ARAGUSPONGINES B, C, D, E, F, G, H, AND J, NEW VASODILATIVE BIS-1-OXAQUINOLIZIDINE ALKALOIDS FROM AN OKINAWAN MARINE SPONGE, XESTOSPONGIA SP.

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Nine new vasodilative alkaloids, araguspongines A, B (1), C (2), D (3), E (4), F (5), G (6), H (7), and J (8), were isolated from an Okinawan marine sponge, Xestospongia sp. On the basis of chemical and physicochemical evidence, the absolute stereostructures of araguspongines B, D, E, F, G, H, and J were determined respectively as 1, 3, 4, 5, 6, 7, 8, and the relative stereostructure of araguspongine C was determined as 2 having two 1-oxaquinolizidine moieties. Araguspongines B, D, and E each comprised a pair of the enantiomers, 1a and 1b, 3a and 3b, and 4a and 4b, respectively.

KEYWORDS marine sponge; Xestospongia sp.; alkaloid; araguspongine; 1-oxaquinolizidine; vasodilative activity; HPLC chiral analysis

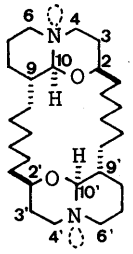
In a continuing search for new bioactive substances from marine organisms,[1] we have isolated nine new macrocyclic alkaloids, araguspongines A, B (1), C (2), D (3), E (4), F (5), G (6), H (7), and J (8) from an Okinawan marine sponge Xestospongia sp. This paper describes their structures.

An acetone extract of the titled fresh sponge (4 kg collected in July at Aragusuku-jima, Okinawa Prefecture) was partitioned into a water-AcOEt mixture and the water phase was further partitioned with 1-butanol. The 1-butanol solute (75 g) was dissolved in aq. $(COOH)_2$ solution (pH 3) and extracted with AcOEt. The aqueous phase was then treated with aq. NH_4OH (to pH 10) and extracted again with AcOEt to furnish an alkaloid fraction (28 g). The alkaloid fraction (4 g) was subjected to silica gel column chromatography (benzene-acetone-NH_4OH) and HPLC (μ-PORASIL, n-hexane-benzene-Et_2NH; COSMOSIL $5C_{18}$, $CHCl_3$-MeOH-CH_3CN-H_2O-NH_4OH) to afford araguspongines A (30 mg)[2], B (1)(85 mg), C (2)(131 mg), D (3)(1 g), E (4) (1.3 g), F (5)(18 mg), G (6)(5 mg), H (7)(16 mg), and J (8)(22 mg).

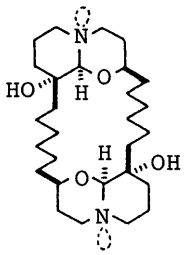
Araguspongine D (3)[3], $C_{28}H_{50}N_2O_2$,[4] $[\alpha]_D$ -5.1° ($CHCl_3$), showed 14 carbon signals in the ^{13}C NMR spectrum, suggesting that 3 had a C_2 symmetry. The IR spectrum of 3 showed Bohlmann absorptions[5] (2810, 2760 cm^{-1}) due to the trans-quinolizidine ring. Based on ^1H (500 MHz) and ^{13}C (125 MHz) NMR analysis, 3 was concluded to be identical with xestospongin A[6] which was previously isolated from the Australian marine sponge Xestospongia exigua and its relative configuration reported. However, the $[\alpha]_D$ value of 3 significantly differed from that ($[\alpha]_D$ +10°) of xestospongin A. So that araguspongine D (3) was subjected to HPLC separation using a chiral column [CHIRALCEL OF (DAICEL), n-hexane-2-PrOH-Et_2NH] to provide (+)-araguspongine D (3b, $[\alpha]_D$ +10°) and (-)-araguspongine D (3a, $[\alpha]_D$ -9°) in 3:7 ratio. The 3b thus obtained was identical with xestospongin A[6] in all respects.

