A potent actomyosin ATPase activator from the Okinawan marine sponge *Agelas* cf. *nemoechinata*

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Summary. A bromine-containing alkaloid, oxysceptrin, has been isolated as a potent actomyosin ATPase activator from the Okinawan marine sponge *Agelas* cf. *nemoechinata*, and the structure elucidated to be **1** on the basis of the 2D NMR spectral data.
Key words. Actomyosin ATPase activator; bromopyrrole alkaloid; oxysceptrin; sponge; *Agelas* cf. *nemoechinata.*

The actin-myosin system is generally accepted to be involved in muscle contraction and many other activities involved in cell-motility and myosin ATPase provides the energy for the events leading to movement. In our continuing studies on bioactive metabolites from Okinawan marine organisms[2–4], we isolated a bromine-containing alkaloid (**1**) which is a potent actomyosin ATPase activator, together with a related compound, sceptrin (**2**)[5,6], from the Okinawan marine sponge *Agelas* cf. *nemoechinata*. The structure of **1** is the same as that of oxysceptrin isolated previously from the sponge *Agelas coniferin*[7], but the detailed structure elucidation has not been reported yet. In this paper we describe the isolation and full characterization of oxysceptrin (**1**) as well as its unique biological activity.

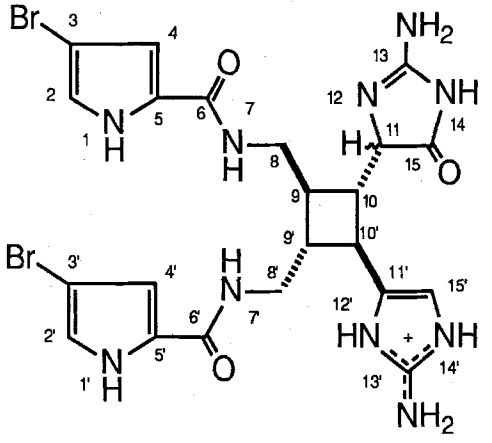
The sponge, collected at Unten Bay ($-70 \sim -80$ m), Motobu Peninsula, Okinawa, was kept frozen at $-20\,^{\circ}C$ until used. The methanolic extracts were partitioned between ethyl acetate and water. The n-butanol-soluble substances from the aqueous layer were separated on a silica gel column with chloroform-n-butanol-acetic acid-water (1.5:6:1:1) to give sceptrin (**2**) (0.2% wet weight of the sponge) and a more polar fraction, which was chromatographed on a C_{18} reversed phase HPLC column (YMC-Pack, AM-323, 5 µm, 10 × 250 mm) with acetonitrile-water-trifluoroacetic acid (40:60:0.2) to yield compound **1** (0.017% wet wt) as a colorless solid[8].

The UV spectrum {MeOH, λ_{max} 268 nm (ε21 000)} and a positive color test of **1** with Fast Red B salt (5-nitro-2-amino-methoxybenzene diazonium chloride zinc sulfate, Tokyo-Kasei) argue for the presence of a substituted pyrrole chromophore[9,10]. The IR spectrum showed absorptions at 3500–3200, 1740 and 1680 cm^{-1} which were attributed to an NH and/or OH group, a γ-lactam and an

Table 1. ^1H NMR (270 MHz) and ^{13}C NMR (67.5 MHz) data of **1**[a]

Position	^1H[b]	m	*J* (Hz)		^{13}C[c]		m[d]
1	11.9		brs				
1′	11.9		brs				
2	6.97		brs		123.0		d
2′	6.97		brs		123.0		d
3					97.6		s
3′					97.6		s
4	6.91		d	1.5	113.8		d
4′	6.91		d	1.5	113.8		d
5					127.3[f]		s
5′					127.0[f]		s
6					163.1[g]		s
6′					163.0[g]		s
7	8.52		t	5.2			
7′	8.42	8.31[e]	t	5.2			
8	3.45		m		43.2	42.6[e,h]	t
8′	3.45		m		42.1	41.8[h]	t
9	2.18		m		45.8		d
9′	2.18		m		40.0		d
10	2.96	2.88	t	8.8	43.9		d
10′	2.57		m		35.1	32.9	d
11	4.38		d	4.9	61.7	61.0	d
11′					129.1	128.7	s
12′	12.2		brs				
13	8.96		br		160.5	160.4	s
13′	7.34		s		148.7		s
14	9.88	9.86	brs				
14′	11.61	11.63	s				
15					174.1		
15′	6.56	6.48	s		110.6	110.5	s

