Mechanistic Studies on the Biomimetic Reduction of Tetrahydroxynaphthalene, a Key Intermediate in Melanin Biosynthesis[1)]

Koji Ichinose, Yutaka Ebizuka,* and Ushio Sankawa*,[2)]

Graduate School of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113–0033, Japan.
Received September 13, 2000; accepted November 6, 2000

1,3,6,8-Tetrahydroxynaphthalene (T4HN) is an aromatic polyketide, serving as a general precursor of fungal melanin. Melanin biosynthesis involves two consecutive deoxygenations of T4HN, consisting of the reduction of a phenolic carbon followed by dehydration. The first reduction to produce scytalone was studied in a biomimetic reduction with sodium borohydride. The reduction required a strong alkaline condition, leading to the tautomerization of T4HN to a reactive species whose structure was clarified by NMR spectroscopy.

Key words 1,3,6,8-tetrahydroxynaphthalene; melanin biosynthesis; scytalone; biomimetic; polyketide

Melanin biosynthesis in a number of pathogenic fungi is an essential step for subsequent infectious procedures such as the formation of appressorium.[3)] Fungal melanin is derived from the polymerization of 1,8-dihydroxynaphthalene (DHN, **1**), which is biosynthesized from a pentaketide intermediate, 1,3,6,8-tetrahydroxynapthalene (T4HN, **2**) by a series of reductions and dehydrations involving scytalone (**3**), 1,3,8-trihydroxynaphthalene (T3HN, **4**) and vermelone (**5**) (Chart 1).[3)] Biosynthesis of **2** is catalyzed by a polyketide synthase (PKS).[4)] Fungal PKS is a multifunctional large protein consisting of catalytic domains such as β-ketoacyl synthase (KS), acyl transferase (AT), acyl carrier protein (ACP), dehydratase (DH), enoyl reductase (ER), ketoreductase (KR) and thioesterase (TE).[4)] The *PKS1* gene was cloned[5)] from the phytopathogenic fungus, *Colletotrichum lagenarium*, by complementation experiments of melanin-deficient albino mutants of *C. lagenarium.* The deduced gene product carries KS, AT, ACP and TE domains, presumed to encode a T4HN synthase. Recently, expression of the *PKS1* gene in a heterologous *Aspergillus oryzae*, demonstrated[6)] that the enzymatic product of the *PKS1* is indeed T4HN (**2**).

The reduction step of T4HN (**2**) is of great interest mechanistically, since it is, together with the subsequent dehydration, an example of post-aromatic deoxygenation of acetate-derived oxygen in the biosynthesis of aromatic polyketides of microbial origin. Other examples are found in the biosynthesis of chrysophanol[7,8)] in *Pyrenochaeta terrestris*, and of sterigmatocystin[9)] in *Aspergillus parasisticus.* From the chemical point of view, the reduction occurring at a phenolic carbon is rather unusual in contrast to the loss of an oxygen atom before aromatization, as seen in the biosynthesis of 6-methylsalicylic acid.[10)] In our earlier biosynthetic studies[11)] on **3**, [1,2-$^{13}C_2$]-acetate was incorporated into **3** with two different labeling patterns in a 1 : 1 ratio, indicating that **3** is biosynthesized *via* a synmetrical intermediate, **2**. It was then demonstrated[12)] that **2** was reduced to **3** by cell-free extracts of *Verticillium dahliae*,[12)] as well as of *Phialophora lagerbergii*[8)] in the presence of NADPH. Recent genetic studies on fungal melanin biosynthesis identified the genes encoding polyhydroxynaphthalene reductase: *ThnR*[13)] from *Magnaporthe grisea*; *THR1*[14)] from *C. lagenarium.* It is also noteworthy that some non-fungicidal anti-blast chemicals, such as tricyclazole, selectively inhibit the reductions.[3)] In the present study we have conducted mechanistic studies on a biomimetic reduction[15)] of **2** to **3** to obtain further chemical information leading to better understanding of post-aromatic deoxygenation.

Results and Discussion

Melanin-precursors, polyhydroxynapththalenes, readily undergo air-oxidation to form quinonic compounds such as flaviolin (**6**) and 2-hydroxyjuglone (**7**), together with polymerized pigments (Chart 1).[3)] Compound **2** is particularly labile and was previously characterized as its stable tetraacetate.[6,15)] The present study required the development of a practical method for the preparation of **2**. A modification (see Experimental) of the method[15)] of Tanaka *et al*., which use alkali fusion of sodium chromotropic acid (**12**), resulted in a significant improvement (58%) (Chart 2).

