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# Total Synthesis and Adjuvant Activity of All Stereoisomers of Pinellic Acid

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**Abstract**—Pinellic acid is a novel and potentially useful oral adjuvant when used in conjunction with intranasal inoculation of influenza HA vaccines. All stereoisomers of pinellic acid have been synthesized via regioselective asymmetric dihydroxylation, regioselective inversion, and stereoselective reduction, and their adjuvant activities were characterized. Among this series of isomers, 9*S*, 12*S*, 13*S* compound has the most potent adjuvant activity. Structure–activity relationships are discussed.

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Influenza is a highly infectious acute respiratory disease caused by specific influenza viruses leading to both worldwide pandemics and local outbreaks.

Influenza virus infection is sometimes critical for patients with respiratory diseases such as bronchial asthma, immunosuppressive syndrome such as AIDS, or for aged persons with cardiopulmonary disease.<sup>1</sup>

Influenza vaccine is useful as prophylaxis of influenza virus infection.<sup>1</sup> Intranasal inoculation of influenza vaccine has been tried to increase its safety and to prevent the 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 influenza vaccine administered intranasally is necessary to enhance the potency of the vaccine.

Pinellic acid (–)-**1** isolated from an oriental medicine, *Pinelliae tuber*, is a novel and potentially useful oral adjuvant when used in conjunction with intranasal inoculation of influenza HA vaccines.<sup>2</sup> (–)-**1** may prove to be a useful and safe oral adjuvant for mucosal vaccines.

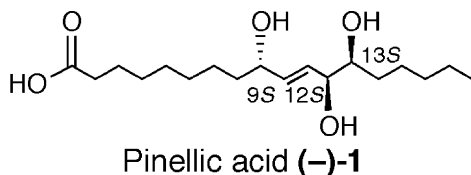
When (–)-**1** was found, the absolute configuration of (–)-**1** was unknown. Thus, we attempted the first total synthesis of (–)-**1** and determined the relative and absolute configuration of (–)-**1**, which is 9*S*, 12*S*, 13*S*. (Fig. 1).<sup>3</sup>

This paper deals with the total synthesis of all stereoisomers of pinellic acid, 9, 12, 13-trihydroxy-10-octadecanoic acid, and their adjuvant activities to clarify the structure–activity relationships.

For the purpose of stereoselective syntheses of all the stereoisomers of (–)-**1**, the following synthetic plan was devised as shown in Figure 2. There are three key points: (1) stereoselective reduction from dienone in order to prepare the allylic alcohol at C-9, (2) regioselective asymmetric dihydroxylation of the dienone to give 12,13-*syn*-diol, (3) inversion of the C-13 hydroxy group from C-12, 13-*syn*-diol for C-12, 13-*anti*-diol (vide infra).

In our previous work,<sup>3</sup> a synthetic route of C-12, 13-*syn*-diol was established the outline of which is shown in Scheme 1. A synthetic problem for C-12, 13-*syn*-diol (–)-**3** was solved by use of regioselective asymmetric dihydroxylation<sup>4</sup> of **2** and stereoselective reduction<sup>5</sup> of (+)-**4**. The 12, 13-*anti*-diol could be constructed via regioselective protection of the C-12 hydroxy group in 12, 13-*syn*-diol followed by inversion of the C-13 hydroxy group.

