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TERPENOID CONIDIATION FACTORS IN *PENICILLIUM CYCLOPIUM*

Yanhong He



LUND UNIVERSITY
BIOORGANIC CHEMISTRY
2004

Akademisk avhandling som för avläggande av teknisk doktorsexamen vid tekniska fakulteten vid Lunds Universitet kommer offentligen försvaras på Kemicentrum, sal K:C, fredagen den 19 november 2004, kl. 13.30.

A doctoral thesis at a university in Sweden is produced as a monograph or as a collection of papers. In the later case, the introductory part constitutes the formal thesis, which summarizes the accompanying papers. These have either already been published or are manuscripts at various stages (*in press, submitted, or in manuscript*).

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献给我亲爱的母亲吴桂芝
谨以此纪念我亲爱的父亲何其猛

List of Papers

This thesis is based on the following papers which are referred to in the text by the roman numerals I-IV. Paper I reproduced by the permission of the publisher.

- I. Tomás Roncal, Shandra Cordobés, Unai Ugalde, Yanhong He and Olov Sterner
Novel diterpenes with potent conidiation inducing activity
Tetrahedron Letters **2002**, 43(38), 6799–6802
- II. Yanhong He, Martin Johansson and Olov Sterner
Mild microwave-assisted hydrolysis of acetals under solvent-free conditions
Synthetic Communications, in press
- III. Yanhong He, Martin Johansson and Olov Sterner
Preparation of analogues of conidiogenone--a novel diterpene with potent conidiation inducing activity
In manuscript
- IV. Yanhong He, Anders Sundin, Martin Johansson, Jose L. Reino and Olov Sterner
Synthesis of a tricyclic analogue of conidiogenone
In manuscript

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婆婆李冰书, 公公官维信, 姨妈李冰珍, 哥哥尹发春, 嫂嫂李美玉, 我永远不会忘记你们为我所做的一切, 深深感谢你们的支持, 理解和关心。

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Abbreviations

Ac	acetyl
COSY	correlation spectroscopy
d	doublet
dd	double doublet
dt	double triplet
DIBAL-H	diisobutyl aluminium hydride
DMF	N,N-dimethylformamide
DMS	dimethylsulfide
DMSO	dimethylsulfoxide
EI	electron impact
ESI	electro-spray ionisation
Et	ethyl
h	hour
HMBC	heteronuclear multiple-bond correlation
HMQC	heteronuclear multiple-quantum correlation
HRMS	high resolution mass spectrometry
HPLC	high-pressure performance liquid chromatography
KHMDS	potassium bis(trimethylsilyl)amide
m	multiple
Me	methyl
MS	mass spectrometry
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser effect spectroscopy
PCC	pyridinium chloro-chromate
Ph	phenyl
ppm	part per million

PPTS	pyridinium <i>p</i> -toluenesulfonate
r.t.	room temperature
s	singlet
TLC	thin-layer chromatography
THF	tetrahydrofuran
TMS	trimethylsilyl
<i>p</i> -TsOH	4-toluenesulfonic acid

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Introduction

1.1 Natural product chemistry

Through the ages, humans have relied on nature for their basic needs for the production of foodstuffs, shelters, clothing, means of transportation, fertilizers, flavors and fragrances, and medicines.¹ Natural product chemistry has developed along with mankind.² Today, it is sometimes viewed as an ancient science, but this wide field is in fact keeping pace with other areas of modern chemistry. Natural product chemistry covers various aspects of research into compounds from different plants, animals, bacteria and fungi: their formation, structures, and biological and pharmacological activities.³ It has, during the last few decades, undergone an explosive growth owing to advances in isolation techniques, synthetic methods, spectroscopic techniques. The importance of natural products, especially when it comes to drugs and pharmaceutical agents, can not be underestimated.

When discussing natural products, it is important to distinguish secondary metabolites from primary metabolites. Primary metabolites are broadly distributed in all living things, and are closely connected with essential life processes. Examples include α -amino acids, proteins, carbohydrates, fats, and nucleic acids. Secondary metabolites are biosynthesised from primary metabolites, and differ and vary between families, genera, and species. Unlike primary metabolites, secondary metabolites are considered to be non-essential to daily life. However, since a large proportion of metabolic resources is invested in their production, it is hard to believe that they are

just waste products. On the contrary, it is generally believed that their production is connected with several external factors. Secondary metabolites could be defined as non-nutritional compounds that influence the biology of an organism as well as that of other species in its environment; they also play an important role in the co-existence and co-evolution of species. Some of their functions have been suggested to be associated with sexual attractants, development agents, defense agents, feedants and antifeedants, repellents, and toxins.⁴

1.2 Natural product based drug discovery

Natural products play a dominant role in the discovery of leads for the development of drugs.⁵ To understand why they are suitable as drugs, it must be remembered that natural products have been formed, not by design, but by selection through evolution. They carry an intrinsic property for acting against biological targets, and this can be related to the enormous impact and importance natural products have had for the treatment of the diseases of mankind for thousands of years.¹ Even today, it has been estimated by the World Health Organization that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care.^{6,7} Natural products also play an important role in the health care systems of the remaining 20% of the population, who mainly reside in developed countries. A study using US-based prescription data from 1993 reported that over 50% of the most-prescribed drugs in the US had a natural product either as the drug, or as a 'forebear' in the synthesis or design of the agent.⁸

A renewed interest in natural products and natural product-derived compounds as a source for new leads in drug discovery programs has been occurring in the recent past.⁹ According to a recent survey,⁵ 61% of the 877 new small-molecular chemical entities introduced as drugs worldwide during the period 1981–2002 can be traced to, or were inspired by, natural products. These include natural products (6%), natural product derivatives (27%), synthetic compounds with natural-product-derived pharmacophores (5%), and synthetic compounds designed on the basis of knowledge

gained from a natural product (i.e. a natural product mimic; 23%). In certain therapeutic areas, the proportion is higher: 78% of antibacterials and 74% of anticancer compounds are natural products or have been derived from, or inspired by, a natural product.

There is a perception that with the emergence of approaches such as advanced genomics, high-throughput screening, combinatorial chemistry and biology, and computer-assisted *de novo* drug design, the role natural products have played historically in drug lead discovery may start to diminish. However, the fact is that not a single *de novo* combinatorially synthesized compound was approved as a drug during the period 1981–2002.⁵ It must be acknowledged that nature provides an unparalleled source of molecular diversity that still is not available from synthetic libraries. A distinct difference, which can be proved statistically, is observed in the structural properties of natural products relative to the synthetic compounds.¹⁰ In addition, the practical difficulties with natural product drug discovery, *e.g.* expensive and difficult isolations, low availability and complex structures, are being overcome by advances in microbiology, chemistry, and gene technology, as well as in instrumentation and automation.¹¹ It is important to be aware that natural product drug discovery is constantly evolving.⁹

1.3 Understanding the biological functions of natural products

Another important and interesting aspect of the study of natural products is to understand their significance for the producing organisms. Since it is reasonable to assume that many biologically active natural products are produced for a specific purpose, there is naturally a general biological interest to expand our knowledge about the producing organisms and their relations to other organisms, from a chemical point of view. It is not only intellectually rewarding but also practically beneficial to try to understand biotic interactions on a molecular level.¹² With the knowledge of chemistry of biotic interactions, mankind could better manage forests, pursue

agriculture, and avoid parasitic diseases and disease vectors.

For example, a unique property of many fungi is their ability to propagate by both sexual and asexual spores. The integration of the teleomorph (meiotic sexual morph) and anamorph (mitotic asexual morph) stages into the fungal life cycle gives great flexibility in dispersal and survival under suboptimal environmental conditions, both of which are very important characteristics for organisms that are essentially sessile in their somatic stage.¹³

Butnick *et al.*¹⁴ identified two mutants (*acoB202* and *acoC193*) in *A. nidulans* that fail to become competent and are blocked in both sexual and asexual sporulation. These mutants overproduce hormone-like fatty acid-derived oxylipins, which are collectively termed the psi factor (precocious sexual inducer). The psi factor serves as a signal that modulate sexual and asexual sporulation by affecting the timing and balance of asexual and sexual spore development.¹⁵⁻¹⁷ Studies carried out for the linoleic acid-derived $\text{psi}\alpha$ molecules (Figure 1.1) showed that $\text{psiB}\alpha$ and $\text{psiC}\alpha$ stimulated sexual and inhibited asexual spore development, whereas $\text{psiA}\alpha$ which is designated for a lactone ring of $\text{psiC}\alpha$ had the opposite effect.^{15,16} These endogenous oxylipins play an important role in integrating sexual and asexual spore development in *A. nidulans*.

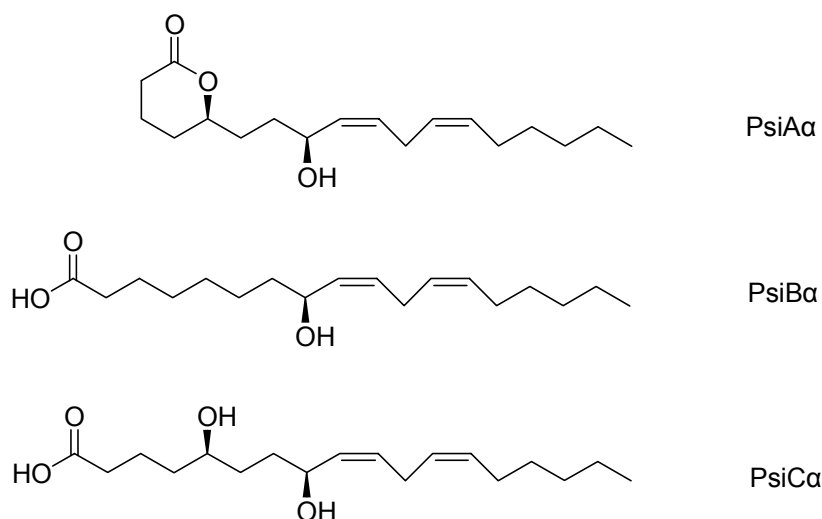


Figure 1.1. Linoleic acid-derived $\text{psi}\alpha$ molecules.

Finally, taking into account that less than 1% of bacterial and less than 5% of fungal species are known and have been studied to any extent underlines that much of nature remains to be explored,¹⁸ and leaves no doubt that a host of novel, bioactive chemotypes await discovery.¹⁹

Biological background

2.1 *Conidiation in Penicillium cyclopium*

Conidia are asexual spores produced by different groups of filamentous fungi, when conditions are not suitable for apical extension growth. Conidiation refers to the process by which conidia are produced. As reviewed²⁰ by Roncal *et.al.*, conidiation in the genus *Penicillium* has attracted considerable interest in different areas, stemming from, for example, the use of *Penicillium* conidia in the food industry,²¹ as biocontrol agents,²² as biotransformation catalysts²³ and as inoculum for antibiotic production.²⁴ In addition, several *Penicillium* species are pathogenic toward humans²⁵ and plants, causing crop spoilage as well as decay processes in which mycotoxins may be produced.²⁶

Conidiation in *Penicillium* species involves the differentiation of the apical compartment into a specialized reproductive cell called a phialide. This then undergoes successive mitotic divisions, each resulting in a new specialised daughter cell: the conidium.²⁷

Although the most detailed morphogenetic accounts of these changes have emerged from studies with surface cultures,²⁷ their timing has been precisely characterised in liquid cultures of *Penicillium cyclopium*, where the morphogenetic change could be synchronously induced by the addition of calcium ions.^{28,29} Four morphogenetic stages have been identified²⁹ (Figure 2.1): Upon induction, apically growing vegetative hyphae immediately arrest extension (stage 1). After four hours, the apical cell is

delimited by a septum and begins to swell, with concomitant formation of subapical branches (stage 2). After six hours, the apical cell differentiates into a phialide (stage 3), which finally buds at its tip, giving rise to the first conidium (stage 4). The overall time period required to complete this process is approximately seven hours. Once the first conidium has formed on the phialide, new conidia appear at approximately hourly intervals, resulting in a chain of conidia. Subapical buds formed at the early stages also differentiate into phialides that produce conidia, resulting in the formation of characteristic brush-like structures called penicilli, which are the origin of the genus name (*Penicillium*).

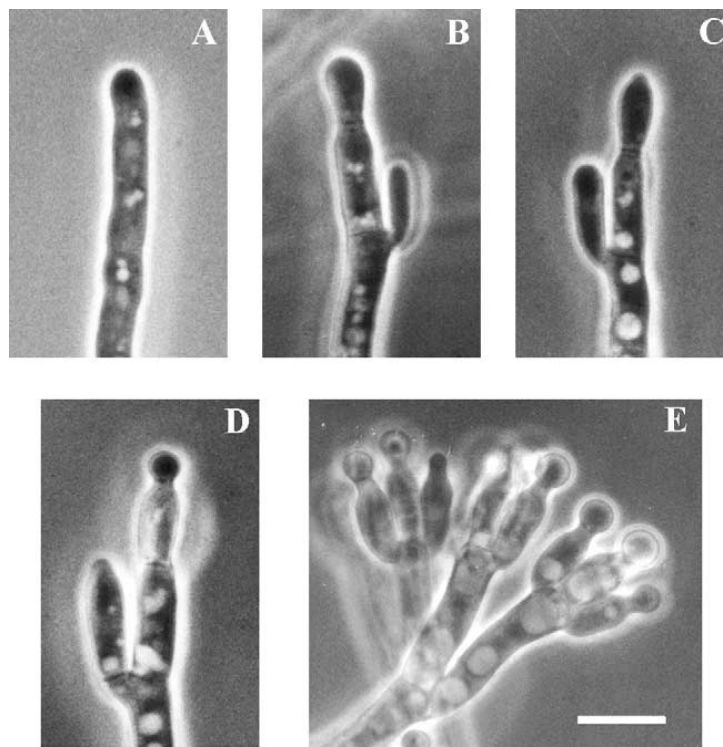


Figure 2.1. Morphological stages of *Penicillium cyclopium* during conidiation in submerged liquid culture. (A) Stage 1: vegetative hypha. (B) Stage 2: apical cell swelling and subapical branching. (C) Stage 3: phialide formation. (D) Stage 4: conidium formation. (E) Penicilli. Scale bar (applicable to all plates): 10 μ m.

