Pergamon

Tetrahedron, Vol. 53, No. 46, pp. 15681-15684, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)10022-9

Konbu'acidin A, a New Bromopyrrole Alkaloid with cdk4 Inhibitory Activity from *Hymeniacidon* Sponge

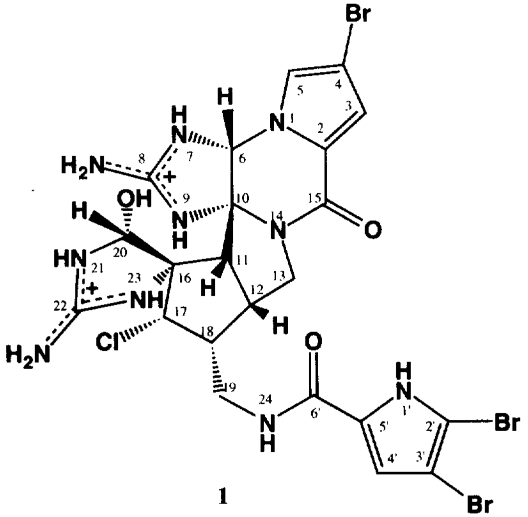
Jun'ichi Kobayashi*, Minako Suzuki, and Masashi Tsuda

Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan

Abstract: A new bromopyrrole alkaloid with a fused-hexacyclic skeleton containing two guanidine units, konbu'acidin A (**1**), has been isolated from an Okinawan marine sponge *Hymeniacidon* sp., and the structure was elucidated from spectroscopic data. Compound **1** exhibited inhibitory activity against cyclin dependent kinase 4 (cdk4). © 1997 Elsevier Science Ltd.

During our continuing studies on bioactive substances from Okinawan marine sponges,[1] we isolated a new fused-hexacyclic alkaloid possessing two bromopyrrole carbonyl groups and two guanidine units, named konbu'acidin A (**1**), with cdk4 inhibitory activity from an Okinawan marine sponge *Hymeniacidon* sp., and the structure was elucidated from spectroscopic data. The relative stereostructure was assigned on the basis of NOE data. This paper describes the isolation and structure elucidation of **1**.

The sponge *Hymeniacidon* sp. collected off Konbu, Okinawa Island, was extracted with MeOH, and the BuOH soluble material of the extract was subjected to a Sephadex LH-20 column (MeOH). The fractions containing bromopyrrole alkaloids were purified by C_{18} MPLC ($MeOH/H_2O/CF_3CO_2H$, 40:60:0.1) and then C_{18} HPLC ($CH_3CN/H_2O/CF_3CO_2H$, 30:70:0.1) to afford konbu'acidin A (**1**, 0.0007 %, wet weight) as bistrifluoroacetate salt.



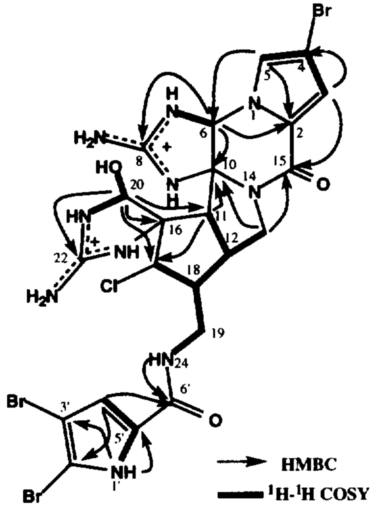
1

Table 1. ^1H and ^{13}C NMR Data of Konbu'acidin A (**1**) in DMSO-d_6.

positn.	δ_H			δ_C		positn.	δ_H		δ_C	
2				125.1	s	18	2.10	m	51.9	d
3	6.78	brs		116.6	d	19	3.56	m	41.0	t
4				101.4	s		3.33	m		
5	7.28	brs		123.8	d	20	5.73	brs	84.5	d
6	6.17	s		69.9	d	20-OH	7.63	brs		
7	8.89	brs				21	9.48	brs		
8				159.1	s	22			159.6	s
8-NH_2	8.24	br				22-NH_2	8.24	br		
9	9.90	brs				23	9.68	brs		
10				84.5	s	24	8.40	brt		
11	2.98	d	14.1	57.7	d	1'	12.73	s		
12	2.52	m		42.7	d	2'			106.9	s
13	3.81	m		47.3	t	3'			100.6	s
	3.03	t	9.6			4'	6.96	s	115.1	d
15				157.8	s	5'			129.1	s
16				72.8	s	6'			162.2	s
17	4.34	d	9.1	75.1	d					

