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Structurally Diverse Diterpenoids from *Isodon scoparius* and Their Bioactivity

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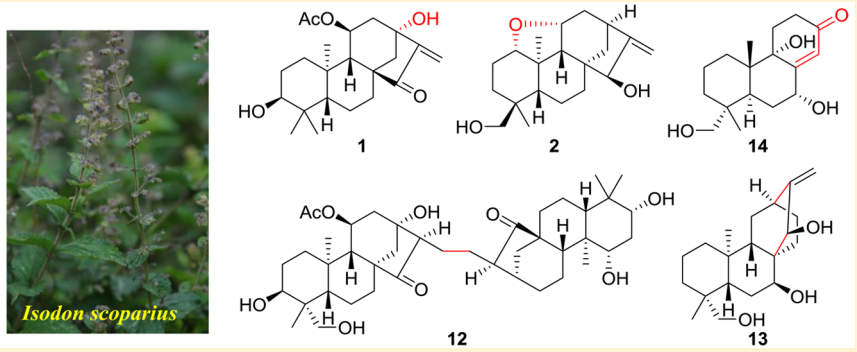
Supporting Information



ABSTRACT: Fourteen new diterpenoids (**1**–**14**) based on four skeletal types and two known analogues (**15** and **16**) were isolated from the aerial parts of *Isodon scoparius*. Compound **2** is the first *ent*-kaurane diterpenoid featuring a 1,11-ether bridge, and the structures of these new compounds were established mainly by NMR and MS methods. The absolute configurations of **1** and **5** and the relative configuration of **3** were determined using single-crystal X-ray diffraction. The absolute configuration of **14** was determined by comparison of the experimental and calculated electronic circular dichroism spectra. Compounds **1**, **4**, and **15** were active against five human tumor cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW-480), and they also inhibited NO production in LPS-stimulated RAW264.7 cells, with IC_{50} values of 1.0, 3.1, and 1.8 μM, respectively.

The genus *Isodon*, with about 150 species, is distributed throughout the world mainly in tropical and subtropical Asia.[1] Hitherto, over 1200 new diterpenoids, which include some compounds featuring fascinating skeletons and/or displaying a broad spectrum of biological activity, have been discovered from *Isodon* plants.[2,3] Especially, eriocalyxin B, an *ent*-kaurane diterpenoid isolated from *I. eriocalyx* var. *laxiflora*, has attracted the attention of biologists and has been regarded as a promising anticancer agent;[4] maoecrystal V, featuring an unprecedented and highly congested pentacyclic framework with adjacent vicinal quaternary stereocenters, has attracted extensive interest among the organic chemistry community.[5]

I. scoparius (C. Y. Wu et H. W. Li) H. Hara, a dwarf shrub, is distributed exclusively in the northwest district of Yunnan Province, People's Republic of China, and has been used as an antipyretic agent by local inhabitants.[6] Previous phytochemical investigations of this species at an altitude of about 2100 m in Shangrila County of Yunnan Province led to the discovery of six novel diterpenoids, which were structurally quite different from those isolated from other *Isodon* species.[7−11] Motivated by this finding and considering that the secondary metabolites of *Isodon* plants are greatly influenced by the ecological environment,[2] we investigated the chemical constituents of *I. scoparius* distributed at an altitude of about 3450 m in the Yulong snow mountain of Lijiang County, Yunnan Province. As a result, 14 new compounds including two *ent*-kauranoids featuring an oxygen bridge (**2** and **3**), nine C-13-oxygenated *ent*-kauranes (**1**, **4**−**11**), a dimeric *ent*-kaurane (**12**), an *ent*-atisane (**13**), and a podocarpane (**14**), as well as the known rosthornins B and C (**15** and **16**),[12] were obtained. This report describes the isolation and structure identification of compounds **1**−**16**, the in vitro cytotoxicity against HL-60, SMMC-7721, A-549, MCF-7, and SW-480 human tumor cell lines, and the inhibitory activity against nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW264.7 cells of selected compounds.

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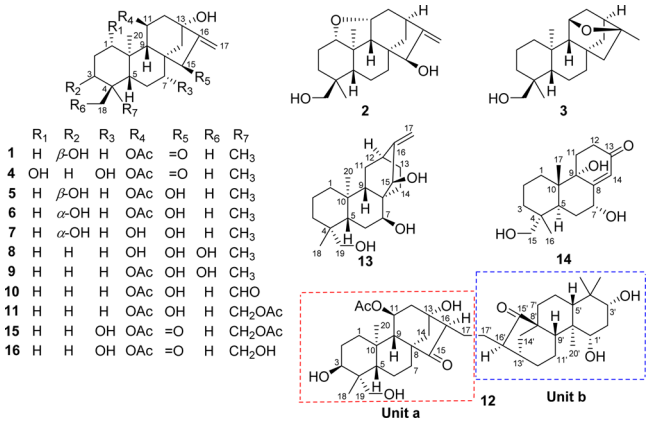

	R_1	R_2	R_3	R_4	R_5	R_6	R_7
1	H	β-OH	H	OAc	=O	H	CH_3
4	OH	H	OH	OAc	=O	H	CH_3
5	H	β-OH	H	OAc	OH	H	CH_3
6	H	α-OH	H	OAc	OH	H	CH_3
7	H	α-OH	H	OH	OH	H	CH_3
8	H	H	H	OH	OH	OH	CH_3
9	H	H	H	OAc	OH	OH	CH_3
10	H	H	H	OAc	OH	H	CHO
11	H	H	H	OAc	OH	H	CH_2OAc
15	H	H	OH	OAc	=O	H	CH_2OAc
16	H	H	OH	OAc	=O	H	CH_2OH

RESULTS AND DISCUSSION

Compound **1** had the molecular formula $C_{22}H_{32}O_5$, as deduced by the sodium adduct (+)-HRESIMS ion at *m/z* 399.2150 $[M + Na]^+$ (calcd 399.2142) and ^{13}C NMR data. The IR spectrum displayed bands for hydroxy (3436 cm^{-1}), carbonyl (1720 cm^{-1}), and olefinic (1633 cm^{-1}) functionalities. The ^1H NMR data (Table 1) showed resonances for four methyl groups at δ_H 1.82, 1.14, 1.03, and 0.83, two oxygenated methines at δ_H 5.50 (br d, J = 3.9 Hz) and 3.58 (t, J = 2.5 Hz), and two olefinic protons at δ_H 6.23 (d, J = 1.3 Hz) and 5.72 (d, J = 1.3 Hz). ^{13}C NMR and DEPT (Table 2) data exhibited 22 carbon resonances corresponding to four methyls, seven methylenes (one olefinic), four methines (two oxygenated), four quaternary carbons (one olefinic), an oxygenated tertiary carbon, and two carbonyl carbons. The aforementioned spectroscopic data of **1** revealed that its structural features were similar to those of the *ent*-kaurane diterpenoid isodopharicin A,[13] differing only in the orientation of HO-3 (β-orientation in **1** and α-orientation in isodopharicin A). This was verified by the shielding of C-5 ($\Delta\delta_C$ −5.6) in **1** compared with that of isodopharicin A, which is caused by the γ-steric compression effect between HO-3β and H-5β.[14] Furthermore, the acetoxy group at C-11 was assigned to be β-oriented based on the ROESY correlation of H-11/Me-20α. However, since C-13 is an oxygenated tertiary carbon, it was difficult to determine the orientation of HO-13 only by analysis of the NMR data. To solve this issue and to determine the absolute configuration of **1**, a single-crystal X-ray diffraction analysis using Cu Kα radiation was carried out. The Flack parameter [0.07(7)] permitted assignment of its (3*S*, 5*S*, 8*R*, 9*S*, 10*R*, 11*S*, 13*R*) absolute configuration (Figure 2).[15] Thus, the structure of **1**, 3-*epi*-isodopharicin A, was defined as 11β-acetoxy-3β,13α-dihydroxy-*ent*-kaur-16-en-15-one. Only five C-20 deoxy-*ent*-kauranoids bearing a C-13 hydroxy group have been obtained from *Isodon* plants previously.[2,3,14]

Compound **2** had the molecular formula $C_{20}H_{30}O_3$, as deduced by the sodium adduct (+)-HRESIMS ion at *m/z* 341.2084 $[M + Na]^+$ (calcd 341.2087) and ^{13}C NMR data, indicating six indices of hydrogen deficiency. The NMR (Tables 1 and 2) and HRESIMS data indicated that **2** was an *ent*-kaurane diterpenoid and structurally related to the known compound *ent*-15α,18-dihydroxykaur-16-ene.[16] The differences between these two structures were the replacement of two methylenes (δ_C 39.9, 18.0) in *ent*-15α,18-dihydroxykaur-16-ene by two methines (δ_C 88.7, 74.6) in **2** and the presence of one more oxygen atom in **2**, suggesting an ether linkage between C-1 and C-11 of **2**. This was supported by (i) $^1H-^1H$ COSY correlations of H-1/H_2-2/H_2-3 and H-9/H-11/H_2-12/H-13/H_2-14 (Figure 3); (ii) HMBC correlations (Figure 3) from H-3b (δ_H 1.58), H-9 (δ_H 2.28), and Me-20 (δ_H 1.22) to C-1 (δ_C 88.7) and from H-11 (δ_H 4.62) to C-9 (δ_C 51.0) and C-10 (δ_C 42.7); (iii) deshielding of C-2 ($\Delta\delta_C$ 5.7 ppm) and C-9 ($\Delta\delta_C$ 4.6 ppm) in **2** compared with those of *ent*-15α,18-dihydroxykaur-16-ene; and (iv) the requirement for one index of hydrogen deficiency. Thus, **2** was determined to be a C-20 deoxy-*ent*-kauranoid featuring a unique ether linkage spanning C-1 and C-11.

