

Regeneration of Pinusolide from Its 17-Nor-8-oxo Derivative

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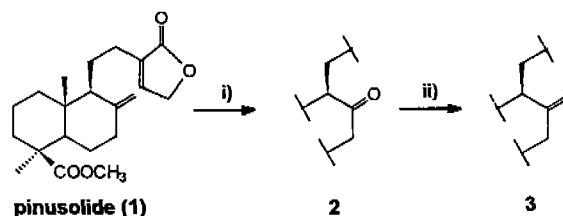
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Abstract - For metabolic study of pinusolide, a naturally occurring platelet activating factor antagonist, a synthetic method for preparation of radiolabeled pinusolide was studied. Pinusolide was first oxidized with OsO₄/NaIO₄ to 17-nor-8-oxo compound (**2**), which was subsequently converted to pinusolide by treatment with the Lombardo reagent (Zn/CH₂Br₂/TiCl₄). The Wittig reaction was unsuccessful in the latter carbonyl methylenation of **2**.

Keywords □ pinusolide, PAF antagonist, wittig reaction, Lombardo reagent.

Pinusolide (**1**) is a platelet activating factor antagonist, which was isolated from the leaves of *Biota orientalis* (Yang *et al.*, 1995a). It showed a potent *in vivo* protective activity in mice when challenged by PAF injection and also possessed a strong topical anti-inflammatory activity (Yang *et al.*, 1995b). In this study, a synthetic method for radiolabeled pinusolide required for ADME study was investigated. In synthesis of a radiolabeled compound, selection of a suitable functional group in which radioactivity is incorporated with ease is important. We envisioned that the exocyclic olefin group in pinusolide may be oxidized to a ketone group and, in subsequent carbonyl methylenation, radioactivity can be incorporated with suitable reagents (Scheme 1). For the latter carbonyl methylenation, various methods are known including the Wittig reaction (March, 1992) and Peterson olefination (Ager, 1984). This can also be accomplished by reactions with the Lombardo reagent (Lombardo, 1982) or Tebbe reagent (Nicolaou *et al.*, 1996).

In the Wittig reaction an aldehyde or ketone is treated with a phosphorus ylide to give an olefin. Phosphorus ylides are normally prepared by treatment of a phosphonium salt with a base such as *n*-BuLi (Lee *et al.*, 1994) or methylsulfinyl carbanion, which is reported to be highly selective towards ketonic function of keto esters (Greenwald *et al.*, 1963). Alternatively, an organometallic reagent prepared from CH₂Br₂ (or CH₂I₂), Zn and TiCl₄, the Lombardo reagent, can be used in methyle-



Scheme 1. Synthetic strategy for radiolabeled pinusolide.

nation of, especially, easily enolizable ketones where the Wittig reaction often fails (Takai *et al.*, 1978; Lombardo, 1982). Reaction with CH₂Br₂, Zn and TiCl₄ is known for its selectivity to ketones (Takai *et al.*, 1978).

In this study, the Wittig reaction and olefination with the Lombardo reagent were applied to convert the 17-nor-8-oxo compound (**2**) to pinusolide (**1**) (reaction (ii) in Scheme 1).

MATERIALS AND METHODS

Chemicals

Pinusolide was isolated from the leaves of *Biota orientalis* as described earlier (Yang *et al.*, 1995a). All other chemicals were obtained from Aldrich (Milwaukee, WI, USA) and Sigma (St. Louis, MO, USA).