The most effective and widespread stimulus of conidiation is exposure of the mycelium to the air.³⁰ Nutrient starvation,^{30,31} oxidative stress,³² light,³³ and chemical signals^{15,16,34,35} have been proposed to have a role in this complex environmental change for the emerging hypha. However, the precise mechanism by which conidiation induction is triggered has proved to be elusive.

2.2 *An endogenous conidiation inducer in *Penicillium cyclopium**

2.2.1 Accumulation of a fungal metabolite in the medium is required for induction of conidiation

Conidiation may be induced in liquid culture under controlled conditions.^{28,31,36} *Penicillium cyclopium* behaves in a certain way which regards to the timing and pattern of conidiation as well as susceptibility to calcium induction.²⁹

Roncal *et.al.* reported the following experiments:³⁷ in a culture growing in Ca^{2+} supplemented fresh F medium (inoculum, 10^5 conidia/ml), conidia germinate after 11 h of culture and conidiation follows at 24 h (Figure 2.2). The morphogenetic program leading to conidium formation invariably requires seven hours²⁹ Thus, induction can be accurately estimated to have occurred at 17 h of culture, after a six hour period of vegetative growth following germination.

When the same experiment was conducted using mF medium (mature F medium, *i.e.* medium in which the fungus had previously grown for 36 h in the absence of added calcium), conidiation could be observed at 18 h (Figure 2.2). Hence, conidiation induction should have coincided with germination (11 h), and the culture did not require any period of vegetative growth for the attainment of competence.

The results obtained in both experiments indicate that the growing mycelium caused a modification in the surrounding medium that accounted for induction of conidiation. The possibility that nutrient starvation was the inducing stimulus was dismissed, since the levels of nutrient depletion observed for mature and fresh medium were irrelevant in terms of growth kinetics. Moreover, when mF medium was supplemented with nutrients, the resulting time and pattern of conidiation remained identical to those with the unsupplemented medium.

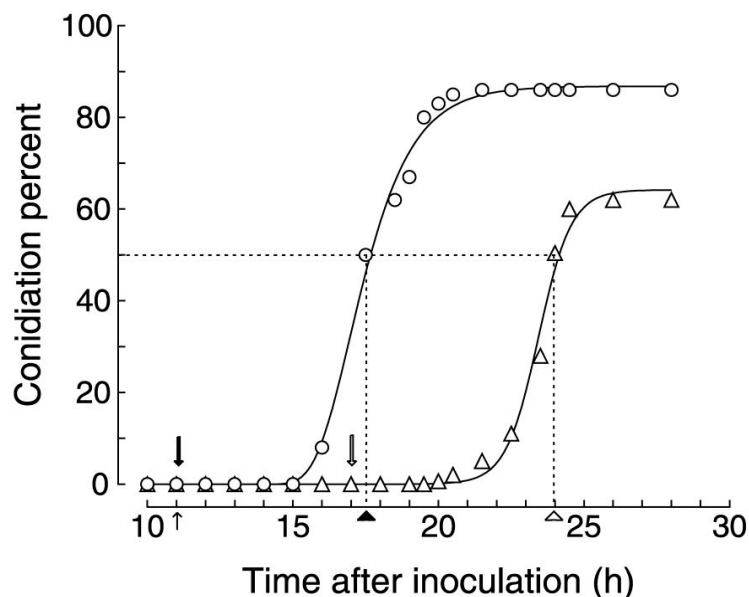


Figure 2.2. Temporal appearance of conidium-bearing tips in cultures of *P. cyclopium*. The percentage of conidiating tips is expressed as the conidiation percent. Assays were carried out in shaken flask cultures inoculated with 10^{13} conidia/ml. The points of 50% conidiation, taken as the reference point at which conidiation was considered to have been reached, are indicated by dotted lines. Triangles represent growth in fresh F medium and circles represent growth in mF medium. The mF medium was prepared by growing the fungus in a 10 liter fermenter for 36 h with an inoculum of 10^6 conidia/ml.

Further experiments showed that the conidiation-inducing activity present in mF medium disappeared after autoclaving, was protease insensitive, was dialyzable through a 3500Da cutoff dialysis membrane, and was extractable by liquid-liquid or liquid-solid-phase extraction procedures. This evidence supports the view that the modification of the medium accounting for the observed conidiation-inducing activity was due to the presence of a metabolic product or inducer secreted by the mycelium.

Therefore, the conidiation-induction bioassay was used to track the active compounds. It was found that the conidiogenic activity was localised in the extracts of medium rather than the mycelium. Fractionation of extracts of the fermentation broth guided by bioassay eventually led to the isolation of two active compounds,³⁸ which were named conidiogenol (1) and conidiogenone (2) (Figure 2.3). The compounds are related tetracyclic diterpenes with a novel carbon skeleton (The isolation and structure determination is described in paper I).

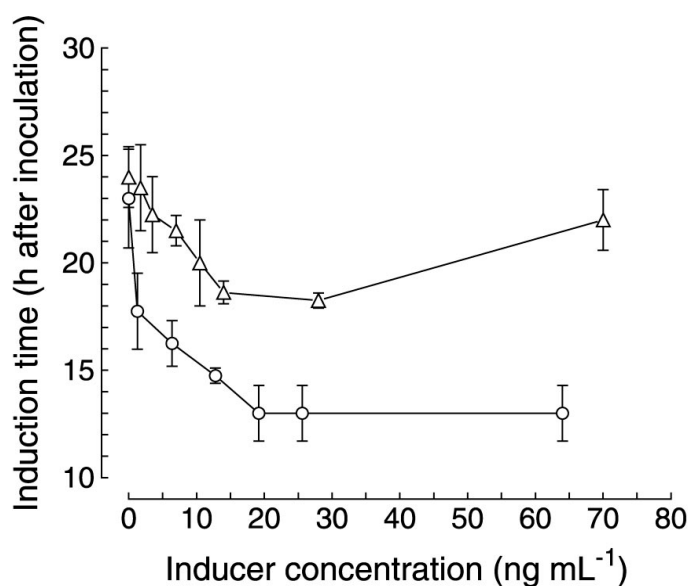


Figure 2.4. Effect of inducer concentration on conidiation induction time. Increasing concentrations of conidiogenol (triangles) and conidiogenone (circles) were added to fresh F medium. The time at which conidiation induction occurred was determined by subtracting the 7-h period required for completion of morphogenesis from the measured conidiation time. The threshold concentrations required to induce conidiation were determined as the lowest concentrations which allowed induction to occur at the earliest time.

Two major differences were observed in the action of the two molecules. Firstly, conidiation induced by conidiogenol was always delayed (by 5 h) relative to that induced by equivalent conidiogenone levels. As their activity concentration ranges were the same, this result suggests that the truly active compound is conidiogenone and that conidiogenol may be an inactive derivative or precursor which requires oxidative transformation into the active form. Secondly, an inhibitory effect of conidiogenol was observed for concentrations higher than the threshold concentration (concentrations up to 350 ng/ml were assayed), resulting in a dose-dependent delay in conidiation. This phenomenon was not observed for conidiogenone, which showed inducing activity only, irrespective of the amount by which its concentration exceeded the threshold concentration.

2.2.3 Conidiogenone is necessary and sufficient to induce conidiation

Solid-phase extraction of small volumes of mF medium effectively removed its conidiation-induction potential without any detectable influence on growth rate. It

was therefore concluded that the extraction step eliminated substances responsible for the induction of conidiation.

Further reverse-phase HPLC fractionation of the crude extract revealed conidiation-inducing activity in only two adjacent fractions, containing conidiogenol and conidiogenone, respectively. When the crude extract was deprived of both active fractions (that is, when the HPLC effluent fractions other than the two active fractions were pooled and assayed), no conidiation induction activity was detected.

This evidence strongly suggested that conidiogenone was the only conidiation inducer present in mF medium, and that its presence was necessary for the induction of conidiogenesis.

In addition, it was believed that calcium is a necessary constituent for morphogenesis to be manifested. But when increased concentrations of conidiogenone in fresh F medium were assayed in the absence of added calcium, conidiation also took place (Figure 2.5), reaching conidiation induction times identical to the earliest times obtained in calcium-supplemented cultures. The threshold concentration was about 125 ng/ml (4×10^{-7} M), five to six times higher than that found when calcium was present in the medium.

This result indicated that calcium was unnecessary for the induction step and rather acted indirectly, by decreasing the conidiogenone threshold concentration required to efficiently induce conidiation, in a manner not yet understood.

Both experiments indicate that the presence of conidiogenone is necessary and sufficient to induce conidiation in *P. cyclopium* growing in liquid culture and that calcium or other factors do not play a direct role in the induction of conidiation.

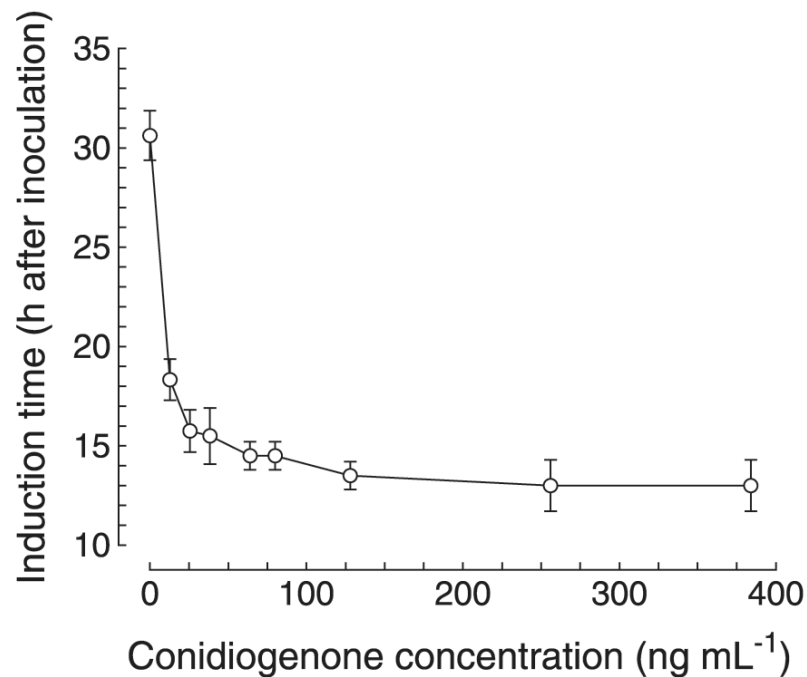


Figure 2.5. Effect of conidiogenone concentration on the conidiation induction time in the absence of calcium in the culture medium. Increasing concentrations of conidiogenone were added to fresh F medium (with no calcium added), and the time at which conidiation induction occurred was determined. The threshold concentration required to induce conidiation was determined as the lowest concentration which allowed induction to occur at the earliest time.

In conclusion, conidiogenone and conidiogenol are produced from the very earliest stages of growth and are continuously released into the culture medium, where they accumulate until a threshold concentration is reached, triggering conidiation. Conidiogenone would therefore act as a hormone-like molecule at extremely low concentrations (around 10^{-7} to 10^{-8} M), which is proof of its high specificity and potency.

Conidiogenol and conidiogenone

3.1 Isolation and structure determination

Two diterpenes, which we have named³⁸ conidiogenol (1) and conidiogenone (2) (Figure 3.1), were isolated by bioassay-guided fractionation of extracts of *P. cyclopium*. Both compounds were obtained in submilligram amounts from 300 L medium. The ability of the extracts of the medium, which had supported growth for 48 h, to trigger the induction of conidiation in fresh liquid cultures of *P. cyclopium* was used as the activity assay.³⁷

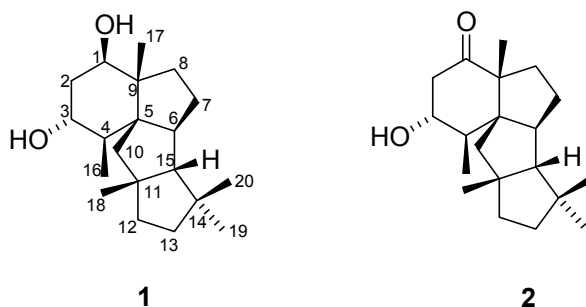


Figure 3.1. Conidiogenol (1) and conidiogenone (2)

Structure determination of conidiogenol (1) and conidiogenone (2) is described in Paper I. The compounds are related tetracyclic diterpenes with a novel carbon skeleton, which to our knowledge has not been previously reported. We have therefore suggested the general name cyclopiane for this type of diterpene.³⁸

3.2 Calculations

3.2.1 Computational methods³⁹

3.2.1.1 Semi-empirical methods

Semi-empirical methods take all the valence electrons into account, and this is an advantage because the outer electrons are often involved in interactions with other electrons in the molecule, other molecules, or with the solvent. Semi-empirical methods are much faster than *Ab initio* methods (see Section 3.2.1.2). They are parameterised to reproduce various results, most often, geometry and energy (usually the heat of formation). Semi-empirical calculations have been very successful in describing organic molecules, in which only a few elements used extensively and the molecules are of moderate size. AM1 (Austin Model 1) is a popular semi-empirical method.

3.2.1.2 *Ab initio* methods

The term *ab initio* is Latin for “from the beginning”. This name is given to computations that are derived directly from theoretical principles with no inclusion of experimental data. This is an approximate quantum mechanical non-parameterised molecular orbital treatment for the description of chemical behavior taking into account nuclei and all electrons. In general, *ab initio* calculations give very good qualitative results, and can yield increasingly accurate quantitative results as the molecules in question become smaller. The advantage of *ab initio* methods is that they eventually converge to the exact solution once all the approximations are made sufficiently small in magnitude. The disadvantage is that they are expensive. These methods often take enormous amounts of computer CPU time, memory, and disk space.

The most common type of *ab initio* calculation is called a Hartree-Fock calculation (HF), in which the primary approximation is the central field approximation. This means that the Coulombic electron-electron repulsion is taken into account by integrating the repulsion term. This gives the average effect of the repulsion, but not

the explicit repulsion interaction. The steps in a HF calculation start with an initial guess for the orbital coefficients, usually using a semi-empirical method.

