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Caulerpin; Biosynthetic Significance and Cytotoxic Activity Against *Artemia salina* Leach.

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ABSTRACT

Caulerpin (**1**) is one of the bisindole alkaloid compounds isolated from the many macroalga species of the *Halimeda* and *Caulerpa* genera. The biosynthetic significance of **1** is suggested by the biotransformation of indole-3-acetic acid to indole-3-carboxaldehyde via tryptophan from the shikimate pathway. This compound has many biological activities reported and this time, the **1** were evaluated for their cytotoxicity in the brine shrimp assay against *A. salina* Leach. in vitro and showed moderate activity with IC_{50} value 81.28 µg/mL.

Keywords: Caulerpin, Halimeda, Caulerpa

I. INTRODUCTION

Halimeda and *Caulerpa* are two of the green macroalga genera, which are distributed in subtropical seawaters of the Pacific, Atlantic, and Hindi oceans. More than half of the species are found growing and thriving on coral and muddy sand in the tropical water of the Indo-Pacific and the Indonesian ocean (Hillis-Colinvaux, L, 1980; Guiry, M.D. & Guiry G.M. 2020). Species of the genus found in the coral islands of the Gulf of Buni, South Sulawesi, Indonesia. Species in the genus are rich in alkaloids as chemical defence compounds (Paul, V.J., Van Alstyne, K.L., 1992). It the interesting that bisindole alkaloids and related compounds are an important class of alkaloids from secondary metabolite compounds are found in the structures are many biological activities (Zhang, D. et al, 2011; Li, X. N. et al, 201; Zhang, W. et al, 2012; Denizot, N. et al, 2015.). Bisindole alkaloid compounds from the *Halimeda* genus are the only two compounds reported to date. Caulerpin (1) (**Figure 1**) was isolated for the first time from *H. stuposa* and *Halimeda* spp. (Yan S. et al 1999; Ovenden, S.PB et al, 2012; Mahomoodally, M.F. et al, 2020), then from *Halimeda cylindracea* Decaisne (Iwan D. et al, 2021a).

Caulerpin was previously reported from the green alga Caulerpa (Maiti & Thomson 1977). Caulerpin is a more common compound from the genus Caulerpa in green macroalgae and is mostly reported to be isolated from Caulerpa racemosa and also one species of red alga, Chondrus armatus (Patterson, GW, 1974). Caulerpin has been known to have various biological activities, include the activity against *A. salina* Leach. will be report bellow.

The biosynthesis pathway of this compound has long been suggested from the acrylic ester (methyl 2-(3-formyl-1H-indol-2-yl) acetate) via a condensation reaction (El-Sawy, E. et al,

2017). This suggest needs to be considered, because the natural metabolite formation of methyl 2-(3-formyl-1H-indol-2-yl) acetate are not yet reported. Therefore, the biosynthesis pathway of this compound is possible from indol-3-carboxaldehyde which will be described.

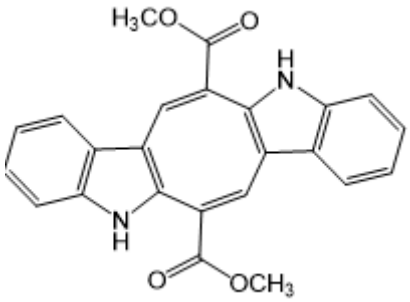


Figure 1. Caulerpin.

II. ISOLATION OF CAULERPIN

The fresh sample (10.47 kg dry weight) of *H. Cylindracea* was extracted with n-hexane at room temperature. Trough evaporating in the reduced pressure, to give crude extracts (12.87 g). The n-hexane crude extract (11.0 g) was separated by vacuum liquid chromatography on silica gel 60 with eluent n-hexane, ethyl acetate, acetone, and methanol as a solvent with gradient polarity to produced six sub-fraction (A-F). Sub-fraction D (830 mg) was fractionation with column chromatography on silica G60 (catalog 7734) eluted with hexane:ethylacetate (9:1) as a solvent constant gradient polarity to produce 75 fractions. Finally, subfraction 33-40 (109 mg) was separated using column chromatography on silica G60 (catalog 7734) eluted with hexane:ethylacetate (8:2) to give compound Caulerpin **1** (12.7 mg). The caulerpin was optaned froum precipitation of ethylacetat extrect of Caulerpa *racemos* (Forsskal) J. Agardh. (Iwan D. 2021b). Analyzing the ^{1}H-NMR of **1** showed of signal proton at δ_H (ppm) 7.38 and 7.45 (doublet); 7.05 and 7.13 (triplet); 8.21, 10.58 and 3.80 (singlet). The ^{13}C-NMR showed of twelve carbon double signals at δ_C (ppm) 132.31, 111.27, 127.06, 137.39, 125.50, 165.27, 117.08, 119.46, 122.12 (C-6), 110.94, 141.24, and 50.95. All of signal were compared literature, such signals are characteristic of the natural caulerpin product (Anjaneyulu ASR et al, 1991; Esteves, P. O. et al, 2019).

