REVIEWS ON SYNTHESIS AND CHARACTERIZATION

OF

(+)- CHINENSIOLIDE B

A MSc Dissertation report by:-

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APRIL 2022

REVIEWS ON SYNTHESIS AND CHARACTERIZATION

OF

(+)- CHINENSIOLIDE B

A DISSERTATION REPORT

Submitted in Partial Fulfillment

of

The Degree of M.Sc. (Organic Chemistry)

By

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To the

School of Chemical Sciences

Goa University

Goa 403206

APRIL 2022

CERTIFICATE

This is to certify that , "***reviews on synthesis and characterization of (+)- chinensiolide B*** '' is the bonafide work carried out by Ms. Yogit G. Kowlekar under the supervision in partial fulfilment of the requirement for the award of the degree of Master of Sciences in Chemistry at the School of chemical Sciences, Goa University, Goa .

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STATEMENT

I hereby declare that the matter presented in this dissertation entitled , "***reviews on synthesis and characterization of (+)- chinensiolide B*** '' is based on results of investigation carried out by me in the School of Chemical Sciences, Goa University , Goa under the supervision of Dr. Rupesh A. Kunkalkar and the same has not been submitted elsewhere for the award of a degree or diploma.

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ACKNOWLEDGEMENT

I would like to express my special thanks to my teacher Dr. Rupesh Kunkalkar for giving me opportunity to work under his guidance , for his patience and invaluable help and assistance during the course of work on this literature review. I am also grateful to our respected Dean Dr. Vidhyadatta Verenkar for his support and providing us the opportunity to work in the School of Chemical Sciences.

Secondly , I would also like to thank to my parents and friends who helped me a lot in finalizing this project within the limited time frame.

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1. INTRODUCTION

(+)- Chinensiolide B is a large and diverse subgroup of naturally occurring Sesquiterpene lactones. Sesquiterpene lactones attracts considerable interest for their biological activities, such as antibiotics, insecticides, antitumor activities.[1] Studies on other species of this genus revealed the presence of sesquiterpene lactones such as guaianolides, eudesmanolides, germacranolides, and their glycosides.[2]

Chinensiolide was isolated from plants *Ixeris chinensis Nakai*, used in the Chinese folk as a medicine as for its haemostatic and anti-flammatory effects. Used as the treatment of Bronchitis, Pharyngitis , Dysentery and poisonous indigestion[3] on the basics of anti-febrile , anti-dotal and analgesis effects. R-Methylene γ-lactones are privileged structures present in a large number of natural products displaying a wide scope of biological activities.[4]

There are four types of Chinensiolides (figure 1) found in nature. That's is Chinensiolides A, Chinensiolide B, Chinensiolide C and Chinensiolide D. Among these groups of Chinensiolide, (+)-Chinensiolides B (figure 2) shows a cytotoxic behaviour against human primary liver cancer (HepG2) and two human lung fibroblast (WI-38 and VA-13) cell lines. [5]

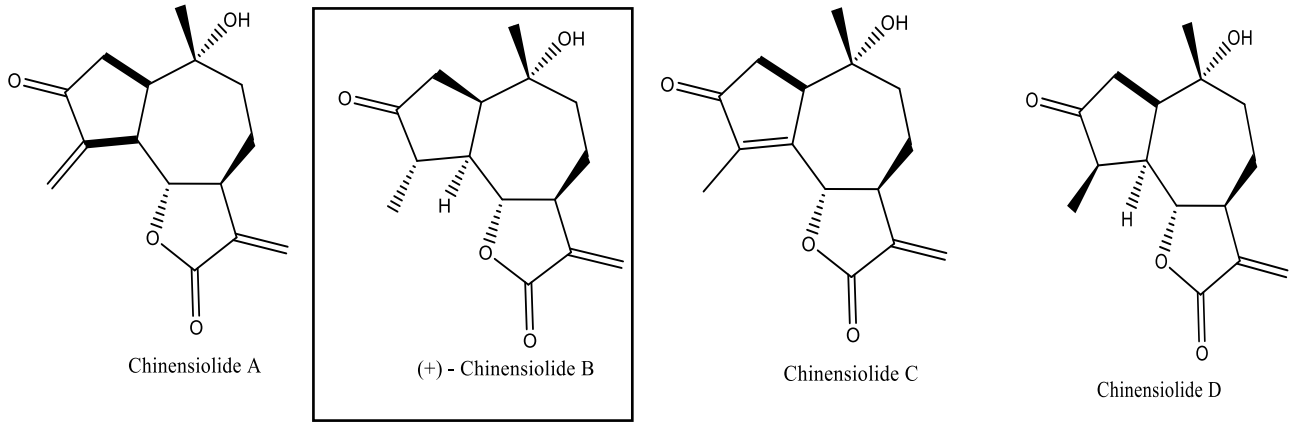


Figure 1.1 Structure of Chinensiolides A-D

These natural products have shown to be useful as DNA Selected natural products containing an a-methylene g-lactone.[6] Their Strategies and Tactics in Organic Synthesis polymerase inhibitors,

