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Trigocherrin A, the First Natural Chlorinated Daphnane Diterpene Orthoester from *Trigonostemon cherrieri*

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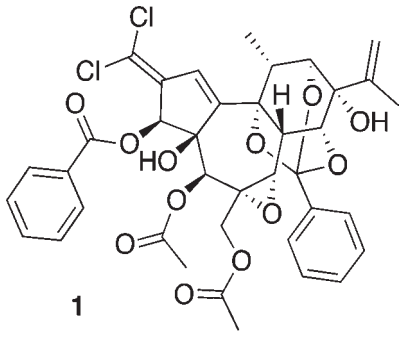
ABSTRACT



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Trigocherrin A, a chlorinated and highly oxygenated daphnane diterpenoid orthoester (DDO), was isolated from the bark of *Trigonostemon cherrieri*. Trigocherrin A is the first example of a naturally occurring halogenated DDO. Its structure was elucidated by comprehensive analysis of NMR spectroscopic data, and its absolute configuration was determined by comparison of experimental and theoretically calculated ECD spectra. Trigocherrin A exhibited a potent and selective effect against Chikungunya virus in Vero cells.

In an effort to identify selective inhibitors of virus replication from tropical plants, the bark of *Trigonostemon cherrieri* Veillon, a tree collected in the sclerophyllous forest of New-Caledonia, was chemically investigated. This species, which is classified as critically endangered by the IUCN red list,[1] is the unique representative of the genus *Trigonostemon* (Euphorbiaceae) in New Caledonia. It only grows in a small parcel of ca. 20 ha of dry forest located on the west coast of the main island. This genus comprises about 80 species occurring in tropical Asia, from India and Sri Lanka to New Guinea.[2] Previous investigations of species of this genus led to the isolation of numerous structurally interesting compounds such as flavonoidal alkaloids,[3] alkaloids,[4] phenanthrenes,[4a,5] and an array of daphnane-type diterpenoids.[6] Daphnane-type diterpenoids have been reported to possess insecticidal,[6a,b] acaricidal,[6c] cytotoxic,[6g,h,j] and antiviral[6d,e] properties.

In this study, trigocherrin A (**1**),[7] an unusual chlorinated daphnane diterpenoid orthoester (DDO) was isolated from the bark of *Trigonostemon cherrieri*. Theoretical calculation of electronic circular dichroism (ECD) spectra and comparison with experimental data allowed determination of the absolute configuration of **1**. Herein, we report the isolation, structure determination, configurational

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study, and antiviral activity in a Chikungunya virus-cell-based assay of trigocherrin A (**1**).

The air-dried powder of the bark of *Trigonostemon cherrieri* (1.2 kg) was extracted manually at room temperature with EtOAc (5 L) and MeOH (5 L). The EtOAc extract (23 g) was partioned between *n*-hexanes and aqueous MeOH. The MeOH-soluble fraction (4.2 g) was then subjected to C18 Flash chromatography using a H_2O/MeOH (0.1% HCO_2H) gradient (80:20 to 0:100) to afford 20 fractions. Fraction 11 (20:80, 315 mg) was then repeatedly purified by preparative and analytical HPLC (H_2O:CH_3CN + 0.1% HCO_2H) to yield compound **1** (1.9 mg).

Compound **1** was obtained as an amorphous powder. The HRESIMS of the quasi-molecular positive ion peak at *m*/*z* 755.1657 established the molecular formula of **1** as $C_{38}H_{36}Cl_2O_{12}$ (calcd. 775.1662), thus requiring a 20 double-bond equivalent. The 3:2 ratio of $[M + H]^+$ and $[M + H + 2]^+$ confirmed the presence of two chlorine atoms in **1**. In accordance with the molecular formula, 38 carbons were resolved in the ^{13}C NMR spectrum (Table 1) and were further characterized by HSQC experiment as four methyls, two methylenes (one olefinic), 18 methines (5 oxygenated and 11 olefinic), and 14 quaternary carbons (3 ester carbonyls, 5 oxygenated, and 6 olefinic). The ^1H NMR spectrum (Table 1) clearly showed the presence of an isopropenyl (C-15 (∂_C 141.8), C-16 (∂_C 116.1), C-17 (∂_C 18.5); H-16 (∂_H 4.93 and 5.19, s, 2H), H-17 (∂_H 1.82, s, 3H)), an oxymethylene (C-20 (∂_C 66.5); H-20 (∂_H 3.52 and 4.76, d, 12.2 Hz, 2H)), two acetyls (∂_H 2.06, s, 3H and 2.32, s, 3H), two hydroxyls (∂_H 2.97, s and ∂_H 3.88, br s), and a benzoyloxy group. The quaternary carbon C-1′ at ∂_C 108.5 is typical of an orthobenzoate.[8] Considering its biological source, the aforementioned data suggested that **1** possessed a modified DDO backbone. The DDO nature and the relative configuration of **1** were established by comprehensive 1D and 2D NMR data analysis (Figures 1 and 2).

