Gentiocrucines A–E, Five Unusual Lactonic Enamino Ketones from *Swertia macrosperma* and *Swertia angustifolia*

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Key words
- gentiocrucines A–E
- *Swertia macrosperma*
- *Swertia angustifolia*
- Gentianaceae
- lactonic enamino ketones
- anti-HBV activity

Abstract

Five unusual lactonic enamino ketones, gentiocrucines A–E (**1**–**5**), were isolated from *Swertia macrosperma* and *S. angustifolia*. Their structures were determined based on extensive spectroscopic analyses (IR, UV, MS, 1D- and 2D-NMR). By anti-HBV assay on the HepG 2.2.15 cell line *in vitro*, compound **1** inhibited both the secretion of hepatitis B virus surface antigen (HBsAg) and hepatitis B virus e antigen (HBeAg) with IC_{50} values of 3.14 and 3.35 mM, and compound **5** only inhibited the secretion of HBsAg with an IC_{50} value of 2.07 mM.

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

Introduction

Swertia macrosperma and *S. angustifolia* are congeneric species of *S. mileensis* (Gentianaceae) documented in the Chinese Pharmacopoeia (1977–2010 editions) for the treatment of hepatitis [1] and have also been used to cure hepatitis in Yunnan Province, China. Previous phytochemical studies demonstrated that xanthones, triterpenoids, and secoiridoid glycosides were their main components [2–10]. However, their anti-hepatitis B virus (HBV) constituents remain unclear. In order to search for active anti-HBV compounds from natural sources, gentiocrucines A–E and a known gentiocrucine were isolated from *S. macrosperma* and *S. angustifolia*. Extensive spectroscopic analyses led to the identification of gentiocrucines A–E belonging to lactonic enamino ketones.

Lactonic enamino ketones composed of a conjugated lactone with an enamino ketone, usually exist as mixtures of stereoisomers because of the presence of intramolecular H-bonds. Until now, only one lactonic enamine, gentiocrucine [11–16], has been reported as a natural product and unambiguously confirmed by a concise synthesis [17], favoring the isomeric enamines **6** (***E***) and **6** (***Z***) as shown in **Fig. 1**. In our report, gentiocrucines A–E were also shown to be mixtures of *E/Z* stereoisomers. The *E/Z* ratio in gentiocrucines A–E was approximately 8 : 5 determined by relative peak heights in the ^{1}H-NMR spectrum. The lower-field proton at C-7 was *cis* with respect to the *α,β*-unsaturated ester (*E* isomer) and the higher-field proton at C-7 was *trans* to the keto (*Z* isomer) [18], but they behaved like a single compound by TLC check (silica gel) in three different solvent systems and by reversed-phase HPLC. Their ^{1}H- and ^{13}C-NMR spectra showed doubling of all signals, but MS indicated monomers. More interestingly, the anti-HBV assay on the HepG 2.2.15 cell *in vitro* demonstrated that compound **1** showed inhibitory activities on HBsAg and HBeAg with IC_{50} values of 3.14 and 3.35 mM, and compound **5** on HBsAg with an IC_{50} value of 2.07 mM. Herein, we described the isolation, structural determination, and anti-HBV activities of compounds **1**–**6**, which not only expanded the group of lactonic enamino ketones interestingly from both the phytochemical and biosynthetic perspectives but also provided information on the anti-HBV constituents of *S. macrosperma* and *S. angustifolia*.

