A New Diterpene from *Dictyota crenulata*

H. Soto, J. Rovirosa, and A. San-Martín

Departamento de Química, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

Reprint requests to Dra. J. Rovirosa; Fax 56/2/2713888. E-mail: aurelio@uchile.cl

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Chemical analysis of the brown alga *Dictyota crenulata*, family Dictyotaceae, collected at Easter Island, yielded a new diterpene. The structure was determined on the basis of spectroscopic evidence. The insecticidal activity of two diterpenes towards the tomato moth *Tuta absolute (Povolny)* was tested.

Key words: Dictyota crenulata, Diterpene

Introduction

Brown algae of the family Dictyotaceae are characterized by the production of diterpenoids belonging to different skeletal classes. Within the brown algae, representatives of the order *Dictyotales* are distinguished by high contents of terpenoids and phenolic compounds. This order is constituted by the family *Dictyotaceae*, which is formed by 15 genera. It has been demonstrated that species of the genus *Dilophus* belong to *Dictyota* genus [1 – 3]. The genus *Dictyota*, with some 30 species, has been the most extensively investigated. More than 90 diterpenes of 17 skeletal classes have been isolated from 18 species from all oceans [4, 5]. Among the diterpenes found in this family of seaweeds, the xenicanes containing a cyclononane skeleton constitute one of the most intriguing groups. Some of these diterpenoids function as chemical deterrents against marine herbivores such as fishes and sea urchins [6 – 10].

D. crenulata has proved to be a rich source of diterpenoids. From the chemical study of the brown alga *D. crenulata* collected in Easter Island, we have reported the isolation of five diterpenoids [11], all possessing a perhydroazulene skeleton. Specimens of this alga from Hawaii and the Gulf of California have been studied on several occasions, leading to the isolation of six xenicane diterpenoids. An exception is pachydictyol A, which possesses a perhydroazulenoid skeleton [12 – 16]. Continuing with our studies of *D. crenulata* from Easter Island, we now report the isolation of the new metabolite **1**, together with two other compounds (**2** and **3**) previously isolated by us [11].

The tomato moth *Tuta absoluta* is a microlepidopteran that affects tomato crops. The female oviposites a large number of eggs on the leaf or fruits approximately 10 hours after copula. The larvae feed on parenchyma tissue, transform in pupae and become adults after 12 or 15 days. In this work we tested the insecticidal activity of dictyotriol A diacetate (**2**) and dictyotriol A triacetate (**3**) against the tomato moth.

Results and Discussion

From the acetylated extract of *D. crenulata*, we isolated compound **1** as colorless oil (20.0 mg). Optical rotation measured for **1** was $[\alpha]_D^{25} - 61.7$ ($CHCl_3$). The ^{13}C NMR spectrum (Table 1) showed well-resolved resonances for 24 carbons and together with LRMS and HRMS indicated the molecular formula $C_{24}H_{38}0_5$ for the compound **1**, which requires six degrees of unsaturation. The IR and ^{13}C NMR data showed the presence of a tertiary hydroxyl group [3390 cm^{-1} and δ_c 73.2 (C)] and two carbonyl groups (1736 cm^{-1} and δ_c 169.2, 170.5). Two carbon resonances at δ_c 134.0 (CH) and 137.5 (CH), and two coupled proton resonances at δ_H 5.28 d ($J = 16.5$ Hz) and 5.67 brd ($J = 16.5$ Hz), were assigned to a disubstituted olefinic double bond. Two deshielded carbon resonances at δ_c 115.4 (CH_2) and 142.0 (C) and two coupled proton resonances at δ_H 5.17 br and 5.20 br were assigned to an exocyclic double bond. The lack of other olefinic or carbonyl resonances in the ^{13}C NMR spectrum suggested that the molecule contains two rings. The ^1H NMR and ^{13}C NMR spectra (Table 1), together with the ^1H COSY, HMQC and HMBC experiments, revealed the presence

