Five New Chromones Possessing Monoamine Oxidase Inhibitory Activity from an Ascomycete, *Chaetomium quadrangulatum*

Haruhiro FUJIMOTO,* Masato NOZAWA, Emi OKUYAMA, and Masami ISHIBASHI

Graduate School of Pharmaceutical Sciences, Chiba University, 1–33 Yayoi-cho, Inage-ku, Chiba 263–8522, Japan.
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Five novel chromones (1,4-benzopyran-4-ones), among which three are tetracyclic and one contains a sulfonyl group, have been isolated from an Ascomycete, *Chaetomium quadrangulatum*, as monoamine oxidase inhibitory features, and named chaetoquadrins A (1)—E (5).

Key words *Chaetomium quadrangulatum*; chromone; monoamine oxidase (MAO) inhibitory activity; chaetoquadrin; fungal metabolite; Ascomycete

In our screening project for monoamine oxidase (MAO) inhibitory constituents from fungi, several metabolites have been isolated from *Talaromyces luteus*,[1a] *Talaromyces helicus*,[1b] Mycelia Sterilia from *Gelasinospora pseudoreticulata*,[1c] *Monascus anka*,[1d] *Coniochaeta tetraspora*,[1e] and *Anixiella* (*Gelasinospora*) *micropertusa*.[1f] We have found that the AcOEt extract of an Ascomycete, *Chaetomium quadrangulatum* CHIVERS strain 71-NG-22,[2] appreciably inhibited mouse liver MAO on the modified Kraml assay.[3] Fractionation guided by the MAO inhibitory activity afforded five new chromones (1,4-benzopyran-4-ones). This paper deals with the isolation, structure elucidation, and MAO inhibitory activity of these five constituents recently isolated from *C. quadrangulatum*.

Results and Discussion

The AcOEt extract of *C. quadrangulatum* strain 71-NG-22[2] cultivated on sterilized rice medium inhibited mouse liver MAO by 30.8% at 1.0×10^{-4} g/ml. The AcOEt extract was partitioned between *n*-hexane and water. The aqueous suspension was further partitioned with AcOEt into an AcOEt layer and an aqueous layer (yields [%] of the *n*-hexane, AcOEt, and aqueous layers after evaporation of the solvents from the AcOEt extract: 38.3, 38.7, and 18.3, respectively). The *n*-hexane, AcOEt, and aqueous layers inhibited 10.3, 29.6, and 21.9% at 1.0×10^{-4} g/ml, respectively. Repeated chromatographic fractionation of the *n*-hexane layer guided by the MAO inhibitory activity afforded five constituents tentatively named CQ-1 (**1**)—5 (**5**) as the MAO inhibitory features of this fungus (yields [%] of **1**—**5** from the AcOEt extract: 0.14, 0.033, 0.025, 0.076, and 0.22, respectively).

CQ-1 (**1**), $C_{20}H_{24}O_6$, was obtained as an optically active, colorless, amorphous, circular dichroism (CD) (1.11 mM, MeOH) $\Delta\varepsilon$ (nm): −2.8 (324), +3.3 (298), +0.38 (267), +3.8 (254), −4.0 (228), +2.8 (206), IR (KBr) ν_{max} cm^{-1}: 3448 (OH), 1654 (C=O), 1612 (C=C), 1456, 1344, 1247 (C–O). The UV spectrum of **1** (in MeOH), λ_{max} nm (log ε): 209 (4.34), 233 (4.21), 249 (4.12), 255 (4.12), 285 (3.87), was similar to that of a chromone (1,4-benzopyran-4-one), 6-hydroxymethyleugenitin (5-hydroxy-6-hydroxymethyl-7-methoxy-2-methylchromone) (**6**) isolated from the lichen *Roccella fuciformis*[4] and the Ascomycete *Chaetomium minutum*[5] (see Chart 1). The ^{1}H- and ^{13}C-NMR data showed the presence of four methyls (C$\underline{H}_3$–CH<×2, C$\underline{H}_3$–C(=C)–×1, C$\underline{H}_3$–O–×1), three methylenes (C–C$\underline{H}_2$–C×3), five methines (>C$\underline{H}$–×1, >C$\underline{H}$–O×2, –C$\underline{H}$=C×2), eight quaternary carbons (>$\underline{C}$(–O)–O×1, >$\underline{C}$=C×2, –$\underline{C}$(–O)=C×4, >$\underline{C}$=O ×1) in **1** (see Table 1). The ^{1}H- and ^{13}C-NMR data including spin-decoupling ^{1}H-NMR, two-dimensional ^{1}H–^{1}H shift correlation (COSY), and ^{1}H-detected single-bond heteronuclear correlation through multiple quantum coherence (HSQC) data indicated that **1** might be composed of four partial structures *a*—*d*, among which *a* was created with the ^{1}H-detected heteronuclear multiple-bond correlation (HMBC) NMR data. Construction of the entire molecular structure of CQ-1 from *a*—*d* was achieved with the aid of the HMBC data to afford a tetracyclic structure containing a 6-substituted 5-oxy-7-methoxy-2-methylchromone skeleton (**1**) as the plane structure (see Chart 1). On acetylation with acetic anhydride and pyridine, **1** gave a monoacetate (**7**), ^{1}H- and ^{13}C-NMR (in $CDCl_3$) δ_H: 2.01 (3H, s), δ_C: 21.3 (q), 170.0 ppm (s) (CH_3CO). Comparison of the ^{13}C-NMR spectrum of **7** with that of **1** showed that C-4′, C-5′, and C-6′ were shifted to δ 35.5 (−3.9), 68.3 (+3.8), and 38.7 (−3.7) ppm, respectively, in accordance with the acetylation shift rule,[6] indicating that the hydroxyl group at position 5′ (OH-5′) in **1** was acetylated to provide **7** (see Table 1).

In the ^{1}H-NMR spectrum of **1**, H-5′ (δ 4.67) was coupled to H_a-4′ (δ 2.43), H_b-4′ (δ 1.32), and H_b-6′ (δ 1.25) with J=4.8, 11.2, 12.0 Hz, respectively, and H-7′ (δ 4.00) was coupled to H_a-6′ (δ 2.04) and H_b-6′ with J=3.0, 12.0 Hz, respectively, indicating that the dihedral angles between H_b-4′ and H-5′ [ϕ(H_b-4′/H-5′)], ϕ(H-5′/H_b-6′), and ϕ(H_b-6′/H-7′) were about 180° (quasi-*trans* 1,2-diaxial), while ϕ(H_a-4′/H-5′) and ϕ(H_a-6′/H-7′) were 50—70° (quasi-*gauche* 1,2-axial-equatorial). Furthermore, a long-range coupling with J= 1.7 Hz was present between H_a-4′ and H_a-6′ (a co-planar W-type long-range coupling) (see Chart 2). In the differential nuclear Overhauser effect (NOEDF) NMR experiment on **1**, an NOE between H_b-1′ (δ 2.91) and H-2′ (δ 2.11) [NOE(H_b-1′ (δ 2.91)/H-2′ (δ 2.11))] was observed of 8%, and seven other NOEs (H-2′/H_b-4′), (H_a-1′ (δ 2.39)/CH_3-2′ (δ 1.00)), (CH_3-2′/H_a-4′), (H_a-4′/H-5′), (H-5′/H_a-6′), (H_a-6′/H-7′), and (H-5′/H-7′) were observed of 5%, 4%, 5%, 3%, 3%, 1%, and 6%, respectively, suggesting that the relative configuration of the moiety of rings C and D in **1** was expressed as shown in Chart 2.