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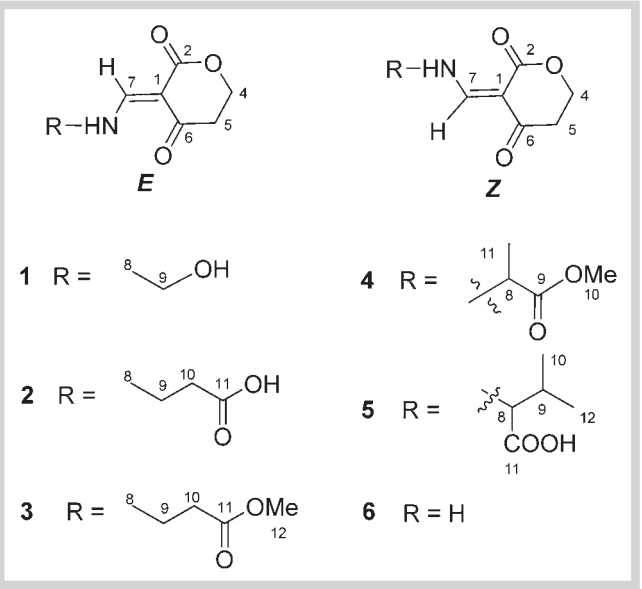

Fig. 1 Structures of compounds **1**–**6**.

Materials and Methods

General experimental procedures

Optical rotations were determined on a Jasco model 1020 polarimeter (Horiba). UV spectra were measured on a Shimadzu UV2401PC spectrophotometer (Shimadzu). IR (KBr) spectra were recorded on a Bio-Rad FTS-135 spectrometer (Bio-Rad). 1D and 2D NMR were recorded on Bruker AM-400, Bruker DRX-500, or AVANCE III-600 spectrometers (Bruker) with TMS as the internal standard. Mass spectra were run on a VG Spec-3000 spectrometer (VG) and Waters AutoSpec Premier P776 (Waters). Silica gel (200–300 mesh) for column chromatography and TLC plates (GF_{254}) were obtained from Qingdao Makall Chemical Company. Semipreparative HPLC was performed on a Waters Alliance 2695 liquid chromatograph with an Eclipse XDB-C_{18} column (9.4 × 250 mm; Waters). Sephadex LH-20 (20–150 µm) was purchased from Pharmacia Fine Chemical Co., Ltd. (Pharmacia). Fractions were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant material

The whole plants of *Swertia macrosperma* (C.B. Clarke) C.B. Clarke (No. 20081102) and *S. angustifolia* Buch.-Ham. ex D. Don (No. 20081127) were collected in Yuxi and Luquan, Yunnan Province, PR China, respectively, in November 2008, and were identified by Professor Li-Gong Lei (Kunming Institute of Botany, Chinese Academy of Sciences). Their voucher specimens were deposited in the Laboratory of Antivirus and Natural Medicine Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The dried and powered plant material (5 kg) of *Swertia macrosperma* was extracted with boiling water (12 L) for three times, 1 h for each time. The extract was concentrated under *vacuum* to give a residue (585 g), which was suspended in H_2O and partitioned among petroleum ether, EtOAc, and *n*-BuOH for three times (2 L × 3), successively. The EtOAc fraction (80 g) was subjected to silica column chromatography (Si CC, 800 g, 8 × 50 cm) and eluted with a $CHCl_3$-MeOH-H_2O (100 : 0 : 0, 95 : 5 : 0, 8 : 2 : 0.2, 7 : 3 : 0.3, 6 : 4 : 0.4, 0 : 100 : 0, v/v, each 10 L) gradient to afford five fractions (A–E). Fraction A (15 g) was further separated to obtain four subfractions (A1–A4) by Si CC with $CHCl_3$-Me_2CO (70 : 30, 60 : 40, 50 : 50, 40 : 60, 30 : 70, 20 : 80, each 1.8 L) as the eluent. Fraction A1 (2.2 g) was subjected to Si CC (100 g, 4 × 19 cm) and eluted with petroleum ether (PE)-Me_2CO (80 : 20, 70 : 30, 60 : 40, 50 : 50, 40 : 60, each 250 mL) to get five fractions A1-1 to A1–5. Fraction A1-1 (0.8 g) was chromatographed over Si CC (48 g, 2 × 34 cm) with an eluent of PE-EtOAc (85 : 15, 80 : 20, 75 : 25, 70 : 30, each 200 mL), then followed by Sephadex LH-20 (50.0 g, 1.4 × 145.0 cm, MeOH) to yield compounds **1** (5 mg) and **2** (8 mg). The fraction A1–2 (60 mg) was loaded on Si CC (24 g, 1.7 × 24.0 cm) with an elution of $CHCl_3$-EtOAc (65 : 35, 100 mL) to afford compound **3** (6 mg). Compound **4** (12 mg) was isolated from the fraction A1–3 (35 mg) with an eluent of PE-Me_2CO (60 : 40, 80 mL) by Si CC (15 g, 1.7 × 15.5 cm). The fraction A1–4 (120 mg) was purified with Si CC (28 g, 1.7 × 27.0 cm, PE-Me_2CO, 60 : 40, 120 mL) to afford compounds **5** (6 mg) and **6** (22 mg). At the same time, gentiocrucines A–E and a known gentiocrucine were obtained from *S. angustifolia* by the similar separation procedure. All purified compounds had a degree of purity > 90%; based on the TLC method in three different solvent systems, they exhibited one spot both under UV radiation and when visualized with H_2SO_4, and NMR spectra with a smooth baseline and no impurity peaks.