nuclear vitamin D receptor inhibitors, cellular steroidal inhibitors, blockers of tumour necrosis factor in production, and have many other uses. The wide inhibitory action of these natural products makes them potential drug medicines due to their cytotoxic, antiallergenic, anti-inflammatory, phytotoxic, and antimicrobial properties.[7] In fact, arglabin has been used successfully in Kazakhstan for the treatment of breast, colon, ovarian, and lung cancers. [8]Chinensiolide B constitutes another fascinating example of a natural product containing the a-methylene g-lactone unit as part of a tricyclic 5-7-5-ring structure.[9] α-methylene - γ -lactone ia a building block of the many natural prodcuts such as in sesquiterpene.[10]

The (+)- Chinensiolide B contains a cis fused 5,7,5- tricyclic ring. It contains six contiguous stereocenters, including five along a flexible seven membered ring, 2 and the sixth stereocenter may be subject to epimerization due to a neighboring ketone.[4] The complex structure of (+)-Chinensiolide B with six continuous streocenters and a double bond in lactones ring make their synthesis a very challenging. [5]

LITERATURE REVIEW

2. SYNTHESIS OF (+)- CHINENSIOLIDE B

2.1 Total synthesis of (+)-Chinensiolide B via Tandem Allylboration/Lactonization

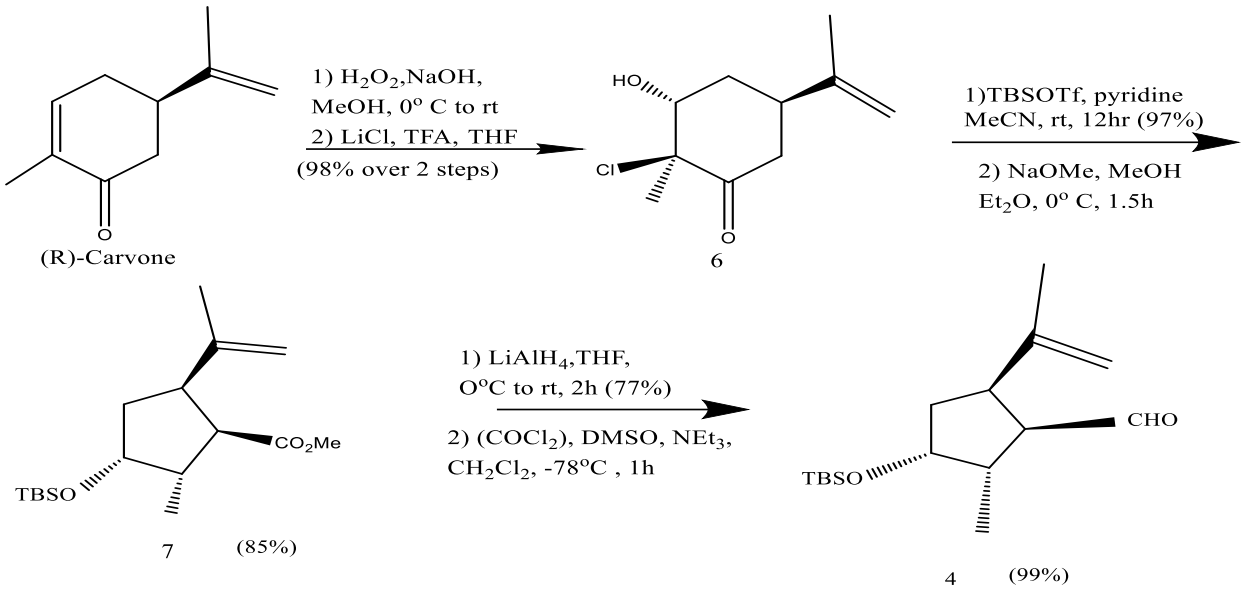
The first synthesis of (+)-Chinensiolide B was reported by Elford et al.[4] 2010 via Tandem Allylboration/Lactonization. The synthesis started with the two-step conversion of Carvone into ketone protocol reported by Ley and coworkers (scheme 1). Using Ketone **6**, protection of the secondary alcohols was followed by Favorskii rearrangement[11] provide a tetra-substituted cyclopentane **7**. The ester **7** then fully reduced and then reoxidized under swern condition [12] providing aldehyde **4**. Conversion of 4-pentyn-1-ol into allylboration **5** was achieved by standard literature procedure[13] and result in isomeric mixture of allylboration[6] of aldehyde **4**.

