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Six insecticidal isoryanodane diterpenoids from the bark and twigs of *Itoa orientalis*

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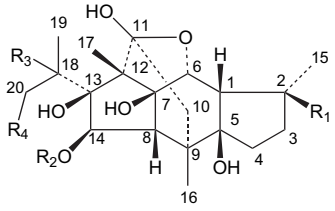
ABSTRACT

Four rare isoryanodane diterpenoids namely itols A–D (**1**–**4**) and two isoryanodane glucosides (**5** and **6**) were isolated from the bark and twigs of *Itoa orientalis*. Their structures were determined by NMR and MS techniques and the structure of **1** was confirmed by a single-crystal X-ray diffraction. These six diterpenoids obviously showed insecticidal activity against *Spodoptera exigua*, with LC_{50} 28.62 ppm for **1** and 52.76 ppm for **2**, respectively. In anti-inflammatory assay, compounds **1** and **4**–**6** showed anti-COX-2 activity, with inhibitory rates of 54.7–78.3% at 10 μM.

1. Introduction

Flacourtiaceae is an age-old family but an aggregate of loosely connected tribes on the view of taxonomy, and there were all kinds of disputations at all times about its diversity of family and genus.[1–3] More than 50 species of Flacourtiaceae have been found in China,[4] but only few chemical and biological researches on them were reported. In order to find new natural bioactive compounds and to provide valuable evidences for plants taxonomy in this family, several Flacourtiaceae plants were investigated in our research group, which resulted in the isolation and elucidation of a series of new phenolic glycosides.[5–7]

As a part of our systematic investigation of Flacourtiaceae plants found in China, the constituents of *Itoa orientalis* were investigated. According to our knowledge, there was no report on constituents of this plant and other *Itoa* species before our research. In a previous paper, we reported the isolation and elucidation of nine new phenolic glycosides from this plant and in present paper, we describe the on-going investigation about the isolation and structural elucidation of four new isoryanodane diterpenoids namely itols A–D (**1**–**4**) and two new isoryanodane glucosides, namely itol A-14-*O*-β-D-glucopyranoside (**5**) and itol B-20-*O*-β-D-glucopyranoside (**6**), as well as their insecticidal activity against *Spodoptera exigua* and anti-COX-2 activity (Fig. 1).



	R_1	R_2	R_3	R_4
1	H	H	H	H
2	H	H	H	OH
3	OH	H	H	H
4	H	H	OH	H
5	H	β-D-glc	H	H
6	H	H	H	O-β-D-glc

Figure 1. Structure of compounds **1**–**6**.

Isoryanodane diterpenoid has a rare diterpenoid skeleton, up to now, only eight of them have been reported and proven to possess anti-complementary and antifeedant activities.[8,9] However, there is no paper reported on its total synthesis and its derivatives' researches due to its complex structure.

2. Results and discussion

An 80% ethanol extract of the bark and twigs of *I. orientalis* was suspended in H_2O and then partitioned successively with $CHCl_3$,

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EtOAc, and *n*-BuOH. The *n*-BuOH-soluble material was repeatedly subjected to columns of silica gel, Sephadex LH-20, and octadecylsilyl silica gel (ODS) to afford four new isoryanodane diterpenoids (**1**–**4**), and two isoryanodane glucosides (**5** and **6**).

The NMR data of the new compounds are presented in Tables 1 and 2, and the bioactivity results are summarized in Tables 3 and 4.

Itol A (**1**) was obtained as a colorless quadrate crystal. Its HRFABMS exhibited an $[M+Na]^+$ ion peak at *m*/*z* 391.2089 (calcd 391.2090), compatible with the molecular formula $C_{20}H_{32}O_6$, indicating five unsaturation degrees. Its ^1H (Table 1) and ^{13}C NMR spectra (Table 2) displayed signals for three methyl groups [(δ_H 1.05, d, *J*=7.0 Hz, δ_C 15.5)(C-15); (δ_H 1.11, s, δ_C 22.3)(C-16); (δ_H 1.29, s, δ_C 10.4) (C-17)], an isopropyl group [δ_H 0.95 (d, *J*=6.5 Hz), δ_C 18.3; δ_H 1.07 (d, *J*=6.5 Hz), δ_C 18.8; δ_H 1.91 (m), δ_C 35.0], one hydroxymethine (δ_H 3.95, s, δ_C 79.2) (C-14), four oxygenated quaternary carbons at δ 88.3 (C-5), 75.9 (C-6), 90.8 (C-7), and δ 83.5 (C-13), and one carbon for a semi-ketal at δ 105.7 (C-11). These assignments were achieved by HSQC and HMBC experiments.

