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NEOISOPRELAUREFUCIN, A HALOGENATED C_{15} NON-TERPENOID COMPOUND FROM *LAURENCIA NIPPONICA*

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Key Word Index—*Laurencia nipponica*; Rhodomelaceae; red alga; C_{15} non-terpenoid; halogenated metabolite; chemotaxonomy; chemical race.

Abstract—A new C_{15} nonterpenoid acetylenic compound, named neoisoprelaurefucin, has been isolated from *Laurencia nipponica* and its structure deduced from spectroscopic data. Neoisoprelaurefucin is a stereoisomer of (3*Z*)-isoprelaurefucin, which has previously been isolated from this species. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

In our continuing taxonomic studies of Japanese species of the red algal genus *Laurencia*, based upon morphological and chemical features as well as genetic affinities, we have recently reported the structures of novel brominated compounds, which are characteristic metabolites of the new species, *Laurencia japonensis* Masuda et Abe sp. ined. [1] and *L. omaezakiana* Masuda sp. ined. [2]. Among the Japanese species of the genus *Laurencia*, *L. nipponica* Yamada displayed a marked variation in its major metabolites [3]. As part of further chemotaxonomic studies, we collected further samples of this species off the coast of Naoetsu and Kashiwazaki, Niigata prefecture. These contained a new acetogenin, which we named neoisoprelaurefucin, as the major metabolite. In this paper we report the isolation and structural elucidation of neoisoprelaurefucin (**1**), which is a stereoisomer of (3*Z*)-isoprelaurefucin (**2**), previously obtained from *L. nipponica* [4].

RESULTS AND DISCUSSION

The neutral methanol extract was fractionated by column chromatography over silica gel with a step-wise gradient of hexane, benzene and ethyl acetate. The fraction eluted with benzene was further subjected to preparative TLC to give **1** in 15% yield (based on the neutral extract) along with (3*E*)-laureatin (**3**) (5%) [5].

Compound **1**, an oil, possessed the molecular formula $C_{15}H_{20}Br_2O_2$ determined by the high resolution mass spectrum (*m/z* 391.9788 [M]). Its IR, mass and ^{1}H NMR spectra showed the presence of a *cis*-2-penten-4-ynyl grouping (ν_{max} 3286 and 2092 cm^{-1}; *m/z* 329, 327 and 325 $[M-C_5H_5]^+$; δ_H 3.10 (1H, *br d*, $J = 2.0$ Hz), 5.57 (1H, *br d*, $J = 11.2$ Hz) and 6.12 (1H, *br ddd*, $J = 11.2$, 7.3 and 7.3 Hz)). Moreover, the IR spectrum revealed no absorptions indicative of hydroxyl or carbonyl groups, thus indicating that the two oxygen atoms in **1** were involved in ether linkages. As the ^{13}C NMR spectrum showed that there were no other double bonds, apart from that of the pentenyne moiety, **1** must consist of two oxide rings. These data were typical for cyclic ethers having a C_{15} straight-chain skeleton found in the species of the genus *Laurencia* [6].

The ^{1}H–^{1}H COSY spectrum of **1** indicated the presence of the partial structure **A** for **1** (Fig. 1). The assignments of the carbons bearing hydrogen(s) were established from the HSQC spectrum (Table 1). Placement of two bromine atoms at C-10 and C-13 was evident from the chemical shifts (δ_C 51.1 and 62.3, respectively) and, therefore, the remaining substituents at C-6, C-7, C-9 and C-12 were verified to be ethereal oxygen atoms based upon the chemical shifts of the pertinent carbons (δ_C 85.7, 75.6, 80.6 and 72.1, respectively).

In view of the above data, three possible structures with two oxide rings, **1a**, **1b** and **1c** (Fig. 1), for neoisoprelaurefucin are given by the following ether closures: (a) bonding between C-6/C-7 and between C-9/C-12, (b) bonding between C-6/C-9 and between C-7/C-12, and (c) bonding between C-6/C-12 and between C-7/C-9, respectively. As the ^{1}H NMR spectrum showed no signals due to epoxide protons [7], structure **1a** can be ruled out. Compound **2** [4] and laureatin (**4**) [8, 9], which have previously been isolated from *L. nipponica*, have the skeletons **1b** or **1c**,

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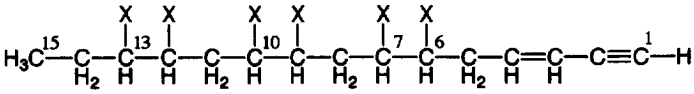
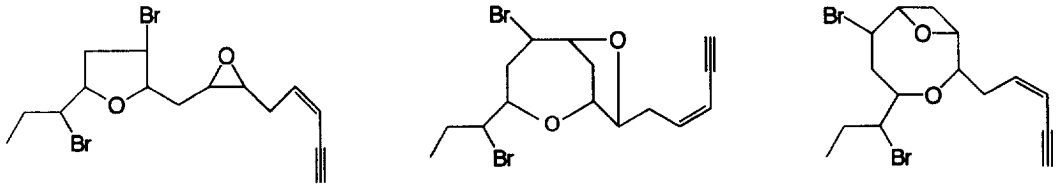


A (X=Br or O)



1a **1b** **1c**

Fig. 1. Partial and possible structures for neoisoprelaurefucin (**1**).