In (scheme 2) **4** and **5** is the key tandem allylboration/lactonization which was attempted with thermal and catalytic method using $BF_3.OEt_2$ at 0 c to give trans- γ -lactone product **3**. Further selective deprotonation of the primary TBDPS[7] groups on **3** was carried out followed by Grieco elimination[8] of the alcohols to give triene **9**. Chemoselctive RCM was performed on **9** using Grubbs catalyst **2** to provide a desired tricyclic **10**.

The final stage of synthesis involved diastrereoselective epoxidation of this **10**. Followed by the treatment of mCPBA to gave epoxide **2** as an unseperable 4:1 mixture of diastereoisomers. The one-pot double reduction protocol whereby the γ-lactone moiety of **2** was first reduced to the diol with DIBALH, and then LiEt3BH was added to regio- and chemoselectively opening the epoxide. This unusual protocol allowed for protection of the R-methylene γ-lactone group from undergoing conjugate addition with the highly reactive LiEt3BH. Simple treatment of the crude triol with MnO2 easily reformed the R-methylene γ-lactone. The two diastereomers originating from the epoxidation could now be separated to provide the desired γ-lactone **11**. Finally, oxidative cleavage of the secondary TBS [14] protecting group **16** was achieved in (+)- Chinensiolide B **1**.

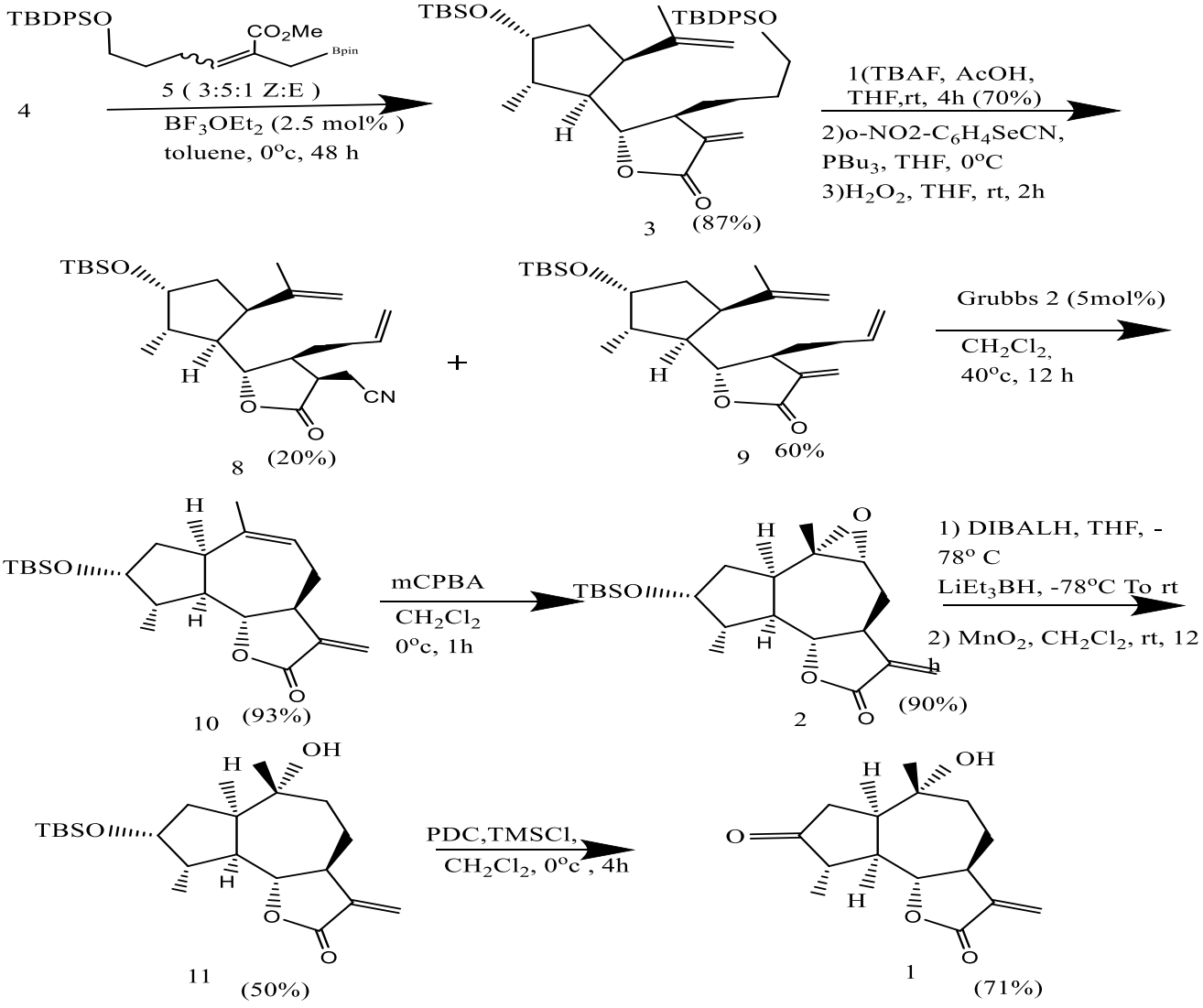
The synthesis required 14 steps for the longest linear process resulting in overall yield of 6.7%.[4]