COSY connectivities of H-2/H-15, H-6/H-1/H-2/H-3/H-4, cooperated by HMBC correlations formed the fragment I (Fig. 2). In HMBC spectrum, the key correlations can be observed as from H-15 to signal at δ 53.9 (C-1) and δ 35.2 (C-3); from signal at δ 3.73 (H-6) to C-5; from H-4 to C-1 and signal at δ 42.1 (C-9); and from signals at δ 1.66/1.79 (H-3) to C-5 and C-1. Also, fragment II (Fig. 2), a cyclopentane connected with an isopropyl group, an angle methyl (δ 1.29/10.4, C-17) and three hydroxyls, was determined by ^1H–^1H COSY and HMBC spectra, where key correlations could be observed from H-17 to C-7 and C-13; from H-19 to C-18 and C-13; from H-14 to C-18, C-12, C-8, and C-7, as well as from H-8 to C-12 and C-13. Further, other observed key HMBC correlations of H-17 to C-11; H-1 to C-7; H-8 to signals at δ 45.3(C-10), C-5, and C-16, and from signal at δ 1.11(s, H-16) to C-10, C-5, and C-8 established the connectivity of two fragments (I and II). All these data and reasoning indicated that **1** possesses a rare isoryanodane skeleton similar to that of cinncassiol D_1, which had been firstly isolated from *Cinnamomum cassis*.[8]

The relative stereochemistry of **1** was determined on the basis of the results of NOESY spectrum, depicted on a three-dimensional structure model (Fig. 3) generated from the computer model, in which MM_2 force field calculations was used for energy minimization (CS Chem. 3D Pro Version 9.0). The observed NOESY correlations between H-8 and δ 1.88 (H-1) indicated that they were in the same side and defined as β-orientation. Correlations between signal at δ 3.95 (H-14) with signal at δ 0.95 (H-20) and H-18 suggested H-14 to be α, whereas OH-14 to be in β-orientation. Signals at δ 1.79 showed NOESY effects with H-2 and δ 1.57, indicating that they were in the same side and assigned signal at δ 1.79 to be H-3β and δ 1.57 to be H-4β, and the correlation between signal at δ 2.18 (H-4α) with δ 1.84 (H-10) indicated that δ 1.84 to be H-10α and δ 1.50 to be H-10β. Finally, X-ray crystallography (Fig. 4) provided unequivocal evidence of the structure and relative stereochemistry of **1**, and named itol A.

Itol B (**2**) was obtained as colorless crystals, whose molecular formula was deduced to be $C_{20}H_{32}O_7$ by HRFABMS at *m*/*z* 407.2044

Table 2
^{13}C NMR (in CD_3OD, 125 MHz) data for **1**–**6**, δ in parts per million

No.	1	2	3	4	5	6
1	53.9	53.9	61.6	53.8	54.2	54.0
2	36.9	36.8	82.5	37.1	36.9	36.9
3	35.2	35.2	41.4	35.5	35.2	35.2
4	37.3	37.4	35.9	37.8	37.4	37.4
5	88.3	88.2	87.7	87.9	88.3	88.2
6	75.9	76.0	75.4	76.9	75.8	76.0
7	90.8	90.5	90.8	90.5	90.8	90.4
8	57.4	57.3	57.4	57.6	52.9	57.1
9	42.1	42.2	42.0	42.8	42.2	42.3
10	45.3	45.3	45.4	45.6	45.1	45.3
11	105.7	105.6	105.9	106.3	105.8	105.7
12	62.5	62.9	62.7	62.7	62.9	62.9
13	83.5	83.7	83.5	85.9	83.8	83.0
14	79.2	79.5	79.1	74.8	83.1	78.6
15	15.5	15.4	24.2	15.3	15.5	15.5
16	22.3	22.1	21.9	21.8	21.8	22.2
17	10.4	10.4	10.3	11.0	9.9	10.5
18	35.0	42.7	35.0	76.7	35.6	40.5
19	18.8	13.9	18.8	28.0	18.4	14.6
20	18.3	66.2	18.3	26.2	19.1	75.1
1′					100.6	104.5
2′					74.9	74.4
3′					78.3	78.1
4′					71.8	71.7
5′					78.0	78.1
6′					62.9	63.0

