New sesquiterpenes from the Okinawan red alga Laurencia luzonensis

New sesquiterpenes from the Okinawan red alga *Laurencia luzonensis*

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Abstract

Compounds **1** and **2** possessing a new rearranged snyderane skeleton together with three new sesquiterpenes **5**, **6** and **7** have been isolated from the red alga *Laurencia luzonensis* and their structures elucidated by the analysis of 1D and 2D NMR spectroscopic experiments.

Introduction

The red algal genus *Laurencia* (Rhodomelaceae, Ceremiales) is marked by species discrimination due to complications arising by the high degree of morphological variation within individual species.[1] As it is well documented, species of *Laurencia* are the most prolific producers of halogenated secondary metabolites with diverse and unique structural features depending on species, localities and season.[1,2] A large number of these compounds have been found from *Laurencia* species having an intracellular refractile inclusion known as "corps en cerise" which is considered to be the site of synthesis and or storage of halogenated secondary metabolites.[3] *Laurencia* species without "corps en cerise" does not produce any halometabolites.[4-6]

The previously uninvestigated *Laurencia luzonensis*, which thrives abundantly on the coral reefs of Okinawa seasonally, produces sesquiterpenes mostly of the snyderane skeleton,[1] though rare diterpenes have been reported.[7-10] In the continuing study of *L. luzonensis*, new sesquiterpenes **1**, **2**, **5-7**, two of which present a new skeleton as a result of a 1,2 methyl migration, and their related known compounds have been isolated and their structures elucidated.

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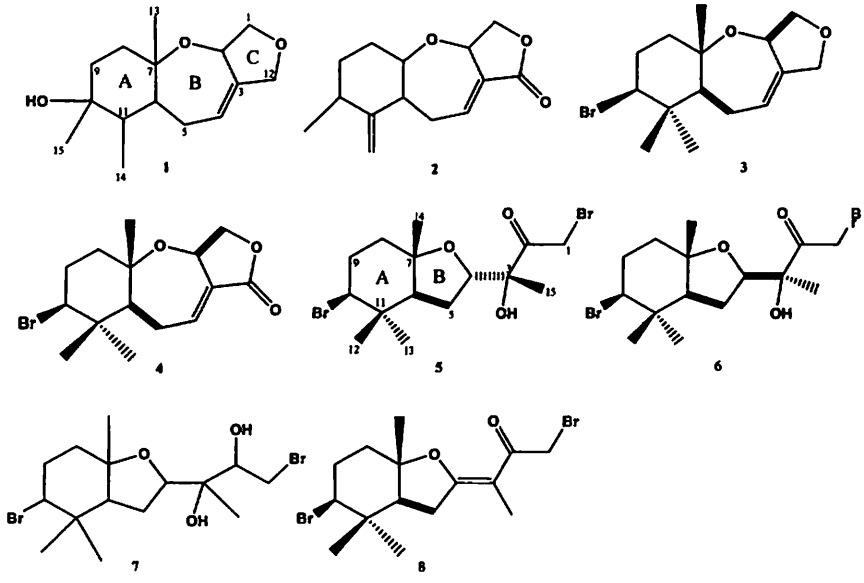
Results and discussion

Compound **1** was isolated as colorless oil. Comparison of its NMR data with those of palisadin A (**3**)[8,9] revealed their close structural similarity. The lack of the bromo methine (C-10) coupled with the oxy-quaternary carbon at δ_C 71.0 and the doublet methyl peak at δ_H 0.98 (C-14) suggested a methyl rearrangement in ring A. This rearrangement was confirmed by HMBC data, which displayed a 1,2-methyl shift from C-11 to C-10 and the presence of a hydroxy group at C-10. To the best of our knowledge this is the first non-halogenated compound with a rearranged snyderane skeleton from the *Laurencia* genus.
Compound **2** was isolated as a colorless amorphous solid. Comparison of its NMR data with those of aplystatin (**4**)[8,9] revealed structural similarities for rings B and C. From the two singlets at δ_H 4.92, 4.56 and δ_C 108.2, a terminal methylene was deduced while the sp^2 quaternary carbon at δ_C 153.4 and a doublet methyl peak at δ_C 19.4 suggested a similar methyl rearrangement as in compound **1**. From the HMBC data, a 1,2-methyl shift from C-11 to C-10 was confirmed. Though this rearrangement is similar to the one observed in compound **1** the mechanistic pathway seems different. The rearrangement in compound **2** can be easily rationalized as debromination, followed by a 1,2-methyl migration and finally deprotonation while in compound **1** a more complicated mechanism is employed after debromination that we are still to determine.

