Chromone Derivatives from the Filamentous Fungus *Lachnum* sp. BCC 2424

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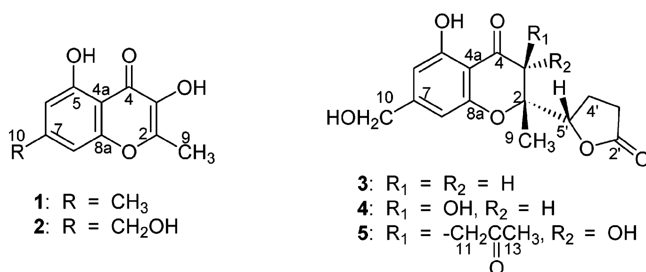
Five new chromones, named lachnones A−E (**1**−**5**), were isolated from the filamentous fungus *Lachnum* sp. BCC 2424 along with known (2*E*,6*S*)-2,6-dimethyl-2,7-octadiene-1,6-diol. Their structures were elucidated by spectroscopic methods. Lachnones B (**2**) and D (**4**) mildly inhibited the growth of *Mycobacterium tuberculosis*, both with MIC values of 200 μg/mL.

The genus *Lachnum* is a rich source of biologically active secondary metabolites including antimicrobial naphthalenones,[1] nematicidal and antimicrobial lachnumones,[2] and antibiotic papyracons.[3] We describe herein the isolation and structural elucidation of five new chromone derivatives, lachnones A−E (**1**−**5**), from the broth EtOAc extract of *Lachnum* sp. BCC 2424 together with known (2*E*,6*S*)-2,6-dimethyl-2,7-octadiene-1,6-diol.[4]



Lachnone A (**1**) was obtained as a yellow gum. The HREIMS showed the molecular formula $C_{11}H_{10}O_4$, indicating that **1** had seven degrees of unsaturation, which was consistent with apparent chromone absorption bands at λ_{max} 245, 277, and 342 nm in the UV spectrum. The IR spectrum exhibited hydroxyl and conjugated carbonyl absorption bands at 3346 and 1639 cm^{-1}, respectively. The ^{1}H NMR spectrum showed resonances for one chelated hydroxy proton (δ 11.58, s), two aromatic protons (δ 6.71, s br and 6.59, s br), one OH proton (δ 5.78, s br), and two methyl groups (δ 2.45, s and 2.40, s). The ^{13}C NMR spectrum showed signals indicating one carbonyl (δ 175.3), six quaternary (δ 159.4, 155.8, 149.7, 146.8, 136.7, and 107.3), two methine (δ 111.0 and 107.7), and two methyl (δ 22.4 and 15.1) carbons. The chelated hydroxyl group that was placed at C-5 (δ 159.4), peri position to the carbonyl group, gave HMBC cross-peaks with C-4a (δ 107.3), C-5 and C-6 (δ 111.0) (see Supporting Information). The aromatic proton at δ 6.59 was attributed to H-6 due to its correlation with C-6 in the HMQC spectrum and ^3J HMBC correlations with C-4a, C-8 (δ 107.7) and C-10 (δ 22.4). HMQC correlations from the methyl protons at δ 2.40 to C-10 and from the other aromatic proton at δ 6.71 to C-8 indicated the location of the methyl group and the aromatic proton at C-7 (δ 146.8) and C-8, respectively. Irradiation of H_3-10 in the NOEDIFF spectrum enhanced the intensity of the H-6 and H-8 resonances, supporting the above assignment. The other hydroxyl group was located at C-3 (δ 136.7) on the basis of HMBC correlations from the OH proton (δ 5.78) to C-2 (δ 149.7), C-3 and C-4 (δ 175.3). HMBC correlations between the remaining methyl protons at δ 2.45 and C-2 and C-3 established the attachment of the methyl group at C-2. Therefore, lachnone A was assigned to be **1**, a 3-hydroxy derivative of altechromone A.[5]

