Pergamon

PII: S0031-9422(98)00099-5

Phytochemistry, Vol. 48, No. 6, pp. 987–990, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

ANHYDROAPLYSIADIOL FROM *LAURENCIA JAPONENSIS*

YOSHINORI TAKAHASHI, MINORU SUZUKI,* TSUYOSHI ABE† and MICHIO MASUDA†

Division of Material Science, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060, Japan; † Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060, Japan

(*Received in revised form* 5 *January* 1998)

Key Word Index—*Laurencia japonensis*; Rhodomelaceae; red alga; diterpenoid; halogenated compound; chemotaxonomy.

Abstract—A new brominated, anhydroaplysiadiol has been isolated from a new *Laurencia* species, *L. japonensis* Abe et Masuda sp. ined., along with aplysiadiol and 2,10-dibromo-3-chloro-α-chamigrene. The last two compounds are commonly found in this species collected from various localities. The structure of anhydroaplysiadiol was deduced from spectral evidence. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Species discrimination in the red algal genus *Laurencia* (Rhodomelaceae, Ceramiales) is complicated by the high degree of morphological variation within individual species. As is well documented, species of *Laurencia* produce diverse, unique, halogenated secondary metabolites [1]. Most species of *Laurencia* are characterized by at least one specific secondary metabolite not found in any others [2, 3] or a specific set of such metabolites [4]. The stability of this specificity and its chemotaxonomic value are supported by chemical studies based on field-collected and cultured plants of several species. Indeed, the chemistry of these species remained constant under varying field and culture conditions [5, 6]. Thus, secondary metabolite chemistry can provide criteria for the taxonomy in this troublesome genus [4]. In our continuing taxonomic studies of Japanese species of *Laurencia* based upon morphological and chemical features as well as genetic affinities [4, 6–8], we examined a new species, *L. japonensis* Abe et Masuda sp. ined. [unpublished results], collected off the coast of several locations in Japan. These samples contained two common metabolites, a halogenated diterpenoid, aplysiadiol (**2**), which has previously been isolated from the sea hare *Aplysia kurodai* [9], and a halogenated sesquiterpenoid, 2,10-dibromo-3-chloro-α-chamigrene (**3**), which has previously been obtained from *Laurencia nipponica* Yamada in Japan [10, 11] and an unidentified species of *Laurencia* in Mexico [12]. Previously, we reported on the structures of two C_{15} acetogenins, laurenenyne-A and -B, which had been isolated from an undescribed species of *Laurencia* collected at Kamishima, Mie Prefecture in Japan [13]. Re-examination of the remaining fractions of the Kamishima sample showed the presence of **2** and **3**. Further taxonomical examination confirmed that the alga collected at Kamishima is also *Laurencia japonensis*.

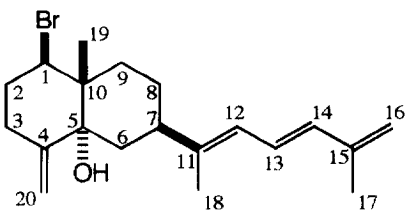
In addition to **2** and **3**, *L. japonensis* contained novel C_{15} acetogenins [unpublished data]. Moreover, a new brominated compound, which we named anhydroaplysiadiol, was isolated from material collected at Chinzei, Saga Prefecture, southern Japan. In this paper we report the isolation and structural elucidation of anhydroaplysiadiol (**1**).

RESULTS AND DISCUSSION

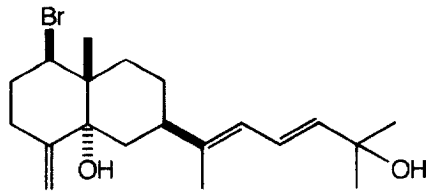
L. japonensis collected at Chinzei, Saga Prefecture, on April 1, 1995, was extracted with methanol. The methanol extract was fractionated by CC over silica gel with a step gradient (hexane and EtOAc). The fraction eluted with hexane–EtOAc (9:1) was further subjected to prep. TLC with toluene to give 2,10-dibromo-3-chloro-α-chamigrene (**3**) [10–12] in 1.3% yield based on the extract. In addition, the fraction eluted with hexane–EtOAc (1:1) was further subjected to prep. TLC with a toluene-EtOAc (4:1) to give anhydroaplysiadiol (**1**) (1.2%) together with aplysiadiol (**2**) (9.7%), the latter of which has previously been isolated from the sea hare *Aplysia kurodai* [9].

