JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Dimeric *ent*-Kaurane Diterpenoids from *Isodon excisus*

Seong Su Hong,[†,‡] Seon A Lee,[†] Chul Lee,[†] Xiang Hua Han,[†] Sanggil Choe,[†] Nahyun Kim,[§] Dongho Lee,[§] Chong-Kil Lee,[†] Youngsoo Kim,[†] Jin Tae Hong,[†] Mi Kyeong Lee,[†] and Bang Yeon Hwang*[,†]

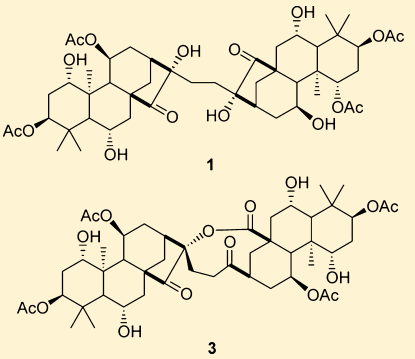
[†]College of Pharmacy, Chungbuk National University, Cheongju 361-763, Korea

[‡]Gyeonggi Pharmaceutical Research Center, Gyeonggi Institute of Science & Technology Promotion, Suwon 443-759, Korea

[§]School of Life Sciences and Biotechnology, Korea University, Seoul 136-713, Korea

Supporting Information

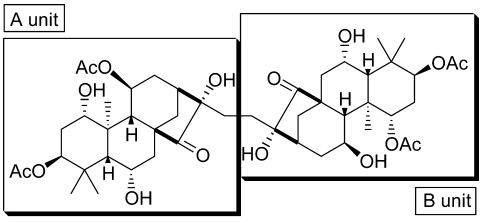
ABSTRACT: Five new dimeric *ent*-kauranoids, biexcisusins A–E (**1**–**5**), were isolated from the aerial parts of *Isodon excisus*. The structures and relative configurations of these compounds were determined on the basis of spectroscopic data interpretation. Of these, biexcisusins C–E (**3**–**5**) are dimeric *ent*-kaurane diterpenoids exhibiting an unprecedented linkage through a nine-membered lactone ring between two *ent*-kaurane subunits. Compounds **1**–**5** showed no inhibitory effects on the LPS-induced production of nitric oxide in murine macrophage RAW264.7 cells, up to a dose of 50 μM.



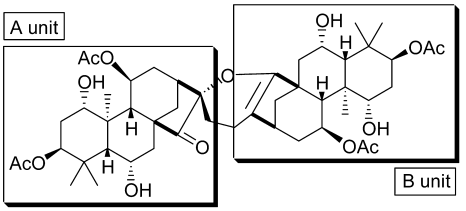
The genus *Isodon*, comprising about 150 species of undershrubs, sub-undershrubs, or perennial herbs, is one of the most widespread taxa of the family Lamiaceae. *Isodon* species are a rich source of diterpenoids such as 7,20-epoxy-*ent*-kauranes, 6,7-seco-*ent*-kauranes, 8,9-seco-*ent*-kauranes, *ent*-gibberellanes, abietanes, *ent*-abietanes, *ent*-pimaranes, and *ent*-kaurane dimers.[1−5] *Isodon excisus* (Max.) Kudo has been used as folk medicine for the treatment of respiratory and gastrointestinal bacterial infections, sore throats, inflammation, and cancer in Korea, mainland China, and Japan.[6] Previous phytochemical investigations of this plant have led to the isolation and characterization of 3-(4-hydroxy-3-methoxyphenyl)-*N*-[2-(4-hydroxyphenyl)-2-methoxyethyl]acrylamide, 3-(3,4-dihydroxyphenyl)acrylic acid 1-(3,4-dihydroxyphenyl)-2-methoxycarbonylethyl ester, corchoionol A, corchorifatty acid B, and *ent*-kauranoids such as excisusins A–F.[7−9] In a continuing search for bioactive constituents from plants in the genus *Isodon*, five new asymmetric dimeric *ent*-kauranoids, biexcisusins A–E (**1**–**5**), were isolated from the aerial parts of *Isodon excisus*. Biexcisusins C–E (**3**–**5**) were characterized as dimeric *ent*-kauranoids possessing an unprecedented linkage of a nine-membered lactone ring between two *ent*-kaurane subunits. This report describes the isolation and structure determination of **1**–**5**, a plausible biogenetic pathway for these substances, and their inhibitory effects on nitric oxide (NO) production.

RESULTS AND DISCUSSION

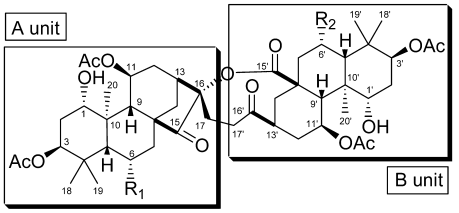
The methanol extract of the aerial parts of *I. excisus* was partitioned successively between *n*-hexane and aqueous methanol and then dichloromethane and water. The dichloromethane-soluble fraction was subjected repeatedly to column chromatography on silica gel, RP-18, and semipreparative HPLC to afford five new *ent*-kaurane dimers, biexcisusins A–E (**1**–**5**).