[a] δ in ppm; [b] DMSO-d_6; [c] MeOH-d_4 containing a few drops of 2N HCl; [d] multiplicity in DEPT; [e] these columns denote minor signals; [f–h] interchangeable.



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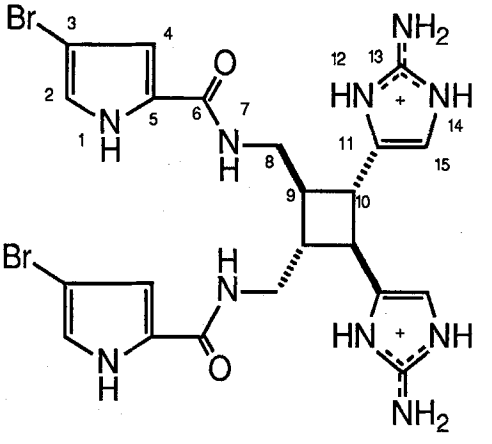

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Figure 1. Structures of oxysceptrin (**1**) and sceptrin (**2**).

amide carbonyl group, respectively. The FAB mass spectrum exhibited molecular ion peaks at *m/z* 635, 637 and 639 (1:2:1), indicating that **1** is a dibrominated compound. The molecular formula $C_{22}H_{24}N_{10}O_3Br_2$ was established by high resolution FABMS (*m/z* 635.0505, $M^+ + H$, $\Delta + 2.7$ nm). The ^1H NMR spectrum showed a total of 24 protons (table 1), 11 of which were not attached to carbons as shown by a heteronuclear multiple quantum coherence (HMQC) experiment[11]; two imidazole NHs (δ 12.2 and 11.6), two pyrrole NHs (δ 11.8), two primary amine NHs (δ 8.96 and 7.34) and three amide NHs (δ 9.87, 8.52, and 8.42). The ^{13}C NMR[12, 13] data, including a DEPT experiment (table 1), disclosed three amide carbonyl carbons (δ 174.1, 163.1 and 163.0), six sp^2 quaternary carbons (δ 160.5, 148.7, 129.1, 127.3, 127.0 and 97.6), five sp^3 methines (δ 61.7, 45.8, 43.9, 40.0 and 35.1), five sp^2 methines (δ 123.0 (2C), 113.8 (2C), and 110.6), and two sp^3 methylenes (δ 43.2 and 42.6).

The detailed analyses of ^1H and ^{13}C NMR data (table 1) including heteronuclear proton-carbon correlation experiments (table 2) revealed that the structure (fig. 1) of **1** was the C-15 monooxygenated form of **2**. The ^{13}C chemical shifts of the two pyrrole units (C-2 and 2′, δ 123.0; C-3 and 3′, 97.6; C-4 and 4′, 113.8; C-5 and 5′, 127.3 and 127.2; C-6 and 6′, 163.1 and 163.0) and an amino imidazole moiety (C-11′, δ 129.1; C-13′, 148.7; C-15′, 110.6) of **1** correlated well with those of the corresponding carbons of **2** (C-2, δ 123.3; C-3, 98.3; C-4, 111.1; C-5, 129.5; C-6, 163.7; C-11, 128.0; C-13, 149.6; C-15, 108.4). The aliphatic carbon resonances of **1** (C-8 and 8′, δ 43.2 and 42.6; C-9 and 9′, 45.8 and 40.0; C-10, 43.9) also corresponded to those of **2** (C-8, δ 43.1; C-9, 39.7; C-10, 45.1). The methine carbon at δ 35.1 was assigned to C-10′ by the HMQC and proton-proton correlation experiments[14]. The remaining carbon resonances of **1** (C-11, δ 61.7; C-13, 160.5; C-15, 174.1) were assignable to those of a 3-aminoimidazolone ring on the basis of a heteronuclear multiple-bond ^1H-^{13}C correlation (HMBC) experiments (table 2)[15, 16]. Since the H-11 (δ 4.38) was coupled to H-10 (δ 2.96), the C-11 was located between an imidazole nitrogen (N-12) and a ketone carbonyl (C-15). In the ^1H and ^{13}C NMR (table 1) and ^1H-^1H COSY spectra (fig. 2) two sets of signals were observed, indicating that epimerization at C-11 generates the two sets of stereostructures of **1**. The absolute configuration of the four-membered ring system of **1** should be the same as that of sceptrin **2** isolated from this sponge, since the optical rotation of **1** {$[\alpha]_D$ −19.7° (*c* 1.02, MeOH)} was very close to that of **2** {$[\alpha]_D$ −14.5° (*c* 0.23, MeOH)}.

