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α-Keto tetrahydrofuran lignan glucosides from the Bangladeshi medicinal plant *Terminalia citrina* inhibit estradiol (E2) induced proliferation in cancer cells

Md. Abdul Muhit [a, b], Kaoru Umehara [a, *], Hiroshi Noguchi [a]

[a] School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan
[b] Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh

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ABSTRACT

EtOAc extract from the leaves of *Terminalia citrina* collected in Bangladesh were separated, and seven previously undescribed α-keto tetrahydrofuran lignan glucosides (terminalosides Q to W) were isolated and characterized. NOESY analysis of ^{1}H NMR spectra and ECD spectroscopic data analysis revealed the absolute stereochemistry of the tetrahydrofuran ring of the isolated constituents as being a (7*S*,8*R*,8′*S*)-configuration in terminalosides Q to U and a (7*R*,8*R*,8′*S*)- configuration in terminalosides V and W. All of the isolated compounds were evaluated for their estrogenic and anti-estrogenic properties using two types of estrogen-responsive human breast cancer cell lines (MCF-7 and T47D). Terminaloside R, which has a dioxymethylene group in its aromatic ring, inhibited 90% of estradiol-enhanced cell proliferation in T47D and MCF-7 cells at concentrations of 0.01 μM and 0.1 μM, respectively. On the other hand, terminaloside T, the analogous compound which has two oxymethyl groups in place of dioxymethylene, suppressed 90% of cell proliferation selectively in T47D cells at a concentration of 0.01 μM. However, terminaloside W, the 7*R*-stereoisomer of terminaloside R, only showed moderate activity.

1. Introduction

Terminalia citrina (Gaertn.) Roxb. is a traditional medicinal plant of the Combretaceae family that is widely distributed in southeast Asia and Africa, including in Bangladesh. Its bark and fruit are used in a similar way to *Terminalia chebula*, and both plants have been used in Ayurvedic medicines such as Haritaki since ancient times (Yusuf et al., 2009). Usage of the various parts of the plant for menstrual pain, dysentery, constipation, and heart diseases is well documented. In addition, anti-nociceptive, anti-inflammatory, anxiolytic, and anthelmintic activity from its leaf extract have also been previously reported (Das et al., 2015). Phytochemical study has produced the isolation of antimicrobial tannins from the fruit of the plant (Burapadaja and Bunchoo, 1995). We previously reported thirteen furofuran (Muhit et al., 2016a) and five furofuranone lignan glucosides (Muhit et al., 2016b) from the EtOAc extract of the leaves of *T. citrina* with significant antiestrogenic properties.

This paper reports on the isolation and characterization of seven previously undescribed α-keto tetrahydrofuran lignan monoglucosides (**1**–**7**) from the EtOAc extract of the leaves of *T. citrina*. In addition, all of the isolated compounds were evaluated for their estrogenic and anti-estrogenic activity using estrogen responsive breast cancer cell lines (MCF-7, T47D). To our knowledge, this is the first report of α-keto tetrahydrofuran lignans from any of the *Terminalia* species demonstrating chemotaxonomic significance among the plants of the Combretaceae family.

2. Results and discussion

2.1. Isolation and structure identification

The leaves of *Terminalia citrina* (Combretaceae) were collected in the Rangamati District of Bangladeshin May 2013 and were identified by Mr. Sardar Nasir Uddin, Senior Scientific Officer, National Herbarium, Mirpur, Dhaka, Bangladesh. The crude methanolic extract was fractionated with EtOAc and H_2O. The EtOAc soluble fraction was subjected to silica-gel column chromatography, followed by repeated reversed-phase HPLC analysis, affording seven previously undescribed lignan glucosides (**1**–**7**) as

* Corresponding author. Present address: Yokohama University of Pharmacy, 601 Matano-cho, Totsuka-ward, Yokohama 245-0066, Japan.
E-mail address: kaoru.umehara@hamayaku.ac.jp (K. Umehara).

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amorphous powders (Fig. 1). The planar structures of the isolated compounds were established primarily on the basis of ^{1}H and ^{13}C NMR spectra. All of the isolated constituents showed the presence of an α-keto tetrahydrofuran skeleton in their structures. ROESY correlations and ECD spectra were also used to determine the absolute configurations.

