Mem. Fac. Fish. Kagoshima Univ.,
Vol. 57, pp. 29~35（2008）

Chemical Structures of Quinolinol Compounds Produced by Marine *Pseudoalteromonas* sp. A1-J11

Taizo Sakata,[1*] Carmelo Segovia del Castillo,[2] Takeshi Yoshikawa,[1]
and Masahito Hashimoto[3]

***Key words*:** butyl-quinolinol, pentyl-quinolinol, NMR, anti-*Vibrio* substance, algicidal activity, *Pseudoalteromonas*

Abstract

Three kinds of quinolinol compounds were produced extracellularly by a marine anti-bacterial strain, *Pseudoalteromonas* sp. A1-J11. The NMR spectra of ^{13}C and ^{1}H and HMBC for major two compounds among them were measured by using a NMR spectrometer (JEOL ECA 600). The chemical structures of these compounds, AVS-03*a* and AVS-03*d*, were identified as 2-*n*-butyl-4-quinolinol and 2-*n*-pentyl-4-quinolinol, respectively. AVS-03*d* showed algicidal activity against a diatom strain *Chaetoceros ceratosporum* as well as *Vibrio* strains.

Marine *Pseudoalteromonas* and *Pseudomonas* spp. are well known to produce various antibiotic and biologically active substances extracellularly.[1-5)] They are frequently found to be associated with eukaryotic hosts in the marine environments and synthesize biologically active agents against a variety of target organisms. Marine *Pseudoalteromonas* sp. A1-J11 isolated from sea water in Kagoshima Bay, Japan was also reported to produce three kinds of quinolinol compounds showing anti-bacterial activity against *Vibri*o strains in previous papers.[6-8)] The chemical structure of AVS-03*d* among them was identified as 2-*n*-pentyl-4-quinolinol on the basis of mass spectrometry and NMR spectroscopy.[8)] In this study, the authors describe ^{13}C and ^{1}H data and HMBC (Heteronuclear multiple bond correlation) for major quinolinol compounds produced by *Pseudoalteromonas* sp. A1-J11 based on NMR analysis and growth inhibitory activity against a diatom strain, *Chaetoceros ceratosporum* C-16.

Materials and Methods

Test strains and media

Antagonistic strain *Pseudoalteromonas* sp. A1-J11 was isolated from sea water in Kagoshima Bay, Kagoshima Prefecture, Japan as described in the previous paper.[7)] Diatom strain *Chaetoceros ceratosporum* C-16 was provided by Dr. K. Fukami of Kochi University.

Bacterial strains were cultured in a modified ZoBell medium (Z-CII)[9)] containing Polypepton (Nippon Seiyaku, Tokyo, Japan) 5 g/l and yeast extract (Nippon Seiyaku) 1g/l in 3/4 strength artificial seawater (ASW, Herbst's formula composed of NaCl 30.0 g, KCl 7.0 g, $MgCl_2 \cdot 6H_2O$ 10.8 g, $MgSO_4 \cdot 7H_2O$ 5.4 g, and $CaCl_2 \cdot 2H_2O$ 1.0 g per l). *Chaetoceros ceratosporum* C-16 was cultivated in 300 ml of Provasoli's modified liquid medium (ESS) with aeration under illumination of 5,000 lx (12L:12D light cycle). Diatom cells were harvested with centrifugation after 2 weeks of incubation and then resuspended in fresh ESS liquid medium at a density

[1] 鹿児島大学水産学部食品・資源利用学分野 (Department of Biochemistry and Technology of Marine Food and Resources, Faculty of Fisheries, Kagoshima University, 4-50-20 Shimoarata, Kagoshima 890-0056, Japan)
[2] 鹿児島大学大学院連合農学研究科 (The United Graduate School of Agricultural Sciences, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan)
[3] 鹿児島大学大学院理工学研究科・ナノ構造先端材料工学専攻 (Department of Nanostructure and Advanced Materials, Graduate School of Science and Engineering, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan)
* Corresponding Author, Email: sakata@fish.kagoshima-u.ac.jp

of 10 times higher than the culture medium. Double layer agar plates were prepared by mixing one ml of the diatom cell suspension with 2 ml of ESS soft agar (0.8% of agar concentration) maintained 50°C to be poured on an ESS basal agar plate as described in the previous reports.[10, 11]

