Enshuol, a Novel Squalene-derived Pentacyclic Triterpene Alcohol from a New Species of the Red Algal Genus *Laurencia*

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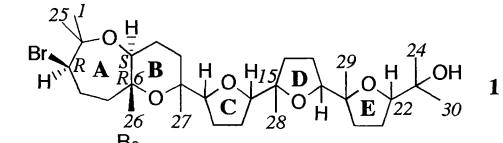
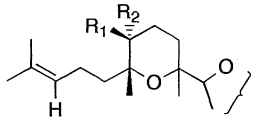
The structure of a novel brominated triterpenoid, designated enshuol, from the red alga *Laurencia omaezakiana* Masuda sp. ined. (Enshu-sozo) was elucidated by spectroscopic analysis and chemical reactions of a degradation product.

Our continuous investigations on constituents of species of the genus *Laurencia* have established the structures of many interesting halogenated products, particularly terpenoids and C_{15}-acetogenins. As part of our chemotaxonomic studies of the Japanese *Laurencia* species, *L. omaezakiana* Masuda sp. ined. was newly collected and examined to isolate a novel squalene-derived metabolite, designated enshuol. We wish to report herein the structural elucidation of enshuol that seems to be characteristic of this species.

Laurencia omaezakiana was collected at Omaezaki, Omaezaki town, Shizuoka Prefecture, on the Pacific coast of central Japan on August 16, 1994, and the whole body was extracted with methanol in the usual manner. Repeated silica gel column chromatography of the methanol extracts has led to the isolation of enshuol (**1**) in 14% yield based on the extracts along with fatty acids, diacylglycerols, and sterols.

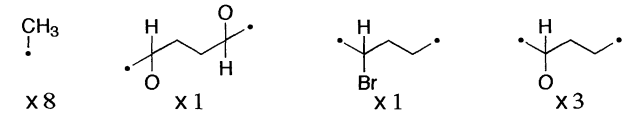


1



2: R_1=OH, R_2=H
3: R_1=R_2=O

Enshuol (**1**),[1] oil, $[\alpha]_D^{22}$ +22.7° (*c* 1.00, $CHCl_3$), had the molecular formula $C_{30}H_{51}BrO_6$ established by high resolution mass spectrum. The IR spectrum showed absorption at ν_{max} 3430 cm^{-1} revealing the presence of hydroxyl group(s). The ^1H and ^{13}C NMR spectral data indicated that enshuol had 8 tertiary methyls, 6 oxygenated quaternary carbons, 5 oxygenated tertiary carbons and a brominated tertiary carbon (δ_C 60.11). The COSY, HOHAHA and HSQC spectra of enshuol suggested the presence of the following partial structural units in the molecule.



(• : Quaternary carbon atom)

These consequences indicated that enshuol was a squalene-derived compound like a series of thyrsiferol,[2] venustatriol,[3] magireol,[4] teurilene,[5] and intricatetraol[6] from genus *Laurencia*. Furthermore, the ^1H-^{13}C long range correlations that were determined through HMBC proved enshuol was a pentacyclic compound including an oxepane ring (Figure 1).

Treatment of **1** with zinc powder and acetic acid in methanol resulted in dehalogenation to afford a tetracyclic compound **2**,[7] $C_{30}H_{52}O_6$, which possessed a secondary hydroxyl group. Oxidation of **2** with pyridinium chlorochromate in methylene chloride gave a ketone **3**,[8] $C_{30}H_{50}O_6$, whose IR spectrum showed absorption at ν_{max} 1715 cm^{-1} due to six-membered ring ketone. Furthermore, the presence of 4-methyl-3-pentenyl group in **2** and **3** strongly supported that enshuol (**1**) had 2,8-dioxabicyclo[5.4.0]undecane ring (A-B ring). The other ether linkages were connected by the specific fragment ions in its low resolution FD mass spectrum (Figure 1).

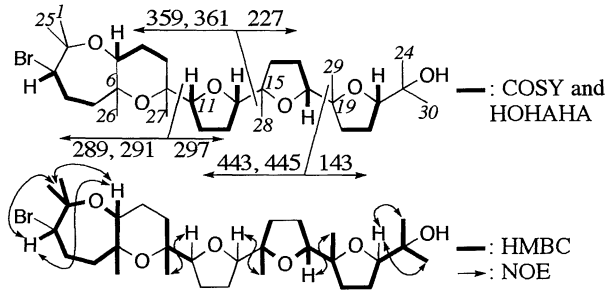


Figure 1. The fragment ions (*m/z*) in FD mass spectrum, and the correlations of *J* coupling from COSY and HOHAHA (upper). NOEs from NOESY and NOE difference spectra, and the long range correlations from HMBC (lower) of **1**.