III. BRINE SHRIMP LETHALITY TEST.

Cytotoxic activity of compound 1 was carried out by the Brine Shrimp Lethality Test (BSLT) method. The test procedure used the concentrations of the sample test used 3.69, 7.81, 15.63, 31.25, 62.50, 125.00, and 250.00 µg/mL. Then, 4 mL of each concentration of sample in vial bottles were inserted by 7-10 shrimp larvae using a micropipette with 1 mL each addition to become 5 mL for all experiments. Mortality of shrimp larvae after 24 hours was observed, and the LC_{50} value was determined by using a regression curve between log concentration (X) and probit value (Y). The probit value (Y) was obtained by changing the percentage of mortality into the probit table. The Categorized active value refers to *Meyer, B. N.* et al, 1982.

IV. BIOSYNTHETIC SUGGESTED

The biosynthesis pathway of bisindole caulerpin has long been suggested from tryptophan via of shikimate pathway, possibly by way of the acrylic ester. Through a synthesis, caulerpin can obtained from two unit of methyl 2-(3-formyl-1H-indol-2-yl) acetate via a condensation

reaction (**Figure 2**). From the synthesis, the condensation in the cis configuration providing of caulerpin 5% yield (Maiti B.C., & Thomson R.H., 1977), and the identical reaction principle but with a different strategy exhibit 43.0% yield (Li, H. et al, 2018). By the Aldol condensation reaction obtained caulerpin 32% yield (Canche Chay, C.I et al, 2014).

Until now it has been known that the key to the formation of bisindole caulerpin is condensation of two indole methyl 2-(3-formyl-1H-indol-2-yl) acetate. Through synthesis, this compound can be obtained from the ester 2-indolasetate via formylation reaction, or from indole-3-carboxaldehyde with xanthate via radical oxidative aromatic substitution reaction. Methyl 2-(3-formyl-1H-indol-2-yl) acetate also possible obtained via addition methylacetate enolate to the indol-3-carboxaldehyde as α,β-unsaturated carbonyl via Michael addition reaction and this reaction type can catalyzed by lipase variant (Kazlauskas, R. J., & Bornscheuer, U. T., 2012). The natural metabolite formation of methyl 2-(3-formyl-1H-indol-2-yl) acetate are not yet reported. Therefore, the biosynthesis pathway of this compound are possible from indol-3-carboxaldehyde were are have been isolated from the *H. stuposa* (Ovenden, S.PB et al, 2012). Indole-3-carboxaldehyde known in vivo forming by the biotransformation of indole-3-acretat acid from the L-tryptophan, were are all of indole alkaloid class are derived from L-tryptophan via the shikimate pathway (**Figure 2**) (Maeda, H., & Dudareva N. 2012; Lin, G.-H. et al, 2015; El-Sawy, E. et al, 2017).

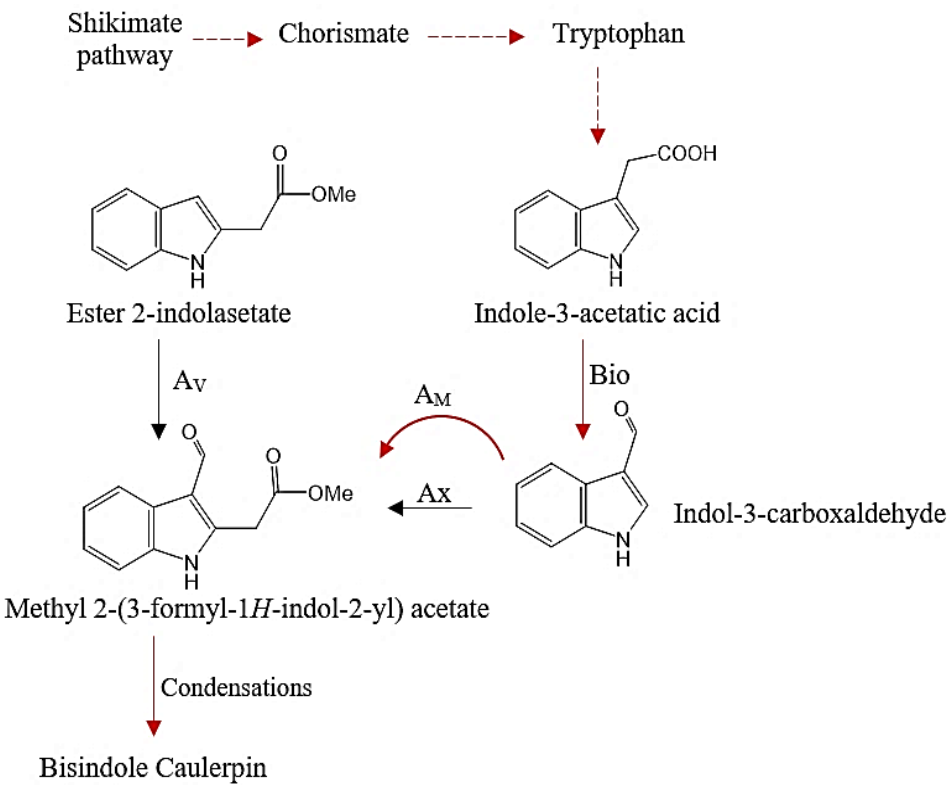


Figure 2. Formation and biosynthetic significance of Caulerpin; biogenesis (red arrow), synthesis (black arrow). Bio, biotransformation; Ax, radical substitution; A_M, Michael addition; A_V, Vilsmeier addition

