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Antioxidant and Tyrosinase Inhibitory Constituents from a Desugared Sugar Cane Extract, a Byproduct of Sugar Production

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ABSTRACT: Recycling agricultural resources has become an important issue worldwide promoting the economical value of agricultural production processes. Desugared sugar cane extract (DSE) from *Saccharum officinarum* is a byproduct obtained during sugar production. Two new neolignan glucosides, saccharnan A (**1**) and saccharnan B (**2**), together with 10 known phenolics (**3**−**12**) were isolated from DSE, and their structures were elucidated on the basis of NMR spectral analysis. Compounds **3**, **4**, **8**, and **9** showed good activity against DPPH radical ($IC_{50} \leq 51.20\ \mu M$) and compounds **3**−**8** and **12** exhibited strong $ABTS^{+}$ free radical scavenging activity ($IC_{50} \leq 51.57\ \mu M$) compared to those of the positive controls, ascorbic acid and Trolox. Moreover, compounds **7** and **12** acted as potent tyrosinase inhibitors ($IC_{50} \leq 42.59\ \mu M$) compared to the positive control arbutin. Our results highlighted the economical value of recycling DSE for the future development of natural antioxidants and/or tyrosinase inhibitors.

KEYWORDS: desugared sugar cane extract, *Saccharum officinarum*, sugar cane byproduct recycling, neolignans, saccharnan A, saccharnan B, antioxidant, free radical scavengers, tyrosinase inhibitors

INTRODUCTION

Sugar cane (*Saccharum officinarum*), one of the major economic crops in the tropics and subtropics, is widely cultivated and consumed throughout the whole world. Taiwan used to be one of the most important sugar producing countries since World War II.[1] In past decades, the research in the sugar industry almost focused on developing new harvesting technologies and improving the yield of current sugar production techniques. Less attention has been devoted to improving the economical and environmental value of the production procedures. Industrial scale sugar production produces tons of wastes and byproducts which are difficult to handle and are considered as an additional burden to the environment. Limited resources and recent environmental legislations forced sugar companies to start active research aiming to develop greener procedures through recycling wastes and byproducts of sugar manufacturing processes.

Sugar cane products and isolated components have displayed a wide range of biological activities, including anti-inflammatory, antioxidant, antiatherosclerotic, and acetylcholine release modulatory activities.[2−5] A number of polyphenolics, including flavonoids, benzenoids, lignans, and neolignans, were isolated from several sugar cane products such as brown sugar, juice, and molasses.[3,6,7]

Higher levels of reactive oxygen species (ROS) and oxidative stress have been linked to a long list of life threatening diseases such as cancers, cardiovascular diseases, osteoporosis, and degenerative diseases suggesting the potential health benefits of antioxidants. Bioactive polyphenolics are able to protect living cells from the oxidative stress effects caused by large amounts of endogenous and exogenous of ROS exceeding the cells' antioxidant defense capacity.[8] Therefore, capturing harmful ROS by natural antioxidants is one of the effective ways to reduce oxidative stress damage.[9] Because of the importance of antioxidants as food preservatives and the limited supplies of natural antioxidants, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been widely used despite their proposed carcinogenic effects.[10] However, several other studies claimed the beneficial effects of using such synthetic antioxidants and denied their relationship to cancer development.[11] This debate and other health safety concerns are

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the driving forces for the discovery of new inexpensive sources of natural antioxidants.

In recent years, tyrosinase inhibitors from natural plant extracts have been utilized in the cosmetic industry due to their skin whitening effect. In the food industry, the tyrosinase mediated browning effect is directly linked to fruit injuries during harvesting; therefore, controlling this reaction with tyrosinase inhibitors is considered an essential step in the fruit pulp manufacturing process.[12] While a number of natural compounds have been described to inhibit the activity of tyrosinase, phenolic compounds retain the ability to be the most effective tyrosinase inhibitors, due to their structural similarities to endogenous tyrosinase substrates.[12]

Desugared sugar cane extract (DSE), a byproduct of the sugar refining process, is produced before sucrose crystallization from sugar cane juice. After squeezing sugar cane, resin is used as a matrix to absorb polyphenols and pigments from sugar cane juice. Most of the sucrose will pass through the resin and then is condensed into edible sucrose. The phenolics and pigments sticking on the resin can be washed and desorbed using ethanolic solution, which is then evaporated to give DSE with a total yield of 0.035% of the raw sugar cane material. In other words, about 350 g of DSE can be produced from 1 ton of sugar cane. Of course with the global production of sugar cane, a considerable amount of DSE is produced every year. In the past, DSE was regarded as a waste byproduct and usually discarded. Recently, food production procedures are developing greener methods, and this new trend cannot be ignored any more. Agricultural models using eco-friendly food production are resulting in less pollution and efficient waste recycling techniques. The richness of sugar cane in polyphenolics and the lack of studies on the biological activities of sugar cane byproducts motivated us to investigate the presence of natural antioxidants and tyrosinase inhibitors in the DSE aiming to improve the economical and environmental value of sugar production processes.

MATERIALS AND METHODS

General Procedures. The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), ascorbic acid, potassium persulfate, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), mushroom tyrosinase, tyrosine, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), and arbutin were purchased from the Sigma chemical Co. (St. Louis, MO, USA). Ultrapure water obtained using the Milli-Q system (Millipore, Bedford, USA) and utilized for HPLC analyses. All solvents used for chromatography were of HPLC grade. All other chemicals were of analytical reagent grade. Optical rotations were measured utilizing a JASCO DIP-370 digital polarimeter. UV spectra were obtained on a Hitachi 220-20 spectrophotometer. IR spectra were measured using a Hitachi 260-30 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Inova 600, Varian Unity Plus 400 MHz, or Varian Gemini 200 MHz spectrometers using TMS as an internal standard. Chemical shifts were reported in ppm (parts per million), and coupling constants (J) were expressed in Hz. HRESI-MS measurements were performed on a Shimadzu LCMS-IT-TOF liquid chromatography mass spectrometer with an ESI interface. TLC was performed on Kieselgel 60, F254 (0.20 nm, Merck), and spots were viewed under ultraviolet light at 254 and 365 nm and/or stained by spraying with 50% H_2SO_4 and heating on a hot plate. For column chromatography, silica gel (Kieselgel 60, 70-230 and 230-400 mesh, Merck), Diaion HP20 (Mitsubishi Chemical Co., Japan), and Sephadex LH-20 were used. Further purification of some compounds was achieved by a preparative HPLC (Shimadzu LC-10AT) system equipped with a Thermo ODS Hypersil column (10 × 250 mm, 5 μm).