Table 1
^1H NMR data (in CD_3OD, 500 MHz) for **1**–**6**, δ in parts per million and *J* in hertz

No.	1	2	3	4	5	6
1	1.88, br d (2.0)	1.89, br d (1.5)	1.87, br d (2.0)	1.88, br d (2.0)	1.90, br d (2.0)	1.87, br d (2.0)
2	2.46, m	2.46, m	—	2.45, m	2.48, m	2.48, m
3α	1.66, m	1.66, m	1.83, m	1.65, m	1.67, m (α)	1.65, m (α)
3β	1.79, m	1.79 m	1.95, m	1.78, m	1.78, m (β)	1.78, m (β)
4α	2.18, m	2.18, m	2.18, m	2.18, m	2.19 m (α)	2.18 m (α)
4β	1.57, m	1.57, m	1.57, m	1.56, m	1.58 m (β)	1.57 m (β)
6	3.73, d (2.0)	3.73, d (2.0)	3.74, d (2.0)	3.81, d (2.0)	3.78, d (2.0)	3.73, d (2.0)
8	2.06, br d (2.0)	2.09, br d (2.0)	2.09, br d (1.5)	2.07, br d (2.0)	2.24, br d (2.0)	2.08, br d (1.5)
10α	1.84, d (14.5)	1.84, d (14.5)	1.84, d (14.5)	1.72, d (15.0)	1.83, d (15.0)	1.83, d (15.0)
10β	1.50, d (14.5)	1.50, d (14.5)	1.51, d (14.5)	2.15, d (15.0)	1.54, d (15.0)	1.51, d (14.5)
14	3.95, s	4.12, s	3.95, s	4.41, br s	4.32, s	4.32, s
15	1.05, d (7.0)	1.05, d (7.0)	1.30, s	1.05, d (7.0)	1.06, d (7.0)	1.04, d (7.0)
16	1.11, s	1.12, s	1.11, s	1.15, s	1.09, s	1.12, s
17	1.29, s	1.30, s	1.29, s	1.36, s	1.29, s	1.29, s
18	1.91, m	1.85, m	1.91, m	—	1.94, m	1.78, m
19	1.07, d (6.5)	1.19, d (7.0)	1.06, d (6.5)	1.37, s	1.05, d (7.0)	1.16, d (7.0)
20	0.95, d (6.5)	3.53, dd (4.0, 10.5) 3.75, dd (6.5, 10.5)	0.94, d (6.5)	1.20, s	0.99, d (7.0)	4.12, 3.59 (7.0)
1′					4.41, d (7.5)	4.23, d (7.5)
2′					3.15, m	3.12
3′					3.43, m	3.40
4′					3.26, m	3.26
5′					3.32, m	3.30
6′					3.87, dd (2.0, 11.0); 3.64, m	3.85, dd (2.0, 11.0); 3.61, m

Table 3
Insecticidal activity of **1** and **2** against *Spodoptera exigua*[a]

Compd	Concentration (ppm)	Dead insects (heads)	Live insects (heads)	Corrected rate of death (%)	LC_{50}	LC_{90}
1	32.00	27	21	56	28.62	67.68
	42.63	35	13	73		
	56.19	40	8	83		
	73.63	45	3	94		
	96.88	46	2	96		
2	31.56	6	37	26	52.76	131.7
	42.08	15	33	31		
	55.64	27	19	59		
	72.68	33	15	69		
	95.65	36	11	77		

[a] Significantly inhibited by **1** with the length of live tested insects less than 1 mm, however, as to **2**, the length to be 3–4 mm, no inhibition was shown.

Table 4
Anti-COX-2 inhibitory effects of **1**–**6**[a]

Compd	IR (%)
1	61.0±1.0
2	16.7±0.6
3	42.3±0.6
4	78.3±1.5
5	54.7±0.6
6	57.0±1.0
NS398	85.3±0.6

[a] Measured in concentration of 1×10^{-5} mol/L and given in mean±SD, n=3.

