Chaetochromins B, C and D, Bis(naphtho-γ-pyrone) Derivatives
from Chaetomium gracile

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Besides chaetochromin A (1), a known mycotoxin and antitumor agent, three related
bis(naphtho-γ-pyrone) derivatives named chaetochromins B (2), C (3), and D (4) were isolated from
Chaetomium gracile. The structures were elucidated as the diastereomer, the 3-demethyl derivative,
and the 2,3-dehydro derivative of 1, respectively, chiefly on the basis of nuclear magnetic resonance
data.

Keywords—Chaetomium gracile; chaetochromin; bis(naphtho-γ-pyrone); mycotoxin; anti-
tumor agent; 1H-NMR; 13C-NMR

Chaetochromin (1) is a mycotoxin, first isolated from Chaetomium thielavioides CHEN
NHL2829, and was identified as 2,2′,3,3′-tetrahydro-5,5′,6,6′,8,8′-hexahydroxy-2,2′,3,3′-
tetramethyl-[9,9′-bi-4H-naphtho[2,3-b]pyran-4,4′-dione].1 The synonymy of the species, Chaetot-
omicum cellulolyticum CHAHAL and Achaetomiella virescens v. ARX with C. virescens  (v. ARX) UDAGAWA was later  proved2 and the species was characterized by the production of the set of mycotoxins, chaetochromin (1), chaetocin, and sterigmatocystin.3 Chaetochrom-
min (1) was also isolated from Chaetomium caprinum BAINIER, C. gracile UDAGAWA, and C. tetrasporum HUGHES.3 Chaetochromin was demonstrated to induce delayed liver injuries,
brone marrow aplasia, and atrophy of lymphatic tissue in mice,4 and to exhibit teratogenicity (e.g., inducing exencephalus) on administration to pregnant mice.5 The compound was effective in the treatment of MX-1 breast xenograft, M5076 ovarian carcinoma, and P388 lymphocytic leukemia in mice.5

To perform detailed biological testing, large amounts of the material were required and
the strain (73-S-T-Y-3)3 of C. gracile was selected among the available strains as the best
producer of chaetochromin (1).

This paper presents the structures of three congeners of chaetochromin isolated in the
course of the study and also the relative stereochemistry of chaetochromin (1), which
remained unsettled at the time of the structure elucidation.1 Preparation of some derivatives
of 1 for the examination of structure–activity relationship is also presented.

Dichloromethane extract of the strain of C. gracile cultured on wheat grains afforded,
besides the major metabolite chaetochromin (this compound will hereafter be referred to as
chaetochromin A (1)), three congeners named chaetochromins B (2), C (3), and D (4) by silica
gel chromatography and high-performance liquid chromatography (HPLC) on Nucleosil 50-
5. Chaetochromins B (2), C (3), and D (4) showed nearly the same ultraviolet (UV) and infra-
red (IR) absorptions as chaetochromin A (1), indicating the presence of the same naphtho-
γ-pyrone chromophores in the molecules. Molecular formulae determined by high-resolution
mass spectroscopy (MS) indicated C_{30}H_{26}O_{16}, C_{29}H_{24}O_{10}, and C_{30}H_{24}O_{10} for 2, 3, and
4, respectively. The values correspond to an isomer and demethyl and dehydro derivatives of
1, respectively.
The proton and carbon-13 nuclear magnetic resonance (1H- and 13C-NMR) spectra of these compounds (Tables I and II) clearly indicated the structural differences of these compounds. Chaetochromin A (1) is a symmetric dimer and all the NMR signals represent the summation of two identical halves in the molecule. The NMR spectra of the three congeners

