Supplemental Observations on Atropisomerism of Fungal Bis(naphtho-γ-pyrone)s

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Epimerization reactions of bis(naphtho-γ-pyrone) derivatives, ustilaginoidin A (2) and dihydroisoustilaginoidin A (6), were examined by refluxing the compounds in acetic acid and in *p*-xylene. The epimerization occurred in acetic acid but not in *p*-xylene. From the reaction mixture in the case of dihydroisoustilaginoidin A, the starting material and the epimer at the biaryl linkage, dihydroustilaginoidin A (7), were isolated and characterized. The circular dichroism Cotton effects due to chiral exciton coupling of biaryl compounds is discussed.

Keywords epimerization; atropisomerism; naphtho-γ-pyrone; ustilaginoidin A; dihydroisoustilaginoidin A; circular dichroism; exciton chirality; stereochemistry

Chaetochromin A (**1**) is a bis(naphtho-γ-pyrone) produced by *Chaetomium* spp., and has cytotoxic and antitumor activities.[1] In the previous paper,[2] the absolute configuration of chaetochromin A (**1**) due to the biaryl linkage was established by the anomalous dispersion method and the result was in agreement with that obtained by the application of the exciton chirality method, removing the contradiction concerning the stereochemistry of bis(naphtho-γ-pyrone) mold metabolites.[3] Subsequently the absolute configurations of the congeners of chaetochromin A (**1**) from *Chaetomium* spp. and of cephalochromin and its derivatives from *Acremonium butyri* were proved to be *S*, while those of ustilaginoidin A (**2**) and the congeners from *Claviceps virens* were *R*.[2,4] This paper provides supplemental results on the atropisomerism of the mold bis(naphtho-γ-pyrone)s and related compounds.

Racemization involving biaryl bonds subject to restricted rotation owing to bulky functions at the *ortho*-positions has been reported for some bis(naphtho-γ-pyrone)s; the optical rotations of aurasperone A (**3**) and aurasperone C dianhydride (**4**) were reduced to half by boiling in acetic acid,[5] while such a reaction was not observed upon the same treatment of nigerone (**5**).[3] In the case of (−)-ustilaginoidin A (**2**), inversion of the optical rotation by boiling in formic acid[6] and racemization by sublimation[7] were reported. To clarify the nature of such reactions, treatments of two bis(naphtho-γ-pyrone)s, (−)-ustilaginoidin A (**2**),[4] which has only one chiral center due to atropisomerism, and (+)-dihydroisoustilaginoidin A (**6**), a metabolite of *Acremonium butyri* having another chiral carbon at the 2-position in the pyrone ring, were performed by refluxing in acetic acid and in formic acid as acidic solvents and in *p*-xylene as a neutral solvent (basic solvents were not used because of the instability of the pyrone derivatives to alkali). The sequence of reactions was followed by the measurement of the optical rotations. The epimerization of the biaryl bond took place during refluxing in the acidic solvents for both compounds and the $[\alpha]_D$ values approached zero within 8 h. The reaction product from (−)-ustilaginoidin A (**2**) was (±)-ustilaginoidin A. The reaction mixture of (+)-dihydroisoustilaginoidin A (**6**) showed the presence of two diastereomeric compounds by high-performance liquid chromatography (HPLC) and they were separated and identified as the starting material (**6**)