Bycroft *et al.*[16)] reported that **2** was prepared from methyl curvulinate (**11**) in a sodium methoxide solution, where *in situ* reduction with sodium borhydride proceeded to afford **3**. This led us to try a reduction of **2**, which had been prepared separately, with sodium borohydride in methanol, which turned out to be unsuccessful. The reported method, therefore, was reinvestigated. Compound **11** was synthesized from commercially available **8**, as shown in Chart 2. Treatment of **11** with sodium methoxide, followed by the addition of sodium borohydride, gave **3** (20%) 6,8-dihydroxy-1-methylisochroman-3-one (5%, **13**), along with quinonic com-

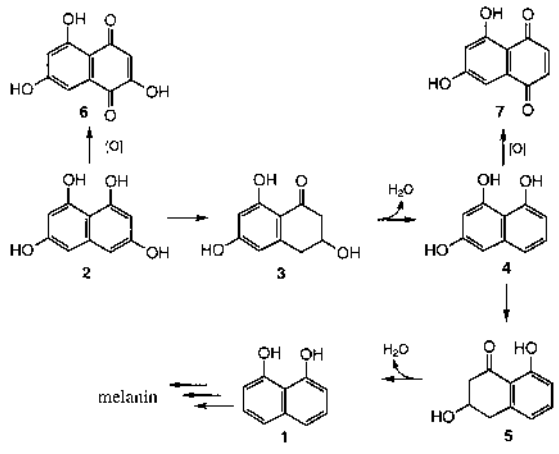

Chart 1. Biosynthesis of Fungal Melanin and Related Metabolites Derived from 1,3,6,8-Tetrahydroxynaphthalene (T4HN, **2**)

* To whom correspondence should be addressed. e-mail: yebiz@mol.f.u-tokyo.ac.jp

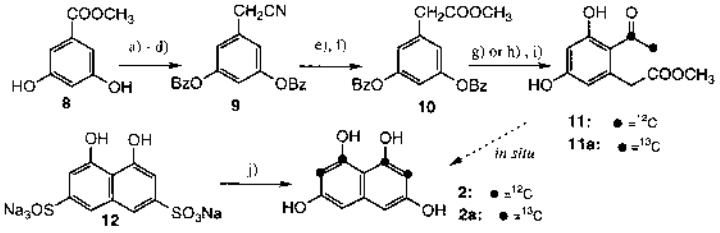

Chart 2. Synthesis of T4HN and Related Materials Used for the Present Study

a) BzBr, K_2CO_3 (96%); b) $LiAlH_4$ (98%); c) CH_3SO_2Cl, NEt_3 (84%); d) NaCN, $BzNEt_3Cl$ (69%); e) $Ba(OH)_2$ (92%); f) CH_2N_2 (100%); g) Ac_2O–60% $HClO_4$ aq. (99%) for **11**; h) [1,2-$^{13}C_2$] AcOH-$(CF_3CO)_2CO$ (89%) for **11a**; i) Pd/C (97%); j) NaOH–$Ba(OH)_2$ (58%).

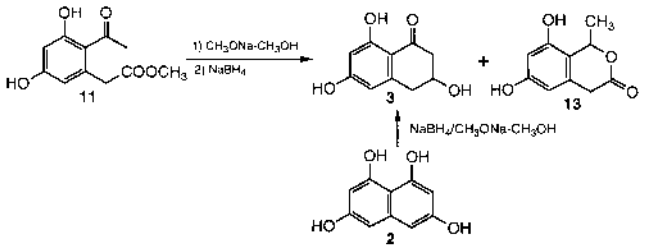

Chart 3. Reaction of T4HN (**2**) with $NaBH_4$ in CH_3ONa–CH_3OH

pounds formed by air oxidation (Chart 3). Apparently, the presence of sodium methoxide is a key factor for the successful reduction. Sodium borohydride reduction of **2** in methanol was conducted in the presence of sodium methoxide, and **3** was obtained in 25% yield, along with the quinonic compounds (Chart 3). The results indicate that **2** was converted into its reactive species only under a strong alkaline condition. We then tried to identify the reactive species using NMR spectroscopy.