Anhydroaplysiadiol (**1**) was assigned the molecular formula $C_{20}H_{29}BrO$ (HREIMS). Its IR spectrum showed the presence of a hydroxyl group (3450 cm^{-1}), an *exo*-methylene group (1680 and 880 cm^{-1}) and a *trans*-double bond (930 cm^{-1}). The presence of two *exo*-methylene groups was evident from the ^1H and ^{13}C NMR spectra (Table 1), which showed signals at

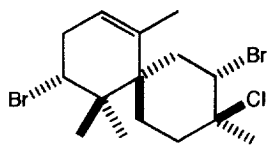
* Author to whom correspondence should be addressed.



1



2



3

δ_H 4.78, 4.88, 4.95 and 4.96 (each 1H, *br s*) and δ_C 109.7 (*t*), 115.9 (*t*), 149.6 (*s*) and 142.4 (*s*). Furthermore, the ^{1}H NMR spectrum indicated the presence of two vinyl methyl groups at δ_H 1.83 and 1.90 (each 3H, *br s*), one tertiary methyl group at δ_H 0.97 (3H, *s*), a bromomethine group at δ_H 4.77 (1H, *dd*, J = 10.7 and 4.9 Hz), a *trans*-double bond at δ_H 6.45 (1H, *dd*, J = 15.1 and 10.7 Hz) and 6.28 (1H, *d*, J = 15.1 Hz) and a trisubstituted double bond at δ_H 5.98 (1H, *br d*, J = 10.7 Hz). The ^{1}H and ^{13}C NMR spectra of **1** were very similar to those of aplysiadiol (**2**). Detailed analysis of the ^{1}H-^{1}H COSY, from which only limited information was provided, and HSQC data, as well as a comparison of the spectral data with those of **2**, led us to assign a planar structure **1** for anhydroaplysiadiol. This assignment was confirmed by the ^{1}H-^{13}C long range correlations determined by HMBC (Table 1). The presence of a 1,5-dimethyl-1,3,5-hexatriene moiety was indicated by cross peaks of H-12 to C-7, C-18 and C-14 and H-14 to C-12, C-13, C-15, C-16 and C-17.

The relative configuration of four chiral centres in **1** could not completely be established from the NOESY spectrum because of the overlapping of several proton

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

No.	^{13}C† δ	^{1}H δ (J in Hz)	Long range correlation
1	63.75	4.77 (*dd*, 10.7, 4.9)	H_2-2
2	34.04	2.1–2.3 (*m*)	H_2-3
3	32.57	2.75 (*m*) and 2.22 (*m*)	H_2-2
4	149.56		H_2-3, H_2-20
5	76.85		H_2-3, H_2-20, H_3-19
6	36.47	1.7–1.8 (*m*) and 1.4–1.6 (*m*)	
7	41.70	2.55 (*dddd*, 12.7, 12.7, 3.9, 3.9)	H-12, H_3-18
8	25.82	1.4–1.6 (*m*)	
9	32.74	1.7–1.8 (*m*)	H_3-19
10	43.09		H-1, H_2-2, H_3-19
11	143.12		H_3-18
12	123.98	5.98 (*br d*, 10.7)	H_3-18, H-14
13	125.46	6.45 (*dd*, 15.1, 10.7)	H-14
14	133.94	6.28 (*d*, 15.1)	H-12, H-13, H_3-16, H_3-17
15	142.41		H-13, H-14, H_3-17
16	115.87	4.96 (*br s*), 4.95 (*br s*)	H-14, H_3-17
17	18.66	1.90 (*br s*)	H-14, H_2-16
18	15.38	1.83 (*br s*)	H-7, H-12
19	14.89	0.97 (*s*)	H-1
20	109.66	4.88 (*br s*) and 4.78 (*br s*)	H_2-3

* Measured in chloroform-d_1.

† Assignments and multiplicities were determined by HSQC and DEPT experiments.

signals. However, since the ^1H and ^{13}C NMR spectra of anhydroaplysiadiol (**1**) were almost identical to those of aplysiadiol (**2**), except for the signals due to the conjugated triene or diene side chain in **1** and **2**, anhydroaplysiadiol should possess the same stereochemistry about the prenylated eudesmane ring as aplysiadiol. Moreover, the *E*-configuration of the double bond at C-11 was indicated by the chemical shift value (δ_C 15.38) of C-18 in the ^{13}C NMR spectrum of **1**.

