Organic Synthesis and Chemical Ecology

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ABSTRACT
The roles of organic synthesis in chemical ecology are discussed, with many examples. The structure, including the absolute configuration, of a semiochemical (signal substance) can be established by enantioselective synthesis. Only through synthesis can a semiochemical be obtained in an amount sufficient for decisive biological evaluation. Rigorous enantioselective synthesis of semiochemicals to provide their pure enantiomers has shown that they are not always enantiomerically pure. Synthesis of the stereoisomers of semiochemicals has clarified their structure−bioactivity relationships to reveal the unprecedented diversity in the stereochemical aspects of pheromone communications.

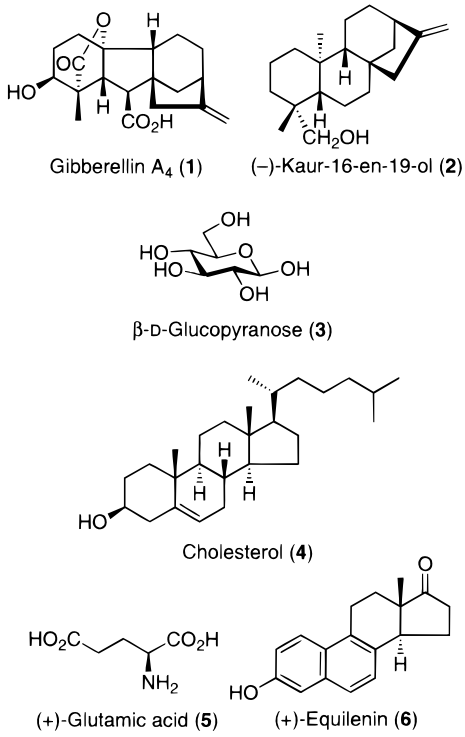
Introduction

This Account summarizes my attempt to find answers to the following three questions: (i) What kind of synthetic work is meaningful to me according to my own taste? (ii) Are bioactive natural products always enantiomerically pure? (iii) Is a single enantiomer of a bioactive natural product always responsible for its bioactivity? Through my pilgrimage to various phenomena in chemical ecology, I can now give you some of my answers.

Since the early days of Berthelot, Liebig, and Wöhler, organic synthesis has remained an important branch of chemistry. Chemical ecology, on the other hand, is a new and interdisciplinary branch of science developed since the 1970s. Indeed, the launch of a periodical named *Journal of Chemical Ecology* took place as late as 1975. Chemical ecology deals with the chemistry and biology of inter- and intraspecific interactions among organisms by means of semiochemicals.

Semiochemicals are biomolecules that spread information between individuals. The word is synonymous with "signal substances" and is derived from "semio" (Greek = sign). They are divided into two groups: pheromones and allelochemicals. Pheromones are used for communication between individuals within the same species. The term "pheromone" was coined by Karlson and Lüscher and is derived from the Greek "pherein" (= to transfer) and "hormon" (= to excite).[1] On the other hand, allelochemicals are used for communication between individuals belonging to different species. The word was derived from "allelon" (Greek = of each other). Isolation, structure determination, and synthesis of semiochemicals are the essential parts of chemical ecology.

Scheme 1. Structures of Some Natural Products



For over two decades we have concentrated our efforts on the synthesis of semiochemicals, because we regarded organic synthesis as one of the key disciplines in chemical ecology on the basis of the following considerations: (i) Semiochemicals are usually extremely minor components of organisms, and therefore the limited availability (milligrams to micrograms) of the natural product renders their structure determination difficult unless X-ray analysis is applicable. Accordingly, their proposed structures must be confirmed by unambiguous synthesis. To make their further study possible, their abundant supply must be obtained by synthesis. (ii) Semiochemicals are usually optically active compounds, and determination of their absolute configuration and enantiomeric purity is feasible only after their reference samples are synthesized with known absolute configuration and high enantiomeric purity. (iii) Availability of the synthetic stereoisomers of semiochemicals enables clarification of stereochemistry−bioactivity relationships among semiochemicals.[2]

Background

In 1968, just after my completion of the synthesis of gibberellin A_4 (**1**, Scheme 1),[3] a plant growth hormone, a

Kenji Mori was born in Seoul, Korea, in 1935, and is the son of a Japanese Christian pastor. He obtained his B.Sc. (1957, agricultural chemistry), M.Sc. (1959, biochemistry), and Ph.D. (1962, organic chemistry) degrees from the University of Tokyo. He remained there until March 1995 (1962−1968, assistant; 1968−1978, associate professor; 1978−1995, professor), and then moved to Science University of Tokyo as a professor. His research interests include the enantioselective synthesis of pheromones and other biofunctional molecules, biotransformations, and chemical ecology. He has been honored by awards from the Japan Academy (in 1981 in the presence of the late Emperor Hirohito), the International Society of Chemical Ecology (Silver Medal in 1996 in Prague), and the American Chemical Society (The Ernest Guenther Award in the Chemistry of Natural Products in 1999 in Anaheim).

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famous microbiology professor in our Department approached me and said, "Congratulations, Dr. Mori! You finally synthesized the gibberellins. But it took nine years of your life. Don't forget that the fungus *Gibberella fujikuroi* will make them within a couple of days." This was indeed a serious criticism and advice about what I had done. Chemists can be respected by biologists only when they provide compounds which are difficult to prepare by biological means. I therefore turned my way to synthesize rare semiochemicals whose supply would benefit biologists.

