Diterpenoids From the Argentine and Malaysian Liverworts Anastrophyllum and Jungermannia Species

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Abstract

We are engaged in the ongoing investigation into the chemical constituents of liverworts in our search for novel compounds and biologically active substances. In the present study, two new rosane diterpenoids were isolated from the Argentine liverwort Anastrophyllum species, together with known aromadendrane sesqui- and rosane diterpenoids. Two new ent-kaurene and 4 ent-kaurene diterpenoids were isolated from the Malaysian liverwort Jungermannia species. Their structures were determined using nuclear magnetic resonance spectroscopy techniques, circular dichroism spectroscopy, and chemical transformation.

Keywords

liverworts, Anastrophyllum, Jungermannia, rosane, ent-kaurene, diterpenoids, terpenoids

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In our work on the isolation of novel natural organic compounds and evaluation of their biological activity, we are continuing studies on the phytochemicals of liverworts.1-3 We have reported the distribution of sesqui- and diterpenoids from the genus Jungermannia4 and diterpenoids from the Anastrophyllum species.5 We reinvestigated phytochemicals of the unknown Argentine and Malaysian Anastrophyllum and Jungermannia species. Here we wish to report the newly isolated lipophilic terpenoids and their structural elucidation and biological activity. ent-5,15-Rosadien-3-one (1) and (3S)-ent-5,15-rosadien-3-ol (2) were isolated from the ether extract of the unknown Argentine Anastrophyllum species by chromatographic separation, together with two known rosanes, (3S)-ent-3-hydroxy-5,15-rosadien-11-one (3)5 and ent-5,15-rosadiene-3,11-dione (4),6 and a known aromadendrane sesquiterpene ketone, ent-cyclocolorone (5),7,8 as shown in Figure 1. Physical and spectral data were determined by spectral analyses and chemical derivatives.

The stereostructure of compound 3 has already been reported as 3α-hydroxy-5,15-rosadien-11-one by our group from circular dichroism (CD) spectroscopy measurements, which showed a positive Cotton effect (λmax = 297); however, the absolute configuration remains to be clarified.7 Therefore, compound 3 was derived to both (R)- (6) and (S)-2-methoxy-2-trifluoromethylphenylacetic acid (MTPA) (7) esters, and the 1H nuclear magnetic resonance (NMR) spectrum of each derivative was measured.9 The δΔ values in the 1H NMR spectra are shown in Figure 2. The results reveal that the absolute configuration of the hydroxyl group at C-3 is S. Accordingly, the absolute configuration of 3 is (3S)-ent-3-hydroxy-5,15-rosadien-11-one.

The stereostructure of compound 4 isolated from the liverwort Tyrimanthus renifolius has already been reported without its absolute configuration.6 The reduction of 4 with lithium aluminum hydride (LiAlH4) gave a monoalcohol, the CD and the other spectral data of which were identical to those of 3, and diol 8. Thus, the absolute structure of 4 was established to be ent-5,15-rosadiene-3,11-dione, as shown in Figure 1.

Compound 1 showed the presence of a carbonyl group (1713 cm−1) from infrared (IR) spectroscopy measurements. The molecular formula, C20H30O, was determined from electron ionization mass spectrometry (EIMS) analysis (m/z 286 [M]+) and high resolution-EIMS (HR-EIMS; calcld: 286.2297 and found: 286.2299). The 1H NMR spectrum of 1 showed a vinyl group at δ 4.87, 4.94, and 5.82; an olefinic proton at δ 5.64, and 4 tertiary methyls at δ 0.69, 1.04, 1.24, and 1.26. 13C NMR and distortionless enhancement by polarization transfer (DEPT) spectra showed the presence of the ketone carbonyl at δ 215.3, vinyl carbons at δ 109.1 t, 151.3 d, and trisubstituted

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The 1H and 13C NMR spectral data were similar to those for 3 or 4, which suggested that the structure of 1 was that of a rosane diterpenoid. The 1H-1H correlation spectrum (COSY) of 1 indicated the presence of four partial segments, as shown by the bold lines in Figure 3. The heteronuclear multiple bond coherence (HMBC) spectrum (Figure 3) shows the geminal methyl protons (H-18 and H-19) which were correlated with the carbonyl carbon (C-3), quaternary carbon (C-4), and a quaternary carbon (C-5) from the trisubstituted olefin. The methyl proton (H-17) was correlated with two methylene carbons (C-12 and C-14), a quaternary carbon (C-13), and a methine carbon (C-15) from a vinyl group. The other methyl proton (H-20) was also correlated with a methylene carbon (C-11), 2 methine carbon (C-8 and C-10), and a quaternary carbon (C-9). Moreover, the other 1H-13C correlations showed that 1 was the rosane diterpenoid with a ketone at C-3 and a C5=C6 double bond.

