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Auriculol, a cytotoxic oxygenated squalene from the Japanese sea hare *Dolabella auricularia*: isolation, stereostructure, and synthesis

Hideo Kigoshi,[a,*] Takashi Itoh,[b] Takafumi Ogawa,[b] Kenya Ochi,[b] Mayuko Okada,[b] Kiyotake Suenaga[b] and Kiyoyuki Yamada[b]

[a]*Department of Chemistry, University of Tsukuba, Tsukuba, Ibaraki 305-8571, Japan*
[b]*Department of Chemistry, Graduate School of Science, Nagoya University, Chikusa, Nagoya 464-8602, Japan*

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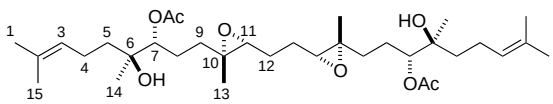
Abstract—Auriculol, a cytotoxic oxygenated squalene, was isolated from the Japanese sea hare *Dolabella auricularia*, and its stereostructure was elucidated by spectral analysis and organic synthesis.

The sea hare *Dolabella auricularia* is known to be a rich source of bioactive and structurally unique organic compounds.[1] Recently, we examined the constituents of Japanese specimens of this animal, resulting in the isolation of new cytotoxic depsipeptides, peptides, macrolides, and other unique metabolites.[2] We report herein the isolation, structural elucidation, and synthesis of auriculol (**1**), an oxygenated squalene from the Japanese sea hare *D. auricularia*.



auriculol (**1**)

The MeOH extract of the internal organs (72.0 kg, wet wt) of the sea hare *D. auricularia* (117 kg, wet wt), collected in Mie Prefecture, Japan, was partitioned between EtOAc and water. The EtOAc-soluble material was further partitioned between 90% aqueous MeOH and hexane. The material obtained from the aqueous MeOH portion was successively subjected to bioassay-guided fractionation using silica gel (i. toluene/EtOAc then EtOAc; ii. hexane/acetone then acetone/MeOH) to afford a cytotoxic fraction ($IC_{50}=3.3$ μg/mL, HeLa S_3 cells). The fraction was further separated by reversed-phase MPLC (i. ODS silica gel, 75:25 to 100:0 MeOH–H_2O; ii. ODS silica gel, 80:20 to 100:0 MeOH–H_2O), silica gel column chromatography (15:1 to 3:1 $CHCl_3$–acetone), and reversed-phase HPLC (i. ODS silica gel, 75:5:40 to 75:5:0 MeCN–MeOH–H_2O; ii. ODS silica gel, 65:35 MeCN–H_2O) to afford 1.8 mg (1.5×10^{-6} % based on wet weight) of auriculol (**1**)[3] as a colorless oil: $[\alpha]_D^{25}$ +0.12 (*c* 0.10, $CHCl_3$).

The IR absorption bands (3430 and 1730 cm^{-1}) of auriculol (**1**) indicated the presence of hydroxy and ester groups in **1**. Resonances in the ^1H and ^{13}C NMR spectra were assigned by the COSY, HSQC, and HMBC spectra, as shown in Table 1, which suggested the terpenoid nature of **1**. The NMR signals due to 29 hydrogens and 17 carbons were observed in the ^1H and ^{13}C NMR spectra of auriculol (**1**), whereas the molecular weight of **1** was found to be 594 by the FABMS spectrum, suggesting that **1** has the symmetric structure. Based on these findings, the molecular formula of auriculol (**1**) was deduced to be $C_{34}H_{58}O_8$.[4] The detailed analysis of the COSY spectrum of **1** provided partial structures, one $(CH_3)_2C{=}CHCH_2$- (**A**) and two -CH(O-)-CH_2- units (Fig. 1). The HMBC correlations summarized in Table 1 allowed the foregoing two -CH(O-)CH_2- units, three methyl groups, two methylenes, two quaternary carbons bearing an oxygen atom, and one carbonyl to be connected, giving the partial structure **B** (Fig. 1). The oxygenated carbon signals of C10 and C11 were observed in a relatively high-field (δ 60.5 s, 62.6 d), suggesting the presence of an epoxide ring at C10 and C11. Considering the molecular formula of auriculol (**1**), the remaining oxygen functional group at C6 was deduced to be a hydroxy group.