My synthetic work on (±)-kaur-16-en-19-ol (**2**) also gave me an important lesson. This diterpene **2** is a biosynthetic precursor of the gibberellins and promotes the growth of dwarf maize d_5, which is a mutant unable to synthesize **2**. The natural **2** is the pure (−)-enantiomer, but what I synthesized by starting from naphthalene was the racemate [(±)-**2**].[4] Bioassay of both the natural (−)-**2** and synthetic (±)-**2** was carried out, and the results clearly indicated that (±)-**2** was only half as active as the natural (−)-**2**.[4] The unnatural (+)-**2** must be inactive. This experience taught me the importance of enantioselective synthesis to prepare only the bioactive enantiomer.

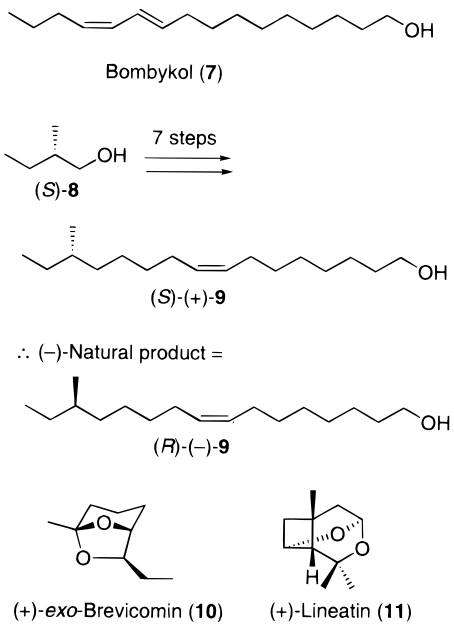
At the time when I started my enantioselective syntheses of semiochemicals in the early 1970s, natural products were generally thought to be enantiomerically pure, like D-glucose (**3**) and cholesterol (**4**), although lactic acid and some simple monoterpenes were known to exist as their racemates. What about the enantiomeric purity of semiochemicals? In early 1970s, it was generally believed that only a single enantiomer is bioactive, and its opposite enantiomer is inactive in the case of a chiral and bioactive natural product. (*S*)-(+)-Glutamic acid (**5**) has taste, while its opposite enantiomer is completely devoid of taste. (+)-Equilenin (**6**) works as a female sex hormone, while its opposite enantiomer is only 7.5% as active as (+)-**6**.[5] What about the stereochemistry−bioactivity relationships among semiochemicals? To solve these questions, we had to synthesize semiochemicals of high enantiomeric purity.

Early Work on Pheromone Stereochemistry

Although the first identified female sex pheromone of the silkworm moth (*Bombyx mori*), bombykol (**7**, Scheme 2), was achiral, a number of chiral pheromones were discovered in late 1960s. For example, (−)-**9** was isolated as the pheromone of the dermestid beetle (*Trogoderma inclusum*). In 1973, I determined the absolute configuration of (−)-**9** as *R* by synthesizing (*S*)-(+)-**9** from the known (*S*)-**8**.[6] This work was the first successful identification of the absolute configuration of an insect pheromone by its enantioselective synthesis.

Subsequently, in 1974, I synthesized both the enantiomers of *exo*-brevicomin (**10**),[7] and only the (+)-isomer was found to be bioactive as the aggregation pheromone of the western pine beetle (*Dendroctonus brevicomis*).[8] This result was in accord with the generally accepted belief that a single enantiomer is important. But you will later see many cases that differ.

Scheme 2. Structures of Some Pheromones



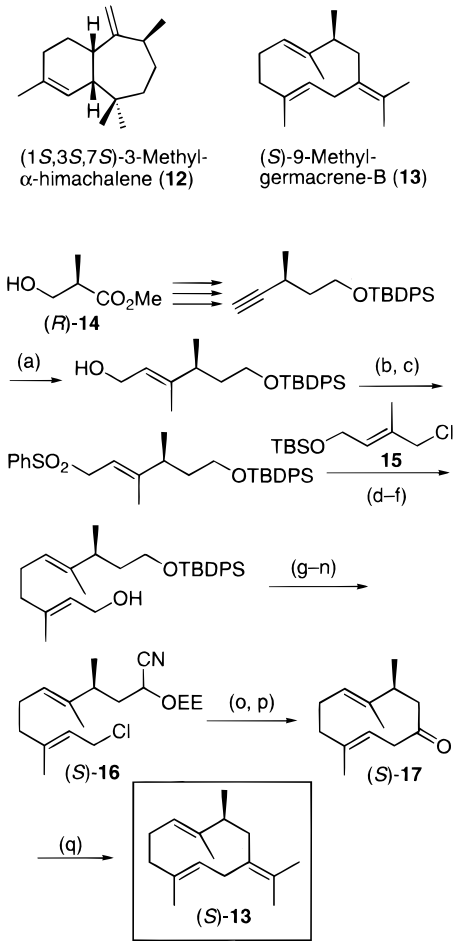
In 1982, Schurig's invention of chiral stationary phases for gas chromatographic analysis was applied to achieve the first precise determination of the enantiomeric purity of a pheromone.[9] By employing our synthetic enantiomers of lineatin as the reference samples, (+)-lineatin (**11**) of 99.0 ± 0.5% ee was found to be produced by the ambrosia beetles *Trypodendron lineatum*, *T. domesticum*, and *T. signatum*.[9] Methods for the determination of the absolute configuration and enantiomeric purity of semiochemicals have been reviewed twice.[10,11]