**Table 1.** 1H-NMR Data for Chaetochromins (in CDCl3)\(^{a}\)

<table>
<thead>
<tr>
<th>Proton</th>
<th>A (1)</th>
<th>B (2)</th>
<th>C (3)</th>
<th>D (4)</th>
</tr>
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<tr>
<td>2, 2'</td>
<td>4.17 (dq, (J = 11.0, 6.1))</td>
<td>4.18 (dq, (J = 11.0, 6.1))</td>
<td>4.17 (dq, (J = 11.0, 6.1))</td>
<td>4.16 (dq, (J = 11.3, 6.1))</td>
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<tr>
<td></td>
<td>4.53 (dq, (J = 3.1, 6.7))</td>
<td>4.49 (dq, (J = 9.8, 5.5, 6.1))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3, 3'</td>
<td>2.62 (dq, (J = 11.0, 6.7))</td>
<td>2.64 (dq, (J = 11.0, 6.7))</td>
<td>2.62 (dq, (J = 11.0, 6.7))</td>
<td>2.61 (dq, (J = 11.3, 6.7))</td>
</tr>
<tr>
<td></td>
<td>2.62 (dq, (J = 3.1, 7.3))</td>
<td></td>
<td>2.681 (d, (J = 9.8))</td>
<td>2.676 (d, (J = 5.5))</td>
</tr>
<tr>
<td>7, 7'</td>
<td>6.48 (s)</td>
<td>6.50 (s)</td>
<td>6.50 (s)</td>
<td>6.55 (s)</td>
</tr>
<tr>
<td>10, 10'</td>
<td>6.49 (s)</td>
<td>6.47 (s)</td>
<td>6.47 (s)</td>
<td>6.47 (s)</td>
</tr>
<tr>
<td>2-CH₃, 2'-CH₃</td>
<td>5.93 (s)</td>
<td>5.93 (s)</td>
<td>5.93 (s)</td>
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</tr>
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<td></td>
<td>5.93 (s)</td>
<td></td>
<td>6.33 (s)</td>
<td></td>
</tr>
<tr>
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<td>1.25 (d, (J = 6.7))</td>
<td>1.24 (d, (J = 6.7))</td>
<td>1.24 (d, (J = 6.7))</td>
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<tr>
<td></td>
<td>1.31 (d, (J = 6.7))</td>
<td>1.38 (d, (J = 6.1))</td>
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<td>2.29 (s)</td>
</tr>
<tr>
<td>5-OH, 5'-OH</td>
<td>15.27 (s)</td>
<td>15.28 (s)</td>
<td>15.25 (s)</td>
<td>16.26 (s)</td>
</tr>
<tr>
<td></td>
<td>15.18 (s)</td>
<td>15.06 (s)</td>
<td>15.26 (s)</td>
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<tr>
<td>6-OH, 6'-OH</td>
<td>9.65 (s)</td>
<td>9.68 (s)</td>
<td>9.63 (s)</td>
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<td>9.63 (s)</td>
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<td>9.62 (s)</td>
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<td>8-OH, 8'-OH</td>
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<td>5.76 (br)</td>
<td>5.84 (br)</td>
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<td></td>
<td>5.84 (br)</td>
<td>5.80 (br)</td>
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</tbody>
</table>

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\(a\) Chemical shifts are given on the \(\delta\) (ppm) scale with tetramethylsilane as an internal standard and coupling constants are given in Hz (s, singlet; d, doublet; t, triplet; q, quartet; br, broad).
(2—4) showed that the two halves in these molecules are not the same. In the $^{13}$C-NMR spectrum of chaetochromin A (1) fifteen signals appear, while in that of chaetochromin B (2) thirty signals, two corresponding to each signal in 1, were observed. The $^1$H-NMR spectrum of 2 showed that the relative stereochemistry of the two secondary methyl groups at the 2- and 3-positions in one half of the molecule is the same as that of the two methyl groups in 1 ($J_{2H-3H}$, 11.0 Hz both in 1 and 2), but the other 2,3-dimethyl group in 2 showed a different coupling constant ($J_{2H-3H}$, 3.1 Hz). In the previous paper$^{11}$ the stereochemistry of chaetochromin A (1) remained unsettled. Comparison of these coupling constants clearly indicated that both of the vicinal methyl groups in 1 and one of the two in 2 are trans, while the other in 2 is cis. Reexamination of the $^{13}$C-NMR data, such as $J_{CH}$ of the C-3 proton and C-2 methyl carbon (1.8 Hz) in 1$^{7}$ did not show any discrepancy in the assignments. Thus, the structure of chaetochromin B (2) was established as the stereoisomer of chaetochromin A (1), where one of the two trans-2,3-dimethyl groups was replaced by a cis-2,3-dimethyl group.

