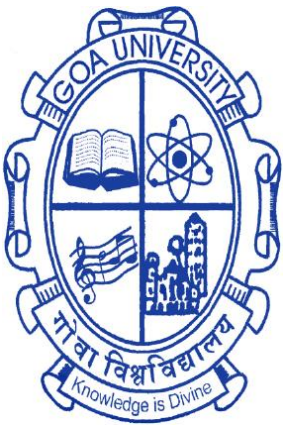
TOTAL SYNTHESIS OF

(-)-LEPENINE

MSc Dissertation by:

MOHIT B. KHANDEPARKAR



SCHOOL OF CHEMICAL SCIENCES
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Total Synthesis Of (-)-Lepenine

DISSERTATION

Submitted in partial fulfilment of
The degree of MSc (Organic Chemistry)

By

Mr. Mohit B Khandeparkar

To

School of chemical sciences
Goa University
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April 2022

CERTIFICATE

This is to certify that the dissertation entitled ***'Total Synthesis of (-)-Lepenine'*** submitted to the School of Chemical Sciences, Goa university in partial fulfilment of the award of the degree of Master of Science in Organic chemistry, is a bonafide literature review done by Mr. Mohit B Khandeparkar, during the year 2021-2022 under the supervision and guidance of Dr. Rupesh Kunkalkar.

Dr. Rupesh Kunkalkar
Project Guide

Dr. Vidyadatta Verenka
Dean
School of Chemical Sciences,
Goa University

DECLARATION

I hereby declare that the matter presented in this dissertation entitled ***'Total Synthesis of (-)-Lepenine'*** was carried out by me during the year 2021-2022 under the guidance of Dr Rupesh Kunkalkar. In keeping with the general practice of reporting scientific observations, due acknowledgements have been made wherever the work described is based on the findings of other investigation.

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ACKNOWLEDGEMENT

It gives me an opportunity to express my views, opinions, and experiences during the course of study. It also brings me great pleasure to express my heartfelt gratitude to Dr. Rupesh Kunkalkar, my mentor for guiding me through the process and improving my knowledge of chemistry. I would want to offer my heartfelt gratitude to Dr. Vidhyadatta Verenkar, Dean of Goa University's School of Chemical Sciences. Last but not least, I would like to thank my family and friends for their continued support and encouragement.

INDEX

Introduction

It is difficult to synthesise architecturally complicated secondary metabolites utilising an iterative approach in which a specific bond formation mechanism (e.g., aldol reaction, crosscoupling, etc.) is prominent. The synthesis approach used for topologically complicated frameworks becomes even more important. Many highly complex, bioactive secondary metabolites co-occur with congeners that have interesting and desirable bioactivity in the producing organism. As a result, unified techniques based on a versatile intermediate are frequently the most efficient way to deal with these topologically complicated, structurally linked molecules..[1,2] Diterpenoid alkaloids are a group of chemicals that are extremely important in this case. These secondary metabolites are extracted from the botanicals *aconite, consolidum*, and *delphinium*, which are used to treat pain and cardiovascular disease in traditional medicine in China. In some cases, it regulates Na + and/or K + ion channels, and it may be subtype specific..[3,4]

The first attempts to synthesise C20 alkaloids resulted in the emergence of atisine, garryine, veatchine, napelline, and nominine. Baran et al. showed that (-)-methyl atisenoate, its alkaloidal equivalent (-)-isoatisine, and the hetidine skeleton may all be approached in the same way. *Aconitum*, a medicinal herb found only in the central and western parts of China which contains several diterpenoid and norditerpenoid alkaloids, including lappaconitine *Aconitum* and *Delphinium* have been used as poisons and medications because to their exceptionally high bioactivities. Figure 1 depicts the Aconitine skeleton, which serves as the backbone for diterpenoids under study.[2,5]