Table 2. Protons of oxysceptrin (**1**) to which correlations were observed in the HMBC and HMQC experiments

Position	HMQC (^1H)[a]	HMBC (^1H)[b]
2	H-2	H-1, H-4
2′	H-2′	H-1′, H-4′
3		H-1, H-2
3′		H-1′, H-2′
4	H-4	H-1, H-2
4′	H-4′	H-1′, H-2′
5		H-1, H-2, H-4
5′		H-1′, H-2′, H-4′
6		H-7
6′		H-7′
8	H-8	H-9[a]
8′	H-8′	H-9′[a]
9	H-9	H-8, H-8′, H-9′[a], H-10′[a]
9′	H-9′	H-8, H-8′, H-9[a], H-10[a]
10	H-10	H-8, H-10′, H-11
10′	H-10′	H-8′, H-11
11	H-11	H-10′, H-14
11′		H-10′, H-15′
13		H-11, H-14
13′		H-12′, H-14′, H-15′
15		H-11, H-14
15′	H-15′	H-10′, H-14′

[a] measurement in MeOH-d_4; [b] measurement in DMSO-d_6.

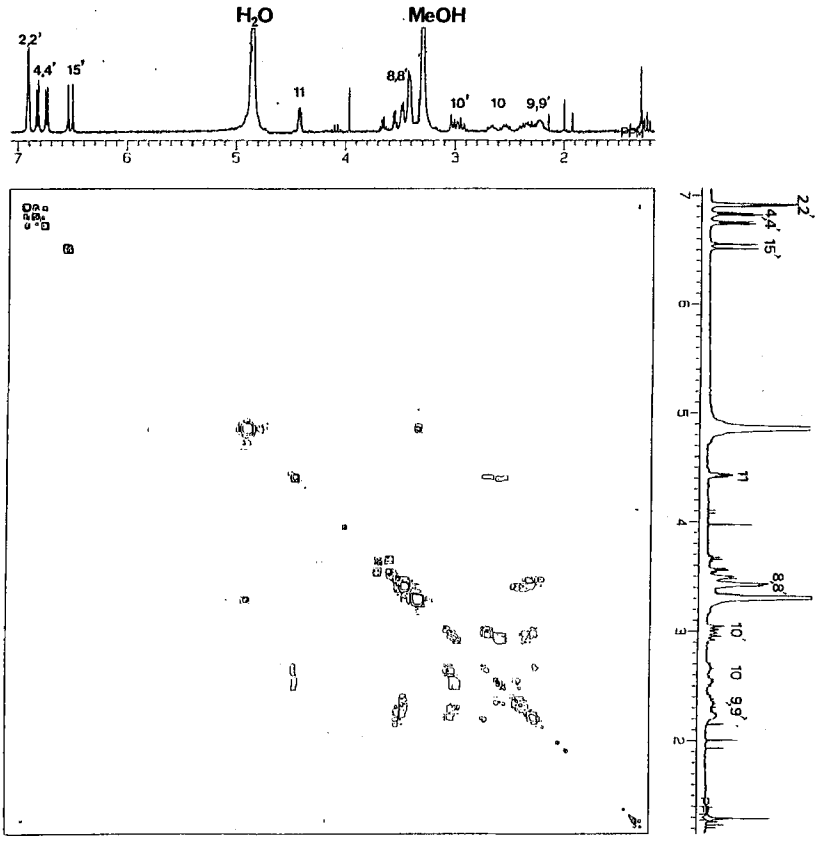

Figure 2. ^{1}H-^{1}H COSY spectrum of oxysceptrin (**1**) in MeOH-d_4.