Konbu'acidin A {**1**, $[\alpha]_D^{24}$ -45° (*c* 0.47, MeOH)} was revealed to possess the molecular formula, $C_{22}H_{22}N_{10}O_3Br_3Cl$, by HRESIMS [*m/z* 748.9169, $(M+H)^+$, Δ -2.4 mmu]. IR absorptions indicated the presence of OH and/or NH (3420 cm^{-1}) and amide carbonyl (1685 cm^{-1}) groups. The ^1H NMR (Table 1) spectrum showed signals due to eleven D_2O-exchangeable, three *sp*^{2} methine, six *sp*^{3} methine, and four *sp*^{3} methylene protons. Six of eight D_2O-exchangeable proton resonances [δ 9.90 (1H), 9.68 (1H), 9.48 (1H), 8.89 (1H), 8.24 (4H), and 7.63 (1H)] were reminiscent of those of palau'amine[2] or styloguanidines,[3] while the other two resonances [δ 12.73 (1H) and 8.40 (1H)] corresponded to a pyrrole NH and amide NH proton signals, respectively, of oroidin[4] or hymenidin.[5] Analysis of the ^1H-^1H COSY spectrum of **1** showed the connectivities from H-3 to H-5, from H-6 to NH-7, from H-11 to H-13, from H-17 to NH-24 from H-12 to H-18, from 20-OH to NH-21, and from NH-1' to H-4' (Fig. 1). Detailed analyses of the ^{13}C NMR data (Table 1) as well as the HMQC and HMBC spectra indicated the presence of a fused-hexacyclic skeleton (C-1 ~ N-24) and a dibromopyrrole carbonyl unit (C-1' ~ C-6') (Fig. 1). The chlorine atom at C-17 was assigned by the chemical shifts of H-17 (δ_H 4.34) and C-17 (δ_C 75.1) which were similar to the chemical shifts at C-17 (δ_H 4.39; δ_C 73.5) of 2,3-dibromostyloguanidine in DMSO-d_6,[3] while the bromine substituent at C-4 was assigned by comparison of the carbon chemical shift at C-4 (δ 101.4) of **1** with the C-4 chemical shift (δ 98.2) of monobromophakeline.[6] Connection of the dibromopyrrole carbonyl unit to NH-24 was elucidated by HMBC correlations for H-4'/C-6' and NH-24/C-6'. Relative stereochemistry of konbu'acidin A (**1**) was deduced from NOESY data as shown in Fig. 2. Thus the structure of konbu'acidin A was elucidated to be **1**.

Konbu'acidin A (**1**) is a new fused-hexacyclic bromopyrrole alkaloid related to palau'amine[2] and styloguanidines,[3] the latter of which was isomeric to palau'amine at the pyrrole portion. Palau'amine and styloguanidines have a primary amino group at C-19, while konbu'acidin A (**1**) possesses a dibromopyrrole



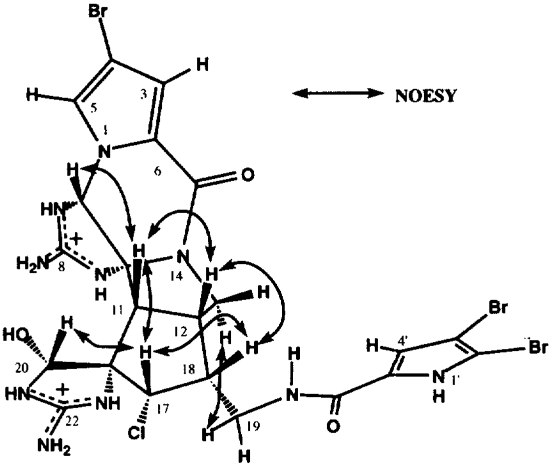


Fig. 1. 2D NMR Correlations of Konbu'acidin A (1) **Fig. 2. Relative Streochemistry of Konbu'acidin A (1)**

carbonyl unit at C-19 via an amide bond. The backbone structure of **1** corresponds to a hybrid of phakeline- and oroidin-type skeleton formed by connection of C-11 and C-16, and C-12 and C-18, although known bromopyrrole alkaloids such as sceptrins,[7-9] ageliferins,[8,10] and mauritiamine[11] apparently seem to be dimers of oroidin-type skeleton. Konbu'acidin A (**1**) exhibited inhibitory activity against cdk4 (IC_{50} 20 μg/mL), although compound **1** did not show cytotoxicity (L1210 and KB cells, IC_{50} >20 μg/mL).

EXPERIMENTAL

General Procedure. Optical rotation was recorded on a JASCO DIP-360 polarimeter. The IR and UV spectra were taken on a JASCO FT/IR-5300 and a JASCO Ubest-35 spectrophotometers, respectively. ^1H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 and a Bruker ARX-500 spectrometers, respectively. ESI mass spectra were obtained on a JEOL JMX-SX120A spectrometers, respectively.