3.2.1.3 Density functional theory calculations

The premise behind density functional theory (DFT) is that the energy of a molecule can be determined from the electron density instead of a wave function. The advantage of using electron density is that the integrals for Coulomb repulsion need be done only over the electron density, which is a three-dimensional function. Furthermore, at least some electron correlation can be included in the calculation. This results in faster calculations than HF and computations that are a bit more accurate as well. The B3LYP hybrid functional (also called Becke3LYP) is most widely used for molecular calculations. This is due to the accuracy of the B3LYP results obtained for a large range of compounds, particularly organic molecules.

3.2.2 The conformations of conidiogenol and conidiogenone

In our conformational analysis of conidiogenol and conidiogenone, the molecules were built from the energy minimized templates in Spartan pro, and minimized with Semi-empirical AM1, then use Spartan pro Hartree-Fock 6-31G* program, and further calculated with the Density-Functional B3LYP 6-31* program.

The lowest energy conformation of conidiogenol was found to have the six-membered ring in a chair conformation and the three five-membered rings in envelope conformations (Figure 3.2); this agrees well with the NMR data. The C-1 hydroxyl group is axial, while both the C-3 hydroxyl group and the C-4 methyl group are equatorial. 1-H is equatorial, and the dihedral angle between 1-H and 2-H α is 51°, and between 1-H and 2-H β 66°; this is in agreement with the observed NMR coupling constants of 1-H $J_1 = J_2 = 3$ Hz. Both 3-H and 4-H are axial, and the dihedral angle between them is 179°, which is in agreement with the NMR coupling constant $J = 11$ Hz.

The energy cost between the global minimum energy chair conformation and a low-energy boat conformation (Figure 3.2) is 1.97 kcal/mol (Table 3.1). The small

energy difference indicates that the boat conformation could coexist with the chair conformation as a small fraction in solution, although it was not detected by NMR spectroscopy.

Table 3.1. The energies of conidiogenol

Conformation	Energy (au)	Relative energy (kcal/mol)
chair	-933.033042	0
boat	-933.029905	+ 1.97

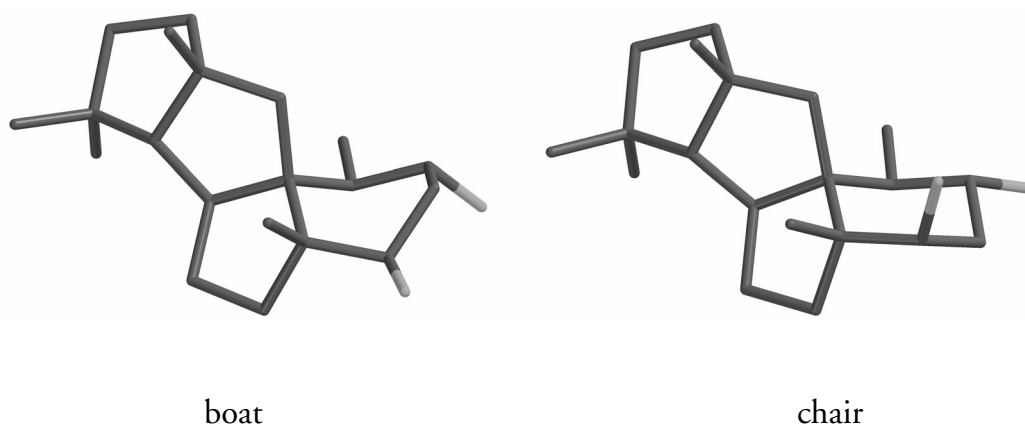


Figure 3.2. The global minimum energy conformations of conidiogenol (1).

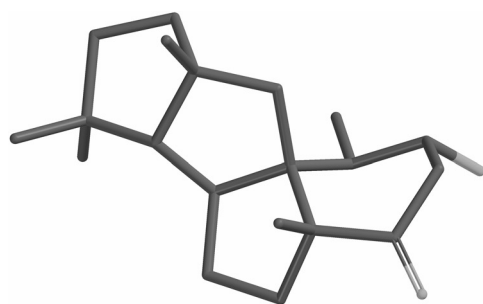
For conidiogenone, similar results to those for conidiogenol were obtained. In the lowest energy conformation, the six-membered ring adopts a chair-like conformation and the three five-membered rings envelope conformations (Figure 3.3); this was also supported by NMR data. Both C-3 hydroxyl group and C-4 methyl group are equatorial; 3-H and 4-H both are axial, and the dihedral angle between them is 175° , which is in agreement with the NMR coupling constant $J = 10.2$ Hz. The dihedral angle between 3-H and 2-H α is 175° , and between 3-H and 2-H β 57° , which are in agreement with their respective NMR coupling constants, $J = 9.0$ Hz and $J = 5.8$ Hz.

The energy cost between the global minimum energy chair conformation and a boat conformation (Figure 3.3) was 1.58 kcal/mol (Table 3.2). This small energy difference suggests that the chair and the boat conformations may be in equilibrium with each

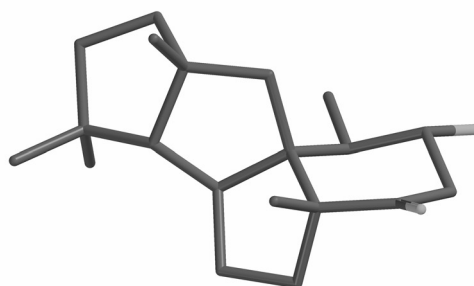
other, although the boat conformer could not be observed by NMR spectroscopy. Therefore, the conidiogenesis inducing activity could reasonably be provoked by either the chair or the boat conformation.

Table 3.2. The energies of conidiogenone

Conformation	Energy (au)	Relative energy (kcal/mol)
chair	-931.839083	0
boat	-931.836568	+1.58



boat



chair

Figure 3.3. The global minimum energy conformation of conidiogenone(2).

Synthetic analogues of conidiogenone

In order to initiate a structure-activity relationship study, and to obtain analogues of the natural product with similar or more potent biological activity, but more available, we decided to undertake the synthesis of simplified analogues of conidiogenone (**2**) (Figure 4.1). Since the two functional groups of the natural product, a carbonyl group and a hydroxyl group, are on the six-membered ring A, we took ring A as an appropriate starting point to establish the minimum structural requirements for inducing conidiogenesis. Thus, three types of analogues were designed and synthesized: ring A (**3**), ring AB (**4**) and (**5**), and tricyclic (**6**) analogues (Figure 4.1)

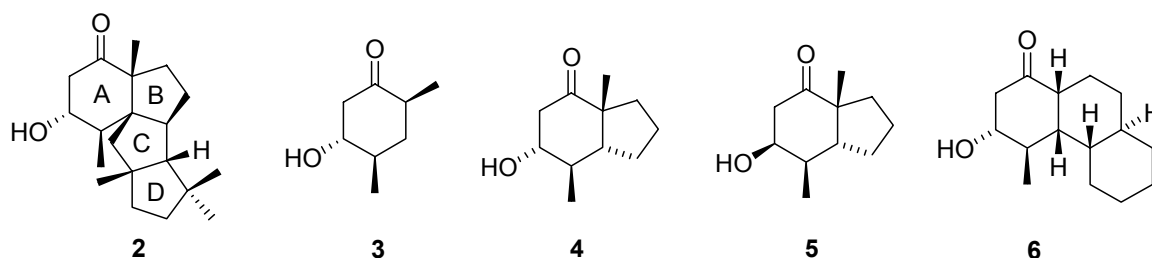


Figure 4.1. conidiogenone (**2**) and analogues.

4.1 Synthesis of ring A analogue of conidiogenone (Paper III)

The β -hydroxyl cyclohexanone **3** (Figure 4.2), which bears the same functional groups and two methyl groups as the natural product **2**, was conceived as a simplified

ring A analogue. It has the same relative configuration between the three substituents as the natural product, but completely lacks the lipophilic moiety of rings B, C and D. Analogue **3** is obviously much smaller than the natural product, and has a much higher polarity, so it might be interesting to see how it performs in the biological test for conidiogenesis.

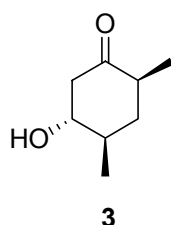
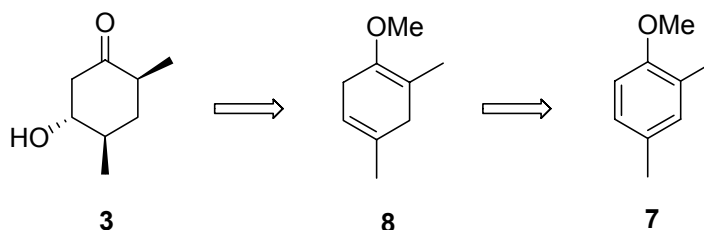


Figure 4.2. Ring A analogue of conidiogenone (**3**).

4.1.1 Retrosynthetic analysis of ring A analogue **3**

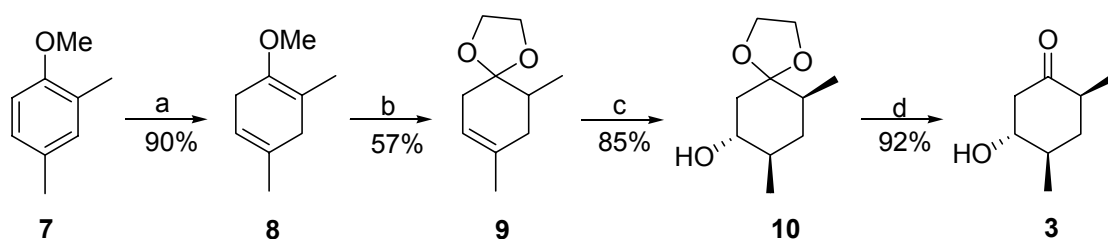
The two disconnections were identified in the structure of compound **3**: the OH bond and the formation of carbonyl group (Scheme 4.1).



Scheme 4.1. Retrosynthetic analysis of ring A analogue **3**.

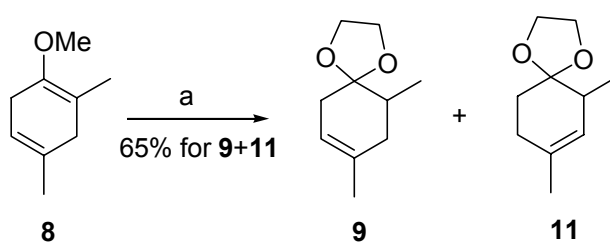
The hydroxyl group could be derived from a double bond, and the carbonyl group could come from an enol ether, so the enol ether olefin **8** would be a perfect key intermediate. Manipulation of the functional groups of a pre-formed six-membered ring with the two methyl groups already in place would be an easy choice, and therefore the readily available 2,4-dimethylanisole (**7**) was used as a starting material.

4.1.2 Synthesis of ring A analogue 3



Scheme 4.2. Synthesis of ring A analogue 3. Reaction conditions: (a) Li, NH_3 (liquid), ether, MeOH; (b) ethylene glycol, PPTS, benzene, Dean-Stark conditions; (c) i. $\text{BH}_3 \cdot \text{THF}$, THF, -15°C then r.t.; ii. NaOH, H_2O , 35% H_2O_2 , 0°C ; (d) Method A: $\text{PdCl}_2(\text{CH}_3\text{CN})_2$, acetone, r.t.; Method B: PPTS, H_2O , silica gel, microwave heating.

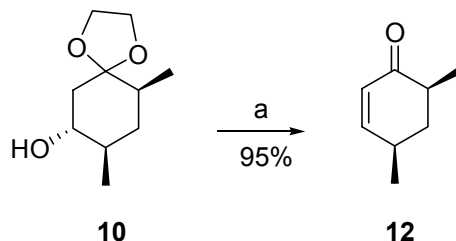
Birch reduction of 2,4-dimethylanisole (**7**) (Scheme 4.2) easily furnished the desired diene **8** according to a previously reported procedure.⁴⁰ Both of the resulting double bonds are useful for the introduction of functional groups. However, an attempt to convert enol ether **8** to the ethylene ketal **9** in the presence of *p*-toluenesulfonic acid⁴⁰ yielded an approximately 1:1 mixture of the two olefins **9** and **11**, owing to isomerization of the trisubstituted double bond (Scheme 4.3). This problem was solved by exchanging *p*-toluenesulfonic acid for the less acidic pyridinium *p*-toluenesulfonate (PPTS);⁴¹ when this reagent was used, compound **9** was obtained in 57% yield and only a trace of **11** was observed.



Scheme 4.3. Preparation of ketal 9. Reaction conditions: ethylene glycol, TsOH, benzene, Dean-Stark conditions

The addition of the borane reagent to olefin **9** proceeded in a completely diastereoselective manner to give the desired alcohol **10** (Scheme 4.2). The stereochemical outcome of the addition is due to the attack of the borane from the less

hindered face of the substituted cyclohexene, yielding the product with the hydroxyl group and the two methyl groups equatorial in the chair conformation.



Scheme 4.4. hydrolysis of 10. Reaction conditions: HCl, THF, H₂O, r.t.

Removal of the dioxolane protecting group of **10** with HCl yielded a conjugated ketone **12** owing to β -elimination (Scheme 4.4). However, treatment with $\text{PdCl}_2(\text{CH}_3\text{CN})_2$ ⁴² in acetone gave the desired cyclohexanone **3**. Alternatively, we have developed a mild method⁴³ to hydrolyze β -hydroxyl ketones with silica-gel-supported PPTS moistened with water in solvent-free conditions under microwave heating (the method is described in paper II). This method gave compound **3** in 92% yield after three minutes. Thereby the synthesis of ring A analogue **3** was completed in four steps, and the synthetic route is concise and efficient.

4.2 Synthesis of ring AB analogues of conidiogenone (Paper III)

Simplified ring AB analogues of conidiogenone (**2**) were designed as four enantiomerically pure compounds: two diastereomers and their enantiomers (Figure 4.3). They have similar ring AB systems to the natural product: fused 6- and 5-membered rings, two functional groups, two methyl groups, but lack lipophilic ring CD moiety. Analogues (+)-**4** and (-)-**4** have the same relative configuration of the rings and the three substitutes as the natural product, whereas their diastereomers (+)-**5** and (-)-**5** differ only in the stereochemistry of the hydroxyl group.