Synthesis as Structure Proof or Material Supply

Because enantioselective syntheses of semiochemicals must precede other studies where the synthetic materials are to be used, it is appropriate here to briefly describe examples. Comprehensive reviews on pheromone synthesis are available.[12−14]

(a) Synthesis of 9-Methylgermacrene-B, the Sandfly Pheromone. The leishmaniases are parasitic diseases which threaten 350 million people in the world. The sandfly *Lutzomyia longipalpis* is the only vector of the protozoan parasite *Leishmania chagasi*, the causative agent of leishmaniasis in South and Central America. Two male-produced pheromones of the sandfly were isolated and identified as **12** and **13** (Scheme 3).[15,16] Our recent synthesis of (*S*)-9-methylgermacrene-B (**13**) is summarized in Scheme 3.[17] The commercially available chiral building block (*R*)-**14** was converted to (*S*)-**16**, whose cyclization to (*S*)-**17** was followed by its isopropylidenation to give (*S*)-**13**. Similarly, (*R*)-**13** was prepared from (*S*)-**14**. Gas chromatographic comparison of (*R*)- and (*S*)-**13** with the natural pheromone employing a chiral stationary phase

Scheme 3. Synthesis of the Sandfly Pheromone[a]



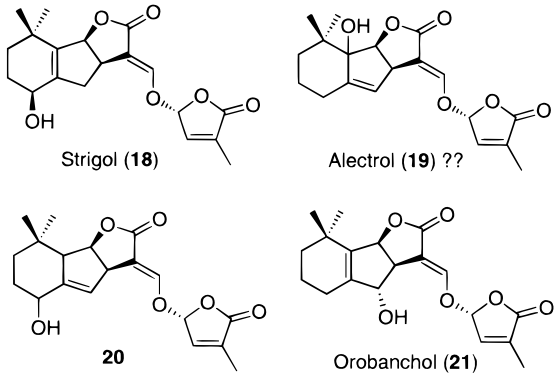
[a] Conditions: (a) (i) Me_3Al, Cp_2ZrCl_2, CH_2Cl_2, H_2O; (ii) *n*-BuLi, hexane; (iii) $(CH_2O)_n$, THF, 80%. (b) Ph_3P, CCl_4. (c) $PhSO_2Na$, DMF, 83% (two steps). (d) *n*-BuLi, THF, HMPA, **15**, 87%. (e) AcOH, THF, H_2O, 81%. (f) $LiBEt_3H$, $PdCl_2(dppp)$, THF, 81%. (g) Ac_2O, C_5H_5N, 95%. (h) $(n\text{-}Bu)_4NF$, THF, 83%. (i) Dess−Martin periodinane, CH_2Cl_2, 87%. (j) TMSCN, KCN, 18-crown-6. (k) $BnMe_3NF$, THF, H_2O. (l) $CH_2{=}CHOEt$, TsOH, C_6H_6, 94% (three steps). (m) K_2CO_3, MeOH, 89%. (n) MsCl, LiCl, DMF, *s*-collidine, 90%. (o) $NaN(SiMe_3)_2$, THF, 42%. (p) PPTS, MeOH, then aqueous NaOH, Et_2O, 50%. (q) CBr_2Me_2, Sm, SmI_2, $CrCl_3$, THF (64%).

established the absolute configuration of the natural product as *S*.

(b) Synthesis of Orobanchol, the Germination Stimulant. Parasitic weeds of the genera *Orobanche* and *Striga* are known to cause severe yield crop losses in grains and legumes in Africa, Asia, and the United States. The seeds of such weeds remain dormant in soil until exudates from their host plant induce germination. Active principles of the exudates have been isolated and are called by the general name of strigolactones. Strigol (**18**, Scheme 4) was the first strigolactone to be isolated, and its structure was solved by X-ray analysis.

Due to the scarcity of the natural products (less than 1 mg), the structures of strigolactones are very difficult to elucidate. For example, the structure **19** was proposed for alectrol, the germination stimulant for the seeds of the root parasites *Alectra vogelii* and *Striga gesnerioides*. About 300 μg of alectrol was isolated from 300 000 *Vigna unguiculate*, the genuine host plant. We synthesized eight stereoisomers of **19** as the racemate and found none of them to show the ^{1}H NMR data reported for alectrol.[18] The structure of alectrol therefore remains obscure.