Compound **11** was treated with a deuteromethanol (CD_3OD) solution containing sodium deuteromethoxide (CD_3OD) under Ar, and the reaction mixture was directly submitted to NMR measurement. Separately, NMR spectra of **2** were measured in a CD_3ONa–CD_3OD solution. The ^{13}C-NMR spectra of the two samples were essentially identical. The ^1H-decoupled ^{13}C-NMR spectra showed ten significant signals, indicating that the reactive species is not a symmetrical compound (Fig. 1a). Two signals at δ: 192.2 and 192.6, were attributed to conjugated carbonyl and enol carbons, respectively. Four broad signals at δ: 41.0, 102.1, 105.3 and 112.2 were assignable to carbon atoms bearing deuterium, which were formed by ^1H–^2H exchange. This is consistent with the following data: (i) ^1H-NMR monitoring experiments of the cyclization of **11** under stepwise elevations in temperature, where signals derived from the product disappear as the reaction proceeds (Fig. 2); (ii) The ^1H, ^2H-decoupled ^{13}C-NMR spectrum showed that respective signals changed into sharp singlets (Fig. 1b).

To assign all the NMR signals, we measured 2D-NMR spectra using CD_3OD–CH_3OH (1 : 1) as a solvent. Three singlet ^1H signals were observed at δ: 5.16, 5.88 and 6.02, which correlated with the signals at δ: 102.1, 105.3 and 112.2 in a C–H COSY spectrum, respectively. The HMBC spectrum revealed key correlations based on $^3J_{C-H}$ involving quarternary carbons (Table 1). A definite assignment for the closely observed signals at δ: 192.2 and 192.6 was made using a doubly ^{13}C-labeled T4HN. Methyl curvulinate doubly labeled at the acetyl moiety (**11a**) was synthesized by the acetylation of **10** with [1,2-$^{13}C_2$] acetic acid. The ^1H-decoupled ^{13}C-NMR spectrum (Fig. 3) of the cyclized **11a** gave two pairs of ^{13}C–^{13}C coupled signals, δ: 102.1 and 192.6 (J=63 Hz) and δ: 105.3 and 165.0 (J=68 Hz), clearly indicating that the C-3 carbon was assignable to the upper-field signal (192.2 ppm). Thus, complete assignments of the ^1H- and ^{13}C-NMR signals were made (Table 1).

In conclusion, the reactive species is a keto-tautomer represented by **14a** and **14b**, being readily formed from **2** under strong alkaline conditions. The fact that **3** is formed as a single product in the biomimetic reduction of **2** suggests **14b** to be an active species for the reduction at C-3 with sodium borohydride. This was rigorously supported by our theoretical calculations: **2** forms a stable keto-tautomer trianion represented by **14a** or **14b** under alkaline conditions induced by sodium methoxide;[17] a hydride attack at C-3 of the trianionic species is more favorable than at the other possible reactive position, C-1.[18]

A plausible mechanism of the enzymatic reduction of T4HN involves its efficient deprotonation steps in the enzyme complex to generate a reactive keto-tautomer that subsequently undergoes hydride transfer from NADPH. Under neutral conditions, NMR spectral profiles of T4HN (**2**) in an aprotic solvent (acetone-d_6), and in a protic one (CD_3OD) are significantly different (see Experimental). The former gave signals solely derived from a symmetrical naphthalene form, but the latter provided a mixture of naphthalene (87.5%) and keto-tautomer (12.5%) species. Extensive NMR analysis established full assignments of the signals derived from the keto-tautomer. The key signal of C-3 at 178.0 ppm is shifted to upfield by *ca.* 14 ppm from that in strong alkali, suggesting the C-3 carbon is less reactive to the reducing reagent. Sodium borohydride reduction in the presence of CH_3ONa was also attempted for the second substrate, **4**, but no reduced product was obtained. The ^{13}C-NMR spectrum of **4** in CD_3ONa–CD_3OD gave ten signals, all assignable to a naphthalene type species (see Experimental), showing that **4** disfavors the formation of a reactive keto-tautomer under the present alakaline conditons.

Previous biochemical studies[8,13] suggested that the enzymatic reductions of **2** and **4** are catalyzed by the same enzyme. The present results, however, indicate that the chemical properties of **2** and **4** are significantly different, presenting a very interesting biochemical problem: how a single enzyme accommodates the substrate, either **2** or **4**, in the catalytic pocket in such a way that an efficient hydride transfer occurs at the C-3 carbon.

