Pergamon

Tetrahedron Letters 43 (2002) 4095–4098

TETRAHEDRON LETTERS

# Toward the total synthesis of pseudolaric acid B. Preparation of a key intermediate by degradation and its use in the reassembly of the natural product

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Received 13 March 2002; revised 20 March 2002; accepted 25 March 2002

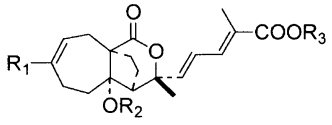
**Abstract**—Synthetic studies of pseudolaric acid B, **2**, provided a relay synthesis of pseudolaric acid B (PLAB) via aldehyde **5**. The aldehyde **5** can serve: to complete the total synthesis of PLAB; as a precursor for the synthesis of PLAB analogs; or as a substrate for the generation of radiolabeled PLAB for mechanistic studies. 

Given the increase in the number of immuno-compromised patients in the last decade, especially from the AIDS epidemic, there has been a concomitant increase in the number of life-threatening mycoses.[1,2] While a number of useful methods exist for the treatment of various fungal infections, as a general rule, these approaches suffer from the all-to-usual drawbacks common to most forms of chemotherapy: poor bioavailability, rapid metabolism, inefficacy due to resistant strains, and in many cases, toxicity to the host.[3] Therefore, there is a critical need for new antifungal agents that are more potent, fungicidal, and less toxic.

The extract of the root bark of *Pseudolarix kaempferi* is a Chinese herbal medicine called Tu Jin Pi, which has been used for many years against fungal infections of the skin and nails. Studies on the bark of this plant have led to the isolation of several novel diterpene acids, namely pseudolaric acids A (PLAA, **1**), B (PLAB, **2**), C (PLAC, **3**) and B-Glycoside (PLAB-Gly, **4**)[4–6] (Fig. 1).

Recent studies have revealed that pseudolaric acid B displays strong activities against *Candida* species, *Totulopsis petrophilum*, *Trichophyton mentagrophytes*, and *Microsporum gypseum*.[7] PLAB shows in vitro cytotoxicity against several human tumor cell lines.[8] In preliminary studies, we have demonstrated that the mechanism of action of PLAB may be unique and involve a direct interaction and/or an action on the phosphorylation state of PPAR isoforms.[9] PLAB has also shown contraceptive effects in mice.[10]

The structure of the pseudolaric acids was elucidated by spectroscopic analysis and X-ray diffraction.[11,12] The unusual structural feature of these diterpenoic acids is the tertiary acetoxy group (or hydroxyl group), which is *trans* to the lactone with a fused hydroazulene skeleton (Fig. 1). Because of its structural features and biological activity, PLAB has attracted considerable interest. To date, three synthetic studies on the total synthesis of pseudolaric acids have been reported.[13–15] However, the total synthesis of the pseudolaric acids remains unaccomplished.




| | | | | |
|---|---|---|---|---|
| **1** | $R_1$ = Me | $R_2$ = Ac | $R_3$ = H | PLAA |
| **2** | $R_1$ = COOMe | $R_2$ = Ac | $R_3$ = H | PLAB |
| **3** | $R_1$ = COOMe | $R_2$ = H | $R_3$ = H | PLAC |
| **4** | $R_1$ = COOMe | $R_2$ = Ac | $R_3$ = *D*-Gly | PLAB-Gly |

