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Bromopyrrole-Imidazole Alkaloids (oroidin), and its Conversion Molecule (Dihydrooroidin) Isolated from a Marine Sponge (*Agelas nemoecinata*)

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Abstract: ***Oroidin (1) is a bromopyrrole-imidazole alkaloid widely distributed in marine sponges of the order Agelasida. The compound exhibits potent cytotoxicity against several tumor cells. Oroidin serves as a prototype building block, giving rise to many bromopyrrole alkaloids. This study aims to re-analysis of the isolated bromopyrrole-related alkaloids derived from oroidin and propose the conversion mechanism. Marine sponge was collected by SCUBA diving at the depth of 15 meters. It was macerated in methanol before extracted by ethyl acetate to yield 2 grams. Crud extract was subsequently isolated by column chromatography method using to RP18 and Si-gel as adsorption material. Structure determination of the isolated compounds were re-analyzed by spectroscopic methods. By comparison the spectroscopic data both mass and ^{1}H-NMR with the literature, compound 1 and 2 was confirmed to be oroidin and dihydrooroidin, respectively.***

Keywords: *Agelas neomiecinata*, oroidin, bromopyrrole-alkaloid, marine, conversion

1. Introduction

Since the discovery of oroidin (**1**) from a marine sponge *Agelas oroides* in 1971, more than 150 bromopyrrole alkaloids have been isolated from various marine sponges. These types of molecules are exclusively synthesized via secondary metabolisms of marine sponges in various genera belonging mainly to the families Agelasidae, Axinellidae and Halichondridae [1]. The structural features of oroidin are constructed by a $C_{11}N_5$ skeleton, which contains pyrrole and imidazole ring systems attached at both ends of a linear chain. The structural diversity of this group of compounds is derived from oroidin through: (i) isomerization of double bonds and oxidation or reduction, (ii) dimerization, and (iii) cyclization [2], as some typical examples including dispacamide, 9-hydroxydihydrooroidin, and hymenidin [3]. Therefore, it has been postulated as a key precursor of bromopyrrole-imidazole alkaloids in marine sponges. Some of them exhibited several promising biological activities and play the important ecological roles particularly chemical defense against predators [4]. In this article, the isolation of oroidin and its conversion molecule dihydrooroidin from a marine sponge, *Agelas nemoecinata* was revised.

2. Methodology

2.1 General experiment procedure

NMR spectra obtained on 500 MHz proton NMR spectrometer (Avance NEO, Bruker, Bremen, Germany), TMS was used as internal standard. LCMS measurement was carried out by Revident LC/Q-TOF (Agilent Technologies, Santa Clara, USA).

2.2 Animal material:

Marine sponges were by SCUBA diving at the depth of 10 meters. The samples were kept in sealed plastic bags then preserved in a freezer at a temperature of -20 °C. The sponge was identified by following to the sponge guide manual namely systerma porifera [5]. Its skeletons are made up of fibers cored by a verticilliate megascleres. These sponges have large spongin fibers with unique style of spicules. They are four growth forms including ramose, lamellate, tubular, and massive growth form. Internal textures are extremely tough. Normal color of these sponges are orange or red.

2.3 Extraction isolation

Sponge sample (wet weight 700 g) was minced and macerated in methanol for 72 hours. After concentrated, it was extract by ethyl acetate to yield 3 g. Crude extract was subjected to RP-18 column eluted by the mixture of dichloromethane and methanol (2:8, v/v) to give oroidin (3 mg). A dragentdroff-positive fraction was separated using a silica gel column, with a mobile phase composed of dichloromethane and methanol (1:4, v/v). Finally, dihydrooroidin was purified by semi-preparative HPLC (UltimateTM 3000, Thermo Scientific™, Massachusetts, USA) eluting with 20% methanol in water, yielding 3 mg.