The relative stereochemistry about 2,8-dioxabicyclo[5.4.0] undecane ring was determined by the NOESY and NOE difference spectra, whose results were depicted in Figure 1. No NOE was detected between H-11/H-14, H_3-28/H-18, and H_3-29/H-22, thus suggesting that the stereochemistries of three oxolane rings were *trans*. However, the relative configurations between H_3-26/H_3-27, H_3-27/H-11, H-14/H_3-28, and H-18/H_3-29 remained uncertain.

Since the stereochemistry at C-7 in enshuol (**1**) was retained in diol **2**, the absolute configuration of secondary hydroxyl group was determined by the application of the advanced Mosher's method.[9] Positive $\Delta\delta$ values were found for all the protons on the C1-C6 side of the MTPA plane, whereas negative values were found for protons on the other side (Figure 2). This meant that C-7 had to have the *S*-configuration and hence the absolute stereochemistry of A-B ring should be 3*R*, 6*R* and 7*S*.

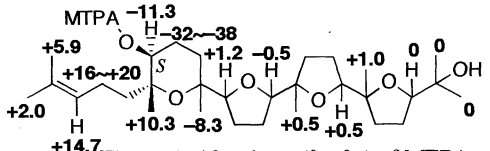
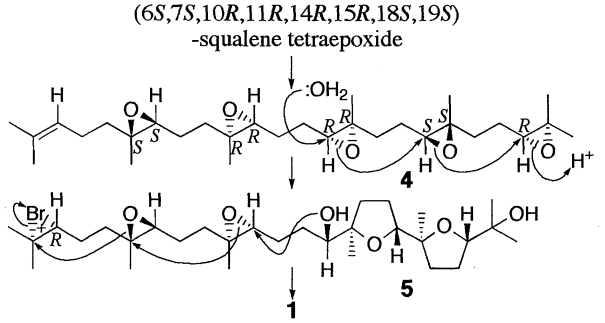


Figure 2. $\Delta\delta$ values (δ_S–δ_R) of MTPA esters of **2** are shown in Hz (400 MHz).

Table 1. ^{13}C (100 MHz) NMR, ^{1}H (400 MHz) NMR, and HMBC data (in $CDCl_3$) for enshuol (**1**)

Pos.	^{13}C δ	^{1}H δ, *J* in Hz	Long range correlations
1	25.42 q	1.35 (*s*)	H_3-25, H-3
2	77.69 s		H_3-1, H_3-25, H-3, H-7
3	60.11 d	4.13 (*br d*), *J*=11.7	H_3-1, H_3-25
4	31.44 t	2.06 (*m*) and 2.17 (*m*)	H-3
5	44.53 t	1.44 (*m*) and 1.67 (*m*)	H-3, H_3-26
6	75.66 s		H_3-26, H-7
7	72.97 d	3.18 (*dd*), *J*=4.2, 11.5	
8	23.90 t	1.55 (*m*) and 1.74 (*m*)	
9	32.61 t	1.59 (*m*) and 1.61 (*m*)	H-11
10	74.91 s		H_3-27
11	87.84 d	3.62 (*dd*), *J*=5.9, 9.3	H_3-27
12	27.12 t	1.71 (*m*) and 1.87 (*m*)	
13	28.51 t	1.52 (*m*) and 1.89 (*m*)	
14	85.13 d	3.89 (*dd*), *J*=6.4, 6.8	H-16
15	85.70 s		H-18, H_3-28
16	33.54 t	1.58 (*m*) and 2.05 (*m*)	H_3-28, H-14
17	29.54 t	1.58 (*m*) and 1.82 (*m*)	H-16
18	84.25 d	4.01 (*dd*), *J*=5.4, 9.8	H_3-29, H_2-20
19	85.83 s		H_3-29
20	31.04 t	1.46 (*m*) and 2.40 (*m*)	H_3-29, H-18
21	26.67 t	1.89 (*m*) and 2.14 (*m*)	H_2-20
22	85.83 d	3.87 (*dd*), *J*=5.4, 5.9	H_3-24, H_2-20
23	71.60 s		H_3-30, H_3-24, H-22
24	25.42 q	1.06 (*s*)	H_3-30
25	24.76 q	1.35 (*s*)	H-3
26	20.47 q	1.19 (*s*)	H-7
27	21.53 q	1.16 (*s*)	
28	23.26 q	1.12 (*s*)	
29	25.26 q	1.15 (*s*)	H_2-20
30	28.15 q	1.25 (*s*)	H_3-24

Enshuol (**1**) represents the first example of a new class of pentacyclic bromotriterpenoids. Scheme 1 outlines a proposed biogenesis for **1**. Oxygenated squalene-derived polyethers isolated from *Laurencia* species may arise from (6*S*,7*S*,10*R*, 11*R*,14*R*,15*R*,18*S*,19*S*)-squalene tetraepoxide as a common precursor[6] that has not yet been isolated. If enshuol (**1**) has also been biosynthesized from pentaepoxide **4** derived from squalene tetraepoxide, an intermediate **5** may arise with all *trans*-configuration on two oxolane rings. Subsequent nucleophilic reaction of hydroxyl group at C-14 with 3*R*-bromonium ion of **5** could then afford enshuol (**1**), for which the (3*R*,6*R*,7*S*,10*R*, 11*S*,14*S*,15*R*,18*R*,19*S*,22*S*)-configuration would tentatively be assigned as one of the possible absolute configurations.



