Synthesis of tribulusterine, a potent toxic alkaloid from Tribulus terrestris

Waya Sengpracha
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Synthesis of Tribulusterine, A Potent Toxic Alkaloid from *Tribulus terrestris*

A thesis submitted in fulfilment of the requirements for the award of the degree

Honours Master of Science

From

University of Wollongong

By

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February 2001
Declaration

I, Waya Sengpracha, declare that this thesis, submitted in fulfilment of the requirements for the award of Honours Master of Science, in the Department of Chemistry, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The work presented in this thesis has not been submitted for the award of any other degree or diploma in any university.

Waya Sengpracha

23 February 2001

Synthesis of Tribulusterine, A Potent Toxic Alkaloid from Tribulus terrestris.
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Synthesis of Tribulusterine, A Potent Toxic Alkaloid from Tribulus terrestris.
Abstract

Synthetic approaches to tribulusterine (15), a suspected toxic alkaloid from *Tribulus terrestris*, have been investigated via nucleophilic and electrophilic substitution reactions, and the Pictet-Spengler cyclisation reaction. Nucleophilic substitution reactions of the 9-(N,N-dimethylsulfamoyl)-β-carboline-N-oxide (50) with the furyllithium (46a) yielded the new 1-substituted β-carboline, 1-(3-furyl)methoxy-9-(N,N-dimethylsulfamoyl)-β-carboline (52). Bromination of the β-carboline derivative (51) afforded 1,3,6,8-tetrabromo-β-carboline and 3,6,8-tribromo-β-carboline as major products when the lithiated β-carboline (51) was treated with N-bromosuccinimide (NBS) and bromine, respectively. The Pictet-Spengler reaction approach may have yielded the required alkaloid, tribulusterine (15), based on some spectroscopic evidence. The new furan derivative, 3-(hydroxymethyl)-2-furaldehyde (66), required for the Pictet-Spengler approach, was synthesised via a lithiation-mediated procedure. A precursor (33) for a palladium-catalysed Negishi-type cross-coupling approach was also prepared.
Chapter 1: Introduction

1.1 General Introduction

Many higher plants are of interest as sources of industrial and medicinal materials since they produce useful organic compounds called natural products. Many of the drugs currently in use are plant-derived or they are produced from natural products. Natural products are often classified as primary and secondary metabolites. Primary metabolites are substances that are distributed in all organisms. These compounds are vital for living organisms as they have a primary function in life processes of the organism. For example, carbohydrates, amino acids and fatty acids are synthesised through the primary metabolic pathways. On the other hand, secondary metabolites are substances that have no primary function in life processes. Secondary metabolites are unique to a particular species and are often of relatively limited occurrence since these compounds provide defence against micro-organisms, insects, and higher predators in order to enhance the survival of the next generation.

As a result, secondary metabolites are often biologically active compounds. Some biologically active secondary metabolites are used as drug entities or as model compounds for drug syntheses and semi-synthesis. In 1985, 119 secondary metabolites from higher plants were used as drugs and many of them are currently used. Alkaloids are one of the major recognised classes of secondary metabolites and usually show significant biological activity.
1.2 Alkaloids as Secondary Metabolites

Alkaloids can be defined as nitrogenous bases, often cyclic, which are derived from either animals or plants. In addition, alkaloids are often quite complex in structure and usually show specific pharmacological activity. A particular alkaloid type is usually restricted to certain genera and families of the plant kingdom. Biosynthetically, the alkaloids are derived from amino acids. At present, more than 10,000 different alkaloids have been isolated from more than 300 plant families and many species have still not been examined. Alkaloids have been isolated from vascular cryptograms, for example Equisetum and Lycopodium, and from monocotyledons and dicotyledons. In addition, the ergot alkaloids are produced by a fungus. Alkaloids can be isolated from roots, seeds, leaves or bark of a mature plant. Sometimes, more than one alkaloid can be found from one organ. The main components are frequently accompanied by small-quantities of a number of biogenetically related compounds. The same species or genus may contain different alkaloids, but often related in structure, or they may not contain alkaloids. Secondary metabolites, like alkaloids have been studied for many years because of their physiological and psychological effects on humans and other animals. Medicinal plants used as traditional medicines have been examined for bio-active compounds for drug development. For example, morphine was the first alkaloid to be isolated and was crystallised from an extract of the opium poppy. Quinine, an antimalarial drug, is an alkaloid found in cinchona bark. Even though some alkaloids can be useful to some organisms as defensive compounds against many predators, alkaloids are fatal to some organisms. Pyrrolizidine alkaloids exemplify this role in mammals and insects. Some butterflies, such as Daniad butterflies, use pyrrolizidine alkaloids as materials for

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for pheromone production, however these alkaloids cause death in livestock which ingest the plants producing them.

Alkaloids have been subdivided into classes, such as indole, isoquinoline, or quinoline alkaloids. The classification of alkaloids is based on their nitrogen-containing structural features, which in turn are normally amino-acid based. Indole alkaloids have been of interest not only to the chemist, but also to pharmacologists, physiologists, and physicians because of their physiological properties. Hesse reported in 1981 that there were about 1400 indole alkaloids known. These included simple indole derivatives and complex indole alkaloids. The simple indole derivatives are comprised of a pyrrole ring fused with a benzene ring such as in tryptamine (1) itself, and in bufotenine (2) (5-hydroxydimethyltryptamine). The complex indole alkaloids, apart from simple indole bases, contain a fused benzene or pyridine ring, such as carbazole (3), β-carboline (4), and γ-carboline (5) (Fig. 1). Both simple indole alkaloids and alkaloids which have a carbazole and β-carboline nucleus, are derived biogenetically from tryptamine (1), which is derived in turn from the essential amino acid L-tryptophan (6) (Fig. 2).

![Fig. 1 Structures of indole derivatives](image-url)
There has been a tremendous number of alkaloids derived from the amino acid tryptophan isolated during the past twenty years. Moreover, this group of compounds has proved to be a prolific source of biologically important compounds (e.g. vinblastine\textsuperscript{9}, an anti-tumour agent).

![Fig. 2 Structure of the amino acid L-tryptophan (6)](image)

1.3 Toxicity of a Medicinal Plant: Tribulus terrestris

The use of medicinal plants has become an important part of daily life in many countries. The World Health Organisation (WHO) reported that about 80\% of people living in developing countries use medicinal plants as traditional medicines. In addition, the developed countries use medicinal plants as a significant source\textsuperscript{10} in modern chemical and pharmaceutical research. Increasing knowledge of metabolic processes, and the effects of plants metabolites on human physiology, has broadened the application of medicinal plants. The result is a significant increase in the use of plant-based medicines all over the world.

However, limited knowledge regarding the proper usage of medicinal herbs, such as dosage, frequency, and usage period, physical condition and sensitivity of the user and possible interaction with any prescribed drugs, may lead to adverse effects of the medicinal herbs. Some medicinal plants that are widely available should not be taken internally because the safety of their prolonged use is in question\textsuperscript{11}. Others are

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very poisonous. The toxicity does not only refer to lethal effects but also to minor body reactions such as, allergy, irritation and sensitivity. Therefore, emphasis should be placed upon preventing children and livestock from ingesting them. One of the medicinal plants that contain potent toxic alkaloids is *Tribulus terrestris* L., commonly known as ‘Puncture vine’ (Plates 1-4).

Plate 1ª: *Tribulus terrestris*  
Plate 2ª: *T. terrestris*  
Plate 3ª: Small pinnate leaves  
Plate 4ª: large spined fruits

ª from www.wa.gov/agr/weedboard/weed_info/weed_photos/puncturevine.html  
ª from www.agf.gov.bc.ca/croplive/cropprot/weedguid/puncture.htm

_Synthesis of Tribulusterine, A Potent Toxic Alkaloid from Tribulus terrestris._
T. terrestris L., from the plant family Zygophyllaceae, is a weed with long stems, small pinnate leaves, and small yellow and large spined fruits. It is widely distributed in the arid and semiarid zones of the Middle East, Africa, Asia Minor and Australia. In traditional Chinese medicine, the fruit of T. terrestris, which is known as ‘Ci Ji Li’ has been used for the treatment of eye trouble, edema, and abdominal distention, emission, and morbid leucorrhea, and also vitiligo. Other properties, such as antimicrobial, antihypertensive, diuretic, anticholinergic, and haemolytic activity and, stimulation of spermatogenesis, have also been cited. The investigation of T. terrestris by a number of workers has recorded the presence of steroidal glycosides, steroidal saponins, flavonoids, and alkaloids. Also lignanamides and cinnamic amide were isolated from this plant. The testing of a saponin mixture from the plant in vivo caused a significant decrease in peristaltic movements of isolated sheep ureter and rabbit jejunum preparations in a dose-dependent manner (p<0.05). These results imply that the saponin mixture of T. terrestris may be useful to treat some smooth muscle spasms or colic pains. The flavonoids, quercetin and kaempferol, were found to possess antibacterial activity after testing against different bacterial and fungal strains. New hepatoprotective lignanamides, tribulusamide A and B from fruits of T. terrestris have shown cytoprotective activity in murine hepatocyte culture. A diethyl ether extract of T. terrestris has been reported to affect the action of juvenile hormone after testing on the penultimate instar of Dysdercus cingulatus. Increased doses of extracts of T. terrestris resulted in increased mortality and the development of adults with crumpled wings.

In Australia, ingestion of T. terrestris by sheep has resulted in outbreaks of locomotor effects. Using thin layer chromatography (TLC) and high performance
liquid chromatography (HPLC), at least five alkaloids were isolated from the plant and all appeared to be β-carboline derivatives. The β-carbolines, harman and norharman were identified as the main alkaloids contained in Australian *T. terrestris*\(^{31}\). Synthetic harman and norharman were administered subcutaneously to sheep and caused limb paresis similar to naturally occurring cases of *Tribulus terrestris* staggers.

As alkaloids are one of the major constituents in *T. terrestris*, and β-carboline alkaloids may be responsible for the pharmacological effects of this plant, the further study of β-carbolines was of interest since they are found in marine and in terrestrial natural products, and show a range of pharmacological activity\(^{28}\). Therefore, their biosynthesis and total synthesis are of interest.

### 1.4 β-Carboline alkaloids in higher plants

β-Carbolines are a class of indole alkaloids which are structurally similar, and are biosynthetically derived from the amino acid L-tryptophan. The pyridine nitrogen is basic, while the indole NH is acidic. Although, many of the β-carboline alkaloids have recently been isolated from marine organisms, the isolation and identification of the simple β-carboline alkaloids are well known from terrestrial plants.

The simple β-carboline derivatives are found in different oxidation state. Many examples have additional carbon or oxygen substituents on the β-carboline nucleus. The structures of some β-carboline alkaloids are shown in Fig. 3.
The most commonly used nomenclature of β-carboline alkaloids is based on the root “harm” from *Peganum harmala* L., the plant that was first shown to contain β-carbolines. Therefore, the β-carboline alkaloids are called the harmala alkaloids. In addition, β-carbolines have been found in various plant families including the Leguminoseae, Malpighiaceae, and Rubiaceae. The seeds of *Peganum harmala* L. (Rutaceae) contained harman (7), harmine (8) and harmaline (9), which have a paralysing action on the skeletal and cardiac muscles and the seeds have been used as a tapeworm remedy\(^6\). Also, Harman (7) and norharman (β-carboline) have been isolated from tobacco smoke. Eleagnine (10) has been isolated from *Elagnus angustifolia* and, like harmine, it was used at one time therapeutically against tremors in Parkinson’s disease.

The search for new β-carbolines is continuing. For example, 1-(5-hydroxymethyl-2-furyl)-9H-pyrido[3,4-b]indole (11) (Fig. 4) was isolated from Perenial Rye-Grass (*Lolium perenne* L.)\(^{29}\), *Lolium chuanxiong* (Gramineae), Korean ginseng and Japanese soy sauce\(^{30}\). However, the biological activity of this compound has not been reported. Recently, 4,7-dimethoxy-1-vinyl-β-carboline (12) and a dimeric alkaloid biogenetically related to compound (12) has been isolated from stem and root bark of *Perriera madagascariensis* \(^{31}\). The stem bark of this plant is used in indigenous
medicine as a bitter tonic and febrifuge, while the roots are reported to be toxic. The 4-oxygenated-\(\beta\)-carbolines (13) are a large subfamily of \(\beta\)-carboline alkaloids isolated from plants in the family Simaroubaceae. Many of these alkaloids possess an oxygen substituent (a hydroxy or a methoxy group) at the C-8 position. Some of these compounds also show interesting biological activity\(^{32}\). An alkaloid isolated from Picrasma quassioides (Simaroubaceae) was identified as the 3-carboxy-\(\beta\)-carboline (14). This alkaloid showed potent interactions with the benzodiazepine receptor of the central nervous system\(^{33-34}\). Tribulusterine (15, Fig 5), an isomer of (11), has been isolated\(^{14}\) in very low yield from Tribulus terrestris, but the biological activity has not been reported yet. However, it is a suspected toxic agent.

Fig. 4 Structures of some recent \(\beta\)-carboline derivatives found in higher plants.

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Three new alkaloids: (-)-isocyclocapitelline (16), (+)-cyclocapitelline (17), and isochrysotricine (18) (Fig. 6) were isolated from *Hedyotis capitellata* (Rubiaceae)<sup>35</sup>, and also two known alkaloids, namely capitelline (19) and chrysotricine (20), were described. Since plants of this genus *Hedyotis* have been widely used in traditional Chinese and Vietnamese medicine, especially for the treatment of inflammations of the stomach, tongue and throat, a study on the bioactive compounds in *H. capitellata* var. *mollis* has been undertaken. This study resulted in the new β-carboline alkaloids, heydyocapitelline (21) and hedyocapitine (22)<sup>36</sup>, being identified.

The β-carboline alkaloid, vulcanine (1-(2-methyl-1-propenyl)-β-carboline (23) has been isolated from *Haplophylum vulcanium* in Turkey, and is the first report of a β-carboline from this genus<sup>37</sup>.