Scheme 1



(R)-Carvone 6 7 (85%) 4 (99%)

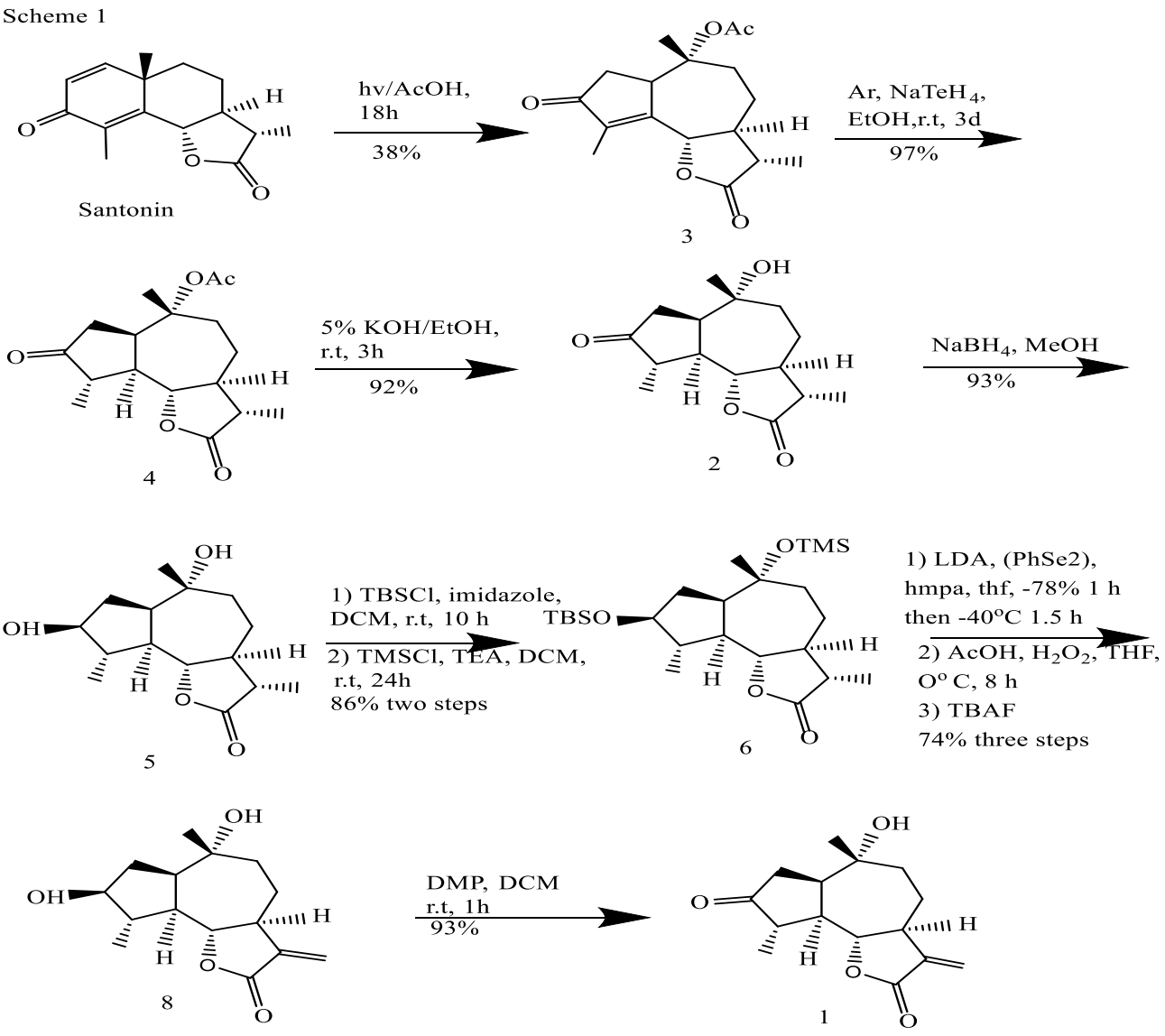
SCHEME 2



2.2 Total synthesis of (+)- Chinensiolide B from α- Santonin

The total synthesis of (+)-Chinensiolide B was reported by Zhang et al. 2017[5]by using a alpha-Santonin. The synthesis started from α- santonin (scheme 1). In this α- santonin undergo photolysis condition described by Barton generated tricyclic lactones **3** then further reduction of enone system in the large excess of $NaTeH_4$ [15]to obtain a trans addition product **4** . Hydrolysis of ester **4** with 5% of aq KOH in ethanol at room temperature generate alcohol **4**, then further treatment with $NaBH_4$ to gave dihydroxyl product **5**. Further to improve the yield of product two different protecting groups used to gave product **6** that is TMSCL and selenylation of **7** with presence of HMPA followed by oxidative elimination of H_2O_2 in AcOH to gave double bond in lactone. Silyl ethers used to removed by treatment with TBAF in THF in solution to give dialcohol in **8** in 3 steps. Finally oxidation of alcohol by Dess Martins Periodine gave (+)- Chinensiolide B. Thus synthesis required 10 steps resulting 18.6% of yield.[5]

