Open Access Library Journal

A New Dolabrane Dinorditerpene from *Ceriops tagal*

Xin Wu[1], Hongbo Liao[2*], Hongyu Lu[3,4], Chaohua Zhang[3,4]

[1]Guangdong Key Laboratory for Research and Development of Natural Drugs, Guangdong Medical University, Zhanjiang, China
[2]Department of Pharmacology, Guangdong Medical University, Zhanjiang, China
[3]Guangdong Provincial Key Laboratory of Aquatic Product Processing and Safety, Guangdong Ocean University, Zhanjiang, China
[4]Key Laboratory of Advanced Processing of Aquatic Products of Guangdong Higher Education Institution, Guangdong Ocean University, Zhanjiang, China
Email: *sarahliao@gdmu.edu.cn

Received 7 August 2016; accepted 15 August 2016; published 19 August 2016

Abstract

A new dolabrane dinorditerpene, tagalsine X(1), was isolated from the leaves of *Ceriops tagal*, along with five known analogues (2-6). Their structures were established on the basis of spectroscopic data or comparison with the literatural data. Their cytotoxic activities against four human carcinoma cell lines (CNE-2, HCT-116, HepG2and A549) were also evaluated, which displayed that only compound 2 had significant cytotoxicity against those cell lines with IC50 values of 13.57, 42.32, 11.21 and 15.23 μM, respectively.

Keywords

***Ceriops tagal*, Dolabrane, Tagalsine X, Cytotoxicity**

Subject Areas: **Plant Science**

1. Introduction

The plant *Ceriops tagal* (Rhizophoraceae), which has been used to treat malaria [1], infected wounds and obstetric and hemorrhagic conditions [2], is the only species of genus Ceriops distributed in southern coastal zone of China [1]. Its previous chemical studies have yielded a series of diterpenoids and triterpenoids [3] with antifouling [4], antifeedant [5] and anticancer [6] activities. In this paper, we reported the isolation and structural eluci-

*Corresponding author.

How to cite this paper: Wu, X., Liao, H.B., Lu, H.Y. and Zhang, C.H. (2016) A New Dolabrane Dinorditerpene from *Ceriops tagal*. *Open Access Library Journal*, **3**: e2957. http://dx.doi.org/10.4236/oalib.1102957

dation of a new dolabrane dinorditerpene, tagalsine X(**1**), along with five known analogues (**2-6**), as well as their cytotoxic activities against four human carcinoma cell lines (CNE-2, A549, HepG2 and HCT-116).

2. Materials and Methods

2.1. Plant Material

The leaves of *Ceriops tagal* (**Figure 1**) were collected in Haikou City, Hainan Province of China, and authenticated by Prof. Weidong Han (College of Agriculture, Guangdong Ocean University). A voucher specimen (No. 20130513) was deposited in the Guangdong Key Laboratory for Research and Development of Natural Drugs, Guangdong Medical College, Zhanjiang, China.

2.2. General

Human carcinoma cell lines (CNE-2, A549, HepG2 and HCT-116) all came from ATCC. Column chromatographies (CC) were carried out byD101 macroporous resin (Beilianchem, China), silica gel (Qingdaohaiyang, China) and reversed-phase C_{18} silica gel (YMC, Japan). 1D and 2D NMR spectra were acquired on Bruker AV-400 spectrometer.HR-ESI-MS was recorded by an Agilent 6210 LC/MSD TOF mass spectrometer. Analytical high-performance liquid chromatography (HPLC) were carried out on a Agilent 1200 series and a C_{18} reversed-phase column (Cosmosil, 4.6 mm × 250 mm, 5.0 μm). Preparative HPLC were carried out on a Gilson 305 pump, a Varian Prostar 345 UV detector and a C_{18} reversed-phase column (Cosmosil, 20 mm × 250 mm, 5.0 μm).

2.3. Extraction and Isolation

The air dried leaves of *Ceriops tagal* (10.0 kg) were powdered and extracted three times (24 h for each) with 95% EtOH at room temperature (3 × 30 L). The tannins were precipitated and filtrated after keeping the solution standing for 48 hours at room temperature. Crude extract (800 g) was yielded by concentrating the filtration under vacuum. The residue was suspended in water, and then partitioned with EtOAc. After removing the solvent, the EtOAc extract (300 g) was separated by D101 macroporous resin column chromatography (CC) using gradient ethanol aqueous solutions (60%, 80% and 95%) as eluants to give three fractions (A-C). Fraction B (80% ethanol, 100 g) was then subjected to silica gel CC eluting with n-hexane-ethyl acetate (100:0→10:1) to yield seven subfractions B1-B7. Subfraction B4 (H:E 9:1, 7.0 g) was chromatographed on ODS column eluting with CH_3CN-H_2O (1:9→7:3) to afford eight subfractionsB4a-B4h. B4b(CH_3CN:H_2O 3:7, 0.6 g) was purified repeatedly by prepared HPLC (CH_3CN:H_2O 4:6) to yield compound **1** (13.0 mg), **2** (14.8 mg), **3** (65.1 mg), **4** (28.3 mg), **5** (50.1 mg) and **6** (61.3 mg) (**Figure 2**).

