Review

Exploratory research on bioactive natural products with a focus on biological phenomena

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Abstract: The discovery of new basic compounds holds the key for advancing material sciences. We have focused on the identification and characterization of natural key compounds that control biologically and physiologically intriguing phenomena. The discovery of new bioactive molecules, facilitated by a deeper understanding of nature, should advance our knowledge of biological processes and lead to new strategies to treat disease. The structure and function of natural compounds are sometimes unexpectedly original. Based on our past experience and results, we have carried out research to find new directions for compound exploration by directly learning from dynamic biological phenomena in the field, and have succeeded in creating a new research field in biological molecular sciences.

Keywords: bioactive natural products, palytoxin, halichondrins, shrew, platypus, spider wasp

1. Introduction

Various natural products with extraordinary chemical structures and significant biological activities have been isolated and characterized from both marine and terrestrial creatures. The discovery of a new chemical substance frequently triggers a breakthrough in basic science. Moreover, these bioactive compounds often become candidates for drugs or biological probes for physiological studies. From the standpoint of natural product chemistry, we have focused on the development of a new paradigm for searching for physiologically and biologically intriguing compounds, and have sought to acquire a deeper understanding of natural product-related biological phenomena. To overcome difficulties in the isolation and characterization of novel biologically attractive natural products with unique structures, careful observation in dynamic ecological systems has been

combined with new chemical approaches together with methods in organic chemistry and spectral analysis. This report presents the attractive structures and physiological activities of major natural products which we have isolated and characterized.

2. Palytoxin, a deadly poison

The three most deadly poisons in marine organisms are tetrodotoxin (pufferfish toxin), saxitoxin (paralytic shellfish poison) and ciguatoxin (ciguatera toxin). The structures of tetrodotoxin and saxitoxin have been elucidated. Ciguatera poisoning, which occurs infrequently in coral reef areas with a low rate of mortality, remains a social issue. Researchers worldwide have been eagerly tackling this issue. In Hawaii, the University of Hawaii took a leading role in conducting large-scale research in cooperation with biologists. In 1971, with the help of A. H. Banner, they looked for clues in the local legend of "limu-make-o-Hana", which means "deadly seaweed of Hana", and identified a specific area to reveal the legendary organism. The area was only known to the chief of a local tribe for the purpose of collecting arrow-poison, and researchers were told that locals did not go near the area, believing it to be cursed. It became clear that the responsible organism was a coelenterate, not seaweed.

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Fig. 1. Colony of *Palythoa tuberculosa* on a coral reef at Ishigaki Island.

P. J. Scheuer and R. E. Moore were the first to chemically study the toxin, and they reported palytoxin, a deadly toxin with a molecular weight of 3,300 without any repeated structure.^{1),2)} In Japan, Y. Hashimoto at the Department of Agriculture, the University of Tokyo, who was conducting research on ciguatera, learned of an oral tradition on Ishigaki Island that the intestine of the filefish *Aluterus scriptus* must not be given to pigs. He determined that the intestine was filled with *Palythoa tuberculosa* (Fig. 1), and found a toxin with a large molecular weight without any repeated structure. Our research was initiated in 1974 to elucidate the toxin's structure. Initially, *P. tuberculosa* was collected on the main island of Okinawa, but, due to unsuccessful results, *P. tuberculosa* was later collected on Ishigaki Island.

