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PANNOSALLENE, A BROMINATED C_{15} NONTERPENOID FROM *LAURENCIA PANNOSA*

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Key Word Index—*Laurencia pannosa*; Rhodomelaceae; red alga; C_{15} nonterpenoid; halogenated metabolite.

Abstract—A new C_{15} nonterpenoid bromoallene, pannosallene, was isolated from *Laurencia pannosa*, a Vietnamese species of red algae. Its structure was deduced from spectroscopic data.

INTRODUCTION

Red algae of the genus *Laurencia* are well known to be prolific sources of diverse halogenated secondary metabolities, particularly terpenoids and C_{15} acetogenins [1, 2]. However, to date there has been no report on the chemical composition of species of *Laurencia* found in Vietnamese waters. As part of our chemotaxonomic investigations of this genus, we collected species at several locations in Vietnamese waters. One of them, *L. pannosa* collected at An Thoi, Phu Quoc Island, Kien Giang Province, was examined. This species also produced a brominated metabolite, which we named pannosallene. We report herein the isolation and structural elucidation of this new compound.

RESULTS AND DISCUSSION

A combination of column and thin-layer chromatography of methanol extracts led to the isolation of pannosallene (**1**) as needles in 1% yield based on extracts, along with chlorofucin (**2**) previously isolated from *L. snyderae* (La Jolla, CA, USA) [3] and bromofucin (**3**) previously isolated from *L. implicata* (Magnetic Island, North Queensland, Australia) [4].

The molecular formula of pannosallene (**1**), $[\alpha]_D^{26}$ + 64.3° (*c* 0.070; $CHCl_3$), was determined as $C_{15}H_{20}Br_2O_2$ on the basis of a high-resolution mass spectrum (*m/z* 273.0482 [M − C_3H_2Br], Δ 0.6 mmu). The melting point of **1** could not be measured because of decomposition of all samples during storage at −18°. The ^1H and ^{13}C NMR spectra of **1** (δ_H 6.06 (1H, *dd*, *J* = 5.9, 1.0 Hz) and 5.56 (1H, *dd*, *J* = 7.8, 5.9 Hz); δ_C 201.1(*s*), 102.4 (*d*) and 74.7 (*d*)) indicated the presence of a terminal bromoallene moiety characteristic of C_{15} acetogenins found in red algae of the genus *Laurencia* [1]. Moreover, the ^1H NMR spectrum showed the presence of another terminal methyl group (δ_H 1.09 (3H, *t*, *J* = 7.3 Hz)), a 1,2-disubstituted double bond [δ_H 5.81 (1H, *br ddd*, *J* = 10.7, 6.4, 6.4 Hz) and 5.77 (1H, *br ddd*, *J* = 10.7, 7.3, 7.3 Hz)) and five methine protons adjacent to a bromine or oxygen atom (δ_H 4.50 (1H, *dddd*, *J* = 7.8, 7.8, 6.4, 1.0 Hz), 4.21 (1H, *ddd*, *J* = 6.4, 4.4, 3.9 Hz), 4.09 (2H, *m*) and 3.96 (1H, *ddd*, *J* = 11.2, 4.4, 4.4 Hz)). The geometry of the double bond was shown to be *Z* from the coupling constant (*J* = 10.7 Hz, each) of the pertinent olefinic protons.

Detailed analysis of ^1H–^1H COSY spectra (chloroform-d_1 or benzene-d_6) of **1** revealed the presence of partial structure **A** (Fig. 1), comprising all the elements implied by the molecular formula. Because the IR spectrum exhibited the absence of hydroxyl and carbonyl functions, the two oxygen atoms were assumed to be involved as ether linkages.