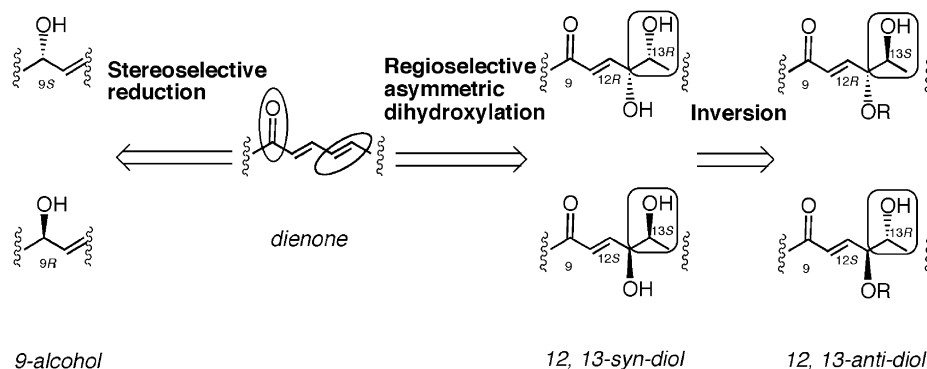
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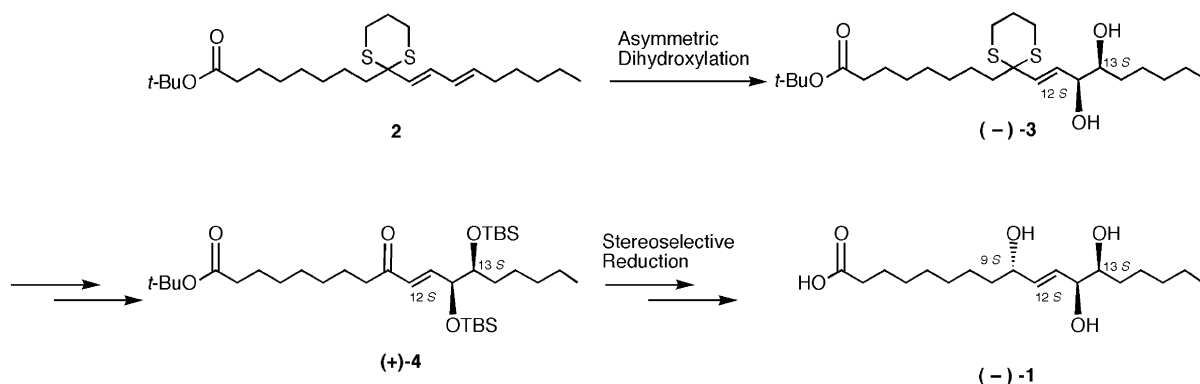
**Figure 1.** Structure of pinellic acid (**(-)-1**).

The preparation of **(+)-10** which has 9*S*, 12*S*, 13*R* configuration is shown in [Scheme 2](#).<sup>3</sup> The selection of the protecting group for this plan is important because only the C12 hydroxy group should be protected. The chemoselective protection of the C-12 hydroxy group in **(-)-3**<sup>3</sup> was accomplished by the use of the TIPS group

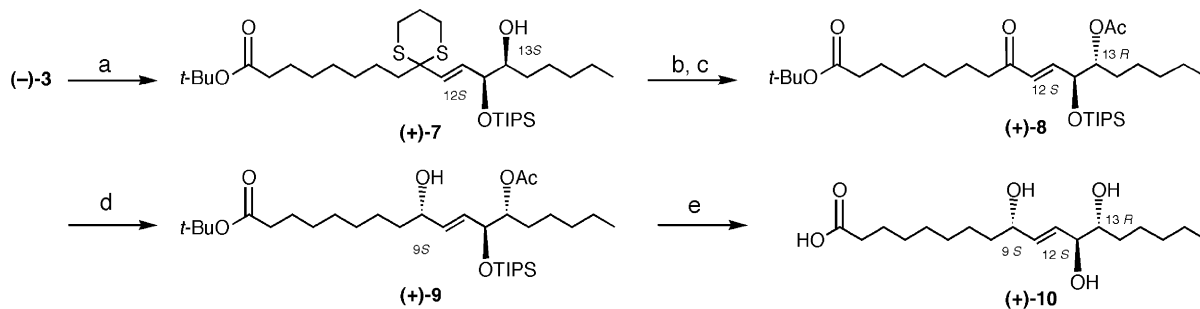
(TIPSOTf, 2,6-lutidine) in good yield without formation of the C-13-*O*-TIPS compound because of high reactivity of the allylic alcohol. Next, we attempted inversion of the hydroxy group at C-13. While the Mitsunobu inversion<sup>6</sup> was not successful because of the hindered *O*-TIPS group, Nakata's method,<sup>7</sup> using monochloromethanesulfonyl (ClSO<sub>2</sub>CH<sub>2</sub>Cl, pyridine) group followed by treatment with CsOAc, gave C-12, 13-*anti*-diol in good yield. Deprotection of dithioacetal furnished enone **(+)-8**. The stereoselective reduction of **(+)-8** by (*S*)-BINAL-H gave 9*S* alcohol **(+)-9**, which was easily separated as a single isomer by silica gel chromatography (diastereoselectivity; 13:1). Finally deprotection of acetyl and *tert*-butyl groups by hydrolysis and desilylation with TBAF gave **(+)-10** ([Scheme 2](#)).