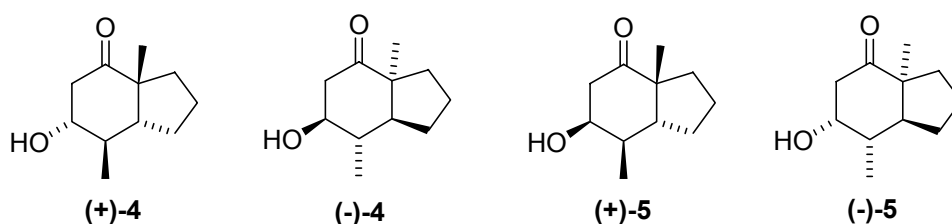
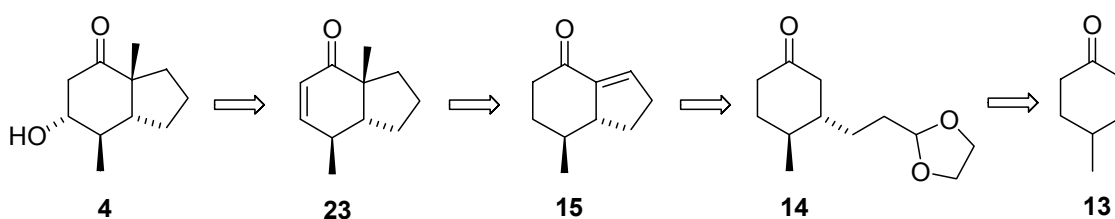


Figure 4.3. Ring AB analogues of conidiogenone.

4.2.1 Retrosynthetic analysis of ring AB analogues

The retrosynthetic analysis of the ring AB analogues is illustrated with compound **4** (Scheme 4.5). Three disconnections were identified from the structure of **4**: the OH bond, the introduction of angular methyl group and the construction of the bicyclic structure.



Scheme 4.5. Retrosynthetic analysis of ring AB analogue **4**.

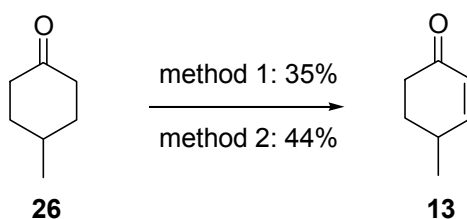
The hydroxyl group of **4** could be developed from the double bond of **23**, the angular methyl group of **23** could be introduced from a conjugated ketone system of **15**, and the bicyclic structure could be constructed by direct annulation of a second ring onto a pre-existing cyclic framework by using carbon-carbon double bond as a site of attachment. 4-methyl-2-cyclohexenone (**13**) was therefore deemed as a key starting material. Furthermore, in order to obtain the analogues in enantiomerically pure form, optical resolution was judged to be an easier and quicker route than asymmetric synthesis.

4.2.2 Synthetic studies of ring AB analogues of conidiogenone

4.2.2.1 Preparation of the key starting material 4-methyl-2-cyclohexenone (13)

To carry out the synthesis of ring AB analogues, we needed large amounts of 4-methyl-2-cyclohexenone (**13**) as a key starting material. It was not available from commercial sources, however several methods for the synthesis of α,β -unsaturated carbonyl compounds have been developed.⁴⁴⁻⁵⁰ Some of these protocols rely on selenium reagents in one- or two-step procedures,⁵⁰ but these are not suitable for large scale preparations as the selenium reagent are highly toxic and expensive. We investigated four different methods in order to find the best way to make **13** on a large scale.

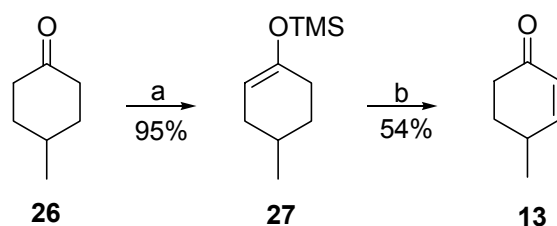
Method 1. using allyl diethyl phosphate (ADP): ADP was easily prepared from diethyl chlorophosphate and allyl alcohol according to a literature procedure.⁵¹ The dehydrogenation of 4-methylcyclohexanone (**26**) with ADP in the presence of $\text{Pd}(\text{OAc})_2$ gave 4-methyl-2-cyclohexenone (**13**) in 20-35% yield⁴⁴ (Scheme 4.6).



Scheme 4.6. preparation of 13 with ADP and IBX. Reaction conditions: Method 1. ADP, $\text{Pd}(\text{OAc})_2$, Na_2CO_3 , THF, reflux; Method 2. IBX, toluene-DMSO, 70 °C.

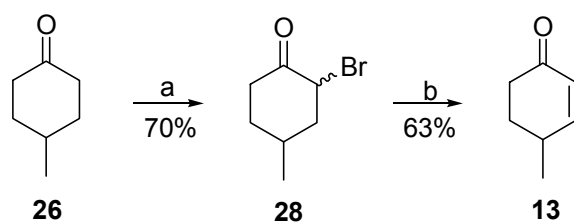
Method 2. using 2-iodoxybenzoic acid (IBX): IBX was readily prepared from 2-iodobenzoic acid and oxone according to a previously reported procedure.⁵² The oxidation of 4-methylcyclohexanone (**26**) afforded **13** in 33-44% yield⁴⁶ (Scheme 4.6).

Method 3. using $\text{Pd}(\text{OAc})_2$: palladium-catalysed oxidation of the silyl enol-ether **27** derived from 4-methylcyclohexanone⁵³ gave **13** in 51% yield over the two steps^{45,47} (Scheme 4.7).



Scheme 4.7. Preparation of 13 with Pd(OAc)₂. Reaction conditions: (a) TMSCl, triethylamine, DMF, reflux; (b) Pd(OAc)₂, MeCN, r.t..

Method 4. using bromine: 4-methylcyclohexanone was treated with bromine to yield the α -bromo ketone **28**.⁵⁴ Dehydrobromination of this compound produced **13** in 44% yield over the two steps^{48,49} (Scheme 4.8).



Scheme 4.8. Preparation of 13 with bromine. Reaction conditions: (a) Br₂, H₂O; 30 °C; (b) Li₂CO₃, DMF, reflux.

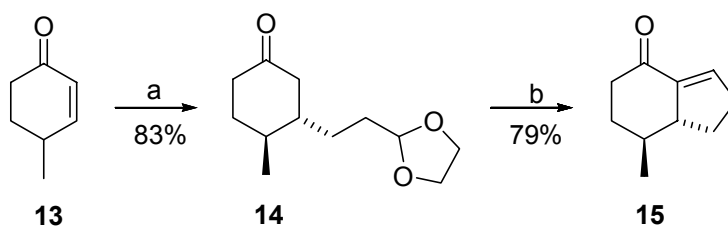
Since the yields from the four methods were not much different varying only between 30-50%, we chose method 4 (bromination and dehydrobromination of **26**) to prepare 4-methyl-2-cyclohexenone (**13**) in large amounts based on the availability and cost of the reagents.

4.2.2.2 Construction of bicyclic structure

The carbon-carbon double bond of 4-methyl-2-cyclohexenone (**13**) could be used as a site of attachment for a functionalized carbon chain, in order to build the bicyclic structure. The second ring could be formed by direct annulation of this chain onto the pre-existing six-membered ring. The bifunctional acetal-containing Grignard reagent derived from 2-(2-bromoethyl)-1,3-dioxolane has been reported as an efficient reagent

for annulating cyclopentene rings onto cyclic α,β -unsaturated ketones through a sequence of conjugate addition, hydrolysis, and intramolecular aldol condensation.⁵⁵ This approach has been used in many total syntheses of natural products.⁵⁶⁻⁵⁸

Grignard reagents usually react in a conjugate manner in the presence of a copper (I) salt. Sworin found that 2-(2-magnesiumbromoethyl)-1,3-dioxolane Grignard reagent can, however, give the conjugate addition products with cyclic enones even in the absence of copper (I) salt.⁵⁹ At $-78\text{ }^{\circ}\text{C}$ and with 2 equivalents of the Grignard reagent, the ratio of 1,2-addition and 1,4-addition product is 1:20 while at $0\text{ }^{\circ}\text{C}$ or with 1 equivalent of the nucleophile it is 1:1. This can be explained as the Schlenk equilibrium at low temperature is shifted toward a dialkylmagnesium intermediate and this active species is probably a softer carbon nucleophile so its reactivity will more closely resemble an organocopper reagent.



Scheme 4.9. Preparation of 15. Reaction conditions: (a) 2-(2-bromoethyl)-1,3-dioxolane, Mg, I_2 , $\text{CuBr}\cdot\text{SMe}_2$, SMe_2 , THF; (b) 1M HCl, THF, r.t..

The conjugate addition of 2-(2-magnesiumbromoethyl)-1,3-dioxolane Grignard reagent to 4-methyl-2-cyclohexenone (**13**) proceeded in a completely diastereoselective manner giving the desired *anti* product **14**^{57,60} (Scheme 4.9). The stereochemical outcome was due to the nucleophile attacking the 4-substituted cyclohexenone axially from the least hindered face.⁵⁶ Ring annulation to form the bicyclic enone **15** was best done slowly in dilute hydrochloric acid for several days. It was sometimes necessary to raise the temperature to reflux at the end to ensure complete dehydration.

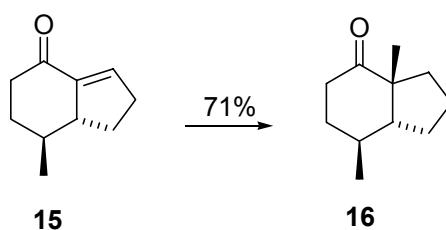
4.2.2.3 Introduction of the angular methyl group

The introduction of the angular methyl group to the bicyclic enone **15** could be

done by a reduction-alkylation procedure. The reduction-alkylation procedure developed by Stork and co-workers⁶¹ often provides an excellent method for directing alkylation to relatively inaccessible α positions of conjugated ketones, and it has been applied successfully in a number of natural product syntheses.⁶²⁻⁶⁴ In general terms, the procedure involves generation of a specific lithium enolate by reduction of an α,β -unsaturated ketone with lithium in liquid ammonia, and then reaction of this enolate with an alkylating agent either in liquid ammonia or other solvent systems.⁶⁵

One equivalent of a proton donor is usually added to bring about more complete reduction of the enones. Smith suggested that the role of the proton donor is probably to prevent competing side-reactions of the enone with lithium amide, which is formed as the reduction proceeds. Such side-reaction would lead to deactivation of the enone toward reduction. Conjugate enolate formation, or 1,2 or 1,4 addition, are possible reactions of the enone with lithium amide that would convert it into a species that resists reduction.⁶⁵

Ashby has studied the reaction mechanism of enolates with alkyl halides,⁶⁶ and found that the reaction of primary iodides with enolate anions is by single electron transfer (SET), and the alkyl bromide and tosylate appear to be reacting via an S_N2 pathway. The effects of leaving group, solvent, and hydrogen atom donors on product distribution and reaction rate were thoroughly investigated. The role of proton donor was shown, in addition, to induce a free-radical chain process in the case of alkyl iodides.

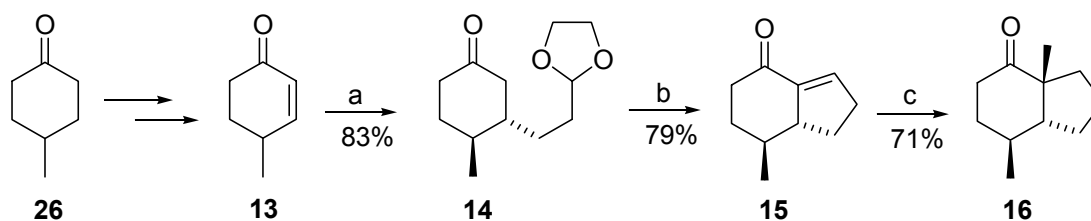


Scheme 4.10. Introduction of the angular methyl group to 15. Reaction conditions: Li, NH_3 (liquid), Ph_3COH , CH_3I , ether.

The angular methyl group was introduced onto bicyclic enone **15** (Scheme 4.10) by

a tandem reduction-alkylation procedure with methyl iodide in a solution of lithium in liquid ammonia. In the absence of a proton donor, the yield was never higher than 36%, and starting material was always recovered, even when the reaction was left overnight.⁶⁴ when 1 equivalent of Ph_3COH was used as a proton donor, the reaction complete in 2 hours in 71% yield.⁶⁶ The methylation was carried out with complete stereocontrol, yielding the desired *cis* product **16** as a single diastereomer due to methyl iodide approaching the enolate from the less hindered convex face of the rings.⁶⁷

4.2.2.4 Convergent preparation of bicyclic ketone **16**



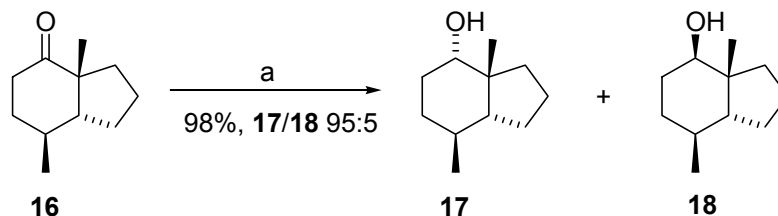
Scheme 4.11. Preparation of bicyclic ketone **16.** Reaction conditions: (a) 2-(2-bromoethyl)-1,3-dioxolane, Mg, I_2 , $\text{CuBr}\cdot\text{SMe}_2$, SMe_2 , THF, $-78\text{ }^\circ\text{C}$; (b) 1M HCl, THF, r.t.; (c) Li, NH_3 (liquid), Ph_3COH , CH_3I , ether.

As discussed above, we could now complete the preparation of bicyclic ketone **16** (Scheme 4.11).