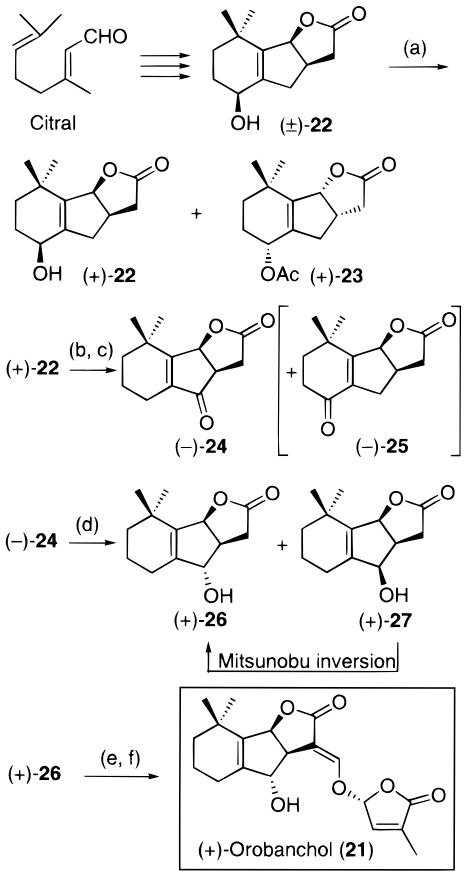
Scheme 4. Structures of Strigolactones



Orobanchol was most recently isolated together with alectrol as the germination stimulant for clover broomrape (*Orobanche minor*) in Japan from its host red clover (*Trifolium pratense*), and the structure **20** was first proposed for it. Our synthesis of the eight stereoisomers of **20** as the racemates disproved the correctness of the proposal.[19] We then synthesized (±)-**21**, compared its TMS ether with that of orobanchol by GC−MS, and found them to be identical.[19] Our synthesis of (+)-orobanchol (**21**) is shown in Scheme 5.[20] The racemic lactone (±)-**22** was prepared from citral and subjected to enzymatic resolution with lipase AK (Amano). The resulting (+)-**22** was converted to (+)-**26** via (+)-**24**, and (+)-orobanchol (**21**) could be derived from (+)-**26**. In this particular case, our fortuitous synthesis of (±)-**21** solved the structural problem.

(c) Synthesis of Persoons's Periplanone-A (= Isoperiplanone-A). In 1974, Persoons isolated periplanone-A and periplanone-B as the female-produced sex pheromone of the American cockroach (*Periplaneta americana*) and proposed the structure **28** (Scheme 6) for the former and **29** (stereochemistry was later clarified) for the latter. Although the structure **29** for periplanone-B was correct, correctness of the structure **28** for periplanone-A was challenged by many people. In 1986, Hauptmann isolated a pheromone component of the American cockroach, spectral properties of which were different from those of Persoons's periplanone-A, and yet gave it the same name, periplanone-A. Its structure was proved to be **30** by Hauptmann's synthesis of (±)-**30**. We synthesized the enantiomers of **30** and thermolyzed 80 mg of (−)-**30** under the conditions employed by Persoons for the purification of his periplanone-A. The thermolysis product was reduced to give an alcohol, the structure of which was established as **32** by X-ray analysis.[21] The thermolysis product, which showed spectral properties identical to those of Persoons's periplanone-A, was therefore **31**. The purified **31** turned out to be biologically inactive. Persoons's periplanone-A was thus the thermal decomposition product of genuine periplanone-A [(−)-**30**] and was renamed as isoperiplanone-A.[22] In this case, a deliberate synthetic work solved the long-standing structural

Scheme 5. Synthesis of Orobanchol[a]



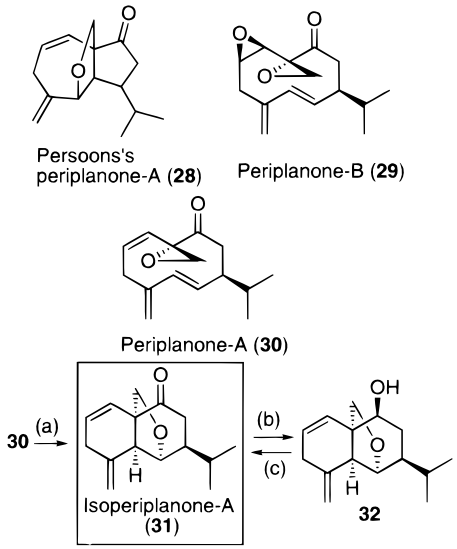
[a] Conditions: (a) CH_2═CHOAc, lipase AK, THF; 48% of (+)-**22** (99% ee) and 52% of **23** (87% ee). (b) (i) Ph_3P, CBr_4, CH_2Cl_2; (ii) Zn−Cu, THF; (iii) CSA, CH_2Cl_2, 76%. (c) CrO_3, 3,5-dimethylpyrazole, CH_2Cl_2; 18% of **24** and 51% of **25**. (d) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, EtOH; 4% of **26** and 81% of **27**. (e) NaH, HCO_2Et, Et_2O. (f) (i) K_2CO_3, (±)-4-bromo-2-methyl-2-buten-4-olide, *N*-methylpyrrrolidone, 30%.

problem. The chemistry of periplanones was reviewed in 1990.[23]

(d) Synthesis of Glycinoeclepin A. Cyst nematodes are well known as serious pests of many crops. They generally have a limited host range, and the specificity is thought to be based on a response to a chemical hatching stimulus secreted by the host plants. In 1985, Masamune et al. isolated less than 1 mg of a degraded triterpenoid, glycinoeclepin A (**33**, Scheme 7), as a potent hatching stimulus for the soybean cyst nematode (*Heteropdera glycines*) from 1 ton of the dried roots of the kidney bean (*Phaseolus vulgaris*) harvested from 10 ha of a field.

Scheme 7 summarizes our synthesis of glycinoeclepin A.[24] The chiral building blocks **34** and **35** were prepared by reduction of the corresponding β-diketones with baker's yeast and converted to **36** and **37**, respectively. Coupling of **36** with **37** was achieved by aldol reaction, and the product **38** was further processed to give **39**. Treatment of **39** with lithium dimethylcuprate furnished **40** in a single step, which finally gave glycinoeclepin A (**33**). By this synthesis we obtained 220 mg of the crystalline hatching stimulus **33**, which showed remarkable hatch-stimulating effect in vitro.

