ent-Clerodanoids from *Isodon scoparius*

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Three new *ent*-clerodane diterpenoids, isoscoparin A, B, and C (**1**–**3**), were isolated from *Isodon scoparius*. Their structures were determined by NMR-spectra analysis. The biogenetic implication of the diterpenes from *Isodon* genus is discussed.

1. Introduction. – *Isodon scoparius* (C. Y. WU et H. W. LI) H. HARA (Labiatae), a rare herbage, was found growing in the rocky mountains in Zhongdiang County of Yunnan Province, P. R. China [1][2]. Similar to other *Isodon*-genus plants [3], *I. scoparius* has been used as an antipyretic in local folk medicine. In previous phytochemical investigations of *Isodon*-genus plants, the tetracyclic diterpenoids, *ent*-kauranes, which frequently exhibited antitumor and antibacterial activities, were shown to be widespread, and besides, tricyclic diterpenoids, *ent*-abietanes, were also found in several *Isodon* plants [4], but bicyclic diterpenoids, such as *ent*-clerodane or *ent*-labdane, were never isolated [3].

Our study on the diterpenoids from the acetone/H_2O 7:3 extract of the whole plant *I. scoparius* led to the discovery of three unprecedented bicyclic diterpenoids, *ent*-clerodanes **1**–**3**. This report describes the structure elucidation of **1**–**3**, and a possible biogenetic implication of *ent*-clerodane.

2. Results and Discussion. – Three new *ent*-clerodane diterpenoids, isoscoparin A–C (**1**–**3**), along with six known compounds, (13*E*)-*ent*-labda-7,13-dien-15-oic acid (**4**) [5–7], pomolic acid (**5**) [8], β-stiosterol (**6**), stigmasterol (**7**), 2-hydroxybenzoic acid (**8**), and genkwanin (**9**) [9] were isolated from *I. scoparius* (*Fig.*).

Isoscoparin A (**1**) was isolated as an optically active colorless gum. The HR-FAB-MS showed a molecular ion at *m*/*z* 361.2367 in agreement with the molecular formula $C_{22}H_{34}O_4$ ($[M-1]^+$; calc. 361.2379), requiring six double-bond equivalents. Further spectral data (^{1}H- and ^{13}C-NMR (*Table*), ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOE) were consistent with the structure of an (acetyloxy)-substituted (13*E*)-cleroda-4(19),13-dien-15-oic acid for **1**. As all diterpenoids found in the *Isodon* genus were of the *ent*-type configuration [3], the biogenesis and negative optical rotation [10] suggested an *ent*-configuration of **1**. Thus, the structure of **1** was established as (11α,13*E*)-11-(acetyloxy)-*ent*-cleroda-4(19),13-dien-15-oic acid, named isoscoparin A.