To apply the modified Mosher's method[7] to **1**, the (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetate ((*R*)-

* To whom correspondence should be addressed. e-mail: fujimoto@p.chiba-u.ac.jp

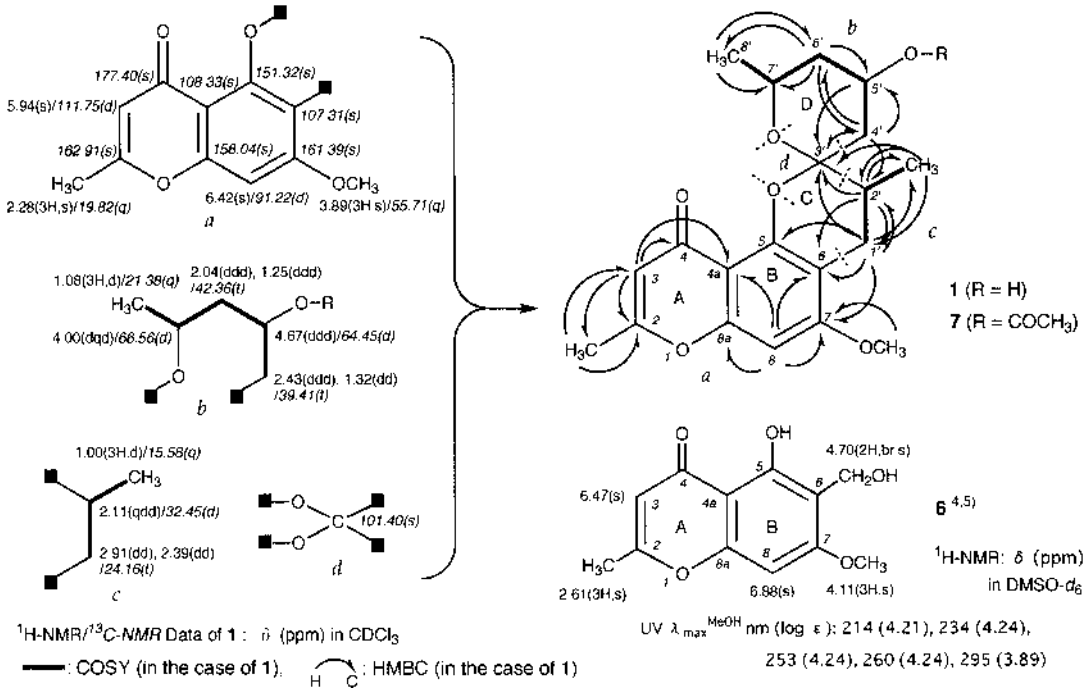

Chart 1. Construction of the Plane Structure of Chaetoquadrin A (**1**) from the Four Partial Structures *a*—*d*, and the Structure of a Chromone, 6-Hydroxymethyleugenitin (**6**)

Table 1. ^{1}H- and ^{13}C-NMR Data for Chaetoquadrin A (**1**), and Chaetoquadrin A Acetate (**7**), δ (ppm) from TMS as an Internal Standard in $CDCl_3$ [Coupling Constants (Hz) in Parentheses]

Position	**1**		**7**	
	δ_H	δ_C	δ_H	δ_C
2		162.9 (s)		162.7 (s)
2-CH_3	2.28 (3H, s)	19.8 (q)	2.27 (3H, s)	19.8 (q)
3	5.94 (s)	111.8 (d)	5.94 (s)	111.9 (d)
4		177.4 (s)		177.0 (s)
4a		108.3 (s)		108.5 (s)
5		151.3 (s)		151.2 (s)
6		107.3 (s)		107.0 (s)
7		161.4 (s)		161.1 (s)
7-OCH_3	3.89 (3H, s)	55.7 (q)	3.89 (3H, s)	55.7 (q)
8	6.42 (s)	91.2 (d)	6.40 (s)	91.3 (d)
8a		158.0 (s)		158.0 (s)
1′	2.91 (dd, 16.9, 6.6), 2.39 (dd, 16.9, 3.2)	24.2 (t)	2.91 (dd, 16.8, 6.5), 2.38 (dd, 16.8, 4.0)	24.3 (t)
2′	2.11 (qdd, 7.1, 6.6, 3.2)	32.5 (d)	2.11 (qdd, 7.0, 6.5, 4.0)	32.6 (d)
2′-CH_3	1.00 (3H, d, 7.1)	15.6 (q)	1.00 (3H, d, 7.0)	15.6 (q)
3′		101.4 (s)		101.3 (s)
4′	2.43 (ddd, 12.7, 4.8, 1.7), 1.32 (dd, 12.7, 11.2)	39.4 (t)	2.43 (ddd, 12.4, 4.6, 1.7), 1.38 (dd, 12.4, 11.3)	35.5 (t)
5′	4.67 (ddd, 12.0, 11.2, 4.8)	64.5 (d)	5.63 (ddd, 11.4, 11.3, 4.6)	68.3 (d)
6′	2.04 (ddd, 12.0, 3.0, 1.7)	42.4 (t)	2.18 (ddd, 11.4, 2.1, 1.7)	38.7 (t)
	1.25 (ddd, 12.0, 12.0, 12.0)		1.25 (ddd, 11.4, 11.4, 11.4)	
7′	4.00 (dqd, 12.0, 6.8, 3.0)	66.6 (d)	4.13 (dqd, 11.4, 6.4, 2.1)	66.3 (d)
8′	1.08 (3H, d, 6.8)	21.4 (q)	1.07 (3H, d, 6.4)	21.3 (q)
5′-OCO<u>C</u>H_3			2.01 (3H, s)	21.3 (q)
5′-O<u>C</u>OCH_3				170.0 (s)

MTPA ester) (**8**) and (*S*)-MTPA ester (**9**) were prepared from **1**. Comparison of the ^{1}H-NMR spectra of **8** and **9** with that of **1** showed that OH-5′ in **1** was (*R*)- and (*S*)-MTPA-esterified to give **8** and **9**, respectively. The $\Delta\delta$ values (δ_9–δ_8) were calculated as shown in Chart 2, indicating that the absolute configuration at position 5′ in **1** was (*R*). Accordingly, CQ-1 was deduced to be (2′*R*,3′*R*,5′*R*,7′*S*)-6-[(5,3′:3′,7′-diepoxy-5′-hydroxy-2′-methyl)octyl]-7-methoxy-2-methylchromone (**1**) (see Chart 2). To our knowledge, this is the first time that **1** has been isolated as a MAO inhibitory constituent from a natural source. Thus we propose the name CQ-1 chaetoquadrin A (**1**).