Scheme 6. Synthesis of Persoons's Periplanone-A (= Isoperiplanone-A)[a]



[a] Conditions: (a) heat, 200 °C, 71%. (b) $NaBH_4$, MeOH, 80%. (c) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, 87%.

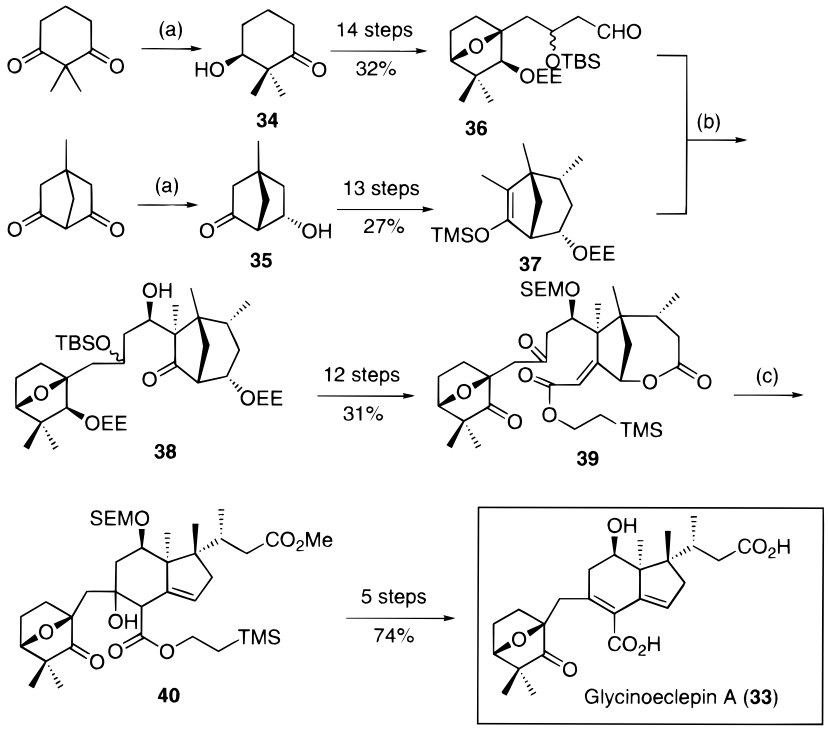
There was an idea that the administration of **33** to the field in the absence of the host plants such as soybeans would cause the hatching of the cysts, and the resulting nematodes would starve to death. Unfortunately, however, in laboratory pots and also in a soybean field, **33** showed no nematocidal effect at all. Perhaps the administered **33** was adsorbed by soil particles or degraded by soil microorganisms before it affected the cysts. The reason **33** works in the real ecosystem may be that the release of **33** by the host root takes place in extreme vicinity of the cysts to make the eggs hatch prior to the disappearance of **33** by soil adsorption or microbial degradation. The very subtle communication mechanisms in the action of semiochemicals often make their application in agriculture difficult. Nevertheless, it is true that only after the synthetic preparation of a substantial amount of **33** was it possible to exactly evaluate the practicality of glycinoeclepin A (**33**) in soybean production.

Semiochemicals and Enantiomerism—Are They Pure Enantiomers?

Semiochemicals are not always pure enantiomers. Scheme 8 shows three examples of stereochemical heterogeneity among semiochemicals. Sulcatol (**41**) is the male-produced aggregation pheromone of the ambrosia beetle, *Gnathotrichus sulcatus*. Its enantiomeric composition was estimated as $R/S = 35{:}65$ by ^{1}H NMR analysis of its Mosher ester.[25] Implication of this heterogeneity will become clear when we discuss the unusual stereochemistry−bioactivity relationship of sulcatol enantiomers.

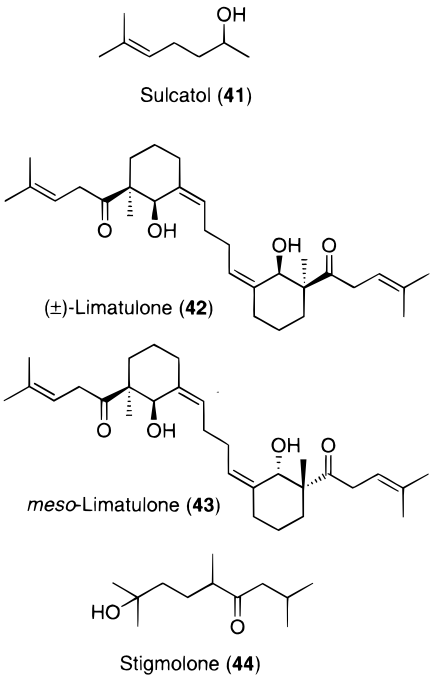
Limatulone is a unique triterpene that occurs as its racemate [(±)-**42**] and also as the *meso*-isomer (**43**).[26] In 1985, Faulkner and co-workers reported the isolation of an allelochemical limatulone as a defensive metabolite of the limpet *Achmeia* (*Collisella*) *limatula*.[27] This triterpene is a potent feeding inhibitor (antifeedant) against fish and

Scheme 7. Synthesis of Glycinoeclepin A[a]



[a] Conditions: (a) baker's yeast, sugar, H_2O. (b) MeLi, $ZnCl_2$, Et_2O, 82%. (c) (i) Me_2CuLi, THF, (ii) CH_2N_2, 72%.

