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# Synthesis of Piericidin Analogues, Inhibitors on Electron Transport System in Mitochondria

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Varoius piericidin analogues (PS-I, -II and -III in Fig. 2) were synthesized from three 4-acetoxy-6-formylpyridines by Wittig reaction to determine the structure-activity relationships. New type inhibitors, 5-alkenyl-2, 3-dimethoxy-4-hydroxy-6-methylpyridines (PS-IV) were synthesized by intramolecular cyclization.

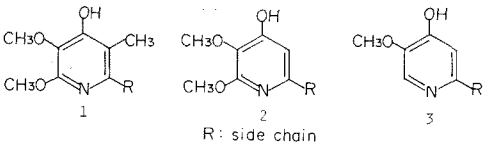
Piericidins A and B, the metabolites of *Streptomyces mobaraensis*,[1] have been well-known as very unique inhibitors against the mitochondrial transport system.[2] Recently, many novel types of piericidins were isolated from the metabolites of *Streptomyces pactum*, and named piericidins An, Bn, Cn and Dn (n=1, 2, 3, and 4).[3] Chemical structures of these new piericidins were established by means of various spectroscopic data as shown in Fig. 1.[4] These naturally occurring piericidins contain in common a fully substituted pyridine ring whose substitution pattern is quite similar to that of benzoquinone in ubiquinones (coenzyme Q), but their side chain structures are different. The structural resemblance between piericidins and ubiquinones prompts us to clarify the structure-activity relationship of piericidins and their analogues as respiratory inhibitors.

Natural piericidins and their derivatives provided much information on the relationship between the activity and the side chain structure, so that our synthetic plan for analogues was mainly based on comparison of the inhibitory effect *vs.* ring structure in piericidin.

After establishing the active part of piericidin structures for respiratory inhibition, another interesting type of compounds was thought out as new inhibitors. To synthesize these compounds, we had to develop a new route. Hence, this paper consists of two parts; one is synthesis of various analogues (PS–I, II and III series) used for the clarification of the structural unit for inhibition of the electron transport system, and the other concerns a new type of inhibitors (PS–IV series).

i) *Synthesis of PS–I, II and III series compounds*

Since preliminary studies on the structure-activity relationship of piericidins[5] suggested that a free phenolic hydroxyl at the 4-position in the pyridine ring should be necessary for inhibition, three types of 4-hydroxypyridine derivatives (**1**～**3**) were chosen to compare the effect due to methoxyls and methyl substituents.




The easiest way to obtain a starting material for **1** might be utilization of piericidin's degradative products. Some pyridyl carboxylic acids (**4a**～**d**) have been obtained as well-known products from oxidative degradation of ozonide of piericidin $A_1$ diacetate (**5**),[6] but these acids seemed not suitable for construction of the side chain. Therefore, reductive degradation of the ozonide was investigated, using various reductants such as triphenylphosphine, dimethylsulfide and zinc-acetic acid. Under any kind of condition, only two

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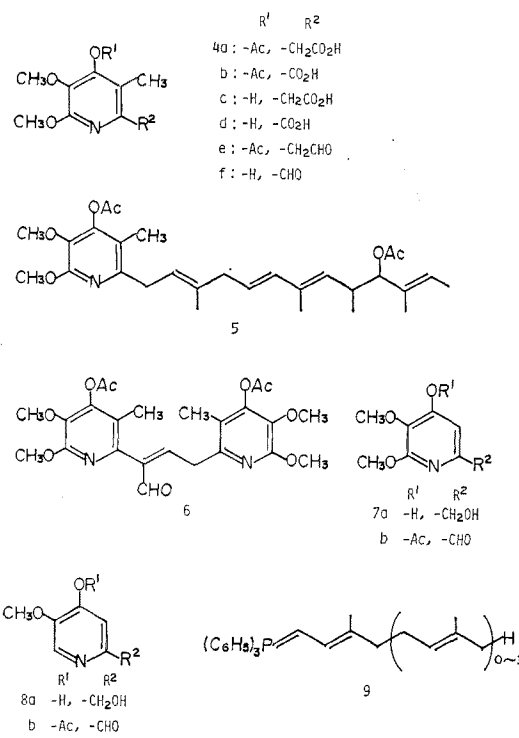
compounds containing the ring moiety of piericidin were produced. One, a major product, was a dimer (**6**) of 4-acetoxy-2,3-dimethoxy-5-methyl-6-(2-oxoethyl)-pyridine (**4e**), considered to arise from aldol condensation, followed by dehydration during the work-up procedure. This result means that **4e**, an attractive candidate for starting material, is unavailable by normal methods due to its remarkable instability. The minor product was 4-acetoxy-6-formyl-2,3-dimethoxy-5-methylpyridine (**4f**), which came from an abnormal cleavage in ozonolysis of **5** as in the cases of **4b**,**d**. Although the yeild of **4f** was rather low (about 20%), this compound contains quite suitable functionality for construction of the side chain.




Starting materials for both **2** and **3** corresponding to **4f** were accessible from kojic acid, because synthesis of 2,3-dimethoxy-4-hydroxy-6-hydroxymethylpyridine (**7a**)[7] and 4-hydroxy-6-hydroxymethyl-3-methoxypyridine (**8a**)[8] had been established. Oxidation of **7a** and **8a** by manganese dioxide, followed by acetylation, afforded 4-acetoxy-2,3-dimethoxy-6-formylpyridine (**7b**) and 4-acetoxy-6-formyl-3-methoxypyridine (**8b**) respectively in good yield.