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*Synthesis of Tribulusterine, A Potent Toxic Alkaloid from Tribulus terrestris.*
(16) (-)-isocyclocapitelline; R = α-CMe₂OH
(17) (+)-cyclocapitelline; R = β-CMe₂OH
(18) isochrysotricine, R = α-CMe₂OH
(20) chrysotricine, R = β-CMe₂OH
(19) capitelline

Fig. 6 Structures of alkaloids from *Hedyotis capitellata*

(21) hedyocapitelline
(22) hedyocapitine

Vulcanine (23)

Fig. 7 Structures of new β-carboline alkaloids

*Synthesis of Tribulusterine, A Potent Toxic Alkaloid from Tribulus terrestris.*
1.5 Biosynthesis of β-carboline alkaloids

The β-carbolines are among the simplest of indole alkaloids and are distributed among a large number of genera. To date, few reported studies have investigated β-carboline alkaloid biosyntheses. The early study of β-carboline biosynthesis in 1991 by Perkin and Robinson\(^3\) proposed that β-carboline was derived by the Mannich condensation of tryptophan or tryptamine with an aldehyde (e.g. acetaldehyde), and was suggested on the basis of the efficiency of this reaction *in vivo*. This has been substantiated by the incorporation of the \(^{14}\)C into C-4 of eleagnine (10) in *Eleagus angustifolia*\(^3\), on administration of DL-[3'-\(^{14}\)C]tryptophan. Tryptophan, which has been decarboxylated to tryptamine, has also been found to be incorporated in harman (7) in *Passiflora edulis*\(^4\). This study proposed that *N*-acetylation of tryptamine by acetyl CoA resulted in the formation of an *N*-acetyltryptamine intermediate (24) (Fig. 8). However, *N*-acetyltryptamine was found neither to be an intermediate for eleagnine (10) in *E. angustifolia* nor a constituent of this plant\(^4\). However, Stolle and Gröger\(^5\) concluded in their study of the biosynthesis of harman that the β-carboline ring arises by the condensation of tryptamine with pyruvic acid to give 1-methyl-1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid (25) (Fig. 9). Recently, compound (25) was also shown to be a natural precursor in the biosynthesis of harman in *P. edulis* and for eleagnine in *E. angustifolia*\(^6\). It was found to be an eight-fold better precursor than *N*-acetyltryptamine. Therefore, it appears that the pathway of biosynthesis of β-carboline alkaloids involves tryptophan, tryptamine, and then 1-methyl-1,2,3,4-β-carboline-1-carboxylic acid.
Fig. 8 Structure of $N$-acetyltryptamine intermediate

(1) Tryptamine, $R = H$
(6) Tryptophan, $R = COOH$

(10) Eleagnine

(25)

Fig. 9 Pyruvate mechanism for the $\beta$-carbolines

(7) Harman

Synthesis of Tribulusterine, A Potent Toxic Alkaloid from Tribulus terrestris.
1.6 Total synthesis of \(\beta\)-carboline alkaloids

Numerous naturally occurring alkaloids embodying the \(\beta\)-carboline nucleus mediate pharmacologically useful physiological effects. Thus, the synthesis of these natural products as well as analogues is of widespread interest to both organic synthesis and medicinal chemistry. The classical synthetic methods for the \(\beta\)-carboline ring are the cyclisation of tryptamines via Bischler-Napieralski and Pictet-Spengler reactions. The resulting products are then aromatised to give the \(\beta\)-carboline framework.

Using the Pictet-Spengler condensation, tryptamines are reacted with aldehydes either in aqueous acid or in aprotic acid conditions in the presence or absence of acids to yield 1,2,3,4-tetrahydro-\(\beta\)-carbolines (26), which are then oxidized to the \(\beta\)-carbolines (Scheme 1).

Scheme 1

Using Bischler-Napieralski cyclisation, amides of tryptamines are cyclised to form dihydro-\(\beta\)-carboline (Scheme 2) in the presence of phosphorus oxychloride or

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phosphorus pentoxide. The dihydro-$\beta$-carboline (27) may then be either oxidised to give the $\beta$-carboline or reduced to form 1,2,3,4-tetrahydro-$\beta$-carboline (26).

However, both the Pictet-Spengler and Bischler-Napieralski condensation methods suffer from lack of convergence, since the R group had been introduced into the molecule in the first step of the reaction. Additionally, 1-substituted-$\beta$-carbolines show a large class of biological activity.$^{44}$

Scheme 2

$\beta$-carbolines substituted in the 1-position can be prepared through either 1-substituted-1,2,3,4-tetrahydro-$\beta$-carboline derivatives or $\beta$-carboline derivatives. However, there are few examples in which the $\beta$-carboline moiety is directly derivatised by an addition reaction of nucleophiles to give 1-substituted-$\beta$-carbolines. Itoh et al.$^{45}$ reported the acylation of the 9-position of the $\beta$-carboline by a chiral acyl chloride, followed by reaction with allyltributyltin and 2,2,2-trichloroethyl chloroformate to afford a 1-allyl-1,2-dihydro-$\beta$-carboline carbamate, and then this product was transformed to 1-allyl-1,2,3,4-tetrahydro-$\beta$-carboline. Suzuki et al.$^{46}$

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*Synthesis of Tribulusterine, A Potent Toxic Alkaloid from Tribulus terrestris.*
reported the functionalisation of the C-1 position of the β-carboline nucleus by cyanation. Modification of the cyano group resulted in various 1-substituted-β-carbolines. Choshi et al.\textsuperscript{47} and Kanekiyo et al.\textsuperscript{48} reported the preparation of the triflate derivative from β-carboline-N-oxide, which was then converted to the desired 1-substituted-β-carboline using a palladium catalysed cross-coupling reaction. Bracher et al.,\textsuperscript{49-50} reported another palladium cross-coupling of 1 halo-β-carboline with electrophiles, which are key steps in the syntheses of various 1-substituted-β-carboline alkaloids.

1.7 Aims of the project

Tribulusterine (15) is of great interest in the agricultural industry. The plant (Tribulus terrestris) containing this alkaloid affects the central nervous system (CNS) of sheep when ingested, and it has been suggested\textsuperscript{72} that tribulusterine may be an important alkaloid in mediating these effects, particularly an asymmetric locomotor disorder. With no previous work on the synthesis of tribulusterine having been reported, direct synthetic approaches towards this alkaloid were of interest.

It is possible that the observed dysfunction caused by T. terrestris may shed some light also on Parkinson’s Syndrome. However, only a very small quantity of tribulusterine was isolated from T. terrestris. The aim of this project was therefore to synthesise tribulusterine, by a direct and efficient route, in order to provide material to examine its biological properties more fully, particularly in sheep.
1.8 Proposed synthetic approaches to tribulusterine

Four methods for the synthesis of tribulusterine were proposed. Approaches one and two involved the synthesis of tribulusterine using aromatic substitution reactions. Other approaches used the Pictet-Spengler reaction by cyclisation of tryptamines with a furaldehyde. The last approach involved palladium-catalysed Negishi cross-coupling reaction of the triflate and a furylzinc.

The first approach involved the nucleophilic addition of furyllithium (28) directly to the C-1 of β-carboline (Scheme 3).

Scheme 3

The second approach involved reactions of lithiated β-carbolines. Lithiation of β-carbolines with butyllithium could result in C-1 lithiation, which could then undergo substitution reactions. In Scheme 4a, lithiation of intermediate (29) would promote intramolecular cyclisation by nucleophilic displacement of bromide. Aromatisation and hydrolysis, followed by reduction of the carboxylic group could

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yield tribulusterine. In Scheme 4b, lithiation of β-carboline followed by bromination could yield 1-bromo-β-carboline (30). The 1-bromo-β-carboline (30) would be converted to tribulusterine through a palladium-catalysed Negishi-type cross coupling reaction with furylzinc (31). The intermediate (31) may be prepared by the lithiation of 3-furanmethanol, followed by quenching with zinc chloride.

The third approach involved a three step synthesis of the tribulusterine using the Pictet-Spengler cyclisation reaction, followed by aromatisation (Scheme 5). This approach was based on the method used by Agarwal et al.\textsuperscript{52}
The last approach involved a palladium-catalysed cross coupling reaction between the triflate (33) and furylzinc (31). The triflate (33) could be prepared as described by Choshi et al.53 by the treatment of the 1-hydroxy-β-carboline (32)49 with trifluoromethanesulfonic anhydride (Tf₂O). The resulting triflate (33) could then be converted to tribulusterine via a palladium catalysed cross coupling reaction (Scheme 6).

Scheme 6

Synthesis of Tribulusterine, A Potent Toxic Alkaloid from Tribulus terrestris.
Chapter 2: Results and Discussion

2.1 Introduction

There were four synthetic approaches proposed for the synthesis of tribulusterine (15), a tryptamine-based alkaloid. All methods proposed were based on direct syntheses. The direct addition of furan substituents into the β-carboline ring via either nucleophilic or electrophilic substitution reactions has not been explored extensively. Normally, substituents are first introduced in tryptamine prior to ring cyclisation to afford a 1-substituted-β-carboline. There have been many studies on the synthesis of β-carboline derivatives using ring cyclisation.52,54-55

2.2 Regiospecific lithiation at C-2 of 3-furoic acid and 3-furanmethanol

Before beginning the synthetic approach to tribulusterine via nucleophilic substitution reactions, it was decided to investigate the regiospecific lithiation of 3-furoic acid and 3-furanmethanol and their potential substitution into the β-carboline moiety. The addition of electrophiles to the anions of 3-furoic acid and 3-furanmethanol would provide a short and direct synthetic pathway to tribulusterine. Other studies showed that the C-2 of both furan derivatives can be lithiated57,60 and the resulting anions were attacked with various electrophiles.

2.2.1 Study of formation and reaction of the bis-anion derived from 3-furoic acid (32)

Early studies on the C-2 lithiation of 3-substituted furans found that a mixture of C-2 and C-5 mono-anions was usually formed56. Recently, the C-2 lithiation of 3-substituted furans has been successfully achieved through the metallation of 3-furoic
acid. The bis-anion (33) of 3-furoic acid can be prepared via the direct metallation of the furan nucleus using either lithium diisopropylamide (LDA) or n-butyllithium (n-BuLi) (Scheme 7). Knight et al. successfully lithiated 3-furoic acid regiospecifically in the C-2 position by treatment of the acid with 2.2 equivalents of LDA in anhydrous tetrahydrofuran (THF) at -78 °C. The use of n-butyllithium to achieve direct ortho-lithiation of the acid (32) has also been reported after treatment with 2 equivalents of n-butyllithium in THF at -20 °C for 1 hour. Addition of the bis-lithiated furoic acid with reactive electrophiles (i.e. iodomethane, aldehydes and ketones) reportedly provides good yields of products (80-90 %). However, the bis-anions are known to give poor alkylation product yields when reacted with allylic and benzylic halides (yields < 42 %). In this work, n-butyllithium (2 equivalents) was added to the solution of the acid (32) in THF at -20 °C.

Scheme 7

![Scheme 7](image)

Reagent: a = LDA (2.2 equiv.), THF -78 °C, b = n-BuLi (2 equiv.), THF -20 °C

In the present study, a confirmatory study of the regioselective lithiation of 3-furoic acid (32) was based on the method used by Bures et al. Deuteration with deuterium oxide (excess) of the solution of the bis-anion (33), followed by acidification with dilute hydrochloric acid, provided 2-deutério-3-furoic acid (34), a deuterated analogue of a known compound, in good yield (80%). The spectroscopic data (^1H-NMR and CI-MS) is consistent with the structure of 2-deutério-3-furoic acid

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(34). The $^1$H-NMR revealed a complete absence of the proton at the C-2 position of the furan ring, which in the known 3-furoic acid itself at $\delta$ 8.17; in addition the two remaining furyl protons appeared at $\delta$ 6.78 and $\delta$ 7.47, which are at the same chemical shift as for H-4 and H-5, respectively, in 3-furoic acid (32) obtained from a commercial source. The mass spectral fragmentation pattern showed 100% relative intensity at m/z 114 (MH$^+$), which confirmed the molecular weight of 2-deuterio-3-furoic acid.

The reaction of the bis-anion (33) with iodomethane was examined next. The 2-methyl-3-furoic acid$^{58}$ product (35) was obtained in very high yield (91%). The structure was indicated by the mass spectrum and $^1$H-NMR. The $^1$H-NMR showed a three proton singlet for the methyl group at $\delta$ 2.60, together with two singlets at $\delta$ 6.78 and $\delta$ 7.46 for the hydrogens on the furan ring, in agreement with the published$^{58}$ $^1$H-NMR data. The mass spectrum exhibited an ion of 64% relative intensity at m/z 126 (MH$^+$), which was consistent with 2-methyl-3-furoic acid.

The addition of bromine in anhydrous THF to the solution of the bis-anion (33), however, failed to give the desired 2-bromo-3-furoic acid, but rather 3-furoic acid was recovered in high yield (83%). There was also no evidence for bromination of the furoic acid.

The results of this preliminary work suggested that butyllithium could be used directly on some C-3 furoic acid to direct lithiation.
2.2.2 Study of formation and reaction of the bis-anion derived from 3-furanmethanol (36)

In order to examine the possible addition of other furans to the β-carbolines, reactions of 3-furanmethanol (36) and its derivatives were studied. Since Knight\textsuperscript{57} reported regioselective α-lithiations of 3-substituted furans, 3-furanmethanol (36) has also been reported to undergo regiospecific lithiation after treatment with 2.2 equivalents of n-BuLi in THF (-78 °C, 2 hrs, 0°C, 1 hr), affording 2-substituted-3-(hydroxymethyl)furan (39) after quenching the dianion with electrophiles. However, the tert-butylidimethylsilylether of 3-hydroxymethylfuran (37) has been reported to undergo rearrangement in an intramolecular reaction to give 3-hydroxymethyl-2-tert-butylidimethylsilylfuran (41) on treatment with n-butyllithium in THF at either 0°C or -20°C in the presence of hexamethylphosphoric triamide (HMPA)\textsuperscript{59} (Scheme 7). To avoid the silyl rearrangement, Goldsmith \textit{et al.}\textsuperscript{60} have reported that the 3-\{[(tert-butyldimethylsilyl)oxy]methyl\}furan (37) can be regiospecifically lithiated at C-2 (1 equiv. of n-BuLi, ether, rt, 6 hrs) to provide 2-substituted-3-silylated furan (40).

