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Dysidotronic acid, a new and selective human phospholipase A_2 inhibitor from the sponge *Dysidea* sp.

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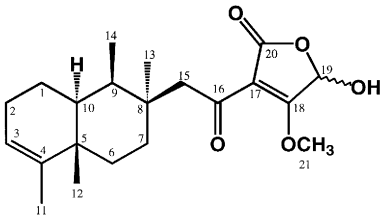
Abstract

A new bioactive sesquiterpenoid, named dysidotronic acid **1**, with a rearranged drimane skeleton has been isolated from the sponge *Dysidea* sp. from Vanuatu islands, along with bolinaquinone **2**. The chemical structure of **1** was determined on the basis of spectroscopic data. Dysidotronic acid significantly inhibited human synovial phospholipase A_2 (PLA_2) at 10 μM, with an IC_{50} value of 2.6 μM and a higher selectivity and potency towards this enzyme than the reference inhibitor manoalide.



Dysidotronic Acid (1)

Sesquiterpene quinones or hydroquinones and related compounds represent a prominent class of biologically active metabolites.[1] They appear to be restricted to sponges belonging to three families (Spongiidae, Thorectidae, Dysideidae) of the order Dictyoceratida and to one family (Niphatidae) of the order Haplosclerida and to one alga species (*Dictyopteris undulata*).[2] The sesquiterpene moiety of these metabolites usually has a bicyclic drimane or a 4,9-friedodrimane skeleton, even if several examples of monocyclic derivatives are also known.[3] A wide range of remarkable biological activity has been reported

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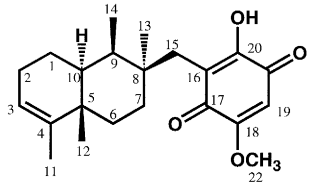
for many of these metabolites, ranging from antibacterial, cytotoxic, inhibitory activity against protein tyrosine kinase, and anti-HIV-1 activity.[4]

As part of an ongoing program devoted to the discovery of new bioactive metabolites from sponges collected in the Vanuatu islands we had the opportunity to study the sponge *Dysidea* sp. whose ethanolic extracts exhibited good cytotoxic activity against NSCLC-N6 (IC_{50}=5.5 μg/mL) cancer cell lines. The CCl_4 extract obtained by a Kupchan solvent partitioning procedure[5] on the crude methanolic extract was fractionated by silica gel MPLC (CH_2Cl_2:MeOH, 998:2) followed by reverse phase HPLC (MeOH/H_2O 75% 0.1% TFA). The major component (0.34% w/w) was bolinaquinone **2**, a cytotoxic sesquiterpene hydroxy quinone with a novel rearranged drimane skeleton recently isolated by Ireland from a Philippine *Dysidea* sponge.[6] In this paper we report the isolation, chemical characterization and the in vitro pharmacological evaluation of a new tetronic acid derivative **1** (colorless solid, $[\alpha]_D$=+44.7, 0.0009% w/w, dry weight) which we have named dysidotronic acid.



Bolinaquinone (2)

^1H and ^{13}C NMR spectra of **1** showed several pairs of analogous signals, indicating the existence of a slightly asymmetric dimeric molecule, a 1:1 mixture of compounds or an equilibrium between two species. HRFABMS established the monomeric nature of **1** and determined its molecular formula to be $C_{21}H_{30}O_5$ [$(M+H)^+$: 363.2148; calcd value for $C_{21}H_{31}O_5$: 363.2171]. Because several attempts to separate the isomeric mixture were unsuccessful, we continued the structural analysis on the mixture.

The same rearranged drimane skeleton as in bolinaquinone was inferred from 2D NMR data, mainly COSY, HMQC and HMBC measurements (Table 1). Particularly diagnostic for this assignment were the ^{13}C NMR chemical shifts of methyl groups 13 and 14 (δ 23.4 and 12.0, respectively) which were close to those reported for bolinaquinone (23.8 and 12.2) but very different from values reported for compounds containing the 4,9-friedodrimane skeleton (ca. 18.1 and 17.9 in isospongiaquinone).[7] The localization of a substituted group at C-8 instead of commonly found substitution at C-9 was also supported by a ^1H–^{13}C long range correlation observed in the HMBC spectrum between one of two diastereotopic protons H15 and the carbon assigned to the C-7 position. The ^{13}C NMR chemical shift of the isolated methylene at C-15 (δ_C 44.9/45.3) was indicative of the presence of adjacent α,β unsatured carbonyl functionality (δ_C 199.8). The linkage to a carbonyl group was also confirmed by the HMBC correlation of the H_2-15 protons to the C-16 carbonyl group and by the ^1H NMR chemical shifts (δ_H 2.72/2.11; 2.52/2.40) which were indicative of a vicinal group with a strong anisotropic effect. The different substitution at C-15 with respect to bolinaquinone resulted in differences in the ^{13}C NMR chemical shifts of carbons C-7–C-10. Having subtracted all the signals relative to the sesquiterpene moiety and to the carbonyl group, the ^{13}C NMR spectrum contained an additional four signals at 176.7, 168.6, 99.0, 91.3, consistent with the presence of a tetronic acid moiety and a methoxy signal at δ 60.1. IR (3410, 1770, 1730 and 1640 cm^{-1}) and UV data (λ_{max}=225 nm) confirmed this hypothesis. The HMBC correlations between the methoxyl group (δ_H 3.94) and the carbon at δ_C 176.7 and between the anomeric signal at δ 5.30 with the carbons at δ_C 176.7 and 99.0 allowed us to assign the same methoxy substituted tetronic acid moiety found in smenotronic acid,[8] which is isomeric to that found in dactyltronic acids.[9]