4.2.2.5 Optical resolution of intermediate alcohol **17**

In order to obtain the analogues in enantiomerically pure form, optical resolution, rather than asymmetric synthesis, was judged to be the easiest and the quickest way. However, it was predicted that target β -hydroxyl ketones would not be stable enough for the derivatisation allowing for separation due to a competing β -elimination of the hydroxyl group. Therefore, we decided to carry out the optical resolution in the middle of the synthesis. An optical resolution can be carried out either on the ketone **16**, or on an intermediate alcohol. Comparing the availability and cost of the optical reagents, and considering that the conversions between ketones and alcohols are

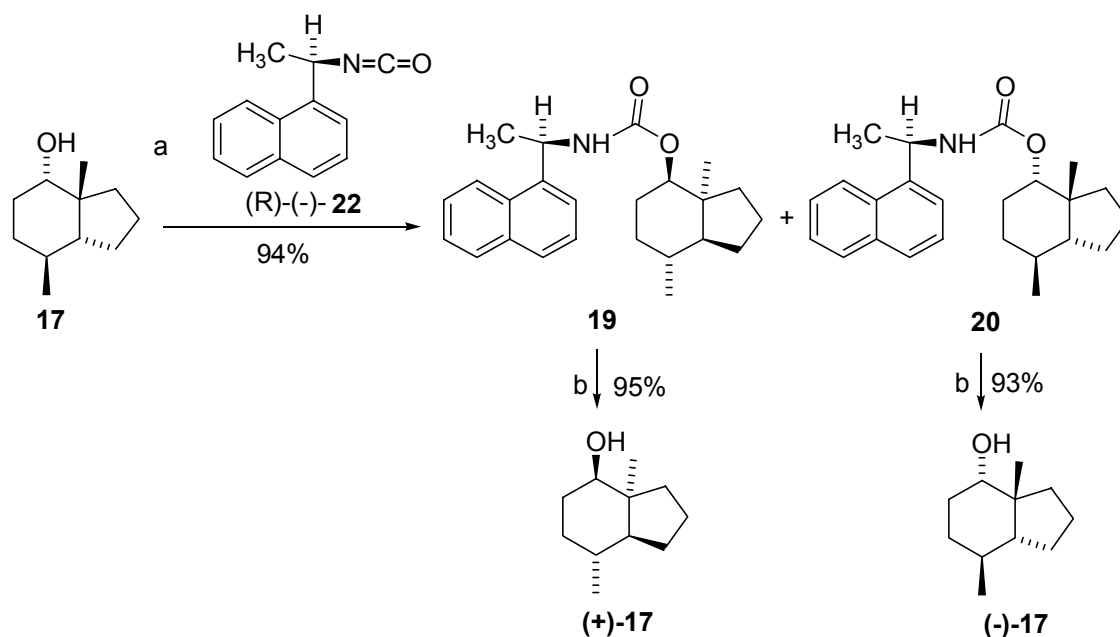
usually easy and high yielding, we decided to use an alcohol as a candidate for optical solution.



Scheme 4.12. Reduction of 16. Reaction conditions: (a) Dibal-H, THF, CH_2Cl_2 , $-78\text{ }^\circ\text{C}$.

The alcohol **17** (Scheme 4.12) was easily obtained from the reduction of ketone **16**. Two reductants, Dibal-H and L-selectride, were compared in order to get the stereoselectivity as high as possible. Interestingly, we found that Dibal-H gave **17** as the major product with a ratio **17** : **18** of 95 : 5, whereas L-selectride gave **18** as the major product with a ratio **17** : **18** of 12 : 88.

In our efforts to carry out the optical resolution of **17**, we tried different reagents. Our initial attempts with (-)-(R)-menthyl chloroformate⁶⁸⁻⁷⁰ and (-)-menthoxyacetyl chloride⁷¹ failed as the resulting diastereomers could not be separated by HPLC. However, pleasingly, R-(-)-1-(1-naphthyl)ethyl isocyanate served as a good reagent for the resolution of **17**;^{72,73} the resulted carbamates **19** and **20** can be separated on HPLC successfully (Scheme 4.13). Cleavage of the carbamate group with lithium aluminum hydride provided enantiomerically pure alcohol (+)-**17** and (-)-**17**.



Scheme 4.13. Preparation of (+)-17 and (-)-17. Reaction conditions: (a) **22**, pyridine, 85 °C; (b) LiAlH₄, THF, reflux.

4.2.2.6 Determination of the absolute configuration of (-)-17

There are a few physical methods, for example, the exciton chirality method, X-ray crystallography, and several chemical methods that can be used to predict the absolute configurations of organic compounds. Among them, Mosher's method^{74,75} using 2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid (MTPA) esters has been most frequently used. The high-field FT NMR application of Mosher's method has been reported to elucidate the absolute configurations of secondary alcohols by using high-field ¹H NMR spectroscopy.⁷⁶⁻⁷⁸

Mosher proposed^{74,75} that in solution, the carbinyl proton, ester carbonyl and the trifluoromethyl group of the MTPA moiety lie in the same plane (Figure 4.4 A). The idealized conformation is depicted in Figure 4.4 B. Due to the diamagnetic effect of the benzene ring, the H_{A,B,C...} NMR signals of the (R)-MTPA ester should appear upfield relative to those of the (S)-MTPA ester. The reverse should hold true for H_{X,Y,Z...}. Therefore, when $\Delta\delta = \delta_S - \delta_R$, protons on the right side of the MTPA plane (Figure 4.4 B) must have positive values ($\Delta\delta > 0$) and protons on the left side of the plane must have negative values ($\Delta\delta < 0$). This is illustrated in Figure 4.4 C.

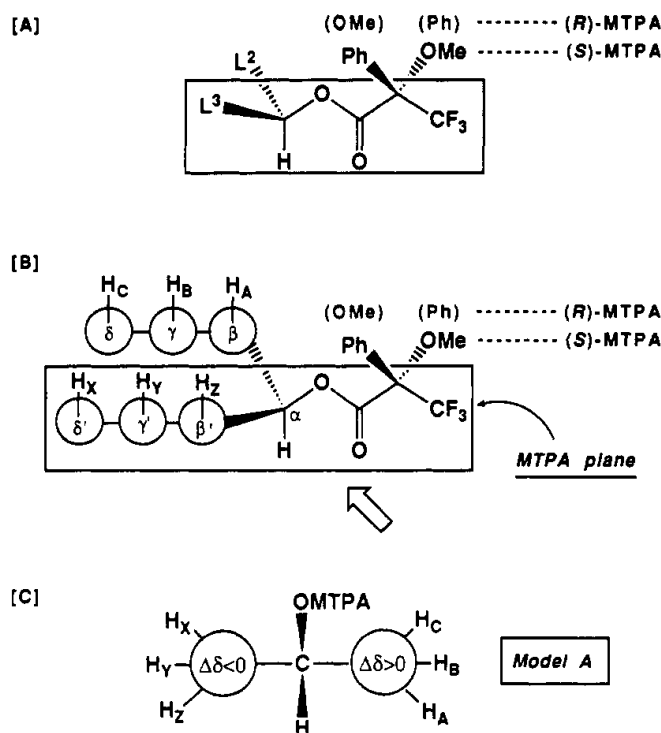


Figure 4.4. (A) Configurational correlation model for the (R)-MTPA derivatives and the (S)-MTPA derivatives proposed by Mosher. (B) MTPA plane of an MTPA ester is shown. H_{A,B,C...} and H_{X,Y,Z...} are on the right and left sides of the plane, respectively. (C) Model A to determine the absolute configurations of secondary alcohols is illustrated. Model A is a view of the MTPA ester drawn in (B) from the direction indicated by the outlined arrow.

Ohtani extended^{76,77} Mosher's method as follows: (1) Assign as many proton signals as possible with respect to each of the (R)- and (S)-MTPA esters. (2) Obtain $\Delta\delta$ values for the protons. (3) Put the protons with positive $\Delta\delta$ on the right side and those with negative $\Delta\delta$ on the left side of model A (Figure 4.4 C). (4) Construct a molecular model of the compound in question and confirm that all the assigned protons with positive and negative $\Delta\delta$ values are actually found on the right and left sides of the MTPA plane, respectively. The absolute values of $\Delta\delta$ must be proportional to the distance from the MTPA moiety. When these conditions are all satisfied, model A will indicate the correct absolute configuration of the compound.

The absolute configuration of (-)-17 was determined according to Mosher's method by converting to the (R)-MTPA and (S)-MTPA esters and comparing their ¹H NMR spectrum to find out the $\Delta\delta$ (Figure 4.5 A). Hence the stereochemistry of (-)-17 was elucidated to be 3aR,4S,7S,7aR (Figure 4.5 B).

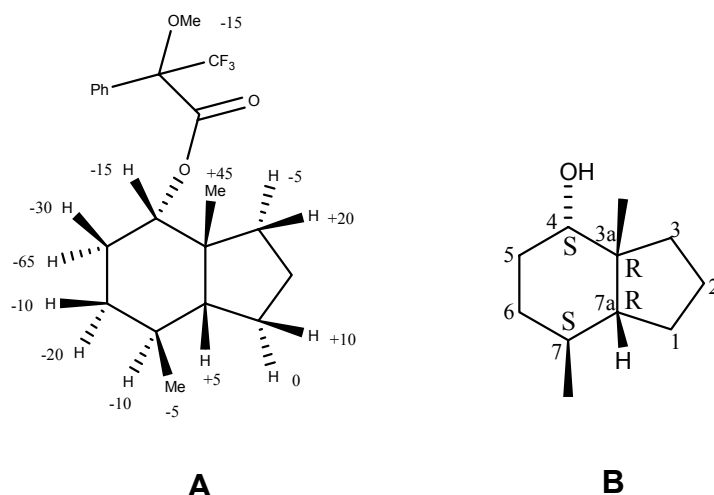


Figure 4.5. (A) $\Delta\delta$ values ($\Delta\delta = \delta_S - \delta_R$ in Hz at 500 MHz) obtained for MTPA esters of (-)-17; (B) Absolute configuration of (-)-17.

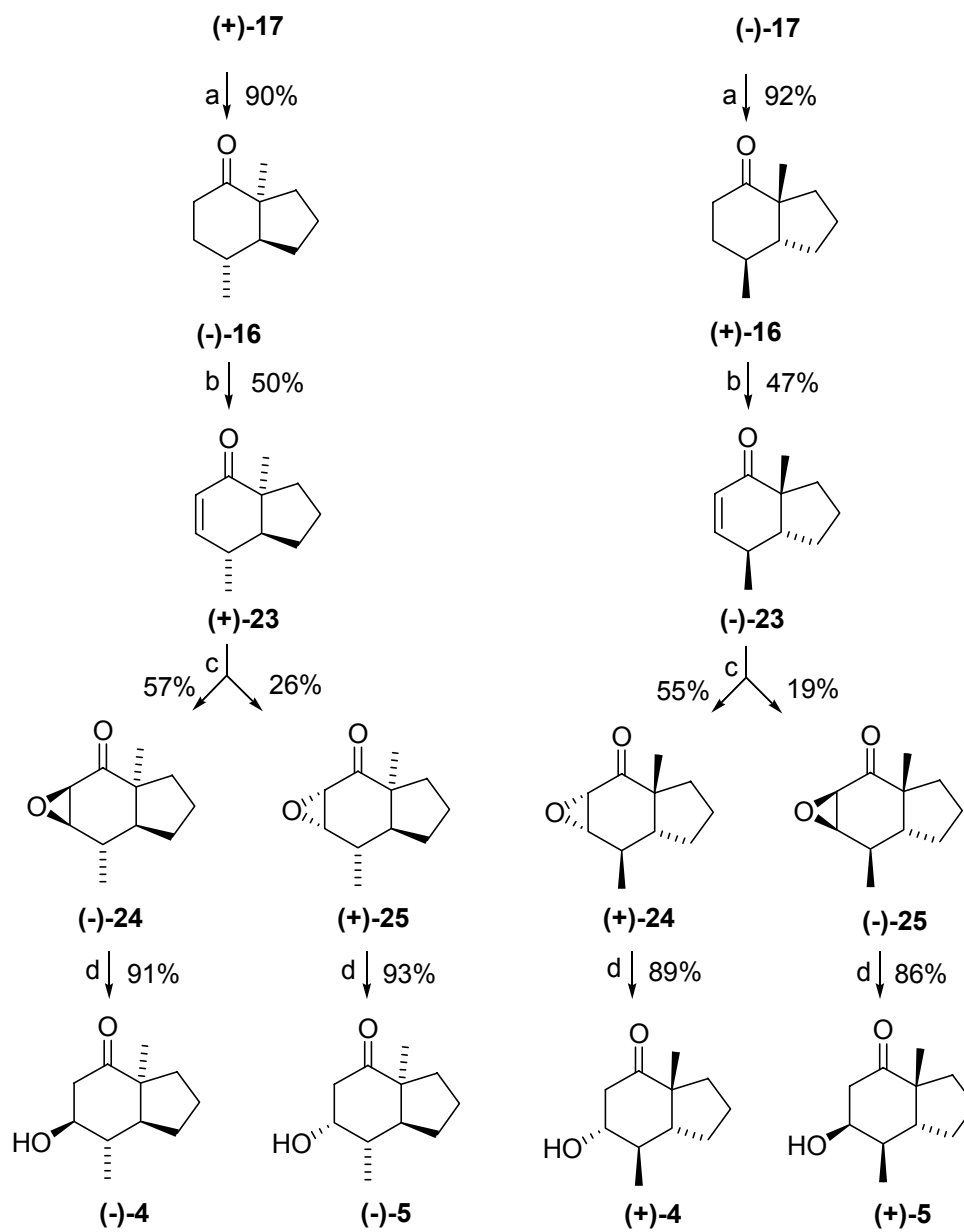
4.2.2.7 Preparation of enantiomerically pure analogues of conidiogenone

We now could prepare the optically pure analogues of conidiogenone from the two enantiomers (+)-17 and (-)-17. Converting the intermediate alcohols back unto the ketones (-)-16 and (+)-16 was easily done by oxidation with pyridinium chlorochromate (PCC) (Scheme 4.14). The ketones were then transformed into α,β -unsaturated ketones (+)-23 and (-)-23 by sequentially treating with PhSeCl followed by hydrogen peroxide.

Attempted epoxidation of (+)-23 and (-)-23 with hydrogen peroxide gave the *syn* addition products **25** with very good stereoselectivity along with only about 5% (as judged by ¹H NMR spectroscopy) of the *anti* addition products **24**. This can be explained by that the small size of hydrogen peroxide makes it possible for it to approach the bicyclic enone from the more hindered face of the rings to form the thermodynamically preferred *cyn* addition products. In order to obtain as many analogues as possible, we wanted to get some *anti* addition products. Therefore, two larger peroxides: triphenyl methyl hydroperoxide and cumene hydroperoxide were used. As expected, both of them yielded the *anti* products **24** as the major products, as well as a minor amount of the *syn* products **25**. The ratio of *anti* to *syn* was 3:1 for the bulkier triphenyl methyl hydroperoxide, and 2.2:1 for cumene hydroperoxide.