Gentiocrucine A (**1**): white amorphous powder; UV (MeOH) λ_{max} (log ε): 295 (5.1), 238 (3.8), 215 (3.6) nm; IR (KBr) ν_{max}: 3395, 3207, 2950, 1684, 1614, 1444, 869 cm^{-1}; ^{1}H-NMR (500 MHz) and ^{13}C-NMR (100 MHz) in CD_3OD, see **Table 1**; EI-MS (70 ev): *m/z* = 185 (74), 167 (42), 154 (100), 126 (66), 82 (24), 70 (23); HR-EI-MS: *m/z* = 185.0685 ($C_8H_{11}NO_4$, calcd. for 185.0688).

Gentiocrucine B (**2**): white amorphous powder; UV (MeOH) λ_{max} (log ε): 296 (4.9), 237 (3.4), 216 (5.6) nm; IR (KBr) ν_{max}: 3432, 3216, 3012, 2912, 1738, 1682, 1640, 1583, 1443, 1172, 1049, 858 cm^{-1}; ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) in C_5D_5N, see **Table 1**; EI-MS (70 ev): *m/z* = 227 (73), 168 (100), 154 (38), 126 (42), 82 (68), 55 (88); HR-EI-MS: *m/z* = 227.0792 ($C_{10}H_{13}NO_5$, calcd. for 227.0794).

Gentiocrucine C (**3**): white amorphous powder; UV (MeOH) λ_{max} (log ε): 299 (4.2), 239 (4.0), 211 (3.6) nm; IR (KBr) ν_{max}: 3207, 2953, 2922, 1734, 1696, 1625, 1603, 1442, 1352, 1176 cm^{-1}; ^{1}H-NMR (500 MHz) and ^{13}C-NMR (100 MHz) in $CDCl_3$, see **Table 2**; EI-MS (70 ev): *m/z* = 241 (93), 168 (100), 154 (38), 126 (42), 82 (40); HR-EI-MS: *m/z* = 241.0949 ($C_{11}H_{15}NO_5$, calcd. for 241.0950).

Gentiocrucine D (**4**): white amorphous powder; $[\alpha]_D^{13.0}$ + 2.60 (*c* 0.24, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 299 (4.2), 239 (3.9), 211 (3.6) nm; IR (KBr) ν_{max}: 3260, 2958, 2925, 1740, 1689, 1652, 1594, 1443, 1366, 1146, 807 cm^{-1}; ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) in $CDCl_3$, see **Table 2**; (−) ESI-MS: *m/z* = 226 [M − H]$^-$; (−) HR-ESI-MS: *m/z* = 226.0717 [M − H]$^-$ ($C_{10}H_{12}NO_5$, calcd. for 226.0715).