Lachnone B (**2**) was obtained as an orange solid. The molecular formula $C_{11}H_{10}O_5$ deduced by HREIMS was higher than that of **1** by one additional oxygen atom. The UV and IR spectra were almost identical to those of **1**. The ^{1}H and ^{13}C NMR spectral data were similar to those of **1**. The difference between **1** and **2** was that the C-10 signal, observed as a methyl carbon signal (δ 22.4) in **1**, was observed as an oxymethylene carbon signal (δ 63.9) in **2**. The replacement of H_3-10 in **1** with H_2-10 resonating at δ 4.69 (s br) in **2** was supported by signal enhancement of H-6 (δ 6.70, s br) and H-8 (δ 6.97, s br) in the NOEDIFF experiment after irradiation of H_2-10. Therefore, lachnone B had the chromone structure **2**.

Lachnone C (**3**) was obtained as a yellow gum, whose molecular formula $C_{15}H_{16}O_6$ was determined by HREIMS. The UV spectrum showed bands at 211, 278, and 348 nm, while the IR spectrum displayed bands at 3384, 1776, and 1642 cm^{-1} characteristic for hydroxyl, γ-lactone carbonyl, and conjugated ketone carbonyl groups, respectively. Carbon resonances at δ 176.1 and 195.8 supported the presence of the γ-lactone and ketone carbonyl groups. The ^{1}H NMR spectrum exhibited resonances for one hydrogen-bonded hydroxyl group (δ 11.57, s), two aromatic protons (δ 6.52, s br and 6.45, s br), hydroxymethyl protons (δ 4.63, s), one oxymethine proton (δ 4.58, t, J = 7.2 Hz), three methylene groups [δ 2.98 (d, J = 17.1 Hz, 1H), 2.61 (d, J = 17.1 Hz, 1H), 2.60 (m, 2H), and 2.32 (m, 2H)], and one oxyquaternary methyl group (δ 1.43, s). HMBC and NOEDIFF results (see Supporting Information) indicated that **3** possessed the left-hand aromatic ring identical to **2**. The nonequivalent methylene protons at δ 2.98 and 2.61 were attributed to H_2-3 of the chromanone skeleton due to their correlations with C-2 (δ 80.9), C-4 (δ 195.8), and C-9 (δ 19.1) in the HMBC spectrum. The oxyquaternary methyl group resonating at δ 1.43 gave a HMQC cross-peak with C-9 and HMBC correlations from the methyl protons to C-2 and C-3 (δ 42.9), revealing the linkage of the methyl group at C-2 of the chromanone unit. In the COSY spectrum, the methylene protons at δ 2.32 (H_2-4′) were coupled with the methylene protons at δ 2.60 (H_2-3′) and the oxymethine proton at δ 4.58 (H-5′). These results together with a ^3J HMBC cross-peak from H-5′ to the carbonyl carbon at δ 176.1 (C-2′) confirmed the presence of the γ-lactone moiety. Bond formation between C-5′ of the γ-lactone unit and C-2 of the chromanone skeleton was established on the basis of ^3J HMBC correlations of H-5′/C-3 and C-9. The NOEDIFF results of H-5′/H_3-9 and H-3α (δ 2.98, d, J = 17.1 Hz) and those of H_3-9/H-5′ and H-3β (δ 2.61, d, J = 17.1 Hz) revealed that both H-5′ and H_3-9 had the β-orientation. Accordingly, lachnone C was elucidated to be **3**.