N° C/H	δ_C	δ_H[a]	DEPT	HMBC	ROESY
1	47.9		C		
2	51.2	2.11*	CH	C_1. C_{11}	H-3, Me-15
3	71.2	4.94 m	CH	C_2.C_4.C_5	H-2, H-6, Me-15
4	39.5	2.28 dd (14.0)	CH_2	C_3.C_5	
		2.57 dd (14.0)			
5	142.0		C		
6	73.1	5.44 m	CH	C_5.C_{19}	H-2, H-3
7	29.4	1.58*	CH_2	C_6	
		1.93*			
8	40.5	1.47*	CH_2	C_6.C_7.C_9	
9	27.6	1.93*[a]	CH_2		Me-15,H-11
		1.43*[b]			
10	31.4	1.53*	CH_2	C_8.C_9.C_{11}	H-12
11	49.5	2.16*	CH	C_1.C_2	Me-17, Me-18, Me-20
12	35.7	2.23 m	CH	C_{11}.C_{13}.C_{20}-Me	H-10, Me-15
13	134.0	5.67 dd (16.5)	CH		
14	137.5	5.28 brd (16.5)	CH	C_1.C_{12}.C_{13}.C_{15}	
15	18.4	0.95 s	CH_3	C_1.C_2.C_{11}.C_{14}	H-9a, H-12
16	73.2		C		
17	32.1	1.25 s	CH_3	C_2.C_{16}.C_{18-}Me	H-11, H-19
18	23.4	1.19 s	CH_3	C_2.C_{16}	H-11, H-19, Me-20
19	115.4	5.17 brd	CH_2	C_4.C_6	Me-17, Me-18
		5.20 brd			Me-17, Me-18
20	22.2	0.97 d (6.9)	CH_3		H-11, Me-17, Me-18
CH_3-C=O	21.4	2.04 s	CH_3		
CH_3-C=O	21.6	2.05 s	CH_3		
CH_3-C=O	169.2		C=O		
CH_3-C=O	170.5		C=O		

Table 1. ^{1}H and ^{13}C NMR (300 and 75 MHz, $CDCl_3$) data of compound **1**.

[a] Coupling constants (J in Hz) in parentheses; * assignments may be interchanged.

of a hydroxyisopropyl group [δ_c 73.2 (C), 32.1 (CH_3), 23.4 (CH_3), δ_H 1.25 s, 1.19 s], a tertiary methyl (δ_c 18.4, δ_H 0.95 s), a secondary methyl [δ_c 22.2, δ_H 0.97 d ($J = 6.9$ Hz)] and two acetate methyl groups (δ_c 21.4, 21.6, δ_H 2.04 s and 2.05 s). The NMR spectra showed signals assignable to two oxygenated methylenes (δ_c 72.2 (CH), 73.1 (CH), δ_H 4.24 m, 5.44 m respectively). Also, there are five methylenes, three methines and one additional quaternary carbon atom.

All the above data for the compound **1** are in agreement with the carbon skeleton depicted in Fig. 1. In the HMBC spectra (Table 1) correlations were observed between the signal at δ_H 0.95 s, 3H, assigned to Me-15 protons with the signals at δ_c 47.9 s (C-1), 51.2 d (C-2), 49.5 (C-11) and 137.5 (C-14). The signal at δ_H 5.28 d, 1H, assigned to H-14 olefinic proton with the signal at δ_c 134.0 (C-13), 47.9 (C-1), and δ_c 35.6 (C-12). The signal at δ_H 2.23 (1H, m, H-12) correlated with the signal at δ_c 22.2 (C-20), C-11 and C-13 (at δ_c 49.5 and 134.0 respectively). Both signals at δ_H 1.25 (3H, Me-17) and 1.19 (3H, Me-18) showed correlations with C-16 and C-2 (at δ_c 73.2 and 51.2 respectively). These facts confirmed the location of the hydroxyl group in C-16. The signal at δ_H 4.94 (1H, m, H-3), assigned to a proton geminal to -OAc showed correlations with

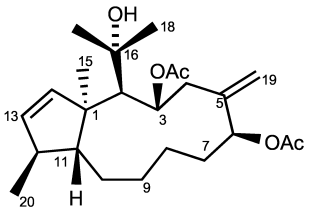

Fig. 1. Dictyocrenulol.

C-2, C-4 and C-5 (at δ_c 51.2, 39.5 and 142.0 d, respectively). The signal at δ_H 1.93, assigned to H-7 b proton correlated with C-6 (at δ_c 73.1 t). The signal at δ_H 1.47, (2H m, H-8) showed correlation with C-7, C-9 and C-6, (at δ_c 29.4, 27.6 and 31.4 respectively). The signal at δ_H 1.53, 2H, assigned to H-10 correlated with C-8, C-9 and C-11 (at δ_c 40.5, 27.6 and 49.5 respectively). The signal at δ_H 2.16, (1H), assigned to H-11 showed correlations with the signals at δ_c 47.9 (C-1) and 51.2 (C-2) and the signal at δ_H 2.11, 1H, assigned to H-2 correlated with C-1 and C-11 (at δ_c 47.9 and 49.5). These facts demonstrated that the molecule had a bond between C-1 and C-11.