CQ-2 (**2**), $C_{20}H_{24}O_6$, was obtained as an optically active, colorless, amorphous substance, of which the UV and IR

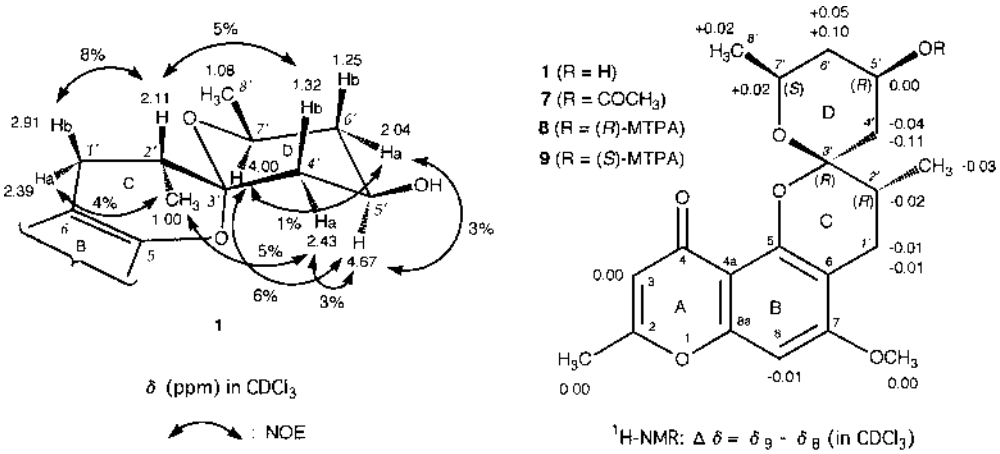

Chart 2. Relative Configuration of the Moiety of Rings C and D in Chaetoquadrin A (**1**) and Absolute Configurations of Chaetoquadrin A (**1**) and Its Derivatives, **7**—**9**

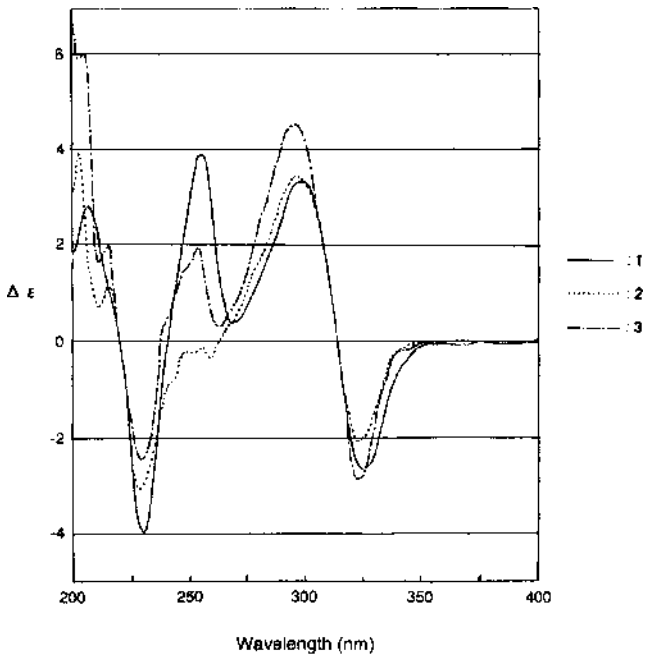

Fig. 1. CD Spectra of Chaetoquadrins A (**1**), B (**2**), and C (**3**) (in MeOH)

spectra were similar to those of **1**. The CD spectrum of **2** (1.11 mM, MeOH), $\Delta\varepsilon$ (nm): −2.1 (322), +3.5 (296), −0.32 (259), −0.18 (255), −3.1 (228), +1.1 (215), +0.63 (211), +3.9 (202), was similar to that of **1** except for the peaks at around 254 and 215 nm (see Fig. 1). These data suggested that **2** might be a stereoisomer of **1**. The CD behavior of **2** and the fact that **2** was obtained from the fungus together with **1** suggested that the absolute configurations of the majority of asymmetric carbons in **2** might be the same as those in **1**. Comparison of the ^{1}H-NMR spectrum of **2** (in $CDCl_3$) with that of **1** indicated that the signals of H_b-4′, H-5′, and H_b-6′ were largely shifted to δ 1.65 (+0.33), 4.17 (−0.50), and 1.49 (+0.24) ppm, respectively (see Table 2). In the ^{1}H-NMR spectrum of **2**, H-5′ was coupled to H_a-4′ (δ 2.35), H_b-4′, H_a-6′ (δ 1.92), and H_b-6′ with J=2.0, 4.1, 4.5, 3.0 Hz, respectively, and H_b-6′ was also coupled to H-7′ with J=11.9 Hz, suggesting that ϕ(H_a-4′/H-5′), ϕ(H_b-4′/H-5′), ϕ(H-5′/H_a-6′), and ϕ(H-5′/H_b-6′) were 50—70° (quasi-*gauche* 1,2-equatorial-equatorial and 1,2-axial-equatorial), and ϕ(H_b-6′/H-7′) was about 180° (quasi-*trans* 1,2-diaxial), as shown in Chart 3. In the NOEDF NMR experiment on **2**, the three NOEs (H_a-4′/H-5′), (H-5′/H_a-6′), and (H-5′/H-7′) which were observed on **1** disappeared, but two NOEs (H_b-4′/H-5′) and (H-5′/H_b-6′) newly appeared of 7% and 10%, respectively, indicating that the configuration of OH-5′ was changed from β quasi-equatorial in **1** to α quasi-axial in **2** (see Chart 3). This was also supported by the fact that although the ^{1}H-NMR signal of OH-5′ of **1** was not observed due to rapid change into OD-5′ in $CDCl_3$ solution, that of **2** was clearly observed at δ 6.32 ppm in $CDCl_3$ solution (see Table 2). Accordingly, CQ-2 was deduced to be the stereoisomer of **1** at position 5′, namely, (2′*R*,3′*R*,5′*S*,7′*S*)-6-[(5,3′:3′,7′-diepoxy-5′-hydroxy-2′-methyl)octyl]-7-methoxy-2-methylchromone (**2**), as shown in Chart 3. To our knowledge, this is the first time that **2** has been isolated from a natural source as a MAO inhibitory constituent. Thus we propose the name CQ-2 chaetoquadrin B (**2**).

