Lignans and Other Compounds From the Roots of Pandanus tonkinensis and Their Lipid Peroxidation Inhibitory Activity

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Abstract
A new phenylpropane (1) and 9 known (2-10) compounds were isolated from the methanol extract of Pandanus tonkinensis roots. Their chemical structures were determined as (7S)-2,6-dimethoxyphenyl-7,9-propanediol-1-O-β-D-glucopyranoside (1), isorhapontigenin (2), pinoresinol-4,4′-di-O-β-D-glucoside (3), isoeucommin A (4), pinoresinol-4′-O-β-D-glucoside (5), acanthoside B (6), eucommin A (7), urolignoside (8), benzyl O-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (9), and (6S,9S)-roseoside (10) by comprehensive analysis of high-resolution electron spray ionization mass spectrum and nuclear magnetic resonance spectral data, as well as by comparison of their spectral data with those reported in the literature. In addition, the stereochemistry of 1 was successfully determined by both theoretical and calculated CD spectra. All the isolates were tested for their lipid peroxidation inhibitory effects by in vitro assay. Compounds 2-7 exhibited significantly lipid peroxidation inhibitory effects with IC50 values of 21.3 ± 1.7, 61.9 ± 3.9, 57.5 ± 5.5, 10.4 ± 0.7, 28.9 ± 0.3, 54.2 ± 3.5 µM, respectively, compared to that of the positive control, trolox (31.4 ± 2.2 µM).

Keywords
Pandanus tonkinensis, pandanaceae, phenylpropanoid, lignan, lipid peroxidation inhibitory

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Introduction
The plant Pandanus tonkinensis Mart. ex B. Stone belongs to Pandanaceae family, which is widely distributed in the northern provinces of Vietnam, such as Hoa Binh, Vinh Phuc, Bac Giang, and Ninh Binh, and is used in folk medicine to treat liver diseases very effectively.1,2 However, up to now, there has been no publication on the chemical composition of this plant. In the program to search for antioxidant components from Vietnamese medicinal plants, the roots of Pandanus tonkinensis were selected for research. This paper reports the isolation and structure determination of 1 new and 9 known compounds from the methanol extract of the roots of this plant. In addition, the lipid peroxidation inhibitory effects of the isolated compounds were also evaluated by in vitro assay.