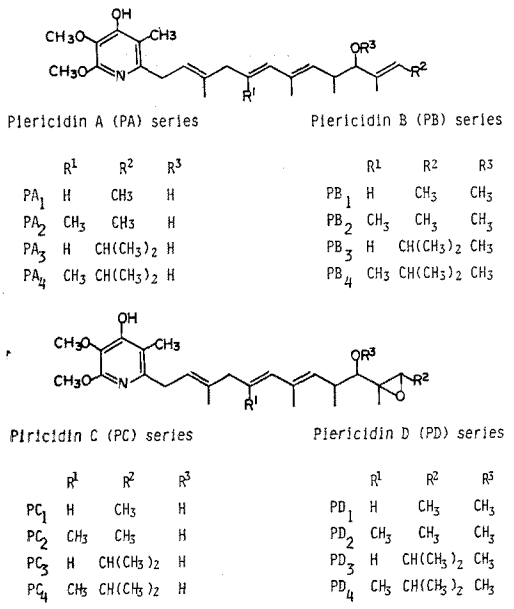



FIG. 1. Structure of Natural Piericidins.

Thus, three 4-acetoxy-6-formylpyridines were available, allowing synthesis of all the analogues **1**, **2** and **3** by a single method such as Wittig reaction, which might be the most convenient way to build up various side chains. Generally Wittig reaction between these aldehydes and poly-(or mono-)prenylidene triphenylphosphorane (**9**) proceeded smoothly at ambient temperature in ether (for **4f** and **7b**) or THF (for **8b**), and gave the desired compounds (**10**~**12**) and their acetates. After acetylation or solvolysis of crude products, purification was simply achieved by preparative TLC, since **10**, **11** and acetate of **12** were easily separated from triphenylphosphine oxide. Finally twelve analogues were synthesized and named PS-I, PS-II and PS-III for **10**, **11** and **12**, respectively; the suffix indicates number of carbons in a side chain. By catalytic hydrogenation, all the compounds of PS series were readily converted into HPS series (**13**~**15**), which has a saturated side chain. Their structures and abbreviated names are summarized in Fig. 2.

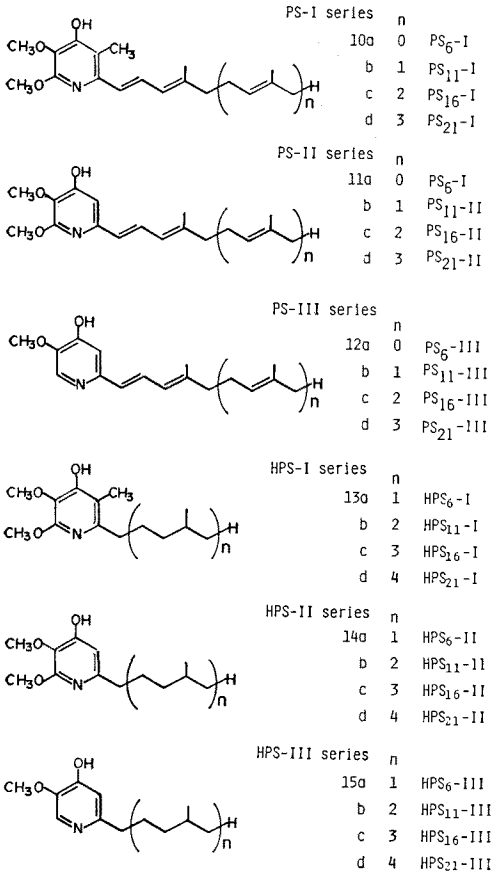



FIG. 2. Structures of Synthetic Analogues, PS-I II and III and their Abbreviated Names.

ii) *Synthesis of PS-IV series*

In order to determine whether side chain position in piericidins (all α-) influenced respiratory inhibition, the structure (**16**) was considered to be worthy of study. Since it was impossible to get any convenient starting materials for this structure (corresponding to kojic acid for the synthesis of II and III series), a new approach to the ring system had to be investigated.

Consideration of retro-synthesis of **16** led us to α,β-dihydroxypyridine derivative (**17**) as a key intermediate. The discrimination between α- and β-hydroxyls seemed to be possible as in the case of the synthesis of **7a**. On the other hand, an attempt to the use of an acyclic starting material for **17** received some support from the work on intramolecular cyclization yielding 4-amino-2-hydroxy-3-methoxy-5-methylpyridine[9]; the synthetic plan shown in Fig. 3 was therefore considered as the most promising approach.

Alkylation of commercially available ethyl 3-aminocrotonate (**18**) was smoothly achieved by the combination of allyl halides (geranyl, farnesyl and phytyl bromides) and sodium hydride in THF. The reaction products, ethyl 2-alkenyl-3-aminocrotonates (**19**), consisted of two geometric isomers due to the conjugated ester part. Since **19** is rather unstable on standing and easily hydrolyzed into ethyl 2-alkenylacetoacetate during chromatography, the crude products were *N*-acylated by methoxyacetyl chloride and *n*-butyllithium to obtain the amides (**20**). NMR spectra of these amides clearly indicated that **20** also consisted of *E*- and *Z*-isomers in almost 1:1 ratio (see EXPERIMENTAL section). Although **20** are more stable than **19**, isolation of individual isomer was unsuccessful due to their similar properties.

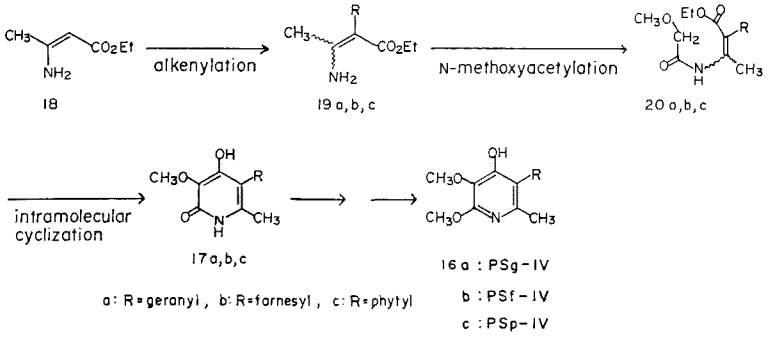
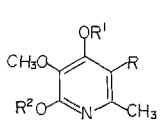



FIG. 3. Synthetic Scheme of PS-IV.



