Chrotacumines A−D, Chromone Alkaloids from *Dysoxylum acutangulum*

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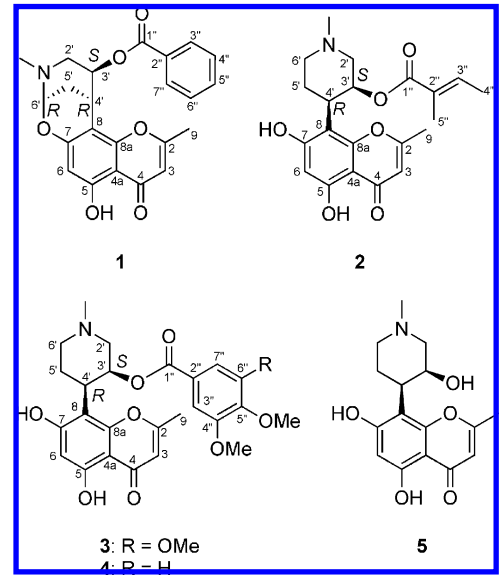
Four new chromone alkaloids, chrotacumines A−D (**1**−**4**), consisting of a 5,7-dihydroxy-2-methylchromone, an *N*-Me piperidine ring, and an ester side chain were isolated from *Dysoxylum acutangulum*, and their structures including absolute configurations were elucidated on the basis of spectroscopic data interpretation including 2D NMR, CD spectra, and X-ray analysis. The known compound rohitukine (**5**) showed moderate cytotoxicity against human HL-60 promyelocytic leukemia and HCT-116 colon cancer cells.

Chromone alkaloids such as rohitukine[1] from *Dysoxylum binectariferum* (Meliaceae) and schumanniophytine[2] from *Schumanniophyton problematicum* (Rubiaceae), with two common structure units of a 5,7-dihydroxy-2-methylchromone and a piperidine ring, have attracted great interest from both biological and biogenetic points of view.[3,4] This unique skeleton when coupled with two different structural units has also been a challenging target for total synthesis.[5−7] Biological activities such as anti-inflammatory and antiviral effects have also been reported.[3,8] Chromone alkaloids have been isolated only from two different families to date, Meliaceae and Rubiaceae.[3,4] We have recently isolated the novel chromone alkaloids cassiarins A and B, which showed potent antiplasmodial activity, from the leaves of *Cassia siamea*.[9,10]

In our search for structurally and biologically interesting natural products from tropical plants belonging to Meliaceae that are found in Malaysia,[11−14] four new chromone alkaloids, chrotacumines A−D (**1**−**4**), consisting of a 5,7-dihydroxy-2-methylchromone (noreugenin), an *N*-Me piperidine ring, and an ester side chain, were isolated from either the leaves or bark of *Dysoxylum acutangulum* Miq. together with rohitukine (**5**),[1] schumanniofioside A, and noreugenin.[15,16] This plant has been used commercially as a timber tree, and (+)-8-hydroxycalamenene, showing antibacterial activity as well as fish toxicity, has been isolated from the seeds.[17] In this paper, we describe the isolation of four new and three known compounds from the leaves and bark of *D. acutangulum* and the structure elucidation including the absolute configurations of chrotacumines A−D (**1**−**4**). The compounds isolated were evaluated against a small panel of cancer cell lines.

Leaves of *D. acutangulum* were extracted with MeOH, and a part of the extract was partitioned between hexane and water. The water-soluble material was extracted with EtOAc, and the EtOAc-soluble part was worked up to give **1** (2.0 mg, 0.004%) and **2** (2.3 mg, 0.005%). The bark of *D. acutangulum* when treated similarly gave **3** (2.9 mg, 0.002%) and **4** (1.9 mg, 0.001%), together with the known chromone alkaloids rohitukine (**5**)[1] and schumanniofioside A, along with noreugenin.[15,16]

Compound **1**, $[\alpha]^{22}_{D}$ −159 (*c* 1.0, MeOH), showed a pseudomolecular ion peak at *m*/*z* 408 $(M + H)^+$ in the ESIMS, and the molecular formula, $C_{23}H_{21}NO_6$, was established by HRESIMS [*m*/*z* 408.1456 $(M + H)^+$]. IR absorptions implied the presence of hydroxy (3420 cm^{-1}), ester carbonyl (1720 cm^{-1}), and conjugated carbonyl (1660 cm^{-1}) functionalities. Analysis of the ^1H and ^{13}C NMR data (Table 1) and the HMQC spectrum of **1** revealed the presence of three sp^3 methines, two sp^3 methylenes, two methyls, seven sp^2 methines, and nine sp^2 quaternary carbons.



The gross structure of **1** was deduced from extensive analysis of the two-dimensional NMR data, including the $^1H-^1H$ COSY, HMQC, and HMBC spectra in CD_3OD (Figure 1).

