Pergamon

Tetrahedron 55 (1999) 2487–2492

TETRAHEDRON

Echinoclathrines A-C: a New Class of Pyridine Alkaloids from an Okinawan Sponge, *Echinoclathria* sp.

Atsushi Kitamura, Junichi Tanaka, Ikuko I. Ohtani, and Tatsuo Higa*

Department of Chemistry, Biology, and Marine Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan

Received 6 November 1998; accepted 28 December 1998

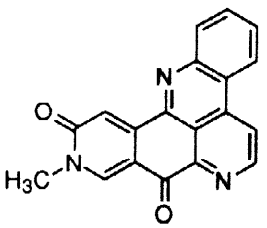
Abstract: Echinoclathrines A-C (**3**-**5**) have been isolated from an Okinawan sponge, *Echinoclathria* sp. and the structures elucidated by interpretation of spectral data. These compounds are a new class of pyridine alkaloids possessing an 4-aryl-2-methylpyridine moiety as a common structural element. Echinoclathrines A and B exhibited weak immunosuppressive activity in a mixed lymphocyte reaction assay. © 1999 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

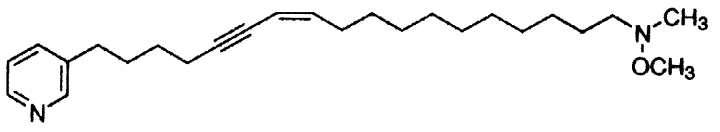
Some one hundred pyridine alkaloids have so far been described from marine sources. The majority of them can be classified into two structural types, either condensed polynuclear aromatics based on a pyrido[2,3,4-*k*,*l*]acridine nucleus or 3-alkylpyridines. The formers encompassing about fifty compounds are mainly metabolites isolated from sponges and ascidians. Examples are amphimedine (**1**),[1] the first such compound reported, from a sponge and shermilamine A[2] from an ascidian. On the other hand, 3-alkylpyridines[3] are mainly reported from sponges and have relatively simple structures as exemplified by xestamine A (**2**).[4]

In our continuing search for novel bioactive substances from marine organisms, we have isolated a new class of pyridine alkaloids, echinoclathrines A-C (**3**-**5**), from a new species of the sponge genus *Echinoclathria*.[5] Echinoclathrines contain an unprecedented 4-aryl-2-methylpyridine as the common structural element. We have briefly described the isolation and structures of these compounds in a preliminary account.[6] However in the earlier report we incorrectly assigned the position of a hydroxyl and acylamino group on the phenyl ring. We now established correct structures and herein describe the isolation, structure elucidation, and biological activity in detail.

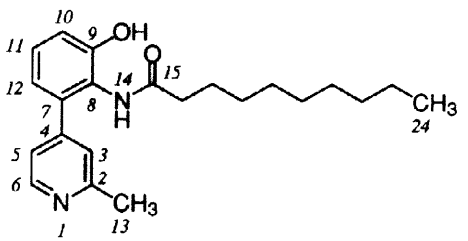
PII: S0040-4020(99)00022-8



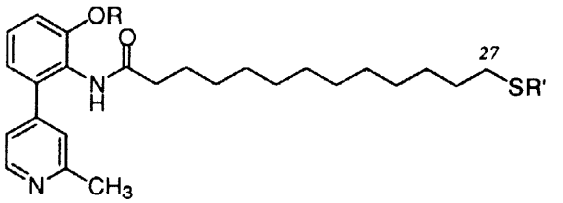
Amphimedine (**1**)



Xestamine A (**2**)



Echinoclathrine A (**3**)



Echinoclathrine B (**4**): R = H, R' = Ac
Echinoclathrine C (**5**): R = H, R' = H
6 : R = Ac, R' = Ac

RESULTS AND DISCUSSION

A sample (3.3 kg, wet weight) of *Echinoclathria* sp. was extracted with acetone, and the concentrated extract was partitioned between ethyl acetate and water. The ethyl acetate soluble material (115 g) was separated on a silica gel column followed by reversed phase HPLC to give echinoclathrines A (**3**, 20 mg), B (**4**, 6 mg), and C (**5**, 2 mg) as white solids.