Finally, the reductive ring-opening of the α,β -epoxy ketones **24** and **25** was

performed by samarium diiodide,⁷⁹ which served as a reducing agent to give the desired products (+)-4, (-)-4, (+)-5 and (-)-5 respectively, without causing β -elimination.



Scheme 4.14. Preparation of ring AB analogues. Reaction conditions: (a) PCC, CH_2Cl_2 , r.t.; (b) i. PhSeCl, EtOAc, r.t.; ii. 35% H_2O_2 , THF- H_2O , r.t.; (c) Cumene hydroperoxide, 1M KOH, THF, r.t.; (d) SmI_2 , MeOH, THF, -78°C .

4.3 Synthesis of a tricyclic analogue of conidiogenone (Paper IV)

Having synthesised the simplified ring A and ring AB analogues, we now wanted to make a more lipophilic analogue with molecular size and shape more like the natural product, for this purpose, a computational modeling was carried out (described in Paper IV), and compound **6** was found as a simplified tricyclic analogue of conidiogenone (**2**) (Figure 4.6). It has a perhydrophenanthrene framework containing a similar ring A moiety as well as the same functional groups and the same relative configuration as the natural product.

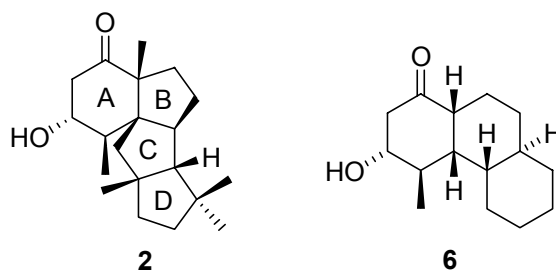
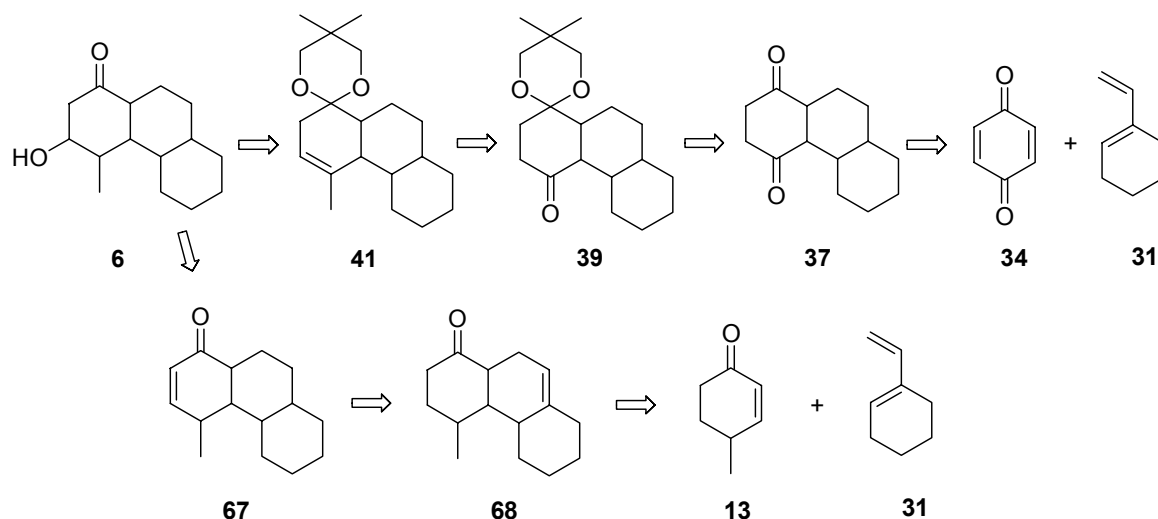


Figure 4.6. conidiogenone (**2**) and tricyclic analogue (**6**).

4.3.1 Retrosynthetic analysis of tricyclic analogue **6**

Initially, two disconnections were identified in the structure of **6**: the OH bond, the construction of perhydrophenanthrene framework (Scheme 4.15).



Scheme 4.15. Retrosynthetic analysis of tricyclic analogue **6**.

The methyl group of **6** could be acquired either from the starting material or being introduced through a carbonyl group, therefore two approaches toward **6** were considered.

Route 1: The hydroxyl group of **6** could be derived from the conjugated ketone **67** by epoxidation and ring-opening reduction, which could be obtained from **68** by hydrogenation and formation of conjugated double bond. The perhydrophenanthrene framework of **68** could be formed by the Diels-Alder reaction between 4-methyl-2-cyclohexenone (**13**) and 1-ethenylcyclohexene (**31**).

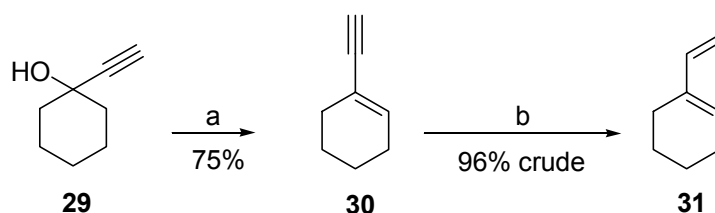
Route 2: the hydroxyl group of **6** could be developed from a carbon-carbon double bond of **41** which could be obtained by introducing the methyl group onto the ketone **39**. The monoketone **39** could be furnished by selective protection of diketone **37**. The perhydrophenanthrene framework of **37** could be formed by the Diels-Alder reaction between 1,4-benzoquinone (**34**) and 1-ethenylcyclohexene (**31**).

Route 1 is obviously shorter than route 2, hence the Diels-Alder reaction between **13** and 1-ethenylcyclohexene (**31**) was attempted first.

4.3.2 Synthesis of tricyclic analogue 6

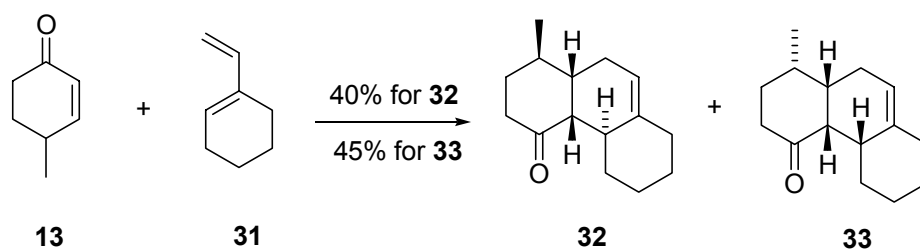
4.3.2.1 The Diels-Alder reaction between 13 and 31

4-Methyl-2-cyclohexenone (**13**) was prepared from 4-methylcyclohexanone (**26**)⁴⁴⁻⁵⁰ (see Section 4.2.2.1). 1-ethenylcyclohexene (**31**) was easily prepared according to the previously reported procedures from 1-ethynyl-1-cyclohexanol (**29**) in 2 steps (Scheme 4.16).^{80,81}



Scheme 4.16. Preparation of 31. Reaction conditions: (a) POCl₃, pyridine, 90 °C; (b) H₂, Lindlar catalyst, MeOH.

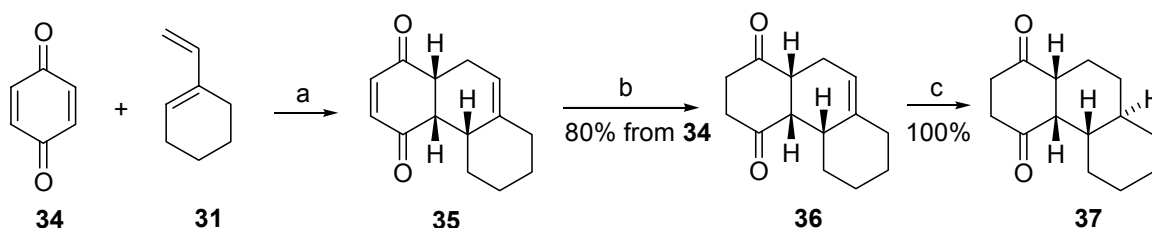
We found that the Diels-Alder reaction between 4-methyl-2-cyclohexenone (**13**) and 1-ethenylcyclohexene (**31**) was very resistant to heating, even at 250 °C for 6 hours, no obvious reaction was observed. However, when treated with 0.8 equivalents of AlEtCl₂, the reaction was complete in 4 hours at room temperature, giving two diastereomeric products **32** and **33**⁸² (Scheme 4.17), which could be separated on HPLC. Unfortunately, they were not the desired products, and none of the desired compound **68** was isolated from the reaction. The reaction outcome was decided by steric factors: the formation of **32** and **33** is sterically preferred as in this case, the bulky cyclohexane ring of the diene approaches the dienophile from the opposite side of the methyl group to form the exo (**32**) and endo (**33**) addition products.



Scheme 4.17. Diels-Alder reaction between **13** and **31**. Reaction conditions: AlEtCl_2 , CH_2Cl_2 , r.t..

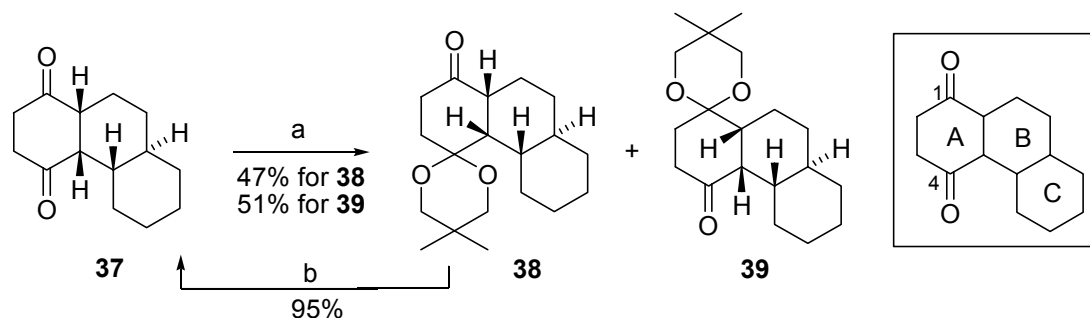
4.3.2.2 Synthesis of tricyclic analogue **6** from 1,4-benzoquinone

Another approach towards **6** involved starting from a symmetrical enone, 1,4-benzoquinone (**34**), which has been reported to cyclise with 1-ethenylcyclohexene (**31**) to form the perhydrophenanthrene framework⁸³ (Scheme 4.18).



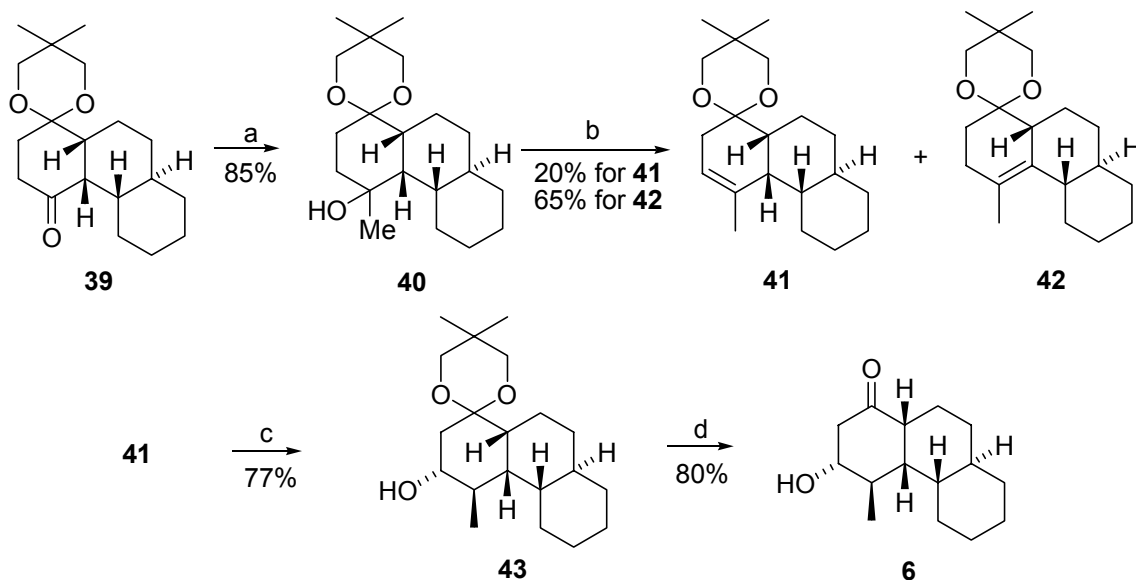
Scheme 4.18. Preparation of **37**. reaction conditions: (a) toluene, 80 °C; (b) Zn , HOAc , r.t.; (c) H_2 , Pd/C , EtOH , 100%;

The Diels-Alder reaction was easily performed in toluene, and only an *endo* addition product **35** was obtained, which was unstable owing to easy aromatization. Thus, without purification, it was reduced with Zn and HOAc to give a stable diketone **36** according to literature.⁸³ Hydrogenation of this compound afforded the saturated diketone **37** as a single diastereomer. The configuration of three fused rings was assigned as *cis*, *trans*.



Scheme 4.19. Preparation of 39. Reaction conditions: (a) 1,3-dihydroxy-2,2-dimethylpropane, benzene, TsOH, Dean-Stark conditions; (b) TsOH, acetone, H₂O, reflux.

In order to introduce a methyl group onto ketone-4 of **37** (Scheme 4.19), we needed to selectively protect ketone-1, which seems to be more exposed to attack than ketone-4. However, when treated with 1 equivalent of 1,3-dihydroxy-2,2-dimethylpropane, which was used as a bulky diol, the two mono ketals **38** and **39** were obtained in an approximately 1:1 ratio.⁸⁴ The desired product **39** can be easily separated, and the undesired ketal **38** could be easily converted back to diketone **37**.⁸⁵



Scheme 4.20. Preparation of 6. Reaction conditions: (a) MeLi, THF, ether, -78 °C; (b) SOCl₂, pyridine, 0 °C; (c) (i) BH₃-SMe₂, THF, -15 °C then r.t., (ii) NaOH, H₂O, 35% H₂O₂, 0 °C; (d) PdCl₂, acetone, r.t..