Purification of anti-*Vibrio* substances from strain A1-J11

The extraction and purification of anti-*Vibrio* substances was made by use of the same procedure as described in the previous papers.[6, 8]

The supernatant of 4 day culture (300 ml x 4 flasks) of strain A1-J11 was separated by centrifugation at 10,000 x g for 15 min and extracted with an equal volume of ethyl acetate (EtAc). Ethyl acetate fraction was applied to a silica gel column (Silica Gel 60, Merck, Darmstadt, Germany, 260 x 25 mm column) and eluted with mobile phase of chloroform: ethyl acetate: acetone (12:1:1, v/v/v). After visible yellow fractions were eluted, about 200 ml of eluent mixture was collected and concentrated. Then, the silica gel purified fraction concentrated and dissolved in methanol was applied to high performance liquid chromatograph (HPLC) column (Mightsyl RP-18GP, 250 x 10 mm, Kanto Kagaku, Tokyo, Japan) and eluted with 25% aqueous acetonitrile at a flow rate of 2.5 ml/min. Eluted fractions were monitored by UV absorption at 325 nm.

NMR analyses

Two isolated compounds and one standard compound, 2-methyl-4-quinolinol (Sigma-Aldrich, St. Louis, USA) were dissolved in methanol-*d*4 (Wako Chemical, Osaka, Japan) and applied to a NMR spectrometer (JEOL ECA 600, JEOL, Tokyo, Japan). The chemical shifts were expressed as δ values with methanol (^{13}C; δ 49.0) as the internal standards.

Algicidal activity assay

Algicidal activity against diatom cells of *Chaetoceros ceratosporum* C-16 was conducted by disk diffusion assay in which the test substances dissolved in methanol (MeOH) were put onto a paper disk (50 μg/8 mm paper disk), dried, and applied to an algal double-layer plate. Algicidal activity was confirmed by observing growth inhibition zone around the paper disks on the double-layer agar plate.

Results

NMR chemical shifts and HMBC correlation for quinolinol compounds

The NMR spectra of major two anti-bacterial compounds (AVS-03*a* and AVS-03*d*) isolated from *Pseudoalteromonas* sp. A1-J11 and 2-methyl-4-quinolinol (2MQ) were measured by using a NMR spectrometer. A butyl group (C4) with lower chemical shifts than δ 49.0 (methanol) was observed for AVS-03*a*, while a pentyl group (C5) was done for AVS-03*d* in ^{13}C NMR spectra as shown in Figs 1, 2 and 3. From the chemical shifts and HMBC data of these compounds (Tables 1, 2 and 3), AVS-03*a* and AVS-03*d* were deduced to be 2-*n*-butyl-4-quinolinol (BQ) and 2-*n*-penthyl-4-quinolinol (PQ), respectively. The chemical structures of three quinolinol compounds are shown in Fig. 4.

Algicidal activity of quinolinol compounds

The algicidal activity of quinolinol compounds was tested by using the paper disk method. The result shown in Fig. 5 indicates that only AVS-03*d* (2-*n*-pentyl-4-quinolinol) has algicidal activity against *Chaetoceros ceratosporum* C-16, but other two quinolinol compounds do not.

Discussion

Three anti-bacterial substances against *V. harveyi* from the culture supernatant of a marine *Pseudoaltermonas* sp. A1-J11 were purified and the chemical structures of major two compounds were identified in the previous paper.[8] In this paper, ^{1}H and ^{13}C NMR spectra and HMBC data were obtained for these compounds dissolved in methanol-*d*4. The authors compared the chemical shifts and HMBC correlation of three test compounds. These analytical data reconfirmed that isolated compounds are 2-*n*-butyl-4-quinilinol (BQ) and 2-*n*-pentyl-4-quinolinol (PQ). Two isolated compounds were shown to strong inhibitory activity against *Vibrio harveyi* strains. The result obtained in this experiment indicates that only AVS-03*d* (2-*n*-pentyl-4-quinolinol) has algicidal activity against *Chaetoceros ceratosporum* C-16 as well as anti-bacterial activity against *V. harveyi*. Recently, synthetic 2-*n*-pentyl-4-quinolinol (PQ) has been reported to be useful as an antifoulant agent which inhibits growth of diatoms, *Amphora* and *Navicula* spp.[5, 12] However, other quinolinol compounds have not been examined on algicidal activity.