In the nuclear Overhauser effect spectroscopy (NOESY) spectrum of 1 shown in Figure 4(a), NOE correlations were observed between: (i) H-18 and H-2β, H-10; (ii) H-10 and
H-1β, H-8, H-11β; (iii) H-19 and H-6; (iv) H-20 and H-1α, H-11α, H-12α and H-14α; and (v) H-17 and H-8, H-11β, H-12β and H-14β. As a consequence, the stereo structure of 1 was determined to be 5,15-rosadien-3-one. The absolute configuration of 1, which is ent-5,15-rosadien-3-one, was confirmed by the positive Cotton effect at 296 nm in the CD spectrum, and application of the back octant rule to 1, as shown in Figure 4(b).10

The 1H NMR spectrum of compound 2 was similar to that of 1, which indicates that 2 was a rosane diterpenoid. The EIMS spectrum of 2 exhibited an M−18 peak at m/z 270 for a loss of H2O. Unexpectedly, chemical ionization mass spectrometry (CIMS) and fast atom bombardment-MS (FAB-MS) measurements in positive mode produced an M−H ion peak at m/z 287. The IR spectrum confirmed the presence of a hydroxyl group (3319 cm−1). 13C NMR and DEPT spectra of 2 showed 20 carbons: 4 methyls, 6 methylenes, 2 methines and 3 quaternary carbons, and oxygenated methine, trisubstituted olefinic and vinyl carbons. From detailed analyses of COSY and HMBC spectra, it was concluded that compound 2 was 5,15-rosadien-3-ol. The NOE correlations were observed between (i) H-19 and H-3α, H-18; (ii) H-18 and H-2β, H-10; and (iii) H-20 and H-1α, H-7α, H-14α. Therefore, the stereochemistry of the hydroxyl group at C-3 was revealed to be a β configuration. Subsequent oxidation of 2 by pyridinium dichromate (PDC) gave a ketone, the physical data (NMR, IR, CD and [α]D) for which were completely identical with those for ent-rosa-5,15-dien-3-one (1). Thus, compound 2 is confirmed to be (3S)-ent-5,15-rosadien-3-ol.

The unknown Malaysian Jungermannia species produced ent-16-kauren-15-one (9),11 (16R)-ent-kauren-16-ol (10),12 ent-16-kauren-15α-ol (11),11 ent-15-oxo-16-kauren-20-oic acid (12)13 and isolated two new ent-15-oxo-16-kauren-20-al (13) and ent-20-hydroxy-16-kauren-15-one (14) as shown in Figure 5. Their known structures were determined as an ent-kaurene series by comparison of their spectral data with those of authentic samples and reference data.1-4

The EIMS spectrum of unstable compound 13 showed m/z 302 [M]+, and the IR spectrum confirmed the presence of two carbonyl groups. (1726 and 1707 cm −1). The molecular formula, C20H28O2 (calcd 300.2089), of 13 was clarified by HR-EIMS analysis. The 1H NMR spectrum of 13 resembled those of ent-kaurene series 9 to 12, except for the presence of an aldehyde proton at δ 10.43. The structure of 13 was determined to be an ent-kaurene series as follows. 13C NMR showed the presence of 20 carbons, including 2 carbonyl and exomethylene carbons.

The 1H-1H COSY spectrum of 13 showed 3 substructures: (i) -CH2-CH2-CH2-, (ii) -CH-CH2-CH2-, and (iii) -CH2-CH2-CH-CH2-. These substructures clarified the correlations between the aldehyde proton at H-20 and C-10, H-5 and the aldehyde carbon at C-20 with the HMBC spectrum, and the other correlations as shown in Figure 6(a). As a result, 13 was revealed to be 16-kauren-15-one possessing an aldehyde group.
bonded at the C-10 position. The NOESY spectrum in Figure 6(b) revealed NOEs between the aldehyde proton at H-20 and H-14α, H-19. Thus, the structure of 13 was determined as 15-oxo-16-kauren-20-al.

