Tetrahedron Vol. 46, No. 23, pp. 7699-7702, 1990
Printed in Great Britain

0040-4020/90 $3.00+.00

HYRTIOSINS A AND B, NEW INDOLE ALKALOIDS FROM THE OKINAWAN MARINE SPONGE *HYRTIOS ERECTA*

Jun'ichi Kobayashi*, Tetsuya Murayama[a], Masami Ishibashi, Sumiko Kosuge[b], Masako Takamatsu[a], Yasushi Ohizumi[c], Hiroshi Kobayashi[b], Tomihisa Ohta[c], Shigeo Nozoe[c], and Takuma Sasaki[d]

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan, [a]Mitsubishi Kasei Institute of Life Sciences, Machida, Tokyo 194, Japan, [b]School of Hygienic Sciences, Kitasato University, Kanagawa 228, Japan, [c]Pharmaceutical Institute, Tohoku University, Sendai 980, Japan, and [d]Cancer Research Institute, Kanazawa University, Kanazawa 920, Japan

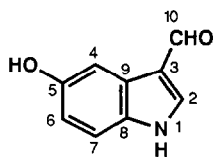
(*Received in Japan* 6 *June* 1990)

Summary: Two new indole alkaloids, hyrtiosins A (**2**) and B (**3**), together with known 5-hydroxyindole-3-aldehyde (**1**) have been isolated from the Okinawan marine sponge *Hyrtios erecta* and their structures elucidated on the basis of the spectroscopic data.

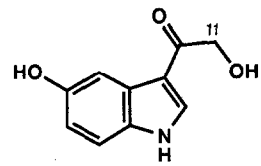
Recently several indole alkaloids have been found from a variety of marine sources like sponges,[1] tunicates,[2] red alga,[3] acorn worms,[4] and symbiotic bacteria.[5] During our studies on bioactive substances from Okinawan marine organisms,[6] methanol extracts of the Okinawan sponge *Hyrtios erecta* were investigated and two new indole derivatives, named hyrtiosins A (**2**) and B (**3**), were isolated in addition to previously reported 5-hydroxyindole-3-aldehyde (**1**). In this paper we describe the isolation and structure elucidation of compounds **1** ~ **3**.

The sponge *H. erecta* was collected at Ishigaki Island, Okinawa, and kept frozen until required. The methanol extract of this sponge was partitioned between ethyl acetate and water. The ethyl acetate-soluble fraction was subjected to silica gel flash column chromatography eluted with 5% chloroform in methanol followed by reversed-phase HPLC (ODS; MeOH/H_2O/AcOH, 50:50:0.5) to give compounds **1** (0.00024% yield, wet weight), **2** (0.00012%), and **3** (0.00024%).

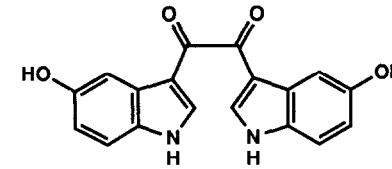
The molecular formula of compound **1** was deduced to be $C_9H_7NO_2$ on the basis of high resolution FABMS in combination with ^1H and ^{13}C NMR data. The UV absorption maxima of **1** at λ_{max} 252, 270, and 298 nm were characteristic of the 3-acyl indole derivatives.[7] The ^1H and ^{13}C NMR spectra of **1** clearly showed the presence of an aldehyde group (δ_H 9.80, 1H, s; δ_C 187.1 d) and suggested the presence of the indole nucleus to which two substituents were attached. To account for the molecular formula ($C_9H_7NO_2$) the other substituent has to be a hydroxyl group. The ^1H and ^{13}C NMR signals were firmly assigned on the basis of the HMBC experiment[8] in which the following long-range C-H correlations were observed (H/C): H-2/C-3, H-2/C-8, H-2/C-9, H-4/C-6, H-4/C-8, H-6/C-4, H-6/C-8, H-7/C-5, H-7/C-9, H-10/C-3, and H-10/C-9. These long-range connectivities clearly revealed the position of the formyl and hydroxyl groups to be on C-3 and C-5, respectively. The ^{13}C chemical shift of C-5 (δ_C 154.8 s) implied that this carbon bore the oxygenated functionality. Thus compound **1** was determined to be 5-hydroxyindole-3-aldehyde. Compound **1** has been tentatively identified as a metabolite of 5-hydroxytryptamine by the nuclear fraction of rat-liver



1



2



3

homogenate.[9] However, the full characterization of this compound (**1**) has never been reported and this is the first veritable isolation of compound **1** from a natural source.