Chart 1



3*R**, 4*S**-Luzonolone (**5**) was isolated as a colorless oil. The ^{13}C NMR spectrum contained resonances characteristic of a ketone (δ_C 204), three oxy-carbons (δ_C 80.2, 80.6 and 81.0) and a secondary bromo-function (δ_C 65.1). From the DEPT spectrum it was established that the molecule consists of four methyls, four methylenes, three methines and four quaternary carbons. Comparison of the NMR data with those of luzonenone (**8**),[10] revealed similarity in the fusion of the cyclohexane ring onto the five-membered cyclic ether by HMBC data. The downfield shifted bromo methyl protons (δ_H 4.24, 4.43), a quaternary carbon (δ_C 80.6) and methyl (δ_H 1.37, δ_C 22.1) indicated the presence of a hydroxyl group (δ_H 3.35) at C-3. From the HMBC data the connectivity of the fragment comprising a bromo methyl, ketone, oxy-quaternary carbon, hydroxyl and methyl was established together with its attachment to the five-member cyclic ether ring at C-4. The relative stereochemistry of the ring portions was determined by NOE measurements (H-13/ H-6, H-6/H-10, H-12/H-14) while the configurations at C-4 and C-3 were established from NOE correlations (H-14/H-4, H-4/H-15, H-15/H-1) and lack of correlation (OH/H-4). The conformation at C-4 locates the bulk fragment at the stable equatorial position while at C-3 the configuration permits the hydroxyl proton to form a hydrogen bond with the ether ring oxygen that hinders the free rotation of the sigma bond between C-3 and C-4.

3*S**, 4*R**-Luzonolone (**6**) was isolated as a colorless oil. From the comparison of the NMR data to those of **5**, it was established that these two compounds were isomers. The configurations at C-3 and C-4 differed as determined by NOE correlations (H-14/OH, H-4/H-15, H-15/H-1). The conformation at C-4 locates the bulky fragment to the unstable axial position while at C-3 the configuration is inverted unlike in **5** to allow a hydrogen bond to form between the hydroxyl proton and the ether ring oxygen. This intramolecular hydrogen bonding allows the unstable conformation at C-4 to exist, which is further supported by the small quantity of this isolated compound as compared to the relatively stable **5**.

Luzondiol (**7**) was isolated as colorless oil. Comparison of its NMR data with those of **5** revealed structural similarities. From the DEPT spectrum it was established the molecule consisted of four methyls, four methylenes, four methines and three quaternary carbons. Lack of the carbonyl resonance and the presence of an oxymethine at δ_C 74.2, δ_H 3.87 suggested the reduction of the ketone functionality to hydroxyl. HMBC data further confirmed this, thus determining that luzondiol is a reduced form of luzonolone.

This preliminary results is part of the on going work on isolation of biological active metabolites from this alga and at the moment these new compounds are being further subjected to other spectroscopic techniques to determine their stereochemistry and molecular weight precisely. In addition to this various bioassays like antifungal, antibacterial and brine shrimp lethality test will be carried out on these compounds to

offer a more concrete contribution to an international journal.

Experimental Section

General experimental procedures. NMR spectra were recorded on a JEOL 500 MHz FT NMR spectrometer with TMS as an internal standard. HPLC was performed on a JASCO 880 PU (pump) and 830 RI (refractometer) using the normal phase LiChrosorb Si60 (7um) column. Cica-MERCK silica gel (Si-60, mesh 200~300) was used for open column chromatography. MERCK 25 TLC plates 20x20 cm silica gel 60 F254 thin-layer chromatography sheets were used for TLC.

Plant material. The red alga *Laurencia luzonensis* Masuda (Rhodomelaceae) was collected on the reef of Sesoko Island, Okinawa, in October 2004. After washing it with tap water and air dried for 2 days the partially dry material (1.8kg) was obtained.

Extraction and Isolation. The combined extracts, after quadruple extraction with methanol, were concentrated and the residue partitioned between EtOAc and H_2O. The organic layer (EtOAc) gave an oil (6.8g) that was subjected to open column chromatography using silica gel and a stepped gradient elution of hexane, EtOAc, and MeOH to give 11 fractions. Fraction 4 was further subjected to separation on silica gel using the same solvent system to give four subfractions. The first subfraction (188 mg) was purified twice by HPLC (Si60, 4% and 10 % EtOAc/hexane) to give palisadin B (57.5 mg),[8,9] a-epoxypalisadin B (3.1mg)[10] and a mixed fraction. The mixed fraction was further purified by HPLC using 15% EtOAc/hexane to give compounds **7** (1.2mg),[5] (2.5mg), **6** (2.5mg) and **5** (16.9mg). Fraction 6 (263.9mg) was separated on silica gel and purified twice by HPLC (Si60, 25% and 35% EtOAc/hexane) to give compounds luzonensol (19.4mg),[11] palisadin A (27.3mg),[8,9] **2** (5.8mg) and **4** (47.8mg). Fraction 9 (299.2mg) was subjected to another silica gal separation (20% EtOAc/hexane) followed by HPLC purification (Si60, 2:1:2 EtOAc/CHCl3/hexane) to afford compound **1** (10.5mg).