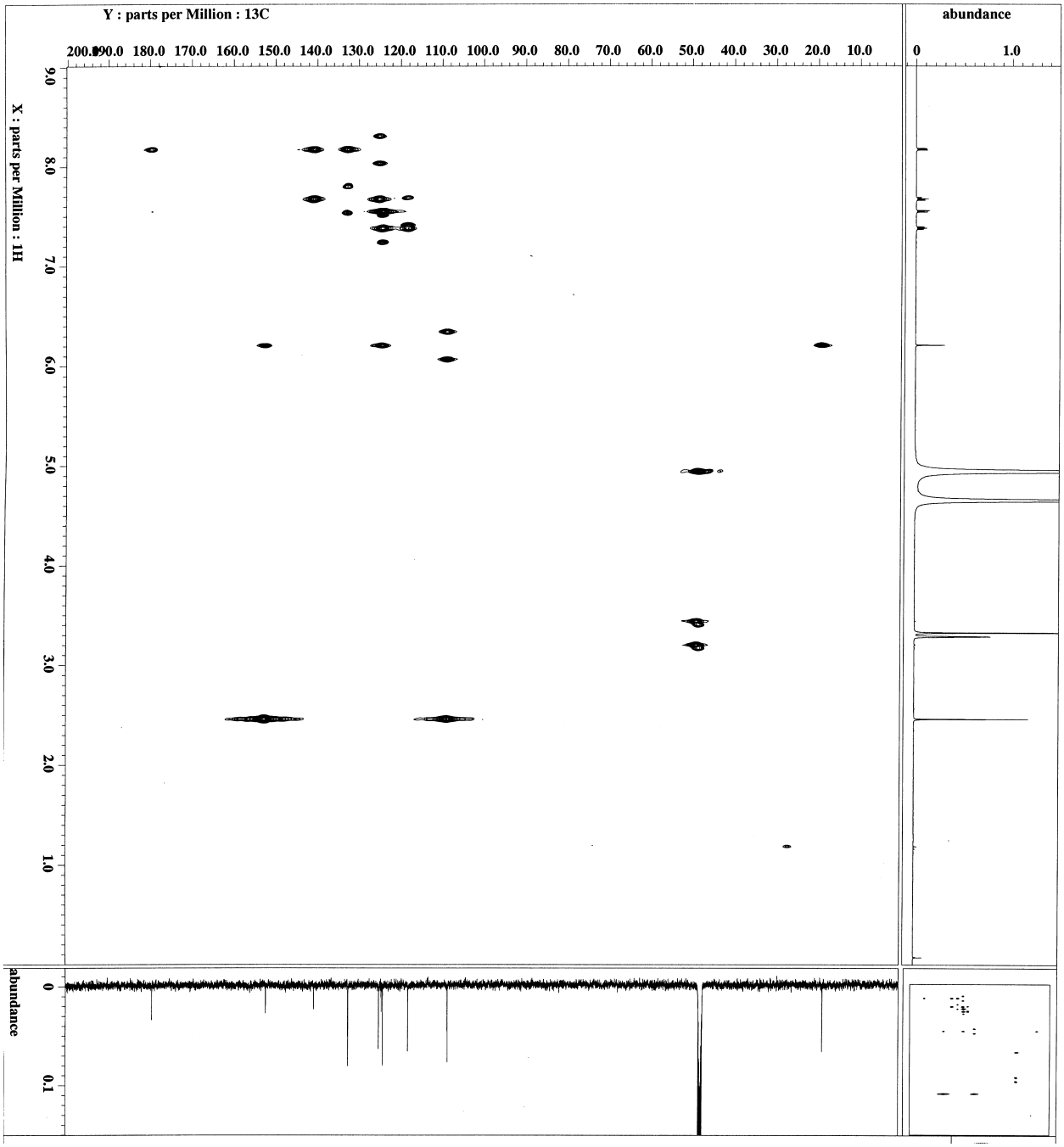

Fig. 1. HMBC analysis of synthetic 2MQ in methanol-*d*4 as deduced from ^{1}H NMR (vertical) and ^{13}C NMR (horizontal) spectra.