The HMBC cross-peaks of H_3-9 to C-2 and C-3, H-3 to C-4 and C-4a, and H-6 to C-4a, C-5, C-7, and C-8 revealed the presence of a 5,7-dihydroxy-2-methylchromone substituted at C-8. The presence of the *N*-methylpiperidine ring was indicated by the HMBC correlations of the *N*-methyl protons to C-2′ and C-6′. In addition, the HMBC cross-peaks of H-6′ to C-7 and H_2-5′ to C-8 established a connectivity between C-6′ and C-7 through an oxygen atom and a connectivity between C-4′ and C-8. Another partial structure (C-3″−C-7″) including a benzoate at C-3′ was verified by the HMBC correlations of H-3′, H-3″, and H-7″ to C-1″. These data suggested that **1** possesses a schumagnine skeleton,[18] with a chromone linked to a piperidine-related moiety. Thus, the gross structure of chrotacumine A was determined as **1** shown in Figure 1.

The relative configuration of **1** was assigned using NOESY correlations as shown in a computer-generated 3D diagram (Figure 2). A NOESY correlation between H-3′/H-5′a indicated that these protons were in a 1,3-diaxial relationship. The equatorially oriented H-4′ and H-6′ were assigned by the NOESY correlations of H-4′/

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Table 1. ^{1}H NMR Data (*J*, Hz) of **1**−**4** in CD_3OD at 300 K[a]

position	1	2	3	4
3	5.86 (1H, s)	6.03 (1H, s)	5.82 (1H, s)	5.78 (1H, s)
6	6.29 (1H, s)	6.20 (1H, s)	6.19 (1H, s)	6.15 (1H, s)
9	1.63 (3H, s)	2.41 (3H, s)	2.23 (3H, s)	2.23 (3H, s)
N-Me	2.61 (3H, s)	2.33 (3H, s)	2.41 (3H, s)	2.32 (3H, s)
2′a	2.45 (1H, t, 11.0)	2.45 (1H, d, 12.7)	2.56 (1H, d, 12.8)	2.50 (1H, d, 12.7)
2′b	2.92 (1H, dd, 11.0, 5.2)	3.14 (1H, d, 12.7)	3.19 (1H, d, 12.8)	3.18 (1H, brd, 12.7)
3′	5.51 (1H, ddd, 11.0, 5.2, 5.2)	5.17 (1H, brs)	5.53 (1H, brs)	5.40 (1H, brs)
4′	3.96 (1H, brs)	3.48 (1H, d, 13.1)	3.58 (1H, d, 13.6)	3.49 (1H, d, 12.8)
5′a	2.29 (1H, brd, 13.3)	1.81 (1H, d, 13.1)	1.81 (1H, d, 13.6)	1.77 (1H, d, 12.4)
5′b	2.03 (1H, ddd, 13.3, 3.7, 3.7)	3.24 (1H, m)	3.30 (1H, m)	3.27 (1H, m)
6′a	5.11 (1H, brs)	2.21 (1H, t, 11.5)	2.29 (1H, d, 12.8)	2.21 (1H, m)
6′b		3.11 (1H, m)	3.19 (1H, d, 12.8)	3.12 (1H, 10.6)
3″	7.79 (1H, d, 7.9)	6.89 (1H, brq, 6.8)	7.33 (1H, s)	7.48 (1H, brs)
4″	7.42 (1H, t, 7.9)	1.72 (3H, d, 6.8)		
5″	7.60 (1H, t, 7.9)	1.65 (3H, s)		
6″	7.42 (1H, t, 7.9)			6.82 (1H, d, 8.4)
7″	7.79 (1H, d, 7.9)		7.33 (1H, s)	7.60 (1H, d, 8.4)
−OCH_3 4″			3.86 (3H, s)	3.79 (3H, s)
−OCH_3 5″			3.81 (3H, s)	3.79 (3H, s)
−OCH_3 6″			3.86 (3H, s)	

[a] δ in ppm.

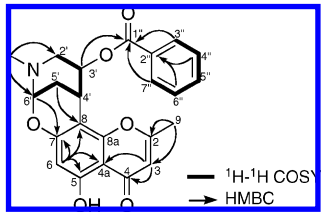

Figure 1. Selected 2D NMR correlations for chrotacumine A (**1**).