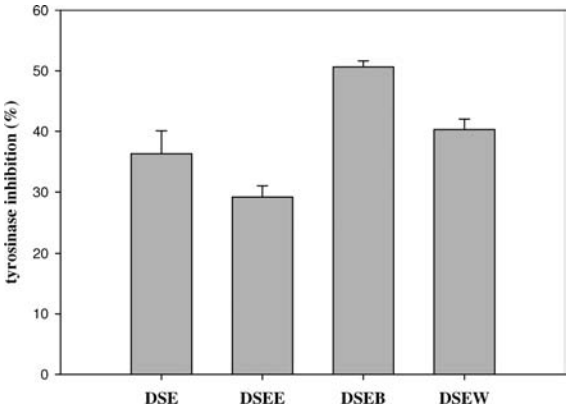

Figure 1. Tyrosinase inhibitory activity of DSE and its soluble layers. DSE, desugared sugar cane extract; DSEE, desugared sugar cane extract ethyl acetate soluble layer; DSEB, desugared sugar cane extract *n*-butanol soluble layer; DSEW, desugared sugar cane extract water-soluble layer. Percentage of tyrosinase inhibition (%) was measured at a concentration of 1 mg/mL. Results are presented as the mean ± SD (n = 3).

Extraction and Isolation. DSE was donated by the Taiwan Sugar Corporation, Tainan, Taiwan, in August, 2008. In brief, DSE (228.1 g) was suspended in 2.5 L of water and was partitioned with the same volume of ethyl acetate, *n*-butanol, and water, successively, yielding ethyl acetate (DSEE, 11.2 g), *n*-butanol (DSEB, 49.2 g), and water (DSEW, 167.5 g) soluble layers. The DSEB layer exhibited a better tyrosinase inhibitory activity with 50.66% inhibition at 1 mg/mL compared to that of the other soluble layers (Figure 1). The DSEB layer was chromatographed over a Diaion HP20 column (5.0 cm × 30 cm) using a methanol and water mixture with gradual decrease in polarity resulting in the separation of seven fractions (A1−A7).

Fraction (Fr.) A3 (2.3 g) was passed through a Sephadex LH-20 column (2.5 cm × 30 cm) and eluted with methanol to obtain five fractions (A3-1−A3-5). Fraction A3-2 (120.5 mg) was purified further using silica gel (1.5 cm × 20 cm, 70-230 mesh, 100 g) eluted with a dichloromethane and methanol mixture (6:1 v/v) followed by preparative reversed-phase HPLC on a Thermo ODS Hypersil column (10 × 250 mm, 5 μm) using methanol and water (1:9 v/v; flow rate, 2 mL/min) as the eluent to yield compounds **5** (8.6 mg) and **6** (7.2 mg). Fraction A3-4 (520.5 mg) was chromatographed further on silica gel (2.0 × 20 cm, 230−400 mesh, 100 g) eluted with a dichloromethane and methanol mixture (6:1 v/v) to give four subfractions (A3-4-1−A3-4-4). Fraction A3-4-2 (150 mg) was purified further by preparative reversed-phase HPLC on a Thermo ODS Hypersil column (10 × 250 mm, 5 μm) with a methanol and water mixture (1:3 v/v, flow rate: 2 mL/min) as the eluent to afford compounds **9** (7.3 mg), **10** (11.6 mg), **11** (12.3 mg), and **12** (4.0 mg).

Fraction A6 (1.6 g) was subjected to a Sephadex LH-20 column and eluted with methanol to give five fractions (A6-1−A6-5). Compounds **1** (1.8 mg), **2** (3.7 mg), **3** (8.5 mg), **4** (5.5 mg), **7** (3.6 mg), and **8** (51.3 mg) were isolated from Fr. A6-4 (588.2 mg) using a silica gel column (2.0 cm × 20 cm, 230−400 mesh, 150 g). The column was eluted with a mixture of dichloromethane and methanol (9:1 v/v), and the compounds were further purified by preparative reversed-phase HPLC on a Thermo ODS Hypersil column (10 × 250 mm, 5 μm) with methanol and water (1:4 v/v; flow rate, 2 mL/min) as the eluent.

*Saccharnan A (**1**).* Colorless gum; $[\alpha]_D^{25}$ +52.5 (c 0.08, CH_3OH); IR (Neat) ν_{max} 3367, 1734, 1637, 1601, 1509, 1169 cm^{-1}. UV (CH_3OH) λ_{max} nm (log ε): 229 (3.20), 330 (3.32), 320 (3.35); ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data are given in Table 1;

Table 1. ^{1}H (600 MHz) and ^{13}C-NMR (150 MHz) Data of Compounds 1 and 2 in CD_3OD