CQ-3 (**3**), $C_{20}H_{24}O_6$, was obtained as an optically active, colorless, amorphous substance, of which the UV and IR spectra were similar to those of **1**. The CD spectrum of **3** (1.11 mM, MeOH), $\Delta\varepsilon$ (nm): −2.9 (322), +4.6 (296), +0.28 (264), +1.9 (255), −2.6 (228), +2.0 (215), +1.6 (212), +6.0 (204), was similar to that of **1** except for the peaks at around 254 and 215 nm (see Fig. 1). These data suggested that **3** might be a stereoisomer of **1**. The CD behavior of **3** and the fact that **3** was obtained from the fungus together with **1** suggested that the absolute configurations of the majority of asymmetric carbons in **3** might be the same as those in **1**. In the ^{1}H-NMR spectrum of **3** (in $CDCl_3$), the signal of OH-5′ was clearly observed at δ 6.44 ppm like that in the spectrum of **2**, suggesting that the configuration of OH-5′ in **3** was considered to be also α quasi-axial. In the spectrum, H-5′ (δ 4.12) was coupled to H_a-6′ (δ 1.84) and H_b-6′ (δ 1.38) with J=2.2, 3.2 Hz, respectively, implying that ϕ(H-5′/H_a-6′) and ϕ(H-5′/H_b-6′) were 50—70° (quasi-*gauche* 1,2-equatorial-equatorial, and 1,2-axial-equatorial), but H-5′ was not coupled to H_a-4′ (δ 1.98) and H_b-4′ (δ 2.03), suggesting that ϕ(H_a-4′/H-5′) and ϕ(H_b-4′/H-5′) were about 90°. In the NOEDF NMR experiment on **3**, the three NOEs (CH_3-2′/H_a-4′), (H-2′/H_b-4′), and (H_b-1′/H-2′) which were

Table 2. ^{1}H- and ^{13}C-NMR Data for Chaetoquadrins B (**2**) and C (**3**), δ (ppm) from TMS as an Internal Standard in $CDCl_3$ [Coupling Constants (Hz) in Parentheses]

Position	**2**		**3**	
	δ_H	δ_C	δ_H	δ_C
2		163.4 (s)		163.4 (s)
2-CH_3	2.29 (3H, s)	19.9 (q)	2.21 (3H, s)	19.9 (q)
3	6.00 (s)	111.5 (d)	5.92 (s)	111.5 (d)
4		177.6 (s)		177.6 (s)
4a		107.9 (s)		108.0 (s)
5		150.3 (s)		150.8 (s)
6		107.2 (s)		109.2 (s)
7		161.7 (s)		160.8 (s)
7-OCH_3	3.90 (3H, s)	55.8 (q)	3.82 (3H, s)	55.8 (q)
8	6.46 (s)	91.5 (d)	6.36 (s)	91.3 (d)
8a		157.9 (s)		157.9 (s)
1′	2.92 (dd, 16.7, 6.5), 2.40 (dd, 16.7, 1.6)	23.3 (t)	2.55 (dd, 16.8, 6.0), 2.30 (dd, 16.8, 12.4)	23.5 (t)
2′	2.05 (qdd, 7.0, 6.5, 1.6)	32.3 (d)	1.89 (qdd, 6.6, 12.4, 6.0)	33.3 (d)
2′-CH_3	0.95 (3H, d, 7.0)	15.3 (q)	1.03 (3H, d, 6.6)	16.0 (q)
3′		100.5 (s)		101.1 (s)
4′	2.35 (dd, 14.3, 2.0), 1.65 (dd, 14.3, 4.1)	36.7 (t)	2.03 (d, 16.4), 1.98 (d, 16.4)	35.9 (t)
5′	4.17 (m)	63.5 (d)	4.12 (ddd, 11.9, 3.2, 2.2)	63.8 (d)
5′-OH	6.32 (d, 11.7)		6.44 (d, 11.9)	
6′	1.92 (ddd, 13.8, 4.5, 2.2), 1.49 (ddd, 13.8, 11.9, 3.0)	39.9 (t)	1.84 (ddd, 14.0, 2.2, 2.2), 1.38 (ddd, 14.0, 12.0, 3.2)	40.1 (t)
7′	4.14 (m)	62.5 (d)	4.03 (dqd, 12.0, 6.1, 2.2)	62.4 (d)
8′	1.04 (3H, d, 6.1)	21.4 (q)	0.94 (3H, d, 6.1)	21.4 (q)

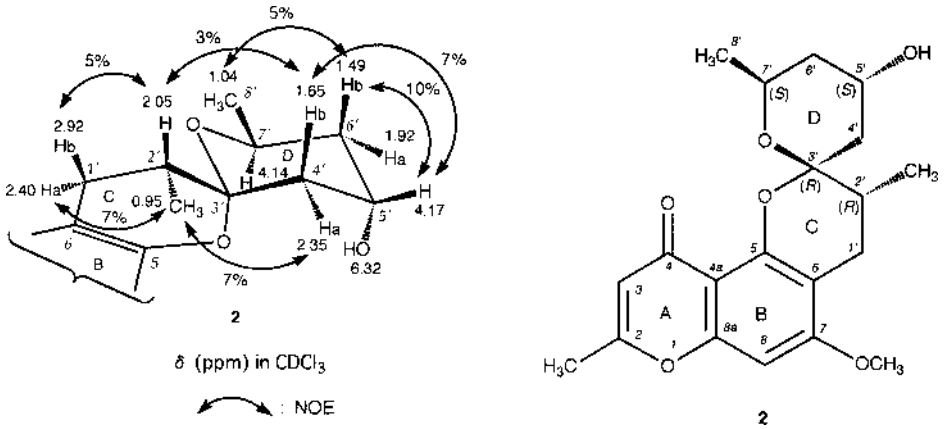

Chart 3. Relative Configuration of the Moiety of Rings C and D in Chaetoquadrin B (**2**) and Proposed Absolute Configuration of Chaetoquadrin B (**2**)

observed on both **1** and **2** disappeared, but three NOEs (H_b-4′/H-5′), (CH_3-2′(δ 1.03)/H_b-4′), and (H_b-1′(δ 2.30)/CH_3-2′) newly appeared of 2%, 5%, and 5%, respectively, suggesting that the configuration of CH_3-2′ was changed from α quasi-equatorial in both **1** and **2** to β quasi-axial in **3**, as shown in Chart 4. This was also supported by the fact that, on **1** and **2**, H-2′ (δ 2.11, 2.05) were coupled to H_a-1′ (δ 2.91, 2.92) with J=6.6, 6.5 Hz, and H_b-1′ (δ 2.39, 2.40) with J=3.2, 1.6 Hz, respectively, but on **3**, H-2′ (δ 1.89) was coupled to H_a-1′ (δ 2.55) and H_b-1′ (δ 2.30) with J=6.0, 12.4 Hz, respectively (see Table 2 and Chart 4). Accordingly, CQ-3 was deduced to be a stereoisomer of **1** at both positions 2′ and 5′, namely, (2′*S*,3′*R*,5′*S*,7′*S*)-6-[(5,3′:3′,7′-diepoxy-5′-hydroxy-2′-methyl)octyl]-7-methoxy-2-methylchromone (**3**), as shown in Chart 4. To our knowledge, this is the first time that CQ-3 has been isolated from a natural source as a MAO inhibitory constituent. Thus we propose the name CQ-3 chaetoquadrin C (**3**).