$[M+Na]^+$. Its ^1H (Table 1) and ^{13}C NMR spectra (Table 2) were quite similar to those of **1**, except that a methyl group (δ_H 0.95; δ_C 18.3) in **1** was replaced by a hydroxymethylene (δ_H 3.53, 3.75; δ_C 66.2) in **2**, implying that a hydroxylation occurs on the isopropyl group, which was further supported by the HMBC correlations of H-20 (δ_H 3.53, δ_C 3.75) to δ 42.7(C-18), 13.9(C-19), and δ 83.7(C-13). The structural assignment of **2** was fully achieved by interpretation of 2D NMR including HSQC, HMBC, and NOESY. In the NOESY spectrum, NOESY correlations of H-20/H-18 and H-14/19 were observed.

Itol C (**3**) was obtained as white amorphous powder, which was identified to be one isomer of **2** by a pseudomolecular ion peak $[M+Na]^+$ at m/z 407.2043 in the HRFABMS spectrum. The spectral data, including its stereochemistry of **3** resembled also those of **1**, and the only difference is the absence of a methenyl group (δ_H 2.46; δ_C 36.9) and the appearance of a quaternary carbon resonance (δ_C 82.5, C-2) in **3**, suggesting that a hydroxylation occurs on C-2, which was further confirmed by the HMBC correlations from H-1 (δ 1.87), H-15 (δ 1.30), H-3 (δ 1.83/1.95), and H-4 (δ 1.57/2.18) all to C-2, and a NOESY correlation of H-15 with H-6 suggested the OH-2 to be in β-orientation. The structural assignment of **3** was fully achieved by interpretation of 2D NMR including HSQC, HMBC, and NOESY.

Itol D (**4**) was obtained as white amorphous powder, whose molecular formula was determined to be $C_{20}H_{32}O_7$ by HRFABMS at m/z 407.2041 $[M+Na]^+$. Its ^1H (Table 1) and ^{13}C NMR spectra (Table 2) were also similar to those of **1**, including their main HMBC and NOESY correlations, except the difference of the isopropyl group. The downfield signal of C-18 (δ_C 76.7) in **4** compared to signal at δ_C 35.0 in **1** suggested a hydroxylation occurrence on C-18, which was further supported by the HMBC correlation between these protons (H-19, H-20, and H-14) all to C-18 (δ_C 76.7). Therefore, the structure of **4** was elucidated and named itol D.

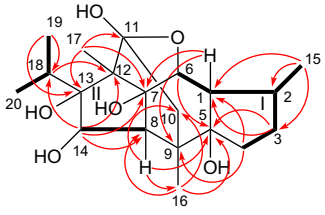

Figure 2. Selected HMBC of fragments I, II and compound **1**.

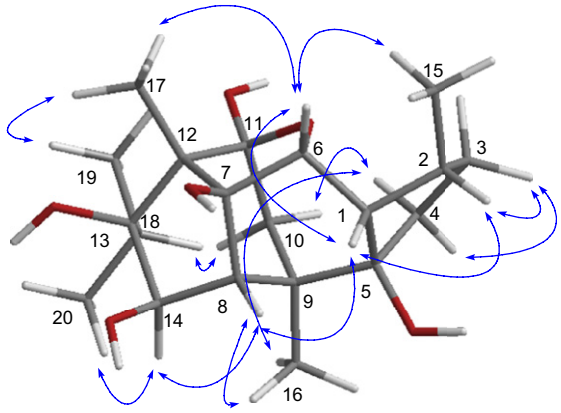

Figure 3. Key NOESY correlations (↔) and relative stereochemistry of **1**.

