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New Truxinic and Truxillic Acid Sucrose Diesters From the Leaves of *Trigonostemon honbaensis*

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Abstract

A new *δ*-truxinic acid sucrose diester and a new *ε*-truxillic acid sucrose diester (named trigohonbanosides E and F) were isolated from the leaves of *Trigonostemon honbaensis*. Their chemical structures were determined by extensive analysis of their HR-ESI-MS and NMR spectra. At a concentration of 20 μM, trigohonbanosides E and F exhibited weak inhibitory effects on NO production in LPS-activated RAW264.7 cells with inhibitory percentages of 22.7% ± 1.1% and 18.5% ± 1.4%, respectively.

Keywords

Trigonostemon honbaensis, truxinic acid, truxillic acid, sucrose diesters, nitric oxide inhibitor

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The chemical constituents of *Trigonostemon* species have attracted the attention of pharmaceutical chemists because of their unique structure backbones and interesting biological activities.[1-4] To date, over 200 compounds have been identified from *Trigonostemon* species; diterpenoids, *β*-carboline alkaloids, and phenolics are the major and most important constituents.[1] A number of these compounds have been reported to have potent anti-viral, insecticidal, and cytotoxic activities.[2] Several of these bioactive compounds and their analogs have been synthesized, such as daphnane diterpenoid orthoesters and β-carboline alkaloids.[5-7] *T. honbaensis* Tagane & Yahara is recently recorded as a new and endemic species of Vietnam. Our previous chemical investigation of this plant identified four new sucrose diesters of *β*-truxinic acid derivatives.[8] Sucrose diesters of truxinic acid derivatives are rarely found in natural sources. A few have been previously isolated from *Imperata cylindrica*, *Coix lachryma-jobi*, *Bidens parviflora*, and oat grains.[9-12] Derivatives of truxinic acid and its isomer, truxillic acid, are naturally occurring compounds formed by [2 + 2] cyclodimerization of cinnamic acid derivatives. Their structures are interesting because of containing a cyclobutane ring and a variety of stereoisomers.[13,14] In our continuing study, this paper describes the isolation from the leaves of *T. honbaensis* and structure elucidation of a new stereoisomer of a *δ*-truxinic acid derivative, and a new *ε*-truxillic acid derivative (Figure 1). The anti-inflammatory activity of the isolated compounds was evaluated by determining the inhibition of NO production in LPS-stimulated RAW264.7 cells.

Results and Discussion

Compound **1** was obtained as a white amorphous powder. The molecular formula of **1** was determined to be $C_{32}H_{38}O_{17}$ based on the quasi-molecular ion peak at m/z 695.2183 $[M + H]^+$ (Calcd. for $C_{32}H_{39}O_{17}$, 695.2187) in the high resolution electron spray ionization mass spectrum (HR-ESI-MS) (Supplemental Figure S1). The ^{1}H NMR and HSQC spectra of **1** exhibited proton signals including 6 aromatic protons [δ_H 6.92 (1H, br s), 6.88 (1H, br s), and 6.77 (4H, overlapped signals)], 1 anomeric proton [δ_H 5.34 (1H, d, J = 3.5 Hz)], and 2 methoxy groups [δ_H 3.84 and 3.83 (each 3 hours, s)] (Supplemental Figure S2–S4, S7 and S8). The ^{13}C NMR and HSQC spectra of **1** (Supplemental Figure S5–S8) revealed the presence of 32 carbon atoms, which were categorized into 9 non-protonated carbons, 18 methine carbons, 3 methylene carbons, and 2 methyl carbons. Twelve aromatic carbons (δ_C 111.7

