How can we develop marine natural products chemistry in Indonesia?

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Abstract. In this article, a brief overview of marine natural products (MNPs) is given along with some useful references. The minimum required instruments and information for conducting the related research are reviewed, and the benefits of searching for MNPs in Indonesia are discussed. Several MNPs that are known from Indonesia are then mentioned. In addition to knowing the existing resources, we should place emphasis on the discovery by Indonesian researchers of new molecules having a chemical or biological interest. Finally, some additional information related to natural products is presented.

1. Introduction

Natural products chemistry is probably the oldest field in organic chemistry. Due to the lack of access to most marine organisms, marine natural products chemistry did not start developing until the 1950s [1]. This presentation introduces reviews on this field, representative MNPs applied to medical and life science studies, the instruments and information needed to conduct the research, the utilization of Indonesian marine resources, and several other topics related to MNPs.

The discovery of penicillin in 1928 by Fleming in the UK stimulated the search for antibiotics or anticancer agents in a large number of fungi and soil microbes. Work on marine metabolites started in the 1960s, led by pioneer researchers including the late Prof. P. J. Scheuer of the University of Hawai‘i, deservedly called the father of marine natural products. Among reasons to focus on marine organisms are: 1) the huge number of species (~500,000) in the marine environment; 2) the evolution of marine organisms under different conditions from terrestrial, such as aquatic media, salinity and high pressure; 3) different biosynthetic routes from terrestrial life; 4) various special ecological relationships such as predation, defense, symbiosis, parasitism, and so on. In consequence more than 29,000 molecules have been reported so far from marine sources [2].

To stay up-to-date on MNPs reported so far, it is recommended to refer to the special issue Marine Natural Products published annually in the review journal Natural Product Reports by the Royal Society of Chemistry [2]. The review was started in 1984 by the late Prof. D. J. Faulkner, another pioneer, and other mainly New Zealand researchers after him, and has been open access since 2014. Another resource that focuses on Indonesian MNPs is a review titled Marine Natural Products from Indonesian Waters by Dr. N. Hanif of IPB (Bogor Agricultural University), in the open-access journal Marine Drugs [3]. In addition to the review, he organized a summer school, the International Summer Course on Marine Natural Products, held early August 2019 at IPB and Pulau Seribu with the help of three international lecturers. As he hopes to have another one next year if a budget is provided, more Indonesian graduate students will have a chance to join.
2. Representative molecules discovered/developed so far

Figure 1 shows the molecular structures of representative USFDA approved drugs based on MNPs [4]. Among the molecules, both the powerful analgesic Ziconotide and the anticancer agent Trabectedin have exactly the same structures as discovered. Two nucleoside analogues Vidarabine and Cytarabine are the first approved drugs based on the marine metabolites spongothymidine and spongouridine, retaining arabinose instead of ribose. Halaven is used for the treatment of relapsed breast cancer, and the molecule is based on the anti-tumor molecule halichondrin B. In spite of the complicated structure, Halaven is supplied by total synthesis. Brentuximab-vedotin belongs to antibody-drug conjugate (ADC) and its peptidic moiety is a derivative of a cytotoxicin dolastatin 10.

![Figure 1. Approved drugs based on MNPs.](image)

In Figure 2, four molecules under clinical trials are shown [4]. Aplidin has exactly the same molecular structures as found from tunicates, while Lurbinectedin is an analog of Trabectedin. Plinabulin and Salinosporamide A were originally discovered from a marine fungus and also from actinomycete, respectively.

![Figure 2. Drug candidates under clinical trial.](image)

In addition to drugs developed for medicines, molecules shown in Figure 3 with specific biological activity are used as reagents for life science studies. Among the molecules, okadaic acid and calyculin A are known as potent and selective phosphatase inhibitors, latrunculin A and jaspamide (jasplakinolide)
are actin-targeting drugs with different activities, kainic acid is an agonist for kainate-type glutamate receptor, pseudopterosin A is an anti-inflammatory agent, and tetrodotoxin (TTX) is a voltage-gated sodium channel (Na,) inhibitor. Pufferfish poisoning caused by TTX has been long known, with evidence even found at an archaeological site near Tokyo. As well as structure determination of the toxic principle in 1964, TTX has been utilized for investigations on the mechanisms of Na,.