Itol A-14-*O*-β-D-glucopyranoside (**5**) was obtained as white amorphous powder. Its molecular formula was determined as $C_{26}H_{42}O_{11}$ by HRFABMS with the $[M+Na]^+$ ion peak at m/z 553.2618. The ^1H and ^{13}C NMR data of **5** showed close resemblance to those of **1** by comparing their NMR data, except for an additional β-D-glucopyranosyl moiety with characteristic NMR signals (δ_H 4.41, d, J=7.5 Hz, H-1′; δ_C 100.6), which was further identified by acid hydrolysis with GC analyses. The glycosidation site was deduced at C-14 by the HMBC correlations from H-1′ to C-14 (δ 83.1) and from H-14 to C-1′. The configuration of H-14 was identified to be α-orientation by the key NOESY correlations of H-14 with H-18 (δ 1.94, m). By analysis of full 2D NMR experiments, compound **5** was finally elucidated as itol A-14-*O*-β-D-glucopyranoside.

Itol B-20-*O*-β-D-glucopyranoside (**6**) was obtained as white amorphous powder with high polarity. Its molecular formula was determined as $C_{26}H_{42}O_{12}$ by HRFABMS with pseudomolecular ion peak at 569.2568 $[M+Na]^+$. Comparison of the ^1H and ^{13}C NMR data of **6** with those of **2** suggested that they were similar with an exception of an additional β-D-glucopyranosyl moiety (δ_H 4.23, d, J=7.5 Hz, H-1′; δ_C 104.5), which was identified by acid hydrolysis with GC analyses. Similarly, the glycosidation position was deduced at C-20 by the HMBC correlation of signal at δ 4.23 (H-1′) to δ 75.1 (C-20). Interpretation of full 2D NMR experiments established **6** to be itol B-20-*O*-β-D-glucopyranoside.

These six new isoryanodane diterpenoids (**1**–**6**) are of special interests as they are thirdly reported on this skeleton diterpenoid.

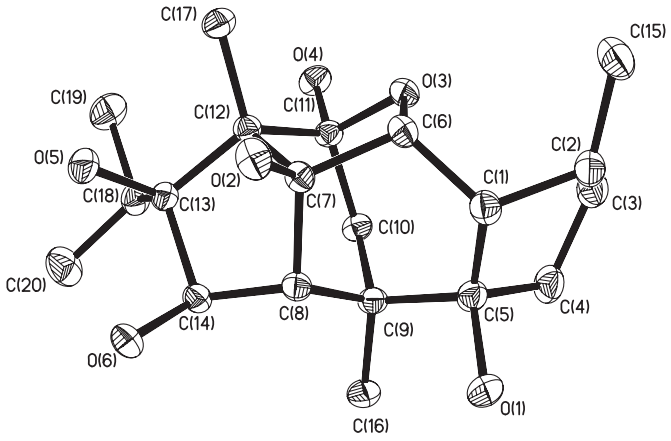

Figure 4. Single-crystal X-ray structure of **1**.

Furthermore, these new isolated diterpenoids all contained the *cis*-13,14-dihydroxyls, which will provide advantages for the studies of derivation or total synthesis. In addition, the discovery of this type of diterpenoid in *itoa* genus showed certain relationships as chemotaxonomic marker between Flacourtiaceae and Lauraceae, considering that all previously reported eight isoryanodane diterpenoids were all from Lauraceae.[8,9]

In the insecticidal assay, compounds **1**–**6** were tested against *S. exigua* and *Heliothis armigera*. The LC_{50} was used to evaluate the insecticidal activity. It was proved that six diterpenoids showed no inhibition effect against *H. armigera*, but obviously exhibited insecticidal activity against *S. exigua*, with LC_{50} of 28.6 μg/mL for **1** and 52.7 μg/mL for **2**, respectively (Table 3). The LC50 values for **3**–**6** were unable to be obtained for less amounts. Additionally, compounds **1**, **4**, **5**, and **6** exhibited inhibitory COX-2 in anti-inflammatory assay, with its inhibitory rates (%) of 61.0, 78.3, 54.7, and 57.0 at 10 μM, respectively (Table 4). However, they (all at 10 μM) barely exhibited cytotoxic activities against 4 human tumor cell lines including HL-60, BGC-823, Bel-7402, and KB.