Biexcisusin A (**1**)



Biexcisusin B (**2**)



Biexcisusin C (**3**) R_1 : -OH, R_2 : -OH
Biexcisusin D (**4**) R_1 : =O, R_2 : -OH
Biexcisusin E (**5**) R_1 : -OH, R_2 : =O

Received: May 27, 2011
Published: November 8, 2011

Biexcisusin A (**1**) was obtained as a white, amorphous powder, and its molecular formula was determined as $C_{48}H_{70}O_{16}$ by HRESIMS (*m/z* 901.4561 $[M - H]^-$; calcd 901.4586), which required 14 degrees of unsaturation in the molecule. The IR spectrum displayed absorption bands diagnostic of hydroxy (3434 cm^{-1}) and ketone (1725 cm^{-1}) functionalities. The ^1H and ^{13}C NMR spectra of **1** were similar to those of xindongnin M ($C_{48}H_{70}O_{15}$, *m/z* 886.4715),[2] except for an additional signal arising from a hydroxy group and the location of the substituents. The ^{13}C NMR and DEPT spectra supported the molecular formula obtained and showed 48 carbon signals due to two *ent*-kaurane diterpene units (**1A** and **1B**) including four acetoxy groups (eight carbon signals). The ^1H and ^{13}C NMR spectra of **1A** and **1B** were consistent with both being based on an *ent*-kaurane skeleton, from the characteristic signals of three methyl groups (**1A**: δ_H 1.06, 1.54, 1.88, δ_C 28.7, 24.3, 15.5; **1B**: δ_H 1.05, 1.56, 2.01, δ_C 29.9, 24.1, 16.1), three methine carbons (**1A**: δ_C 49.5, 61.8, 40.97; **1B**: δ_C 49.7, 64.98, 41.7), and three quaternary carbons (**1A**: δ_C 38.27, 50.4, 44.8; **1B**: δ_C 38.3, 50.1, 43.8). Comparison of the ^{13}C NMR and DEPT data of **1A** with those of the known *ent*-kauranoid inflexinol[10] revealed the only difference to be the absence of a double bond at C-16 and C-17, which was confirmed by the chemical shift value of C-16 (oxygenated quaternary carbon, δ_C 79.84) and C-17 (methylene carbon, δ_C 26.4). Furthermore, the ^1H, ^{13}C, and DEPT NMR spectroscopic features of **1B** were similar to those of inflexinol, except for the position of an acetoxy group at C-1′ rather than at C-11′, which was confirmed by the HMBC correlations between H-1′ (δ_H 5.38) and the acetoxy carbonyl carbon (δ_C 170.8). Also, an exomethylene carbon (C-16 and 17) in inflexinol was replaced by an oxygenated quaternary carbon (C-16′, δ_C 80.2) and a methylene carbon (C-17′, δ_C 26.2) in **1B**. The $^1H-^1H$ COSY correlations of these two methylene groups (H-17: δ_H 3.15 and 3.10; H-17′: δ_H 3.10 and 2.52) and HMBC correlations from H-17 to C-13 (δ_C 40.97), C-16 (δ_C 79.84), and C-17′ (δ_C 26.2) and from H-17′ to C-13′ (δ_C 41.7), C-16′ (δ_C 80.2), and C-17 (δ_C 26.4) clearly indicated C-17 to be linked to C-17′ through a single carbon−carbon bond.[1−3] The locations and relative configurations of the hydroxy and acetoxy groups were assigned as OH-1α, OAc-3β, OH-6α, OAc-11β, OAc-1′α, OAc-3′β, OH-6′α, and OH-11′β, respectively, by HMBC and ROESY correlations, as shown in Figure 1. The hydroxy groups at C-16 and C-16′ were found to be α-oriented by key NOESY correlations between H-12β and H-17, and H-12′β and H-17′ (Figure 1). These results were confirmed by the chemical shifts of C-16 and C-16′ (δ_C 79.84 and 80.2, respectively) in **1**, which resembled those of xindongnim M (δ_C 80.5) and bisrubescensin B (δ_C 80.1).[2,4] Therefore, the structure of compound **1** was determined as shown, and has been given the trivial name biexcisusin A.

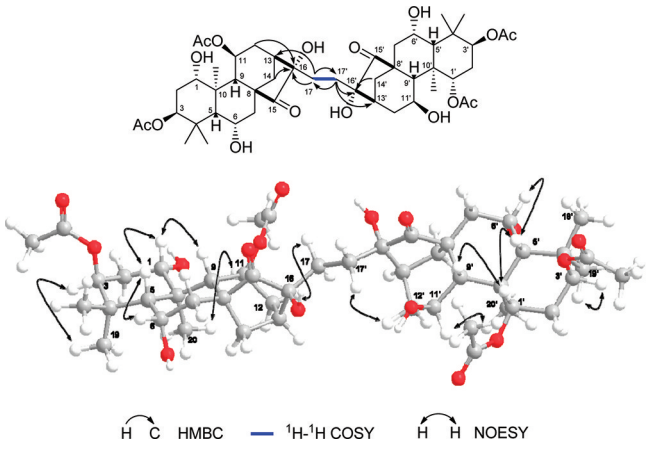


Figure 1. Key HMBC, COSY, and NOESY correlations of biexcisusin A (**1**).

