Preface

This book is dedicated to new and important research in the field of phytochemistry which is in the strict sense of the word the study of phytochemicals. These are chemicals derived from plants. In a narrower sense the terms are often used to describe the large number of secondary metabolic compounds found in plants. Many of these are known to provide protection against insect attacks and plant diseases. They also exhibit a number of protective functions for human consumers.

Techniques commonly used in the field of phytochemistry are extraction, isolation and structural elucidation (MS,1Dand 2D NMR) of natural products, as well as various chromatography techniques (MPLC, HPLC, LC-MS).

Short Communication - When millet (*Panicum miliaceum* L.) seeds were soaked in 20 mM acetate buffer, pH 3.5, and grown on moist absorbent cotton at 28 ^{0}C for 3 days, about 40% of the seeds (germinated seeds) grew normally after germination and the rest (ungerminated seeds) did not grow normally even if they had germinated. While the activities of α, β-amylases obtained from germinated seeds were about three times higher than those from ungerminated seeds, there was little difference in the activities of other starch-degrading enzymes. The optimum pH and isoelectric point of β-amylase from germinated seeds were different from those of ungerminated seeds. Ungerminated seeds produced inadequate β-amylase due to acidic stress. β-Amylase in germinated seeds was isolated by a procedure that included ammonium sulfate fractionation, ethanol fractionation, CM-cellulofine column chromatography, preparative isoelectric focusing, and preparative disc gel electrophoresis. The enzyme was homogeneous by SDS-PAGE. The molecular weight of the enzyme was estimated to be 58,000 based on its mobility on SDS-PAGE and 53,000 by gel filtration with TSKgel G4000SW$_{XL}$, which showed that it was composed of a single unit. The isoelectric point of the enzyme was 4.62. The enzyme readily hydrolyzed amylose EX-I, amylose EX-III, amylopectin and soluble starch. However, the Km values of the enzyme for amylopectin and soluble starch were about three times higher than those of β-amylase from millet seeds that had been germinated with water.

Therefore, when millet seeds germinate under acidic stress, starch will be hydrolyzed more slowly than starch in millet seeds that have been germinated with water, and this will result in slow germination and growth.

Chapter I - Perilla is a common culinary herb in Asia and also used as a natural medicine used in Japanese Kampo and Chinese Traditional Medicine. The perilla plants in common use belong to a cultivated species, *Perilla frutescens*, while there are three wild species in the genus, *P. citriodora*, *P. hirtella*, and *P. setoyensis*. The cultivated and wild species differ in their chromosome number, 2n=40 in the former and 2n=20 in the latter. The cultivated species of perilla is supposed to be an amphidiploid of the wild species, and its course of formation as well as its ancestral species is under investigation using various experiments on the biosynthesis of essential oil compounds as keys to the answer. Studies performed in the course of the project, namely GC-MS analysis of each oil type, analysis of oil constituents, seed fertility of artificial amphidiploids, RFLP (restriction fragment length polymorphism) and RAPD (randomly amplified polymorphic DNA analyses of the wild and cultivated species, and cloning and functional expression of geraniol synthase and limonene synthase, each of which catalizes the initial step of monoterpene synthesis in perilla, are discussed. More information on perilla collected through fieldwork, which has been carried out in Indochina, and the results of further experiments using the samples collected in the fieldwork are also described.

Chapter II - Appearance of various kinds of drug-resistant bacteria became serious problems in hospitals. Among them, methicillin-resistant *Staphylococcus aureus* (MRSA) have one of the most frequently found ones in Japan. The authors investigation on the natural products with antibacterial effects revealed that a series of polyphenolic compounds with low or high (tannin) molecular weight have direct antibacterial effects on MRSA, and several of them have suppressive effects on the antibiotic resistance. Among those polyphenols, epigallocatechin gallate (EGCG) is unstable in solution, but its decrease does not mean the decrease of the antibacterial effect directly, and theasinensin A, an oxidation product from EGCG, also showed direct antibacterial effect and suppressive effect on the antibiotic resistance. However, the prolonged treatment of theasinensin A weakened the suppressive effect. The effects of the addition of food additive to EGCG solution for prolongation or enhancement of the effects of EGCG were examined, and ascorbic acid was the effective one for this purpose. Since mechanistic study suggested that some interference of enzyme proteins such as penicillin binding protein (PBP) 2a due to polyphenols and disturbance of bacterial lipid membrane participate in the effects of those polyphenolic compounds, interaction of polyphenols with biomolecules was also investigated, to clarify the fundamental properties of polyphenols related to the antibacterial effect, employing bovine serum albumin (BSA). The size and stability of the complex formed from polyphenols and BSA varied dependent on the structures of polyphenols. Although the differences in the properties of polyphenols in complex formation were not correlated with the antibacterial effect directly, this approach will give useful information for explaining the differences in the effects on the bacterial biomolecules.