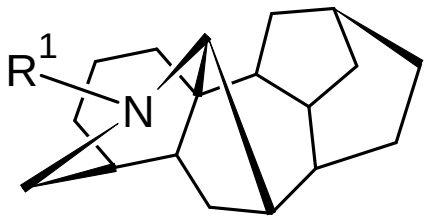


Fig (1) Aconitine Skeleton

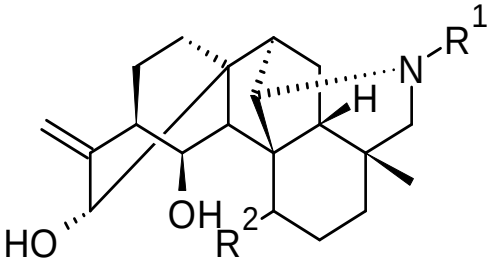


Fig (2) Denudatine and Derivatives

R1=Et , R2=H: Denudatine

R1= Et, R2=OH: Lepenine

R1=Me, R=OH: Stenocarpine

Fukuyama and co-workers were the first to complete a synthesis of (-)-lepenine. Lepenine, which belongs to the denutatine family of diterpenoid alkaloids, was initially isolated from Aconitum Kusnezoffii.[1,6] Because of their wide biological activity and structural complexity, these nitrogen-containing diterpenoids have caught the interest of the synthetic community. Previously documented synthesis procedures either target one diterpenoid alkaloid or multiple biogenetically related natural products within the C20 family; however, given study focused on a strategy that would allow access to C20, C19, and C18 congeners.[1,3,7] *Aconitine*, a known toxin from the same genus for its acute toxicity has been reported; (Oral LD50 LD50 Oral - mouse - 1 mg/k, targeting heart and CNS. While handling proper PPE kit is advised).[8] Chun-Lan Yuan and Xiao-Ling Wang identified the significance of lepenine, which was isolated from the genus Aconitum's *Aconitum sinomontanum*. They discovered that Lepenine had pesticidal that is, antifungal and antibacterial effects against *S. aureus*, *B. mycoides*, and *P. vulgaris*.[2]

A hexacyclic system including tetradecahydrophenanthrene, a polycyclic system having a nitrogen atom, and a bicyclo [2.2.2] skeleton compose the framework of denudatine type alkaloids. There has been no formal complete synthesis of denudatinr type alkaloids. Figure 2 displays the Denudatine skeleton and its synthesised modifications..[9,10]

The schemes that have been reported are as follows:

Scheme 1. Retrosynthetic Analysis

Scheme 2. Construction of the Phenanthrene Skeleton

Scheme 3. Intramolecular Mannich Reaction

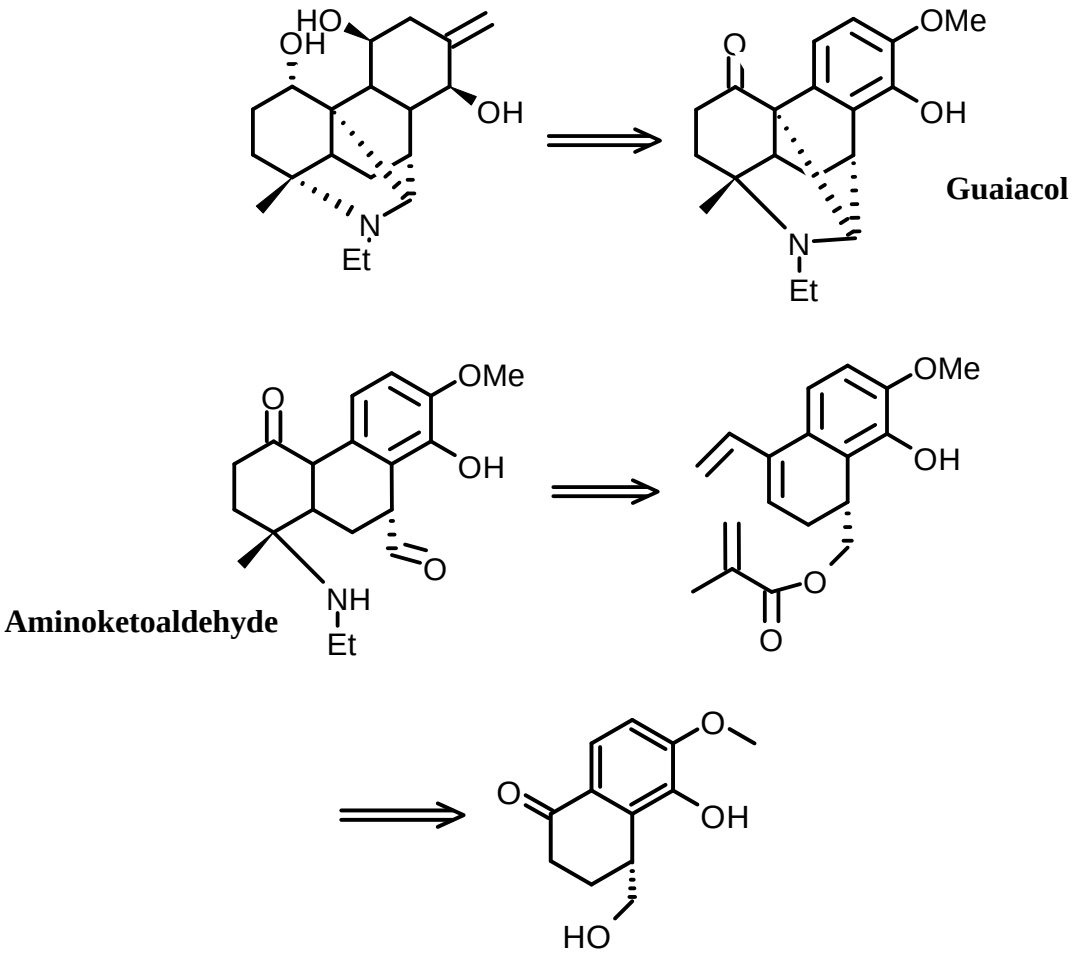
Scheme 4. Construction of the Bicyclo [2.2.2] Skeleton