21 a,b,c : $R^1=R^2=Ac$
22 a,b,c : $R^1=Ac$, $R^2=H$
23 a,b,c : $R^1=H$, $R^2=Ac$
24 a,b,c : $R^1=Ac$, $R^2=CH_3$
25 a,b,c : $R^1=CH_3$, $R^2=Ac$
26 a,b,c : $R^1=CH_3$, $R^2=H$

a:R geranyl, b:R farnesyl, c:R phytyl

Intermolecular cyclization of **20** was examined under various conditions, and was finally effected by using 3 eq. lithium diisopropylamide in THF under reflux to form **17**. Although the yield of **17** was rather low (less than 20%), the purification of this compounds was readily achieved by silica gel chromatography. All the compounds **17** show positive $FeCl_3$-coloration and show no signal due to the ester part of **20** in IR and NMR spectra. Since we did not succeed in selective acetylation of the 4-hydroxyl of **17**, 5-alkenyl-2,4-diacetoxy-3-methoxy-6-methylpyridines (**21**) were prepared by acetic anhydride-pyridine to investigate selective solvolysis. The diacetates (**21**) were very sensitive to acidic condition, even in silica gel TLC. A short treatment of **21** with 0.5 N methanolic HCL gave a single product, which may have a structure **22** or an alternative one **23**. The selection between two possible structures has been done in the latest stage of synthesis. The monoacetate was methylated by diazomethane and a catalytic amount of $BF_3$ etherate. Fortunately, this methylation afforded only dimethoxylated products, which were converted into dimethoxypyridinols by alkaline solvolysis.

Thus, the fully substituted pyridinols were synthesized; however, it was necessary to confirm the substitution pattern of the final products. As mentioned above, there were two possible structures, **16** and its isomer (**26**). Obviously, UV spectra of pyridinols should give information about the position of a free hydroxyl on the pyridine ring.[10] The UV spectra of the final products, dimethoxypyridinols, showed very close feature to the hydrogenated piericidin analogue and derivative, namely $PS_g$-IV (**16a**) and octahydropiericidin $A_1$ ($H_8PA_1$) as shown in Fig. 4. Thus, these compounds should be 4-hydroxpyridine derivatives (PS-IV series).

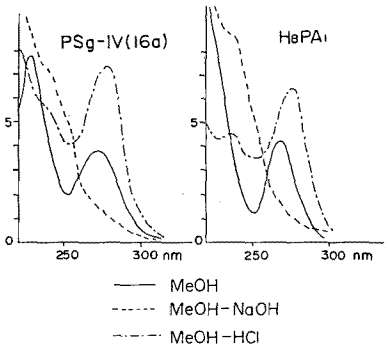



FIG. 4. UV Spectra of PSg-IV (**16a**) and Octahydropiericidin $A_1$ ($H_8PA_1$).

Our synthetic route for PS-IV series is reasonably simple but it included a problem about the intramolecular cyclization, where we used mixture of geometric isomers as reactants. It is of some interest to clarify whether the *E*- or the *Z*-isomers is preferentially cyclized under such condition. The most effective way of isolating the individual isomers is being surveyed for this reason.

## EXPERIMENTAL

Nuclear magnetic resonance spectra were obtained on a Jeol MH-100 spectrometer. Low and high resolution mass spectra were obtained on a Hitachi RMU-6L and RMH-2 spectrometers respectively. Infrared spectra were obtained on a Jasco KCl spectrophotometer and ultraviolet spectra on a Cary spectrophotometer. Unless specified otherwise, NMR were measured in $CCl_4$ solution.

*4-Acetoxy-2,3-dimethoxy-6-formyl-5-methylpyridine* (**4f**). Ozone was passed into a solution of 10 g of piericidin $A_1$ diacetate (**5**) in 70 ml of chloroform at −78°C until pale blue color had been charged. The solution was warmed to room temperature to mix with a solution of 21 g of triphenylphosphine in 100 ml of chloroform. After refluxing for 2 hr, the mixture was concentrated under reduced pressure. The residue was dissolved in hot ethyl acetate to precipitate triphenylphosphine oxide by cooling. The ethyl acetate layer was filtered and evaporation of the solvent afforded 13.5 g of syrup, which was purified by silica gel column chromatography. Elution with benzene afforded 892 mg (18.7%) of **4f**: mp 85°C (recrystallized from hot *n*-

hexane); IR 1785 (ester), 1715 (fomyl), 1605 (pyridine) $cm^{-1}$; NMR $\delta$ 2.28 (3H, s), 2.34 (3H, s), 3.82 (3H, s), 3.99 (3H, s), 9.95 (1H, s); MS *m/e* 239 ($M^+$), 197 (M–42, base peak). *Anal.* Calcd for $C_{11}H_{13}O_6N$: C, 55.23; H, 5.48; N, 5.86. Found: C, 55.01; H, 5.50; N, 5.82.

Elution with 2% ethyl acetate-benzene afforded 3.27 g of yellow oil of the dimer (**6**): IR 1785 (ester), 1705 (formyl), 1600 (pyridine), 1570 (C=C) $cm^{-1}$; NMR $\delta$ 2.05 (3H, s), 2.11 (3H, s), 2.18 (3H, s), 2.21 (3H, s), 3.25 (2H, d), 3.86 (3H, s), 3.91 (3H, s), 3.95 (3H, s), 4.00 (3H, s), 6.32 (1H, t), 9.82 (1H, s); MS *m/e* 488 ($M^+$), 446 (M–42, base peak).