H_3-9 and H-6′/H-6. The chair conformation of piperidine ring was also supported by the NOESY correlations of H_3-9/H-6″ and H-7″. The absolute configuration of **1** was elucidated by applying the exciton chirality method.[19] The sign of the first Cotton effect [λ_{max} 257 nm ($\Delta\varepsilon$ +0.83)] was positive, while that of the second one [λ_{max} 239 nm (−9.67)] was negative, indicating that the chirality between the benzoate (ca. 230 nm) at C-3′ and chromone (ca. 250 nm) at C-4′ chromophores in **1** was in the form of a right-handed screw, as shown in Figure 2. Therefore, the configurations of C-3′, C-4′, and C-6′ were assigned as *S*, *R*, and *R*, with the configurations at C-3′ and C-4′ being the same as rohitukine (**5**).[20]

Compound **2**, $[\alpha]^{22}_D$ −84 (*c* 1.0, MeOH), showed a pseudomolecular ion peak at *m*/*z* 388 $(M + H)^+$ in the ESIMS, and the molecular formula, $C_{21}H_{25}NO_6$, was established by HRESIMS [*m*/*z* 388.1740 $(M + H)^+$]. IR absorptions implied the presence of hydroxy (3420 cm^{-1}), ester (1710 cm^{-1}), and conjugated (1660 cm^{-1}) carbonyl functionalities. The ^{13}C NMR data of **2** revealed the presence of two sp^3 methines, three sp^3 methylenes, four methyls, three sp^2 methines, and nine sp^2 quaternary carbons (Table 2).

The gross structure of **2** was deduced from the ^{13}C NMR data, which were found to be similar to those of rohitukine (**5**).[1] The noticeable differences of the chemical shifts of H-3′ (δ_H 5.17) and C-3′ (δ_C 72.3) of a piperidine ring compared to those of rohitukine (δ_H 4.15; δ_C 69.5) and observation of an ester carbonyl carbon (δ_C 169.2) and two olefinic carbons (δ_C 130.0 and 138.8) with two methyls (δ_C 12.0 and 14.3) could be explained by the presence of a tiglate moiety at C-3′.[21] The placement of the tiglate group was proved to be at C-3′ by HMBC correlations of H-3′, H-3″, and H-5″ to C-1″ (Figure 3).

The relative configuration of **2** was elucidated by NOESY correlations (Figure 4). NOESY correlations between H-2′a, H-4′, and H-6′a indicated that the chair conformation of the piperidine ring and that between H_3-4″ and H_3-5″ supported the presence of the tiglate. The absolute stereochemistry of **2** was assigned by applying the exciton chirality method.[19] The sign of the first Cotton

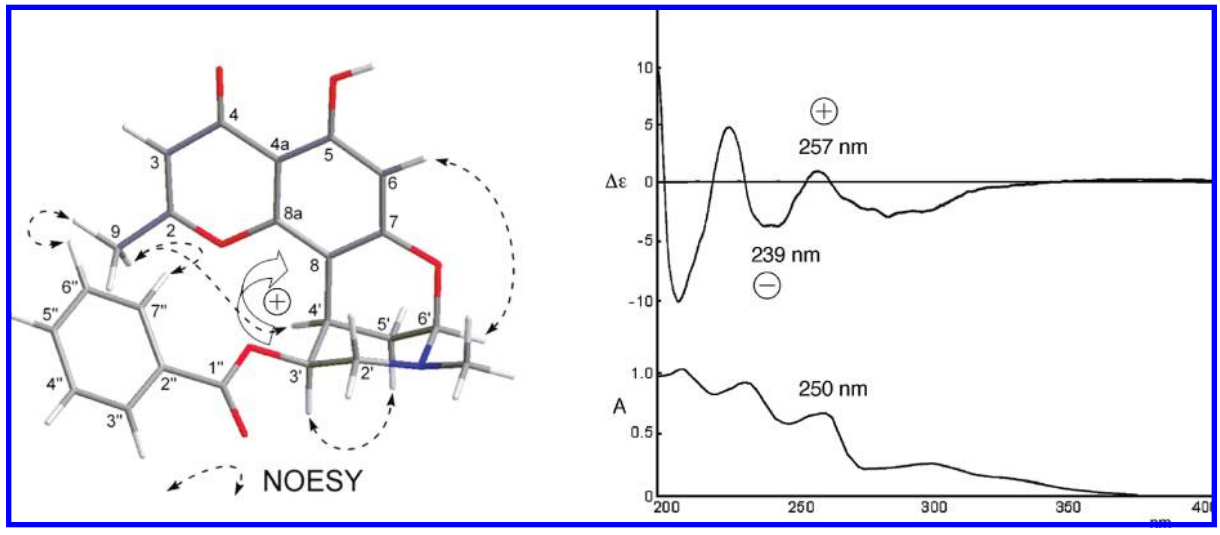

Figure 2. Selected NOESY correlations, CD, and UV spectra for chrotacumine A (**1**).

