9.05 Determination of Structure including Absolute Configuration of Bioactive Natural Products

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9.05.1 Introduction

Since the advent of modern physical tools such as UV, IR, ^{1}H-NMR, ^{13}C-NMR, mass spectrometry (MS), circular dichroism (CD), and X-ray analysis, the structure determination of bioactive small molecules is often regarded as a routine operation for natural products chemists.[1] Such a view by biologists and many chemists is contestable, and two reviews have appeared recently, both treating incorrectly assigned structures of many natural products.[2,3] Even X-ray analysis can be erroneous.[3] There are some cases in which the correctly proposed structures of the presumably bioactive molecules do not represent the structures of genuinely bioactive molecules, as shown by the bioassay of synthetic compounds with the proposed structures.[2] This type of error usually stems from the incorrect and nonreproducible bioassay methods employed for the biological phenomena in discussion.

In the case of the complex marine polyether brevenal (**1**), the structure as shown in the upper part of **Figure 1** was proposed by Bourdelais *et al.*[4] through extensive spectroscopic studies. After completing the synthesis of the proposed structure, Fuwa *et al.*[5] revised the structure of brevenal as **1**, because there were subtly distinct discrepancies of the chemical shifts in the ^{1}H- and ^{13}C-NMR spectra of the left-hand region of the synthetic material compared with those of the natural brevenal. It may therefore be important to evaluate the proposed structure by means of its synthesis.

9.05.2 Absolute Configuration and Sign of Optical Rotation

Semiochemicals are usually compounds with structures much simpler than that of brevenal. But even with simple compounds, there are possibilities of misassigning their absolute configuration. Male-produced pheromone components of the flea beetle *Aphthona flava* were isolated and identified in 2001 by Bartelt *et al.*[6] They proposed himachalene-type sesquiterpene structures **2**–**5** (**Figure 2**) to the components. In 2004, Mori and coworkers synthesized **2**–**5** and their enantiomers *ent*-**2**–*ent*-**5** from enantiomers of citronellal, and the

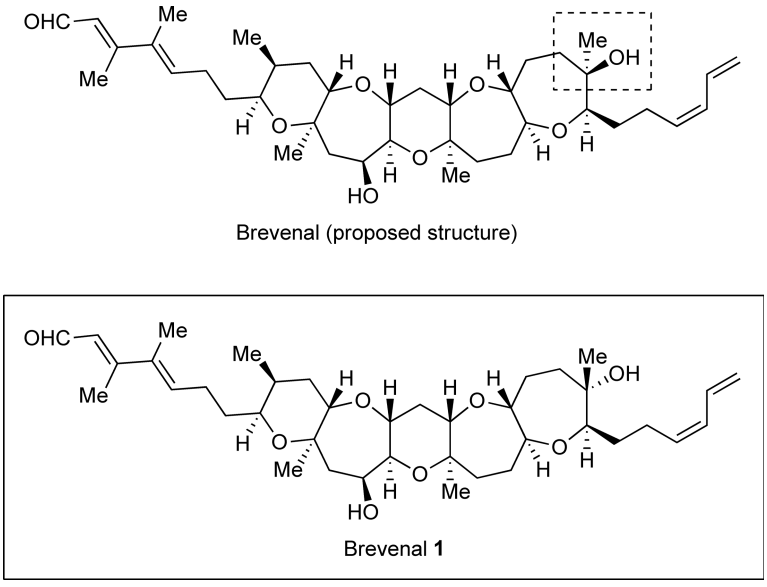


Figure 1 Structure of brevenal (**1**).

pheromone components were found to possess the absolute configuration as depicted in *ent*-**2**–*ent*-**5**.[7] Mori's assignments were opposite to those proposed by Bartelt *et al.*, and indeed *ent*-**2**–*ent*-**4** were pheromonally active against the Hungarian flea beetle *Phyllotreta cruciferae*, while **2**–**4** were inactive.[8]

Bartelt *et al.*[6] proposed the absolute configuration **5** for their pheromone component on the basis of its positive rotation (in hexane), because Pandey and Dev[9] reported positive rotation (in chloroform) of their synthetic **5**. Mori[10] synthesized *ent*-**5** by employing (*R*)-*ar*-turmerone (**6**) as the key intermediate, and found it to be dextrorotatory in hexane while levorotatory in chloroform. A simple mistake of using hexane as the solvent, instead of the reported chloroform, for measuring the optical rotation resulted in stereochemical misassignment of the absolute configuration of their pheromone components.

A similar example had been reported in 1976.[11] (1*S*,4*S*,5*S*)-*cis*-Verbenol (**7**) is a pheromone component of *Ips* bark beetles. Prior to Mori's work,[11] some researchers had called **7** (+)-*cis*-verbenol, while others referred to it as (−)-*cis*-verbenol. After synthesis of **7** and measurements of its optical rotations in different solvents, it became clear that **7** was dextrorotatory in acetone or methanol but levorotatory in chloroform. It is therefore of utmost importance to use the same solvent as reported by others, when one compares the sign of the optical rotation of a new sample with the previous data.

9.05.3 Elucidating the Structure of Pheromones of Stink Bugs

A simple example of the examination of a proposed structure through synthesis is provided in this section. In 2005, Takita[12] proposed the structure of the male-produced aggregation pheromone of the stink bug *Eysarcoris lewisi* as the sesquisabinene alcohol, (*E*)-2-methyl-6-(4′-methylenebicyclo[3.1.0]hexyl)hept-2-en-1-ol (**8**) (**Scheme 1**). Mori[13] synthesized (6*R*)-**8** and (6*S*)-**8** from the enantiomers of citronellal (**10**). The key steps were the intramolecular addition of an α-keto carbene to the alkene bond (**11** → **12**) and (*E*)-selective olefination of **13** to give **14**. The ^{1}H- and ^{13}C-NMR spectra of **8** around the trisubstituted double bond at C-2 were different from those of the natural pheromone.

(*Z*)-Alcohol **9** was therefore synthesized by (*Z*)-selective olefination of **13** with Ando's reagent **15**, giving **16**. ^{1}H- and ^{13}C-NMR spectra of synthetic (*R*,*Z*)-**9** and (*S*,*Z*)-**9** (both mixtures of diastereomers of C-1′ and C-5′) were very similar to those of the natural pheromone, and (*R*,*Z*)-**9** was pheromonally active against *E. lewisi*.[13] Mori's synthesis, however, could not determine the relative configuration at C-1′ and C-5′ of the pheromone.