Starting the following year, 500-600 kg of P. tuberculosa was harvested every year, frozen and transported by air to Nagova. This laboriously harvested P. tuberculosa was subjected to extraction. and we succeeded in efficiently obtaining palytoxin. Palytoxin is amphipathic, which means that it is both water-soluble and hydrophobic. Therefore, we obtained good results by adsorbing palytoxin in solution to polystyrene resin, and then desorbed it based on ethanol concentration. Amazingly, we found that purified palytoxin was 50 times more toxic than tetrodotoxin. Since its molecular weight is approximately 3,000 and it is different from polysaccharides and proteins, it was very difficult to determine its structure. In 1978, ²⁵²Cf-plasma desorption mass spectrometry clarified that its molecular weight was 2,681 and it had eight double bonds.³⁾ Its molecular formula, C₁₂₉H₂₂₃N₃O₅₄, was astonishing, and drew considerable attention as the largest single-structure natural product. Since the carbon chain of palytoxin was unusually long and contained many hydroxy groups, its structure was determined by oxidative degradation based on partial ozone oxidation and restrictive periodate oxidation in a polystyrene resin column. The resulting crystallized fragment was subjected to X-ray crystal structure analysis, and other structures were analyzed by NMR. Its planar structure was clarified in $1981,^{(4),(5)}$ and its stereostructure was identified in $1982.^{6)-9)}$



palytoxin



Fig. 2. 3D-views of palytoxin and N-acetypalytoxin.



Fig. 3. The black sponge *Halichondria okadai* in a tide pool at Aburatsubo (Kanagawa Pref.).

A high-field Fourier transform 600 MHz NMR spectrometer at Carnegie Mellon University in the U.S. was used to determine the overall planar structure from each degraded product. The spectrometer was not commercially available at the time. We successfully determined the stereochemistry by not only performing a detailed NMR spectral analysis of the degraded compounds, but also by combining organic synthesis chemistry techniques established by Y. Kishi at Harvard University. Metabolites that were unique to marine organisms and made of long carbon chains, such as palytoxin, were called "super-carbonchain compounds",¹⁰⁾ and triggered a period of research and development. In 1984, with I. Muramatsu and his colleagues, we found that palytoxin induced potent coronary artery contraction and peripheral vessel contraction by facilitating the absorption of sodium ions by neurons, and that it was not antagonistic to tetrodotoxin, a sodium channel inhibitor, which suggested the existence of a new sodium channel.^{11),12)} An electrophysiological study of palytoxin

showed that Na⁺, K⁺-ATPase was the target molecule, and it was proposed that there was a channel structure inside the ion pump. Subsequently, it has been generally accepted that a channel structure exists in ATPase.

In 2003, D. C. Gadsby reported the channel structure inside Na⁺, K⁺-ATPase in detail,¹³⁾ and K. Itoh's group and I reached the same conclusion later that year.¹⁴) Many researchers participated in this research, which markedly increased the demand for palytoxin as a biochemical reagent. However, the true form of palytoxin posed a new challenge. The question was whether compounds with a long carbon chain and many hydroxy groups have defined or random conformations. We envisaged that the molecular shape of palytoxin would facilitate our understanding of its interaction with ATPase. However, conventional techniques used for conformational analysis in natural product chemistry, such as X-ray crystallographic analysis and NMR, were not applicable, due to the non-crystalline nature of palytoxin and the heavy overlapping of NMR signals. Thus, in collaboration with a group at RIKEN, we examined whether a technique used in protein science was suitable for our purpose. This was indeed a challenge because even the biggest natural product is much smaller than proteins, and to our knowledge this had never been tried before. Synchrotron radiation X-ray small angle scattering (SAXS) was used at the SPring-8 synchrotron radiation machine.¹⁵ Briefly, in aqueous solution, palytoxin has a horseshoe-like shape and exists as a dimer (Fig. 2). Interestingly, N-acetyl palytoxin exists as a monomer with a similar horseshoe-like shape, in which the amino group at the end of the molecule is acetylated, and its toxicity is less than 1/100 that of palytoxin. A further analysis of SAXS data required optimization by a modeling program that was originally developed for proteins. Additional information was obtained from our subsequent NMR study.¹⁶ While more studies are needed, we believe that other super-carbon-chain compounds also do not exist in random conformations.