Compound **1**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.98 (3H d, J = 6.5 Hz), 1.20 (3H, s), 1.22 (3H, s), 1.34 (1H, m), 1.38 (1H, m), 1.62 (1H, m), 1.74 (1H, m), 2.13 (1H, m), 2.14 (1H, m), 2.29 (1H, m), 2.33 (1H, m), 3.49 (1H, t, J = 8.5 Hz), 4.09 (1H, t, J = 8.5 Hz), 4.33 (1H, dd, J = 2.0 Hz, 12.5 Hz), 4.41 (1H, dd, J = 2.0 Hz, 12.5 Hz), 4.99 (1H, brs), 5.52 (1H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ 12.6, 19.6, 26.9, 29.5, 32.5, 38.2, 41.9, 43.7, 69.8, 71.0, 71.2, 72.4, 79.0, 121.0, 141.8.

Compound **2**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.09 (3H, s), 1.14 (3H, d, J = 7.5 Hz), 1.45 (1H, m), 1.55 (1H, m), 1.74 (1H, m), 2.12 (1H, m), 2.54 (1H, m), 2.61 (1H, m), 2.65 (1H, m), 3.03 (1H, dd, J = 2.0 Hz, 11.5 Hz), 3.93 (1H, dd, J = 7.0 Hz, 9.0 Hz), 4.55 (1H, dd, J = 7.0 Hz, 9.0 Hz), 4.56 (1H, s), 4.92 (1H, s) 5.32 (1H, m). ^{13}C NMR (125 MHz, $CDCl_3$)

δ 19.0, 19.4, 28.7, 29.1, 32.1, 38.0, 42.8, 66.8, 69.9, 81.3, 108.2, 131.5, 142.8, 153.4, 169.4.

3*R**, 4*S**-Luzonolone (5). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.93 (3H, s), 1.05 (3H, s), 1.15 (3H, s), 1.37 (3H, s), 1.45 (1H, m), 1.46 (1H, m), 1.80 (1H, m), 1.89 (1H, m), 2.02 (1H, m), 2.23 (1H, m), 3.35 (OH, s), 3.87 (1H, dd, *J* = 4.5 Hz, 12.5 Hz), 4.24 (1H, dd, J = 4.5 Hz, 8.5 Hz), 4.24 (1H, d, J = 15 Hz), 4.43 (1H, d, J = 14.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.8, 20.1, 22.1, 25.5, 30.1, 32.6, 32.7, 38.8, 39.0, 55.8, 65.1, 80.2, 80.6, 81.0, 204.7.

3*S**, 4*R**-Luzonolone (6). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.96 (3H, s), 1.10 (3H, s), 1.19 (3H, s), 1.32 (3H, s), 1.48 (1H, m), 1.68 (1H, m), 1.78 (1H, m), 1.85 (1H, m), 2.01 (1H, m), 2.01 (1H, m), 2.25 (1H, m), 3.33 (OH, s), 3.85 (1H, dd, *J* = 5.0 Hz, 13 Hz), 4.25 (1H, dd, *J* = 4.5 Hz, 12.5 Hz), 4.28 (1H, d, *J* = 14 Hz), 4.36 (1H, d, *J* = 14 Hz). ^{13}C NMR (125 MHz, CDCl3) δ 17.7, 22.1, 23.8, 25.3, 30.5, 32.1, 33.4, 38.9, 40.2, 65.0, 79.6, 80.8, 82.6, 204.7.

Luzondiol (7). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.90 (3H, s), 1.18 (3H, s), 1.24 (3H, s), 1.28 (3H, s), 1.50 (1 H, d, *J* = 10 Hz), 1.73 (1H, m), 2.03 (1H, m), 2.07 (1H, m), 2.17 (1H, m), 2.27 (1H, m), 2.15 (1H, m), 2.20 (OH, s) 3.87 (1H, dd, *J* = 4.5 Hz, 12.0 Hz), 3.87 (1H, brs), 3.25 (1H, t, *J* = 10.0 Hz), 3.93 (1H, dd, *J* = 2.5 Hz, 10.0 Hz), 4.07 (1H, dd, *J* = 3.5 Hz, 12.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.1, 18.1, 22.9, 30.7, 32.9, 33.3, 34.3, 39.3, 41.3, 53.3, 65.0, 70.5, 74.2, 77.1, 77.4.

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