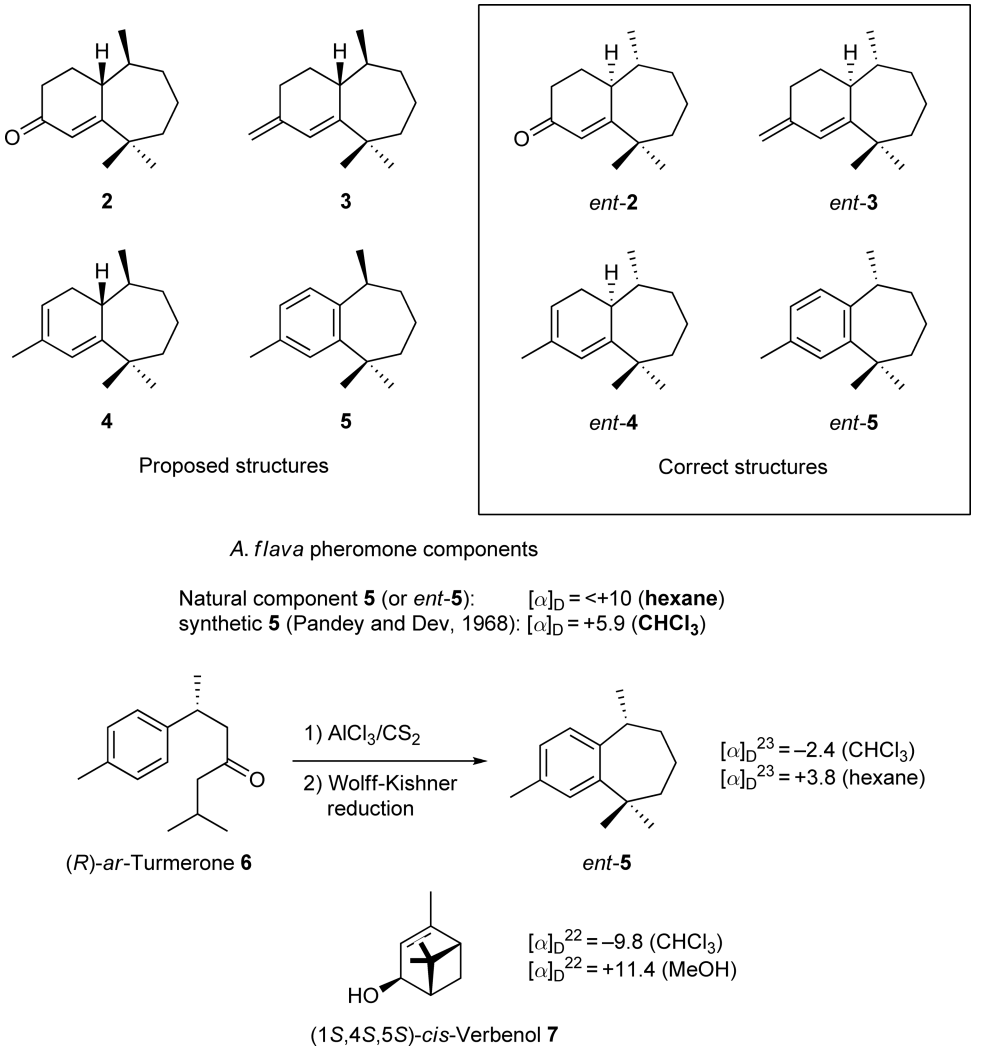


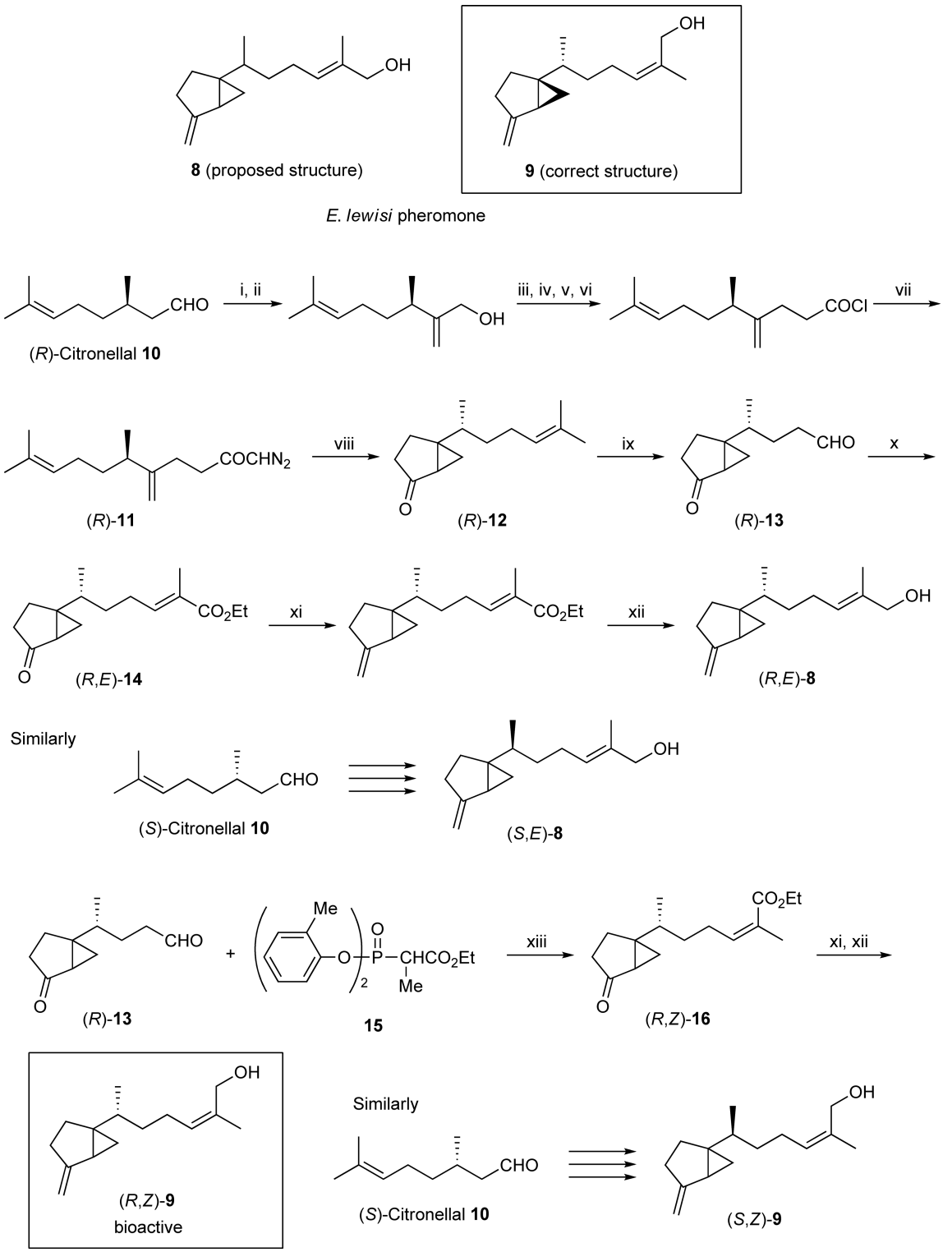
Figure 2 Absolute configuration of *Aphthona flava* pheromone components.

Mori *et al.*[14] finally determined the absolute configuration of the pheromone as (2*Z*,6*R*,1′*S*,5′*S*)-**9** by employing lipase-catalyzed asymmetric acetylation of **17′** as the key step (**Scheme 2**). Reduction of (6*R*)-**12** with L-selectride® afforded a mixture of **17** and **17′**, the latter of which could be acetylated with vinyl acetate in the presence of lipase PS-D (Amano) to give **18**. The remaining **17** was oxidized to give (6*R*,1′*S*,5′*R*)-**12**. Its absolute configuration was determined as depicted by CD comparison with (−)-sabina ketone **19** with a known absolute configuration. (2*Z*,6*R*,1′*S*,5′*S*)-**9** was synthesized from (6*R*,1′*S*,5′*R*)-**12**, while (6*R*,1′*R*,5′*S*)-**12** yielded (2*Z*,6*R*,1′*R*,5′*R*)-**9**. NMR and GC comparisons of these two products with the natural pheromone revealed (2*Z*,6*R*,1′*S*,5′*S*)-**9** to be the correct structure of the pheromone. Synthetic (2*Z*,6*R*,1′*S*,5′*S*)-**9** was biologically active, and none of its stereoisomers was either active or inhibitory.

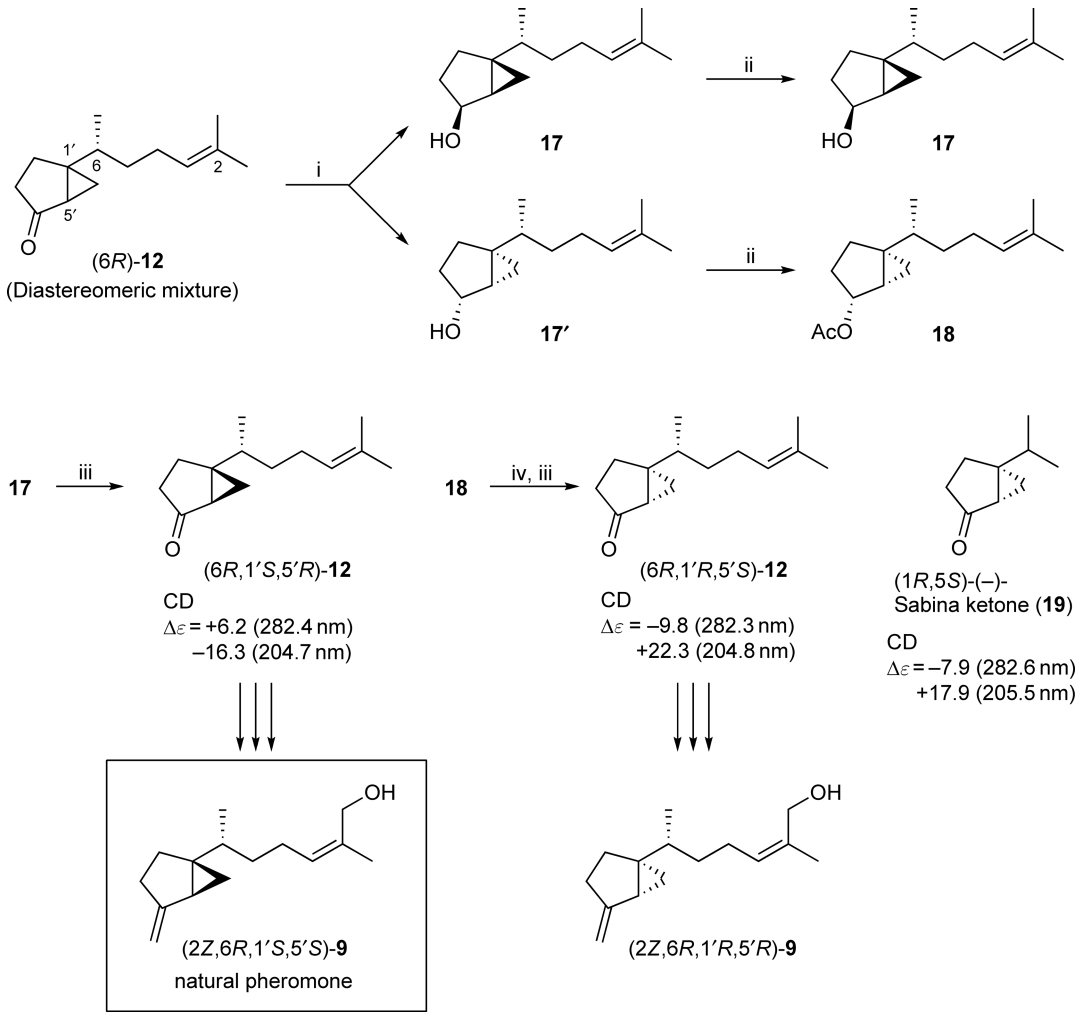
9.05.4 Absolute Configuration Involving Remote Stereocenters

9.05.4.1 German Cockroach Pheromone

In 1974, Nishida *et al.*[15–17] isolated and identified the components of the contact sex pheromone of the German cockroach *Blattella germanica*. They proposed the structures of the three components as **20**, **21**, and **22** (**Figure 3**). Their isolated amounts are shown in parentheses. As to the absolute configuration of **20** and **21**,



Scheme 1 Synthesis of the possible structures of the male-produced aggregation pheromone of the stink bug *Eysarcoris lewisi*. Reagents: (i) 37% CH_2O, $EtCO_2H$, pyrrolidine, Pr^iOH (90%); (ii) $LiAlH_4$, Et_2O (91%); (iii) $MeC(OEt)_3$, $EtCO_2H$, heat (95%); (iv) KOH, aq. EtOH (83%); (v) NaOEt, EtOH; (vi) $(COCl)_2$, C_5H_5N, hexane (quant., 2 steps); (vii) CH_2N_2, Et_2O (quant.); (viii) Cu, $CuSO_4$, cyclohexane, heat (58%); (ix) OsO_4, $NaIO_4$, THF, Bu^tOH, H_2O (quant.); (x) $Ph_3P{=}C(Me)CO_2Et$, THF, CH_2Cl_2 (57%); (xi) $Ph_3P(Me)Br$, Bu^nLi, THF (96%); (xii) Bu^i_2AlH, toluene (55%).