CQ-4 (**4**), $C_{16}H_{19}NO_7S$, was obtained as an optically inactive white powder, IR (KBr) ν_{max} cm^{-1}: 3300 (OH), 1660 (C=O), 1626 (C=C), 1448, 1344, 1182, 1126 (C–O, S=O). The UV spectrum of **4** (in MeOH), λ_{max} nm (log ε): 205 (4.23), 234 (4.21), 250 (4.19), 258 (4.19), 289 (3.69), also suggested the presence of a 6-substituted 5-hydroxy-7-methoxy-2-methylchromone skeleton in **4**. The ^{1}H- and ^{13}C-NMR data (in $CDCl_3$ and in DMSO-d_6) including spin-decoupling ^{1}H-NMR, COSY, HSQC, and HMBC data showed the presence of two partial structures *e* ($C_{12}H_{11}O_4$) and *f* (C_4H_8NO) in **4** (Table 4). Considering the molecular formula, the molecule of **4** should be constructed with *e*, *f*, and a sulfonyl group, SO_2 (partial structure *g*). Construction of the entire molecule of CQ-4 from *e*—*g* was achieved to link both carbons at δ_C 48.6 (t) in *e* and at δ_C 52.7 (t) in *f* (in $CDCl_3$) to the sulfur in *g*, because the methylene carbon at the α-position to a sulfonyl group resonates at 46—55 ppm in the ^{13}C-NMR spectrum,[8] providing the entire molecular structure of

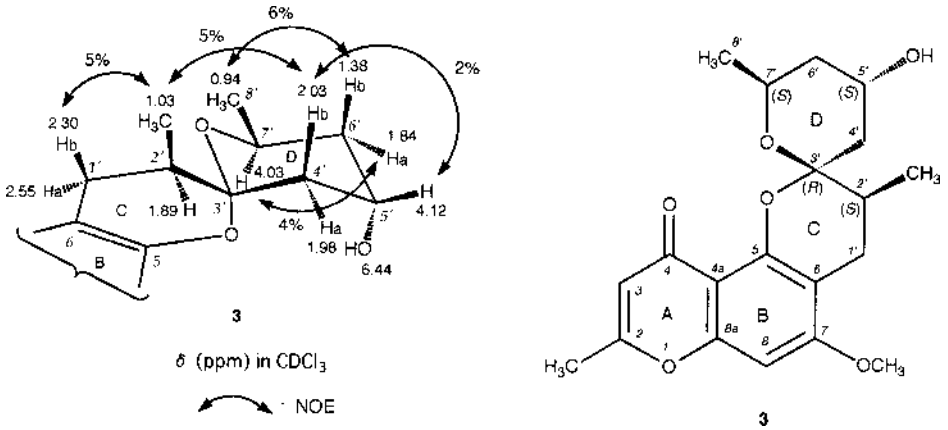

Chart 4. Relative Configuration of the Moiety of Rings C and D in Chaetoquadrin C (**3**) and Proposed Absolute Configuration of Chaetoquadrin C (**3**)

Table 3. ^{1}H- and ^{13}C-NMR Data for Chaetoquadrins D (**4**) and E (**5**), and Chaetoquadrin E Acetonide (**10**), δ (ppm) from TMS as an Internal Standard [Coupling Constants (Hz) in Parentheses]

Position	**4**				**5**		**10**	
	in $CDCl_3$		in DMSO-d_6		in $CDCl_3$		in $CDCl_3$	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2		167.3 (s)		168.5 (s)		166.7 (s)		166.3 (s)
2-CH_3	2.36 (3H, s)	20.5 (q)	2.40 (3H, s)	19.9 (q)	2.33 (3H, s)	20.4 (q)	2.32 (3H, s)	20.4 (q)
3	6.08 (s)	109.2 (d)	6.31 (s)	108.4 (d)	6.03 (s)	108.8 (d)	6.01 (s)	108.9 (d)
4		182.2 (s)		181.9 (s)		182.4 (s)		182.5 (s)
4a		105.1 (s)		104.1 (s)		104.9 (s)		105.1 (s)
5		160.0 (s)		159.4 (s)		158.3 (s)		158.7 (s)
5-OH	13.40 (s)		13.42 (s)		13.09 (s)		12.76 (s)	
6		100.2 (s)		100.4 (s)		112.2 (s)		113.1 (s)
7		163.6 (s)		163.3 (s)		163.2 (s)		163.2 (s)
7-OCH_3	3.93 (3H, s)	56.5 (q)	3.91 (3H, s)	56.8 (q)	3.86 (3H, s)	56.0 (q)	3.85 (3H, s)	55.8 (q)
8	6.43 (s)	90.4 (d)	6.78 (s)	90.9 (d)	6.37 (s)	89.8 (d)	6.33 (s)	89.3 (d)
8a		158.5 (s)		157.8 (s)		156.6 (s)		156.6 (s)
1′	4.43 (2H, s)	48.6 (t)	4.36 (2H, s)	48.1 (t)	2.74 (2H, m)	17.8 (t)	2.69 (m), 2.64 (m)	18.2 (t)
2′					1.71 (m), 1.63 (m)	35.9 (t)	1.63 (m), 1.60 (m)	34.8 (t)
3′	3.20 (2H, m)	52.7 (t)	3.19 (2H, dd, 7.6, 6.6)	52.4 (t)	3.74 (m)	67.7 (d)	3.80 (m)	66.7 (d)
4′	3.78 (2H, m)	32.8 (t)	3.45 (2H, m)	32.4 (t)	1.55 (2H, m)	43.9 (t)	1.60 (2H m)	39.9 (t)
5′	6.31 (br s)		8.06 (t, 5.5)		4.10 (m)	65.3 (d)	3.95 (m)	62.8 (d)
6′		170.2 (s)		169.5 (s)	1.15 (3H, d, 6.4)	23.4 (q)	1.17 (3H, d, 6.1)	21.8 (q)
7′	1.94 (3H, s)	23.2 (q)	1.80 (3H, s)	22.5 (q)				
–O(–O)C(C$\underline{H}_3$)C$\underline{H}_3$							1.35 (3H, s)	25.2 (q)
							1.34 (3H, s)	25.1 (q)
–O(–O)$\underline{C}$(CH_3)CH_3								100.0 (s)

CQ-4, namely, 5-hydroxy-7-methoxy-6-(2-thia-2,2,6-trioxo-5-azaheptyl)-2-methylchromone (**4**), as shown in Chart 5. To our knowledge, this is the first time that CQ-4 has been isolated from a natural source as a MAO inhibitory constituent. Thus we propose the name CQ-4 chaetoquadrin D (**4**).