Assignments of the carbons bearing hydrogen(s) were established from an HSQC spectrum (Table 1). The chemical shift (δ_C 58.1) of the methine carbon at C-13 suggested that a bromine atom was attached to this carbon. Moreover, substituents at C-4, C-6, C-7 and C-12 were verified as ethereal oxygen atoms based upon the chemical shifts of the pertinent carbons (δ_C 74.7, 73.7, 83.9 and 79.8, respectively). Pannosallene (**1**), which has five degrees of unsaturation, must contain two oxide rings. Thus, three possible structures with two oxide rings, **1a**, **1b** and **1c** (Fig. 1), are given by the following ether closures: (1) bonding between C-4/C-7 and between C-6/C-12, (2) bonding between C-4/C-12 and between C-6/C-7 and (3) bonding between C-4/C-6 and between C-7/C-12. As the ^1H NMR spectrum showed no signals due to

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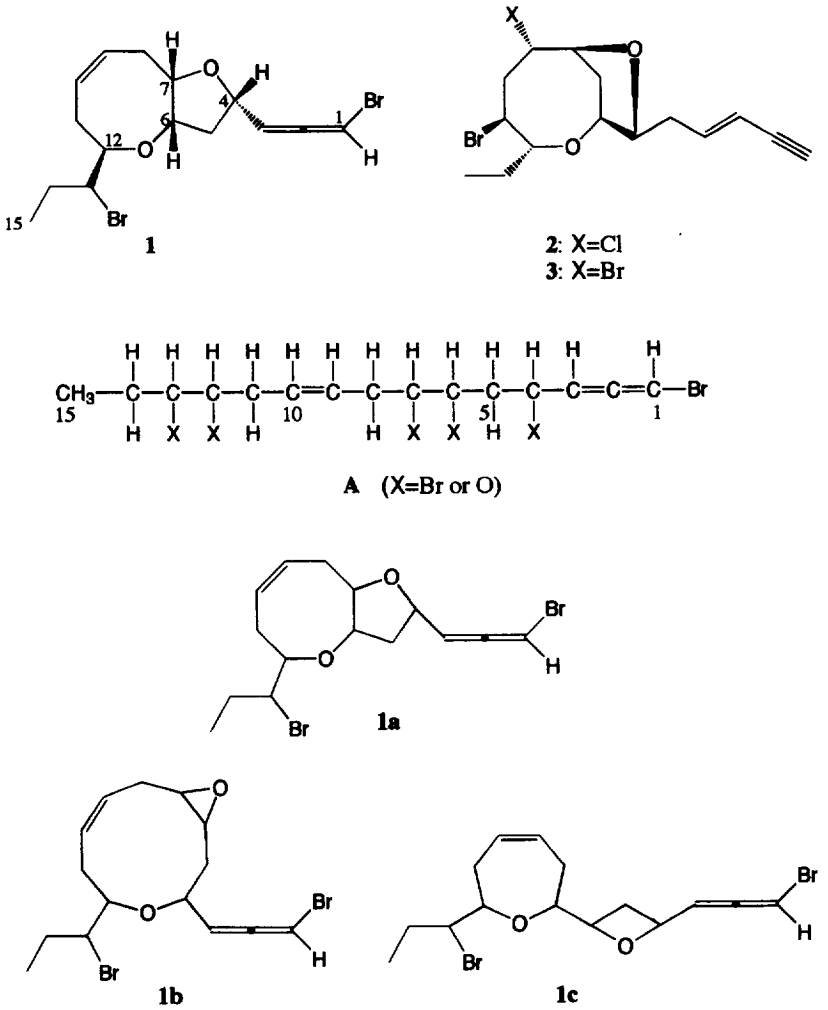

Fig. 1. Partial and possible structures for pannosallene (1).

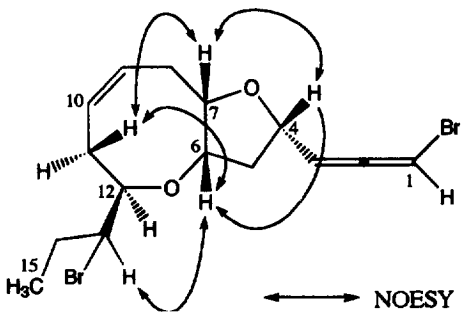

Fig. 2. NOESY spectrum of pannosallene (1).

expoxide protons [5] and methylene protons on a 1,3-disubstituted oxetane ring [6], the two structures **1b** and **1c** can be ruled out and, hence, pannosallene is represented by formula **1a** as a planar structure.