The principal results from ROESY NMR experiments (Table 1), suggested that **1** has the stereochemistry shown in Fig. 1. These experiments showed cor-

relations between the signal at δ_H 2.23 (H-12) and the signals at δ_H 0.95 (Me-15) and 1.53 (H-10) which should have a α configuration. The signal at δ_H 2.16 (H-11), showed correlations with the signals at δ_H 0.97 (Me-20), 1.25 (Me-17) and 1.19 (Me-18), indicating that the hydroxyisopropyl group is in the β position. Finally, the signal at δ_H 4.94 (H-3), showed correlation with the signals at δ_H 2.11 (H-2), 5.44 (H-6) and 0.95 (Me-15), indicating that they are in the same relative configuration. The absolute stereochemistry was not determined. Therefore, we suggest the structure shown in Fig. 1 for this new diterpene and we propose the name dictyocrenulol (**1**).

The results show that the amount of leaf eaten by larvae in those sprayed with compound **3** at 100 ppm was just slightly lower than controls. Compound **2** did not protect the tomato leafs at the concentration tested.

Experimental Section

General experimental procedures. – Optical rotations were measured on a Perkin-Elmer 241 polarimeter. ^{1}H and ^{13}C NMR, HMQC, HMBC, NOESY and ^{1}H-^{1}H COSY spectra were measured employing a Bruker AMX-300 instrument operating at 300 MHz for ^{1}H NMR and at 75 MHz for ^{13}C NMR. Mass spectra were recorded on a V.G. Micromass, ZAB-2R. Infrared spectra were measured on a Bruker IFS-25 spectrometer. Merck Si gels 7734 and 7741 were used in column chromatography. The spray reagent for TLC was H_2SO_4-H_2O-AcOH (1:4:20).

Plant material. – *D. crenulata* was collected off at Vaihú, Easter Island, Chile, by SCUBA diving. The alga was identified by Prof. M. Eliana Ramírez and a voucher specimen has been deposited at the collection of the Faculty of Science, University of Chile, Santiago.

The dried and ground material (450 g) was extracted to exhaustion with methanol. The solvent was then removed at reduced pressure, affording an oily extract which was acetylated in dry pyridine with acetic anhydride and stirred at room temperature for 2 h and then poured into 10% aqueous HCl and extracted with $CHCl_3$. The organic layer was washed with water and brine, dried and concentrated. The organic residue was separated by a column chromatography on coarse-grained silica gel of Merck, with petroleum ether-ethyl acetate gradient with increasing amounts of ethyl acetate as eluent. From the more polar fractions, we obtained a complex mixture that was further separated on a column of Sephadex LH-20, with *n*-hexane-$CHCl_3$-MeOH (5:2:0.5) as solvent and then on HPLC with hexane-isopropanol (97-3), affording 15 mg of compound **1**, 16 mg of **2** and 23 mg of **3**.

Compound **1**. – Colorless oil. $[\alpha]_D^{25}$ – 61.7° (0.25% g/100 ml, $CHCl_3$). – IR (KBr): ν_{max} = 3390 (OH), 3023, 2961, 2927, 1736 cm^{-1}. – ^{1}H and ^{13}C NMR (Table 1). HREIMS: found 406.2740, (calculated 406.2719 for $C_{24}H_{38}O_5$). EIMS (70 eV, direct inlet): *m/z* (%) = 406 (1) $[M]^+$, 304 (13), 286 (63), 228 (52), 213 (36), 149 (24), 107 (34), 84 (74), 59 (100), 55 (66), 51 (40).

Bioassay. – Tomato plants (cultivar Rome) were grown in greenhouse under 15 – 20° and a 12/12 h light/dark photoperiod. When plants had 3 or 4 leaves they were transplanted to pots, situated into entomological cages and infected with larvae of the tomato moth. Barley plants (cultivar Aramir) were grown in pots under control conditions of temperature (25°) of 12/12 h light/dark photoperiod and irrigated with Hoagland solution. Tomato larvae of *T. absolute* were collected from colonies maintained on tomato plants cv Rome in a grown in chambers under 12/12 h, light/dark a photoperiod and 15 – 20°. Larvae were fed with leaves taken from tomato variety Rome sprayed with different concentration of compounds dissolved in acetone. The leaves were placed in a Petri disc that contains a moistened filter paper. The solution (1 ml) was sprayed on the leaves faces. After 15 minutes, when the acetone was evaporated, one larva was placed on each leaf. Each treatment was repeated 10 times. The quantity of leaf eaten by the larva and the mortality of insect were measured after 24 h. The quantity of leaf eaten was determined as follow: each leaf was weighed. The contour of the leaf was drawn on a milimetric paper. After the assay the damage area of the leaf was measured. Quantity of leaf eaten = Damaged area × Weigh of the leaf / Total area of the leaf.

Statistical analysis of data: We performed ANOVAS and Tukey test to evaluate the existence of differences among treatments.

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