Gentiocrucine E (**5**): white amorphous powder; $[\alpha]_D^{17.8}$ − 45.0 (*c* 2.32, $CHCl_3$: MeOH = 1 : 1, *v/v*); UV (MeOH) λ_{max} (log ε): 299 (4.5), 238 (2.9), 215 (2.9) nm; IR (KBr) ν_{max}: 3435, 3130, 2879, 1730, 1680, 1627, 1561, 1446, 1249, 860 cm^{-1}; ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) in CD_3OD, see **Table 3**; EI-MS (70 ev): *m/z* = 241 (78), 196 (100), 150 (57), 124 (48), 69 (36), 55 (63); HR-EI-MS: *m/z* = 241.0948 ($C_{11}H_{15}NO_5$, calcd. for 241.0950).

Table 1 ^{1}H- and ^{13}C-NMR data for gentiocrucines A [a] in CD_3OD and B [b] in C_5D_5N.

Position	1				2			
	E		*Z*		*E*		*Z*	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	–	97.7 (s)	–	98.1 (s)	–	97.3 (s)	–	97.9 (s)
2	–	169.4 (s)	–	168.0 (s)	–	169.9 (s)	–	168.2 (s)
4	4.37 (2H, t, 6.2)	65.5 (t)	4.39 (2H, t, 6.2)	65.4 (t)	4.32 (2H, t, 6.2)	64.4 (t)	4.32 (2H, t, 6.2)	64.1 (t)
5	2.59 (2H, t, 6.2)	36.6 (t)	2.57 (2H, t, 6.2)	36.9 (t)	2.61 (2H, t, 6.2)	36.5 (t)	2.61 (2H, t, 6.2)	36.8 (t)
6	–	192.4 (s)	–	195.6 (s)	–	190.6 (s)	–	193.9 (s)
7	8.28 (1H, s)	160.9 (d)	8.14 (1H, s)	162.4 (d)	8.58 (1H, d, 14.8)	159.0 (d)	8.48 (1H, d, 14.8)	160.7 (d)
8	3.58 (2H, m)	53.5 (t)	3.58 (2H, m)	53.6 (t)	3.57 (2H, m)	49.9 (t)	3.57 (2H, m)	49.9 (t)
9	3.69 (2H, t, 5.0)	61.5 (t)	3.69 (2H, t, 5.0)	61.5 (t)	2.04 (2H, m)	26.5 (t)	2.04 (2H, m)	26.5 (t)
10					2.55 (2H, t, 7.2)	31.6 (t)	2.55 (2H, t, 7.2)	31.6 (t)
11					–	175.1 (s)	–	175.1 (s)

[a] ^{1}H-NMR recorded at 500 MHz; ^{13}C-NMR recorded at 100 MHz; [b] ^{1}H-NMR recorded at 400 MHz; ^{13}C-NMR recorded at 100 MHZ

Table 2 ^{1}H- and ^{13}C-NMR data for gentiocrucines C [c] and D [d] in $CDCl_3$.

Position	3				4			
	E		*Z*		*E*		*Z*	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	–	96.8 (s)	–	97.4 (s)	–	97.8 (s)	–	98.2 (s)
2	–	169.9 (s)	–	168.2 (s)	–	169.5 (s)	–	168.0 (s)
4	4.34 (2H, t, 6.2)	64.2 (t)	4.34 (2H, t, 6.2)	63.9 (t)	4.35 (2H, t, 6.2)	64.2 (t)	4.37 (2H, t, 6.2)	63.9 (t)
5	2.57 (2H, t, 6.2)	35.8 (t)	2.57 (2H, t, 6.2)	36.1 (t)	2.60 (2H, t, 6.2)	35.9 (t)	2.56 (2H, t, 6.2)	36.2 (t)
6	–	191.0 (s)	–	194.2 (s)	–	191.0 (s)	–	194.3 (s)
7	8.14 (1H, d, 14.0)	158.6 (d)	8.03 (1H, d, 14.0)	160.4 (d)	8.15 (1H, d, 14.0)	157.1 (d)	8.04 (1H, d, 14.0)	158.8 (d)
8	3.46 (2H, m)	49.5 (t)	3.46 (2H, m)	49.5 (t)	4.22 (2H, m)	57.1 (t)	4.22 (2H, m)	57.2 (t)
9	1.95 (2H, m)	25.4 (t)	1.95 (2H, m)	25.4 (t)	–	170.6 (s)	–	170.6 (s)
10	2.36 (2H, t, 7.0)	30.4 (t)	2.36 (2H, t, 7.0)	30.4 (t)	3.76 (3H, s)	53.0 (q)	3.76 (3H, s)	53.0 (q)
11	–	172.6 (s)	–	172.6 (s)	1.57 (3H, d, 7.2)	18.7 (q)	1.57 (3H, d, 7.2)	18.8 (q)
12	3.65 (3H, s)	51.8 (q)	3.65 (3H, s)	51.8 (q)				