![Chemical structures](image)

**Figure 3.** Several MNPs for life science studies.

3. How do you carry out MNPs studies in Indonesia?

To conduct MNPs studies, at least the following equipments are required in a lab: evaporators with water chiller and diaphragm pump, high-performance liquid chromatography (HPLC) in addition to thin-layer chromatography (TLC) and column chromatography (CC), a vacuum manifold to dry material and also to conduct some reactions, freezers to keep extracted molecules, nitrogen gas to remove small amount of solvents, fume hood for safety, and a few other items.

More expensive instruments such as nuclear magnetic resonance spectrometer (NMR), mass spectrometer (MS), X-ray crystallography machine, and electric circular dichroism spectrometer (ECD) are to be installed in a common, centralized analytical instrument center, where any researcher can access the required instrument. NMR is the most powerful equipment to characterize molecular structures, however in Indonesia it is currently installed only at LIPI and a few universities in Jawa Island. The number of machines appears too few considering the number of researchers in organic chemistry and related fields. To overcome this bottleneck without having your own NMR, a good approach is to send younger researchers to an instrument center frequently to be trained in running NMR. MS is also an indispensable machine to characterize molecules not only by knowing their molecular weight and formula, but to determine new, or known, compounds by using MS/MS with global natural product social (GNPS) molecular networking [5]. X-ray crystallography is a very useful instrument if the molecule gives a single (transparent) crystal. It used to be a specialist work, however, thanks to instrument and software developments, you can now analyze 3D structures after overnight measurement. If the molecule is stable, you can obtain the structure quickly after sending tiny crystals to a collaborator. ECD is used for determination of absolute configuration if there is UV-absorbing chromophore. In addition to the measurement, you may need to ask a calculation chemist for calculated ECD spectra and obtain a conclusion after comparing the data.

In addition to this chemical equipment, it is necessary to install basic biological instruments such as clean bench, CO₂ incubator, microscope, autoclave, safety cabinet, microplate reader, centrifuge, electrophoresis, and so on, to find fractions or materials that are relevant to the biological target of interest. In the case of genetically modified microbes or other pathogenic resources which could become biohazards, you had better ask specialized labs for collaboration.
As new molecules are published every year, it will become more and more difficult to discover new molecules. Therefore, a dereplication process to avoid wasting your time and resources by identifying known molecules is important. Database search with SciFinder, provided by Chemical Abstract Service can be used for any molecules, while Marinlit can be used for specific search within MNPs. The database Marinlit was originated by the pioneering New Zealand professors M. Munro and J. Blunt and is currently available through the Royal Society of Chemistry. In addition, GNPS molecular networking for learning whether the molecule is new or known is available to any researchers if you submit MS/MS data of your compounds [5].

Looking at labs in UNSYIAH for MNPs, it seems the conditions may not yet be adequate to work on MNPs from Indonesian waters, particularly for obtaining molecular information. With the current economic growth in Indonesia, it is expected that several expensive instruments such as NMR may be installed at UNSYIAH in the near future, but it will be a waste for younger researchers to simply wait. Therefore, research groups should prepare small budgets for sending young members to run instruments at other institutions.

Nowadays at natural product symposia held in industrial countries, it is rare to see structure determination of small molecules in an oral presentation. The reason may be that anyone with access to modern instruments can do the job, except for some stereochemical assignments, molecules with many heteroatoms or condensed ring structures, or with quite high molecular weight. Therefore, even if your lab is equipped with the above instruments, you will just be catching up with the conditions in other countries. What you have to do to stand out and advance, is discovery.

4. Utilization of Indonesian marine bioresources
The Coral Triangle (CT) is defined by Prof. J. E. N. Veron based on his expertise on stony corals [6]. The region comprises the Philippines, the eastern part of Indonesia, and Papua New Guinea. Outside of this focus, the number of species decreases in all directions. Since the CT is based on the concentration of stony corals and fishes, the distribution of the groups now targeted for MNP study, sponges and tunicates, might not overlap with the corals. Aceh is located in the far west of the CT, but you should not be disappointed. During 2000-2009, 50–100 and 25–50 MNPs were reported from eastern and western Indonesia, respectively [7].