The relative configuration of **2** was established by a ROESY experiment (Figure 3). The ROESY correlations of H-9/H-5/H_2-18 indicated their cofacial arrangement, and they were assigned β-orientations in accordance with the structural features of the *ent*-kauranoids previously obtained from *Isodon* plants.[2,14] ROESY correlations of H-11/H-1/H-9β disclosed the α-orientation of the 1,11-epoxy group. The HO-15 was assigned to be β-oriented by the ROESY correlation of H-15/H-14β (δ_H 1.08).[17] Thus, the structure of compound **2**, scopariusol A, was defined as 1α,11α-epoxy-15β,18-dihydroxy-*ent*-kaur-16-ene.

Compound **3** displayed a sodium adduct ion at *m/z* 327.2291 $[M + Na]^+$ (calcd 327.2295) in the HRESIMS, in accordance with a molecular formula of $C_{20}H_{32}O_2$. Its NMR data (Tables 1 and 2) displayed signals typical for an *ent*-kauranoid, except for the presence of a deshielded oxygenated tertiary carbon (δ_C 85.3), indicating that **3** was a modified *ent*-kaurane diterpenoid. Analysis of the NMR and HRESIMS data established the linkage of C-11 and C-16 via an oxygen atom: (i) the HSQC and $^1H-^1H$ COSY correlations indicated a C(9)H−C(11)H−C(12)H_2−C(13)H−C(14)H_2 fragment; (ii) the HMBC correlations from H-11 (δ_H 4.40) to C-13 (δ_C 46.1) and C-16 (δ_C 85.3) and from H_2-15 (δ_H 1.56 and 1.26) and Me-17 (δ_H 1.36) to C-16, along with the indices of hydrogen deficiency of **3**, disclosed the presence of a 11,16-oxygen bridge. The HMBC correlations from H_2-18 (δ_H 3.61, 3.28) to C-3 (δ_C 35.8), C-5 (δ_C 49.4), and C-19 (δ_C 18.5) in conjuction with the ROESY correlations of H-18a/H-5β and Me-19/Me-20 showed the location of an OH group at C-18. The β-orientation of the 11,16-epoxy group of **3** was deduced by the coupling pattern of H-9 (δ_H 1.61), which displayed a broad singlet in the ^1H NMR spectrum.[14] The relative configuration was confirmed by the single-crystal X-ray diffraction analysis using Cu Kα radiation (Figure 4). Hence, the structure of compound **3**, scopariusol B, was established as 11β,16β-epoxy-18-hydroxy-*ent*-kaurane.

Compound **4** had the molecular formula $C_{22}H_{32}O_6$ based on the HRESIMS and ^{13}C NMR data, indicating that it had one more oxygen atom than **1**. The NMR data (Tables 1 and 2) resembled those of **1**, except for the deshielding of a methine (δ_C 74.7 to δ_C 80.0) and the presence of an additional methine (δ_C 69.8) in **4** instead of the methylene (δ_C 33.6) in **1**. In the HMBC spectrum, H-7 (δ_H 4.62) correlated to C-9 (δ_C 59.6), C-14 (δ_C 37.3), and C-15 (δ_C 207.7), demonstrating that C-7 constituted the additional oxymethine group, which was supported by the $^1H-^1H$ COSY correlations of H-5/H_2-6/H-7. Similarly, the deshielded oxymethine was ascribed to C-1 on the basis of $^1H-^1H$ COSY correlations of H-1/H_2-2/H_2-3, along with the HMBC correlations from H-9 (δ_H 1.99) and Me-20 (δ_H 1.38) to C-1. ROESY correlations of H-5β/H-1/H-9β and H-7/H-5β established the α-orientation of the hydroxy groups at C-1 and C-7. Thus, the structure of compound **4**, scopariusol C, was defined as 11β-acetoxy-1α,7α,13α-trihydroxy-*ent*-kaur-16-en-15-one.

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Table 1. ^{1}H NMR Spectroscopic Data for Compounds 1−7 (δ in ppm, J in Hz)

position	1[a,c]	2[a,b]	3[a,b]	4[a,b]	5[a,b]	6[a,b]	7[a,b]
1a	1.89, m	3.30, dd (11.5, 4.5)	1.64, br t (2.5)	3.66, m	1.96, overlap	1.97, td (13.0, 3.6)	2.02, m
1b	1.72, overlap		0.97, m		1.78, m	1.08, dt (13.0, 5.2)	1.30, m
2a	1.93, m	2.00, m	1.40, overlap	1.91, m	1.96, overlap	1.80, m	1.85, m
2b	1.72, overlap	1.76, m		1.72, m	1.73, m		
3a	3.58, t (2.5)	1.83, m	1.80, dt (12.5, 4.5)	1.30, m	3.58, br s	3.37, dd (10.7, 5.6)	3.41, br d (10.7)
3b		1.58, m	1.40, overlap	1.21, dt (14.1, 4.0)			
5	1.79, overlap	1.72, dd (6.5, 3.0)	1.49, dd (12.0, 2.0)	0.97, dd (12.3, 1.8)	1.75, dd (12.3, 2.3)	0.84, dd (12.1, 2.5)	0.94, dd (12.1, 2.0)
6a	1.55, m	1.69, m	1.58, m	2.04, m	1.52, overlap	1.59, br d (12.1)	1.62, br d (12.1)
6b	1.28, m		1.22, m	1.78, t (12.3)	1.32, m	1.34, m	1.38, m
7a	2.34, dt (11.4, 3.4)	2.16, m	1.54, overlap	4.62, dd (12.3, 4.2)	2.08, dt (13.1, 4.3)	2.02, dd (13.3, 3.9)	2.06, dd (13.1, 3.9)
7b	1.47, td (11.4, 3.5)	1.51, ddd (14.0, 5.0, 1.5)	1.31, m		1.46, td (13.1, 3.1)	1.47, td (13.3, 3.1)	1.53, br t (13.1)
9	1.71, br s	2.28, d (9.0)	1.61, br s	1.99, br s	1.91, br s	1.72, br s	1.94, br s
11	5.50, br d (3.9)	4.62, q (9.0)	4.40, br t (3.5)	7.13, dd (4.8, 1.6)	5.51, overlap	5.44, dd (5.0, 2.2)	4.39, t (5.2)
12a	2.56, dd (12.0, 3.9)	2.27, m	1.96, br d (11.0)	2.76, dd (14.4, 5.0)	2.36, br d (5.1)	2.30, br d (5.0)	2.50, br d (13.5)
12b	2.43, br d (12.0)	1.92, m	1.89, m	2.57, br d (14.4)			2.43, dd (13.5, 5.2)
13		2.74, br s	2.11, br t (3.5)				
14a	2.75, d (9.0)	2.15, d (12.0)	1.90, dd (11.0, 3.5)	3.04, dd (11.3, 3.1)	2.33, d (11.4)	2.30, d (11.5)	2.39, d (11.5)
14b	1.79, overlap	1.08, dd (12.0, 5.0)	1.13, m	2.62, d (11.3)	1.52, overlap	1.53, d (11.5)	1.55, dd (11.5, 2.6)
15a		4.06, br d (7.0)	1.56, d (11.0)		4.19, td (10.5, 2.8)	4.24, br d (10.2)	4.27, td (10.1, 2.6)
15b			1.26, d (11.0)				
17a	6.23, d (1.3)	5.54, br s	1.36, s	6.19, d (1.7)	5.59, dd (2.8, 1.3)	5.62, dd (3.0, 1.4)	5.69, dd (2.8, 1.6)
17b	5.72, d (1.3)	5.18, br s		5.68, d (1.7)	5.51, overlap	5.54, dd (2.3, 1.4)	5.56, t (1.6)
18a	1.14, s	3.47, br d (10.0)	3.61, d (10.5)	0.78, s	1.15, s	1.17, s	1.17, s
18b		3.36, br d (10.0)	3.28, d (10.5)				
19	0.83, s	1.01, s	0.85, s	0.78, s	0.83, s	0.97, s	1.01, s
20	1.03, s	1.22, s	1.05, s	1.38, s	0.98, s	0.94, s	1.02, s
AcO-11	1.82, s			1.93, s	1.93, s	2.06, s	

[a]Recorded in pyridine-d_5. [b]Recorded at 500 MHz. [c]Recorded at 400 MHz.