Araguspongine J (8),[7] $C_{30}H_{54}N_2O_2$, $[\alpha]_D$ -23.4° ($CHCl_3$) showed Bohlmann absorptions (2800, 2750 cm^{-1}) in the IR spectrum and 15 carbon signals in the ^{13}C NMR spectrum due to the C_2 symmetrical trans-1-oxaquinolizidine structure. The ^1H NMR spectrum ($CDCl_3$) of 8 showed a doublet methyl signal (δ1.10, J=6.7 Hz) together with signals due to 10α-H_{ax} (δ3.03, d, J=8.9 Hz), 6α-H_{ax} (δ1.90, ddd, J=12.2,12.2,2.8 Hz), and 6β-H_{eq} (δ2.66, brd, J=ca 12.2 Hz) which were very similar to those observed in the ^1H NMR spectrum of 3. The location of the secondary methyl was determined at 3β axial from the coupling pattern of the signals due to 2α-H_{ax} (δ3.46, ddd, J=10.4,2.3,2.3 Hz), 4α-H_{ax} (δ2.30, dd, J=11.0,3.4 Hz), and 4β-H_{eq} (δ2.73, dd, J=11.0, 1.8 Hz). Consequently, the relative stereostructure of araguspongine J was revealed to be 8, which corresponded to a 3β,3'β-dimethyl analogue of araguspongine D (3).

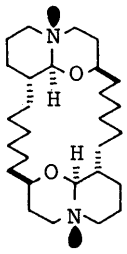
Araguspongine G (6),[8] $C_{29}H_{52}N_2O_2$, $[\alpha]_D$ -16° ($CHCl_3$) showed ^1H and ^{13}C signals ascribable to one half moiety of both araguspongines D (3) and J (8) in its ^1H and ^{13}C NMR spectra. So the relative stereostructure of araguspongine G was clarified as 6 which had a hybrid structure of 3 and 8.



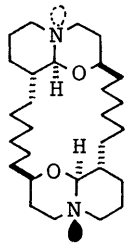
(+)-aragu. B (1a)
[(-)-aragu. B (1b) =enantiomer of 1a]



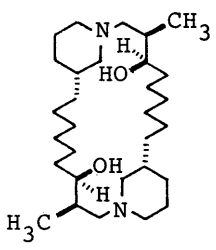
aragu. C (2)
[relative structure]



(-)-aragu. D (3a)
[(+)-aragu. D (3b) =enantiomer of 3a =xestospongin A]



(+)-aragu. E (4a)
[(-)-aragu. E (4b) =enantiomer of 4a]



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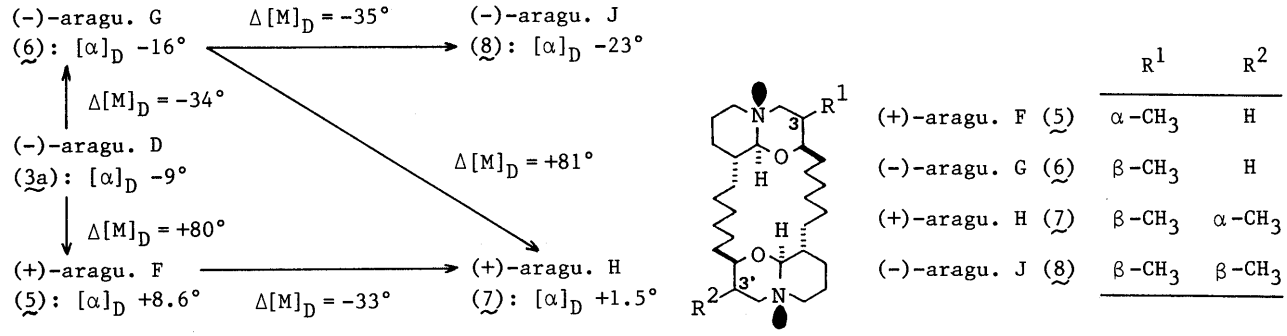

	R^1	R^2
(+)-aragu. F (5)	α-CH_3	H
(-)-aragu. G (6)	β-CH_3	H
(+)-aragu. H (7)	β-CH_3	α-CH_3
(-)-aragu. J (8)	β-CH_3	β-CH_3

Chart [aragu. = araguspongine]