*4-Acetoxy-2,3-dimethoxy-6-formylpyridine* (**7b**). Into a chloroform solution (30 ml) of 2.0 g of 2,3-dimethoxy-4-hydroxy-6-hydroxymethylpyridine (**7a**)[7] was added 10 g of active manganese oxide, and the reaction mixture was stirred for 3 hr under reflux. After filtration, the resulting clear solution was concentrated under reduced pressure. The residue was dissolved in a mixture of 5 ml of acetic anhydride and 10 ml of dry pyridine. After standing overnight, the reaction solution was poured onto a mixture of crashed ice and 2 N HCl. The product was extracted with ethyl acetate, and the extract was washed with aq. $NaHCO_3$ and dried over anhydrous $Na_2SO_4$. Evaporation of ethyl acetate under reduced pressure gave 1.8 g of solid, which was crystallized from hot *n*-hexane to yield 1.6 g (80%) of **7b**: mp 104°C; IR 1775 (ester), 1706 (formyl), 1604 (pyridine) $cm^{-1}$; NMR $\delta$ 2.22 (3H, s), 3.69 (3H, s), 4.00 (3H, s), 6.75 (1H, s), 9.85 (1H, s). *Anal.* Calcd for $C_{10}H_{11}O_5N$: C, 53.33; H, 4.92; N, 6.22. Found: C, 53.17; H, 4.98; N, 6.05.

*4-Acetoxy-6-formyl-3-methoxypyridine* (**8b**). A mixture of 15 g of 6-hydroxymethyl-3-methoxypyrid-4-one[8] in 200 ml of DMF and 75 g of active manganese dioxide was stirred for 4 hr at 80°C. According to the similar procedure for acetylation as above 9.6 g of tarry residue was obtained. The residue was crystallized from ethyl acetate-*n*-hexane to yield 7.1 g (36%) of colorless crystal **8b**: mp 138~139°C; IR 1772 (ester), 1698 (formyl), 1578 (pyridine) $cm^{-1}$; NMR $\delta$ 2.25 (3H, s), 3.90 (3H, s), 6.90 (1H, s), 8.20 (1H, s), 9.85 (1H, s). *Anal.* Calcd for $C_9H_9O_4N$: C, 55.38; H, 4.65; N, 7.18. Found: C, 55.36; H, 4.71; N, 7.15.

*PS-I, II and III series* (**10~12**). To a suspension of 12 mM of phosphonium salts in 200 ml of dry ether was added a solution of 12 mM of *n*-butyllithium in *n*-hexane at room temperature with vigorous stirring under $N_2$ atmosphere. To the resulting red solution of phosphorane (**9**) was added dropwise a solution of 10 mM of formyl pyridine in ether (for **4f**, **7b**) or THF (for **8b**) at room temperature, and the reaction mixture was stirred for 1 hr at that temperature. After standing overnight, the mixture was filtrated to remove precipitates, and the filtrate was concentrated to afford crude materials. For purification of PS-I and II series, the crude residue was treated with 0.5 N sodium methoxide for 1 hr at ambient temperature. After evaporation of methanol, the residue was dissolved into ether, and the solution was washed with water and dried over anhydrous $Na_2SO_4$. Concentration of the solvent afforded an oily product, which was purified with preparative TLC (solvent system: 7% ethyl acetate-benzene). For purification of PS-III, the crude products of Wittig reaction were acetylated with acetic anhydride-pyridine. After the usual work-up, the acetates were purified with preparative TLC (solvent system: 10% ethyl acetate-benzene), and the resulting pure acetates were treated with 0.5 N sodium methoxide according to the similar procedure as above. The overall yield of these compounds was in the range between 40 to 75%. The properties of products are as follows; $PS_6$-I (**10a**): pale yellow wax, mp 35°C; IR 3420 (broad, OH), 2900, 1580 $cm^{-1}$; NMR $\delta$ 1.86 (6H, s), 2.02 (3H, s), 3.44 (3H, s), 3.92 (3H, s), 6.0~7.1 (3H, m); MS *m/e* 249 ($M^+$, base peak). $PS_{11}$-I (**10b**): pale yellow oil; IR 3500, 2960, 1572 $cm^{-1}$; NMR $\delta$ 1.62 (3H, s), 1.68 (3H, s), 1.88 (3H, s), 2.10 (3H, s), 2.15~2.30 (4H, m), 3.80 (3H, s), 3.96 (3H, s), 5.30 (1H, m), 6.0~7.1 (3H, m); MS *m/e* 317 ($M^+$), 248 (M–69, base peak). $PS_{16}$-I (**10c**): pale yellow oil; IR 3500, 2960, 1572 $cm^{-1}$; NMR $\delta$ 1.6~1.8 (9H), 1.86 (3H, s), 2.08 (3H, s), 2.10~2.35 (8H), 3.84 (3H, s), 3.94 (3H, s), 5.3~7.1 (5H); MS *m/e* 385 ($M^+$), 248 (M–137). $PS_{21}$-I (**10d**): pale yellow oil; IR 3500, 2960, 1570 $cm^{-1}$; NMR 1.6~1.8 (12H), 1.88 (3H, s), 2.08 (3H, s), 2.10~2.35 (12 H), 3.82 (3H, s), 3.92 (3H, s), 5.3~7.1 (6H); MS *m/e* 453 ($M^+$), 384 (M–69), 248 (M–205, base peak). $PS_6$-II (**11a**): yellow wax, mp 42~44°C; IR 3480, 2960, 1580 $cm^{-1}$; NMR $\delta$ 1.92 (6H, s), 3.95 (3H, s), 4.01 (3H, s), 6.0~7.1 (3H), 7.15 (1H, s); MS *m/e* 235 ($M^+$, base peak). $PS_{11}$-II (**11b**): pale yellow oil; IR 3450, 2960, 1584 $cm^{-1}$; NMR $\delta$ 1.59 (3H, s), 1.66 (3H, s), 1.85 (3H, s), 2.10~2.30 (4H), 3.79 (3H, s), 3.97 (3H, s), 5.3~7.1 (4H), 7.18 (1H, s); MS *m/e* 303 ($M^+$), 234 (M–69, base peak). $PS_{16}$-II (**11c**): pale yellow oil; IR 3450, 2960, 1580 $cm^{-1}$ NMR $\delta$ 1.60 (6H, s), 1.65 (3H, s), 1.85 (3H s), 2.1~2.3 (8H), 3.84 (3H, s), 3.96 (3H, s), 5.3~7.1 (5H), 7.15 (1H, s). $PS_{21}$-II (**11d**): pale yellow oil; IR 3500, 3000, 1580 $cm^{-1}$; NMR $\delta$ 1.60 (9H, s), 1.67 (3H, s), 1.86 (3H, s), 2.1~2.3 (12H), 3.84 (3H, s), 4.00 (3H, s), 5.3~7.1 (6H), 7.18 (1H, s); MS *m/e* 439 ($M^+$), 234 (M–205, base peak). $PS_6$-III (**12a**): yellow plate, mp 173°C; IR 3420, 1610, 1565 $cm^{-1}$; NMR $\delta$ 1.87 (3H, s), 1.98 (3H, s), 4.00 (3H, s), 6.28 (1H, s), 5.3~7.1 (3H), 7.53 (1H, s); MS *m/e* 205 ($M^+$, base peak). $PS_{11}$-III (**12b**): yellow oil; IR 2960, 2800 (broad), 1635, 1600, 1535 $cm^{-1}$; NMR $\delta$ 1.55 (3H, s), 1.63 (3H, s), 1.84 (3H, s), 2.1~2.2 (4H), 3.74 (3H, s), 5.3~7.1 (4H), 6.30 (1H, s), 7.50 (1H, s); MS *m/e* 273 ($M^+$), 205 (M–68, base peak). $PS_{16}$-III (**12c**): yellow oil; IR 2960, 2800 (broad),

