行政院國家科學委員會補助專題研究計畫成果報告

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※　天然物抗真菌抗生素 Pterulinic acid 和 Pterulone 的全合成　※
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計畫類別：☒個別型計畫　　□整合型計畫

計畫編號：**NSC89－2113－M－038－007－**

執行期間：**89 年 12 月 01　日至 91 年 7 月 31 日**

計畫主持人：黃聲東

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計畫參與人員：鄭文斌

執行單位：台北醫學大學醫學系生化學科

中　華　民　國　　91 年 9　月　20 日

Total Synthesis of Novel Antifungal Antibiotic and NADH:Ubiquinone Oxidoreductase Inhibitors, Pterulinic Acid and Pterulone

計畫編號：NSC89－2113－M－038－007－
執行期限： 89 年 12 月 01 日至 91 年 7 月 31 日
主持人：黃聲東
執行機構及單位名稱: 台北醫學大學醫學系生化學科

中文摘要: 從 Pterula sp. 82168 發酵槽中萃取出兩天然物。Pterulinic acid 和 Pterulone. Pterulinic acid 和 Pterulone 主要的化學結構是由一個七環型鏈的 1-benzoxepin 組合而成,爾且含有氯元素,這兩個天然物雖然具有有效的抗真菌功效,卻只有微弱的細胞毒性,而抗真菌的活性是抑止真菌的呼吸系統,因爲這兩個天然物是粒線體菸鹼醯胺泛氧化還原酵素(NADH: ubiquinone oxidoreductase)的抗化劑(inhibitor)。天然物 Pterulinic acid 和 Pterulone 的全合成可由容易取得之起始物 salicylaldehyde，Pterulinic acid 全合成只需五部而 Pterulone 的全合成只需四部。

關鍵詞：粒線體菸鹼醯胺泛氧化還原酵素 pterulone, pterulinic acid, Tandem S_N2/Witting Reaction

Abstract: A concise synthesis of NADH:Ubiquinone Oxidoreductase (Complex I) antagonist pterulone (**1**) and pterulinic acid (**2a** and **b**) is reported. Natural products pterulone and pterulinic acid were prepared in 4 and 5 steps, respectively, known salicylaldehyde.

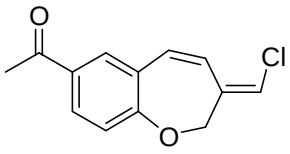
Keywords: NADH:Ubiquinone Oxidoreductase , pterulone, pterulinic acid, Tandem S_N2/Witting Reaction

Introduction

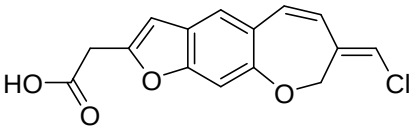
NADH:Ubiquinone oxidoreductase comprises the first phosphorylation site of mitochondria and is the energy-conserving enzyme complex that is commonly known as "complex I".[1] There are a wide variety of natural and synthetic inhibitors of complex I which have found multiple applications.[2] Complex I inhibitors have been used to elucidate the role of this enzyme in normal cell physiology and also have been used to mimic complex I deficiencies in order to study mitochondrial diseases.[3] Inhibitors of complex I have also been a preferred targeted for the development of commercial insecticides and acaricides for years.[4] Recently, it has been shown that inhibition of complex I causes concomitant reduction in the activity of orthine decarboxylase (ODC).[5] ODC is responsible for the biosynthesis of polyamine growth

factors required for cellular prolification.[6] Since the overexpression of ODC in tumor cell contributes to aberrant proliferation, the ability of complex I inhibitors to reduce ODC activity makes them promising candidates as next generation antitumor agents.[7]

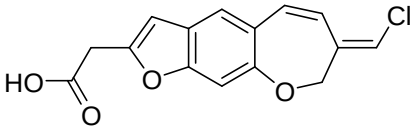
The fungal metabolites pterulone (**1**) and pterulinic acid (**2a** and **b)** were isolated from fermentations of a *Pterula sp* 82168 species.[8] The structures of **1**, **2** were assigned based on their physical and spectral characteresitcs.[8,9] The architectural framework that is common to pterulone and pterulinic acid is a monochlorinated 2,3-dihydro-1-benzoxepine ring skeleton. Pterulinic acid (**2** and **3**) as a 1:5 inseparable mixture of the two isomers (*Z*)-**2a** and (*E*)-**2b**, in addition contains a furan. Pterulone (**1**) and pterulinic acid (**2a** and **b**) exhibited significant antifungal activity, and it is a highly potent inhibitor of complex I with an IC_{50} value of 36 μM and 450μM respectivitly.[8]



