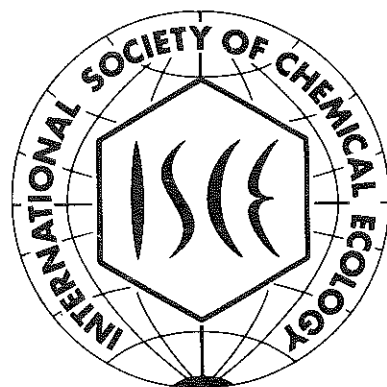


9th Annual Meeting  
of  
International Society of Chemical Ecology

第9回 国際化学生態学会議



July 6-9, 1992  
Kyoto, Japan



## PROGRAMME

### July 6th, Monday

- 15:00 - 17:00 Registration
- 17:30 - 19:30 Welcoming Reception

### July 7th, Tuesday

- 8:30 - Registration
- 9:00 - 9:45 Opening Plenary Lecture
- 10:00 - 12:30 **Session 1.** Indigenous Medicinal Plants
- 12:30 - 14:00 Poster Session begins (through July 9th)
- 14:00 - 17:30 **Session 2.** Allelopathy

### July 8th, Wednesday

- 9:00 - 12:00 **Session 3.** Chemical Ecology of Aqueous  
Biosphere
- 13:30 - 15:00 **Session 4.** Plenary Lectures on Natural Product  
Chemistry
- 15:00 - 17:00 Poster Discussions
- 18:00 - 20:30 Banquet at Holiday Hall, Holiday Inn Kyoto

### July 9th, Thursday

- 9:00 - 11:00 **Session 5.** Chemical Ecology of Fruit Flies
- 12:00 - 13:00 Poster Presentation closing
- 13:15 - 16:30 **Session 6.** Utilization of Natural Products in  
Pest Control
- 19:00 - Evening Tour (Participants and Accompanying Persons)



## **ABSTRACTS**



## Opening Plenary Lecture

Chairperson: K. Fuji

July 7, 1992

9:05 - 9:45 Her Royal Highness Princess Chulabhorn Mahidol  
Bioactive Natural Products from Thai Plants.





## **Bioactive Natural Products from Thai Plants**

**Chulabhorn Mahidol**

**Chulabhorn Research Institute, Bangkok, Thailand**

Thailand is uniquely located to represent the fauna and flora which characterizes the biogeographic province of Indo-Burma. A number of the eastern Himalaya temperate taxa penetrate south into the northern mountains of Thailand while the southern part is evergreen forest thus making this area one of the richest floristic regions of the world. It has been estimated that the vascular plants in Thailand are not less than 10,000 species of about 1,763 genera from 245 families. Thailand is endowed with a great diversity of indigenous medicinal plants species, and the Thais have a long tradition of using medicinal herbs and plants in the folklore medicine but many of the claimed curative properties have not been scientifically proven. Research is urgently needed to explore the potential of these plants as a source of medicinal natural products before opportunities become forever lost through advancing deforestation. The lecture will highlight the search of therapeutic agents from Thai plants as well as some interesting natural products isolated from some plants of Thailand.



## Session 1. Indigenous Medicinal Plants

Chairperson: K. Fuji

### July 7 [Morning]

- S-1-1** 10:00-10:35 H. Itokawa  
Discovery of new antitumor agents from medicinal plants.
- S-1-2** 10:35-10:50 E. Yesilada  
Traditional medicine in Turkey.
- S-1-3** 10:50-11:05 S. Ueda  
A global view of Gardenieae iridoid production.
- S-1-4** 11:05-11:20 P. Yutisri  
Anthelmintic effect of Ma-Klua against on some intestinal nematodes of animals and man.  
I. A morphological observation on the parasites affected with Ma-Klua (*Diospyros mollis*).
- S-1-5** 11:20-11:35 H. Ohigashi  
Do wild chimpanzees use *Vernonia amygdalina* for parasite diseases?
- S-1-6** 11:35-12:10 A. Marston  
The search for antifungal, molluscicidal, and larvicidal compounds from tropical plants.
- 12:10-12:20 General Discussion



S-1-1

#### DISCOVERY OF NEW ANTITUMOR AGENTS FROM MEDICINAL PLANTS

H. ITOKAWA  
TOKYO COLLEGE OF PHARMACY  
HACHIOJI, TOKYO, JAPAN

Many types of compounds isolated from higher plants. One of them, named as RA series Cyclic hexapeptides isolated from *Rubia akane* and *R. cordifolia* also have strong antitumor activity against various types of tumors. Till now 10 kinds of RA series compounds were isolated and named as RA-I, II, III, IV, V, VI, VII, VIII, IX and X. Moreover, monoglucoside of RA-V newly isolated from same plant.

Many kinds of derivatives including natural RA compounds were tested for QSAR, and one of them, RA-VII was screened up as a most suitable substance as an antitumor agent. RA-VII (=RA-700) has strong cytotoxic activity against KB cells, P388 lymphocytic leukemia and MM2 mammary carcinoma cells.

RA-VII has been under investigation for Phase I clinical trials.

S-1-2

#### TRADITIONAL MEDICINE IN TURKEY

YESILADA Erdem  
Gazi University, Faculty of Pharmacy, Dept. of Pharmacognosy,  
Ankara, Turkey

As a result of the cultural diversity and rich flora, there is a great potency of folk medicine in Turkey. In this presentation, the past and present situation of traditional medicine in Anatolia (Asiatic part of Turkey) will be discussed on the basis of a recent comprehensive field study. Various utilizations of some plants will be mentioned as well as the results of biological activity studies concerning the claimed medicinal usages.

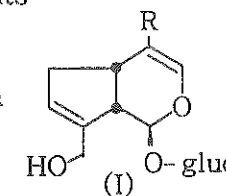
### S - 1 - 3

#### A Global View of Gardenieae Iridoid Production

Ueda, Shinichi

Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606 - 01, Japan

*Gardenia* species indigenous to the temperate East Asia and Australia produce iridoid glycosides in high levels, while those indigenous to the tropical areas of Southeast Asia and Australia as well as those indigenous to tropical and temperate Africa have low iridoid productivity. On callus induction, both temperate and tropical *Gardenia* plants remarkably increased the iridoid production. Tropical American *Genipa* plant also enhanced the iridoid production through callus induction. *Tarenna* plants indigenous to subtropical Okinawa and the Bonin islands and their cell cultures produce iridoid glucosides tarenoside (I, R = CHO) and geniposidic acid (I, R = CO<sub>2</sub>H) in high levels. Cell cultures of the Southeast Asian *Gardenia* and tropical American *Genipa* plants do not accumulate germination - inhibitory geniposide (I, R = CO<sub>2</sub>CH<sub>3</sub>).



### S - 1 - 4

#### ANTHELMINTIC EFFECT OF MA-KLUA AGAINST ON SOME INTESTINAL NEMATODES OF ANIMALS AND OF MAN I. A MORPHOLOGICAL OBSERVATION ON THE PARASITES AFFECTED WITH MA-KLUA (DIOSPYROS MOLLIS)

Piboon YUTISRI

Department of Zoology Faculty of Science Kasetsart University,  
Bangkok, 10900 Thailand

A morphological observation was carried out on Necator americanus and Enterobius vermicularis affected with Ma-Klua to elucidate the mechanism of the action of the drug upon the parasites.

It was so easy to find out the Ma-Klua substance, because it changed in colour from pale yellow to very strong brownish black after its going into the parasites. The substance was found in almost all cellular parts, that is, body wall cells, hypodermal cells, intestinal cells, lateral cords, gonadal cells, and others, being stained yellow to brown. And it was found very densely in the buccal cavity and the lumen of oesophagus too. Many dark brown granules were also recognized in the cytoplasmic part of the body-wall cells, intestinal cells, body cavity, and the lumen of intestine, especially being remarkable in intestinal cells.

DO WILD CHIMPANZEES USE Vernonia amygdalina  
FOR PARASITE DISEASES?

OHIGASHI Hajime<sup>1</sup>, Michael A. HUFFMAN<sup>2</sup>, Masanori KAWANAKA<sup>3</sup> and  
Koichi KOSHIMIZU<sup>1</sup>, <sup>1</sup>Department of Food Science and Technology,  
<sup>2</sup>Department of Zoology, Kyoto University, <sup>3</sup>Department of  
Parasitology, National Institute of Health, Japan

Vernonia amygdalina (Compositae) is a plant widely occurring in tropical Africa. The possible medicinal use of this plant by wild chimpanzees was first observed in 1987 by Huffman and Seifu. The bitter tasting plant was found to contain two classes of bitter compounds; sesquiterpene lactones (eg. vernodalin, vernolide and hydroxyvernolide) and steroid glucosides (vernoniosides). The sesquiterpene lactones and some of the steroid glucosides showed *in vitro* antischistosomal activities against Schistosoma japonicum. However, vernodalin, a major sesquiterpene lactone, was highly toxic to the cercaria-infected mouse. Interestingly, the level of vernodalin in the pith used by the chimpanzee was very low, while active steroid glucosides such as vernonioside B<sub>1</sub> were present in significant concentrations. The phytochemical significance of V. amygdalina will be discussed from the standpoint of its use by chimpanzees, possibly for parasite-related diseases.

THE SEARCH FOR ANTIFUNGAL, MOLLUSCIDAL, AND LARVICIDAL  
COMPOUNDS FROM TROPICAL PLANTS

Andrew MARSTON and Kurt HOSTETTMANN\*

Institute of Pharmacognosy and Phytochemistry, University of Lausanne,  
1015 Lausanne, Switzerland.

Many plant species from tropical regions are used in folk medicine. They provide a valuable reservoir of compounds with interesting bioactivities. Largely based on information provided by traditional healers, a selection of plants from Africa, Panama and Indonesia has been screened for fungicidal, larvicidal and molluscicidal activities. Bioautography with the fungus *Cladosporium cucumerinum* has been used in the search for antifungal compounds, while larvicidal activity has been bioassayed with *Aedes aegyptii*, the vector of yellow fever. Snail killing activity with *Biomphalaria glabrata*, the intermediate host of schistosomiasis, has been employed for the discovery of new and potent plant molluscicides. Isolation of active compounds has been achieved by a variety of modern separation techniques, including centrifugal partition chromatography and HPLC. By this approach, the following bioactive compounds have, for example, been isolated: polyphenols from *Bauhinia rufescens* (Leguminosae), biphenyls from *Monnina sylvatica* (Polygalaceae), xanthenes from *Garcinia livingstonei* (Guttiferae), saponins from *Tetrapleura tetraptera* (Leguminosae) and *Sapindus rarak* (Sapindaceae).





## Session 2. Allelopathy

Chairperson: C. H. Chou

### July 7 [Afternoon]

- |       |            |  |
|-------|------------|--|
|       | 2:00- 2:05 | C. H. Chou<br>Opening Remarks  |
| S-2-1 | 2:05-2:35  | F. A. Einhellig<br>Inhibitory mechanisms and mode of action of<br>allelopathic chemicals from sorghum.                                     |
| S-2-2 | 2:35-3:05  | J. V. Lovett<br>Allelopathy and self defence in barley.  |
| S-2-3 | 3:05-3:25  | J. Mizutani<br>Recent findings on plant ecochemicals from the<br>viewpoint of allelopathy.   |
| S-2-4 | 3:25-3:45  | A. D. Worsham<br>Allelopathic cover crops in weed management to<br>improve environmental quality.  |
| S-2-5 | 3:45-4:05  | J. D. Weidenhamer<br>The effect of phytotoxins on competitive outcome.   |
| S-2-6 | 4:05-4:25  | H. Nishimura<br>Allelochemicals from <i>Polygonum sachalinense</i><br>(Polygonaceae).  |
| S-2-7 | 4:25-4:45  | J. Miersch<br>Ecological significance of canavanine and<br>medicagenic acid as allelochemicals in alfalfa<br>( <i>Medicago sativa</i> L.). |
| S-2-8 | 4:45-5:05  | B. -S. Kil<br>Allelopathic effects of <i>Artemisia princeps</i> var.<br><i>orientalis</i> on selected species.                             |
| S-2-9 | 5:05-5:25  | H. H. Cheng<br>Modeling and experimental considerations for<br>characterization of the transport of allelochemicals in<br>the soil.        |
|       | 5:25-5:30  | General Discussion   |



S-2-1

INHIBITORY MECHANISMS AND MODE OF ACTION OF ALLELOPATHIC CHEMICALS FROM SORGHUM. Einhellig, Frank A., Dept. of Biology, University of South Dakota, Vermillion, SD 57069, USA.

Field data has shown the efficacy of a Sorghum bicolor crop in suppressing weeds. Sorghum species contain and release several different water-soluble and hydrophobic compounds which inhibit seed germination and seedling growth. Apparently Sorghum allelopathy is mediated by a complex of compounds that include numerous phenolic acids, cyanogenic glycosides, and sorgoleone, and p-benzoquinone found in root exudates. These compounds can alter energy metabolism and water relationships. Sorgoleone inhibits mitochondrial oxygen uptake and chloroplast oxygen evolution at micromolar levels ( $I_{50} = 0.5$  and  $0.2 \mu\text{M}$ , respectively) and blocks mitochondrial electron transport. Soybean mitochondrial respiration was inhibited by several phenolic allelochemicals tested;  $I_{50}$  of  $10 \mu\text{M}$ ,  $6 \text{ mM}$ ,  $7 \text{ mM}$ , and  $27 \text{ mM}$  for tannic acid, salicylic acid, gentisic acid, and p-hydroxybenzaldehyde. Using soybean as a bioassay species, several phenolic compounds, singly and in combination, caused an enhancement of tissue  $^{13}\text{C}:^{12}\text{C}$  that paralleled growth reductions. This reduction in discrimination against  $^{13}\text{C}$  indicates that another mechanism of action of Sorghum allelochemicals is alteration of long-term plant-water status.

S-2-2

#### ALLELOPATHY AND SELF DEFENCE IN BARLEY

Lovett, John V.

Department of Agronomy & Soil Science, University of New England, Armidale, NSW 2351, Australia

In common with other temperate cereals, barley contains secondary metabolites which are biologically active. Allelopathic activity of barley against several weed species is documented in the literature and is identified with two alkaloids, gramine and hordenine. In recent experiments the release of these compounds from growing barley plants has been monitored and, in a hydroponics system, found to continue from the seedling stage to more than 70 days. The compounds were inhibitory to radicle development of white mustard (*Sinapis alba* L.) over this period. Further studies have shown that barley allelochemicals can also inhibit growth and/or development in plant pathogens and insect larvae.

The hypothesis that production of gramine, synthesized in shoots of barley, has been diminished by selection for other characteristics has been tested. Data from 43 lines of *Hordeum* spp. tend to confirm this hypothesis, indicating that the capacity for self defence has been diminished over time.

## S - 2 - 3

### RECENT FINDINGS ON PLANT ECOCHEMICALS FROM THE VIEWPOINT OF ALLELOPATHY.

Mizutani, Junya  
Dept. of Applied Bioscience, Fac. of Agriculture, Hokkaido Univ., Sapporo 060,  
and Plant Ecochemicals Project, JRDC, Eniwa 061-13, Japan.

We have been working on the so-called secondary metabolites of green plants, which might be part of defense mechanisms against competing plants, pathogenic organisms and herbivorous insects. Various kinds of terpenoids, phenolics and isothiocyanates have been isolated, and identified as allelochemical candidates from many different species of wild plants. To detect allelopathic compounds and evaluate their roles in plant-plant interactions, we have devised and developed bioassay methods.

Hirsutin (8-methylsulfinyloctyl isothiocyanate) and 4-methoxy-3-indolylacetonitrile produced by *Rorippa sylvestris* (Cruciferae) seem to play important roles to invade the territories of its neighboring plant species. Seasonal variations of the glucosinolates which are precursors of hirsutin and 4-methoxy-3-indolylacetonitrile have been pursued for further understanding of allelopathic phenomenon of *R. sylvestris*.

Anthraquinones (emodin and physcion) produced by *Polygonum sachalinense* (Polygonaceae) are considered to be allelochemicals. Their glucosides have also been found in *P. sachalinense*. Emodin occurred in the soil under its community with effective concentrations for interfering the growth of nearby plant species especially in the fall.

## S - 2 - 4

### ALLELOPATHIC COVER CROPS IN WEED MANAGEMENT TO IMPROVE ENVIRONMENTAL QUALITY. Worsham, A. Douglas, Udo Blum, and Joe Yenish, N.C. State Univ., Raleigh, NC 27695-7620, USA.

In the U.S., there is increasing public pressure to move to a more "sustainable" agriculture using less off-farm inputs, including pesticides. We and other researchers and farmers have found that use of certain allelopathic cover crops in no-tillage crop production lessen, and in some cases, eliminate the need for preemergence herbicides. Winter rye and subterranean clover have been the most weed-suppressive cover crop mulches. In studies to learn more about the causes for weed suppression, *Amaranthus* spp. control increased with increasing pH of soil water extracts and total phenolic acid content of soils under various mulches. Weed control was related convexly to soil water content and mulch debris amount. It appears that it will still be most practical in the Southeast to use a non-selective herbicide for cover crop kill and selective postemergence herbicides as needed, especially for grasses and perennial weeds. This approach will still enhance agricultural sustainability because: (a) soil will be conserved, (b) herbicide use (especially preemergence herbicides) should be reduced, (c) herbicides for cover crop kill have little to no potential for contaminating surface or groundwater, and (d) postemergence selective herbicides have little potential for environmental contamination. More work is still needed on: (a) the most weed-suppressive cover crops, (b) factors responsible for the weed suppression by the mulches, and (c) how to integrate this approach into current cropping systems.