Table 2. ^{13}C NMR Data of **1**−**4** in CD_3OD at 300 K[a]

carbon	1	2	3	4
2	169.1	168.7	168.7	168.4
3	109.0	108.5	108.2	108.3
4	184.1	184.2	184.1	183.8
4a	105.5	104.9	104.9	104.7
5	161.7	161.7	161.7	161.5
6	99.4	99.9	100.0	100.0
7	164.2	164.6	164.8	164.6
8	101.8	106.8	106.8	106.7
8a	156.8	158.3	158.1	158.0
9	19.4	20.3	20.3	20.3
N-Me	42.2	46.5	46.6	46.4
2′	49.7	60.0	60.3	60.1
3′	72.7	72.3	72.7	72.3
4′	27.4	38.4	38.7	38.4
5′	27.8	26.5	26.4	26.2
6′	88.2	57.9	58.0	57.8
1″	166.7	169.2	167.5	167.4
2″	131.4	130.0	126.9	123.7
3″	130.4	138.8	108.7	114.0
4″	129.8	14.3	154.4	149.8
5″	134.7	12.0	143.5	154.6
6″	129.8		154.4	111.4
7″	130.4		108.7	125.0
−OCH_3 4″			57.0	56.3
−OCH_3 5″			61.1	56.7
−OCH_3 6″			57.0	

[a] δ in ppm.

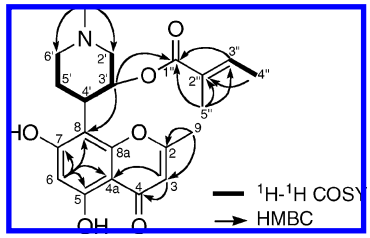

Figure 3. Selected 2D NMR correlations for chrotacumine B (**2**).

effect [λ_{max} 257 nm ($\Delta\varepsilon$ −6.38)] was negative, while that of the second one [λ_{max} 236 nm (+3.18)] was positive, indicating that the chirality between the two chromophores at C-3′ and C-4′ in **2** was a left-handed screw, as shown in Figure 4. Thus, the structure of **2** was elucidated as the 3′-tiglate derivative of rohitukine (**5**) with 3′*S*, 4′*R* configurations and was named chrotacumine B.

Compound **3**, $[\alpha]^{22}_D$ −124 (*c* 1.0, MeOH), showed a pseudomolecular ion peak at *m*/*z* 500 $(M + H)^+$ in the ESIMS, and the

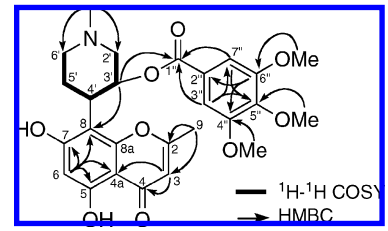

Figure 5. Selected 2D NMR correlations for chrotacumine C (**3**).

molecular formula, $C_{26}H_{29}NO_9$, was established by HRESIMS [*m*/*z* 500.1917, $(M + H)^+$, Δ −0.3 mmu]. In turn, compound **4**, $[\alpha]^{22}_D$ −177 (*c* 1.0, MeOH), showed a pseudomolecular ion peak at *m*/*z* 470 $(M + H)^+$ in the ESIMS, and the molecular formula, $C_{25}H_{27}NO_8$, was established by HRESIMS [*m*/*z* 470.1819, $(M + H)^+$, Δ +0.4 mmu]. IR absorptions of **3** and **4** implied the presence of hydroxy (3420 cm^{-1}), ester (1710 cm^{-1}), and conjugated (1660 cm^{-1}) carbonyl functionalities. The ^1H and ^{13}C NMR spectra of **3** and **4** were very similar to each other except for the addition of a methoxy group for **3** (**3**, δ_C 57.0 × 2 and 61.1; **4**, δ_C 56.3, 56.7 × 2). The presence of a 3,4,5-trimethoxybenzoate unit for **3** and a 3,4-dimethoxybenzoate moiety for **4** was assigned by 2D NMR analysis (Figure 5). The position of this ester function of **3** and **4** was verified by HMBC correlations of H-3′ and H-3″ to C-1″. Thus, chrotacumine C (**3**) was assigned as the 3,4,5-trimethoxybenzoate of rohitukine (**5**) and **4** (chrotacumine D) as the des-methoxy derivative of **3**.

The relative configurations of **3** and **4** were elucidated by NOESY correlations. The same NOESY correlations among H-2′, H-4′, and H-6′ in **3** and **4** as those in **2** indicated the chair conformation of the piperidine ring (Figure 6). The absolute configurations of **3** and **4** were assigned on the basis of the exciton chirality method.[19] The sign of the first Cotton effect [λ_{max} 270 nm ($\Delta\varepsilon$ −23.69)] was negative, while that of the second one [λ_{max} 252 nm (+13.11)] was positive, indicating that the chirality between two chromophores at C-3′ and C-4′ in **3** and **4** was a left-handed screw in each case, as shown in Figure 6. Therefore, the configurations at C-3′ and C-4′ of **3** and **4** were assigned as 3′*S*, 4′*R*, respectively. X-ray analysis of a crystal of chrotacumine C (**3**) obtained from MeOH confirmed the proposed absolute structure through the Flack parameter,[22] $x = -0.03(17)$ (Figure 7).