The IR spectrum of 14 showed the presence of hydroxyl (3514 cm⁻¹) and carbonyl groups (1726 cm⁻¹). The molecular formula, C₂₀H₃₀O₂ (calcd 302.2246), was clarified by HR-EIMS analysis. The ¹H NMR spectrum resembled those of the ent-kaurene series 9 to 12, except for the loss of one methyl. Moreover, the methylene proton connected to the hydroxyl group was confirmed at δ 4.06 and 4.11. The ¹³C NMR and DEPT spectra showed an exo-methylene, a carbonyl, a methylene carbon connected with a hydroxyl group, 2 tertiary methyls, 8 methylenes, 3 methines, and 3 quaternary carbons. The ¹H-¹H COSY of 14 indicated the presence of 3 partial segments, as shown in Figure 7(a). HMBC correlations were observed between an isolated methylene proton (H-20)-bounded hydroxyl group and C-1, C-5, C-9, and C-10, between an exo-methylene proton (H-17) and C-13, C-16, and ketone carbon at C-15, and the other correlations shown in Figure 7(b). NOE correlations were observed between (i) H-19 and H-3α, H-6α, H-20 and (ii) H-18 and H-3β, H-5, H-6β, as shown in Figure 7(b). Consequently, the structure of 14 was determined to be 20-hydroxy-16-kauren-15-one.

The CD spectrum of 13 and 14 exhibited Cotton effects similar to those in the reported ent-kaurene.¹⁴¹⁵ Thus, the absolute structures of 13 and 14 were established to be ent-15-oxo-16-kauren-20-al and ent-20-hydroxy-16-kauren-15-one, respectively, as shown in Figure 5.

The absolute configuration of 3 that was isolated from the unknown Argentine Anastrophyllum species was re-examined and its absolute structure was determined. The rosane diterpenoids isolated in this study were the same as those isolated from an Anastrophyllum species reported by us.³ The isolation of sphenolobane, fusicoccane, and rosane diterpenoids from the genus Anastrophyllum has been reported to date.¹³ This, therefore, suggests that sphenolobane, fusicoccane, and rosane are characteristic diterpenoids produced by species in the genus Anastrophyllum.

So far, many isolations of ent-kaurane, labdane, rosane, and verticillane diterpenoids have been reported from the genus Jungermannia.⁴ ent-Kaurane diterpenoids were isolated from the present unknown Malaysian Jungermannia species produce. Particularly, it is suggested that ent-kaurane and labdane diterpenoids are characteristic chemical markers of species in the genus Jungermannia.

Compounds 9, 10, 12, and 14 were tested for capase-1 inhibitory activity; however, none of them exhibited strong activity. Combinations of 9 and 10, 9 and 12, 10 and 12, 9 and 14, and 12 and 14 were also tested; however, they also did not exhibit any caspase-1 inhibitory activity. Cytotoxic activity of 9, 10, and 12 was conducted against HL-60 cells. As a result, the
cytotoxic activity of compound 9 was confirmed at 0.1 µg/mL, but no other potent activity was observed. The combination of 9 and 12 was also tested against HL-60 cells; however, the cytotoxic activity was less than that of 9 itself.

**Experimental**

**General**

1H and 13C NMR: 400 and 600 MHz (1H NMR), and 100 and 150 MHz (13C NMR). Chemical shift values are expressed in δ 7.26 (ppm) from CDCl3 as a standard (1H NMR) and δ 77.03 (ppm) from CDCl3 as a standard (13C NMR). Thin layer chromatography: visualized under UV (254 nm) light and by spraying with 15% H2SO4 and Godin’s reagent16 followed by heating at 120°C-130°C. MeOH-CH2Cl2 (1:1) was used for Sephadex LH-20.

**Plant Materials**

Anastrophyllum and Jungermannia were collected in Argentina and Malaysia, respectively. These species were identified by Professor S. R. Gradstein and Dr. T. Furuki. The voucher specimens were deposited at the Laboratory of Natural Products Chemistry, Daiichi University of Pharmacy.