2-Trimethylsilyl-3-\{[(tert-butyldimethylsilyl)oxy]methyl\}furan\textsuperscript{60} (40, $E = \text{TMS}$) (42%) was prepared from the silylated furan (37), quenching the anion with trimethylsilyl chloride (Scheme 8) by a similar procedure to Goldsmith's. The $^1$H-NMR and mass spectra (CI-MS) were consistent with the known\textsuperscript{60} compound (40, $E = \text{TMS}$). Two singlets of 15 protons of silyl tert-butyl groups and 9 protons of methyl groups were observed at δ 0.08 and δ 0.92, respectively. The two protons of the furan ring were ascribed to the two singlets at typical chemical shifts of δ 6.37 and δ 7.36. Chemical ionisation mass spectrometry (CI-MS) displayed a molecular ion peak at m/z 286.

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For the 3-furanmethanol (36) and its derivative, 3-methoxymethylfuran (38), lithiation attempts at C-2 were successful using 2 equivalents of n-butyllithium for (36) and 1 equivalent of n-butyllithium for (38) in THF at -78 °C, 2 hours and 0 °C for 1 hour. 3-(Hydroxymethyl)-2-furaldehyde (39, E=CHO) was prepared in 23 % yield after quenching the dianion from compound (36) with N,N-dimethylformamide (DMF). The 1H-NMR of the resulting aldehyde (39, E=CHO) showed the aldehyde singlet at δ 9.80, and two singlets representing the hydrogens in the furan ring at δ 6.58 and δ 7.60. Mass spectral analysis (Cl-MS) displayed the molecular ion of the product at m/z [MH]+ 127, consistent with 3-(hydroxymethyl)-2-furaldehyde. However, 3-(hydroxymethyl)-2-furoic acid (39, E=COOH) could not be prepared after quenching the dianion from compound (36) with saturated carbon dioxide. 2-Deuterio-3-methoxymethylfuran (42) was prepared, however, in 46% yield via the formation of the dianion of compound (38) (n-BuLi, -78 °C, 2 hrs, 0°C, 1 hr), followed by deuteration with deuterium oxide. The structure was confirmed by 1H-NMR, which showed a singlet of three protons for the methyl group at δ 3.33, a singlet of two protons of the methylene group at δ 4.33, and two singlets for the hydrogens in the furan ring at δ 6.43 and δ 7.42.
2.3 Nucleophilic substitution reactions of β-carbolines

As the initial investigation into lithiation of both 3-furoic acid and 3-furanmethanediol proved promising for attack in the 2-position of the furans, introducing a β-carboline moiety in this position was of interest for the direct preparation of tribulusterine. Comparison of the resonance stabilisation in the pyridine ring to benzene reveals that the pyridine ring is much more reactive than benzene, and it is also susceptible to nucleophilic attack at the carbon alpha to the nitrogen, resulting ultimately in substitution. Thus the C-1 of the β-carboline moiety could be susceptible to the furyllithium attack. This section of the work will investigate this hypothesis.

The indole NH was protected using N,N-dimethylsulfamoyl chloride, as the sulfamoyl group is stable in the presence of strong bases and may be easily removed in high yield under acidic conditions. Moreover, the sulfamoyl moiety as an electron withdrawing group makes C-1 more vulnerable to nucleophilic attack.

The 9-N,N-(dimethylsulfamoyl)-β-carboline (43) was prepared by the treatment of norharman (4) with sodium hydride and N,N-dimethylsulfamoyl chloride in dry THF (Scheme 9). Purification using flash silica chromatography, incorporating triethylamine in the eluent gave compound (43) in high yield (91%).

Scheme 9

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Without the use of triethylamine in the solvent mixture, the base (43) was difficult to elute from the silica gel. Trying to improve the yield of (43) by using alumina instead of silica gel, the eluent (30 % dichloromethane in hexane) caused some precipitation. This gave pure 9-N,N-(dimethylsulfamoyl)-β-carboline (43) but only in moderate yield (46 %). Reaction of (43) with the bis-anion of 3-furoic acid (33) for an hour at −78°C, followed by warming to room temperature and stirring overnight, failed to give (45) or show any addition of the nucleophile either at C-1 or any other positions (Scheme 10a). The 3-furoic acid (32) and norharman (4) were recovered after the experimental work-up.

Scheme 10a

In order to examine further possible addition of furans to the activated 1,2-position of β-carboline, 3-furanmethanol (36) and its tert-butyldimethylsilyl ether (37) were investigated (Scheme 10b). To allow the one-step addition of compound (37) to C-1 of β-carboline, compound (37) was initially lithiated in ether at room temperature, since silyl rearrangement has been observed in THF in the presence of HMPA59. After 6 hours, the furyllithium (46b) was added to the solution of (43) at 0 °C under nitrogen. However, the reaction mixture contained only starting materials. It appears that the bulky steric properties of the protective tert-butyldimethylsilyl group obstructed the addition. Therefore, introducing furyllithium (46a) was attempted.

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Ortho-lithiation of (36) using standard conditions (2 equivalents of n-butyllithium in THF, 2 hrs -78 °C, 1 hr 0 °C) was used and the dianion then added to a solution of (43). After hydrolytic work-up, however, only starting materials were recovered.

Scheme 10b

![Scheme 10b](image)

2.4 Nucleophilic substitution reactions of activated β-carbolines

Since the initial attempted addition of furyllithium to the C-1 of β-carbolines without activation of the C=N moiety failed and no adduct was obtained, it appeared that activation of the C=N double bond was required. This section of the work reports the nucleophilic addition of organometallic reagents to C=N activated β-carbolines.

β-Carbolines may be activated to nucleophilic attack by quaternisation on the pyridine nitrogen. The most useful reaction of this type has been either the transformation of the pyridine nitrogen into an iminium salt or coordination with a Lewis acid. Boron trifluoride diethyl etherate (BF$_3$.Et$_2$O) is a Lewis acid catalyst and has been used to facilitate the addition of basic nucleophiles such as alkyl- or aryl-lithium to various electrophiles$^{61}$. Some examples the coordination of BF$_3$.Et$_2$O with the C=N double bond to activate imines to undergo 1,2-addition reactions$^{62-63}$. Kawate et al.$^{63}$ reported that the coordination of BF$_3$.Et$_2$O with the C=N double bond of 3,4-dihydro-β-carboline occurred smoothly to give 1-substituted-1,2,3,4-tetrahydro-β-
carbolines. In this reaction, 3,4-dihydro-β-carboline was alkylated with alkyllithium species in the presence of BF$_3$.Et$_2$O to give the corresponding 1-substituted-1,2,3,4-tetrahydro-β-carbolines in high yields. However, there have been no reports on coordination with β-carbolines to yield 1-substituted-3,4-dihydro-β-carbolines. Thus, the anion addition to the β-carboline moiety in the presence of BF$_3$.Et$_2$O was investigated.

The activated β-carboline (48) was prepared as described by Pyne et al. by treating the β-carboline (43) in tetrahydrofuran (THF) with 1.2 equivalents of BF$_3$.Et$_2$O at -20 °C for 15 minutes. The resulting BF$_3$-iminium salt (48) was immediately treated with methyllithium (1 molar equivalent) at -20 °C, however, none of the desired product was formed, with only starting material being recovered. Increasing the equivalents of methyllithium (3 molar equivalents) and then partially purification using preparative layer chromatography afforded in a yellow solid (0.8 mg), however, the structure could not be clearly identified by $^1$H-NMR due to the small amount obtained. Chemical ionisation mass spectroscopy (CI-MS) indicated the presence of the mixture of the desired product (49) (m/z 290 [MH$^+$]) and the starting material (43) (Scheme 11). In the case of compound (49), elimination occurred after the initial nucleophilic addition, resulting in the aromatised product.

Scheme 11

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2.5 Nucleophilic substitution reactions of activated β-carboline-N-oxide

In order to improve the alkylation of (43), a refated approach using a BF$_3$ iminium salt of β-carboline-N-oxide was examined.

An aromatic N-oxide group increases the activity of its α and γ positions to electrophilic and nucleophilic reagents at the same time$^{64-65}$. Consequently, a large number of nucleophilic and electrophilic substitutions have been described. The oxygen in the aromatic N-oxide is basic and undergoes addition of a proton, metal ions, Lewis acids, alkyl halides, alkyl sulfonates, and acyl halides to form complex compounds$^{64}$. An aromatic N-oxide forms a stable salt with a strong acid and also with a Lewis acid such as BF$_3$.Et$_2$O. As has already been stated, BF$_3$.Et$_2$O induces the 1,2-addition reactions. Thus, the complexation of β-carboline-N-oxide with BF$_3$ was expected to regiospecifically induce nucleophilic substitution at the position α to the nitrogen.

The β-carboline-N-oxide was prepared based on the method of Suzuki et al.$^{46}$ The β-carboline (43) was converted to the corresponding N-oxide (50) by reaction with m-chloroperbenzoic acid (m-CPBA) in dichloromethane at room temperature. After the reaction work-up, the β-carboline-N-oxide (50) was obtained in 51% yield after purification via column chromatography. The N-oxide-BF$_3$ complex (51) was prepared by the addition of BF$_3$.Et$_2$O (1.2 equivalents) in THF at -20 °C for 15 minutes, and was then quenched with methylithium. After the nucleophilic addition was complete, the deoxygenation of the N-oxide occurred, resulting in the aromatisation of the pyridine ring, and yielding (49) in a 17 % yield (Scheme 12).
NMR spectroscopic analysis of (49) revealed the addition of a three-proton singlet at δ 3.05, for the C-1 methyl substitutent and the absence of the one-proton singlet at C-1 of the β-carboline. Mass spectral analysis (CI-MS) revealed a molecular ion peak at m/z 290, consistent with the desired product composition of C₁₄H₁₅N₃O₂S. Comparison of ¹H-NMR and mass spectral analysis of (49) with the commercially available harman, which was protected at the NH with the sulfonamide protecting group (7, Scheme 9), revealed that the newly synthesised (49) to satisfactionly mimic the molecular fragmentation pattern and proton spectrum of harman.

Scheme 12

The investigation of nucleophilic substitutions at C-1 of the N-oxide-BF₃ complex thus showed that small nucleophiles such as methyllithium can be successfully substituted in the pyridine ring. The treatment of the N-oxide-BF₃ complex with organolithium reagents from furan derivatives was therefore considered a viable approach.
3-Furanmethanol (36) was lithiated using the standard method (-78 °C, 2 hrs; 0 °C, 1 hr) by treatment with n-BuLi. The resulting organometallic species (2 molar equivalents) was added to the solution of the N-oxide-BF$_3$ complex (51) at -20 °C. After a period of 2 hours at -20 °C, no evidence of the furan derivative addition was obtained. However, when 3 molar equivalents of furyllithium (46a) was used, followed by preparative layer chromatography, it was clear that the furyl group had added to the C-1-position of the β-carboline. Preliminary $^1$H-NMR data suggested the product was compound (52). Analysis of the $^1$H-NMR spectrum showed the loss of the proton at the C-1 (δ 9.48) position and the protons of the furan ring at δ 6.50 (H-3') and δ 7.57 (H-2' and H-5') were present. The other protons of the β-carboline ring and the methyls of the sulfonamide protecting group were clearly seen. Oxygen addition to the C-1 of β-carboline was evident from the 2 proton singlet seen for the furanyl methylene at δ 4.75. This oxygen addition might result from steric hindrance of the sulfonamide protecting group and the bulky furyllithium, aided by the fact that alkoxide is a good nucleophile. Consequently, the oxygen attacked the C-1 position in preference to attack by carbon-2 in the lithiated furan.

![Image]

(52)

Treatment of lithiated 3-furoic acid (33) with the N-oxide-BF$_3$ complex (51) was also examined, since the carboxylate group is not as a good nucleophile as the alkoxide, and thus, might promote carbon rather oxygen addition to the C-1 of β-carboline. However, although increasing the molar equivalent of furyllithium (33) up

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to 3 equivalents, there was no evidence for any addition (Scheme 13). The $^1$H-NMR of the product mixture displayed protons for both β-carboline and 3-furoic acid but after separation by preparative layer chromatography, the starting materials were obtained. The same result was confirmed when checking by CI-MS. The addition of lithiated methoxymethylfuran (54) to compound (51) was also investigated. No evidence was found, however, that the furyl group had substituted the β-carboline ring (Scheme 13).

Scheme 13

Avoiding the steric hindrance of the sulfonamide protecting group, the β-carboline-N-oxide (56) was prepared. With similar conditions to those in the preparation of (52), and adding additional lithiated reagent to allow for proton removal at N-9, the carbon-carbon bond was expected to form since no steric hindrance from the bulky protecting group. However, only the starting materials only were recovered (Scheme 14).

Scheme 14

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2.6 Other substitution reactions of β-carbolines

The dimetalated species of 1-halo-β-carbolines described by Bracher et al.\textsuperscript{50} and prepared by deprotonation with KH in THF and halogen-lithium exchange with \( t-\text{BuLi} \), have been reported as useful building blocks for the synthesis of 1-substituted-β-carbolines. Thus, in order to synthesise tribulusterine as described earlier in Scheme 4b, compound (43) was chosen as a starting material for preparing 1-bromo-β-carboline (30).

An initial attempt to prepare the 1-substituted-β-carboline from (43) by lithiation with \( t-\text{BuLi} \), followed by the addition of iodomethane, gave the 1-methyl-β-carboline (49, scheme 15) but in a poor yield. Bromination of the lithiated derivative of (43) failed to yield the 1-bromo-β-carboline (57) however.