Chapter III - A large number of halogenated organic compounds have been isolated from plants, microorganisms, insects and animals. However, not much is known about the mechanisms by which the halogen atoms are incorporated into organic compounds. Cultured roots of *Menispermum dauricum*, the Asian vine, produce four structurally related chlorinated isoquinoline alkaloids; acutumine (1), acutumidine (2), dauricumine (3) and dauricumidine (4). Additionally *M. dauricum* roots produce their dechloro-derivatives dechloroacutumine

(5) and dechlorodauricumine (6). The structures of these alkaloids were established by spectroscopic, crystallographic and chemical methods. These alkaloids were labeled with ^{3}H, ^{13}C, ^{15}N, or ^{36}Cl, isolated, and fed independently to the *Menispermum* root culture. Biosynthetic relationships among the chlorinated alkaloids and their dechloro-derivatives were studied by monitoring incorporation of tracers derived from one alkaloid to the others, using a liquid scintillation counter for the radio isotopes ^{3}H and ^{36}Cl, an NMR spectrometer for ^{13}C, and a mass spectrometer for ^{15}N. Based on the results obtained from these tracer experiments, biosynthetic pathway from tyrosine to the chlorinated alkaloids are proposed, with special attention to steps related to introduction of a chlorine atom into the alkaloid molecules.

Chapter IV - Sorbitol is important in translocating photosynthate in fruit trees of the Rosaceae. Sorbitol-6-phosphate dehydrogenase (S6PDH) (or aldose-6-phosphate reductase) catalyzes the NADPH-dependent reduction of glucose-6-phosphate to sorbitol-6-phosphate, and S6PDH likely plays a key role in the biosynthesis of sorbitol in leaves. NAD-dependent sorbitol dehydrogenase (SDH) catalyzes the NAD-dependent oxidation of sorbitol to fructose, and thus SDH is believed to play a key role in the conversion of sorbitol to fructose in sink organs. cDNAs encoding S6PDH and SDH have been cloned for detailed analyses, and molecular genetic approaches for S6PDH have revealed that S6PDH activity regulates the ratio of sorbitol to sucrose in fruit trees of the Rosaceae. In addition, efforts have been made to engineer stress tolerance by introducing S6PDH (and SDH) into plants that do not synthesize sorbitol. Interestingly, S6PDH- and SDH-like sequences are widespread in the plant kingdom; that is, the sequences are found not only in the Rosaceae, but also in other plants that synthesize sucrose instead of sorbitol. In this review, the authors describe recent progress in the molecular aspects of S6PDH and SDH in plants.

Chapter V - Although the glucosinolate composition of *Brassica oleracea* and *Brassica rapa* varieties is known for some time, their phenolic composition was still unknown until recently. It was only understand that *B. oleracea* and *B. rapa* varieties were rich in very complex flavonoid heterosides and phenolic acid derivatives, but only the aglycones were described and no compounds were totally identified. That was possible with the use of liquid chromatography–UV diode-array detection–electrospray ionization mass spectrometry (HPLC-DAD-ESI-MS^n).

In this work the authors report the application of HPLC-DAD-ESI-MS^n on the determination of the phenolic profile (flavonoids and hydroxycinnamic derivatives) of several *B. oleracea* varieties, namely *Brassica oleracea* L. var. *costata* DC (tronchuda cabbage) and *Brassica oleracea* L. var. *italica* Plenck (broccoli), and of *B. rapa* varieties, like *Brassica rapa* L. var. *rapa* (turnip), *Brassica rapa* L. subsp. *sylvestris* (L.) Janch var. *esculenta* Hort. (turnip), and *Brassica rapa* L. subsp. *chinensis* L. (Hanelt.) (Chinese cabbage).

HPLC-DAD-ESI-MS^n allows the differentiation between *B. oleracea* and *B. rapa* varieties. In these last species, isorhamnetin glycosides were the main flavonoid derivatives, differing from those found in the vegetables belonging to the *B. oleracea* group.