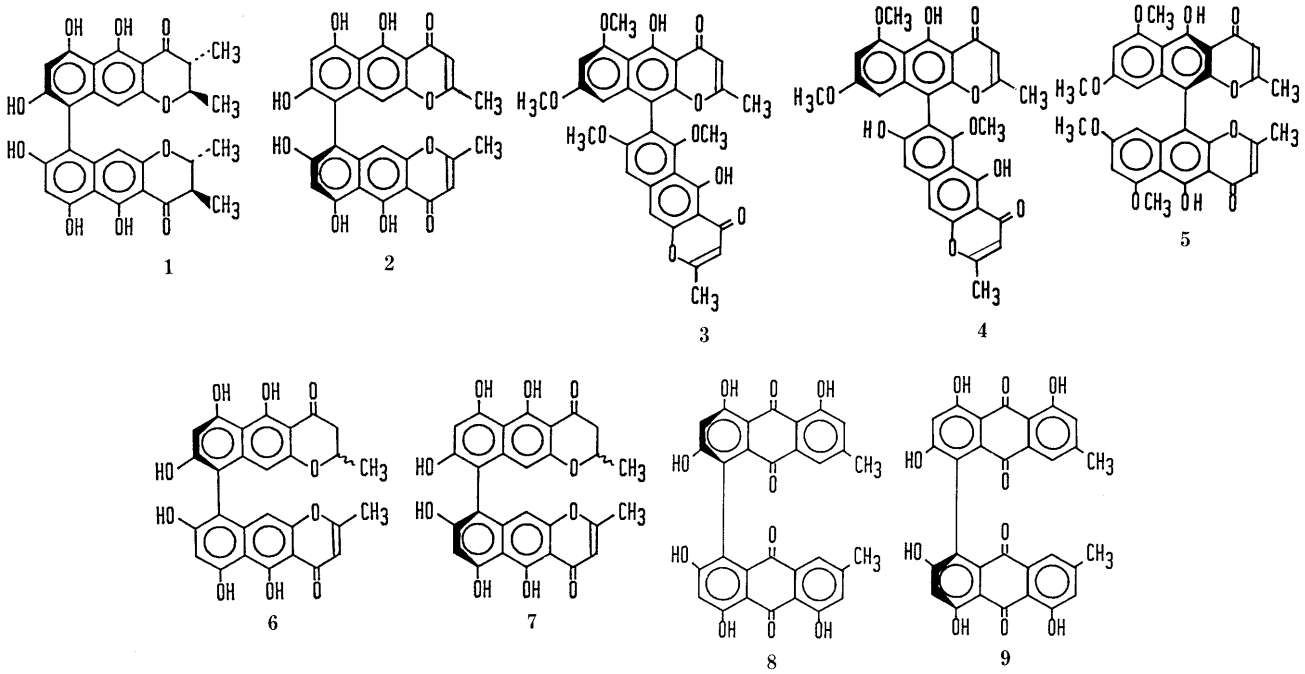

Chart 1

and the epimer of the biaryl linkage, (−)-dihydroustilaginoidin A (**7**). Ustilaginoidin G is one of the bis(naphtho-γ-pyrone)s from *Claviceps virens* (*Ustilaginoidea virens*).[4] From the spectral data, the compound was characterized as a diastereomer of dihydroisoustilaginoidin A (**6**) from *Verticillium* sp.[8] Direct comparison of the physical properties and by HPLC revealed that dihydroustilaginoidin A (**7**) derived from dihydroisoustilaginoidin A (**6**) is identical with ustilaginoidin G.

Time-course studies on the reactions revealed that the reaction was first-order (ustilaginoidin A (**2**), $k=0.316$, $t_{1/2}=2.19$ h; dihydroisoustilaginoidin A (**6**), $k=0.398$, $t_{1/2}=1.74$ h).

On the other hand, when these compounds were refluxed in *p*-xylene, the epimerization did not occur. These results suggest that intramolecular hydrogen bonds between the hydroxyl functions at the *ortho*-positions of the binaphthyl linkage may be formed in *p*-xylene, preventing the epimerization, while, in acetic acid, intermolecular hydrogen bonds between the hydroxyl functions at the *ortho*-position and the solvent molecules were formed rather than the intramolecular hydrogen bonds, so that the racemization is facilitated.

Our results reported in the previous paper[2] showed the applicability of the exciton chirality method to bis(naphtho-γ-pyrone) derivatives. Now we wished to examine the feasibility of the method for an anthraquinone dimer. Skyrin, a 6,6′-dimer of emodin, occurs in several fungi and lichens as (−)- and (+)-enantiomers in different optical purities.[9] Recently the stereochemistry of (+)-skyrin (**8**) was established as *S* by making use of an extension of the Horeau method.[10,11] The circular dichroism (CD) of both enantiomers, (+)-skyrin (**8**) isolated from *Penicillium islandicum*[12] and (−)-skyrin (**9**) from *Preussia multispora*,[13] were determined and the two showed antipodal split bands around 255 nm. (+)-Skyrin (**8**) showed strong positive first and negative second Cotton effects. This indicates that the two long axes of the anthraquinone chromophores are twisted in a clockwise manner, suggesting *S*-configuration of the compound. On the other hand, (−)-skyrin (**9**) showed negative first and positive second Cotton effects, suggesting *R*-configuration. The results are in agreement with those obtained by the Horeau method.[10,11] These facts indicated again that, though the empirical application of CD data has limitations, the exciton chirality method is applicable for the dimeric quinones.

