

ISOLATION AND IDENTIFICATION OF NOVEL ALLELOCHEMICALS AND UTILIZATION OF ALLELOPATHIC COVER PLANTS FOR SUSTAINABLE AGRICULTURE

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ABSTRACT

We are now operating a National Project, entitled "Screening of innovative allelochemicals and their utilization" from 2008 to 2012. We have screened about 4000 plants all around the world, mainly from Japan and Asian countries, and evaluated these by specific bioassays for allelopathy, including "Plant Box Method", "Sandwich Method", "Dish-pack Method", and "Rhizosphere Soil Method". Our final target is to isolate novel bioactive natural chemicals from allelochemicals. Strategies for screening new allelochemicals are based on "Total Activity Method", carefully calculating the concentration of allelochemicals in plants. We have already isolated cis-cinnamic acid derivatives as potent allelochemicals, and obtained a patent and now are performing organic synthesis of derivatives to increase activity. Mode of action of new allelochemicals are evaluated by DNA microarray technique using Arabidopsis as the receiver plant. Volatile allelochemicals are another target of our screen for allelochemicals. Final goal of this study is the utilization of allelochemicals as new agrochemicals. Another goal of our study is to use allelopathic plants as ground covers for agricultural and natural settings. Some promising domestic and alien plants with allelopathic activity were screened, and field tested to demonstrate their potential; utilization of these new ground cover plants will be discussed.

Key words: Allelopathy, allelochemicals, cover plants, DNA microarray, organic synthesis

INTRODUCTION

For the development of new herbicides and potential ground cover plants to suppress weeds, we have started a National Project,

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entitled "Screening of innovative allelochemicals and their utilization". We have developed specific bioassay methods for allelopathy, named "Plant Box Method", "Sandwich Method", "Dish-pack Method", and "Rhizosphere Soil Method". We have also developed a new strategy for the isolation of allelochemicals based on "Total Activity". By using these methods, trials for the isolation and identification of novel allelochemicals, and mode of action studies using DNA microarray will be performed for promising allelochemicals. Organic synthesis and organically synthesized derivatives were developed. Bioassay and field tests for the evaluation of chemicals and cover plants with allelopathic activity will also be performed.

RESULTS AND DISCUSSION

Research was directed to three sub-groups. Group A, National Institute for Agro-Environmental Sciences (NIAES), will select allelopathic plants and perform isolation and identification of allelochemicals. Up to now, potent allelopathic plants include *Cleome aculeate* and *Wendtia calycina* from Paraguai, *Trigonella foenum-graecum* from Egypt, and *Kelussia odoratissima* from Iran. We have isolated methyl isothiocyanate, (S)-(+)-carvone, *trans*-2-hexenal and other potent novel allelochemicals in action. We are also conducting transcriptome analysis for selected allelochemical candidates. We have also found from *Bletilla striata*, a Japanese ground cover, militarin and dactylorhin A as allelochemicals (Fig. 1). *Geranium carolinianum*, a noxious invasive weed from North America now in Japan, produces ethyl gallate as an allelopathic and fungi-static chemical. The other candidates we are now focusing are *Enterolobium contortisiliquum*, *Pachysandra terminalis*, *Goniothalamus andersonii*, *Albizia saman*, *Albizia guachapele*. As for mode of action of these chemicals, specific genes responsible for their activity were checked by database system such as APASD-II. We have already reported on specific genes that are up or down regulated by L-DOPA and allelochemicals from buckwheat.

Group B (University of Tokushima) is focusing on the organic synthesis of potent allelochemical candidates for novel herbicide or agrochemicals. Organic synthesis of sundifensifolide, a helianan type sesquiterpenoid, and brevion were established. Helibisabonol A and B were newly synthesized and absolute structure of these compounds were fixed (Fig. 2). Xanthatine, a xanthanolide terpenoide, was also synthesized. Bioactivity of these synthesized compounds were sent to Group A at NIAES, and some potent candidates were re-evaluated. In this study, total organic synthesis of helibisabonol A and B were reported for the first time and their absolute configurations were specified.