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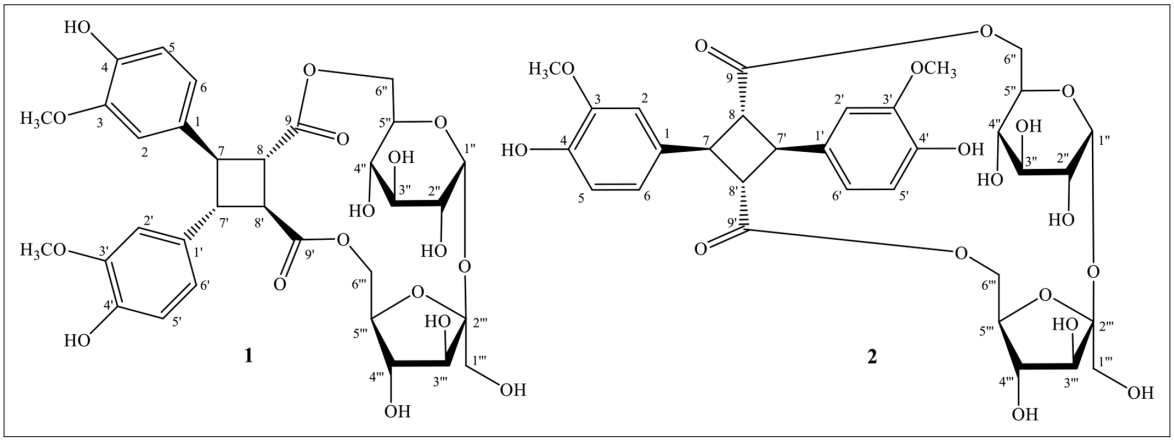

Figure 1. Chemical structures of compounds **1** and **2**.

~ 149.1) were assigned to 2 benzene rings. Two de-shielded carbon signals (δ_C 173.9 and 174.5) indicated the presence of 2 carbonyl groups. One methine anomeric carbon (δ_C 93.5), 1 non-protonated anomeric carbon (δ_C 104.4), and 10 other carbinol carbons (δ_C 62.9 ~ 80.4) characterized the presence of a sucrose moiety. The 4 saturated methine groups (δ_C/δ_H: 48.3/3.59, 47.1/3.37, 46.4/3.45, and 48.0/3.64) and their H-H COSY (Supplemental Figures S10 and S11) cross peaks (H-7 (δ_H 3.59)/ H-8 (δ_H 3.37)/ H-8' (δ_H 3.45)/ H-7' (δ_H 3.64)/ H-7) indicated the presence of a cyclobutane ring (binding order C-7/C-8/C-8'/C-7'/C-7). HMBC correlations between H-7 (δ_H 3.59) and C-1 (δ_C 134.2)/ C-2 (δ_C 111.7)/ C-6 (δ_C 120.3), H-7' (δ_H 3.64) and C-1' (δ_C 134.4)/ C-2' (δ_C 111.7)/ C-6' (δ_C 120.4) indicated that 2 aryl groups were correspondingly attached to C-7 and C-7' (Figure 2 and Supplemental Figure S9). Two carboxyl groups locating at C-8 and C-8' formed a truxinic acid derivative, which was then elucidated by HMBC correlations between H-7 (δ_H 3.59) and C-9 (173.9), H-7' (δ_H