Scheme 2 Synthesis of the male-produced aggregation pheromone of the stink bug *Eysarcoris lewisi*. Reagents: (i) (a) $LiBBu^{s}_{3}H$, THF; (b) 30% H_2O_2, dil. NaOH (94%); (ii) (a) lipase PS-D (Amano), $CH_2{=}CHOAc$, Et_2O, room temperature, 10–13 h, repeat three times; (b) SiO_2 chromatography; (iii) $Pr^{n}_{4}NRuO_4$, NMO, MS 4A CH_2Cl_2, room temperature, 5 h (quant.); (iv) K_2CO_3, MeOH (quant.).

Nishida *et al.*[18] proposed the 3*S*-configuration on the basis of their optical rotatory dispersion (ORD) spectra coupled with NMR studies employing a chiral shift reagent. No information was available to assign the absolute configuration at C-11, because the stereocenter at C-11 was separated from the C-3 stereocenter by seven methylene groups.

Mori *et al.*[19] established the absolute configuration of **20** and **21** as 3*S*,11*S* by synthesizing all four stereoisomers of **20** and **21** and comparing their physical properties with those of the natural products. As shown in **Figure 3**, the stereoisomers of **20** and **21** were synthesized from (*R*)-isopulegol (**23**) via (*R*)-citronellic acid (**24**) of 92% ee (enantiomeric excess). Because the two stereocenters of **20** and **21** were separated, their stereoisomers showed identical ^{1}H- and ^{13}C-NMR spectra. However, their IR spectra as nujol mulls (i.e., as solid state and not as solutions) showed differences. Their optical rotations and melting points (mp's) were also very important in assigning the absolute configuration of natural **20** as shown in **Table 1**.

The natural ketone **20** was dextrorotatory in hexane, and (3*S*,11*S*)-**20** as well as (3*S*,11*R*)-**20** showed positive rotations, while (3*R*,11*R*)- and (3*R*,11*S*)-**20** were levorotatory. The natural **20** must therefore be either (3*S*,11*S*)- or (3*S*,11*R*)-**20**. As chloroform solutions, all the stereoisomers of **20** showed IR spectra that were identical to

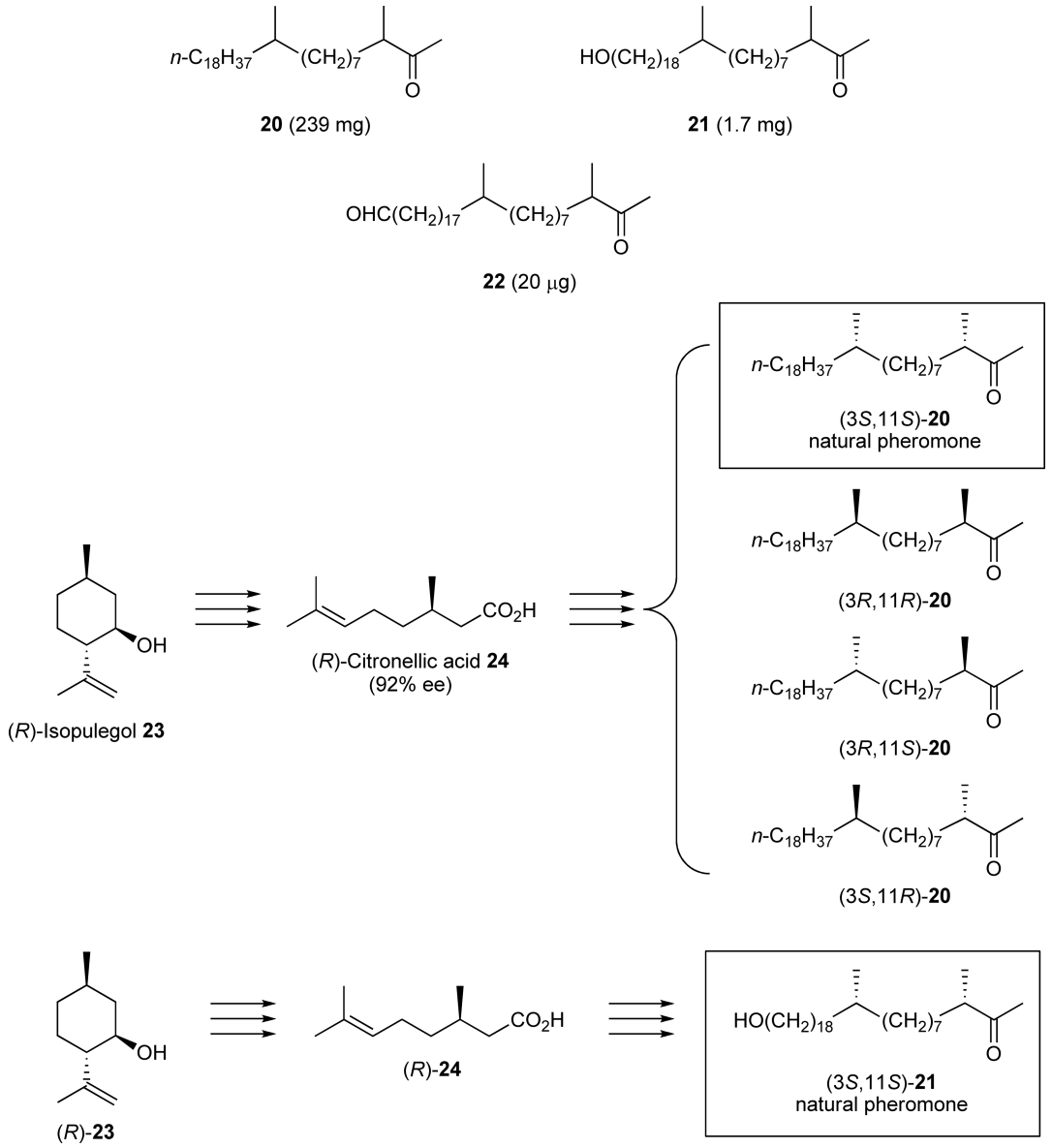


Figure 3 Structures of the sex pheromone components of the German cockroach *Blattella germanica* and related compounds.

each other. Their ^{1}H- and ^{13}C-NMR spectra were also indistinguishable. However, when their IR spectra were measured as nujol mulls, the stereoisomeric and crystalline ketones **20** showed subtle differences in the spectra due to the difference in their crystalline lattice structures. Thus, the IR spectrum of natural **20** was identical to those of (3*S*,11*S*)- and (3*R*,11*R*)-**20**. The natural **20** seemed to be (3*S*,11*S*)-**20** at this stage. To confirm this conclusion, the mp's of the four stereomers of **20** were measured, and the mixture mp determinations of the four isomers with the natural **20** were carried out. As can be seen from **Table 1**, (3*S*,11*S*)- and (3*R*,11*R*)-**20** showed the same mp as the natural **20**. Mixture mp determinations revealed (3*S*,11*S*)-**20** to be the natural **20**, because it showed no depression.[19] The classical method of mixture mp test is still useful in establishing the identity of two like samples. Similarly, the absolute configuration of the natural **21** could be established as 3*S*,11*S*.[19]

Later in 1990, highly pure (>99% ee) stereoisomers of **20** were synthesized from (*R*)-citronellal and ethyl (*R*)-3-hydroxybutanoate.[20] Bioassay of the four pure isomers of **20** by Schal and coworkers[21] showed that the natural pheromone (3*S*,11*S*)-**20** was the least effective of the four stereoisomers at eliciting courtship

Table 1 Specific rotations, IR spectra, and mp's of the natural and synthetic stereoisomers of **20** and their mixture mp's with natural **20**

Sample	$[\alpha]_D$ *(in hexane)*	*IR (nujol)*	*mp (°C)*	*Mixture mp with the natural* **20**
Natural **20**	+5.1 ($c = 3.54$)		45–46	–
(3*S*,11*S*)-**20**	+5.98 ($c = 0.9$)	same	44–44.5	44–45
(3*R*,11*R*)-**20**	–5.63 ($c = 4.1$)		44.5–45	35–37.5
(3*R*,11*S*)-**20**	–5.68 ($c = 4.0$)	different	39–39.5	34.5–35.5
(3*S*,11*R*)-**20**	+5.73 ($c = 2.04$)	same	38–38.5	33.5–35

responses in males. The German cockroach produces the least active (3*S*,11*S*)-**20** due to the stereochemical restriction in the course of its biosynthesis.

9.05.4.2 Plakoside A

In 1997, plakoside A (**25**) (**Figure 4**) was isolated by Fattorusso and coworkers[22] as an immunosuppressive metabolite of the Carribean sponge *Plakortis simplex*. It is a structurally unique glycosphingolipid with a prenylated D-galactose moiety and cyclopropane-containing alkyl chains. Its 2*S*,3*R*,2‴*R* stereochemistry was proposed on the basis of the CD measurements of its degradation products.[22] The absolute configuration at the two cyclopropane moieties of **25**, however, remained unknown, although the *cis*-stereochemistry was suggested by detailed ^{1}H-NMR analysis of **25**.[22]

In 2000, Nicolaou *et al.*[23] accomplished the synthesis of (2*S*,3*R*,11*R*,12*S*,2‴*R*,5‴*Z*,11‴*R*,12‴*S*)-**25**, and found its ^{1}H- and ^{13}C-NMR data to be identical to those reported for the natural **25**. They therefore claimed their synthetic product to be identical to the natural product. However, in 2001, Seki and Mori[24] synthesized both (2*S*,3*R*,11*R*,12*S*,2‴*R*,5‴*Z*,11‴*R*,12‴*S*)- and (2*S*,3*R*,11*S*,12*R*,2‴*R*,5‴*Z*,11‴*S*,12‴*R*)-**25**, both of which were spectroscopically indistinguishable from natural **25**. Then, which stereoisomer of **25** is plakoside A? In order to solve this problem, degradation studies of natural plakoside A (**25**) were executed as shown in **Figure 5**, and the degradation products were compared with the synthetic samples of known absolute configuration.[25]

Lipase-catalyzed asymmetric acetylation of *meso*-diol **26** gave enantiomerically pure **27**, which was converted to the enantiomers of the reference acids **28** and **29**. These acids were derivatized and analyzed by high-performance liquid chromatography (HPLC) according to Ohrui and coworkers.[26–28] Esterification of acids **28** and **29** with Ohrui's chiral and fluorescent reagent R*OH **30** yielded esters **31** and **32**. All of these derivatives were separable by reversed-phase HPLC at a column temperature of −50 °C. Owing to the presence of the anthracene system in **31** and **32**, their picogram quantities were detectable by fluorescence, and therefore minute amounts of degradation products could be analyzed.