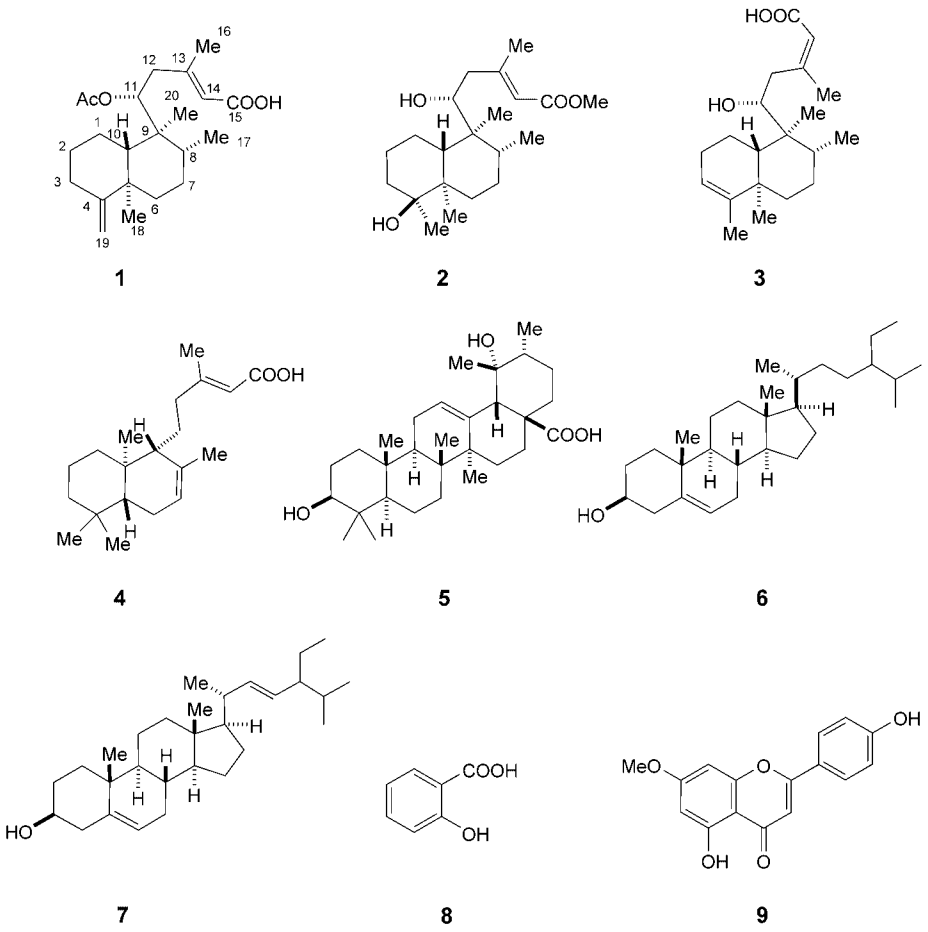


Fig. 1. *Compounds isolated from* Isodon scoparius

The ^{1}H- and ^{13}C-NMR (including DEPT) spectra of **1** indicated the presence of an (acetyloxy) group (δ(C) 174.6, 22.8) and a carboxylic C-atom (δ(C) 169.6), 4 Me (δ(C) 14.1, 16.8, 18.5, and 20.3), 6 CH_2 (δ(C) 23.2, 28.0 (2 C), 32.9, 36.8, and 42.9), and 3 CH groups (δ(C) 36.5, 48.1, and 74.2), 2 quaternary C-atoms (δ(C) 39.0 and 43.7), an exocyclic CH_2=C (δ(H) 4.43 (*s*) and 4.44 (*s*); δ(C) 102.8 (*t*) and 159.8 (*s*)), and a trisubstituted olefinic group (δ(H) 5.56 (*s*); δ(C) 120.6 (*d*) and 169.6 (*s*)). Two C=O and two olefin moieties accounted for four sites of unsaturation, and the lack of NMR signals for further unsaturated functionality indicated the presence of 2 additional rings in **1**. The ^{1}H,^{1}H COSY and HMQC plots revealed the following partial structures; Me(17)–CH(8)–CH_2(7)–CH_2(6), CH_2(3)–CH_2(2)–CH_2(1)–CH(10), and CH(11)–CH_2(12). In the HMBC experiment, cross-peaks appeared for Me(16) (δ(H) 2.06) with C(12) (δ(C) 42.9), C(13) (δ(C) 155.4), and C(14) (δ(C) 120.0), Me(17) (δ(H) 0.77) with C(7) (δ(C) 28.0), C(8) (δ(C) 36.5), and C(9) (δ(C) 43.7), Me(18) (δ(H) 0.97) with C(4) (δ(C) 159.8), C(5) (δ(C) 39.0), C(6) (δ(C) 36.8), and C(10) (δ(C) 48.1), CH_2(19) (δ(H) 4.43 and 4.44) with C(3) (δ(C) 32.9), C(4) (δ(C) 159.8), and C(5) (δ(C) 39.0), Me(20) (δ(H) 0.80) with C(8) (δ(C) 36.5), C(9) (δ(C) 43.7), C(10) (δ(C) 48.1), and C(11) (δ(C) 74.2), CH_2(3) (δ(H) 2.03 and 2.18) with C(1) (δ(C) 23.2), C(4) (δ(C) 159.8), and C(5) (δ(C) 39.0), H–C(14) (δ(H) 5.56) with C(12) (δ(C) 42.9), C(13) (δ(C) 155.4), C(15) (δ(C) 169.6), and C(16) (δ(C) 18.5), and MeCO (δ(H) 1.82) with C(11) (δ(C) 74.2). These NMR data suggested the presence of a clerodane skeleton bearing a CH_2(19)=C(4) and a C(13)=C(14)