Scheme 15

Another attempt to synthesise 1-bromo-β-carboline (57) involved the bromination of 9-(\( t-\)butoxycarbonyl)-β-carboline (58). Compound (58) was prepared by the treatment of norharman (4) with di-\( tert-\)butyl dicarbonate and triethylamine in chloroform (Scheme 16). After purification using flash column chromatography (silica gel, 1% methanol in dichloromethane), compound (58) was obtained in moderate yield (64%). \(^1\)H-NMR analysis showed a singlet of nine protons at \( \delta 1.78 \) representing the addition of the \( t-\)butyl carbamate moiety, along with other peaks for

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protons on the β-carboline ring. Mass spectral analysis confirmed the structure with CI-MS revealing a molecular ion peak at (m/z) 269, consistent with the molecular formular C_{16}H_{16}N_{2}O_{2}.

Methylation of the Boc-protected derivative (58) with iodomethane (excess) in THF at -78 °C, after reaction with t-BuLi, failed to add the methyl moiety onto the C-1 of the β-carboline ring; from ^1H-NMR and CI-MS evidence, the reaction product appeared to contain the starting material (58), together with some (4) from loss of the protecting group.

Bromination of the Boc-protected derivative (58) with 2 molar equivalents of N-bromosuccinimide (NBS) yielded the 1,3,6,8-tetrabromo-β-carboline (60a) as a major product and 3-bromo-9-(t-butoxycarbonyl)-β-carboline (60b) as a minor product (Scheme 16).

Scheme 16

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Investigation of the $^1$H-NMR of the major compound (60a) revealed three singlets at $\delta$ 7.90, 8.02, 8.13 for the protons on the β-carboline ring and a singlet at $\delta$ 8.43 for the proton on nitrogen. The mass spectrum showed the relative intensities of isotope peaks for combinations of four bromine atoms, which presented the MH$^+$ ion peaks at m/z 481, 483, 485, 487, and 489. The $^1$H-NMR of the minor compound (60b) showed a singlet of nine protons representing the t-butyl group at $\delta$ 1.80, along with the loss of a one proton doublet for the H-4 proton on the β-carboline ring. Mass spectral analysis confirmed the presence of one bromine atom, displaying molecular ion isotopic peaks at m/z $\delta$ 347 and $\delta$ 349.

The loss of the Boc protecting group in (60a) might result from the use of a strong base, t-BuLi. Tetra-bromination then occurred at susceptible carbons in the β-carboline ring. To avoid using such a strong base, n-BuLi was used to form the anion, followed by bromination with bromine (Scheme 17). Again, a mixture of products (61a) and (61b) were obtained. Their structures were confirmed by $^1$H-NMR and mass spectral analysis. The major product obtained was a tri-brominated derivative (61a), in which the Boc protecting group was lost. The fact that no bromine was added to the C-1 of (61a) suggests that the Boc group was lost after the bromination had occurred. Due to the steric hindrance of the Boc group, C-1 may not be lithiated. Instead, C-3 may be lithiated and then brominated by bromine. Bromine has then involved in further electrophilic substitution reactions on the benzene ring. The minor product of the reaction, (60b), resulted for the mono-substitution only of bromine at C-3 after lithiation.
The investigation of the $^1$H-NMR of the major compound (61a) revealed four singlets at δ 7.85, 8.06, 8.15, and 8.74 for the protons on the β-carboline ring and a singlet at δ 8.55 for the proton on nitrogen. The mass spectrum showed the relative intensities of isotope peaks for combinations of three bromine atoms, which presented the molecular ion isotopic peaks at m/z 402, 403, 404, 405, 406. The $^1$H-NMR of the minor compound (61b) showed a singlet of nine protons representing the tert-butyl group at δ 1.80, along with the loss of a one-doublet for the proton H-4 on the β-carboline ring. Mass spectral analysis confirmed the presence of one bromine atom, displaying molecular ion isotopic peaks at 347 and 349.

Due to the lack of success in achieving the synthesis of a 1-bromo-β-carboline via the lithiation method, further synthesis towards tribulusterine utilising this approach could not be achieved. Therefore the synthesis of tribulusterine using ring cyclisation via the Pictet-Spengler reaction was investigated.

2.7 Possible synthesis of tribulusterine via Pictet-Spengler cyclisation

There have been many examples of the synthesis of β-carboline analogues based on the chemistry of the Pictet-Spengler reaction which have been used for the synthesis of both indole and isoquinoline alkaloids. In this reaction, the cyclisation

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of tryptamine analogues and aldehydes afforded tetrahydro-β-carbolines, which could then be dehydrogenated to yield β-carboline derivatives.

Scheme 18

In a model study, the L-(2-furyl)-β-carboline (64) was prepared based on the method of Agarwal et al. Tryptamine hydrochloride was condensed with 2-furaldehyde (65) in absolute ethanol under a nitrogen atmosphere at room temperature for 16 hours (Scheme 18). The 1,2,3,4-tetrahydro-1-(2-furyl)-β-carboline (62) was obtained in a very high yield (98%), and it was then dehydrogenated using Pd/C in xylenes to afford 1-(2-furyl)-β-carboline (64). The structure of (64) was established from the $^1$H-NMR and $^{13}$C-NMR data and the mass spectrum ([MH]$^+$ with m/z 235), and by comparison with literature data.

A similar reaction of the synthetic 3-(hydroxymethyl)-2-furaldehyde (66) (see page 24) with tryptamine hydrochloride was then attempted, in a direct two step approach to tribulusterine. However, the $^1$H-NMR of the crude mixture showed that a
poor yield was obtained after stirring for 16 hours at room temperature, presumably due to the greater steric hindrance of (66) in the region of the aldehyde. To improve the yield, the reaction was heated under reflux for 3 hours. The $^1$H-NMR of the product showed a mixture of tryptamine and the required 1,2,3,4-tetrahydro-1-(3-hydroxymethyl-2-furyl)-β-carboline (63) in a ratio of 3:1 on the basis of integration ratios in the $^1$H-NMR. Purification using flash column chromatography (silica gel) eluting with 10% methanol in dichloromethane (with 0.1% ammonium hydroxide) proved to be difficult, due to the basicity of both tryptamine and (63), and the presence of the hydroxyl group in (63). Thus, dehydrogenation with Pd/C was undertaken on the crude mixture. Purification using preparative layer chromatography yielded six different bands. The bands were isolated and analysed via $^1$H-NMR and mass spectrometry. The middle band (7.5 mg, $R_f=0.4$) showed on [MH]$^+$ ion at m/z 265 in the CI-MS, however, the $^1$H-NMR could not clearly confirm its structure due to the dilute concentration and the fact that the band was not completely pure. Comparison with the $^1$H-NMR spectrum of authentic tribulusterine (kindly provided by Professor T-S. Wu, National Cheng Kung University, Taiwan) indicated that tribulusterine may have been present in the band. High resolution mass spectrometry confirmed the molecular formular (C$_{16}$H$_{12}$N$_2$O$_2$) for tribulusterine (15). To improve the yield of (15), the alcohol (66) may have to be protected as its acetate for Pictet-Spengler-Dehydrogenation sequence, and then deacetylated at the end.

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2.8 Attempted synthesis of tribulusterine via palladium cross coupling reaction of trifluoromethanesulfonates

Palladium cross coupling reactions of aryl compounds with organometallic species are important and versatile methods for carbon-carbon bond formation and are well documented\textsuperscript{69-70}. Aryl triflates are increasingly involved in the process due to their highly effective promotion of carbon-carbon bond formation\textsuperscript{70}. It was therefore to be expected that the triflate (33) might undergo a palladium cross coupling reaction with furylzinc (31) to give tribulusterine (Scheme 19).

Scheme 19

The 1-hydroxy-β-carboline\textsuperscript{49} (32), prepared by the condensation of tryptamine with triphosgene, followed by cyclisation, was chosen as a starting material to prepare the triflate (33). Treatment of (32) with trifluoromethanesulfonic anhydride [(CF\textsubscript{3}SO\textsubscript{2})\textsubscript{2}O] in pyridine gave the desired triflate (33) (Scheme 19). The \textsuperscript{1}H-NMR showed a mixture of the starting material (32) and the triflate (33). Also the mass spectrum (CI-MS) revealed ions at m/z 317 (15\%) for the [MH]\textsuperscript{+} of the triflate (33).
and at m/z 185 (100%) for (32) and also $[\text{M-SO}_2\text{CF}_3]^+$ . However, due to the limitation of time, purification of (33) was not completed. Thus, the palladium cross coupling reaction between the triflate (33) and furylzinc (31), with protection of the N9-H, was not examined.

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Chapter 3: Conclusion

3.1 Conclusion

Due to the toxicity of Tribulus terrestris to sheep in Australia, the synthesis of tribulusterine, an alkaloid present in T. terrestris, has been studied in order to examine its biological effects. In this thesis, approaches to the synthesis of tribulusterine involving nucleophilic and electrophilic substitution reactions of β-carbolines, and the traditional Pictet-Spengler condensation reaction, have been examined. The last approach has indicated that tribulusterine may have been formed, but in low yield.

Initial studies on a precursor for a palladium-catalysed cross coupling approach to tribulusterine were also undertaken.

The addition of nucleophiles to unactivated-β-carbolines (Table 1, Appendix) failed to give any 1-substituted-β-carbolines. The methylation with methyllithium of the activated-β-carboline with BF$_3$ gave 1-methyl-β-carboline, although in poor yield (5 %) (Table 2, Appendix). The methylation was increased to 17% yield by the treatment of methyl lithium with a β-carboline-N-oxide.BF$_3$ complex (Table 3, Appendix). Similar reactions have been examined using furyllithium. Only the bis-anion of 3-furanmethanol was added to the C-1 position. Unfortunately, the reaction proceeds via carbon-oxygen bond formation, rather than the required carbon-carbon bond formation, affording the new β-carboline (52). This is thought to be due to the steric hindrance of the sulfonamide protecting group and the bulky furanmethanol. Neither the methyl ether of the 3-furanmethanol nor the bis-anion of furan-3-carboxylic acid underwent any additions.

The synthesis of tribulusterine was attempted through the two step syntheses of electrophilic substitution reactions and a palladium catalysed Negishi-type cross...
coupling reaction. However, the bromination of β-carboline did not yield the desired 1-bromo-β-carboline, therefore the palladium catalysed Negishi-cross coupling reaction was unable to be attempted. Substitution reactions of lithiated β-carboline through bromination via N-bromosuccinimide (NBS) afforded 1,3,6,8-tetrahydroxy-β-carboline as a major product and 3-bromo-9-(t-butoxyacetylen)-β-carboline as a minor product. Bromination using bromine yielded 3,6,8-tribromo-β-carboline as a major product. No evidence for the 1-bromo-β-carboline being formed was seen. Therefore, the palladium-catalysed Negishi-cross coupling reaction has not been investigated.

Tribulusterine was synthesised via the traditional Pictet-Spengler condensation reactions. Tryptamine hydrochloride was condensed with synthetic 3-(hydroxymethyl)-2-furaldehyde (66) under reflux. However, the purification caused some difficulty. The mixture of 1,2,3,4-tetrahydro-1-(3-hydromethyl-2-furyl)-β-carboline (63) and tryptamine was then dehydrogenated. One of the bands obtained from preparative layer chromatography had the correct molecular weight and formular for tribulusterine (15), but the ¹H-NMR was not sufficiently resolved to confirm the structure.

Another palladium-catalysed Negishi-cross coupling reaction was expected to yield tribulusterine via triflate (33) and furylzinc (31). The triflate (33) has been prepared by the reaction of 1-hydroxy-β-carboline (32) with trifluoromethanesulfonic anhydride in pyridine. However, due to the limitation of time, the triflate was not purified and the palladium-cross coupling reaction was not studied.
3.2 Future work

The synthesis of tribulusterine through the Pictet-Spengler-Dehydrogenation sequence may be increased in scale in order to confirmed the structure of the substituted β-carboline product using $^1$H-NMR and $^{13}$C-NMR. Alternatively, nanoprobe $^1$H-NMR may be used to elucidate the structure. The reaction of palladium cross coupling reactions should also be investigated after the triflate has been purified. This reaction should also yield the desired tribulusterine. Once the tribulusterine has been obtained, the biological assessment of the alkaloid could be undertaken.
Chapter 4: Experimental

4.1 General Procedures

Melting points

Melting points were determined by a Reichert hot stage melting point apparatus and are uncorrected.

$^1$H and $^{13}$C Nuclear Magnetic Resonance (NMR) spectra

$^1$H-NMR (300 MHz) and $^{13}$C-NMR (75 MHz) spectra were recorded on a Varian Unity 300 or a Mercury 300 Fourier transform NMR spectrometer. The spectra were measured in deuterated chloroform (CDCl$_3$), unless otherwise stated. All chemical shifts were measured relative to internal tetramethylsilane (TMS) or the solvent signal (CDCl$_3$). Resonances are quoted in ppm.

Mass Spectra (MS)

Chemical ionisation mass spectra (MS-CI) were determined using a Shimadzu QP-5000 by the direct insertion technique. High resolution CI mass spectra were determined using a Fisons/VG Autospec-TOF-oa Mass Spectrometer.

Preparative-layer Chromatography

Preparative-layer chromatography was carried out on 20 x 20 cm glass plates coated with Kieselgel 60 F$^{254}$ (Merck)

Flash column Chromatography

Flash column chromatography was carried out with 60 mesh Merck silica gel, 0.063-0.200 nm particle size.

Solvents

Evaporation of solvents from the extracts was done using rotary evaporation (Büchi rotary evaporator) at reduced pressure (water pump). All solvent extracts were Synthesis of Tribulusterine, A Potent Toxic Alkaloid from Tribulus terrestris.
dried over anhydrous sodium sulfate prior to evaporation. All solvent ratios are v/v.

Tetrahydrofuran (THF) was dried over sodium metal/benzophenone and distilled under nitrogen.

Starting Materials

Norharman was purchased from Sigma Chemical Co. 3-Furoic acid and 3-furanmethanol were purchased from Aldrich Chemical Co.

All reactions required anhydrous conditions. Glassware was dried by heating and then cooled under an anhydrous nitrogen atmosphere.