Keywords: sea hare; *Dolabella auricularia*; triterpenoid; isolation; stereostructure; synthesis; cytotoxicity.

* Corresponding author.

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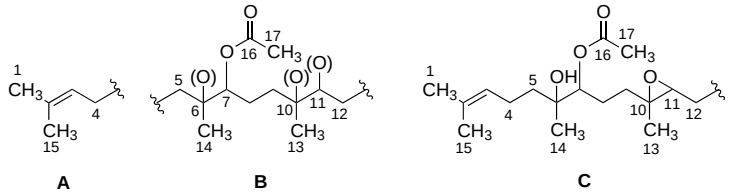


Figure 1.

Table 1. NMR data and HMBC correlations for auriculol (**1**) in $CDCl_3$

Position	^1H[a]	^{13}C[b]	HMBC[c]
1	1.69 (br s)	25.7 (q)	H-3, 15
2		132.2 (s)	H-1, 15
3	5.10 (t, 7.1)	124.1 (d)	H-1, 15
4	1.99–2.15 (m)	22.0 (t)	H-3
5	1.37–1.60	38.0 (t)	H-14
6		74.1 (s)	H-14
7	4.83 (dd, 9.8, 2.4)	78.6 (d)	H-14
8	1.52–1.80 (m)	24.4 (t)	H-7
9	1.39–1.60 (m)	34.9 (t)	H-7, 11, 13
10		60.5 (s)	H-13
11	2.79 (t, 5.4)	62.6 (d)	H-13
12	1.58–1.78 (m)	25.7 (t)	H-11
13	1.27 (s)	16.6 (q)	
14	1.17 (s)	23.2 (q)	
15	1.62 (br s)	17.7 (q)	H-1, 3
16		170.9 (s)	H-7, 17
17	2.09 (s)	21.1 (q)	
OH	2.85 (br s)		

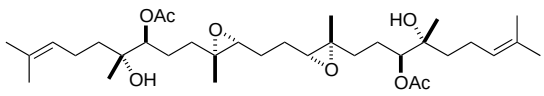
[a] The spectrum was recorded at 400 MHz. Coupling constants are in hertz.

[b] The spectrum was recorded at 100 MHz. Multiplicities were determined by the DEPT experiments.

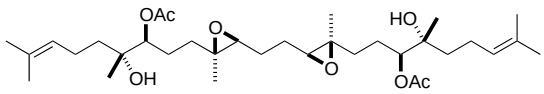
[c] Protons correlated to carbon resonances in ^{13}C column. Parameters were optimized for $J_{CH}=6$ and 8 Hz.

Further evidence for the connectivity between these two partial structures, **A** and **B**, could not be obtained from the spectral data. However, considering the symmetric structure of **1** as well as the triterpenoid nature of **1**, the methylene carbon (C4) of **A** must be bonded to the methylene carbon (C5) of **B**, leading to the partial structure **C**, which must be dimerized at C12. Thus, the gross structure of auriculol (**1**) was elucidated to be 7,7′-diacetoxy-6,6′-dihydroxysqualene-10,11,10′,11′-diepoxide.

Although many types of oxygenated squalenes were isolated, it is difficult to determine their stereostructures only by spectroscopic analysis. For example, the originally assigned stereostructure[5] of glabrescol based on spectral data was recently revised using the organic synthetic method.[6] In the case of **1**, we had some information about the stereochemistry: (1) **1** has a symmetric structure and is optically active, $[\alpha]_D^{25}$ +0.12 (*c* 0.10, $CHCl_3$), indicating that **1** possesses C_2 symmetry. (2) The NOE correlation between H-12 and H-13 indicated that the two substituents on the epoxide ring, C9 and C12, were *trans*. Assuming that **1** was derived from a tetraepoxysqualene and that the monoacetylated glycol unit (C6–C7) was generated by ring opening of the corresponding epoxide moiety, the relative stereochemistry would be *erythro*,[7] and the absolute stereostructure of **1** is deduced to be represented by structure **1a** or **1b**, or their enantiomers. For the determination of the complete stereostructure of **1**, we have employed the organic synthetic method coupled with the spectroscopic method. Thus, we decided to enantioselectively synthesize **1a** and **1b** and to compare the spectral data and the optical rotation of synthetic **1a** and **1b** with those of natural **1**.