	1		2	
position	δ_C	δ_H	δ_C	δ_H
1	138.1		132.0	
2, 6	106.3	6.57 (2H, s)	105.5	6.50 (2H, s)
3, 5	154.8		149.8	
4	138.1		135.9	
7	47.5	4.54 (1H, br dd, J = 3.6, 2.4 Hz)	47.3	4.51 (1H, br dd, J = 3.6, 2.4 Hz)
8	87.6	4.41 (1H, dd, J = 7.6, 3.6 Hz)	88.1	4.42 (1H, dd, J = 6.6, 3.6 Hz)
9	63.9	3.80−3.88 (2H, m)	63.9	3.79−3.82 (2H, m)
1′	126.0		129.8	
2′,6′	134.2	7.28 (2H, d, J = 8.8 Hz)	133.7	7.40 (2H, d, J = 8.8 Hz)
3′,5′	116.6	6.66 (2H, d, J = 8.8 Hz)	117.5	7.00 (2H, d, J = 8.8 Hz)
4′	161.5		160.4	
7′	140.9	7.69 (1H, d, J = 2.4 Hz)	139.9	7.72 (1H, d, J = 2.4 Hz)
8′	124.2		127.0	
9′	174.8		174.6	
1″	105.3	4.82 (1H, d, J = 7.2 Hz)	101.8	4.91 (1H, d, J = 7.2 Hz)
2″	75.7	3.46 (1H, dd, J = 8.8, 7.2 Hz)	74.8	3.41 (1H, m)
3″	77.8	3.39 (1H, m)	77.9	3.37 (1H, m)
4″	71.2	3.41 (1H, m)	71.3	3.45 (1H, m)
5″	78.2	3.18 (1H, m)	78.2	3.45 (1H, m)
6″	62.5	3.65 (1H,dd, J = 12.0, 6.6 Hz) 3.72 (1H, m)	62.4	3.67 (1H, dd, J = 12.0, 6.6 Hz) 3.87 (1H, m)
3, 5-OMe	57.0	3.76 (6H, s)	56.8	3.75 (6H, s)

ESI-MS m/z 557 $[M + Na]^+$; HRESI-MS m/z 557.1638 $[M + Na]^+$ (calcd for $C_{26}H_{30}O_{12}Na$, 557.1635).

*Saccharnan B (**2**).* Colorless gum; $[\alpha]_D^{25}$ +61.2 (c 0.1, CH_3OH); IR (Neat) ν_{max} 3362, 1731, 1644, 1601, 1511, 1072 cm^{-1}. UV (CH_3OH) λ_{max} nm (log ε): 230 (3.36), 288 (3.46), 309 (3.49); ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data are given in Table 1; ESI-MS m/z 557 $[M + Na]^+$; HRESI-MS m/z 557.1637 $[M + Na]^+$ (calcd for $C_{26}H_{30}O_{12}Na$, 557.1635).

DPPH Assay. Radical scavenging capacity of all compounds against the stable DPPH radical was determined spectrometrically according to the reported method by Aniya et al.[13] Each stock solution of the tested samples (100 μL, 500 μM) was mixed with 100 μL of 1 mM DPPH solution for further examination. Methanol (200 μL) was used as a control, and a mixture of methanol (100 μL) and DPPH (100 μL, 1 mM) was used as a blank making the total volume for each reaction mixture in each well 200 μL. The solution was mixed, and the scavenging capacity was measured by monitoring the decrease in absorbance at 517 nm measured after 30 min. Measurements were performed at least in triplicate. Radical scavenging capacity was calculated by using the following formula:

$$\%DPPH = 1 - \left(\frac{A_{sample} - A_{control}}{A_{blank} - A_{control}}\right) \times 100$$

The calculated IC_{50} value represents the concentration of a sample required to decrease the absorbance at 517 nm by 50%.

ABTS$^+$ Assay. The $ABTS^+$ assay was performed according to Thana et al.[14] The ABTS radical cation ($ABTS^+$) was produced by mixing ABTS (7 mM) with potassium persulfate (2.45 mM) dissolved in H_2O (final volume 25 mL), and the mixture was kept it in the dark at room temperature for 12−16 h. For analysis, the reagent was diluted in methanol, and the absorbance was adjusted to 0.70 ± 0.02 at 734 nm. For the photometric assay, 100 μL of the sample solution (500 μM) was mixed with 100 μL of $ABTS^+$ solution, methanol (200 μL) was used as a control, and 100 μL methanol with 100 μL $ABTS^+$ solution was used as a blank. The total volume for each reaction mixture in each well was 200 μL, and the absorbance was measured after 6 min at 734 nm. Measurements were performed at least in triplicate. The IC_{50} was calculated as described in the DPPH assay.

Tyrosinase Inhibitory Assay. The method employed by Lai et al. was followed with a slight modification.[15] Each tested sample (1 mM) was mixed with the following substances: 40 μL of 10% aqueous DMSO, 80 μL of phosphate buffer solution (PBS, pH 6.8), and 40 μL of 150 U/mL mushroom tyrosinase. The mixture was incubated at 25 °C for 10 min and then followed by the addition of 40 μL of tyrosine (2.5 mM, PBS pH 6.8). The reaction mixture was incubated for 15 min. The inhibitory activity was determined by measuring the optical density (OD) at 475 nm using a Thermo Multiskan Ascent microplate reader. Arbutin was used as a positive control, and measurements were performed at least in triplicate. The percentage of tyrosinase inhibitory activity was calculated as follows:

$$\%\text{inhibition} = \left\{\frac{[(A - B) - (C - D)]}{(A - B)}\right\} \times 100$$

where A, OD at 475 nm without the test substance; B, OD at 475 nm without the test substance and tyrosinase; C, OD at 475 nm with the test substance; and D, OD at 475 nm with the test substance, but without tyrosinase. The IC_{50} value represents the concentration of the tested sample needed to inhibit 50% of tyrosinase activity.