Pterulone (1)



(*Z*)-Pterulinic acid (2a)



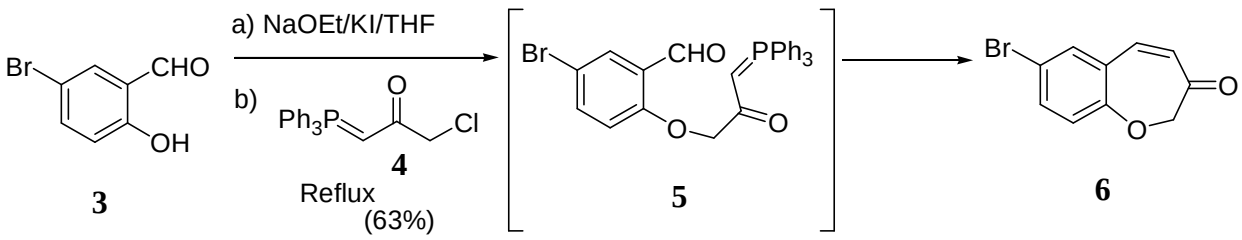
(*E*)-Pterulinic acid (2b)

Synthesis of pterulone and pterulinic acid is compelling due to their complex I antagonist activity, the synthetic challenges posed by their structure, and their status as potential new leads in drug discovery efforts. An extensive survey of the literature did not reveal any efficient methods for the preparation of the 2,3-dihydro-1-benzoxepine ring skeleton, the architectural framework resident in **1**, and **2**. Disclosed herein is the first total synthesis of **1,** and **2** requiring only 4 and 5 steps, respectively, from known salicylaldehyde. Key to the synthesis is a tandem S_N2/ Wittig reaction sequence for construction of the 2,3-dihydro-1-benzoxepine ring skeleton. We believe this approach is very efficient for preparation of the 2,3-dihydro-1-benzoxepine ring skeleton and holds promise for providing future analogues of **1**,and **2**, if desired.

Result and Discussion

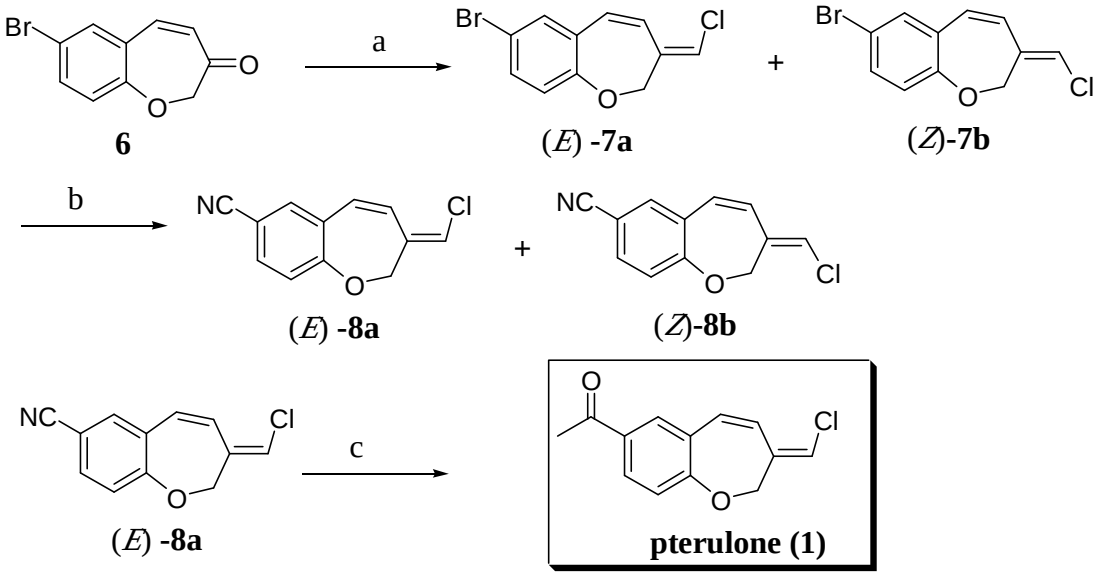
The first step in the synthesis of **1** was the preparation of 7-bromobenzoxepine-3-one (**6**) via a tandem S_N2/Wittig reaction (Scheme I). Treatment of 5-bromosalicylaldehyde (**3**) with 1.2 equivalent of sodium ethoxide generated the corresponding sodium salt of 5-bromosalicylaldehyde; it's subsequent O-alkylation with α-chloroketone (**4**)[10] produced **5**. Intramolecular ring formation via Witting olefination between the tethered triphenylacetophosphorane and the formyl group in **5** gave **6** in 63% overall yield based on **3**.