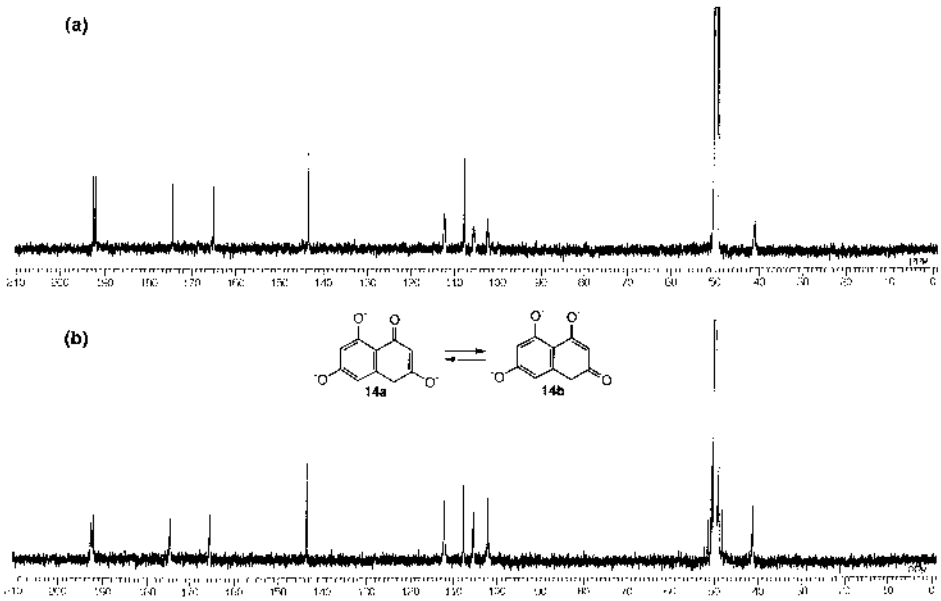

Fig. 1. ^{13}C-NMR Spectra of T4HN (**2**) in CH_3ONa–CH_3OH: (a) ^{1}H-Decoupled; (b) ^{1}H, ^{2}H-Decoupled

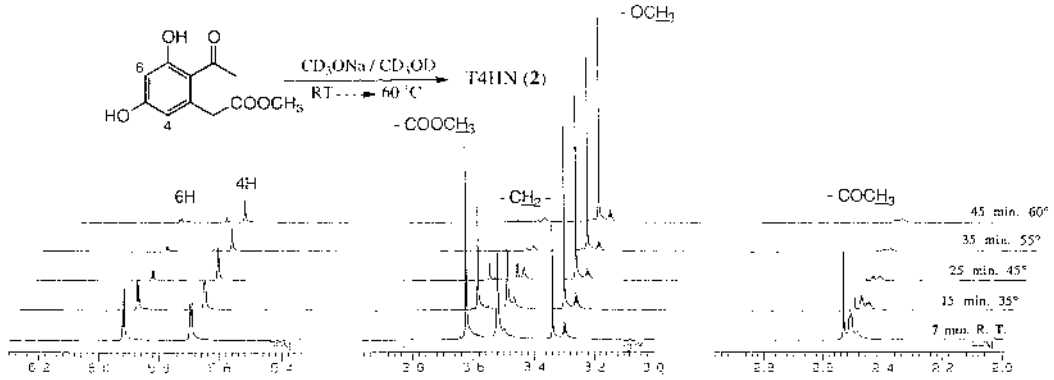

Fig. 2. Changes of ^{1}H-NMR Spectra during the Formation of T4HN (**2**) from Methyl Curvulinate (**11**)