**Extraction and Isolation**

The dried and ground material of the unidentified Anastrophyllum species and Jungermannia species was each extracted with Et2O for 1 month. The crude Et2O extract (1.1 g) of an unidentified Anastrophyllum species collected in Tucuman, Argentina, in 2005, was chromatographed on silica gel (n-hexane-EtOAc gradient) and divided into 8 fractions. Fraction (Fr.) 3 was rechromatographed on Sephadex LH-20, silica gel, and preparative high pressure liquid chromatography (prep. HPLC; Unison UK-silica, n-hexane-EtOAc 19:1) to give ent-5,15-rosadien-3-one (1) (2.6 mg, 0.2%). Fr. 5 was rechromatographed on Sephadex LH-20 with medium pressure liquid chromatography (MPLC; LiChroprep DIOL, n-hexane-EtOAc 9:1) and prep. HPLC (Unison UK-Silica, n-hexane-EtOAc 9:1) to give ent-5,15-rosadien-3,11-dione (4) (66.5 mg, 6.0%), (3S)-ent-3-hydroxy-5,15-rosadien-11-one (3) (72.2 mg, 6.6%), and ent-cyclocolorenone (5) (4.9 mg, 0.4%). (3S)-ent-5,15-Rosadien-3-ol (2) (36.1 mg, 3.2%) was purified by the chromatography on Sephadex LH-20 and silica gel from Fr. 6.

The crude Et2O extract (1.72 g) of the Jungermannia species collected in Cameron Highland, Malaysia, in 2005, was chromatographed on Sephadex LH-20 to give 3 fractions. Frs. 1-2 was rechromatographed on Sephadex LH-20 and silica gel to give ent-16-kauren-15-one (9) (111.2 mg, 6.5%), ent-16-kauren-15α-ol (11) (36.7 mg, 2.1%), and ent-15-oxo-16-kauren-20-al (13) (11.0 mg, 0.6%).

Frs. 1-3 was chromatographed by silica gel and divided into 7 fractions. Frs. 1-3-4 and 1-3-5 were rechromatographed on silica gel and MPLC (Ultra Pack Si-40A, n-Hexane-EtOAc 9:1) to give ent-kauren-16β-ol (10) (31.2 mg, 1.8%) and ent-20-hydroxy-16-kauren-15-one (14) (29.0 mg, 1.7%). The ent-15-oxo-16-kauren-20-oic acid (12) (174.3 mg, 10.1%) was purified from Fr. 2-8-3 by prep. HPLC (ZORBAX BP-SIL n-hexane-EtOAc 17:5).

**ent-5,15-Rosadien-3-One (1)**

[α]D28°: −64.3 (c 0.24, CHCl3).

FTIR νmax cm−1: 1713.

1H NMR (600 MHz, CDCl3): δ 1.59 (1H, m, H-1α), 1.99 (1H, dddd, J = 13.5, 5.5, 5.5 Hz, H-1β), 2.40 (1H, ddd, J = 15.4, 5.5, 5.5 Hz, H-2α), 2.53 (1H, ddd, J = 15.4, 13.5, 5.5 Hz, H-2β), 5.64 (1H, dd, J = 5.6, 2.2 Hz, H-6), 1.69 (1H, m, H-7α), 1.83 (1H, dddd, J = 17.7, 5.1, 5.1, 5.1, 2.5 Hz, H-7β), 1.62 (1H, m, H-8), 2.24 (1H, m, H-10), 1.71 (1H, m, H-11α), 1.35 (1H, ddd, J = 13.5, 13.5, 3.8 Hz, H-11β), 1.52 (1H, ddd, J = 13.5, 13.5, 3.8 Hz, H-12α), 1.27-1.31 (2H, m, H-12β and H-14α), 1.16 (1H, ddd, J = 13.5, 3.8, 2.5 Hz, H-14β), 5.82 (1H, dd, J = 17.6, 10.8 Hz, H-15), 4.87 (1H, dd, J = 10.8, 1.4 Hz, H-16), 4.94 (1H, dd, J = 17.6, 1.4 Hz, H-16), 1.04 (3H, s, H-17), 1.24 (3H, s, H-19), 1.26 (3H, s, H-18), 0.69 (3H, s, H-20); 13C NMR: Table 1.