Scheme I



Next, the vinyl chloride moiety was installed (scheme II). Benzoxepin-3-one (**6)** was treated with chloromethylphosphonium ylide (generated *in situ* with *n*-BuLi) to give **7**, in 78% yield, as an inseparable mixture of 2 diastereomers in a ratio of 1:4, *E*:*Z* (calculated from the integrals in the ^{1}H-NMR spectrum). Treatment of aryl bromides **7** with 10 equivalent of CuCN in refluxing DMF gave aryl nitrile **8** in 64% yield.[11] At this point, the diastereomeric mixture of aryl nitriles **8** could be separated by silica column chromatography (ethyl acetate:hexane, 1:35), and the final assignment of the configuration for purified (*E*)-**8a** and (*Z*)-**8b** was determined by NOE and 2D-heteronuclear correlation experiments. The final transformation to complete the synthesis is outlined in the latter half of scheme II. Treatment of aryl nitrile (*E*)–**8a** with methyl lithium in THF at –30^{o}C produced pterulone (**1**) in 78% yield.[12]

Scheme II

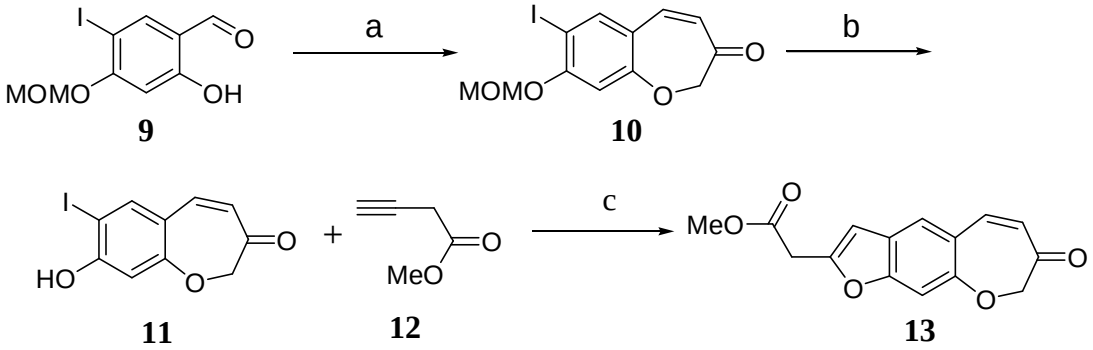


Condition: (a) n-BuLi, $Ph_3PCH_2Cl_2$, THF, rt 78%;

(b) 10 eq. CuCN, DMF, reflux 64%; (c) MeLi,THF,-30^{o}C (78%).

The synthesis of pterulinic acid (**2a** and **b**) was outline in scheme III and IV. The synthesis started with the preparation of benzoxepine-3-one (**10**) via a tandem S_N2/Wittig reaction (Scheme III) from known salicylaldehyde **9**.[13] Treatment of salicylaldehyde **9** with sodium ethoxide, KI, and phosphorane **4** produced **10** in 61% overall yield based on **9**. Removing the MOM group on **10** with 2N HCl gave o-iodophenol **11** in 99% yield. The o-iodophenol **11** underwent palladium-catalyzed heteroannulation with methyl 3-butynoate (**12**)[14] to give the tricyclic **13** in 53% yield.[15] The reaction sequence, tandem S_N2/Wittig reaction followed by palladium-catalyzed heteroannulation effetely constructed the tricyclic **13** which composed the core structure of the pterulinic acid and the essential functional handle for the completion of the synthesis.