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Table 2. ^{13}C NMR Spectroscopic Data for Compounds 1−7 (δ in ppm)[a]

position	1	2	3	4	5	6	7
1	33.1, CH_2	88.7, CH	41.0, CH_2	80.0, CH	33.5, CH_2	38.6, CH_2	38.8, CH_2
2	26.1, CH_2	23.8, CH_2	18.4, CH_2	29.7, CH_2	26.4, CH_2	28.1, CH_2	28.2, CH_2
3	74.7, CH	36.0, CH_2	35.8, CH_2	39.8, CH_2	74.7, CH	77.7, CH	77.9, CH
4	38.1, C	38.1, C	37.9, C	33.3, C	38.1, C	39.4, C	39.7, C
5	48.6, CH	46.7, CH	49.4, CH	51.3, CH	48.7, CH	54.8, CH	55.1, CH
6	18.4, CH_2	22.0, CH_2	20.0, CH_2	29.2, CH_2	19.7, CH_2	20.0, CH_2	20.2, CH_2
7	33.6, CH_2	31.2, CH_2	38.0, CH_2	69.8, CH	38.8, CH_2	38.8, CH_2	39.5, CH_2
8	53.6, C	46.1, C	45.1, C	60.5, C	45.5, C	45.3, C	45.3, C
9	59.4, CH	51.0, CH	59.6, CH	59.6, CH	52.5, CH	52.3, CH	55.6, CH
10	39.1, C	42.7, C	36.8, C	44.4, C	38.1, C	37.9, C	37.7, C
11	69.7, CH	74.6, CH	76.8, CH	73.3, CH	70.8, CH	70.8, CH	66.8, CH
12	46.8, CH_2	39.0, CH_2	40.7, CH_2	47.1, CH_2	47.7, CH_2	47.7, CH_2	50.7, CH_2
13	75.0, C	39.2, CH	46.1, CH	75.5, C	75.8, C	75.8, C	76.3, C
14	45.3, CH_2	34.5, CH_2	44.0, CH_2	37.3, CH_2	44.8, CH_2	44.8, CH_2	45.1, CH_2
15	207.5, C	81.0, CH	57.6, CH_2	207.7, C	81.8, CH	81.7, CH	81.6, CH
16	154.4, C	157.1, C	85.3, C	155.5, C	161.0, C	160.9, C	161.8, C
17	112.7, CH_2	107.3, CH_2	23.6, CH_3	111.4, CH_2	105.4, CH_2	105.4, CH_2	105.6, CH_2
18	29.4, CH_3	71.6, CH_2	71.6, CH_2	33.1, CH_3	29.4, CH_3	28.9, CH_3	28.9, CH_3
19	22.3, CH_3	20.0, CH_3	18.5, CH_3	21.4, CH_3	22.4, CH_3	16.4, CH_3	16.4, CH_3
20	17.8, CH_3	16.9, CH_3	19.2, CH_3	14.8, CH_3	17.5, CH_3	17.5, CH_3	17.7, CH_3
AcO-11	169.1, 21.2			169.9, 21.5	169.0, 21.5	169.3, 21.6	

[a]Recorded in pyridine-d_5 at 125 MHz.

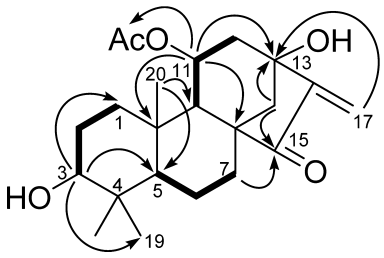

Figure 1. ^{1}H−^{1}H COSY (bold) and selected HMBC (arrow) correlations of 1.

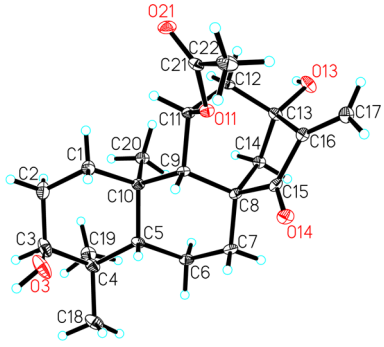

Figure 2. ORTEP drawing of compound 1.

Compound 5 possessed the molecular formula $C_{22}H_{34}O_5$, indicating that it had one less index of hydrogen deficiency than 1. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) showed similarities to those of 1, but a signal of an oxygenated methine (δ_C 81.8) was observed instead of the carbonyl carbon (δ_C 207.5) in 1. In the HMBC spectrum, H-15 (δ_H 4.19) correlated to C-7 (δ_C 38.8), C-9 (δ_C 52.5), and C-17 (δ_C 105.4), demonstrating the location of a hydroxy group at C-15 in 5 rather than the carbonyl group in 1. The β-orientation of HO-15 was determined by the ROESY correlation of H-15/H-14β (δ_H 1.52). Similar ROESY correlations observed for 5 and 1 showed the identity of their relative configurations. To determine the absolute configuration, a single-crystal diffraction analysis of 5 was performed. The final refinement on Cu Kα data resulted in a Flack parameter of 0.03(4), allowing unambiguous assignment of the absolute configuration of 5 as (3*S*, 5*S*, 8*R*, 9*S*, 10*R*, 11*S*, 13*R*, 15*S*) (Figure 5).[15] Thus, the structure of 5, scopariusol D, was established as 11β-acetoxy-3β,13α,15β-trihydroxy-*ent*-kaur-16-ene.

Compound 6 shared a molecular formula of $C_{22}H_{34}O_5$ with 5. The ^{13}C NMR spectra of compounds 6 and 5 were similar, except for differences in the chemical shifts of C-1 ($\Delta\delta_C$ 5.1), C-3 ($\Delta\delta_C$ 3.0), C-5 ($\Delta\delta_C$ 6.1), and C-19 ($\Delta\delta_C$ −6.0) compared with those of 5. In combination with the 2D NMR spectra, compound 6 possessed the same 2D structure as that of 5, only differing in the orientation of the C-3 hydroxy group. The ROESY correlation of H-3/H-5β confirmed the α-orientation of HO-3 in 6. Thus, compound 6 was structurally characterized as 3-*epi*-scopariusol D.

Compound 7 had the molecular formula $C_{20}H_{32}O_4$. The similarities of its NMR data with those of 6 revealed that these compounds were structural analogues and that the only difference was the presence of a hydroxy group at C-11 in 7, which replaced the acetoxy group in 6. This was corroborated by the shielding of C-11 ($\Delta\delta_C$ −4.0) in 7 compared with that of 6, as well as the HMBC correlations from H-11 (δ_H 4.39) to C-8 (δ_C 45.3), C-10 (δ_C 37.7), and C-13 (δ_C 76.3). Similar ROESY correlations showed that the relative configuration of 7 was the same as that of 6. Accordingly, the structure of compound 7, scopariusol E, was defined as 3α,11β,13α,15β-tetrahydroxy-*ent*-kaur-16-ene.

Scopariusol F (8) had the molecular formula $C_{20}H_{32}O_4$ based on the sodium adduct (+)-HRESIMS ion at m/z 359.2199 [M + Na]$^+$ (calcd 359.2193). The NMR data (Tables 3 and 4) resembled those of 7, differing in that signals for an oxymethylene (δ_C 71.5) and a methylene (δ_C 35.9) were observed in 8 instead of the oxygenated methine (δ_C 77.9) and methyl (δ_C 28.9) in 7. HMBC correlations from H_2-3 (δ_H 1.82

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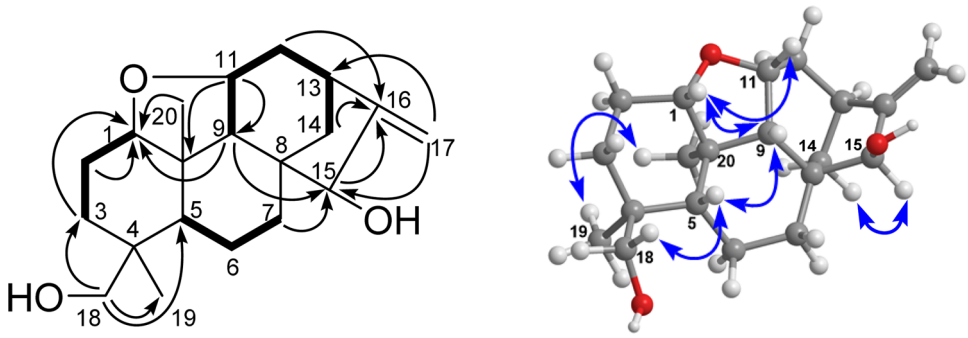

Figure 3. $^{1}H-^{1}H$ COSY (bold), selected HMBC (arrow), and key ROESY (double arrow) correlations of **2**.

