JOURNAL OF NATURAL PRODUCTS

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N-Cinnamoyltetraketide Derivatives from the Leaves of *Toussaintia orientalis*

Stephen S. Nyandoro,*[,†] Joseph Ndanu,[†] Joan J. E. Munissi,[†] Amra Gruhonjic,[‡] Paul A. Fitzpatrick,[‡] Göran Landberg,[‡] Yu Lu,[§] Bin Wang,[§] Fangfang Pan,[⊥] Kari Rissanen,[⊥] and Máté Erdélyi*[,∥,#]

[†]Chemistry Department, College of Natural and Applied Sciences, University of Dar es Salaam, P.O. Box 35061, Dar es Salaam, Tanzania

[‡]Sahlgrenska Cancer Centre, University of Gothenburg, Gothenburg SE-405 30, Sweden

[§]Beijing Key Laboratory of Drug Resistance Tuberculosis Research, Department of Pharmacology, Beijing Chest Hospital, Capital Medical University, Beijing Tuberculosis and Thoracic Tumour Research Institute, Beijing 101149, People's Republic of China

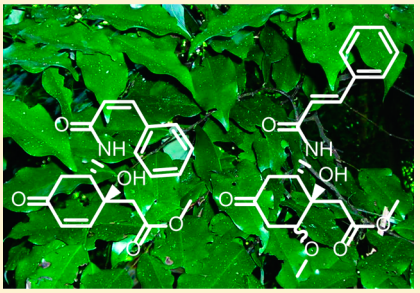
[⊥]Department of Chemistry, Nanoscience Center, University of Jyvaskyla, P.O. Box 35, FI-40014, Jyvaskyla, Finland

[∥]Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg SE-412 96, Sweden

[#]Swedish NMR Centre, University of Gothenburg, Gothenburg SE-405 30, Sweden

Supporting Information

ABSTRACT: Seven *N*-cinnamoyltetraketides (**1**–**7**), including the new *Z*-toussaintine E (**2**), toussaintine F (**6**), and toussaintine G (**7**), were isolated from the methanol extract of the leaves of *Toussaintia orientalis* using column chromatography and HPLC. The configurations of *E*-toussaintine E (**1**) and toussaintines A (**3**) and D (**5**) are revised based on single-crystal X-ray diffraction data from racemic crystals. Both the crude methanol extract and the isolated constituents exhibit antimycobacterial activities (MIC 83.3–107.7 μM) against the H37Rv strain of *Mycobacterium tuberculosis*. Compounds **1**, **3**, **4**, and **5** are cytotoxic (ED_{50} 15.3–105.7 μM) against the MDA-MB-231 triple negative aggressive breast cancer cell line.



Toussaintia orientalis Verdc. (Annonaceae) is endemic to Tanzania[1] and is one of the four *Toussaintia* species that are found only in tropical Africa.[2] It occurs in the Tanzanian coastal forests and in the Eastern Arc Mountains and is classified as an endangered species.[3] Controversially, *T. orientalis* is used in traditional medicinal. Its roots mixed with lion skin are boiled to produce a concoction that is applied as a remedy for the treatment of asthma and skin rashes arising from abdominal worms, whereas its leaves mixed with chicken feathers are burnt, with the resulting ashes being mixed with coconut oil to treat irritating rashes.[2]

Recent phytochemical investigations of *T. orientalis* serendipitously uncovered myriads of new bioactive metabolites,[1,4,5] among others bioactive *N*-cinnamoyltetraketides having α,β-unsaturated cyclohexenoid, tetrahydrobenzofuranoid, or indolidinoid skeletons amidated with a cinnamoyl moiety.[1,6] These structural units are commonly found in antituberculotic,[7] cytostatic,[8] antimalarial, and cardioprotective compounds.[9] As part of our ongoing work on the phytochemical characterization of East African medicinal plants, we report the isolation, identification, and the cytotoxic and antimycobacterial activities of three new and four known *N*-cinnamoyltetraketide derivatives isolated from the leaves of *T. orientalis*. Moreover, the configurations[1] of the known toussaintines A, D, and E are corrected based on X-ray crystallographic data.

RESULTS AND DISCUSSION

Vacuum liquid chromatographic (VLC) fractionation of the methanol extract of *T. orientalis* leaves, followed by repeated gravitational column chromatographic separation on silica gel 60 and Sephadex LH-20, afforded *E*-toussaintine E (**1**),[1] *Z*-toussaintine E (**2**), and toussaintines A (**3**), C (**4**), D (**5**), F (**6**), and G (**7**). Of these, **2**, **6**, and **7** are new natural products. The compounds were identified using NMR, MS, and single-crystal X-ray studies as well as by comparison of their observed and reported spectroscopic data.