Experimental

All melting points were determined on a Yanagimoto MP micromelting point apparatus and are uncorrected. The proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra were recorded on a JEOL GSX-400 spectrometer (^{1}H 400 MHz and ^{13}C 100 MHz) with tetramethylsilane as an internal standard. Chemical shifts are recorded in ppm (δ). Mass spectra (MS) were taken on JEOL JMS-D300 and JEOL JMS-HX100 instruments. The $[\alpha]_D$ values were measured with a JASCO DIP-140 digital polarimeter. CD spectra were measured in 0.01—0.1 mg/ml dioxane solutions for ustilaginoidin A (**2**), dihydroisoustilaginoidin A (**6**), and dihydroustilaginoidin A (**7**), and in CH_3OH solutions for (+)- (**8**) and (−)-skyrin (**9**) using a 10 mm cell on a JASCO J-20 spectropolarimeter.

Kieselgel 60 F_{254} (Merck) precoated plates were used for thin-layer chromatography (TLC) and the spots were detected by ultraviolet (UV) illumination. Column chromatography was carried out on 70—230 mesh silica gel (Merck). HPLC was carried out by using a Waters M45J pump with an Oyo-Bunko Uvilog 7 UV detector.

Ustilaginoidin A (2) from *Claviceps virens* (*Ustilaginoidea virens*) Isolation and the physical properties were reported in the previous paper.[4]

Isolation of Dihydroisoustilaginoidin A (6) from *Acremonium butyri* (*Nectoria viridescens*) The strain (CBS 479.69) was incubated in a stationary culture on sterilized wheat (110 g) at 26 °C for 30 d. The moldy wheat was extracted three times with EtOAc (200 ml) for 24 h at room temperature. The extract was chromatographed over silica gel (treated with 3% oxalic acid) using CH_2Cl_2 as the developing solvent and purification by HPLC (Nucleosil 50-5, treated with 3% oxalic acid) using hexane–EtOAc (4:1) as the developing solvent gave cephalochromin (tetrahydroisoustilaginoidin A) as the major metabolite[2] and dihydroisoustilaginoidin A (**6**) (2.8 mg) as the minor metabolite, orange powder (from EtOAc–hexane), mp > 300 °C, $[\alpha]_D^{20}$ +518° ($c=0.06$, dioxane). UV $\lambda_{max}^{dioxane}$ nm (ε): 228 (36000), 270 (48400), 290 (58700), 325 (9600), 336 (10200), 411 (8900). IR ν_{max}^{KBr} cm^{-1}: 3400, 1640, 1632, 1588, 1450, 1385, 1364, 1346, 1150, 1120, 1084, 870, 840..CD (dioxane) $[\theta]^{20}$ (nm): +51400 (226), 0 (234), −68500 (242), −376900 (265), 0 (275), +428300 (294), +24800 (324), 0 (333), −30400 (341), 0 (358), +3400 (425). Its identity with **6** from *Verticillium* sp.[8] was confirmed by ^{1}H- and ^{13}C-NMR and CD.

Epimerization of (−)-Ustilaginoidin A (2) and (+)-Dihydroisoustilaginoidin A (6) in Boiling Acetic Acid and *p*-Xylene The optically active natural naphtho-γ-pyrones (**2** or **6**) (25—50 mg) in acetic acid or *p*-xylene (50—100 ml) were refluxed for 11 h in an oil bath and aliquots of the solution were taken each hour for measurement of the optical rotations.

Epimerization of (−)-Ustilaginoidin A (2) (−)-Ustilaginoidin A (**2**) (29.3 mg) in acetic acid (50 ml) was refluxed in an oil bath for 10 h. EtOAc and H_2O were added to the reaction mixture and the organic layer, after being washed with H_2O and dried over Na_2SO_4, was evaporated to dryness. (±)-Ustilaginoidin A (29.1 mg) was obtained from the residue by HPLC (Nucleosil 50-5 treated with 3% oxalic acid) with EtOAc–hexane (1:4) as the developer. It showed good agreement with starting material as regards physical data except for the $[\alpha]_D^{20}$ value and the CD spectra; $[\alpha]_D^{20}$ 0° ($c=0.08$, dioxane); CD (dioxane) $[\theta]^{20}$ (nm) = no peak. In the case of the treatment with formic acid, similar results were obtained.