Degradation of plakoside A pentaacetate (**33**) was executed by first treating it with nitrous acid in acetic anhydride through N-nitrosation at the amide nitrogen of **33** to give **34** and **35**, which were further cleaved to give **28** and **29**, respectively. A mixture of **28** and **29** was derivatized with **30**, and the products were subjected to HPLC analysis to show them to be (6*S*,7*R*)-**31** and (9*S*,10*R*)-**32**. Accordingly, the absolute configuration of plakoside A must be (2*S*,3*R*,11*S*,12*R*,2‴*R*,5‴*Z*,11‴*S*,12‴*R*)-**25**. The synthetic product (2*S*,3*R*,11*R*,12*S*,2‴*R*,5‴*Z*,11‴*R*,12‴*S*)-**25** of Nicolaou *et al.* turned out to be a diastereomer of plakoside A.[25] A combination of enantioselective synthesis and HPLC analysis is a powerful method for the determination of the absolute configuration of a compound with stereogenic centers remote from other functionalities and stereogenic centers.

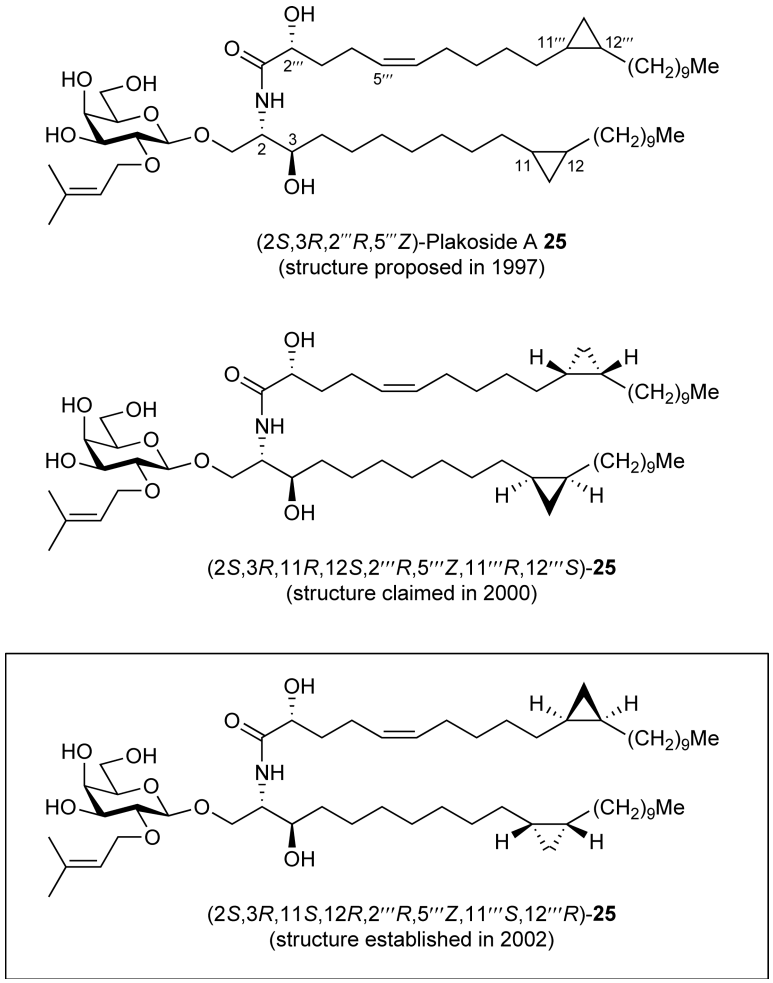


Figure 4 Structure of plakoside A (**25**).

9.05.5 Absolute Configuration Involving Stereocenters Separated by a Polymethylene Spacer

9.05.5.1 *cis*-Solamin

Annonaceus acetogenins, isolated from the plant species belonging to Annonaceae (custard apple family), are waxy solids with cytotoxic and antitumor activity. They are characterized by the presence of one or more 2,5-disubstituted tetrahydrofuran rings connected to a butenolide through a polymethylene spacer. As exemplified by *cis*-solamin A (**36**) and *cis*-solamin B (**37**) (**Figure 6**), metabolites of tropic fruit tree *Annona muricata*, two stereogenic moieties are separated by a polymethylene spacer. Within the tetrahydrofuran moiety, its relative configuration could be determined by NMR analysis as depicted, but its absolute configuration was difficult to determine. Brown and coworkers[29] synthesized four possible stereoisomers (**36**, *ent*-**36**, **37**, and *ent*-**37**) of *cis*-solamin by the route summarized in **Figure 6**.

The four synthetic isomers of *cis*-solamin were indistinguishable from each other and from natural *cis*-solamin on the basis of their IR, MS, ^{1}H-NMR, and ^{13}C-NMR spectra, owing to the length and flexibility of the spacer connecting the tetrahydrofurandiol and butenolide moieties. Optical rotation values obtained for each of the pairs of diastereomers were also very similar, and consistent with the known fact that the contribution to optical rotation from the butenolide moiety dominates that from a pseudosymmetrical

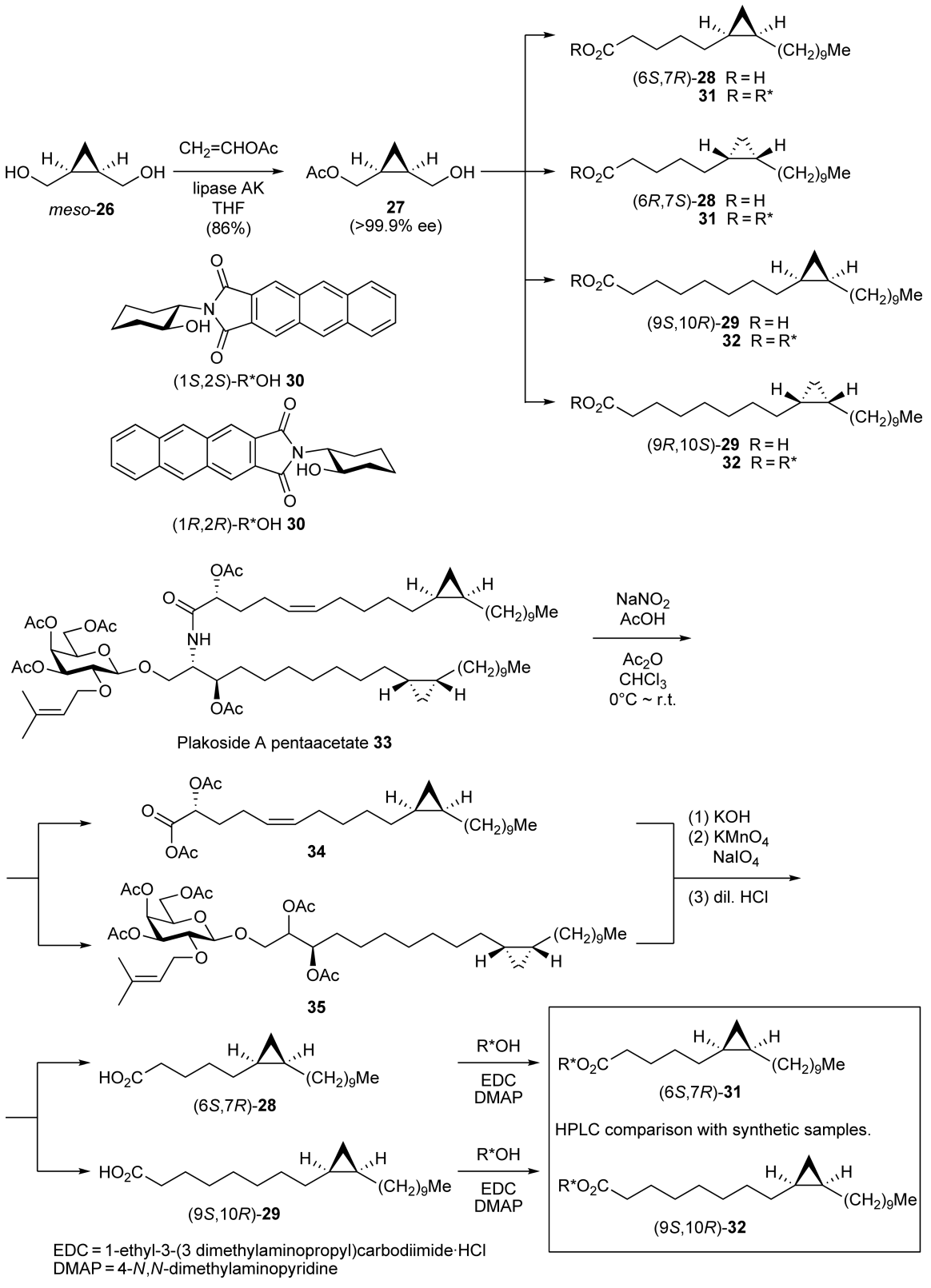


Figure 5 Determination of the absolute configuration of plakoside A.