[c] ^{1}H-NMR recorded at 500 MHz; ^{13}C-NMR recorded at 100 MHz; [d] ^{1}H-NMR recorded at 500 MHz; ^{13}C-NMR recorded at 125 MHZ

Table 3 ^{1}H- and ^{13}C-NMR data for gentiocrucine E [e] in CD_3OD.

Position	5			
	E		*Z*	
	δ_H	δ_C	δ_H	δ_C
1	–	98.1 (s)	–	98.6 (s)
2	–	172.1 (s)	–	171.1 (s)
4	4.43 (2H, t, 6.0)	65.8 (t)	4.41 (2H, t, 6.0)	65.6 (t)
5	2.64 (2H, t, 6.0)	36.8 (t)	2.65 (2H, t, 6.0)	37.1 (t)
6	–	194.0 (s)	–	196.4 (s)
7	8.28 (1H, s)	160.0 (d)	8.14 (1H, s)	161.6 (d)
8	4.26 (1H, m)	68.5 (d)	4.24 (1H, m)	68.9 (d)
9	2.33 (1H, m)	33.0 (d)	2.33 (1H, m)	33.0 (d)
10	0.97 (3H, d, 6.9)	17.3 (q)	0.97 (3H, d, 6.9)	17.3 (q)
11	–	172.8 (s)	–	172.8 (s)
12	1.00 (3H, d, 6.9)	19.3 (q)	1.00 (3H, d, 6.9)	19.3 (q)

[e] ^{1}H-NMR recorded at 600 MHz; ^{13}C-NMR recorded at 125 MHz

Anti-HBV assay on HepG 2.2.15 cell line *in vitro*

The anti-HBV assay was performed according to our previous report [14]. Tenofovir, purchased from Jiangxi Chenyang Pharmaceutical Co. Ltd, China (purity > 97.6%, NO. 200904009), was used as the positive control.

Supporting information

1D- and 2D-NMR, HR-ESI-MS, HR-EI-MS, IR, and UV spectra of compounds **1**–**5** as well as the procedure for the anti-HBV assay and the anti-HBV data of compounds **1**–**6** from two independent experiments are available as Supporting Information.