E-Toussaintine E (**1**) and *Z*-toussaintine E (**2**) were obtained as white solids and showed similar ^{1}H and ^{13}C NMR spectra, with the main difference being the chemical shifts and the $^{3}J_{HH}$ values of the olefinic protons of their cinnamoyl groups (Table 1, Supporting Information). Compound **1** was identified as *E*-toussaintine E by comparison of its experimental and published spectroscopic data.[1] Previously, it was obtained as a minor component of the leaves of *T. orientalis*,[1] and its relative configuration was proposed based on NMR data. Good-quality single crystals were obtained from a 1:1 MeOH/MeCN solvent mixture. The X-ray crystallographic analysis confirms the previously established main conformation of its cyclohexenoyl

Received: April 24, 2015
Published: July 27, 2015

DOI: 10.1021/acs.jnatprod.5b00356
J. Nat. Prod. 2015, 78, 2045–2050

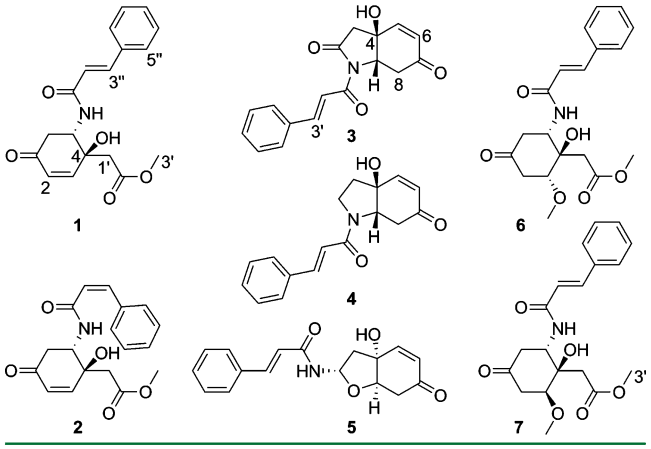


Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for *Z*-Toussaintine E (2) Acquired in $CDCl_3$ at 25 °C (δ_H, Multiplicity (*J* in Hz))

position	δ_C, type	δ_H	m (*J* in Hz)
1	195.3, C=O		
2	128.0, CH	5.96	d (10.5)
3	152.4, CH	6.92	d (10.5)
4	72.1, C−O		
5	54.6, CH	4.53	ddd (4.8, 8.0, 11.2)
6	39.7, CH_2	2.58, 2.18	dd (4.8, 17.6), dd (11.2, 17.6)
1′	40.6, CH_2	2.62	d (15.1)
		2.49	d (15.1)
2′	171.4, C=O		
3′	52.4, OCH_3	3.73	s
1″	168.5, C=O		
2″	124.1, CH	6.06	d (12.7)
3″	138.9, CH	7.01	d (12.7)
4″	134.7, C		
5″	128.8, CH	7.39	m
6″	129.0, CH	7.39	m
7″	129.4, CH	7.37−7.36	m
NH		5.60	d (8.0)

core and the relative configuration of C-5 (Figure 1); however, it did not support the previously suggested[1] C-4 relative configuration. On the basis of the X-ray diffraction structure, the correct relative configuration of racemic *E*-toussaintine E (**1**) is methyl 2-[6α-(1-*E*-cinnamido-1β-hydroxy-4-oxacyclohex-2-en-1-yl)]acetate.

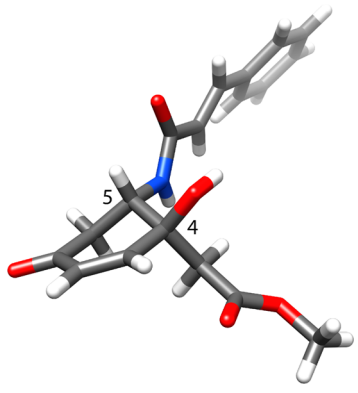


Figure 1. X-ray crystal structure of racemic *E*-toussaintine E (**1**) with its C-4 and C-5 stereocenters being highlighted.