4.2 Synthetic Reactions

4.2.1 Studies of the regiospecific lithiation at C-2 of 3-furoic acid and 3-furanmethanol

Synthesis of 2-deuterio-3-furoic acid (34)

To a solution of 3-furoic acid (32) (65 mg, 0.58 mmol) in dry THF (5 mL) at -78 °C under nitrogen was added n-butyllithium (2.5 M in hexane) (0.46 mL, 1.16 mmol) and the mixture stirred for an hour at -78 °C. A portion (1 mL) of the reaction mixture was quenched with deuterium oxide (excess). This solution was then stirred at room temperature for 10 minutes and acidified with hydrochloric acid (5% v/v). The organic layer was dried and the solvent was concentrated to afford 2-deuterio-3-furoic acid (52 mg, 80 %) as a colourless solid.

$^1$H-NMR (CDCl$_3$) δ 6.78 (d, 1H, $J= 3.3$ Hz, H-4), 7.47 (d, 1H, $J= 4.8$ Hz, H-5);

MS-Cl (m/z) 114 [MH]$^+$ 100 %
Synthesis of 2-methyl-3-furoic acid (35)

To a solution of 3-furoic acid (32) (0.29 g, 2.58 mmol) in dry THF (10 mL) at -78 °C under nitrogen was added n-butyllithium (2.5 M) in hexane (2.00 mL, 5.16 mmol). The mixture was stirred at -78 °C for 1 hour and then at -20 °C for 1 hour. The reaction mixture was then treated with iodomethane (0.5 mL, excess). The mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and diethyl ether (10 mL) and saturated ammonium chloride (10 mL) were added. Aqueous hydrochloric acid (10% v/v, 10 mL) was added, and the ether layer was separated, dried, and evaporated. The residue (0.25g) was subjected to flash column chromatography (silica gel, dichloromethane:hexane, 1:1) to give 2-methyl-3-furoic acid (0.12 g, 37 %) as a yellow solid, mp 99-105 °C (lit. mp 102-105 °C).

^1H-NMR (CDCl₃) δ 2.60 (s, 3H, CH₃), 6.78 (s, 1H, H-4), 7.46 (s, 1H, H-5);

MS-Cl (m/z) 126 [MH]^+ 64 %, 111 [M-CH₃]^+ 100%

Attempted synthesis of 2-bromo-3-furoic acid

To a solution of 3-furoic acid (32) (0.24 g, 2.14 mmol) in dry THF (10 mL) at -78 °C under nitrogen was added n-butyllithium (2.5 M) in hexane (1.71 mL, 4.28 mmol). The mixture was stirred at -78 °C for 1 hour and -20 °C for 1 hour. The reaction mixture was treated bromine (0.5 mL) in dry THF (3 mL). The mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and ethyl acetate (10 mL) and saturated ammonium chloride (10 mL) were added. Aqueous hydrochloric acid (10%
v/v, 10 mL) was added. The organic layer was separated, dried, and removed to give the starting 3-furoic acid (0.20 g).

Synthesis of 3-\{(tert-butyldimethylsilyl)oxy\}methyl\}furan (37)

To a solution of imidazole (1.50 g, 21.0 mmol) in DMF (5 mL) at 0°C under nitrogen was added tert-butyl-dimethylsilyl chloride (1.55 g, 10.0 mmol). After 10 minutes, 3-furanmethanol (36) (0.88 mL, 10.0 mmol) was added and the reaction mixture stirred for 12 hours at room temperature. The mixture was concentrated, and the residue dissolved in diethyl ether (30 mL). The solution was washed with water (3 x 20 mL) and then dried with anhydrous sodium sulfate. The crude product was distilled under reduced pressure to give the title compound (70 mg, 40 %) as an oil, bp 109-110 °C/ 20 Torr (lit.59 bp 106-109 °C/20 Torr).

^1H-NMR (CDCl₃) δ 0.087 (s, 6H, CH₃), 0.92 (s, 9H, C-CH₃), 4.58 (s, 2H, CH₂), 6.37 (d, 1H, J=0.9 Hz, H-4), 7.35-7.38 (m, 2H, H-2, H-5);

MS-CI (m/z) 213 [MH]+ 5 %, 97 [M-TBDMS]+ 100%

Synthesis of 2-trimethylsilyl-3-\{(tert-butyldimethylsilyl)oxy\}methyl\}furan (40)

To a solution of silylated furan (37) (0.032 g, 0.15 mmol) in ether (5 mL) at room temperature under a nitrogen atmosphere was added n-butyllithium in hexane (60 µL, 0.15 mmol). The stirring was continued for 6 hours. Then the reaction mixture was cooled to 0°C and quenched with trimethylsilyl chloride (20 µL, 0.15 mmol). After 2 hours, the solution was allowed to warm to room temperature and stirred for 48 hours.
Saturated ammonium chloride (10 mL) was added and the solution extracted with diethyl ether (2 x 10 mL). The organic layer was washed with water (2 x 10 mL), dried and the solvent then removed in vacuo to afford the silylated furan (40) as a yellow oil (18 mg, 42 %) after preparative layer chromatography (silica gel, dichloromethane/hexane, 1:1).

\(^1H\)-NMR (CDCl\textsubscript{3}) \( \delta \) 0.08 (s, 15H, CH\textsubscript{3}), 0.92 (s, 9H, C-CH\textsubscript{3}), 4.60 (s, 2H, CH\textsubscript{2}), 6.37 (s, 1H, H-4), 7.36 (s, 1H, H-5)

MS-CI (m/z) 285 [MH]\textsuperscript{+} 10 %, 211 [M-SiMe\textsubscript{3}]\textsuperscript{+} 29%

**Synthesis of 3-methoxymethylfuran (38)**

To a solution of 3-furanmethanol (36) (0.50 mL, 2.79 mmol) in acetonitrile (5 mL) was added iodomethane (0.7 mL, excess). Silver oxide (0.5 g) was added and the suspension was refluxed and stirring was continued overnight. The solid was removed by filtration and the solvent was removed under reduced pressure. The residue was subjected to flash column chromatography (silica gel, petroleum ether (bp 40-60 °C)/ diethyl ether, 3:2). 3-Methoxymethylfuran\textsuperscript{71} was isolated as a clear oil (0.13 g, 42 %).

\(^1H\)-NMR (CDCl\textsubscript{3}) \( \delta \) 3.38 (s, 3H, CH\textsubscript{3}), 4.33 (s, 2H, CH\textsubscript{2}), 6.42 (s, 1H, H-4), 7.42 (d, 2H, J= 2.1 Hz, H-2, H-5);

MS-CI (m/z) 113 [MH]\textsuperscript{+} 26 %, 97 [M-Me]\textsuperscript{+} 66%
Synthesis of 2-deuterio-3-methoxymethylfuran (42)

To a solution of 3-methoxymethylfuran (38) (0.064 g, 0.57 mmol) in dry THF (5 mL) at -78 °C under nitrogen was added n-butyllithium (2.5 M in hexane) (0.23 mL, 0.57 mmol). The solution was stirred at -78 °C for 1 hour and then quenched with deuterium oxide (0.1 mL, excess). The resulting mixture was allowed to warm to room temperature and stirred for a further 2 hours. Water (10 mL) was added and extracted with dichloromethane (2 x 10 mL). The combined extracts were dried and evaporated to give a yellow crude oil. The oil was subjected to preparative layer chromatography (silica gel, dichloromethane) to afford the title compound (0.030 g, 46%) as a yellow oil.

\[^1\text{H}-\text{NMR} \text{(CDCl}_3\text{)} \delta: 3.38 \text{ (s, 3H, CH}_3\text{)}, 4.33 \text{ (s, 2H, CH}_2\text{)}, 6.43 \text{ (s, 1H, H-4), 7.42 (s, 1H, H-5)}\]

MS-CI (m/z): 114 [MH]^+ 10 %, 98 [M-Me]^+ 100%

Synthesis of 3-(hydroxymethyl)-2-furaldehyde (66)

To a solution of 3-furanmethanol (36) (0.30 g, 3.11 mmol) in dry THF (10 mL) at -78°C under nitrogen was added n-butyllithium in hexane (2.49 mL, 6.22 mmol). The mixture was stirred at -78 °C for 2 hours and at then 0 °C for 1 hour. A solution of anhydrous N,N-dimethylformamide (1.06 mL, 13.68 mmol) in anhydrous THF (5 mL) was added dropwise at - 78 °C. Then the solution was allowed to warm to room temperature and stirring was continued overnight. After addition of saturated ammonium chloride solution (20 mL), extraction with ethyl acetate (3 x 20 mL), drying, and solvent

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evaporation gave the crude product (1.72 g). This was purified by flash column chromatography (silica gel, dichloromethane) to give 3-(hydroxymethyl)-2-furaldehyde (0.09 g, 23%) as a yellow oil (measured MH+ 127.0396, calculated for C₆H₆O₃+H 127.0395).

^1H-NMR (CDCl₃): δ 4.79 (s, 2H, CH₂), 6.58 (s, 1H, H-4), 7.60 (s, 1H, H-5), 9.80 ( s, 1H, CHO);

^13C-NMR (CDCl₃): δ 57.3 (CH₂), 110.0 (C-4), 113.4 (C-3), 145.0 (C-5), 147.8 (C-2), 181.0 (CHO);

MS-Cl (m/z) 127 [MH]^+ 100%

**Attempted synthesis of 3-(hydroxymethyl)-2-furoic acid (39)**

To a solution of 3-furanmethanol (36) (0.43 g, 4.42 mmol) in dry THF (20 mL) at -78 °C under nitrogen was added n-butyllithium (2.0 equivalents of 2.5 M in hexane). The mixture was stirred at -78 °C for 2 hours and -20 °C for 1 hour. The solution was then treated with THF (12 mL) which had previously been saturated with carbon dioxide; during the reaction more carbon dioxide was also passed through the solution. The mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed under reduced pressure and ethyl acetate (15 mL) and saturated ammonium chloride (15 mL) were added. Aqueous hydrochloric acid (10%, 15 mL) was added, and ethyl acetate was separated, dried, and removed to give the starting 3-furanmethanol (0.23 g).
4.2.2 Studies of nucleophilic substitution reactions of β-carbolines

Synthesis of 9-(N,N-Dimethylsulfamoyl)-β-carboline (43)

Method A

A mixture of N,N-dimethylsulfamoyl chloride (0.15 mL, 1.4 mmol), norharman (4) (0.14 g, 0.82 mmol), triethylamine (0.1 mL, 1.0 mmol) and benzene (4mL) was stirred under a nitrogen atmosphere for 10 hours. The resulting solution was filtered and the precipitate was washed with benzene. The filtrate and the washing were combined. The benzene solvent was evaporated and the product was purified by column chromatography (silica gel) eluting with 1% (v/v) methanol in dichloromethane to yield 9-(N,N-dimethylsulfamoyl)-β-carboline (24.1 mg, 10.7 %), m.p. 95-99 °C (measured MH+ 276.0805, calculated for C_{13}H_{13}N_{3}O_{2}S+H 276.0807);

^1H-NMR (CDCl3) δ 2.90 (s, 6H, CH3), 7.45 (t, 1H, J= 7.4 Hz, H-6), 7.59 (t, 1H, J= 7.8 Hz, H-7), 7.82 (d, 1H, J= 6.9 Hz, H-4), 7.97 (d, 1H, J= 7.8 Hz, H-5), 8.11 (d, 1H, J= 8.1 Hz, H-8), 8.26 (d, 1H, J=5.4 Hz, H-3), 9.16 (s, 1H, H-1);

MS-CI (m/z) 276 [MH]+ 100 %

Method B

To a solution of norharman (4) (0.43 g, 2.5 mmol) in dry THF (20 mL) at 0 °C under argon was added sodium hydride (0.20 g of a 60 % dispersion in oil, 5 mmol). The reaction mixture was stirred at 0 °C for 30 minutes. N,N-Dimethylsulfamoyl chloride (0.54 mL, 5 mmol) was added and the mixture was warmed to room temperature and stirred for 3 hours. The solution was concentrated and the residue dissolved in diethyl ether (30 mL) before being washed with water (3 x 15 mL), dried

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and concentrated. The product was column chromatographed on silica gel (dichloromethane: hexane: triethylamine; 95:5:1) and 9-(N,N-dimethylsulfamoyl)-β-carboline was crystallised (0.63 g, 91 %) as a colourless solid, mp 99-100 °C (measured MH⁺ 276.0812, calculated for C₁₃H₁₃N₃O₂S+H 276.0807).

¹H-NMR (CDCl₃) δ 2.86 (s, 6H, CH₃), 7.45(t, 1H, J= 7.2 Hz, H-6), 7.64 (t, 1H, J=7.5 Hz, H-7), 7.93 (d, 1H, J= 4.2 Hz, H-4), 8.08 (d, 1H, J= 8.4 Hz, H-5), 8.20 (d, 1H, J= 8.7 Hz, H-8), 8.62 (d, 1H, J= 5.1 Hz, H-3), 9.48 (s, 1H, H-1);

¹³C-NMR (CDCl₃) δ: 39.0 (CH₃), 114.5 (C-4), 115.2 (C-8), 121.7, 122.4, 122.6 (C-4b, C-5, C-6), 123.8 (C-7), 130.1, 131.5 (C-4a, C-9a), 137.2 (C-3), 139.8 (C-8a), 143.0 (C-1);

MS-Cl (m/z) 276 [MH⁺] 100 %

Attempted synthesis of 2-[9-(N,N-dimethylsulfamoyl)-β-carboline-1-yl]furan-3-carboxylic acid (45)

To a solution of 3-furoic acid (32) (40 mg, 0.36 mmol) in dry THF (3 mL) at -78 °C under nitrogen was added n-butyllithium (2.5 M in hexane) (290 µL, 0.72 mmol). The solution was stirred at -78 °C for 1 hour and then at -20 °C for 1 hour. Then a solution of 9-(N,N-dimethylsulfamoyl)-β-carboline (43) (93 mg, 0.36 mmol) in dry THF (5 mL) was added dropwise. The reaction mixture was stirred at -78 °C for 1 hour and then overnight at room temperature. The solution was then concentrated. Hydrochloric acid (5 % v/v, 5 mL) was added and then the mixture extracted with diethyl ether (3 x 5 mL). The organic
layer was dried and concentrated to give 3-furoic acid (43 mg). The aqueous layer was concentrated to give 9-(N,N-dimethylsulfamoyl)-β-carboline (69 mg).