Echinoclathrine A (**3**) exhibited a prominent peak at *m/z* 354 in the EIMS, which on exact mass measurement gave a formula $C_{22}H_{30}N_2O_2$ (*m/z* 354.2304, Δ -0.3 mmu). Interpretation of the NMR spectra (Table 1) including HOHAHA and COLOC data led to the formulation of a 2-methyl-4-substituted pyridine (C-2 to C-6) and a 1,2,3-trisubstituted benzene (C-7 to C-12) moiety (Fig. 1). A broad signal at δ 1.26 suggested the presence of a long methylene chain. A decanoyl group (C-15 to C-24) was deduced from an EIMS fragment ion at *m/z* 200 (base peak), corresponding to the loss of a decanoyl group ($C_{10}H_{18}O$) from the molecular ion. The COLOC correlations (H-3,5/C-7; H-12/C-4) indicated the connectivity of the pyridine and benzene rings as shown in Fig. 1. The NH signal (δ 7.20) showed the COLOC correlations to two carbon resonances at δ 174.0 and 150.8. The former was assigned to the carbonyl carbon (C-15) of the decanoyl moiety establishing its connectivity. The latter, assigned to C-9, also showed the COLOC correlation with H-11. In order to assign the position of the substituents on the benzene ring, ^{13}C chemical shifts observed for **3** was compared with those calculated[7] for model compounds **A** and **B** (Fig. 2). Similarity of the chemical shifts with those of **A** but not of **B** indicated that **3** has an 2-acylamino-3-hydroxyphenyl moiety, concluding the structure of echinoclathrine A as shown in **3**.

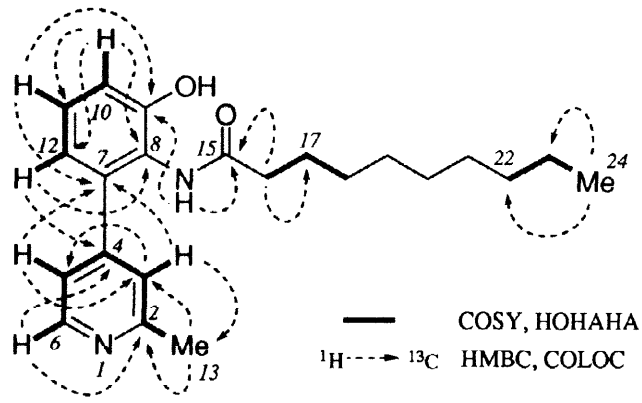


Fig. 1. ^{1}H-^{1}H COSY, HOHAHA, HMBC, and COLOC correlations of echinoclathrine A (**3**).

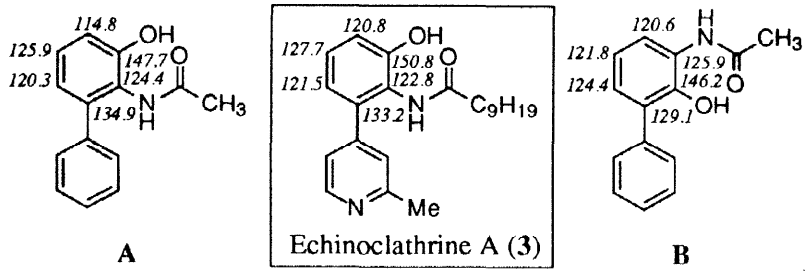


Fig. 2. Comparison of ^{13}C chemical shifts of echinoclathrine A (**3**, observed) with calculated values for model compounds **A** and **B**.

Echinoclathrine B (**4**) has a molecular formula $C_{27}H_{38}N_2O_3S$, which differed from that of **3** by C_5H_8OS, as determined by the high-resolution EIMS (M^+, *m/z* 470.2606, Δ +0.3 mmu). The ^{1}H and ^{13}C NMR signals (Table 1) for the aromatic region were superimposable on those of **3**, while those for the side chain portion were different. The NMR data indicated the presence of an acetyl group (δ_H 2.31, δ_C 196.1, 30.6). The low-field signal of the acetyl carbonyl and its HMBC correlation to the methylene signal at δ 2.85 suggested the presence of an acetylthio group (CH_3COS) at the end of the side chain, which was supported by the IR band at 1694 cm^{-1}.[8] The remaining three-carbon unit must be incorporated into the side chain to complete the structure (**4**). This conclusion was also supported by the fragment ion peaks at *m/z* 427 ($[M\text{-}Ac]^+$), 395 ($[M\text{-}SAc]^+$), and 200 ($[M\text{-}CO(CH_2)_{12}SAc]^+$) in the EIMS.