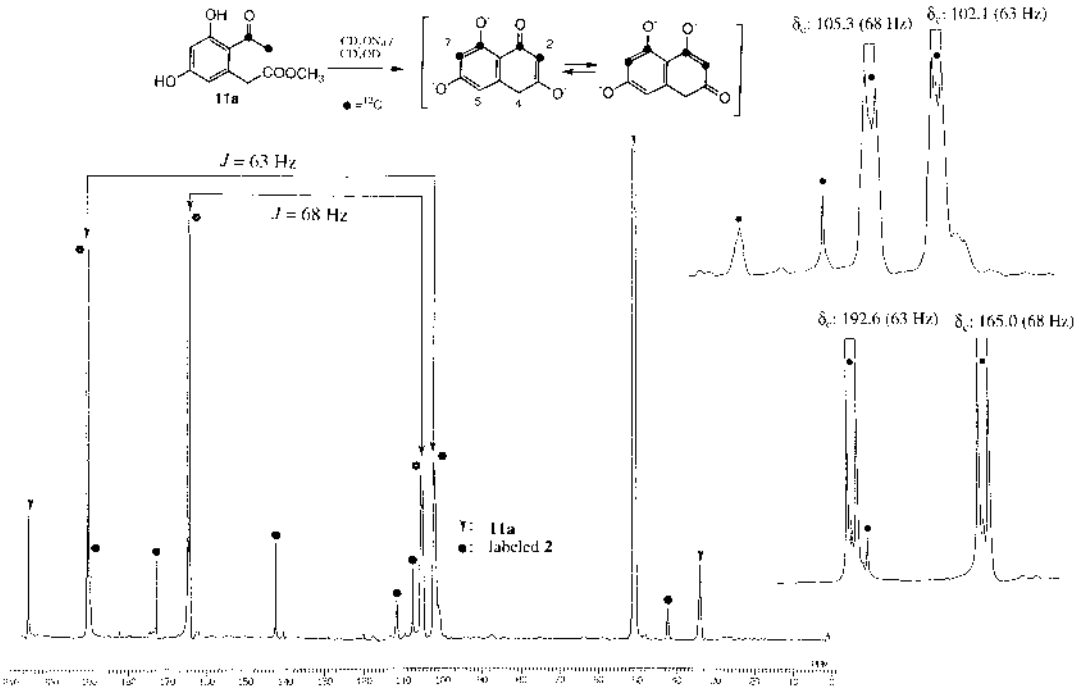

Fig. 3. ^{1}H-Decoupled ^{13}C-NMR Spectrum of T4HN Prepared from ^{13}C-Labeled Methyl Curvulinate (**11a**)

Table 1. NMR Spectral Data for T4HN (**2**) in CD_3ONa–CD_3OD

Position	δ_C (ppm)	δ_H (ppm)[a]	Carbons correlated in HMBC spectrum[a]
C-1	192.6	—	—
C-2	102.1	5.16	4, 8a
C-3	192.2	—	—
C-4	41.0	[b]	—
C-4a	143.4	—	—
C-5	112.2	6.02	4, 6, 7, 8a
C-6	174.5	—	—
C-7	105.3	5.88	5, 6, 8, 8a
C-8	165.0	—	—
C-8a	107.7	—	—

a) Measured in a CD_3OD–CH_3OH (1 : 1) solution of sodium methoxide. *b*) Overlapped with methanol signal.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and uncorrected. Column chromatography was carried out with Wakogel C-200. TLC was conducted on a 0.25 or 2 mm precoated silica gel plate ($60F_{254}$, Merck). NMR and MS spectra were measured on JEOL GSX-400 or 500 and JEOL JMS DX-300, respectively. [1,2-$^{13}C_2$] Acetic acid was purchased from MSD Isotopes Co., Ltd.

(3′,5′-Dibenzyloxy)phenylacetonitrile (9) Potassium carbonate (103 g) and benzyl bromide (75 ml) were added to an anhydrous acetone (400 ml) solution of methyl 3,5-dihydroxybenzoate (**8**) (50.4 g). The mixture was refluxed for 12 h. After cooling, the precipitate was removed by filtration and the filtrate was evaporated. The residue was crystallized from hexane to give methyl 3,5-dibenzyloxybenzoate (100.4 g, 96%) as a white powder (mp 67—68 °C). Part (99 g) was reduced with lithium aluminum hydride to afford 3,5-dibenzyloxybenzyl alcohol (89 g, 97%). A typical reaction condition for the subsequent step includes the dropwise addition of methanesufonyl chloride (0.17 ml) to a dichloromethane solution of the alcohol (640 mg) in the presence of trimethylamine (0.42 ml) at 4 °C. The mixture was stirred for 20 min and poured over iced water and extracted with dichloromethane. The extract was washed with 10% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, followed by drying over Na_2SO_4. The residue, after evaporation of the solvent, was crystallized from dichloromethane–hexane to give methanesulfonyl 3,5-dibenzyloxybenzyl alcohol (670 mg, 84%) as colorless needles (mp 80—81 °C). The product (20 g) was dissolved in chloroform (25 ml), and an aqueous solution (8.5 ml) of sodium cyanide (3.5 g) was added. The mixture was heated under reflux in the presence of benzyl trimethylammonium (813 mg) for 2 h. The cooled mixture was poured into water and extracted with chloroform. The extract was washed with water and dried over Na_2SO_4. The residue, after evaporation of the solvent, was subjected to chromatography on silica gel with chloroform–hexane (4 : 1), followed by crystallization from ethyl acetate–hexane, to give **9** (11.4 g, 69%) as pale yellow plates. mp 85—86 °C. *Anal.* Calcd for $C_{22}H_{19}O_2N$: C, 80.22; H, 5.81; N, 4.25. Found: C, 79.94; H, 5.81; N, 4.34. EI-MS *m/z*: 329 (M^+). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.67 (2H, d, J=0.7 Hz, $-CH_2CN$), 5.03 (4H, s, $-C\underline{H}_2C_6H_5$), 6.15 (3H, br s, 2, 4, 6-H), 7.2—7.4 (10H, m, $2\times C_6H_5$).