**Figure 2.** Synthetic strategy of construction of three hydroxy groups.



**Scheme 1.** Total synthesis of **(-)-1**, 12, 13-*syn*-diol.



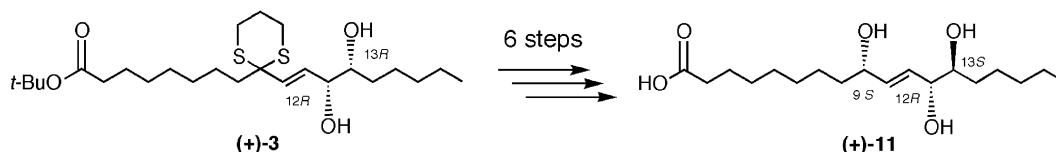
**Scheme 2.** Synthesis of **(+)-10**. Reagent and conditions: (a) TIPSOTf, 2, 6-lutidine, CH<sub>2</sub>Cl<sub>2</sub>, -78 °C, 8 h, (79%); (b) (1) ClCH<sub>2</sub>SO<sub>2</sub>Cl, pyridine, 0 °C, 1 h; (2) CsOAc, 18-crown-6, benzene, 80 °C, 20 h, (75%); (c) Hg(ClO<sub>4</sub>)<sub>2</sub>, CaCO<sub>3</sub>, THF/H<sub>2</sub>O (5:1), rt, 5 min, (89%); (d) (*S*)-BINAL-H, THF, -78 °C, 1 h, (99%, d.r.; 13:1); (e) (1) 1.0 N KOH in EtOH/H<sub>2</sub>O (4:1), rt, 5 day; (2) TBAF, THF, 45 h, (98%).

Synthesis of (+)-**11** having 9*S*, 12*R*, 13*S* was also successful according to the following synthetic route, utilizing (+)-**3**<sup>3</sup> as the starting material (Scheme 3).

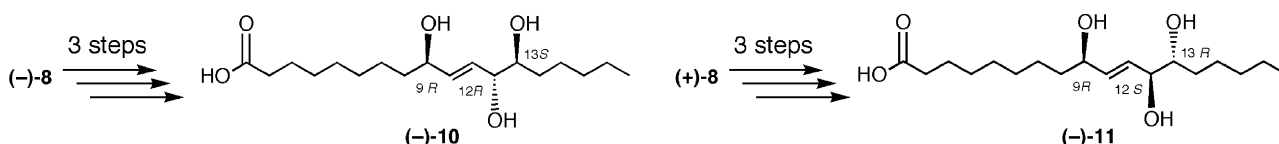
According to the synthetic routes for C-12, 13-*syn*- and *anti*-diol as shown in Schemes 4 and 5, all the stereo isomers of pinellic acid [(–)-**10**, (–)-**11**, (–)-**12** and (+)-**1**] could be prepared from the corresponding intermediates [(–)-**8**, (+)-**8**, (+)-**4** and (–)-**4**].<sup>8</sup> The

structures and the  $[\alpha]_D$  values of all isomers are shown in Figure 3.