Compound **1**, $[\alpha]_D$ +16, was obtained as a colorless amorphous powder, and its molecular formula was assigned as $C_{28}H_{34}O_{13}$, based on the protonated ion peak $[M + H]^+$ 579.2060 (calcd 579.2078) that appeared in the HRFABMS data, indicating 12 indices of unsaturation. UV spectrum revealed the presence of a carbonyl group conjugated with aromatic rings (227, 272 and 306 nm) in its structure, which was in accordance with the presence of a carbonyl group at δ_C 199.9 (C-7′) in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum of **1** showed the characteristic signals of an α-keto tetrahydrofuran lignan, including a benzylic oxymethine proton [δ_H 4.81 (1H, d, J = 8.5 Hz, H-7)], two methines [δ_H 4.27 (1H, overlapped, H-8′) and 2.74 (1H, m, H-8)], and two pairs of oxymethylenes [δ_H 4.22 (1H, dd, J = 10.0, 8.0 Hz, H-9′a) and 4.18 (1H, dd, J = 10.0, 5.0 Hz, H-9′b); 4.06 (1H, dd, J = 10.5, 4.5 Hz, H-9a) and 3.66 (1H, overlapped, H-9b)] (Table 1). The ^{1}H NMR spectrum also displayed ABX-type aromatic proton signals [δ_H 7.70 (1H, dd, J = 8.0, 2.0 Hz, H-6′), 7.48 (1H, d, J = 2.0 Hz, H-2′) and 6.96 (1H, d, J = 8.0 Hz, H-5′)] and one dioxymethylene proton signal at δ_H 6.06 (2H, s), suggesting the presence of a 3,4-methylenedioxyphenyl moiety. This was confirmed with HMBC spectra, where dioxymethylene protons showed clear correlations to two aromatic carbon resonances at δ_C 149.9 (C-3′) and 153.8 (C-4′). In addition to an anomeric proton signal [δ_H 4.28 (1H, d, J = 8.0 Hz, H-1″)], the ^{1}H NMR spectrum of **1** also displayed a singlet aromatic proton [δ_H 6.78 (2H, H-2,6)], along with two oxymethyl group signals at δ_H 3.86 (6H, s) and 3.75 (3H, s), indicating the presence of a 3,4,5-trimethoxyphenyl moiety.

From the above data, the structure of **1** was proposed to be a 7,8,8′-trisubstituted α-keto tetrahydrofuran lignan, with a glucopyranosyl moiety attached to one of the oxymethylene groups. This type of α-keto tetrahydrofuran lignan glucoside (aketrilignoside B) has also been reported in *Akebia trifoliata* (Guan et al., 2008). The connectivity of the partial structures was elucidated by three H-C long-range correlations in the HMBC spectrum: i) from two *meta*-coupled protons [δ_H 7.48 (H-2′) and 7.70 (H-6′)] to a carbonyl carbon at δ_C 199.9 (C-7′), ii) from a singlet aromatic proton signal [δ_H 6.78 (H-2,6)] to a benzylic oxymethine at δ_C 85.1 (C-7), and iii) from an anomeric proton signal [δ_H 4.28 (H-1″)] and a benzylic oxymethine signal [δ_H 4.81 (H-7)] to an oxymethylene carbon at δ_C 69.2 (C-9). Acid hydrolysis of **1**–**7** gave a sugar moiety, which was identified as D-glucose by HPLC analysis, and the anomeric center of the D-glucose was found to have a β-configuration based on the coupling constant of an anomeric proton signal (H-1″, J = 7.5 Hz).

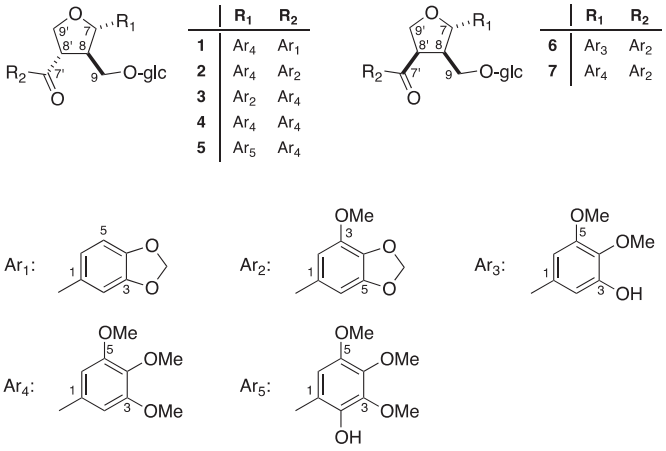

Fig. 1. α-Keto tetrahydrofuran lignan glucosides (**1**–**7**) from *T. citrina*.

The relative configurations of C-7 and 8 were deduced as *trans* from NOE correlations between a benzylic oxymethine proton [δ_H 4.81 (H-7)] and oxymethylene protons [δ_H 4.06 (H-9a), 3.66 (H-9a)], and between a methine proton in the highest field [δ_H 2.74 (H-8)] and aromatic protons [δ_H 6.78 (H-2,6)] in the ROESY spectrum of **1** (Fig. 2). These correlations were observed in the ROESY spectra of all of the isolated compounds (**1**–**7**). ECD spectra analysis revealed a positive Cotton effect at 276 ($\Delta\varepsilon$ +2.61) and a negative Cotton effect at 322 ($\Delta\varepsilon$ −1.3) nm (Fig. 3), which are similar to those of wikstrone (Liao et al., 2006; Lee et al., 2016; Xiong et al., 2011). Therefore, the structure of **1** (terminaloside Q) was deduced as (+)-(7*S*,8*R*,8′*S*)-tetrahydro-7-(3,4,5-trimethoxyphenyl)- 8′-(3′,4′-methylenedioxybenzoyl)- furan- 8-methyl-*O*-β-D-glucopyranoside.