Biexcisusin B (**2**), a white, amorphous powder, showed a molecular ion peak at *m/z* 891 $[M + Na]^+$ in the ESIMS, and the molecular formula $C_{48}H_{68}O_{14}$ was assigned on the basis of the HRESIMS (*m/z* 913.4571 $[M + COOH]^-$; calcd 913.4586), indicating 15 degrees of unsaturation. Its ^{13}C NMR spectrum showed 48 carbon signals (Table 1). Thus, **2** was assigned tentatively as an asymmetric *ent*-kaurane dimer. The ^1H NMR, ^{13}C NMR, and DEPT data of two substructures of **2** suggested its structure to be similar to that of inflexinol.[10] The α,β-unsaturated ketone groups of the two inflexinol moieties were replaced by a ketone carbon (C-15, δ_C 215.3), an oxygenated quaternary carbon (C-16, δ_C 83.1), a tetrasubstituted double bond (C-15′, δ_C 154.7; C-16′, δ_C 112.5), and two methylene carbons (C-17, δ_C 24.9; C-17′, δ_C 18.9), of parts **2A** and **2B**. The positions of the acetoxy groups were determined at C-3, 3′, 11, and 11′ according to the HMBC correlations of H-3, 3′ (δ_H 4.98), H-11 (δ_H 6.77), and H-11′ (δ_H 6.80) to the carbonyl carbons δ_C 170.7, 170.75, 170.83, and 171.4. Furthermore, the locations of the hydroxy groups could be assigned as C-1, 1′, 6, and 6′ by HMBC correlations, as shown in Figure S1. $^1H-^1H$ COSY correlations of these two methylene groups were observed (H-17: δ_H 2.59 and 2.10; H-17′: δ_H 2.88 and 2.06), with key HMBC correlations from H-17 to C-16 (δ_C 83.1), C-16′ (δ_C 112.5), and C-17′ (δ_C 18.9) and from H-17′ to C-15′ (δ_C 154.7), C-16′ (δ_C 112.5), C-16 (δ_C 83.1), and C-17 (δ_C 24.9) evident (Figure S1, Supporting Information). These indicated that the C-16−O−C-15′−C-16′−C-17′−C-17 unit is linked by a six-membered dihydropyran ring.[2,4,11,12] The relative configuration of **2** was confirmed by a NOESY experiment, wherein correlations were observed from H-1β (1′β) to H-5β (5′β) and H-9β (9′β), from H-3α (3′α) to H-2α (2′α) and Me-19 (19′), from H-6β (6′β) to H-5β (5′β) and H-7β (7′β), and from H-11α (11′α) to the Me-20 (20′) protons (Figure S1, Supporting Information). These results indicated that the C-1 (C-1′)-OH, C-3 (C-3′)-OAc, C-6 (C-6′)-OH, and C-11 (C-11′)-OAc substituents have α-, β-, α-, and β-orientations, respectively. The relative configuration at C-16 was deduced from the NOESY correlations between H-12β and H-17, which indicated the β-orientation of the methylene at C-16. This configuration was confirmed by the upfield shift of C-12 (δ_C 35.1, $\Delta\delta$ −3.4) compared to that in inflexinol caused by the γ-steric compression effect between 16-β methylene and H-12β.[2] Therefore, biexcisusin B (**2**) was determined to be a dimer of the inflexinol derivative linked by a six-membered dihydropyran ring, as shown.

Biexcisusin C (**3**) was obtained as a white, amorphous powder, and the molecular formula $C_{48}H_{68}O_{16}$ was determined on the basis of negative HRESIMS (*m/z* 945.4449 $[M + COOH]^-$; calcd 945.4484), indicating 15 degrees of unsaturation. Its IR spectrum showed absorption bands diagnostic of hydroxy (3442 cm^{-1}), ketone (1720 cm^{-1}), and ester carbonyl (1680 cm^{-1}) functionalities. The ^{13}C NMR and DEPT spectra confirmed that the molecule contained 48 carbons including two ketone carbons (δ_C 218.2, 212.2), one ester carbonyl carbon (δ_C 176.0), six methyl groups (δ_C 28.6, 24.2, 15.3, 28.1, 26.2, 12.7), one oxygenated quaternary carbon (δ_C 88.1), eight oxygenated methine carbons (δ_C 77.6, 80.4, 66.3, 71.2, 77.6,

dx.doi.org/10.1021/np200439n | J. Nat. Prod. 2011, 74, 2382−2387

Table 1. ^{1}H and ^{13}C NMR Data of Compounds 1 and 2[a]