It is recommended to use all the possible methods for collection of marine bioresources, from beach walking for algae and cyanobacteria living in intertidal zones, skin and scuba diving for marine organisms in shallow coral reefs, trimix rebreathers for the mesophotic coral ecosystems (deeper coral reefs), dredge or trawling for sessile organisms on sea bottoms, and even remotely operated vehicle (ROV) if possible. It is difficult for a small lab to do all of these, but you can supply various bioresources for discovery by establishing a network with other researchers in Indonesia and by sharing the collected material and study projects.

As the lecturer has some small experience on Indonesian marine organisms, several known molecules for life science reagents which can be supplied with the source organisms from Indonesian waters are shown in the lecture. They are: latrunculin B from Negombata sp., latrunculin A from Cacospongia mycofijiensis, swinholide A from Theonella swinhoei, jaspamide (jasplakinolide) from Jaspis sp., ilimaquinone from an unidentified sponge, xestospongine C from Neopetrosia exigua, and psammplanin A from an unidentified sponge.

In addition to the natural harvest, the culture of microorganisms may provide useful molecules. Ciguatera food poisoning (CFP) is caused by eating coral reef fishes, which incorporate toxic principles, ciguatoxins (CTXs), through the food chain. The original producers are a group of dinoflagellates of the genera Gambierdiscus or Fukuyoa. The structures of CTXs were revealed as P-CTX-1B and P-CTX-3C in the Pacific and C-CTX for the Caribbean as in figure 4, while I-CTX of the Indian Ocean remains to be solved. LCMS analysis of these toxins requires a supply of chemical standards, but these are difficult to obtain. In principle, cases of ciguatera poisoning in Aceh offer a chance to identify I-CTX as well as to produce the toxin standard in future. However, you would need very good skills in the purification of the potent toxic molecules, in addition to careful manipulation.
5. What other researchers do?
In this section, a few example molecules are discussed.

From an endemic sponge *Cortinium simplex* found only in the Flores and Kupang regions, a joint research group of Osaka University and UNILA reported a series of unique steroids named cortistatins as anti-angiogenesis molecules [8]. After the discovery, the molecules attracted synthesis and pharmacology researchers.

Manzamine A was originally discovered as an alkaloid from two Okinawan sponges [9]. One of the sponges, *Acanthostrongylophora ingens*, is widely distributed from Sabang to Kupang and Sorong in Indonesia, though the underwater appearance can be slightly different. Specimens collected near Manado were reported by UNSRAT and a Kumamoto University group [10] to contain new analogs. Although it is a very rare case, the true producer of manzamine A was identified by researchers at University of Mississippi and UGM as an autotrophic microbe *Micromonospora* sp. [11].

In addition to macroorganisms, there are a large number of publications on the metabolites of marine microorganisms such as fungi and bacteria. It is a well-known phenomenon that the microbial in axenic culture do not produce all the molecules they could otherwise do. These cryptic biosynthetic genes may be divided into silent or orphan genes, which researchers have tried to stimulate into activity in several ways. One method is co-culture, because in nature the microorganisms are surrounded by many microbes and could be expected to produce molecules in response to them. Some researchers simply mix the microbes, while other researchers separate two kinds of microbes by a dialysis membrane which allows small chemicals to pass through and activate.

The expanding interest in MNP research also raises issues of structural revision and competition. If you report a wrong structure, it will be revised by other researchers sooner or later. We also must think about the inadvertent competition with other research groups. Exactly the same molecule may be found by coincidence from other resources or from the same species at a different location. This increases the pressure to publish the discovery quickly.

6. Conclusion
Judging from the many Jamu vendors on the city streets, it is probably still common for Indonesian people to use traditional medicines based on herbs. There are many new opportunities to propel this tradition into the modern era based on scientific evidence. Indonesia clearly still has large undiscovered marine bioresources. Areas like islands off Sumatra and Cendrawasih Bay in West Papua are attractive destinations where we may encounter new bioresources easily. A base of minimum equipment needs to exist at MNP labs, while expensive instruments can be maintained by an instrument center of a university. Before installation of the machines, young researchers should visit other labs to obtain necessary data. By forming strong research networks, you can accumulate various macro- and microorganisms, work jointly with chemists and biologists, and discover exciting molecules to be
published in international journals. From the viewpoints to nurture new researchers for MNPs, it is recommended to learn not only organic chemistry and instrumental analyses, but all other basic chemistry subjects, molecular biology, biochemistry, statistics, etc. Therefore, general scientific training like STEM education is desirable.

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