The vicinal coupling constant of the protons of the cinnamoyl double bond indicates a *Z*-configuration for **2** ($^{3}J_{HH}$ = 12.7 Hz), but an *E*-configuration for **1** ($^{3}J_{HH}$ = 15.8 Hz). The shielding of the olefinic protons of **2** ($\delta_{H\text{-}2''}$ 6.06, $\delta_{H\text{-}3''}$ 7.01) relative to those of **1** ($\delta_{H\text{-}2''}$ 6.44, $\delta_{H\text{-}3''}$ 7.71) is likely due to the difference in their relative orientation as compared to the carbonyl moiety that possesses a strong magnetic anisotropy. The chemical shifts of the proximal H-1′, H-5, and H-6ab are also influenced by the *E*−*Z* geometrical isomerism, yet to a lesser degree. The 2D NMR and MS data, shown in the Supporting Information, are consistent with the proposed structures of **1** and **2**. Thus, on the basis of its spectroscopic data **2** was characterized as the *Z*-isomer of the known **1**, i.e., methyl 2-[6α-(1-*Z*-cinnamido-1β-hydroxy-4-oxacyclohex-2-en-1-yl)]acetate, and was assigned the trivial name *Z*-toussaintine E.

Toussaintines A (**3**), C (**4**), and D (**5**) were isolated as white solids and were identified by comparison of their NMR spectroscopic data (Supporting Information) with reported data.[1] Single crystals were obtained for **3** and **5** by recrystallization from MeOH/MeCN (1:1). On the basis of the X-ray structures (Figure 2), the relative configurations at C-4 of toussaintine A and at C-2 and C-9 for toussaintine D are here unambiguously determined. As for toussaintine E (**1**), the X-ray diffraction structures of toussaintine A (**3**) and toussaintine D (**5**) were based on racemic crystals.

Toussaintine F (**6**) was obtained as a white solid and was assigned the molecular formula $C_{19}H_{23}NO_6$ based on HRMS (ESI) data ($[M + H]^+$ *m*/*z* obs 362.1616, calcd 362.1604). It was identified as a cinnamide derivative upon observation of the *E*-olefinic protons H-3″ (δ_H 7.57, *J* = 15.7 Hz) and H-2″ (δ_H 6.27, *J* = 15.7 Hz), the H-5″/9″ ortho (δ_H 7.51−7.47), H-6″/8″, and H-7″ (δ_H 7.40−7.34) aromatic protons, and the amido proton at δ 6.67 (1H) (Table 2) by ^{1}H NMR data. The ^{13}C NMR signals of C-1″ (δ_C 165.0), CH-3″ (δ_C 141.6), CH-6″/8″ (δ_C 129.0), C-4″ (δ_C 134.8), C-5″/9″ (δ_C 128.0), CH-7″ (δ_C 129.9), and CH-2″ (δ_C 120.7), giving HSQC and HMBC correlations to the above protons, further confirmed this assumption (Table 2, Table S1).

Unlike the recently reported *N*-cinnamoyltetraketide derivatives,[1] the ^{1}H NMR spectrum of **6** does not show olefinic signals typical of a cyclohexenoyl unit, but instead aliphatic resonances corresponding to a trisubstituted cyclohexanone ring, as confirmed by $^{1}H,^{1}H$ COSY (Figure S43). The cinnamide amido proton (δ_H 6.67) couples to methine H-5 (δ_H 4.78), which in turn couples to the diastereotopic methylene H-6a and H-6b (δ_H 3.20, 2.40) and to the oxymethine H-3 (δ_H 3.81) through a *W*-coupling, frequently observed for 1,3-diequatorial protons of cyclohexanes. Thus, this ^{4}J coupling reveals the equatorial orientation of both H-3 and H-5 in the preferred conformation of the cyclohexanone ring of **6** (Figure 3). Vicinal coupling is observed between H-3 (δ_H 3.81) and the diastereotopic methylene H-2a (δ_H 3.08) and H-2b (δ_H 2.69).

The methylene H-2b (δ_H 2.69) shows a *W*-coupling to H-6b (δ_H 2.40), revealing the equatorial orientation of both protons (Figure 3). The position of OCH_3-3 (δ_H 3.41) is revealed by its $^{3}J_{CH}$ correlation to C-3 (δ_C 85.9), whereas that of the methoxycarbonyl methyl substituent is indicated by the HMBC correlations of the diastereotopic CH_2-1′ (δ_H 2.88 and 2.80) to C-4 (δ_C 71.2). The composition of this substituent is indicated by the HMBC cross-peaks of OCH_3-3′ (δ_H 3.75) and of H-1′ (δ_H 2.88 and 2.80) to the ester carbonyl C-2′ (δ_C