Synthesis of 17-nor-8-oxo-13-labden-16,15-olid-19-oic acid methyl ester (**2**)

2 was prepared by a known procedure (Demuth *et al.*, 1986). **1** (1 g, 2.9 mmol) and NaIO₄ (1.24 g, 5.8 mmol) were dissolved in a mixture of dioxane-H₂O (1:1, 40 mL), and 2.8 mL of an OsO₄ solution (1 g of OsO₄ in

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100 mL of dioxane-H₂O, 1:1) was added. After 24 hr stirring at room temperature, the reaction was quenched by addition of 20 mL of saturated Na₂SO₃ solution, and following filtration with celite, the filtrate was extracted with EtOAc (50 mL × 3). The organic layer was then washed with brine and dried over Na₂SO₄. Chromatography on silica gel (CHCl₃:MeOH=10:1) afforded white crystals of **2** (0.65 g, 64.3% yield): mp 131-132°C. Anal. (C₂₀H₂₈O₅) C, H, N: C: calcd, 68.94; found, 69.19; H: calcd, 8.10; found, 8.30. Mass (EI, m/z, 70 eV, rel. int. %): 348 (M⁺, 4.1), 333 (9.1), 289 ([M-CH₃COO]⁺, 4.4), 273 (10.4), 251 (32.2), 238 (43.8), 223 (100), 191 (11.2), 121 (38.0). HRMS: calcd, 348.1929; found, 348.1942. ¹H-NMR (CDCl₃, 300 MHz): δ 0.54 (3H, s, -CH₃), 1.26 (3H, s, -CH₃), 3.62 (3H, s, -OCH₃), 4.76 (2H, dd, *J*=0.8, 2.1 Hz, lactonyl=CH-CH₂-O-), 7.10 (1H, t, *J*=0.8 Hz, lactonyl -C=CH-). ¹³C-NMR (CDCl₃, 125 MHz): 13.12, 19.71, 20.19, 24.95, 25.71, 28.84, 38.03, 39.56, 43.11, 43.67, 44.43, 51.50, 55.01, 62.62, 70.29, 134.58 (lactonyl -C=CH-), 144.72 (lactonyl -C=CH-), 174.68 (lactonyl -COO-), 177.38 (-COOCH₃), 211.66 (-C(=O)-).

Synthesis of pinusolide from compound **2** : the Wittig reaction

(1) *n*-BuLi as the base: To a solution of Ph₃P⁺CH₃I⁻ (0.13 g, 0.32 mmol) in THF was added dropwise 0.18 mL of *n*-BuLi (0.32 mmol, 1.6 M hexane solution) at 0°C under N₂ atmosphere. After 30 min stirring at room temperature, **2** (0.1 g, 0.29 mmol) in THF was slowly added to the reaction mixture. Production of pinusolide was monitored by TLC (*n*-hexane:EtOAc=2:1).

(2) Methylsulfinyl carbanion as the base: NaH (0.01 g, 0.37 mmol) was thoroughly washed with hexane under N₂ and dissolved in 2 mL DMSO at 30°C. To this solution, Ph₃P⁺CH₃I⁻ (0.13 g, 0.32 mmol) in THF was added while stirring. After 30 min at room temperature, **2** (0.1 g, 0.29 mmol) in THF was slowly added to the reaction mixture. Production of pinusolide was monitored by TLC (*n*-hexane:EtOAc=2:1).

Synthesis of pinusolide from compound **2** with the Lombardo reagent

CH₂Br₂ (0.03 mL, 0.43 mmol) and TiCl₄ (0.086 mL, 0.086 mmol, 1.0 M CH₂Cl₂ solution) were added to zinc (0.05 g, 0.77 mmol) suspension in 1 mL THF under N₂. The reaction mixture was stirred for 15 min until it turned dark brown, and **2** (0.03 g, 0.086 mmol) in 1 mL THF was added. After 2 hr, 10 mL of 1 N HCl was added to the reaction mixture and the solution was further stirred

for 10 min. The ether extract was washed with brine and dried over Na₂SO₄. Chromatography on silica gel (*n*-hexane:EtOAc=5:1, R_f=0.2) afforded compound **3** (0.02 g, 33.6 % yield), of which ¹H-NMR, Mass and HPLC data were identical to those of authentic pinusolide.