Methyl(3′,5′-dibenzyloxy)phenylacetic Acid (10) 15 grams of **9** was dissolved in dioxane (150 ml) and mixed with 0.5 M $Ba(OH)_2$ (150 ml). The mixture was heated at 115 °C for 15 h. After cooling, the mixture was acidified with 1 N aqueous HCl and extracted with ethyl acetate. The usual work-up and crystallization from ethyl acetate–hexane gave (3′,5′-dibenzyloxy) phenylacetic acid (14.5 g, 92%) as colorless needles (mp 106—107 °C). The product was converted quantitatively to **10** by treatment with diazomethane. mp 63—64 °C (methanol). *Anal.* Calcd for $C_{23}H_{22}O_4$: C, 76.22; H, 6.12. Found: C, 76.27; H, 6.20. EI-MS *m/z*: 362 (M^+). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.56 (2H, s, $-C\underline{H}_2COOCH_3$), 3.68 (3H, s, OCH_3), 5.02 (4H, s, $-C\underline{H}_2C_6H_5$), 6.54 (3H, s, 2, 4, 6-H), 7.3—7.4 (10H, m, $2\times C_6H_5$).

Methyl Curvulinate (11) Acetic anhydride (3.6 ml) was added to an acetic acid solution (7.5 ml) of **9** (1.1 g), and the mixture was heated at 35 °C for 7 min, followed by the dropwise addition of 60% aqueous $HClO_4$ (6.5 ml). The mixture was further heated at 35 °C for 5 min, poured into water, and extracted with ethyl acetate. The extract was washed with saturated aqueous $NaHCO_3$ and water. The usual work-up and crystallization from benzene–hexane yielded methyl curvulinate dibenzylether (1.2 g, 99%) as colorless prisms (mp 82—83 °C). The product in ethanol was treated with 5% Pd/C under H_2 to afford **11** (0.47 g, 97%) as a white powder. mp 135—136 °C. *Anal.* Calcd for $C_{11}H_{12}O_5$: C, 58.92; H, 5.40. Found: C, 58.63; H, 5.41. EI-MS *m/z*: 224 (M^+), 182 (M−Ac). ^{1}H-NMR (400 MHz, acetone-d_6) δ: 2.53 (3H, s, CH_3CO–), 3.63 (3H, s, OCH_3), 3.79 (2H, s, $-C\underline{H}_2COOCH_3$), 6.33 (1H, d, J=2.3 Hz, 4-H), 6.37 (1H, d, J=2.3 Hz, 6-H), 9—10 (br –OH). ^{13}C-NMR (100 MHz, acetone-d_6) δ: 32.6, 40.9, 52.4, 103.2, 112.8, 119.2, 138.3, 161.9, 162.2, 172.6, 204.0.

^{13}C-Doubly Labeled Methyl Curvulinate (11a) 868 mg of **10** was dissolved in a mixture of [1,2-$^{13}C_2$] acetic acid (99.7 atom % ^{13}C) (0.1 ml) and acetic acid (0.1 ml). Trifluoroacetic anhydride (0.6 ml) was added to the mixture at 4 °C, and it was then stirred for 45 min at room temperature, poured into saturated aqueous $NaHCO_3$ and extracted with ethyl acetate. The usual work-up and crystallization from benzene–hexane gave a dibenzylether of the product (863 mg, 89%). Enrichment of ^{13}C was evaluated to be 51% based on the inverse gated decoupled ^{13}C-NMR spectrum. **11a** was prepared by quantitative hydrogenation of the dibenzylether with Pd/C, as described above.