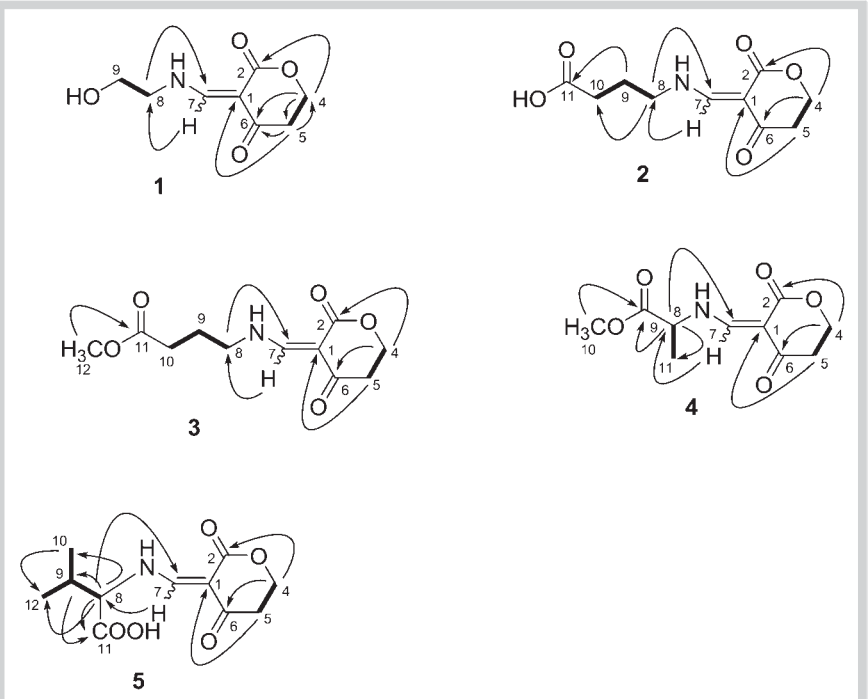

Fig. 2 Selected COSY (—) and HMBC (→) correlations of compounds **1**–**5**.

Results and Discussion

Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined to be $C_8H_{11}NO_4$ by HR-EI-MS at *m/z* 185.0685 $[M]^+$ (calcd. 185.0688). The IR spectrum showed the absorptions attributable to hydroxyl (3395 cm^{-1}), amino (3207 cm^{-1}), conjugated lactonic carbonyl (1684 cm^{-1}), and conjugated double bond (1614 cm^{-1}) groups. The ^{1}H-NMR (**Table 1**) spectrum showed a one-proton signal at δ_H 8.28 (***E***) or 8.14 (***Z***) due to a stereoisomeric vinyl proton. The ^{13}C-NMR (DEPT) spectrum exhibited eight pairs of carbons, including eight methylenes (four oxygenated), two methines, and six quaternary carbons which included two trisubstituted double bonds and four carbonyl signals. In the comparison of its NMR data with those of gentiocrucine [12], one more hydroxyethyl moiety was observed in compound **1**, which was inferred to be placed at the N atom of the enamine group. This assumption was confirmed by the ^{1}H-^{1}H COSY (H-8/H-9) and HMBC (H-8/C-7) experiments. The configuration of **1** was defined by the HMBC spectrum (**Fig. 2**), in which the correlations of H-4/C-2, C-5, C-6 and H-5/C-1, C-4, C-6 were demonstrated. Thus, the structure of compound **1** was characterized as shown in **Fig. 1**.

Compound **2**, a white amorphous powder, was assigned to have a molecular formula of $C_{10}H_{13}NO_5$ based on HR-EI-MS (*m/z* 227.0792 $[M]^+$). Its IR spectrum revealed the presence of carboxyl (3432 cm^{-1}), amino (3216 cm^{-1}), carbonyl (1738 cm^{-1}), conjugated lactonic carbonyl (1682 cm^{-1}), and conjugated double bond (1640 cm^{-1}) functions. The ^{13}C-NMR (DEPT) spectrum displayed ten pairs of carbons ascribed as ten methylenes, two methines, and eight quaternary carbons. Comparison of its NMR data with those of gentiocrucine [12] indicated that they possessed a similar skeleton. The main difference was that **2** had one more butyrate moiety confirmed by the correlations of H-8/C-9, C-10 and H-9/C-8, C-10, C-11 in the HMBC experiment (**Fig. 2**). The butyrate unit was proposed to be connected with the N atom of the enamine unit based on the HMBC analysis (from H-8 to C-7). Therefore, compound **2** was elucidated as gentiocrucine B.