Scheme 5. Total Synthesis of (-)- Lepenine

Literature Survey

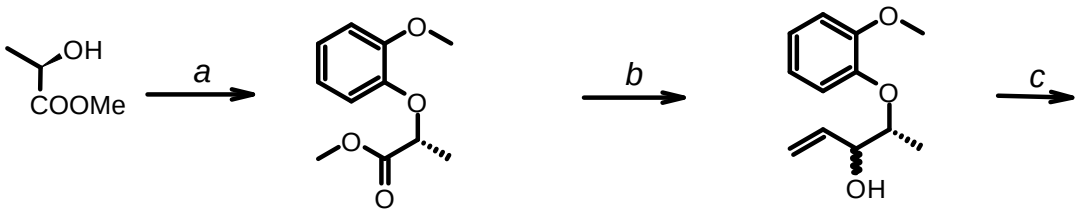
The Total synthesis of (-)-Lepenine is the subject of this study. This was accomplished through the simple asymmetric synthesis of lepenine, the first member of the denudatine-type alkaloids to have complete synthesis. The over 1200 known diterpenoid alkaloids are categorized into C20, C19, and C18 families depending on the number of contiguous carbon atoms comprising the framework.[1,6]

Scheme 1: The retrosynthetic approach has been examined as follows

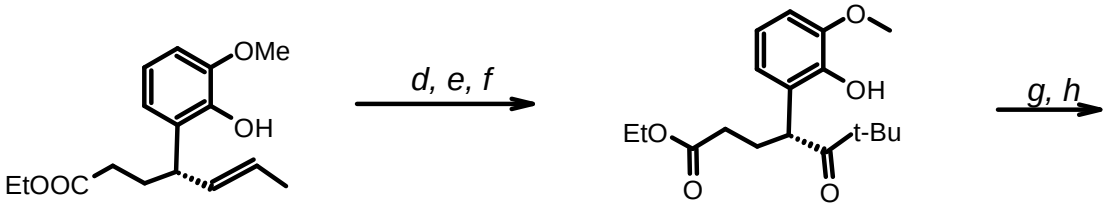


Construction of bicyclo [2.2.2] skeleton could be achieved from Guaiacol by oxidative dearomatization followed by a Diels−Alder reaction. Which could be indeed constructed via intramolecular Mannich reaction of Aminoketoaldehyde.[6]

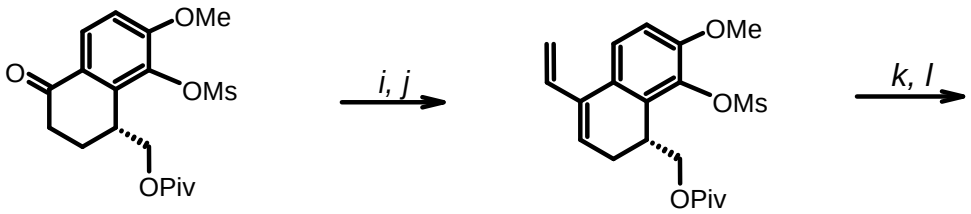
<u>Scheme 2</u>: Construction of the Phenanthrene Skeleton



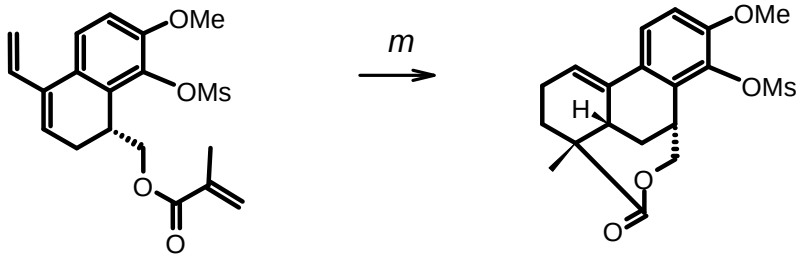
(1) (2) (3)