3. Halichondrins, antitumor compounds

Halichondria okadai Kadota (Fig. 3) is a sponge that is abundant in the tidal zone in the Pacific Ocean south of the Bosoh Peninsula in Japan. Sponges take seawater into their bodies, gather food by filtration, and coexist with symbiotic microorganisms, which account for about 40% of the sponge's

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norhalichondrin A: R1=R2= OH, R3= H norhalichondrin B: R1=R2=R3= H norhalichondrin C: R1=R3= H. R2= OH p-bromophenacyl derivative: R1=R2= OH, R3= CH2COC6H4Br



halichondrin B: R= H halichondrin C: R= OH



volume.¹⁷⁾ Thus, sponges might be aggregates of marine microorganisms. Since sponges have existed for about five hundred million years without being able to physically move, they must possess some hidden enduring capability. After the oil crisis in the 1970's, it was found that Gorgonians contain large quantities of prostaglandins, and this increased the level of interest in natural marine resources. This was an example of a shift from mass chemicals to fine chemicals. Under these circumstances, the level of interest in marine organisms, and particularly sponges, increased, and research on antitumor compounds in *H. okadai* Kadota was started. Inhibitory effects were closely examined based on the proliferation of B16 melanoma cells in vivo, and eight halichondrin homologs were identified.¹⁸⁾

Based on the genus name of this particular sponge, the antitumor substances were named halichondrins. However, from 600 kg of the sponge, less than 15 mg of the most active homolog, halichondrin B, was obtained. Fortunately, the experience gained by determining the structure of palytoxin was useful, and we concluded that its structure could not be determined based solely on detailed NMR data. In particular, halichondrin B was very fragile and was obtained in small quantities, which made our task very difficult. Thus, norhalichondrin A, which was present in greater quantities, was purified in the form of a *p*-bromophenacyl ester, and a high-quality

crystal was obtained. X-ray crystal structure analyses were performed, and extremely complex and specific structures were seen.¹⁹⁾ The 2,6,9-trioxatricy $clo[3.3.2.0^{3,7}]$ decane ring system inside the molecule was the most complex. Both ends of the molecule were diverse: at one end, the number and length of the carbon chains varied, and at the other end the number and oxidation state of the hydroxy groups varied.

We suspected that there are a total of nine types of change, but we have not identified all of these changes in halichondrin A mostly due to issues with quantity. The antitumor activities of halichondrin B have been clarified by in vivo experiments, and it appears to be a promising compound. However, toxicity has always been an issue. In a Japan-U.S. science seminar on cancer research held in Hawaii in 1987, B. A. Chabner at NCI and G. R. Pettit at Arizona State University were very excited by our research. In fact, Pettit and M. Munro at Canterbury University in New Zealand had obtained halichondrin B from different sponges. However, they had not been able to determine its structure.

When the framework of a complicated natural product has been elucidated, whole of the structure can be determined using various analytical techniques. Therefore, it is extremely important to elucidate the skeleton, and being the first to ascertain a skeleton is a joy that only researchers can appreciate.



Fig. 4. Proposed biogenesis of pinnatoxin A.

In 1992, Y. Kishi, a leading researcher in the total synthesis of complex natural products, who had already successfully synthesized palytoxin carboxylate, synthesized halichondrin B.²⁰ Based on these findings, the antitumor activities of the intermediates were tested at Eisai's U.S. laboratory, and the results clarified that the right half of the molecule was active.²¹⁾ While these results were expected, they were very encouraging because this meant that such compounds could be produced at total synthesis laboratories. Subsequently, many researchers studied the molecule and fine-tuned its structure to develop eribulin mesylate.²²) With the help of process chemistry, which is at the center of modern organic synthesis chemistry, they succeeded in mass production, and there are high expectations for its application in breast cancer therapy.²³⁾

It is not easy to supply halichondrin B from living organisms. Munro's group has planned and implemented sponge cultivation and has conducted studies, but the process has not always gone smoothly. As mentioned above, sponges coexist with many microorganisms and filter food from their environment. We do not know which organism actually produces halichondrins, although many laboratories have isolated and cultured microorganisms from inside sponges. However, less than 1% of the microorganisms can be cultured,²⁴⁾ and the vast majority of microorganisms in sponges cannot be cultivated. Subsequently, with the use of a metagenome technique, gene groups were viewed as compound sources, and fosmid libraries were prepared. In total, about 150,000 libraries were made, and research is currently focused on compound production and genomic information.²⁵⁾ In the near future, we believe that a new biosynthesis system will be elucidated. In any case, we hope that eribulin mesulate will soon be commercially available.