1637, 1600, 1540 $cm^{-1}$; NMR $\delta$ 1.55 (6H, s), 1.63 (3H, s), 1.84 (3H, s), 2.0~2.2 (8H), 3.74 (3H, s), 5.3~7.2 (5H), 6.54 (1H, s), 7.50 (1H, s); MS *m/e* 341 ($M^+$), 204 (M–69, base peak). $PS_{21}$–III (**12d**): yellow oil; IR 2960, 2800 (broad), 1635, 1602, 1540 $cm^{-1}$; NMR $\delta$ 1.56 (9H, s), 1.65 (3H, s), 1.86 (3H, s), 2.0~2.3 (12H), 3.74 (3H, s), 5.3~7.3 (6H), 6.55 (1H, s), 7.48 (1H, s); MS *m/e* 409 ($M^+$), 204 (M–69).

*HPS–I, II and III series* (**13**~**15**). A solution of 1 mM of PS series compound in ethanol (20 ml) was mixed with a suspension of platinum catalyst (activated from 50 mg of platinum oxide) in 10 ml of ethanol, and the reaction mixture was vigorously stirred under $H_2$ atmosphere for 3 hr. After removal of the catalyst, the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate in order to remove insoluble materials. Evaporation of ethyl acetate afforded crude HPS, which was purified with preparative TLC (solvent system; 7% ethyl acetate-benzene for **13** and **14**, 25% ethanol-chloroform for **15**). $HPS_6$–I (**13a**): colorless oil; IR 3500 (broad), 2960, 1590 $cm^{-1}$; NMR $\delta$ 0.88 (6H, d), 1.1~1.7 (5H), 2.09 (3H, s), 3.72 (3H, s), 3.96 (3H, s); MS *m/e* 253 ($M^+$), 183 (M–70, base peak); High resolution MS Calcd for $C_{14}H_{23}O_3N$: 253.1677; Found: 253.1698. $HPS_{11}$–I (**13b**): colorless oil; IR 3500 (broad) 2960 1589 $cm^{-1}$; NMR $\delta$ 0.84 (9H, d), 1.1~1.7 (12H), 2.10 (3H, s), 2.60 (2H, t), 3.74 (3H, s), 3.98 (3H, s); MS *m/e* 323 ($M^+$), 183 (M–140, base peak); High resolution MS Calcd for $C_{19}H_{33}O_3N$: 323.2458, Found: 323.2450. $HPS_{16}$–I (**13c**): colorless oil; IR 3500 (broad), 2960, 1590 $cm^{-1}$; NMR $\delta$ 0.84 (12H, d), 1.1~1.7 (19H), 2.10 (3H, s). 2.60 (2H, t), 3.75 (3H, s), 3.98 (3H, s); MS *m/e* 393 ($M^+$), 183 (M–210, base peak); High resolution MS Calcd for $C_{24}H_{43}O_3N$: 393.3239, Found: 393.3232. $HPS_{21}$–I (**13d**): colorless oil; IR 3500 (broad), 2960, 1588 $cm^{-1}$; NMR $\delta$ 0.84 (15H, d), 1.1~1.7 (26H), 2.08 (3H, s), 2.60 (2H, t), 3.74 (3H, s), 4.00 (3H, s); MS *m/e* 463 ($M^+$), 183 (M–280, base peak); High resolution MS Calcd for $C_{29}H_{53}O_3N$: 463.4021, Found: 463.4035. $HPS_6$–II (**14a**): colorless oil; IR 3450 (broad), 2960, 1590 $cm^{-1}$; NMR $\delta$ 0.84 (6H, d), 1.1~1.7 (5H), 2.60 (2H, t), 3.79 (3H, s), 3.95 (3H, s), 6.40 (1H, s); MS *m/e* 239 ($M^+$), 169 (M–70, base peak); High resolution MS Calcd for $C_{13}H_{21}O_3N$: 239.1520. Found: 239.1483. $HPS_{11}$–II (**14b**): colorless oil; IR 3450 (broad), 2960, 1588 $cm^{-1}$; NMR $\delta$ 0.84 (9H, d), 1.1~1.7 (12H), 2.60 (2H, t), 3.79 (3H, s), 3.95 (3H, s), 6.40 (1H, s); MS *m/e* 309 ($M^+$), 169 (M–140); High resolution MS Calcd for $C_{18}H_{31}O_3N$: 309.2302. Found: 309.2316. $HPS_{16}$–II (**14c**): colorless oil; IR 3450 (broad), 2960, 1588 $cm^{-1}$; NMR $\delta$ 0.84 (12H, d), 1.1~1.7 (19H), 2.60 (2H, t), 3.78 (3H, s), 3.95 (3H, s), 6.40 (1H, s); MS *m/e* 379 ($M^+$), 169 (M–210, base peak); High resolution MS Calcd for $C_{23}H_{41}O_3N$: 379.3084. Found: 379.3076. $HPS_{21}$–II (**14d**): colorless oil; IR 3500 (broad), 2960, 1590 $cm^{-1}$; NMR $\delta$ 0.84 (15H, d), 1.1~1.7 (26H), 2.60 (2H, t), 3.79 (3H, s), 3.99 (3H, s), 6.45 (1H, s); MS *m/e* 449 ($M^+$), 169 (M–280, base peak); High resolution MS Calcd for $C_{28}H_{51}O_3N$: 449.3866. Found: 449.3891. $HPS_6$–III (**15a**); colorless oil; IR 2960, 2800 (broad), 1630, 1550 $cm^{-1}$; NMR $\delta$ 0.88 (6H, d), 1.1~1.7 (5H), 2.65 (2H, t), 3.74 (3H, s), 6.32 (1H, s), 7.45 (1H, s); MS *m/e* 209 ($M^+$), 139 (base peak); High resolution MS Calcd for $C_{12}H_{19}O_2N$: 209.1414. Found: 209. 1395. $HPS_{11}$–III (**15b**): colorless oil; IR 2960, 2800 (broad), 1630, 1540 $cm^{-1}$; NMR $\delta$ 0.90 (9H, d), 1.1~1.7 (12H), 2.65 (2H, t), 3.74 (3H, s), 6.30 (1H, s), 7.40 (1H, s); MS *m/e* 279 ($M^+$), 139 (M–140, base peak); High resolution MS Calcd for $C_{17}H_{29}O_2N$: 279.2196. Found: 279.2177. $HPS_{16}$–III (**15c**): colorless oil; IR 2960, 2800 (broad), 1630, 1540 $cm^{-1}$; NMR $\delta$ 0.90 (12H, d), 1.1~1.7 (19H), 2.65 (2H, t), 3.75 (3H, s), 6.30 (1H, s), 7.40 (1H, s), MS *m/e* 349 ($M^+$), 139 (M–210, base peak); High resolution MS Calcd for $C_{22}H_{39}O_2N$: 349.2978. Found: 349.2954. $HPS_{21}$–III (**15d**): colorless oil; IR 2960, 2800 (broad), 1630, 1540 $cm^{-1}$; NMR $\delta$ 0.90 (15H, d), 1.1~1.7 (26H), 2.65 (2H, t), 3.75 (3H, s), 6.30 (1H, s), 7.40 (1H, s); MS *m/e* 419 ($M^+$), 139 (M–280, base peak); High resolution MS Calcd for $C_{27}H_{49}O_2N$: 419.3760. Found: 419.3781.