(4) (5)



(6) (7)



(8) (9)

Reagents and conditions:

(a) Guaiacol, Ph_3P, DEAD, toluene, 0 °C, 87%, >99% ee; (b) i-Bu_2AlH, Et_2O, hexane, −78 to −40 °C; vinylmagnesium chloride, THF, −40 to 0 °C, 94% (1:1.6 mixture); (c) 4-$O_2NC_6H_4OH$ (5 mol %), $(EtO)_3CMe$, reflux, 9 d, 85%;

(d) MsCl, Et_3N, DCM ,0°C, 85%; (e) O_3, DCM, MeOH, −78 °C; NaBH4, −78 to 0 °C, 86%; (f) PivCl, pyridine, DMAP, DCM, rt, 80%, 91% ee;

(g) aq LiOH, THF, MeOH, 0 °C; (h) TFAA, TFA, DCM, rt, 82% (two steps); (i) vinylmagnesium chloride, THF, −40 °C, 85%;

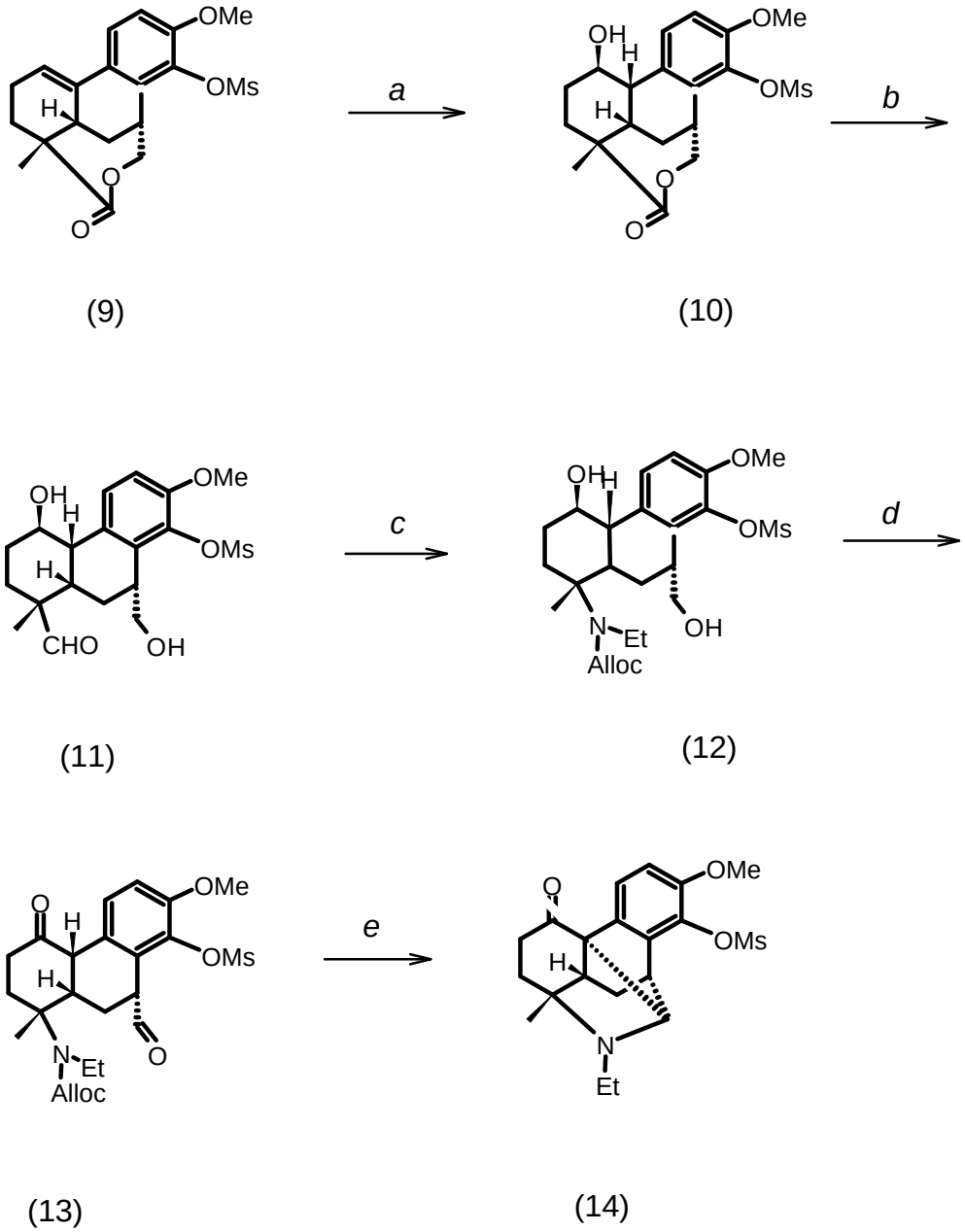
(j) AgOTf (5 mol %), toluene (20 mM), reflux, 1 h, 63%; (k) i-Bu_2AlH, hexane, DCM,0°C, 89%; (l) methacrylic acid, DCC, DMAP, DCM, rt, 85%;