Lachnone D (**4**) was obtained as a yellow gum. The HREIMS showed the molecular formula $C_{15}H_{16}O_7$, 16 mass units higher than

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that of **3**. The UV and IR spectra were almost identical to those of **3**, indicating the presence of an identical chromophore. The ^{1}H NMR data were similar to those of **3**, except for the replacement of H_2-3 resonances in **3** with an oxymethine signal (δ 4.59, s) in **4**, thus revealing the location of the oxymethine proton at C-3 (δ 71.3). This assignment was confirmed by HMBC correlations from H-3 to C-2 (δ 84.3), C-4 (δ 196.4), C-9 (δ 13.4), and C-5′ (δ 82.3). Irradiation of H-5′ (δ 4.78, dd, J = 7.8 and 6.3 Hz), in the NOEDIFF experiment, enhanced only the signal intensity of H-3 and H_3-9 (δ 1.42), suggesting that H-5′, H_3-9, and 3-OH were β-oriented. Therefore, lachnone D (**4**) was a 3-hydroxy derivative of **3**.

Lachnone E (**5**) was obtained as a yellow gum, whose molecular formula $C_{18}H_{20}O_8$ was deduced by HREIMS. The UV and IR spectra were similar to those of **4**, with an additional ketone carbonyl stretching frequency at 1731 cm^{-1}. The ^{1}H and ^{13}C NMR data were similar to those of **4**, implying that **4** and **5** had a similar chromanone unit with a γ-lactone moiety attached at C-2. The differences were the replacement of the methine unit (δ_H 4.59, δ_C 71.3) in **4** with an oxyquaternary carbon (δ_C 78.7) in **5**, thus revealing the absence of the hydrogen atom at C-3. In addition, the ^{1}H and ^{13}C NMR spectra displayed additional resonances belonging to one methylene unit [(H_2-11, δ_H 2.86, d, J = 16.5 Hz, and 2.79, d, J = 16.5 Hz) and (C-11, δ_C 39.1)], one methyl group [(H_3-13, δ_H 2.45, s) and (C-13, δ_C 31.9)], and one ketone carbonyl carbon (C-12, δ_C 212.7). The HMBC correlations from H_2-11 to C-2 (δ 84.3), C-3, C-4 (δ 195.5), and C-12 and from H_3-13 to C-12 linked a 2-propanoyl group at C-3. This assignment was further supported by the HMBC cross-peaks between 3-OH (δ 6.78) with C-2, C-3, and C-11. The orientation of 3-OH and the 2-propanoyl group at the α- and β-positions, respectively, was established due to the following NOEDIFF results: H_3-9/H_2-11 and H-5′ (δ 5.36, dd, J = 9.0 and 7.0 Hz)/3-OH. Therefore, lachnone E was determined to be **5**.

All compounds were inactive in antimalarial (*Plasmodium falciparum* K1), antifungal (*Candida albicans*), and cytotoxicity (KB cells, BC-1 cells) assays. Lachnones B (**2**) and D (**4**) were weakly active against *M. tuberculosis* H37Ra, both with minimum inhibitory concentration (MIC) values of 200 μg/mL, while the other compounds were inactive.

Experimental Section

General Experimental Procedures. Melting points were measured on an electrothermal melting point apparatus (Electrothermal 9100). Infrared spectra (IR) were determined neat on a Perkin-Elmer 783 FTS165 FT-IR spectrometer. Ultraviolet (UV) absorption spectra were determined in MeOH on a Shimadzu UV-160A spectrophotometer. ^{1}H and ^{13}C NMR spectra were run on either a 300 MHz Bruker FTNMR Ultra Shield spectrometer or a 500 MHz Varian UNITY INOVA spectrometer in $CDCl_3$, or as otherwise stated. Mass spectra were obtained on a MAT 95 XL mass spectrometer (Thermofinnigan). Optical rotations were measured in MeOH on a JASCO P-1020 polarimeter. Thin-layer chromatography (TLC) and precoated TLC were performed on silica gel GF_{254} (Merck). Column chromatography (CC) was carried out on Sephadex LH-20 or silica gel (Merck) type 100 (70-230 mesh ASTM).