Chrotacumines A−D (**1**−**4**) were all inactive against HL-60 (human blood promyelocytic leukemia) (IC_{50} > 10 μM), whereas rohitukine (**5**) was active when evaluated against various human cancer cell lines (IC_{50} 7.5 μM against HL-60, 8.8 μM against HCT-

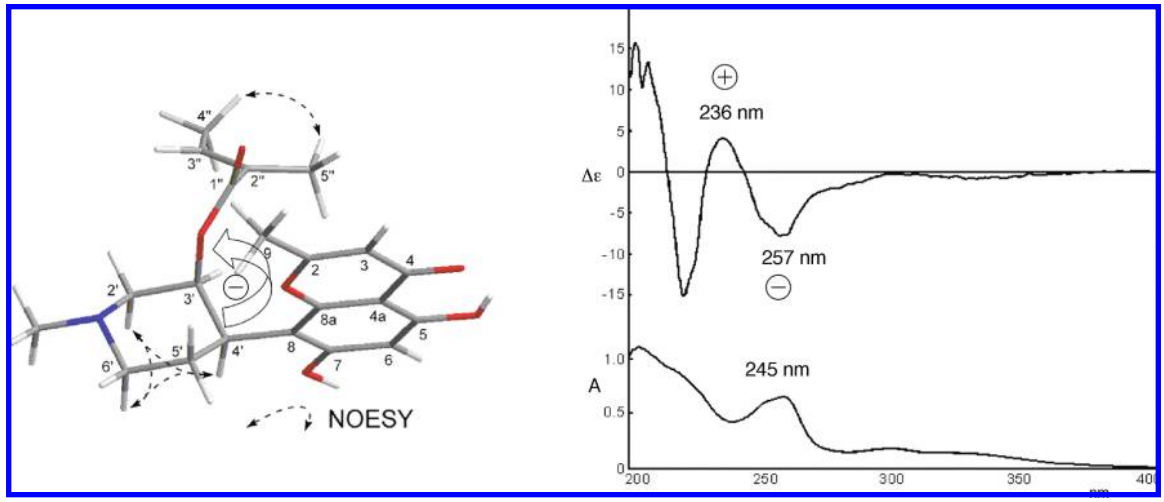

Figure 4. Selected NOESY correlations, CD, and UV spectra for chrotacumine B (**2**).

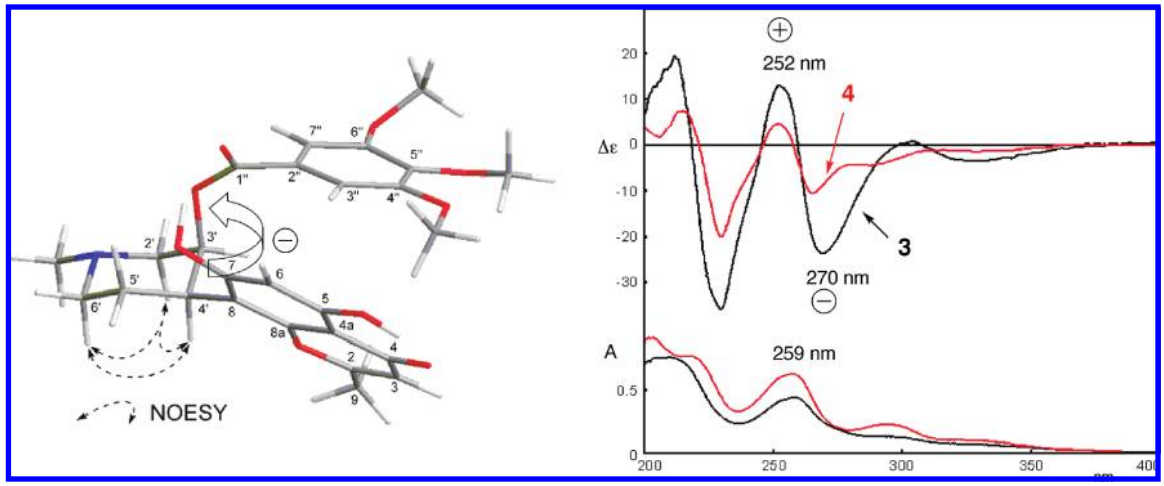

Figure 6. Selected NOESY correlations for chrotacumine C (**3**) and CD and UV spectra for chrotacumines C and D (**3** and **4**).