(m) BHT, PhCN (20 mM), 160 °C, 6 h, 90%; (n) crystallization from $CHCl_3$/hexane (1:2), 84%.[6,9]

Claisen rearrangements of (3) resulted in phenol (4). The protection of phenol with a mesyl group, followed by oxidative cleavage of the double bond and reduction with NaBH4, produced primary alcohol that was preserved as its pivalate to furnish (5). After hydrolysis of the ethyl ester moiety with LiOH, the resultant carboxylic acid was subjected to an intramolecular Friedel−Crafts reaction to afford tetralone (6). Intermolecular Diels−Alder reaction.of Ketone gave diene (7) in a two-step procedure involving addition of vinylmagnesium chloride followed dehydration of the resulting tertiary alcohol. The pivaloyl group of was then removed and replaced with a methacryloyl group in (8). The critical intramolecular DielsAlder reaction proceeded easily after heating (8) at 160 °C in benzonitrile with radical scavenger, yielding tetracyclic lactone (9) in 90% yields.. At this stage, crystallization of lactone (9) from chloroform/hexane gave enantiomerically pure material in sufficient yield.[9]

The optical purity of (5) was reported to be 91 % ee, implying that L-lactic acid's chirality was efficiently transmitted during the Claisen rearrangement.[11,6]

<u>Scheme 3</u>: Intramolecular Mannich Reaction

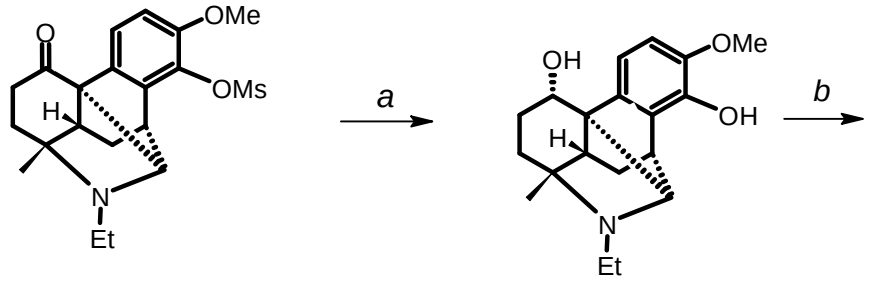


Reagents and conditions:

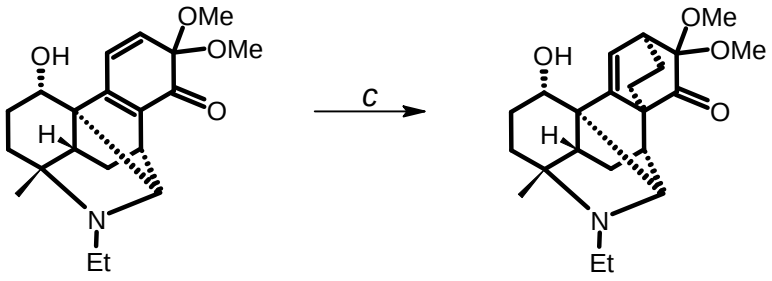
(a) BH_3·THF, THF, rt; MeOH, 0 °C; aq NaOH, aq H2O2, 97%; (b) i-Bu_2AlH, hexane, CH_2Cl_2, −40 °C, 97%; (c) $EtNH_2$·HCl, Et_3N, AcOH, MeCN, rt; $NaBH(OAc)_3$; aq NaOH, 0 °C; AllocCl, 93%; (d) Dess−Martin periodinane, CH_2Cl_2, rt, 79%; (e) $Pd(PPh_3)_4$, AcOH, CH_2Cl_2, reflux, 75%.[6]

Hydroboration of (9) gave (10), having secondary alcohol. Subsequent reduction of seven-membered lactone with diisobutylaluminum hydride yielded aldehyde (11). The aldehyde was subjected to reductive amination with ethylamine to give a secondary amine, which was masked with an Alloc group. Oxidation of the resultant diol (12) with Dess−Martin periodinane gave ketoaldehyde (13). Interestingly, after treatment with acetic acid and a palladium catalyst, (13) underwent smooth deprotection of the Alloc group and the intramolecular Mannich reaction, affording (14), a polycyclic system containing a nitrogen atom,[9,12,6] next task is establishing bicyclo [2.2.2] skeleton.