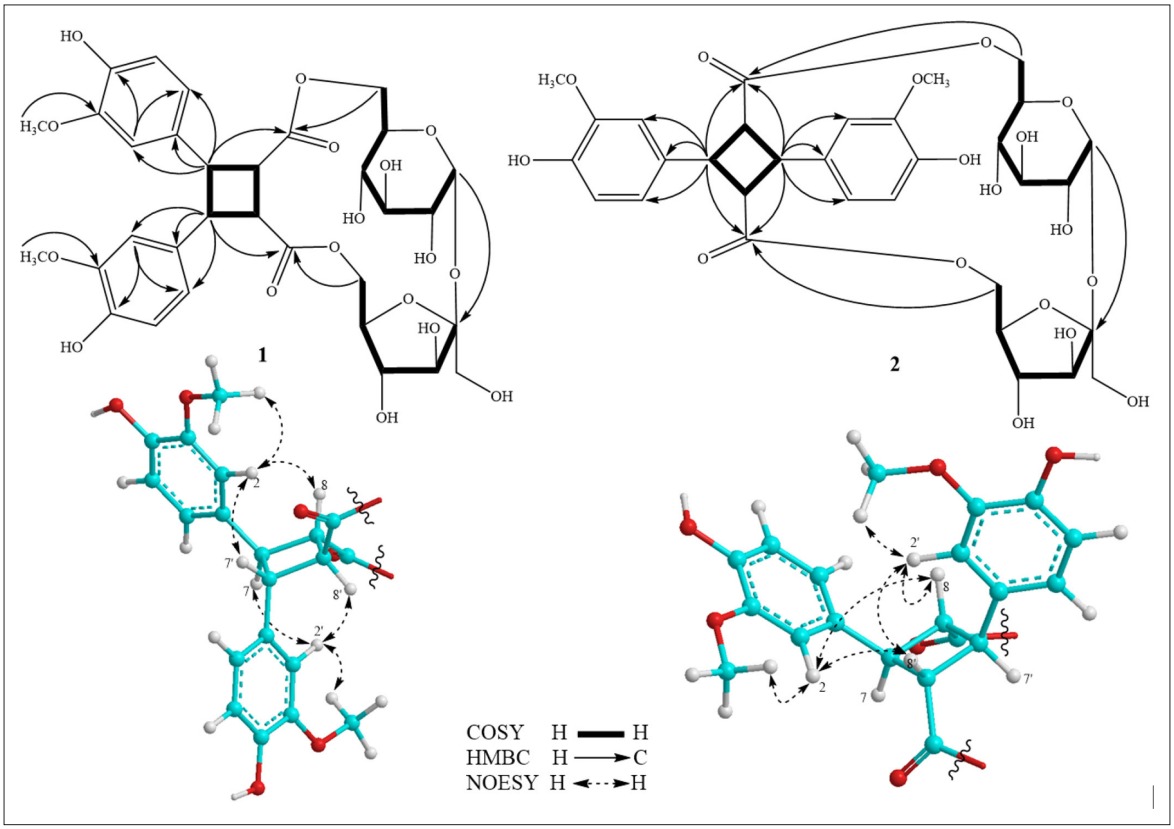

Figure 2. Important HMBC, COSY and NOESY correlations of compounds **1** and **2**.

3.64) and C-9' (δ_C 174.5). Although the proton signals of H-5/ H-6/H-5'/ H-6' overlapped each other (δ_H 6.77), NOESY correlations between H-2 (δ_H 6.88) and 3-OC$\underline{H}_3$ (δ_H 3.84), H-2' (δ_H 6.92) and 3'-OC$\underline{H}_3$ (δ_H 3.83) indicated the presence of methoxy groups at C-3 and C-3' (Figure 2 and Supplemental Figures S12 and S13). Diester linkages were established between C-9 and C-6" and between C-9' and C-6‴, which were confirmed by HMBC correlations between H-6" (δ_H 4.56) and C-9 (δ_C 173.9), and H-6‴ (δ_H 4.47) and C-9' (δ_C 174.5). The planar structure of **1** was then determined and revealed to be similar to trigohonbanosides A and B.[8] However, the NOESY spectrum of **1** exhibited interactions between H-2' (δ_H 6.92) and H-7 (δ_H 3.59)/ H-8' (δ_H 3.45), and H-2 (δ_H 6.88) and H-8 (δ_H 3.37)/ H-7' (δ_H 3.64), indicating the close proximity between H-2' and H-7/H-8', H-2 and H-8/H-7', respectively. The presence of a sucrose disaccharide in **1** was also confirmed after alkaline hydrolysis and TLC analysis by comparison with authentic sucrose.[8] Consequently, compound **1** was determined to be 3,3'-dimethoxy-4,4'-dihydroxy-*δ*-truxinoyl 6",6‴-sucrose diester and named as trigohonbanoside E.

Compound **2** was obtained as a white amorphous powder. HR-ESI-MS analysis of **2** exhibited a quasi-molecular ion peak at *m/z* 695.2177 $[M + H]^+$ (Calcd. for $C_{32}H_{39}O_{17}$, 695.2187), indicating the same molecular formula as that of compound **1**, $C_{32}H_{38}O_{17}$ (Supplemental Figure S14). The ^1H, ^{13}C NMR, and HSQC spectral data of **2** were quite similar to those of **1**, except the assigned signals for the cyclobutane ring (Table 1, Supplemental Figures S15–S21). The H-H COSY spectrum of **2** showed cross peaks of H-7 (δ_H 4.32)/ H-8 (δ_H 3.19)/ H-7' (δ_H 4.06)/ H-8' (δ_H 3.21)/ H-7, which indicated the binding of C-7/C-8/C-7'/C-8'/C-7 to form a cyclobutane ring (Supplemental Figures S23–24). Therefore, compound **2** was determined to be a truxillic acid derivative instead of a truxinic acid derivative, as in compound **1**. A diester formed at C-6" and C-6‴ of sucrose was confirmed by HMBC correlations from H-6" (δ_H 4.75) to C-9 (δ_C 173.7) and from H-6‴ (δ_H 4.56) to C-9' (δ_C 173.7) (Figure 2 and Supplemental Figure S22). The presence of a sucrose disaccharide in **2** was also confirmed by TLC analysis of the alkaline hydrolysis product by comparison with authentic sucrose.[8] NOESY (Figure 2 and Supplemental Figures S25, S26). correlations between H-2 (δ_H 6.93) and H-8 (δ_H 3.19)/ H-8' (δ_H 3.21), and H-2' (δ_H 6.89) and H-8/H-8' indicated that the 2 aryl groups were located on the same side of the cyclobutane ring (assumed *β*-orientation), and 2 carboxylic groups were located on the opposite side with aryl groups (*α*-orientation). On the other hand, NOESY correlations from H-2 (δ_H 6.93) to 3-OC$\underline{H}_3$ (δ_H 3.87) and from H-2' (δ_H 6.89) to 3'-OC$\underline{H}_3$ (δ_H 3.89) indicated the presence of methoxy groups at C-3 and C-3'. Consequently, compound **2** was determined to be 3,3'-dimethoxy-4,4'-dihydroxy-*ε*-truxilloyl 6",6‴-sucrose diester, and named as trigohonbanoside F.