Scheme III

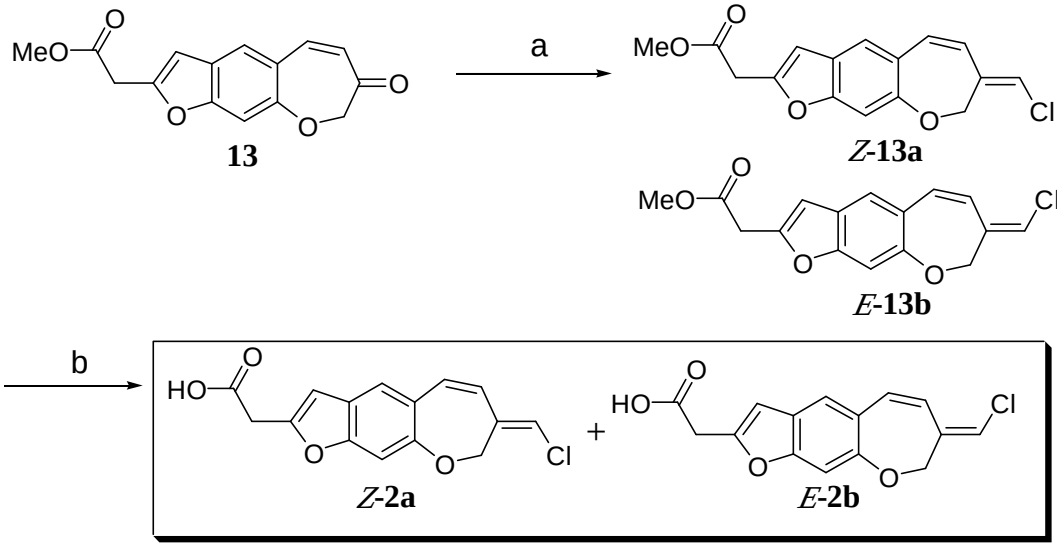


Condition: (a) EtONa, THF, KI, **4** Reflux, 61%; (b) 2N HCl, THF, Rt 99%

(c) 10 mol% $(Ph_3)_3PPdCl_2$, 2eq. CuI, 3eq. Et_3N, DMF, 60^oC 53%

Finally, the vinyl chloride moiety was installed (scheme IV). Benzoxepin-3-one (**13)** was treated with chloromethylphosphonium ylide in 86% yield, as an inseparable mixture of 2 diastereomers in a ratio of 1:5, *E*:*Z* (calculated from the integrals in the ^{1}H-NMR spectrum). Hydrolyzed the methyl ester **13** under basic followed by acidic work up gave pterulinic acid (**2a** and **2b**) in 99% yield.

Scheme IV



Condition: (a) n-BuLi, $(Ph_3)_3PCH_2Cl_2$, THF, Rt, 86%;

(b) 1N NaOH, THF, reflux then HCl, 99%

Conclusion

The spectral and physical characteristics (IR, ^{1}H NMR, ^{13}C NMR, and melting points) of synthetic **1**, **2** were identical to the published data.[8] The synthesis reported herein provide pterulone **1** and pterulinic acid **2** in 4 and 5 steps respectively. This one-pot, tandem S_N2/Witting reaction of salicylaldehyde and phosphorane **4** producing the 1-benzoxepine ring

skeleton is an efficient approach for its construction and this synthetic strategy may be exploited for the preparation of analogues of both natural products.

計畫成果自評

本人的第一件國科會計畫案『天然物抗真菌抗生素 Pterulinic acid 和 Pterulone 的全合成』執行期限爲一年七個月。在有限的計畫執行期間及經費支助下，本人於最短的期間內於台北醫學大學完成建立個人實驗室並期有效的運作。此計畫之最初提案執行並不如預期的順利，雖然花費不少經費於失敗的合成途徑中，在多次的實驗失敗裡學取經驗並修改合成途徑，最終方能運用有限的經費找到最有效率的合成途徑完成此案，並將此案之結果發表第一篇論文於 2001 年 tetrahedron letters，第二篇相關論文正在籌備中。從此次有效的經費及時間管理之下完成此計畫案，使本人更有自信面對未來的計畫挑戰。

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