4. Pinnatoxins, food poisoning-causing shellfish toxins

There have been reports of poisoning due to fanmussels [Atrina (Pinna) pectinata Linnaeus] along the coast of the Ariake Sea in Japan, and in China people are told not to eat the midgut gland of bicolor pen shells. They have a well-developed adductor muscle and make good sushi cuisine in Japan. We isolated fan-mussel pinnatoxins and identified as a Ca^{2+} channel activator. Since it was difficult to obtain a large amount of fan-mussels, which are expensive, we decided to collect the toxin from *Pinna* muricata Linnaeus, a type of fan-mussel that Okinawan people never eat due to the risk of poisoning. P. muricata lives in the sand among seaweeds. We isolated pinnatoxins A–D and determined their structures as Ca²⁺ channel activators from the bivalve P. muricata. Pinnatoxin is a water-soluble amphoteric ion compound with iminium and carboxylate within the molecule.^{26)–29)}

Its structure suggests that it is a carbocycle, but if we consider the Diels-Alder reaction for cyclohexene synthesis, we can hypothesize that the formation of a 6,7-spirocycle is based on the Diels-Alder reaction and Schiff base formation (Fig. 4). Based on this biogenesis, Y. Kishi first synthesized an enantiomer of pinnatoxin, followed by true pinnatoxin.³⁰ This specific Diels-Alder reaction can be viewed as a retro Diels-Alder reaction³¹ within charge-remote fragmentation in the mode of mass spectrum segmentation. Pinnatoxins are highly toxic and are about one-eighth as active as tetrodotoxin.

In this research, a methodology for determining the structures of compounds with the use of only very small quantities could be established. By using this methodology, we attempted to study other toxins. We next focused on Okinawan *Pteria pen*- Exploration of bioactive natural products



guin. Although this shellfish has a large scallop, the local people never eat it, and the toxin is strong enough to turn away larger predators such as moray eel. We found that the toxins were pteriatoxins A, B and $C^{(32)-34}$ The structures of pteriatoxins B and C were determined even though only nanomole quantities were available. Recently, all eight possible stereo-isomers of pteriatoxins were synthesized, and their stereochemistries have been established.

5. Compound exploration focusing on ecosystem dynamism

The discovery of new biologically active compounds often triggers the development of basic scientific concepts in the field of biological sciences, since these compounds have direct physiological and behavioral effects on other living organisms. The discovery of new bioactive molecules, facilitated by a deeper understanding of nature, will advance our knowledge of biological processes and give us new leads to treat disease. To discover new compounds that impact the life sciences based on compound exploration in the ecosystem, we focused our attention on intriguing marine phenomena and paralytic neurotoxins from land animals.

5.1. Compounds related to coral communities. Coral reefs are rich resources as primary producers in tropical and subtropical areas. However, coral is being destroyed by many different external factors, including overgrowth by organisms that cover coral and feeding by coral predators. From the black sponge, *Terpios hoshinota*, which covers corals along Okinawan coasts, we isolated nakiterpiosin, a substance that kills coral cells.^{35),36)} Notably, nakiterpiosin and its analog have a unique highly oxidized ring that corresponds to the steroid A-ring, and contain bromine and chlorine atoms. While its steroidal skeleton, which consists of C-nor and D-homo, is found on land, this is the first example to be found in the marine environment. The first proposed structure was corrected using synthetic chemistry techniques devised by other researchers.³⁷⁾

The sea star Acanthaster planci (crown-ofthorns starfish) is a well-known coral predator. We have been interested in determining what attracts A. planci to coral. The fishermen of Okinawa found that A. *planci* gather around the internal organs of a sea urchin, Toxopneustidae. We focused on this phenomenon and found that a battalion of A. planci gathers to feed on the organs. From extracts of Toxopneustidae internal organs, arachidonic acid and α -linolenic acid have been identified as feeding attractants, and a study was conducted to harvest the creature in the sea.³⁸⁾ Since considerable effort was required to capture Drupella cornus, which is a relatively small snail that is also a predator of coral, traps and monitoring devices were required. Montiporic acids were found to be attractants of the coral Montipora sp. and field experiments were successful.³⁹⁾ These compounds function as information messengers for the survival of predators to attach to marine organisms, such as corals.