Scheme 8. Examples of Bioactive Natural Products That Are Enantiomerically Impure



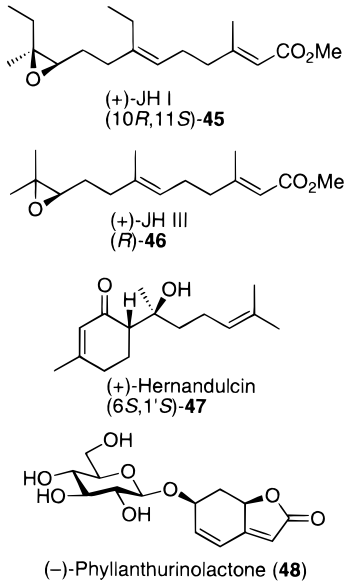
crab. The natural product was reported to be optically inactive, implying that it must be either (±)-**42** or *meso*-**43**. We synthesized both (±)-**42** and **43**. The ^1H and ^{13}C NMR spectra of the natural product were identical with those of (±)-**42**. Moreover, the ^1H NMR spectrum of a less bioactive fraction from the HPLC separation of *A. limatula* metabolite coincided with that of the synthetic *meso*-**43**. It is clear that the limpet *A. limatula* produces both (±)-**42** and *meso*-**43**.

Myxobacteria are unique procaryotes that undergo multicellular development, including swarming and aggregation of their cells and formation of fruiting bodies. Stigmolone (**44**) is the pheromone to aggregate the starving cells of a myxobacterium, *Stigmatella aurantiaca*. We synthesized both of the enantiomers of **44**,[28] and they were separately bioactive.[29] The natural stigmolone (**44**) was shown to be a 1:1 mixture of (*R*)- and (*S*)-**44**.[29] It is now apparent that nature does not always utilize enantiomerically pure semiochemicals.

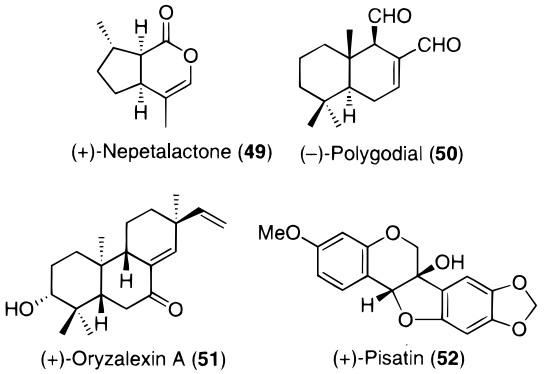
Stereochemistry—Bioactivity Relationships among Bioactive Natural Products—Is a Single Enantiomer Always Responsible for Bioactivity?

As mentioned earlier, it has generally been believed that only one enantiomer is bioactive, and its opposite enantiomer is inactive in the case of a chiral and bioactive natural product. When our synthetic enantiomers of insect juvenile hormone (JH) I (**45**, Scheme 9) were bioassayed, only the naturally occurring (+)-JH I was extremely active [ca. 1.2×10^4 times more active than (−)-**45**].[30] Similarly, (+)-JH III (**46**) was ca. 5×10^3 times more active than (−)-**46**.[31] Out of the four stereoisomers of hernandulcin (**47**), only the naturally occurring (6*S*,1′*S*)-**47** is a sweetener (1000 times as sweet as sucrose).[32] Out of the four stereoisomers of phyllanthurinolactone (**48**) with different stereochemistry in the aglycon part, only the naturally occurring isomer **48** is active as the leaf-closing factor of

Scheme 9. Examples of Bioactive Natural Products, a Single Enantiomer of Which Is Extremely Active



Scheme 10. Examples of Bioactive Natural Products, Both Enantiomers of Which Are Bioactive



the nyctinastic plant *Phyllanthus urinaria*.[33] All of these cases indicate the importance of the correct stereochemistry for the expression of bioactivity.

There are, however, less rigorous cases, as shown in Scheme 10. (+)-Nepetalactone (**49**) is the well-known cat attractant in catnip. It was reisolated in 1987 as the sex pheromone of the vetch aphid (*Megoura viciae*) by Pickett and co-workers, and its opposite enantiomer was devoid of the pheromone activity.[34] However, when the enantiomers of **49** were bioassayed on Japanese cats, both of them were extremely active at the 0.01-mg dosage level.[35]

(−)-Polygodial (**50**) is a potent insect antifeedant. We synthesized the enantiomers of **50**,[36] and bioassay revealed both to be strong antifeedants.[37] The proposed mechanism of action of polygodial, reaction with lysine residues to form pyrroles, is essentially independent of absolute stereochemistry. Our synthesis of the enantiomers of oryzalexin A (**51**)[38] and also of pisatin (**52**),[39] the phytoalexins, was followed by their bioassays. The results showed that both of the enantiomers are active as phytoalexins, although the natural enantiomers (+)-**51** and (+)-**52** were slightly more active. It should be added that even nonnatural strigolactones **19** and **20**, including all their stereoisomers, were strong germination stimulants for parasitic weeds. In these cases, recognition of chirality of semiochemicals by organisms is not so strict.

Stereochemistry—Bioactivity Relationships among Pheromones—Diversity Is the Keyword of Pheromone Response

The relationships between stereochemistry and bioactivity have been extensively studied in the case of pheromones since the early 1970s, when their pure enantiomers became available by synthesis. A detailed review is available on this subject.[40] The relationships are far from straightforward. Organisms utilize chirality to enrich and diversify their communication system. The stereochemistry−bioactivity relationships are classified into 10 categories as explained below.