*Ethyl 2-alkenyl-3-aminocrotonate* (**19**). To a suspension of 25 mM of sodium hydride in 50 ml of THF was added a THF (20 ml) solution of ethyl 3-aminocrotonate (**18**, 2.84 g, 22 mM) at 0°C under $N_2$ atmosphere, and the reaction mixture was stirred for 15 min at that temperature. A solution of 20 mM of alkenyl bromide in THF was added dropwise to the mixture. After overnight stirring at room temperature, the reaction mixture was poured into 200 ml of cold brine. The organic layer was extracted with three portions of 100 ml of ethyl acetate, and the combined extract was washed with cold brine and dried over anhydrous $Na_2SO_4$. Evaporation of the solvent gave crude **19**, which was used for the next step without any purification. IR 3500~2800 ($NH_2$), 1705 (C=O) $cm^{-1}$; NMR[11] $\delta$ 1.18 (3H, t), 1.82 (3H, s), 2.80 (2H, d) 3.88 (2H, q) due to *E* isomer, and 1.20 (3H, t), 1.77 (3H, s), 2.80 (2H, d), 3.98 (2H, q) due to *Z*.

*Ethyl 2-alkenyl-3-methoxyacetylaminocrotonate* (**20**). To a magnetically stirred solution of 15 mM of **19** in 150 ml of THF under $N_2$ at −78°C was added dropwise a solution of 18 mM of *n*-butyllithium in *n*-hexane. After stirring the reaction mixture for 15 min, a solution of 18 mM (1.95 g) of methoxyacetyl chloride in 20 ml of THF was added. The resulted clear solution was refluxed for 3 hours. The reaction mixture was poured into 200 ml of cold 2 N HCl solution and 100 ml of ethyl acetate. The aqueous layer was washed twice with 100 ml of ethyl acetate, and the combined organic layer was washed with saturated $NaHCO_3$ solution,

