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

Results and Discussion

Compound 1 was obtained as a white amorphous powder. The molecular formula of 1 was determined to be C₃₂H₃₈O₁₇ based on the quasi-molecular ion peak at m/z 695.2183 [M + H]+ (Calcd. for C₃₂H₃₉O₁₇, 695.2187) in the high resolution electron spray ionization mass spectrum (HR-ESI-MS) (Supplemental Figure S1). The ¹H NMR and HSQC spectra of 1 exhibited proton signals including 6 aromatic protons [δH 6.92 (1H, br s), 6.88 (1H, br s), and 6.77 (4H, overlapped signals)], 1 anomeric proton [δH 5.34 (1H, d, J = 3.5 Hz)], and 2 methoxy groups [δH 3.84 and 3.83 (each 3 hours, s)] (Supplemental Figure S2–S4, S7 and S8). The ¹³C NMR and HSQC spectra of 1 (Supplemental Figure S2–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 (δC 111.7...
~ 149.1) were assigned to 2 benzene rings. Two de-shielded carbon signals ($\delta_C$ 173.9 and 174.5) indicated the presence of 2 carbonyl groups. One methine anomic carbon ($\delta_C$ 93.5), 1 non-protonated anomic carbon ($\delta_C$ 104.4), and 10 other carbonyl carbons ($\delta_C$ 62.9 ~ 80.4) characterized the presence of a sucrose moiety. The 4 saturated methine groups ($\delta_C/\delta_H$: 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 ($\delta_H$ 3.59)/ H-8 ($\delta_H$ 3.37)/ H-8′ ($\delta_H$ 3.45)/ H-7′ ($\delta_H$ 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 ($\delta_H$ 3.59) and C-1 ($\delta_C$ 134.2)/ C-2 ($\delta_C$ 111.7)/ C-6 ($\delta_C$ 120.3), H-7′ ($\delta_H$ 3.64) and C-1′ ($\delta_C$ 134.4)/ C-2′ ($\delta_C$ 111.7)/ C-6′ ($\delta_C$ 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 ($\delta_H$ 3.59) and C-9 (173.9), H-7′ ($\delta_H$ 3.64) and C-9′ (173.9). Two de-shielded carbon signals ($\delta_C$ 173.9 and 174.5) indicated the presence of 2 carbonyl groups. One methine anomic carbon ($\delta_C$ 93.5), 1 non-protonated anomic carbon ($\delta_C$ 104.4), and 10 other carbonyl carbons ($\delta_C$ 62.9 ~ 80.4) characterized the presence of a sucrose moiety. The 4 saturated methine groups ($\delta_C/\delta_H$: 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 ($\delta_H$ 3.59)/ H-8 ($\delta_H$ 3.37)/ H-8′ ($\delta_H$ 3.45)/ H-7′ ($\delta_H$ 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 ($\delta_H$ 3.59) and C-1 ($\delta_C$ 134.2)/ C-2 ($\delta_C$ 111.7)/ C-6 ($\delta_C$ 120.3), H-7′ ($\delta_H$ 3.64) and C-1′ ($\delta_C$ 134.4)/ C-2′ ($\delta_C$ 111.7)/ C-6′ ($\delta_C$ 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 ($\delta_H$ 3.59) and C-9 (173.9), H-7′ ($\delta_H$ 3.64) and C-9′ (173.9).
namic acid, while truxillic acid is obtained by head- to- tail head- to- head cyclodimerization of cinnamic acid. Truxinic acid diester, and named as trigohonbanoside F.

The planar structure of 1 was then determined and revealed to be similar to trigohonbanosides A and B. However, the NOESY spectrum of 1 exhibited interactions between H-2 (δH 6.92) and H-7 (δH 3.59) / H-8 (δH 3.45), and H-2 (δH 6.88) and H-8 (δH 3.37) / H-7 (δH 3.64), indicating the close proximity between H-2′ and H-7′, H-2 and H-8′, respectively.