Corals engage in simultaneous mass spawning at around the time of the full moon. These gametes become planktonic larvae after fertilization. Microscopic larvae are widely dispersed and transported by currents. After days to months, they begin to swim toward the bottom and search for suitable substrates for settlement and metamorphosis. We have tried to identify what compounds enable coral larvae to settle and metamorphose into the adult form. Extensive studies on coral larva colonization and metamorphosis-inducers led to the isolation of 11deoxyfitularin-3, a metamorphosis-inducer of *Pseu*-



No. 3]

dosiderastrea tayamai larva originating from crustose coralline red algae (CCA), which is active at concentrations of 10^{-8} and 10^{-7} M. Interestingly, the metamorphosis-inducing activity of this compound was enhanced by the addition of CCA carotenoids, such as fucoxanthin (10^{-9} M) and fucoxanthinol (10⁻⁹ M). Additionally, β -carotene (10⁻⁹ M) and lycopene (10^{-9} M) were also effective.⁴⁰ Furthermore, in collaboration with Guam University, we investigated the chemical constituents of the CCA Hydrolithion sp. We found that several coral larvae, i.e., A. digitifera, A. nobilis, A. surculosa and L. purpurea, could be induced to settle and metamorphose by extracts of this CCA species. We recently isolated a novel macrolide, luminaolide, which induces colonization and metamorphosis in hermatypic coral larvae.⁴¹⁾ These results are extremely important and are directly linked to the conservation of coral reefs.

5.2. Compound exploration focusing on the actual producers of marine natural products. Compounds with similar skeletons are occasionally discovered in different marine organisms, e.g., halichlorine, a VCAM (vascular cell adhesion molecule)-1 synthesis inhibitor from H. okadai, and pinnaic acid, a cytosolic phospholipase A_2 inhibitor from $P. muricata.^{10}$ This suggests that a common producer exists and that such compounds are concentrated through food chains, perhaps via symbiotic dinoflagellates. Therefore, dinoflagellates that coexist with marine invertebrates were isolated and cultured to search for useful compounds. Some of the more remarkable molecules produced by dinoflagellates are "super-carbon-chain compounds," whose long carbon chains are polyoxygenated, which differ greatly from most metabolic products of terrestrial organisms. Symbiodinolide, a polyol macrolide with a molecular weight of 2,859, was isolated from a symbiotic dinoflagellate of a flatworm harvested in Okinawa.⁴²⁾ Symbiodinolide has a 62-membered mono-sulfated macrolactone moiety, a bis-epoxide





symbioimine

moiety, and 6.6-spiroacetal and hemiacetal rings. To date, we have confirmed the relative configurations of C26–C32, C44–C51, and C64–C66 as well as the absolute configurations of C69-73, C83-C103, and C3'-C18' in symbiodinolide by a detailed spectroscopic analysis and synthetic studies. Symbiodinolide was found to exhibit significant voltage-dependent N-type Ca²⁺ channel-opening activity. Interestingly, symbiodinolide caused flatworms, the host animals, to excrete symbiotic algae. Thus, it may serve as a defense substance to prevent digestion of their host animals. We also discovered symbioimines, new tricyclic iminiums,^{43),44)} and symbiospirols with two specific [4.4]-spiroketal rings⁴⁵ from the same strain of dinoflagellate. Since symbioimines suppress the differentiation of osteoclasts, they will serve as lead compounds for drugs to treat osteoporosis. Since symbiospirol A blocked the activation of protein kinase C induced by phosphatidylserine, it may be useful as an anti-inflammatory agent. Durinskiol A,¹⁰) a unique linear carbon-chain polyol compound with a molecular weight of 2,128, was isolated from the symbiotic dinoflagellate Durinskia sp., which was



Fig. 5. Blarina brevicauda eating a grasshopper.