Compounds **2**, $[\alpha]_D$ +54, **3**, $[\alpha]_D$ +25, and **4**, $[\alpha]_D$ +22, were isolated as colorless amorphous powders, and their molecular formulas were determined to be $C_{29}H_{36}O_{14}$, $C_{29}H_{36}O_{14}$, and $C_{30}H_{40}O_{14}$, based on ion peaks in HRFABMS data at *m/z* 609.2191 $[M + H]^+$ (calcd 609.2183), 631.1982 $[M + Na]^+$ (calcd 631.2002), and 625.2486 $[M + H]^+$ (calcd 625.2496), respectively. The ^{1}H and ^{13}C NMR spectroscopic features of **2** and **3** were congruent with **1** (Tables 1 and 2). However, also apparent were a pair of *meta*-coupled aromatic protons signals [**2**: δ 7.35 (1H, d, J = 2.0 Hz, H-2′) and 7.24 (1H, d, J = 2.0 Hz, H-6′); **3**: δ 6.70 (1H, d, J = 1.5 Hz, H-2) and 6.64 (1H, d, J = 1.5 Hz, H-6)] and signals for an additional oxymethyl group [**2**: δ_H 3.95 (3H, s), δ_C 57.5; **3**: δ_H 3.92 (3H, s), δ_C 57.4], while ABX-type aromatic proton signals were observed in **1**. The above NMR spectra suggested the presence of another oxymethyl group next to the dioxymethylene group (Tables 1 and 2), which was confirmed in the HMBC spectra. On the other hand, while no dioxymethylene signal was observed in the ^{1}H NMR data for **4**, two sets of singlet aromatic protons [δ 7.32 (2H, s, H-2′,6′) and 6.78 (2H, s, H-2,6)] were observed in it. Although **2** and **3** were shown to have common partial structures, the singlet aromatic proton signal of **3** [δ 7.32 (2H, s, H-2′,6′)] appeared in the lower field of the NMR spectrum when compared with that of **2** [δ 6.78 (2H, s, H-2,6)]. The singlet aromatic proton [**2**: δ 6.78 (2H, s, H-2,6)] showed a correlation with an oxymethine carbon [δ_C 85.1 (C-7)] in the HMBC spectrum of **2**, whereas a homologous signal [**3**: δ 7.32 (2H, s, H-2′)] was correlated with a carbonyl carbon signal [δ_C 200.6 (C-7′)] in the case of **3**. Based on further analysis of HMBC and the ECD spectra, **2** (terminaloside R), **3** (terminaloside S), and **4** (terminaloside T) were identified as (+)-(7*S*,8*R*,8′*S*)-tetrahydro-7-(3,4,5-trimethoxyphenyl)-8′-(3′-methoxy-4′,5′ -methylenedioxybenzoyl) -furan-8-methyl-*O*-β-D-glucopyranoside (**2**), (+)-(7*S*,8*R*,8′*S*)-tetrahydro-7-(3′-methoxy-4′,5′-methylenedioxybenzoyl)-8′-(3,4,5-trimeth- oxyphenyl)-furan-8-methyl-*O*-β-D-glucopyranoside (**3**), and (+)-(7*S*,8*R*,8′*S*)-tetrahydro-7- (3,4,5-trimethoxyphenyl)-8′-(3′,4′,5′-trimethoxyphenyl)-furan-8-methyl-*O*-β-D-gluco- pyranoside (**4**), respectively.

Compound **5**, $[\alpha]_D$ +37, was obtained as a colorless amorphous powder and was assigned the molecular formula $C_{30}H_{41}O_{15}$, based on its protonated ion at *m/z* 641.2462 $[M + H]^+$ (calcd 641.2445) in HRFABMS data, which suggested an additional oxygen atom to that of **4**. The NMR spectra showed similar characteristics to those of **4**, indicating a common partial structure of a α-keto tetrahydrofuran glucoside based on an oxymethine signal [δ_H 5.14 (1H, d, J = 7.0 Hz, H-7)/δ_C 79.9 (C-7)], two oxymethylene signals [δ_H 4.13 (1H, dd, J = 10, 6.5 Hz, H-9a) and 3.75 (1H, dd, J = 10, 6.5 Hz, H-9b)/δ_C 69.8 (C-9); 4.33 (1H, overlapped, H-9a′) and 4.24 (1H, t, J = 8 Hz, H-9b′)/δ_C 71.2 (C-9′)], a carbonyl carbon signal [δ_C 201.1 (C-7)], and sugar anomeric proton and carbon signals [δ_H 4.35 (1H, d, J = 7.5 Hz, H-