## THE EFFECT OF PHYTOTOXINS ON COMPETITIVE OUTCOME

<sup>1</sup>Thijs, Heleen, <sup>2</sup>Jodi R. Shann and <sup>1</sup>Jeffrey D. Weidenhamer<sup>1</sup>Ashland University, Ashland, Ohio 44805 USA; <sup>2</sup>University of Cincinnati, Cincinnati, Ohio USA

Atrazine, a commonly used herbicide for broadleaf weeds, was applied to corn-soybean mixtures to model the effects of allelopathic substances on competitive outcome. A target-neighbor design was used, in which differing densities of a neighbor species are planted around one individual of the target species. This approach proved more useful than the De Wit replacement series, due to the density-dependent nature of phytotoxic effects. Neighbor density greatly influenced the response to atrazine. At corn densities of 0, 3, 6, 9 and 12 plants per pot and atrazine treatment of 3.0 ppm, the dry weight of the soybean (target) plant increased from 0.2 g with no neighbors to 0.5 g with 9-12 neighboring corn plants. This is due to uptake of atrazine by the corn plants, which decreased the amount available to the soybean target.

Allelochemicals from Polygonum sachalinense (Polygonaceae)Hiroyuki NISHIMURA<sup>1</sup>, Masafumi INOUE<sup>2</sup>, Hai-Hang LI<sup>2</sup> and Junya MIZUTANI<sup>2</sup><sup>1</sup>Department of Bioscience and Technology, School of Engineering, Hokkaido Tokai University, Sapporo 005, JAPAN<sup>2</sup>Research Development Corporation of Japan, Mizutani Plant Ecochemicals Project, Eniwa 061-13, JAPAN

The root exudates from Polygonum sachalinense in a recirculating system significantly inhibited lettuce seedling growth. Bioassay of the neutral-acidic fraction on the TLC-agar-plate showed the inhibitory activity corresponded to the two yellow pigment bands. Two orange needles were isolated and identified as anthraquinone compounds, emodin and physcion. Both compounds exhibited inhibitory activities against the seedling growth of several testing plant species. The concentrations of emodin, physcion and their glucosides from rhizome with roots, aerial part, fallen leaves and soil were determined. The evaluation of anthraquinones as allelochemicals will be discussed.

S-2-7

Ecological significance of canavanine and medicagenic acid as allelochemicals in alfalfa, (*Medicago sativa* L.)

MIERSCH, J.\*, R. BAUMBACH\*, A. KROLL\*, G.-J. KRAUSS\*, I. ROSCHE\*\* and P.M. GORSKI\*\*\*

\*Martin-Luther-University, Institute of Biochemistry, \*\*Institute of Agronomy and Crop Science, Halle, Germany and \*\*\*Institute of Soil Science and Plant Cultivation, Pulawy, Poland

Alfalfa contains some biologically active compounds, like the toxic non-protein amino acid canavanine, and the saponin medicagenic acid. Canavanine is stored in the seeds of alfalfa varieties to concentrations at 0.6 to 1.6 % per dry mass, whereas medicagenic acid is absent. In general, during imbibition and germination canavanine was mobilized and degraded yielding the toxic canaline and urea. Contrary, the level of canavanine is increased in young seedlings. Canavanine got in the environment of swelled seeds or in the rhizosphere of seedlings (cv. Verko) up to 1 % of the total canavanine. We can calculate in the milieu (sandy bed etc.) to concentrations at 3 to 57 µM. In such concentrations the growth of some seedlings (cabbage, amaranth, cress, weeds) is inhibited. The maximal amounts of canavanine and medicagenic acid are different in various stages of alfalfa development.

S-2-8

ALLELOPATHIC EFFECTS OF ARTEMISIA PRINCEPS VAR. ORIENTALIS ON SELECTED SPECIES

Yun, Kyeong Won and Bong-Seop Kil, Dept. of Biology Educ., Wonkwang University, Iri, 570-749, Republic of Korea

The allelopathic effects of the wormwood plant (Artemisia princeps var. orientalis) on receptor species and possible phytotoxic chemicals were investigated. The aqueous extracts of the wormwood plant caused significant inhibition in the germination, seedling elongation and dry weight growth of the receptor plants. The germination and growth of receptor plants were also suppressed by volatile substances and essential oil from the wormwood leaf at concentration-dependent. The leaf extract and the essential oil inhibited the growth of selected microbes and callus induction of the receptor species. It was, therefore, confirmed that the chemical substances from the wormwood plant had biologically toxic activity. The GC/MS method was employed for analysis and identification of phytotoxic substances from the wormwood. Seventeen phenolic compounds and thirty-six terpenoids were identified from the leaf extract and from the essential oil, respectively. By the bioassays with the ten commercial compounds it was found that terpinen-4-ol, cineole and (-)-thujone were acted as the strongest growth inhibitors. These phenolic compounds and terpenoids would be responsible for allelopathic potential.

Modeling and Experimental Considerations for Characterization of the Transport of Allelochemicals in the Soil. CHENG, H. H., Department of Soil Science, University of Minnesota, St. Paul, MN 55108-6028, U.S.A.

Cheng (1989,1992) has proposed a conceptual framework for depicting the fate and transport of allelochemicals in soils, especially in relation to the root environment. The interactive nature of the retention, transformation, and transport processes and the chemical, soil, ambience, and plant factors affecting the fate of allelochemicals in the root environment has been outlined. Mathematical relationships accounting for the interactions of the various processes and factors can now be established to construct simulation models to characterize the transport process and assess the potential of any chemical emitted from the roots of one plant influencing the growth of surrounding plants. Appropriate experimental approaches to characterize the the transport of allelochemicals from the producing plant to the affected plants in the surrounding are needed, as most of the conventional methods are inadequate to provide realistic measurements. Special attention should be given to developing sampling and sample handling procedures which preserve the existing conditions of the root environment and account for the proper scale of representation of measured data and the temporal as well as the spatial variations affecting the fate process. Also needed are in-situ, microscale techniques which can monitor the root environment without disturbing the ambient conditions.





### Session 3. Chemical Ecology of Aqueous Biosphere

Chairperson: D. Daloz

#### July 8 [Morning]

- S-3-1 9:00- 9:35 B. M. Olivera  
Conotoxins: Neuropeptides as chemical weapons in marine snails.
- S-3-2 9:35-10:10 H. Nakamura  
Chemical studies on bioluminescent circadian rhythm of a unicellular marine alga.
- S-3-3 10:10-10:25 M. Murata  
Toxic dinoflagellate implicated in ciguatera.
- 10:25-10:40 Break
- S-3-4 10:40-10:55 T. Matsuno  
Relationship between carotenoids in the integument of ayu *Plecoglossus altivelis* and their food.
- S-3-5 10:55-11:10 E. Zeeck  
Reproductive behaviour of marine polychaetes and chemical structures of their sex pheromones.
- S-3-6 11:10-11:25 K. Shibata  
Defensive metabolites of marine octocorals.
- S-3-7 11:25-11:40 H. Kamiya  
Antifoulants of a marine sponge, *Phyllospongia papyracea*.
- S-3-8 11:40-11:55 N. Shimidzu  
Non- or less-toxic antibiofouling substances in marine invertebrates.
- 11:55-12:00 General Discussion



S-3-1



CONOTOXINS: NEUROPEPTIDES AS CHEMICAL WEAPONS IN MARINE SNAILS. Baldomero M. Olivera, Department of Biology, University of Utah, Salt Lake City, Utah.

The cone snails are venomous predators that are found in all tropical marine waters. In their venoms, they have evolved a remarkable diversity of neuropeptides which are used for the rapid paralysis of prey, and in certain cases, defensively against their predators. There are over 500 species of *Conus*, most of which are highly specialized. However, for the genus *Conus* as a whole, a broad phylogenetic range of prey are used including vertebrates (i.e., fish) and several invertebrate phyla (in particular molluscs and polychaete worms). In certain species of *Conus*, there may be well over 100 different peptides in the venom. A biochemical analysis of the venoms of different fish-hunting *Conus* species has revealed a diversity of peptides in venoms from different species. Multiple paralytic toxins are clearly used to speed up the rate of paralysis; in addition, numerous other peptides probably play an accessory role in making paralysis more rapid.

Because of the necessity for rapid prey paralysis, the peptides present in *Conus* venoms have unique characteristics. They are much smaller than other polypeptide toxins, they are remarkably restrained in conformation, with multiple disulfide linkages, and are able to discriminate between closely related subtypes of receptors and ion channels. Presumably, rapid paralysis requires that the peptides show no binding affinity for closely related targets. Recently, a number of *Conus* peptides have been used for medically related applications. For example, the  $\omega$ -conotoxins have become important as reagents for characterizing subtypes of calcium channels, in particular those that control neurotransmitter release. Thus,  $\omega$ -conotoxins have already been used as diagnostic tools (i.e., the Lambert-Eaton syndrome) as well as potential therapeutic agents in stroke.

S-3-2

Chemical Studies on Bioluminescent Circadian Rhythm  
of a Unicellular Marine Alga

Hideshi Nakamura

Department of Chemistry, Hokkaido University, Sapporo 060

Dinoflagellates are known as symbionts of various marine invertebrates and play important roles such as CO<sub>2</sub> fixation and production of organic molecules in marine environments. On the other hand, they often form blooms, so called red tide, and caused sea food poisoning. Although it has been shown that they produce numerous bioactive materials including neurotoxins, substances related to biological phenomena displayed by themselves have not been well documented.

A bioluminescent dinoflagellate *Gonyaulax polyedra* shows circadian rhythmicities in spontaneous glow as well as in cell division, photosynthetic ability and motility. We have isolated a cyclopropanecarboxylic acid, gonyauline, as an endogenous substance shortening the period of bioluminescent circadian rhythm. I will present our recent results on uptake, metabolisms, biogenesis and activity of gonyauline.

## Toxic Dinoflagellate Implicated in Ciguatera

Michio Murata,<sup>1</sup> Hiroshi Nagai,<sup>2</sup> Anne-Marie Legrand<sup>3</sup>  
and Takeshi Yasumoto<sup>1</sup>

<sup>1</sup>Faculty of Agriculture, Tohoku University, Sendai 981; <sup>2</sup>Central Research Institute, Taiyo Fishery Co., Tsukuba 300-42, Japan; <sup>3</sup>Institut de Malarde, Papeete, Tahiti, French Polynesia

Ciguatera is a food poisoning caused by ingestion of coral-reef fish. The causative toxins are produced by the dinoflagellate Gambierdiscus toxicus; a unicellular alga, which dwells on macro-algae (ca. calcareous alga) or corals. The algal toxins were transformed and concentrated in fish through the food chain in coral ecosystem.

The dinoflagellate has been found to produce three groups of bioactive compounds, all of which belong to polyether class. Gambiertoxins, precursors of ciguatoxins, are activators of voltage sensitive sodium channels and have potent toxicity particularly against higher animals. The second group includes maitotoxin, the most potent non-proteinaceous toxin known so far. The toxin also has ichthyotoxicity, inferring its role as an antifeedant against herbivorous animals.

Gambieric acids, which bear unprecedentedly potent antifungal activity, are released from the cells, while maitotoxin being retained. Poor solubility of the acids in water implies short-range allelopathy against other epiphytic micro-organisms.

## RELATIONSHIP BETWEEN CAROTENOIDS IN THE INTEGUMENT OF AYU PLECOGLOSSUS ALTIVELIS AND THEIR FOOD

Matsuno T., M. Katsuyama and T. Maoka

Department of Natural Products Research, Kyoto Pharmaceutical University, Yamashina-Ku, Kyoto 607, Japan

The fresh water fish ayu, like some other salmonids, is usually anadromous and spawning must take place in the river. The young fish live in the sea, feeding on small crustacea (copepod) until they reach 6-8cm in length, ascending to the river after the onset of spring.

On the other hand, the adults are herbivorous, taking diatoms and blue green algae in the river.

The major characteristic carotenoid zeaxanthin in the integument of both young and adult ayu will be discussed on the basis of carotenoid from their food.

Chemical test - For epoxides = HCl  
B,β-carotene, luteins A+B, (BR, BR') - Zeaxanthin (major).

\* Get carotenoid stds for pollen, sunflower.

Carotenoids sequenced from diet in adults.

Young fish do some biosynthesis steps, but do use precursors from food (copepods).

S-(+)-5Me-3-heptanone (♂ to ♀) P. dumerilii  
R-(-)-5Me-3-heptanone (♀ to ♂)

Example for  
class

S-3-5 With other compounds, one sex produces one geometric isomer, another produces another isomer.

Zeeck, Erich; Jörg D. Hardege; Helga Bartels-Hardege; Carsten Müller. Institut f. Chemie und Biologie des Meeres, University of Oldenburg, D 2900 Oldenburg, Germany.

**Reproductive behaviour of marine polychaetes and chemical structures of their sex pheromones.**

The reproductive behaviour of many marine polychaetes, especially nereids, is controlled not only by temperature, moon phase and day time, but also by a bouquet of different sex pheromones. Some of these sex pheromones are volatile, hydrophobic substances of low molecular weight (C<sub>8</sub> - C<sub>11</sub>), other pheromones are hydrophilic substances of higher molecular weight. Whereas pheromones of the latter type have the function of controlling the gamete release, the volatile substances serve for the recognition of the presence of the mating partner and for some species initiate the nuptial dance behaviour, dependent on concentration as a species criterion. The both enantiomers of 5-methyl-3-heptanone belong to these volatile pheromones, produced by different Platynereis spec., Perinereis nuntia, Nereis succinea, N. virens and others, whereas the group N. japonica, N. diversicolor and others not contain this compound but use 3,5-octadien-2-one as a pheromone. It is of some interest in evolution theory that volatile substances such as methyl-heptanones are pheromones for marine polychaetes as well as for terrestrial insects.

~~Some~~ species use a lot of the same compounds, but in different concentrations, use concentrations to keep separate.

S-3-6

Defensive Metabolites of Marine Octocorals

Kozo Shibata and Masamitsu Ochi<sup>o</sup>

Faculty of Science, Osaka City University, Sumiyoshi-ku, Osaka Japan

<sup>o</sup>Faculty of Science, Kochi University, Akebono-cho, Kochi 780 Japan

It is currently known that aggregates of muricid gastropods of the genus Drupella are capable of significant destruction of scleractinian corals. On the other hand, most of octocorals, which are devoid of physical means of defense, are relatively free from Drupella attack in the same marine habitats. In our preliminary screening, five species of octocorals were found to possess molluscidal and repellent activities against the gastropod Drupella fragum. Bioassay directed fractionation of crude extracts of these animals has led to the isolation of six classes of compounds. We report the structure and biological activities of these compounds.

Many structures, terpenoid & further mostly.

Unsat. Fatty acids.

S-3-7

Antifoulants of a marine sponge, *Phyllospongia papyracea*

Kamiya, H.<sup>a</sup>, Goto, R.<sup>a</sup>, Murata, M.<sup>b</sup>, Kado, R.<sup>a</sup>, Muramoto, K.<sup>a</sup>

<sup>a</sup>School of Fisheries Sciences, Kitasato University, Sanriku, Iwate 022-01, Japan and <sup>b</sup>Faculty of Agriculture, Tohoku University, Sendai, Miyagi 981, Japan

In the subtidal and intertidal zones, a variety of non-motile marine animals are subject to fouling. There is tremendous opportunity that invertebrates larvae and algae settle on and overgrow these marine organisms.

A sponge *Phyllospongia papyracea* (Order Dictyocoeatida, Family Spongidae) is leaf-like in Kuchierabu Island, Kagoshima. *P. papyracea* is free from fouling organisms. This suggests that the sponge could produce metabolites which prevent fouling. The fat-soluble fraction of *P. papyracea* showed potent antifouling activity. Bioassay-directed separation using barnacle larvae and mussels gave fatty acids and a C<sub>21</sub>-furanoterpene as antifoulants. The latter is identical to Furospongolide isolated from *Dysidea herbacea*. Furospongolide, together with the free fatty acids, may be responsible for the antifouling mechanism in the *P. papyracea*.

S-3-8

Non- or Less-Toxic Antibiofouling Substances in Marine Invertebrates

Shimidzu, Nobuyoshi, Shigeyuki Mizobuchi, Kazumi Kon-ya, Masayuki Katsuoka, Mutsumi Kawamata, Akihiro Yokoyama, Nobuko Ohtaki, Kyoko Adachi, and Wataru Miki  
Marine Biotechnology Institute Co., Ltd. (MBI), Shimizu Institute,  
1900 Sodeshi, Shimizu, Shizuoka 424, Japan.

Marine attaching organisms such as blue mussels and barnacles, have damaged ship bottoms, etc. Organotin compounds have been developed against these organisms, however, stern warnings have been issued to their effects on the marine environment. On the other hand, we considered that some chemical signals in marine invertebrates show repellent activities against attaching organisms as seen from the basis of their defence mechanisms. Therefore, we started to search for effective antifouling substances with non- or less-toxic properties from these marine invertebrates.

Aqueous acetone extracts of over hundred species of marine invertebrates which had been collected from the sea of Suruga Bay, Okinawa, Ogasawara and Palau, were examined using the repellent assay system against blue mussels *Mytilus edulis*<sup>1)</sup> and the attaching inhibition assay system against the cypris larva of barnacle *Balanus amphitrite*. (-)- $\beta$ -Bisabolene, fatty acids and steroids, and seco-manolide were isolated from the octocoral *Simularia* sp., *Dendronephthya* sp. and the sponge *Hyrtios erecta*, respectively, with high repellent activities against blue mussels. On the other hand, compounds exhibiting high inhibitory activities against barnacles, such as halocynthiaxanthin, trigonelline and ubiquinone<sub>8</sub> were isolated from bryozoa *Zoobotryon pellucidum*, octocoral *Dendronephthya* sp. and the symbiotic bacteria *Alteromonas* sp. KK10304, of the marine sponge *Halichondria okadae*, respectively.