DOI: 10.1021/acs.jnatprod.5b00356
J. Nat. Prod. 2015, 78, 2045−2050

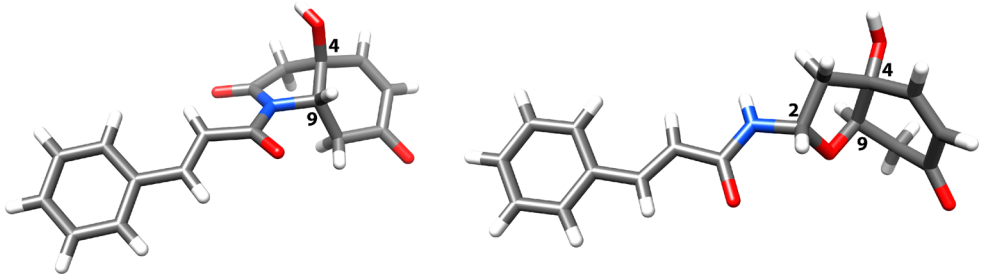


Figure 2. X-ray crystal structures of racemic toussaintines A (**3**) and D (**5**), to the left and to the right, respectively, with their stereocenters being highlighted.

Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data for Toussaintines F (6) and G (7) Acquired in $CDCl_3$ at 25 °C (δ_H, Multiplicity (J in Hz))

	6			7		
position	δ_C, type	δ_H	m (J in Hz)	δ_C, type	δ_H	m (J in Hz)
1	207.8, C=O			207.0, C=O		
2	40.3, CH_2	3.08, 2.69	dd (2.9, 14.7), ddd (2.8, 2.8, 14.7)	42.3, CH_2	2.85, 2.77	dd (9.7, 14.0), ddd (1.9, 4.8, 14.0)
3	85.9, CH−O	3.81	ddd (2.5, 2.8, 2.9)	80.4, CH−O	3.50	dd (4.8, 9.7)
4	71.2, C−O			74.5, C−O		
5	54.7, CH	4.78	dddd (2.4, 2.5, 5.8, 10.2)	52.2, CH	4.66	ddd (4.3, 5.8, 9.0)
6	43.8, CH_2	3.20	dd (5.8, 14.6)	43.1, CH_2	3.24	dd (5.8, 14.8)
		2.40	ddd (2.4, 2.8, 14.6)		2.31	ddd (1.9, 4.3, 14.8)
3-OMe	57.9, OCH_3	3.41	s	57.5, OCH_3	3.38	s
1′	37.8, CH_2	2.87	d (16.7)	38.3, CH_2	2.79	d (15.5)
		2.80	d (16.7)		2.71	d (15.5)
2′	173.8, C=O			173.4, C=O		
3′	52.4, OCH_3	3.75	s	52.4, OCH_3	3.76	s
1″	165.0, C=O			166.1, C=O		
2″	120.7, CH	6.27	d (15.7)	119.7, CH	6.33	d (15.5)
3″	141.6, CH	7.57	d (15.7)	142.7, CH	7.62	d (15.5)
4″	134.8, C			134.5, C		
5″	128.0, CH	7.51−7.47	m	128.1, CH	7.49	7.4, 2.2
6″	129.0, CH	7.40−7.34	m	129.1, CH	7.40−7.36	m
7″	129.9, CH	7.40−7.34	m	130.2, CH	7.40−7.36	m
NH		6.67	d (10.2)		5.71	d (8.9)

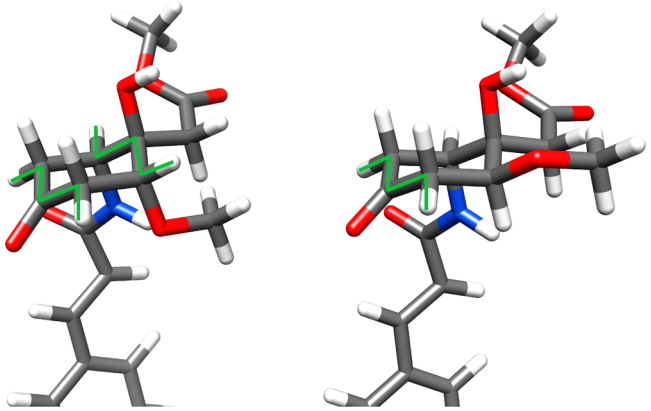


Figure 3. *W*-Couplings (^4J) observed for the equatorial protons of toussaintines F (**6**) and G (**7**) reveal the preferred conformation of their cyclohexanone cores.