Fungal Material. The fungus used in this study was collected on wood in leaf litter at Khao Yai National Park, Central Thailand, by Dr. Nigel. L. Hywel-Jones, BIOTEC. On the basis of the sequence data, it was identified as *Lachnum* sp., by Dr. Janet Jennifer Laungsaard, BIOTEC. This fungus was deposited in the BIOTEC Culture Collection as BCC 2424 on May 25, 1999.

Fermentation and Isolation. BCC 2424 was maintained on potato dextrose agar at 25 °C, which was cut into pieces (1 × 1 cm), and inoculated into 2 × 250 mL Erlenmeyer flasks containing 25 mL of Difco potato dextrose broth (PDB; composition, potato starch 4.0 g, dextrose 20.0 g, per liter) (15 pieces for each flask). After incubation at 25 °C for 7 days on a rotary shaker (200 rpm), each primary culture was transferred into a 1000 mL Erlenmeyer flask containing 250 mL of the same liquid medium (PDB) and incubated at 25 °C for 7 days on a rotary shaker (200 rpm). Each 25 mL portion of the secondary seed cultures (in 2 flasks) was transferred into 20 × 1000 mL Erlenmeyer flasks each containing 250 mL of PDB. Fermentation was carried out on rotary shakers at 25 °C for 14 days. The cultures were separated by filtration into the mycelia and filtrate. The filtrate was extracted three times with equal volumes of EtOAc (5 L). The EtOAc layer was dried over anhydrous $MgSO_4$ and evaporated to dryness under reduced pressure to obtain a brown gum (572.4 mg), which was fractionated by column chromatography (CC) over Sephadex LH20 using MeOH as eluent to afford five fractions (A−E). Fraction B (64.1 mg) was further purified by CC on silica gel with a gradient of CH_2Cl_2−MeOH to give four subfractions (B1−B4). Subfraction B2 contained (2*E*,6*S*)-2,6-dimethyl-2,7-octadiene-1,6-diol[4] (9.3 mg). Fraction C (146.5 mg) was then separated by CC with a gradient of CH_2Cl_2−MeOH to give four subfractions (C1−C4). Subfraction C2 (7.2 mg, 2−3% MeOH−CH_2Cl_2) was further purified by precoated TLC using 100% CH_2Cl_2 as mobile phase (15 runs) to give **3** (3.6 mg) and **5** (2.9 mg). Subfraction C3 (22.7 mg, 5% MeOH−CH_2Cl_2) was subjected to CC using a gradient of CH_2Cl_2-MeOH to give three subfractions. The second subfraction (8.2 mg) was purified by TLC using 30% EtOAc−light petroleum as mobile phase (21 runs) to afford **4** (3.5 mg). Fraction D (154.6 mg) was subjected to CC using a gradient of CH_2Cl_2−MeOH to give **1** (1.8 mg) and **2** (13.3 mg).

Lachnone A (1): yellow gum; UV(MeOH) λ_{max} nm (log ϵ) 245 (3.54), 277 (3.10), 342 (3.00); FT-IR (neat) $\nu_{cm^{-1}}$ 3346, 1639; ^{1}H NMR (300 MHz) 11.58 (1H, s, 5-OH), 6.71 (1H, s br, H-8), 6.59 (1H, s br, H-6), 5.78 (1H, s br, 3-OH), 2.45 (3H, s, H-9), 2.40 (3H, s, H-10); ^{13}C NMR (125 MHz) 175.3 (C, C-4), 159.4 (C, C-5), 155.8 (C, C-8a), 149.7 (C, C-2), 146.8 (C, C-7), 136.7 (C, C-3), 111.0 (CH, C-6), 107.7 (CH, C-8), 107.3 (C, C-4a), 22.4 (CH_3, C-10), 15.1 (CH_3, C-9); EIMS *m/z* (% relative intensity) 206 (100), 191 (23), 177 (23), 151 (26), 135 (23); HREIMS *m/z* 206.0590 $[M]^+$ (calcd for $C_{11}H_{10}O_4$ 206.0579).