V. CYTOTOXIC ACTIVITY OF CAULERPIN

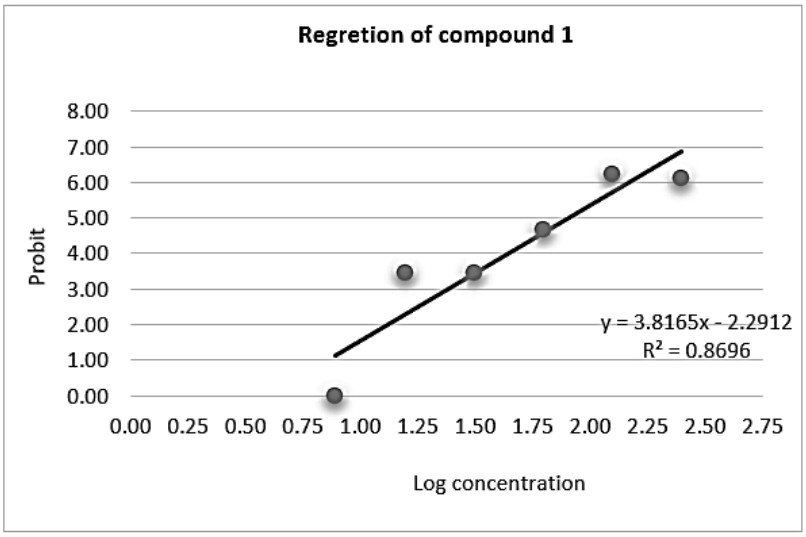
Caulerpin has been reported as anti-nociceptive and inflammatory activity (De Souza, et al, 209), protein-tyrosine phosphatase 1B (*PTP1B) inhibitory activity* with IC_{50} values 5.86 µM (Yang, H. et al, 2014), active to several human cancers cell line; breast cancer SK-BR-3, lung cancer A549, colon cancer HT29, cervical cancer HeLa, leukemia K562 and liver cancer Huh7

with IC_{50} cytotoxicity values of 3.71, 4.20, 4.04, 1.95, 4.67 and 0.72 µM respectively (Li, H. et al, 2018), anti-proliferation to cancer cell HCT-116 and HT-29 (Yu, H et al 2017), have potential as antiviral against virus HSV-1, CHIKV line cell, and active to *bovine viral diarrhea virus* with EC_{50} 2.0 µM (Macedo, N.R.P.V. et al, 2012; Pinto, A. M. V. et al, 2012; Esteves, P. O. et al, 2019), have potential as anti-diabetic with inhibited protein hPTP1B with IC_{50} 3.77 µM (Mao, S. C., Guo, Y.-W., & Shen, X., 2006), inhibited the growth *Mycobacterium tuberculosis* cell strain H37Rv with IC_{50} 0.24 µM (Canche Chay, C.I et al, 2014).

In this study, caulerpin is active against *A. salina* Leach. in the BSLT assay, with LC_{50} value 81.28 µg/mL. This criterion is in agreement with the American National Cancer Institute, which fixed the limitation of IC_{50} must be lower than 30 µg/mL. The compound 1 as moderately toxic it have correlation as anticancer. The data calculations are shown in **Table 1** and **Fig. 3**.

BSLT Data % death of *A. Salina* Leach. versus concentration of compound **1**

Con. (µg/mL)	log con.	% death		% death corrected	Probit value	LC_{50} (µg/mL)
		Compound 1	Control			
3.69	0.57	0.00	0.33	0	0.00	
7.81	0.89	5.56	5.88	0	0.00	
15.63	1.19	5.56	0.00	6	3.45	
31.25	1.49	6.25	0.00	6	3.45	**81.28**
62.50	1.80	47.06	10.53	37	4.67	
125.00	2.10	100.00	11.11	89	6.23	
250.00	2.40	100.00	13.33	87	6.13	



Subtitut Y = 5
5= 3,8165x - 2,2912
X = 1,91
LC50 = inv log 1,91
81.28

Figure 3. Graph of probity %lethality versus log10 concentration for chronic (24 hours) toxicities of compound **1**

VI. CONCLUSION

The biosynthesis significance of bisindole alkaloid caulerpin are suggested from methyl 2-(3-formyl-1H-indol-2-yl) acetate via of indol-3-carboxaldehyde, in vivo was desirable. In the recent study, Caulerpin are active in the brine shrimp Lethality Test (BSLT) assay with LC_{50} value 81.28 µg/mL.

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