1) K. Ina et al. (1989) *Agric. Biol. Chem.*, 53, 3319.

## Session 4. Plenary Lectures on Natural Product Chemistry

Chairperson: K. Mori

### July 8 [Afternoon]

1:30-2:10 K. Nakanishi

Chemical studies of tunichromes.

2:15-2:55 A. B. Smith, III

The trienomycins: A family of novel antitumor antibiotics.  
Structural elucidation and synthetic studies.





S-4-1

Summarized in *Acad. Chem Research* in 1991.**Chemical studies of tunichromes**

Nakanishi, Koji; Ryan, Daniel

Department of Chemistry, Columbia University, New York, New York 10027, U.S.A.

It is well known that some species of ascidians, e.g., *Ascidia nigra*, accumulate vanadium to unusually high concentrations - from 1 to 10 million-fold from the surrounding sea water, and that a family of yellow pigments, called tunichromes, are prevalent in their blood cells, comprising 50% of dry weight in *A. nigra* blood cells. Although the natural state of vanadium in sea water is monomeric vanadate in the +5 oxidation state, the vanadium accumulated in ascidian blood cells is present in the +3 and/or +4 states. The phlebobranch species store vanadium primarily as oxygen-sensitive  $V^{III}$ , e.g., *A. nigra* stores at least 90% of its vanadium in the +3 state! An accurate determination of the cellular pH in vanadium-laden blood cells, or vanadocytes, has been controversial. However, such high concentrations of  $V^{III}$  or  $V^{IV}$  are widely thought to necessitate sequestration by an organic ligand, thus inhibiting vanadium precipitation. After much effort, an anaerobic purification scheme was developed to isolate these oxygen-sensitive pigments which turned out to be polyphenolic trimeric peptides, the structures of which suggested that they could be ligands for vanadium. They have also been synthesized in the unprotected forms, while the synthetic protection/deprotection methodology has led to a greatly simplified isolation scheme. In studies of the reactivity of tunichrome *in vitro*, we have obtained the first evidence showing the ability of tunichrome to reduce  $V^V$ , and also  $V^{IV}$ , to the +3 oxidation state,  $V^{III}$ . EPR has shown that Mm-1 completely reduces  $V^V$  to  $V^{III}$ . Moreover, Mm-1 reduces  $V^{IV}$  to  $V^{III}$ , apparently after complexation of Mm-1 is complete. The results could explain how marine ascidians convert  $V^V$  (from sea water) to the highly unstable  $V^{III}$  species in their blood cells under neutral pH.

Protect TBMSCl/Imidazole, and  $SOCl_2$ ,  $CH_2Cl_2$ . Once blocked stable. Deblock by TFA and HF/py complex. Purify by ppt'n repeatedly.

S-4-2

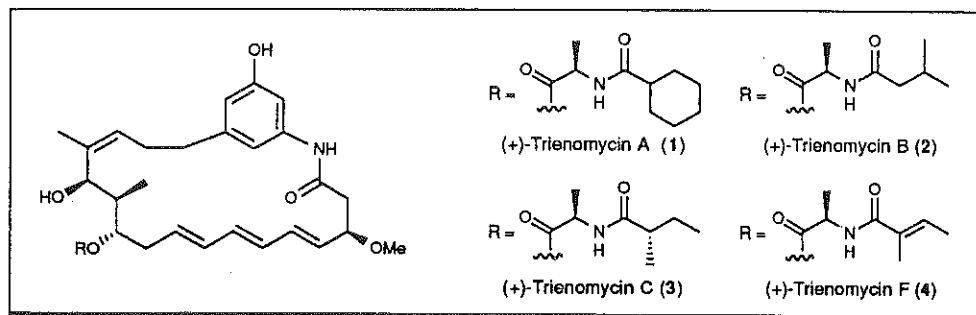
Good method to isolate poly phenols and phenolic amine type compounds.

**The Trienomycins: A Family of Novel Antitumor Antibiotics.  
Structural Elucidation and Synthetic Studies.**

Amos B. Smith, III

Department of Chemistry, the Monell Chemical Senses Center, and the Laboratory for Research on the Structure of Matter, University of Pennsylvania, Philadelphia, Pennsylvania 19104

As prelude to total synthesis, we assigned the complete relative and absolute stereochemistry of (+)-trienomycins A, B, C, and F (1-4). A detailed account of the structural elucidation studies as well as progress directed at the total synthesis of trienomycin A (1) will be presented.



From esters readily. Use ROP



## Session 5. Chemical Ecology of Fruit Flies

Chairperson: J. L. Nation

### July 9 [Morning]

- 9:00- 9:05 J. L. Nation  
Opening Remarks
- S-5-1 9:05- 9:40 K. -H. Tan  
Chemical ecology of Malaysian Dacinae fruit flies –  
Plant-fruit fly-predator interrelationships.
- S-5-2 9:40-10:15 K. Y. Kaneshiro  
Behavioral ecology of tephritid fruit flies.
- S-5-3 10:15-10:30 J. -M. Jallon  
Olfactory and other physiological responses of  
*Drosophila* to alcohols, acids and acetates.
- S-5-4 10:30-10:45 H. Kuba  
Sex pheromone of Dacinae fruit fly.
- S-5-5 10:45-11:00 R. Nishida  
Accumulation of orchid flower fragrance in the rectal  
glands by males of Melon fly, *Dacus cucurbitae*.
- 11:00-11:05 General Discussion

Lets form at same sites repeatedly.  
Physical factors (sunlight, wind, etc.) vary ab.  
males set up territories on undersides of leaves.  
Best lek sites (citrus) are not best oviposition sites.  
Courtship behavior bias based on pheromone over female.  
\* Attractive diet: hexane extractable.  $\therefore$  Hexane doesn't mess them up.

### Hexane

Acetaldehyde, acetone, acetic acid, EtOH, IPA, Ammonia  $\text{Me}_3\text{N}$  and similar  
Sulfur acids, alds, alcohols, amines, esters, cyclic amines

Do male secretions change depending on host?  
Selective sequestration biosynthesis?

S-5-1 *Dacus = Bactrocera*

Chemical ecology of Malaysian Dacinae fruit flies -  
Plant-fruit fly-predator interrelationships.

Tan, K.H., School of Biological Sciences,  
Univ. Sci. Malaysia, 11800 Penang, Malaysia.

Methyl eugenol (ME) and raspberry ketone (RK) are known to be strong attractants for many Bactrocera species. Both the compounds have also been detected in several plants, especially orchid species (Orchidaceae) of Malaysia. RK is sequestered by B. cucurbitae which in turn may use it as male aggregation pheromone. ME may be used by plants either as deterrent to plant feeding insects or as an attractant to fruit flies which may act as pollinating agents. Males of B. dorsalis complex and B. umbrosa are strongly attracted to and compulsively feed on ME. The consumed ME is then converted to other phenyl propanoid(s) in the male fly's rectum. The rectal compounds are ejaculated under stress. They may act as anti-predation agent and/or male aggregation pheromone. They may protect conspecific females through automimicry or when they are sprayed on to females by courting males during courtship. The complex interrelationship between plants, fruit flies and predators will be discussed using Malaysian examples.

*D. dorsalis* - Cariferyl alcohol, bunch of other phenyl propanoids.  
Compounds rep methyl eugenol detested by birds.

S-5-2 Check papers by Ritsuo Nishida re. chemistry (1991 papers)

**Behavioral Ecology of Tephritid Fruit Flies.**

**Kaneshiro, Kenneth Y.**

Hawaiian Evolutionary Biology Program, University of Hawaii, Honolulu, Hawaii 96822

Many species of tephritid fruit flies are serious agricultural pests throughout tropical, subtropical, as well as temperate regions of the world. Control techniques for these economically important species include: 1) use of chemical attractants for male annihilation and 2) the Sterile Insect Technique (SIT). For the SIT program, the target species is reared in large mass-rearing facilities, sterilized by use of gamma radiation, and then released into areas of infestation. Sterile flies mating with individuals in the natural population reduce the fitness of succeeding generations until the pest population is totally eradicated.

Field studies of natural populations of fruit flies indicate that there are specific mating arenas (leks) where matings occur. Mass-reared flies which have been maintained in extremely crowded conditions must be able to locate and penetrate natural mating arenas in order for the SIT program to be successful. Quality control of the SIT program is dependent on an understanding of the complex mating system of the natural population. Pheromones, sexual selection via female choice, and mating success in the Mediterranean fruit fly are discussed to illustrate the importance of understanding the basic biology of these pest species in order to develop more effective control techniques.

Males preexposed to trimedlure are less attracted and mating success of males exposed to the eugenol at trimedlure have much higher rate of mating success.  
Attraction dirt: 1) Activity extracts with hexane-35-2) Activity bleeds through plastic bags  
3) Attraction dissipates rapidly.

S - 5 - 3

OLFACTORY AND OTHER PHYSIOLOGICAL RESPONSES OF DROSOPHILA TO ALCOHOLS, ACIDS AND ACETATES.

M. COBB, L. LEGAL AND J.-M. JALLON, Laboratoire de Biologie et Génétique Evolutive. CNRS, 91198 Gif-sur-Yvette, France

Many fruitflies are sensitive to alcohols, acids and/or their esters of various sizes produced by host-plants. These compounds may modify different aspects of their behavior and physiology. *Drosophila* are good model systems to dissect these complex interactions.

Olfactory responses of *D. melanogaster* larvae to a series of alcohols, acids or acetates with various chain lengths were tested. Shorter chains are attractive while longer chains are repulsive. Three mutant strains were isolated following EMS mutagenesis on chromosome III. These strains show partial or total anosmia when stimulated with nonanol.

The behaviors of adult flies, either wild type or mutants were then compared, as well as their viability after exposure to the same compounds. Results are discussed in the light of the ecology of *Drosophila* larval and imaginal flies, and the present knowledge about receptor conformation and number.

S - 5 - 4

SEX PHEROMONE OF DACINAE FRUIT FLY

KUBA Hiroyuki and Yutaka NAKAMOTO  
Okinawa Prefectural Fruit Fly Eradication Project Office,  
123 Maji, Naha 902 Japan

Most Dacinae fruit flies, which mate at dusk, are sexually dimorphic (wing and targa bristles). The pheromone release of the male melon fly, *Bactrocera* (= *Dacus*) *cucurbitae* associates with this sexual dimorphism (KUBA & SOKEI, 1988). When the male *B. cucurbitae* engages in calling behavior, some droplets are excreted from his anus. This excretion is picked up with the tarsi of his hind legs. Then it is deposited on hairs in a cubital cell of the wing. During wing vibrations, the targa bristles on the 3rd abdominal segment, which are peculiar to the male, are rubbed against specialized hairs of the cubital cell. Thus, calling males spray a pheromone cloud with these actions. We have investigated the mating behaviors of other Dacinae as well as *B. cucurbitae*. The other 4 species of dusk mating Dacinae release their pheromone clouds as *B. cucurbitae* does (*B. dorsalis*, *B. scutellatus*, *B. latifrons*, *B. depressus*).

The suspected pheromone gland of Dacinae is the rectal gland of the male (KOBAYASHI et al., 1978). We have also analyzed the compounds in the rectal glands of some Dacinae species by GC-MS.

1,3-nonanediol, Compound on next page, Me. of the pentanoate  
Different strains have similar qualitative 36 - rectal gland secretions

**Accumulation of Orchid Flower Fragrance in the Rectal Glands by Males of Melon Fly, *Dacus cucurbitae***

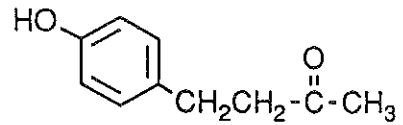
Nishida, Ritsuo, Hiroshi Fukami, Osamu Iwahashi<sup>1</sup> and Keng Hong Tan<sup>2</sup>

*Pesticide Research Institute, Kyoto University, Kyoto, 606-01 Japan*

<sup>1</sup>*College of Agriculture, The University of Ryukyus, Okinawa, 903-01, Japan*

<sup>2</sup>*School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia*

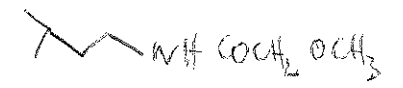
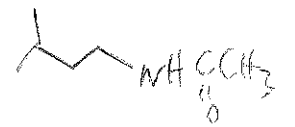
Males of the melon fly, *Dacus cucurbitae* Coquillett, show strong affinity to the blossoms of the orchid, *Dendrobium superbum* Rchb. f. A potent attractant was isolated from the flower petals and identified as 4-(4-hydroxyphenyl)-2-butanone (I). The male flies fed compulsively on the flower petals selectively sequestered compound I, in significant quantities, in the rectal glands. The compound was found within 6 hours after ingestion, and was retained for more than 6 days in the gland sacs. The males secrete a series of volatile components as a smoke from the rectal glands during courtship period. Selective accumulation of the orchid fragrance in the rectal glands suggested an additional pheromonal function of the compound in nature where the chemical source is available.



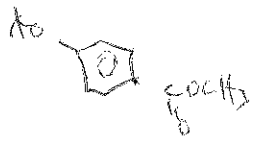
Compound I

Raspberry ketone

Rasp. Ketone

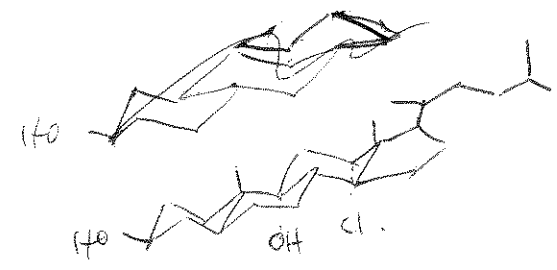
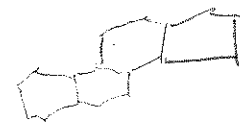


1,3 hexanediol



Ethyl & propyl parahydroxybenzoate

Other comp.





## Session 6. Utilization of Natural Products in Pest Control

Chairperson: W. S. Bowers

### July 9 [Afternoon]

- S-6-1 1:15-1:50 M. B. Isman  
Prospects for the development of new botanical insecticides.
- S-6-2 1:50-2:05 J. -L. Clement  
Novel insect antifeedant limonoid from Meliaceae.
- S-6-3 2:05-2:20 M. Serit  
Termite antifeedant limonoids from neem, *Azadirachta indica*.
- S-6-4 2:20-2:35 W. Kraus  
Insecticidal constituents of *Azadirachta indica* and other Meliaceae.
- 2:35-2:50 Break
- S-6-5 2:50-3:05 S. Hasegawa  
Practical methods for biological control of soil-borne diseases by *Pseudomonas* species.
- S-6-6 3:05-3:20 A. -I. David-Henriet  
Utilization of the fungus *Sporothrix insectorum* (Hoog and Evans) on the lace bug *Leptopharsa gibbicularina* (Froeschner) in African palm *Elaeis guineensis* (JACQ).
- S-6-7 3:20-3:35 H. M. Niemeyer  
Hydroxamic acids: Chemical defences in wheat against aphids.
- S-6-8 3:35-3:50 A. Shani  
Role of pheromones in integrated pest management.
- S-6-9 3:50-4:25 J. A. Pickett  
Host and non-host plant chemicals in pest control.
- 4:25-4:30 General Discussion



## PROSPECTS FOR THE DEVELOPMENT OF NEW BOTANICAL INSECTICIDES

Isman, Murray B.

Department of Plant Science, University of British Columbia, Vancouver, Canada V6T 1Z4

Although plants produce a vast range of natural products with significant physiological and behavioral effects on pest insects, only a handful of botanical preparations are in current use as commercial insecticides. The wide gap between the observation of biological activity against insects in the laboratory and the ultimate production and registration of a novel botanical insect control product exists because there are a large number of criteria which such a product must meet to ensure its commercial viability. Some of these criteria are biological, including the spectrum-of-action against pests, effects on non-target arthropods (e.g. natural enemies), potential phytotoxicity and toxicity to vertebrates. Other criteria are chemical, including stability of the active ingredients, mixtures of active ingredients, and resulting quality-control specifications.

However, many plant natural products which satisfy most or all of the biological criteria may nonetheless be unsuitable for commercial development because they fail to meet practical and economic criteria, in particular their continuous availability in reasonable quantity. Examples will be given of plant natural products which fail to meet sufficient criteria for commercialization and will be compared to a recent product which has been successful commercialized (neem). The potential for some natural products currently under investigation will also be discussed.

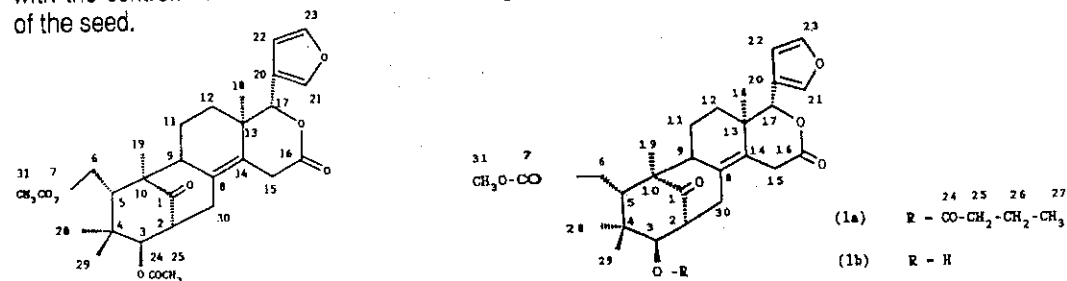
## NOVEL INSECT ANTIFEEDANT LIMONOID FROM MELLIACEA

CLEMENT Jean-Luc\*, LAMAQUE Dominique\*\*, LHOMMET Gérard\*\*, VAUCHOT Blandine\*, LANGE Catherine\*\*.