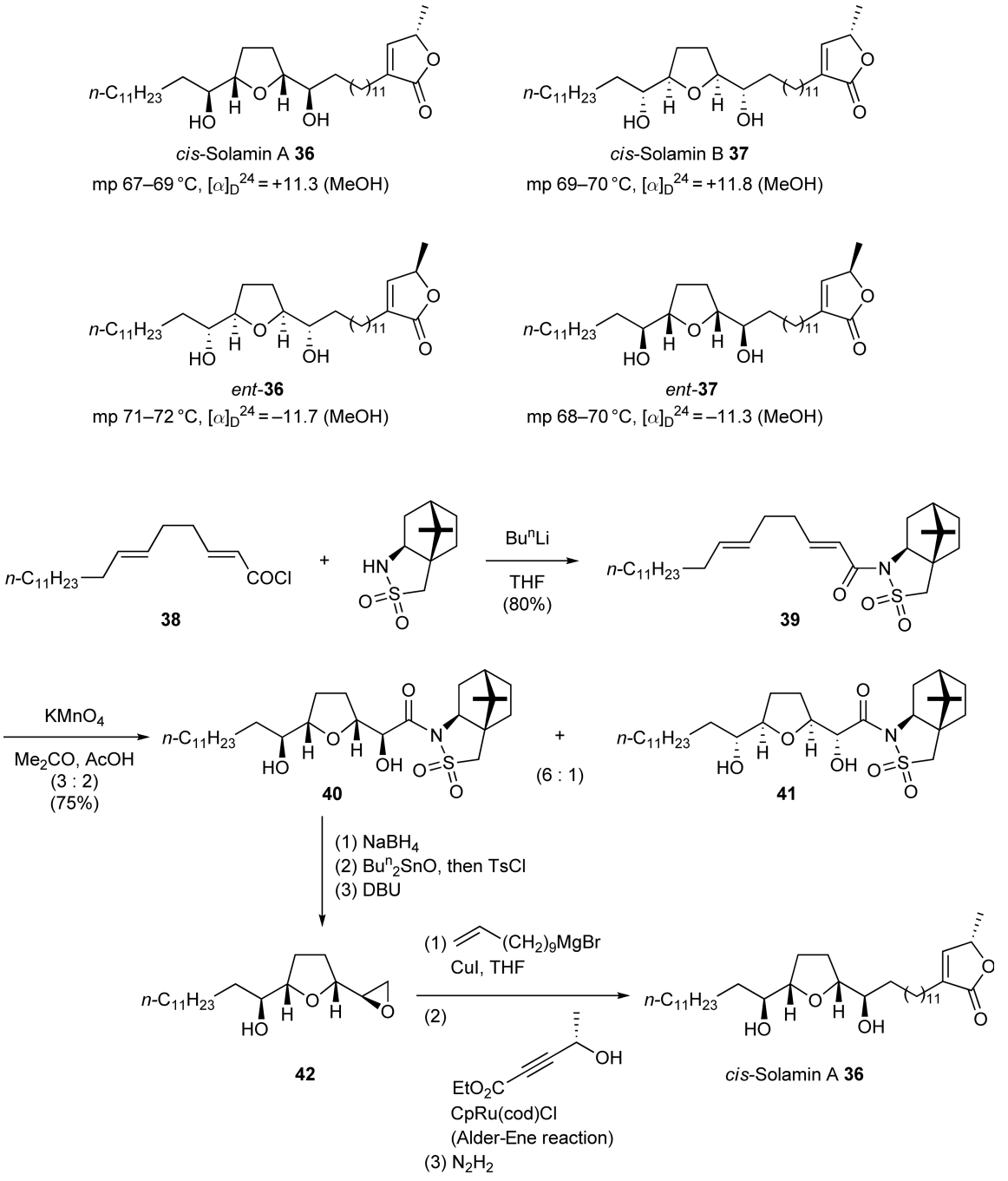


Figure 6 Structure and synthesis of *cis*-solamin A (**36**).

tetrahydrofurandiol region in acetogenins. In the course of this study, Brown and coworkers[29] found that all four isomers (**36**, *ent*-**36**, **37**, and *ent*-**37**) were separable by enantioselective HPLC employing a cyclodextrin-based stationary phase.

Subsequently, Figadére and coworkers[30] analyzed natural *cis*-solamin by enantioselective HPLC, and found it to be a 9:8 mixture of *cis*-solamin A (**36**) and *cis*-solamin B (**37**). Thus, natural *cis*-solamin was stereochemically heterogeneous demonstrating that enantioselective chromatography is indeed a powerful technique in

stereochemical studies of natural products. Although NMR and X-ray analysis are regarded as the most powerful techniques for structure elucidation, a chromatographic method gave the decisive evidence to show the heterogeneity of *cis*-solamin.

9.05.5.2 Murisolin

In 2006, Curran *et al.*[31] published an important paper on murisolin (**43**; **Figure 7**), another acetogenin, entitled 'On the proof and disproof of natural product stereostructures'. They synthesized two 16-member stereoisomer libraries of murisolin isomers that provided 24 of the 32 possible diastereomers of murisolin (**43**). Each member of the 16-member sublibrary of murisolins was subjected to NMR analysis at 600 MHz (^{1}H) and 150 MHz (^{13}C). The library members have 4*R*,34*S* configurations in the butenolide moiety with all possible configurations at the remaining stereogenic centers in the tetrahydrofurandiol fragment. Every NMR spectrum belongs to one of only six groups, and the spectra within each group are substantially identical. Symmetry considerations of simple model compounds **44** as shown in **Figure 7** help us to understand why there are only six groups. The butenolide group in **43** is substantially separated from the tetrahydrofurandiol moiety, and therefore ^{1}H-NMR spectrum of the former cannot be affected sufficiently to show differences due to the stereochemistry of the latter.

The six groups of the ^{1}H-NMR spectra were organized according to the local symmetry of the tetrahydrofurandiol moiety. On the basis of this NMR information, inspection of the NMR spectrum of a murisolin stereoisomer enables users to assign the relative configuration to its tetrahydrofurandiol region. Very small ($\leq$0.1 ppm) differences were observed in the hydroxybutenolide region of the 150 MHz ^{13}C-NMR spectra of

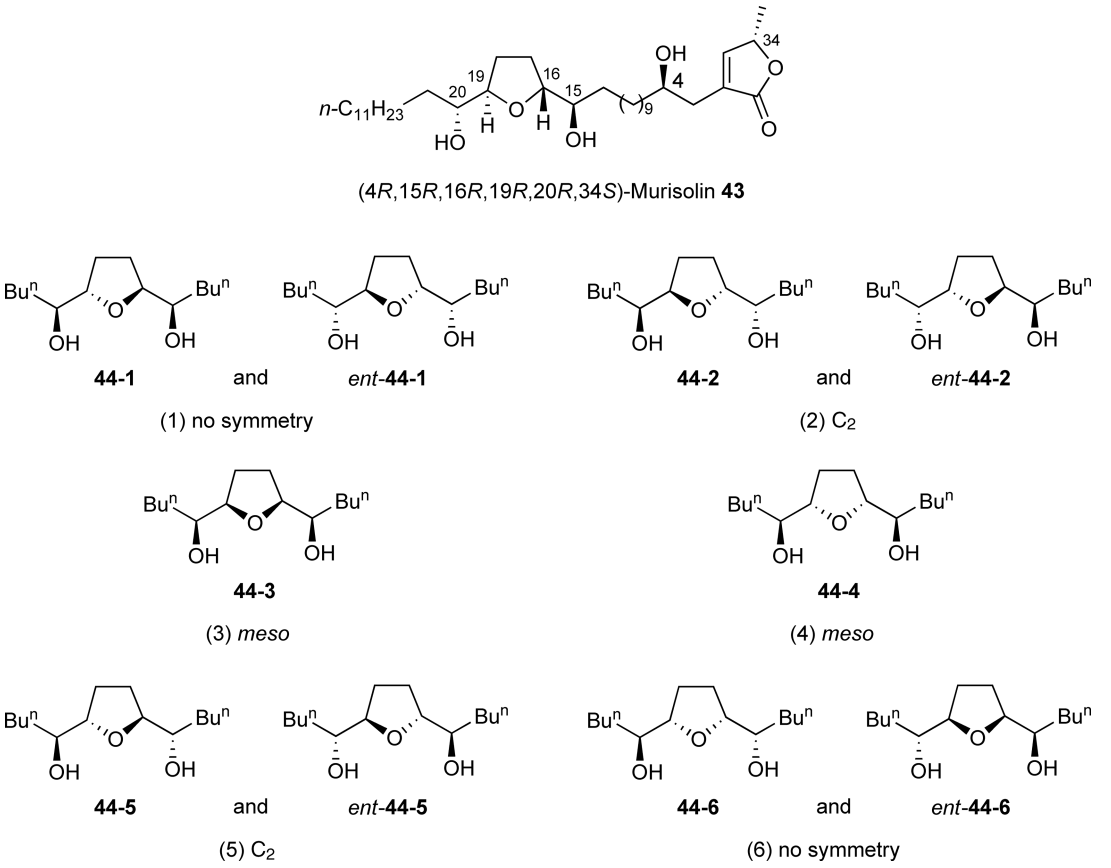


Figure 7 Structure of murisolin (**43**) and group classifications of its stereoisomers on the basis of simple model compounds **44**.

murisolin stereoisomers, based on the *syn*/*anti* relative configuration at C-4 and C-34. Derivatization of **43** and its stereoisomers to tris-(*S*)-Mosher esters followed by NMR measurements revealed that murisolin-Mosher ester stereoisomers exhibited one of only 10 sets of ^{1}H-NMR spectra. Through these observations it was possible to assign 4*R*,15*R*,16*R*,19*R*,20*R*,34*S* configuration to murisolin (**43**), which was in accord with the previous proposals. Curran *et al.* also comment that enantioselective HPLC is superior to either optical rotation or melting point comparisons to prove or disprove structures, if all the candidate isomers are available.

Curran *et al.*'s work informs us that construction of stereoisomer libraries followed by thorough studies on their NMR and enantioselective HPLC behaviors is an especially reliable way of elucidating the stereostructure of natural products. This type of thorough stereochemical analysis is likely to become more popular in the future in connection with the advances in parallel synthesis.