Lachnone B (2): orange solid; mp 148.5−149.2 °C; UV(MeOH) λ_{max} nm (log ϵ) 245 (4.30), 344 (3.70); FT-IR (neat) $\nu_{cm^{-1}}$ 3346, 1639; ^{1}H NMR (300 MHz) (acetone-d_6) 12.10 (1H, s, 5-OH), 7.85 (1H, s br, 3-OH), 6.97 (1H, s br, H-8), 6.70 (1H, s br, H-6), 4.69 (2H, s br, H-10), 2.43 (3H, s, H-9); ^{13}C NMR (75 MHz) (acetone-d_6) 176.9 (C, C-4), 160.8 (C, C-5), 156.6 (C, C-8a), 152.2 (C, C-2), 152.1 (C, C-7), 138.1 (C, C-3), 109.3 (C, C-4a), 107.8 (CH, C-6), 105.0 (CH, C-8), 63.9 (CH_2, C-10), 15.1 (CH_3, C-9); EIMS *m/z* (% relative intensity) 222 (100), 193 (29); HREIMS *m/z* 222.0511 $[M]^+$ (calcd for $C_{11}H_{10}O_5$ 222.0528).

Lachnone C (3): yellow gum; $[\alpha]^{29}_D$ +43.4 (*c* 0.13, MeOH); UV (MeOH) λ_{max} nm (log ϵ) 211 (3.64), 278 (3.36), 348 (2.77); FT-IR (neat) $\nu_{cm^{-1}}$ 3384, 1776, 1642; ^{1}H NMR (300 MHz) 11.57 (1H, s, 5-OH), 6.52 (1H, s br, H-6), 6.45 (1H, s br, H-8), 4.63 (2H, s br, H-10), 4.58 (1H, t, J = 7.2 Hz, H-5′), 2.98 (1H, d, J = 17.1 Hz, H-3α), 2.61 (1H, d, J = 17.1 Hz, H-3β), 2.60 (2H, m, H-3′), 2.32 (2H, m, H-4′), 1.43 (3H, s, H-9); ^{13}C NMR (125 MHz) 195.8 (C, C-4), 176.1 (C, C-2′), 162.0 (C, C-5), 158.9 (C, C-8a), 153.2 (C, C-7), 106.9 (C, C-4a), 106.4 (CH, C-6), 105.3 (CH, C-8), 82.6 (CH, C-5′), 80.9 (C, C-2), 64.5 (CH_2, C-10), 42.9 (CH_2, C-3), 28.2 (CH_2, C-3′), 22.3 (CH_2, C-4′), 19.1 (CH_3, C-9); EIMS *m/z* (% relative intensity) 292 (19), 207 (100), 167 (35), 149 (55), 72 (37), 69 (25), 59 (58); HREIMS *m/z* 292.0949 $[M]^+$ (calcd for $C_{15}H_{16}O_6$ 292.0947).

Lachnone D (4): yellow gum; $[\alpha]^{29}_D$ +27.1 (*c* 0.22, MeOH); UV (MeOH) λ_{max} nm (log ϵ) 211 (4.42), 244 (3.97), 279 (4.18), 346 (3.70); FT-IR (neat) $\nu_{cm^{-1}}$ 3388, 1770, 1644; ^{1}H NMR (300 MHz) 10.60 (1H, s, 5-OH), 6.56 (1H, dt, J = 1.2, 0.9 Hz, H-6), 6.48 (1H, dt, J = 1.2, 0.9 Hz, H-8), 4.78 (1H, dd, J = 7.8, 6.3 Hz, H-5′), 4.65 (2H, t, J = 0.9 Hz, H-10), 4.59 (1H, s, H-3), 2.73 (1H, m, H-3′a), 2.61 (2H, m, H-3′b, H-4′a), 2.37 (1H, m, H-4′b), 1.42 (3H, s, H-9); ^{13}C NMR (125 MHz) 196.4 (C, C-4), 176.7 (C, C-2′), 161.2 (C, C-5), 158.7 (C, C-8a), 154.4 (C, C-7), 106.9 (CH, C-6), 106.1 (CH, C-8), 104.6 (C, C-4a), 84.3 (C, C-2), 82.3 (C, C-5′), 71.3 (CH, C-3), 64.4 (CH_2, C-10), 28.3 (CH_2, C-3′), 21.9 (CH_2, C-4′), 13.4 (CH_3, C-9); EIMS *m/z* (% relative intensity) 308 (6), 284 (41), 223 (28), 185 (46), 133 (32), 81 (28), 69 (61), 61 (43); HREIMS *m/z* 308.0863 $[M]^+$ (calcd for $C_{15}H_{16}O_7$ 308.0896).