173.8). The substitution pattern of the cyclohexanone core of **6** is further indicated by the $^3J_{CH}$ HMBC correlations of OH-4 (δ_H 4.70) to C-3 (δ_C 85.9) and C-5 (δ_C 54.7) and the very weak $^3J_{CH}$ to C-4 (δ_C 71.2). The resonance of C-1 (δ_C 207.8) is confirmed by its $^2J_{CH}$ correlation to H-6a (δ_H 3.20), for instance. The *E*-configuration of the C-2″−C-3″ olefinic bond is indicated by the $^3J_{H\text{-}2'',H\text{-}3''}$ value of 15.7 Hz. NOESY correlations (Figure 4) confirm the proposed constitution and relative configuration. Hence, the cynnamide (δ_H 6.67) shows NOEs to H-6ab (δ_H 3.20 and 2.40), H-5 (δ_H 4.78), OCH_3-3 (δ_H 3.41), CH_2-1′ab (δ_H 280 and 2.87), and H-2″ (δ_H 6.27). In turn, H-5 (δ_H 4.78) shows further NOEs to CH_2-6ab (δ_H 3.20 and 2.40) and CH_2-1′ab (δ_H 280 and 2.87), whereas the latter protons give NOEs to OH-4 (δ_H 4.70) and the equatorial H-3 (δ_H 3.81) as well. Overall, the relative configuration and the main conformation of the cyclohexanone core of **6** are revealed by the $^3J_{H\text{-}6b,H\text{-}5}$ (2.4 Hz), $^3J_{H\text{-}6a,H\text{-}5}$ (5.8 Hz), $^4J_{H\text{-}5,H\text{-}3}$ (2.5 Hz), $^3J_{H\text{-}3,H\text{-}2a}$ (2.9 Hz), $^3J_{H\text{-}3,H\text{-}2b}$ (2.8 Hz), and $^3J_{H\text{-}2b,H\text{-}6b}$ (2.8 Hz) gauche couplings and are corroborated by the corresponding set of NOEs shown in Figure 4. No crystals for X-ray analysis were obtained; however, based on NOE interactions and presuming a common biosynthetic route to **1**, **2**, and **6**, the relative configuration is presumably that shown in Figure 4. On the basis of the above data, **6** was characterized as methyl 2-[6α-(1-*E*-cinnamido-1β-hydroxy-2α-methoxy-4-oxacyclohex-1-yl)]acetate and was assigned the trivial name toussaintine F.

Toussaintine G (**7**) was obtained as a white solid and was assigned the molecular formula $C_{19}H_{23}NO_6$ based on HRMS (ESI) data ($[M + H]^+$ m/z obs 362.1429, calcd 362.1604). It

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J. Nat. Prod. 2015, 78, 2045−2050

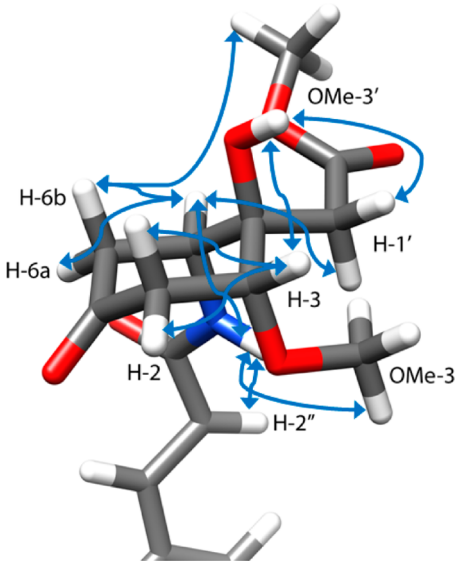

Figure 4. Key NOE correlations (blue arrows) observed for compound **6** (mixing time 700 ms, $CDCl_3$, 25 °C, 800 MHz, spectrum S44, Supporting Information).

exhibited spectroscopic features similar to those of **6**, suggesting a closely related cinnamide-based structure, with significant chemical shift differences observed only for their C-3 to C-5 region (Table 2, Supporting Information). The $^3J_{H\text{-}5,H\text{-}6a}$ (5.8 Hz) and $^3J_{H\text{-}5,H\text{-}6b}$ (4.3 Hz) gauche couplings along with the $^4J_{H\text{-}2b,H\text{-}6b}$ (1.9 Hz, Figure 3) indicate that the cyclohexanone ring of **7** prefers a main conformation that is comparable to that of **6**. However, its H-3 is axial, as revealed by its trans $^3J_{H\text{-}2a,H\text{-}3}$ (9.7 Hz) and cis $^3J_{H\text{-}2b,H\text{-}3}$ (4.8 Hz) values, in contrast to **6**, whose H-3 is equatorial and exhibits two gauche vicinal couplings. The absence of $^4J_{H\text{-}3,H\text{-}5}$ *W*-coupling (Figure 3) and the observation of an NOE between the axial H-3 (δ_H 3.50) and the amido proton (δ_H 5.71) of the axial substituent further corroborate the above conclusion. The similar $^3J_{HH}$ value and NOE pattern of the CH_2-6ab–H-5–H-1′ region of **6** and **7** suggest their comparable relative configurations at C-4 and C-5 as well as their similar overall conformation. On the basis of the NMR data and presuming a common biosynthetic route to **1**, **2**, and **6**, compound **7** was characterized as methyl 2-[6*α*-(1-*E*-cinnamido-1*β*-hydroxy-2*β*-methoxy-4-oxacyclohex-1-yl)] and was given the trivial name toussaintine G.