In chaetochromin C (3), the next congener, one of the four secondary methyl groups is lacking ($^1$H- and $^{13}$C-NMR), and the $^1$H-NMR spectrum indicated the presence of one methylene group at the 3-position (δ 2.681 and 2.676, each 1H). The spectral data indicated that the other part of the molecule is the same as in 1. Thus, chaetochromin C (3) is composed

<table>
<thead>
<tr>
<th>Carbon atom at</th>
<th>Chaetochromins</th>
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<td></td>
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<td></td>
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<td>200.8</td>
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<tr>
<td>4a, 4a'</td>
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<td></td>
<td>101.8</td>
</tr>
<tr>
<td>5, 5'</td>
<td>164.4</td>
</tr>
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<td>164.5</td>
</tr>
<tr>
<td>5a, 5a'</td>
<td>105.6</td>
</tr>
<tr>
<td></td>
<td>105.6</td>
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<tr>
<td>6, 6'</td>
<td>159.8</td>
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<td></td>
<td>159.9</td>
</tr>
<tr>
<td>7, 7'</td>
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<td></td>
<td>99.8</td>
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<td>8, 8'</td>
<td>160.8</td>
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<td>160.8</td>
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<tr>
<td>9, 9'</td>
<td>102.0</td>
</tr>
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<td></td>
<td>101.4</td>
</tr>
<tr>
<td>9a, 9a'</td>
<td>141.9</td>
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<td>141.9</td>
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<tr>
<td>10, 10'</td>
<td>99.3</td>
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<td>99.3</td>
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<td>10a, 10a'</td>
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<td>16.6</td>
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<td>3-CH₃, 3'-CH₃</td>
<td>9.9</td>
</tr>
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<td>9.5</td>
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</tbody>
</table>

(a) Assignments may be interchanged.
of half of 1 having a 2,3-dimethyl group and half having a 2-methyl group but lacking the 3-methyl group. Cephalochromin (5), a metabolite of Cephalosporium sp., \(^8,9\) Verticillium sp., \(^10\) Nectria viridescens, \(^11\) and N. flavoviridis, \(^11\) is a known dimer of the latter half of chaetochromin C (3).

The third compound, chaetochromin D (4), showed the presence of one \(\gamma\)-pyrone moiety instead of one dihydro-\(\gamma\)-pyrone unit in the molecule of chaetochromin A (1): one of the two pairs of two sp\(^3\) carbons at the 2- and 3-positions is replaced by one pair of two sp\(^2\) carbons (\(^{13}\)C-NMR of 4, \(\delta\) : 162.4 and 113.1 ppm) and one of the two pairs of vicinal secondary methyl groups is replaced by a dimethyl group on a double bond (\(^1\)H-NMR of 4, \(\delta\) : 1.99 (3H, s) and 2.29 (3H, s)). These facts indicated that chaetochromin D (4) corresponds to the 2,3-dehydro compound of chaetochromin A (1). In the case of cephalochromin (5), the bisdemethyl compound of chaetochromin A (1), coproduction of the bisdehydro compound (6) and the tetrakis-dehydro compound (isoustilaginoidin A) (7) by Verticillium sp. was reported.\(^10\)

The circular dichroism (CD) curves of these compounds (1—4) showed positive Cotton effects around 295 nm and negative effects around 265 nm, as shown in Fig. 1, indicating the same stereostructure around the 9-9’ positions, which is antipodal to that in ustilaginoidsins,

![Fig. 1. CD Spectra of Chaetochromins (in Dioxane)](image)

--- chaetochromin A (1); -----, chaetochromin B (2); ---, chaetochromin C (3); ---, chaetochromin D (4); ----, ustilaginoidin A (7).