HPLC analysis

The reactions of **2** with the Lombardo reagent using varying amounts of TiCl₄ were analyzed by HPLC to find the molar ratio of the reagents resulting in high yields. After 2 hr reaction, 0.05 mL of the reaction solution was mixed with 0.05 mL of 1 N HCl and 0.9 mL of EtOAc. A 10 μL aliquot of the resulting solution was injected onto a reversed-phase column (COSMOSIL 5C18, 4.6 × 150 mm, nacalai tesque, Kyoto, Japan). The samples were eluted in isocratic conditions with a mobile phase of 33% (v/v) acetonitrile in water at 1.0 mL/min flow rate. Detection was made with UV at 220 nm. The retention times of **2** and pinusolide were 4.5 min and 13 min, respectively. Calibration curves of the compounds were prepared and linearity was found over the investigated concentration range.

RESULTS AND DISCUSSION

Synthesis of compound **2**

The exocyclic double bond in pinusolide was first oxidized to a glycol, which was then cleaved to yield a ketone in **2**. Two olefinic proton peaks at δ 4.56 and 4.87 of pinusolide (Yang *et al.*, 1995a) were absent in **2**. In ¹³C-NMR of **2**, a peak at 108 ppm corresponding the exocyclic methylene carbon (=CH₂) in pinusolide disappeared and a peak at 148 ppm was shifted to 207 ppm. High resolution mass spectra and elemental analysis confirmed the structure of **2**.

Conversion of compound **2** to pinusolide

Compound **2** remained unreacted when subjected to the Wittig reaction under various conditions. When increased amounts of CH₃Ph₃P⁺I⁻ and base (*n*-BuLi and methylsulfinyl carbanion) were used to force the reaction, **2** was converted to unidentified byproducts. The Wittig reagent can function as a base to remove the α-protons of easily enolizable ketones (Hibino *et al.*, 1985) and, when used in excess, it can also react with esters (Uijtewaal *et al.*, 1978).

Therefore, it was thought that steric hindrance due to the axial methyl group at β-position and the bulky alkyl branch at α-position prohibited the access of ylide to the carbonyl carbon and that excessive base might have promoted enolization giving rise to side reactions.

Table I. Regeneration of pinusolide from compound **2** (carbonyl methylenation) with Zn/CH₂Br₂/TiCl₄.

run	Zn (eq.)	CH ₂ Br ₂ (eq.)	TiCl ₄ (eq.)	Yield (%) ^a	Recovery of 2
1	9	5	0.5	7.0	37
2	9	5	1	30	12
3	9	5	2	7.0	1.0

^aThe amounts of product (pinusolide) and the remaining, unreacted compound **2** were determined by HPLC using standard curves. Detailed method for HPLC analysis is described in the text. Yield (%)=(amt. of pinusolide produced/amt. of **2** added)×100. Recovery (%)=(amt. of **2** recovered/amt. of **2** added)×100.

On the other hand, **2** was successfully converted to pinusolide with the Lombardo reagent, albeit the yield was not high. It was reported that the Lombardo reagent, which is related to the Simmons-Smith reaction (Simmons *et al.*, 1973; Friedrich *et al.*, 1989), did not proceed to give cyclopropanes from olefins, but, molar ratio of TiCl₄ to the substrate should be carefully controlled especially when the substrate bears Lewis acid sensitive functional groups (Takai *et al.*, 1978). In fact, major byproducts of the reaction of pinusolide with the Lombardo reagent were UV-positive but Dragendorff-negative, indicating destruction of the lactone ring. The conjugated lactone group in pinusolide gave a strong false-positive alkaloid reaction with Dragendorff's spray reagent (Habib, 1980).