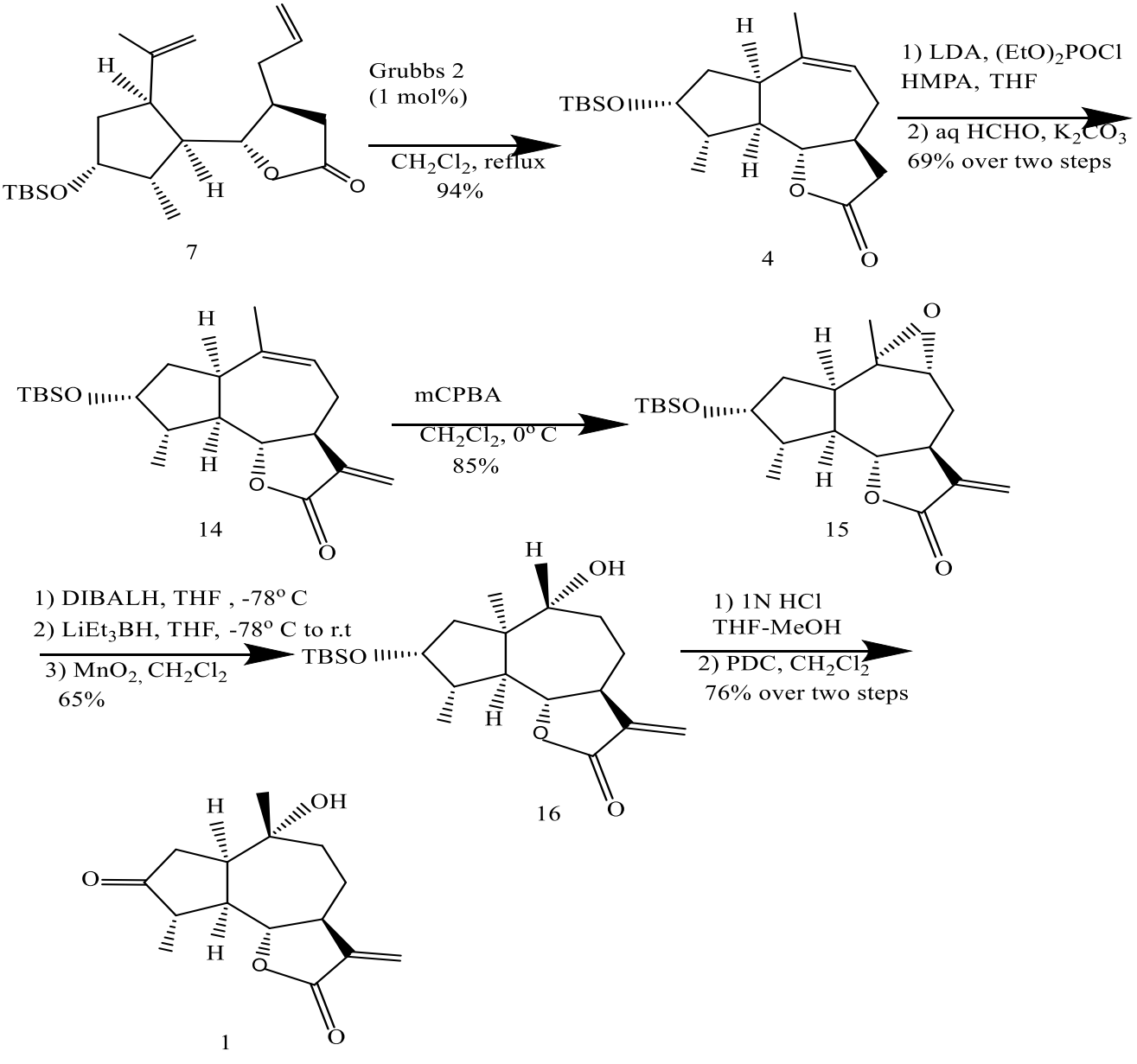
Scheme 1



2.3 Unified Total Synthesis of (+)- Chinensiolide B (2017)

Total synthesis of (+)-Chinensiolide B was reported by Hajra et al. 2017 . The synthesis include the unified total synthesis of (+)-Chinensiolide B and (+)-8-epigrosheimin from a standard precursor **6**, which has been prepared in multi-gram quantity from easily accessible R-(-)-carvone derived aldehyde **1** and succinyl substrate 3b in three steps via highly stereoselective Evans syn-aldol reaction, one-pot chemoselective reduction-lactonization and Swern oxidation respectively. The key reactions to achieve the gram-scale synthesis of Hall's intermediate **14** include chemoselective addition of vinyl Grignard reagent, Pd-catalyzed allylic deacetylation, RCM and α-methylenation.[1]

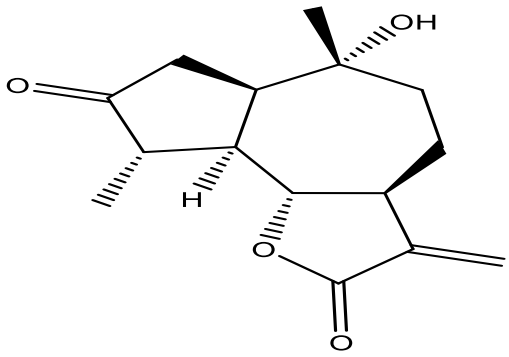
Total synthesis of (+)-Chinensiolide B required **14** steps ranging from **1** and 3b with an overall yield of 11.7%.



3. SPECTROSCOPY DATA OF (+)- CHINENSIOLIDE B

In the three new guaianolides research paper, named (+)- Chinensiolide B were isolated from the whole plant of *Siyekucai (Ixeris chinensis)*. The structures were determined by HREIMS, UV, IR, and one- and two-dimensional NMR techniques (1H and 13C NMR, COSY, HMQC, HMBC, and NOE difference and NOESY experiments). Studies on other species of this genus revealed the presence of sesquiterpene lactones like guaianolides, eudesmanolides, germacra- nolides, and their glycosides. As a part of their studies on bitter substances, they investigated I. chinensis and now report the isolation of three new guaianolides from this plant.