In the plant kingdom, truxinic acid and truxillic acid are generated by cyclodimerization of cinnamic acid. Truxinic acid is formed by head-to-head [2 + 2] cyclodimerization of cinnamic acid, while truxillic acid is obtained by head-to-tail [2 + 2] cyclodimerization.[14] Therefore, compounds **1** and **2** were correspondingly expected by head-to-head and head-to-tail [2 + 2] cyclodimerization of either caffeic acid or ferulic acid derivatives. Compounds **1** and **2** were evaluated for their anti-inflammatory activity by inhibition of NO production in LPS-stimulated RAW264.7 macrophages, as previously described.[15] At a concentration of 20 μM, compounds **1** and **2** weakly inhibited NO production (inhibitory percentages of 22.7% ± 1.1% and 18.5% ± 1.4%, respectively) in comparison with the positive control L-NMMA (N^G-monomethyl-L-arginine, inhibitory percentage of 82.6% ± 1.9%).

Table 1. ^1H NMR and ^{13}C NMR Spectroscopic Data for Compounds **1** and **2** in Deuterated Methanol.

	1		2	
Pos.	δ_C [a]	δ_H [b] (mult., *J* in Hz)	δ_C [a]	δ_H [b] (mult., *J* in Hz)
1	134.2	-	134.2	-
2	111.7	6.88 (br s)	111.8	6.93 (br s)
3	149.1	-	149.2	-
4	146.7	-	146.8	-
5	116.8	6.77*	116.5	6.80*
6	120.3	6.77*	120.1	6.80*
7	48.3	3.59 (dd, 9.5, 9.5)	44.1	4.32 (dd, 9.0, 9.0)
8	47.1	3.37 (dd, 9.5, 9.5)	52.2	3.19 (dd, 9.0, 9.0)
9	173.9	-	173.7	-
3-OCH_3	56.4	3.84 (s)	56.6	3.87 (s)
1'	134.4	-	134.2	-
2'	111.7	6.92 (br s)	111.3	6.89 (br s)
3'	149.1	-	149.2	-
4'	146.7	-	146.8	-
5'	116.3	6.77*	116.4	6.80*
6'	120.4	6.77*	119.9	6.80*
7'	48.0	3.64 (dd, 9.5, 9.5)	42.9	4.06 (dd, 9.0, 9.0)
8'	46.4	3.45 (dd, 9.5, 9.5)	51.5	3.21 (dd, 9.0, 9.0)
9'	174.5	-	173.7	-
3'-OCH_3	56.4	3.83 (s)	56.4	3.89 (s)
Glc				
1"	93.5	5.34 (d, 3.5)	94.3	5.49 (d, 3.5)
2"	73.4	3.48 (dd, 3.5, 9.5)	73.5	3.54 (dd, 3.5, 9.5)
3"	74.5	3.75 (dd, 9.0, 9.5)	74.4	3.81 (dd, 9.0, 9.5)
4"	73.2	3.14 (dd, 9.0, 9.5)	72.6	3.20 (dd, 9.0, 9.5)
5"	71.9	4.22*	72.8	4.33 (m)
6"	66.5	4.56 (dd, 2.5, 9.5) 4.22*	66.3	4.75 (dd, 1.5, 11.5) 4.00 (dd, 9.5, 11.5)
Fru				
1‴	65.4	3.67 (d, 12.0) 3.58 (d, 12.0)	64.6	3.79 (d, 12.5) 3.60 (d, 12.5)
2‴	104.4	-	105.1	-
3‴	79.8	4.10 (d, 7.5)	81.1	4.16 (d, 6.0)
4‴	74.7	4.08 (dd, 7.5, 7.5)	76.5	4.20 (dd, 6.0, 8.0)
5‴	80.4	3.86 (m)	80.0	3.83 (m)
6‴	62.9	4.47 (dd, 4.0, 13.0) 4.26 (dd, 2.0, 13.0)	62.6	4.56 (br d, 12.5) 4.30 (br d, 12.5)