\* CNRS - Communication Chimique UPR 27 - LNB - 31, chemin J. Aiguier 13402 MARSEILLE, France

\*\*Université P.M. Curie - Chimie Organique Structurale - 4, place Jussieu 75005 PARIS, France

*Khaya ivorensis* (Meliaceae) is known to present a good resistance against insects attack. Seeds of this tree have an antifeeding activity against pest moth caterpillars. We identified the responsible active principles by separating seeds extracts. A known limonoid : fassinolide (1) and a novel limonoid (2) were determined to be a very powerful antifeedant against *Spodoptera frugiperda*, *Agrotis segetium* and *Scotia ipsilon* at the concentration of 100 ppm (1,5 % of the real concentration of each compound in the seeds), when incorporated into the artificial diet. The growth of larvae being reduced by half compared with the control. These limonoid are not lethal against insects, thus they play an effective protecting role of the seed.

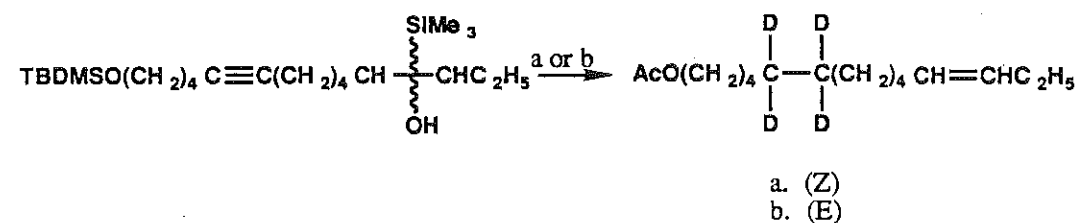




P - 1

**A B-Hydroxysilane as an Olefin-Protecting Group in the Synthesis and Labeling of Unsaturated Insect Pheromones.** Oliver, James E. Insect Chemical Ecology Laboratory, Agricultural Research Service, USDA, Beltsville, MD USA 20705.

Toward the preparation of both radiolabeled geometric isomers of 11-tetradecenyl acetate, a (Z)-ene-yne ((E) could also have been used) was converted, via its epoxide, to a mixture of β-hydroxysilanes; after hydrogenation (deuteration, tritiation) of the triple bond, either geometric isomer of the double bond can be regenerated by appropriate treatment of the hydroxysilanes (conversion of the TBDMS group to acetate is smoothly achieved with Nafion H in the presence of acetic anhydride).



a. for (Z) - pheromone: 1. D<sub>2</sub>, catalyst; 2. Nafion H, Ac<sub>2</sub>O

b. for (E) - pheromone: 1. D<sub>2</sub>, catalyst; 2. KO<sup>t</sup>Bu; 3. Nafion H, Ac<sub>2</sub>O

*Deuteration with Pt/D<sub>2</sub> gave some deuteration scrambling  
Use homogeneous catalysis instead, Wilkinson's catalyst in carefully degassed benzene. Slower, but no scrambling.*

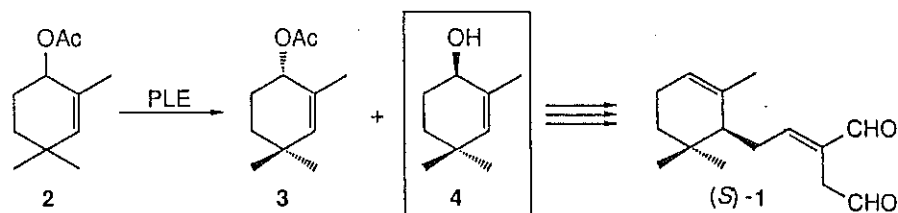
P - 2

**Synthesis and Absolute Configuration of (E)-3-Formyl-(2,6,6-trimethyl-2-cyclohexenyl)-3-pentenal, a Sesquiterpenoid from a Marine Alga, *Caulerpa ashmeadii*.**

Shinji Aki and Kenji Mori\*

Department of Agricultural Chemistry, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan

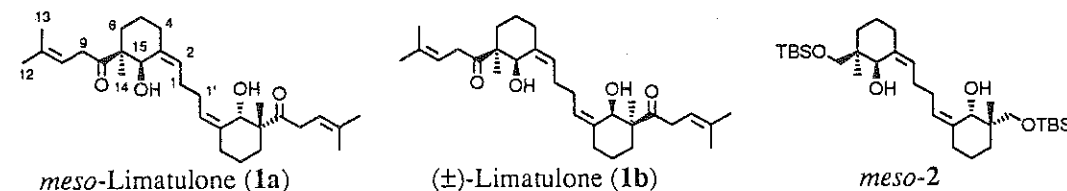
A six-step synthesis of the enantiomers of (E)-3-formyl-(2,6,6-trimethyl-2-cyclohexenyl)-3-pentenal(1) was achieved by starting from the enantiomers of 2,4,4-trimethyl-2-cyclohexenol(4). By comparing the sign of the optical rotation of the naturally occurring material isolated from *Caulerpa ashmeadii* with those of the synthetic samples, the (S)-absolute configuration was assigned to the natural product.



SYNTHESIS OF (±)- AND *meso*-LIMATULONE, DEFENSIVE TRITERPENE METABOLITES OF THE LIMPET *Collisella limatula*

H. TAKIKAWA,<sup>a</sup> K. MORI,<sup>\*a</sup> M. KIDO,<sup>b</sup> K. F. ALBIZATI<sup>c</sup> and D. J. FAULKNER<sup>c</sup>  
<sup>a</sup>Department of Agricultural Chemistry, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan <sup>b</sup>Second Tokushima Institute of New Drug Research, Otsuka Pharmaceutical Co., Ltd., Kawauchi, Tokushima 770-01, Japan <sup>c</sup>Scripps Institution of Oceanography, University of California San Diego, La Jolla, California, U.S.A.

In 1985, limatulone (**1a** and **1b**) was isolated from the intertidal limpet *Collisella limatula* as the most potent fish feeding inhibitor.<sup>1</sup> Their structures were confirmed by a total synthesis coupled with X-ray crystallographic analysis of the intermediate *meso*-2.



<sup>1</sup>) K. F. Albizati, J. R. Pawlik and D. J. Faulkner *J. Org. Chem.*, **50**, 3428-3430 (1985).

CHEMICAL TRANSFORMATION OF ECDYSTEROIDS FOR ANALYTICAL AND CHEMOECOLOGICAL USE.

Harmatha Juraj, Jaroslav Piš and Miloš Buděšínský

Institute of Organic Chemistry and Biochemistry,  
 Czechoslovak Academy of Sciences, 166 10 Prague, Czechoslovakia

A combination of protective groups (cyclic boronates and acetates) was applied in order to prepare analogues of natural ecdysteroid conjugates: fatty acid esters, benzoates and glycosides. Reaction of phenylboronic acid exclusively with the diol in the side chain was used for selective protection. Regioselectivity, quantitative yield and reaction rate as well as the properties of formed boronates determined a wide use of this reaction in chemistry and the analysis of ecdysteroids. Change of chromatographic properties was used for a simple prove of 20,22-diol in ecdysteroids by TLC or HPLC as well as for special chromatographic separations and purifications. The *in situ* acylation of hydroxyls with trichloroacetyl isocyanate (TAI-method) in the NMR sample tube was used for structural analysis of a series of ecdysteroid derivatives by <sup>1</sup>H-NMR spectroscopy. The use of TAI-method is demonstrated on paxillosterone, the first natural ecdysteroid from fungi.

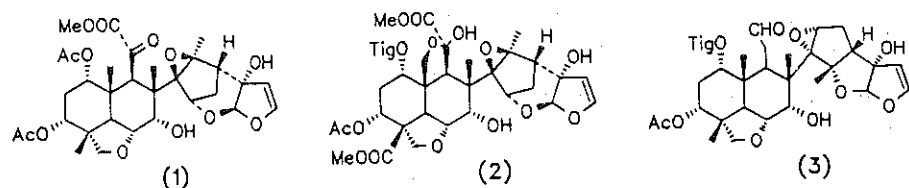
## DIOXATRIQUINANE DERIVATIVES AS INTERMEDIATES IN THE BIOSYNTHESIS OF AZADIRACHTIN

W. Kraus,<sup>a</sup> M. Bokel,<sup>a</sup> R. Soellner,<sup>b</sup> B. Vogler,<sup>a</sup> D. Wendisch,<sup>b</sup> and Y. Zhou-Halwart<sup>a</sup>

<sup>a</sup>Department of Chemistry, University of Hohenheim, 7000 Stuttgart 70,

<sup>b</sup>Bayer AG, 5090 Leverkusen, Germany

Recently we reported on keto ester (1) as a possible intermediate in the biosynthesis of azadirachtin (2).<sup>1</sup> Further work up of methanolic extracts from *Azadirachta indica* seeds yielded compound (3), which can be considered to be a very early precursor of (1) and (2). The structure of (3) has been established by n.m.r. and molecular modelling. The conversion of the dioxatriquinane moiety attached to C-8 into the bridged acetale present in azadirachtin (2), and also in keto ester (1), will be discussed.



<sup>1</sup>W. Kraus, H. Gutzeit, and M. Bokel, *Tetrahedron Lett.* **30**, 1797 (1989)

## Barrier of Biotransformation Experiments

Ohnishi, Mayumi\*, Hiroaki Noda\*\* and Yoko Naya\*

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\*\*National Institute of Sericultural and Entomological Science, Owashi, Tsukuba, Ibaraki 305, Japan

Previously we have reported that 24-methylenecholesterol (I), the major sterol in the host insect *Nilaparvata lugens*, should originate from ergosta-5,7,24(28)-trien-3 $\beta$ -ol (II) produced by intracellular symbionts<sup>1</sup>. To test our hypothesis, the deuterated trienols (d<sub>3</sub>-II, d<sub>7</sub>-II) were synthesized with modification to the reported methods<sup>2,3</sup>. The deuterated compounds were then dissolved in olive oil containing Emulgen 913 (0.5 %) and injected into the insect. In contrast to the conversion of I to cholesterol<sup>3</sup>, the expected biotransformation of the deuterated trienols into I and/or cholesterol was not observed to a significant extent. When the separated symbiont<sup>4</sup> was incubated in an MGM medium at 28°C for 3 h, II was the only product and increased obviously overtime, however none of these products were secreted into the medium. The presence of a specific transport mechanism of II at the symbiont membrane is required for the utilization by the host insect. An additional approach using stable isotope ratio analysis (SIRA) of the sterols will also be discussed.

1. Y. Naya et al., *J. Chem. Ecol.*, submitted.
2. M. Fryberg, A. C. Oelschlager and A. M. Unrau, *J. Amer. Chem. Soc.*, **95**, 5747-57 (1973).
3. B. K. Eya et al., *J. Chem. Ecol.*, **15**, 373-80 (1989).
4. H. Noda, T. Ohmura, *J. Invertebr. Pathol.*, **59**, 104-5 (1992).

P - 7

## BIOTRANSFORMATION OF MONOTERPENOIDS WITH p-MENTHAN-1-ONE SKELETON.

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Kinki University, Kowakae, Higashiosaka-shi, Osaka 577, JAPAN.

Monoterpenoids with p-menthan-1-one skeleton can be metabolized by a strain of *Rhizoctonia solani*, which is a parasite of the farm crops. Three monoterpenoids, ( $\pm$ )-Piperitone (1), (-)-Piperitone oxide(2), and (+)-Piperitenone oxide(3) were examined, 12 varieties products were produced by stereoselective hydroxylation, reduction, hydrolysis, and enzymic Baeyer-Villiger type oxidation. (1) was biotransformed to 5 products on enantioselectivity hydroxylation. The biotransformation of (2) was formed to 1,2-diols. The main site of epoxide cleavage was at the C(1) carbone, opposite to an acid-catalyzed epoxide opening. (3) was transformed to  $\epsilon$ -lactone and another.

P - 8

Chemical structure of carbonyl compounds related to natural antifeedants from Artemisia capillaris and the antifeedant activity for larvae of Pieris rapae crucivora

Yano, Katsumi

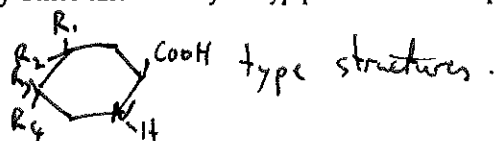
Fukuoka University of Education, Department of Chemistry  
811-41 729 Akama, Munakata, Fukuoka, Japan

The antifeedant activity of carbonyl compounds related to capillin from the growing buds of Artemisia capillaris was assessed by the bioassay against larvae of Pieris rapae crucivora. In this study, it was observed that the insect antifeedants possessing  $\overset{\text{O}}{\parallel}\text{C}-\text{CH}_3$  group showed stronger activity than that possessing  $\overset{\text{O}}{\parallel}\text{C}-\text{H}$  group. These interesting facts were the same with a phenomenon that the antifeedant activity of a compound possessing  $\text{C}=\text{C}-\text{CH}_3$  group was stronger than that of a compound possessing  $\text{C}=\text{C}-\text{H}$  group.



POTENTIAL NEW INSECTICIDAL IMINO ACIDS FROM COSTA RICAN INGA SPECIES. John T. Romeo and Timothy C. Morton. University of South Florida, Tampa, FL 33620 USA.

Nonprotein amino acids are characteristic of a large number of Mimosoid legumes. Several derivatives of the imino acid, pipercolic acid, are common in the genera Inga, Calliandra, and Zapoteca. All compounds which have been tested show varying degrees of toxicity to Lepidopterans and aphids and are particularly toxic in combinations (Simmonds & Romeo, 1988). They negatively affect growth and survival by interfering with nutritional physiology (Shea & Romeo, 1991). Along a latitudinal gradient in Costa Rica, extending from lowlands (650m) to the cloud forest (1650m), 7 species of Inga were sampled for nonprotein imino acids (NPIA). Within species, NPIAs generally were identical, showing relatively consistent concentrations. Between species, the chemical patterns were distinct. Three NPIAs, not previously known from plants, were discovered. One species, Inga oerstediana, which spans the latitudinal gradient, showed trans-4-methoxypipercolic acid, a unique compound, present only in the high elevation plants. We hypothesize that this more lipophilic compound is playing an important role in defense at high elevations. There, ants, which also probably contribute to plant defense in Inga, are lacking. Another species, I. densiflora, also showed distinctive differences between lowland and upland populations. In this species a unique trihydroxypipercolic acid is present. The only other known trihydroxypipercolic acid is a potent glycosidase inhibitor in insects.



LIGNAN AS A BOTANICAL INSECTICIDE: STRUCTURE-ACTIVITY RELATIONSHIP OF HAEDOXAN ANALOGS

Eiji TANIGUCHI, Satoshi YAMAUCHI, Hiroshi GOTANDA, and Rumiko URASHI Department of Agricultural Chemistry, Faculty of Agriculture, Kyushu University, Hakozaki 6-10-1, Higashi-Ku, Fukuoka 812.

Lignan biosynthesis may provide us an ecochemical measure of plant resistance against herbivorous insects.

A sesquiliglan, haedoxan A,<sup>1)</sup> had been isolated from Phryma leptostachya L. ("Hae-doku-sou") as a potentially-insecticidal phytochemical to a variety of insects. By synthesis of its stereoisomers and assessment of their activities, it was made evident that 1S,2R,5R,6S,2'R,3'R-configuration of the natural product was indispensable for the insecticidal activity.<sup>2)</sup> Modification of the substituents and molecular framework of haedoxan have revealed some structural requirements<sup>3)</sup> for the biological activity. Structure-activity relationship of the neolignan will be discussed on the basis of the results from incremental experiments.

1) E. Taniguchi et al., Agric. Biol. Chem., 53, 631 (1989).

2) F. Ishibashi and E. Taniguchi, Agric. Biol. Chem., 53, 1565 (1989).

3) S. Yamauchi and E. Taniguchi, Biosci. Biotech. Biochem., 56, 418 (1992).

## P - 1 1

New Okaramine Congeners Produced by Strains of *Penicillium simplicissimum*

Hideo HAYASHI, Teruaki FUJIWARA, Yoshihide ASABU, Sawao MURAO, Mitsuru NAKAYAMA, and Motoo ARAI

Department of Agricultural Chemistry, University of Osaka Prefecture, 1-1 Gakuen-cho, Sakai, Osaka 593, Japan

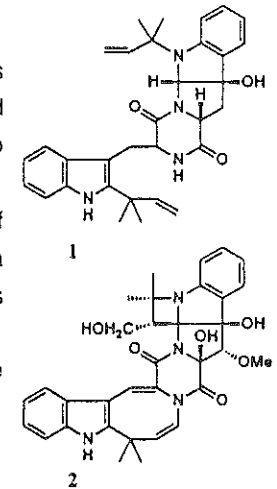
Okaramines A and B are new insecticidal indole alkaloids produced by *Penicillium simplicissimum* AK-40.<sup>1)</sup> Since we had an interest in the unique structures of okaramines, we tried to obtain okaramine analogues.

From the acetone extract of okara (the insoluble residue of whole soybeans) fermented with *P. simplicissimum* AHU 8402, a new congener, okaramine C (1), was isolated and its structure was deduced from spectral data.<sup>2)</sup>

We also found that AK-40 produced an additional active compound, which was isolated and termed okaramine D (2).

1) H. Hayashi *et al.*, *Agric. Biol. Chem.*, **53**, 461 (1989).

2) H. Hayashi *et al.*, *Agric. Biol. Chem.*, **55**, 3143 (1991).



## P - 1 2

**Insecticidal activity screening of evergreen Lauraceae present in Japan.**

Gonzalez-Coloma Azucena<sup>1,2</sup>, Pierre Escoubas<sup>2</sup>, Labunmi Lajide<sup>2</sup>, Junya Mizutani<sup>2</sup> and Osamu Saito<sup>1</sup>

<sup>1</sup>Hokkaido National Agricultural Experiment Station, Toyohira-ku, Sapporo 001  
<sup>2</sup>Research and Development Corporation of Japan, Eniwa RBP, Meguro Kita 3-1-1, Eniwa Shi, Hokkaido 061-13 Japan.