1a

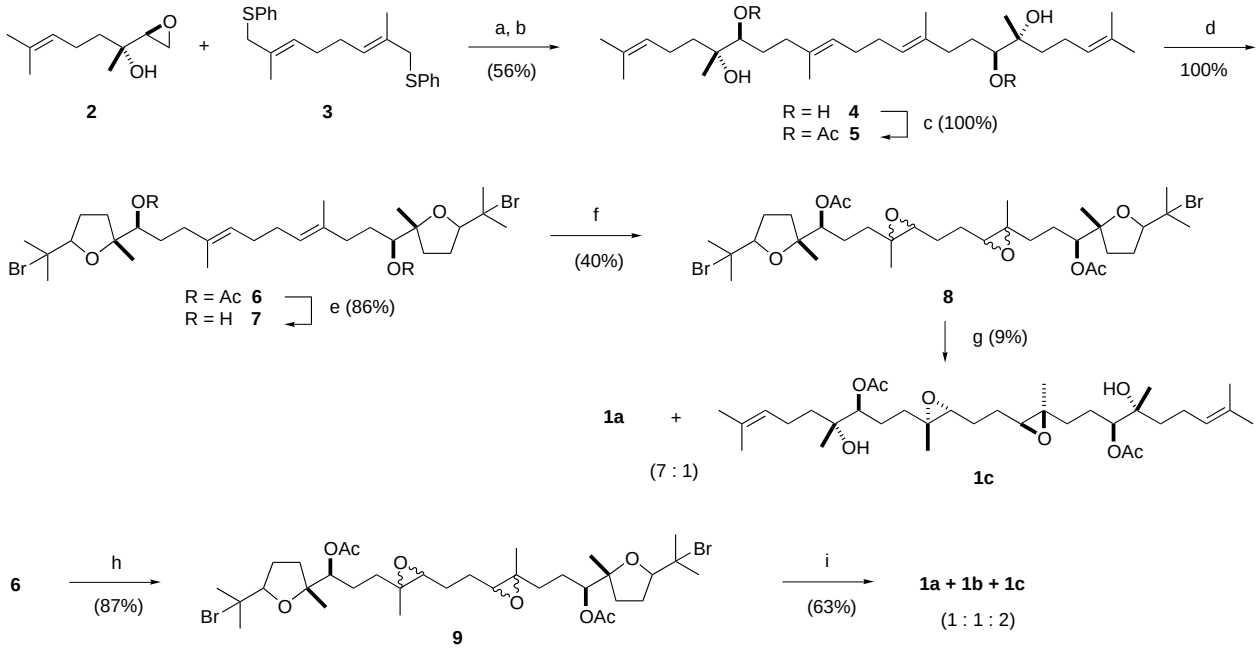


1b

The synthesis of **1a** and **1b** started with linalool oxide **2** prepared from geraniol using Shirahama–Matsumoto's procedure (Scheme 1).[8] The coupling reaction of linalool oxide **2** and the disulfide **3**[8] with *n*-BuLi–TMEDA gave a diastereomeric mixture of hydroxy sulfides, which was desulfurized with sodium to afford tetraol **4**. The secondary hydroxy groups in **4** were acetylated to give diol **5**. To protect the outside olefins in **5** during the next epoxidation, bromoetherification with 2,4,4,6-tetrabromo-2,5-cyclohexadienone (TBCO) was effected to provide a diastereomeric mixture of bromo ether **6**. The acetyl groups in **6** were removed by DIBAL reduction to give diol **7**.