3. Experimental

3.1. General experimental procedures

Melting points were determined on an XT4A digital micro-melting point apparatus and are uncorrected. Optical rotations were recorded on a Perkin–Elmer 243B digital polarimeter. UV Spectra was measured on a Shimadzu UV-2450 spectrophotometer. NMR spectra were recorded in CD_3OD using Inova 500 MHz NMR spectrometers with tetramethylsilane as internal standard. HRFABMS was measured on an AutoSpec Ultima-TOF mass spectrometer in positive ion mode and HRESIMS on Bruker APEX IV FTMS mass spectrometer; in *m/z*. GC: Agilent 6890N (HP-5 capillary column (28 m×0.32 mm, id); detection, FID; detector temperature, 260 °C; column temperature, 180 °C; carrier gas, N_2; flow rate, 40 mL/min). All solvents used were of analytical grade (Beijing Chemical and Industry Factory). Silica gel (200–300 mesh, Qingdao Mar. Chem. Ind. Co., Ltd.), Sephadex LH-20 gel (Pharmacia), and C_{18} reverse-phased silica gel (150–200 mesh, Merck, performed by applying a N_2 pressure of 0.12 MPa) were used for column chromatography.

3.2. Plant material

The bark and twigs of *I. orientalis* Hemsl. were collected in December 2004 from Xiashi trees park of Chinese Academy of Forestry (CAF) in Guangxi Province of China, and authenticated by Mr. Maojing Yang, an engineer in Xiashi trees Park, CAF. A voucher specimen was kept in the herbarium of Peking University Modern Research Centre for Traditional Chinese Medicine (IO20041205).

3.3. Extraction and isolation

The dried bark (18 kg) and twigs (22 kg) of *I. orientalis* were extracted twice with 80% ethanol (2×400 L), each for 2 h. After removal of the solvent under reduced pressure at 60 °C, the residue was suspended in water (1.8 L) and extracted with $CHCl_3$ (2×5 L), EtOAc (2×5 L), and *n*-BuOH (2×5 L) successively.

A portion of ethyl acetate extract (100 g) was subjected to silica gel CC and eluted with petroleum ether/Me_2CO (3:1–0:1) to get five fractions (E-1–E-5), and **1** was obtained (1350 mg) by recrystallizing in $CHCl_3$/CH_3OH (10:1) from Fr. E-2. The Fr. E-3 was rechromatographed by silica gel CC and eluted with $CHCl_3$/CH_3OH (20:1–5:1) affording five portions (E-3a–e). The E-3c was subjected to Sephadex LH-20 CC (MeOH) to obtain **4** (16 mg). The *n*-butanol extract (520 g) was subjected to silica gel CC and eluted with $CHCl_3$/MeOH (20:1–0:1) to yield seven fractions (Fr. B-1–7). Rechromatography of the Fr. B-4 (60 g) by silica gel CC eluted with EtOAc/MeOH (30:1, 5:1) afforded eight fractions (Fr. B-4I–VIII), followed by further silica gel CC of Fr. B-4II (3 g) eluted with $CHCl_3$/MeOH (15:1), the fractions 6–11 (30 mL/Fr.) were subjected to Sephadex LH-20 (MeOH) to provide **2** (220 mg). Fr. B-4I (2 g) was isolated by Sephadex LH-20 (MeOH/H_2O, 8:2) and further purified by ODS eluted with MeOH/H_2O (1:1) to yield **3** (30 mg). Fr. B-6 (50 g) was subjected to silica gel CC eluted with $CHCl_3$/MeOH (6:1, 2:1, 0:1) to get Fr. B-6i–viii. Rechromatography of the Fr. B-6v (5.5 g) by silica gel CC eluted with EtOAc/MeOH/H_2O (10:1:0.1) gave seven portions (P. a–f), and further isolation of P. d (1.6 g) by ODS (MeOH/H_2O 4:6) afforded **5** (21 mg).

The Fr. B-7 (17 g) was subjected to silica gel CC eluted with EtOAc/MeOH/H_2O (8:2:0.5) to get Fr. B-7A–C. The Fr. B-7B (10.5 g) was rechromatographed by silica gel CC and eluted with $CHCl_3$/MeOH/H_2O (3:1:0.1) to yield four fractions (Fr. $7B_1$–$7B_4$). The Fr. $7B_3$ was rechromatographed by silica gel CC and eluted with EtOAc/EtOH/H_2O (8:3:0.5), followed by ODS (MeOH/H_2O, 1:1) and purified by Sephadex LH-20 (MeOH) to afford **6** (44 mg).