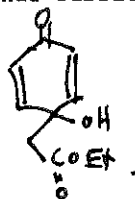
Naturally occurring combinations of substances exert a multifactorial selective pressure on insect herbivores and therefore these products are potential botanical insecticides. Within this framework, we have screened several evergreen Lauraceae growing in Japan for insecticidal activity. We have extracted the following plants: *Cinnamomum camphora*, *C. japonicum*, *C. loureirii*, *Machilus thunbergii*, *M. japonica*, *Litsea glauca*, *Actinodaphne lancifolia*, *A. acuminata* and *Laurus nobilis*; and bioassayed their ethanolic foliar extracts against the following target species: *Spodoptera litura* (leaf-disk choice bioassay for antifeedant activity and diet incorporation bioassay for toxicity), *Aedes aegyptii* larvae (toxicity) and *Drosophila melanogaster* (contact toxicity).

Among the plants studied, *M. japonica* showed a significant negative effect on *S. litura* larval growth; and *L. glauca* proved to be antifeedant. The results of the investigation regarding the natural products involved in the above mentioned activities will be discussed.

FOLIAR SURFACE CHEMISTRY: A BIOACTIVE CYCLOHEXADIENONE FROM *Senecio cannabifolius*.

Lajide Labunmi, Pierre Escoubas, Azucena Gonzalez-Coloma and Junya Mizutani. Plant Ecochemicals Project, JRDC, Eniwa RBP, Megumino Kita 3-1-1, Eniwa-shi, Hokkaido 061-13, Japan.

In continuation of our study of the role of plant allelochemicals in plant-insect interactions, we have examined the foliar surface chemistry of selected Hokkaido plants for antifeedant and insecticidal activities. Leaf surface extracts were prepared by dipping fresh leaves in chloroform for 3 min., then evaporated, redissolved in acetone and dewaxed by cooling to 0°C. The filtrate was then used in a choice leaf-disk bioassay against tobacco cutworm larvae (*Spodoptera litura*). Activity was found only in the extract of *Senecio cannabifolius*, a very successful weed growing luxuriantly in Hokkaido. When individual fractions were tested for antifeedant activity, no significant activity was observed. Incorporation of the individual fractions in an artificial diet fed to neonate *S. litura*, led to the isolation of ethyl (1-hydroxy-4-oxo-2,5-cyclohexadienyl) acetate as the active compound. To our knowledge, this is the first report of the insecticidal activity of this compound. The results of the bioassay, as well as antimicrobial activities, contact toxicity against *Drosophila melanogaster*, and seed germination and plant growth inhibition of the compound will be presented. This compound seems to represent a multifunctional surface defensive agent for the plant.



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Faculty of Agriculture, Tokyo University of Agriculture,  
Tokyo, 156 JAPAN

INSECT GROWTH REGULATOR FROM ANNONACEAE

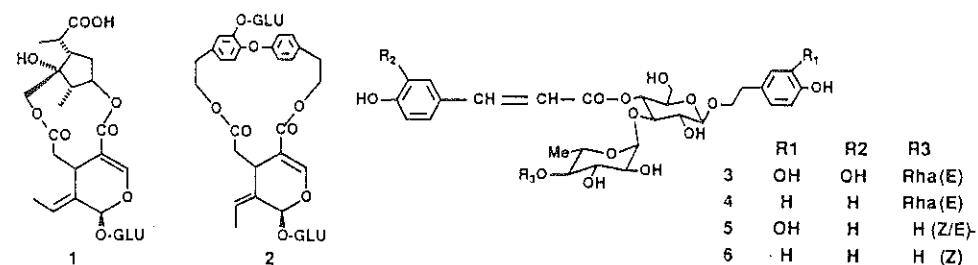
Annonaceae family are used in folk medicine and also as pesticide in tropical country. We report the results obtained from a comparative investigation on the pesticidal activity of some natural acetogenin substances from the collected Annonaceae. Squamocin, Asimicin and desmethylyvaricin are isolated from *A. squamosa* and *A. glabra*. *A. montana*, *A. reticulata* and *A. muricata* also contain asimicin-like compounds. All of the extracts from collected seeds of Annonaceae and isolated compounds showed insecticidal activity against the azuki bean weevil. Squamocin was lethal to larvae of diamondback moth at high concentration and inhibited growth at low concentration. When 2nd instar larvae of the cabbage cluster-caterpillar was offered treated leaf disks, they survived for 3 to 4 days but never molted to the 3rd instar. Ether extract of *Polyalthia litoralis* showed insecticidal activity against the brown planthopper.

## P-15

### PHENYLPROPANOID AND IRIDOID GLYCOSIDES OF OLEACEOUS PLANTS IN CHINA

He Zhen-Dan, Chong-Ren Yang, Tetsuro Fujita\*, Shinichi Ueda\* and Kenichiro Ioue\*\*  
 Laboratory of Phytochemistry, Kunming Institute of Botany, Academia Sinica, Kunming 650204, Yunnan, China  
 \* Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606-01, Japan  
 \*\*Gifu Pharmaceutical University, Gifu 502, Japan

Examination of the glycoside constituents of three oleaceous plants growing in Yunnan, China, has led to the isolation of the following new compounds: *Jasminum mesnyi* (leaves): jasmisnyiroside (1); *Olea yunnanensis* (stem), a folk medicine for inflammation: oleayunnanoside (2); *Ligustrum purpurascens*, a plant for traditional drinking tea, Ku-Ding-Cha (leaves): ligupurpurosides A (3), B (4), the Z/E epimer mixture of 2-(3,4-dihydroxyphenyl) ethyl (3-O- $\alpha$ -L-rhamnopyranosyl) (4-O-coumaroyl)-O- $\beta$ -glycopyranoside (5) and the cis of epimer of osmanthuside B (6) obtained as a 1:1 mixture with the known trans epimer.

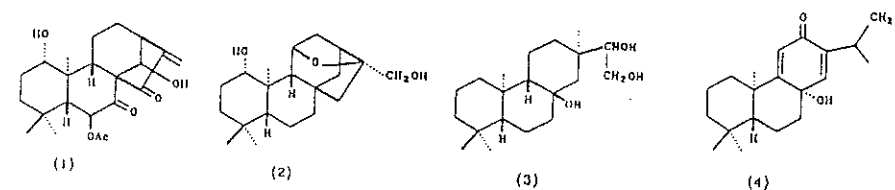


## P-16

### DITERPENOIDS OF LABIATE PLANT IN CHINA

Chen Yiping, Lin Zhongwen, Sun Handong and Tetsuro Fujita\*  
 Laboratory of Phytochemistry, Kunming Institute of Botany, Academia Sinica, Kunming 650204, Yunnan, China  
 \* Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606-01, Japan

Examination of the diterpenoid constituents of Labiate plants, *Isodon glutinosa* C. Y. Wu et H. W. Li, growing in Yunnan, China, has led to the isolation of the following four new compounds, glutiorabdals A - D (1 - 4), along with three known compounds. The structures of glutiorabdals were determined by the spectral analysis of their  $^1\text{H}$  and  $^{13}\text{C}$  NMR,  $^1\text{H}-^1\text{H}$  COSY,  $^1\text{H}-^{13}\text{C}$  COSY and NOESY spectra.



Cytotoxic Substances from *Eurycoma longifolia*

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Horunouchi 1432-1, Hachioji, Tokyo 192-03, JAPAN

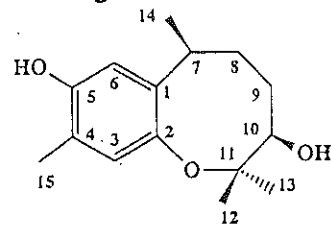
*Eurycoma longifolia* (Simaroubaceae) is one of famous folk medicine in the Southeast Asia. During the survey of novel antitumor compounds from higher plants, the alcohol extract showed significant antitumor activity against Sarcoma 180 ascites in mice, V-79, KB and P388 cultured cells. The active principles were found to be various C<sub>19</sub>- and C<sub>20</sub>-skeleton quassinoids, squalene-type and tirucallane-type triterpenes. These structures were established by spectroscopic data, chemical evidences and X-ray analysis etc. The isolation, characterization and cytotoxic activity of above compounds are reported.

## NOVEL SESQUITERPENES FROM BIOACTIVE FRACTIONS OF CULTIVAR SUNFLOWERS.

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Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, Apdo. 40,  
11510 Puerto Real, Cádiz, Spain

The existence of allelopathy has been well documented over the past few decades, particularly in relation to its significance in both natural and agroecosystems. Total yield of sunflower kernels in the EC (1986-90) was 3.4-4.3 million L/year (ca. 20% of world production). The potentiality of sunflower (*Helianthus annuus*) as source of allelochemicals is well known. Laboratory bioassays of leaves aqueous extracts from different cultivated sunflower varieties from the Andalusian region (Spain) have shown strong inhibition on germination and root length of mono- and dicotyledone species, as well as stimulatory effects on shoot length.

In addition to several new sesquiterpene lactones, we have isolated two sesquiterpenes (heliannuol A and B) from the medium polar active fractions. Heliannuol A and B are of particular interest since they contain a previously unknown skeleton, heliannuol (see figure), whose structural elucidation was made based on spectroscopic techniques, X-Ray diffraction analysis and chemical correlations.



Heliannuol A  
Heliannuol B  $\Delta^{8,9}$

*Also guananolides.*

ALLELOPATHIC POTENTIAL OF MENTHOFURAN MONOTERPENES FROM  
CALAMINTHA ASHEI

<sup>1,3</sup>Weidenhamer, Jeffrey D., <sup>1</sup>Marios Menelaou, <sup>1</sup>Francisco A. Macias, <sup>1</sup>Nikolaus H. Fischer and <sup>2</sup>G. Bruce Williamson.

<sup>1</sup>Dept. of Chemistry and <sup>2</sup>Dept. of Botany, Louisiana State University, Baton Rouge, Louisiana 70803 USA; <sup>3</sup>Current address: Ashland University, Ashland, Ohio 44805 USA.

A reversed-phase HPLC analysis was used to separate and quantify five menthofuran monoterpenes in Calamintha leaf soaks and washes. Epi-evodone and desacetylcalaminthone were the major constituents of both soaks and washes. Concentrations of epi-evodone and desacetylcalaminthone were as high as 109 and 133 ppm, respectively, in leaf soaks. Total concentrations of monoterpenes in leaf washes by misting were in no case higher than 4 ppm. Bioassays of Calamintha monoterpenes have demonstrated effects on germination as low as 8 ppm for epi-evodone. The analytical results are evaluated against the bioassay data with respect to the allelopathic potential of Calamintha.

ALLELOPATHIC EFFECT OF RED PEPPER (CAPSICUM ANNUUM L.)

Tsuchiya, Kazunari<sup>1</sup>, Jae-Wook, Lee<sup>2</sup> & Tsuguo, Hoshina<sup>3</sup> (<sup>1</sup>Hokkaido Natl. Agric. Exp. Stn., Memuro, Hokkaido, 082 Japan, <sup>2</sup>Hort. Exp. Stn., Suwon, 440-310 Korea, <sup>3</sup>Nat. Res. Inst. Veg. Ornam. Plts. & Tea, Ano, Mie, 514-23 Japan)

The yield of red pepper has decreased and the quality has become worse because of continuous cropping in Korea. The main reasons for this decline are considered to be diseases. But, we guessed that allelopathy was one of the reasons. Therefore, we assayed the growth inhibition of red pepper by using the germination test with water or organic solvent extracts of leaf, stem, root and cultivated soil. In addition, we discriminated the allelopathy of red pepper from the other competitions by using continuous root exudate trapping system.

(1) Methanol extracts of stem and root of red pepper strongly inhibited the radicle growth of red pepper, respectively, and methanol extracts of leaf and root inhibited the hypocotyl growth. In the methanol extract of red pepper root, there were several phenolic acids such as p-hydroxybenzoic acid, vanillic acid. (2) Without the column of XAD-4 resin, the growth of red pepper was inhibited by the root exudate as compared with the column. In addition, this exudate inhibited the germination of red pepper, and it had a characteristic absorption peak same as the methanol extract of cultivated soil at 254nm. (3) We presumed that the allelopathic effect is one of the reasons for red pepper decline problem in continuous cropping.

Screening of allelopathic candidates by new assessment methods; Allelopathy of *Mucuna pruriens*, *Vicia villosa* and allelopathic strains of *Oryza sativa*

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and Okuno Masakazu \*\*\*

\* Shikoku National Agricultural Experiment Station (Zentsuji, Kagawa, Japan)

\*\* National Institute of Agro-Environmental Sciences (Tsukuba, Ibaraki, Japan)

\*\*\* National Institute for Agricultural Resources (Tsukuba, Ibaraki, Japan)

A series of laboratory assessments and field tests to search for allelopathic plants indicated that velvetbean (*Mucuna pruriens* var. utilis) and hairy vetch (*Vicia villosa*) were the promising candidates. The allelopathic substance of velvetbean was identified as L-3,4-dihydroxyphenylalanine (L-DOPA). L-DOPA, contained in a large amount (about 1% of the fresh weight in leaves and roots of velvetbean) inhibited the growth of some broad leaf weeds, while little effect was observed on gramineous plants.

A new assessment method to prove allelopathy by root exudates has been developed. This method is a sort of mixed culture in agar-medium. Main merits of this method are, 1) to assess the allelopathic activity of many allelopathic candidates without other smothering factor, 2) to extract allelopathic chemicals from agar medium. By this method, we checked over 500 plant species and found some promising plants. Velvetbean and hairy vetch showed strong allelopathic activity. In order to suppress paddy weeds, some rice varieties which have allelopathic activity were surveyed from 189 species by this method.

### Modelling Biological Reactions to Allelochemicals

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<sup>1</sup> School of Agriculture, Charles Sturt University, Wagga 2650, Australia

<sup>2</sup> Department of Agronomy, University of New England, Armidale 2351, Australia

The characteristic responses of an organism to an allelochemical, i.e. stimulation / attraction at low concentrations and inhibition / repellence as the concentration increases, have been widely recognized in allelopathy studies. It is hypothesized that such reaction results from the character of allelochemicals themselves. An allelochemical is assumed to have two attributes, stimulation and inhibition, both occurring simultaneously. As concentration changes, the relative dominance of stimulation and inhibition of the allelochemical alters. This can only be observed through the biological responses when an allelochemical acts on an organism. It is defined as a biological property of an allelochemical. Based on this assumption, a mathematical model has been developed to interpret such biological reactions. Several sets of experimental data from the literature have been compared with the model predictions, and a good agreement has been gained.

## P - 2 3

The effect of the weed Pluchea lanceolata (DC) C B Clarke (Asteraceae) on some soil characteristics.

Inderjit\* and K.M.M.Dakshini, Department of Botany, University of Delhi, Delhi 110007, INDIA.

A comparative analysis of twelve chemical variables of soils with and without Pluchea lanceolata, a rapidly spreading perennial weed of semiarid tracts of North West India, was carried out. Results were examined to detect the changes in the soil characteristics that might be responsible for the allelopathic interference success of the weed. Discriminant function analysis as well as factor analysis of the two groups of the soils suggested that the discriminant between the two groups was brought about by five, electrical conductivity, total phenolics, pH,  $Cl^-$  and  $K^+$ , of the twelve chemical variables analysed. Further, it was noted that total phenolics, contributed by the weed, was an important discriminant variable and it influenced other four variables. The weed on one hand, improved the soil by lowering pH, electrical conductivity,  $Cl^-$  and increasing  $K^+$  but by incorporating total phenolics in the soil, made it unsuitable for the growth of crop plants usually grown in these semiarid regions. It has been concluded that the weed, by maintaining continuous availability of phenolics, possesses an allelopathic interference mechanism.

## P - 2 4

The Endogenous Elicitor for Phytoalexin Accumulation of Potato  
NAKAMURA Hideshi, Tsutomu MONDEN, and Akio MURAI  
(Hokkaido Univ., Sapporo, JAPAN)

Phytoalexin accumulation can be induced by the endogenous elicitors which are originated from plants themselves. We have reported that  $H_2O_2$  is formed when potatoes suffer some stresses. As a result, we could detect formation of the endogenous elicitors of potatoes by treating with  $H_2O_2$ .

The bioassays were based on the amount of rishitin, the representative phytoalexin of potato. The water soluble extracts from potato tissues (cv Rishiri) treated with  $H_2O_2$  were purified on a Sephadex G-25, a Sephacryl S-100, a DEAE Sephacel, and a Sephadex G-75 columns. Using 206 kg of the potatoes, we isolated 14 mg of an acidic polysaccharide as a major endogenous elicitor.

The elicitor obtained thus was found to be homogenous by a Sephadex G-75 column and cellulose acetate membrane electrophoreses. The molecular weight of the purified elicitor was estimated to be 9,200. It was shown that the compound was composed of hexoses, uronic acids, pentoses, and 6-deoxyhexoses by colorimetric assays. The elicitor activity appeared at lower concentration than  $50 \mu g/3 ml$ .

The studies on the structure elucidation are now in progress.