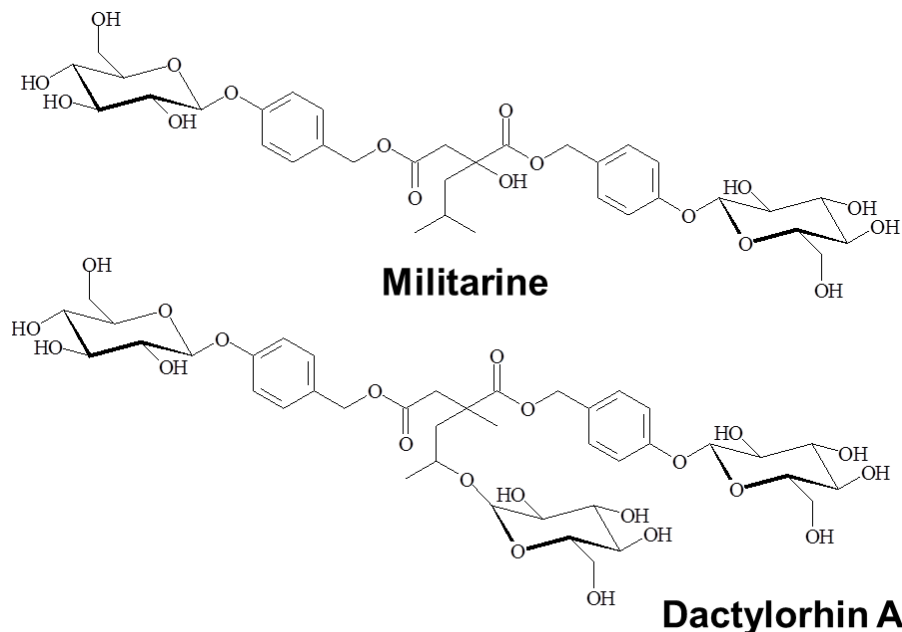


Figure 1. Militarine and Dactylorhin A from *Bletilla striata* Shi-ran).

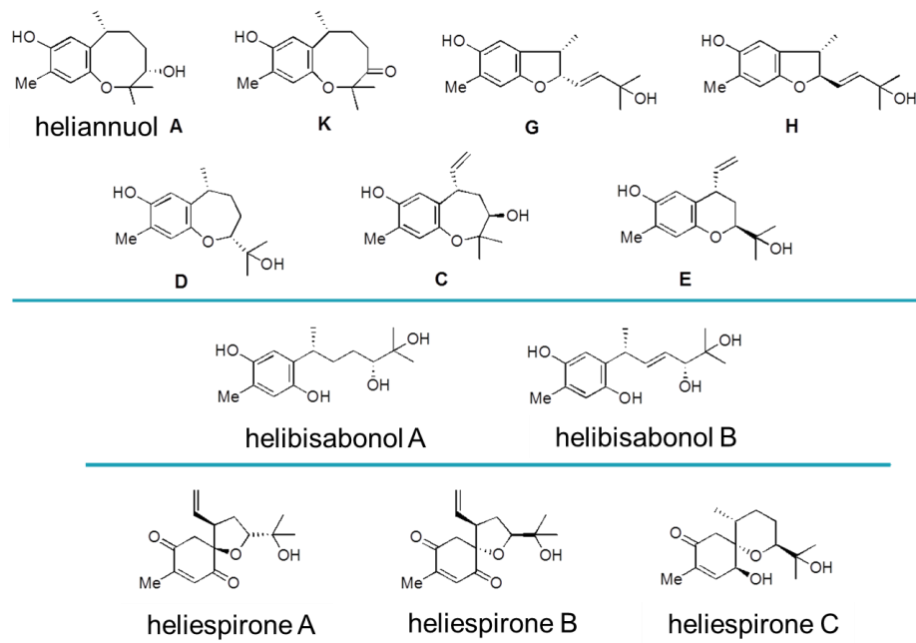


Figure 2. Organically synthesized allelochemicals.