Figure 1. The twigs of *C. tagal* with leaves and fruit.

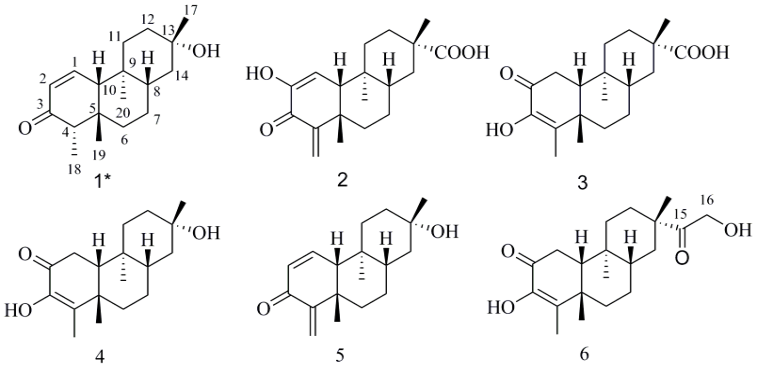

Figure 2. The structures of compounds **1-6**.

2.4. Cytotoxicity Assay by MTT Method

The cytotoxicity effects of compounds **1-6** were tested against four human carcinoma cell lines (CNE-2, A549, HepG2 and HCT-116) by the MTT method as described previously [7]. Generally, the cell suspensions were platedinto 96-well plates and cultured in RPMI-1640 at 37˚C, with 5% CO_2 in incubator overnight. The test compound solutions (in 0.1% DMSO) at different concentrations were added to the corresponding wells. After exposure for 68 h, MTT was added to each well and the plates were incubated for 4 h. Finally, the supernatant was discarded and 200 μL of DMSO was added to the well to dissolve the blue-violet crystal, then the optical-density (OD) values were read on the microplate reader at 570 nm. All tests and analyses were carried out in triplicate. DMSO and doxorubicin were applied as the blank control and positive control, respectively.

3. Results and Discussion

3.1. Structure Elucidation

Compound **1**, obtained as pale yellow amorphous powder in methanol, had the molecular formula $C_{18}H_{28}O_2$ as established by its HR-ESI-MS at *m/z* 299.1992 $[M + Na]^+$ (calcd for $C_{18}H_{28}O_2Na$: 299.1982), which implied that **1** had five degrees of unsaturation. The ^{13}CNMR and DEPT-135 spectra of **1** displayed a carbonyl carbon, and two olefinic carbons, as well as 15 aliphatic carbons including four methyls, five methylenes, three methines, and three quaternary carbons. The ^{1}HNMRspectrum of **1** showed fourmethyls at δ_H 0.87 (3H, s), 0.92 (3H, s), 1.03 (3H, d, J = 5.2 Hz), 1.31 (3H, s), a pair of olefinic protons at δ_H 6.84 (1 H, dd, J = 10.1, 5.9 Hz) and6.13 (1H, d, J = 10.2 Hz), a methine at δ_H 2.8 (1H, dd, 13.3, 6.6)and a set of aliphatic protons ranging from δ_H 1.22 to 1.96. The above spectroscopic features revealed that compound **1** was a dolabrane-type dinorditerpenoid. The ^{13}C NMR and ^{1}H NMR spectra of compound **1** were very similar to those of tagalsin Q (**5**) [8] except for the positions at 3-5, 18 and 19, which suggested that their B/C rings were the same, while their A rings were different. All the ^{1}H and ^{13}C NMR signals of **1** were assigned as shown in **Table 1** with the aid of ^{1}H-^{1}H COSY, HSQC and HMBC experiments. The bond between C-4 and C-18 changed from olefinic double-bond of tagalsin Q (**5**) into aliphatic single-bond of **1**, which was further supported by the HMBC correlations between H-18 (δ_H 1.03) and C-3 (δ_C202.7)/C-5 (δ_C39.0) (**Figure 3**). The relative configuration of **1** was proposed on the basis of ROESY correlations (**Figure 3**). The *β*-orientation of H-8, H-10, H_3-19 and H_3-17, and the *α*-orientation of H_3-18 and were H_3-20 deduced from the presence of ROESY interactions between H-8/H-6*β*, H-6*β*/H-10, H-10/H_3-19, H-19/H_3-4, H-10/H-6*β*, H-6*β*/H-8, H-10/H-8, H-10/H-11*β*, H-11*β*/H-17 and H-18/H-6*a*, and the absence of ROESY interactions between H_3-18/H-19, H_3-18/H-10, H_3-20/H-8 and H_3-20/H-19. On the basis of the above results, compound **1** was identified as (4*S**, 5*S**, 8*S**, 9*S**, 10*R**)-13*S**-hydroxy-15, 16-dinorlabr-1(2)-en-3-one, and named tagals in X.