**Attempted synthesis of 1-(3-{[(t-butyldimethylsilyl)oxy)methyl]-2-furyl}-9-(N,N-dimethylsulfamoyl)-β-carboline (47b)**

To a solution of the silyl ether (37) (0.087 g, 0.4 mmol) in dry diethyl ether at room temperature under nitrogen was added n-butyllithium (2.5 M in hexane) (0.16 mL, 0.4 mmol). The reaction mixture was stirred for 6 hours and cooled to 0 °C. 9-(N,N-dimethylsulfamoyl)-β-carboline (43) (0.10 g, 0.36 mmol) was added and the reaction mixture stirred for a further 48 hours. Saturated ammonium chloride was then added. Hydrochloric acid (5% v/v, 5 mL) was added and then extracted with diethyl ether (2 x 10 mL). The organic layer was dried and concentrated to afford the silyl ether (37) (0.082 g). The aqueous layer was neutralised with sodium carbonate and the solution was extracted with diethyl ether (5 mL). The organic extract was dried, concentrated, and the solvent removed to provide starting material, the β-carboline (43) (0.072 g).

**Attempted synthesis of 1-(3-hydroxymethyl-2-furyl)-9-(N,N-dimethylsulfamoyl)-β-carboline (47a)**

To a solution of 3-furanmethanol (36) (0.43 mL, 0.5 mmol) in dry THF (5 mL) at −78 °C under nitrogen was added n-butyllithium (2.5 M in hexane) (0.4 mL, 1.0 mmol). The solution was stirred for 2 hours.
at -78 °C and at 0 °C for 1 hour. Then a solution of 9-(N,N-dimethylsulfamoyl)-β-carboline (43) (0.14 g, 0.5 mmol) was added dropwise. The reaction mixture was stirred for a further 2 hours at 0 °C and then at room temperature overnight. The reaction was worked up as for compound (47b). Only starting materials, the β-carboline (43) (0.079 g) and the furan (36) (0.038 g), were obtained at the end of the work-up.

4.2.3 Studies of nucleophilic substitution reactions of activated β-carbolines

Attempted synthesis of 1-methyl-9-(N,N-dimethylsulfamoyl)-β-carboline (49)

To a solution of 9-(N,N-dimethylsulfamoyl)-β-carboline (43) (0.054 g, 0.20 mmol) in dry THF (3 mL) at 0 °C under nitrogen was added boron trifluoride diethyl etherate (30 μL, 0.23 mmol). The solution was stirred at 0 °C for 15 minutes and then methyllithium in ether (140 μL, 0.20 mmol) was added. The reaction mixture was stirred at 0 °C for an hour and at room temperature overnight. The solution was concentrated and dichloromethane (5 mL) was added. The solution was washed with 10% potassium carbonate (3 x 5 mL). The organic layer was dried and concentrated under reduced pressure to give 9-(N,N-dimethylsulfamoyl)-β-carboline (43) as a yellow solid (0.060 g).
Synthesis of 1-methyl-9-(N,N-dimethylsulfamoyl)-β-carboline (49)

To a solution of 9-(N,N-dimethylsulfamoyl)-β-carboline (43) (0.024 g, 0.035 mmol) in dry THF (5 mL) at −20 °C under nitrogen was added boron trifluoride diethyl etherate (12 μL, 0.094 mmol). The solution was stirred at −20 °C for 15 minutes. Methyl lithium in ether (180 μL, 0.255 mmol) was added and stirred at −20 °C for 2 hours and then allowed to warm to room temperature and further stirred overnight. Sodium hydroxide (10 % w/v) was added and extracted with dichloromethane (3 x 10 mL). The dichloromethane extract was dried and removed under reduced pressure to give a crude brown solid (45 mg). The crude mixture was subjected to preparative layer chromatography (silica gel, 3% methanol in dichloromethane) to yield a yellow solid (0.8 mg), which from the mass spectral evidence indicated the presence of the mixture of the title compound (49) and the starting material (43).

MS-CI (m/z) 290 [MH]+ for compound (49) 11 %, 276 [MH]+ for starting material (43) 100 %, 169 [M-SO₂NMe₂]+ 14 %.

4.2.4 Studies of nucleophilic substitution reactions of activated β-Carboline-N-oxide

Preparation of the 9-(N,N-dimethylsulfamoyl)-β-carboline-N-oxide (50)

To a solution of 9-(N,N-dimethylsulfamoyl)-β-carboline (43) (0.27 g, 0.96 mmol) in dichloromethane (10 mL) at room temperature under nitrogen was added m-chloroperbenzoic acid (0.43 g, 1.45 mmol). The solution was stirred for 48 hours.
Hexane was added to the solution and then washed with 10% potassium carbonate (3 x 10 mL) and water (3 x 10 mL). The aqueous layers were combined and washed with dichloromethane (2 x 20 mL). The organic layers were combined, dried and evaporated to give a yellow solid (0.24 g). The solid was subjected to silica column chromatography (5% methanol in dichloromethane) to yield the title compound (50) (0.14g, 51%) as a yellow solid, mp 141-145 °C (measured MH⁺ 292.0756, calculated for C_{13}H_{13}N_3O_3S+H 292.0756).

^1H-NMR (CDCl₃) δ: 2.90 (s, 6H, CH₃), 7.45 (t, 1H, J=7.8 Hz, H-6), 7.59 (t, 1H, J=7.2 Hz, H-7), 7.82 (d, 1H, J= 6.6 Hz, H-4), 7.97 (d, 1H, J=7.8 Hz, H-5), 8.10 (d, 1H, J=7.8 Hz, H-8), 8.26 (d, 1H, J=5.4 Hz, H-3), 9.16 (s, 1H, H-1);

^13C-NMR (CDCl₃): 38.9 (CH₃), 115.0, 116.1 (C-4 and C-8), 121.0 (C-6), 123.0 (C-4a), 124.5, (C-5), 125.5 (C-7), 128.0 (C-4b), 129.4 (C-8a), 135.0 (C-9a), 140.5 (C-1)

MS-CI (m/z); 292 [MH]⁺ 76%, 276 [M-O]⁺ 100%, 169 [M-SO₂NMe₂]⁺ 49 %.

Synthesis of 1-methyl-9-\(N,N\)-dimethylsulfamoyl)-\(β\)-carboline (49)

To a solution of 9-\(N,N\)-dimethylsulfamoyl)-\(β\)-carboline-\(N\)-oxide (50) (0.047 g, 0.16 mmol) in dry THF (5mL) at -20 °C under nitrogen was added boron trifluoride diethyl etherate (24 μL, 0.19 mmol). The reaction mixture was stirred at -20 °C for 15 minutes. Then methyllithium in ether (340 μL, 0.48 mmol) was added and further stirred at -20 °C for 2 hours. The solution was allowed to warm to room temperature and stirred overnight. The solution was concentrated to dryness and dichloromethane (5mL) was added. The solution was washed with sodium hydroxide (10 % w/v) (2 x 5 mL). The dichloromethane extract was dried and the
solvent removed under reduced pressure to give a yellow solid. The solid was further purified using preparative layer chromatography (silica gel, 3 % methanol in dichloromethane) to yield the title compound (49) (8 mg, 17.3 %) as a brown solid mp 234-235 °C (measured \( \text{MH}^+ 290.0970 \), calculated for \( \text{C}_{14}\text{H}_{15}\text{N}_3\text{O}_2 + \text{H} 290.0970 \)).

\[ ^1\text{H-NMR} (\text{CDCl}_3) \delta 2.54 \text{ (s, 6H, CH}_3\text{)}, 3.05 \text{ (s, 3H, CH}_3\text{)}, 7.45 \text{ (t, 1H, } J= 7.8 \text{ Hz, H-6)}, 7.58 \text{ (t, 1H, } J= 7.8 \text{ Hz, H-7)}, 7.70 \text{ (d, 1H, } J= 5.1 \text{ Hz, H-4)}, 7.97 \text{ (d, 1H, } J= 7.8 \text{ Hz, H-5)}, 8.20 \text{ (d, 1H, } J= 8.4 \text{ Hz, H-8)}, 8.55 \text{ (d, 1H, } J= 5.1 \text{ Hz, H-3)} \]

\[ ^{13}\text{C-NMR} (\text{CDCl}_3) \delta: 25.3 \text{ (CH}_3\text{)}, 38.7 \text{ (N-CH}_3\text{)}, 112.0 \text{ (C-4)}, 115.0 \text{ (C-8)}, 118.6 \text{ (C-6)}, 121.2 \text{ (C-5)}, 125.1 \text{, (C-4b)}, 125.2 \text{ (C-4a)}, 126.0 \text{ (C-4a)}, 129.8 \text{ (C-7)}, 135.6 \text{ (C-8a)}, 139.5 \text{ (C-9a)}, 144.1 \text{ (C-3)}, 149.1 \text{ (C-1)} \]

\[ \text{MS-CI (m/z): 290 [MH}\text{]+ 45\%, 183 \text{ [M-SO}_2\text{NMe}_2\text{]} 100\%, 169 \text{ [M-CH}_3\text{-SO}_2\text{NMe}_2\text{]+ 25\%}.} \]

**Synthesis of 1-(3'-furyl)methoxy-9-(N, N-dimethylsulfamoyl)-β-carboline (52)**

To a solution of 3-furanmethanol (36) (0.096 mL, 1.1 mmol) in dry THF (3mL) at -78°C under nitrogen was added \( n\)-butyllithium in THF (0.9 mL, 2.3 mmol). The reaction mixture was stirred at -78 °C for 2 hours and at 0 °C for 1 hour. The solution was added to the solution of the \( N\)-oxide.BF\(_3\) complex (51), prepared by adding boron trifluoride diethyl etherate (0.06 mL, 0.46 mmol) to a solution of the \( N\)-oxide (50) (0.11g, 0.38 mmol) in dry THF (3 mL) at -20 °C under nitrogen and stirred for 15 minutes, in THF at -20 °C under nitrogen. The reaction mixture was stirred at -20 °C for 2 hours. Then the solution was allowed to warm to room temperature and stirred overnight. The solvent was

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removed to dryness and dichloromethane (5 mL) was added. Sodium hydroxide (10% w/v) was added and the organic layer was dried and concentrated to yield a yellow solid. The solid was subjected to preparative layer chromatography (silica gel, 3% methanol in dichloromethane) to give three bands. The highest band \((R_f^* = 0.8)\) contained the title compound (52) (3 mg, 2%) as a yellow solid mp 133-135°C (measured \(MH^+ 372.0999\), calculated for \(\text{C}_{18}\text{H}_{17}\text{N}_3\text{O}_4\text{S}^+\text{H} 372.0998\);)

\[^1\text{H}-\text{NMR (CDCl}_3\delta:\text{ 2.37 (s, 6H, CH}_3\text{), 4.75 (s, 2H, CH}_2\text{), 6.50 (d, 1H, } J= 1.8\text{ Hz H-4'), 7.51 (t, 1H, } J= 7.5\text{ Hz, H-6), 7.57 (d, 2H, } J= 1.8\text{ Hz, H-2' and H-5'), 7.64 (t, 1H, } J= 8.1\text{ Hz, H-7), 7.75 (d, 1H, } J= 5.1\text{ Hz, H-4), 8.04 (d, 1H, } J= 8.4\text{ Hz, H-5), 8.09 (d, 1H, } J=8.1\text{ Hz, H-8), 8.61 (d, 1H, } J= 5.1\text{ Hz, H-3);}\n
\[^{13}\text{C}-\text{NMR (CDCl}_3\delta:\text{ 39.0 (CH}_3\text{), 59.0 (CH}_2\text{), 112.8, 113.2 (C-4', C-8), 118.6 (C-4), 121.8 (C-6), 123.9 (C-5), 125.5, 126.1, 126.3 (C-3', C-4b, C-7), 128.1 (C-4a), 130.1 (C-9a), 133.1 (C-8a), 137.3 (C-5'), 142.4 (C-1'), 143.8 (C-1)\n
MS-CI (m/z): \(372 [MH]^+ 100\%\)

\((R_f^* = \text{the distance between the band (developed 2 times) and the base line} / \text{the distance between the base line and the solvent front})\n
**Attempted synthesis of 2-[9-(N,N-dimethylsulfamoyl)-β-carboline-1-yl]furan-3-carboxylic acid (45)**

To a solution of 3-furoic acid (32) (0.12g, 1.00 mmol) in dry THF at -78 °C under nitrogen was added n-butyllithium (0.80 mL, 2.03 mmol). The solution was stirred at -78 °C for 1 hour and then transferred via syringe to a solution of the N-oxide (50) (0.08g, 0.29 mmol)
complexed with boron trifluoride diethyl etherate (0.04 mL, 0.35 mmol) in dry THF at -20 °C. The reaction mixture was then stirred at -20 °C for 2 hour and room temperature for 3 hours. Solvent was removed and dichloromethane (15 mL) was added and then washed with water (3 × 15 mL). The organic layer was dried, evaporated under reduced pressure. The product was purified by preparative layer chromatography (silica gel, 3% methanol in dichloromethane). However, only starting materials were obtained.

**Attempted synthesis of 1-(3-methoxymethyl-2-furyl)-9-(N,N-dimethylsulfamoyl)-β-carboline (55)**

Lithiated 3-methoxymethylfuran (54) (0.072 g, 0.64 mmol) in THF (5 mL) was added to the suspension of precomplexed boron trifluoride diethyl etherate (0.04 mL, 0.32 mmol) and the N-oxide (50) (0.061 g, 0.21 mmol) at -78 °C under nitrogen. The reaction mixture was worked-up as described above (in the attempted synthesis of compound (45)). The solvent was removed to yield a mixture of the N-oxide (50), methoxymethylfuran (38) and 9-(N,N-dimethylsulfamoyl)-β-carboline (43).

