

Indolyl Alkaloid Derivatives, *N_b*-Acetyltryptamine and Oxaline from a Marine-Derived Fungus

Yong Li, Xi Feng Li, Dong Soo Kim¹, Hong Dae Choi², and Byeng Wha Son

Department of Chemistry, Pukyong National University, Busan 608-737, Korea, ¹Department of Food Science and Technology, Kyungsoong University, Busan 608-736, Korea, and ²Department of Chemistry, Donggeui University, Busan 614-714, Korea

(Received October 16, 2002)

Indolyl alkaloids, *N_b*-acetyltryptamine (**1**) and the known oxaline (**2**) have been isolated from the organic extract of the broth of an unidentified fungus collected from the surface of the marine red alga *Gracilaria verrucosa*. The structure of *N_b*-acetyltryptamine (**1**) was assigned on the basis of comprehensive spectroscopic analyses.

Key words: Marine fungus, Indolyl alkaloid, *N_b*-Acetyltryptamine, Oxaline, *Gracilaria verrucosa*

INTRODUCTION

Marine microorganisms are receiving increasing attention as sources of bioactive compounds, and expanded research can be expected in this area. Marine natural product research is now focusing more on marine microorganisms, mainly bacteria and fungi that can be cultured (Faulkner, 2002; Pietra, 1997). The marine fungi, particularly those associated with marine animals and plants, appear to be an unusually rich resource for secondary metabolites. As part of a program to explore the bioactive metabolites produced by fungi isolated from marine habitats (Son *et al.*, 2002), we investigated the chemical constituents of an unidentified marine algicolous fungus, which was separated from the red alga *Gracilaria verrucosa*, and isolated indolyl alkaloids, *N_b*-acetyltryptamine (**1**) and the known oxaline (**2**) (Nagel *et al.*, 1976). This paper deals with the structure elucidation of these alkaloids having an indolyl moiety.

MATERIALS AND METHODS

General experimental

IR spectrum was recorded on a Bruker FT-IR model IFS-88 spectrometer. ¹H (400 MHz) and ¹³C NMR (100 MHz) spectra were obtained on a JEOL JNM-ECP 400 NMR

spectrometer, using TMS or solvent peaks as reference standard. MS spectra were obtained on a JEOL JMS-700 spectrometer. UV/visible spectra were measured on a Hitachi U-2001 UV/Vis spectrometer.

Fungal isolation and culture

An unidentified fungal strain (culture # MFA 936) was isolated from the surface of the marine red alga *Gracilaria verrucosa* collected in Hamdeok Beach, Cheju Island in 2001. The fungus was cultured (20 L) for 30 days (static) at 29°C in SWS medium : soytone (0.1%), soluble starch (1.0%), and seawater (100%).

Isolation of *N_b*-acetyltryptamine (**1**) and oxaline (**2**)

The mycelium and broth were separated by filtration. The broth was extracted twice with EtOAc. The combined extract (1.9 g) was subjected to silica gel flash column chromatography, eluting with *n*-hexane/EtOAc (from 100% to 0%), to obtain 8 fractions. Further purification of fraction 7 (400 mg) by silica gel column chromatography using CH₂Cl₂ / MeOH (20:1), followed by HPLC (YMC ODS-A, 10×250 mm) (MeOH) yielded a *N_b*-acetyltryptamine (**1**, 13 mg) and oxaline (**2**, 6.0 mg).

1 : Yellowish oil; IR (neat): 3400 (NH), 1638 (amide), 1384, 744 cm⁻¹; UV (MeOH): 222 (log ε 4.0), 283 (2.3) nm; HREIMS *m/z* 202.1060 (calcd for C₁₂H₁₄N₂O, 202.1106); LREIMS *m/z* 202[M]⁺ (rel. int., 7), 154 (47), 143 (62), 130 (58), 86 (18), 70 (100).

See Table I for NMR spectral data.

Correspondence to: Byeng Wha Son, Department of Chemistry, Pukyong National University, Busan 608-737, Korea
E-mail: sonbw@pknu.ac.kr

Table I. ^1H (δ , mult, J) and ^{13}C (δ , mult) NMR data of N_b -acetyltryptamine (**1**)^a