Acid Hydrolysis of Compounds 1 and 2. A solution of the tested compound (1 mg/50 μL THF) was dissolved in 6 N HCl (150 μL), then refluxed for 4 h. The solution was diluted with water and extracted with ethyl acetate (1 mL × 3). The water layer was evaporated and then vacuum-dried to offer a monosaccharide residue. From the residue, glucose was proposed as the sugar part through comparing to an authentic sample using TLC [$CHCl_3$−CH_3OH−H_2O (66:33:1)], and the R_f value of glucose is 0.18. Regarding the absolute configuration of the

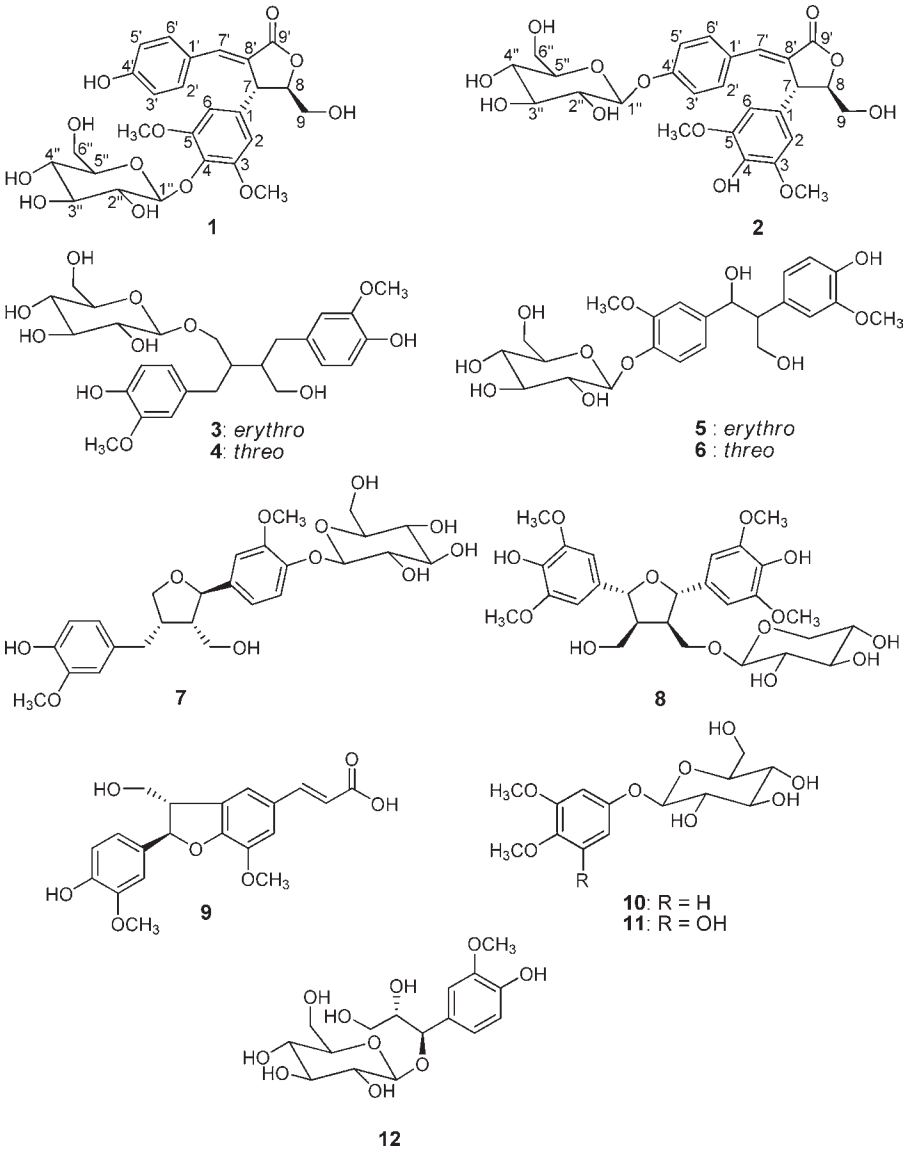

Figure 2. Structures of compounds **1**−**12** isolated from DSE.

glucose moiety, the glucose was confirmed as a D-glucose on the basis of the positive optical rotation.

RESULTS AND DISCUSSION

General. Desugared sugar cane extract (DSE) was suspended in water and was partitioned with ethyl acetate, *n*-butanol, and water, successively, yielding ethyl acetate (DSEE), *n*-butanol (DSEB), and water (DSEW)-soluble layers. The higher tyrosinase inhibitory activity exhibited by DSEB compared to that of other soluble layer encouraged us to further purify this layer utilizing bioassay-guided fractionation. The DSEB layer was fractioned on Diaion HP20, Sephadex LH-20, silica gel columns, and was finally purified using preparative reversed-phase HPLC. Twelve compounds (**1**−**12**) were isolated from the DSEB layer (Figure 2). Compounds **1** and **2** are new neolignan glucosides (**1**−**2**), and compounds **3**−**7**, **9**, **11**, and **12** are identified for the first time from the genus *Saccharum*.

Identification of Two New Neolignan Glucosides. Saccharnan A (**1**) was obtained as a colorless gum, and its molecular formula, $C_{26}H_{30}O_{12}$, was determined by the HRESI-MS at *m*/*z* 557.1638 $[M + Na]^+$. The IR spectrum of **1** indicated the presence of a hydroxyl group at 3367 cm^{-1} and an α,β-unsaturated γ-lactone ring at 1637 and 1734 cm^{-1}.[16] In ^1H NMR spectra, the aromatic region exhibited the presence of a typical AA′BB′ system at δ_H 7.28 (2H, d, *J* = 8.8 Hz, H-2′, 6′) and δ_H 6.66 (2H, d, *J* = 8.8 Hz, H-3′, 5′), which suggested the presence of a *p*-substituted benzoyl group. The 1,3,4,5-tetrasubstituted phenyl moiety was expected due to protons showing at δ_H 6.57 (2H, s, H-2, 6). Two methoxy groups at δ_H 3.76 (6H, s) and an anomeric proton at δ_H 4.82 (1H, d, *J* = 7.2 Hz, H-1″) were also detected. Additionally, a ^1H NMR signal at δ_H 7.69 (1H, d, *J* = 2.4 Hz, H-7′) was assigned to an olefinic proton conjugated with a carbonyl group, which showed a long-range coupling with H-7. Twenty-six signals, including 8 quaternary, 14 methine, 2 methylene, and 2 methyl carbons, were observed in the ^{13}C NMR and DEPT spectra. In the HSQC experiment, two additional proton signals δ_H 4.54 (1H, *br* dd, *J* = 3.6, 2.4 Hz, H-7) and δ_H 4.41 (1H, dd, *J* = 7.6, 3.6 Hz, H-8) were observed and correlated