1,3,6,8-Tetrahydroxynaphthalene (T4HN) (2) Sodium chromotropic acid (12 g) was heated at 260 °C for 7 h under N_2 in the presence of NaOH (48 g), $Ba(OH)_28H_2O$ (72 g), and KOH (72 g). After cooling under reduced pressure, the resultant solid was triturated with 10% H_2SO_4 to give an aqueous suspension, followed by extraction with ether. The concentrated extract was immediately subjected to flash chromatography on silica gel with ether–hexane (4 : 1). The eluent containing the product was concentrated to precipitate **2** (3.7 g, 58%) as a yellowish powder. EI-MS *m/z*: 194 (M^+). HRMS *m/z*: 192.0404 (Calcd for $C_{10}H_8O_4$: 192.0404). ^{1}H-NMR (400 MHz, acetone-d_6) δ: 6.23 and 6.45 (each 2H, d, J=2.1, 2.0 Hz, 2, 7-H and 4, 5-H). The ^{1}H-NMR spectrum of **2** in CD_3OD indicates the presence of a mixture of naphthalene type (87.5%) and keto-tautomer type (12.5%) under neutral conditions. Extensive NMR studies established the following assignments of both species.

The Naphthalene Type: δ_H: 6.11 (2, 7-H), 6.35 (4, 5-H); δ_C: 99.86 (C-2), 102.0 (C-4), 107.2 (C-8a), 141.4 (C-4a), 157. 8 (C-1), 157.9 (C-3).

The Keto-Tautomer Type: δ_H: 3.67 (4-H), 5.56 (2-H), 6.15 (5-H), 6.28 (7-H); δ_C: 35.0 (C-4), 103.0 (C-7), 104.5 (C-2), 108.6 (C-5), 110.8 (C-8a), 143.2 (C-4a), 164.9 (C-6), 165.5 (C-8), 178.0 (C-3), 194.7 (C-1).

Compound **2** was derivatized to its tetraacetate by treatment with acetic anhydride in the presence of pyridine. mp 127—129 °C (methanol). *Anal.* Calcd for $C_{18}H_{16}O_8$: C, 60.00; H, 4.48. Found: C, 59.82; H, 4.48. EI-MS *m/z*: 360 (M^+), 192 (M−4×Ac). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.33 and 2.38 (each 3H, s, $C\underline{H}_3CO$–), 6.99 and 7.51 (each 2H, d, J=2.2, 2.0 Hz, 4-H and 5, 7-H).

1,3,8-Trihydroxynaphthalene (T3HN) (4) Scytalone (**3**) (3.65 g) was treated with 1 N NaOH (50 ml) at 45 °C under N_2 for 3 h. The mixture was poured over iced water, acidified with 0.5 M H_3PO_4, and extracted with ethyl acetate. The extract was washed with water and dried. The residue after evaporation of the solvent was chromatographed on silica gel to give **4** (2.54 g, 76%) as a greenish yellow powder. EI-MS *m/z*: 176 (M^+). HRMS *m/z*: 176.0476 (Calcd for $C_{10}H_8O_3$: 176.0474). The other spectral data were identical with those described in the ref. 3*a*).

Reduction of T4HN (1) (a) 90 mg of **11** in a methanol (7 ml) solution of CH_3ONa (7.8 mmol, prepared from 180 mg of sodium) was heated at 60 °C under N_2 for 20 min. After cooling to room temperature, sodium borohydride (80 mg) was added to the mixture. It was further heated at 60 °C under N_2 for 4 h, poured into 1 N HCl, and extracted with ethyl acetate. The extract was washed with water and dried. The residue after evaporation of the solvent was chromatographed on consecutive TLCs with benzene–acetone (6 : 4) and ether–benzene (5 : 1) to give **3** (18 mg, 20%, the spectral data were identical with those previously described[11]) and 6,8-dihydroxy-1-methylisochroman-3-one (**13**) (4.5 mg, 5%). EI-MS *m/z*: 194 (M^+). HRMS *m/z*: 194.0576 (Calcd for $C_{10}H_{10}O_4$: 194.0579). ^{1}H-NMR (400 MHz, acetone-d_6) δ: 1.53 (3H, d, J=6.8 Hz, 7-H), 3.48 (1H, d, J=9.3 Hz, 2-H), 3.79 (1H, d, J=9.3 Hz, 2-H), 5.69 (1H, J=6.8 Hz, 7-H), 6.24 (1H, br s, 3-H), 6.35 (1H, dd, J=2.1, 0.8 Hz, 5-H), 8.40 and 8.79 (each 1H, br, –OH).

(b) 38 mg of **2** was treated in essentially the same manner to give scytalone (9.5 mg, 25%). The foregoing reduction conditions gave quinonic compounds in over 60% yield, in which flaviolin (**6**) was identified by a series of spectroscopic studies, as described in ref. 3*a*).