9.05.5.3 New World Screwworm Fly

Female-produced sex pheromones of the New World screwworm fly *Cochliomyia hominivorax* were first studied by Pomonis *et al.*[32] in 1993. They isolated 16 pheromone candidates from the female flies, but they were unable to identify the pheromonally active compounds. In 2002, Mori and coworkers[33] synthesized stereoisomeric mixtures of **45** and **46** (**Figure 8**), and they were found to be pheromonally active. Subsequently, all four stereoisomers of the more potent acetate **45** were synthesized as shown in **Figure 8**.[34] In the course of the synthesis, the parent alcohol **50** was esterified with the anthracene-containing acid (1*S*,2*S*)-**51**, and the derived ester **52** was analyzed by HPLC at −25 °C by the method of Ohrui and coworkers.[26–28] All four diastereomers of **52** were separable, and therefore the stereochemical purities of the four isomers of **45** could be estimated as depicted.[34]

The four isomers of **45** showed identical IR, ^{1}H-NMR, and ^{13}C-NMR spectra. In addition, all of them were equally bioactive as the sex pheromone. Accordingly, their derivatization to **52** followed by HPLC analysis was the only way to distinguish the stereoisomers. Finally, the natural pheromone component was shown to be (6*R*,19*R*)-**45** by its derivatization to **52** followed by HPLC analysis.[35] Enantioselective HPLC or gas chromatography (GC) and chromatographic analysis after derivatization with Ohrui's reagent seems to be the most sensitive method for discrimination of stereoisomers. Thus, it may be concluded that structural analysis must, from time to time, be carried out by employing various kinds of different analytical methods. Otherwise, mistakes are likely to occur.

9.05.6 Origin of Biological Homochirality

A characteristic hallmark of life is believed to be its 'homochirality'.[36] In general, it is true, although natural products are not always enantiomerically pure.[37] The origin of biomolecular homochirality is discussed in depth by MacDermott.[36] Those who are interested to see whether the parity-violating weak force is the cosmic dissymmetry that Pasteur was looking for should read her chapter in the book entitled '*Chirality in Natural and Applied Science*'.

Soai *et al.*[38] discovered and developed asymmetric autocatalysis (**Figure 9**), in which the structures of the chiral catalyst (*S*)-**54** and the chiral product (*S*)-**54** are the same after the addition of diisopropylzinc to aldehyde **53**. Consecutive asymmetric autocatalysis starting with (*S*)-**54** of 0.6% ee amplifies its ee, and yields itself as the product with >99.5% ee. Even chiral inorganic crystals, such as quartz or sodium chlorate, act as chiral inducers in this reaction. Soai *et al.*'s asymmetric autocatalysis gives us an insight to speculate on the early asymmetric reactions on this planet Earth. However, it can be argued whether such strictly anhydrous organometallic reactions are possible under the nonartificial conditions or not.

A phenomenon that may be related to the origin of biological homochirality was recently reported by Cooks and coworkers:[39] Serine sublimes with spontaneous chiral amplification. Sublimation of near racemic sample of serine **55** (**Figure 9**) yields a sublimate that is enriched in the major enantiomer. The chiral purity maximizes at 190–210 °C, and then falls as thermolysis becomes favorable. This simple one-step sublimation may represent a possible mechanism for the chiral amplification step to explain the origin of biological homochirality.

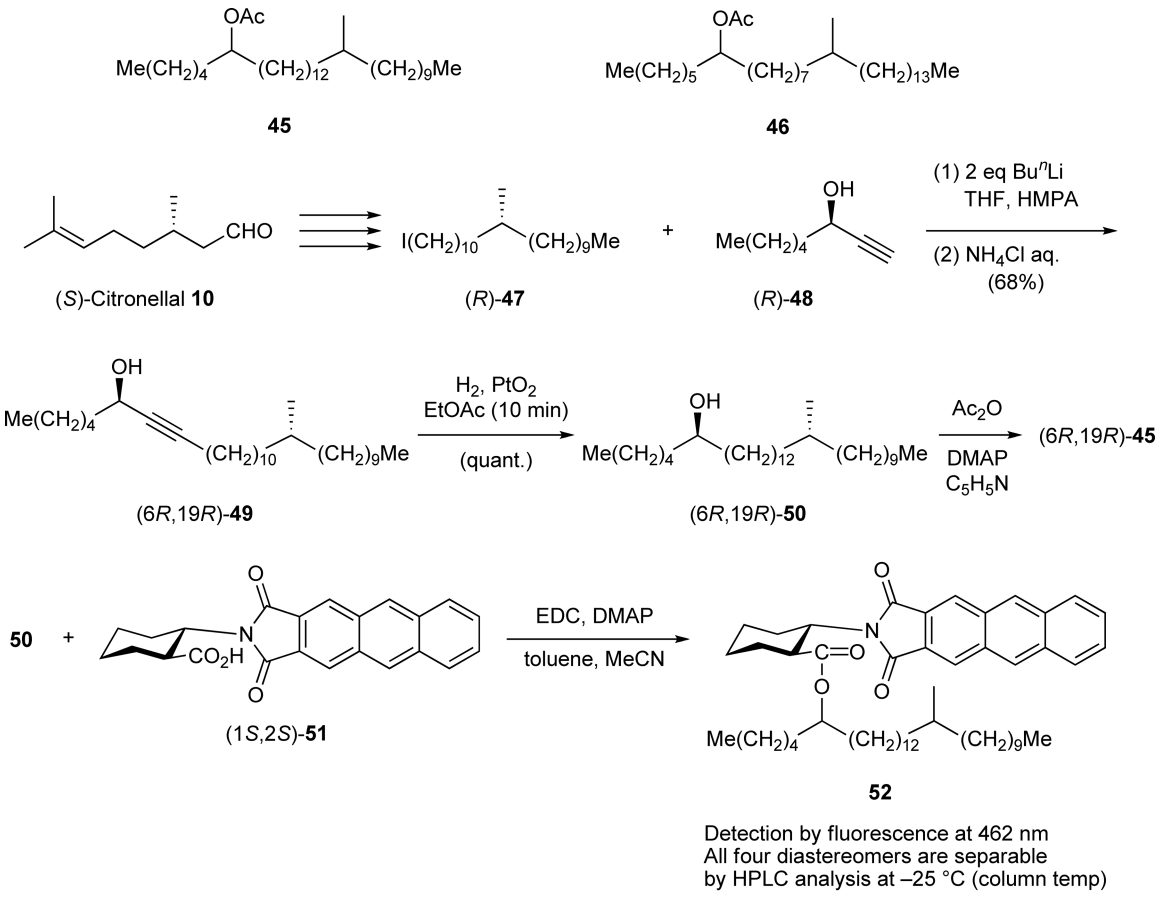


Figure 8 Synthesis of four stereoisomers of the most potent component **45** of the female sex pheromone of the New World screwworm fly *Cochliomyia hominivorax*.

9.05.7 Exceptions to Biological Homochirality

Until recently, we believed our human bodies to be constituted from L-amino acids only. Advances in analytical methods now indicate that there are a number of D-amino acids in human bodies as detailed in the review by Fujii and Saito.[40] Free D-serine was observed predominantly in mammalian brain, and free D-aspartic acid

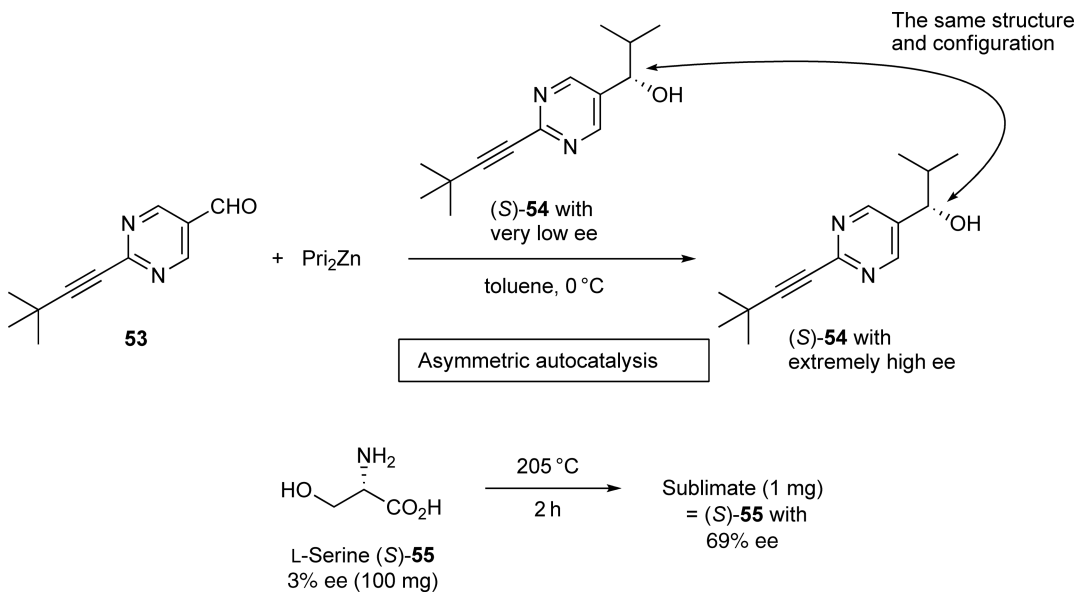


Figure 9 Structures of compounds in connection with biological homochirality (1).