Scheme 1. Possible biogenesis for enshuol (**1**).

We are currently attempting to prepare a crystalline derivative from **2** for X-ray crystallographic analysis to confirm the proposed structure **1** for enshuol.

References and Notes

1 Enshuol (**1**); IR ($CHCl_3$), ν_{max} 3430, 3000, 2900, 1460, 1380, and 1090 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1); HR-EIMS *m/z*: 588.2889, Calcd for $C_{30}H_{51}{}^{81}BrO_6$, 588.2848 [M].

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7 **2**; Colorless amorphous solid; $[\alpha]_D{}^{21}$ +5.2° (*c* 0.58, $CHCl_3$); IR (neat), ν_{max} 3460, 3000, 2960, 2900, 1460, 1380, and 1080 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (3H, *s*, H_3-24), 1.13 (3H, *s*, H_3-28), 1.14 (3H, *s*, H_3-29), 1.15 (3H, *s*, H_3-27), 1.19 (3H, *s*, H_3-26), 1.24 (3H, *s*, H_3-30), 1.60 (3H, *s*, H_3-1), 1.67 (3H, *s*, H_3-25), 3.44 (1H, *dd*, *J* =5.1 and 11.5 Hz, H-7), 3.63 (1H, *dd*, *J*=6.1 and 9.0 Hz, H-11), 3.84 (1H, *dd*, *J* =6.8 and 6.3 Hz, H-22), 3.87 (1H, *dd*, *J* =5.6 and 9.5 Hz, H-14), 4.00 (1H, *dd*, *J* =5.6 and 10.0 Hz, H-18), 5.11 (1H, *m*, H-3), and following methylene protons were assigned with the aid of HSQC spectrum, 2.05 (2H, *m*, H_2-4), 1.34, 1.48 (each 1H, *m*, H_2-5), 1.72 (2H, *m*, H_2-8), 1.51, 1.64 (each 1H, *m*, H_2-9), 1.77, 1.85 (each 1H, *m*, H_2-12), 1.52, 1.88 (each 1H, *m*, H_2-13), 1.58, 2.04 (each 1H, *m*, H_2-16), 1.58, 1.79 (each 1H, *m*, H_2-17), 1.44, 2.32 (each 1H, *m*, H_2-20), and 1.85, 2.09 (each 1H, *m*, H_2-21); ^{13}C NMR (100 MHz, $CDCl_3$) *C*H_3: δ 17.61 (C-1), 20.19 (C-26), 20.72 (C-27), 22.88 (C-28), 25.18 (C-29), 25.40 (C-24), 25.71 (C-25), and 27.03 (C-30), *C*H_2: δ 21.65 (C-4), 24.87 (C-8), 26.74 (C-21), 27.03 (C-12), 28.29 (C-13), 29.39 (C-17), 31.15 (C-20), 33.49 (C-9), 34.04 (C-16), and 42.30 (C-5), *C*H: δ 73.43 (C-7), 84.40 (C-18), 85.13 (C-14), 85.79 (C-22), 88.06 (C-11), and 125.18 (C-3), *C*: δ 71.53 (C-23), 74.65 (C-10), 75.95 (C-6), 85.57 (C-15), 85.85 (C-19), and 131.00 (C-2); HR-FABMS *m/z*: 509.3840, Calcd for $C_{30}H_{53}O_6$, 509.3842 [M+H].

8 **3**; Colorless oil; $[\alpha]_D{}^{19}$ +42.1° (*c* 0.10, $CHCl_3$); IR ($CHCl_3$), ν_{max} 3420, 3000, 2900, 1715, 1380, and 1260 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05, 1.14, 1.15, 1.21, 1.24, 1.31, 1.57, and 1.65 (each 3H, *s*), 3.84 (1H, *dd*, *J*=6.3 and 7.3 Hz), 3.88 (1H, *dd*, *J* =5.6 and 9.5 Hz), 3.99 (1H, *dd*, *J*=6.4 and 6.4 Hz), 4.02 (1H, *dd*, *J* =5.9 and 7.8 Hz), and 5.04 (1H, *m*); ^{13}C NMR (100 MHz, $CDCl_3$) *C*H_3: δ 17.59, 22.61, 22.73, 25.17, 25.35, 25.64, 26.63, and 28.18, *C*H_2: δ 22.39, 27.25, 28.37, 29.24, 29.81, 31.04, and 34.05, *C*H: δ 84.18, 84.27, 85.17, 85.79, and 124.16, *C*: δ 71.60, 82.00, 85.31, 85.68, 131.64, and 215.53; HR-FABMS *m/z*: 507.3659, Calcd for $C_{30}H_{51}O_6$, 507.3685 [M+H].

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