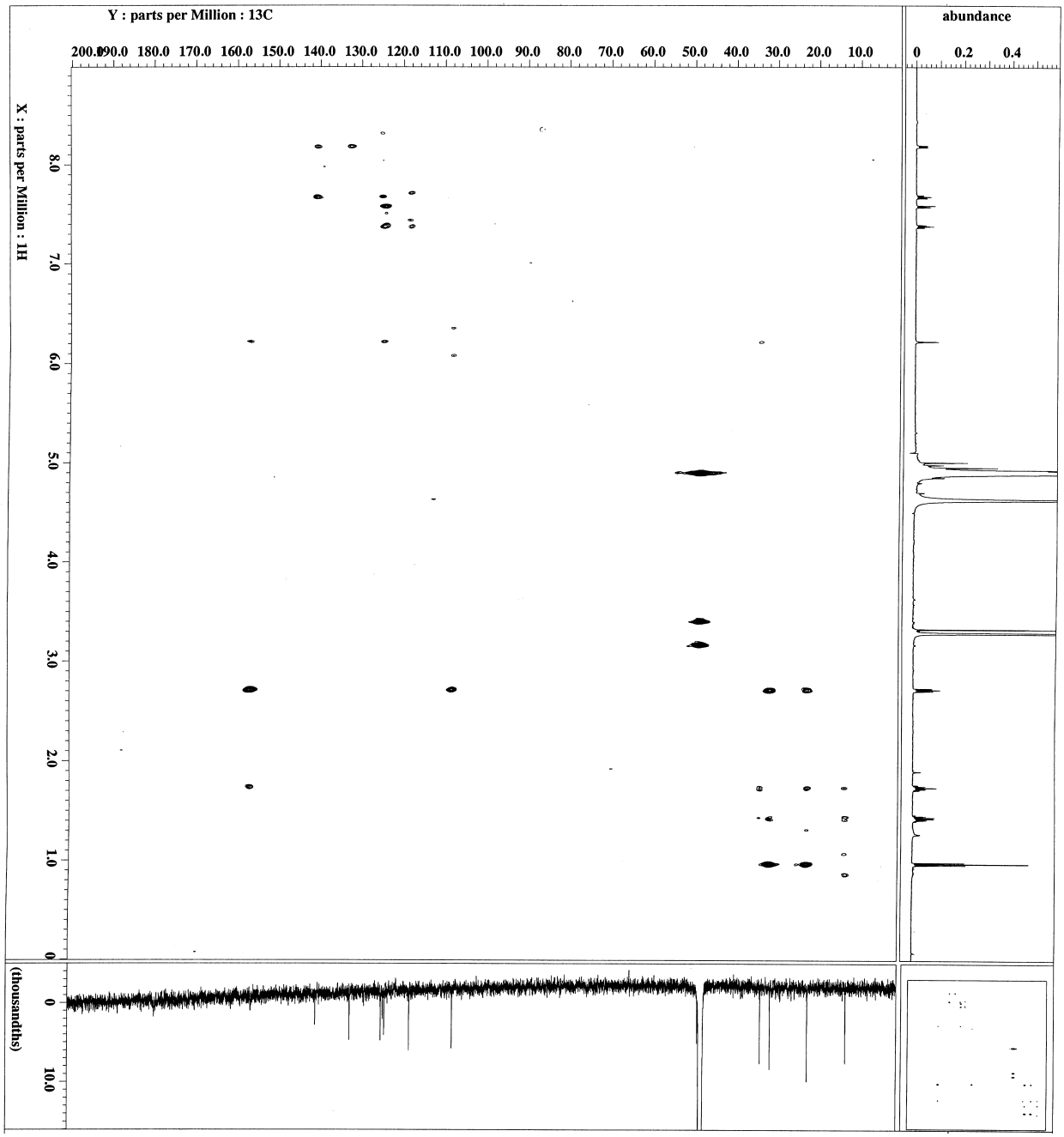

Fig. 2. HMBC analysis of AVS-03*a* in methanol-*d*4 as deduced from ^{1}H NMR (vertical) and ^{13}C NMR (horizontal) spectra.