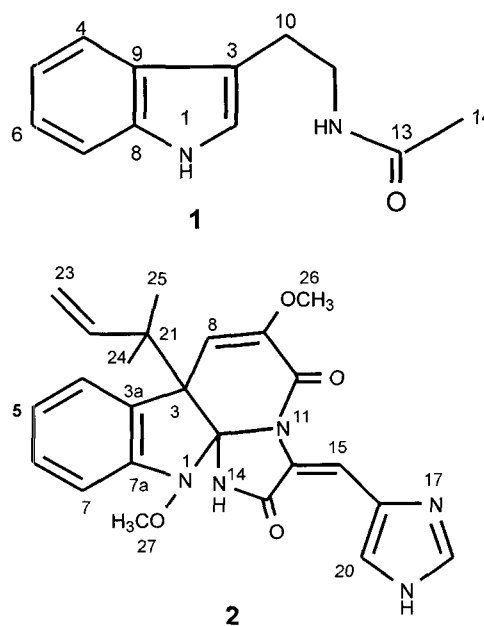
Carbon No.	δ_{H}	δ_{C}	HMBC (H to C)
1	8.14 (br, s)		
2	7.04 (d, 2.2)	122.0 (d)	8, 9
3		113.1 (s)	
4	7.60 (d, 8.0)	118.7 (d)	9, 11
5	7.13 (dd, 8.0 8.0)	119.5 (d)	7, 9
6	7.21 (dd, 8.0 8.0)	122.2 (d)	4, 8
7	7.38 (d, 8.0)	111.2 (d)	5, 9
8		136.4 (s)	
9		127.3 (s)	
10	2.98 (t, 6.5)	25.3 (t)	2, 3, 9, 11
11	3.60 (dt, 6.5, 6.0)	39.8 (t)	3, 10, 13
12	5.53 (br, s)		
13		170.0 (s)	
14	1.92 (s)	23.4 (q)	11, 13

^aRecorded in CDCl_3 at 400 MHz (^1H) and 100 MHz (^{13}C).

2: Yellowish oil; IR (neat): 3186 (NH), 3089, 3013, 1702 (δ -lactam), 1632 (amide) cm^{-1} ; UV (MeOH): 208 ($\log \epsilon$ 4.4), 228 (4.0), 284 (3.6), 344 (4.0), 360 (4.0) nm; HRFABMS m/z 448.1985 (calcd for $\text{C}_{24}\text{H}_{26}\text{N}_5\text{O}_4$, 448.5015); LRFABMS m/z 448[M+H]⁺ (rel. int., 100), 379 (34), 154 (12), 136 (23); ^1H NMR (400 MHz, CDCl_3) δ_{H} 7.58 (1H, d, J = 7.6 Hz, H-4), 7.09 (1H, dd, J = 7.6, 7.5 Hz, H-5), 7.29 (1H, dd, J = 7.6, 7.5 Hz, H-6), 6.98 (1H, d, J = 7.6 Hz, H-7), 5.13 (1H, s, H-8), 12.88 (1H, br. s, 14-NH), 8.37 (1H, s, H-15), 7.58 (1H, br. s, H-18), 7.23 (1H, br. s, H-20), 6.11 (1H, br. s, H-22), 5.10 (1H, br. d, J = 18.3 Hz, H-23a), 5.06 (1H, br. d, J = 14.0 Hz, H-23b), 1.25 (3H, s, H_3 -24), 1.32 (3H, s, H_3 -25), 3.64 (3H, s, H_3 -26), 3.73 (3H, s, H_3 -27); ^{13}C NMR (100 MHz, CDCl_3) δ_{C} 101.3 (C-2), 52.4 (C-3), 146.4 (C-3a), 124.7 (C-4), 123.4 (C-5), 128.5 (C-6), 112.1 (C-7), 146.5 (C-7a), 106.8 (C-8), 125.9 (C-9), 157.4 (C-10), 122.8 (C-12), 165.7 (C-13), 110.0 (C-15), 126.2 (C-16), 136.8 (C-18), 134.6 (C-20), 42.4 (C-21), 142.6 (C-22), 114.1 (C-23), 24.1 (C-24), 23.7 (C-25), 55.7 (C-26), 65.2 (C-27).

RESULTS AND DISCUSSION

N_b -Acetyltryptamine (**1**) was isolated as a yellowish oil and was found to have an elemental composition $\text{C}_{12}\text{H}_{14}\text{N}_2\text{O}$ on the basis of HREIMS and ^{13}C NMR methods. The IR spectrum of **1** revealed absorption bands for amine (3400 cm^{-1}) and amide (1638 cm^{-1}). The ^1H and ^{13}C NMR data for **1**, including results from DEPT, TOCSY, HMQC, and HMBC experiments, showed indol and ethylamine acetate moieties (Table I). These moieties were further supported

**Fig. 1.** Structures of N_b -acetyltryptamine (**1**) and oxaline (**2**)

by UV spectral data [222 ($\log \epsilon$ 4.0), 283 (2.3) nm], and by MS fragment m/z 143 [$\text{M}-\text{CH}_3\text{CONH}_2$]⁺. The position of the ethylamine acetate group was deduced by HMBC correlations from H-4 to C-3, and H_2 -10 to C-2, C-3 and C-9, and by TOCSY correlations between H-2 and H-1 and H_2 -10, and between H_2 -10 and H-2, H_2 -11 and H-12.