Abbreviation: NMR, Nuclear magnetic resonance.
Asterisk indicates overlapped signals.
[a]Measured at 125 MHz.
[b]Measured at 500 MHz.

Material and Methods

General Experimental Procedures

Optical rotation was recorded on a Jasco P-2000 polarimeter. HR-ESI-MS were acquired on an Agilent 6530 Accurate Mass Q-TOF system, and NMR spectra on a Bruker Avance III 500 MHz spectrometer. Column chromatography was performed using either silica gel or reversed phase (C-18) resins as adsorbent. Thin layer chromatography was carried out on pre-coated silica gel 60 F_{254} and/or RP-18 F_{254S} plates. Compounds were visualized under UV irradiation (254 nm and 365 nm) and by spraying with H_2SO_4 solution (5%), followed by heating with a heat gun.

Plant Material

The leaves of *Trigonostemon honbaensis* Tagane & Yahara were collected at Nui Chua National Park, Ninh Thuan Province, Vietnam in December 2018. Its scientific name was identified by one of the authors, Prof. Ninh Khac Ban. A voucher specimen (No. NCCT-P79) is kept at the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology.

Extraction and Isolation

The leaves of *Trigonostemon honbaensis* (dried powder, 4 kg) were ultrasonically extracted with methanol at room temperature, 3 times (each 10 L MeOH, 60 minutes). After removal of the solvent under vacuum, the methanol extract (450 g) was suspended in distilled water (3.0 L) and successively partitioned with dichloromethane and ethyl acetate to give dichloromethane (80.7 g), ethyl acetate (4.1 g), and water-soluble portions. The water layer was separated on a Diaion HP-20 column, eluting with methanol/water (1/3, 1/1, 3/1, 1/0, stepwise each 1.5 L, v/v) to give 4 fractions (TH1-TH4). Fraction TH2 was chromatographed on a reversed phase C18 (RP-18) column, eluting with methanol/water (1/1, v/v, 2 L) to yield 6 fractions (TH2A- TH2F). Fraction TH2D was then separated on a Sephadex LH-20 column, eluting with methanol/water (1/1, v/v, 1 L) to give four fractions (TH2D1-TH2D4). Fraction TH2D2 was purified by preparative HPLC using a J'Sphere ODS-H80 column (20 × 250 mm, 4 μm) and an isocratic mobile phase of acetonitrile (20%) in water to give compounds **1** (4.3 mg, t_R 47.6 minutes) and **2** (3.2 mg t_R 50.8 minutes).

*Trigohonbanoside E (**1**).* White amorphous powder, $[\alpha]_D^{25}$: +67.6° (*c* 0.1, MeOH); **HR-ESI-MS: *m/z*** 695.2183 $[M + H]^+$ (Calcd. for $C_{32}H_{39}O_{17}$, 695.2187)**; ^{1} hours NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data are given in Table 1**.

*Trigohonbanoside F (**2**).* White amorphous powder, $[\alpha]_D^{25}$: +43.5° (*c* 0.1, MeOH); **HR-ESI-MS: *m/z*** 695.2177 $[M + H]^+$ (Calcd. for $C_{32}H_{39}O_{17}$, 695.2187)**; ^{1} hours NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data are given in Table 1**.

Alkaline Hydrolysis

Refer to Supplemental Material.

Nitric Oxide Assay

Refer to Supplemental Material.

Conclusions

Two new sucrose diesters of *δ*-truxinic acid and *ε*-truxillic acid derivatives (named trigohonbanosides E and F) were isolated from the leaves of *Trigonostemon honbaensis*. At a concentration of 20 μM, trigohonbanosides E and F exhibited weak inhibitory effects on NO production in LPS-activated RAW264.7 cells with inhibitory percentages of 22.7% ± 1.1% and 18.5% ± 1.4%, respectively.

Declaration of Conflicting Interests

The author(s) 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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