Stereoselective epoxidation of bishomoallylic alcohols with *t*-BuOOH–$VO(acac)_2$ has been reported,[9,10] and Shirahama and co-workers disclosed the stereoselectivity of this epoxidation.[10] According to their prediction, the epoxidation of diol **7** would lead to **1a** as a major product along with **1c** as a minor product. Actually, oxidation of diol **7** with *t*-BuOOH–$VO(acac)_2$ followed by acetylation gave a diastereomeric mixture of diepoxide **8**, which was reduced with zinc to afford a 7:1 mixture of diepoxides, **1a** and **1c**.[11] These diastereomers could be separated by HPLC using chiral columns.[12,13] Although diepoxide **1a** proved to possess C_2 symmetry based on the spectral data, **1a** was shown to be different from natural **1** by spectral comparison.

On the other hand, epoxidation of **6** with *m*CPBA gave a diastereomeric mixture of epoxide **9**, which was

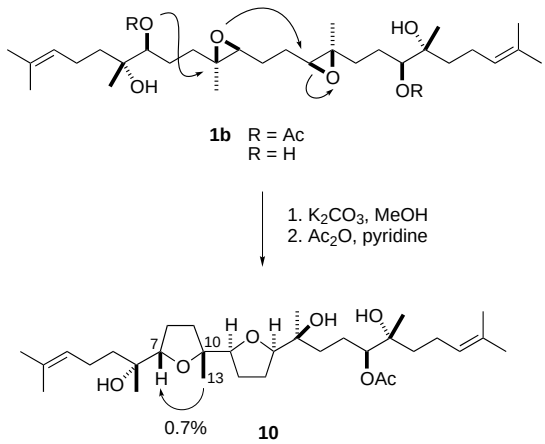


Scheme 1. (a) *n*-BuLi, TMEDA, THF, −78°C, 8 h; (b) Na, 2-propanol, THF, reflux, 5 h; (c) Ac_2O, pyridine, rt, 17.5 h; (d) TBCO, CH_2Cl_2, 0°C, 4 h; (e) DIBAL, CH_2Cl_2, −78°C, 2 h; (f) $VO(acac)_2$, TBHP, NaOAc, benzene, rt, 3.5 h; Ac_2O, pyridine, DMAP, rt, 15.5 h; (g) Zn, NH_4OAc (aq.), THF, 50°C, 12.5 h (**1a**:**1c** = 7:1); (h) *m*CPBA, CH_2Cl_2, 0°C, 1.5 h; (i) Zn, NH_4OAc (aq.), THF, 50°C, 9.5 h (**1a**:**1b**:**1c** = 1:1:2).

reduced with zinc to give a 1:1:2 mixture of diepoxides **1a**, **1b**, and **1c**, as statistically expected.[12,13] While diepoxides **1a** and **1b** were shown to possess C_2 symmetry based on the spectral data, **1c** was not. Comparison of the spectroscopic data and the optical rotations of synthetic **1a** and **1b** (**1a**: $[\alpha]_D^{25}$ −0.44; **1b**: $[\alpha]_D^{25}$ −0.14) with those of natural **1** ($[\alpha]_D^{25}$ +0.12) indicated that natural **1** was the enantiomer of **1b**.

To confirm the stereostructure of compound **1b**, **1b** was transformed into the tetrahydrofuran derivative **10** as follows (Scheme 2). Methanolysis of **1b** under basic conditions and subsequent acetylation afforded the cyclic ether **10** (24%). The observed NOE of **10** illustrated in Scheme 2 disclosed the relative stereochemistry between H7 and C13 in **10** to be *cis*. Thus, considering the S_N2 nature of the basic cyclization of an epoxy alcohol derived from **1b**, the assignment of stereochemistry of synthetic **1a** and **1b** was unambiguously confirmed.

In summary, we have isolated auriculol (**1**) from the Japanese sea hare *D. auricularia* and determined its stereostructure by spectral analysis and organic synthesis. Auriculol (**1**) exhibited cytotoxicity against HeLa S_3 cells with an IC_{50} value of 6.7 μg/mL. As related compounds of **1**, three kinds of monoepoxysqualenes from a fungus[14] and from marine algae[15] and a monoepoxysqualene glycoside from a fungus[16] were reported. Because various epoxysqualenes are known to be important biogenetic precursors of triterpene polyethers, the determination of the stereochemistry of the oxygenated squalenes is significant for investigating the biogenesis of triterpene polyethers. It is noteworthy