THE METABOLISM OF PURINE NUCLEOTIDES AND PURINE ALKALOIDS IN CAMELLIA AND COFFEA PLANTS

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Department of Applied Biology, Faculty of Textile Science,  
Kyoto Institute of Technology, Matsugasaki, Kyoto 606, Japan

\* Department of Biology, Faculty of Science, Ochanomizu University, Otsuka,  
Tokyo 112, Japan

\*\*Department of Biochemistry, Oklahoma Agricultural Experiment Station,  
Oklahoma State Univ., Stillwater, OK 74078, USA

The metabolism of purine nucleotides and purine alkaloids (e.g. caffeine and theobromine) are reviewed in tea and coffee plants (1). Purine metabolism in these plants is similar to that in plants, which do not contain caffeine (1,2). Tea and coffee plants have purine nucleotides, including those formed directly by the pathway of purine biosynthesis de novo, as effective precursors of caffeine and this will be outlined. Xanthosine is the first methyl acceptor from S-adenosylmethionine in caffeine biosynthesis, and it is metabolized by the purine degradation pathway via xanthine and uric acid. The regulation of purine alkaloid biosynthesis remains elusive, but the activity of the three N-methyltransferases is considered as methods for controlling the amount of caffeine in tea and coffee. Ecological roles of the alkaloids have been reported, but their physiological significance in tea and coffee plants remains uncertain. 1) Suzuki, T., Ashihara, H. and Waller, G. R. (1992) *Phytochemistry* 31, in press; 2) Suzuki, T. (1992) *Seikagaku* 64, in press.

Host Cell Recognizing Protein of Endosymbiont of Brown Planthopper

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Kyoto Institute of Technology, Matsugasaki, Kyoto 606,  
\*National Institute of Agrobiological Resources, Tsukuba  
Science City, Ibaraki 305, Japan

Brown planthoppers harbor intracellular bacteria that are transmitted through eggs from their mother insects. Some of those bacteria have been successfully cultivated. Recently, it has been revealed that brown planthoppers incorporate bacteria on leaves of rice plant into their own cells, suggesting a recognizing mechanism between the host cell and the symbiont. We purified the host cell recognizing protein (HCRP) from cell walls of symbiotic bacteria, Enterobacter sp. N1-T-C1-1W.

Enterobacter sp. isolated from an egg of brown planthopper was cultivated in liquid culture. Bacterial cells were degraded by sonication 0.1 M PBS, pH7.4 and cell walls were purified by centrifugation. HCRPs were extracted from the cell wall with 0.1 M PBS containing 1% octyl  $\beta$ -glucoside, and purified by DEAE-chromatography, affinity chromatography using membrane proteins of brown planthoppers as ligands, and gel-filtration. Two HCRPs thus obtained showed ability for specific binding to some organs of brown planthopper.

Detection and Enumeration of Microorganisms in Activated Sludge  
Using 16S rRNA Targeted Oligonucleotide Probe

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Due to ecological complexity, there have been few reports on the study of population structure and dynamics in biological treatment systems. There is currently great interest in the new staining method, based on rRNA sequences that is sufficiently sensitive to identify single microbial cells. We determined a partial 16S rRNA sequence of *Paracoccus kocurii*<sup>(1)</sup>, which was capable of degrading tetramethylammonium contained in waste water from semiconductor manufacturing processes, and synthesized a specific fluorescent-dye-conjugated oligonucleotide probe for *P. kocurii*. This probe was highly species-specific and could differentiate *Paracoccus denitrificans*, having only one base mismatch to the probe, from *P. kocurii*. The growth rate for *P. kocurii* was very slow. More than two weeks were required for the formation of visible colonies on agar plates. Using the fluorescent-dye-conjugated probe, *P. kocurii* in activated sludge could be detected and enumerated by fluorescence microscopy in one day.

(1) Ohara, M. *et al.* 1990. *Int. J. Syst. Bacteriol.* 40:292-296.

Are cardiac glycosides feeding deterrents/transport inhibitors?  
Richardson, David P., Forney, Robert, and Adam C. Messer, Department of Chemistry, Williams College, Williamstown, MA 01267 USA, and University of Shizuoka, Yada 52-1, Shizuoka 422 JAPAN  
The bitter taste of cardiac glycosides functions as a feeding deterrent in several plant groups. However many of these compounds are also potent ion transport inhibitors in vertebrates. We are testing the hypothesis that disruption of insect ion transport processes may represent another mode of action of these plant defensive chemicals, and that unrelated plant groups have converged on similar defensive chemicals for this reason. Ouabain, a specific transport inhibitor from the Apocynaceae, and antiarins and other compounds isolated from Dyak dart poisons prepared from *Antiaris toxicaria* (Moraceae), have been tested in an *in vitro* transport system. The transepithelial potential difference of isolated, everted *Periplaneta americana* recta, which had been stimulated with 8 mM acetate, was measured after treatment of the mucosal (i.e. interior) surface with test solutions. Hindgut tissues did not respond to any of the cardiac glycosides or extracts tested. These results indicate that in insects the cardiac glycosides may inhibit transport by other tissues (i.e., midgut), or that the known serosal effects of compounds like ouabain are the result of structural/functional similarities between ion pumps and taste receptors.

SEASONAL VARIATION IN THE IMPORTANCE OF SUNFLOWER POLLEN ON THE REPRODUCTIVE BIOLOGY OF THE SUNFLOWER MOTH. **Jeremy N. McNeil & Johanne Delisle** Département de biologie, Université Laval, Ste-Foy, P. Q., Canada .

Under long-day conditions (16L:8D) virgin females in the presence of sunflower pollen (known to contain an oviposition stimulant) call at a significantly younger age than those without pollen, at both 15 and 25°C. However, the importance of pollen on calling behaviour was less pronounced when tested under short-day conditions (12L:12D). These findings will be discussed with respect to the seasonal biology of the sunflower moth.

#### ANALYSIS AND IDENTIFICATION OF PHEROMONES FROM MALE GOATS

Noortwijk, Claudia<sup>a</sup>, Over, Robert<sup>b</sup>, Kraus, Wolfgang<sup>a</sup>, and Claus, Rolf<sup>b</sup>  
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Olfactory stimuli (pheromones) extracted from hairs of the parietal region of male goats are effective in inducing cyclic ovarian activity in seasonally anoestrous goats<sup>1</sup>. We report on the analysis of the dichloromethane extract by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) after separation by low-pressure and high-pressure liquid chromatography.

The main fractions consist of fatty acid and 4-ethyl substituted fatty acid esters, and of steroids. Long chain fatty acid - fatty alcohol esters seem to be the matrix of fixatives for the pheromones. The branched fatty acids and esters found in the extract did not elevate the luteinizing hormone pulse frequency in goats.

The steroid fraction mainly consists of cholesterol, its biogenetic precursors, oxidation products, and of 16 $\alpha$ -methylpregnenolone, 5 $\beta$ -pregnane-3 $\alpha$ ,11 $\beta$ -diol-20-one, and androst-5-en-3 $\beta$ -ol.

Biological responses were evoked by the fraction containing the main part of steroids.

<sup>1</sup>R.Claus, R.Over, and M.Denhard, *Anim.Reprod.Sci.*, 22, 27 (1990).

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✓ FATTY ACID PRECURSORS OF UNUSUAL HYDROCARBONS FOUND AS THE ALARM PHEROMONE AND ITS RELATED COMPONENTS IN THE ACARID MITE Tortonia sp.

Kuwahara, Yasumasa, Muneaki Samejima, Zyun-ichi Fukushima, Atsushi Morino, Masashi Sato, Shigeru Matsuyama and Takahisa Suzuki  
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The acarid mite Tortonia sp. is known as a noxious pest attacking nests of Osmia cornifrons (Radozkowski), an important pollinator of apple orchard in Japan. The presence of the alarm pheromone was demonstrated in the mite's extract. The active compound was purified by a conventional method. The structure was established as (Z,Z)-6,9-heptadecadiene and identified by synthesis. Two other uncommon hydrocarbons were detected in the mite, whose structure was concluded as (Z,Z)-4,8-heptadecadiene and (Z,Z,Z)-4,8,11-heptadecatriene by a combination of partial reduction and GC/MS analysis of dimethyldisulfide derivatization.

Because structures of these hydrocarbons were unusual as natural products, fatty acid precursors corresponding to hydrocarbons were surveyed in the mite extract (6.8 g), after hydrolysis and methylation. Unusual methyl dienoate (62 mg) and methyl trienoate (1.9 mg) were isolated by repeated chromatographic separation. Structures of these alkenoates were determined as methyl 5,9-octadecadienoate and methyl 5,9,12-octadecatrienoate, respectively. Z-Geometry were suggested, based on NMR and product's hydrocarbons. (Z,Z)-5,9-octadecadienoic acid is a new compound and (Z,Z,Z)-5,9,12-octadecatrienoic acid is known as pinoleic acid in the plant kingdom. From the present results, it may be reasonable to conclude that hydrocarbons found as the major components of acarid mites were biosynthesized from corresponding fatty acids.

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ROLE OF HALOPHENOLS IN THE TICK RHPICEPHALUS APPENDICULATUS:  
A REAPPRAISAL

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Several halophenols are present in the tick R. appendiculatus, of which 2,6-dichlorophenol was previously shown to play the role of a sex attractant. However, their presence in both sexes of unfed ticks and in immature stages led us to reinvestigate their role. We have shown that the halophenols are attractive to all unfed stages of the tick i.e. they probably play an aggregation role. Feeding by the adults leads to a reduction of the amounts of 2,6-dichlorophenol in males and an increase in females i.e. it becomes a sex attractant.

\* What has ratio data mean from wild moths?

**Effect of Rearing Temperature on Pheromone  
Component Ratio in Potato Tuberworm Moth,  
*Phthorimaea operculella*.**

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The ratio of two components in the sex pheromone of the potato tuberworm moth, *Phthorimaea operculella*, was influenced by the rearing temperature. The percentage of the (E,Z,Z)-4,7,10-tridecatrienyl acetate dropped as the rearing temperature was raised. The total amount of the pheromone did not change parallel with the change of ratio. The critical period sensitive to temperature seemed to be the pupal stage. The temperature in larval stage may also influence the ratio slightly. The mechanism or the meaning of the phenomenon will be also discussed.

Males have broad response range.

Olfactory Modulation of Pheromone-Mediated Flight in Moths

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The response of males to pheromone that results in sustained upwind flight and source location is, for at least some moths, a reiterative process, as recently proposed by our laboratory. We previously hypothesized that each iteration would involve a rapid, phasic, anemotactic response to the onset and offset of a single filament of pheromone in the finely structured plume, the response consisting of an upwind surge. It would also involve a tonic, long-lasting behavioral response to the large parcels of pheromone-free air during a large-scale wind shift, called casting flight, which we only recently recognized as being pheromone-mediated despite its being performed in clean air. Casting is driven by a program of counterturning. In an attempt to understand the behavior of male moths during each iterational response to pheromone ON and OFF, we experimentally manipulated the filament frequencies of *Heliothis virescens* pheromone, and determined the reaction times of *H. virescens* males in response to a single filament of the natural blend of pheromone components. These experiments demonstrated for the first time that moths intersecting a single filament of odor respond by exhibiting a surge of upwind movement. The latency of the response to onset of odor seems to be somewhat variable, but in most cases the moths reacted in less than 0.30 sec. The response consisted of an upwind surge lasting ca. 0.30 sec., and a decrease of the course angles (and resultant track angles) to point the moth more upwind. The single pheromone filament did not extinguish counterturning, and thus the surge retained a zig-zag shape. The surge was also comprised of a slight slowing of the airspeed (and resultant groundspeed), and altitude was much better regulated during the surge as opposed to during casting, before and after the surge. These results predicts that the male must contact, on average, at least 3-4 filaments per second in a natural plume or in experimentally generated filaments in order to maintain an upwind flight track.

Composition, bioactivity and practical application of the female sex pheromone of the Oriental tobacco budworm, *Helicoverpa assulta* (Lepidoptera: Noctuidae). K.S. Boo, K.C. Park, A. Cork, D.R. Hall, and D.K. Reed. Department of Agricultural Biology, College of Agriculture and Life Sciences, Seoul National University, Suwon 441-744, Republic of Korea

Composition and activity of female sex pheromone were studied for the Oriental tobacco budworm, *Helicoverpa assulta*. Extract of *H. assulta* female sex pheromone gland gave 9 different chemicals: hexadecanal, cis-9-hexadecenal (Z9-16:Ald), cis-11-hexadecenal (Z11-16:Ald), hexadecyl acetate, cis-9-hexadecenyl acetate (Z9-16:Ac), cis-11-hexadecenyl acetate, hexadecanol, cis-9-hexadecenol (Z9-16:OH), and cis-11-hexadecenol. Approximate ratio of the two major components, Z9-16:Ald and Z11-16:Ald, was 13.3:1. The best ratio of the two components for attracting *H. assulta* male adults was 20-25:1. Addition of Z9-16:Ac enhanced attracting power of the two-component mixture when tested in hot pepper fields. But, when the lure included Z9-16:OH, its attracting activity greatly decreased. Anyway, the two- or three-component mixture was much more powerful in attracting male adults when tested against 2 live female adults. PVC-based controlled release resin dispenser loaded with synthetic sex pheromone components showed a good biological activity in disrupting adult mating in the fields. And their attractancy was stable for a considerable length of time under varying weather conditions outdoors. This pheromone mixture was also more effective in forecasting their first appearance date than black-light traps. For this species cone trap was much better than universal trap.

**FUNCTION OF CONTACT SEX PHEROMONE IN THE MATING BEHAVIOR OF THE SUGI BARK BORER (*Semanotus japonicus* Lacordair)**

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<sup>1</sup>Kansai Research Center, Forestry and Forest Products Research Institute, Momoyama, Fushimi-Ku, Kyoto 612, Japan.

We observed males of the sugi bark borer mount females to copulate after recognizing a sex pheromone in female cuticular wax through their antennae. The wax was extracted with hexane and purified on a silica gel column. The active fraction inducing males to mount on an extract-treated glass rod was separated by hexane elution. The active eluate was further fractionated on a gel permeation chromatograph and analyzed with gas chromatography-mass spectrometry.

Males perceive the sex pheromone with their antennae and mouthparts (maxillary and labial palpi). Findings on the chemoreceptors of the contact sex pheromone both by electron microscopy and behavioral response will be discussed.

Compounds branched hydrocarbons. Detected by antennae + mouthparts.  
n-C<sub>25</sub> 13Me-C<sub>25</sub>, 7,11e-C<sub>25</sub>, + many others  
Found in both sexes, but males also produce inhibitory compounds.

**Identification of Anomala schonfeldti OHAUS Sex Pheromone Using a High Resolution GC-Behavior Bioassay**

LEAL, Walter Soares (National Institute of Sericultural and Entomological Science, Tsukuba, Ibaraki 305 Japan), Makoto HASEGAWA and Masaaki SAWADA (Chiba Prefectural Agricultural Experiment Station, Daizenno-cho, Midori-ku, Chiba 266 Japan)

Despite their economic importance as agricultural pests and the high demand for safer agrochemicals, our knowledge of the pheromone chemistry of scarab beetles is still very limited, mainly due to the lack of uniform and consistent laboratory bioassays. Recently, this barrier has been overcome by coupling GC-EAD with simplified laboratory- or field-bioassays. Nevertheless, attempts to apply GC-EAD to beetles with small antenna, such as Anomala schonfeldti (body length, 10-12 mm), were unrewarding. We used, instead, high resolution gas chromatography-behavior bioassay (GC-BB), which played a pivotal role in the characterization of the active compound. The attractancy of the synthetic sex pheromone was tested in a wind tunnel (2m long, 30 cm I.D.) in a clear room. With one female equivalent, 100% of the males took upwind flight, 80% approached the pheromone source and 60% landed on the edge of the FP where the pheromone was applied. The pheromone is unlikely to be biosynthetically related to the ones previously identified in scarab beetles.

*E2-nonanal. Identified by bioassay chamber attached to GC outlet. Excellent.*

*Get papers*

**Pheromone Perception in Scarab Beetles. Are the Pheromone-Sensitive Sensilla Pore-Less?**

LEAL, Walter Soares, Fumiaki MOCHIZUKI, Masuhiro TSUKADA (National Institute of Sericultural and Entomological Science, Tsukuba, Ibaraki 305 Japan) and Makoto HASEGAWA (Chiba Prefectural Agricultural Experiment Station, Daizenno-cho, Midori-ku, Chiba 266 Japan)

As in other scarab beetles, a single component has been identified as the sex pheromone of Anomala cuprea, namely, (R,Z)-5-(-)-(oct-1-enyl)oxa-cyclopentan-2-one. In order to investigate the occurrence of minor component(s) (which could not be elucidated by GC-EAD or behavioral observations), we carried out an electrophysiological study of the chemoreception in the beetle. In marked contrast to the hair-like sensilla of other insects, A. cuprea possesses four types of sensilla placodea and coeloconica on the inner surfaces of the lamellae. Although the pores of three types of plates could be easily visualized by SEM, we failed to identify any openings in the fourth type, which covers most of the inner area of the lamellae. Nevertheless, this type of plate turned to be the pheromone-sensitive, as revealed by single cell recordings. It responded to the (R)- (the naturally occurring pheromone) but not to the (S)-enantiomer even at a 10 ng level. However no inhibitory effect of the latter was found.

**Antimicrobial Activity of Some Sex Pheromones**

BS LEAL, Walter Soares and Akira SHIRATA (National Institute of Sericultural and Entomological Science, Tsukuba, Ibaraki 305 Japan)

Apart from communication, semiochemicals such as alarm pheromones are known to play other important roles in defense, for example. However, it has been generally considered that sex pheromones play the single role of behavior-modifying and their other ecological significances have been mostly overlooked. Although the beetle *Holotrichia parallela* utilizes its sex pheromone for ca. 10 min every other day, it possess large amounts of the chemical even during the photophase. Therefore, we hypothesized a defensive role of the chemical and investigated its antimicrobial activity against the fungi and bacteria which attack the roots of the host plants, *Morus* spp., where the insects remain during day time. The pheromone was active against all microorganisms tested whereas the pheromone of another scarab beetle (which is not a pest of mulberry) was inactive against most of the same microorganisms. Furthermore, vapors of these and other sex pheromones demonstrated antifungal activity against insects parasites such as *Beauveria bassiana* and *Metarhizium anisoplae*. This raises the question whether it is environmentally safe to utilize sex pheromones at high concentrations in the field for mating disruption.