Oxysceptrin **1** is a bromo-containing alkaloid closely related to oroidin[17, 18], keramadine[19] and dibromoagelaspongin[20] from marine sponges of the genus *Agelas*, mono and dibromophakelins[21] from *Phakelia frabellata*, hymenin[22] and hymenidin[23] from *Hymeniacidon* sp., hymenialdisine[24, 25] from *Hymeniacidon aldis*, *Axinella verrucosa* and *Acanthella aurantiaca*, and stevensine[26, 27] from *Pseudaxinysaa cantharella*. Oxysceptrin (**1**) and sceptrin (**2**) are both considered to be 2 + 2 cycloaddition products of hymenidin, in which the oxidation at C-15 may occur after the cycloaddition. The ATPase activity of myofibrils from rabbit skeletal muscle[28] was elevated to 150% of the control value by compound **1** (3×10^{-5} M). Oxysceptrin **1** may provide a useful chemical tool for the study of the molecular mechanisms of actin-myosin contractile systems, since few substances have been reported which modulate the ATPase activities of myosin and actomyosin.

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6 Sceptrin **(2):** a colorless solid; $[\alpha]_D^{22} -14.5°$ (*c* 0.23, MeOH); ^{1}H NMR (DMSO-d_6) δ 12.2 (brs, H-14), 11.8 (brs, H-1), 11.7 (brs, H-12), 8.18 (brt, J = 5.2 Hz, H-7), 7.36 (brs, H-13), 6.98 (dd, J = 1.1 and 2.7, Hz H-2), 6.80 (d, J = 1.1 Hz, H-4), 6.60 (s, H-15), 3.43 (m, H-8), 2.93 (d, J = 8.4 Hz, H-10) and 2.27 (m, H-9); ^{13}C NMR (MeOH-d_4) δ 163.7 (s, C-6), 149.6 (s, C-13), 129.5 (s, C-11), 128.0 (s, C-5), 123.3 (d, C-2), 111.1 (d, C-4), 108.4 (d, C-15), 98.3 (s, C-3), 45.1 (t, C-8), 43.1 (d, C-10) and 39.7 (d, C-9); FABMS (positive ion), *m/z* 619 ($M^+ + H$), 621 ($M^+ + 2 + H$), 623 ($M^+ + 4 + H$), 541 ($M^+ - Br + H$) 543 ($M^+ - Br + 2 + H$). HRFABMS *m/z* 619.0590 ($M^+ + H$; calcd for $C_{22}H_{25}N_{10}O_2Br_2$, 619.0583).

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8 Oxysceptrin **(1):** a colorless solid; $[\alpha]_D^{22} -19.7°$ (*c* 1.02, MeOH); IR (nujol) 3500-3100 (br), 3160, 1740, 1680, 1610, 1570, 1490, 1420, and 1350 cm^{-1}; UV (MeOH) 214 (ε 21 000), and 268 nm (21 000); ^{1}H NMR (in DMSO-d_6) and ^{13}C NMR (see table 1); ^{1}H NMR in MeOH-d_4 (signals for major diastereomer) δ 6.94 (2H, s; H-2 and 2'), 6.82 (1H, d, J = 1.5 Hz; H-4 or 4'), 6.73 (1H, d, J = 1.5 Hz; H-4' or 4), 6.54 (1H, s; H-15'), 4.43 (1H, d, J = 4 Hz; H-11), 3.49 ~ 3.42 (4H, m; H_2-8 and H_2-8'), 3.01 (1H, t, J = 9 Hz; H-10'), 2.66 (1H, dt, J = 4 and 9 Hz; H-10), and 2.25 (2H, m; H-9 and 9'); ^{1}H-^{1}H COSY correlations in MeOH-d_4 (H/H):2/4, 2'/4', 8/9, 8'/9', 9/10, 9'/10', 10/10', and 10/11; FABMS (positive ion), *m/z* 635 ($M^+ + H$), 637 ($M^+ + 2 + H$), 639 ($M^+ + 4 + H$), 557 ($M^+ - Br + H$), 559 ($M^+ - Br + 2 + H$). HRFABMS *m/z* 635.0505 ($M^+ + H$; calcd for $C_{22}H_{25}N_{10}O_3Br_2$, 635.0532).