To improve the reaction yield, the reaction was carried out using varying amounts of the reagents and the reaction products were analyzed by HPLC. Addition of increased amounts of CH₂Br₂ in the reaction mixture showed little effects on consumption of **2** and production of pinusolide. However, the molar ratio of TiCl₄ to **2** proved to be critical. As shown in Table I, at molar ratio of 1 to 1 (run 2), the yield of pinusolide was highest at 30%. Use of greater than 1 eq. TiCl₄ resulted in decreased yield of pinusolide and increased byproducts production. Efforts to significantly increase the yields of the reaction by changing reaction temperature and duration were unsuccessful.

In conclusion, a simple two step reaction scheme involving OsO₄/NaIO₄ oxidation and carbonyl methylenation with the Lombardo reagent (¹⁴CH₂Br₂ or CT₂Br₂, Zn and TiCl₄) was proposed for the synthesis of radiolabeled pinusolide.

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REFERENCES

- Ager, D. J. (1984). The Peterson reaction. *Synthesis* 384-398.
- Demuth, M., Ritterskamp, P., Weigt, E. and Schaffner, K. (1986). Total synthesis of (-)-coriolin. *J. Am. Chem. Soc.* **108**, 4149-4154.
- Friedrich, E. C., Lunetta, S. E. and Lewis, E. J. (1989). Titanium(IV) chloride catalyzed cyclopropanations of alkenes using zinc dust, copper(I) chloride and dihalomethanes. *J. Org. Chem.* **54**, 2388-2390.
- Greenwald, R., Chaykovsky, M. and Corey, E. J. (1963). The Wittig reaction using methylsulfinyl carbanion-dimethyl sulfoxide. *J. Org. Chem.* **28**, 1128-1129.
- Habib, A. M. (1980). False-positive alkaloid reactions. *J. Pharm. Sci.* **69**, 37-43.
- Hibino, J. I., Okazoe, T., Takai, K. and Nozaki, H. (1985). Carbonyl methylenation of easily enolizable ketones. *Tetrahedron Lett.* **26**, 5579-5580.
- Lee, E., Lee, Y. R., Moon, B., Kwon, O., Shim, M. S. and Yun, J. S. (1994). Oxyanion orientation in anionic oxycope rearrangements. *J. Org. Chem.* **59**, 1444-1456.
- Lombardo, L. (1982). Methylenation of carbonyl compounds with Zn-CH₂Br₂-TiCl₄. Application to gibberellins. *Tetrahedron Lett.* **23**, 4293-4296.
- March, J., *Advanced Organic Chemistry*, 4th ed., pp. 956-960, John Wiley & Sons, New York, 1992.
- Nicolaou, K. C., Postema, M. H. D. and Claiborne, C. F. (1996). Olefin metathesis in cyclic ether formation. Direct conversion of olefinic esters to cyclic enol ethers with Tebbe-type reagents. *J. Am. Chem. Soc.* **118**, 1565-1566.
- Simmons, H. E., Cairns, T. L. and Vladuchick, S. A. (1973). Cyclopropanes from unsaturated compounds, methylene iodide, and zinc-copper couple. *Org. React.* **20**, 1-83.
- Takai, K., Hotta, Y., Oshima, K. and Nozaki, H. (1978). Effective methods of carbonyl methylenation using CH₂I₂-Zn-Me₃Al and CH₂Br₂-Zn-TiCl₄ system. *Tetrahedron Lett.* 2417-2420.
- Ujttewaal, A. P., Jonkers, F. L. and van der Gen, A. (1978). Reactions of esters with phosphorus ylides. *J. Org. Chem.* **43**, 3306-3311.
- Yang, H. O., Suh, D. Y. and Han, B. H. (1995a). Isolation and characterization of platelet-activating factor receptor binding antagonists from *Biota orientalis*. *Planta Med.* **61**, 37-40.
- Yang, H. O., Kang, Y. H., Suh, D. Y., Kim, Y. C. and Han, B. H. (1995b). Biological and pharmacological effects of pinusolide, a novel platelet activating factor antagonist. *Planta Med.* **61**, 519-522.