Pergamon

Tetrahedron Letters 43 (2002) 1265–1268

TETRAHEDRON LETTERS

Total synthesis of pinellic acid, a potent oral adjuvant for nasal influenza vaccine. Determination of the relative and absolute configuration

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Received 12 November 2001; revised 3 December 2001; accepted 7 December 2001

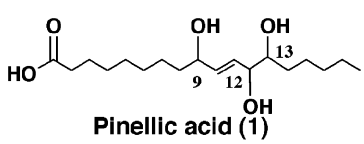
Abstract—Pinellic acid (**1**), isolated from a medicinal plant *Pinelliae tuber*, has potent adjuvant activity. The absolute configuration of pinellic acid was expected by derivatization of this compound and CD exciton chirality method. A convergent synthetic route to pinellic acid has been developed via regioselective asymmetric dihydroxylation and stereoselective reduction. The absolute configuration of pinellic acid was determined to be 9*S*,12*S*,13*S*, by comparing with the spectra data of natural and synthetic compounds.

Influenza virus infection is epidemic, and sometimes critical for patients with respiratory diseases and aged persons with respiratory diseases. Influenza vaccine is useful as prophylaxis of influenza virus infection. Intranasal inoculation of influenza vaccine has been tried to increase its safety and to prevent antigenic variation of influenza viruses. Nonetheless, it has generally been considered that intranasal inoculation of the vaccine alone cannot readily induce high levels of antibodies. Therefore, the development of effective adjuvants for nasal influenza vaccine is necessary to enhance the potency of the vaccine.

In our program to discover effective adjuvants, Kampo medicine, 'Sho-seiryu-to' was found to possess potent adjuvant activity by oral administration on nasal influenza infection and nasal influenza vaccination.[1–3] Furthermore, our research has made it clear that the activity of 'Sho-seiryu-to is expressed by ingredients from *Pinelliae tuber*, one of the component herbs of 'Sho-seiryu-to'. Pinellic acid **1** has been isolated from *Pinelliae tuber* as an active compound of the adjuvant activity[4] (Fig. 1).

Although **1** is an effective oral adjuvant for nasal influenza vaccine, *Pinelliae tuber* contains a small amount of **1** and the stereochemistry remained unknown. Although 10*E*-9,12,13-trihydroxyoctadecenoic acid as **1** has been isolated from natural sources, we were not able to determine its relative and absolute stereochemistry because of lack of detailed reports regarding its stereochemistry.[5] Herein, we report the enantioselective total synthesis and assignment of the absolute stereochemistry of **1**.

First, to determinate the relative configuration of 12,13-diol, esterification of **1** followed by dimethylacetalization gave acetonide **2** (Scheme 1). The coupling



Pinellic acid (1)

Figure 1. Structure of pinellic acid **1**.

Keywords: adjuvant; 10*E*-9,12,13-trihydroxyoctadecenoic acid; asymmetric dihydroxylation; BINAL-H.

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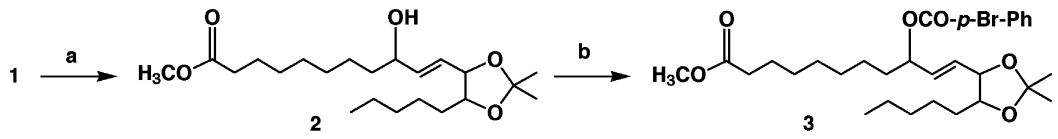
0040-4039/02/$ - see front matter
PII: S0040-4039(01)02348-6



Scheme 1. Derivatization of **1**. *Reagents and conditions*: (a) (1) $TMSCHN_2$, benzene:MeOH (10:1), rt, 2.5 h, (2) 2,2-dimethoxypropane, PPTS, CH_2Cl_2, 60°C, 48 h, (100% from **1**); (b) *p*-Br-BzCl, DMAP, pyridine, (68%).

constant ($J_{12,13}=8.0$ Hz) in the ^{1}H NMR spectrum of **2** and NOE analysis indicates *syn* configuration at 12,13-diol (Fig. 2).