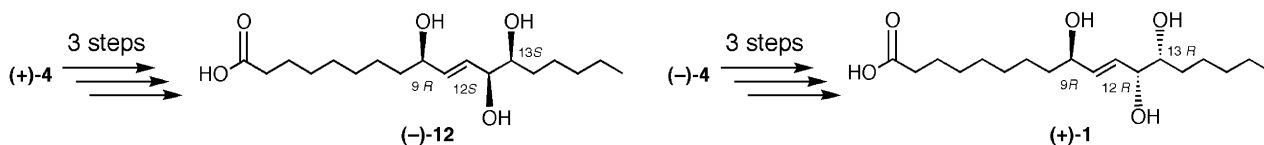
Oral administration of pinellic acid analogues as an adjuvant for the intranasal inoculation of influenza HA vaccine was investigated.<sup>2</sup> Mice were orally administered pinellic acid analogues (1 microgram/mouse) with intragastric gavage followed by intranasal inoculation of HA vaccine (1 microgram/mouse), and 3 weeks later,



Scheme 3. Synthesis of (+)-**12**.



Scheme 4. Synthesis of (–)-**11** and (–)-**13**.



Scheme 5. Synthesis of (+)-**1** and (+)-**15**.

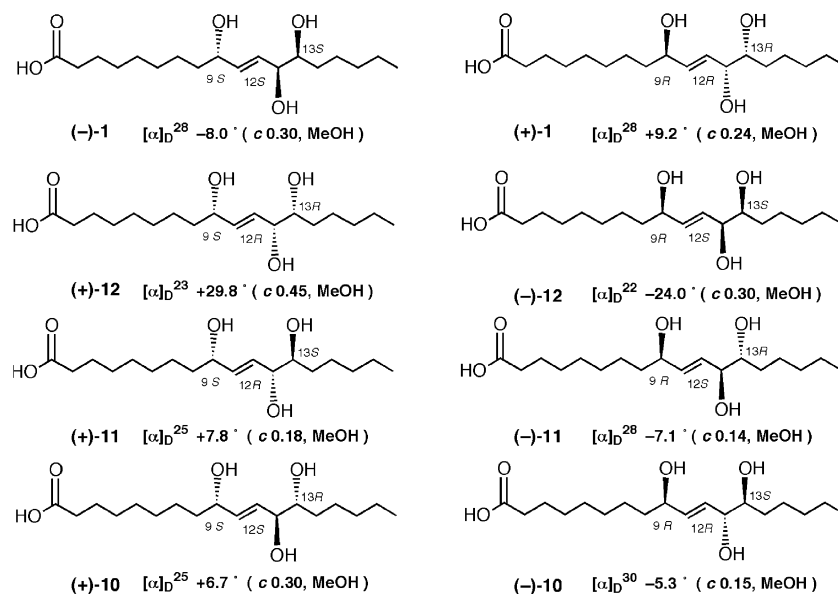


Figure 3. The structures and the  $[\alpha]_D$  values of all stereoisomers of pinellic acid.