**Figure 1.** Structure of pseudolaric acids (PLA's).

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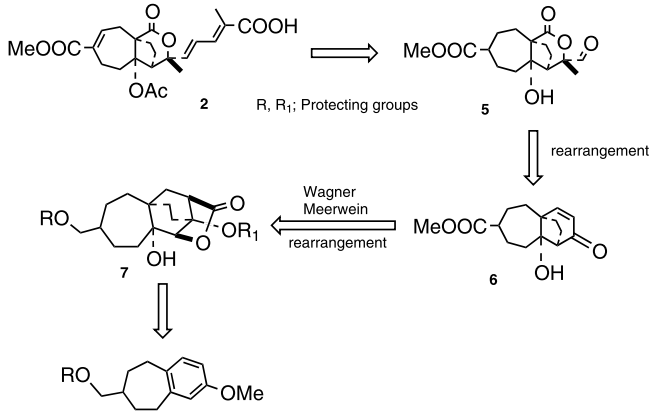
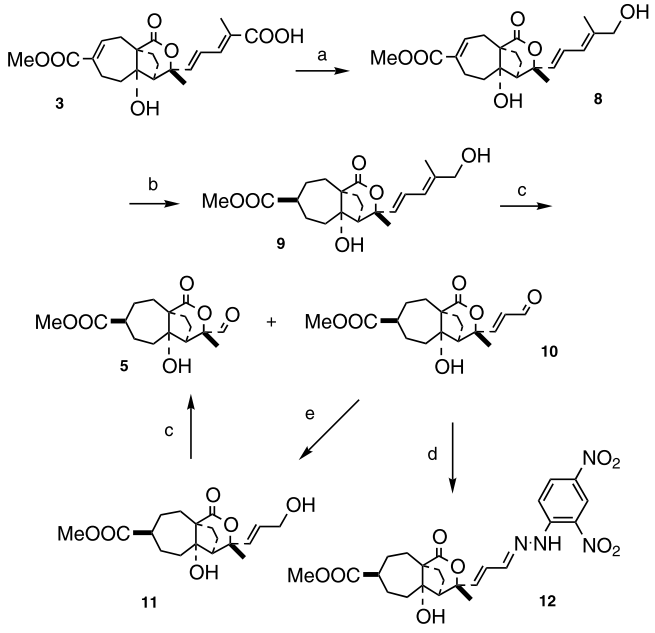
0040-4039/02/$ - see front matter 
PII: S0040-4039(02)00596-8

Based on these observations, we have been engaged in the semi-synthesis and total synthesis of PLAB (**2**) and numerous analogs, with the goal of performing structure–activity relationships in the pursuit of potent antifungals. Our total synthetic plan was based on the fact that the tricyclic core of PLAB (**5**) could be accessed through a Wagner–Meerwein-type rearrangement[16] from intermediate **7**, followed by an oxidative ring opening and subsequent rearrangement of **6** (Scheme 1). Herein, we disclose the first part of our relay synthesis of PLAB, employing **5** as the key intermediate.

Intermediate **5** could be obtained by degradation of PLAC (**3**) as shown in Scheme 2. (We chose PLAC for degradation to **5** over PLAB because it was found in higher yields during isolation of *P. kaempferi* bark). Selective reduction of acid **3** to alcohol **8** in the presence of an ester was accomplished by conversion of the acid to the acid chloride ($SOCl_2$), followed by reduction with 2 equiv. of $LiAlH(OCMe_3)_3$ (72%, two steps).[17] Direct ozonolysis of triene **8** gave a complex mixture of products even in the presence of pyridine.[18] Therefore, the *endo* double bond of **8** was selectively reduced with Mg in dry methanol[19] (70%). (The stereochemistry of alcohol **9** was assigned accordingly at a later stage). Subsequent ozonolysis of diene **9** provided aldehyde **10** after reductive workup (63%), along with a small amount of aldehyde **5** (9%). Excess ozone and longer reaction times resulted in an inferior yield of **10** with no subsequent increase in the yield of **5**. Aldehyde **10** was then reduced with $LiAlH(OCMe_3)_3$ to give allylic alcohol **11**, which was subjected to ozonolysis to give intermediate **5** (67%, two steps), thus marking the completion of the degradation stage of our relay synthesis.




**Scheme 1.** Retrosynthetic analysis of PLAB (**2**).




**Scheme 2.** *Reagents and conditions*: (a) (i) $SOCl_2$, 70°C; 15 min, (ii) THF, −78°C, 2 equiv. $LiAlH(OCMe_3)_3$; (b) Mg, MeOH, rt, 12 h; (c) $O_3$, $CH_2Cl_2$, −78°C, then $Me_2S$; (d) $2,4\text{-}(NO_2)_2C_6H_3NHNH_2$, DMF, cat. HCl; (e) THF, −78°C, 1.1 equiv. $LiAlH(OCMe_3)_3$.