On the other hand, to establish the absolute configuration of C9 by CD exciton,[6] the corresponding *p*-bromobenzoate **3** was prepared. The coupling constant between 9*H* and 10*H* in the ^{1}H NMR spectrum of **3** was 7.0 Hz, indicating antiperiplanar conformation of these two protons. Moreover, the CD spectrum of **3** shows a positive Cotton effect [λ_{max} ($\Delta\varepsilon$): 244.8 (+6.97), 220.8 (+2.13), 209.1 (+5.97) (MeOH)], which suggested 9*S* configuration (Fig. 3).[7]

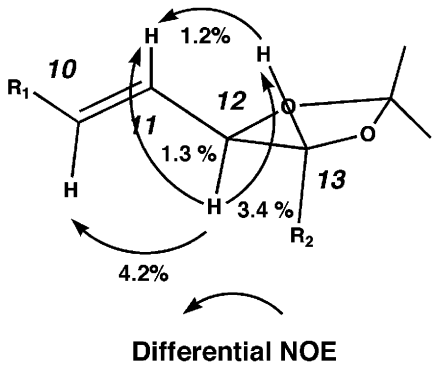


Figure 2. NOE experiment of **2**.

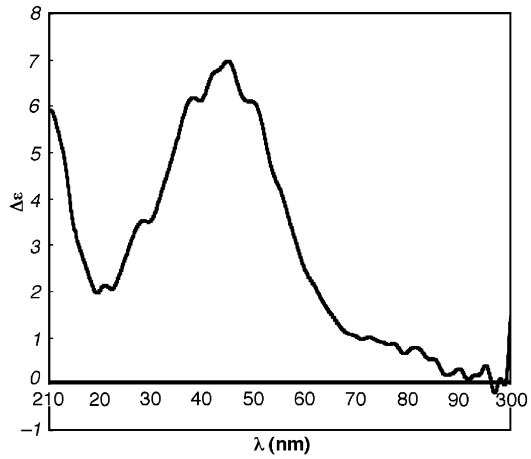


Figure 3. CD spectrum of **3**.

Based on these results the absolute configuration of **1** was assumed as **4** (9*S*,12*S*,13*S*) or **5** (9*S*,12*R*,13*R*) (Fig. 4). Accordingly, we tried to establish a convergent synthetic route to **4** and **5**.

The strategic disconnection is outlined in Fig. 5. The main problem should be the construction of stereochemistry of three hydroxy groups. We planned to construct the *syn* diol at C12-13 by regioselective asymmetric dihydroxylation.[8] The allylic alcohol at C9 could be prepared from enone by stereoselective reduction.

The synthesis of C18 skeleton **11** is shown in Scheme 2. The esterification of suberic acid mono methylester **6** with $(Boc)_2O$, DMAP in *t*-BuOH furnished *tert*-butylester **7**. The diester **7** was converted to iodide **8** in good yield via three steps: (1) hydrolysis of methyl ester, (2) reduction of carboxylic acid[9] and (3) iodination of the prepared primary alcohol. The dithiane coupling[10] of **10** derived from commercially available **9** with **8** gave diene **11** in high yield. We next introduced hydroxy groups to the C18 skeleton (Scheme 3).

The regioselective asymmetric dihydroxylation of **11** by using modified Sharpless ligand (DHQ(PHAL)DHQ·Me^+I^-)[8] furnished 12,13 syn diol (−)-**12** which has 12*S*,13*S* in 75% yield with 95% e.e. followed by protection of diol with TBSOTf.[12] Deprotection of dithioacetal (+)-**13** provided enone (+)-**14**. The reduction of enone (+)-**14** was reported.[13] We found (*S*)-BINAL-H[14] was the best reagent for stereoselective reduction of (+)-**14** to furnish 9*S* alcohol[11] (diastereoselectivity; >20:1). The desilylation with TBAF gave triol (+)-**15** as a single isomer. The hydrolysis of *tert*-butyl ester by a highly concentrated alkaline solution afforded (−)-**4**[15] which has 9*S*,12*S*,13*S* configuration. (−)-**4**[15] was identical in all respects with natural product **1**. [400 MHz ^{1}H and 100 MHz ^{13}C NMR, IR, HRMS, optical rotation {$[\alpha]_D^{25}$ −8.0 (*c* 0.30, MeOH), natural;[4] $[\alpha]_D^{28}$ −8.1 (*c* 0.32, MeOH)}, and oral adjuvant activity].