Results and Discussion
Compound 1 (Figure 1) was obtained as a white amorphous powder. Its molecular formula was determined to be C17H26O9 from the pseudo-molecular ion peaks at m/z 397.1470 [M + Na]+ (calcd. for [C17H26O9Na]+, 397.1469, Δ 1.4 ppm).
HPLC analysis and comparison with the ring, one methyl doublet signal at (2H, s) suggesting a 1,3,4,5-substituted symmetrical aromatic D/L-glucose.4 All the above evidence corresponded to that of the propane moiety attached to C-1, methoxy groups linked to 15 degrees of unsaturation. The 1H nuclear magnetic resonance (NMR) spectrum of \([\text{C}_{17}\text{H}_{30}\text{O}_{9}\text{N}]\) showed signals of 17 carbon atoms, including 4 nonprotonated, 8 methines, 2 methylenes, and 3 methyls. Of these, 6 methoxy protons (δC 76.6), H-8 (δC 10.6), as well as the proton and carbon chemical shifts, δH 0.94 (3H, d, J = 7.2 Hz), 2 methoxy groups at δH 3.89 (6H, s), and an anomeric proton at δH 4.85 (1H, d, J = 7.8 Hz). The 13C NMR and HSQC spectra of \(\text{N}^{+}\) showed signals of 17 carbon atoms, including 4 nonprotonated, 8 methines, 2 methylenes, and 3 methyls. Of these, 6 carbon signals (δC 105.6, 75.8, 77.8, 71.4, 78.3, and 62.6) were assigned to a glucopyranosyl group. The above evidence suggested that 1 was a phenylpropane glycoside having 2 methoxy groups at C-3 and C-5 of the aromatic ring.3 In the propane side chain, the observed HSQC correlations of H-7 (δH 4.51)/C-7 (δC 76.6), H-8 (δH 1.76)/C-8 (δC 30.0), and H-9 (δH 0.94)/C-9 (δC 10.6), as well as the proton and carbon chemical shifts, the multiplets, and 1H-1H coupling constants indicated the presence of a -CH(OH)-CH2-CH3 moiety.3 In the HMBC spectrum of 1, H-8 correlated to C-1 (δC 143.4)/C-2 and C-5 (δC 105.0), methoxy protons (δH 3.89) correlated to C-3/C-5 (δC 154.1), and the anomeric proton (H-1′, δH 4.85) correlated to C-4 (δC 135.3) (Supplemental Figures S1). This evidence confirmed the propane moiety attached to C-1, 2 methoxy groups linked to C-3 and C-5, and the glucose attached to C-4. Furthermore, the coupling constant of the anomeric proton, JH1′/H2, = 7.8 Hz, indicated a β-glucopyranosyl linkage. Later, the presence of a D-glucose moiety was identified by acid hydrolysis, treatment with cysteine methyl ester and O-tolyl isothiocyanate, followed by HPLC analysis and comparison with the \(\text{R}_{t}\) values of authentic D/L-glucose.4 All the above evidence corresponded to that of the (7S)-configuration of 1. This suggestion was confirmed based on both theoretical and calculated CD spectra. The 2 possible configurations 7S/7R of 1 were submitted for calculation of their theoretical ECD spectra and compared with experimental results (Figure 2). The experimental CD analysis of 1 was in good agreements with the 7S-configuration. Consequently, the complete structure of compound 1 was elucidated as (7S)-2,6-dimethoxyphenyl-7,9-propanediol-1-O-β-D-glucopyranoside (Supplemental Figures S2-S7).

The other compounds were identified as isorhapontigenin (2),5 pinoresinol-4,4’-di-O-β-D-glucoside (3),6 isoeucumin A (4),7 pinoresinol-4’-O-β-D-glucoside (5),8 acanthoside B (6),9 eucommin A (7),10 urolignoside (8),11 benzyl O-α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside (9),13 and (6S,9S)-roseoside (10)14 by comparisons of their NMR spectroscopic data with those reported in the literature (Supplemental Figures S8-S43). Furthermore, the (6S,9S)-configuration of 10 was determined based on the positive Cotton effect at 240 nm in its CD spectrum and the lower carbon chemical shift of C-9 (δC 74.6 ppm).14

Phenolic compounds, including lignans, are a type of secondary plant metabolite exhibiting diverse structures, which exhibit potentially beneficial bioactive properties due to antioxidant activity.15-18 Therefore, all the isolates were tested for their lipid peroxidation inhibitory effects by \(\text{in vitro}\) assay.19,20 At a concentration as high as 100 µg/mL, compounds 2-7 exhibited significantly lipid peroxidation inhibitory effects with inhibition in the range from 72.7% to 88.4%, compared to the positive control, trolox (31.4 ± 0.7, 61.9 ± 3.9, 57.5 ± 5.5, 10.4 ± 0.7, 28.9 ± 0.3, 54.2 ± 3.5 µM, respectively, compared to that of the positive control, trolox (31.4 ± 2.2 µM). Regarding structure activity relationship, our results suggested that the lignan glycoside compounds having a furofuran structure showed the most lipid peroxidation inhibitory effects (Table 1).
Materials and Methods

