d, J = 2.0 Hz, H-2′), 7.14 (1H, d, J = 8.2 Hz, H-5′); ^{13}C-NMR (CD_3OD): Table 2.

*Methylation of **2***

To a solution of **2** (2.0 mg) in MeOH (2 mL) was added (trimethylsilyl) diazomethane (2.0 M solution in *n*-hexane, 0.5 mL). The reaction mixture was stirred at room temperature for 2 h. The mixture evaporated to give a residue that purified by prep. HPLC [column, Cosmosil 5SL; CH_2Cl_2-MeOH-H_2O (60:10:1)] to afford **7** (1.6 mg), which was identified by comparison of the spectral data including the optical rotation [6, 7].

*Acid hydrolysis of **1** and **2***

Each of the compounds (ca. 1.0 mg) was refluxed with 1 M HCl (1 mL) for 5 h. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The solution was concentrated in vacuo and dried to give a sugar fraction. The sugar fraction was analyzed by HPLC under the following conditions: column, TSK gel Amide-80; column temperature, 45°C; mobile phase, CH_3CN-H_2O (4:1); flow rate, 1.0 mL/min; chiral detection (JASCO OR-2090). Identification of D-glucose present in the sugar fraction was carried out by the comparison of the retention time and optical rotation with that of authentic sample; t_R (min) 39.0 (D-glucose, positive optical rotation).

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References

1. Machida K, Sagawa H, Onoguchi R, Kikuchi M (2010) Three new glycosides from *Viburnum plicatum* Thunb. var. *tomentosum*. Miq Helv Chim Acta 93:290–297
2. Jensen SR, Nielsen BJ, Norn V (1979) Phenolic glucosides in *Viburnun henryi*. Phytochemistry 18:904–906
3. Fukuoka M (1982) Chemical and toxicological studies on the bracken fern, *Pteridium aquilinum* var. *latiusculum*. VI. Isolation of 5-*O*-caffeoyl shikimic acid as an antithiamine factor. Chem Pharm Bull 30:3219–3224
4. Nagaoka I, Kikuchi M (1990) Structural analysis on the constituents of *Ligustrum* species. XVI. The components from the leaves of *Ligustrum ovalifolium* Hassk. J Tohoku Pharm Univ 37:51–56
5. Inoue Y, Aoyagi S, Nakanishi K (1965) Isolation of 4,5-di-*O*-caffeoyl quinic acid from coffee beans. Chem Pharm Bull 13:101–104
6. Deyama T, Ikaw T, Nishibe S (1983) The constituents of *Eucommia ulmoides* olive. I. Isolation of (+)-medioredinol di-*O*-β-D-glucopyranoside. Chem Parm Bull 31:2993–2997
7. Sakamoto S, Machida K, Kikuchi M (2008) Lignan glycosides from the leaves of *Osmanthus heterophyllus*. J Nat Med 62:362–363
8. Machida K, Kikuchi M (1990) Studies on the constituents of *Viburnum* species. IV. On the flavonoids from the leaves of *Viburnum dilatatum* Thunb. J Tohoku Pharm Univ 37:57–62
9. Sugiyama M, Machida K, Matsuda N, Kikuchi M (1993) A secoiridoid glycoside from *Osmanthus asiaticus*. Phytochemistry 34:1169–1170
10. Kakuda R, Yaoita Y, Machida K, Kikuchi M (2000) Structural analysis on the constituents of *Lonicera* species. XVI. On the chemical constituents of the flower buds of *Lonicera japonica* Thunb. J Tohoku Pharm Univ 47:55–60