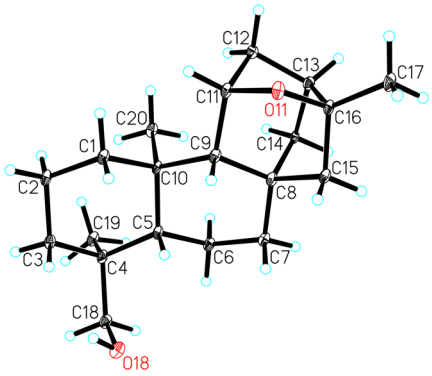

Figure 4. ORTEP drawing of compound **3**.

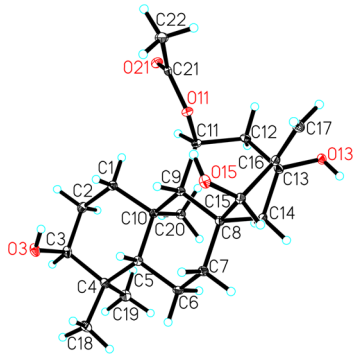

Figure 5. ORTEP drawing of compound **5**.

and 1.40), H-5 (δ_H 1.68), and Me-19 (δ_H 0.83) to the oxygenated methylene implied that the OH group connected to C-3 in **7** was attached to C-18 in **8**, which was supported by the ROESY correlation of Me-19/Me-20. Thus, the structure of compound **8**, scopariusol F, was defined as 11β,13α,15β,18-tetrahydroxy-*ent*-kaur-16-ene.

Compound **9** had the molecular formula $C_{22}H_{34}O_5$ according to the HRESIMS and ^{13}C NMR data, indicating six indices of hydrogen deficiency. The NMR data (Tables 3 and 4) suggested that **9** was structurally similar to **8**, except for the presence of an acetoxy group at C-11 in **9** rather than the hydroxy group in **8**. This was confirmed by the HMBC correlation from H-11 (δ_H 5.45) to the acetoxy carbonyl carbon (δ_C 169.0) and the deshielding of C-11 ($\Delta\delta_C$ +4.0) compared with **8**. The ROESY spectrum showed that the relative configuration of **9** was identical to **8**. Thus, the structure of **9**, 11-*O*-acetylscopariusol F, was identified as shown.

Compound **10** had the molecular formula $C_{22}H_{32}O_5$ as deduced by the sodium (+)-HRESIMS ion at *m*/*z* 399.2150 [M + Na]$^+$ (calcd 399.2142), which was two mass units less than that of **9**. Analysis of the NMR data of **10** (Tables 3 and 4) revealed that it was a structural analogue of **9**, except for the presence of a formyl group (δ_C 205.4 and δ_H 9.77) in **10** rather than the oxygenated methylene (δ_C 71.2) in **9**, which caused deshielding of a methyl group (from δ_C 18.1 to 24.1) compared with that of **9**. The C-19 formyl group was assigned via the HMBC cross-peaks from H-5 (δ_H 1.10) and Me-18 (δ_H 0.86) to the corresponding formyl carbon and the ROESY correlation of Me-18/H-5β. Therefore, the structure of **10**, scopariusol G, was defined as 11β-acetoxy-13α,15β-dihydroxy-*ent*-kaur-16-en-19-al.

The molecular formula of **11** was assigned as $C_{24}H_{36}O_6$ based on HRESIMS and ^{13}C NMR data. The NMR data (Tables 3 and 4) analysis revealed that its structure was closely related to that of **10**, and the only distinction was the replacement of the formyl group in **10** by an acetoxy methylene group in **11**. This was confirmed by the HMBC cross-peaks from H_2-19 (δ_H 4.30, 3.95) to C-3 (δ_C 36.1) and C-5 (δ_C 55.9) and an acetoxy carbonyl group (δ_C 171.0). On the basis of the ROESY and ^{13}C NMR data, **11** was assigned the same relative configuration as **10**. Hence, the structure of compound **11**, scopariusol H, was defined as 11β,19-diacetoxy-13α,15β-dihydroxy-*ent*-kaur-16-ene.

The molecular formula of compound **12** was established as $C_{42}H_{64}O_9$ according to HRESIMS and ^{13}C NMR data, indicative of 11 indices of hydrogen deficiency. The ^{1}H and ^{13}C NMR data (Table 5) exhibited signals of two diterpenoid units (**12a** and **12b**), implying the presence of a dimer similar to biexcisusin A.[18] The NMR data revealed that unit **12a** was structurally related to **1**, with differences being the presence of a HO-19 group, a C-16 methine, and a C-17 methylene in **12a** rather than the C-19 methyl and the exocyclic $\Delta^{16,17}$ double bond in **1**. The presence of HO-19 was verified by HMBC correlations from H-5 (δ_H 2.00) and Me-18 (δ_H 1.54) to C-19 (δ_C 65.5) and by a ROESY correlation of H_2-19/Me-20. The resonance at δ_C 60.8, which showed HMBC correlations with H_2-12 (δ_H 2.51, 2.36) and H_2-14 (δ_H 2.70, 1.87), was ascribed to C-16. HMBC correlations from H-11 (δ_H 5.50) and H_2-14 to an oxygenated tertiary carbon (C-13, δ_C 75.6) confirmed the presence of a HO-13 in **12a**, similar to **1**. Analysis of the NMR data of **12b** implied that its structural features were similar to those of the known compound (16*S*)-3α-hydroxy-15-oxo-*ent*-kaurane,[19] with the differences being at C-1′ and C-17′ (respectively δ_C 79.6 and 25.0 for **12b** and δ_C 37.7 and 10.0 for the latter), indicating the presence of HO-1′ and the replacement of the Me-17′ by a methylene in **12b**. The conclusion was confirmed by the HMBC correlations from H_2-2′ (δ_H 2.30), H-9′ (δ_H 1.63), and Me-20′ (δ_H 1.41) to C-1′ (δ_C

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Table 3. ^{1}H NMR Spectroscopic Data for Compounds 8−11, 13, and 14 (δ in ppm, J in Hz)[a]

position	8	9	10	11	13	14
1a	2.00, td (13.0, 3.4)	1.93, m	1.92, m	1.93, m	1.52, m	2.07, m
1b	1.20, dt (13.0, 3.8)	0.97, overlap	0.89, m	0.88, m	0.96, m	1.38, br d (12.7)
2a	1.72, overlap	1.61, overlap	1.61, overlap	1.62, m	1.64, m	1.57, m
2b	1.47, overlap	1.41, overlap	1.31, m	1.29, m	1.34, m	
3a	1.82, dt (13.2, 4.2)	1.81, m	2.14, m	1.68, td (13.7, 3.6)	2.18, dt (8.2, 2.1)	1.83, dt (12.2, 5.5)
3b	1.40, td (13.2, 3.3)	1.35, td (13.1, 2.7)	2.00, m	0.89, overlap	1.07, dt (8.2, 2.6)	1.53, br d (12.2)
5	1.68, dd (12.1, 2.1)	1.61, overlap	1.10, dd (12.8, 2.3)	0.94, dd (13.9, 1.8)	2.08, dd (8.3, 1.3)	3.06, br d (13.2)
6a	1.72, m	1.70, br d (12.9)	1.78, m	1.53, td (13.9, 3.5)	1.93, td (8.3, 1.6)	2.19, br d (13.2)
6b	1.33, m	1.28, m	1.61, overlap	1.29, m	1.85, dt (8.3, 1.3)	1.70, m
7a	2.17, dt (13.4, 4.0)	2.16, dt (13.5, 4.2)	1.98, m	1.95, dd (13.2, 4.2)	3.94, t (1.6)	4.61, br s
7b	1.47, overlap	1.41, overlap	1.48, td (13.2, 3.2)	1.42, td (13.2, 3.5)		
9	2.05, br s	1.80, br s	1.73, br s	1.72, br s	2.52, dd (7.5, 3.2)	
11a	4.39, br s	5.45, dd (4.8, 2.3)	5.37, dd (5.4, 1.6)	5.40, dd (4.8, 3.0)	1.76, m	2.09, m
11b					1.34, m	1.95, m
12a	2.51, br d (13.4)	2.36, m	2.31, m	2.32, br d (4.8)	2.31, m	2.88, m
12b	2.44, br d (13.4)					2.38, td (15.2, 3.5)
13					1.48, m	
14a	2.42, br d (11.3)	2.32, d (11.4)	2.23, d (11.5)	2.23, d (11.4)	1.67, td (7.8, 2.0)	6.13, s
14b	1.54, br d (11.3)	1.51, br d (11.4)	1.52, dd (11.5, 2.4)	1.49, br d (11.4)	0.96, m	
15a	5.94, d (10.1)	4.18, td (10.4, 2.8)	4.24, td (10.1, 2.6)	4.20, td (10.3, 2.8)	4.32, s	3.68, d (10.5)
15b						3.47, d (10.5)
16						0.97, s
17a	5.68, dd (2.8, 1.5)	5.60, dd (2.8, 1.3)	5.61, dd (2.6, 1.2)	5.60, dd (2.8, 1.2)	5.32, br s	0.89, s
17b	5.54, t (1.5)	5.51, dd (2.8, 1.3)	5.53, dd (2.6, 1.2)	5.52, dd (2.8, 1.2)	5.11, br s	
18a	3.64, d (10.6)	3.63, dd (10.6, 4.1)	0.86, s	0.92, s	1.26, s	
18b	3.31, d (10.6)	3.28, dd (10.6, 4.0)				
19a	0.83, s	0.78, s	9.77, d (1.4)	4.30, d (11.0)	4.05, d (6.7)	
19b				3.95, d (11.0)	3.73, d (6.7)	
20	1.05, s	0.97, s	0.78, s	0.90, s	1.02, s	
HO-9						5.53, s
AcO-11		2.02, s	2.02, s	2.03, s		
AcO-19				2.03, s		

[a]Recorded in pyridine-d_5 at 500 MHz.