Motivated by the use of *T. orientalis* in traditional medicine and by the previously reported antimycobacterial[6] and cytostatic activities[8] of cinnamoyl derivatives, compounds **1**–**7** and the crude leaf extract of *T. orientalis* were evaluated for in vitro activity against *Mycobacterium tuberculosis* (H37Rv strain) and for cytotoxicity against the aggressive human breast cancer cell line (MDA-MB-231, Table 3). Both the crude methanol extract and its isolated constituents exhibit weak antimycobacterial activities (MIC 83.3–107.7 μM) against the H37Rv strain of *M. tuberculosis*. Compounds **1**, **3**, **4**, and **5** are cytotoxic, with ED_{50} values of 15.3–105.7 μM. Sample limitations precluded assessment of **2**, **6**, and **7** for cytotoxicity.

Thus, seven compounds were isolated from the leaves of *T. orientalis*. Three of these are new natural products, whereas the relative configurations of three additional compounds are revised here. The crude extract and its isolated constituents exhibit weak antitubercular activity and weak to moderate toxicity against MDA-MB-231 breast cancer cells. The above observations provide useful information for the understanding of the phytochemistry of *Toussaintia* species and may provide insight into the antituberculotic and cytostatic properties of cynnamoyltetraketides.

Table 3. Antimycobacterial (MIC μM) and Cytotoxic (ED_{50} μM) Activities of Selected Toussaintines and of the Crude Leaf Extract of *Toussaintine orientalis*

sample	MIC[a]	ED_{50}[a]
T. orientalis leaf extract	>32	53.7
E-toussaintine E (**1**)	96.9	15.3
Z-toussaintine E (**2**)	96.9	n.d.
toussaintine A (**3**)	107.7	105.7
toussaintine C (**4**)	83.3	23.6
toussaintine D (**5**)	107.0	70.6
toussaintine F (**6**)	88.4	n.d.
toussaintine G (**7**)	88.4	n.d.
isoniazide (control)	0.3	
rifampicin (control)	0.9	

[a]MIC and ED_{50} are given in μg/mL for crude and in μM for pure compounds; n.d., not determined.

EXPERIMENTAL SECTION

General Experimental Procedures. Column chromatography (CC) was carried out using silica gel 60 (230–400 mesh). NMR spectra were acquired on Varian VNMR-S 500 and Bruker Avance III HD 800 MHz spectrometers and were processed using MestReNova-10.0. Structural assignment was based on ^1H and ^{13}C NMR, COSY,[10] NOESY,[11] HSQC,[12] and HMBC[13] spectra. The solvent residual peak was used for chemical shift referencing ($CHCl_3$, δ_H 7.26 and δ_C 77.16). LC-MS (ESI) chromatograms were acquired on a PerkinElmer PE SCIEX API 150 EX instrument equipped with a Turbolon spray ion source and a Gemini 5 mm RPC_{18} 110 Å column, applying a H_2O/MeCN, 80:20–20:80 (0.2% formic acid), gradient solvent system with a separation time of 8 min. HRMS (ESI) was obtained with a Q-TOF-LC/MS spectrometer (Stenhagen Analys Lab AB, Gothenburg, Sweden) using a 2.1 × 30 mm, 1.7 μm, RPC_{18} column and a H_2O/MeCN gradient system (5:95–95:5 gradient and 0.2% HCOOH). Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} (Merck) precoated aluminum plates, which after development with an appropriate solvent system were evaluated under UV light (254 and 366 nm) and then sprayed with 4-anisaldehyde reagent (prepared by mixing 3.5 mL of 4-anisaldehyde with 2.5 mL of concentrated H_2SO_4, 4 mL of glacial HOAc, and 90 mL of MeOH) followed by heating for identification of UV-negative compounds and detection of the color change of the UV positive spots. Gel filtration was carried out over Sephadex LH-20 (Pharmacia) suspended in CH_2Cl_2/MeOH (1:1) or 100% MeOH. Preparative HPLC was performed on a Waters 600E system using the Chromulan (Pikron Ltd.) software and an RP-C_8 Kromasil column (250 mm × 25 mm) with the solvent system H_2O/MeOH (gradient, 70:30 to 100% MeOH, for 20–40 min, flow rate of 8–15 mL/min).