<table>
<thead>
<tr>
<th>Proton at</th>
<th>5,5’-Dimethyl ether (8)</th>
<th>5,5’,8,8’-Tetramethyl ether (9)</th>
<th>5,5’,6,6’,8,8’-Hexamethyl ether (10)</th>
<th>6,6’,8,8’-Tetra-acetate (11)</th>
</tr>
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<tbody>
<tr>
<td>2, 2’</td>
<td>4.18 (dq, (J = 11.0, 6.1))</td>
<td>4.13 (dq, (J = 11.0, 6.1))</td>
<td>4.11 (dq, (J = 11.0, 6.1))</td>
<td>4.14 (dq, (J = 11.0, 6.1))</td>
</tr>
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<td>3, 3’</td>
<td>2.50 (dq, (J = 11.0, 6.6))</td>
<td>2.50 (dq, (J = 11.0, 7.0))</td>
<td>2.51 (dq, (J = 11.0, 7.0))</td>
<td>2.63 (dq, (J = 11.0, 7.2))</td>
</tr>
<tr>
<td>7, 7’</td>
<td>6.54 (s)</td>
<td>6.69 (s)</td>
<td>6.63 (s)</td>
<td>6.92 (s)</td>
</tr>
<tr>
<td>10, 10’</td>
<td>6.35 (s)</td>
<td>6.32 (s)</td>
<td>6.32 (s)</td>
<td>6.08 (s)</td>
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<td>2, 2’-CH(_3)</td>
<td>1.42 (d, (J = 6.1))</td>
<td>1.39 (d, (J = 6.1))</td>
<td>1.37 (d, (J = 6.1))</td>
<td>1.41 (d, (J = 6.1))</td>
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<tr>
<td>3, 3’-CH(_3)</td>
<td>1.20 (d, (J = 6.6))</td>
<td>1.19 (d, (J = 7.0))</td>
<td>1.17 (d, (J = 7.0))</td>
<td>1.21 (d, (J = 7.2))</td>
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<td>10.17 (s)</td>
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<td>14.19 (s)</td>
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<tr>
<td>6, 6’-OH</td>
<td>5.69 (br)</td>
<td></td>
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<td>8, 8’-OH</td>
<td>5.69 (br)</td>
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<td>5, 5’-OCH(_3)</td>
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<td>4.14 (s)</td>
<td>4.07 (s)</td>
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<tr>
<td>6, 6’-OCH(_3)</td>
<td>4.00 (s)</td>
<td>4.00 (s)</td>
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<tr>
<td>8, 8’-OCH(_3)</td>
<td>3.76 (s)</td>
<td>3.79 (s)</td>
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<td>1.93 (s)</td>
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<td>6, 6’-OCOCH(_3)</td>
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<td></td>
<td>2.41 (s)</td>
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</table>
metabolites of Claviceps virens (anamorph state: Ustilaginoidea virens).\textsuperscript{12)} The absolute configurations of these compounds will be the subject of a forthcoming paper.

To examine the structure–activity relationship of the chaetochromin and related compounds, partial methylation and acetylation of chaetochromin A (1) were attempted. The 5,5'-di- (8), 5,5',8,8'-tetra- (9), and 5,5',6,6',8,8'-hexa-methyl ethers (10) and the 6,6',8,8'-tetra-acetate (11) were prepared and the structures were confirmed by \textsuperscript{1}H- and \textsuperscript{13}C-NMR as shown in Tables III and IV. The results of biological tests will also be reported in a forthcoming paper.