Table 4. ^{13}C NMR Spectroscopic Data for Compounds 8−11, 13, and 14 (δ in ppm)[a]

position	8	9	10	11	13	14
1	40.1, CH_2	39.8, CH_2	39.3, CH_2	39.8, CH_2	40.2, CH_2	31.6, CH_2
2	18.6, CH_2	18.4, CH_2	18.6, CH_2	20.1, CH_2	18.4, CH_2	18.4, CH_2
3	35.9, CH_2	35.6, CH_2	34.1, CH_2	36.1, CH_2	36.6, CH_2	35.9, CH_2
4	38.2, C	37.9, C	48.4, C	37.3, C	38.6, C	38.2, C
5	49.1, CH	48.4, CH	55.7, CH	55.9, CH	47.6, CH	33.4, CH
6	20.1, CH_2	19.8, CH_2	19.5, CH_2	18.3, CH_2	28.3, CH_2	30.4, CH_2
7	39.4, CH_2	38.4, CH_2	38.7, CH_2	38.9, CH_2	75.7, CH	72.6, CH
8	45.2, C	45.4, C	45.3, C	45.4, C	39.7, C	160.3, C
9	55.8, CH	52.4, CH	50.9, CH	52.4, CH	40.0, CH	76.0, C
10	37.8, C	38.1, C	38.1, C	37.9, C	37.8, C	43.0, C
11	66.8, CH	70.8, CH	70.7, CH	70.6, CH	27.4, CH_2	27.9, CH_2
12	50.7, CH_2	47.7, CH_2	47.3, CH_2	47.6, CH_2	36.4, CH	34.7, CH_2
13	76.4, C	75.8, C	75.6, C	75.7, C	26.8, CH_2	200.1, C
14	45.4, CH_2	44.9, CH_2	44.9, CH_2	44.6, CH_2	27.2, CH_2	127.6, CH
15	81.7, CH	81.9, CH	81.7, CH	81.7, CH	78.0, CH	71.8, CH_2
16	161.8, C	161.0, C	160.8, C	160.8, C	155.7, C	18.1, CH_3
17	105.6, CH_2	105.3, CH_2	105.5, CH_2	105.4, CH_2	109.3, CH_2	18.7, CH_3
18	71.5, CH_2	71.2, CH_2	24.1, CH_3	27.5, CH_3	27.8, CH_3	
19	18.1, CH_3	18.1, CH_3	205.4, CH	66.7, CH_2	64.7, CH_2	
20	18.2, CH_3	17.9, CH_3	16.1, CH_3	17.8, CH_3	14.8, CH_3	
AcO-11		169.0, 21.5	169.1, 21.5	169.0, 21.6		
AcO-19				171.0, 20.8		

[a]Recorded in pyridine-d_5 at 125 MHz.

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Table 5. ^{1}H and ^{13}C NMR Spectroscopic Data for Compound 12 (δ in ppm)[a]

position	^{1}H[b]	^{13}C[c]	position	^{1}H	^{13}C
1	2.00, overlap 1.81, overlap	33.2, CH_2	1′	3.64, t (8.4)	79.6, CH
2	2.36, m 1.81, overlap	26.2, CH_2	2′	2.30, m	39.4, CH_2
3	4.36, br s	69.7, CH	3′	3.49, t (8.3)	75.3, CH
4		44.2, C	4′		39.7, C
5	2.00, overlap	49.3, CH	5′	0.81, br d (11.4)	52.9, CH
6	1.81, overlap	19.1, CH_2	6′	1.72, m 1.48, m	18.7, CH_2
7	2.24, dd (13.4, 4.0) 1.59, td (13.4, 3.4)	34.8, CH_2	7′	2.49, overlap 1.38, overlap	38.1, CH_2
8		55.0, C	8′		53.7, C
9	1.68, br s	58.9, CH	9′	1.63, d (8.3)	54.0, CH
10		38.8, C	10′		45.6, C
11	5.50, d (6.2)	69.3, CH	11′	3.55, dd (15.5, 6.1) 1.54, overlap	21.3, CH_2
12	2.51, br d (12.4) 2.36, dd (12.4, 6.2)	40.7, CH_2	12′	1.72, m	25.7, CH
13		75.6, C	13′	2.60, m	33.9, CH
14	2.70, d (11.4) 1.87, d (11.4)	46.2, CH_2	14′	2.04, overlap 1.38, overlap	35.3, CH_2
15		219.3, C	15′		224.2, C
16	2.64, t (6.2)	60.8, CH	16′	2.43, m	54.2, CH
17	2.55, m 1.93, m	25.9, CH_2	17′	2.33, m 2.07, overlap	25.0, CH_2
18	1.54, s	23.8, CH_3	18′	1.16, s	28.8, CH_3
19	3.99, d (10.9) 3.78, d (10.9)	65.5, CH_2	19′	1.05, s	16.1, CH_3
20	1.08, s	18.3, CH_3	20′	1.41, s	14.9, CH_3
AcO-11	2.04, s	169.2, 21.4			

[a]Recorded in pyridine-d_5. [b]Recorded at 500 MHz. [c]Recorded at 125 MHz.

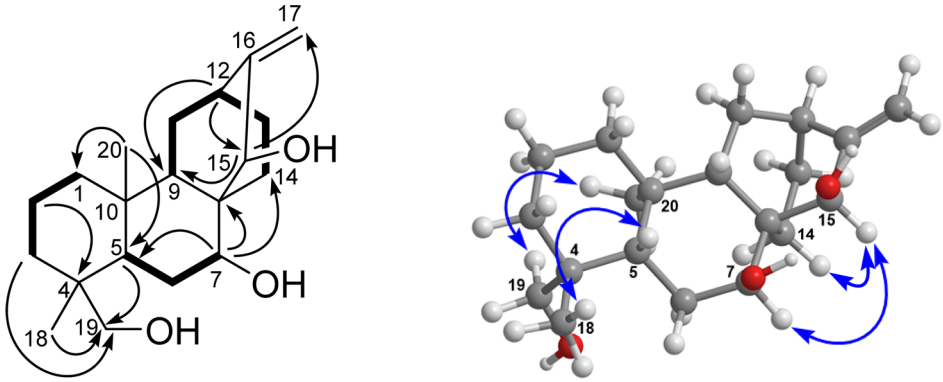

Figure 6. $^{1}H-^{1}H$ COSY (bold), selected HMBC (arrow), and key ROESY (double arrow) correlations of 13.

79.6), as well as the HSQC-TOCSY correlation of H-16′ (δ_H 2.43) to C-17′ (δ_C 25.0). Hence, the 2D structures of the monomeric units **12a** and **12b** were clearly deduced, and their linkage through the C-17−C-17′ single bond was established by the HSQC-TOCSY correlations from H_2-17, H_2-17′ (δ_H 2.33 and 2.07), and H-16′ to C-16, which indicated a C(16)H−C(17)H_2−C(17′)H_2−C(16′)H fragment, along with the HMBC correlations from H-16′ to C-17 and C-17′. The ROESY correlations of H-11/Me-20 and H-16/H-14a (δ_H 2.70) indicated that AcO-11 was β-oriented and H-16 was α-oriented. The HO-3 was assigned to be β-oriented due to the shielding of C-5 ($\Delta\delta_C$ −5.5) in **12** compared with that of **6**, which is caused by the γ-steric compression effect between HO-3β and H-5β in **12**.[14] Similarly, HO-1′, HO-3′, and H-16′ were α-oriented according to the ROESY correlations of H-3′/H-5′β/H-1′ and H-16′/H-14′a (δ_H 2.04). Thus, the structure of **12**, scopariusol I, was defined as shown.