Plant Material. The leaves of *T. orientalis* were collected on January 15, 2013, from the Gongo village at the edge of Saadan National Park, Bagamoyo District, Pwani Region, Tanzania. The plant was identified in the field by Frank M. Mbago and confirmed in the Herbarium of the Botany Department, University of Dar-es-salaam, where a voucher specimen is preserved as FMM 3330.

Extraction and Isolation. The air-dried and pulverized leaves (1 kg) were extracted with MeOH for 48 h (2 × 500 mL) at room temperature. The extracts were concentrated using a rotary evaporator at 40 °C to give 113 g of crude extract. Vacuum liquid chromatography (silica gel, 30–100% v/v EtOAc/petroleum ether gradient) of the leaf extract (80 g) gave 10 fractions, ca. 250 mL each, which were concentrated and analyzed by TLC using 4-anisaldehyde spray reagent. The combined fractions 3–4, obtained by eluting with 50%

DOI: 10.1021/acs.jnatprod.5b00356
J. Nat. Prod. 2015, 78, 2045–2050

EtOAc/petroleum ether, were passed through an activated charcoal column using CH_2Cl_2 eluent to remove chlorophyll, providing 10 fractions, ca. 50 mL each. Subfractions 3−10 were subjected to CC on silica gel using a 50−75% EtOAc/petroleum ether gradient to yield 30 subfractions, ca. 20 mL each. The contents of subfractions 9−13 were white solids following evaporation. These were combined and recrystallized from a 1:1 mixture of MeOH/MeCN, yielding *E*-toussaintine E (**1**, 87.2 mg, anisaldehyde: yellow-orange), whereas subfractions 18−21 yielded *Z*-toussaintine E (**2**, 9.3 mg, anisaldehyde: yellow-orange) following CC using Sephadex LH-20 and MeOH as eluent, followed by HPLC (C-8 column, 70:30 to 100:0 H_2O/MeOH gradient for 20 min, flow rate of 8 mL/min). The combined VLC fractions 6 and 7, upon removal of chlorophyll using activated charcoal, were subjected to repeated CC purification on silica gel 60, with 75% EtOAc/petroleum ether as eluent, followed by HPLC (C-8 column, 70:30 to 100:0 H_2O/MeOH gradient for 20 min, flow rate of 8 mL/min), affording toussaintine F (**6**, 8.5 mg, anisaldehyde: yellow) and toussaintine G (**7**, 6.4 mg, anisaldehyde: yellow). Following the removal of chlorophyll using activated charcoal and CH_2Cl_2, the combined VLC fractions 7−8 were subjected to repeated column chromatographic purification using silica gel 60 and 75% EtOAc/petroleum ether eluent, followed by gel filtration on Sephadex LH-20, with MeOH as eluent, yielding toussaintine C (**4**, 22.4 mg, anisaldehyde: orange). Semipurified fractions[1] containing **3**, **4**, and **5** were purified by CC using silica gel 60 with 30−50% EtOAc/isohexane as eluent, to afford toussaintine A (**3**, 30 mg, anisaldehyde: yellow), whereas with 50−75% EtOAc/isohexane as eluent a mixture yielding toussaintine C (**4**) and toussaintine D (**5**) was obtained. Fractions containing **4** and **5** were further purified by HPLC using a C-8 column with a 70:30 to 100:0 H_2O/MeOH gradient for 20 min with a flow rate of 8 mL/min, providing toussaintine C (**4**, 12 mg, anisaldehyde: orange) and toussaintine D (**5**, 22 mg, anisaldehyde: pink). Compounds **3** and **5** were crystallized from a 1:1 mixture of MeCN/MeOH.

*E-Toussaintine E (**1**) (ref 1):* white solid, MS (ESI) m/z 330.3 $[M + H]^+$; ^1H and ^{13}C NMR data, see Supporting Information.

*Z-Toussaintine E (**2**):* white solid, $[\alpha]_D = -8$ (CH_3OH, c 0.0015); HRESIMS m/z 330.1134 $[M + H]^+$ (calcd for $C_{18}H_{19}NO_5$ 330.3472); ^1H and ^{13}C NMR data, see Table 1 and Supporting Information.

*Toussaintine A (**3**) (ref 1):* white, amorphous solid; MS (ESI) m/z 298.6 $[M + H]^+$; ^1H and ^{13}C NMR data, see Tables S2 and S3, Supporting Information.

*Toussaintine C (**4**) (ref 1):* white, amorphous solid; MS (ESI) m/z 284.4 $[M + H]^+$; ^1H and ^{13}C NMR data, see Tables S2 and S3, Supporting Information.