**Synthesis of norharman-N-oxide (56)**

To a solution of norharman (4) (0.17 g, 1.0 mmol) in CHCl₃ (5 mL) at room temperature was added m-chloroperbenzoic acid (64%) (0.46 g, 1.5 mmol) and then the mixture was stirred at room temperature for 24 hours. Sodium bisulfite (0.04 g, 0.35 mmol) in dry THF at -20 °C. The reaction mixture was then stirred at -20 °C for 2 hours and room temperature for 3 hours. Solvent was removed and dichloromethane (15 mL) was added and then washed with water (3 × 15 mL). The organic layer was dried, evaporated under reduced pressure. The product was purified by preparative layer chromatography (silica gel, 3% methanol in dichloromethane). However, only starting materials were obtained.

**Attempted synthesis of 1-(3-methoxymethyl-2-furyl)-9-(N,N-dimethylsulfamoyl)-β-carboline (55)**

Lithiated 3-methoxymethylfuran (54) (0.072 g, 0.64 mmol) in THF (5 mL) was added to the suspension of precomplexed boron trifluoride diethyl etherate (0.04 mL, 0.32 mmol) and the N-oxide (50) (0.061 g, 0.21 mmol) at -78 °C under nitrogen. The reaction mixture was worked-up as described above (in the attempted synthesis of compound (45)). The solvent was removed to yield a mixture of the N-oxide (50), methoxymethylfuran (38) and 9-(N,N-dimethylsulfamoyl)-β-carboline (43).

**Synthesis of norharman-N-oxide (56)**

To a solution of norharman (4) (0.17 g, 1.0 mmol) in CHCl₃ (5 mL) at room temperature was added m-chloroperbenzoic acid (64%) (0.46 g, 1.5 mmol) and then the mixture was stirred at room temperature for 24 hours. Sodium bisulfite (0.04 g,
0.19 mmol) was added and stirred for 15 minutes. Saturated sodium bicarbonate solution (15 mL) was then added. The organic layer was separated, and the aqueous layer was extracted with CHCl₃ (15 x 3 mL). The combine organic layers were dried, filtered, and evaporated in vacuo to yield norharman-N-oxide (56) (0.0641 g, 34 %) as a yellow powder, mp. 211-214 °C ;

¹H-NMR (DMSO-d₆) δ: 7.22 (td, 1H, J= 7.2, 0.9 Hz, H-6), 7.45 (td, 1H, J= 8.1 , 1.2 Hz, H-7), 7.54 (d, 1H, J=8.4 Hz, H-8), 8.00 (dd, J= 6.6, 1.5 Hz, H-4), 8.10 (d, 1H, J= 6.9 Hz, H-3), 8.13 (d, 1H, J= 7.8 Hz, H-5), 8.53 (d, 1H, J= 1.2 Hz, H-1), 12.10 (bd s, 1H, NH)

¹³C-NMR (CDCl₃) δ: 111.9 (C-4), 112.4 (C-8), 115.1 (C-6), 116.7 (C-5), 120.2 (C-4b), 122.0 (C-4a), 123.9 (C-7), 128.7 (C-8a), 130.7 (C-9a), 133.7 (C-3), 139.0 (C-1); MS/CI (m/z) 185 [MH]+ 4%, 169 [M-O]+ 100%

Attempted synthesis of tribulusterine (15)

The lithiated dianion of 3-furanmethanol (46a) (0.16g, 1.62 mmol) in THF (5 mL) was added to the suspension of precomplexed boron trifluoride diethyl etherate (0.10 mL, 0.82 mmol) and norharman-N-oxide (56) (0.10 g, 0.54 mmol) at -78 °C under nitrogen. The reaction mixture was stirred at -78°C for 1 hour and then at -20 °C for 2 hours, and the solution was then allowed to warm to room temperature and stirred overnight. The solvent was removed to dryness. Chloroform (15 mL) was added and washed with 10 % NaOH (3 x 5 mL). The solvent was dried and evaporated to give a yellow
crude solid. The solid was purified by preparative layer chromatography (silica gel, 1\% methanol in dichloromethane). However, only starting materials were obtained.

**Attempted synthesis of 1-(3-methoxymethyl-2-furyl)-9-(N,N-dimethylsulfamoyl)-\(\beta\)-carboline (55)**

![Chemical structure of 1-(3-methoxymethyl-2-furyl)-9-(N,N-dimethylsulfamoyl)-\(\beta\)-carboline (55)](image)

Lithiated 3-methoxymethylfuran (54) (0.072g, 0.64 mmol) in THF (5 mL) was added to the suspension of boron trifluoride diethyl etherate (0.10 mL, 0.82 mmol) and norharman-\(N\)-oxide (56) (0.10g, 0.54 mmol) at -78 °C under nitrogen. The reaction mixture was worked-up as described in the attempted synthesis of tribulusterine. The solvent was removed to yield a mixture of starting materials.

**4.2.5 Studies of electrophilic substitution reactions of \(\beta\)-carbolines**

**Synthesis of 1-methyl-9-(N,N-dimethylsulfamoyl)-\(\beta\)-carboline (49)**

![Chemical structure of 1-methyl-9-(N,N-dimethylsulfamoyl)-\(\beta\)-carboline (49)](image)

To a solution of 9-(N,N-dimethylsulfamoyl)-\(\beta\)-carboline (43) (70 mg, 0.25 mmol) in THF (10 mL) at -78 °C under nitrogen was added \(t\)-butyllithium (1.38 M) in hexane (0.18 mL, 0.25). The reaction mixture was stirred at -78 °C for 1 hour. Iodomethane (0.15 mL, excess) was added dropwise and then the mixture stirred for 30 minutes at -78 °C and for 2 hours at room temperature. Then saturated aqueous sodium thiosulfate (10 mL) was added, followed by extraction with ethyl acetate (3 x 10 mL). The combined organic layers were dried and concentrated. The crude product was purified by preparative layer chromatography (silica gel, 3%
methanol in dichloromethane) to give the title compound (49) (10 mg, 12%) as a yellow solid, mp 233-235 °C.

1H-NMR (CDCl₃) δ 2.62 (s, 6H, N-CH₃), 3.00 (s, 3H, CH₃), 7.45 (t, 1H, J=7.5 Hz, H-6), 7.63 (t, 1H, J= 7.5 Hz, H-7), 7.72 (d, 1H, J=5.4 Hz, H-4), 8.09 (d, 1H, J= 7.5 Hz, H-5), 8.20 (d, 1H, J=8.4 Hz, H-8), 8.62 (d, 1H, J=5.1 Hz, H-3)

MS-Cl (m/z) 290 [MH]+14%, 289 [M]+ 97%

Attempted synthesis of 1-bromo-9-(N,N-dimethylsulfamoyl)-β-carboline (57)

To a solution of 9-(N,N-dimethylsulfamoyl)-β-carboline (43) (0.37 g, 1.36 mmol) in THF (10 mL) at -78 °C under nitrogen was added t-butyllithium (1.38 M) in hexane (0.99 mL, 1.36 mmol). The reaction mixture was stirred at -78 °C for 1 hour. Bromine (0.18 mL, 3.4 mmol) was then added dropwise and the mixture stirred for 30 minutes at -78 °C and for 2 hours at room temperature. Then saturated aqueous sodium thiosulfate (10 mL) was added, followed by extraction with ethyl acetate (3 x 10 mL). The combined organic layers were dried and concentrated. The crude product was purified by flash column chromatography (silica gel, 3% methanol in dichloromethane) to give the starting materials.

Synthesis of 9-(t-butoxycarbonyl)-β-carboline (58)

A mixture of di-tert-butyldicarbonate (0.13 g, 0.53 mmol), norharman (4) (0.18 g, 1.06 mmol), triethylamine (5 mL) and chloroform (10 mL) was stirred at 0 °C for 1 hour.
and allowed to warm to room temperature and further stirred for 2 hours. Then the solvent was removed to dryness and sodium hydrogen carbonate (10% w/v, 10 mL) was added followed by extraction with diethyl ether (3 x 10 mL). The combined organic layers were washed with brine (3 x 5 mL). The organic layer was dried and concentrated under reduced pressure to yield a pale yellow solid (0.16 g). The crude product was purified by flash column chromatography to give the title compound (58) as a colourless solid (0.091 g), mp 130-133 °C (measured MH⁺ 269.1295, calculated for C₁₆H₁₆N₂O₂+H 269.1290).

¹H-NMR (CDCl₃) δ 1.79 (s, 9H, CH₃), 7.42 (t, 1H, J= 7.8 Hz, H-6), 7.62 (t, 1H, J= 7.8 Hz, H-7), 7.90 (d, 1H, J= 4.2 Hz, H-4), 8.06 (d, 1H, J= 7.5 Hz, H-5), 8.40 (d, 1H, J= 8.4 Hz, H-8), 8.60 (d, 1H, J= 5.1 Hz, H-3), 9.59 (s, 1H, H-1)

¹³C-NMR (CDCl₃) δ 28.5 (CH₃), 30.0 (C), 85.9 (C-4), 114.2 (C-8), 121.0, 116.8 (C-6), 121.2 (C-5), 123.6 (C-4b), 129.9 (C-7), 132.0 (C-4a), 135.0 (C-8a), 138.7 (C-9a), 139.1 (C-3), 143.0 (C-1), 150.9 (C=O)

MS-Cl (m/z): 269 [MH]+ 100 %

Attempted synthesis of 1-methyl-9-(t-butoxycarbonyl)-β-carboline (59)

To a solution of 9-(t-butoxycarbonyl)-β-carboline (58) (0.062 g, 0.24 mmol) in THF (5 ml) at -78 °C under nitrogen was added t-butyllithium in hexane (1.35 M) (0.17 mL, 0.24 mmol). The reaction mixture was stirred at -78 °C for 1 hour. Iodomethane (excess) was added and the solution was stirred at -78 °C and then allowed to warm to room temperature. The reaction was stirred for a further period of 12 hours. Then saturated aqueous sodium
thiosulfate (10 mL) was added, followed by extraction with ethyl acetate (3 x 10 mL). The combined organic layers were dried and concentrated to give the 9-(t-butoxycarbonyl)-β-carboline (58) (0.054 g).

**Synthesis of 1,3,6,8-tetrabromo-β-carboline (60a) and 3-bromo-9-(t-butoxycarbonyl)-β-carboline (60b)**

To a solution of 9-(t-butoxycarbonyl)-β-carboline (58) (0.11 g, 0.43 mmol) in THF (10 ml) at –78 °C under nitrogen was added t-butyllithium (1.38 M in hexane) (0.30 mL, 0.43 mmol). The reaction mixture was stirred at –78°C for 1 hour and then N-bromosuccinimide (NBS) (0.15 g, 0.86 mmol) in THF (2 mL) was added dropwise and stirred for 30 minutes at –78 °C. The solution was allowed to warm to room temperature and stirred for a further 3 hours. Saturated sodium thiosulfate (10 mL) was added, followed by extraction with ethyl acetate (3 x 10 mL). The combined organic layers was dried and concentrated to give a crude brown residue (0.13 g). The residue was subjected to preparative layer chromatography (silica gel, dichloromethane) to give 3 major bands. The highest band (Rf = 0.8) gave the compound (60a) (20 mg, 10 %) as a yellow solid, m.p. 222-226 °C. (measured MH+ 480.7178, calculated for C_{11}H_{79}Br_4N_2+H 480.7181).

^1H-NMR (CDCl3) δ 7.90 (d, 1H, J= 1.5 Hz, H-5), 8.02 (s, 1H, H-4), 8.13 (s, 1H, H-7), 8.43 (br.s, 1H, NH);

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$^{13}$C-NMR (CDCl$_3$) δ: 106.2 (C-4), 114.2 (C-8), 118.9 (C-5), 123.3, 123.4 (C-6 and C-4b), 124.3 (C-7), 128.9 (C-4a), 131.9, 134.5, 134.7 (C-3, C-8a and C-9a), 138.3 (C-1);

MS-CI (m/z); 485 (plus 481, 483, 487, and 489 for the other Br isotopic combinations), [MH, $^{79}$Br$_2$, $^{80}$Br$_2$]$^+$ 55%, 405 [485 - $^{80}$Br]$^+$ 39%, 327 [483 - 2$^{80}$Br]$^+$, 247 [487 - 3$^{80}$Br]$^+$ 11%.

The middle band (R$_f$ = 0.7) gave 3-bromo-9-(t-butoxycarbonyl)-β-carboline (60b) (3 mg, 2%) as a yellow solid. (measured MH$^+$ 347.0395, calculated for C$_{16}$H$_{15}$$^{79}$BrN$_2$O$_2$ + H 347.0395)

$^1$H-NMR (CDCl$_3$) δ: 1.78 (s, 9H, CH$_3$), 7.47 (td, 1H, J= 7.8, 1.8 Hz, H-6), 7.68 (td, 1H, J= 8.7, 1.2 Hz, H-7), 8.18 (s, 1H, H-4), 8.43 (d, 1H, J= 8.1 Hz, H-5), 8.78 (d, 1H, J= 7.2 Hz), 9.54 (s, 1H, H-1)

MS-CI (m/z); 347 [MH, $^{79}$Br]$^+$ 100 %

Synthesis of 3,6,8-tribromo-β-carboline (61a)

To a solution of 9-(t-butoxycarbonyl)-β-carboline (58) (0.08 g, 0.30 mmol) in THF (5 ml) at −78 °C under nitrogen was added n-butyllithium 2.5 M in hexane (0.12 mL, 0.3 mmol). The reaction mixture was stirred at −78°C for 1 hour and then bromine (0.03 mL) in THF (1 mL) was added dropwise and stirred for 30 minutes at −78 °C. The solution was allowed to warm to room temperature and stirred for a further 3 hours. Saturated sodium thiosulfate (10 mL) was added, followed by extraction with ethyl acetate (3 x 10 mL).
The combined organic layers was dried and concentrated. The residue was subjected to preparative layer chromatography (silica gel, dichloromethane) to give five major bands. The lowest band ($R_f = 0.5$) gave the compound (61a) as a yellow powder, mp 202-203 °C (measured $\text{MH}^+ 402.8066$, calculated for $\text{C}_{11}\text{H}_{57}\text{Br}_3\text{N}_3\text{H} 402.8054$),

$^1\text{H-NMR (CDCl}_3 \delta$: 7.85 (dd, 1H, $J= 1.5$ Hz, H-5), 8.06 (s, 1H, H-4), 8.15 (s, 1H, H-7), 8.55 (br. s, 1H, NH), 8.74 (s, 1H, H-1)

$^{13}\text{C-NMR (CDCl}_3 \delta$: 105.8 (C-4), 114.6 (C-8), 119.3 (C-5), 123.6 (C-6), 124.1 (C-7), 131.0 (C-4b), 132.7 (C-4a), 134.0 (C-8a), 134.3 (C-1), 135.2 (C-9a), 138.7 (C-3)

MS-Cl (m/z) 405 [MH, $^{79}\text{Br}_2$, $^{80}\text{Br}]+ 100$

4.2.6 Attempted synthesis of tribulusterine via palladium cross coupling reaction of aryl fluorosulfonates

Synthesis of 2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-one (32a)

To a solution of tryptamine base (0.91 g, 5.7 mmol) in warm toluene (100 mL) was added triethylamine (1.5 mL). To this solution was added, dropwise, triphosgen (0.73 g, 2.5 mmol) in toluene (10 mL) with strong stirring and then the mixture was stirred for a further 20 minutes at room temperature. Hydrogen bromide solution (0.8 mL) (45% in glacial acetic acid) was added and the mixture was heated under reflux for 30 minutes. The mixture was cooled and water (100 mL) was added. Ethyl acetate (100 mL) was added and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (100 mL). The combined organic layers were dried and the solvent was evaporated under reduced pressure. The residue was passed through small flash column chromatography (silica gel, EtOAc) and the solvent was
evaporated and recrystallised (MeOH/ EtOAc, 1:1 v/v) to give the title compound (32a) (0.66 g, 62 %) as colourless needles, mp 180-185 °C (lit. 184 °C) (measured MH+ 187.0871, calculated for C11H10N2O+H 187.0870).