Oroidin (1) was isolated as a slight yellow**.** ESIMS *m/z* 386, 388 and 390 (1:2:1) $[M+H]^+$, λ_{max} 276 nm, ^{1}H-NMR (DMSO-d_6,500 MHz) δ 10.43 (NH, br t, J = 5.6, 5.5, H-1), 7.06 (H, s, H-4), 8.53 (NH, br t, J = 5.0, 10.1, H-7), 3.93 (2H, br t, 5.0,11.3, H-8), 6.12 (H, td, J = 10.0, 15.7, 5.0, H-9), 6.21 (H, br d, J = 16.3, H-10), 12.70 (NH, br s, H-12), 6.81 (H, s, H-15), 7.60 (NH_2, br s)

Dihydrooroidin (**2**) was obtained as a colorless oil. ESIMS *m/z* 388, 390, and 392 (1:2:1) $[M+H]^+$, λ_{max} 210 nm, ^{1}H-NMR (DMSO-d_6,500 MHz) δ 12.70 (NH, br s, H-1), 8.10 (1H, br s, H-4), 8.21 (NH, br s, H-7), 3.55 (2H, br t, J = 2.5, 13.2, H-8), 2.22 (2H, td, 6.3, 13.3, H-9), 2.0 (2H, m, H-10), 4.10 (2H, m, H-11), 8.21 (NH, br s, H-12), 5.20 (1H, br s, H-15), 7.90 (NH, br s).

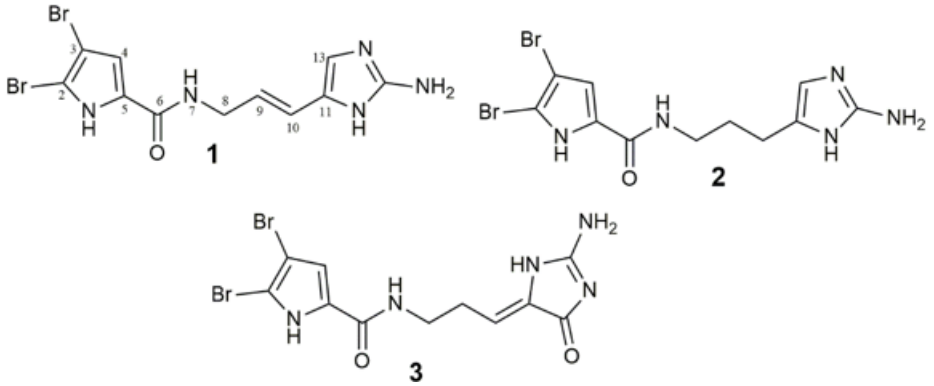

Figure 1 Molecular structure of compounds isolated from *Agelas* sponge

3. Results and discussion

Oroidin was isolated as a slight yellow oil containing two bromine substituents in the molecule as shown by the quasimolecular ion clusters at *m/z* 386, 388 and 390 $[M+H]^+$. Its ^{1}H-NMR showed four sp^2 signals in the molecule, which contained the typical singlet protons in a pyrrole ring and an imidazole ring at δ_H 7.06 and 6.81, respectively. Moreover, the allylic spin system at δ_H 3.39 (H-8, br t, 5.0, 11.3), δ_H 6.12 (H-9, dd d, 10.0, 15.7, 5.0) and 6.21 (H-10, br d, 16.3) was also defined. Typical $^2J_{NH}$ triplet with coupling constant of 5.5 Hz at δ_H 8.53 to the methylene protons at δ_H 3.39 ppm was confirmed by HMBC correlations. Its HMBC correlation was used to establish the connectivity of the pyrrole ring, imidazole moiety, and allylic system in order to complete the structure. The pyrrole ring was connected with the side chain through the correlation of the amine carbonyl carbon at C-6 with the proton at δ_H 7.06 (H-4) which also correlated with C-3 (δ_C 104.4) and C-5 (δ_C 128.1). The C-H long range correlation of the methylene proton at δ_H 3.93 (H-8) with C-6 (δ_C 158.6) also strongly supported this assignment and its attachment to the amide moiety. An imidazole ring was directly attached to the allylic system at C-11 (δ_C 124.1) by using the correlation of the singlet at δ_H 6.81 ppm (H-15) to sp^2 carbon at δ_C 117.1 ppm (C-10).