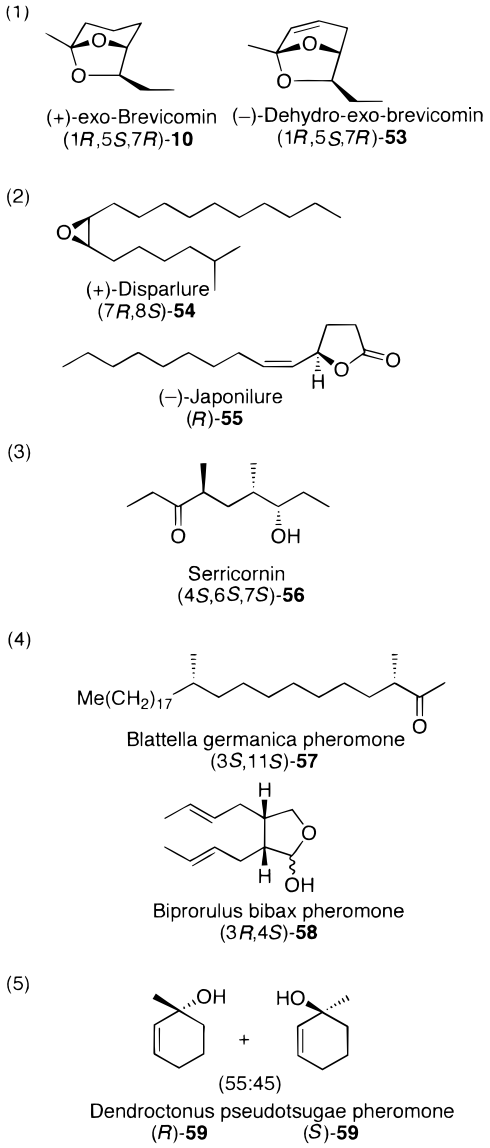
(1) Only a single enantiomer is bioactive, and its opposite enantiomer does not inhibit the response to the active stereoisomer. This is the most common relationship, and the majority (about 60%) of the chiral pheromones belong to this category. As described earlier in this Account, (+)-*exo*-brevicomin (**10**, Scheme 11) was bioactive, while (−)-**10** was inactive.[8] (−)-Dehydro-*exo*-brevicomin (**53**) is the pheromone of the male house mouse (*Mus musculus*) that induces his aggressive behavior.[41] It is interesting to note that animals as different as the mouse and the pine beetle biosynthesize these acetals with the same absolute configuration.

(2) Only one enantiomer is bioactive, and its opposite enantiomer inhibits the response to the pheromone. The enantiomers of disparlure (**54**), the pheromone of the gypsy moth (*Lymantria dispar*), were first prepared by Marumo and co-workers,[42] and then by us.[43] Electroantennographic and behavioral responses of the gypsy moth to the enantiomers showed that (7*R*,8*S*)-(+)-**54** was the most effective, and (±)-**54** was second most active, while (−)-**54** inhibited the activity of the (+)-isomer.[42,44,45]

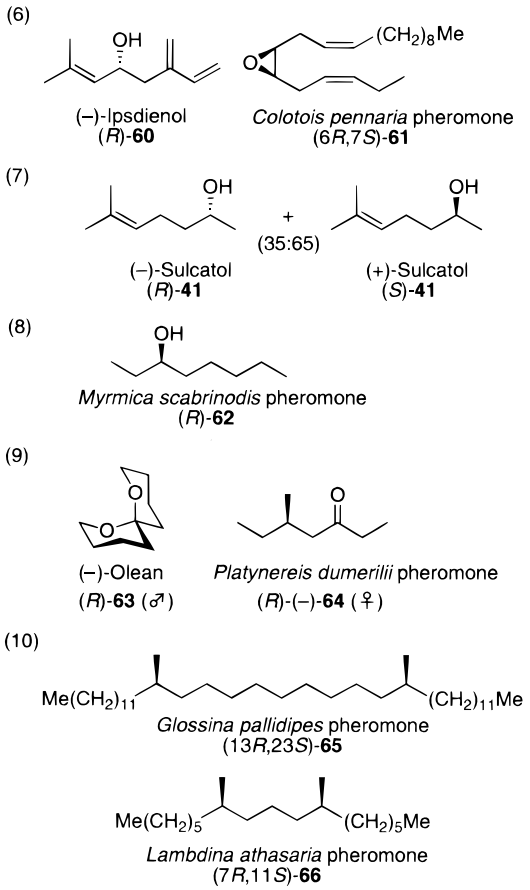
Very strong inhibitory action of the opposite (*S*)-enantiomer of japonilure (*R*)-**55**, the female-produced sex pheromone of the Japanese beetle (*Popillia japonica*), was found by Tumlinson et al.[46] As a result, (*R*)-**55** of 99% ee was about two-thirds as active as pure (*R*)-**55**, that of 90% ee was about one-third as active, that of 80% ee was about one-fifth as active, and both (*R*)-**55** of 60% ee and (±)-**55** were inactive.[46] One must develop a highly enantioselective synthesis for the practical use of these pheromones.

(3) Only one enantiomer is bioactive, and its diastereomer inhibits the response to the pheromone. Serricornin (**56**) is the female-produced sex pheromone of the cigarette beetle (*Lasioderma serricorne*). Only (4*S*,6*S*,7*S*)-**56** was bioactive, and the opposite enantiomer did not inhibit the response to the pheromone. However, the (4*S*,6*S*,7*R*)-isomer was inhibitory. Accordingly, the commercial pheromone lure must be manufactured diastereoselectively to avoid the presence of the (4*S*,6*S*,7*R*)-isomer.[47] In this and similar cases, one must achieve diastereoselective synthesis of the pheromone to manufacture potent pheromone lures.