*Toussaintine D (**5**) (ref 1):* white, amorphous solid; MS (ESI) m/z 300.5 $[M + H]^+$; ^1H and ^{13}C NMR data, see Tables S2 and S3, Supporting Information.

*Toussaintine F (**6**):* white, amorphous solid; $[\alpha]_D$ −14 (MeOH, c 0.001); HRESIMS $[M + H]^+$ m/z 362.1616 (calcd for $C_{19}H_{23}NO_6$, 362.1604); ^1H and ^{13}C NMR data see Table 2.

*Toussaintine G (**7**):* white, amorphous solid; $[\alpha]_D$ −10 (MeOH, c 0.001); HRESIMS $[M + H]^+$ m/z 362.1429 (calcd for $C_{19}H_{23}NO_6$, 362.1604); ^1H and ^{13}C NMR data see Table 2.

Cytotoxicity Assay. The cytotoxic activity of the methanol extract and of its constituents was evaluated against human breast cancer cells (MDA-MB-231) as previously reported by Irungu.[14] MDA-MB-231 human breast cancer cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, 2 mM L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 °C in humidified 5% CO_2. For cytotoxicity assays, cells were seeded in 96-well plates at optimal cell density (10 000 cells per well) to ensure exponential growth for the duration of the assay. After a 24 h preincubation growth, the medium was replaced with experimental medium containing the appropriate drug concentrations or vehicle controls (0.1% or 1.0% v/v DMSO). After 72 h of incubation, cell viability was measured using Alamar Blue reagent (Invitrogen Ab, Lidingö, Sweden) according to the manufacturer's instructions. Absorbance was measured at 570 nm with 600 nm as a reference wavelength. Results were expressed as the mean ± standard error for six replicates as a percentage of vehicle control (taken as 100%). Experiments were carried out three independent times with six replicates each time. Statistical analyses were performed using a two-tailed Student's t-test. $p < 0.05$ was considered to be statistically significant.

Antimycobacterial Activity Assay. Antimycobacterial activity of the MeOH extract and its purified constituents was evaluated against the H37Rv strain of *Mycobacterium tuberculosis* using rifampicin (RIF) and isoniazide (INH) as positive controls. The minimum inhibitory concentration against replicating *M. tuberculosis* was determined by the microplate Alamar Blue assay (MABA). Compound stock solutions and the range of final testing concentrations were 64 μg/mL and 32−0.5 μg/mL, respectively. *M. tuberculosis* (H37Rv) was grown to late log phase (70 to 100 Klett units) in Difco Middlebrook 7H9 broth (catalog no. 271310) supplemented with 0.2% (v/v) glycerol, 0.05% Tween 80, and 10% (v/v) albumin−dextrose catalase (BBL Middlebrook ADC Enrichment, catalogue no. 212352, 7H9-ADCTG). The cultures were centrifuged, washed twice, and then resuspended in phosphate-buffered saline. Suspensions were passed through an 8 μm pore-size filter to remove clumps, and the aliquots were frozen at −80 °C. Twofold dilutions of the studied compounds were prepared in 7H9-ADC-TG in a volume of 100 μL in 96-well, black, clear-bottom microplates (BD Biosciences, Franklin Lakes, NJ, USA). *M. tuberculosis*, 100 μL containing 2×10^5 CFU, was added, yielding a final testing volume of 200 μL. The plates were incubated at 37 °C. On the seventh day of incubation, 12.5 μL of 20% Tween 80 and 20 μL of Alamar Blue were added to all wells. Following incubation at 37 °C for 16−24 h, the fluorescence was read at excitation λ = 530 nm and emission λ = 590 nm. The MIC was defined as the lowest concentration effecting a reduction in fluorescence of ≥90%, relative to the mean of replicate bacterium-only controls.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

1D and 2D NMR, MS spectra, X-ray crystallography. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00356.

■ AUTHOR INFORMATION

Corresponding Authors

*Tel: +255-754-206560. E-mail: nyandoro@udsm.ac.tz (S. S. Nyandoro).

*Tel: +46-31-786-9033. E-mail: mate@chem.gu.se (M. Erdélyi).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The Swedish Research Council (Swedish Research Links, 2012-6074) and the Academy of Finland (K.R., grant nos. 263256 and 265328) are gratefully acknowledged for financial support. S.S.N. is thankful to the Swedish Institute for a postdoctoral research award (00045/2014). We thank Mr. F. M. Mbago, a curator at the Herbarium of the Department of Botany, University of Dar es Salaam, for locating and identifying the investigated plant species.

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DOI: 10.1021/acs.jnatprod.5b00356
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