and dried over $Na_2SO_3$. After solvent removal, the oily residue was purified by column chromatography. The pure product was eluted with 20% ethyl acetate-*n*-hexane in 25~35% yield. Ethyl 2-geranyl-3-methoxyacetylcrotonate (**20a**): IR 3240 (NH), 1715 (ester), 1675, 1615 (amide) $cm^{-1}$; NMR[11] $\delta$ 1.32 (3H, t), 2.50 (3H, s), 3.06 (2H. d) 3.60 (3H, s), 4.00 (2H, s) for *E* isomer, 1.40 (3H, t), 2.48 (3H, s), 3.06 (2H, d), 3.63 (3H, s), 4.05 (2H, s) for *Z*, 1.5~1.8 (9H), 2.0~2.3 (4H), 4.35 (2H, q), 5.16 (2H, m); MS *m/e* 337 ($M^+$), 291 (M–46). Ethyl 2-farnesyl-3-methoxyacetylcrotonate (**20b**): IR 3160 (NH), 1710 (ester), 1670, 1610 (amide) $cm^{-1}$; NMR[11] $\delta$ 1.30 (3H, t) 2.37 (3H, s), 3.54 (3H, s), 3.98 (2H, s) for *E*, 1.37 (3H, s), 2.34 (3H, s), 3.58 (3H, s), 4.02 (2H, s) for *Z*, 1.5~1.8 (12H), 2.0~2.2 (8H), 3.00 (2H, d), 4.26 (2H, q), 5.12 (3H, m); MS *m/e* 405 ($M^+$), 359 (M–46). Ethyl 3-methoxyacetyl-2-phytylcrotonate (**20c**): IR 3240 (NH), 1710 (ester), 1675, 1610 (amide) $cm^{-1}$; NMR[11] $\delta$ 1.28 (3H, s), 2.42 (3H, s), 3.50 (3H, s), 3.98 (2H, s) for *E*, 1.35 (3H, s), 2.40 (3H, s), 3.56 (3H, s), 4.03 (2H, s) for *Z*, 0.88 (12H, d), 1.64 (3H, s), 1.2~1.8 (19H), 2.25 (2H, t), 3.00 (2H, d), 4.12 (2H, q), 4.92 (1H, m); MS *m/e* 479 ($M^+$), 433 (M–46).

*5-Alkenyl-2,4-dihydroxy-3-methoxy-6-methylpyridine* (**17**). To a THF (50 ml) solution of **20** (5 mM) was added dropwise a THF (30 ml) solution of lithium diisopropylamide (15 mM) under $N_2$ with magnetical stirring at −78°C, and the reaction mixture was refluxed for 10 hr. After the similar work-up as described above, the resulting mixture was chromatographed on silica gel. Less polar impurity was eluted with a mixture of ethyl acetate and *n*-hexane (1:1), and elution with ethanol gave pure **17** in 12~15% yield. 5-Geranyl-2,4-dihydroxy-3-methoxy-6-methylpyridine (**17a**): IR 3000 (OH, broad), 2920, 1620 $cm^{-1}$; NMR $\delta$ 1.60 (3H, s), 1.70 (6H, s), 2.04 (4H, s) 2.18 (3H, s), 2.98 (2H, d) 3.96 (3H. s) 5.06 (2H, m); MS *m/e* 291 ($M^+$). 2,4-Dihydroxy-5-farnesyl-3-methoxy-6-methylpyridine (**17a**): IR 3000 (broad), 2920, 1620 $cm^{-1}$; NMR $\delta$ 1.60 (3H, s), 1.68 (9H, s), 2.04 (8H, s), 2.25 (3H, s), 3.06 (2H, d), 3.92 (3H, s), 5.12 (3H, m); MS *m/e* 359 ($M^+$). 2,4-Dihydroxy-3-methoxy-6-methyl-5-phytylpyridine (**17c**): IR 3000 (broad), 2960, 1615 $cm^{-1}$; NMR $\delta$ 0.88 (12H, d), 1.60 (3H, s), 1.90~2.10 (2H, m), 2.20 (3H, s), 3.00 (2H, d), 3.99 (3H, s), 5.06 (1H, m).

*5-Alkenyl-2,4-diacetoxy-3-methoxy-6-methylpyridine* (**21**). A solution of **20** (1 mM) in 2 ml of acetic anhydride was refluxed for 2 hr, and the usual work-up afforded **21** in quantitative yield. 2,4-Diacetoxy-5-geranyl-3-methoxy-6-methylpyridine (**21a**): IR 2920, 1780, 1600 $cm^{-1}$; NMR $\delta$ 1.60 (3H, s), 1.68 (6H, s), 2.00 (4H, s), 2.26 (6H, s), 2.40 (3H, s), 3.18 (2H, d), 3.76 (3H, s), 5.00 (2H, s). 2,4-Diacetoxy-5-farnesyl-3-methoxy-6-methylpyridine (**21b**): IR 2920, 1780, 1600 $cm^{-1}$; NMR $\delta$ 1.60 (3H, s), 1.70 (9H, s), 2.05 (8H, s), 2.28 (6H, s), 2.40 (3H, s), 3.20 (2H, d), 3.78 (3H, s), 5.06 (3H m). 2,4-Diacetoxy-3-methoxy-6-methyl-5-phytylpyridine (**21c**): IR 2960, 1780, 1596 $cm^{-1}$; NMR $\delta$ 0.88 (12H, d), 1.05~1.45 (19H, broad), 1.70 (3H s) 1.92 (2H, t), 2.28 (6H, s), 2.40 (3H, s), 3.22 (2H, d), 3.76 (3H, s), 5.00 (1H, m).

*4-Acetoxy-5-alkenyl-2-hydroxy-3-methoxy-6-methylpyridine* (**22**). A solution of 0.5 mM of **21** in 20 ml of 0.5 N methanolic HCl was stirred for 20 min at room temperature. After neutralization, methanol was removed under reduced pressure, and the residue was diluted with 10 ml of water. The aqueous mixture was extracted with ethyl acetate (10 ml×3), and the combined extract was evaporated under reduced pressure after drying. The resulted crude monoacetate (**22**) was used in the following methylation without purification. 4-Acetoxy-5-geranyl-2-hydroxy-3-methoxy-6-methyl pyridine (**22a**): IR 3000 (OH or NH, broad), 2960, 1780, 1575 $cm^{-1}$; NMR $\delta$ 1.60 (3H, s), 1.70 (6H, s), 2.00 (4H, s), 2.28 (6H, s), 3.00 (2H, d), 3.88 (3H, s), 5.04 (2H, m). 4-Acetoxy-5-farnesyl-2-hydroxy-3-methoxy-6-methylpyridine (**22b**): IR 3000, 2960, 1780, 1580 $cm^{-1}$; NMR $\delta$ 1.60 (3H, s), 1.68 (9H, s), 2.00 (8H, broad), 2.20 (6H, s), 2.96 (2H, d), 3.80 (3H, s), 5.00 (3H, m). 4-Acetoxy-2-hydroxy-3-methoxy-6-methyl-5-phytylpyridine (**22c**): IR 3000, 2960, 1775, 1580 $cm^{-1}$; NMR $\delta$ 0.84 (12H, d), 1.1~1.5 (19H, broad), 1.96 (2H, t), 2.20 (6H, s), 3.00 (2H, d), 3.80 (3H, s), 4.96 (1H, m).