Compound **3** had a molecular formula of $C_{11}H_{15}NO_5$, as determined by HR-EI-MS (*m/z* 241.0949 $[M]^+$), suggesting five degrees of unsaturation. The presence of amino (3207 cm^{-1}), carbonyl (1734 cm^{-1}), conjugated lactonic carbonyl (1696 cm^{-1}), and conjugated double bond (1625 cm^{-1}) groups was deduced from the IR spectrum. The careful analyses of ^{1}H-NMR and ^{13}C-NMR spectroscopic data suggested that the structure of **3** was similar to gentiocrucine B, except for one more MeO moiety deduced to be linked at the C-11 position by the HMBC correlated signal (**Fig. 2**) from H-12 to C-11. From the above evidence, the structure of compound **3** was established as shown.

Compound **4** had a molecular formula of $C_{10}H_{13}NO_5$ based on negative HR-ESI-MS (*m/z* 226.0717, $[M - H]^-$), indicating five degrees of unsaturation. The IR spectrum showed absorptions of amino (3260 cm^{-1}), carbonyl (1740 cm^{-1}), conjugated lactonic carbonyl (1689 cm^{-1}), and conjugated double bond (1652 cm^{-1}) groups. The ^{13}C-NMR (DEPT) spectrum showed ten pairs of carbon resonances, assigned to four methyl, four methylene, four methine, and eight quaternary groups. The ^{1}H- and ^{13}C-NMR data (**Table 2**) of compound **4** were similar to those of gentiocrucine [12], except for one more $C_4H_7O_2$ fragment which was deduced as a 2-methyl propionate group located at the N atom of the enamine unit on the basis of the HMBC correlations (**Fig. 2**) of H-8 to C-7, C-9, C-11; H-10 to C-9, in combination with the ^{1}H-^{1}H COSY correlation of H-8 to H-11. The structure of compound **4** was thus determined to be gentiocrucine D.

Compound **5** was isolated as a white amorphous powder, with a molecular formula of $C_{11}H_{15}NO_5$ determined by HR-EI-MS (*m/z* 241.0948, calcd. for $C_{11}H_{15}NO_5$, 241.0950). The IR absorptions were indicative of carboxyl (3435 cm^{-1}), amino (3130 cm^{-1}), carbonyl (1730 cm^{-1}), conjugated lactonic carbonyl (1680 cm^{-1}), and conjugated double bond (1627 cm^{-1}) groups. The NMR spectra (**Table 3**) showed a 2-(3-methyl) butyric acid fragment, and the residual signals were in accordance with those of gentiocru-

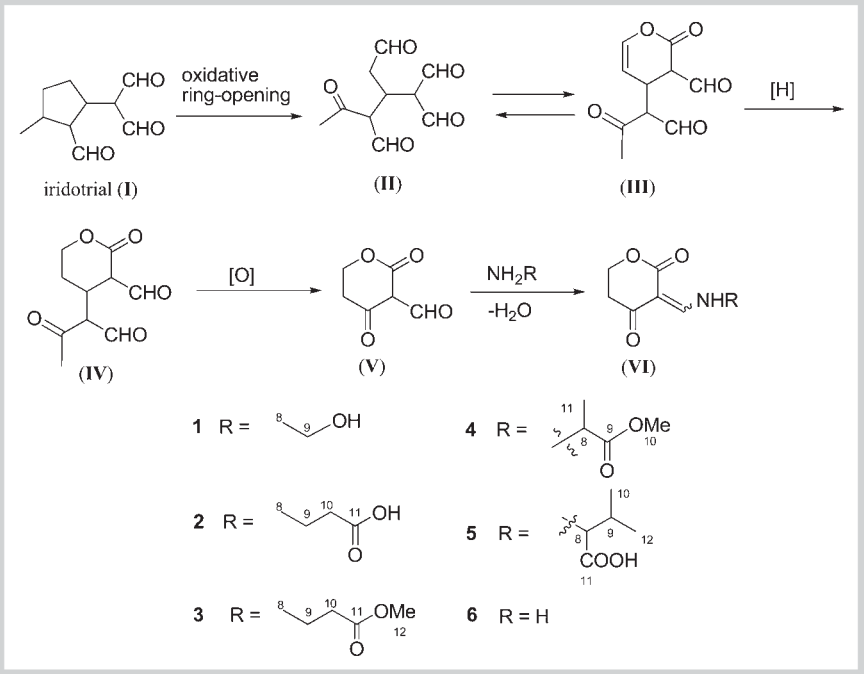

Fig. 3 A plausible biosynthetic route of compounds **1**–**6**.