For some species the differences between several materials were enhanced. For instance, for *B. oleracea* L. var. *costata* DC, the differences between external and internal leaves, seeds and sprouts were described. The influence of two fertilization regimens (conventional and

organic practices) and collection date on phenolics profiles of internal and external leaves was also discussed.

Chapter VI - Recently, there has been a renewal of interest concerning the study of photochemical and free radical-mediated degradation of lipid components during the senescence of phototrophic organisms. The present paper reviews the results obtained in the course of these studies.

In a first part, the photo-oxidation of the main lipid cell components (chlorophyll phytyl chain, carotenoids, sterols, unsaturated fatty acids, alkenones and unsaturated alkenes) in senescent phototrophic organisms (phytoplankton, cyanobacteria, higher plants, purple sulfur bacteria and aerobic anoxygenic phototrophic bacteria) is examined. Probably due to its long lifetime in hydrophobic micro-environments and thus in senescent cells, singlet oxygen plays a key role in the photodegradation of most of the lipid components. Emphasis is given to the structure and the tracer potentialities of the photoproducts formed. This part also describes the induction of visible light-induced photoprocesses in attached heterotrophic bacteria during the senescence of phytoplankton, which was recently demonstrated.

In senescent phototrophic organisms, the mechanism of initiation of free-radical oxidation, which has been debated for many years, seems to be the homolytic cleavage (catalyzed by some metal ions) of photochemically produced hydroperoxides. It was also demonstrated recently that viral infection and autocatalytic programmed cell death of phytoplanktonic cells could also lead to elevated production of reactive oxygen species (ROS) able to induce the degradation of cell components. The second part of this paper thus describes the free radical oxidation of lipid components (chlorophyll phytyl side-chain, sterols, unsaturated fatty acids, alkenones...) during the senescence of phototrophic organisms.

Chapter VII - Auraptene is the most abundant prenyloxycoumarin that occurs in nature. It has been isolated for the first time at the beginning of 30's by Komatsu and cowokers from *Citrus aurantium* L. and then found to be a common coumarin-based component of plants mainly belonging to the family of Rutaceae, many of which used for proven or supposed therapeutic properties according to some ancient ethnomedical traditions. Although known for a long time, only in the last decade auraptene was seen to exert *in vivo* and *in vitro* valuable pharmacological properties as orally active cancer chemopreventive, immunomodulatory, anti-bacterial, anti-protozoal, anti-inflammatory, anti-platelet aggregation, spasmolytic and anti-oxidant agent. The aim of this review is to examine in detail the properties of auraptene so far reported in the literature from a phytochemical and pharmacological point of view.

Chapter VIII - Brassinosteroids have attracted considerable interest since 1979, when the isolation and structure determination of brassinolide was reported. Some brassinosteroids display powerful plant growth-regulating activity at doses as low as 1 ng *per* individual plant. Numerous studies of the chemistry and biology of brassinosteroids, as well as of their potential applications, have been reported.

Organofluorine compounds have recently drawn considerable attention in the fields of agrochemistry, pharmaceutical and material science. Fluorinated analogues have been recognized as useful tools for pharmacological and physiological studies of natural products since the introduction of a fluorine atom into a molecule often leads to a significant change in

its physical and biological properties. In view of their unique biological properties, fluorinated steroids have been widely studied and several fluoro-substituted compounds are now considered as analogues of plant hormones.

This report reviews recent advances on the field of brassinosteroid research, with emphasis made in the work developed by the authors group and others on fluorinated analogues. The synthetic strategies leading to the introduction of fluorine atoms in the steroidal frame will be discussed. Finally, the biological properties of these fluorinated brassinosteroids, compared to their natural counterparts, will be analysed.

Chapter IX - Although there have been large improvements in cancer treatment over the last twenty years, the lack of cancer chemotherapeutic drugs is still a major cause of death in this century. Medicinal plants play an important role in the treatment of cancer by offering unique active drugs or their templates for clinical uses, as exemplified by paclitaxel (Taxol®), vinca alkaloids (vincristine, vinblastine), and flavopiridol. The strategies for developing anticancer agents from medicinal plants have changed in the last decade for a number of reasons, including advances in technology, changes in the plant selection mode and biological activity testing. This review reflects and discusses the newest methods applied for searching anticancer agents from medicinal plants including plant selection and extraction, the active principle isolation, structure elucidation and biological testing. This review reflects and discusses the newest methods applied for searching anticancer agents from medicinal plants including plant selection and extraction, the active principle isolation, structure elucidation and biological testing.