	1[b]		2[b]	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1β	4.19 dd (11.7, 4.5)	77.6 d	4.22 dd (11.7, 4.5)	77.4 d
2α	2.46 m	34.3 t	2.48 m	34.2 t
2β	2.13[c]		2.14[c]	
3α	4.97 t (2.7)	80.4 d	4.98[c]	80.3 d
4		38.3 s		38.3 s
5β	1.65 br s	49.5 d	1.66 br s	49.5 d
6β	4.65 br s	66.6 d	4.68 br s	66.7 d
7α	2.53[c]	44.6 t	2.00 m	44.3 t
7β	2.08 dd (13.5, 2.7)		2.45 d (14.4, 2.7)	
8		50.4 s		51.2 s
9β	2.25 s	61.8 d	2.22[c]	62.4 d
10		44.8 s		44.8 s
11α	6.66 d (5.4)	72.4 d	6.77 d (6.3)	72.5 d
12α	2.02 m	35.1 t	2.33 td (15.3, 4.5)	34.3 t
12β	2.39 br d (16.0)		2.27 m	
13α	2.58 d-like (2.7)	40.97 d	2.53 m	42.3 d
14α	2.94 d (11.7)	36.2 t	3.04 d (12.6)	36.0 t
14β	2.84 br d (11.7)		2.71 br d (11.7)	
15		220.8 s		215.3 s
16		79.84 s		83.1 s
17a	3.15 m	26.4 t	2.59 m	24.9 t
17b	3.10 m		2.10 m	
18	1.06 s	28.7 q	1.07 s	28.7 q
19	1.54 s	24.3 q	1.56 s	24.3 q
20	1.88 s	15.5 q	1.94 s	15.7 q
1′β	5.38 dd (11.4, 4.2)	81.4 d	4.19 dd (11.7, 4.5)	78.0 d
2′α	2.20[c]	29.9 t	2.49 m	34.5 t
2′β	2.20[c]		2.14[c]	
3′α	4.90 t (2.7)	79.8 d	4.98[c]	80.6 d
4′α		38.27 s		38.5 s
5′β	1.71 br s	49.7 d	1.55 br s	49.6 d
6′β	4.68 br s	66.7 d	4.59 br s	67.2 d
7′α	2.59 dd (13.5, 2.7)	45.0 t	1.81 dd (13.5, 2.7)	43.6 t
7′β	2.13[c]		2.21 dd (13.5, 1.8)	
8′		50.1 s		45.18 s
9′β	2.50 s	64.98 d	2.22[c]	55.6 d
10′		43.8 s		45.1 s
11′α	5.13 br d (5.4)	66.5 d	6.80 d (6.3)	73.9 d
12′α	2.52[c]	38.3 t	2.07 m	33.3 t
12′β	2.52[c]		1.97 m	
13′α	2.77 d-like (2.7)	41.7 d	2.42 m	38.2 d
14′	3.05 d (12.5)	37.0 t	2.82 d (10.8)	45.2 t
14′β	2.93 m		2.02 br d (10.8)	
15′		220.5 s		154.7 s
16′		80.2 s		112.5 s
17′a	3.10 m	26.2 t	2.88 m	18.9
17′b	2.52[c]		2.06 m	
18′	1.05 s	29.9 q	1.10 s	29.1 q
19′	1.56 s	24.1 q	1.58 s	24.5 q
20′	2.01 s	16.1 q	1.95 s	15.6 q
OAc		170.86 s		170.75 s
	1.93 s	21.3 q	1.78 s	21.3 q
		170.9 s		170.7 s
	2.03 s	22.1 q	1.99 s	21.9 q
		170. s		170.83 s
	1.92 s	21.9 q	1.93 s	21.4 q
		170.8 s		171.4 s
	1.97 s	21.4 q	2.09 s	22.5 q

[a]Assignments were based on the DEPT, COSY, HMQC, and HMBC experiments. [b]^{1}H NMR spectra were recorded at 900 MHz, and ^{13}C NMR spectra at 225 MHz in pyridine-d_5. [c]Multiplicity patterns were unclear due to signal overlapping.

80.2, 65.9, 70.7), six nonoxygenated methine carbons (δ_C 49.4, 61.8, 43.3, 46.3, 49.1, 45.2), six quaternary carbons (δ_C 38.3, 52.4, 44.8, 38.9, 46.6, 43.9), ten methylene carbons (δ_C 34.5, 45.6, 34.3, 36.9, 31.8, 32.8, 41.3, 29.2, 37.3, 32.6), and four acetoxy groups (δ_C 171.6, 21.6, 170.5, 22.1, 170.8, 21.4, 170.1, 21.9). Comparison of the ^{1}H and ^{13}C NMR data of **3** with those of biexcisusin B (**2**) indicated that both compounds have the same *ent*-kaurane diterpene skeletons. The substructural units of **3** were both determined as an inflexinol derivative by COSY, HMQC, HMBC, and ROESY experiments. Differences were observed in the olefinic carbon signals of C-15′ (δ_C 154.7) and C-16′ (δ_C 112.5) of biexcisusin B (**2**), which were replaced by an ester carbonyl carbon (δ_C 176.0) and a ketonic carbon (δ_C 212.2) in **3**. The $^{1}H-^{1}H$ COSY spectrum showed connectivities between H-17 (δ_H 3.03 and 2.79) and H-17′ (δ_H 3.59 and 2.15). The HMBC correlations from H-17 to C-15 (δ_C 218.2), C-16 (δ_C 88.1), C-16′ (δ_C 212.2), and C-17′ (δ_C 32.6), from H-17′ to C-16′ (δ_C 212.2), C-16 (δ_C 88.1), and C-17 (δ_C 31.8), from H-14 to C-15 (δ_C 218.2) and C-16 (δ_C 88.1), and from H-7′, H-9′, and H-14′ to C-15′ (δ_C 176.0) and the degree of unsaturation indicated clearly the presence of a C-16−O−C-15′−C-8′−C-14′−C-13′−C-16′−C-17′−C-17 unit forming a nine-membered ring (Figure S2, Supporting Information). The relative configuration of C-16 was also confirmed by a NOESY experiment, wherein correlations were observed from H-12β to H-17, which indicated the β-orientation of the methylene at C-16. Therefore, biexcisusin C (**3**) was determined to be a dimer of an inflexinol derivative linked by a nine-membered ring, as shown.