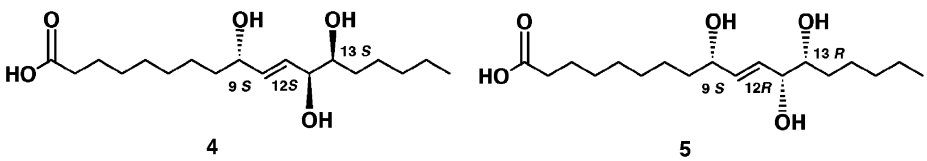


Figure 4. Assumable structure of **1**.

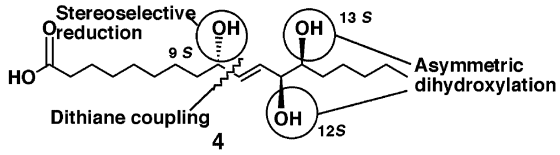
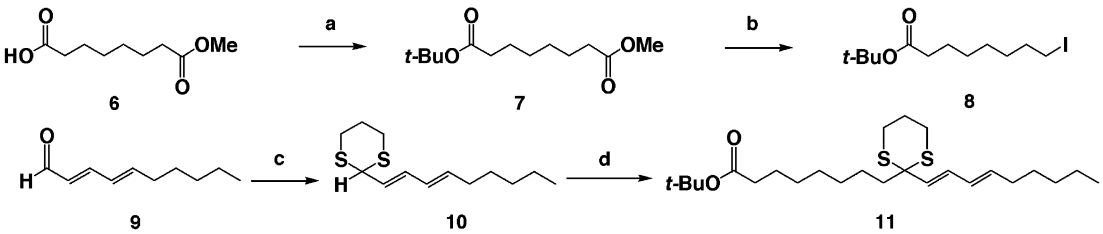
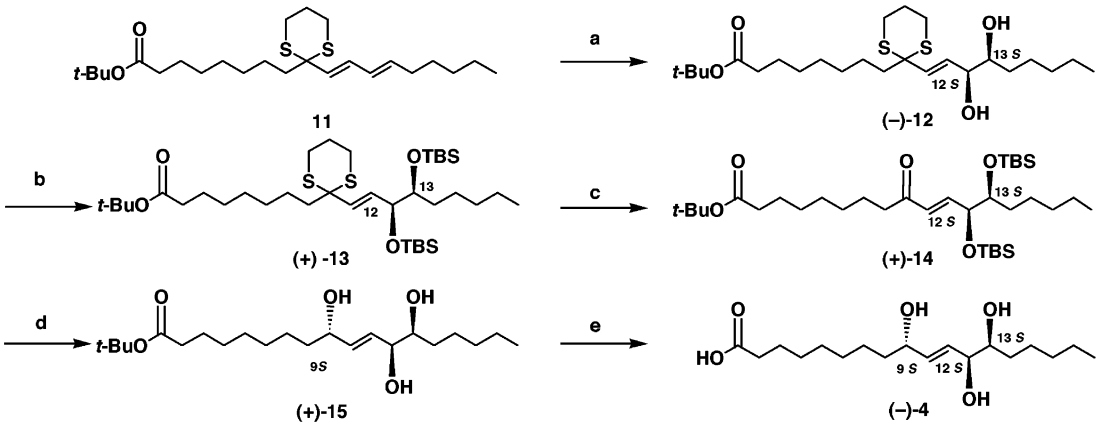


Figure 5. Synthetic strategy of **4**.