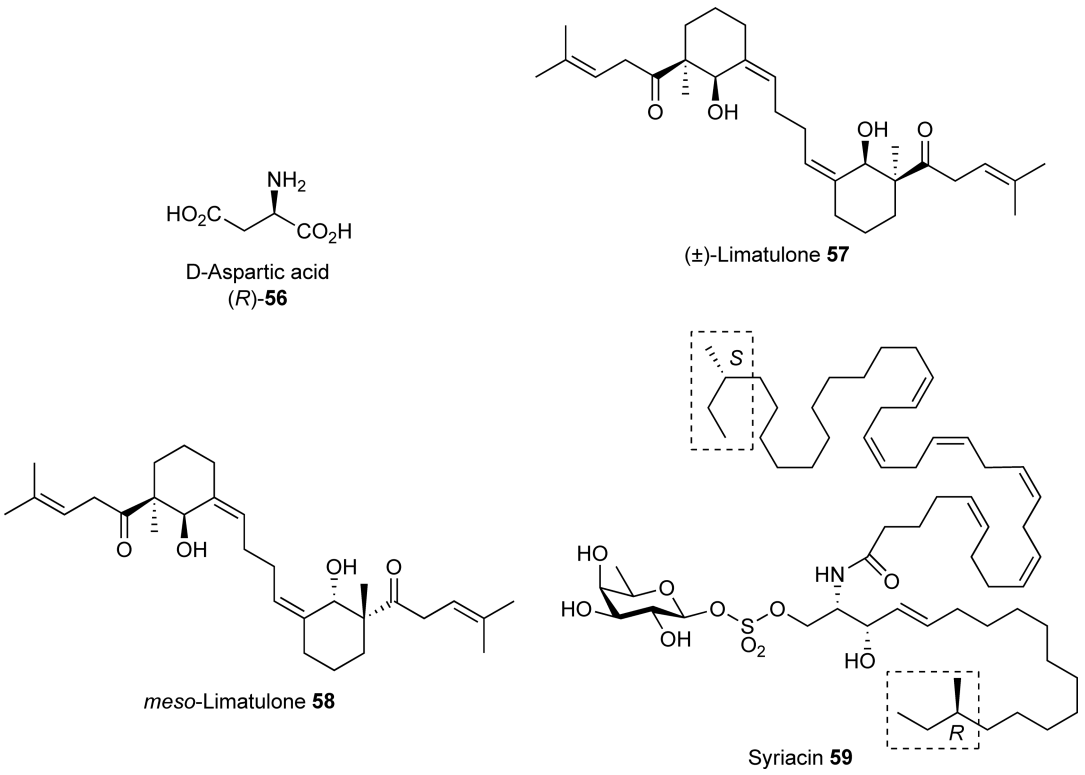


Figure 10 Structures of compounds in connection with biological homochirality (2).

(**56**; **Figure 10**) exists in various mammalian tissues. For example, in the prefrontal cortex of human brain, as much as 60% of the total aspartic acid was in the D-form at week 14 of gestation, but rapidly decreased to trace levels by the time of birth.

D-Amino acids were detected in various aged human tissues such as tooth, bone, aorta, brain, erythrocytes, eye lens, skin, ligament, and lung. Especially D-serine was found in the β-amyloid protein of Alzheimer's disease. Stereoinversion of L-aspartic acid to D-aspartic acid takes place in alpha A and alpha B crystallins of human lens. Thus, aged persons possess more D-aspartic acid in the lens. This phenomenon seems to be related to cataract.[40]

The fluctuation of the amount of free D-amino acids in living bodies suggests that D-amino acids might be one of the factors controlling the generation and differentiation of cells or tissues. D-Amino acids in proteins can be interpreted as molecular markers of aging.[40] Another review is available concerning the occurrence and functions of free D-aspartic acid and its metabolizing enzymes.[41]

As already described in the first edition of this Comprehensive Series,[42] the limpet *Acmeia (Collisella) limatula* produces a defensive metabolite, limatulone, both as the racemate (**57** and its enantiomer) and as the *meso*-isomer **58**.[43] This example demonstrates that nature does not always produce enantiomerically pure compounds.

In 2006, Rezanka *et al.*[44] isolated an antifeedant, syriacin (**59**), from the freshwater sponge *Ephydatia syriaca* in the Jordan river. It is an unusual sulfated ceramide glycoside with branched-chain sphingosine and also a branched-chain fatty acid. From the viewpoint of absolute configuration, **59** is very unusual, because it contains both (*R*)- and (*S*)-configured *sec*-butyl terminals in its alkyl chains. It seems to be biosynthesized from precursors with opposite absolute configuration.

9.05.8 Mimics of Bioactive Natural Products and Bioisosterism

There are practical demands for the invention of pheromone mimics, because pheromones are often too labile to be used in pest control. Various mimics have been prepared to date, several of which will be described in this section.

Tacke *et al.*[45] synthesized the enantiomers of sila-linalool (**61**) as shown in **Figure 11**. The starting material **60** was converted into (±)-**61**, which was resolved by GC to give both (+)-**61** and (−)-**61**. Both enantiomers were bioactive as tested by electroantennographic detection (EAD) on the males of the vernal solitary bee *Colletes cunicularius*. There was no major difference between the bioactivity of the sila-pheromone **61** and the natural linalool. The substitution of a carbon atom by silicon provides a good example of bioisosterism.

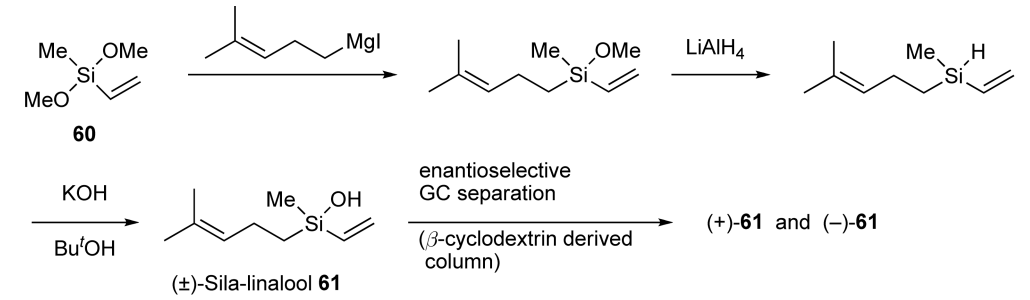
(1*S*,5*R*)-Frontalin (**62**) is the aggregation pheromone of bark beetles such as *Dendroctonus brevicomis* and *D. frontalis*. Strunz *et al.*[46] synthesized its isomer **63**, which was shown to be pheromonally active. Bravo *et al.*[47] synthesized the trifluoro analogue **64** of frontalin. Its bioactivity, however, was not reported.

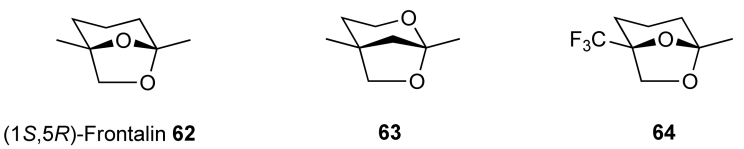
(4*S*,5*R*)-Eldanolide (**65**) is the male-produced sex pheromone of the African sugarcane borer *Eldana saccharina*. Itoh *et al.*[48,49] reported the synthesis and pheromonal activity of its fluorinated analogues **66**–**68**. Two analogues, **66** and *ent*-**66**, were bioactive, while the remaining four analogues showed no activity as revealed by EAD.

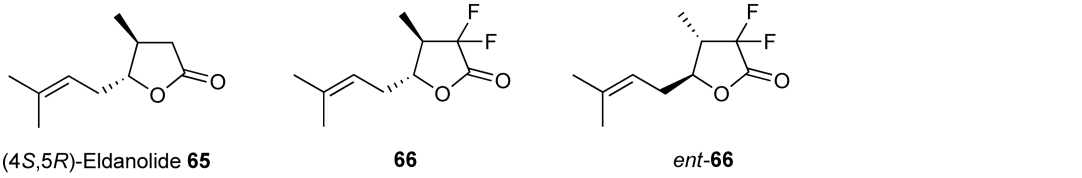
(7*R*,8*S*)-Disparlure (**69**) is the female-produced sex pheromone of the gypsy moth *Lymantria dispar*. Plettner and coworkers[50] synthesized and bioassayed its 5-oxa analogues **70** and *ent*-**70**. GC-EAD bioassay revealed both **70** and *ent*-**70** to be bioactive. The dose–response curve for **70** and that for *ent*-**70** were similar. Interestingly, pheromone-binding protein 1 (PBP1), which binds (7*S*,8*R*)-*ent*-**69** strongly, binds **70** and *ent*-**70** with nearly the same affinity as *ent*-**69**. The affinity of PBP1 for naturally occurring (7*R*,8*S*)-**69** is known to be much weaker than for *ent*-**69**. Neither **70** nor *ent*-**70** functioned as a pheromone inhibitor. The concept of bioisosterism works in this case, too, although with a subtle difference.

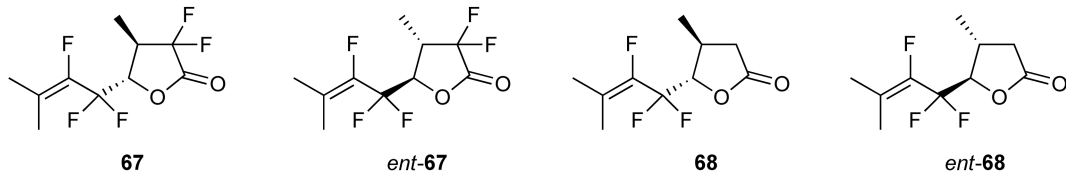
(4*R*,8*R*)-4,8-Dimethyldecanal (**71**; tribolure) is the aggregation pheromone of the flour beetle *Tribolium castaneum* and *T. confusum*. Due to the air sensitivity of **71** as an aldehyde, the more stable formate ester **72** was synthesized. This was found to be bioactive and was used in commercial pheromone traps.[51] This is an example of bioisosterism by which a carbon atom is replaced by an oxygen atom.

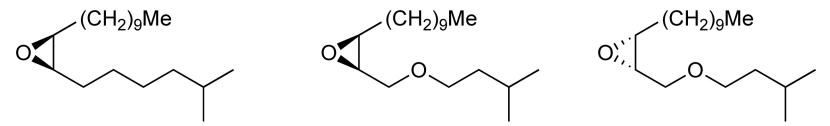
(2*S*,3*R*,1′*R*)-Stegobinone (**73**) is the female sex pheromone of the drugstore beetle *Stegobium paniceum*. Its (2*S*,3*R*,1′*S*) isomer is a strong inhibitor of pheromone action. The methyl group at C-1′ of **73** is so readily epimerizable that the natural **73** soon becomes biologically inactive, and **73** cannot be used practically.