The known compounds were identified as (5*S**, 8*S**, 9*S**, 10*R**, 13*S**)-2-hydroxy-16-nor-3-oxodolabr-1,4(18)-dien-15-oic acid (**2**) [8], (5*S**, 8*S**, 9*S**, 10*R**, 13*S**)-3-hydroxy-16-nor-2-oxodolabr-3-en-15-oic acid (**3**) [8], tagalsin P (**4**) [8], tagalsin Q (**5**) [8] and (5*S**, 8*S**, 9*S**, 10*R**, 13*S**)-3,16-dihydroxydolabr-3-ene-2,15-dione (**6**) [8], respectively, on the basis of their ^{1}H NMR and ^{13}C NMR spectra analysis and comparison with those reported data in the related literatures (**Figure 2**).

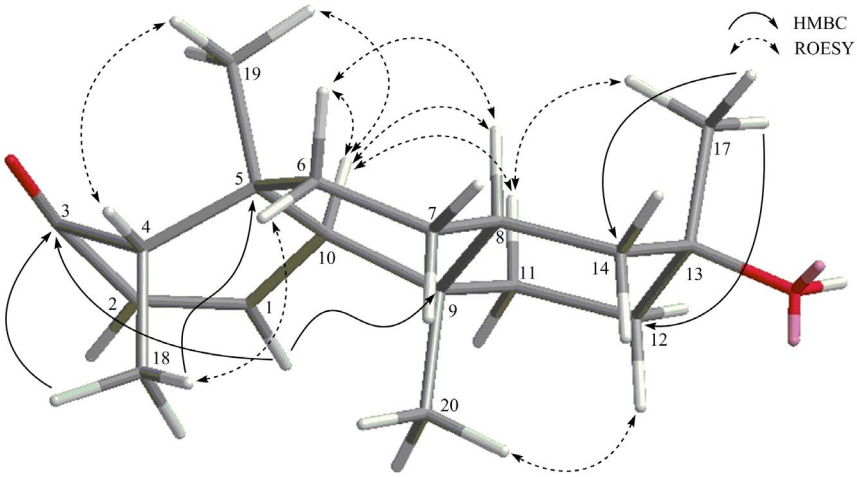

Figure 3. The key HMBC and ROESY correlations of compound **1**.

Table 1. NMR data of compound 1 (at 500 MHz in $CDCl_3$, in ppm, J in Hz)[a,b].

Position	δ_C	δ_H (HSQC)	COSY	HMBC	ROESY
1	148.0CH	6.84 (dd,10.1,5.9)	2/10	3/10/9	2/10/11*α*
2	130.2CH	6.13 (d,10.2)	1	10	-
3	202.7C	-	-	-	-
4	45.0CH	2.83 (dd,13.3,6.6)	18	3/5/18/19	18/19
5	39.0C	-	-	-	-
6	37.5CH_2	1.96 (*α*) 1.25 (*β*)	6/7	5/7/8/10	18 8/10
7	25.3CH_2	1.22 (*α*) 1.36 (*β*)	-	-	-
8	44.5CH	1.31	-	13/14/20	6*β*/10/14*β*
9	39.3C	-	-	-	-
10	57.4CH	1.86 (d,5.5)	1	1/2/4/5/19/20	6*β*/8/11*β*/19
11	37.4CH_2	1.72 (*α*) 1.25 (*β*)	11/12	13/20 8/10/12/13/20	- 10/17
12	35.5CH_2	1.69 (*α*) 1.58 (*β*)	11/12	11/13/17	20 11*β*
13	71.2C	-	-	-	-
14	42.9CH_2	1.53 (d,13) (*α*) 1.43 (d,13) (*β*)	8	8/9/13/17 8/9/12/13/17	20/7*α* -
17	26.9CH_3	1.31(s)	-	12/13/14	11*β*
18	7.9CH_3	1.03 (d,5.2)	4	3/4/5	6*a*
19	26.3CH_3	0.87 (s)	-	4/6/10	4/6*a*
20	13.5CH_3	0.92 (s)	-	10/8/11	14*a*

[a]Assignments were established by interpretation of the ^1H-^1H COSY, HSQC, and HMBC spectra; [b]Overlapped signals are reported without designating multiplicity.