Scheme 4: Construction of the Bicyclo [2.2.2] Skeleton



(14) (15)



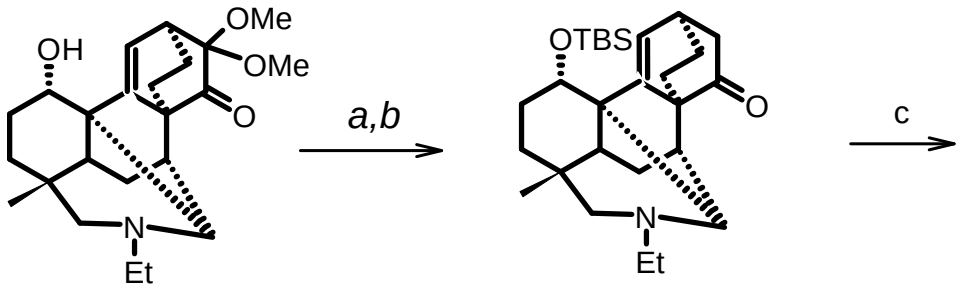
(16) (17)

Reagents and conditions:

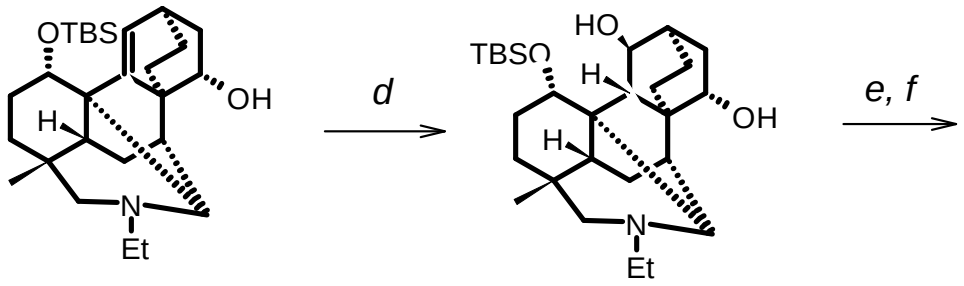
(a)KOH, MeOH, 60°C, 3h; $NaBH_4$,0 °C, 95%; (b) methyl red, AcCl, MeOH, rt; $PhI(OAc)_2$,0°C, 88%; (c) ethylene (70 bar), DCM, 70°C, 5 d, 84%.

To produce phenol, the mesyl group in (14) was removed with potassium hydroxide and the ketone moiety was reduced with sodium borohydride in a single pot in methanol (15). The intermediate orthoquinone monoketal (16) was obtained by treating (15) with methanolic hydrogen chloride and then oxidising it with iodobenzene diacetate. Upon heating (16) under an ethylene atmosphere, the desired Diels−Alder reaction proceeded smoothly to give (17) with complete control of stereochemistry.[6,9]

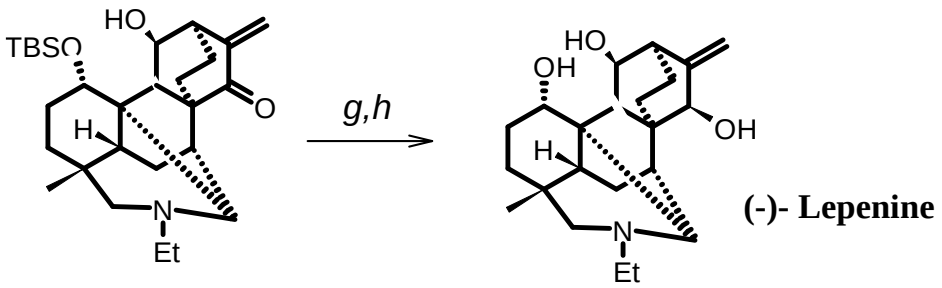
Scheme 5: Total Synthesis of (-) –Lepenine



(17) (18)



(19) (20)



(-)- Lepenine

(21) (22)

Reagents and conditions:

(a) TBSOTf, 2,6-lutidine, DCM, rt, 91%; (b) SmI_2, MeOH, THF, 0 °C, 96%;

(c) Red-Al, toluene, 0 °C, 88%; (d) BH_3·THF, THF, rt; H_2O, 0 °C; $NaBO_3$·H_2O, 0 °C to rt, 54%; (e) Dess−Martin periodinane, TFA, DCM, rt, 72%;