General Experimental Procedures

Optical rotation was measured on a Jasco P-2000 polarimeter, IR spectra on a Spectrum Two FT-IR spectrometer, CD spectra on a Chirascan spectrometer (Applied Photophysics), NMR spectra on a Bruker Avance NEO 600 MHz spectrometer, and HRESIMS on a SCIEX X500 QTOF LC/MS. Flash column chromatography was performed using either silica gel or reversed phase (RP-18) resins as adsorbent. The ratio column chromatography was performed using either silica gel CC eluting with dichloromethane/methanol (7/1, v/v) to obtain 6 subfractions, PT6A-PT6F. PT6A (83.0 mg) was chromatographed by HPLC (j’sphere H-80 column, 250 mm length x 20 mm ID, eluting with 15% acetonitrile in water, a flow rate of 2.5 mL/min) to give compounds 1 (R<sub>t</sub> 30.689, 8.7 mg) and 10 (R<sub>t</sub> 35.024, 20.2 mg). PT6D (172.6 mg) was separated by HPLC (j’sphere H-80 column, 250 mm length x 20 mm ID, eluting with 15% acetonitrile in water, a flow rate of 2.5 mL/min) to give compound 9 (R<sub>t</sub> 37.50, 36.0 mg). PT6E (151.3 mg) was chromatographed by HPLC (j’sphere H-80 column, 250 mm length x 20 mm ID, eluting with 15% acetonitrile in water, a flow rate of 2.5 mL/min) to give compound 9 (R<sub>t</sub> 37.50, 36.0 mg). PT6E (151.3 mg) was chromatographed by HPLC (j’sphere H-80 column, 250 mm length x 20 mm ID, eluting with 20% acetonitrile in water, a flow rate of 2.5 mL/min) to give compounds 4 (R<sub>t</sub> 48.319, 4.9 mg), 5 (R<sub>t</sub> 50.933, 40.4 mg), 6 (R<sub>t</sub> 53.606, 9.6 mg), and 7 (R<sub>t</sub> 57.438, 4.5 mg). PT7E (17.8 mg) was chromatographed by HPLC using the same conditions to give compound 8 (R<sub>t</sub> 24.60, 4.1 mg). 7(S)-2,6-Dimethoxyphenyl-7,9-Propanediol-1-O-D-Glucopyranoside (1). Colorless amorphous powder, [α]<sub>D</sub> +9.0 (c 0.1, MeOH); IR (KBr) ν<sub>max</sub>: 3428, 2935, 1650, 1078 cm<sup>−1</sup>. HRESIMS m/z 397.1470 [M + Na]<sup>+</sup> (calcd. for [C<sub>17</sub>H<sub>26</sub>O<sub>9</sub>Na]<sup>+</sup>; 397.1469, Δ = +0.2 ppm) and m/z 392.1910 [M + NH<sub>4</sub>]<sup>+</sup> (calcd. for [C<sub>17</sub>H<sub>26</sub>O<sub>9</sub>NH<sub>4</sub>]<sup>+</sup>; 392.1915, Δ = −1.2 ppm).

<sup>1</sup>H NMR (CD<sub>3</sub>OD, 600 MHz) δ (ppm): 6.71 (2H, s, H-2, H-6), 4.51 (1H, t, J = 7.2 Hz, H-7), 1.76 (2H, m, H-8), 0.94 (3H, d, J = 7.2 Hz, H-9), 4.85 (1H, d, J = 7.8 Hz, H-1′), 3.50 (1H, dd, J = 9.0, 7.8 Hz, H-2′), 3.43 (1H, dd, J = 9.0, 9.0 Hz, H-3′), 3.44 (1H, dd, J = 9.0, 9.0 Hz, H-4′), 3.22 (1H, m, H-5′), 3.68 (1H, dd, J = 12.0, 5.0 Hz, H<sub>2</sub>-6′), 3.79 (1H, dd, J = 12.0, 5.0 Hz, H<sub>1</sub>-6′), 3.89 (6H, s, 2×OCH<sub>3</sub>). <sup>13</sup>C NMR (CD<sub>3</sub>OD, 150 MHz) δ (ppm): 143.4 (C-1), 105.0 (C-2, C-6), 154.1 (C-3, C-5), 135.3 (C-4), 76.6 (C-7), 30.0 (C-8), 10.6 (C-9), 105.6 (C-1′), 75.8 (C-2′), 77.8 (C-3′), 71.4 (C-4′), 78.3 (C-5′), 62.6 (C-6′), 57.0 (2×OCH<sub>3</sub>).