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Dithyreanitrile: an unusual insect antifeedant from *Dithyrea wislizenii*

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Summary. Dithyreanitrile, a novel sulfur-containing indole alkaloid, was isolated from the seeds of *Dithyrea wislizenii* (Cruciferae). Dithyreanitrile inhibits feeding of fall armyworm *(Spodoptera frugiperda)* and European corn borer *(Ostrinia nubilalis)* larvae. Dithyreanitrile, the first natural product with two sulfur atoms and a nitrile attached to the same carbon, was characterized by X-ray diffraction, spectroscopy, and chemical synthesis.
Key words. Insect antifeedant; indole alkaloid; X-ray diffraction.

Over thirty years have passed since Fraenkel's classic paper directed attention to the deterrent effects of plant metabolites towards insects[1]. Our knowledge of the chemistry and biology of plant-insect interactions has increased dramatically in the intervening years, and a large number of compounds representing virtually every structural type has been implicated in plant defense[2]. Even with all of the results accumulated by the systematic study of plant-insect interactions, the search for insect antifeedants can still produce surprising new compounds. We wish to report the isolation, structural characterization, and biological activity of dithyreanitrile, a most unusual insect antifeedant from *Dithyrea wislizenii*, a member of the Cruciferae native to the southwestern U.S. and northern Mexico[3]. Dithyreanitrile is a sulfur-containing indole alkaloid with a novel functional group – a carbon bonded to two S-methyls and a nitrile.

Seeds of *D. wislizenii* Engelm. were collected and authenticated by USDA botanists. The ground seeds (4.8 kg) were steeped for 24 h in water/ethanol (1:4), the solvent was removed, and the process repeated two more times. The collected solvent was partitioned three times with hexane (200 ml of hexane per 800 ml of solvent). The hexane layers were combined and evaporated to yield 78 g of oil, and the aqueous ethanol layer was evaporated to dryness yielding 263 g of residue. The ethanolic residue was partitioned between ether and water, yielding 44 g of ether-soluble material. The ether-soluble material was chromatographed on silica gel with chloroform and increasing amounts of methanol to yield 570 mg of active material that was further purified by HPLC using a Whatman partisil 10 PAC 20 Magnum column and chloroform elution. Final purification was accomplished on a Rainin Dynamax Macro 12″ silica column with chloroform as the eluting solvent. This procedure yielded 44 mg of dithyreanitrile (1×10^{-3}% yield).

The isolation of dithyreanitrile was monitored by a two-choice insect antifeedant bioassay[4]. Disposable Petri dishes with a moistened filter paper on the bottom had 12 1-cm diameter green bean leaf disks, half treated and half control, placed around the circumference. The disks had been dipped for 5 s either in a 1% or a 10% w/v solution of the extract being tested, or in solvent (control). The solvents used for the controls – hexane, chloroform, methanol or 95% ethanol – were selected to correspond to the solubility characteristics of the material being tested. After allowing 5 min for the solvent to evaporate the disks were arranged alternately in each Petri dish. Six fall armyworm *(Spodoptera frugiperda)* larvae, of roughly equal size, that had been reared on pinto bean artificial diet[5] for 9 days posteclosion and starved overnight, were placed in the center of each Petri dish and allowed to feed for 3 h. Assays were conducted in darkness at 27 °C and 60% relative humidity in three simulta-