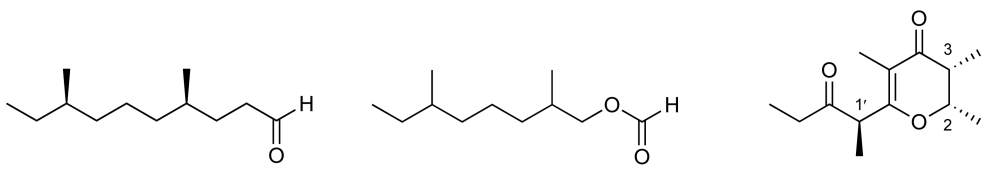












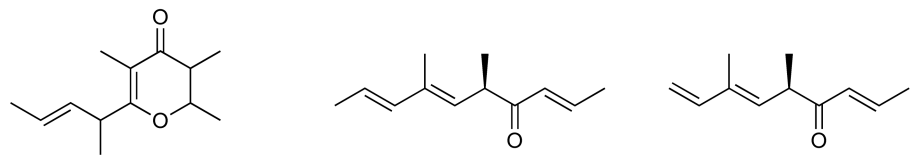


Figure 11 Structures of pheromones and their mimics.

Scientists at Fuji Flavor Co. synthesized stegobiene (**74**), which showed pheromone activity and could be used commercially to monitor the population of the drugstore beetle.

The female sex pheromone (*R*)-**75** of the Israeli pine bast scale *Matsucoccus josephi* is also a potent kairomone that attracts the scale insect's predator *Elatophilus hebraicus*. A mimic **76** of the pheromone **75** shows only the pheromone activity with no kairomone activity.[52,53] Accordingly, **76** is a more useful population-monitoring agent for *M. josephi* than the pheromone itself, which also catches the beneficial predator *E. hebraicus*.

9.05.9 Inventions of Pesticides and Medicinals

Natural products continue to be prototypes of pesticides and medicinals. Chemists' creativity and efforts have brought about many new mimics that are more potent, more economical, and more stable or safer than the original natural products.

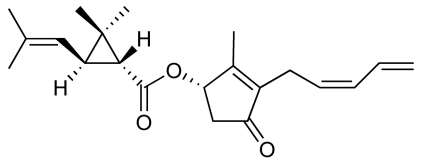
Pyrethrum powder is the dried flowerheads of *Chrysanthemum cinerariaefolium* and has been used widely as an insecticide. Its active principle was studied by L. Ruzicka, H. Staudinger, R. Yamamoto, and others, and pyrethrin I (**77**) (**Figure 12**) was identified as the major component.[54] Even now, after 80 years following the elucidation of the structure, modification of **77** continues to generate a group of insecticides called pyrethroids. Allethrin (**78**) was the first mimic to be manufactured in a large scale by Sumitomo Chemical Co. in 1953. Subsequently, in 1979 Sumitomo developed (*S*)-fenvalerate (**79**), while in 1981 etofenprox (**80**) was commercialized by Mitsui Chemical Co. These two compounds are stable in field conditions and are widely used as agricultural insecticides.[55]

Agelasphin 9b (**81**) and its relatives were isolated from the Okinawan marine sponge *Agelas mauritianus* as glycosphingolipids and they exhibited anticancer activity *in vivo* in mice and humans.[56] By simplifying the structure of **81**, researchers at Kirin Brewery Co. developed KRN7000 (**82**) as an anticancer drug candidate.[57]

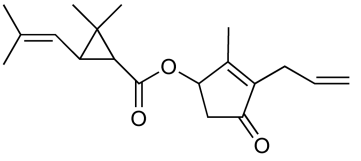
It has been shown that KRN7000 (**82**) is a ligand that forms a complex with CD1d protein, a glycolipid presentation protein on the surface of the antigen-presenting cells of the immune system. Lipid alkyl chains of **82** are bound in the grooves in the interior of the CD1d protein and the galactose head group of **82** is presented to the invariant Vα14 antigen receptors of natural killer (NK)T cells. After activation by recognition of the CD1d–**82** complex, NKT cells release both helper T1 (Th1) and Th2 types of cytokines simultaneously in large quantities. Th1-type cytokines such as interferon (IFN)-γ mediate protective immune functions like tumor rejection, whereas Th2-type cytokines such as interleukin (IL)-4 mediate regulatory immune functions to ameliorate autoimmune diseases. Th1- and Th2-type cytokines can antagonize each other's biological actions. Because of this antagonism, the use of **82** for clinical therapy has not been successful yet. To circumvent this problem, many research groups modified the structure of KRN7000 (**82**) to develop new analogues of **82** that induce NKT cells to produce either Th1- or Th2-type cytokines.

Modification of the α-galactosyl part of **82** afforded α-*C*-GalCer (**83**)[58] and RCA1-56 (**84**).[59] These two compounds showed an enhanced Th1-type response *in vivo* to generate IFN-γ. Modification of the phytosphingosine part of **82** by shortening the alkyl chain to give OCH (**85**) resulted in an enhanced Th2-type response *in vivo* to produce IL-4.[60] Introduction of an aromatic ring at the end of the fatty acid chain to give **86** caused enhanced IFN-γ production.[61] Chemical modification of the parent KRN7000 (**82**) turned out to be a promising way to invent a more specific anticancer drug candidate.

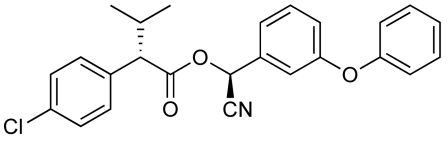
It is true that we can see computer-generated docking models of the bioactive prototype compounds and their receptors. Even so, however, invention of mimics is restricted by the limited human capacity to imagine only conventional changes in functional groups and skeletons of the parent compounds. Natural products certainly will give us new and vast opportunities to find out unusual structures beyond our imagination. There are many mimics of natural products in the areas of taste, flavor, and fragrance. These interesting topics have been treated in Volume 4.



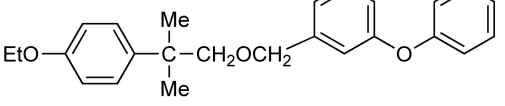
Pyrethrin I **77** (1924)



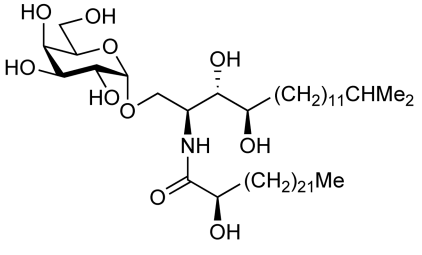
Allethrin **78** (1953)



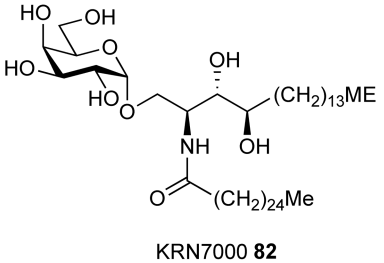
(*S*)-Fenvalerate **79** (1979)
Sumitomo Chemical Co.



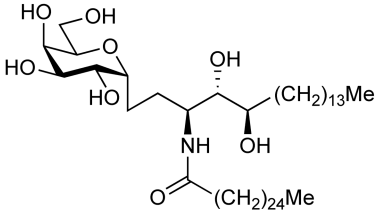
Etofenprox **80** (1981)
Mitsui Chemical Co.



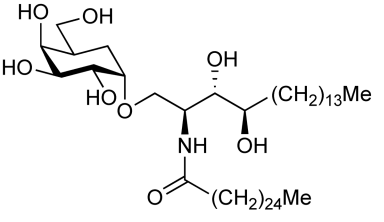
Agelasphin 9b **81**



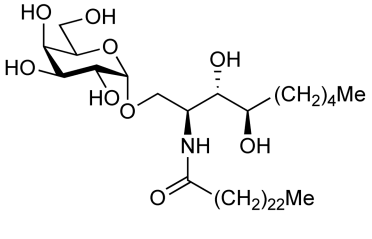
KRN7000 **82**
Kirin Brewery Co.



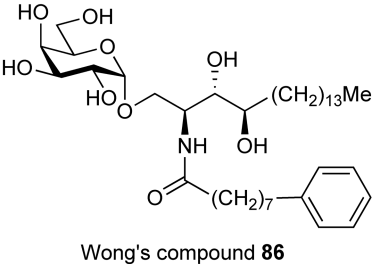
α-*C*-GalCer **83**



RCAI-56 **84**



OCH **85**



Wong's compound **86**

Figure 12 Structures of natural product-inspired pesticides and medicinals.

9.05.10 Conclusion

This chapter has treated the three important points in the studies of bioactive natural products.

First, recent examples of the determination of structure including the absolute configuration of bioactive natural products have been discussed, emphasizing the techniques to solve stereochemical problems among compounds with remote stereogenic moieties separated by a polymethylene spacer. Case studies with pheromones and acetogenins have been given to illustrate the problems and solutions.

Second, problems related to biological homochirality have been discussed to contemplate its origin and also to see exceptions to the homochirality principle. The presence and roles of D-amino acids in organisms as well as the presence of stereochemically heterogeneous compounds have been illustrated with examples.

Third, invention of mimics of bioactive natural products has been briefly discussed to show the importance of natural products as prototypes of pesticides and medicinals.

There are so many new discoveries in natural products research that no one can be an expert in all the areas unless they were content to be superficial. Thus, we have to remember the following words of Apostle Paul, "The person who thinks he knows something really does not know as he ought to know." (I Corinthians 8:2).

Abbreviations

EAD	electroantennographic detection
HPLC	high-performance liquid chromatography
IFN	interferon
IL	interleukin
NK	natural killer
mp	melting point
ORD	optical rotatory dispersion
PBP1	pheromone-binding protein 1

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Biographical Sketch

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