Table. *^{13}C-NMR Data* (100 MHz, C_5D_5N) *of Compounds* **1**–**4**. δ in ppm.

	1	**2**	**3**	**4**
C(1)	23.2 (*t*)	22.9 (*t*)	26.4 (*t*)	39.2 (*t*)
C(2)	28.0 (*t*)	23.2 (*t*)	20.1 (*t*)	19.0 (*t*)
C(3)	32.9 (*t*)	38.4 (*t*)	121.1 (*d*)	42.5 (*t*)
C(4)	159.8 (*s*)	85.9 (*s*)	143.6 (*s*)	33.1 (*s*)
C(5)	39.0 (*s*)	44.1 (*s*)	38.7 (*s*)	50.2 (*d*)
C(6)	36.8 (*t*)	28.3 (*t*)	36.7 (*t*)	24.1 (*t*)
C(7)	28.0 (*t*)	33.8 (*t*)	28.0 (*t*)	122.9 (*d*)
C(8)	36.5 (*d*)	36.9 (*d*)	36.9 (*d*)	135.0 (*s*)
C(9)	43.7 (*s*)	43.7 (*s*)	42.6 (*s*)	54.6 (*d*)
C(10)	48.1 (*d*)	41.3 (*d*)	46.9 (*d*)	37.0 (*s*)
C(11)	74.2 (*d*)	76.1 (*d*)	84.2 (*d*)	25.8 (*t*)
C(12)	42.9 (*t*)	44.5 (*t*)	29.9 (*t*)	43.6 (*t*)
C(13)	155.4 (*s*)	160.3 (*s*)	159.0 (*s*)	159.0 (*s*)
C(14)	120.0 (*d*)	117.8 (*d*)	116.7 (*d*)	117.8 (*d*)
C(15)	169.6 (*s*)	167.1 (*s*)	165.4 (*s*)	169.3 (*s*)
C(16)	18.5 (*q*)	19.5 (*q*)	22.7 (*q*)	19.1 (*q*)
C(17)	16.8 (*q*)	17.1 (*q*)	16.6 (*q*)	22.4 (*q*)
C(18)	20.3 (*q*)	16.8 (*q*)	19.9 (*q*)	33.3 (*q*)
C(19)	102.8 (*t*)	27.5 (*q*)	18.3 (*q*)	22.0 (*q*)
C(20)	14.1 (*q*)	14.7 (*q*)	14.1 (*q*)	13.8 (*q*)
Me*C*O	174.5 (*s*)			
*Me*CO	22.8 (*q*)			
MeO		50.6 (*q*)		