**THE OLFACTORY RESPONSE OF THE CIGARETTE BEETLE, *Lasioderma serricorne* Fabricius, TO THE SEX PHEROMONE COMPONENT AND ITS BEHAVIORAL INHIBITOR**

Okada, Kentaro, Akihiko Watanabe, Masataka Mori, Kazuko Shimazaki, Yoshisuke Nishi, Tatsuji Chuman, Shigeru Sato,<sup>†</sup> Fumiaki Mochizuki<sup>††</sup> and Tatsuaki Shibuya,<sup>††</sup> Life Science Research Laboratory, Japan Tobacco Inc., 6-2, Umegaoka, Midori-ku, Yokohama, 227, Japan, <sup>†</sup>Central Institute for Electron Microscopic Researches, Nippon Medical School, Tokyo 113, Japan, <sup>††</sup>Institute of Biological Sciences, University of Tsukuba, Ibaraki 305, Japan.

Electroantennogram (EAG) and single sensillum recordings were performed on the male cigarette beetle, *Lasioderma serricorne*, antennae. Strong EAG responses were elicited by not only natural sex pheromone component (4S, 5S, 7S)-serricornin (SSS-serricornin) but also non-natural behavioral inhibitor, (4S, 5S, 7R)-serricornin (SSR-serricornin). In the case of mixture application of the SSS- and SSR-serricornin, integrated EAG responses were observed. Single sensillum recording from male flagella sensilla showed that majority of basiconic type sensilla responded to the behavioral inhibitor as well as to the sex pheromone. However, a few of them responded only to the inhibitor. Microscopic observation revealed that the basiconic sensilla contained two olfactory receptor cells. These findings suggest that one of the two receptor cells in the basiconic sensilla responds to the natural pheromone and the other cell responds to its behavioral inhibitor. The behavioral inhibitory action of SSR-serricornin toward the SSS-serricornin results from central integration of the information from separate receptor cells rather than blockage at the peripheral receptors.



**INHIBITION OF IPSDIENOL AND *E*-MYRCENOL PRODUCTION BY COMPACTIN: DEMONSTRATION OF *DE NOVO* PHEROMONE BIOSYNTHESIS IN *IPS DUPLICATUS* (SCOLYTIDAE: COLEOPTERA)**

Per Ivarsson, Göran Birgersson

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The biosynthesis of ipsdienol and *E*-myrcenol was studied by using the enzyme inhibitor compactin (mevilonin). The inhibitor blocks the action of hydroxymethylglutaryl-coenzyme A reductase, an early enzyme in the terpenebiosynthesis. The compactin were injected in the beetle's abdomen using a thin tapered glass needle. Controls were, using the same technique, injected with physiologic salt solution (0.9 % NaCl). To rule out the possibility that the inhibitor causes an unspecific metabolic depression of the beetles, *Ips typographus* was subjected to the same treatment and monitored for the hydroxylation of (-)- $\alpha$ -pinene, giving *cis*-verbenol. The production of *cis*-verbenol is not affected by compactin. By showing that the production of ipsdienol, and *E*-myrcenol is reduced by approximately 70 % and 40% respectively, as the beetles are injected with compactin, we conclude that ipsdienol and *E*-myrcenol can be synthesised *de novo* and not only as a hydroxylation of myrcene, present in the resin of the attacked trees.

**AGGREGATION BEHAVIOR OF THE PINE ENGRAVER BEETLE, *IPS PINI*, IN RESPONSE TO IPSDIENOL AND LANIERONE** Steven J. Seybold<sup>1</sup>, Yoshihiro Yokokawa<sup>1</sup>, Isao Kubo<sup>1</sup>, Toshikazu Ohtsuka<sup>1</sup>, Masato Nomura<sup>1</sup>, Stephen A. Teale<sup>2</sup>, and David L. Wood<sup>1</sup>, <sup>1</sup>Department of Entomological Sciences, University of California at Berkeley, Berkeley, California 94720, <sup>2</sup>Department of Biology, State University of New York, Syracuse, NY 13210.

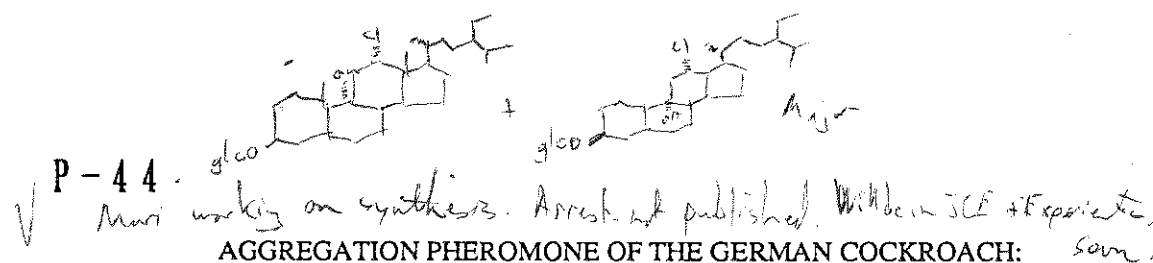
A population of *Ips pini* in California was analyzed for production of and flight response to two aggregation pheromone components, ipsdienol (2-methyl-6-methylene-2,7-octadien-4-ol) and lanierone (2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one). Males produced predominantly (*R*)-(-)-ipsdienol (92%-98%-(*R*)-(-)), but did not produce detectable levels of lanierone (found in *I. pini* in eastern North America). The enantiomers of ipsdienol (>99.7% e.e.) were separated by normal phase, preparative R-HPLC of synthetic racemic ipsdienol and (*R*)-(-)-ipsdienol attracted both sexes of *I. pini* dose-dependently in the field with a detection level between 10 and 100  $\mu$ g/day. (*S*)-(+)-ipsdienol inhibited the response dose dependently (significant inhibition between 1% and 10% (*S*)-(+)-ipsdienol). The absolute configurations of synthetic and naturally derived ipsdienol were confirmed by CD spectroscopy of the benzoates. Though lanierone is not produced by this population, adding four doses of it to 99.4%-(*R*)-(-)-ipsdienol elicited a significantly higher flight response, perhaps indicating a relictual behavior that relates populations of *I. pini* from eastern and western North America.

- ✓ Localization of Sex Pheromone Gland in Females of Ascogaster reticulatus Watanabe (Hymenoptera: Braconidae), an Egg-Larval Parasitoid of the Smaller Tea Tortrix, Adoxophyes sp. (Lepidoptera: Tortricidae)

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A sex pheromone of Ascogaster reticulatus Watanabe (Hymenoptera: Braconidae) was identified as (Z)-9-hexadecenal (Kainoh et al., 1991), which elicits antennal mate-searching behavior in males. In order to localize the source of sex pheromone production, each body part was separated and extracted and the extract was subjected to 9-cm petri dish bioassay. The highest activity was found in thorax. Among legs, wings, and thorax, legs have the highest activity. Among fore, middle, and hind legs, hind legs have the highest activity. Among coxa and trochanter, femur, tibia, and tarsus, tibia has the highest activity. From these results, the presence of tibial sex pheromone gland was suggested.



#### ATTRACTANTS AND ARRESTANTS IN THEIR SHELTER

Masayuki Sakuma and Hiroshi Fukami

(Pesticide Research Institute, Kyoto University, Japan)

The German cockroaches, *Blattella germanica* (L.), were known to produce aggregation pheromone with their frass. Usually they harbor in a shelter and sometimes come out for searching food and water, but return to it again. The shelter is contaminated with their frass, so the pheromone helps them to find and settle in it. Several attempts were made for the isolation, but no conclusive results have been reported. We started with re-designing the behavioral assay system instead of a popular vertical paper assay. We developed both olfactometer and choice-chamber assays to distinguish attractant and arrestant components. The attractants were given as odor, and 'chemotaxis' and 'odor modulated anemotaxis' were evaluated with the olfactometer. From the methanol extract of the shelter, several alkylamines and 1-dimethylamino-2-methyl-2-propanol were isolated. The arrestants were, on the other hand, detected with choice-chamber assay as 'contact chemicals'. They were found in a different fraction from that for the attractants and eventually estimated as 12- $\alpha$ -chloro-9,11 $\alpha$ -epoxy-5 $\alpha$ -stigmast-3 $\beta$ -yl- $\beta$ -D-glucopyranoside and 12- $\alpha$ -chloro-9 $\alpha$ -hydroxy-5 $\alpha$ -stigmast-3 $\beta$ -yl- $\beta$ -D-glucopyranoside denoted as Blattellastanoside A, B, respectively.

≡ App. Ent + Zool. 1990

Arrestant, smell, host/sex,  $\text{NaOH}$ -water, Acid base, 1990.

P - 4 5

- ✓ **Chemical Aspects of Sex and Nymph Discrimination by the Male Cockroach, *Nauphoeta cinerea* (Olivier)** FUKUI, Masao and Shozo TAKAHASHI  
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Kyoto, 606-01, Japan

Sex and nymph discrimination by males was investigated in the cockroach, *Nauphoeta cinerea*. A sexually mature male takes a courting position (wing-raising) toward a sexually mature female when he comes into contact with her and recognizes the female on a chemical basis through antennal contact. The males also show wing-raising behavior toward teneral adults and nymphs. However, mature males often behave aggressively toward each other. Mature males secrete an intermale recognition pheromone as they mature. The pheromone was identified in the cuticular wax of mature males to be octadecyl (Z)-9-tetracosenoate.

In contrast, mature males do not show conspicuous behavior toward nearby nymphs, although they showed wing-raising behavior toward teneral nymphs. At post-teneral period, nymphs produce a wing-raising depressant in their cuticular wax. The depressant was found to be a mixture of *cis*-25,26-epoxy-henpentacontadlenes.

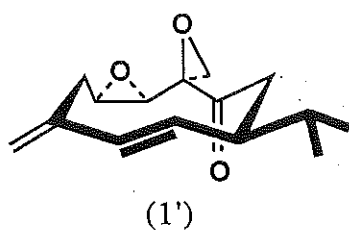
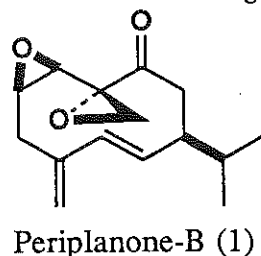
The mode of sex and nymph recognition by mature males is discussed.

P - 4 6

- ✓ **The Importance of Conformational Property on the Biological Activity of Periplanone Analogs**

Mori, Masataka, Kentaro Okada, Kazuko Shimazaki, Akihiko Watanabe, and Tatsuji Chuman, Life Science Research Laboratory, Japan Tobacco Inc., 6-2 Umegaoka, Midori-ku, Yokohama, Kanagawa 227, Japan.

Periplanone-B (1) is a major sex pheromone component of American cockroach, *Periplaneta americana* L. It was shown that (1) preferentially exists in a conformer (1'). In our study on the structure-activity relationships of natural pheromones and their synthetic analogs, we have pointed out the conformational resemblance between (1') and each of the major conformers of bio-active analogs. Computational chemical methods (molecular mechanics and molecular orbital calculations) could provide the quantitative evaluations of the conformational and electronic properties, and good correlation was obtained between these parameters and the biological activities. The structural factors for generating the biological activity will be discussed.



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Chemistry and Biology of Sex Pheromone Volatiles from Caribbean and Mexican Fruit Flies. Nation, James. Dept. of Ent. & Nem., Univ. of Florida, Gainesville, FL 32611-0620.

The volatile compounds released by male Caribbean fruit flies, A. suspensa, and the Mexican fruit fly, Anastrepha ludens, have been identified. Males of both species typically form leks and attract females by releasing a multi-component volatile pheromone. Males of both species emitted the isomeric lactones, anastrephin, epianastrephin, and suspensolide, (Z)-3-nonenol and (Z,Z)-3,6-nonadienol,  $\beta$ -bisabolene,  $\alpha$ -farnesene, and  $\alpha$ -trans-bergamotene. A. suspensa released the monoterpene cis- $\beta$ -ocimene, while A. ludens released limonene. There are also differences in the ratio of the other components between the 2 species. An aliquot of a solution of volatiles from Caribbean males equal to about 15 sec of release time by one male ( $3 \times 10^{-3}$  Male Hour Equivalent) attracted females in a bioassay. A light entrained circadian rhythm determines the peak of pheromone release, but small amounts of pheromone volatiles were released during the scotophase. Exposure to continuous light for even one 24-hour period, as well as for several days, greatly altered the quantity and ratio of pheromonal compounds released.

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VOLATILE COMPONENTS EMITTED FROM THE EUROPEAN HONEYBEE COLONY AND THEIR DAILY FLUCTUATION

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Dispersion of a characteristic flavor is noticed by the authors from the colony of the Japanese honeybee Apis cerana Japonica during the hiving-off, however, suitable colony of the species was not available to study. Therefore, daily volatiles from a colony of the European honeybee Apis mellifera L. was examined, especially focusing on the season of hiving-off.

Volatiles of the colony was trapped by a Porapak Q column and was eluted by hexane. The eluate was analyzed by GLC (100-200°C, 4°C/min, at temperature programed condition, using a CP-Sil 19CB fused silica capillary column) and by GC/MS.

More than 70 peaks were observed by GLC; parts of major peaks appeared constantly in the chromatogram, while several other peaks appeared and disappeared at daily basis. At present no relationships were conclusive to the activity of bees in the hive.

Monoterpene hydrocarbons,  $\alpha$ - and  $\beta$ -pinene ( $M^+$ ,  $m/z$  136) were suggested by GC/MS analysis and their structures were identified by co-chromatography to authentic samples. Presence of at least 6 sesquiterpene hydrocarbons ( $M^+$ ,  $m/z$  204 and  $m/z$  202) were detected, among which muurolene, cadinene, cubebene and calamene were suggested by comparison of mass spectra with reported data. Further analysis on volatile components are now in progress.

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✓ HYDROCARBON PROFILES IN THE JAPANESE, THE EUROPEAN AND THE ASIAN HONEYBEES,  
FOCUSING ON THEIR ANNUAL AND SEASONAL CHANGES ALONG WITH THEIR HIVING OFF.

Sasagawa, Hiromi\*, Yoshie Kizawa and Yasumasa Kuwahara  
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When a group of the Japanese honeybee *Apis cerana japonica* Rad.(Acj) were artificially transferred to the other colony, almost all bees were accepted without any problem, while such a transfer was impossible for the European honeybee *Apis mellifera* L.(Am). As the representative components of the body surface, therefore, hydrocarbon(HC) profiles of workers and drones were compared among three species for four years, to obtain some clues of hive-mate recognition using GLC and GC/MS. These species were distinguishable not only by the combination of components, but also by the occurrences of specific components to each species.

HCs consisted of normal C<sub>23</sub>-C<sub>33</sub> with various degree of unsaturation (0 to 3). Presence of alka-6,9-dienes(C<sub>25</sub> to C<sub>29</sub>) was characteristic of Acj, while Am possessed more than three methylene unit divided dienes. HC profiles among individual workers and drones of a hive were different in Acj, Am and the Asian bee *Apis cerana indica* as well. The HC profiles from drones were divided into two patterns, workers were divided into three to ten patterns, depending possibly on the queen's multiple mating. Although the number of patterns remained unchanged, profiles differed seasonally in Acj workers and, possibly in Am worker as well. Numbers of patterns differed yearly from the Acj colony at the same site, possibly due to multiple hiving off. Possible mother-daughter relationships among colonies of Acj and Am were also discussed.

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IMPORTANCE OF SIMILARITY IN CUTICULAR HYDROCARBON COMPOSITION  
ON COMMENSALISM BETWEEN ANTS AND THE MYRMECOPHILES.

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Several commensals are found in ant nest. In some cases, the ant is so carniborous that the organisms living with should develop some special way to avoid its predation. We speculate that the special magic shown by the commensals might be similarity of cuticular hydrocarbon composition that had been pointed out as a species recognition signal candidate.

According to our observation of several combinations of ants and myrmecophiles before living together, the myrmecophil had its original CHC which was completely different from that of the ant afterward it will live together. But after living together the CHC changed dramatically and became similar to that of the ant. The similarity of CHC is the reason why the commensals can avoid the attack of predacious ants. We shall also show how do the commensals obtain the CHC from the ants.

CHEMICAL MIMICRY BETWEEN A PARASITIC ANT  
(*FORMICOXENUS PROVANCHERI*) AND ITS HOST (*MYRMICA  
INCOMPLETA*)

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*Formicoxenus provancheri* is an ant species living in close relation to its host *Myrmica incompleta*. This form of parasitism is called *Xenobiosis*. To understand how the parasites can introduce into the *Myrmica* colony, the cuticular hydrocarbons were compared. It appeared that they are very similar between the two species. We will discuss also the origin of the guest hydrocarbons.

ISOLATION AND IDENTIFICATION OF AN OVIPOSITION STIMULANT  
FOR *APHYTIS MELINUS* FROM ITS HOST, CALIFORNIA RED SCALE

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Luck and Uygun (1986) reported that the parasitoid, *Aphytis melinus* Debach (Hymenoptera: Aphelinidae) oviposited on disks of filter paper treated with either water or ethanol extracts of covers of the California Red Scale, *Aonidiella aurantii* (Homoptera: Diaspididae), and other diaspidid scale species. We will describe the isolation, identification, and synthesis of the kairomone, which is an ester of tyrosine and caffeic acid.

*A. melinus* shows a curvilinear response to increasing concentrations of the kairomone in laboratory bioassays on filter paper disks mimicking scale covers. Such a response may account in part for the apparent variation in behavioral acceptability of scales of different age or size by *A. melinus*.