Acid hydrolysis and sugar analyses were carried out according the procedure described in the literature.[7]

3.3.1. Itol A (*1*)

Colorless quadrated crystal; $[\alpha]_D^{18}$ +4.7 (*c* 1.0, MeOH); mp: 241–243 °C; UV (MeOH) λ_{max} (log ε) 280.0 (0.13), 229.0 (0.67) nm; IR (KBr) ν_{max} 3384, 2970, 2953, 2919, 2876, 1723, 1630, 1467, 1330, 1019, 843 cm^{-1}. ^1H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2. ESIMS *m/z* 391 $[M+Na]^+$, 386 $[M+NH_4]^+$, 351, 333, 315, 192. HRFABMS *m/z* 391.2089 $[M+Na]^+$ ($C_{20}H_{32}O_6Na$, calcd 391.2090).

3.3.2. Itol B (*2*)

Colorless crystalline solid; $[\alpha]_D^{18}$ +3.3 (*c* 0.6, MeOH); mp: 143–145 °C; UV (MeOH) λ_{max} (log ε) 280.0 (0.94), 227.0 (0.31) nm; IR (KBr) ν_{max} 3510, 3438, 3316, 3003, 2964, 2940, 2925, 2904, 1650, 1455, 1332, 1046, 949 cm^{-1}. ^1H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2. ESIMS *m/z* 407 $[M+Na]^+$, 402 $[M+NH_4]^+$, 385, 367, 349, 258. HRFABMS *m/z* 407.2044 $[M+Na]^+$ ($C_{20}H_{32}O_7Na$, calcd 407.2040).

3.3.3. Itol C (*3*)

White amorphous powder; $[\alpha]_D^{18}$ +7.1 (*c* 0.60, MeOH); mp: 206–208 °C; UV (MeOH) λ_{max} (log ε) 280.0 (0.71), 223.0 (0.34) nm; IR (KBr) ν_{max} 3416, 2935, 1608, 1518, 1274, 1032 cm^{-1}. ^1H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2. ESIMS *m/z* 407 $[M+Na]^+$, 402 $[M+NH_4]^+$, 391, 367, 349, 279. HRFABMS *m/z* 407.2043 $[M+Na]^+$ ($C_{20}H_{32}O_7Na$, calcd 407.2040).

3.3.4. Itol D (*4*)

White amorphous powder; $[\alpha]_D^{18}$ +4.6 (*c* 0.50, MeOH); mp: 156–158 °C; UV (MeOH) λ_{max} (log ε) 277.0 (1.13) nm; IR (KBr) ν_{max} 3419, 2962, 2876, 1717, 1467, 1385, 1063 cm^{-1}. ^1H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2. ESIMS *m/z* 407 $[M+Na]^+$, 402 $[M+NH_4]^+$, 367, 349, 295, 259. HRFABMS *m/z* 407.2041 $[M+Na]^+$ ($C_{20}H_{32}O_7Na$, calcd 407.2040).

3.3.5. Itol A-14-O-β-D-glucopyranoside (*5*)

White amorphous powder; $[\alpha]_D^{18}$ −18.7 (*c* 0.10, MeOH); mp: 136–138 °C; UV (MeOH) λ_{max} (log ε) 281.0 (0.96), 230 (0.54) nm; IR (KBr) ν_{max} 3362, 2943, 2948, 2870, 1052, 1019 cm^{-1}. ^1H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2. ESIMS *m/z* 553 $[M+Na]^+$, 548 $[M+NH_4]^+$, 333. HRFABMS *m/z* 553.2618 $[M+Na]^+$ ($C_{26}H_{42}O_{11}Na$, calcd 553.2619).

*3.3.6. Itol B-20-O-β-D-glucopyranoside (**6**)*

White amorphous powder; $[\alpha]_D^{18}$ −19.7 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 281.0 (0.64) nm; IR (KBr) ν_{max} 3419, 2958, 2881, 1600, 1075, 1031 cm^{-1}. ^1H NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C NMR (CD_3OD, 125 MHz), see Table 2. ESIMS *m*/*z* 569 $[M+Na]^+$, 349. HRESIMS *m*/*z* 569.2568 $[M+Na]^+$ ($C_{26}H_{42}O_{12}Na$, calcd 569.2568).