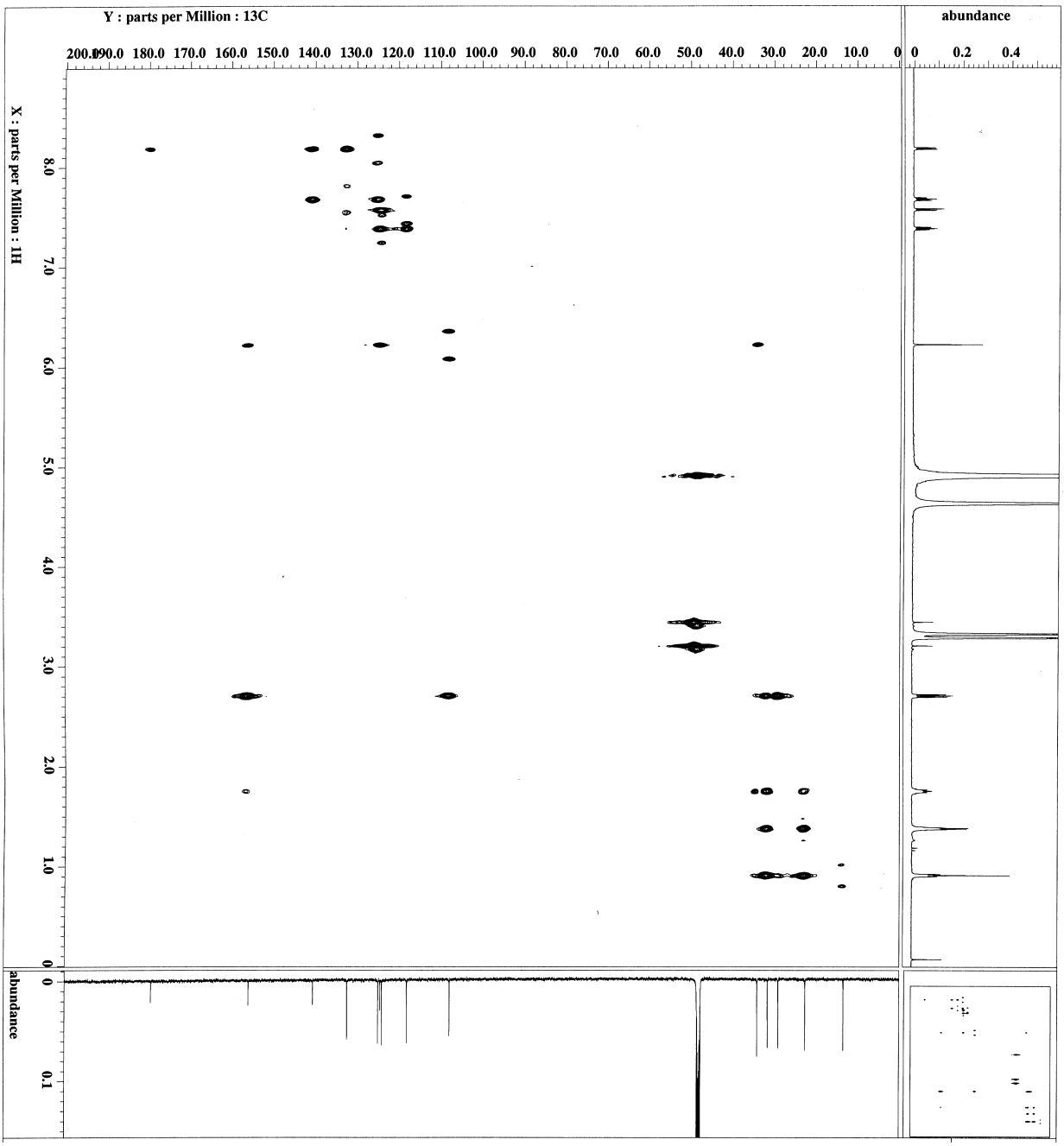

Fig. 3. HMBC analysis of AVS-03*d* in methanol-*d*4 as deduced from ^{1}H NMR (vertical) and ^{13}C NMR (horizontal) spectra.