Based on all of the foregoing evidence, the structure of N_b -acetyltryptamine was determined to be 2-(3-indolyl)ethylamine acetate (**1**) (Fig. 1). N_b -Acetyltryptamine (**1**) has not previously been found to occur in nature but had been obtained as a biotransformed-metabolite derived from tryptamine by *Streptomyces staurosporeus* (Yang and Cordell, 1997).

Tryptamine and its derivatives are widely distributed in animals, plants and fungi (Buckingham *et al.*, 1994; Morales-Rios *et al.*, 1987; Salmoun *et al.*, 2002). Tryptamine has been discovered to be present in several edible fruits, namely, tomato, plum, and eggplant, and also in traces in oranges (Saxton *et al.*, 1965). N,N -Dimethyltryptamine was first identified as a constituent of the seeds and pods of *Piptadenia peregrina* and *P. macrocarpa* (Leguminosae) during an attempt to isolate the hallucinogenic principles present in the narcotic snuff prepared from these plants by certain American Indian tribes.

In later investigations, N,N -dimethyltryptamine occurs more widely in nature, and is the simplest of several naturally occurring tryptamine derivatives which exhibit psychotomimetic activity (Fish *et al.*, 1955)

On account of the activity of N,N -dimethyltryptamine, the physiological activity of N_b -acetyltryptamine in humans is of interest.

ACKNOWLEDGEMENTS

NMR and Mass spectral data were kindly provided by the Korea Basic Science Institute, Taejeon. This study was supported by a grant of the "2002 Korea Sea Grant Program (2002 KSGP)", Ministry of Maritime Affairs and Fisheries (2002).

REFERENCES

- Buckingham, J., Macdonald, F. M., and Bradley, H. M. (Eds.). *Dictionary of Natural Products*, Vol. 10, Chapman & Hall, London, pp. 5932-5933, (1994).
- Faustine, D. J., Marine natural products. *Nat. Prod. Rep.*, 19, 1-48 (2002).
- Fish, M. S., Johnson, N. M., and Horning, E. C., Piptadenia alkaloids. Indole bases of *P. peregrina* (L.) Benth. and related species. *J. Am. Chem. Soc.*, 77, 5892 (1955).
- Moraes-Rios, M. S., Espineira, J., and Joseph-Nathan, P., ¹³C NMR spectroscopy of indole derivatives. *Magn. Resonance Chem.*, 25, 377-395 (1987).
- Nage, C. W., Pachler, K. G. R., Steyn, P. S., Vlegaar, R., and Weissels, P. L., The chemistry and ¹³C NMR assignments of oxaline, a novel alkaloid from *Penicillium oxalicum*. *Tetrahedron*, 32, 2625-2631 (1976).
- Pietra, F., Secondary metabolites from marine microorganisms: bacteria, protozoa, algae and fungi. Achievements and prospects. *Nat. Prod. Rep.*, 14, 453-464 (1997).
- Salmoun, M., Devijver, C., Daloz, D., Braekman, J. C., and van Soest, R. W. M., 5-Hydroxytryptamine-derived alkaloids from two marine sponges of the genus *Hyrtios*. *J. Nat. Prod.*, 65, 1173-1176 (2002).
- Saxton, J. E., The simple bases, In Manske, R. H. F. (Ed.). *The alkaloids, Chemistry and Physiology*, Vol. VIII, Academic Press, New York, pp. 8-10, (1965) and references therein.
- Son, B. W., Choi, J. S., Kim, J. C., Nam, K. W., Kim, D. S., Chung, H. Y., Kang, J. S., and Choi, H. D., Parasitenone, a new epoxycyclohexenone related to gabosine from the marine-derived fungus *Aspergillus parasiticus*. *J. Nat. Prod.*, 65, 794-795 (2002).
- Yang, S. W. and Cordell, G. A., Metabolism studies of indole derivatives using a staurosporine producer, *Streptomyces staurosporeus*. *J. Nat. Prod.*, 60, 44-48 (1997).
- Yang, S. W. and Cordell, G. A., Further metabolic studies of indole and sugar derivatives using the staurosporine producer *Streptomyces staurosporeus*. *J. Nat. Prod.*, 60, 230-235 (1997).