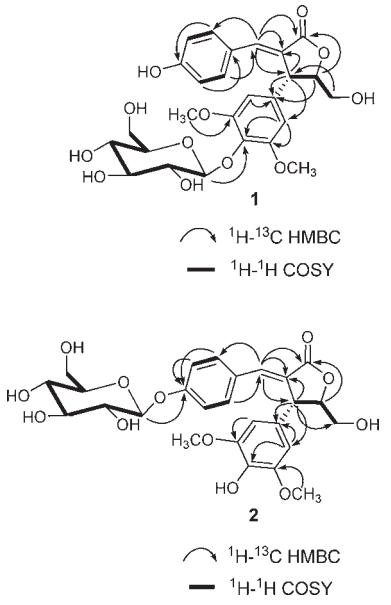

Figure 3. HMBC correlations of compounds **1** and **2**.

with the corresponding carbon signals at δ_C 47.5 (C-7) and δ_C 87.6 (C-8), respectively. The ^{1}H−^{1}H COSY and HMBC correlations of compound **1** are shown in Figure 3. The HMBC correlations of H-7/C-1, C-2, C-6, C-9; H-8/C-1, C-9′; H-7′/C-2′, C-6′, C-8′, C-9′, C-7; and CH_3O-/C-3, C-5 indicated that the 1,3,4,5-tetrasubstituted phenyl moiety is linked to C-7 and that the *p*-substituted benzoyl group is linked to C-7′ of olefinic carbon. Furthermore, two methoxy groups were located symmetrically at C-3 and C-5 in the 1,3,4,5-tetrasubstituted phenyl ring. Moreover, the HMBC correlation between the anomeric proton at δ_H 4.82 (1H, d, J = 7.2 Hz, H-1″) and C-4 was observed, and six sugar carbon signals at δ_C 105.3, 78.2, 77.8, 75.7, 71.2, and 62.5 indicated that the sugar is β-D-glucose. Furthermore, acid hydrolysis of **1** gave a glucosyl residue, and glucose was confirmed through comparison to an authentic sample using TLC and optical rotation. The stereochemistry was determined by a NOESY experiment shown in Figure 4, and a NOE effect was observed between H-7 and H-2′/ H-6′ indicating that the geometry of the vinyl group is *E* configuration. Another NOE effect was observed between H-2 and H-8 revealing that H-7 and H-8 have a trans relationship. On the basis of the aforementioned analysis, compound **1** was confirmed as a new neolignan glucoside and was named saccharnan A.

Saccharnan B (**2**) was isolated as a colorless gum. The HRESI-MS of **2** displayed a pseudomolecular ion peak at m/z 557.1637 $[M + Na]^+$, corresponding to the formula of $C_{26}H_{30}O_{12}Na$. The IR spectrum of **2** revealed the presence of a hydroxyl group at 3362 cm^{-1} and an α,β-unsaturated γ-lactone ring at 1644 and 1731 cm^{-1}. Comparing the NMR data with those of **1** revealed that compounds **1** and **2** were analogues and that the major difference in NMR spectra was the shift in the anomeric carbon signal of compound **2** (δ_C 101.8) compared to the signal of the same carbon in compound **1** (δ_C 105.3). Six sugar carbon signals at δ_C 105.3, 78.2, 77.8, 75.7, 71.2, and 62.5 and the coupling constant (J = 7.2 Hz) of the anomeric proton suggested that the sugar is β-D-glucose as in compound **1**. The ^{1}H−^{1}H COSY and HMBC correlations of compound **2** are shown in Figure 3. An obvious correlation between the anomeric proton at δ_H 4.91

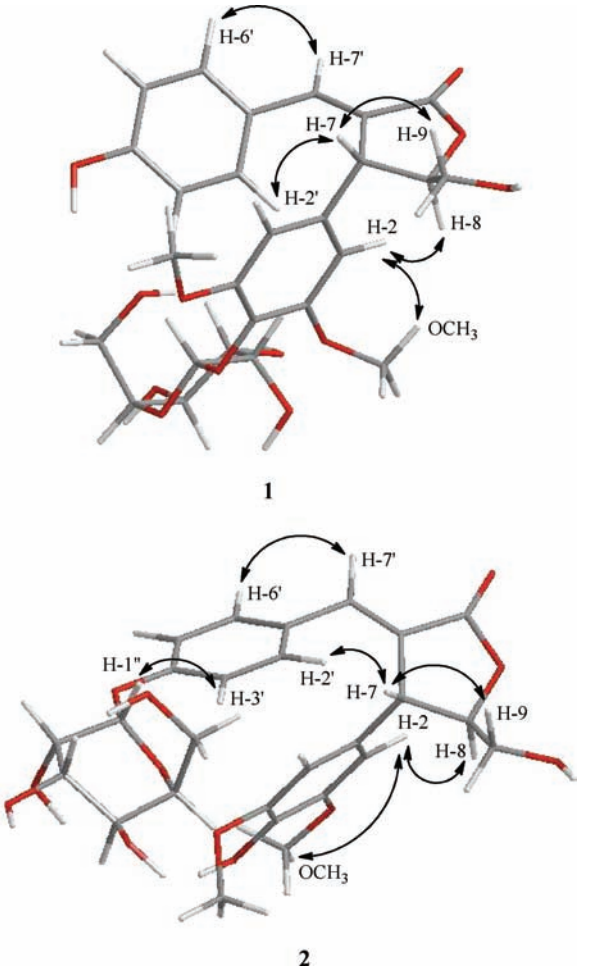

Figure 4. NOESY correlations of compounds **1** and **2**.

(1H, d, J = 7.2 Hz) and C-4′ at δ_C 160.4 was observed in the HMBC experiment indicating that glucose is linked at C-4′ of *p*-hydroxybenzoyl moiety. In NOESY, the NOE correlations of H-7 to H-2′/H-6′ indicated that the vinyl group has an *E* configuration and that another NOE effect was detected between H-8 to H-2 suggesting a trans relationship between H-8 and H-7 (Figure 4). According to the reported literature data and NMR analysis, the structure of compound **2** was deduced as shown and was named saccharnan B.