The I. chinensis plant was collected in Qiqihar City, Heilongjiang Province, China, in summer. The meth- anolic extract of the fresh whole plant was defatted by extraction with hexane. The MeOH layer was concentrated, diluted with H2O, and extracted with ethyl acetate. The ethyl acetate extracts were then subjected to separation with flash chromatography followed by HPLC on silica gel to give three new guaiane type R-methylene-γ-lactone. Following are the observation reported.



1

(+) - Chinensiolide B

Chinensiolide B **1** have the composition C15H20O4, which was determined by a combination of HREIMS and 1H and 13C NMR spectra. The IR spectrum of **1** indicated the existence of a hydroxyl group (3612 cm-1), a five-membered ring carbonyl (1744 cm-1), and an R,â-unsaturated γ-lactone (1770 and 1646 cm-1).

The 13C NMR spectrum displayed 15 carbon resonances. Lactone and ketone carbonyl signals were located at δ 169.9 and 219.3, respectively. Two signals of carbons bearing oxygen were observed at δ 85.8 (d) and 73.1 (s). An exocyclic methylene resonance was observed at 119.4 (t).

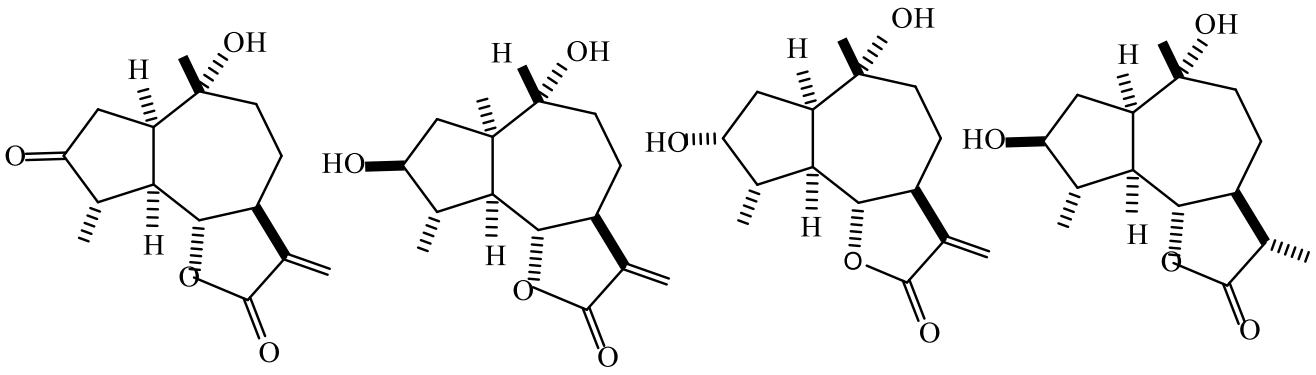
Judging from the DEPT and HMQC spectra, it had been clear that the remaining protonated carbon resonances were due to two methyl carbons, three methylene carbons, and four methine carbons. The 1H NMR showed a singlet methyl, a doublet methyl, an exocyclic methylene, and an oxymethine proton.