CQ-5 (**5**), $C_{17}H_{22}O_6$, was obtained as an optically active white powder, IR (KBr) ν_{max} cm^{-1}: 3392 (OH), 1656 (C=O), 1619 (C=C), 1452, 1344, 1203 (C–O). The UV spectrum of **5** (in MeOH), λ_{max} nm (log ε): 210 (4.42), 232 (4.27), 254 (4.21), 258 (4.21), 292 (3.93), also suggested the presence of a 5-hydroxy-7-methoxy-2-methylchromone skeleton in **5**. The ^{1}H- and ^{13}C-NMR data (in $CDCl_3$) including spin-decoupling ^{1}H-NMR, COSY, HSQC, and HMBC data afforded the molecular structure without stereochemistry of CQ-5 (**5**), which contained a 1,3-diol system at positions 3′ and 5′ (see Chart 5). On the reaction with acetone in the presence of *p*-toluenesulfonic acid, **5** gave an acetonide (**10**), as a white powder. The ^{1}H- and ^{13}C-NMR spectra of **10** (in $CDCl_3$) showed that the signals of two acetal methyls, δ_H 1.35, 1.34 (each 3H, s), δ_C 25.2, 25.1 (each, q), and one tertiary acetal carbon δ_C 100.0 (s) newly appeared, and the signals of H-3′, H-5′, and C-3′, C-5′ were shifted to δ_H 3.80 (+0.06), 3.95 (−0.15), and δ_C 66.7 (−1.0), 62.8 (−2.5) ppm, respectively, indicating that the two hydroxyl groups at positions 3′ and 5′ in **5** were involved in acetonide formation to furnish **10**. It has been known that *anti*-1,3-diol acetonide gives its two acetal methyls at 25, and its acetal carbon at 100.5 ppm. On the other hand, *syn*-1,3-diol acetonide gives its two acetal methyls at 19 and 30, and its acetal carbon at 98.5 ppm in the ^{13}C-NMR spectrum,[9] indicating that **10** is not *syn*-1,3-diol acetonide, but *anti*-1,3-diol acetonide. To determine the absolute configuration of **5**, (*R*)-MTPA ester (**11**) and (*S*)-MTPA ester (**12**) were prepared from **5**. Comparison of the ^{1}H-NMR spectra of **11** and **12** with that of **5** showed that the

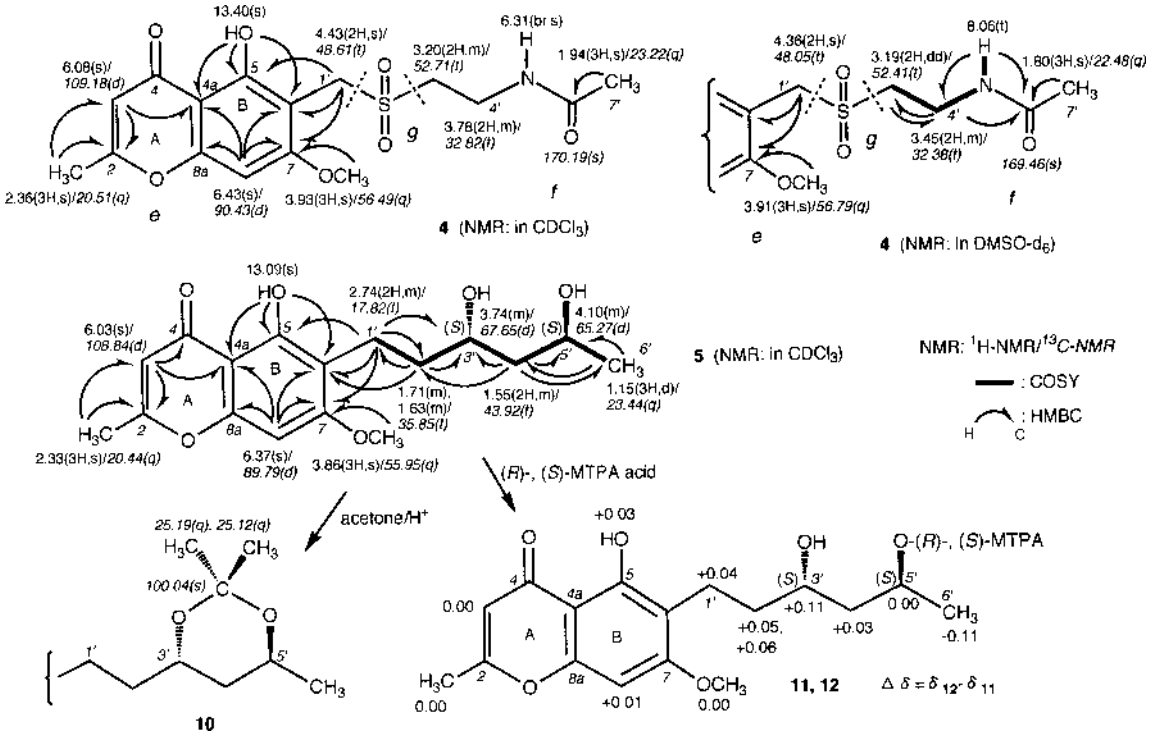

Chart 5

Table 4. Mouse Liver MAO Inhibitory Activities of Chaetoquadrins A (**1**)—E (**5**)

Compound	Inhibitory ratio (%)			
	1.0×10^{-4}	2.5×10^{-5}	1.0×10^{-5}	5.0×10^{-6} g/ml
Chaetoquadrin A (**1**)	n.t.*a)*	7.7	4.8	5.3
Chaetoquadrin B (**2**)	n.t.	17.5	11.0	9.4
Chaetoquadrin C (**3**)	n.t.	31.9	20.0	12.4
Chaetoquadrin D (**4**)	n.t.	62.3	42.5	28.5
Chaetoquadrin E (**5**)	29.8	8.8	6.1	n.t.
Chaetoquadrin D (**4**): $IC_{50}=3.8\times10^{-5}$ M (1.4×10^{-5} g/ml)				

a) n.t.: not tested.

signals of H-3′ and H-5′ were shifted to δ 3.37 (−0.37) and 5.39 (+1.29) in **11**, and shifted to δ 3.48 (−0.26) and 5.39 (+1.29) in **12**, respectively, indicating that the hydroxyl group at position 5′ in **5** was (*R*)- and (*S*)-MTPA-esterified to afford **11** and **12**, respectively. The $\Delta\delta$ values (δ_{12}–δ_{11}) were calculated as shown in Chart 5, showing that the absolute configuration at position 5′ in **5** was (*S*). Therefore the absolute configuration at position 3′ in **5** was determined to be (*S*). Accordingly, CQ-5 was deduced to be (3′*S*,5′*S*)-6-(3′,5′-dihydroxyhexyl)-5-hydroxy-7-methoxy-2-methylchromone (**5**), as shown in Chart 5. To our knowledge, this is the first time that CQ-5 has been isolated from a natural source as a MAO inhibitory constituent, and we propose the name CQ-5 chaetoquadrin E (**5**).