Epimerization of (+)-Dihydroisoustilaginoidin A (6) (+)-Dihydroisoustilaginoidin A (**6**) (40.0 mg) in acetic acid (80 ml) was refluxed in an oil bath for 10 h. EtOAc and H_2O were added to the reaction mixture and the organic layer, after being washed with H_2O and dried over Na_2SO_4, was evaporated to dryness. Dihydroisoustilaginoidin A (**6**) (12.3 mg), the starting material, and dihydroustilaginoidin A (**7**) (12.5 mg), the epimer at the binaphthyl bond, were obtained from the residue by HPLC (Nucleosil 50-5 treated with 3% oxalic acid) with EtOAc–hexane (1:3) as the developer. Dihydroustilaginoidin A (**7**) was recrystallized from EtOAc–hexane as an orange powder, mp 238—242 °C, $[\alpha]_D^{20}$ −288° ($c=0.11$, dioxane). MS *m/z*: 516.1099 (M^+, Calcd for $C_{28}H_{20}O_{10}$; 516.1056). UV $\lambda_{max}^{dioxane}$ nm (ε): 228 (32000), 272 (43400), 291 (52900), 326 (7400), 340 (4600), 414 (8200). IR ν_{max}^{KBr} cm^{-1}: 3390, 1640, 1630, 1585, 1450, 1380, 1360, 1340, 1260, 1130, 1080, 873, 840, 833. CD (dioxane $[\theta]^{20}$ (nm): +171200 (264), 0 (275), −218700 (291), −10500 (323), 0 (331), +16600 (343), +500 (415). ^{1}H-NMR (DMSO-d_6) δ: 1.27 (3H, d, $J=6.0$ Hz, 2-CH_3), 2.26 (3H, s, 2′-CH_3), 2.68 (1H, d, $J=16.7$ Hz, 3-H), 2.81 (1H, dd, $J=11.7$, 16.7 Hz, 3-H), 4.42 (1H, m, 2-H), 5.66 (1H, s, 3′-H), 6.17, 6.18, 6.46, 6.57 (1H × 4, each s, 7,7′,10,10′-H), 9.76 (2H, s), 9.82, 9.95 (1H × 2, each s, 6,6′,8,8′-OH). ^{13}C-NMR (DMSO-d_6) δ: 20.1, 20.4 (2,2′-CH_3), 42.7 (3-C), 72.8 (2-C), 98.0, 98.4, 100.0, 100.7 (7,7′,10,10′-C), 101.6, 101.6 (4a,4′a-C), 105.8 (3′-C), 104.3, 105.8, 106.5, 106.9 (5a,5′a,9,9′-C), 139.7, 141.6 (9a,9′a-C), 151.9, 154.8 (10a,10′a-C), 157.8, 158.8, 159.0, 160.1 (6,6′,8,8′-C), 162.6, 164.7 (5,5′-C), 169.7 (2′-C), 183.3 (4′-C), 198.0 (4-C). It showed good agreement with ustilaginoidin G from *Claviceps virens*[4] as regards physical data and HPLC behavior.

Isolation of (+)-Skyrin (8) from *Penicilliun islandicum* The specimen was obtained from a culture of the mold following the method previously reported.[12] CD(CH_3OH)$[\theta]^{20}$ (nm): −5100 (248), 0 (255), +15200 (262), 0 (276), −1900 (305).

(−)-Skyrin (9) from *Preussia multispora*[13] The authentic sample showed CD (CH_3OH) $[\theta]^{20}$ (nm): +1700 (251), 0 (255), −5500 (264), 0 (279), +700 (303).

Acknowledgement The authors thank Professor K. Suda, Meiji College of Pharmacy, for his suggestion concerning the reaction mechanism. They are indebted to Dr. S. Udagawa, National Institute of Hygienic Sciences, and Prof. U. Sankawa, University of Tokyo, for the supply of the fungal strains.

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