The EIMS of echinoclathrine C (**5**) revealed molecular ion at *m/z* 428, indicating a formula $C_{25}H_{36}N_2O_2S$,[9] which corresponded to the loss of an acetyl group from **4**. Indeed, no acetyl signals were observed in the ^{1}H NMR spectrum of **5**, and the signal for H_2-27 was shifted to δ 2.52 (q, J = 7.5 Hz) from δ 2.85 (t, J = 7.5 Hz) in **4**, suggesting that **5** has a thiol instead of an acetylthio group at the end of the side chain. This was supported by the fragment ions at *m/z* 395 ($[M\text{-}SH]^+$) and 200 ($[M\text{-}CO(CH_2)_{12}SH]^+$) in the EIMS of **5**. The structure was confirmed by acetylation of **5** to form diacetate **6** which was identical with a sample prepared by acetylation of **4**.

Table 1. NMR Data for Echinoclathrines A-C (3-5)

No.	Echinoclathrine A (3)[a] ^{13}C NMR	^{1}H NMR [mult. *J* (Hz)]	HMBC (10 Hz)	COLOC (10 Hz)	No.	Echinoclathrine B (4)[b] ^{13}C NMR	^{1}H NMR [mult. *J* (Hz)]	Echinoclathrine C (5)[a] ^{13}C NMR	^{1}H NMR [mult. *J* (Hz)]
2	δ159.3 s		H6,H13	H6,H13	2	δ159.3 s		δ159.4 s	
3	123.7 d	δ7.15 brs	H5,H13	H5,H13	3	123.7 d	δ7.16 brs	123.8 d	δ7.17 d 1.5
4	146.6 s		H6	H6,H12	4	146.6 s		146.6[f] s	
5	121.2 d	7.09 dd 5.5,1.2	H3,H6	H3,H6	5	121.2 d	7.09 dd 5.0,1.5	121.3 d	7.11 dd 5.5,1.5
6	149.6 d	8.55 d 5.5			6	149.7 d	8.58 d 5.0	149.8 d	8.60 d 5.5
7	133.2 s		H3,H11	H3,H5,H11	7	133.1 s		133.1 s	
8	122.8 s		H10,H12	H10,H12	8	122.8 s		122.8 s	
9	150.8 s		H11	H11,NH	9	150.7 s		150.7 s	
10	120.8 d	7.11 dd 7.9,1.2	H12	H12	10	120.8 d	7.11 dd 8.3,1.3	120.9 d	7.12 dd 8.2,1.5
11	127.7 d	7.24 dd 7.9,7.3		H10,OH	11	127.7 d	7.24 dd 8.3,7.6	127.8 d	7.25 dd 8.2,7.6
12	121.5 d	6.82 dd 7.3,1.2	H10	H10	12	121.5 d	6.81 dd 7.6,1.3	121.5 d	6.82 dd 7.6,1.5
13	24.5 q	2.60 s		H3	13	24.5 q	2.61 s	24.7 q	2.63 s
15	174.0 s		H16	H16,NH	15	174.0 s		174.0 s	
16	36.7 t	2.32 t 7.5			16	36.8 t	2.31 t 7.6	36.9 t	2.32 t 7.5
17	25.5 t	1.62 quint 7.5	H16	H16	17	25.5 t	1.62 quint 7.6	25.5 t	~1.6[g]
18	29.3[c] t	1.26 brs			18				
19	29.23[c] t	1.26 brs			|	[d]	[e]	[h]	[i]
20	29.18[c] t	1.26 brs			26				
21	29.10[c] t	1.26 brs			27	[d]	2.85 t 7.3	34.0 t	2.52 q 7.5
22	31.8 t	1.26 brs	H24	H24,δ1.26					
23	22.6 t	1.26 brs	H24	H24,δ1.26	Ac	196.1 s			
24	14.0 q	0.88 t 7.0				30.6 q	2.31 s		
NH		7.20 brs			NH		7.20 brs		7.03 brs
OH		8.82 brs			OH		8.80 brs		8.80 brs