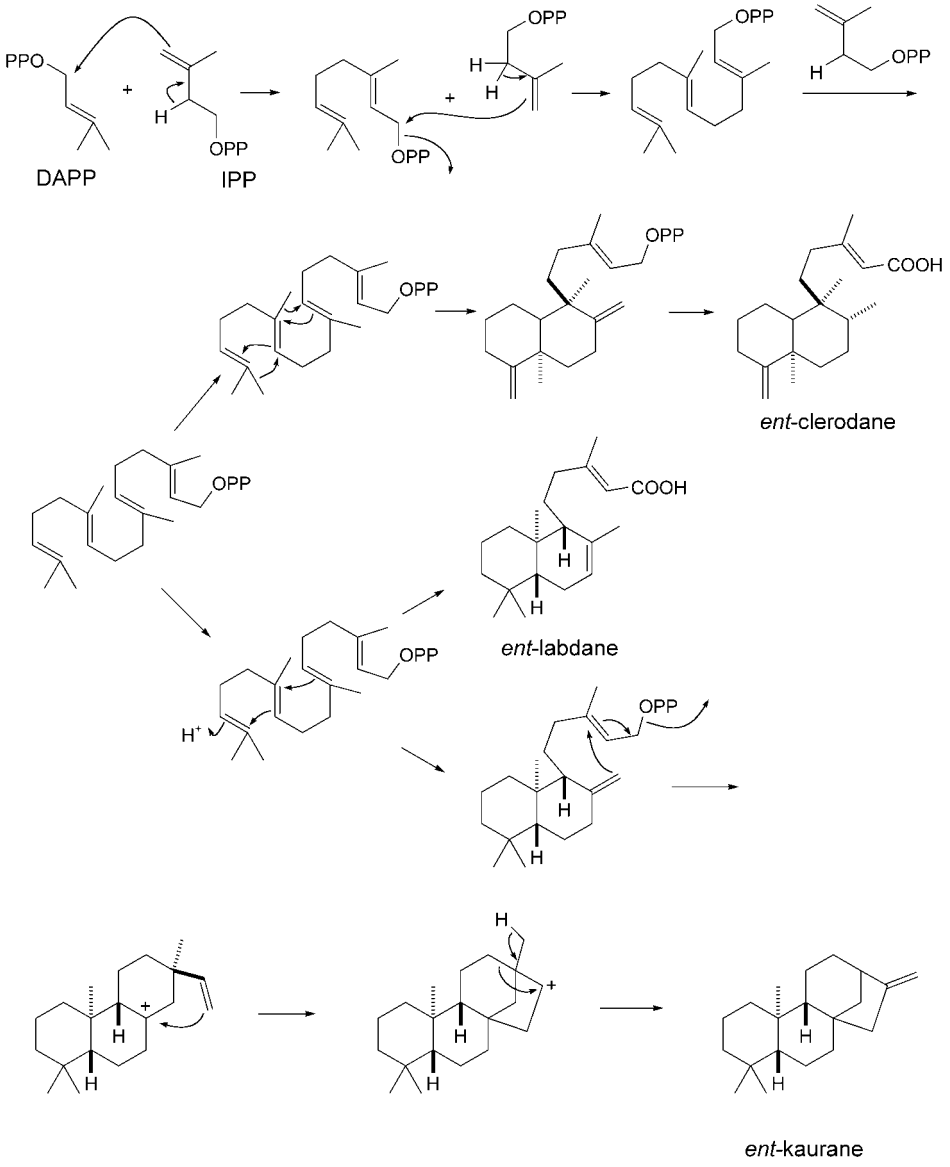
unsaturation, an AcO group at C(11), and a C(15) oxidized to a carboxylic acid function. Furthermore, the NOE correlation H–C(11) (δ(H) 3.52)/H–C(8) (δ(H) 1.32) revealed that AcO was in α-position. A careful analysis of reference compounds [10][11] possessing a C(13)=C(14) bond revealed significant differences in the ^{13}C-NMR data of C(12) and C(16) between the (13*E*)- and (13*Z*)-configuration. In the (*Z*)-type, a *γ-gauche* steric-compression effect between C(15)OR and CH_2(12) shifted C(12) upfield (δ(C) *ca.* 27; note: C(11) was not substituted), and C(16) was unaffected (δ(C) *ca.* 24); similarly, in the (*E*)-type, a *γ-gauche* steric-compression effect between C(15)OR and Me(16) shifted C(16) upfield (δ(C) *ca.* 18), and C(12) was unaffected (δ(C) *ca.* 35; note: C(11) was not substituted). Consequently, the chemical shifts of C(12) (δ(C) 43.7) and C(16) (δ(C) 18.5) revealed a (13*E*)-configuration of **1**, which was supported by the NOE correlation H–C(14) (δ(H) 5.56)/CH_2(12) (δ(H) 2.25 and 1.83).