EIMS m/z (rel. int.): 286 [M]+ (53), 271 (12), 243 (5), 229 (21), 229 (6), 173 (4), 149 (12), 138 (100), 136 (15), 133 (10), 125 (58), 115 (53), 103 (45), 81 (43), 70 (37), 59 (46), 47 (43), 35 (40), 29 (35), 27 (30), 19 (23), 17 (22), 13 (15), 11 (13), 9 (11), 7 (14), 5 (12), 3 (16), 1 (22).

Figure 7. (a) 1H-1H COSY (bold lines) and HMBC correlations (arrows) for 14. (b) Important NOE correlations for 14.
Table 1. $^{13}$C NMR Chemical Shifts for the 20 Carbon Atoms in Compounds 1, 2, 8, 13, and 14 (CDCl$_3$, 100 MHz Except Where Indicated).

| 1  | 2$^a$ | 8  | 13 | 14$^a$ |
|----|------|----|----|-------|
| 1  | 22.9 | 23.5 | 22.7 | 34.1 | 34.4 |
| 2  | 38.2 | 30.2$^b$ | 30.0 | 19.0 | 18.4 |
| 3  | 215.3 | 76.9 | 76.8 | 41.6 | 41.7 |
| 4  | 50.7 | 41.7 | 41.8 | 33.8 | 33.1 |
| 5  | 143.6 | 145.0 | 145.2 | 55.0 | 56.0 |
| 6  | 120.3 | 118.7 | 117.8 | 17.9$^b$ | 18.4 |
| 7  | 30.5 | 30.3$^b$ | 30.5 | 30.0 | 33.6 |
| 8  | 36.1 | 36.0 | 29.9 | 52.2 | 52.3 |
| 9  | 35.4 | 34.7 | 38.5 | 54.1 | 52.7 |
| 10 | 47.5 | 46.3 | 37.1 | 54.2 | 43.1 |
| 11 | 34.2 | 34.3 | 72.4 | 18.0$^b$ | 18.9 |
| 12 | 32.5 | 32.3 | 39.4 | 33.0 | 31.5 |
| 13 | 36.6 | 36.4 | 35.6 | 38.0 | 38.2 |
| 14 | 39.1 | 38.9 | 39.1 | 39.0 | 37.6 |
| 15 | 151.3 | 151.4 | 151.3 | 209.1 | 211.3 |
| 16 | 109.1 | 108.7 | 108.7 | 149.2 | 150.0 |
| 17 | 22.5 | 22.4 | 25.8 | 115.1 | 114.0 |
| 18 | 29.2 | 21.6 | 24.4 | 31.9 | 34.0 |
| 19 | 23.8 | 24.3 | 21.9 | 20.8 | 22.3 |
| 20 | 11.9 | 12.5 | 13.1 | 207.1 | 61.3 |

$^a$Measured at 150 MHz.
$^b$Values in vertical columns may be interchanged.

### FAB-MS $m/z$ 287 [M− H]$^+$. HR-FAB-MS: calcd for C$_{20}$H$_{31}$O: 287.2375; found: 287.2376.

### Preparation of (R)- and (S)-MTPA Esters of 3

(R)-MTPA (5 mg), dicyclohexylcarbodiimide (5 mg), and 4-(dimethylamino)pyridine (8 mg) were added to compound 3 (8.8 mg) in CH$_2$Cl$_2$ (1.5 mL), and the mixture allowed to stand at room temperature overnight. The reaction mixture was chromatographed on Sephadex LH-20, and purified by prep. HPLC (Unison UK-silica, n-hexane-EtOAc 9:1) to give (R)-MTPA ester 6 (6.5 mg). Compound 3 (13.7 mg) was treated with (S)-MTPA (5 mg) in the same manner to afford (S)-MTPA ester 7 (4.5 mg).