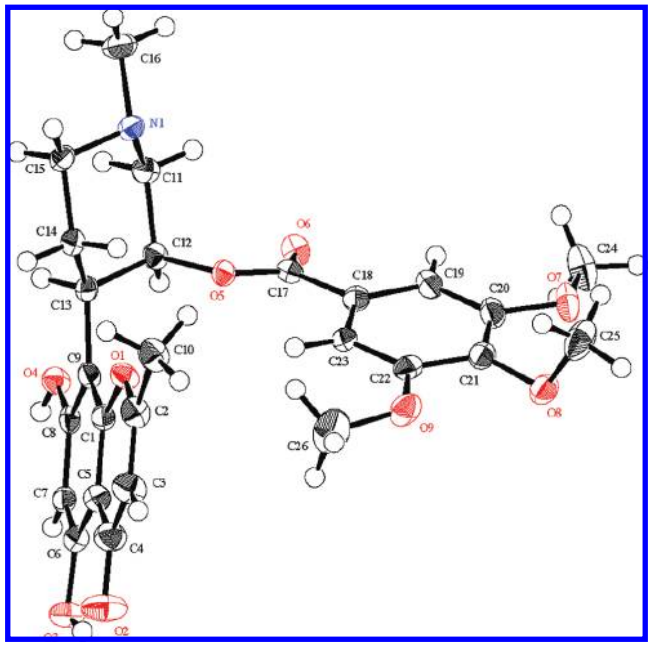

Figure 7. Molecular structure of chrotacumine C (**3**) obtained by X-ray analysis [Flack parameter: $x = -0.03(17)$].

116, >10 μM against NCI-H226, >10 μM against MCF-7, and >10 μM against A-549).

Biogenetically, chrotacumine A (**1**), consisting of a 5,7-dihydroxy-2-methylchromone, an *N*-Me piperidine ring, and an ester side chain, might be generated through an imine intermediate followed by connection between a hydroxy at C-7 as a nucleophile and C-6′ of an iminium carbon, after coupling of a nicotinic acid unit and the chromone skeleton, even though the schumagnine skeleton was proposed to connect between a hydroxy at C-7 and C-2′ of a piperidine ring.[18]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 automatic digital polarimeter. UV spectra were obtained on an Ultrospec 2100 pro spectrophotometer, CD spectra were measured on a JASCO J-820 spectropolarimeter, and IR spectra were recorded on a JASCO FT/IR-4100 spectrophotometer. ^{1}H and 2D NMR spectra were recorded on JEOL ECA600 and Bruker AV 400 spectrometers, and chemical shifts were referenced to the residual solvent peaks (δ_H 3.31 and δ_C 49.0 for CD_3OD). Standard pulse sequences were employed for the 2D NMR experiments. ^{1}H−^{1}H COSY, HOHAHA, and NOESY spectra were measured with spectral widths of both dimensions of 4800 Hz, and 32 scans with two dummy scans were accumulated into 1K data points for each of 256 t_1 increments. NOESY spectra in the phase-sensitive mode were measured with a mixing time of 800 ms. For HMQC spectra in the phase-sensitive mode and HMBC spectra, a total of 256 increments of 1K data points were collected. For HMBC spectra with Z-axis PFG, a 50 ms delay time was used for long-range C−H coupling. Zero-filling to 1K for F_1 and multiplication with squared cosine-bell windows shifted in both dimensions were performed prior to 2D Fourier transformation. High-resolution ESIMS were obtained on a LTQ Orbitrap XL (Thermo Scientific).

Plant Material. The leaves and bark of *Dysoxylum acutangulum* Miq. were collected in Terengganu, Malaysia, in August 2008. The botanical identification was made by Mr. Syamsul Khamis of the Institute of Bioscience, University Putra Malaysia. A voucher specimen (herbarium no. SK1567-08-IBS) is deposited at the Herbarium of Laboratory of Biodiversity, Institute of Bioscience, Universiti Putra Malaysia.

Extraction and Isolation. The leaves (1.19 kg) of *D. acutangulum* were extracted with MeOH (90.8 g), and a part of the extract (3.5 g) was partitioned between hexane and water. The water-soluble material (3.0 g) was extracted with EtOAc, and the EtOAc-soluble material (1.0 g) was subjected to an ODS column chromatography (MeOH−H_2O, 0:1 → 1:0) followed by silica gel column chromatography (hexane−acetone, 7:3 → 0:1, and then toluene−EtOAc, 1:0 → 2:3) to give **1** (2.0 mg, 0.004%) and **2** (2.3 mg, 0.005%). In turn, the bark (250 g) of *D. acutangulum* was extracted with MeOH (5.7 g), and a part of the extract (4.4 g) was partitioned between hexane and water. The water-soluble material (4.1 g) was extracted with EtOAc, and the EtOAc-soluble material (800 mg) was subjected to chromatography over an ODS column (MeOH−H_2O, 0:1 → 1:0) followed by silica gel ($CHCl_3$−EtOAc−MeOH, 8:2:0 → 0:0:1; toluene−EtOAc−MeOH, 1:1:0 → 0:0:1; hexane−acetone, 3:2 → 0:1) to give **3** (2.9 mg, 0.002%) and **4** (1.9 mg, 0.001%) together with rohitukine (**5**),[1] schumanniofioside A, and noreugenin.[15,16]