Thus *L. japonensis* is characterized by its production of a set of two compounds, aplysiadiol (a haloditerpenoid) and 2,10-dibromo-3-chloro-α-chamigrene (a halosesquiterpenoid), along with various types of minor C_{15} acetogenins [unpublished data].

Previously brominated sesquiterpenoids, aplysin and aplysinol [14], and diterpenoids, aplysin-20 [15] and isoaplysin-20 [16], have been isolated from the sea hare *A. kurodai*. Among them aplysin and aplysinol were found in *L. okamurae* Yamada [17, 18]. Moreover, aplysin-20 was also obtained from *L. venusta* Yamada [19]. The present results as well as the previous ones clearly indicate that *A. kurodai* consumes various species of the red algal genus *Larencia*.

EXPERIMENTAL

General

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

Collection

Six samples of *Laurencia japonensis* were collected at Heki, Yamaguchi Prefecture (July 13, 1989), Mihonoseki, Shimane Prefecture (July 15, 1989), Iwami, Tottori Prefecture (July 16, 1989), Toyooka, Hyogo Prefecture (July 16, 1989), Shimoda, Shizuoka Prefecture (July 6, 1993) and Chinzei, Saga Prefecture (April 1, 1995). The voucher specimens are deposited in the Herbarium of the Graduate School of Science, Hokkaido University (SAP).

Extraction and isolation

The partially dried alga (38 g) collected at Chinzei (SAP 062630) was extracted with MeOH, and the MeOH soln was concentrated *in vacuo* and partitioned between Et_2O and water. The Et_2O soln was washed with water, dried over anhydrous Na_2SO_4 and evaporated to leave a brown oil (922 mg). The extract (231 mg) was fractionated by CC on silica gel with a step gradient (hexane and EtOAc). The fraction (30 mg) eluted with hexane–EtOAc (9:1) was further submitted to prep. TLC with toluene to give 2,10-dibromo-3-chloro-α-chamigrene (**3**) [10–12] (3.2 mg, 1.3% based on the weight of the MeOH extract). The fr. eluted with hexane–EtOAc (3:1) (55 mg) gave anhydroaplysiadiol (**1**) (2.7 mg, 1.2%) and new C_{15} acetogenins [unpublished data] by prep. TLC with toluene. Furthermore, the fr. (94 mg) eluted with hexane–EtOAc (1:1) was further purified by prep. TLC with toluene–EtOAc (4:1) to give aplysiadiol (**2**) [9] (22 mg, 9.7%).

The remaining five samples collected from other locations were also extracted with MeOH and each MeOH extract was separated in the same manner. Heki sample (SAP 062611): aplysiadiol (**2**) (15.2%) and 2,10-dibromo-3-chloro-α-chamigrene (**3**) (0.96%); Mihonoseki sample (SAP 062612): **2** (10.1%) and **3** (2.5%), Iwami sample (SAP 062613): **2** (5.5%) and **3** (1.2%); Toyooka sample (SAP 062614): **2** (8.3%) and **3** (1.4%); Shimoda sample (SAP 062629): **2** (7.6%) and **3** (0.9%). The samples also contained a variety of C_{15} acetogenins [unpublished data].

Kamishima sample [13] (SAP 062615–062620); detailed analysis of the previous extract yielded **2** (10.4%) and **3** (9.6%).

Anhydroaplysiadiol (**1**). Colourless oil, $[\alpha]_D^{27}$ −116.2° ($CHCl_3$, *c* 0.10). IR ν_{max} (film) cm^{-1}: 3450, 3020, 1680, 1520, 1225, 1205, 1165, 1020, 1094, 985, 930, 915, 880, 795, 715 and 665; ^1H and ^{13}C NMR: Table 1; LR-EIMS *m/z* (rel. int.): 366, 364 (16:16) $[M]^+$, 348, 346 (21:21) $[M-H_2O]^+$, 333 (19), 331 (16), 267 (61), 211 (11), 187 (18), 159 (42), 145 (40), 119 (41), 107 (100), 91 (40), 81 (35), 55 (30) and 41 (33); HR-EIMS *m/z*: 364.1400. Calcd for $C_{20}H_{29}{}^{79}BrO$, 364.1402 [M].