### (R)-MTPA Ester 6

1H NMR (600 MHz, CDCl$_3$): δ 1.109 (1H, ddd, $J = 13.0, 13.0, 13.0$, 3.7 Hz, H-1α), 2.048 (1H, dq, $J = 13.0, 3.7$ Hz, H-1β), 1.984 (1H, m, H-2α), 1.871 (1H, m, H-2β), 4.6965 (1H, dd, $J = 11.5, 4.5$ Hz, H-3α), 5.582 (1H, m, H-6), 1.991 (1H, m, H-7α), 1.880 (1H, m, H-7β), 1.766 (1H, m, H-8), 2.753 (1H, m, H-10), 2.725 (1H, d, $J = 13.0$ Hz, H-12a), 1.9555 (1H, dd, $J = 13.0, 2.3$ Hz, H-15), 1.757 (1H, m, H-14α), 1.359 (1H, br dd, $J = 9.9, 2.3$ Hz, H-14β), 5.8265 (1H, dd, $J = 17.4, 10.7$ Hz, H-15), 4.9435 (1H, dd, $J = 10.7, 0.8$ Hz, H-16), 4.961 (1H, dd, $J = 17.4, 0.8$ Hz, H-16), 0.937 (3H, s, H-17), 0.968 (3H, s, H-19), 1.024 (3H, s, H-18), 0.996 (3H, s, H-20).

### (S)-MTPA Ester 7

1H NMR (600 MHz, CDCl$_3$): δ 1.106 (1H, ddd, $J = 13.0, 13.0, 13.0$, 3.7 Hz, H-1α), 2.0195 (1H, dq, $J = 13.0, 3.7$ Hz, H-1β), 1.912 (1H, m, H-2α), 1.752 (1H, m, H-2β), 4.6665 (1H, dd, $J = 11.6, 4.5$, 3.7 Hz, H-3α), 5.595 (1H, m, H-6), 1.989 (1H, m, H-7α), 1.883 (1H, m, H-7β), 1.763 (1H, m, H-8), 2.7335 (1H, m, H-10), 2.7215 (1H, d, $J = 12.5$ Hz, H-12a), 1.9495 (1H, dd, $J = 12.5, 2.3$ Hz, H-15), 1.757 (1H, m, H-14α), 1.3575 (1H, br dd, $J = 9.9, 2.3$ Hz, H-14β), 5.8245 (1H, dd, $J = 17.4, 10.7$ Hz, H-15), 4.9415 (1H, dd, $J = 10.7, 0.8$ Hz, H-16), 4.959 (1H, dd, $J = 17.4, 0.8$ Hz, H-16), 0.963 (3H, s, H-17), 1.067 (3H, s, H-19), 1.024 (3H, s, H-18), 0.994 (3H, s, H-20).

### Oxidation of 2

Compound 2 (4.0 mg) was oxidized by PDC (15 mg) in dry CH$_2$Cl$_2$ (10 mL) overnight at room temperature. The reaction mixture was filtered through a short column packed with silica gel to give a crude oil, which was chromatographed on Sephadex LH-20 and purified by prep. HPLC (Unison UK-silica, n-hexane-EtOAc 17:3) to afford compound 1 (2.0 mg). The spectral data for derivative 1 were identical with those for ent-5,15-rosadien-3-one.
Reduction of 4

Compound 4 (23.6 mg) in dry Et₂O (2 mL) was added to a suspension of LiAlH₄ (8 mg) in dry Et₂O (2 mL) and stirred for 50 minutes at room temperature. A few drops of H₂O was added to the reaction solution to obtain a crude oil, which was chromatographed using MPLC (LiChroprep Si60, n-hexane-EtOAc 4:1) and finally prep. HPLC (Unison UK-Silica, n-hexane-EtOAc 7:3) to give monoalcohol 3 (5.8 mg) and diol 8 (10.2 mg). The spectral data for 3 were identical with those for (3γ)-ent-3-hydroxy-5,15-rosadien-11-one, including Cotton effects (CD [MeOH]: $\Delta \varepsilon_{296nm} = 2.37$, $\Delta \varepsilon_{231nm} = 1.29$ ($c = 1.10 \times 10^{-2}$)).

**ent-5,15-Rosadiene-3β,11β-Diol (8)**

$[\alpha]^{D}_{25} = +33.1 (c 1.52, CHCl₃).$

FTIR $\nu_{max}$ cm⁻¹: 3431.