Scheme 11. Stereochemistry and Pheromone Activity (1)



Scheme 12. Stereochemistry and Pheromone Activity (2)



(4) The natural pheromone is a single enantiomer, and its opposite enantiomer or diastereomer is also active. The female German cockroach (*Blattella germanica*) produces (3*S*,11*S*)-**57** as her contact sex pheromone.[48] The males, however, do not discriminate between the four stereoisomers of **57**, and all of them are bioactive.[49] The male spined citrus bug (*Biprorulus bibax*) produces (3*R*,4*S*)-**58** as the aggregation pheromone. The opposite enantiomer was also active.[50]

(5) The natural pheromone is an enantiomeric mixture, and both the enantiomers are separately active. Female Douglas fir beetles (*Dendroctonus pseudotsugae*) produce an average of 55:45 mixture of (*R*)- and (*S*)-**59**.[51] The enantiomers are separately active, but both enantiomers are required for maximum response.[51]

(6) Different enantiomers and diastereomers are employed by different species. (*R*)-Ipsdienol (**60**, Scheme 12) is the pheromone component of the bark beetles *Ips calligraphus* and *I. avulsus*, while the (*S*)-isomer is used by *Ips paraconfusus*.[52] The chirality of pheromones is important to discriminating between two species of geometrid moths in Europe. Thus, (6*R*,7*S*)-**61** is the pheromone of *Colotois pennaria*, while *Erannis defoliaria* uses (6*S*,7*R*)-**61**.[53] In these cases, insects utilize chirality to segregate different species.

(7) Both enantiomers are necessary for bioactivity. Natural sulcatol, the pheromone of *Gnathotrichus sulcatus*, is a mixture of (*R*)- and (*S*)-**41**, as already mentioned.[25] It became clear that the pheromone activity of **41** was observed only in the presence of both of the enantiomers. Neither of the enantiomers was active in isolation.[54] Indeed, this is the reason natural sulcatol is an enantiomeric mixture.

(8) One enantiomer is more active than the other, but an enantiomeric mixture is more active than the enantiomer alone. The ant *Myrmica scabrinodis* uses a mixture of (*R*)-**62** and its (*S*)-isomer (*R*/*S* = 9:1) as its pheromone, and this mixture is more active than the pure (*R*)-**62** or (±)-**62**, while (*S*)-**62** is inactive.[55]

(9) One enantiomer is active on males, while the other is active on females. Olean (**63**) is the female-produced sex pheromone of the olive fruit fly (*Bactrocera oleae*). Bioassay in Greece revealed that (*R*)-**63** was active against the male *B. oleae*, while (*S*)-**63** activates the female. Interestingly, the female *B. oleae* produces (±)-**63** and activates both males and herself.[56]

5-Methyl-3-heptanone (**64**) was isolated as a pheromone in the coelomic fluid of gravid specimens of nereid

marine polychaetes. It is responsible for the induction of the nuptial dance behavior prior to the release of gametes in *Platynereis dumerilii*, and the female-produced (*S*)-**64** activates the males, while the male-produced (*R*)-**64** is active on females.[57]

(10) Only the meso-isomer is active. There are some alkane pheromones with methyl branchings, whose *meso*-isomers are bioactive. Thus, (13*R*,23*S*)-**65** was active as the sex stimulant pheromone of the female tsetse fly (*Glossina pallidipes*).[58] Neither its (13*R*,23*R*)- nor the (13*S*,-23*S*)-isomer was bioactive. Female-produced sex pheromone components of the spring hemlock looper moth (*Lambdina athasaria*) are 7-methylheptadecane and 7,-11-dimethylheptadecane (**66**). We synthesized all of their stereoisomers, and a mixture of (*S*)-7-methylheptadecane and (7*R*,11*S*)-**66** (*meso*-isomer) was found to be bioactive.[59]

It must be emphasized that these 10 categories were found only through experiments by using pure pheromone enantiomers of synthetic origin.

Conclusion

Semiochemicals are often simple compounds. However, their synthesis to provide highly pure enantiomers is not always a simple task. Thanks to advances in both analytical and synthetic methods, we can now provide pure enantiomers with even higher enantiomeric purities than those of the natural semiochemicals themselves. Through synthesis we can verify the structure proposal and determine the absolute configuration of even extremely volatile and scarce semiochemicals. Through synthesis we can clarify the diversity in structure−activity relationships, the discovery of which has been the most unexpected and fascinating outcome of our work.

Enantioselective synthesis is important in practical application of semiochemicals in pest management. Disparlure [(7*R*,8*S*)-**54**] and japonilure [(*R*)-**55**] are manufactured and used as the pure enantiomers to control or monitor the population of the gypsy moth and the Japanese beetle, respectively. Semiochemicals are expected to be used more and more as environmentally benign agents for pest control to protect our health, agriculture, and forestry.

Our knowledge in chemical ecology increased dramatically during the past three decades. We are now overwhelmed by the diversity which can be recognized in our ecosystem.[60] This fact brings me to the conclusion leading to my future prospect: "What we see now is like the dim image in a mirror; then we shall see face to face. What I know now is only partial; then it will be complete (1 Corinthians 13:12)."

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