In order to determine the stereochemistry of alcohol **9**, aldehyde **10** was condensed with 2,4-dinitrophenylhydrazine to form the 2,4-DNP hydrazone **12**. Single crystal X-ray analysis of this derivative revealed that the methoxycarbonyl group of **12** has the relative configuration shown in Fig. 2.[20] Based on these findings, the relative stereochemistry of **9** could be assigned accordingly, as shown in Scheme 2.

With intermediate **5** in hand, we began the process of synthesizing PLAB. The first step involved acetylation of the sterically hindered tertiary hydroxyl group (Scheme 3). Not surprisingly, the acetylation of **5** was not successful using routine conditions ($Ac_2O$, pyridine, DMAP). Under dual activation conditions,[21] the reaction also failed to provide the desired product. However, when **5** was treated with scandium triflate and

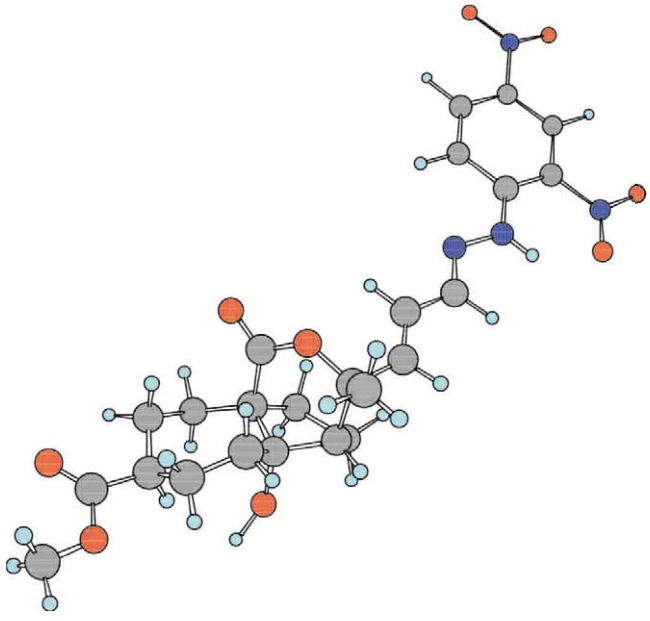
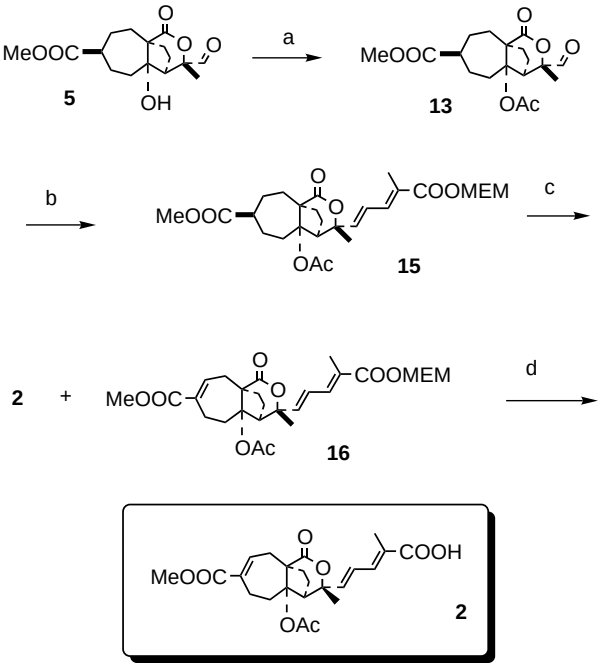


**Figure 2.** X-Ray crystallographic structure of 2,4-DNP-hydrazone **12**. Other than the hydroxyl proton located in the electron density difference map, the hydrogen atoms are shown in idealized positions using CS Chem3D Ultra (CambridgeSoft Corp., v 6.0) for visualization.