Hyrtiosin A (**2**) was shown to have the molecular formula, $C_{10}H_9NO_3$, by high resolution FABMS [negative ion, *m/z* 190.0496 $(M-H)^-$, Δ -0.9 mmu]. The UV absorption maxima of **2** (λ_{max} 252, 270, and 303 nm) were also indicative of the presence of a 3-acyl indole chromophore. The ^1H and ^{13}C NMR spectra were compared with those of compound **1** and suggested that compound **2** also possessed an indole nucleus with two substituents at C-3 and C-5 positions. The ^1H and ^{13}C NMR spectra showed the signals for an oxygenated methylene (δ_H 4.69, 2H, s; δ_C 66.2 t) and a carbonyl (δ_C 195.7 s) groups. These observation accounted for $C_{10}H_7NO$ out of the molecular formula of **2** ($C_{10}H_9NO_3$), thus remaining two OH's. The ^{13}C chemical shift of C-5 (δ_C 154.5 s) indicated that the C-5 position also bore a hydroxyl group in **2**. The other substituent was therefore suggested to be a hydroxyacetyl ($COCH_2OH$) group. In the EIMS of **2** intense ion peaks were observed at *m/z* 160 and 132 that corresponded to the fragment ions generated by loss of CH_2OH and $COCH_2OH$, respectively. Indisputable evidence for these findings was provided by the HMBC spectrum of **2** [correlations (H/C): H-2/C-3, H-2/C-8, H-2/C-9, H-4/C-3, H-4/C-8, H-6/C-4, H-6/C-8, H-7/C-5, H-7/C-9, and H_2-11/C-10], which enabled complete assignment of ^1H and ^{13}C signals. Thus the structure of compound **2** was established to be 2-(5-hydroxyindol-3-yl)-2-oxo-ethan-1-ol.

The molecular formula of hyrtiosin B (**3**), $C_{18}H_{12}N_2O_4$, containing 14 degrees of unsaturation, was determined by high resolution FABMS [negative ion, *m/z* 319.0734 $(M-H)^-$, Δ +1.5 mmu]. The ^1H NMR of **3** (in CD_3OD) showed only four proton signals that were assigned to the indole ring protons (H-2, 4, 6, and 7) by comparison with the ^1H NMR of **1** or **2**. The ^{13}C NMR spectrum of **3**, exhibiting only 9 carbon lines (4 protonated and 5 non-protonated sp^2 carbons by the DEPT experiments), point clearly to a symmetrical dimer structure. In the EIMS of **3** the most intense peak was observed at *m/z* 160 that was attributed to a monomer fragment ion, the dimeric molecular ion also being observed at *m/z* 320. The ^1H and ^{13}C NMR spectra of **3** along with the consideration of the molecular formula indicated that compound **3** is a dimer of an indole with substituents at C-3 and C-5 positions and the substituents consist of two carbonyl and two hydroxyl groups. The ^{13}C NMR spectrum of **3** revealed a signal due to a carbonyl group (δ_C 191.0 s), the chemical shift of which suggested that this carbonyl carbon was not assignable to a carboxyl group but attributed to a conjugated ketone. From the ^{13}C chemical shift of C-5 (δ_C 155.0 s), this carbon was inferred to bear a hydroxyl group as it was also in the case of compounds **1** and **2**. Since the H-2 signal resonated in the fairly low field (δ_H 8.02, 1H, s),[10] a carbonyl group was implied to be attached to C-3. The EIMS of **3** showed a prominent peak at *m/z* 132 due to a fragment ion caused by loss of CO from the monomer ion (*m/z* 160). Thus the structure of hyrtiosin B (**3**) was concluded to be 1,2-bis-(5-hydroxyindol-3-yl)-ethane-1,2-dione.

The three 3-acyl-5-hydroxyindole derivatives (**1** ~ **3**) from the Okinawan sponge *H. erecta*, seemed to be closely related to one another, *e.g.* hyrtiosin B (**3**) is considered to result from condensation of two molecules of

compound **1**. Hyrtiosin A (**2**) appears to be biogenetically related to 4-hydroxy-5-(indol-3-yl)-5-oxo-pentan-2-one previously obtained from the Bermudian sponge *Dysidea etheria*, which was proposed to be an artifact formed by addition of acetone to 2-(indol-3-yl)-2-oxo-ethan-1-one.[1d] This is the first isolation of alkaloids from the sponges belonging to *Hyrtios* sp., although this genus has been shown to contain terpenoids and sterols.[11] Compound **1** exhibited cytotoxic activity against human epidermoid carcinoma KB cells in vitro (IC_{50} 4.3 μg/mL), while hyrtiosins A (**2**) and B (**3**) were less cytotoxic than **1**.[12]

EXPERIMENTAL

General. IR spectra were obtained on a Hitachi 260-50 IR spectrometer and UV spectra on JASCO 660 UV/VIS spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a JEOL GX-500 spectrometer. EI and FAB mass spectra were obtained on a Shimadzu GCMS-QP1000A and a JEOL HX-100 spectrometer, respectively. Wako C-300 silica gel (Wako Pure Chemical) was used for glass column chromatography. TLC was carried out on Merck silica gel GF_{254}.