Thus, compound **1** was a 1:1 mixture of two C-19 epimers, as already found in all compounds containing the configurationally unstable semialdehyde group in the γ-hydroxybutenolide moiety. The

Table 1
^{13}C (125 MHz), ^{1}H (500 MHz) NMR data [δ ppm, (mult.) *J* hertz] with HMBC and NOE correlations of **1**

n°	^{13}C $CDCl_3$	^{1}H $CDCl_3$	HMBC	NOE
1	24.4	1.68-1.22 (m)	C2	
2	26.6	2.04 (m)		H3
3	120.7	5.17 (br s)		H2, H11
4	143.2			
5	37.5			
6	31.5	1.48 (m)	C5, C7, C12	
7	30.7/30.5	1.68 (m)		
8	36.5/36.6			
9	40.7/40.8	1.90/1.88 (m)		
10	39.9	1.83 (m)		
11	17.5	1.55 (s)	C3, C4, C5	H3
12	19.6	0.91 (s)	C4, C5, C6, C10	H14
13	23.3/23.4	1.15/1.12 (s)	C7, C9, C15	H15
14	12.0/12.2	0.78/0.73, (d) 7.0		H12, H15
15	44.9/45.3	2.52-2.40 (d) 19.0 2.77-2.11 (d) 18.5	C7, C8, C9, C16	H13, H14
16	199.8			
17	99.0/98.9			
18	176.7			
19	91.2/91.3	5.30 (s)	C17, C18, C20	OCH_3
20	168.6/169.5			
OCH_3	60.0/60.1	3.94/3.93 (s)	C18	H19

trans-fusion of the bicyclic system was indicated by the chemical shift of the Me-12 group (δ_C 19.6).[2] The relative stereochemistry as indicated in **1** was suggested by the ROESY correlations found between Me-12 and Me-14, and CH_2-15 and Me-14.

Dysidotronic acid represents the second example of a new class of sesquiterpene derivatives with a further rearranged drimane skeleton. It has some structural analogies with manoalide[10] and cacospongiolide B[11] derivatives which have been extensively investigated as potent inhibitors of phospholipase A_2 (PLA_2).[12,13] Therefore, compound **1** was tested for its inhibitory effect on $sPLA_2$,[14] belonging to the groups I (*Naja naja* venom and porcine pancreatic enzymes), II (human synovial recombinant enzyme and rat air pouch PLA_2[15]) and III (bee venom enzyme) as well as on cytosolic PLA_2[16] from macrophage line Raw 264.7 (group IV), using manoalide as reference inhibitor.

Dysidotronic acid inhibited preferentially human synovial PLA_2 in the μM range (Table 2) showing a higher potency and selectivity towards this enzyme than the reference inhibitor, manoalide. In contrast, this compound had no inhibitory effects on cytosolic PLA_2, although this enzyme was partially inhibited by manoalide at 10 μM (44.6±5.6 of inhibition, mean±SEM, *n*=6).

On the other hand, this compound was devoid of significant cytotoxic effects on human neutrophils, peritoneal murine macrophages as well as on macrophage line Raw 264.7 at concentrations up to 10 μM, as assessed by the mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to formazan[17] (data not shown).

Dysidotronic acid is a new and non-complex manoalide analogue lacking the pyranofuranone ring. It is interesting to note that this compound can offer new structural requirements for further studies about mechanistic interactions between PLA_2 enzymes and inhibitors, as has been described for manoalide-like sesterterpene molecules.[12] Further pharmacological studies are currently in progress.

Table 2
Effect of dysidotronic acid **1** on different secretory PLA_2 activities

Compound	*N. Naja* venom	Pancreas	Human synovial		RAP+zymosan	Bee venom	
	% I (10 μM)	% I (10 μM)	%I (10 μM)	IC_{50}(μM)	% I (10 μM)	%I(10 μM)	IC_{50}(μM)
Dysidotronic acid	1.8±1.1	18.8±2.9	80.6±3.3**	2.6	0.6±0.4	26.0±4.1*	
Manoalide	17.0±1.7*	32.3±2.7**	93.2±0.2**	3.9	38.4±0.5**	62.5±3.8**	7.5

Results show percentages of inhibition at 10 μM and IC_{50} (μM) values determined only for those compounds that reach 50 % of inhibition. Mean±S.E.M. (n=6). *P<0.05; ** P<0.01.
RAP: Rat air pouch PLA_2.

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