Isoscoparin B (**2**), an optically active colorless gum, showed an $[M-1]^+$ ion at *m/z* 351.2555 in the HR-FAB-MS, indicating a molecular formula of $C_{21}H_{36}O_4$ ($[M-1]^+$, calc. 351.2535). Similar to compound **1**, the NMR and further spectral data of **2** suggested the presence of an *ent*-clerodane skeleton with an C(13)=C(14) bond. The differences between **1** and **2** were that AcOC(11) of **1** was replaced by OH–C(11) in **2**, that the acid function of **1** was esterified in **2**, and that the exocyclic CH_2=C(4) group of **1** was transformed to a Me–C(4) group in **2**, C(4) being hydroxylated. Thus, compound **2** was elucidated as methyl (4β,11α,13*E*)-4,11-dihydroxy-*ent*-clerod-13-en-15-oate, named isoscoparin B.

In the HMBC experiment with **2**, the observed correlations of Me(19) (δ(H) 1.46) with C(3) (δ(C) 38.4), C(4) (δ(C) 85.9), and C(5) (δ(C) 44.1), and of C(4) (δ(C) 85.9) with CH_2(2) (δ(H) 1.43), CH_2(3) (δ(H) 1.75, and 1.85), CH_2(6) (δ(H) 1.58, and 1.40), H–C(10) (δ(H) 2.30), and Me(18) (δ(H) 0.97) confirmed this deduction. The NOE correlation between Me(19) with Me(18) indicated the β-configuration for OH–C(4).

Isoscoparin C (**3**) was obtained as an optically active colorless gum. Its molecular formula $C_{20}H_{32}O_3$ was revealed by the HR-FAB-MS (*m*/*z* 319.2286 (calc. 319.2273)) and NMR DEPT spectrum. ^{1}H,^{1}H COSY, HMQC, and HMBC experiments also suggested that compound **3** was similar to compounds **2** and **1**, possessing a *ent*-clerodane skeleton with a C(3)=C(4) bond and (13*Z*)-configuration. The structure of

Scheme. *Proposed Biosynthesis of* ent-*Clerodane and* ent-*Kaurane in* Isodon *Plants*



3 was inferred as (11α,13*Z*)-11-hydroxy-*ent*-cleroda-3,13-dien-15-oic acid, named isoscoparin C.

H–C(3) (δ(H) 5.16) of **3** showed cross-peaks with C(1) (δ(C) 26.4), C(5) (δ(C) 38.7), and Me(19) (δ(C) 18.3) in the HMBC spectrum. The chemical shifts of C(12) (δ(C) 29.9) and C(16) (δ(C) 22.7) indicated that the C(13)=C(14) bond had (*Z*)-configuration (see deduction of (*E*)-configuration of **1**), which was supported by the NOESY correlation H–C(14) (δ(H) 5.89)/Me(16) (δ(H) 1.90).

The bioactivity of the *ent*-clerodanes **1**–**3** from *I. scoparius* was not assayed, but some very similar *ent*-clerodanes from *Eetarium microcarpum* showed significant bioactivity as insecticide and insect antifeedant [10].

According to the biogenesis, a bicyclic diterpenoid is a more-primary metabolite then a tricyclic and tetracyclic diterpenoid. Therefore, the actual discovery of bicyclic diterpenoids suggests that *I. scoparius* is a relatively primordial species in the *Isodon* genus, and could enhance comprehension of the biosynthetic pathway of *ent*-kaurane. Herein, we proposed a possible biosynthesis of *ent*-clerodane, *ent*-labdane, and *ent*-kaurane in *Isodon* plants [3] (*Scheme*).

Experimental Part

General. See [12].