Sponge Materials. The medium brown colored sponge *Hymeniacidon* sp. (order Halichondrida; family Halichondriidae) was collected off Konbu, Okinawa Island, and kept frozen until used. Soft and compressible sponge has some membranous tissue around the oscules. Mesohyl skeleton has a plumose to plumoreticulate skeleton with strong fibre development. Fibres are 180 μm across and are cored centrally by approximately seven styles. Meshes between primary fibles are narrow centrally. Spicules form fans at the surface. Megascleres (mean size, 502 x 9 μm) are long and smooth styles with some tylote modifications. Microsclere is not observed. The voucher specimen (SS-964) was deposited at the Faculty of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation. The sponge (1.4 kg, wet weight) was extracted with methanol (1 L x 2). The methanolic extract (53.6 g) was partitioned between EtOAc (500 mL x 3) and water (500 mL), and the aqueous layer was extracted with *n*-BuOH (500 mL x 3). The *n*-BuOH soluble material (6.92 g) was subjected to Sephadex LH-20 (MeOH) and then C_{18} columns (Develosil ODS-LOP, Nomura Chemical, 45 x 490 mm; MeOH/H_2O/CF_3CO_2H, 40:60:0.1 → 100 % MeOH). The fraction eluted with MeOH was purified by C_{18} HPLC (Develosil ODS-HG-5, Nomura Chemical, 10 x 250 mm; CH_3CN/H_2O/CF_3CO_2H, 30:70:0.1; flow rate, 2.5 mL/min; UV detection at 260 nm) to yield konbu'acidin A (**1**, 0.0007 % wet weight, t_R 30.4 min).

Konbu'acidin A (1). A colorless amorphous solid; $[\alpha]_D^{24}$ -45° (*c* 0.47, MeOH); UV (MeOH) λ_{max} 277 (ε 13000) nm; IR (KBr) ν_{max} 3420 (br), 1685, 1385, 1205, and 1140 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); ESIMS (Pos., MeOH) *m/z* 747, 749, 751, 753, and 755 (MH^+, ca. 3:10:10:5:1); HRESIMS *m/z* 746.9169 MH^+, calcd for $C_{22}H_{23}N_{10}O_3{}^{79}Br_3{}^{35}Cl$, 746.9193.

Acknowledgements We thank Dr. K. Watanabe, NMR laboratory, Faculty of Agriculture, Hokkaido University, for measurements of ESIMS, Mr. Z. Nagahama for his help with sponge collection, Dr. J. Fromont, Western Australian Museum, for identification of the sponge, and Banyu Pharmaceutical Co., Ltd., for kinase assay. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan.

References

1. a) Tsuda, M.; Inaba, K.; Kawasaki, N.; Honma, K.; Kobayashi, J. *Tetrahedron*, **1996**, 52, 2139-2144. b) Kobayashi, J.; Yuasa, K.; Kobayashi, T.; Tsuda, M. *Tetrahedron*, **1996**, *52*, 5745-5750. c) Kobayashi, J.; Nakamura, T.; Tsuda, T. *Tetrahedron*, **1996**, *52*, 6355-6360. d) Kobayashi, J.; Tsuda, M.; Fuse, H.; Sasaki, T.; Mikami, Y. *J. Nat. Prod.*, **1997**, *60*, 150-154.
2. Kinnel, R. B.; Gehrken, H.-P.; Scheuer, P. J. *J. Am. Chem. Soc.*, **1993**, *115*, 3376-3377.
3. Kato, T.; Shizuri, Y.; Izumida, H.; Yokoyama, A.; Endo, Y. *Tetrahedron Lett.*, **1995**, *36*, 2133-2136.
4. a) Forenza, S.; Minale, L.; Riccio, R.; Fattorusso, E. *J. Chem. Soc., Chem. Commun.*, **1971**, 1129-1130. b) Garcia, E. E.; Benjamin, L. E.; Fryer, R. I. J. *J. Chem. Soc., Chem. Commun.*, **1973**, 78-79.
5. Kobayashi, J.; Ohizumi, Y.; Nakamura, H.; Hirata, H. *Experientia*, **1986**, *42*, 1176-1177.
6. Sharma, G.; Mangdoff-Fairchild, B. *J. Org. Chem.*, **1977**, *42*, 4118-4124.
7. Walker, R. P.; Faulkner, D. J.; Van Engen, D.; Clardy, J. *J. Am. Chem. Soc.*, **1981**, *103*, 6772-6773.
8. Keifer, P. A.; Schwartz, R. E.; Koker, M. E. S.; Hughes, R. G., Jr.; Rittschof, D.; Rinehart, K. L. *J. Org. Chem.*, **1991**, *56*, 2965-2975.
9. Kobayashi, J.; Tsuda, M.; Ohizumi, Y. *Experientia*, **1991**, *47*, 301-304.
10. Kobayashi, J.; Tsuda, M.; Murayama, T.; Nakamura, H.; Ohizumi, Y.; Ishibashi, M.; Iwamura, M.; Ohta, T.; Nozoe, S. *Tetrahedron* , **1990**, *46*, 5579-5586.
11. Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *J. Nat. Prod.*, **1996**, *59*, 501-503.

(*Received in Japan* 22 *July* 1997; *accepted* 3 *September* 1997)