Biexcisusin D (**4**), a white, amorphous powder, gave the molecular formula $C_{48}H_{66}O_{16}$, corresponding to 16 degrees of unsaturation, on the basis of negative HRESIMS data (m/z 943.4329 $[M + COOH]^-$; calcd 943.4327). The IR absorptions at 3444, 1718, and 1655 cm^{-1} indicated the presence of hydroxy, ketone, and ester carbonyl groups. The ^{13}C NMR and DEPT spectra of **4** (Table 1) displayed 48 carbons assignable to two *ent*-kaurane diterpenoid monomeric units. These data were closely comparable to those of biexcisusin C (**3**), indicating that **4** has the same nine-membered ring skeleton on the basis of the characteristic ^{13}C NMR signals, namely, an oxygenated quaternary carbon (C-16, δ 88.1), three methylene carbons (C-17, C-17′, and C-14′, δ 31.2, 31.8, 37.2), a methine carbon (C-13′, δ 44.7), a ketone carbon (C-16′, δ 211.2), and an ester carbonyl carbon (C-15′, δ 176.0). The molecular weight of **4** was 2 mass units greater than that of biexcisusin C (**3**), indicating instead of the hydroxy group (C-6, δ 66.3) in biexcisusin C (**3**), an exchangeable ketone group (δ 210.2) to be present. Thus, it was concluded that **4** is constructed from two different diterpene units, inflexin (**4A**) and inflexinol (**4B**) derivatives. This was further

supported by HMBC correlations from H-7 (δ 3.07) to C-6 (δ 210.2) and C-15 (δ 213.3) and from H-14 (δ 2.15) to C-15 (δ 213.3) and C-16 (δ 88.1). Therefore, biexcisusin D (**4**) was established to be a dimer of inflexinol (**4B**) and inflexin (**4A**) derivatives linked by a nine-membered ring, with the structure as shown.

Biexcisusin E (**5**) was isolated as a white, amorphous powder. The negative HRESIMS (*m/z* 897.4276, $[M - H]^-$; calcd 897.4273) suggested that this compound is an isomer of biexcisusin D (**4**), with a molecular formula of $C_{48}H_{66}O_{16}$. The ^1H and ^{13}C NMR data of **5** were also similar to those of **4**. However, HMBC correlations from H-7′ (δ 2.28) to C-15′ (δ 175.8) and C-6′ (δ 206.8) and from H-14′ (δ 1.97 and 2.80) to C-15′ (δ 175.8) and C-16′ (δ 210.9) clearly indicated that the **5B** unit was an inflexin derivative. Therefore, biexcisusin E (**5**) was elucidated as shown.

Biexcisusins C–E (**3**–**5**) are the first examples of dimeric *ent*-kauranoids found to possess a nine-membered ring connecting the subunits A and B. In turn, biexcisusins A (**1**) and B (**2**) exhibit, respectively, a single carbon–carbon (C-17 and C-17′) bond and the linkage of a six-membered dihydropyran ring between the two structural subunits.[2,4,11]

A plausible biosynthetic pathway indicates that the six-membered dihydropyran ring (**2**) may be formed by an enzymatic Diels–Alder reaction between the olefin group and the α,β-unsaturated ketone group in inflexinol or inflexin (Scheme S1, Supporting Information),[4] which is one of the major constituents in *I. excisus*. Oxidative cleavage of the ether bond and the double bond between C-15′ and C-16′ of the six-membered dihydropyran ring (**2**) might result in the formation of a single-bond linkage (**1**) between the two subunits and a nine-membered-ring skeleton (**3**–**5**), respectively.

All isolates were evaluated for their inhibitory effects on the LPS-induced production of nitric oxide in murine macrophage RAW264.7 cells. However, the five dimers were inactive in this assay, with IC_{50} values > 50 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-1000 polarimeter. UV and IR spectra were obtained on a JASCO UV-550 and Perkin-Elmer model LE599 spectrometer, respectively. ^1H, ^{13}C, and 2D NMR spectra were recorded using Bruker Biospin Avance II 900 and Bruker DRX 500 NMR spectrometers using $CDCl_3$ and C_5D_5N as solvents. High-resolution electrospray ionization (ESI) mass spectra were obtained on a Q-Tof micro (Waters, Milford, MA, USA) mass spectrometer. Preparative HPLC was carried out on a Waters (515 pump and 2996 photodiode array detector) and a YMC J'sphere ODS-H80 column (4 μm, 20 × 250 mm), using a mixed solvent system of acetonitrile–water at a flow rate of 6.5 mL/min. Open column chromatography was performed using silica gel (Kieselgel 60, 70–230 mesh, Merck) and Lichroprep RP-18 (40–63 μM, Merck) with TLC conducted using precoated silica gel 60 F_{254} (0.25 mm, Merck) plates.