Table 1. ^{13}C NMR (100 MHz, DEPT), ^{1}H NMR (400 MHz) and HMBC data* for neoisoprelaurefucin (**1**)

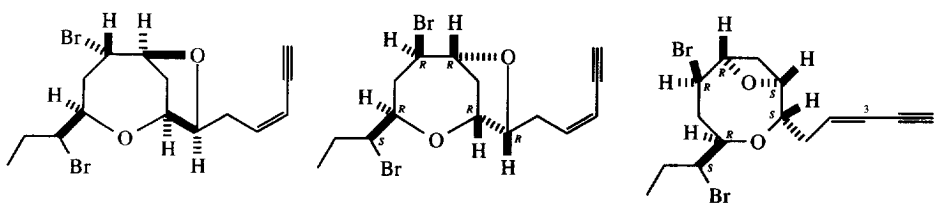
C†	^{13}C (δ)	^{1}H (δ)	*J* (Hz)	Long-range correlations
1	80.2	3.10	*br d* (2.0)	H-3
2	82.0			H-3
3	110.4	5.57	*br d* (11.2)	H-1, H-4, H_2-5
4	141.5	6.12	*ddd* (11.2, 7.3, 7.3)	H-1, H-3, H_2-5
5	30.5	2.93	*ddd* (14.2, 7.3, 5.8)	H-3, H-4
		2.66	*ddd* (14.2, 8.3, 7.3)	
6	85.7	3.91	*ddd* (8.3, 5.8, 2.9)	H_2-5, H-7, H_α-8, H-9
7	75.6	4.36	*dd* (3.9, 2.9)	H_2-5, H-6, H_α-8, H-9
8	30.3	2.78	*d* [15.1 (H_α)]	H-9
		2.12	*ddd* [15.1, 8.3, 3.9 (H_β)]	
9	80.6	4.49	*dd* (8.3, 4.4)	H-7, H_2-8, H-10, H_2-11
10	51.1	4.29	*ddd* (4.4, 3.4, 2.4)	H_2-8, H-10, H_2-11, H-12
11	36.4	2.49	*ddd* [15.1, 9.3, 3.4 (H_β)]	H-9, H-13
		2.18	*dd* [15.1, 2.4 (H_α)]	
12	72.1	3.94	*dd* (9.3, 3.4)	H-7, H-9, H-10, H_2-11, H-13
13	62.3	4.07	*ddd* (8.8, 4.9, 3.4)	H_2-11, H-12, H_2-14, H_3-15
14	28.5	1.91	*m*	H-12, H-13, H_3-15
15	12.5	1.09	*t* (7.3)	H-13, H_2-14

*Measured in chloroform-d_1.

†Assignment made with the aid of the HSQC spectrum.

respectively. However, the ^{1}H and ^{13}C NMR data for **1** were different from those of **2** and **4**.

In order to confirm the gross structure for **1**, we recorded a ^{1}H-detected heteronuclear multiple-bond ^{1}H–^{13}C correlation spectrum (HMBC) [10]. In this spectrum (Table 1), the H-6 at δ_H 3.91 showed a cross-peak to C-9 at δ_C 80.6, leading to a planar structure **1**, which includes a 1,6-dioxabicyclo-[4.2.1]nonane skeleton. A study of Dreiding models indicated that the stereochemistry between H-7 and H-9



1 **2**

3: *trans* at C-3
4: *cis* at C-3

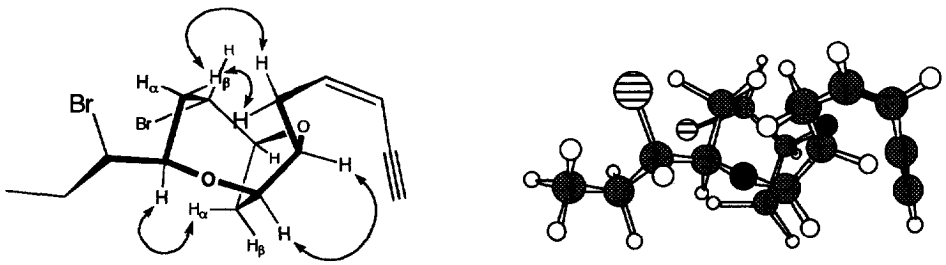


Fig. 2. Selected NOEs observed in the NOESY and NOE difference spectra and perspective view of neoisoprelaurefucin (**1**).

in **1** is *cis*. The remaining relative stereochemistries, excluding that at C-13, were defined by the NOESY and NOE difference spectra, the results of which are depicted in Fig. 2. NOE was observed between H-6 and H-7, indicating that the two methine protons, H-6 and H-7, are *cis*. This was further supported by the correlation between H_2-5 and H_β-11. Furthermore, the correlation between H_α-8 and H-12 indicated that the relative configuration between H-7 and H-12 is *cis*. The relative stereochemistry of the bromine atom at C-10 was deduced to be α from the absence of NOE between H_α-8 and H-10, as well as biogenetic considerations. Thus, the configuration between C-9 and C-10 of the C_{15} non-terpenoid is *threo* (see below).