The relative stereochemistry in pannosallene (1), excluding that at C-13, was defined by a NOESY correlation spectrum. As shown in Fig. 2, the correlation between H-4/H-6 and between H-4/H-7 showed that three methine protons (H-4, H-6 and H-7) on the five-membered ring are all *cis*. Furthermore, the correlation between H-6 and H-13 indicated that the relative configuration at H-6 and H-12 is *trans*. The remaining configuration of C-13 was deduced from a biogenetic viewpoint. It is suggested that many halogenated C_{15} nonterpenoids isolated from various *Laurencia* species arise from (6*S*,7*S*)- or (6*R*,7*R*)-laurediol [7] and, furthermore, possess the (12*R*,13*S*)- or (12*S*,13*R*)-*erythro* configuration [1, 8], reflecting the (12*E*) double-bond in both precursors. Assuming that pannosallene (**1**) is also biosynthesized from (6*S*,7*S*)- or (6*R*,7*R*)-laurediol *via* the (12*S*,13*S*)-bromonium ion, the absolute configuration at C-12 and C-13 may be *R* and *S*, respectively. Furthermore, from the strong positive rotation of **1**, which appeared to be due to its bromoallene moiety, the absolute configuration of the allene moiety would be assigned as *S* by application of Lowe's rule [9]. The relative configuration, however, between H-3 and H-4 remains unclear.

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

C†	^{13}C δ	^1H δ		J (Hz)
1	73.3	6.06	*dd*	5.9, 1.0
2	201.1			
3	102.4	5.56	*dd*	7.8, 5.9
4	74.7	4.50	*dddd*	7.8, 7.8, 6.4, 1.0
5	39.8	2.43	*ddd*	13.7, 7.8, 6.4
		1.88	*ddd*	13.7, 6.4, 3.9
6	73.7	4.21	*ddd*	6.4, 4.4, 3.9
7	83.9	3.96	*ddd*	11.2, 4.4, 4.4
8	29.7	2.63	*ddd*	13.7, 11.2, 3.9
		2.34	*ddd*	13.7, 6.4, 4.4
9	129.6	5.81	*br ddd*	10.7, 6.4, 6.4
10	127.3	5.77	*br ddd*	10.7, 7.3, 7.3
11	30.7	2.72	*br ddd*	15.6, 7.3, 2.0
		2.69	*m*	
12	79.8	4.09	*m*	
13	58.1	4.09	*m*	
14	28.1	2.01	*ddq*	14.7, 2.4, 7.3
		1.78	*ddq*	14.7, 6.8, 7.3
15	11.3	1.09	*t*	7.3

*Measured in chloroform-d_1.
†Assignment was made with the aid of HSQC.

Consequently, the structure of pannosallene must be represented by formula **1** (4*S**,6*S**,7*S**,12*R**,13*S**), which has a 2,6-dioxa [6.3.0]undecene skeleton in the same relative configuration as that of epilaurallene [10], previously isolated from *L. nipponica*, one of the Japanese species. However, the ^1H and ^{13}C NMR spectral data of pannosallene (**1**) were different from those of epilaurallene. Re-examination of the NOESY spectrum of epilaurallene revealed no correlation between H-4 and H-6 and between H-4 and H-7, thus indicating that the assigned structure of epilaurallene was incorrect. Further structural investigation of epilaurallene which may be a polymorph of laurallene [11] would be useful.

EXPERIMENTAL

^1H NMR: 400 MHz and ^{13}C NMR: 100 MHz, $CDCl_3$, TMS as int. standard. Low- and high- resolution MS: 70 eV. CC: silica gel (Merck, Kieselgel 60, 70–230 mesh). Prep. TLC: silica gel (Merck, Kieselgel 60 F_{254S}). Yields based on wts of extracts.