Total organic syntheses of zanthatin and sudiversifolide, both zanthanolide type terpenoides, were also accomplished. Bioassay of these compounds was performed by Group A and promising leads were selected for further consideration. A library of more than 60 derivatives of helianane type sesquiterpenenoids was established. As for brevione A and B, other allelochemicals with antimicrobial activities were synthesized by enantio-regulated synthesis and coupling synthesis for hybrid compounds. Synthesized compounds were sent to NIAES and 37 of them with potent activity were set aside for further consideration as a precursor for herbicides. Total organic synthesis of ionone type terpenoids, 3-hydroxy- β -ionone and 3-oxo- α -ionol, identified at NIAES, was also started.

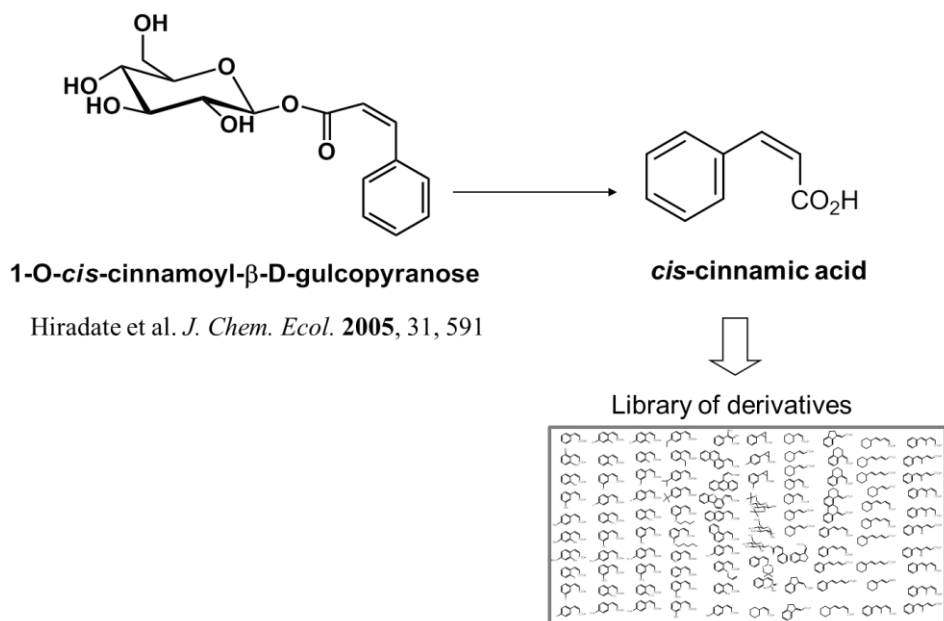


Figure 3. Organic synthesis of *cis*-cinnamic acid derivatives.

Group C (Kyusyu University, Research Institute for Innovative Chemicals), established total organic synthesis of xanthanoid type compounds in connection with Group B. Library construction of *cis*-cinnamic acid derivatives was started (Fig. 3). *Cis*-cinnamic acid is a novel allelochemical with potent activity, identified by Group A from a Japanese plant, yukiyonagi, as first reported. More than 180 derivatives of *cis*-cinnamic acid were synthesized and herbicidal activity for plants were tested by Group A. Some derivatives showed stronger activity than original nonsynthetic compounds; therefore, we have submitted for a Japanese Patent. We are now performing

structural activity studies, and studies of the mode of action of these cis-cinnamic acid derivatives are now on going.

With Group A, 21 allelochemicals were identified while 100 allelochemicals were synthesized by Group B and 180 derivatives of allelochemicals synthesized by group C are now undergoing evaluation as potential herbicides.

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