Isolation. The sponge, *H. erecta*, (2.0 kg, wet weight) collected by SCUBA at Ishigaki Islands, Okinawa, was kept frozen until used. The methanol (2.5 and 1.0 L) extract of the sponge was evaporated under reduced pressure to afford the residue (61.67 g), which was dissolved in a mixed solvent of ethyl acetate (400 mL) and water (400 mL) and then partitioned between ethyl acetate (400 mL x 3) and water (400 mL). The ethyl acetate-soluble material (2.14 g) was partially (0.91 g) subjected to silica gel flash column chromatography eluted with methanol/chloroform (5:95). The fractions eluting 370 ~ 530 mL, 720 ~ 810 mL, and 1040 ~ 1270 mL were evaporated under reduced pressure to give residues of 12.8, 6.3, and 8.1 mg, respectively. Each fraction was further purified by HPLC [CAPCELL-PAK C_{18}, Shiseido, 10 x 250 mm; eluant, methanol/water/acetic acid (50:50:0.5)] to yield compounds **1** (2.0 mg), hyrtiosin A (**2**: 1.0 mg), and hyrtiosin B (**3**: 2.0 mg), respectively.

Compound **1.** Colorless needles; mp 220-221 °C; IR ν_{max} 3340, 3170, 1605, 1140, 810, and 790 cm^{-1}; UV(MeOH) λ_{max} 215 (ε 12500), 252 (11300), 270 (9000), and 298 nm (6500); ^1H NMR (CD_3OD) δ 9.80 (1H, s; H-10), 7.99 (1H, s; H-2), 7.56 (1H, d, *J*=2.4 Hz; H-4), 7.28 (1H, d, *J*=8.8 Hz; H-7), and 6.80 (1H, dd, *J*=2.4 and 8.8 Hz; H-6); ^{13}C NMR (CD_3OD) δ 187.1 d (C-10), 154.8 s (C-5), 139.8 d (C-2), 133.3 s (C-8), 126.9 s (C-9), 119.7 s (C-3), 114.7 d (C-6), 113.7 d (C-7), and 107.0 d (C-4); EIMS *m/z* (relative intensity %) 161 (M^+, 98), 160 (100), 132 (45), 105 (25), and 77 (20); HRFABMS (negative), found *m/z* 160.0477, calcd for $C_9H_6NO_2$ (M-H) 160.0399.

Hyrtiosin A (**2**). Colorless needles; mp 196-197 °C; IR ν_{max} 3300, 3200, 1615, 1060, 910, and 800 cm^{-1}; UV(MeOH) λ_{max} 215 (ε 59200), 252 (35000), 270 (22400), and 303 nm (23300); ^1H NMR (CD_3OD) δ 8.10 (1H, s; H-2), 7.64 (1H, dd, *J*=0.5 and 2.4 Hz; H-4), 7.27 (1H, dd, *J*=0.5 and 8.8 Hz; H-7), 6.77 (1H, dd, *J*=2.4 and 8.8 Hz; H-6), and 4.69 (2H, s; H_2-11); ^{13}C NMR (CD_3OD) δ 195.7 d (C-10), 154.5 s (C-5), 134.2 d (C-2), 132.7 s (C-8), 128.1 s (C-9), 114.4 s (C-3), 114.1 d (C-6), 113.5 d (C-7), 107.2 d (C-4) and 66.2 t (C-11); EIMS *m/z* (relative intensity %) 191 (M^+, 23), 160 (100), and 132 (19); HRFABMS (negative), found *m/z* 190.0496, calcd for $C_{10}H_8NO_3$ (M-H) 190.0505.

Hyrtiosin B (**3**). Colorless needles; mp >310 °C; IR ν_{max} 3400, 3250, 1600, 1425, 1200, and 780 cm^{-1}; UV(MeOH) λ_{max} 212 (ε 30000), 255 (12800), 290 (12100), and 332 nm (11000); ^1H NMR (CD_3OD) δ 8.02 (1H, s; H-2), 7.75 (1H, dd, *J*=0.6 and 2.3 Hz; H-4), 7.31 (1H, dd, *J*=0.6 and 8.7 Hz; H-7), 6.82 (1H,

dd, *J*=2.3 and 8.7 Hz; H-6); ^{13}C NMR (CD_3OD) δ 191.1 d (C-10), 155.0 s (C-5), 138.5 d (C-2), 132.9 s (C-8), 128.4 s (C-9), 114.5 d (C-6), 114.2 s (C-3), 113.7 d (C-7), and 107.6 d (C-4); HMBC correlations (H/C): H-2/C-3, H-2/C-8, H-2/C-9, H-4/C-3, H-4/C-8, H-6/C-4, H-6/C-5, H-6/C-8, H-7/C-5, and H-7/C-9; EIMS *m/z* (relative intensity %) 320 (M^+, 19), 160 (100), 132 (17), and 105 (8); HRFABMS (negative), found *m/z* 319.0734, calcd for $C_{18}H_{11}N_2O_4$ (M-H) 319.0719.