Plant Material. The whole plants of *Isodon scoparius* were collected from Baishuitai of Zhongdian County, Yunnan Province, P. R. China, in October 2002. The identity of the plant material was verified by Prof. *Xi-Wen Li*, and a voucher specimen (KIB 02-11-16 Li) has been deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. The dried and powdered aerial parts of *I. scoparius* (2.1 kg) were extracted with Me_2CO/H_2O 7:3 (3 × 2 l) at r.t. for 72 h. The extract was filtered and the filtrate was concentrated and extracted with petroleum ether and AcOEt. The petroleum ether extract (16 g) was subjected to CC (silica gel (300 g), gradient petroleum ether/acetone 1:0, 9:1, 8:2, 7:3, 1:1): *Fractions 1–5*. *Fr. 2–4* were each resubjected to CC (silica gel (50 g), petroleum ether/acetone 9:1 → 6:4): **6** (165 mg), **7** (12 mg), and **5** (19 mg) resp. *Frs. 5* was combined with the AcOEt extract. The AcOEt extract (28 g) was applied to CC (*D101* (200 g), 100% H_2O, 25% MeOH/H_2O, 85% MeOH/H_2O, and 95% MeOH/H_2O); *Fr. 6* (2 g), *Fr. 7* (4 g), *Fr. 8* (18 g), and *Fr. 9* (1 g), *Fr. 6, 7*, and *9* being devoid of diterpenoids (by TLC). *Fr. 8* was subjected to CC (silica gel (300 g), gradient petroleum ether/acetone 1:0, 9:1, 8:2, 7:3, and 4:6): *Fr. 8.1–8.5*. *Fr. 8.2* was subjected to CC (silica gel (50 g), gradient petroleum ether/acetone): **2** (30 mg) and **4** (125 mg), *Fr. 8.3* was subjected to CC (silica gel (50 g), gradient petroleum ether/acetone): **1** (25 mg). *Fr. 8.4* was resubjected to CC (silica gel (50 g), gradient petroleum ether/acetone): **3** (210 mg) and **9** (21 mg). *Fr. 8.5* was resubjected to CC (silica gel (50 g), gradient $CHCl_3$/MeOH): **8** (110 mg).

Isoscoparin A (=*(2*E*,5*R*)-5-(Acetyloxy)-5-[(1*S*,2*R*,4a*R*,8a*S*)-decahydro-1,2,4a-trimethyl-5-methylene-naphthalen-1-yl]-3-methylpent-2-enoic Acid*; **1**): Colorless gum. $[\alpha]_D^{20} = -25.0$ ($c = 0.10$, MeOH). UV (MeOH): 202 (3.5). IR (KBr): 3443, 2922, 1702, 1652, 1237. ^{1}H-NMR (D_6)DMSO, 400 MHz): 1.75, 2.05 (overlapped, each 1 H, CH_2(1)); 1.43, 1.20 (overlapped, each 1 H, CH_2(2)); 2.03 (overlapped, H_α–C(3)); 2.18 (br. *d*, $J = 10.2$, H_β–C(3)); 1.38, 1.47 (overlapped, each 1 H, CH_2(6)); 1.42, 1.73 (overlapped, each 1 H, CH_2(7)); 1.32 (*m*, H_β–C(8)); 1.11 (*dd*, $J = 1.6$, 9.3, H_β–C(10)); 3.52 (br. *d*, $J = 8.5$, H_β–C(11)); 2.25 (br. *t*, $J = 10.9$, H_α–C(12)); 1.83 (overlapped, H_β–C(12)); 5.56 (*s*, H–C(14)); 2.06 (*s*, Me(16)); 0.77 (*d*, $J = 6.0$, Me(17)); 0.97 (*s*, Me(18)); 4.43, 4.44 (2*s*, each 1 H, CH_2(19)); 0.80 (*s*, Me(20)); 1.82 (*s*, MeCO). ^{13}C-NMR: *Table 1*. FAB-MS: 361 (2, $[M-1]^+$), 301 (100). HR-FAB-MS: 361.2367 ($C_{22}H_{33}O_4^+$; calc. 361.2379).