Compound **13** possessed the molecular formula $C_{20}H_{32}O_3$ according to HRESIMS and ^{13}C NMR data. The ^{13}C NMR and DEPT spectra exhibited 20 carbon signals (Table 4) for two methyls, nine methylenes (one oxygenated and one olefinic), five methines (two oxygenated), and four quaternary carbons (one olefinic), indicating a possible *ent*-kaurane-type diterpenoid according to the reported constituents from this species.[2] Interestingly, the $^{1}H-^{1}H$ COSY spectrum (Figure 6) exhibited correlations of H-9/H_2-11/H-12/H_2-13/H_2-14, which were not

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previously observed in *ent*-kaurane diterpenoids. In the HMBC spectrum (Figure 6), a methine proton (δ_H 2.31, H-12) correlated to C-9 (δ_C 40.0) and C-15 (δ_C 78.0), implying that C-16 was connected to C-12. Considering that all the kauranoids isolated from *Isodon* plants possess an *ent*-configuration,[2,3,14] **13** was assumed to be an *ent*-atisane diterpenoid, similar to the known compound alboatisin C, which was previously obtained from *I. albopilous*.[20] The locations of two hydroxy groups at C-7 and C-19, respectively, were determined by the HMBC correlations from H-7 (δ_H 3.94) to C-5 (δ_C 47.6), C-8 (δ_C 39.7), and C-14 (δ_C 27.2) and from H_2-3 (δ_H 2.18, 1.07), H-5 (δ_H 2.08), and Me-18 (δ_H 1.26) to C-19 (δ_C 64.7). The presence of a β-OH group at C-15 was evident from the HMBC correlations from H-15 (δ_H 4.32) to C-7 (δ_C 75.7), C-9 (δ_C 40.0), and C-17 (δ_C 109.3), along with the ROESY correlation of H-15/H-14β (δ_H 0.96) (Figure 6). In the ROESY spectrum, H-7 correlated to H-15α, implying the β-orientation of HO-7. Thus, the structure of compound **13**, scopariusol J, was defined to be 7β,15β,19-trihydroxy-*ent*-atis-16-ene. Only five diterpenoids based on an *ent*-atisane skeleton have been reported from the genus *Isodon*.[20,21]

Compound **14** had the molecular formula $C_{17}H_{26}O_4$, with five indices of hydrogen deficiency. The ^{13}C NMR and DEPT spectra displayed 17 carbon resonances (Table 4) corresponding to two methyls, seven methylenes (one oxygenated), three methines (one oxygenated and one olefinic), three quaternary carbons (one olefinic), an oxygenated tertiary carbon, and a carbonyl carbon. Apart from two indices of hydrogen deficiency attributed to carbonyl and olefinic functionalities, compound **14** was assumed to have a tricyclic nucleus. Analysis of the NMR data of **14** implied that it was a podocarpane trinorditerpenoid similar to 7α,15-dihydroxypodocarp-8(14)-en-13-one, with the only difference being the presence of an additional OH group at C-9 in **14**.[22] This was evident from the HMBC correlations (Figure 7) from H-5 (δ_H 3.06), H_2-12 (δ_H 2.88, 2.38), and H-14 (δ_H 6.13) to C-9 (δ_C 76.0). HMBC correlations from H-11b (δ_H 1.95) and H_2-12 to a carbonyl carbon (δ_C 200.1) and from H-14 to C-7 (δ_C 72.6) and C-12 (δ_C 34.7) indicated a C-13 conjugated carbonyl group, which was further confirmed by the UV absorption maximum at approximately 226 nm (Figure S81, Supporting Information). The relative configuration was determined by the ROESY spectrum, in which HO-9 (δ_H 5.53) correlated to H-5α, implying the α-orientations of HO-9 and HO-7. The absolute configuration of **14** was determined by comparison of the experimental and calculated electronic circular dichroism (ECD) spectra of **14** and *ent*-**14** (Figure 8). Overall, the calculated ECD spectrum for **14** showed diagnostic positive and negative Cotton effects at 229 and 359 nm, respectively, consistent with the experimental spectrum, to establish its (4*R*, 5*R*, 7*R*, 9*S*, 10*S*) absolute configuration.[23] Thus, the structure of **14**, scopariusol K, was defined as 7α,9α,15-trihydroxypodocarpa-8(14)-en-13-one. Only four podocarpane diterpenoids have previously been reported from *Isodon* plants.[24]

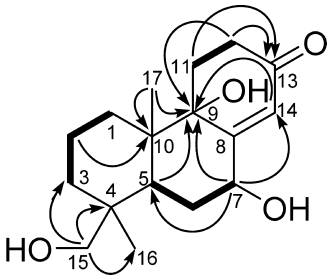

Figure 7. $^1H-^1H$ COSY (bold) and selected HMBC (arrow) correlations of **14**.

The two known compounds rosthornins B and C were identified by comparison of the observed and reported NMR and MS data.[12]

On the basis of the cytotoxic activity of *ent*-kauranoids previously obtained from *Isodon* plants,[2] all isolates except **2**, **3**, **12**, and **13** (due to the sample limitation) were evaluated for their in vitro cytotoxicity against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW-480) using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) method as previously reported,[25] with cisplatin and paclitaxel as positive controls. Compound **1** showed significant cytotoxic activity against all the tumor cell lines, with respective IC_{50} values of 1.0, 1.5, 4.4, 2.9, and 0.9 μM, better than that of cisplatin (Table 6). Compounds **4** and **15** showed stronger cytotoxicity against four of the five tumor cell lines than cisplatin, and the remaining eight compounds (**5**−**11**, **14**, and **16**) were noncytotoxic in these tested systems (IC_{50} > 40 μM). Compound **5** differs from **1** in that the C-15 carbonyl group was reduced to a hydroxy group in **5**. The result, along with those previously reported, suggested that the α-exomethylene-cyclopentanone moiety is an important structural requirement for cytotoxicity.[2] Deacetylation at C-19 of **15** resulting in compound **16** led to a dramatic decrease in cytotoxicity.

The excessive production of NO, which is produced by the inducible NO synthase (iNOS) in macrophages and endothelial cells, is responsible for the inflammatory response and implicated in the pathogenesis of several inflammatory diseases.[26] On the basis of the folk use of *I. scoparius*, the active compounds **1**, **4**, and **15** were tested for their ability to inhibit NO production in LPS-stimulated RAW264.7 cells. All the tested compounds showed significant inhibitory effects, with IC_{50} values of 1.0, 3.1, and 1.8 μM, respectively (Table 7).

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were obtained on an XRC-1 apparatus and were uncorrected. Optical rotations were measured on Horiba SEPA-300 and JASCO P-1020 polarimeters. UV spectra were recorded on a Shimadzu UV-2401A spectrophotometer. Experimental ECD spectra were measured on a Chirascan instrument. IR spectra were obtained on a Tenor 27 FT-IR spectrometer with KBr pellets. HRESIMS data were acquired on an Agilent 6540 QSTAR TOF time-of-flight mass spectrometer. NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with tetramethylsilane as internal standard. All chemical shifts (δ) were expressed in ppm relative to the solvent signals. X-ray data were collected on a Bruker APEX DUO diffractometer using Cu Kα radiation. Column chromatography (CC) was performed on silica gel (80−100 mesh and 100−200 mesh; Qingdao Marine Chemical Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40−63 μm, Merck, Darmstadt, Germany), MCI gel (75−150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), and Sephadex LH-20 (Pharmacia, Uppsala, Sweden). Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18} (Agilent, 9.4 mm × 250 mm) column. TLC was carried out on silica gel 60 F_{254} on glass plates (Qingdao Marine Chemical, Inc.), and spots were visualized by UV light (254 nm) and sprayed with 10% H_2SO_4 in ethanol, followed by heating.

Plant Material. The aerial parts of *I. scoparius* were collected in August 2014 from the Yulong snow mountain of Lijiang, Yunnan

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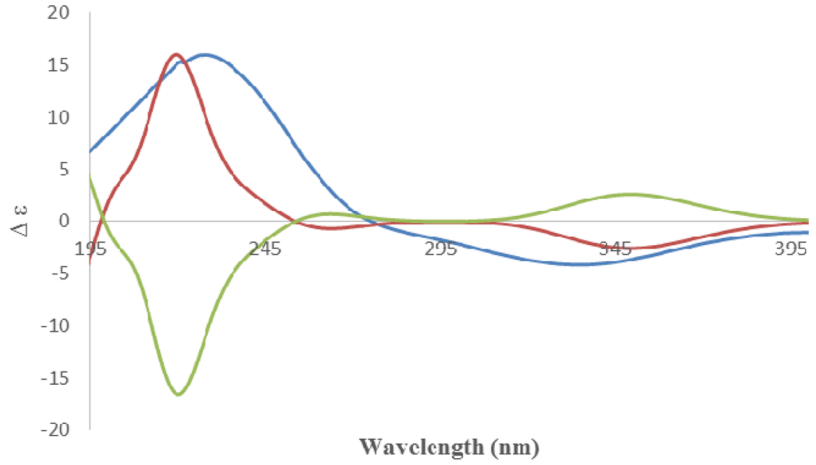

Figure 8. Experimental ECD of **14** (blue), calculated ECD of **14** (red), and calculated ECD of *ent*-**14** (green).