Table 1. ^1H and ^{13}C NMR Data of Trigocherrin A (**1**) ($CDCl_3$)

position	∂_C[a]	∂_H, mult (*J* in Hz)[b]
1	128.4	6.41, s
2	136.6	–
3	79.1	5.62, s
4	84.5	–
5	69.8	5.50, s
6	59.4	–
7	62.8	3.28, s
8	34.9	3.43, s
9	74.6	–
10	146.9	–
11	35.9	1.99, q (7.0)
12	79.7	4.25, br s
13	70.4	–
14	79.3	4.44, br s
15	141.8	–
16	116.1	5.19, s
		4.93, s
17	18.5	1.82, s
18	13.8	1.20, d (7.0)
19	120.2	–
20a	66.5	4.76, d (12.2)
20b		3.52, d (12.2)
1′	108.5	–
2′	137.9	–
3′	125.5	7.74, m
4′	128.4	7.40, m
5′	129.9	7.40, m
6′	128.4	7.40, m
7′	125.5	7.74, m
1″	168.1	–
2″	129.3	–
3″	130.1	7.98, d (7.6)
4″	128.8	7.45, t (7.6)
5″	133.8	7.59, t (7.6)
6″	128.8	7.45, t (7.6)
7″	130.1	7.98, d (7.6)
4-OH	–	2.97, s
13-OH	–	3.88, br s
5-OAc	170.1, 21.2	2.32, s
20-OAc	170.9, 21.0	2.06, s

[a] Data recorded at 150 MHz. [b] Data recorded at 600 MHz.

The secondary methyl at ∂_C 13.8 was assigned to Me-18 by HMBC correlations from Me-18 to C-9, C-11, and C-12. The presence of a trisubstituted 6,7-epoxide was determined by chemical shifts of C-6 and C-7 (∂_C 59.4 and 62.8, respectively) and multiple HMBC correlations from H-7 to C-6, C-8, C-9, and C-20; from H-20 to C-7; and from H-8 to C-6 and C-7. The benzoyloxy group was attached to C-3 (∂_C 79.1) by the HMBC correlation from H-3 (∂_H 5.62, s) to the carbonyl C-1″ (∂_C 168.1). The two acetoxyls were located at C-5 (∂_C 69.8) and C-20 (∂_C 66.5), on the basis of HMBC correlations from H-5 and H-20 to the corresponding carbonyls of the acetyls at ∂_C 170.1 and 170.9, respectively. The two hydroxyl protons at ∂_H 2.97 and 3.88 were assigned as 4-OH and 13-OH on the basis of their correlation with C-4 (∂_C 84.5) and C-13 (∂_C 70.4), respectively. The three remaining oxygenated carbons

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(7) Trigocherrin A (**1**): amorphous powder, $[\alpha]^{25}_D$ −150 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 267 (4.01) 233 (3.87) nm; ^1H and ^{13}C NMR data: see Table1; HRESIMS (pos.) *m*/*z* 755.1657 $[M + H]^+$ (calcd for $C_{38}H_{37}Cl_2O_{12}$, 775.1662).