$^1$H NMR (600 MHz, CDCl₃): $\delta$ 1.14 (1H, dddd, $J = 13.3, 13.3, 4.0$ Hz, H-1α), 1.73 (1H, m, H-1β), 1.80 (1H, m, H-2α), 1.59 (1H, m, H-2γ), 3.25 (1H, dd, $J = 11.5, 4.0$ Hz, H-3), 5.38 (1H, m, H-6), 1.71 (1H, m, H-7), 1.85 (1H, m, H-8), 1.84 (1H, m, H-9), 2.68 (1H, br d, $J = 13.2$ Hz, H-10), 3.80 (1H, t, $J = 3.2$ Hz, H-11), 1.72 (1H, m, H-12α), 1.50 (1H, dt, $J = 14.8, 3.2$ Hz, H-12β), 1.32 (1H, t, $J = 13.6$ Hz, H-14α), 1.21 (1H, m, H-14γ), 5.75 (1H, dd, $J = 17.6, 10.8$ Hz, H-15), 4.84 (1H, dd, $J = 10.8, 1.2$ Hz, H-16), 4.89 (1H, dd, $J = 17.4, 1.2$ Hz, H-16), 1.22 (3H, s, H-17), 1.15 (3H, s, H-18), 0.99 (3H, s, H-19), 0.64 (3H, s, H-20).

$^1$C NMR: Table 1.

EIMS $m/z$ (rel. int.): 286 [M −18]+ (100), 268 (88), 253 (86), 225 (92), 211 (22), 189 (85), 185 (50), 171 (73), 157 (47), 143 (37), 131 (42), 119 (44), 105 (45), 91 (34), 79 (18), 69 (16), 69 (8), 55 (23), 41 (20).

FAB-MS $m/z$: 327 [M+Na]+, 343 [M+K]+.

HR-FAB-MS: calcd for C$_{20}$H$_{28}$O$_2$: 300.2089; found: 300.2086.

**ent-20-Hydroxy-16-Kauren-15-One (14)**

$[\alpha]^{D}_{25} = -147.4 (c 2.21, CHCl₃).$

FTIR $\nu_{max}$ cm⁻¹: 3514, 1726.

$^1$H NMR (600 MHz, CDCl₃): $\delta$ 2.16 (1H, m, H-1α), 0.65 (1H, ddd, $J = 13.5, 13.5, 3.8$ Hz, H-1β), 1.50-1.57 (2H, m, H-2x and 12β), 1.42-1.48 (3H, m, H-2β, 3α and 14β), 1.20 (1H, ddd, $J = 13.0, 13.0, 4.4$ Hz, H-3β), 1.08 (1H, dd, $J = 12.6, 2.0$ Hz, H-5), 1.33-1.41 (2H, m, H-6α, 7α), 1.58 (1H, m, H-6β), 2.03 (1H, ddd, $J = 13.9, 13.9, 4.9$ Hz, H-7β), 1.32 (1H, m, H-9), 1.41 (1H, m, H-11α), 1.81 (1H, dd like, $J = 15.2, 5.9$ Hz, H-11β), 2.19 (1H, ddd, $J = 12.8, 5.9, 2.7$ Hz, H-12α), 3.03 (1H, br s, H-13), 2.56 (1H, d, $J = 11.8$ Hz, H-14β), 5.24 (1H, t, $J = 1.2$ Hz, H-17), 5.92 (1H, t, $J = 1.2$ Hz, H-17), 0.90 (3H, s, H-18), 0.86 (3H, s, H-19), 4.06 (1H, d, $J = 12.6$ Hz, H-20), 4.11 (1H, d, $J = 12.6$ Hz, H-20).

$^1$C NMR: Table 1.

EIMS $m/z$ (rel. int.): 302 [M]+ (86), 284 (24), 271 (100), 253 (51), 245 (79), 243 (81), 215 (19), 201 (48), 189 (22), 175 (40), 161 (21), 157 (26), 147 (28), 131 (27), 119 (29), 105 (45), 91 (67), 79 (43), 69 (32), 55 (25), 41 (27).

HR-EIMS: calcd for C$_{20}$H$_{32}$O$_2$Na: 322.2246; found: 322.2248.

UV (MeOH): $\lambda_{max}$ (log $\varepsilon$): 236 nm (3.84) ($c 4.44 \times 10^{-4}$).

CD (MeOH): $\Delta \varepsilon_{217nm} = 8.31$, $\Delta \varepsilon_{349nm} = 0.77$ ($c 4.44 \times 10^{-4}$).

Bioassay of the Isolated Compounds

Capase-1 inhibitory and cytotoxic activity tests against HL-20 cells of the isolated compounds were conducted by Professor Dr T. Ito, Miss S. Nakamatsu, and Dr Y. Yagi (Tokushima Bunri University), respectively.

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Declaration of Conflicting Interests

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