The presence of a sucrose disaccharide in 1 was also confirmed after alkaline hydrolysis and TLC analysis by comparison with authentic sucrose. Consequently, compound 1 was determined to be 3,3′-dimethoxy-4,4′-dihydroxy-6″,6″-suco 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 C32H38O17, 695.2187), indicating the same molecular formula as that of compound 1, C32H38O17 (Supplemental Figure S14). The 1H, 13C NMR, and HSQC spectral data of 2 were quite similar to those of 1, except for the assigned signals for the cyclobutane ring (Table 1, Supplemental Figures S15–S21). The H-H COSY spectrum of 2 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 (δH 4.32) / H-8 (δH 3.19) / H-7′ (δH 4.06) / H-8′ (δH 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-9′ (δC 174.5) was also confirmed by HMBC correlations from H-2′ (δH 4.56) and H-7/H-8′, H-2 and H-8/H-7′, respectively.

The 1H NMR and 13C NMR Spectroscopic Data for Compounds 1 and 2 in Deuterated Methanol.

| Pos. | δH (mult., J in Hz) | δC (mult., J in Hz) |
|------|----------------------|----------------------|
| 1    | 134.2                | 134.2                |
| 2    | 111.7                | 111.8                |
| 3    | 149.1                | 149.2                |
| 4    | 146.7                | 146.8                |
| 5    | 116.8                | 116.5                |
| 6    | 120.3                | 120.1                |
| 7    | 48.3                 | 44.1                 |
| 8    | 47.1                 | 52.2                 |
| 9    | 173.9                | 173.7                |
| 3-OCH3 | 56.4 (s)            | 56.6 (s)             |
| 1′   | 134.4                | 134.2                |
| 2′   | 111.7                | 111.3                |
| 3′   | 149.1                | 149.2                |
| 4′   | 146.7                | 146.8                |
| 5′   | 116.3                | 116.4                |
| 6′   | 120.4                | 119.9                |
| 7′   | 48.0                 | 42.9                 |
| 8′   | 46.4                 | 51.5                 |
| 9′   | 174.5                | 173.7                |
| 3-OCH3 | 56.4 (s)            | 56.4 (s)             |
| Glc  | 1′                   | 93.5                 |
| 2′   | 73.4                 | 73.5                 |
| 3′   | 74.5                 | 74.4                 |
| 4′   | 73.2                 | 72.6                 |
| 5′   | 71.9                 | 72.8                 |
| 6′   | 66.5                 | 66.3                 |
| 7′   | 4.56 (dd, 2.5, 9.5)  | 4.75 (dd, 1.5, 11.5) |
| Fru  | 1′                   | 65.4                 |
| 2′   | 104.4                | 105.1                |
| 3′   | 79.8                 | 81.1                 |
| 4′   | 74.7                 | 76.5                 |
| 5′   | 80.4                 | 80.0                 |
| 6′   | 62.9                 | 62.6                 |
| Glc  | 1′                   | 64.6                 |
| 2′   | 3.67 (dd, 12.0)      | 3.59 (dd, 12.0)      |
| 3′   | 3.58 (dd, 12.0)      | 3.60 (dd, 12.0)      |
| 4′   | 4.10 (dd, 7.5)       | 4.16 (dd, 6.0)       |
| 5′   | 4.08 (dd, 7.5, 7.5)  | 4.20 (dd, 6.0, 8.0)  |
| 6′   | 3.86 (m)             | 3.83 (m)             |
| 7′   | 4.47 (dd, 4.0, 13.0) | 4.56 (br d, 12.5)    |
| 8′   | 4.26 (dd, 2.0, 13.0) | 4.30 (br d, 12.5)    |

Abbreviation: NMR, Nuclear magnetic resonance.
Asterisk indicates overlapped signals.
*Measured at 125 MHz.
*Measured at 500 MHz.

Therefore, compounds 1 and 2 were correspondingly expected by head-to-head and head-to-tail 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. 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 (Nω-monomethyl-L-arginine, inhibitory percentage of 82.6% ± 1.9%).
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 F254 and/or RP-18 F254S plates. Compounds were visualized under UV irradiation (254 nm and 365 nm) and by spraying with H2SO4 solution (5%), followed by heating with a heat gun.

Plant Material

The leaves of *Trigonostemon bonhaensis* 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 bonhaensis* (dried powder, 4 kg) were ultrasonically extracted with methanol at room temperature, 3 times (each 10 L McOH, 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, tR 47.6 minutes) and 2 (3.2 mg tR 50.8 minutes).

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 bonhaensis*. 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.

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 bonhaensis*. 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

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Supplemental Material

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