[a] $\underline{C}DCl_3$ (77.0 ppm) and TMS (0 ppm) signals were used as internal standards for ^{13}C (125 MHz) and ^{1}H NMR (500 MHz), respectively. [b] $\underline{C}DCl_3$ (77.0 ppm) and residual $C\underline{H}Cl_3$ (7.26 ppm) signals were used as internal standards for ^{13}C (125 MHz) and ^{1}H NMR (500 MHz), respectively. [c] Interchangeable signals [d] δ29.44 (3C), 29.37, 29.32, 29.18, 29.12, 29.08, 29.04, 28.8 (each t) [e] δ1.55 (2H, quint, 7.6), 1.34, 1.28, 1.25 (br) [f] Recorded at 67.5 MHz [g] Obscured by H_2O signal [h] δ29.51 (2C), 29.48, 29.38, 29.23, 29.12 (2C), 29.05, 28.4 (each t) [i] δ1.37 (2H, m), 1.33 (2H, m), 1.26 (brs)

Echinoclathrine A (**3**) showed weak cytotoxicity against P388, A-549, and HT-29 cells at a level of IC_{50} 10 μg/mL. Both **3** and **4** exhibited weak immunosuppressive activity in a mixed lymphocyte reaction assay with IC_{50} 7.9 and 9.7 μg/mL, respectively.

EXPERIMENTAL

General Experimental Procedures

The IR spectra were measured using a Hitachi infrared spectrophotometer 269-10 and a JASCO FT/IR-300. The UV spectra were obtained in methanol using a JASCO UVDEC 610 spectrometer. Low resolution electron impact mass spectra (EIMS) were measured on a Hitachi M-2500 and a VG-70SE magnetic sector mass spectrometer; high resolution EIMS were measured on a Hitachi M-2500. The ^{1}H and ^{13}C NMR spectra were

recorded on a JEOL EX-279, a JEOL α-500, and on a GE QE-599 spectrometer. Merck silica gel 60 H was mainly used for vacuum liquid chromatography (VLC). Merck silica gel 60 was used for column chromatography. HPLC columns were nacalai Cosmosil 5C18-AR (8 x 250 mm) and Waters Prep Nova-pac HR C18 60 Å (6 μm, 19 x 300 mm). The HPLC pumps used were as follows: Waters 510, Hitachi 6000, and Shimadzu LC-9A, while HPLC detectors used were Waters Differential Refractometer R401, JASCO 875-UV detector, Hitachi L-4000 UV detector.

Extraction and Isolation

A sample (3.3 kg wet weight) of *Echinoclathria* sp. was collected in the channel between the islands of Miyako and Irabu, Okinawa in 1991, and kept frozen until used. It was extracted with acetone (14.5 L), and the concentrated extract was partitioned between ethyl acetate and water. The ethyl acetate layer was concentrated under reduced pressure to give an oil (115 g). The oil was separated by silica gel VLC using hexane, ethyl acetate, and MeOH as eluent. The fraction eluted with hexane-EtOAc (1:1) was separated by reversed phase HPLC on ODS (8 x 250 mm, MeOH-H_2O, 5:1; 19 x 300 mm, MeOH-CH_2Cl_2-H_2O, 6:1:3) to afford echinoclathrines A (**3**, 20 mg), B (**4**, 6 mg), and C (**5**, 2 mg).

Echinoclathrine A (3)

A white amorphous solid; mp 143-144 °C (hexane-EtOAc); LREIMS *m/z* 354 (M^+), 336, 307, 293, 279, 266, 251, 237, 224, 200, 185; HREIMS *m/z* 354.2304 (calcd for $C_{22}H_{30}N_2O_2$ 354.2307, Δ -0.3 mmu); UV (MeOH) λ_{max} 256 nm (ε 7500); IR ($CHCl_3$) 3420, 2930, 2850, 1660, 1600, 1470 cm^{-1}. ^1H and ^{13}C NMR data are listed in Table 1.

Echinoclathrine B (4)

A white amorphous solid; mp 135-136 °C (hexane-EtOAc); LREIMS *m/z* 470 (M^+), 427, 409, 395, 237, 224, 200, 181, 169, 132, 119; HREIMS *m/z* 470.2606 (calcd for $C_{27}H_{38}N_2O_3S$ 470.2603, Δ +0.3 mmu); UV (MeOH) λ_{max} 257 nm (ε 4200); IR (film) 3332, 2915, 2847, 1694, 1657, 1611, 1465, 1284, 787 cm^{-1}. ^1H and ^{13}C NMR data are listed in Table 1. HMBC data ($CDCl_3$): C-2/H-3,H-6,H-13; C-3/H-5,H-13; C-4/H-6,H-12; C-5/H-3; C-6/H-5; C-7/H-3,H-5,H-11; C-8/H-10,H-12; C-9/H-10,H-11; C-10/H-12; C-12/H-10; C-13/H-3; C-15/H-16,H-17; C-16/H-17; C-17/H-16; Ac (δ 196.1)/H-27, Ac (δ 2.31).