Dihydrooroidin was obtained as a colorless oil with UV absorbance at λ_{max} 210 nm. The positive ESIMS data showed a pseudomolecular ion pattern at *m/z* 388, 390 and 392, which indicated the presence of two bromines in the molecule and was two mass units higher than the molecular weight of compound oroidin. Its proton NMR data was comparable to that of **1,** but it has a NH-$CH_2CH_2CH_2$ instead of a NH-CH_2CH=CH spin system. This difference in the spin system was revealed by comparison of the chemical shifts at δ_H 6.12 (H-8), 6.21 (H-9) for **1** and at δ_H 3.55 (H-8), 2.22 (H-10) for **2**, respectively. The olefinic peak at δ_H 6.12 and 6.21 in oroidin disappeared in dihydrooroidin. Compound **2** has also the 2,3-dibromo-1*H*pyrrole-2-caboxylic acid amide as substructure which is the same as found in compound **1** as indicated by the pyrrole singlet for H-4 (δ_H 7.06). The imidazole proton at H-15 (δ_H 6.81) was also observed. The NH-$CH_2CH_2CH_2$ spin system was established through its HMBC and proton multiplicity pattern. The proton at δ_H 3.55 ppm (H-8) was directly attached to a hetero atom which exhibited the NH coupling constant of 2.5 Hz with the methylene proton at C-8. The correlation of the methylene proton at δ_H 2.22 (H_2-9) with C-11 (δ_C 83.5) while the imidazole methine singlet at δ_H 6.81 (H-15) correlated with C-10 (δ_C 39.9). This information suggested that the imidazole ring was directly connected to the chain NH$CH_2CH_2CH_2$ at C-11 which was similar to **1**. Compound **2** was transformed from **1** which could be explained by the electron transfer between the nucleophilic C-9 and electrophilic C-10 of **1** that afforded the intermediate skeleton **1.1** which could be transformed to compound **2** as shown in figure 2**.** The intermediate **1.1** is closely related to dispacamide (**3**), a compound isolated from the Carribean *Agelas* sponge [6].

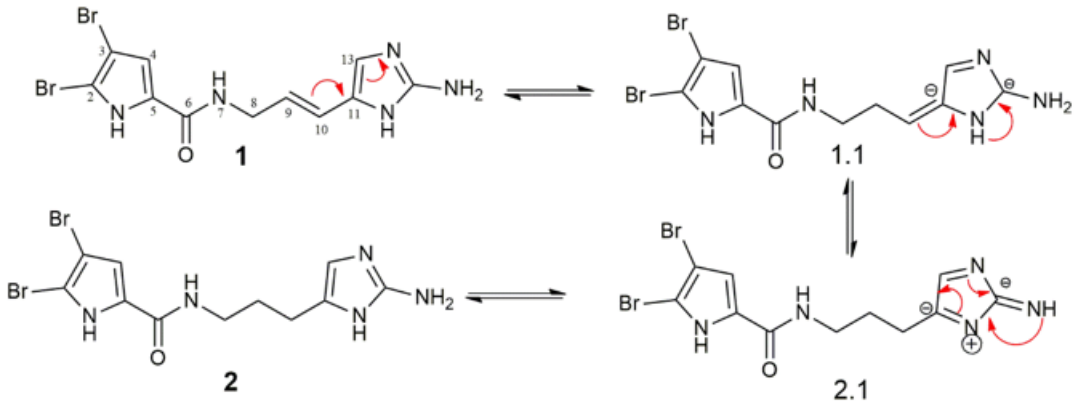

Figure 2: The proposed conversion of oroidin to dihydrooriodin

4. Conclusion

By re-analysis spectroscopic data, it is ensured that oroidin is a key precursor of pyrrole-imidazole compounds synthesized by Agelas spp. Dihydrooroidin is a derivative compound that transforms from the oroidin molecule through electron transfer and rearrangement of the double bond in the imidazole ring. This This exemplifies the diversity of pyrrole-imidazole molecules in marine sponges through isomerization of double bonds and oxidation or reduction processes.

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