Isoscoparin B (=*(2*E*,5*R*)-5-[(1*S*,2*R*,4a*R*,5*S*,8a*R*)-Decahydro-5-hydroxy-1,2,4a,5-tetramethylnaphthalen-1-yl]-5-hydroxy-3-methylpent-2-enoic Acid Methyl Ester*; **2**): Colorless gum. $[\alpha]_D^{20} = -12.5$ ($c = 0.10$, MeOH). UV (MeOH): 221 (3.6). IR (KBr): 3442, 1609, 1558, 1454. ^{1}H-NMR ((D$_5$)pyridine, 400 MHz): 2.62 (br. *d*, $J = 10.2$, H_α–C(1)); 1.99 (overlapped, H_β–C(1)); 1.43 (overlapped, CH_2(2)); 1.75, 1.85 (overlapped, each 1 H, CH_2(3)); 1.58, 1.40 (overlapped, each 1 H, CH_2(6)); 1.90 (overlapped, CH_2(7)); 1.35 (*m*, H_β–C(8)); 2.30 (*d*, $J = 9.5$, H_β–C(10)); 3.93 (*dd*, $J = 5.1$, 8.0, H_β–C(11)); 2.60 (br. *d*, $J = 9.8$, H_α–C(12)); 2.42 (br. *d*, $J = 9.8$, H_β–C(12));

6.10 (*s*, H–C(14)); 2.37 (*s*, Me(16)); 0.80 (*d*, *J* = 5.0, Me(17)); 0.97 (*s*, Me(18)); 1.46 (*s*, Me(19)); 1.09 (*s*, Me(20)); 3.60 (*s*, MeO). ^{13}C-NMR: *Table*. FAB-MS: 351 (19, $[M-1]^+$), 188 (100). HR-FAB-MS: 351.2555 ($C_{21}H_{35}O_4^+$; calc. 351.2535).

Isoscoparin C (= *(2Z,5*R*)-5-Hydroxy-3-methyl-5-[(1*S*,2*R*,4a*R*,8a*S*)-1,2,3,4,4a,7,8,8a-octahydro-1,2,4a,5-tetramethylnaphthalen-1-yl]pent-2-enoic Acid*; **3**): Colorless gum. $[\alpha]_D^{20} = -75.0$ (*c* = 0.06, MeOH). UV (MeOH): 219 (3.8). IR (KBr): 3527, 2918, 1703, 1646, 1239. ^{1}H-NMR ((D$_5$)pyridine, 400 MHz): 1.82, 1.90 (overlapped, each 1 H, CH_2(1)); 1.56 (overlapped, CH_2(2)); 5.16 (br. *s*, H–C(3)); 1.58 (overlapped, H_α–C(6)); 1.10 (overlapped, H_β–C(6)); 1.23–1.33 (overlapped, CH_2(7)); 1.32 (overlapped, H_β–C(8)); 1.51 (overlapped, H_β–C(10)); 4.02 (*dd*, *J* = 2.8, 12.0, H_β–C(11)); 2.39 (*t*, *J* = 9.8, H_α–C(12)); 2.16 (overlapped, H_β–C(12)); 5.89 (*s*, H–C(14)); 1.90 (*s*, Me(16)); 0.72 (*d*, *J* = 6.3, Me(17)); 0.96 (*s*, Me(18)); 1.52 (*s*, Me(19)); 1.00 (*s*,Me(20)). ^{13}C-NMR: *Table*. FAB-MS: 319 (100, $[M-1]^+$), 127 (92). HR-FAB-MS: 319.2286 ($C_{20}H_{31}O_3^+$; calc. 319.2273).

*(13*E*)-*ent*-Labda-7,13-dien-15-oic Acid* (= *(2*E*)-3-Methyl-5-[(1*R*,4a*R*,8a*R*)-1,4,4a,5,6,7,8,8a-octahydro-2,5,5,8a-tetramethylnaphthalen-1-yl]pent-2-enoic Acid*; **4**). White amorphous powder. ^{1}H-NMR (D$_5$)pyridine, 400 MHz): 6.15 (br. *s*, H–C(14)); 5.41 (br. *s*, H–C(7)); 2.42 (*s*, Me(16)); 1.72 (*s*, Me(17)); 0.88 (*s*, Me(18)); 0.84 (*s*, Me(19)); 0.75 (*s*, Me(20)). ^{13}C-NMR: *Table*.

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Received July 14, 2004