Table 1. The data of ^{13}C and ^{1}H and HMBC correlation for 2MQ in methanol-*d*4

Carbon Number	$^{13}C(\delta)$	$^{1}H(\delta)$	HMBC correlation (from H to C)
1	18.50	1.17(3H, t)	2, 3
2	151.72	-	-
3	108.21	6.21(1H, s)	1, 2, 5
4	179.22	-	-
5	123.99	-	-
6	124.63	8.17(1H, d)	4, 8, 9
7	123.70	7.38(1H, m)	5, (8), 9
8	132.06	7.55(1H, d)	6, (7), 10
9	117.65	7.66(1H, m)	(4), 5, 7
10	140.23	-	-

Table 2. The data of ^{13}C and ^{1}H and HMBC correlation for AVS-03*a* in methanol-*d*4

Carbon Number	$^{13}C(\delta)$	$^{1}H(\delta)$	HMBC correlation (from H to C)
1	12.74	0.97(3H, t)	2, 3
2	21.97	1.42(2H, m)	3
3	30.99	1.73(2H, m)	2
4	33.38	1.71(2H, t)	2, 3, 5
5	155.85	-	-
6	107.47	6.22(1H, s)	4, 5, 8
7	179.35	-	-
8	124.12	-	-
9	124.61	8.18(1H, d)	7, 11, 12
10	123.74	7.38(1H, m)	8, (12), 13
11	132.09	7.58(1H, d)	9, (10)*, 13
12	117.77	7.67(1H, m)	(7), 8, 10
13	140.28	-	-

Table 3. The data of ^{13}C and ^{1}H and HMBC correlation for AVS-03*d* in methanol-*d*4

Carbon Number	$^{13}C(\delta)$	$^{1}H(\delta)$	HMBC correlation (from H to C)
1	12.94	0.91(3H, t)	2, 3
2	22.09	1.38(2H, m)	3
3	28.56	1.38(2H, m)	2
4	31.09	1.75(2H, t)	2, 3, 5, 6
5	33.62	2.70(2H, t)	3, 4, 6, 7
6	155.85	-	-
7	107.48	6.22(1H, s)	5, 6, 9
8	179.31	-	-
9	124.13	-	-
10	124.62	8.18(1H, d)	8, 12, 13
11	123.74	7.38(1H, m)	9, (12)*, 13
12	132.08	7.68(1H, m)	10, (11), 14
13	117.73	7.57(1H, d)	(8), 9, 11
14	140.27	-	-

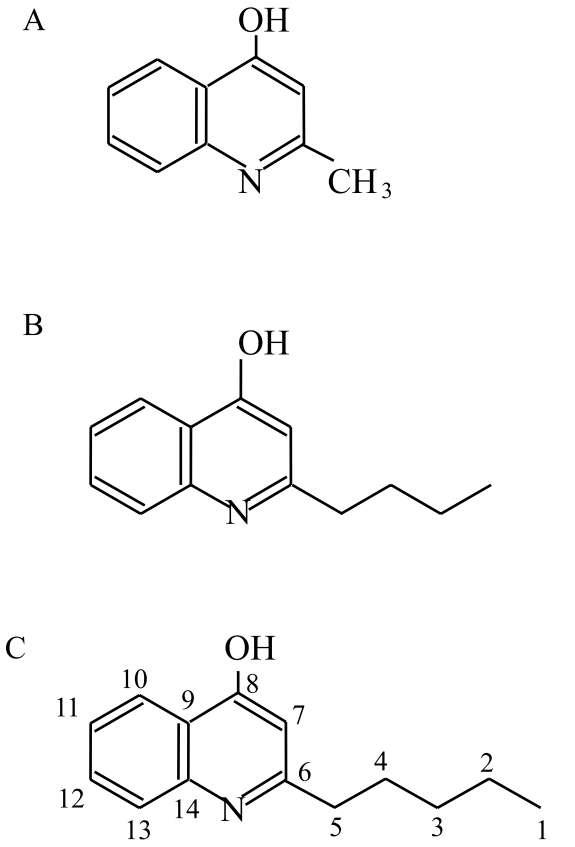

Fig. 4. Chemical structures of 2-methyl-4-quinolinol (A), AVS-03*a* (B), and AVS-03*d* (C). Numbers indicate assignment of carbon positions based on ^{13}C NMR signals.