NMR Measurements of T4HN and T3HN under Alkaline Conditions Compound **2** (20 mg) was dissolved in a CD_3OD (0.6 ml) solution of CD_3ONa (1.0 mmol) under Ar and transferred into a NMR tube, where a mixture of CH_3OH–CD_3OD (1 : 1) was used instead of CD_3OD for 2D-NMR measurements (CH-COSY and HMBC). The tube was sealed under Ar and

subjected to NMR measurement. Alternatively, **2** was prepared *in situ* from **11** or **11a** by treatment with CD_3ONa (2.0 mmol) in CD_3OD (2 ml) at 60 °C under Ar for 15 min. Part of the mixture (0.6 ml) was used for NMR measurements, as described above. Conversion of **11** to **2**, upon stepwise elevations in temperature, was monitored on a NMR spectrometer by changes in the ^{1}H-NMR spectrum of **11** in CD_3ONa–CD_3OD. ^{2}H-decoupling was conducted using a JEOL triple resonance system, G50IR2. ^{1}H-decoupled ^{13}C-NMR spectrum of **4** in CD_3ONa–CD_3OD gave the following ten signals at δ: 104. 5, 105.7, 113.9, 114.3, 128.1, 128.2, 141.3, 164.6, 165.3, 168.3.

Acknowledgements We are grateful to Prof. H. Noguchi, School of Pharmaceutical Sciences, University of Shizuoka, for his helpful advice in NMR measurement. We also thank Ms. R. Hirata for elemental analysis services.

Reference and Notes

1) Preliminary account for this paper was reported: Ichinose K., Ebizuka Y., Sankawa U., *Chem. Pharm. Bull*., **37**, 2873—2875 (1989).
2) Present address: *International Traditional Medicine Research Center*, *Toyama International Health Complex*, *Tomosugi*, *Toyama 939–8224*, *Japan.*
3) *a*) Bell A. A., Stipanovic J. E., Puhalla J. E., *Tetrahedron*, **32**, 1353—1356 (1976); *b*) Bell A. A., Puhalia J. E., Tolmsoff W. J., Meola S., *Can. J. Microbiol*., **22**, 702—711 (1976); *c*) Bell A. A., Wheeler W. H., *Annu. Rev. Phytopathol*., **24**, 411—451 (1986).
4) Hopwood D. A., *Chem. Rev*., **97**, 2465—2497 (1997).
5) Kubo Y., Nakamura H., Kobayashi K., Okuno T., Furusawa I., *Plant-Microbe Interact*., **5**, 440—445 (1991).
6) Fujii I., Mori Y., Watanabe A., Kubo Y., Tsuji G., Ebizuka Y., *Biosci. Biotechnol. Biochem*., **63**, 1445—1452 (1999).
7) Anderson J. A., *Phytochemistry*, **25**, 103—106 (1986).
8) Ichinose K., Kiyono J., Ebizuka Y., Sankawa U., *Chem. Pharm. Bull*., **41**, 2015—2021 (1993).
9) Skory C. D., Chang P. K., Cary J., Linz J. E., *Appl. Environ. Microbiol*., **58**, 3527—3537 (1992).
10) Dimroth P., Walter H., Lynen F., *Eur. J. Biochem*., **13**, 98—110 (1970).
11) Sankawa U., Shimada H., Sato T., Kinoshita T., Yamasaki K., *Chem. Pharm. Bull*., **29**, 3536—3542 (1981).
12) Wheeler M. H., *Exp. Mycol*., **6**, 171—179 (1982).
13) Vidal-Cros A., Viviani F., Labesse G., Boccara M., Gaudry M., *Eur. J. Biochem*., **219**, 985—992 (1994).
14) Perpetua N. S., Kubo Y., Yasuda N., Takano Y., Furusawa I., *Mol. Plant Microbe Interact*., **9**, 323—329 (1996).
15) Tanaka H., Ohne Y., Ogawa N., Tamura T., *Agr. Biol. Chem*., **27**, 48—55 (1963).
16) Bycroft B. W., Cashyap M. M., Leung T. K., *J. Chem. Soc*., *Chem. Commun*., **1974**, 443—444.
17) Ichinose K., Sugimori M., Itai A., Ebizuka Y., Sankawa U., *Tetrahedron Lett*., **31**, 5905—5908 (1990).
18) Ichinose K., Sugimori M., Itai A., Ebizuka Y., Sankawa U., *Chem. Lett*., **1991**, 219—222.