*3.3.7. Crystallographic data for **1***

$C_{20}H_{32}O_6$, *M*=368.23, monoclinic, space group $P2_12_12_1$, *a*=7.699(1), *b*=12.995(1), *c*=18.349(1) Å. *V*=1835.8(4) $Å^3$, *Z*=4, *d*=1.333 g/cm^3, crystal dimensions 0.30×0.30×0.50 mm was used for measurement on a MAC DIP-2030K diffractometer with a graphite monochromator (ω scans, 2θ max=50.0), Mo Kα radiation. The total number of independent reflections measured was 2290, of which 2280 were observed ($|F|^2 \geq 2\sigma|F|^2$). Final indices: R_f=0.0476, R_w=0.1322.

The crystal structure (**1**) was solved by direct methods using SHELX-97 and expanded using difference Fourier techniques, refined by the program NOMCSDP8 and full-matrix least-squares calculations. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 602815). Copies of the data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: +44 1223 336 033 or deposit@ccdc.cam.ac.uk].

3.4. Insect bioassay

S. exigua and *H. armigera* (Noctuidae) larvae, from a laboratory colony, were reared on artificial diet at 25 °C, 70% rh, and 14.5:9.5 h light period. The feedstuff was prepared by the Cs3ab2-1991 (China), and the bioassay procedure all performed by a bioassay method was described by Zeng et al.[10] for the toxicity evaluation of Bt against *S. exigua*. Compounds were dissolved in DMSO and diluted with H_2O. The prepared solution was added to the feedstuff, then the mixed feedstuff was infused to 24-well plates (∅ 15 mm) for bioassay, with a water-mixed feedstuff as control. Newly hatched larvae were put in each well, then they were reared at 25 °C for 72 h. Results were given by counting the amount of dead larvae and determined its LC_{50} values.

The COX-2 assay described by Duan[11] et al. was used for measurement of COX-2 inhibitory activity. Cytotoxicity assay of the isolated compounds was investigated using human cancer cell lines including Bel-742, BGC-823, KB, and HL-60. The growth-inhibitory effects were measured using standard MTT assay.[12]

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.04.022.

References and notes

1. Lai, S. S. *Bull. Bot. Res.* **1994**, *14*, 221–229.
2. Fan, G. S. *J. Southwest Forest College* **1995**, *15*, 1–86.
3. Lu, L.; Wang, H. *Acta Bot. Yunnanica* **2004**, *26*, 73–82.
4. Fu, L. G.; Chen, T. Q.; Lang, K. Y. *Higher Plants of China*; Qing Dao: Qing Dao, China, 1998; Vol. 5, pp 110–129.
5. Shi, H. M.; Wen, J.; Jia, C. Q.; Jin, W.; Zhang, X. F.; Yao, Z. R.; Tu, P. F. *Planta Med.* **2006**, *72*, 948–950.
6. Shi, H. M.; Yao, Z. R.; Chai, X. Y.; Xu, Z. R.; Zhou, Y. H.; Wen, J.; Tu, P. F. *Nat. Prod. Res.*, in press.
7. Chai, X. Y.; Xu, Z. R.; Ren, H. Y.; Shi, H. M.; Lu, Y. N.; Li, F. F.; Tu, P. F. *Helv. Chim. Acta* **2007**, *90*, 2176–2185.
8. Nohara, T.; Kashiwada, Y.; Tomimatsu, T. *Tetrahedron Lett.* **1980**, *21*, 2647–2648.
9. Braulio, M. F.; Azucena, G. C.; Carmen, G.; David, T. *J. Nat. Prod.* **1997**, *60*, 880–883.
10. Zeng, X. H.; Zhang, H. Y.; Yu, Z. N.; Hu, C. *Chin. J. Bio. Control* **1998**, *14*, 172–175.
11. Duan, W. G.; Zhang, L. Y. *Prostaglandins Leukot. Essent. Fatty Acids* **2006**, *74*, 317–321.
12. Wang, W.; Liu, J. Z.; Han, J.; Xu, Z. R.; Liu, R. X.; Wang, W. X.; Ma, X. C.; Guan, S. H.; Guo, D. *Planta Med.* **2006**, *72*, 450–457.