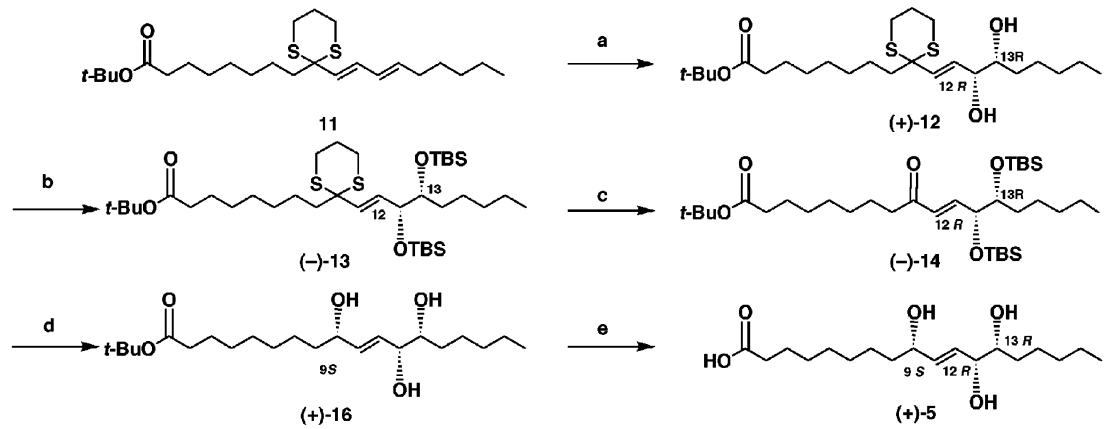
On the other hand, for the synthesis (+)-**5** according to the foregoing synthetic route (Scheme 4), asymmetric dihydroxylation using $(DHQD)_2PHAL$ of **11** provided (+)-**12** which has 12*R*,13*R* configulation[11] in 75% yield with 92% e.e.. After dihydroxylation, (+)-**5**[16] could be synthesized in the same way for synthesis of (−)-**4**. (+)-**5**[16] was not identical in 400 MHz ^1H and 100 MHz



Scheme 2. Synthesis of C18 skeleton **11**. *Reagents and conditions*: (a) $(Boc)_2O$, DMAP, *t*-BuOH, rt, 1 h, (82%), (b) (1) 0.1N NaOH in THF:MeOH:H_2O (3:1:1), rt, 22 h, (2) BH_3·THF, THF, 0°C–rt, 24 h, (3) I_2, PPh_3, imidazole, CH_2Cl_2, 0°C–rt, 1 h, (77% from **7**); (c) 1,3-propanedithiol, BF_3·OEt_2, CH_2Cl_2, 0°C–rt, (96%); (d) *n*-BuLi, THF, −78°C, 1 h, then **8**, −78°C, 1 h (85%).



Scheme 3. Synthesis of **4**. *Reagents and conditions*: (a) DHQ(PHAL)DHQ·Me^+·I^-, $K_3[Fe(CN)_6]$, K_2CO_3, K_2OsO_4·$2H_2O$, methanesulfonamide, *t*-BuOH: H_2O (1:1), 0°C, 1 h, (64%, 95% e.e.); (b) TBSOTf, 2,6-lutidine, −78°C, 10 min, (89%); (c) $Hg(ClO_4)_2$, $CaCO_3$, THF:H_2O (5:1), rt, 30 min, (83%); (d) (1) (*S*)-BINAL-H, THF, −78°C, 1 h, (diastereoselectivity; >20:1), (2) TBAF, THF, 70°C, 3 h, (76% from (+)-**14**); (e) 2.0N KOH in EtOH:H_2O (5:1), rt, 46 h, (82%).