The methylation of ketone **39** (Scheme 4.20) with methyllithium⁸⁶ gave alcohol **40**. Elimination of hydroxyl group of **40** afforded two isomers: the tri-substituted olefin **41** and the tetra-substituted olefin **42** in a ratio of 1:3.⁸⁷⁻⁸⁹ Compound **42** is a useful intermediate for our future work to prepare some analogues with angular methyl groups. Olefin **41** was subjected to a hydroboration-oxidation procedure to provide alcohol **43** with stereocontrol.⁹⁰ Finally, tricyclic analogue **6** was prepared by removal of the dioxolane protecting group with palladium(II) chloride in acetone,⁹¹ which were used instead of usual conditions owing to competing β -elimination.

4.4 Conclusions

Three types of analogue of natural product conidiogenone (**2**) have been prepared. Using in all cases, ring A which bears all the functional groups, as a core for design, the simplified ring A, ring AB, and tricyclic analogues were designed and synthesized. They share the same functional groups and the same relative configuration as the parent molecule but differ on the size, so it would be interesting to investigate their behavior in the biological tests for conidiogenesis-inducing activity.

A route towards the total synthesis of the natural product conidiogenone

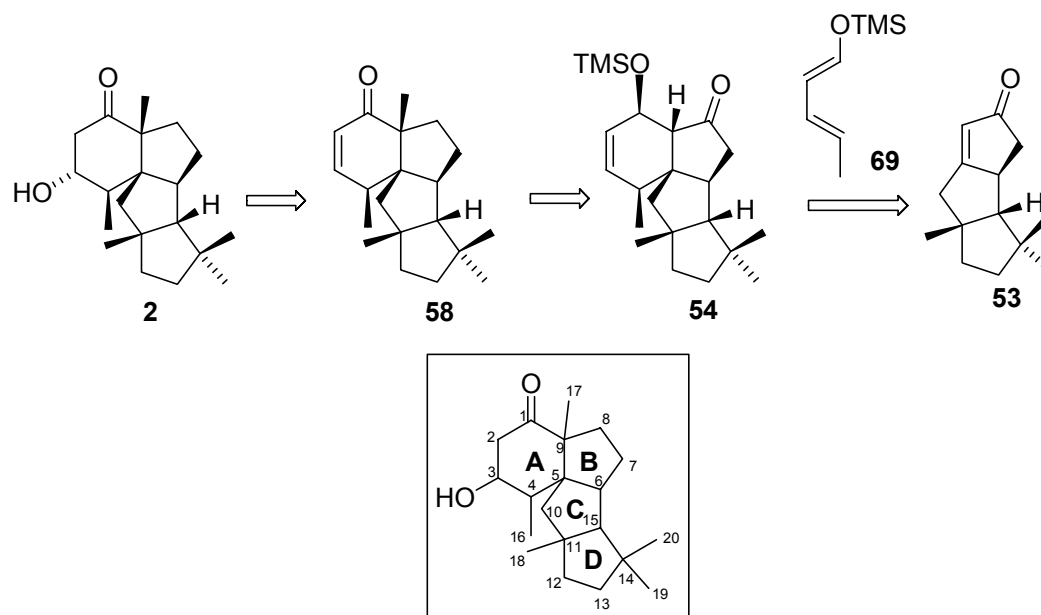
Total synthesis is a practice where all aspects of organic chemistry are combined in both planning and execution to reach a pre-designated target.⁹² The targets of a total synthesis are often biologically active natural products that are available in limited amounts. Total synthesis plays a very important role in organic chemistry, not only as a tool to obtain compounds, but also as a strong stimulant for the development of new synthetic methods. In addition, it can be seen as a test of the skills and imagination of chemists, and also serve as a training ground for young scientists.

Fascinated by the beautiful structure of conidiogenone (2), we decided to propose a route towards its total synthesis.

5.1 Retrosynthetic analysis of conidiogenone 2

5.1.1 The disconnections of ring A

Two disconnections were identified for ring A in the structure of conidiogenone: the OH bond, and formation of cyclohexane ring (Scheme 5.1).

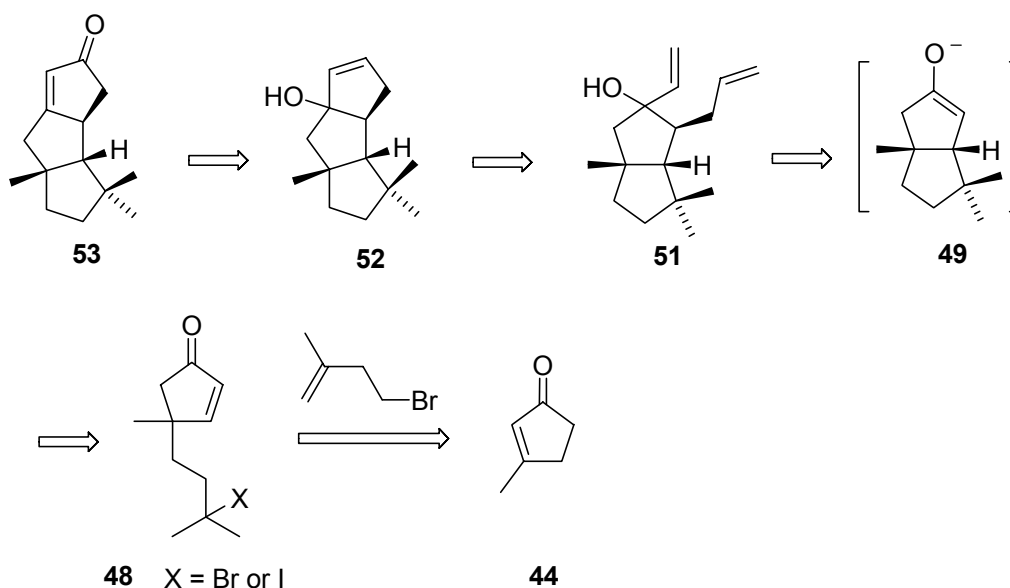


Scheme 5.1. The disconnections of ring A of conidiogenone (2).

The hydroxyl group of conidiogenone (2) could be derived from the carbon-carbon double bond of **58**. Ring A could be formed through an intermolecular [4+2] cycloaddition between the silyl enol ether diene **69** and enone **53**. There are three advantages to the use of a silyl enol ether: Firstly, after the cyclization, the resulting silyl ether can be easily transformed into the desired ketone. Secondly, it is electron-rich diene that makes the Diels-Alder reaction more favourable. Thirdly, the bulky trimethyl silyl group makes the diene **69** prefer to approach the enone **53** from the less sterically hindered face of the ring resulting the product with the correct stereochemistry. The C-17 methyl group could be introduced after the formation of ring A. Therefore, the tricyclic enone **53** is a key intermediate.

5.1.2 Retrosynthetic analysis of tricyclic enone 53

A retrosynthetic analysis of tricyclic enone **53** is proposed below (Scheme 5.2).



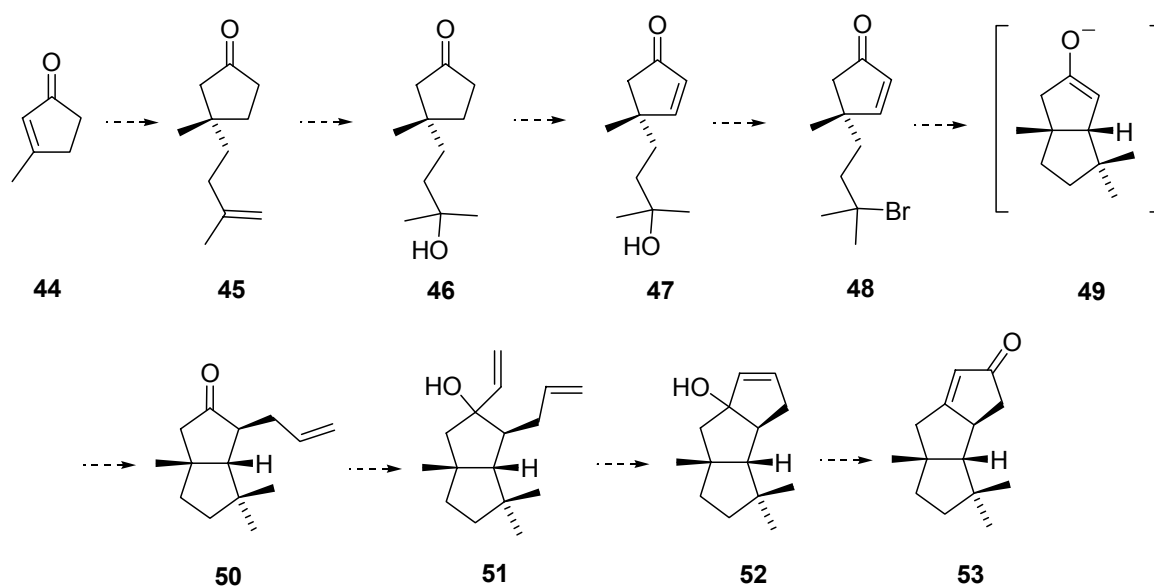
Scheme 5.2. Retrosynthetic analysis of tricyclic enone **53**

The conjugated ketone **53** could be derived from an oxidative rearrangement of tertiary allylic alcohol **52**, which could be formed by a ring-closing metathesis reaction from diene **51**. The allyl side chain of the **51** could be added by trapping enolate **49** to give the correct regiochemistry. The enolate **49** could be formed by an intramolecular conjugate addition from **48**. The attachment of a functionalised five-carbon side-chain to **44** could provide compound **48**. Thus, the retrosynthetic analysis ends at 3-methyl-2-cyclopentenone **44**.

5.2 *A proposed synthetic route towards conidiogenone 2*

5.2.1 Proposed synthetic pathway towards tricyclic enone **53**

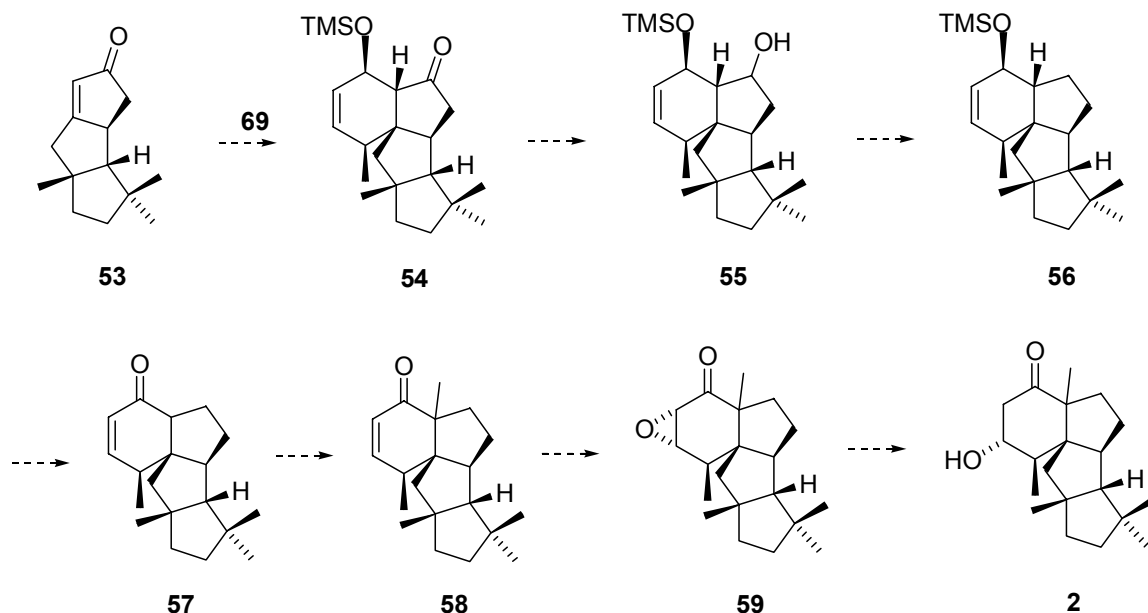
A proposed synthetic pathway is described below with the suggested reagents (Scheme 5.3):



Scheme 5.3. Proposed synthetic pathway towards tricyclic enone 53.

The commercially available conjugated ketone, 3-methyl-2-cyclopentenone **44**, (Scheme 5.3) can be used as a starting material. The attachment of a functionalised five-carbon side-chain to **44** could be achieved by a conjugate addition of a Grignard reagent derived from 2-methyl-4-bromobutene⁹³ in the presence of cuprous bromide to afford **45**, which could be converted to alcohol **46** by treatment with acid.⁹⁴ Dehydrogenation of **46** under palladium-catalysed reaction⁹⁵ could furnish cyclopentenone **47**, which would be expected to yield bromide **48** upon treatment with PBr_3 .⁹⁶ An intramolecular conjugate addition of the Grignard reagent **48** carried out in the presence of cuprous bromide is anticipated to afford an intermediate *cis* enolate **49**.⁹⁷ Quenching enolate **49** with allyl bromide could provide the unsaturated ketone **50**; it is anticipated that the allyl side-chain would be introduced with stereocontrol onto the least sterically hindered convex face of the bicyclic enolate.⁶⁷ Ketone **50** could be attacked by vinyl magnesium bromide to give diene **51**; stereocontrol is not required for this addition since the newly formed hydroxyl group needs to be eliminated later. Diene **51** could undergo ring-closing metathesis with Grubbs' catalyst⁹⁸⁻¹⁰² to give tricyclic alcohol **52**. Finally, oxidative rearrangement of the tertiary allylic alcohol with pyridinium chlorochromate¹⁰³⁻¹⁰⁸ could afford tricyclic enone **53**.

5.2.2 Proposed synthetic pathway from tricyclic enone **53** to conidiogenone (**2**)



Scheme 5.4. Proposed synthetic pathway from **53** to conidiogenone (**2**).

The key step of the route is a Diels-Alder reaction to form the skeleton of conidiogenone. The [4+2] cycloaddition^{109,110} between enone **53** and silyl enol ether diene **69** could produce silyl ether **54** (Scheme 5.4). Since diene **69** and dienophile **53** are not symmetrical, the Diels-Alder reaction could afford eight products theoretically. The possible major products were predicted by calculation (discussed in Section 5.3), which indicate that the desired product **54** is both thermodynamically and kinetically preferred comparing to the other possible products; it could be one of the major products.