Many halogenated C_{15} non-terpenoids isolated from various *Laurencia* species are suggested to arise from (6*R*,7*R*)- or (6*S*,7*S*)-laurediol [11]. As described in a previous paper [3], the configurations between C-9/C-10 and C-12/C-13 of C_{15} non-terpenoids found in *L. nipponica* are *threo* and *erythro*, reflecting the (9*Z*)- and (12*E*)-double bond in both precursors, respectively. In view of the co-existence with (3*E*)-laureatin (**3**) in the same alga, **1** is assumed to be biosynthesized from (6*S*,7*S*)- or (6*R*,7*R*)-laurediol. Therefore, the structure of neoisoprelaurefucin would be assigned as formula **1**, with a relative configuration of 6*S**, 7*S**, 9*S**, 10*S**, 12*R** and 13*S**.

Many morphologically similar, but chemically distinct, populations have been found in *L. nipponica*. Each chemical type is characterized by particular halogenated secondary metabolites, such as chamigrane-type sesquiterpenoids and C_{15} nonterpenoids. These types of secondary metabolite syntheses remained the same in the wild and under various culture conditions. The results of interpopulational crosses between female and male gametophytes of populations producing different metabolites indicate that the diverse chemical types found in *L. nipponica* can be referred to as chemical races (Masuda, M. *et al.*, unpublished data). The specimens collected at Naoetsu and Kashiwazaki appear to be a new chemical race.

EXPERIMENTAL

General. ^{1}H NMR: 400 MHz and ^{13}C NMR: 100 MHz, $CDCl_3$, TMS as int. standard. LR and HR MS: 70 eV. CC: silica gel (Merck, Kieselgel 60, 70–230 mesh). Prep. TLC: silica gel (Merck, Kieselgel 60 F_{254S}).

Collection. Two samples of *L. nipponica* Yamada were collected at Naoetsu (29 April 1992) and Kashiwazaki (1 May 1992), Niigata prefecture.

Extraction and isolation. Dried alga (23 g) collected at Naoetsu was extracted with MeOH, and the MeOH extracts partitioned between Et_2O and H_2O. The Et_2O soln was shaken with 0.5 M aq. KOH, washed with H_2O, dried (Na_2SO_4) and evapd to leave a brown oil (385 mg). The neutral extract (360 mg) was fractionated by silica gel CC with a step-wise gradient of hexane, C_6H_6 and EtOAc. The fr. eluted with C_6H_6 was further submitted to prep. TLC with C_6H_6 to give a mixt. of acetylenic compounds, which was further subjected to prep. TLC with hexane–EtOAc (10:1) to yield **1** (15% based on the neutral extract) along with (**3**) (5%) [5]. The neutral extract of Kashiwazaki's sample showed almost identical TLC profiles to those of Naoetsu's sample. Chromatographic sepn of Kashiwazaki's extract gave **1** (17%).

Neoisoprelaurefucin (**1**). Oil. $[\alpha]_D^{23}$ +17.2° (*c* 1.30; $CHCl_3$). IR ν_{max} (film) cm^{-1}: 3286, 2092, 1233, 1187, 1138, 1094, 1054, 985, 972, 912, 834, 801, 754. ^{1}H and ^{13}C NMR: Table 1. LR-MS *m/z* (rel. int.): 394, 392, 390 (3.8:7.4:3.7) $[M]^+$, 329, 327, 325 (0.8:1.5:0.7) $[M-C_5H_5]^+$, 313, 311 (4.6:4.7) $[M-Br]^+$, 273, 271, 269 (9:18:10) $[M-C_8H_9O]^+$, 189 (11), 179 (14), 177 (15), 121 (100), 107 (33), 93, (34), 81 (36), 67 (54), 65 (34), 55 (47), 41 (56); HR-MS *m/z*: 391.9788, 326.9442, 270.9182, 121.0654. Calc. for $C_{15}H_{20}{}^{79}Br^{81}BrO_2$: 391.9810 [M], $C_{10}H_{15}{}^{79}Br^{81}BrO_2$: 326.9419 $[M-C_5H_5]$, $C_7H_{11}{}^{79}Br^{81}BrO$: 270.9156 $[M-C_8H_9O]$ and C_8H_9O: 121.0653 $[M-C_7H_{11}Br_2O]$.

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