Scheme 4. Synthesis of **5**. *Reagents and conditions*: (a) DHQD(PHAL)DHQD, $K_3[Fe(CN)_6]$, K_2CO_3, K_2OsO_4·$2H_2O$, methanesulfonamide, *t*-BuOH:H_2O (1:1), 0°C, 1 h, (75%, 92% e.e.), (b) TBSOTf, 2,6-lutidine, −78°C, 10 min, (87%); (c) $Hg(ClO_4)_2$, $CaCO_3$, THF:H_2O (5:1), rt, 30 min, (83%); (d) (1) (*S*)-BINAL-H, THF, −78°C, 1 h, (diastereoselectivity; >20:1); (2) TBAF, THF, 70°C, 3 h, (76% from (−)-**14**); (e) 2.0N KOH in EtOH:H_2O (5:1), rt, 46 h, (76%).

^{13}C NMR, and optical rotation {$[\alpha]_D^{23}$ +29.8 (*c* 0.45, MeOH)} with natural product **1**. The oral adjuvant activity of synthetic (−)-**4** was the same as that of natural compound **1**, while that of (+)-**5** was less than **1**.

In summary, we have elucidated that the relative and absolute configuration of pinellic acid **1** is 9*S*,12*S*,13*S*. A synthetic route to pinellic acid was established via asymmetric dihydroxylation and stereoselective reduction by (*S*)-BINAL-H. This route enables preparation of all of the stereoisomers of **1**. Further synthesis of the isomers and the structure–activity relationship study are in progress in our laboratory.

Acknowledgements

This work was supported in part by a grant from the Ministry of Education, Science and Culture of Japan, and Japan Keirin Association.

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15. (−)-**4**: White powder, mp: 104–106°C (MeOH), $[\alpha]_D^{25}$ −8.0 (*c* 0.30, MeOH), {natural;[4] $[\alpha]_D^{28}$ −8.1 (*c* 0.32, MeOH)}, IR (KBr) 3372 (s), 1695 (m), 1637 (m), ^{1}H NMR (400 MHz, CD_3OD) δ : 5.72 (1H, dd, J=15.5, 5.0 Hz), 5.67 (1H, dd, J=15.5, 5.0 Hz), 4.05 (1H, ddd, J=6.5, 6.0, 5.0 Hz), 3.91 (1H, dd, J=5.5, 5.0 Hz), 3.41 (1H, ddd, J=8.5, 5.5, 2.5 Hz), 2.27 (2H, t, J=7.5 Hz), 1.60 (2H, dt, J=7.5, 7.0 Hz), 1.55–1.50 (2H, m), 1.45–1.25 (16H, m), 0.91 (3H, t, J=6.3 Hz), ^{13}C NMR (100 MHz, CD_3OD) δ:177.8, 136.6, 131.1, 76.5, 75.8, 73.0, 38.3, 35.0, 33.6, 33.1, 30.5, 30.4, 30.2, 26.6, 26.5, 26.1, 23.7, 14.4, HR-FABMS m/z: 353.2305 [M+Na], calcd for $C_{18}H_{34}NaO_5$: 353.2304.
16. (+)-**5**: White powder, mp: 68–71°C (MeOH), $[\alpha]_D^{23}$ +29.8 (*c* 0.45, MeOH), IR (KBr) 3430 (s), 1697 (m), 1632 (m), ^{1}H NMR (400 MHz, CD_3OD) δ : 5.70 (1H, dd, J=15.5, 5.5 Hz), 5.64 (1H, dd, J=15.5, 6.0 Hz), 4.03 (1H, ddd, 6.5, 6.0, 5.5 Hz), 3.87 (1H, dd, J=6.0, 5.5 Hz), 3.40 (1H, ddd, J=7.0, 5.5, 2.0 Hz), 2.27 (2H, t, J=7.5 Hz), 1.60 (2H, dt, J=7.5, 7.0 Hz), 1.55–1.50 (2H, m), 1.44–1.25 (16H, m), 0.91 (3H, t, J=6.3 Hz), ^{13}C NMR (100 MHz, CD_3OD) δ:178.2, 136.7, 131.3, 76.7, 75.7, 73.2, 38.3, 36.0, 33.8, 33.1, 30.5, 30.4, 30.2, 26.5, 26.5, 26.2, 23.7, 14.4, HR-FABMS m/z: 353.2309 [M+Na], calcd for $C_{18}H_{34}NaO_5$: 353.2304.