Acid Hydrolysis of Compound 1. Compound 1 (1.5 mg) was treated with 2 N aqueous HCl (2 mL) in sealed flask at 90 °C for 2 h. The acidic aqueous mixture was dried, CHCl<sub>3</sub> (1 mL) was added, and the CHCl<sub>3</sub> solution was extracted with H<sub>2</sub>O.

Table 1. Lipid Peroxidation Inhibitory Activity of Compounds 1-H10.

| Compounds | IC<sub>50</sub> (µM) | Compounds | IC<sub>50</sub> (µM) |
|-----------|-----------------|-----------|-----------------|
| 1         | >100            | 6         | 28.9±0.3        |
| 2         | 21.3±1.7        | 7         | 54.2±3.5        |
| 3         | 61.9±3.9        | 8         | >100            |
| 4         | 57.5±5.5        | 9         | >100            |
| 5         | 10.4±0.7        | 10        | >100            |
| Trolox<sup>a</sup> | 31.4±2.2 |

<sup>a</sup>Positive control.

Plant Material

The roots of *Pandanus tonkinensis* Mart. ex B. Stone were collected in Son Duong District, Hoa Binh Province, Vietnam, in April 2021, and identified by Dr. Do Thi Xuyen, Head of Department of Botanic, Faculty of Biology, VNU University of Science. A voucher specimen (code HNU 024663) is kept at the Herbarium, Vietnam National University, Hanoi, Vietnam.

Extraction and Isolation

Dried powder of *P. tonkinensis* roots (14 kg) was sonicated with methanol (3 times, each 25 L MeOH). After removal of solvent, the MeOH extract (40 g) was suspended in water and then partitioned with n-hexane, dichloromethane, and ethyl acetate to give the corresponding residues (PT1, 2.5 g, PT2, 3.7 g, PT3, 5.3 g), and water (PT4, 27.0 g). After checking by TLC, PT4 (26.5 g) was further chromatographed on a Diaion HP-20 column eluting with water to remove sugar, then with increasing concentration of methanol in water (25, 50, and 100%) to obtain 3 fractions, PT4A (2.4 g), PT4B (2.6 g), and PT4C (21.0 g). PT4C (20.0 g) was chromatographed on a silica gel column eluting with dichloromethane/methanol (v/v) 20:1, 5:1, 1:1, and 0:1 (each 2 L) to give 4 corresponding subfractions (PT4C1-PT4C4). PT4C1 (14.0 g) was further chromatographed on a YMC column eluting with acetone/water (1/3, v/v) to give 7 smaller fractions, PT5A-PT5G. PT5C (5.8 g) was fractionated by silica gel CC eluting with dichloromethane/methanol (7/1, v/v) to obtain 6 subfractions, PT6A-PT6F. PT6A (83.0 mg) was chromatographed by HPLC (j’sphere H-80 column, 250 mm length x 20 mm ID, eluting with 15% acetonitrile in water, a flow rate of 2.5 mL/min) to give compounds 1 (R<sub>t</sub> 30.689, 8.7 mg) and 10 (R<sub>t</sub> 35.024, 20.2 mg). PT6D (172.6 mg) was separated by HPLC (j’sphere H-80 column, 250 mm length x 20 mm ID, eluting with 15% acetonitrile in water, a flow rate of 2.5 mL/min) to give compound 9 (R<sub>t</sub> 37.50, 36.0 mg). PT6E (151.3 mg) was chromatographed by HPLC (j’sphere H-80 column, 250 mm length x 20 mm ID, eluting with 20% acetonitrile in water, a flow rate of 2.5 mL/min) to give compounds 4 (R<sub>t</sub> 48.319, 4.9 mg), 5 (R<sub>t</sub> 50.933, 40.4 mg), 6 (R<sub>t</sub> 53.606, 9.6 mg), and 7 (R<sub>t</sub> 57.438, 4.5 mg). PT7E (17.8 mg) was chromatographed by HPLC using the same conditions to give compound 8 (R<sub>t</sub> 24.60, 4.1 mg).
(1 mL). The aqueous fraction was evaporated to dryness to obtain the liberated sugar. Sugar samples, including the saccharide hydrolysis product of 1, D-glucose and L-glucose (Sigma Aldrich) were separately dissolved in 1 mL pyridine and heated with 2 mg L-cysteine methyl ester at 60 °C for 1 h, and then 2.5 µL O-tolyl isothiocyanate was added to the reaction mixture and further reacted at 60 °C for 1 h. Then, the reaction mixture was analyzed on a 250 × 4.6 mm i.d. Ultimate™ XB-C18 column (Welsh Material, Inc.) at 35 °C with isocratic elution with 25% CH3CN in 0.5% formic acid for 40 min at a flow rate of 0.8 mL/min, and detection with an UV detector (at 250 nm). Under these conditions, the standard sugars gave peaks at tR (min) 22.0 and 23.0 for L-glucose and D-glucose, respectively. A peak at tR (min) 23.0 (D-glucose) for 1 was observed.