The chemical properties of this kairomone differ substantially from those of the kairomonal mixture of wax esters from the cover of *Unaspis vanonensis* (Kuwana), another Diaspidid scale infesting citrus, which is parasitized by the *Aphytis* species, *A. vanonensis* (Takahashi et al., 1990).

## ISOLATION AND IDENTIFICATION OF KAIROMONES UTILISED BY SOUTHERN PINE BEETLE PARASITIDS

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<sup>1</sup>) present address: Chemical Ecology, Göteborg University, Göteborg, Sweden

We are currently studying the volatile compounds that help parasitoids to find their host trees and larvae and pupae under the bark. Our hypothesis is that general compounds, as oxygenated monoterpenes, guide the parasitoid females to an attractive bark beetle infested tree. After the females have landed on the trees, they actively search on the surface for late instar larvae beneath the bark.

Volatiles from bark beetle infested pines, which were attractive or non-attractive to parasitoids, were collected on Porapak Q columns. Extracts from the columns were analysed using combined gas chromatography - mass spectrometry (GC-MS). Chromatograms from different trees, (*i.e.* attractive and non-attractive) were analysed qualitatively and semi-quantitatively. The amount of each compound was based on arbitrary area units in the chromatograms, aeration time, and extract volume.

Although hundreds of compounds were identified or described, most of them were present in all samples. However, there were significant differences in amounts of oxygenated compounds. For example, the releases of oxygenated monoterpenes such as camphor, fenchone, isopinocampone, pinocampone, *trans*-pinocarveol,  $\alpha$ -terpineol, and *trans*-verbenol, are much higher from attractive trees than from non-attractive ones. The release of 4-allyl anisole and bornyl acetate from attractive shortleaf pines is much higher than from non-attractive ones, while their release is very low from all Virginia pines.

Several of these oxygenated compounds have been tested in field bioassays and in electroantennogram analyses. Data indicate that some of the tested compounds are readily detected by parasitoids and that attraction occurs.

## Attractants from Wheat Infested by Sawtoothed Grain Beetle

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The sawtoothed grain beetle, *Oryzaephilus surinamensis* is one of major world-wide pests of stored grain. In 1988, we found that the hexane extract of wheat grain damaged by rice weevil, *Sitophilus oryzae*, contained the hydrocarbons as repellents against the sawtoothed grain beetle. The activity was tested by using the two-choice paper disk method described before. Recently we also found that the hexane extract of wheat flour infested by the sawtoothed grain beetle showed attractant and/or arrestant activities. From the extract two active compounds 1 and 2 were isolated and identified as 13-oxo-9-octadecenoic acid and 15-oxo-11-eicosenoic acid respectively, by IR, NMR, MS and GC analyses of both compounds and their derivatives (methyl esters and ozonolysis products). Compound 1 exhibited higher attractant activity than compound 2. Another active fraction which contained three major components with the similar steroidal structures was obtained from the extract. Based on spectral and GC analyses and syntheses the components were identified as stigmastan-3-one, ergostan-3-one and cholestan-3-one. The functions of these semiochemicals remain to be clarified.

P-55

Cerambycid attractant

Attractants for the Japanese Sugi Twig Borer, Anaglyptus subfasciatus (Coleoptera: Cerambycidae)

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Anaglyptus subfasciatus is one of the most harmful insect pest against Cryptomelia japonica and Chamaecyparis obtusa, the two most abundant tree species in Japan. The larva bores into the trunk via dead twig, following the introduction of microbes. The wood is, therefore, heavily dequalified by the larval excavation and the relating fungus infection causing colored stains. To monitor the population or to control the beetle, we have been studying the trapping method utilizing attractants derived from flowers, as both sexes of adults tend to aggregate to white-yellow small flowers beside the stand. Fifty two candidate compounds originating from flower components were first screened by EAG and on an olfactometer, then by the field trapping. Among six compounds [benzyl acetate(BA), neryl acetate, linalyl acetate, phenylethyl propionate, linalool and citronellal] selected, BA showed the strongest attractiveness in the field trapping. Methylphenyl acetate, a non-natural chemical which structure is very similar to BA, attracted much more beetles and fewer non-target insects than BA.

Add sorbic acid to water in trap to stop decay.  
Prelim. work submitted Jap. J. Forstry.

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Attractancy of Cryptoporus volvatus to Ischnodactylus loripes

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Cryptoporus volvatus(Peck)Shesr occurred in a ponderosa pine forest weakened or killed by fire. When the sporophore of the fungus was nearly mature, the fruits body secretes odoriferous compounds. A beetle, Ischnodactylus loripes Lewis infests the fruits body. In the present study, we considered the chemical relationship between the fungus and insects. The volatile components of the fungus examined by means of GC-MS. The individual components showed no chemical attractancy. It was found that the attractancy of the fungus for Ischnodactylus loripes were found to be the synergistic action of undecatriene together with pinocampnone, pinocarveol.



CHEMICAL BASIS OF DIFFERENTIAL OVIPOSITION BY SOME *PAPILIO* BUTTERFLIES

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- (1) *Ovipositional Responses of Papilio protenor and P. xuthus to Phellodendron amurense (Rutaceae)*: These two sympatric species mainly feed on *Citrus* plants. The latter occasionally utilizes *P. amurense* as a host, while the former never does in nature, though water-soluble components of *P. amurense* strongly stimulate oviposition by both species. It was found that a flavanol glycoside, phellamurin, present in another fraction regulates the host selection in these butterflies.
- (2) *Ovipositional Responses of P. protenor and P. memnon to Fagara ailanthoides (Rutaceae)*: Whereas the latter feeds only on *Citrus* plants, the former infests also *F. ailanthoides*. Chlorogenic acid, which had previously been shown to be one of oviposition stimulants for the former, was found to deter the egg-laying by the latter.

Pharmacophagous Association of the Turnip Saw Fly, *Athalia rosae*, with *Clerodendron trichotomum* (Verbenaceae)Nishida, Ritsuo, Takashi Amano, Kunji Kawai<sup>1</sup> and Hiroshi Fukami<sup>2</sup>

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The turnip saw fly, *Athalia rosae ruficornis*, feeds exclusively on the plant family Cruciferae during the larval stage. However, the adults frequently visit leaves of a shrubby plant, *Clerodendron trichotomum* (Verbenaceae). A series of diterpenoids (clerodendrins B, D and H) were characterized as the potent phagostimulants for the adults. The insect selectively incorporated some of these bitter-tasting analogs (C and D) in the body, and thus obtained a defensive measure against predatory animals (e.g. birds and lizards). In addition, *C. trichotomum* was found to provide a rendezvous site to the turnip saw fly. Females that had been exposed to *Clerodendron*-leaves became more attractive to the males, suggesting a pheromone-like role of the leaf component(s) in their courtship sequence. The pharmacophagous association of *A. rosae* was also observed with some *Ajuga* species (Labiatae) which also contained neo-clerodane diterpenoids. (Present: <sup>1</sup>Mitsubishi Kasei Corp., <sup>2</sup>Ritsumeikan University)

Sawfly - brush leaves, then extend to get trichomes.  
 Feed plants much more attractive. Corp. Fed. rejected by predators. Flies induced to feed by the compounds. Ex. coll. J. CE 1990.

Feeding and Oviposition Deterrence of Globemallow, *Sphaeralcea emoryi* (Malvaceae), to The Boll Weevil, *Anthonomus grandis* BOH. (Coleoptera: Curculionidae) HIROSHI HONDA (University of Tokyo, Tokyo 113, Japan) and WILLIAM S. BOWERS (The University of Arizona, Tucson 85721, USA)

NFG The globemallow, *Sphaeralcea emoryi*, which is native to Arizona, is significant alternate host plant for the boll weevil, *Anthonomus grandis* Boh. This study was initiated to identify globemallow allelochemicals that limit attack by the weevils in order to introduce useful genes of wild resistant plants to cotton lines. Boll weevils were deterred from feeding and oviposition in the flower buds unless the calyx was removed. Even male and virgin female weevils were discouraged from feeding as much as gravid weevils. Deterrent agents in the flower buds may be primarily a feeding deterrent and so as chemicals are preventing oviposition. The feeding deterrents were highly concentrated in the calyx of the buds, and the most potent deterrent activity was found in the methanol extract of the calyx. Boll weevils were able to perceive the deterrents with contact chemosensory organs on antennae, maxillary palpi and labial palpi.

Mechanism by which kidney beans are protected from bean weevil infestation Pitiyont, Bongotrat†, Vlnai Pitiyont†, Hiroshi Honda and Izuru Yamamoto Department of Agricultural Chemistry, Tokyo University of Agriculture Setagaya, Tokyo 158, Japan † Department of Agriculture (Thai Government), Bangkok, Bangkok 10900 Thailand

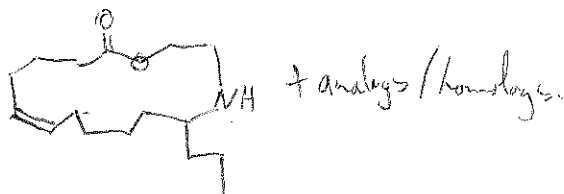
Bean weevils (*Callosobruchus chinensis*, *C. maculatus*) deposit the eggs on kidney beans (*Phaseolus vulgaris*). The hatched larvae penetrate into the beans but die at the first instar. The larval growth inhibitor for *C. chinensis* was isolated and characterized as a N-glycosylprotein having M.W. 48,000 and isoelectric point 4.48 with several subunits. The content was ca. 0.5%. It showed  $\alpha$ -amylase-inhibitory activity. Both carbohydrate and native protein moieties seemed to be required for growth and  $\alpha$ -amylase-inhibitory activities. The carbohydrate content was 15.2% based on 20 mannose, 1 fucose, 2 xylose and 15 N-Ac-glucosamine residues per one mole. It inhibited  $\alpha$ -amylases of animal origins, but not those of plant and microbial origins. In the past for the larval growth inhibitor, saponin was proposed without evidences; trypsin-inhibitory activity was not shown; Janzen et al. (1976) found that lectin added to an artificial diet caused larval growth inhibition of *C. maculatus* and Gatehouse et al. (1984) confirmed it and suggested that the ingested lectin causes disruption of the epithelial cells of the larval midgut leading to a breakdown of the transport of nutrient into these cells. However, Ishimoto and Kitamura (1988) and Smitanond et al. (1990) claimed that lectin was not involved in the larval growth inhibition of *C. chinensis*, implying the unavailability of starch due to the  $\alpha$ -amylase inhibitor.

NOVEL MACROCYCLIC ALKALOIDS FROM A COCCINELID  
BEETLE

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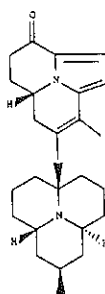
**Abstract**---A new group of macrocyclic alkaloids have been isolated and characterized from pupae of a coccinellid beetle. Structure assignment of the major alkaloid is based on mass spectrometric, infrared, and proton NMR data, as well as on microchemical transformations.



Exochomine, a Dimeric Ladybird Alkaloid isolated from  
*Exochomus quadripustulatus* (Coleoptera: Coccinellidae).

Daloze, Désiré<sup>a</sup>, Timmermans, Michel<sup>a</sup>, Braekman, Jean-Claude<sup>a</sup>,  
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Many species of ladybird beetles are protected against predators by bitter alkaloids present in their haemolymph. Till now, about 15 of these compounds have been described. We report now on the isolation and structure determination of exochomine, a dimeric alkaloid of the European ladybird *Exochomus quadripustulatus*.

The molecular structure including the absolute configuration was solved by an X ray diffraction analysis on exochomine hydrochloride. The NMR assignment of most hydrogen and carbon atoms based on extensive 1D and 2D methods will be discussed. Preliminary data on the biological activity and ecological significance of exochomine will also be presented.

+ other compounds

VARIATION IN DEFENSIVE SECRETION COMPONENTS OF INDIVIDUAL LEAF BEETLES *Oreina gloriosa* (COLEOPTERA: CHRYSOMELIDAE)

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Chemical defense is thought to be the result of evolutionary adaptation which requires (heritable) genetic variation. We examined genetic sources of variation in the chemical defense of individual, adult leaf beetles *Oreina gloriosa*. The chemical defense of this species is based on the exocrine secretion of a complex mixture of cardenolides, ethanolamine and tyrosine betaine. Genetic differences explained a considerable part of the total variation in secretion composition. Thus, predators may be still directing the evolution of the chemical defense in *O. gloriosa*. The average heritability in the concentration of 16 secretion components of 221 laboratory-reared beetles was 0.31 leaving a 69% variation which was partially accounted for by physiological factors. Ageing was correlated with significant increases (up to 3.5-fold) and decreases (up to 2.7-fold) of concentrations of several components. Differences between the sexes were smaller, but concentrations of 7 components were higher in females than in males. The effect of mating on 4 components in females was significant. In these components the concentrations increased up to 1.6-fold after pairing, suggesting that the secretions could also be involved in the reproductive biology of *Oreina gloriosa*.

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THE EFFECTS OF AGE ON CARDIAC GLYCOSIDE CONCENTRATION IN MONARCH BUTTERFLIES.  
Alonso-Mejia, Alfonso.

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Monarch butterflies possess bitter and emetic cardiac glycosides (CGs) that reduce their susceptibility to bird predation. I tested the hypothesis that the concentration of these compounds decreases as the adult ages. Monarchs were reared on two foodplants, one containing uniformly large amounts of CGs (*Asclepias humistrata*), and the other with less but more variable amounts (*A. syriaca*). Recently hatched monarchs were released in a flight cage. Butterflies were then collected over a wide range of ages. I examined CG concentration for different body regions. My results show that the concentration of CGs decreased as individual butterflies age regardless of the initial amounts or the CG types. High rates of change were found in abdomens and wings. Thoraces showed no decrease. The mechanism of this age-related decline in CG content may result from CG denaturation, physical scale loss, excretion, and/or an increased molecular binding of CGs to the exocuticle over time. Consequently, monarchs can change from being unpalatable models to palatable mimics during their lifetime. Since the butterflies breed continuously over the spring and summer, freshly emerged monarchs will serve as noxious models for the older individuals that have become automimics.

CHEMISTRY AND COEVOLUTION AT THE INTERFACE BETWEEN NEOTROPICAL BUTTERFLIES AND THEIR NATURAL HOST PLANTS. Keith S. Brown Jr., Clécio Klitzke, José Roberto Trigo, Silvana A. Henriques, Carina Berlinger, P.E. Rubbo-dos-Santos, André V.L. Freitas and Paulo Cesar Motta (Dept. de Zoologia, Instituto de Biologia, Universidade Estadual de Campinas, C.P. 6109, Campinas, São Paulo 13.081 Brazil)

Using standard mild fractionation methods for fresh material, active chemical classes and compounds were isolated from host plants actually used by local populations of Ithomiinae (Lepidoptera: Nymphalidae; feeding on Apocynaceae and Solanaceae, 5 and 42 species extracted) and Papilionidae (14 Aristolochiaceae, 4 Rutaceae, 3 Annonaceae, 3 Lauraceae, 2 Magnoliaceae, 2 Piperaceae) and also from adult insects whose larvae fed on these plants, in order to test hypotheses of biochemical coevolution, chemical defense, adaptive colonization of hosts, and chemical mediation of host selection. Among the active principles discovered were alkaloids (mostly quaternary), phenolics, glycosides, various acids, and terpenes. Confrontation of the phylogenies of Ithomiinae genera and their hosts suggests that advancing butterflies colonized progressively more primitive host plants, by pre-adaptation to the novel defensive chemicals they contain. Only one species (*Placidula euryanassa*) was found to store defensive chemicals (tropane alkaloids) from the larval host (*Brugmansia*) and pass them on to the adults. In contrast, advancing Troidine swallowtails closely followed the evolutionary progress of their *Aristolochia* host plants, and store a variety of their chemicals (aristolochic acids, alkaloids). More advanced *Aristolochia* produce compounds antifeedant to most Troidini (and all other insects, often toxic) and remove phagostimulants from their leaves.



CHEMISTRY AND COEVOLUTION AT THE INTERFACE BETWEEN NEOTROPICAL BUTTERFLIES AND THEIR NATURAL HOST PLANTS. Keith S. Brown Jr., Clécio Klitzke, José Roberto Trigo, Silvana A. Henriques, Carina Berlinger, P.E. Rubbo-dos-Santos, André V.L. Freitas and Paulo Cesar Motta (Dept. de Zoologia, Instituto de Biologia, Universidade Estadual de Campinas, C.P. 6109, Campinas, São Paulo 13.081 Brazil)

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A Chemical Ecological Approach to the Cause of Pine Wilt Disease  
Yamamoto, Izuru, Hiroshi Honda and Toshio Kozaki  
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The wilt disease of pine trees (*Pinus densiflora*; *P. thunbergii*) has prevailed in Japan. A nematode, *Bursaphelenchus xylophilus*, was said to be the pathogen which is transmitted by a vector beetle, *Monochamus alternatus*. However, certain fungi were also proposed as the major cause of the disease. The situation has affected the protection strategy and aroused much public concern. To solve the problem, the following approach was attempted. 1~3 used the seedlings. 1. Selection of chemical descriptors for the health conditions of pines: ratio of terpene components of pine volatiles. 2. Chemical description of pine wilt by various treatment: the nematode, fungi and chemicals gave different ratios of terpene. 3. Chemical description by pattern recognition: there was no similarity between healthy, nematode-treated and fungi-treated trees based on the pattern similarity rate which implies that 1.00 ~ 0.950 is the indication of close similarity. 4. Comparison of the field-standing tree with the laboratory-grown seedling: a close similarity was obtained between healthy field and laboratory pines, while the wilted field tree was close to the nematode-treated, but not to fungi-treated trees in the pattern similarity rate.





*List of Participants*  
(as of June 15, 1992)



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