In addition to spectral data mentioned above, the 1H-1H correlations of H-4 and H-5 and H-4 and H-15 in the 1H- 1H COSY spectrum suggested that 2 was the 4,15-dihydro derivative of 1. The relationships of the protonated carbons were determined by analysis of the 1H-1H COSY spectrum and the assignments of quaternary and carbonyl carbons, and also the attachment of a lactone ring and a hydroxyl group was determined by analysis of the HMBC spectrum by the analogous discussion for 1. Thus, compound 2 possessed the guaianolide structure 10-hydroxy-3-oxoguaia-11(13)- eno-12,6-lactone. The coupling constant between H-1 and H-5 (J) 8.0 Hz) indicated A,B cis ring fusion, and the coupling constant of H-6 (J5,6) 10.0 Hz and J6,7) 10.0 Hz) indicated the existence of trans-fused γ-lactone. The stereochemistry of C-4 Me and C-10 Me were determined to be the R- and â-configuration, respectively, by the NOESY experiment. Thus, a strong NOESY correlation was observed between H-4 and H-6 and between H-6 and H-14.[2]

4. APPLICATION OF (+)-CHINENSIOLIDE B

4.1 Biotransformation of chinensiolide B and the cytotoxic activities of the transformed products

Biotransformation of (+)-Chinensiolide B by cell cultures of two plants and one fungus, and the cytotoxic activities of the products was reported by Dai et al.[16] in 2017, 10α-hydroxy-1α,5α,15-H-3-oxoguaia-11(13)-en-6α,12-olide (1), yielded three selectively reduced products, 3α,10α-dihydroxy-1α,5α,15α-H-guaia-11(13)-en-6α,12-olide (2), 3α,10α-dihydroxy-1α,5α,15α-H-guaia-11(13)-en-6α,12-olide (3), and 3β,10α-dihydroxy-1α,5α,11β,15α-H-guaia-6α,12-olide (4) by the cell suspension cultures of Catharanthus roseus. 2 and 3 were also obtained from 1 incubated with cell cultures of a fungus Abisidia coerulea IFO 4011 and Platycodon grandiflorum, respectively. Among them, 2, 3 are two new compounds. The three products, 2–4, along with 1 were preliminarily evaluated for their in vitro cytotoxic activity against 3 cell lines (HepG2, WI-38 and VA-13) and all showed potent inhibitory effects on the cell proliferation. Of the four compounds, 3 was the most toxic to the three cell lines tested with IC50 values of 22.7, 0.33 and 3.30 μM, respectively.



Chinensiolide B (1) 2 3 4

The following concluded result of experiment,

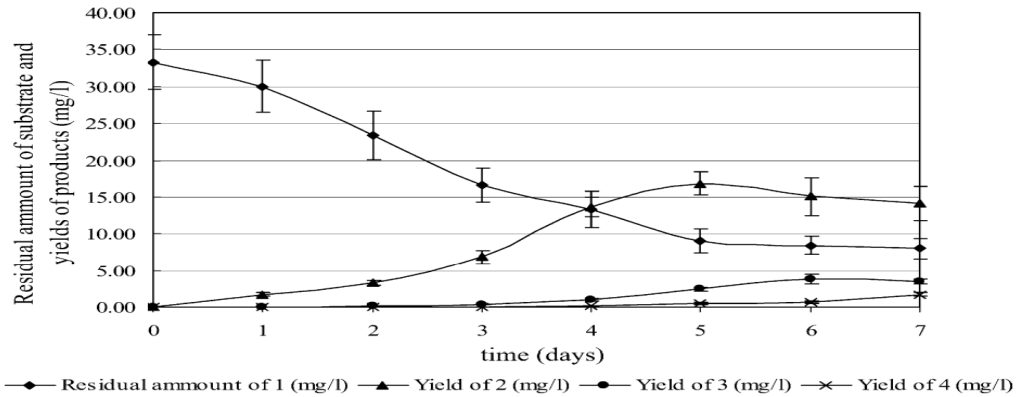


Figure 4.1 :- Time course of the biotransformation of 1 to 2, 3 and 4 by cell suspension cultures of C. roseus[16]

The cytotoxicity of **1** and its metabolites **2**, **3** and **4** in vitro

Compound	IC_{50} in μM ($n = 3$)[a]		
	HepG2	WI-38	VA-13
1	26.5 ± 5.2	0.34 ± 0.05	26.8 ± 3.2
2	23.9 ± 4.8	1.69 ± 0.12	23.4 ± 5.8
3	22.7 ± 5.4	0.33 ± 0.04	3.30 ± 0.15
4	24.2 ± 3.6	1.94 ± 0.13	21.0 ± 4.8
Paclitaxel (control)	8.09 ± 1.2	0.005 ± 0.001	0.040 ± 0.008

[a] The IC_{50} values were the means of triplicates ± standard deviations.