**Scheme 3.** *Reagents and conditions*: (a) $Ac_2O$, MeCN, $Sc(OTf)_3$, −20 to 0°C; (b) $(EtO)_2P(O)CH_2CHC(Me)$-COOMEM (**14**), *n*-BuLi, THF, −20 to 0°C; (c) KHMDS, THF, −78°C, then PhSeCl, −23°C, then AcOH, $H_2O_2$; (d) AcOH, THF, $H_2O$, rt.

$Ac_2O$ in acetonitrile,[22] acetate **13** was obtained in 34% yield (unoptimized) along with starting material. Aldehyde **13** was then subjected to a Wittig reaction to rebuild the side chain, affording diene **15** (53%). Finally, regeneration of the *endo* double bond was accomplished by treatment of ester **15** with potassium hexamethyldisilazane (KHMDS) and PhSeCl followed by oxidative elimination,[23] to give pseudolaric acid B (**2**) as the major product (31%),[24] along with **16** (11%) and unreacted **15** (25%). Recovered **16** was treated with acid to complete the conversion of **15** to PLAB (**2**).[25]

In summary, we have described part of the relay synthesis of PLAB, employing **5** as the key intermediate. The acetyl group was forged onto the hindered tertiary hydroxyl group, and the side chain was built up in a single step via a Wittig reaction. Selenylation followed by oxidative elimination gave the desired *endo* double bond. This relay synthesis is important in that it provides a synthetic route for PLAB (**2**), for the synthesis of PLAB analogs, and for radiolabeling of PLAB (**2**) for upcoming mechanistic studies. The remaining steps in our relay synthesis are forthcoming. Furthermore, we are working to optimize and improve the acetylation of the hindered tertiary alcohol. These findings will be published separately.

## Acknowledgements

The authors would like to thank Dr.(s) Erguang Li and Xing-Cong Li for their assistance with the natural product isolation process.

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24. In regards to the unexpected selectivity in the selenation–deselenation sequence leading from ester **15** to the naturally-configured, unsaturated ester ($\Delta^{1,2}$) **2**, we conducted an extensive comparison of all low energy intermediates, first by MM2, and then by semi-empirical methods (AM1). The respective enolate conformers leading to β-selenation or α-selenation are of similar energies, as are the α- and β-phenylselenyl rotomers (two each), occurring

in boat- and chair-like conformers of the 7-membered ring. Furthermore, the energies ($\Delta H_f$) for natural eneoate ($\Delta^{1,2}$) versus the unnatural ($\Delta^{1,7}$) are almost identical (<0.2 kcal/mol). The only significant difference between the two selenoxide elimination pathways is the bond number from the methylene undergoing elimination to the closest oxygen atom. Thus, C-2 is 4 bonds to the lactone oxygen; while C-7 is 3 bonds to the acetate oxygen atom. Therefore, under transition state control, $\Delta^{1,7}$ formation is less favorable than $\Delta^{1,2}$ formation. While this result is unusual, selenoxide eliminations do occur away from β-oxygen substituents, and heavily oxygenated systems require heating to effect selenoxide elimination. For a discussion on selenoxide eliminations see the following and references cited within: (a) Engman, L. *J. Org. Chem.* **1989**, *54*, 884–890; (b) Reich, H. J.; Wollowitz, S.; Trend, J. E.; Chow, F.; Wendelborn, D. F. *J. Org. Chem.* **1978**, *43*, 1697–1705; (c) Boullais, C.; Zylber, N.; Xylber, J.; Guilhem, J.; Gaudemer, A. *Tetrahedron* **1983**, *39*, 759–765.

25. The synthetic sample of pseudolaric acid B (**2**) was identical to an authentic sample of the natural product as judged by $^{1}H$, $^{13}C$ NMR, FTIR and LCMS.