Chrotacumine A (1): brown, amorphous solid; $[\alpha]^{22}_D$ −159 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3420, 1720, 1660, 1620, 1260, 1110 cm^{-1}; UV (MeOH) λ_{max} 228 (ε 18 600), 258 (ε 13 700), 297 nm (ε 5300); CD (MeOH) λ_{max} 207 ($\Delta\varepsilon$ −24.64), 225 ($\Delta\varepsilon$ 9.77), 239 ($\Delta\varepsilon$ −9.67), 257 ($\Delta\varepsilon$ 0.83), 283 nm ($\Delta\varepsilon$ −7.55); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m*/*z* 408 $(M + H)^+$; HRESIMS *m*/*z* 408.1456 $[(M + H)^+]$ (calcd for $C_{23}H_{22}NO_6$, 408.1447).

Chrotacumine B (2): brown, amorphous solid; $[\alpha]^{22}_D$ −84 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3420, 1710, 1660, 1620, 1260, 1090 cm^{-1}; UV (MeOH) λ_{max} 245 (ε 15 000), 258 (ε 16 600), 299 nm (ε 4600); CD (MeOH) λ_{max} 205 ($\Delta\varepsilon$ 8.50), 223 ($\Delta\varepsilon$ −11.33), 236 ($\Delta\varepsilon$ 3.18), 257 nm ($\Delta\varepsilon$ −6.38); ^{1}H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m*/*z* 388 $(M + H)^+$; HRESIMS *m*/*z* 388.1740 $[(M + H)^+]$ (calcd for $C_{21}H_{26}NO_6$, 388.1760).

Chrotacumine C (3): brown, amorphous solid; $[\alpha]^{22}_D$ −124 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3420, 1710, 1660, 1590, 1270 cm^{-1}; UV (MeOH) λ_{max} 259 nm (ε 29 800); CD (MeOH) λ_{max} 211 ($\Delta\varepsilon$ 19.52), 230 ($\Delta\varepsilon$ −36.02), 252 ($\Delta\varepsilon$ 13.11), 270 nm ($\Delta\varepsilon$ −23.69); ^{1}H and ^{13}C

NMR, see Tables 1 and 2; ESIMS *m/z* 500 $(M + H)^+$; HRESIMS *m/z* 500.1917 $[(M + H)^+]$ (calcd for $C_{26}H_{30}NO_9$, 500.1921).

Chrotacumine D (4): brown, amorphous solid; $[\alpha]^{22}_D$ −177 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3420, 1710, 1660, 1590, 1270 cm^{-1}; UV (MeOH) λ_{max} 217 (ε 32 800), 257 (ε 26700), 294 nm (ε 9700); CD (MeOH) λ_{max} 215 (Δε 7.41), 230 (Δε −19.96), 252 (Δε 4.57), 265 nm (Δε −10.51); ^1H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 470 $(M + H)^+$; HRESIMS *m/z* 470.1819 $[(M + H)^+]$ (calcd for $C_{25}H_{28}NO_8$, 470.1815).

X-ray Analysis of Chrotacumine C (3). Chrotacumine C (**3**) free base was crystallized from MeOH to give colorless needles (mp 140−141 °C). Crystal data: $C_{27.67}H_{35.67}O_{10.67}$, space group *R*3 (#146), $a = 19.2969(4)$ Å, $c = 19.1134(7)$ Å, $V = 6163.7(3)$ Å^3, $Z = 9$, $D_{calc} = 1.341$ g/cm^3, Cu Kα radiation ($\lambda = 1.54187$ Å), T −180(1) °C. The structure was solved by direct methods and expanded using Fourier techniques. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model. The final cycle of full-matrix least-squares refinement on F^2 was based on 4965 observed reflections and converged with unweighted and weighted agreement factors of $R_1 = 0.0495$ [$I > 2.00\sigma(I)$] and $wR_2 = 0.1358$. The absolute configuration was determined on the basis of Flack parameter −0.03(17),[22] refined using 2476 Friedel pairs. Complete crystallographic data of **3** have been deposited in the Cambridge Crystallographic Data Centre (CCDC 736778).[23]