### Experimental

All melting points were determined on a Yanagimoto MP micromelting point apparatus and are uncorrected. The \textsuperscript{1}H- and \textsuperscript{13}C-NMR spectra were recorded on a JEOL GX-400 (\textsuperscript{1}H 400 MHz and \textsuperscript{13}C 100 MHz) spectrometer in CDCl\textsubscript{3} with tetramethylsilane as an internal standard. Chemical shifts are recorded in ppm (\textdelta). MS were taken on a JEOL JMS-D300. UV and IR spectra were measured with a Shimadzu UV-240 spectrophotometer and a JASCO A-102 infrared spectrophotometer. The [\alpha]D values were measured with a JASCO DIP-140 digital polarimeter. CD spectra were recorded on a JASCO J-20 spectropolarimeter.

Kiesel gel 60F\textsubscript{254} (Merck) precoated plates were used for thin-layer chromatography (TLC) and the spots were detected by UV illumination. Column chromatography was carried out on 70–230 mesh silica gel (Merck). HPLC were carried out by using a Waters M45J pump with a Oyo-Bunko Uvilog-5IIIA UV detector.

**Isolation of Metabolites from Chaetomium gracile** —— The strain (73-S-T-Y-3) was incubated in stationary culture on sterilized wheat (150 g) at 26 °C for 5 d. The moldy wheat was extracted twice with CH\textsubscript{2}Cl\textsubscript{2} (250 ml) for 24 h at room temperature. The extract was chromatographed over silica gel (treated with 3% oxalic acid) using CH\textsubscript{2}Cl\textsubscript{2} as the developing solvent to afford fractions 1 and 2. Purification by HPLC (Nucleosil 50-5, treated with 3%
oxalic acid) using EtOAc-hexane (1:5) as the developing solvent gave chaetochromin A (1) (125 mg) from fraction 1 and chaetochromins A (1) (103 mg), B (2) (1 mg), C (3) (15 mg), and D (4) (4 mg) from fraction 2. Chaetochromin A (1) was recrystallized from CHCl₃-hexane as a yellow powder, mp 207—210°C, [α]₂⁰° + 600 (c = 0.06, dioxane). MS m/z: 546.1515 (M⁺, Calcd for C₂₉H₂₆O₁₂: 546.1526). UV λ max nm (c): 235 (43680), 270 (48230), 292 (59150), 325 (15470), 335 (10740), 412 (10470). IR ν absorption cm⁻¹: 3400, 1640, 1630, 1587, 1445, 1383, 1360, 1348, 1257, 1150, 1133, 1091, 1025, 915, 840. CD (dioxane) [θ]₂²⁰° (nm): +91400 (226), 0 (236), -63700 (243), -436800 (266), 0 (275), +196600 (281), +509600 (294), +50100 (326), 0 (333), -49900 (339), 0 (354), 9100 (415). Its identity with an authentic sample of chaetochromin A (1) was confirmed by IR and TLC.

**Chaetochromin B (2)** - Recrystallized from CHCl₃-hexane as a yellow powder, mp 204—206°C, [α]₂⁰° + 524 (c = 0.05, dioxane). MS m/z: 546.1496 (M⁺, Calcd for C₂₉H₂₆O₁₂: 546.1526). UV λ max nm (c): 230 (37200), 270 (52100), 292 (62900), 325 (10300), 340 (6500), 412 (9500). IR ν absorption cm⁻¹: 3400, 1638, 1628, 1585, 1443, 1380, 1357, 1340, 1149, 1140, 1085, 1025, 1018, 908, 839. CD (dioxane) [θ]₂²⁰° (nm): +86500 (226), 0 (235), -64900 (240), -459500 (266), 0 (275), +216200 (283), +46500 (327), 0 (334), -47000 (340), 0 (356), -7600 (415). The authors thank Miss K. Kinoshita for her help in a part of this work.
6) The screening performed under the auspices of the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland, U.S.A.