Plant Material. The aerial parts of *I. excisus* were collected from Hwacheon, Kangwondo, Korea, in August 2003. The plant material was identified by Emeritus Professor Kyong Soon Lee, and a voucher specimen of this plant has been deposited at the Herbarium of the College of Pharmacy, Chungbuk National University, Korea (CBNU0308).

Extraction and Isolation. The air-dried and powdered aerial parts of *I. excisus* (1.6 kg) were extracted with MeOH (3 × 15 L) at room temperature. The extract was filtered and concentrated in vacuo and then suitably diluted with water (1.5 L) and partitioned between *n*-hexane (3 × 1.5 L) and dichloromethane (3 × 1.5 L). The dichloromethane-soluble layer was concentrated, and an aliquot (13.7 g) loaded onto a silica gel column (9 × 25 cm, 70–230 mesh), eluted with a dichloromethane–methanol mixture, with increasing proportions of methanol, to yield seven fractions (IEA–IEG). Subfraction IED (4.1 g) was applied to further column chromatography over silica gel (3 × 25 cm, 70–230 mesh), eluting with *n*-hexane–acetone (5:1, 3:1, 3:2, and 0:1), to yield six fractions (IED1–IED6). Fraction IED-3 (1.7 g) was subjected to column chromatography over silica gel (2 × 25 cm, 70–230 mesh), eluting with dichloromethane–methanol (80:1, 50:1, 30:1, and 10:1), to afford five subfractions (IED31–IED35). Fraction IED33 (100 mg) was subjected to flash column chromatography on RP-18 (2 × 30 cm, 40–63 μm), eluting with acetonitrile–water (3:7, 4:6, 5:5, and 3:7), to give seven subfractions (IED331–IED337). Fractions IED332, IED334, IED336, and IED337 were further purified by means of semi-preparative HPLC (YMC J'sphere ODS-H80 column, 20 × 250 mm), eluting with acetonitrile–water (50:50) at a flow rate of 6.5 mL/min, to yield **3** (8.2 mg), **4** (4.3 mg), **5** (4.8 mg), and **2** (8.9 mg), respectively. Fraction IED35 (50 mg) was subjected to flash column chromatography on RP-18 (2 × 30 cm, 40–63 μm), eluting with acetonitrile–water (3:7, 4:6, 5:5, and 6:4), and finally purified by semipreparative HPLC, eluting with acetonitrile–water (1:1), to give **1** (9.1 mg).

*Biexcisusin A (**1**):* white, amorphous powder; $[\alpha]^{25}_D$ −47.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.52) nm; IR (KBr) ν_{max} 3434, 2940, 1725, 1366, 1214, 1043, 958 cm^{-1}; ^1H and ^{13}C NMR (C_5D_5N, 900 and 225 MHz), see Table 1; ESIMS (positive ion) *m/z* 903 $[M + H]^+$; ESIMS (negative ion) *m/z* 901 $[M - H]^-$; HRESIMS (negative ion) *m/z* 901.4561 (calcd for $C_{48}H_{69}O_{16}$ $[M - H]^-$, 901.4586).

*Biexcisusin B (**2**):* white, amorphous powder; −74.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.78) nm; IR (KBr) ν_{max} 3440, 2943, 1722, 1369, 1210, 1039, 955 cm^{-1}; ^1H and ^{13}C NMR (C_5D_5N, 900 and 225 MHz), see Table 1; ESIMS (positive ion) *m/z* 891 $[M + Na]^+$, 869 $[M + H]^+$, 809 $[M - OAc + H]^+$, ESIMS (negative ion) *m/z* 913 $[M + COOH]^-$; HRESIMS (negative ion) *m/z* 913.4571 (calcd for $C_{49}H_{69}O_{16}$ $[M + COOH]^-$, 913.4586).

*Biexcisusin C (**3**):* white, amorphous powder; −70.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.97) nm; IR (KBr) ν_{max} 3442, 2941, 2875, 1720, 1680, 1361, 1209, 1040, 953 cm^{-1}; ^1H and ^{13}C NMR (C_5D_5N, 900 and 225 MHz), see Table 2; ESIMS (positive ion) *m/z* 923 $[M + Na]^+$, 841 $[M - OAc + H]^+$, ESIMS (negative ion) *m/z* 945 $[M + COOH]^-$; HRESIMS (negative ion) *m/z* 945.4449 (calcd for $C_{49}H_{69}O_{18}$ $[M + COOH]^-$, 945.4484).