*4-Acetoxy-5-alkenyl-2,3-dimethoxy-6-methylpyridine* (**24**). To a solution of **22** (0.3 mM) and $BF_3$ etherate (trace) in 30 ml of ether was added a solution of diazomethane, and the resulted mixture was stirred for 30 min. After evaporation, the residue was repeatedly methylated by the same procedure until TLC of the product showed almost one spot. The resulting substance was purified by preparative TLC (yield 70~85%). 4-Acetoxy-2,3-dimethoxy-5-geranyl-6-methylpyridine (**24a**): IR 2960, 1780, 1580 $cm^{-1}$; NMR $\delta$ 1.60 (3H, s), 1.70 (6H, s), 2.00 (4H, broad), 2.26 (3H, s), 2.32 (3H, s), 3.04 (2H, d), 3.74 (3H, s), 3.90 (3H, s), 4.96 (2H, m); MS *m/e* 347 ($M^+$), 304 (M–43), 224 (M–123, base peak). 4-Acetoxy-2,3-dimethoxy-5-farnesyl-6-methylpyridine (**24b**): IR 2960, 1780, 1580 $cm^{-1}$; NMR $\delta$ 1.58 (3H, s), 1.66 (9H, s), 1.96 (8H, broad), 2.24 (3H, s), 2.30 (3H, s), 3.02 (2H, d), 3.72 (3H, s), 3.88 (3H, s), 5.00 (3H, m); MS *m/e* 415 ($M^+$), 372 (M–43), 224 (M–191, base peak). 4-Acetoxy-2,3-dimethoxy-6-methyl-5-phytylpyridine (**24c**): IR 2970, 1780, 1585 $cm^{-1}$; NMR $\delta$ 0.84 (12H, d), 1.1~1.5 (19H, broad), 1.72 (3H, s), 1.94 (2H, t), 2.30 (3H, s), 2.36 (3H, s), 3.00 (2H, d), 3.78 (3H, s), 3.96 (3H, s), 4.96 (1H, m); MS *m/e* 489 ($M^+$), 446 (M–43), 224 (M–265, base peak).

*5-Alkenyl-2,3-dimethoxy-4-hydroxy-6-methylpyridine* (**16**). A solution of **24** (0.2 mM) in 1 N $CH_3ONa$–$CH_3OH$ (10 ml) was stirred overnight at room temperature. After neutralization followed by evaporation, the residue was diluted with 10 ml of water, and the aqueous mixture was extracted with ethyl acetate (10 ml × 3). The extract was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The crude product was purified by preparative TLC (solvent system: 7% ethyl acetate-benzene) to afford pure **16**. 2,3-Dimethoxy-5-geranyl-4-hydroxy-6-methylpyridine (**16a**, $PS_g$-IV): colorless oil; IR 3400 (OH, broad), 2960 (CH), 1575 (pyridine), 1450, 1110 $cm^{-1}$; NMR $\delta$ 1.52 (3H, s), 1.60 (3H, s), 1.70 (3H, s), 2.00 (4H, broad), 2.32 (3H, s), 3.24 (2H, d), 3.84 (3H, s), 3.94 (3H, s), 5.04 (2H, m); MS *m/e* 305 ($M^+$), 236 (M–69), 182 (M–123, base peak); UV $\lambda_{max}$ 225 ($\varepsilon$ 7750), 268 (3750) in MeOH; High resolution MS Calcd for $C_{18}H_{27}O_3N$: 305.1499. Found: 305.1506. 2,3-Dimethoxy-5-farnesyl-4-hydroxy-6-methylpyridine (**16b**, $PS_f$-IV): colorless oil; IR 3400 (broad). 2960 1580, 1450 (broad), 1115 $cm^{-1}$; NMR $\delta$ 1.60 (3H, s), 1.68 (6H, s), 1.72 (3H, s), 2.00 (8H, broad), 2.32 (3H, s), 3.28 (2H, d), 3.84 (3H, s), 3.92 (3H, s), 5.06 (3H, m); MS *m/e* 373 ($M^+$). 236 (M–137), 182 (M–191, base peak); UV $\lambda_{max}$ 228 ($\varepsilon$ 7500), 269 (5250) in methanol; High resolution MS Calcd for $C_{23}H_{49}O_3N$: 373.2072. Found: 373.2065. 2,3-Dimethoxy-4-hydroxy-6-methyl-5-phytylpyridine (**16c**, $PS_p$-IV): colorless oil; IR 3400 (broad), 2960, 1580, 1450, 1110 $cm^{-1}$; NMR $\delta$ 0.80 (12H, d), 1.0~1.6 (19H, broad), 1.74 (3H, s), 1.96 2H, t), 2.32 (3H, s), 3.24 (2H, d), 3.84 (3H, s), 3.92 (3H, s), 5.04 (1H, m); MS *m/e* 447 ($M^+$), 236 (M–211), 222 (M–225), 182 (M–265, base peak); UV $\lambda_{max}$ 215 ($\varepsilon$ 7200), 272 (4100) in MeOH; High resolution MS Calcd for $C_{28}H_{49}O_3N$: 447.3074. Found: 447.3078.

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