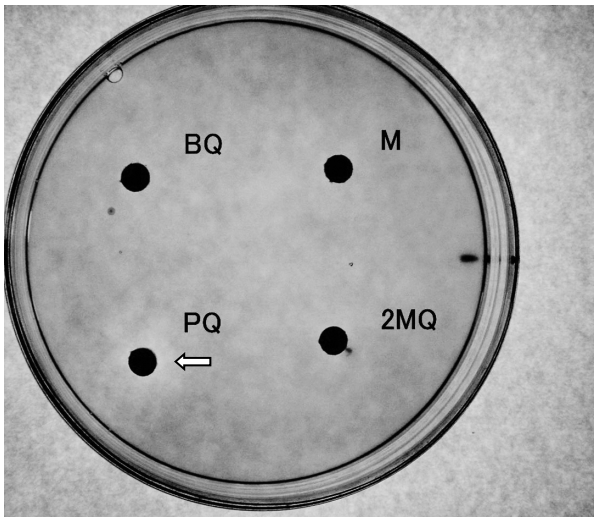

Fig. 5. Growth inhibitory activity of isolated quinolinol compounds on a double layer agar plate containing *Chaetoceros ceratosporum* C-16 cells. Arrow indicates growth inhibition zone around a paper disk. M, methanol; 2MQ, 2-methyl-4-quinolinol (synthetic); BQ, 2-*n*-butyl-4-quinolinol (isolated); PQ, 2-*n*-pentyl-4-quinolinol (isolated).

Further work is needed to determine the action mechanisms of quinolinol compounds as anti-bacterial and anti-algal agents

References

1. Wratten, S. T., M. S. Wolfe, R. J. Andersen, and D. J. Faulkner (1977). Antibiotic metabolism from a marine pseudomonad. *Antimicrob. Agents Ch*., **11**: 411-414.
2. Leisinger, T. and R. Margraff (1979). Secondary metabolites of the fluorescent pseudomonads. *Microbiol. Rev.*, **43**: 422-442.
3. Gauthier, M. J. and V. A. Breittmayer (1979). A new antibiotic-producing bacterium from seawater: *Alteromonas aurentia* sp. nov. *Int. J. Syst. Bacteriol*., **29**: 366-372.
4. Holmström, C. and S. Kjelleberg (1999). Marine *Pseudoalteromonas* species are associated with higher organisms and produce biologically active extra-cellular agents. *FEMS Microbiol. Ecol*., **30**: 285-293.
4. Isnansetyo, A. and Y. Kamei (2003). MC21-A, a bactericidal antibiotic produced by a new marine bacterium, *Pseudoalteromonas phenolica* sp. nov. O-BC30, against methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Ch*., **47**: 480-488.
5. Long, R. A., A. Qureshi, D. J. Faulkner, and F. Azam (2003). 2-*n*-pentyl-4-quinolinol produced by a marine *Alteromonas* sp. and its potential ecological and biogeochemical roles. *Appl. Environ. Microb*., **69**: 568-576.
6. Sakata, T., C. S. del Castillo, Y. Demizu, M. Matsuzaki, and T. Yoshikawa (2007). Purification and characterization of anti-*Vibrio* substances from marine *Pseudoalteromonas* sp. A1-J11. *Mem. Fac. Fish. Kagoshima Univ.*, **56**: 63-68.
7. del Castillo, C. S., M. I. Wahid, T. Yoshikawa, and T. Sakata (2008). Isolation and inhibitory effect of anti-*Vibrio* substances from *Pseudoalteromonas* sp. A1-J11 isolated from the coastal sea water of Kagoshima Bay. *Fisheries Sci*., **74**: 174-179.
8. del Castillo, C. S., T. Yoshikawa, M. Hashimoto, and T. Sakata (2008). Correlation between chemical structures and inhibitory activities of anti-bacterial substances from marine *Pseudoalteromonas* sp. A1-J11. *Fish Pathol*., **43**: 65-71.
9. Sashihara, N., T. Sakata, and D. Kakimoto (1975). Study on the proteases of marine bacteria. *Mem. Fac. Fish. Kagoshima Univ.*, **24**: 149-160.
10. Sakata, T. and K. Iwamoto (1995). Isolation of marine algicidal microorganisms on diatom double layer agar plates. *Fisheries Sci*., **61**: 173-174.
11. Sakata, T., T. Fujisawa, and T. Yoshikawa (2000). Colony formation and fatty acid composition of marine labyrinthulid isolates grown on agar media. *Fisheries Sci*. **66**: 84-90.
12. Wigglesworth-Cooksey, B., K. E. Cooksey, and R. Long (2007). Antibiotic from the marine environment with antimicrobial fouling activity. *Environ. Toxicol*., **22**: 275-280.