Collection. Laurencia pannosa Zanardini was collected at An Thoi, Phu Quoc Island, Kien Giang Province, Vietnam, on February 8, 1993.

Extraction and isolation. Dried alga (9.9 g) was extracted with MeOH and the MeOH extracts partitioned between Et_2O and H_2O. The Et_2O soln was washed with H_2O, dried (Na_2SO_4) and evapd to leave a green oil (238 mg), which was fractionated by silica-gel CC with a step-wise gradient (hexane–EtOAc). The fr. eluted with hexane–EtOAc (9:1) was further submitted to prep. TLC using toluene to give a mixt. of chlorofucin (**2**) [3] and bromofucin (**3**) [4] (1.9%). The fr. eluted with hexane–EtOAc (3:1) was further subjected to prep. TLC with CH_2Cl_2 to yield pannosallene (**1**) (1%), along with sterols (1.9%).

Pannosallene (**1**). Needles. $[\alpha]_D^{26}$ + 64.3° (*c* 0.070; $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3020, 1260 1230, 1200, 1045, 930, 880, 855, 800, 715 and 660. ^1H NMR ($CDCl_3$): Table 1. ^1H NMR (C_6D_6): δ0.97 (3H, *t*, *J* = 7.3 Hz, H_3-15), 1.52 (1H, *ddq*, *J* = 14.7, 8.3 and 7.3 Hz, H-14), 1.56 (1H, *ddd*, *J* = 13.2, 6.4 and 4.4 Hz, H-5), 1.71 (1H, *ddq*, *J* = 14.7, 2.9 and 7.3 Hz, H-14), 1.74 (1H, *ddd*, *J* = 13.2, 7.3 and 6.4 Hz, H-5), 2.22 (1H, *ddd*, *J* = 14.2, 6.4 and 3.4 Hz, H-8), 2.32 (1H, *m*, H-11), 2.49 (1H, *ddd*, *J* = 15.1, 6.4 and 2.9 Hz, H-11), 2.58 (1H, *m*, H-8), 3.51 (1H, *ddd*, *J* = 6.4, 4.4 and 3.9 Hz, H-6), 3.59 (1H, *ddd*, *J* = 11.2, 4.4 and 3.9 Hz, H-7), 3.72 (1H, *ddd*, *J* = 9.8, 8.3 and 2.9 Hz, H-13), 3.90 (1H, *ddd*, *J* = 9.8, 7.3 and 2.9 Hz, H-12), 4.23 (1H, *dddd*, *J* = 7.3, 7.3, 6.4 and 1.0 Hz, H-4), 5.40 (1H, *dd*, *J* = 7.3 and 5.4 Hz, H-3), 5.53 (2H, *m*, H-9 and H-10) and 5.69 (1H, *dd*, *J* = 5.4 and 1.0 Hz, H-1). ^{13}C NMR ($CDCl_3$): Table 1. ^{13}C NMR (C_6D_6): Me: δ11.1 (C-15), CH_2: δ28.1 (C-14), 30.3 (C-8), 30.7 (C-11) and 39.8 (C-5), CH: δ58.1 (C-13), 73.4 (C-1), 73.6 (C-6), 74.5 (C-4), 79.7 (C-12), 83.6 (C-7), 103.2 (C-3) 127.0 (C-10) and 130.0 (C-9), C; δ201.6 (C-2). LR-MS *m/z* (rel. int.): 275, 273 (11:11) $[M - C_3H_2Br]^+$, 214 (10), 149 (15), 123 (13), 121 (18), 109 (23), 107 (49), 105 (20), 95 (24), 93 (30), 91 (33), 81 (34), 79 (96), 78 (34), 77 (57), 69 (33), 67 (77), 65 (46), 55 (73), 53 (32), 44 (54), 43 (68), 41 (100), 40 (40) and 39 (64). HR-MS *m/z*: 273.0482. Calc. for $C_{12}H_{18}{}^{79}BrO_2$ $[M - C_3H_2Br]$: 273.0488.

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