Table 6. Cytotoxic Activities of Diterpenoids from *I. scoparius* against Five Human Tumor Cell Lines[a]

compound	HL-60	SMMC-7721	A-549	MCF-7	SW-480
1	1.0	1.5	4.4	2.9	0.9
4	7.0	4.7	19.6	11.0	2.5
15	3.2	3.9	13.4	4.8	1.3
DDP[b]	3.0	10.2	16.0	15.3	9.3
paclitaxel[b]	<0.008	<0.008	<0.008	<0.008	<0.008

[a]Results are expressed as IC_{50} values in μM. Cell lines: HL-60, acute leukemia; SMMC-7721, hepatie cancer; A-549, lung cancer; MCF-7, breast cancer; SW-480, colon cancer. Compounds **5**−**11**, **14**, and **16** were inactive (IC_{50} > 40 μM) for all the cell lines. [b]DDP (cisplatin) and paclitaxel were used as positive controls.

Table 7. Inhibitory Effects of Compounds 1, 4, and 15 on LPS-Activated NO Production in RAW264.7 Cells

compound	IC_{50} (μM)
1	1.0
4	3.1
15	1.8
L-NMMA[a]	39.9

[a]N^G-Methyl-L-arginine acetate salt was used as a positive control.

Province, People's Republic of China, and identified by Prof. Xi-Wen Li at the Kunming Institute of Botany. A voucher specimen (KIB2014081909) has been deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered aerial parts of *I. scoparius* (10.0 kg) were extracted with 70% aqueous acetone (4 × 35 L, 2 days each) at room temperature and filtered. The filtrate was evaporated under reduced pressure and partitioned by liquid−liquid extraction between EtOAc and H_2O. The EtOAc extract (310 g) was chromatographed over silica gel (1.5 kg, 80−100 mesh), eluted with $CHCl_3$/acetone (1:0−0:1 gradient system). Seven fractions obtained from the silica gel column were individually decolorized using MCI gel and eluted with 90:10 MeOH/H_2O to yield fractions A−G.

Fraction B ($CHCl_3$/acetone, 9:1; 20 g), a brown gum, was subjected to RP-18 silica gel CC (MeOH/H_2O, 35:65−100:0 gradient) to provide seven fractions, B1−B7. Fraction B6 (1.8 g) was purified by repeated silica gel CC (petroleum ether/EtOAc, 30:1−1:1) to give eight subfractions (B6-1−B6-8) based on TLC analysis. Fraction B6-3 (16 mg) was subjected to semipreparative HPLC (MeCN/H_2O, 60:40), to afford compound **3** (1.7 mg, t_R = 18.2 min).

Fraction C (41 g) was further fractionated into eight fractions (C1−C8) by RP-18 silica gel CC (MeOH/H_2O, 30:70−90:10 gradient). Fraction C2 (1.1 g) was subjected to silica gel CC (petroleum ether/acetone, 15:1−1:2 gradient) to provide six subfractions (C2-1−C2-6). Fraction C2-4 was separated by Sephadex LH-20 CC ($CHCl_3$/MeOH, 1:1), followed by semipreparative HPLC (MeCN/H_2O, 45:55), to yield **6** (2.7 mg, t_R = 3.6 min). Fraction C3 (0.9 g) was fractionated by silica gel CC eluted with petroleum ether/acetone (20:1−1:2 gradient) to give six subfractions (C3-1−C3-6). Semipreparative HPLC analysis (MeCN/H_2O, 27:73) of C3-3 afforded compounds **4** (3.6 mg, t_R = 9.4 min), **5** (59.0 mg, t_R = 15.6 min), and **1** (80.0 mg, t_R = 20.5 min). Repeated CC on silica gel, eluted with a petroleum ether/acetone gradient (25:1−1:2), followed by semipreparative HPLC (MeCN/H_2O, 35:65), gave compounds **9** (5.5 mg, t_R = 7.1 min), **10** (2.3 mg, t_R = 13.9 min), and **11** (12.0 mg, t_R = 31.5 min) from C4. Compounds **16** (5.2 mg, t_R = 3.7 min), **13** (1.1 mg, t_R = 8.2 min), and **15** (40.0 mg, t_R = 10.2 min) were obtained from fraction C7 by silica gel CC (petroleum ether/acetone, 30:1−1:1), followed by semipreparative HPLC (MeCN/H_2O, 35:65).

Fraction D (8.7 g) was separated by RP-18 silica gel CC (MeOH/H_2O, 25:75−90:10 gradient) to give fractions D1−D6. Fraction D5 was subjected to repeated silica gel CC, eluted with petroleum ether/acetone (gradient system 7:1−1:2), followed by semipreparative HPLC (MeCN/H_2O, 40:60), to afford compound **2** (1.4 mg, t_R = 7.6 min).

Fraction E (15.0 g) was separated by RP-18 silica gel CC (MeOH/H_2O, 20:80−90:10 gradient) to afford fractions E1−E9. Fraction E5 was purified by silica gel CC ($CHCl_3$/MeOH, 70:1−1:1) to yield compound **7** (5.2 mg). Fraction E6 was separated into six subfractions (E6-1−E6-6) using RP-18 CC (MeOH/H_2O, 30:70−80:20 gradient). Fraction E6-3 was subjected to semipreparative HPLC (MeCN/H_2O, 35:65) to obtain compound **8** (20.0 mg, t_R = 4.1 min). Compounds **12** (1.8 mg, t_R = 8.0 min) and **14** (1.9 mg, t_R = 9.7 min) were obtained from fraction E8 by Sephadex LH-20 CC ($CHCl_3$/MeOH, 1:1) and then by semipreparative HPLC (MeCN/H_2O, 36:64).

3-epi-Isodopharicin A (***1***)*:* colorless, rectangular crystals (MeOH); mp 262−264 °C; $[\alpha]^{19}_D$ −135 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 231 (3.8), 197 (3.5) nm; IR (KBr) ν_{max} 3436, 2926, 1720, 1633, 1452, 1385, 1267, 1075 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS $[M + Na]^+$ *m*/*z* 399.2150 (calcd for $C_{22}H_{32}O_5Na$, 399.2142).

Scopariusol A (***2***)*:* white, amorphous powder; $[\alpha]^{19}_D$ −65 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.7) nm; IR (KBr) ν_{max} 3425, 2926, 2870, 1631, 1466, 1384, 1106, 1049, 968, 871 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS $[M + Na]^+$ *m*/*z* 341.2084 (calcd for $C_{20}H_{30}O_3Na$, 341.2087).

Scopariusol B (***3***)*:* colorless, rectangular crystals (MeOH); mp 206−207 °C; $[\alpha]^{19}_D$ −37 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 201 (2.7), 214 (2.5) nm; IR (KBr) ν_{max} 3437, 2950, 2929, 2866, 1631, 1450, 1382, 1260, 1175, 1112, 1076, 1056, 1038, 818 cm^{-1}; ^1H and

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^{13}C NMR data, see Tables 1 and 2; positive HRESIMS $[M + Na]^+$ m/z 327.2291 (calcd for $C_{20}H_{32}O_2Na$, 327.2295).

Scopariusol C (4): white, amorphous powder; $[\alpha]^{19}_D$ −119 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 232 (3.8), 197 (3.5) nm; IR (KBr) ν_{max} 3439, 2934, 1722, 1647, 1385, 1370, 1265, 1082, 1052, 1022 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS $[M + Na]^+$ m/z 415.2095 (calcd for $C_{22}H_{32}O_6Na$, 415.2091).

Scopariusol D (5): colorless, rectangular crystals (MeOH); mp 216−217 °C; $[\alpha]^{19}_D$ −40 (c 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.7) nm; IR (KBr) ν_{max} 3542, 3517, 3456, 2929, 2876, 1724, 1642, 1448, 1398, 1368, 1258, 1234, 1086, 1050, 1012, 983 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS $[M + Na]^+$ m/z 401.2304 (calcd for $C_{22}H_{34}O_5Na$, 401.2298).

3-epi-Scopariusol D (6): white, amorphous powder; $[\alpha]^{19}_D$ −42 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.7); IR (KBr) ν_{max} 3435, 2935, 2871, 1738, 1638, 1447, 1373, 1241, 1059 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS $[M + Na]^+$ m/z 401.2292 (calcd for $C_{22}H_{34}O_5Na$, 401.2298).

Scopariusol E (7): white, amorphous powder; $[\alpha]^{19}_D$ −37 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.7) nm; IR (KBr) ν_{max} 3425, 2932, 2868, 1705, 1628, 1445, 1385, 1057 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS $[M + Na]^+$ m/z 359.2204 (calcd for $C_{20}H_{32}O_4Na$, 359.2193).