Echinoclathrine C (5)

A white amorphous solid; mp 121-122 °C (hexane-EtOAc); LREIMS *m/z* 428 (M^+), 395, 200, 181, 138; UV (MeOH) λ_{max} 259 nm (ε 11700). ^1H and ^{13}C NMR data are listed in Table 1.

Acetylation of 4 and 5

A sample of **4** was treated with acetic anhydride and pyridine in the usual manner to give echinoclathrine B acetate (**6**) as white amorphous solid. LREIMS *m/z* 512 (M^+), 470, 469, 453, 437, 427, 410, 395, 377, 363,

349, 335, 321, 307, 293, 284, 279, 242, 224, 200; HREIMS *m/z* 512.2672 (calcd for $C_{29}H_{40}N_2O_4S$ 512.2709, Δ -3.7 mmu); IR (film) 3243, 2924, 2853, 1767, 1692, 1601, 1518, 1462, 1368, 1196, 1134, 1013, 953, 840, 788 cm^{-1}; ^1H NMR (270 MHz, CD_3OD) δ 8.41 (1H, d, *J* = 5.1 Hz; H-6), 7.46 (1H, t, *J* = 7.9 Hz; H-11), 7.32-7.25 (3H, m; H-3, 10, 12), 7.22 (1H, dd, *J* = 5.1, 1.5 Hz; H-5), 2.85 (2H, t, *J* = 7.1 Hz; H_2-27), 2.55 (3H, s; H_3-13), 2.29, 2.26 (each 3H, s; Ac), 2.18 (2H, t, *J* = 7.3 Hz; H_2-16), 1.52 (2H, quint, *J* = 7.3 Hz; H_2-16), 1.28 (18H, brs; H_2-18-26). (Residual CHD_2OD, δ 3.30, signal was used as internal standard.)

A sample of **5** was similarly treated with acetic anhydride and pyridine to give a diacetate which was identical with the sample prepared from **4** by MS, IR, and ^1H NMR. HREIMS *m/z* 512.2705 (calcd for $C_{29}H_{40}N_2O_4S$ 512.2709, Δ -0.4 mmu).

ACKNOWLEDGMENT

We thank Professor P. J. Scheuer, Dr. T. Ichiba, and W. Y. Yoshida (Univ. of Hawaii) for recording some of the NMR and EI mass spectra, and PharmaMar Research Institution, Spain for bioassay.

REFERENCES AND NOTES

1. Schmitz, F. J.; Agarwal, S. K.; Gunasekera, S. P.; Schmidt, P. G.; Shoolery, J. N. J. Am. Chem. Soc. 1983, 105, 4835-4836.
2. Cooray, N. M.; Scheuer, P. J.; Parkanyi, L.; Clardy, J. J. Org. Chem. 1988, 53, 4619-4620.
3. Andersen, R. J.; van Soest, R. W. M.; Kong, F. 3-alkylpiperidine Alkaloids isolated from Marine Sponges in the Order Haplosclerida. In Alkaloids: Chemical and Biological Perspectives; Pelletier S. Ed.; Pergamon Press: Oxford, 1998; 302-355.
4. Sakemi, S.; Totton, L. E.; Sun, H. H. J. Nat. Prod. 1990, 53, 995-999. Stierle, D. B.; Faulkner, D. J. J. Nat. Prod. 1991, 54, 1134-1136.
5. Taxonomic examination of the sponge was carried out by Dr. John N. A. Hooper, Queensland Museum, South Brisbane, Qld, Australia, and it was concluded to be a new species of the genus *Echinoclathria*. Voucher specimen (No. QM G301466) has been deposited at Queensland Museum.
6. Higa, T.; Tanaka, J.; Kitamura, A.; Koyama, T.; Takahashi, M.; Uchida, T. Pure Appl. Chem. 1994, 66, 2227-2230.
7. Ewing, D. F. Org. Magn. Reson. 1979, 12, 499.
8. Nakanishi, K.; Solomon, P. H.; Furutachi, N. In Infrared Absorption Spectroscopy; Nankodo: Tokyo, 1960; p. 46.
9. No HRMS was recorded, but its molecular formula was confirmed by HREIMS measurement with its diacetate **6**.