3.2. Cytotoxicity Activity *in Vitro*

As can be seem from **Table 2**, compound **2** showed significant cytotoxicity against CNE-2, A549, HepG2 andHCT-116 cell lines with IC_{50} values of 13.57 ± 1.02, 42.32 ± 2.21, 11.21 ± 1.13 and 15.23 ± 1.42 μM, respectively, while the other five analogues had no obvious effect even with the concentration of 50 μM.

Table 2. Cytotoxicity of compounds **1-6** against four cancer cell lines[a].

Position	IC_{50} μM[a]			
	CNE-2	A549	HepG2	HCT-116
1	>50	>50	>50	>50
2	13.57 ± 1.02	42.32 ± 2.21	11.21 ± 1.13	15.23 ± 1.42
3	>50	>50	>50	>50
4	>50	>50	>50	>50
5	>50	>50	>50	>50
6	>50	>50	>50	>50
Doxorubicin[b]	0.52 ± 0.13	3.12 ± 0.23	0.83 ± 0.22	0.42 ± 0.11

[a]All results are expressed as mean ± SD, n = 3 for each group; [b]Positive control.

3.3. Discussion

Up to now, chemical examinations of the plant *Ceriops tagal* have resulted in the isolation of 27 dolabrane-type diterpenes [3] [9], which could be divided into four sub-types: diterpene (twenty), 16-norditerpene (three), 15, 16-dinorditerpene (two) and ring A-seco-diterpene (two). Interestingly, dolabranes were regarded as a small group of natural products as there were only about fifty dolabranes isolated from plants, and as this papermentioned, most of them existed in *Ceriops tagal*. So we regarded dolabrane as chemoaxonomic marker of *Ceriops tagal*.

4. Conclusion

The present study attempts to explore the chemical constituents of the leaves of *Ceriops tagal*, and their cytotoxicity against four human carcinoma cell lines (CNE-2, HCT-116, HepG2 and A549). The result indicated that a new dolabrane dinorditerpene, tagalsine X, and five known analogues were isolated and identified. Among them, only compound 2 had significant cytotoxicity against the tested cell lines with IC50 values ranging from 11.21 to 42.32 μM, which deserved further studies on its exact mechanisms.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (81503226) and the 863 Program (2013AA092902).

References

[1] Lin, P. and Fu, Q. (1995) Environmental Ecology and Economic Utilization of Mangroves in China. Higher Education Press, Beijing, 1-95.

[2] Bamroongrugsa, N. (1999) Bioactive Substances from Mangrove Resources. *Songklanakarin Journal of Science and Technology*, **21**, 377-386.

[3] Wang, H., Li, M.Y. and Wu, J. (2012) Chemical Constituents and Some Biological Activities of Plants from the Genus Ceriops. *Chemistry & Biodiversity*, **9**, 1-11. http://dx.doi.org/10.1002/cbdv.201000299

[4] Chen, J.D., Feng, D.Q., Yang, Z.W., Wang, Z.C., Qiu, Y. and Lin, Y.M. (2008) Antifouling Metabolites from the Mangrove Plant Ceriops Tagal. *Molecules*, **13**, 212-219. http://dx.doi.org/10.3390/molecules13020212

[5] Du, S.S., Wang, C.F., Li, J., Zhang, H.M., Liu, Q.Z., Liu, Z.L. and Deng, Z.W. (2011) Antifeedant Diterpenoids against *Tribolium castaneum* from the Stems and Twigs of *Ceriops tagal* (Rhizophoraceae). *Molecules*, **16**, 6060-6067. http://dx.doi.org/10.3390/molecules16076060

[6] Ouyang, X.W., Wang, X.C., Yue, Q.X. and Hu, L.H. (2010) A New Dolabrane-Type Diterpene from *Ceriops tagal*. *Natural Product Communication*, **5**, 9-12.

[7] Wu, X., Liao, H.B., Zhu, X.H., Lu, H.Y., Zeng, X.B., Cui, L., Lv, X.H., Li, Y.Q. and Zhang, C.H. (2016) Two New Dammarane Triterpenes from the Leaves of *Ceriops tagal*. *Records of Natural Products*, **10**, 628-632.

[8] Hu, W.M., Li, M.Y., Li, J., Xiao, Q., Feng, G. and Wu, J. (2010) Dolabranes from the Chinese Mangrove, Ceriops tagal. *Journal of Natural Products*, **73**, 1701-1705. http://dx.doi.org/10.1021/np100484w

[9] Yang, Y., Zhang, Y., Liu, D., Li-Weber, M., Shao, B. and Lin, W. (2015) Dolabrane-Type Diterpenes from the Mangrove Plant Ceriops tagal with Antitumor Activities. *Fitoterapia*, **103**, 277-282. http://dx.doi.org/10.1016/j.fitote.2015.04.016