Acknowledgement: We thank Dr. P. Bergquist (Auckland University) for the sponge identification, Mr. Z. Nagahama for his help with collection of the sponge, and Ms. M. Hamashima for her technical assistance.

REFERENCES AND NOTES

1. (a) Tymiak, A. A.; Rinehart, K. L., Jr.; Bakus, G. J. *Tetrahedron* **1985**, *41*, 1039. (b) Djura, P.; Faulkner, D. J. *J. Org. Chem.* **1980**, *45*, 735. (c) Djura, P.; Stierle, D. B.; Sullivan, B.; Faulkner, D. J.; Arnold, E.; Clardy, J. *J. Org. Chem.* **1980**, *45*, 1435. (d) Cardellina II, J. H.; Nigh, D.; Van Wagenen, B. C. *J. Nat. Prod.* **1986**, *49*, 1065. (e) Kohmoto, S.; McConnell, O. J.; Wright, A. *Experientia* **1988**, *44*, 85.
2. (a) Roll, D. M.; Ireland, C. M. *Tetrahedron Lett.* **1985**, *26*, 4303. (b) Moriarty, R. M.; Roll, D. M.; Ku, Y.-Y.; Nelson, C.; Ireland, C. M. *Tetrahedron Lett.* **1987**, *28*, 749.
3. (a) Bano, S.; Bano, N.; Ahmad, V. U.; Shameel, M.; Amjad, S. *J. Nat. Prod.* **1986**, *49*, 549. (b) Tanaka, J.; Higa, T.; Bernardinelli, G.; Jefford, C. W. *Tetrahedron Lett.* **1988**, *29*, 6091. (c) Tanaka, J.; Higa, T.; Bernardinelli, G.; Jefford, C. W. *Tetrahedron* **1989**, *45*, 7301.
4. Higa, T.; Ichiba, T.; Okuda, R. K. *Experientia* **1985**, *41*, 1487.
5. Gil-Turnes, M. S.; Hay, M. E.; Fenical, W. *Science* **1989**, *246*, 116.
6. (a) Kobayashi, J.; Ishibashi, M.; Wälchli, M. R.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Ohizumi, Y. *J. Am. Chem. Soc.* **1988**, *110*, 490. (b) Kobayashi, J.; Ishibashi, M.; Nakamura, H.; Hirata, Y.; Ohizumi, Y. *J. Chem. Soc., Perkin Trans. I* **1989**, 101. (c) Kobayashi, J.; Murayama, T.; Ohizumi, Y.; Sasaki, T.; Ohta, T.; Nozoe, S. *Tetrahedron Lett.* **1989**, *30*, 4833. (d) Kobayashi, J.; Ishibashi, M.; Murayama, T.; Takamatsu, M.; Iwamura, M.; Ohizumi, Y.; Sasaki, T. *J. Org. Chem.* **1990**, *55*, 3421. (e) Kobayashi, J.; Cheng, J.-F.; Ohta, T.; Nozoe, S.; Ohizumi, Y.; Sasaki, T. *J. Org. Chem.* **1990**, *55*, 3666.
7. Scott, A. I. In "Interpretation of the Ultraviolet Spectra of Natural Products", Pergamon Press: New York, 1964, p. 176.
8. Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093.
9. (a) Kveder, S.; Iskric, S. *Biochem. J.* **1965**, *94*, 509. (b) Iskric, S.; Stancic, L.; Kveder, S. *Clin. Chim. Acta* **1969**, *25*, 435.
10. H-2 of 3-methylindole (δ_H 6.96)[2a]; H-2 of 3-formylindole (δ_H 8.3): Chowdhury, B. K.; Chakraborty, D. P. *Phytochemistry* **1971**, *10*, 481.
11. (a) Crews, P.; Bescansa, P. *J. Nat. Prod.* **1986**, *49*, 1041. (b) Crews, P.; Bescansa, P.; Bakus, G. *Experientia*, **1985**, *41*, 690. (b) Koch, P.; Djerassi, C.; Lakshmi, V.; Schmitz, F. J. *Helv. Chim. Acta* **1983**, *66*, 2431. (d) Amade, P.; Chevelot, L.; Perzanowski, H. P.; Scheuer, P. J. *Helv. Chim. Acta* **1983**, *66*, 1672.
12. Hyrtiosins A (**2**) and B (**3**) showed 21% and 16% inhibition, respectively, at 10 μg/mL against KB cells in vitro.