Scopariusol F (8): white, amorphous powder; $[\alpha]^{19}_D$ −50 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.7) nm; IR (KBr) ν_{max} 3341, 2932, 2852, 1702, 1630, 1441, 1385, 1112, 1056 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 3 and 4; positive HRESIMS $[M + Na]^+$ m/z 359.2199 (calcd for $C_{20}H_{32}O_4Na$, 359.2193).

11-O-Acetylscopariusol F (9): white, amorphous powder; $[\alpha]^{19}_D$ −74 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.8) nm; IR (KBr) ν_{max} 3455, 2930, 1724, 1635, 1383, 1244, 1057 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 3 and 4; positive HRESIMS $[M + Na]^+$ m/z 401.2300 (calcd for $C_{22}H_{34}O_5Na$, 401.2298).

Scopariusol G (10): white, amorphous powder; $[\alpha]^{19}_D$ −55 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.7) nm; IR (KBr) ν_{max} 3476, 2934, 1736, 1715, 1632, 1382, 1247, 1060, 1021, 971 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 3 and 4; positive HRESIMS $[M + Na]^+$ m/z 399.2150 (calcd for $C_{22}H_{32}O_5Na$, 399.2142).

Scopariusol H (11): white, amorphous powder; $[\alpha]^{19}_D$ −54 (c 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.8) nm; IR (KBr) ν_{max} 3559, 3494, 2932, 1738, 1634, 1446, 1373, 1246, 1056, 974, 897 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 3 and 4; positive HRESIMS $[M + Na]^+$ m/z 443.2417 (calcd for $C_{24}H_{36}O_6Na$, 443.2404).

Scopariusol I (12): white, amorphous powder; $[\alpha]^{19}_D$ −71 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.5) nm; IR (KBr) ν_{max} 3432, 2930, 2866, 1730, 1634, 1451, 1383, 1250, 1077, 1023, 989 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 5; positive HRESIMS $[M + Na]^+$ m/z 735.4442 (calcd for $C_{42}H_{64}O_9Na$, 735.4443).

Scopariusol J (13): white, amorphous powder; UV (MeOH) λ_{max} (log ε) 203 (3.5); IR (KBr) ν_{max} 3424, 2930, 2869, 1630, 1446, 1384, 1250, 1082, 1054, 1028 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 3 and 4; positive HRESIMS $[M + Na]^+$ m/z 343.2248 (calcd for $C_{20}H_{32}O_3Na$, 343.2244).

Scopariusol K (14): yellow solid; $[\alpha]^{19}_D$ −21 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 226 (3.7); experimental ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 229 (+25.70), 341 (−5.02); IR (KBr) ν_{max} 3432, 2929, 2870, 1633, 1385, 1042 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 3 and 4; positive HRESIMS $[M + Na]^+$ m/z 317.1728 (calcd for $C_{17}H_{26}O_4Na$, 317.1723).

X-ray Crystal Structure Analysis. Crystals of **1**, **3**, and **5** were obtained in MeOH. Intensity data were collected at 100 K on a Bruker APEX DUO diffractometer equipped with an APEX II CCD using Cu Kα radiation. Cell refinement and data reduction were performed with Bruker SAINT. The structures were solved by direct methods using SHELXS-97,[27] and refinements were performed using full-matrix least-squares, with anisotropic displacement parameters for the non-hydrogen atoms. The hydrogen atoms were placed in calculated positions and refined using a riding model. Crystallographic data (excluding structure factor tables) for the reported structures have been deposited with the Cambridge Crystallographic Data Center (CCDC) as supplementary publications no. CCDC 1528177 for **1**, CCDC 1528175 for **3**, and CCDC 1528176 for **5**. Copies of the data can be obtained free of charge from the CCDC, 12 Union Road, Cambridge CB 1EZ, UK [fax: Int. + 44(0) (1223) 336 033; e-mail: deposit@ccdc.cam.ac.uk].

Crystallographic data for 3-epi-isodopharicin A (1): $C_{22}H_{32}O_5$, M = 376.47, a = 6.5935(2) Å, b = 17.1424(4) Å, c = 17.2244(4) Å, α = 90°, β = 90°, γ = 90°, V = 1946.85(9) Å^3, T = 100(2) K, space group $P212121$, Z = 4, μ(Cu Kα) = 0.723 mm^{-1}, 10 717 reflections measured, 3518 independent reflections (R_{int} = 0.0521). The final R_1 values were 0.0489 [$I > 2\sigma(I)$]. The final $wR(F^2)$ values were 0.1267 [$I > 2\sigma(I)$]. The final R_1 values were 0.0491 (all data). The final $wR(F^2)$ values were 0.1269 (all data). The goodness of fit on F^2 was 1.151. Flack parameter = 0.07(7).

Crystallographic data for scopariusol B (3): $C_{20}H_{32}O_2$, M = 304.45, a = 10.6027(2) Å, b = 7.43600(10) Å, c = 21.0823(4) Å, α = 90°, β = 96.4580(10)°, γ = 90°, V = 1651.62(5) Å^3, T = 100(2) K, space group $P21$, Z = 4, μ(Cu Kα) = 0.588 mm^{-1}, 12 135 reflections measured, 5172 independent reflections (R_{int} = 0.0962). The final R_1 values were 0.1227 [$I > 2\sigma(I)$]. The final $wR(F^2)$ values were 0.2865 [$I > 2\sigma(I)$]. The final R_1 values were 0.1370 (all data). The final $wR(F^2)$ values were 0.3106 (all data). The goodness of fit on F^2 was 1.227. Flack parameter = −0.2(2).

Crystallographic data for scopariusol D (5): $C_{22}H_{34}O_5$, M = 378.49, a = 8.1897(2) Å, b = 11.8105(2) Å, c = 20.5092(4) Å, α = 90°, β = 90°, γ = 90°, V = 1983.74(7) Å^3, T = 100(2) K, space group $P212121$, Z = 4, μ(Cu Kα) = 0.710 mm^{-1}, 12 127 reflections measured, 3605 independent reflections (R_{int} = 0.0309). The final R_1 values were 0.0311 [$I > 2\sigma(I)$]. The final $wR(F^2)$ values were 0.0793 [$I > 2\sigma(I)$]. The final R_1 values were 0.0312 (all data). The final $wR(F^2)$ values were 0.0795 (all data). The goodness of fit on F^2 was 1.062. Flack parameter = 0.03(4).

Cytotoxicity Assays. The following human tumor cell lines were used: HL-60 (acute leukemia), SMMC-7721 (hepatic cancer), A-549 (lung cancer), MCF-7 (breast cancer), SW-480 (colon cancer), and HeLa (cervical cancer), which were obtained from ATTC (Manassas, VA, USA). All cells were cultured in RPMI-1640 or DMEM medium (Biological Industries, Kibbutz Beit-Haemek, Israel), which were supplemented with 10% fetal bovine serum (Biological Industries, Kibbutz Beit-Haemek, Israel) at 37 °C in a humidified atmosphere containing 5% CO_2. The cytotoxicity assay was evaluated by the MTS (Promega, Madison, WI, USA) assay.[25] Briefly, cells were seeded into each well of a 96-well cell culture plate. After 12 h of incubation at 37 °C, the test compound (40 μM) was added. After incubation for 48 h, cells were subjected to the MTS assay. Compounds with a growth inhibition rate of 50% were further evaluated at concentrations of 0.064, 0.32, 1.6, 8, and 40 μM in triplicate, with cisplatin and paclitaxel (Sigma, St. Louis, MO, USA) as positive controls. The IC_{50} value of each compound was calculated by Reed and Muench's method.[28]

Nitric Oxide Production in RAW264.7 Macrophages. The murine macrophage cell line RAW264.7 was obtained from Cell Bank of the Chinese Academy of Sciences. RAW264.7 cells were seeded in 96-well cell culture plates (1.5×10^5 cells/well) and treated with serial dilutions of the compounds with a maximum concentration of 20 μM in triplicate, followed by stimulation with 1 μg/mL LPS (Sigma) for 18 h. NO production in the supernatant was assessed by adding 100 μL of Griess reagent (Reagent A and Reagent B, respectively, Sigma). After 5 min of incubation, the absorbance at 570 nm was measured using a microplate reader (Thermo, Waltham, MA, USA). N^G-Methyl-L-arginine acetate salt (L-NMMA, Sigma), a well-known nitric oxide synthase inhibitor, was used as a positive control.[29] The viability of RAW264.7 cells was simultaneously evaluated using the MTS assay to exclude the interference of the cytotoxicity of the test compounds.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00163.

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^{1}H and ^{13}C NMR, DEPT, HSQC, HMBC, COSY, ROESY, HRESIMS, IR, and UV spectra of compounds **1**−**3** and **12**−**14**; ^{1}H and ^{13}C NMR, DEPT, and HRESIMS spectra of compounds **4**−**11**; ECD calculation details of **14**; X-ray data of compounds **1**, **3**, and **5** (PDF)

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Notes

The authors declare no competing financial interest.

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