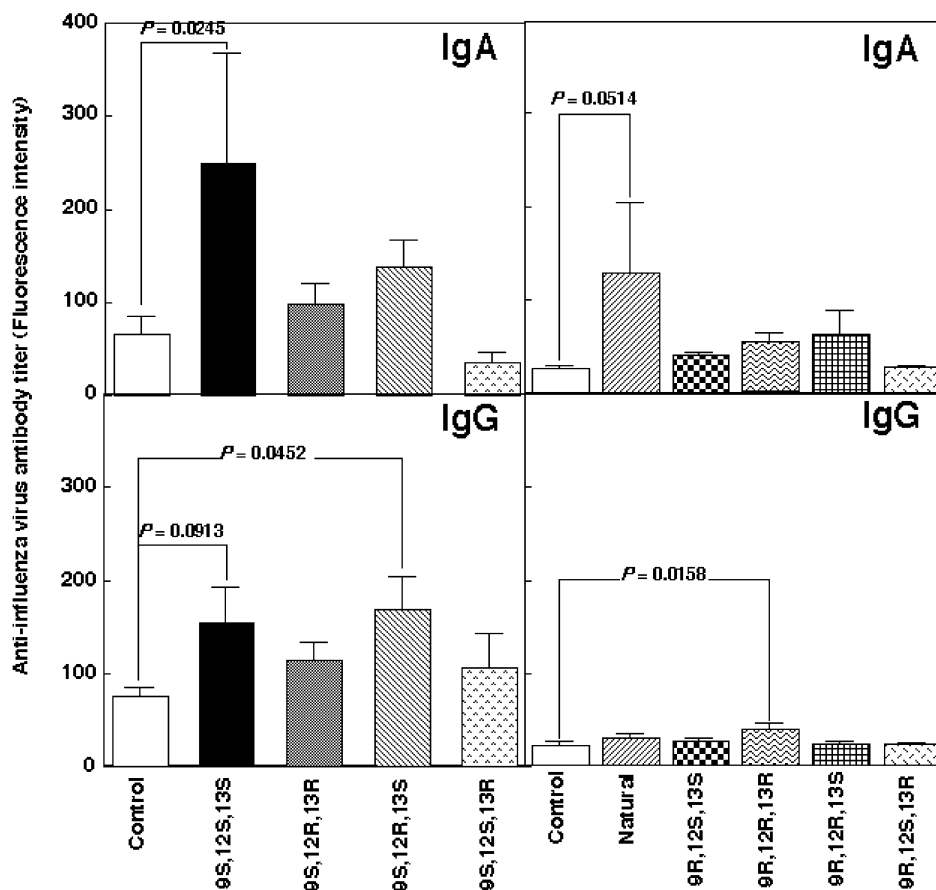


Figure 4. Adjuvant activity of all stereoisomers.

the same procedure was repeated. The IgA and IgG antibody responses against anti-influenza virus in the nasal and serum in the vaccinated mice were examined 1 week after vaccination.

The results of adjuvant activity of all stereoisomers are shown in Figure 4.

The antiviral IgA and IgG antibody responses, induced in the nasal of mice given pinellic acid (–)-**1** with vaccine, were enhanced 5.2- and 2-fold, respectively compared with control mice given the vaccine and solvent alone. Among the C9-isomers of pinellic acid, 9*S*-compounds showed much stronger activity compared with 9*R*-compounds. Thus, stereochemistry at the C-9 hydroxyl group is very important for adjuvant activity. Among the 9*S*-derivatives, the adjuvant activities of 13*S*-compounds are stronger than that of 13*R*-compounds. However, the stereochemistry of the C-12 hydroxyl group is not important for adjuvant activity.

Interestingly, the adjuvant activity of (+)-**1**, the enantiomer of natural pinellic acid, was weaker than that of the natural one.

In the data shown in Figure 4, adjuvant activity of pinellic acid (–)-**1** from a natural source was lower than that of the synthetic one. The result is presumably due to the chemical purity of the available sample.

In summary, we have established synthetic routes to enable preparation of all the stereoisomers of **1** via regioselective asymmetric dihydroxylation, stereoselective inversion, and stereoselective reduction. In this series, 9*S*, 12*S*, 13*S* compound has the most potent adjuvant activity. Studies on the mechanism of adjuvant and protective effects of pinellic acid with nasal influenza HA vaccine against influenza virus infection are currently under way.

#### Acknowledgements

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#### References and Notes

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7. Shimizu, T.; Hiranuma, S.; Nakata, T. *Tetrahedron Lett.* **1996**, *37*, 6145.
8. The non-selective reduction of (+)-**4** (NaBH<sub>4</sub>, CeCl<sub>3</sub>) afforded 1:1 mixture of 9,12-*anti*, and 9, 12-*syn*-triol. So, the

stereoselectivity on the reduction of (+)-**4** depended upon the chiral reagents [(*R*)-BINAL, diastereoselectivity; >20:1, (*S*)-BINAL, diastereoselectivity, 1:>20]. On the other hand, the non-selective reduction of (+)-**8** (NaBH<sub>4</sub>, CeCl<sub>3</sub>) afforded 3:2 mixture of 9,12-*anti*, and 9, 12-*syn*-triol, because of the steric hindrance of 12-OTIPS. The reduction of (+)-**8** by (*S*)-BINAL gave the lower diastereoselectivity; 13:1 (mis-matched), however, by (*R*)-BINAL gave the higher diastereoselectivity; >20:1 (matched).