The mouse liver MAO inhibitory activities of chaetoquadrin A (**1**)—E (**5**) were calculated as shown in Table 5. Among these five compounds, **4** displayed the highest MAO inhibitory activity (IC_{50}: 3.8×10^{-5} M). Comparison of the activity of **4** with those of other MAO inhibitory components, luteusin A (IC_{50}: 6.6×10^{-6} M),[1a] GP-A (IC_{50}: 2.7×10^{-6} M),[1c] monankarin A (IC_{50}: 1.6×10^{-5} M),[1d] and coniochaetone A (IC_{50}: 2.9×10^{-5} M),[1e] which we have previously isolated from fungi, suggested that **4** displays moderate activity.

Experimental

The general procedures for chemical experiments were the same as described in our previous report.[1f] Optical rotations and CD spectra were measured with a JASCO DIP-140 digital polarimeter and a JASCO J-500 spectropolarimeter, respectively. UV and IR spectra were recorded on Hitachi U-3200 and JASCO FT/IR-230 spectrophotometers, respectively. Electron impact (EI)-MS and high-resolution (HR)-FAB-MS spectra were measured with Hitachi M-60 and JEOL JMX-HX-110A spectrometers, respectively. ^{1}H- and ^{13}C-NMR spectra were measured with JEOL JNM-A400 (^{1}H, 399.65; ^{13}C, 100.40 MHz) and -A500 (^{1}H, 500.00; ^{13}C: 125.65 MHz) spectrometers. The procedure for the evaluation of inhibitory activity of samples against mouse liver MAO was also the same as described in our previous report.[1a] Chemical shifts are expressed in δ (ppm) values from tetramethylsilane (TMS) as an internal standard.

Isolation of Chaetoquadrins A (1)—E (5) *C. quadrangulatum* strain 71-NG-22[2] was cultivated on sterilized rice (200 g/flask×100) at 25 °C for 33 d. The moldy rice was extracted with AcOEt (30 l) with shaking at room temperature for 6 h two times to give an AcOEt extract (61.2 g), which inhibited MAO by 30.8% at 1.0×10^{-4} g/ml. The AcOEt extract (41.5 g) was dissolved in MeOH (100 ml) to give a solution, which was then diluted with H_2O (1 l) to give a suspension. The suspension was partitioned with *n*-hexane (600 ml) three times into an *n*-hexane layer (after evaporation *in vacuo*, 15.89 g) and aqueous suspension. The aqueous suspension was further partitioned with AcOEt (600 ml) three times into an AcOEt layer (after evaporation *in vacuo*, 16.07 g) and aqueous layer (after evaporation *in vacuo*, 7.60 g). The AcOEt layer (14.80 g), which inhibited MAO by 29.6% at 1.0×10^{-4} g/ml, was subjected to chromatography on a silica gel column with *n*-hexane–acetone (10 : 1, v/v), (3 : 1), (1 : 1), (1 : 1—0 : 1), and MeOH to give fractions I—V, respectively. Fraction IV (6.38 g), which inhibited MAO by 36% at 1.0×10^{-4} g/ml, was further chromatographed on a silica gel column with $CHCl_3$–MeOH (50 : 1), (30 : 1), (20 : 1), (20 : 1), (10 : 1), and MeOH to give the six fractions IVa—f, respectively. Fraction IVb (667 mg), which inhibited MAO by 31% at 2.5×10^{-5} g/ml, was chromatographed on a silica gel column with *n*-hexane–acetone (2 : 1), and successively on a high-performance liquid chromatographic (HPLC) octadecyl silica gel (ODS) column with CH_3CN–H_2O (55 : 45) at a flow rate of 8 ml/min to afford **2** (10 mg) and **3** (7 mg). Fraction IVc (1.76 g), which inhibited MAO by 19%

at 2.5×10^{-5} g/ml, was further chromatographed on a silica gel column with *n*-hexane–acetone (3 : 1), (2 : 1), (2 : 1), (1 : 1), and MeOH to give fractions IVc1—5, respectively. Fraction IVc3 (406 mg) and IVc4 (402 mg) were then chromatographed on HPLC ODS columns with CH_3CN–H_2O (45 : 55) and (35 : 65) at a flow rate of 4—8 ml/min to afford **1** (55 mg) and **4** (29 mg), respectively. Fraction IVc2 (374 mg) was chromatographed on a silica gel column with $CHCl_3$–MeOH (50 : 1), (50 : 1), (30 : 1), (10 : 1), and MeOH to give the five fractions IVc2a—e. Fraction IVc2b (110 mg) was then chromatographed on an HPLC ODS column with CH_3CN–H_2O (70 : 30) to afford **5** (45 mg), **3** (2.4 mg), and **2** (2.5 mg). Fraction IVc2c (119 mg) was chromatographed on an HPLC ODS column with CH_3CN–H_2O (50 : 50) to afford **5** (38 mg).

Chaetoquadrin A (**1**): Colorless amorphous. HR-FAB-MS *m/z*: 361.1657 ($C_{20}H_{25}O_6$ requires 361.1651 [$(M+H)^+$]).

Chaetoquadrin B (**2**): Colorless amorphous. HR-FAB-MS *m/z*: 361.1660 ($C_{20}H_{25}O_6$ requires 361.1651 [$(M+H)^+$]). IR ν_{max}^{KBr} cm^{-1}: 3423 (OH), 1654 (C=O), 1606 (C=C), 1452, 1346, 1205 (C–O). UV λ_{max}^{MeOH} nm (log ε): 207 (4.30), 234 (4.19), 248 (4.10), 254 (4.09), 285 (3.88).

Chaetoquadrin C (**3**): Colorless amorphous. HR-FAB-MS *m/z*: 361.1672 ($C_{20}H_{25}O_6$ requires 361.1651 [$(M+H)^+$]). IR ν_{max}^{KBr} cm^{-1}: 3421 (OH), 1654 (C=O), 1606 (C=C), 1452, 1346, 1205 (C–O). UV λ_{max}^{MeOH} nm (log ε): 208 (4.31), 233 (4.20), 248 (4.10), 254 (4.09), 286 (3.87).

Chaetoquadrin D (**4**): White powder from aqueous CH_3CN, mp 216—219 °C. HR-FAB-MS *m/z*: 370.0968 ($C_{16}H_{20}NO_7S$ requires 370.0960 [$(M+H)^+$]).

Chaetoquadrin E (**5**): White powder from aqueous CH_3CN, mp 100—102 °C. $[\alpha]_D^{20}$ +11.5° (*c*=0.20, MeOH). HR-FAB-MS *m/z*: 323.1503 ($C_{17}H_{23}O_6$ requires 323.1495 [$(M+H)^+$]).