*Biexcisusin D (**4**):* white, amorphous powder; −128.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.82) nm; IR (KBr) ν_{max} 3444, 2938, 2871, 1718, 1655, 1358, 1220, 1035 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 500 and 125 MHz), see Table 2; ESIMS (positive ion) *m/z* 921 $[M + Na]^+$, 839 $[M - OAc + H]^+$, ESIMS (negative ion) *m/z* 943 $[M + COOH]^-$; HRESIMS (negative ion) *m/z* 943.4329 (calcd for $C_{49}H_{67}O_{18}$ $[M + COOH]^-$, 943.4327).

*Biexcisusin E (**5**):* white, amorphous powder; −78.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.75) nm; IR (KBr) ν_{max} 3446, 2937, 2860, 1723, 1371, 1225, 1043 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 500 and 125 MHz), see Table 2; ESIMS (positive ion) *m/z* 921 $[M + Na]^+$, 839 $[M - OAc + H]^+$; HRESIMS (negative ion) *m/z* 897.4276 (calcd for $C_{48}H_{65}O_{16}$ $[M - H]^-$, 897.4273).

Determination of LPS-Induced NO Production in Murine Macrophage RAW264.7 Cells. The level of nitric oxide production was determined by measuring the amount of nitrite in the cell culture supernatant as previously described.[9] Aminoguanidine was used as a positive control with an IC_{50} value of 27.5 μM.

Table 2. ^{1}H and ^{13}C NMR Data of Compounds 3–5[a]

	3[b]		4[c]		5[c]	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1β	4.22 dd (11.7, 4.5)	77.6 d	3.97 dd (12.0, 4.2)	75.3 d	3.67 dd (11.4, 4.2)	76.7 d
2α	2.47 m	34.5 t		32.1 t	1.97 m	33.7 t
2β	2.15[d]		1.80 br d (4.2)		1.74 br t (4.2)	
3α	4.95[d]	80.4 d	4.62 br s	78.8 d	4.68 br s	79.7 d
4		38.3 s		36.5 s		38.0 s
5β	1.70 br s	49.4 d	2.75 br s	58.7 d	1.31 br s	48.3 d
6β	4.62 d-like (2.7)	66.3 d		210.2	4.36 br s	67.9 d
7α		45.6 t	3.07 d (12.5)	53.0 t	3.07 d (12.5)	36.1 t
7β	2.55[d]		2.26 br d (12.0)		2.26 br d (12.0)	
8		52.4 s		56.8 s		52.0 s
9β	2.38 br s	61.8 d	2.17 br s	61.5 d	1.71 br s	61.9 d
10		44.8 s		51.0 s		44.5 s
11α	6.86 d (6.3)	71.2 d	5.94 d (6.0)	69.6 d	5.92 d (6.0)	71.2 d
12α	2.29 m	34.3 t		33.7 t		33.2 t
12β	2.22 m		2.01 m		2.01 m	
13α	2.55[d]	43.3 d	2.66 m	42.3 d	2.65 m	43.8 d
14α	3.03 m	36.9 t		34.5 t	2.75[d]	36.3 t
14β	2.34 m		2.15[d]		2.15[d]	
15		218.2 s		213.3 s		217.4 s
16		88.1 s		88.1 s		89.1 s
17a	3.03 m	31.8 t		31.2 t		31.4 t
17b	2.79 dd (13.5, 7.2)		2.71 m		2.71 m	
18	1.02 s	28.6 q	0.86 s	26.8 q	0.90 s	28.6 q
19	1.52 s	24.2 q	1.30 s	22.3 q	1.24 s	24.3 q
20	1.92 s	15.3 q	1.08 s	15.3 q	1.42 s	15.1 q
1′β	3.99 t-like (8.1)	77.6 d	3.50 dd (12.0, 4.2)	77.3 d	3.75 dd (11.4, 4.2)	75.8 d
2′α	2.25 dd (8.1, 2.7)	32.8 t		33.1 t		30.4 t
2′β			2.03 m		1.76 br t (4.2)	
3′α	4.95[d]	80.2 d	4.68 br s	80.0 d	4.70 br s	78.6 d
4′α		38.9 s		38.5 s		36.9 s
5′β	1.53 br s	46.3 d	1.07 br s	46.0 d	2.24 br s	56.1 d
6′β	4.88 dd (11.7, 7.2)	65.9 d	4.48 dd (12.5, 7.5)	66.9 d		206.8 s
7′α	2.00 m	41.3 t		40.4 t	2.82 m	44.6 t
7′β	2.51 dd (13.5, 8.1)		1.60 dd (13.5, 7.5)		2.28 d (18.0)	
8′		46.6 s		46.2 s		47.0 s
9′β	2.90 d (10.8)	49.1 d	2.36 d (10.8)	48.8 d	2.69 d (11.4)	48.4 d
10′		43.9 s		43.6 s		44.5 s
11′α	5.52 m	70.7 d	5.02 m	70.2 d	4.88 m	69.9 d
12′α	3.03[d]	29.2 t		28.6 t		28.5 t
12′β	2.44 m		2.64 br d (7.8)		2.64 br d (7.8)	
13′α	2.59 m	45.2 d	2.59 m	44.7 d	2.57 m	44.2 d
14′	2.41 m	37.3 t		37.2 t	2.80 m	31.4 t
14′β	2.03 m		2.33 m		1.97 m	
15′		176.0 s		176.0 s		175.8 s
16′		212.2 s		211.2 s		210.9 s
17′a	3.59 t (13.5)	32.6 t	3.29 m	31.8 t	3.24 t-like (13.2)	32.4 t
17′b	2.15[d]		1.88 m		2.01[d]	
18′	1.16 s	28.1 q	1.02 s	28.1 q	1.02 s	27.8 q
19′	1.66 s	26.2 q	1.40 s	26.4 q	1.25 s	23.0 q
20′	1.52 s	12.7 q	1.10 s	12.3 q	0.83 s	12.3 q
OAc		171.6 s		170.9 s		171.9 s
	2.10 s	21.6 q	2.14 s	22.0 q	2.02 s	22.5 q
		170.5 s		171.5 s		170.6 s
	1.98 s	22.1 q	2.06 s	22.4 q	2.08 s	21.8 q
		170.8 s		170.6 s		171.2 s
	1.93 s	21.4 q	2.00 s	21.9 q	2.10 s	22.1 q
		170.1 s		170.1 s		169.3 s
	2.16 s	21.9 q	2.16 s	22.1 q	2.18 s	22.0 q