The new compounds **1** and **2** possess a rare neolignan skeleton with C7, C8′-linkage, and a γ-lactone function. To the best of our knowledge, only one compound has this type of skeleton.[16] The 10 known isolates were identified by comparing their physical and spectroscopic data with the corresponding authentic samples or literature values, and they are *erytho*-secoisolariciresinol-*O*-β-D-glucoside (**3**),[17] *threo*-secoisolariciresinol-*O*-β-D-glucoside (**4**),[18] *erythro*-1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propanediol 4′-*O*-β-D-glucopyranoside (**5**),[19] *threo*-1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propanediol 4′-*O*-β-D-glucopyranoside (**6**),[19] lariciresinol 4-*O*-β-D-glucoside (**7**),[17] (7*S*,8*R*,7′*R*,8′*S*)-icariol A2-9-*O*-β-xylopyranoside (**8**),[20] 2-(3-methoxyl-4-hydroxyphenyl)-3-hydroxylmethyl-5-*trans*-carboxylethylene-7-methoxyl-2,3-dihydrobenzofuran (**9**),[21] 3,4-dimethoxyphenyl-β-D-glucopyranoside (**10**),[22] 3-hydroxy-4,5-dimethoxyphenyl-β-D-glucopyranoside (**11**),[3] and *threo*-guaiacylglycerol 7-*O*-β-D-glucopyranoside (**12**).[23]

Table 2. Antioxidative and Anti-Tyrosinase Activities of Compounds 1−12 Isolated from DSE[a]

	IC_{50} value (μM)		
compounds	DPPH	ABTS	tyrosinase inhibition
1	N.D.[b]	N.D.[b]	N.D.[b]
2	>500	189.53 ± 1.88	287.95 ± 2.67
3	28.04 ± 0.85	22.20 ± 0.18	114.71 ± 3.03
4	23.48 ± 0.26	16.80 ± 0.01	153.07 ± 0.12
5	177.92 ± 1.15	47.57 ± 0.93	85.87 ± 5.86
6	91.39 ± 1.61	34.18 ± 0.23	95.81 ± 2.83
7	107.7 ± 0.30	43.78 ± 0.30	42.59 ± 3.46
8	30.11 ± 0.73	51.57 ± 0.45	>1000
9	51.20 ± 3.48	123.12 ± 1.30	798.02 ± 13.32
10	>500	>500	417.76 ± 10.12
11	284.61 ± 1.40	95.14 ± 1.05	>1000
12	>500	48.25 ± 0.89	57.72 ± 5.13
ascorbic acid[c]	95.08 ± 0.49	107.44 ± 0.40	N.D.[b]
Trolox[c]	70.07 ± 0.68	89.94 ± 2.12	N.D.[b]
arbutin[d]	N.D.[b]	N.D.[b]	57.56 ± 2.06

[a] Concentration necessary for 50% inhibition (IC_{50}). The data are expressed as the mean ± SD (n = 3), and the results are given in μM. [b] Not determined. [c] Ascorbic acid and Trolox were used as positive controls for antioxidant activities. [d] Arbutin was used as a positive control for tyrosinase inhibitory activity.

DPPH and ABTS$^+$ Radical Scavenging Activities of the Identified Compounds. Several studies have suggested the strong correlation between the phenolic composition of the sample and its free radical-scavenging activity.[24] The DPPH radical-scavenging assay is usually used to evaluate the ability of certain compound or an extract to capture free radicals by producing the reduced form DPPH-H through hydrogen-donating action.[25] ABTS$^+$ is another synthetic radical and is more versatile than DPPH as the ABTS$^+$ model can be used to assess the scavenging activity for both polar and nonpolar samples. The system also holds another advantage as the detected maximum absorption wavelength is far away from those of natural products, resulting in low experimental error.[26] The results of the DPPH and ABTS$^+$ radical scavenging activities for compounds **2**−**12** isolated from DSE are summarized in Table 2. Unfortunately, due to the limited quantity of the isolated new compound **1** (1.8 mg), its biological activity was not tested. Among all tested compounds, **3**, **4**, **8**, and **9** showed DPPH radical-scavenging activity with IC_{50} values of 28.04, 23.48, 30.11, and 51.20, respectively. In addition, compounds **3**, **4**, **5**, **6**, **7**, **8**, and **12** also displayed strong ABTS$^+$ radical-scavenging activity. It was found that the isomeric compounds **3** and **4** having two *ortho*-methoxy *para*-substituted phenols possessed the most potent DPPH/ABTS$^+$ radical-scavenging activity, with IC_{50} values of 28.04/22.20 and 23.48/16.80 μM, respectively. The IC_{50} values of compounds **3** and **4** were 2 to 5-fold more potent than the IC_{50} values of ascorbic acid and Trolox used as positive controls. Furthermore, compound **8** with two *ortho*-dimethoxy *para*-substituted phenols exhibited potent IC_{50} values of DPPH/ABTS$^+$ scavenging assays 30.11/51.57 μM. On the basis of the results, the number of hydroxyl groups on the aromatic rings of the tested compound can be correlated to its antioxidant activity, supporting reported results by Sroka and Cisowski.[27] It can be stated that the greater the number of hydroxyl groups attached to the aromatic rings, the higher the antioxidative activity.[28] Also, these results were in accordance with literature findings, which reported that the ortho- and para-substitution of phenols by electron donating groups reduced the bond dissociation energies and increased the transfer rate of the hydrogen atom to the peroxyl radicals, improving compound antioxidant activity.[29]