Lachnone E (5): yellow gum; $[\alpha]^{29}_D$ +51.4 (*c* 0.035, MeOH); UV (MeOH) λ_{max} nm (log ϵ) 281 (3.90), 354 (3.28); FT-IR (neat) $\nu_{cm^{-1}}$ 3367, 1775, 1731, 1644; ^{1}H (500 MHz) 10.80 (1H, s, 5-OH), 6.78 (1H, s br, 3-OH), 6.53 (1H, q, J = 1.5 Hz, H-6), 6.47 (1H, q, J = 1.5 Hz, H-8), 5.36 (1H, dd, J = 9.0, 7.0 Hz, H-5′), 4.64 (2H, s br, H-10), 2.86

(1H, d, $J = 16.5$ Hz, H-11a), 2.79 (1H, d, $J = 16.5$ Hz, H-11b), 2.60 (2H, m, H-3′), 2.45 (3H, s, H-13), 2.44 (2H, m, H-4′), 1.26 (3H, s, H-9); ^{13}C NMR (125 MHz) 212.7 (C, C-12), 195.5 (C, C-4), 176.4 (C, C-2′), 162.4 (C, C-5), 157.7 (C, C-8a), 153.3 (C, C-7), 106.8 (CH, C-6), 105.2 (CH, C-8), 103.9 (C, C-4a), 84.3 (C, C-2), 78.7 (C, C-3), 77.7 (C, C-5′), 64.5 (CH_2, C-10), 39.1 (CH_2, C-11), 31.9 (CH_3, C-13), 28.0 (CH_2, C-3′), 23.1 (CH_2, C-4′), 12.8 (CH_3, C-9); EIMS *m/z* (% relative intensity) 364 (26), 222 (39), 194 (46), 177 (80), 167 (100), 85 (45); HREIMS *m/z* 364.1151 $[M]^+$ (calcd for $C_{18}H_{20}O_8$ 364.1158).

Bioassay Procedures. Antimalarial activity was evaluated against the parasite *P. falciparum* (K1, multidrug-resistant strain), using the microculture radioisotope technique based on the method described by Desjardins et al.[6] The standard compound was dihydroartemisinin, showing an IC_{50} value of 3.7 nM. Cytotoxicity was determined according to the colorimetric assay described by Skehan and co-workers.[7] The standard compound was ellipticine, exhibiting IC_{50} values of 0.24 and 0.22 μg/mL against human epidermoid carcinoma (KB) and human breast cancer (BC) cell lines, respectively. Antimycobacterial activity was performed against *M. tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA).[8] Standard drugs, isoniazid and kanamycin sulfate, exhibited MIC values of 0.05 and 2.50 μg/mL, respectively. Antifugal activities were assessed against *Candida albicans* (ATCC 90028) by the modified colorimetric method utilizing the tetrazolium salt (MTT).[9]

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Supporting Information Available: Table of HMBC and NOE-DIFF data for lachnones A−E (**1**−**5**) is available free of charge via the Internet at http://pubs.acs.org.

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