Scheme 2.

that aurilol, a brominated squalene polyether, was isolated from the same animal.[2b]

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3. IR ($CHCl_3$) 3430 (br), 1730, 1455, 1375, 1240, 1020 cm^{-1}; FABMS (*m*-nitrobenzyl alcohol) *m*/*z* 617 $[M+Na]^+$.
4. The high-resolution FABMS data of **1** could not be obtained due to the decomposition of the natural sample during storage.
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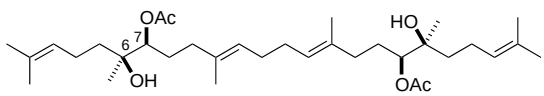
7. This inference was confirmed by the following experiments. Using the same sequence of reactions in Scheme 1, the model compound **11** with *threo* stereochemistry was prepared from linalool oxide diastereomeric with **2** that was synthesized from nerol. The compound **11** was found to be distinguishable from diol **5** with *erythro* stereochemistry by the NMR spectroscopy. The NMR data of auriculol (**1**) resembled those of **5** more closely than those of **11**. Selected NMR data in $CDCl_3$: Compound **5** δ_H 4.84 (1H, dd, $J=9.6$, 3.3 Hz, H-7) and 1.16 (3H, s, H-14), δ_C 37.7 (t, C5) and 23.6 (q, C14). Compound **11** δ_H 4.85 (1H, dd, $J=9.3$, 3.4 Hz, H-7) and 1.14 (3H, s H-14), δ_C 39.1 (t, C5) and 21.9 (q, C14).



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11. About 50% of starting material **8** was recovered. The reaction conditions were not optimized.
12. Separation conditions: (1) Chiralcel OD-H (4.6×250 mm), 9:1 hexane–ethanol, 0.5 mL/min; retention times: **1a** and **1c** 12.7 min, **1b** 11.8 min. (2) Sumichiral OA-4500 (4.0× 250 mm), 90:9:1 hexane–CH_2ClCH_2Cl–ethanol, 1.0 mL/min; retention times: **1a** 13.0 min, **1b** and **1c** 12.2 min.
13. Compound **1a**: colorless oil; $[\alpha]_D^{25}$ −0.44 (*c* 0.042, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.16 (6H, s, H-14), 1.26 (6H, s, H-13), 1.62 (6H, s, H-15), 1.69 (6H, s, H-1), 2.09 (6H, s, H-16), 2.78 (2H, m, H-11), 4.82 (2H, dd, $J=10.0$, 2.2 Hz, H-7), 5.10 (2H, m, H-3); HRFABMS *m*/*z* calcd for $C_{34}H_{58}NaO_6$ (MNa^+) 617.4030, found 617.4026.
Compound **1b**: colorless oil; $[\alpha]_D^{25}$ −0.14 (*c* 0.028, $CHCl_3$); ^1H NMR ($CDCl_3$, 800 MHz) δ 1.17 (6H, s, H-14), 1.27 (6H, s, H-13), 1.30–1.80 (16H, m), 1.62 (6H, s, H-15), 1.69 (6H, s, H-1), 2.00–2.15 (4H, m), 2.09 (6H, s, H-16), 2.79 (2H, t, $J=5.4$ Hz, H-11), 4.83 (2H, dd, $J=9.8$, 2.4 Hz, H-7), 5.10 (2H, t, $J=7.1$ Hz, H-3); HRFABMS *m*/*z* calcd for $C_{34}H_{58}NaO_6$ (MNa^+) 617.4030, found 617.4019.
Compound **1c**: colorless oil; $[\alpha]_D^{25}$ +0.14 (*c* 0.089, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 1.16, 1.17 (3H each, s, H-14), 1.26, 1.27 (3H each, s, H-13), 1.62 (6H, s, H-15), 1.69 (6H, s, H-1), 2.09 (6H, s, H-16), 2.75 (2H, m, H-11), 4.83 (2H, m, H-7), 5.11 (2H, m, H-3); HRFABMS *m*/*z* calcd for $C_{34}H_{58}NaO_6$ (MNa^+) 617.4030, found 617.4003.
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