[a]Assignments were based on the DEPT, COSY, HMQC, and HMBC experiments. [b]^{1}H NMR spectra were recorded at 900 MHz, and ^{13}C NMR spectra at 225 MHz in pyridine-d_5. [c]^{1}H NMR spectra were recorded at 500 MHz, and ^{13}C NMR spectra at 125 MHz in $CDCl_3$. [d]Multiplicity patterns were unclear due to signal overlapping.

dx.doi.org/10.1021/np200439n | J. Nat. Prod. 2011, 74, 2382–2387

■ ASSOCIATED CONTENT

Supporting Information

1D NMR, 2D NMR, and HRESIMS of compounds **1**−**5**, including Figures S1 and S2 and Scheme S1, are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +82-43-261-2814. Fax: +82-43-268-2732. E-mail: byhwang@chungbuk.ac.kr.

■ ACKNOWLEDGMENTS

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (MRC, 2010-0029480) and a grant from the Korean Ministry of Education, Science and Technology (The Regional Core Research Program/Chungbuk BIT Research-Oriented University Consortium). We thank the Korea Basic Science Institute for providing certain instrumentation used in this study.

■ REFERENCES

(1) Sun, H. D.; Huang, S. X.; Han, Q. B. *Nat. Prod. Rep.* **2006**, *23*, 673−698.
(2) Han, Q. B.; Lu, Y.; Zhang, L. L.; Zheng, Q. T.; Sun, H. D. *Tetrahedron Lett.* **2004**, *45*, 2833−2837.
(3) Han, Q. B.; He, Z. D.; Qiao, C. F.; Xu, H. X.; Sun, H. D. *Helv. Chim. Acta* **2005**, *88*, 817−821.
(4) Huang, S. X.; Xiao, W. L.; Li, L. M.; Li, S. H.; Zhou, Y.; Ding, L. S.; Lou, L. G.; Sun, H. D. *Org. Lett.* **2006**, *8*, 1157−1160.
(5) Zhao, Y.; Pu, J. X.; Huang, S. X.; Ding, L. S.; Wu, Y. L.; Li, X.; Yang, L. B.; Xiao, W. L.; Chen, G. Q.; Sun, H. D. *J. Nat. Prod.* **2009**, *72*, 988−993.
(6) Tang, W.; Eisenbrand, G. *Chinese Drugs of Plant Origin*; Springer Verlag: New York, 1992; pp 817−847.
(7) Lee, C.; Kim, J.; Lee, H.; Lee, S.; Kho, Y. *J. Nat. Prod.* **2001**, *64*, 659−660.
(8) Hong, S. S.; Lee, S. A; Han, X. H.; Jin, H. Z.; Lee, J. H.; Lee, D.; Lee, J. J.; Hong, J. T.; Kim, Y.; Ro, J. S.; Hwang, B. Y. *J. Nat. Prod.* **2007**, *70*, 632−636.
(9) Hong, S. S.; Lee, S. A.; Kim, N.; Hwang, J. S.; Han, X. H.; Lee, M. K.; Jung, J. K.; Hong, J. T.; Kim, Y.; Lee, D.; Hwang, B. Y. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1279−1281.
(10) Takeda, Y.; Shingu, T.; Ichihara, T.; Fujita, T.; Yokoi, T.; Kida, K.; Ueno, A. *Chem. Pharm. Bull.* **1988**, *36*, 4576−4580.
(11) Na, Z.; Li, S. H.; Xiang, W.; Zhao, A. H.; Li, C. M.; Sun, H. D. *Chin. J. Chem.* **2002**, *20*, 884−886.
(12) Han, Q. B.; Lu, Y.; Wu, L.; He, Z. D.; Qiao, C. F.; Xu, H. X.; Zheng, Q. T.; Sun, H. D. *Tetrahedron Lett.* **2005**, *46*, 5373−5375.