Tyrosinase Inhibitory Activity of the Identified Compounds. Tyrosinase inhibitors play an important role in the cosmetic industry as they are incorporated in skin whitening creams. Tyrosinase enzyme is one of the key enzymes which takes part in the initial steps of pigment formation pathways, thereby indicating that tyrosinase can block pigment production.[30] The tyrosinase inhibitory activity of the isolated compounds was similar to the antioxidant activity, which can also be correlated to the number of hydroxyl groups on the aromatic rings. These hydroxyl groups can form a hydrogen bond to the active site of the enzyme inhibiting tyrosinase activity. Tyrosinase inhibitory activity results of compounds **2**−**12** isolated from DSE are illustrated in Table 2. The isomeric compounds **5** and **6** displayed similar moderate tyrosinase inhibitory activity with IC_{50} values of 85.87 and 95.81 μM, respectively. The order of tyrosinase inhibitory activity was **7** > arbutin > **12** > **5** > **6** > **3** > **4** > **2** > **10** > **9**. Compounds **7** and **12** showed the most potent tyrosinase inhibitory activity with IC_{50} values of 42.59 and 57.72 μM, respectively, and were equivalent to the positive control arbutin (57.56 μM). One characteristic structural feature of all compounds showing effective tyrosinase inhibitory activity is the presence of the free hydroxyl group at position 4 in the aromatic ring.[31] It seems that this hydroxyl group is essential for forming a hydrogen bond with the active site of the tyrosinase enzyme. Interestingly, the structural similarity of compound **10** with the positive control arbutin did not lead to similar tyrosinase inhibitory activity, but the activity of compound **10** was lower. The absence of the free hydroxyl group in position 4 of the aromatic ring in compound **10** can be correlated to the lower activity of compound **10** compared to that of arbutin.[32] Also, the activity of compounds **7** and **8** were markedly different with lower activity exhibited by compound **8**. The lower activity of compound **8** may be attributed to the increase in the substituents bulkiness hindering the accessibility of compound **8** to the active site of the enzyme.[33] Compound **9** exhibited the lowest tyrosinase inhibitory activity, which may be a direct result of the presence of the free carboxylic group capable of forming internal hydrogen bonding to the free hydroxyl group. The formation of the internal hydrogen bond prevents the hydroxyl group of compound **9** from forming a hydrogen bond with the active sites of the tyrosinase enzyme.

In summary, 2 new neolignan glucosides and 10 known compounds were isolated from desugared sugar cane extract (DSE). It is the second report of the rare neolignan skeleton of compounds **1** and **2** with C7, C8′-linkage and a γ-lactone function to be isolated from natural resources. In the antioxidative assays, compounds **3**, **4**, **8**, and **9** showed good activity against DPPH (IC_{50} 23.48−51.20 μM) and also compounds **3**−**8** and **12** exhibited strong ABTS$^+$ free radical scavenging activity (IC_{50} 16.80−51.57 μM) even more potent than those of positive controls. Moreover, compounds **7** and **12** displayed potent inhibition of tyrosinase with IC_{50} values of 42.59 and 57.72 μM, respectively. These two compounds not only showed potent activity as tyrosinase inhibitors but also as antioxidants. Our research proved the economical value of recycling DSE as a potent source of inexpensive natural antioxidants and tyrosinase

inhibitors which can be incorporated in different food and cosmetic products.

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ABBREVIATIONS USED

DSE, desugared sugar cane extract; DSEE, desugared sugar cane extract ethyl acetate soluble layer; DSEB, desugared sugar cane extract *n*-butanol soluble layer; DSEW, desugared sugar cane extract water-soluble layer; ROS, reactive oxygen species; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); OD, optical density; DEPT, distortion enhancement by polarization transfer; COSY, correlation spectroscopy; HSQC, heteronuclear single quantum coherence; HMBC, heteronuclear multiple bond coherence.

REFERENCES

(1) Yang, Y. C. *Story of Taiwan Cane Sugar*; OWL Publishing House: Taipei, Taiwan, 2001.

(2) Hikosaka, K.; Koyama, Y.; Motobu, M.; Yamada, M.; Nakamura, K.; Koge, K.; Shimura, K.; Isobe, T.; Tsuji, N.; Kang, C. B.; Hayashidani, H.; Wang, P. C.; Matsumura, M.; Hirota, Y. Reduced lipopolysaccharide (LPS)-induced nitric oxide production in peritoneal macrophages and inhibited LPS-induced lethal shock in mice by a sugar cane (*Saccharum officinarum* L.) extract. *Biosci. Biotechnol. Biochem.* **2006**, *70*, 2853–2858.

(3) Takara, K.; Matsui, D.; Wada, K.; Ichiba, T.; Nakasone, Y. New antioxidative phenolic glycosides isolated from *Kokuto* non-centrifuged cane sugar. *Biosci. Biotechnol. Biochem.* **2002**, *66*, 29–35.

(4) Okabe, T.; Toda, T.; Inafuku, M.; Wada, K.; Iwasaki, H.; Oku, H. Antiatherosclerotic function of *Kokuto*, Okinawan noncentrifugal cane sugar. *J. Agric. Food Chem.* **2009**, *57*, 69–75.

(5) Re, L.; Barocci, S.; Capitani, C.; Vivani, C.; Ricci, M.; Rinaldi, L.; Paolucci, G.; Scarpantonio, A.; Leon-Fernandez, O. S.; Morales, M. A. Effects of some natural extracts on the acetylcholine release at the mouse neuromuscular junction. *Pharmacol. Res.* **1999**, *39*, 239–245.

(6) Mabry, T. J.; Liu, Y. L.; Pearce, J.; Dellamonica, G.; Chopin, J.; Markham, K. R.; Paton, N. H.; Smith, P. New flavonoids from sugarcane (*Saccharum*). *J. Nat. Prod.* **1984**, *47*, 127–130.

(7) Takara, K.; Matsui, D.; Wada, K.; Ichiba, T.; Chinen, I.; Nakasone, Y. New phenolic compounds from *Kokuto*, non-centrifuged cane sugar. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 376–379.

(8) Stohs, S. J.; Bagchi, D. Oxidative mechanisms in the toxicity of metal ions. *Free Radical Biol. Med.* **1995**, *18*, 321–336.