Figure :- 4.2 Cytotoxicity and metabolites and in vitro conclude value. [16]

The experiment conclude that (+)-chinensiolide B can be specifically reduced [C-3 ketone to alcohol, and/or 11(13) methylene to methyl] by the cell suspension cultures of C. roseus, P. grandiflorum and a fungus A. coerulea IFO 4011. Bioassays showed that chinensiolide B and its three metabolites exhibited potent in vitro cytotoxicity against 3 cell lines. The results suggested that biotransformation is a useful approach to diversifying bioactive natural product searching for more bioactive natural compounds and to provide various derivatives for biological evaluation, and for the structure-activity relationship (SAR) studies. In addition, with respect to the large-scale production of the most bioactive products, the best choose will be using cell suspension cultures of P. grandiflorum as the biocatalyst.

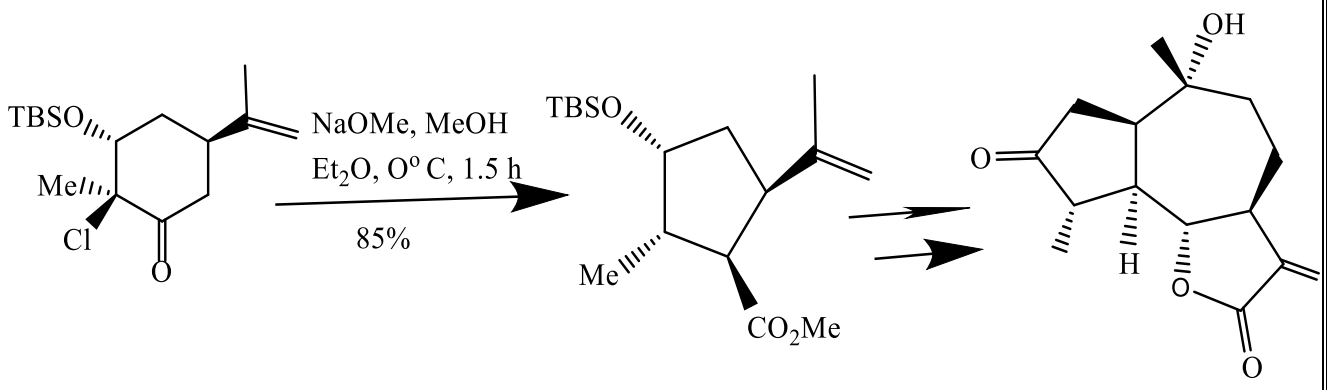
4.2 Ring contraction reaction in the total synthesis of biological active natural products

In 2013 Luiz et al [17] reported a Ring contraction reaction which help in the synthesis of active natural products. Ring contraction reactions can be used in the synthesis of biologically active compounds, including complex natural products. A ring contraction approach opens new applications for the chiral pool and readily available starting materials. Even highly using Favorskii and Wolff rearrangements.

The complex structure of (+)-Chinensiolide B with six continuous streocenters and a double bond in lactones ring make their synthesis a very challenging, thus ring contraction provide a new approach for the chiral pool and readily available starting materials. ring contraction reactions have been used as an efficient tool in the stereoselective synthesis of biologically active natural products. Finally, ring contraction approaches are particularly powerful to prepare cyclopentyl derivatives from six-

membered ring substrates because there are many reliable reactions to obtain six-membered ring compounds. Additionally, this ring is the most available in nature.

The (+)Chinensiolide B synthesis conclude the use of Favorskii rearrangement synthesized from (R)- or (S)-carvone (scheme 1) ,which possesses cytotoxicity against human primary liver cancer and human lung fibroblast cell line.[17]



(+)- Chinensiolide B

CONCLUSION

(+)- Chinensiolide B is the one of the important sesquiterpene which are yet to be explored. It has a amazing medicinal properties which can be benefial for the cancer treatment drugs. (+)-Chinensiolide toxicity and biological properties makes it rare kind of natural products which shows cytotoxic behaviour against human primary liver cancer and two human lung fibroblast. At present the synthesis available are very lengthy and full of protecting reagents as well as longer routes to the final products which need to be change in future for the betterment of the society.

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