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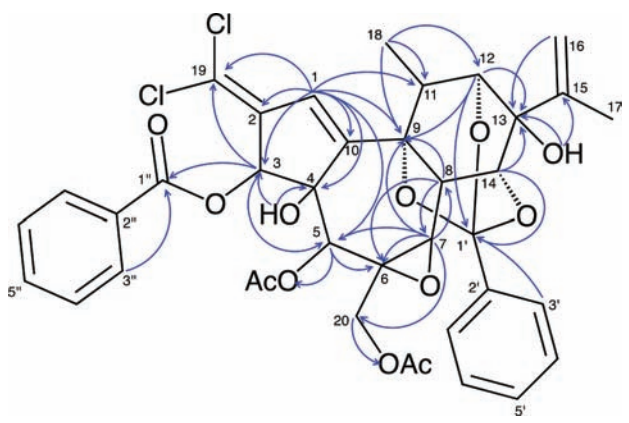

Figure 1. Selected HMBC (blue →) correlations of **1**.

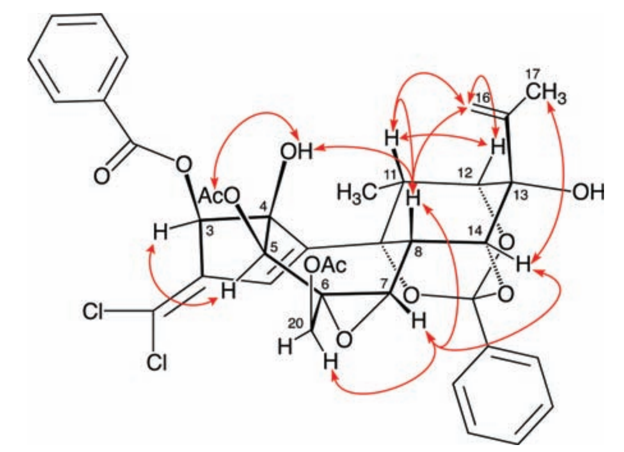

Figure 2. Key (red ↔) ROESY correlations of **1**.

were assigned to C-9 (∂_C 74.6), C-12 (∂_C 79.7), and C-14 (∂_C 79.3), and formed a 9,12,14-orthobenzoate pattern as confirmed by HMBC correlations from H-12, H-14, H-3′, and H-7′ to the characteristic quaternary C-1′ at ∂_C 108.5. The location of the aforementioned substituents left two double-bond equivalents and two chlorine atoms to dispose on the five-membered ring of the daphnane core, thus implying the presence of an insaturation between C-1 and C-10, and an exocyclic double bond bearing the two Cl atoms. Multiple HMBC correlations from H-1 to C-2, C-3, C-4, C-5, C-9, C-10, C-11, and C-19 secured the planar structure of **1**.

The relative configuration of **1** was established by ROESY experiment (Figure 2). The ROESY cross peaks of H-12/H-11, H-11/H-8, H-8/OH-4, OH-4/OAc-5, H-20b/H-7, H-7/H-8, and H-7/H-14 indicated that they were all cofacial and randomly assigned as β-oriented. The strong intensity of the H-3/H-5 correlation allowed us to determine H-3 and H-5 as α-oriented. However, as observed in the case of trigochilide A,[9] a weaker ROESY correlation of H-3 with OH-4 was also observed. Correlation of H-14/Me-17 and multiple correlations of H_2-16 with H-8, H-11, and H-12 suggested the axial position of the isopropenyl at C-13. The relative stereochemistry of **1** was consequently established as 3*S**, 4*R**, 5*R**, 6*S**, 7*S**, 8*S**, 9*R**, 11*R**, 12*S**, 13*S**, 14*R** (Figure 2).

In order to determine the absolute configuration of **1**, its theoretically calculated and experimental ECD curves were compared. A conformational study on the 3*S*, 4*R*, 5*R*, 6*S*, 7*S*, 8*S*, 9*R*, 11*R*, 12*S*, 13*S*, 14*R*-configured trigocherrin A was performed.[10] Hybrid methods taking into account experimental data in the process of quantum mechanics calculations have proven themselves to be successful in the theoretical simulation of ECD spectra.[11] Therefore, 1000 conformations were generated by a Monte Carlo random search method and optimized by a TNCG method using the Macromodel program with the MM2 force field. Among the generated conformations and within a range of 3 kcal/mol from the global minimum, the conformer consistent with ROESY NMR experimental data was retained. ECD calculation was performed after gradient optimization of the selected conformer using the TDDFT method at the B3LYP/6-31G(d,p) level. The calculations were performed on the *Gaussian03* program, and an ECD curve was generated using SpecDis (Figure 3).