1H-NMR (CDCl3) δ 3.07 (t, 2H, CH2), 3.72 (t, 2H, CH2), 5.67 (br. s, 1H, NH), 7.16 (t, 1H, J = 6.3 Hz, H-6), 7.3 (t, 1H, J=7.2 Hz, H-7), 7.45 (d, 1H, J= 8.1, H-5), 7.61(d,1H, J= 8.1 Hz, H-8), 9.14 (br. s, 1H, NH);

13C-NMR (CDCl3) δ 21.1 (C-4), 42.4 (C-3), 113.0 (C-8), 120.2, 120.4, 120.5 (C-4b, C-5, and C-6), 125.3, 125.4 (C-7, C-4a), 126.5 (C-9a), 137.9 (C-8a), 164.2 (C-1)

Synthesis of 2,9-dihydro-1H-pyrido[3,4-b]indol-1-one (32b)

2,3,4,9-Tetrahydro-1H-pyrido[3,4-b]indol-1-one (32a) (0.075 g, 0.40 mmol) was dissolved in hot xylene (5 mL), then 10 % Pd/C (0.03 g) was added and the mixture was heated under reflux under nitrogen for 1 hour. The reaction mixture was then cooled and the product crystallised on the catalyst. The solvent was decanted and the residue was extracted with boiling ethanol (3 x 5 mL). The combined extracts were concentrated to yield the title compound (32b) as a yellow solid (0.03 g, 40 %) m.p. 254-257 °C (lit. 255-257 °C),

1H-NMR (Acetone-d6) δ 7.12 (d, 1H, J= 6.9 Hz, H-4), 7.20-7.36 (m, 2H, H-3 and H-6), 7.43 (t, 1H, J= 7.5 Hz, H-7), 7.69 (d, 1H, J= 8.4 Hz, H-5), 8.06 (d, 1H, J= 7.8 Hz, H-8), 9.64 (br. s, 1H, NH), 10.04 (br. s, 1H, NH)

13C-NMR (Acetone-d6) δ 101.0 (C-4), 112.7 (C-8), 118.7 (C-6), 120.0, 120.4 (C-4b, C-5),124.5, 125.5 (C-3 and C-7), 126.7 (C-9a), 139.6 (C-8a), 156.4 (C-1)

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Synthesis of 1-[(trifluoromethanesulfonyl)oxy]-β-carboline (33)

To a stirred solution of the compound (32b) (80 mg, 0.43 mmol) and pyridine (130 μL, 1.29 mmol) in THF (5 mL) at 0°C under nitrogen was added trifluoromethanesulfonic anhydride (87 μL, 0.52 mmol). After stirring at room temperature for 2 hours, the solution was treated with water. The mixture was then extracted with dichloromethane (2 x 15 mL). The organic layer was washed with water and brine and then dried. The solvent was removed, and the residue was purified by column chromatography (silica gel) using ethyl acetate-hexane (1:19, v/v) as an eluent to give a yellow, crude solid (0.11 g).

MS-CI (m/z): 317 [MH]+ 15%, 185 [M-SO₂CF₃]+ 100%

4.2.7 Synthesis of tribulusterine via Pictet-Spengler cyclisation

Synthesis of 1,2,3,4-tetrahydro-1-(2'-furyl)-9H-pyrido[3,4-b]indole (62)

To a stirred solution of tryptamine hydrochloride (0.26g, 1.34 mmol) in absolute ethanol (10 mL) in dark under nitrogen at room temperature was added 2-furaldehyde (0.11 mL, 1.11 mmol). The solution was stirred for 16 hours and concentrated to dryness in vacuo. The residue was neutralised with 10% aqueous sodium carbonate solution and extracted with dichloromethane (2 x 15 mL). The organic extract was washed with water (2 x 10 mL), dried and concentrated to give a crude brown solid.
The solid was purified over a flash silica column chromatography (3% methanol in dichloromethane) to yield the title compound (62) (0.26, 79%) as a yellow solid, mp 126-128 °C (lit. 52 130-132 °C) (measured MH⁺ 239.1175, calculated for C₁₅H₁₄N₂O+H 239.1171).

¹H-NMR (CDCl₃) δ: 3.18 (t, 2H, J = 7.2 Hz, CH₂, H-4), 3.90 (t, 2H, J = 7.5 Hz, CH₂, H-3), 6.46 (dd, 1H, J = 3.6, 1.8 Hz, H-4'), 6.68 (d, 1H, J = 3.3 Hz, H-3'), 7.02 (s, 1H, H-1), 7.10-7.22 (m, 2H, ArH), 7.36 (d, 1H, J = 7.8 Hz, H-5), 7.51 (br. s, 1H, H-5'), 7.65 (d, 1H, J = 7.8, H-8), 7.96 (s, 1H, NH), 8.00 (br. s, 1H, indole NH);

¹³C-NMR (CDCl₃) δ: 27.5 (C-4), 62.0 (C-3), 110.0 (C-4'), 111.3, 111.8 (C-8, C-3'), 114.2, 119.1, 119.4 (C-4b, C-5, C-6), 122.2, 122.4 (C-4a, C-7), 127.7 (C-9a), 136.5 (C-8a), 139.0 (C-1), 145.0 (C-5'), 150.2 (C-2')

MS-CI (m/z); 239 [MH⁺] 100 %

**Synthesis of 1-(2-furyl)-9H-pyrido[3,4-b]indole (64)**

![Chemical Structure](attachment:image.png)

To a solution of 1,2,3,4-tetrahydro-1-furyl-9H-pyrido[3,4-b]indole (62) (0.055g, 0.23 mmol) in dry xylene (2 mL) was added 10 % Pd/C (0.06g). The mixture was refluxed for 3 hours under nitrogen. The mixture was filtered, and the black mass collected was washed with hot xylene (3 x 5 mL). The combined filtrates were evaporated to give a crude brown solid. The solid was purified using preparative layer chromatography (silica gel, 1% methanol in dichloromethane) to yield the title compound (64) (0.012 g, 22%) as a yellow solid.

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(measured MH$^+$ 235.0872, calculated for C$_{15}$H$_{10}$N$_2$O+H 235.0872) mp 179-181 °C
(lit.$^{52}$ 180 °C);

$^1$H-NMR (CDCl$_3$) δ: 6.68 (dd, 1H, J= 3.6, 1.8 Hz, H-4'), 7.29- 7.34 (m, 2H, ArH),
7.58-7.60 (m, 2H, ArH and H-3'), 7.74 (dd, 1H, J= 1.8, 0.6 Hz, H-5'), 7.89 (d, 1H, J= 4.8 Hz, H-4), 8.14 (d, 1H, J= 7.8 Hz, H-8), 8.46 (d, 1H, J= 5.1 Hz, H-3), 9.40 (br. s, 1H, NH)

$^{13}$C-NMR(CDCl$_3$) δ: 108.9 (C-4'), 111.8, 112.6 (C-8 and C-3'), 113.9 (C-4), 120.4
122.0, 122.1 (C-4b, C-5 and C-6), 128.9 (C-7), 130.8 (C-4a), 132.0 (C-9a), 135.6 (C-
8a), 139.2 (C-3), 143.0 (C-1 and C-5'), 156.0 (C-1')

MS-CI (m/z) 235 [MH]$^+$ 100%

Synthesis of 1,2,3,4-tetrahydro-1-[(3-hydroxymethyl)-2-furyl]-9H-pyrido[3,4-
b]indole (63)

To a stirred solution of tryptamine hydrochloride (0.07g, 0.35 mmol) in absolute ethanol (6 mL) in the dark under nitrogen, at room temperature, was added 3-
(hydroxymethyl)-2-furaldehyde (0.04 g, 0.35 mmol). The solution was heated under reflux for 3 hours. The mixture was cooled and concentrated to dryness in vacuo. The residue was neutralised with 10% aqueous sodium carbonate solution and extracted with dichloromethane (2 x 10 mL). The organic extract was washed with water (2 x 10 mL), dried and concentrated to give a crude brown solid (0.06g). The mixture could not be separated using either flash column or preparative layer chromatography (silica gel, 10% methanol in ethyl
acetate, with 0.1% ammonium hydroxide). A component of the mixture had a MH\(^+\) at m/z 269.1301 (calculated for C\(_{16}\)H\(_{16}\)N\(_2\)O\(_2\)+ H 269.1290) in the high resolution CI-MS.

MS-Cl (m/z) \(\delta\) 269 [MH\(^+\)] 89 %, 161 [tryptamine, MH\(^+\)] 58 %

Possible synthesis of tribulusterine (15)

![Tribulusterine structure](image)

To the crude mixture of compound (63) (0.119 g) in dry xylene (5 mL) was added 10% Pd/C (0.12 g). The mixture was refluxed for 3 hours under nitrogen. The mixture was filtered off, the black mass was washed with hot xylene (3 x 5 mL). The filtrate was evaporated to give a crude brown solid. The solid was partially purified using preparative layer chromatography (silica gel, 3% methanol in ethyl acetate) to yield a yellow solid fraction (7.5 mg, \(R_f = 0.3-0.4\)), which from mass spectral evidence indicated the presence of tribulusterine (15) (measured MH\(^+\) 265.0965, calculated for C\(_{16}\)H\(_{12}\)N\(_2\)O\(_2\)+H 265.0977).

MS-Cl (m/z) 265 [MH\(^+\)] 100 %

Synthesis of Tribulusterine, A Potent Toxic Alkaloid from Tribulus terrestris.
References


*Synthesis of Tribulusterine, A Potent Toxic Alkaloid from Tribulus terrestris.*


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*Synthesis of Tribulusterine, A Potent Toxic Alkaloid from Tribulus terrestris.*
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42. Stolle, K.; Gröger, D., Arch. Pharm., 1968, 301, 561

Synthesis of Tribulusterine, A Potent Toxic Alkaloid from Tribulus terrestris.


*Synthesis of Tribulusterine, A Potent Toxic Alkaloid from Tribulus terrestris.*
Synthesis of Tribulusterine, A Potent Toxic Alkaloid from Tribulus terrestris.


**Appendix**

Table 1: The direct addition of nucleophiles to the C-1 position of β-carbolines.

![Chemical diagram](image)

<table>
<thead>
<tr>
<th>Nucleophiles</th>
<th>Temp. (°C)</th>
<th>Solvent</th>
<th>R</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTBDMS</td>
<td>RT</td>
<td>ether</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Li</td>
<td>-78</td>
<td>THF</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LiO</td>
<td>-78</td>
<td>THF</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Synthesis of Tribulusterine, A Potent Toxic Alkaloid from Tribulus terrestris.*
Table 2: The methylation of methyl lithium to the β-carboline·BF$_3$ complex.

![Diagram showing the reaction of methyl lithium to β-carboline·BF$_3$ complex.]

<table>
<thead>
<tr>
<th>Nucleophiles (molar equiv)</th>
<th>Temp. (°C)</th>
<th>Solvent</th>
<th>R</th>
<th>Yield (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeLi (1)</td>
<td>0</td>
<td>THF</td>
<td>Me</td>
<td></td>
</tr>
<tr>
<td>MeLi (3)</td>
<td>-20</td>
<td>THF</td>
<td>Me</td>
<td>0.8*</td>
</tr>
</tbody>
</table>

* mixture of product and starting material

_Synthesis of Tribulusterine, A Potent Toxic Alkaloid from Tribulus terrestris._
Table 3. Nucleophilic substitution reactions of the β-carboline-N-oxide.BF₃ complex (51).

Table:

<table>
<thead>
<tr>
<th>Nucleophiles (molar equiv)</th>
<th>Temp.(°C)</th>
<th>Solvent</th>
<th>R</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeLi (3)</td>
<td>-20</td>
<td>THF</td>
<td>Me</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>-20</td>
<td>THF</td>
<td>OH</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-20</td>
<td>THF</td>
<td>COOH</td>
<td>-</td>
</tr>
<tr>
<td>(3)</td>
<td>-20</td>
<td>THF</td>
<td>OMe</td>
<td>-</td>
</tr>
</tbody>
</table>

Synthesis of Tribulusterine, A Potent Toxic Alkaloid from Tribulus terrestris.
The $^1$H-NMR Spectrum of tribulusterine (from Professor T-S. Wu, National Cheng Kung University, Taiwan).

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Synthesis of Tribulusterine, A Potent Toxic Alkaloid from Tribulus terrestris.
The IH-NMR of the synthetic reaction