Fig. 6. The platypus *Ornithorhynchus anatinus* (Taronga Zoo photo).

collected from an Okinawan nudibranch. An extensive 2D-NMR analysis allowed us to construct a C_{93} carbon-chain poly-oxygenated skeleton of durinskiol A, which included a 6,5,6-bis-spiroacetal ring, five six- or seven-membered rings, and two sugar moleties. Durinskiol A caused a short body length, abnormal pigment pattern, and pericardiac and volk-sac edema in zebrafish. Symbiotic relationships play critical roles in the marine ecosystem. Further chemical and biological studies on these molecules, especially huge polyol and polyether compounds, should contribute to a deeper understanding of their physiological roles in the marine ecosystem. We are currently very interested in the biological roles and limitations of super-carbon-chain molecules. Amazingly, we found a compound with a molecular weight of 8,150 in a dinoflagellate isolated from P. tuberculosa. 5.3. Paralytic molecules originating from land animals and function clarification. The toxic constituents produced by relatively lower animals have been well studied. Meanwhile, venomous mammals are extremely rare; only a few members of the order Insectivora (shrew and solenodon) and Monotremata (platypus) have been demonstrated to produce toxic venom. However, due to their instability as well as the difficulty of collecting fresh saliva and salivary gland specimens in sufficiently large amounts, their unique venoms have not been well investigated.

Despite its relatively small body length (75 to 105 mm), it has been reported that the extract from the salivary glands of only one *Blarina* shrew is capable of killing 200 mice. Accounts of humans bitten by Blarina describe a local burning sensation around the tooth puncture marks and subsequent swelling. In a recent study on salivary anesthetics in shrews, blarina toxin (BLTX), a lethal protein toxin with a molecular weight of 35 kDa, was isolated from the submandibular gland of *B. brevicauda*, which lives in North America (Fig. 5), and its primary amino acid sequence was determined.^{46),47)} This toxin was highly homologous to tissue kallikrein, and although it lowered blood pressure, it exhibited characteristic neurotoxin symptoms that are not seen with conventional kallikrein in mice, such as hindlimb paralysis, respiratory distress and violent convulsions, followed by death. Furthermore, the acute toxicity and proteolytic activity of BLTX were strongly inhibited by aprotinin, a potent kallikrein inhibitor, suggesting that its toxicity is due to the kallikrein-like activity of the venom. The kallikrein-kinin system is important in blood pressure homeostasis. Kinins relax vascular smooth muscle, increase vasodilation, and enhance vascular permeability. Thus, a type of kallikrein activity-linked vasodilatation, together with another undefined toxicity exerted by BLTX, may contribute to its lethality in pharmacological doses.

The duck-billed platypus Ornithorhynchus anatinus, a unique Australian Monotremata (egg-laying mammals) species, is one of the few living venomous mammals (Fig. 6). The adult male platypus carries a spur on each hind leg, and fighting males are known to use this device to inject their competitors with poison. Envenoming by a platypus causes immediate excruciating pain, which evolves toward a longlasting hyperalgesia (hypersensitivity to pain). Since this poison was shown to be toxic against rabbits at H-His-Asp-His-Pro-Asn-Pro-Arg-OH H-Asp-His-Pro-Asn-Pro-Arg-OH H-Leu-DLeu-His-Asp-His-Pro-Asn-OH H-Leu-DLeu-His-Asp-His-Pro-Asn-Pro-Arg-OH H-Gly-Asp-Lys-Glu-Pro-Lys-Gly-Asp-Arg-Pro-Arg-OH H-Lys-Gly-Asp-Lys-Glu-Pro-Lys-Gly-Asp-Arg-Pro-Arg-OH H-Gly-Gly-Gly-Lys-Lys-Gly-Asp-Lys-Glu-Pro-Lys-Gly-Asp-Arg-Pro-Arg-OH H-Ala-Asn-Gly-Gly-Gly-Lys-Lys-Gly-Asp-Lys-Glu-Pro-Lys-Gly-Asp-Arg-Pro-Arg-OH H-Gly-Asp-Lys-Glu-Pro-Lys-Gly-Asp-Arg-Pro-Arg-Leu-OH H-Ala-Asn-Gly-Gly-Gly-Gly-Lys-Lys-Gly-Asp-Lys-Glu-Pro-Lys-Gly-Asp-Arg-Pro-Arg-Leu-OH