Aplysiadiol (**2**). Colourless oil, $[\alpha]_D^{20}$ −50.8° ($CHCl_3$, *c* 0.44). ^1H NMR ($CDCl_3$): δ 0.97 (3H, *s*, H_3-19), 1.35 (6H, *s*, H_3-16 and H_3-17), 1.52 (3H, *m*, H_2-8 and H-6), 1.76 (1H, *m*, H-9), 1.87 (1H, *dd*, *J* = 13.7 and 12.7 Hz, H-6), 2.12 (2H, *m*, H-3 and H-9), 2.20 (2H, *m*, H_2-2), 2.52 (1H, *dddd*, *J* = 12.7, 12.7, 3.9 and 3.9 Hz, H-7), 2.75 (1H, *m*, H-3), 4.76 (1H, *dd*, *J* = 10.7 and 4.9 Hz, H-1), 4.78 (1H, *br s*, H-20), 4.88 (1H, *br s*, H-20), 5.78 (1H, *d*, *J* = 15.1 Hz, H-14), 5.90 (1H, *br d*, *J* = 10.7 Hz, H-12) and 6.49 (1H, *dd*, *J* = 15.1 and 10.7 Hz, H-13); ^{13}C NMR ($CDCl_3$): Me; δ 14.89 (C-19), 15.25 (C-18), 29.96 × 2 (C-16 and C-17), CH_2; δ 25.81 (C-8), 32.59 (C-3), 32.77 (C-9), 34.05 (C-2), 36.47 (C-6) and 109.62 (C-20), CH; δ 41.55 (C-7), 63.72 (C-1), 123.02 × 2 (C-12 and C-13) and 139.67 (C-14), C; δ 43.11 (C-10), 70.96 (C-15), 76.85 (C-5), 142.56 (C-11) and 149.41 (C-4). These NMR spectra together with the IR spectrum were identical to those of the authentic sample.

2,10-*Dibromo-3-chloro-α-chamigrene* (**3**). Colourless oil, $[\alpha]_D^{21}$ −20.5° ($CHCl_3$, *c* 0.60). IR and NMR data were identical to those of the authentic sample.

Acknowledgements—We are grateful to Prof. Kiyoyuki Yamada, Nagoya University, for providing aplysiadiol and its spectral data. This study was supported in part by a Grant-in-Aid for Scientific Research (No. 09839001 to M.M.) from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES

1. Erickson, K. L. In *Marine Natural Products: Chemical and Biological Perspectives*, Vol. V, ed. P. J. Scheuer, Academic Press, New York, 1983, pp. 131–257.
2. Fenical, W., *Journal of Phycology*, 1975, **11**, 245.
3. Fenical, W. and Norris, J. N., *Journal of Phycology*, 1975, **11**, 104.
4. Masuda, M., Abe, T., Suzuki, T. and Suzuki, M., *Phycologia*, 1996, **35**, 550.
5. Howard, B. M., Nonomura, A. M. and Fenical, W., *Biochemical Systematics and Ecology*, 1980, **8**, 329.
6. Masuda, M., Abe, T., Sato, S., Suzuki, T. and Suzuki, M., *Journal of Phycology*, 1997, **33**, 196.
7. Matsuo, Y., Suzuki, M. and Masuda, M., *Chemistry Letters*, 1995, 1043.
8. Suzuki, M., Mizuno, Y., Matsuo, Y. and Masuda, M., *Phytochemistry*, 1996, **43**, 121.
9. Ojika, M., Yoshida, Y., Okumura, M., Ieda, S. and Yamada, K., *Journal of Natural Products*, 1990, **53**, 1619.
10. Suzuki, M., Furusaki, A. and Kurosawa, E., *Tetrahedron*, 1979, **35**, 823.
11. Suzuki, M., Segawa, M., Kikuchi, H., Suzuki, T. and Kurosawa, E., *Phytochemistry*, 1985, **24**, 2011.
12. Howard, B. M. and Fenical, W., *Tetrahedron Letters*, 1975, 1687.
13. Suzuki, M., Matsuo, Y. and Masuda, M., *Tetrahedron*, 1993, **49**, 2033.
14. Yamamura, S. and Hirata, Y., *Tetrahedron*, 1963, **19**, 1485.
15. Yamamura, S. and Hirata, Y., *Bulletin of the Chemical Society of Japan*, 1971, **44**, 2560.
16. Yamamura, S. and Hirata, Y., *Tetrahedron Letters*, 1977, 2171.
17. Irie, T., Suzuki, M. and Hayakawa, Y., *Bulletin of the Chemical Society of Japan*, 1969, **42**, 843.
18. Suzuki, M. and Kurosawa, E., *Bulletin of the Chemical Society of Japan*, 1979, **52**, 3352.
19. Suzuki, M., Kurosawa, E. and Kurata, K., *Phytochemistry*, 1988, **27**, 1209.