Comparison of theoretically calculated and experimental ECD curves permitted the unambiguous assignment

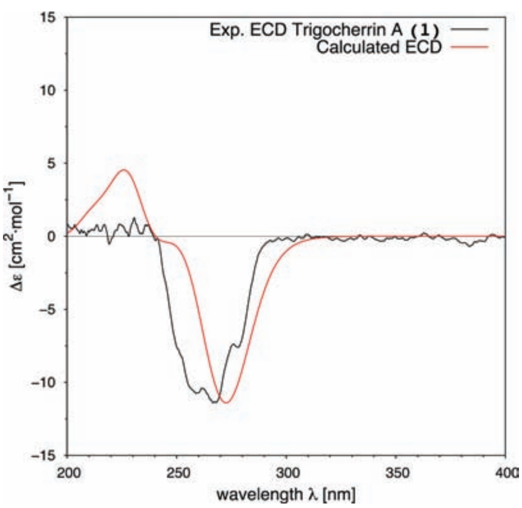

Figure 3. Experimental and calculated ECD for **1**.

of the absolute configuration of **1** as 3*S*, 4*R*, 5*R*, 6*S*, 7*S*, 8*S*, 9*R*, 11*R*, 12*S*, 13*S*, 14*R*.

Since no chlorinated solvent was used for extraction or purification, the nonartifactual nature of **1** could be

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(10) Details about the computational methods as well as the TDDFT-optimized geometry of **1** are available in the Supporting Information.

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established. Furthermore, characteristic M + 2 isotopic patterns were observed in the LC-MS chromatogram of the crude EtOAc extract, indicating the presence of chlorinated compounds in the bark. Halogenation is a structural feature that has been encountered in more than 4500 natural products. If most organohalogens are common in marine organisms, they are more rarely isolated from higher plants.[12] To our knowledge, all halogenated terrestrial diterpenes reported in the literature were chlorohydrins. Thus, trigocherrin A (**1**), possessing an α,β-unsaturated dichlorovinyl moiety, represents the first member of a new class of chlorinated diterpene. While most biohalogenases require aromatic, electron-rich substrates,[13] a class of enzyme recently reported by Vaillancourt et al. is shown to be capable of carrying out halogenation at aliphatic, unactivated carbon centers.[14] These nonheme iron, O_2, α-ketoglutarate-dependent halogenases catalyze the addition of a radical Cl• species on an aliphatic carbon. They can perform multiple chlorinations, as it is the case in the biosynthesis of barbamide,[15] and are thought to be responsible for vinyl chloride formation in the cyanobacterial metabolite jamaicamide.[16] A chlorination mechanism leading to **1** could involve such a nonheme iron halogenase to catalyze radical Cl• addition on carbon C-19. Validation of this biogenetic hypothesis would require a thorough biochemical study of the plant's genetic material.

Compound **1** reproducibly inhibited Chikungunya virus-induced cell death in a virus-cell-based assay with an EC_{50} of 1.5 ± 0.6 μM and only caused a significant antimetabolic effect at a concentration of 35 ± 8 μM (CC_{50}), allowing us to calculate a selectivity index (SI or window for antiviral selectivity) of 24. Following microscopic quality control, several concentrations of compound were found to perfectly protect the host cells from the virus-induced cytopathic effect without showing any adverse effects. For the reference compound chloroquine, a CC_{50} and EC_{50} of respectively 89 ± 28 μM and 11 ± 7 μM were obtained, resulting in an SI of 8, indicating that compound **1** is a 3-fold more potent antiviral.

In conclusion, trigocherrin A is the first member of a new series of chlorinated DDO with a potent and selective effect on Chikungunya virus-induced cell death. The structures and biological activities of this chemical series will be reported later on. The discovery of original bioactive compounds from a threatened biotope highlights the urgency to protect the unique habitat that is the sclerophyllous forests of New Caledonia.

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Supporting Information Available. Experimental procedures, computational methods, TDDFT-optimized geometry, and 1D and 2D NMR spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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