Fig. 7. Amino acid sequences of platypus venom peptides.



Fig. 8. The bioactive heptapeptide contained in platypus venom fluid.



Fig. 9. The spider wasp Cyphononyx dorsalis.

the end of the 19th century, extracts of secreted venom or the poison gland have been investigated. However, the precise mechanism of action that leads to the excruciating pain caused by platypus venom in humans remains unclear. In collaboration with the University of New South Wales and the Taronga Zoo in Australia, we successfully collected fresh venom samples, and discovered for the first time that the toxin facilitated Ca^{2+} absorption by human neuroblasts and exhibited kallikrein-like protease activity. Several kallikrein-related proteases have been isolated as lethal factors from a shrew (BLTX) and lizards. Although platypus venom did not show lethality against mice, its proteolytic activity might synergistically contribute to toxicity through the specific cleavage of venom constituents. Furthermore, through the purification of venom components and MS/MS analysis, 11 new peptides corresponding to the pre-sequence and N-terminus of C-type natriuretic peptide (CNP) were discovered, and their stereostructures were determined (Fig. 7).⁴⁸⁾ CNP is a peptide hormone with vasodilating and diuretic actions, but the present study was the first to identify peptides corresponding to the pre-sequence and fragmented peptide of the N-terminus side. In addition, it has been shown that a heptapeptide (HDHPNPR)

(Fig. 8) facilitated Ca^{2+} absorption by human neuroblasts, induced inflammation when injected subcutaneously into mice and was cytotoxic to HeLa tumor cells. Since no mammalian Ca^{2+} channel agonists have been reported to date, we hope to develop drugs with novel neurotoxin actions and revolutionary anesthetics and analgesics.⁴⁹

With regard to research on paralytic molecules in wasps, we were interested in the oviposition behavior of the solitary spider wasp Cuphononux dorsalis (Fig. 9), which hunts brown huntsman spiders and lay eggs on it. It uses its stinger to paralyze its prey to feed its larva. We collected both wasps and spiders in the field. The venom reservoirs were manually squeezed, and venom droplets were collected from the stingers $(1-2 \ \mu L/specimen)$. When the crude venom fluid was injected into a spider, immediate paralysis was observed. These paralysis symptoms were reproducible, and the induced long-term paralysis lasted for up to 40 days. Venom components were then fractionated by bioassay-guided separation techniques, such as ultrafiltration, ion exchange, and two-dimensional electrophoresis. Two of the major proteins in an active fraction were analyzed by enzymatic digestion and protein sequencing. The results showed that arginine kinase-like proteins exhibited paralytic activities.⁵⁰⁾ Recombinant proteins, prepared according to the sequence, also induced paralysis in spiders with symptoms similar to those of the crude venom.

6. Conclusion

As mentioned above, the discovery of new basic compounds holds the key for advancing material sciences. We have focused on the identification of natural compounds that control biologically and physiologically intriguing phenomena. The discovery of new bioactive molecules, facilitated by a deeper understanding of nature, should advance our knowledge of biological processes and lead to new strategies to treat disease. Recent comprehensive research on material exploration has revised the importance of the element of surprise. The structure and function of natural compounds are sometimes unexpectedly original, and based on past experience and results, we have carried out research to find new directions for compound exploration by directly learning from dynamic biological phenomena in the field. As a consequence, we have succeeded in creating a new research field in biological molecular sciences.

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Profile

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