Cytotoxic Bioassay. Each cell line [HL-60 (human blood premyelocytic leukemia), A549 (lung carcinoma), NCI-H226 (non-small cell lung carcinoma), HCT-116 (human colon cancer), and MCF-7 (human breast adenocarcinoma) cells] was seeded onto 96-well microtiter plates (1×10^4 cells per well for HL-60 and 5×10^3 cells per well for A549, NCI-H226, HCT-116, and MCF-7). Cells were preincubated for 24 h at 37 °C in a humidified atmosphere of 5% CO_2. Different concentrations of each compound (10 μL) were added to the cultures, and then the cells were incubated at 37 °C for 48 h. On the third day, 15 μL of MTT solution (5 mg/mL) was added into each well of the cultured medium. After a further 2 h of incubation, 100 μL of 10% SDS−0.01 N HCl solution was added to each well, and the formazan crystals in each well were dissolved by stirring with a pipet. The optical density measurements were made using a micropipet reader (Benchmark Plus microplate spectrometer, Bio-Rad) equipped with a two wavelength system (550 and 700 nm). In each experiment, three replicates were prepared for each sample. The ratio of the living cells was determined on the basis of the difference of the absorbance between those of samples and controls. These differences are expressed in percentages, and cytotoxic activity was indicated as an IC_{50} value.

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Supporting Information Available: ^1H NMR and ^{13}C NMR spectra of chrotacumines A−D are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Harmon, A. D.; Weiss, U.; Silverton, J. V. *Tetrahedron Lett.* **1979**, 721–724.
(2) Schlittler, E.; Spitaler, U. *Tetrahedron Lett.* **1978**, *19*, 2911–2914.
(3) Houghton, P. J. In *Studies in Natural Products Chemistry*; Rahman, A., Ed.; Elsevier Science B.V.: Amsterdam, 2000; Vol. 21, pp 123−155.
(4) Houghton, P. J. *J. Chromatog. A* **2002**, *967*, 75–84.
(5) Macklin, T. K.; Reed, M.; Snieckus, V. *Eur. J. Org. Chem.* **2008**, *9*, 1507–1509.
(6) Naik, R. G.; Kattige, S. L.; Bhat, S. V.; Alreja, B. B.; Douza, N. J.; Rupp, R. H. *Tetrahedron* **1988**, *44*, 2081–2086.
(7) Kelly, T. R.; Kim, M. H. *J. Org. Chem.* **1992**, *57*, 1593–1597.
(8) Houghton, P. J.; Harvey, A. L. *Planta Med.* **1989**, *55*, 273–275.
(9) Morita, H.; Oshimi, S.; Hirasawa, Y.; Koyama, K.; Honda, T.; Ekasari, W.; Indrayanto, G.; Zaini, N. C. *Org. Lett.* **2007**, *9*, 3691–3693.
(10) Ekasari, W.; Widyawaruyanti, A.; Zaini, N. C.; Syafruddin, D.; Honda, T.; Morita, H. *Heterocycles* **2009**, *78*, 1831–1836.
(11) Awang, K.; Lim, C. S.; Mohamad, K.; Morita, H.; Hirasawa, Y.; Takeya, K.; Thoison, O.; Hadi, A. H. A. *Bioorg. Med. Chem.* **2007**, *15*, 5997–6002.
(12) Mohamad, K.; Hirasawa, Y.; Lim, C. S.; Awang, K.; Hadi, A. H. A.; Takeya, K.; Morita, H. *Tetrahedron Lett.* **2008**, *49*, 4276–4278.
(13) Mohamad, K.; Hirasawa, Y.; Litaudon, M.; Awang, K.; Hadi, A. H. A.; Takeya, K.; Ekasari, W.; Widyawaruyanti, A.; Zaini, N. C.; Morita, H. *Bioorg. Med. Chem.* **2009**, *17*, 727–730.
(14) Ismail, I. S.; Nagakura, Y.; Hirasawa, Y.; Hosoya, T.; Lazim, M. I. M.; Lajis, N. H.; Morita, H. *Tetrahedron Lett.* **2009**, *50*, 4830–4832.
(15) Tane, P.; Ayafor, J. F.; Sondengam, B. L.; Connolly, J. D. *Phytochemistry* **1990**, *3*, 1004–1007.
(16) Harmon, A. D.; Weiss, U. *Tetrahedron Lett.* **1979**, *8*, 721–724.
(17) Nishizawa, M.; Inoue, A.; Sastrapradija, S.; Hayashi, Y. *Phytochemistry* **1983**, *22*, 2083–2085.
(18) Houghton, P. J.; Hairong, Y. *Planta Med.* **1987**, *47*, 262–264.
(19) Harada, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1969**, *91*, 3989–3991.
(20) Yang, D. H.; Cai, S. Q.; Zhao, Y. Y.; Liang, H. *Chin. Chem. Lett.* **2003**, *14*, 720–723.
(21) Li, M.; Wu, J.; Zhang, S.; Xiao, Q.; Li, Q. *Magn. Reson. Chem.* **2007**, *45*, 705–709.
(22) Flack, H. D. *Acta Crystallogr.* **1983**, *A39*, 876–881.
(23) CCDC 736778 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/deposit, or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).

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