Ketone **54** could be reduced to alcohol **55** with NaBH_4 ,¹¹¹ and then converted to **56** by conversion to the bis-methyl xanthate and then reduction with $n\text{-Bu}_3\text{SnH}$.¹¹² Hydrolysis and oxidation of allyl silyl ether **56** with Jones reagent¹¹³ could afford enone **57**. The quaternary methyl group could be introduced via generation of the enolate anion with potassium hexamethyldisilazide¹¹⁴ followed by addition of methyl iodide to obtain enone **58**; the stereochemical outcome is not easy to predict.

Stereoselective epoxidation of the conjugated double bond could be achieved by a bulky peroxide such as cumene hydroperoxide¹¹⁵ to give epoxide **59**; a bulky peroxide prefers to attack the enone onto the less sterically hindered side to give the correct stereochemistry. Finally, the reductive ring opening of the α,β -epoxy ketone **59** with samarium diiodide¹¹⁶ could afford conidiogenone (**2**) with retention of the configuration of β -hydroxyl group.

5.3 Calculations of the Diels-Alder reaction

The Diels-Alder reaction between enone **53** and silyl enol ether diene **69** is the key step in the total synthesis (Scheme 5.4). In order to predict the major products, calculations of the eight theoretically possible products and their transition states were carried out.

5.3.1 Theoretically possible products of the Diels-Alder reaction

Since the diene **69** and dienophile **53** are not symmetrical, the Diels-Alder reaction can give eight possible products, theoretically (Figure 5.1).

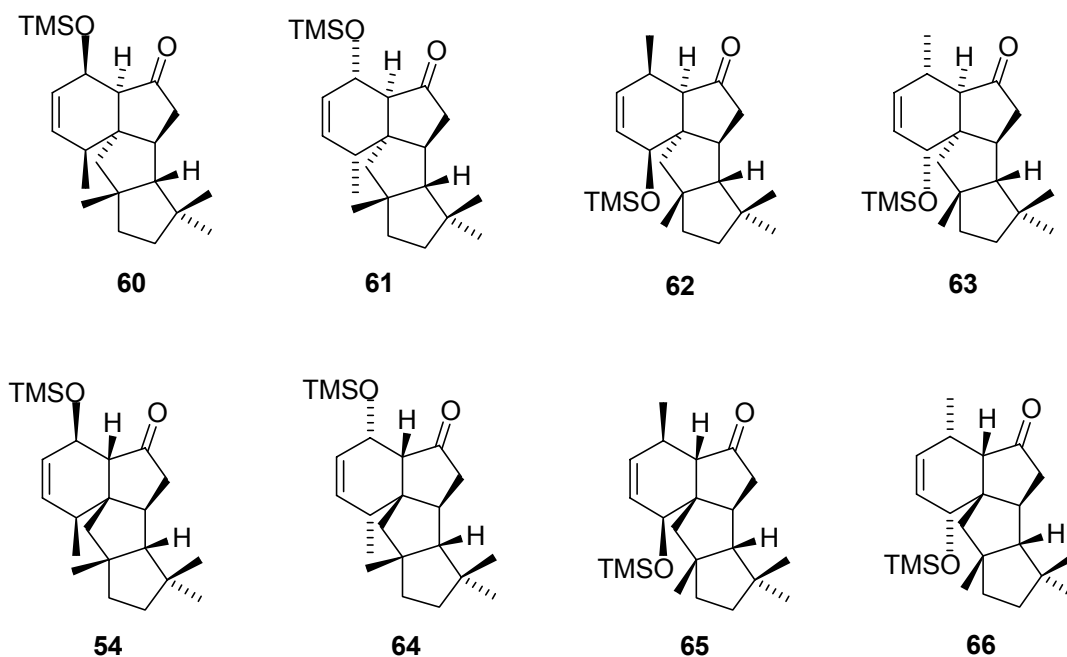


Figure 5.1. Theoretically possible products of the Diels-Alder reaction.

Products **60**, **61**, **62**, and **63** are formed by the addition of diene **69** onto the enone **53** from the top of the enone. **60** and **62** are *exo* products; **61** and **63** are *endo* products. Products **54**, **64**, **65** and **66** are formed by the addition of diene **69** from the bottom of the enone **53**. **54** and **65** are *endo* products, **64** and **66** are *exo* products.

5.3.2 The HF energy comparison of theoretically possible products

The molecules were built from energy minimized templates in PC Spartan pro and were minimized with Spartan pro Hartree-Fock 3.21G*.

The equilibrium geometries of the eight theoretically possible products were calculated and compared (Table 5.1). Products **65**, **54**, **66** and **64**, which are formed by diene **69** approaching enone **53** from the bottom, have much lower energies than **62**, **63**, **61** and **60**, which are formed by addition of diene **69** onto enone **53** from the top. The significant energy difference between these two groups indicates that products **65**, **54**, **66** and **64** are thermodynamically preferred. An *endo* product **65** has the lowest energy, and another *endo* product **54**, the desired product, is only 1.43 kcal/mol higher than **65**. Two *exo* products **64** and **66** are 3.93 and 4.75 kcal/mol higher than **65** respectively.

Table 5.1. The HF energies of products

Product	Energy (au)	Relative energy (kcal/mol)
65	-1285.8477998	0
54	-1285.8455252	+1.43
66	-1285.8415425	+3.93
64	-1285.8402313	+4.75
62	-1285.8287343	+11.96
63	-1285.8246050	+14.55
61	-1285.8188417	+18.17
60	-1285.8180526	+18.67

5.3.3 HF energy comparison of transition states

The transition structures were built from the structures of the products by constraining two new c-c bonds to 2.25 Å assuming that it was the intermediate distance of product and starting material. The HF energy of the eight transition states was minimized with Spartan pro Hartree-Fock 3.21G* and for each case, it was confirmed that there was only one imaginary vibrational frequency, which, when animated, corresponded to the expected reaction coordinates.

The energy differences were compared (Table 5.2). Two *endo* transition states (Figure 5.2) have much lower energies than the others. The transition state **TS54**, which corresponds to the desired product **54**, has the lowest energy. **TS65**, which corresponds to the lowest energy product **65**, is only 1.34 kcal/mol higher than **TS54**. This indicates that the two *endo* transition states which formed by diene **69** approaching from the bottom of enone **53** should be the most probable transition states. Therefore, their corresponding products **54** and **65** would be kinetically preferred.

Table 5.2. HF energy comparison of transition states

Transition state ^a	Energy (au)	Relative energy (kcal/mol)
TS54	-1285.7413659	0
TS65	-1285.7392292	+1.34
TS64	-1285.7101874	+19.56
TS66	-1285.7061629	+22.09
TS60	-1285.6812554	+37.72
TS63	-1285.6805999	+38.13
TS62	-1285.6792446	+38.98
TS61	-1285.6758715	+41.10

a. Transition states are named as TS followed by the Arabic numbers of the corresponding products.

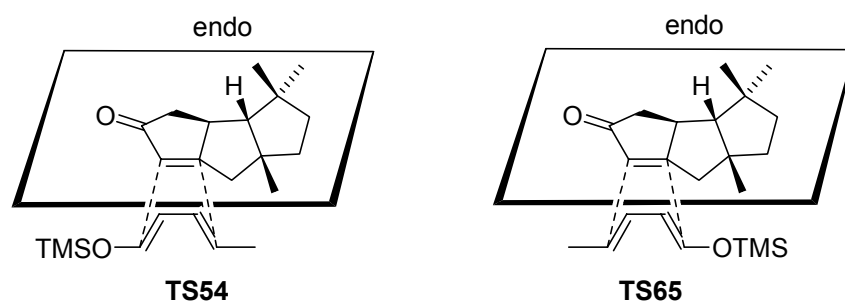


Figure 5.2. Transition states of TS54 and TS65.

The calculations above indicate that the Diels-Alder reaction between diene **69** and enone **53** could provide two major *endo* addition products **54** and **65** because they are both thermodynamically and kinetically preferred compared to the other theoretically possible products. The desired product **54** is one of the major products.

Mild microwave-assisted hydrolysis of acetals under solvent-free conditions

6.1 *Introduction*

6.1.1 Microwave heating in organic reactions

Microwave activation as a non-conventional energy source has become a very popular and useful technology in organic chemistry.¹¹⁷ The most important aspects of microwave-assisted reactions are the short reaction times and enhancements in conversions.^{118,119} Thus, microwave heating has been used in a wide range of organic reactions.¹²⁰

As reviewed by Lidstrom,¹²⁰ the traditional heat-transfer equipment that most organic reactions use, such as oil baths, sand baths and heating jackets, are rather slow, and a temperature gradient can develop within the sample. In addition, local overheating can lead to product, substrate and reagent decomposition. However, in microwave dielectric heating, the microwave energy is introduced into the chemical reactor remotely. The microwave radiation passes through the walls of the vessel and heats only the reactants and solvent, not the reaction vessel itself. If the apparatus is properly designed, the temperature increase will be uniform throughout the sample, which can lead to fewer by-products or decomposition products.

In addition, microwave heating combined with solvent-free conditions has attracted considerable attention as an environmental-friendly approach.

6.1.2 Hydrolysis of acetals

The selective introduction and removal of protecting groups is a very important operation in natural product synthesis. The success largely depends on the stability of the protecting groups towards various acidic or non-acidic reagents and how effectively they can be installed and removed.¹²¹

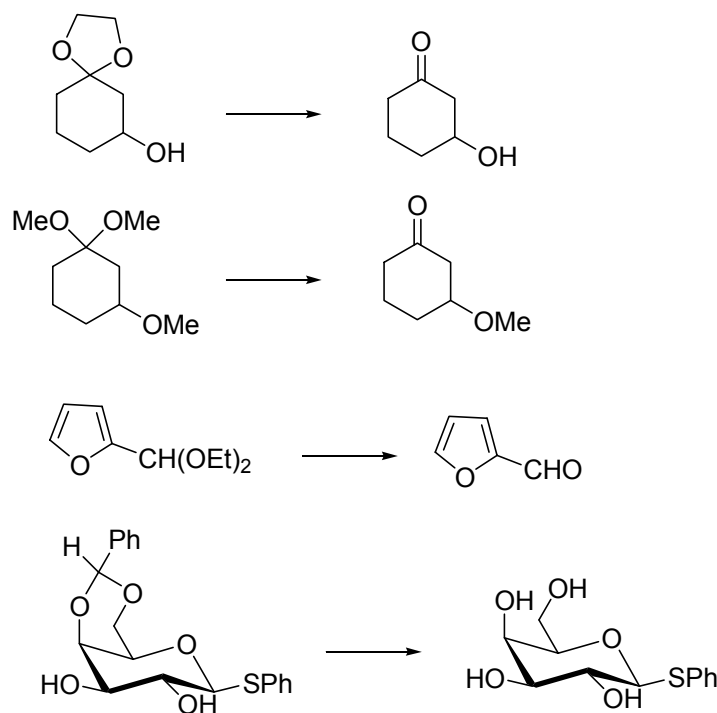
Aldehydes, ketones and diols (1,2- or 1,3-) are frequently protected as acetals in organic synthesis, and a number of methods for their hydrolysis are known.¹²² Most of these use aqueous acid^{122,123} or acid-catalysed exchange with acetone.¹²⁴ These conditions are not suitable when other acid-sensitive functional groups are present in the acetal-containing compounds. Therefore, considerable effort has been directed towards developing mild, selective methods for the hydrolysis of acetals. Some milder methods have been developed, using reagents such as acidified silica gel,¹²⁵ acetone in the presence of pyridinium tosylate,¹²⁶ lithium tetrafluoroborate in wet acetonitrile,¹²⁷ titanium tetrachloride in diethyl ether,¹²⁸ bismuth nitrate in dichloromethane,¹²⁹ bismuth triflate in THF/H₂O,¹³⁰ cerium ammonium nitrate in a 1:1 MeCN/borate-HCl buffer solution,¹³¹ and (trimethylsilyl) bis(fluorosulfonyl)imide.¹³² In addition, a few examples of solvent-free conditions for the cleavage of acetals have been reported, such as using potassium peroxymonosulfate on alumina.¹²¹ Each method has its own advantages.

6.2 *Mild microwave-assisted hydrolysis of acetals under solvent-free conditions*

During our synthesis of the analogues of conidioenone, we need to deprotect some 3-hydroxyacetals to yield a 3-hydroxy ketone. With conventional aqueous acid hydrolysis, such acetals only yield the corresponding α,β -unsaturated ketone (see Scheme 4.4 in Chapter 4). A milder and more selective method was required. We, therefore, developed a procedure for the hydrolysis of acetals with silica gel supported pyridinium tosylate moistened with water in solvent-free conditions under microwave irradiation.

Pyridinium tosylate (pyridinium *p*-toluenesulfonate, PPTS), is a cheap and safe reagent that has been found to be an excellent catalyst for the cleavage of dioxolane-type of acetals by transacetalisation with acetone.¹²⁶ PPTS is a weaker acid (pH 3.0 in 1.0 M aqueous solution) than acetic acid (pH 2.4 in 1.0 M aqueous solution),¹³³ suggesting that it should be useful as a catalyst in the deprotection of acid sensitive compounds.

We investigated the hydrolysis of acetals with PPTS in the absence of acetone and under solvent-free conditions with microwave heating. The method is very simple (described in Paper II).



Scheme 6.1. Hydrolysis of different types of acetals. Reaction conditions: PPTS, H₂O, silica gel, microwave.

A number of different kinds of acetals: 1,3-dioxolanes and dialkylacetals, acetals of aromatic aldehydes, and the benzylidene acetal of a sugar derivatives were efficiently hydrolysed (Scheme 6.1). The acid-labile 3-hydroxyketone was obtained in high yield after only a few minutes without any trace of cyclohex-2-enone.

Summary and future prospects

Three types of analogues of the natural product conidiogenone (**2**) have been designed and synthesised: the simplified ring A, ring AB, and tricyclic analogues. They share the same functional groups and the same relative configurations as the parent molecule, but differ in size.

Computer-assisted analysis of various ring systems indicates that the tricyclic compound **6b** (described in Paper IV) fits the hydrocarbon space of conidiogenone (**2**) very well. Therefore, it would be a good target for the future work.

A reasonable route towards the total synthesis of conidiogenone (**2**) has been proposed. The synthesis would be an exciting challenge for the future.

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