(f) HCO_2Et, KHMDS, toluene, 70 °C; aq HCHO, THF, 50 °C, 70%; (g) $NaBH_4$, $CeCl_3$·$7H_2O$, MeOH, 0 °C, 83%; (h) TBAF, THF, 65 °C, 93%.[6,9]

Protection of the hydroxy group of (17) with a TBS group, followed by reductive removal of the two methoxy groups at the α-position of the ketone using samarium(II) iodide, furnished (18). The ketone in (18) was stereoselectively reduced with Red-Al to produce (19), which was then exposed to a hydroboration-oxidation sequence to produce diol (20). The two hydroxy groups in (20) were clearly distinguished by oxidation with Dess-Martin periodinane in the presence of trifluoroacetic acid, giving hydroxyketone Since direct α-methylenation of the ketone met with only limited success, a formyl group was introduced at the α-position of the ketone. Thus, treatment with KHMDS and ethyl formate, followed by subsequent formalin addition, gave (21) in 70% yield. Luche reduction of enone and removal of the TBS group resulted the desired (-)-lepenine (22), which was indistinguishable from natural Lepenine in all respects..[6,13,14]

Experimental evidence and Characterization:

Nuclear magnetic resonance (1H NMR (400 MHz), 13C NMR (100 MHz)) spectra were determined on a JEOL-ECS400 instrument. Chemical shifts for 1H NMR are reported in parts per million (ppm) downfield from tetramethylsilane (δ) as the internal standard and coupling constants are in hertz (Hz). (-)-Lepenine was obtained as colourless prisms (Me_2CO-n-hexane): mp 120-122 °C, IR ν max (KBr) cm-^{1}.[4]

The 1H-NMR, 13C-NMR, Infra-Red (IR), High-resolution mass spectrometry (HRMS), and optical activity results for the final (-)-Lepenine molecule are shown below. Figure (3) and (4) represents H^1 and ^{13}C NMR spectra, respectively.[4]

^{1}H NMR (CDCl3): 5.23 (s, 1H), 5.03 (s, 1H), 4.45 (d, J = 8.7 Hz, 1H), 4.29 (d, J = 6.8 Hz, 1H), 4.15 (dd, J = 11.0, 6.4 Hz, 1H), 3.67 (s, 1H), 2.70 (dd, J = 13.3, 7.8 Hz, 1H), 2.58-2.46 (m, 2H), 2.44-2.28 (m, 2H), 2.24 (d, J = 11.4 Hz, 1H), 2.22-2.16 (m, 2H), 1.93 (ddd, J = 13.8, 11.4, 6.9 Hz, 1H), 1.79 (m, 1H), 1.77 (m, 1H), 1.74 (m, 1H), 1.71 (m, 1H), 1.67 (m, 1H), 1.58 (m, 1H), 1.47 (m, 1H), 1.36-1.18 (m, 4H), 1.12 (m, 1H), 1.04 (t, J = 7.1 Hz, 3H), 0.70 (s, 3H).

^{13}C NMR (CDCl3): 154.5 (C), 109.3 (CH2), 78.0 (CH), 73.2 (CH), 71.0 (CH), 67.6 (CH), 57.1 (CH2), 54.2 (CH), 52.4 (CH), 50.9 (C), 50.7 (CH2), 46.7 (CH), 43.7 (C), 42.2 (CH), 38.6 (CH2), 33.7 (C), 31.3 (CH2), 27.4 (CH2), 25.9 (CH3), 24.5 (CH2), 23.1 (CH2), 13.7 (CH3).

IR: 3368, 2929, 2871, 1455, 1375, 1292, 1173, 1094, 1072, 1050, 1031, 906. **HRMS**: 382.2355 (Calculated for $C_{22}H_{33}NNaO_3$ +: 382.2353**). $[\alpha]_D^{23}$** = –18° C(0.15, CHCl3).