(9) Halliwell, B.; Gutteridge, J. M. C. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J.* **1984**, *219*, 1–14.

(10) Whysner, J.; Wang, C. X.; Zang, E.; Iatropoulos, M. J.; Williams, G. M. Dose response of promotion of butylated hydroxyanisole in chemically initiated tumors of the rat forestomach. *Food Chem. Toxicol.* **1994**, *32*, 215–222.

(11) Williams, G. M.; Iatropoulos, M. J.; Whysner, J. Safety assessment of butylated hydroxyanisole and butylated hydroxytoluene as antioxidant food additives. *Food Chem. Toxicol.* **1999**, *37*, 1027–1038.

(12) Seo, S. Y.; Sharma, V. K.; Sharma, N. Mushroom tyrosinase: Recent prospects. *J. Agric. Food Chem.* **2003**, *51*, 2837–2853.

(13) Aniya, Y.; Yokomakura, T.; Yonamine, M.; Shimada, K.; Nagamine, T.; Shimabukuro, M.; Gibo, H. Screening of antioxidant action of various molds and protection of *Monascus anka* against experimentally induced liver injuries of rats. *Gen. Pharmacol.* **1999**, *32*, 225–231.

(14) Thana, P.; Machmudah, S.; Goto, M.; Sasaki, M.; Pavasant, P.; Shotipruk, A. Response surface methodology to supercritical carbon dioxide extraction of astaxanthin from *Haematococcus pluvialis*. *Bioresour. Technol.* **2008**, *99*, 3110–3115.

(15) Lai, H. Y.; Lim, Y. Y.; Tan, S. P. Antioxidative, tyrosinase inhibiting and antibacterial activities of leaf extracts from medicinal ferns. *Biosci. Biotechnol. Biochem.* **2009**, *73*, 1362–1366.

(16) Takeda, R.; Hasegawa, J.; Shinozaki, M. The first isolation of lignans, megacerotonic acid and anthocerotonic acid, from non-vascular plants, anthocerotae (hornworts). *Tetrahedron Lett.* **1990**, *31* 4159–4162.

(17) Baderschneider, B.; Winterhalter, P. Isolation and characterization of novel benzoates, cinnamates, flavonoids, and lignans from riesling wine and screening for antioxidant activity. *J. Agric. Food Chem.* **2001**, *49*, 2788–2798.

(18) Achenbach, H.; Waibel, R.; Addae-Mensah, I. Lignans and other constituents from *Carissa edulis*. *Phytochemistry* **1983**, *22*, 749–753.

(19) Warashina, T.; Nagatani, Y.; Noro, T. Constituents from the bark of *Tabebuia impetiginosa*. *Chem. Pharm. Bull.* **2006**, *54*, 14–20.

(20) Chung, Y. M.; Lan, Y. H.; Hwang, T. L.; Leu, Y. L. Anti-inflammatory and antioxidant components from *Hygroryza aristata*. *Molecules* **2011**, *16*, 1917–1927.

(21) Zheng, J.; Chen, G. T.; Gao, H. Y.; Wu, B.; Wu, L. J. Two new lignans from *Mentha spicata* L. *J. Asian Nat. Prod. Res.* **2007**, *9*, 445–449.

(22) Tanimoto, S.; Tominaga, H.; Okada, Y.; Nomura, M. Synthesis and cosmetic whitening effect of glycosides derived from several phenylpropanoids. *Yakugaku Zasshi-J. Pharm. Soc. Jpn.* **2006**, *126*, 173–177.

(23) Sugiyama, M.; Kikuchi, M. Studies on the constituents of *Osmanthus* species. X. Structure of phenolic glucosides from the leaves of *Osmanthus asiaticus* Nakai. *Chem. Pharm. Bull.* **1992**, *40*, 325–326.

(24) Cheung, L. M.; Cheung, P. C. K.; Ooi, V. E. C. Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chem.* **2003**, *81*, 249–255.

(25) Chung, Y. C.; Chien, C. T.; Teng, K. Y.; Chou, S. T. Antioxidative and mutagenic properties of *Zanthoxylum ailanthoides* Sieb & Zucc. *Food Chem.* **2006**, *97*, 418–425.

(26) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231–1237.

(27) Sroka, Z.; Cisowski, W. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. *Food Chem. Toxicol.* **2003**, *41*, 753–758.

(28) Prasad, K. N.; Divakar, S.; Shivamurthy, G. R.; Aradhya, S. M. Isolation of a free radical-scavenging antioxidant from water spinach (*Ipomoea aquatica* Forsk). *J. Sci. Food Agric.* **2005**, *85*, 1461–1468.

(29) Singh, N.; O'Malley, P. J.; Popelier, P. L. A. Mechanistic aspects of hydrogen abstraction for phenolic antioxidants. Electronic structure and topological electron density analysis. *Phys. Chem. Chem. Phys.* **2005**, *7*, 614–619.

(30) Kobayashi, T.; Vieira, W. D.; Potterf, B.; Sakai, C.; Imokawa, G.; Hearing, V. J. Modulation of melanogenic protein expression during the switch from eu- to pheomelanogenesis. *J. Cell Sci.* **1995**, *108*, 2301–2309.

(31) Fais, A.; Corda, M.; Era, B.; Fadda, M.; Matos, M.; Quezada, E.; Santana, L.; Picciau, C.; Podda, G.; Delogu, G. Tyrosinase inhibitor activity of coumarin-resveratrol hybrids. *Molecules* **2009**, *14*, 2514–2520.

(32) Miyazawa, M.; Oshima, T.; Koshio, K.; Itsuzaki, Y.; Anzai, J. Tyrosinase inhibitor from black rice bran. *J. Agric. Food Chem.* **2003**, *51*, 6953–6956.

(33) Shimizu, K. Tyrosinase and 5 α-reductase inhibitory components from *Artocarpus incisus* tree. *Bull. Kyusyu. Univ. For.* **2001**, *82*, 21–63.