Chaetoquadrin A Acetate (7) A solution of **1** (5.0 mg) in acetic anhydride (125 μl) and pyridine (250 μl) was allowed to stand at room temperature for 8 h, and treated as usual to give a crude product, which was then purified on a preparative thin-layer chromatographic (TLC) silica gel plate with $CHCl_3$–MeOH (10 : 1) to afford **7** (3.5 mg) as a colorless amorphous substance. EI-MS *m/z* (%): 402 (6, M^+), 342 (14), 245 (43), 219 (100).

(*R*)- and (*S*)-MTPA Esters of Chaetoquadrin A (8 and 9) A solution of **1** (4.0 mg), (*R*)-MTPA acid (12 mg), and dicyclohexylcarbodiimide (DCC) (10 mg) in pyridine (20 μl) and CH_2Cl_2 (1.0 ml) was allowed to stand at 40 °C for 7 h. The reaction mixture was evaporated *in vacuo* to give a resinous residue, which was purified on a preparative TLC silica gel plate with $CHCl_3$–MeOH (10 : 1) to afford **8** (2.9 mg), as a colorless amorphous substance, ^{1}H-NMR δ (ppm, $CDCl_3$): 1.00 (3H, d, 6.8, CH_3-2′), 1.07 (3H, d, 6.2, CH_3-8′), 1.33 (ddd, 11.6, 11.6, 11.6, H_b-6′), 1.52 (dd, 12.0, 11.6, H_b-4′), 2.12 (qdd, 6.8, 6.4, 3.6, H-2′), 2.20 (ddd, 11.6, 2.4, 2.0, H_a-6′), 2.27 (3H, s, CH_3-2), 2.38 (dd, 16.8, 3.6, H_b-1′), 2.54 (ddd, 12.0, 4.8, 2.0, H_a-4′), 2.91 (dd, 16.8, 6.4, H_a-1′), 3.55 (3H, s, OCOC(OC$\underline{H}_3$)(CF_3)C_6H_5-5′), 3.88 (3H, s, CH_3O-7), 4.12 (dqd, 11.6, 6.2, 2.4, H-7′), 5.95 (ddd, 11.6, 11.6, 4.8, H-5′), 5.97 (s, H-3), 6.43 (s, H-8), 7.39 (3H, m) and 7.55 (2H, m) (OCOC(OCH_3)(CF_3)$C_6\underline{H}_5$-5′). A solution of **1** (4.0 mg), (*S*)-MTPA acid (12 mg), and DCC (10 mg) in pyridine (20 μl) and CH_2Cl_2 (1.0 ml) was allowed to stand at 40 °C for 5 h. The reaction mixture was treated in the same way as described for the preparation of **8** from **1** to afford **9** (1.7 mg), as a colorless amorphous substance, ^{1}H-NMR δ (ppm, $CDCl_3$): 0.97 (3H, d, 7.1, CH_3-2′), 1.09 (3H, d, 6.3, CH_3-8′), 1.41 (dd, 12.4, 8.0, H_b-4′), 1.43 (ddd, 11.6, 11.6, 11.6, H_b-6′), 2.10 (qdd, 7.1, 7.6, 3.6, H-2′), 2.25 (ddd, 11.6, 4.0, 1.6, H_a-6′), 2.27 (3H, s, CH_3-2), 2.37 (dd, 17.0, 3.6, H_b-1′), 2.50 (ddd, 12.4, 4.8, 1.6, H_a-4′), 2.90 (dd, 17.0, 7.6, H_a-1′), 3.56 (3H, s, OCOC(OC$\underline{H}_3$)(CF_3)C_6H_5-5′), 3.88 (3H, s, CH_3O-7), 4.14 (dqd, 11.6, 6.3, 4.0, H-7′), 5.95 (ddd, 11.6, 8.0, 4.8, H-5′), 5.97 (s, H-3), 6.42 (s, H-8), 7.39 (3H, m) and 7.55 (2H, m) (OCOC(OCH_3)(CF_3)$C_6\underline{H}_5$-5′).

Acetonide of Chaetoquadrin E (10) A suspension of **5** (5 mg), *p*-toluenesulfonic acid monohydrate (1 mg), and K_2CO_3 (10 mg) in dry acetone (1.0 ml) was allowed to stand with stirring at room temperature for 12 h. The reaction mixture was purified on a preparative TLC silica gel plate with $CHCl_3$–MeOH (10 : 1) to afford **10** (2 mg), white powder.

(*R*)- and (*S*)-MTPA Esters of Chaetoquadrin E (11 and 12) A solution of **5** (6.3 mg), (*R*)-MTPA acid (31.5 mg), and DCC (16 mg) in pyridine (25 μl) and CH_2Cl_2 (1.0 ml) was allowed to stand at room temperature for 1 h and at 40 °C for 4 h. The reaction mixture was evaporated *in vacuo* to give a resinous residue, which was purified on a preparative TLC silica gel plate with $CHCl_3$–MeOH (10 : 1) to afford **11** (1.7 mg), as a colorless amorphous substance, ^{1}H-NMR δ (ppm, $CDCl_3$): 1.36 (3H, d, 6.4, CH_3-6′), 1.60 (2H, m, CH_2-4′), 1.61 (m) and 1.65 (m) (CH_2-2′), 2.37 (3H, s, CH_3-2), 2.73 (2H, m, CH_2-1′), 3.37 (m, H-3′), 3.45 (3H, s, OCOC(OC$\underline{H}_3$)(CF_3)C_6H_5-5′), 3.87 (3H, s, CH_3O-7), 5.39 (m, H-5′), 6.06 (s, H-3), 6.37 (s, H-8), 7.33 (3H, m), and 7.47 (2H, m) (OCOC(OCH_3)(CF_3)$C_6\underline{H}_5$-5′), 13.08 (s, OH-5). A solution of **5** (5.0 mg), (*S*)-MTPA acid (26.5 mg), and DCC (15 mg) in pyridine (25 μl) and CH_2Cl_2 (1.0 ml) was allowed to stand at room temperature for 3 h and at 40 °C for 1 h. The reaction mixture was treated in the same way as described for the preparation of **11** from **5** to afford **12** (1.3 mg), colorless amorphous, ^{1}H-NMR δ (ppm, $CDCl_3$): 1.25 (3H, d, 6.4, CH_3-6′), 1.63 (2H, m, CH_2-4′), 1.67 (m) and 1.70 (m) (CH_2-2′), 2.37 (3H, s, CH_3-2), 2.77 (2H, m, CH_2-1′), 3.36 (3H, s, OCOC(OC$\underline{H}_3$)(CF_3)C_6H_5-5′), 3.48 (m, H-3′), 3.87 (3H, s, CH_3O-7), 5.39 (m, H-5′), 6.06 (s, H-3), 6.38 (s, H-8), 7.34 (3H, m) and 7.45 (2H, m), (OCOC(OCH_3)(CF_3)$C_6\underline{H}_5$-5′), 13.11 (s, OH-5).

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