**Antioxidant TBARS assay**

Thiobarbituric acid reactive substances (TBARS) assay values are usually reported in MDA equivalents, a compound formed from the decomposition of polyunsaturated fatty acid lipid peroxides. Briefly, a 0.1 mL sample at difference doses was added to 1 mL of mice brain homogenate (10%) and 0.8 mL phosphate buffer in the presence of 0.1 mL Fenton reagent (FeSO4 0.1 mM: H2O2 15 mM at a ratio of 1:1). After incubating the mixture at 37 °C for 15 min, 1 mL of trichloroacetic acid 10% was added to each tube and the tubes were centrifuged at 12000 rpm for 5 min. The supernatant was then mixed with 1 mL of 0.8% thiobarbituric acid at a ratio of 2:1 and heated at 100 °C for 15 min. After cooling, the absorbance of the mixture was determined at 532 nm by using a microplate reader (BioRad). The percentage inhibition was calculated by the following formula:

\[
\% \text{ Inhibition of lipid peroxidation} = \left(\frac{OD_{\text{control}} - OD_{\text{sample}}}{OD_{\text{control}}}\right) \times 100
\]

**Conclusions**

Phytochemical study of the methanol extract of *P. tonkinensis* roots led to the isolation of a new phenyl propane, (7,5)-2,6-dimethoxyphenyl-7,9-propanediol-1-O-β-D-glucopyranoside (I), and 9 known (2-10) compounds. The chemical structure of these compounds were determined by comprehensive analysis of HRESIMS and NMR spectral data, as well as by comparison of their spectral data with those reported in the literature. The stereochemistry of 1 was successfully determined by both theoretical and calculated CD spectra. Compounds 2-7 exhibited significant lipid peroxidation inhibitory effects in an *in vitro* antioxidant TBARS assay with IC50 values of 21.3 ± 1.7, 61.9 ± 3.9, 57.5 ± 5.5, 10.4 ± 0.7, 28.9 ± 0.3, 54.2 ± 3.5 μM, respectively, compared to that of the positive control, trolox, (31.4 ± 2.2 μM).

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**Author Contribution**

Research idea: PH Viet, DH Anh, NX Nhim, PV Kiem. Isolation: DTH Trang. Structure elucidation and writing: BH Tai, DTH Trang, DH Anh, PV Kiem.

**Declaration of Conflicting Interests**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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**Supplemental material**

Supplemental material for this article is available online.

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List of Abbreviations

NMR nuclear magnetic resonance
COSY correlation spectroscopy
HRESIMS high-resolution electrospray ionization mass spectrometry
HMBC heteronuclear multiple bond correlation
HSQC heteronuclear single quantum coherence
NOESY nuclear overhauser effect spectroscopy
ECD electronic circular dichroism
TBARS thiobarbituric acid reactive substances
MDA malonyl dialdehyde