Table 4 Anti-HBV activities of compounds **1**–**6** [a].

No.	CC_{50} (mM) and the cell destroy rate (%) [b]	HBsAg [c]		HBeAg [d]	
		IC_{50} (mM) or the inhibitory rate (%) [b]	SI [b]	IC_{50} (mM) or the inhibitory rate (%) [b]	SI
1	> 3.89 (9.7%)	3.14 (3.07 ~ 3.21)	> 1.24	3.35 (3.26 ~ 3.44)	> 1.16
2	> 3.78 (− 18.4%)	> 3.78 (7.0%)	–	> 3.78 (0.8%)	–
3	> 4.59 (− 52.6%)	> 4.59 (5.9%)	–	> 4.59 (− 4.5%)	–
4	> 4.01 (− 153.5%)	> 4.01 (3.4%)	–	> 4.01 (0.2%)	–
5	> 3.90 (21.3%)	2.07 (1.96 ~ 2.18)	> 1.88	–	–
6	> 7.85 (9.0%)	> 7.85 (8.1%)	–	> 7.85 (13.1%)	–
Tenofovir [e]	> 4.18 (3.6%)	3.98 (3.89 ~ 4.07)	> 1.05	2.80 (2.68 ~ 2.92)	> 1.49

[a] All values are the mean of two independent experiments; [b] CC_{50} = 50% cytotoxic concentration, IC_{50} = 50% inhibition concentration, if CC_{50} or IC_{50} was not reached at the tested concentration, the cell destroy rate or the inhibitory rate was given; SI = CC_{50}/IC_{50}; [c] HBsAg: HBV surface antigen; [d] HBeAg: HBV e antigen; [e] Tenofovir: an antiviral agent used as the positive control

cine [12]. The 2-(3-methyl) butyric acid fragment was located at the N atom of the enamine group, which was confirmed by the correlations of H-8 to C-7, C-9, C-10, C-11, C-12; H-9 to C-8, C-11; H-10 to C-8, C-12; and H-12 to C-8, C-10 in the HMBC experiment (**Fig. 2**), and the correlations of H-9 to H-8, H-10, H-12 in the ^{1}H-^{1}H COSY experiment. Consequently, compound **5** was determined to be gentiocrucine E.

A plausible biogenetic pathway of compounds **1**–**6** was postulated to explain their origins (**Fig. 3**). Briefly, iridotrial (**I**) generated the intermediate **V**, possibly involving a putative oxidative, an oxidative decarboxylation, a hemiacetal cyclization, a hydrogenation and oxidation reaction [19]. Then, attacked by NH_2R as the nucleophilic reagent, **V** was further changed to form the target products, gentiocrucines A–E.

In order to evaluate their anti-HBV activities, compounds **1**–**6** were assayed on the HepG 2.2.15 cell line *in vitro* (**Table 4**). The anti-HBV assay was performed according to a previous report [19] by using tenofovir as the positive control. Gentiocrucine A (**1**) exhibited moderate inhibitory activity on the secretion of HBsAg (IC_{50} 3.14 mM) and HBeAg (IC_{50} 3.35 mM). Gentiocrucine D (**5**) also displayed inhibitory activity on HBsAg secretion with an IC_{50} value of 2.07 mM but no inhibitory activity on HBeAg. Compounds **2**, **3**, **4**, and **6** showed no anti-HBV activities at the tested concentrations of 3.78, 4.59, 4.01, and 7.85 mM, respectively.

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Conflict of Interest

There are no conflicts of interest among all authors in this manuscript.

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