FIG (3) H^1-NMR Spectra

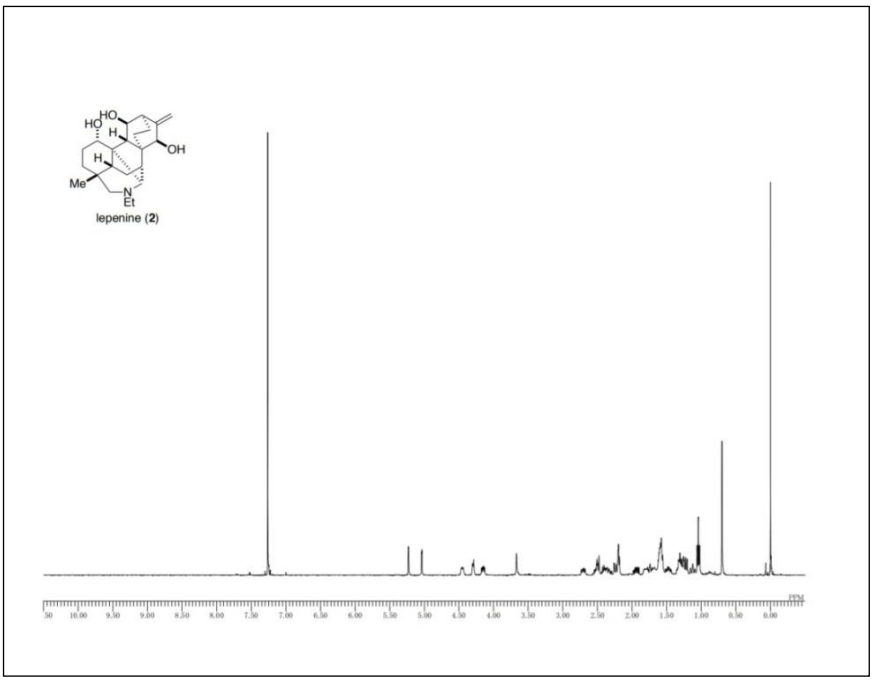


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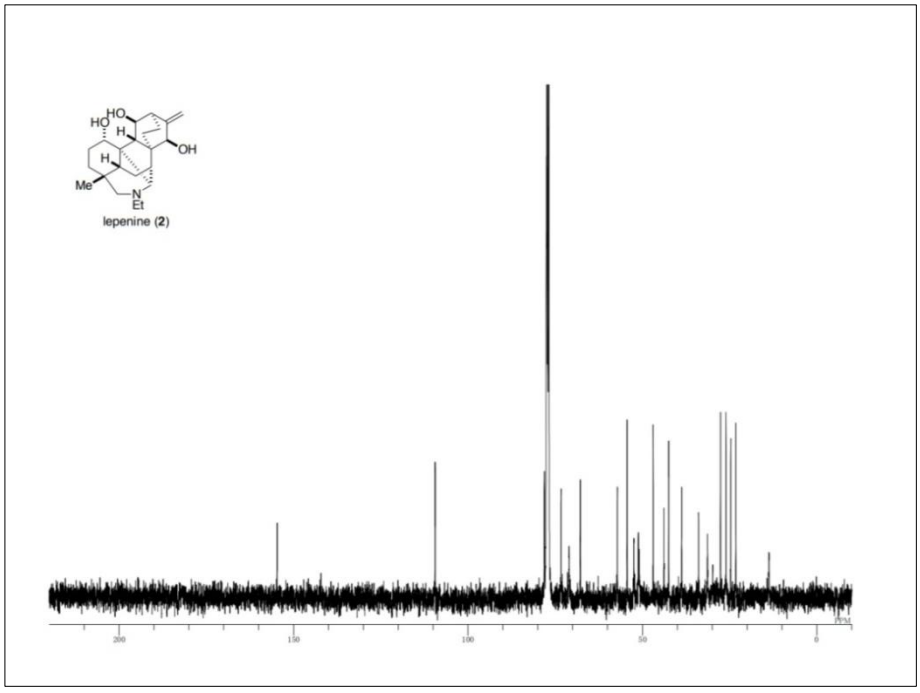
FIG (4) ^{13}C-NMR Spectra



Key features of the synthesis-

1. Synthesis of tetralone core.
2. Chirality transfer from L-lactic acid methyl ester via Claisen rearrangement.
3. Construction of hexacyclic ring system via tethered intramolecular Diels-Alder reaction, Mannich reaction and Diels-Alder reaction between an ortho-quinone and ethylene.[9]

Conclusion

To summarise, Fukuyama's synthesis of (-)-lepenine includes an intramolecular Mannich reaction to generate the essential five-membered ring. The synthesis begins with a Mitsunobu reaction with an enantiopure lactic acid derivative and guaiacol to produce a product with complete stereochemistry inversion.[14] The complex hexacyclic system is effectively constructed via a tethered intramolecular Diels-Alder reaction, an intramolecular Mannich reaction, and a Diels-Alder reaction between an ortho-quinone monoketal and ethylene.-lepenine, the first member of the denudatine-type alkaloids to succumb to total synthesis.

The transfer of chirality from L-lactic acid methyl ester via a Claisen rearrangement is another crucial part of the process..[6,1]

The characterization was done using H^1-NMR, ^{13}C-NMR and Infra Red, Mass spectrometry and polarimetric techniques which confirmed the formation of (-)-Lepenine in all aspects.[4]

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