The ^1H NMR spectrum of araguspongine F (5),[9] $C_{29}H_{52}N_2O_2$, $[\alpha]_D$ +8.6° ($CHCl_3$) showed ^1H signals ascribable to the 3α-methyl-trans-1-oxaquinolizidine structure (δ0.76, 3H, d, J=6.4 Hz; 2.95, 1H, brt, J=ca 9.5 Hz, 2α-H_{ax}; 1.80, 1H, dd, J=11.0,11.0 Hz, 4α-H_{ax}; 2.85, 1H, dd, J=11.0,4.0 Hz, 4β-H_{eq}; 3.02, 1H, d, J=8.5 Hz, 10α-H_{ax}) together with those observed in the ^1H NMR spectrum of araguspongine D (3). Araguspongine H (7),[10] $C_{30}H_{54}N_2O_2$, $[\alpha]_D$ -9° ($CHCl_3$) showed ^1H and ^{13}C signals ascribable to one each of the 3β-methyl-trans-1-oxaquinolizidine and 3α-methyl-trans-1-oxaquinolizidine moieties in its ^1H and ^{13}C NMR spectra. Therefore, the relative stereostructures of araguspongines F and H were 5 and 7, respectively.

Araguspongines F (5), G (6), H (7), and J (8) were shown to be optically pure by HPLC analysis using a chiral column. Based on the ^1H and ^{13}C NMR analysis, it was concluded that 5, 6, 7, and 8 have the same skeletal conformation as araguspongine D (3) regardless of the presence of 3α- and/or 3β-methyl group(s) which may contribute independently to the optical rotatory property. We then applied the Hudson rule[11] to 5, 6, 7, 8 and araguspongine D (3a or 3b) and the results were as shown in the Chart. Thus, the comparison of $[M]_D$ values among 5, 6, 7, 8, and (-)-araguspongine D (3a) disclosed that 5, 6, 7, 8, and 3a have the same absolute stereostructures. To determine the absolute stereostructures of these alkaloids, we next applied Horeau's method[12] to a diol 9, which was obtained by $NaBH_3CN$ reduction of araguspongine J (8) in THF-MeOH (1:1) under reflux. The recovered α-phenylbutylic acid showed $[\alpha]_D$ +1.6° (benzene), so that the 2R configuration in 8 and consequently the absolute stereostructures of (-)- and (+)-araguspongines D (3a and 3b), (+)-araguspongine F (5), (-)-araguspongine G (6), (+)-araguspongine H (7), and (-)-araguspongine J (8) were determined as shown. In addition, the absolute stereostructure of xestospongin A[6], which was identified with (+)-araguspongine D (3b), was clarified.

Araguspongine B (1),[13] $C_{28}H_{50}N_2O_2$, $[\alpha]_D$ -0.8° ($CHCl_3$) showed 14 carbon signals in the ^{13}C NMR spectrum but lacked Bohlmann absorption in its IR spectrum. Treatment with Al_2O_3 60 F_{254} (Type E, Merck)(80°C, 5h) selectively isomerized 1 to furnish 3, so that it was concluded that 1 had a bis-cis-1-oxaquinolizidine structure while possessing the same relative configurations at 2,2' and 9,9' as those in 3. Upon irradiation of 4α-H_{ax} in the ^1H NMR of 1, 7% NOE was observed on 10α-H whereas irradiation of 10α-H resulted in 8% NOE on 2α-H_{ax}. So the relative stereostructure of araguspongine B was shown to be 1 having a bis-cis-1-oxaquinolizidine structure.

Araguspongine E (4),[14] $C_{28}H_{50}N_2O_2$, $[\alpha]_D$ -1.1° ($CHCl_3$) showed ^1H and ^{13}C signals ascribable to one half moiety of both araguspongines B (1) and D (3) in the ^1H and ^{13}C NMR spectra and was selectively converted to 3 on Al_2O_3 treatment. So that the relative stereostructure of araguspongine E was shown to be 4 which had a hybrid structure of 1 and 3. HPLC analysis using a chiral column showed that both 1 and 4 were mixtures of the respective enantiomers 1a and 1b (1:1) and 4a and 4b (3:2), respectively: $[\alpha]_D$ ($CHCl_3$) +17° (1a), -15° (1b), +1°(4a), and -2° (4b). Al_2O_3 treatment of 1a and 4a furnished 3a, whereas the similar treatment of 1b and 4b furnished 3b. Thus, the absolute stereostructures of 1a, 1b, 4a, and 4b were elucidated as shown.

Finally, araguspongine C (2),[15] $C_{28}H_{50}N_2O_4$, $[\alpha]_D$ +11.1° ($CHCl_3$) showed 14 carbon signals in the ^{13}C NMR spectrum, suggesting 2 to have a C_2 symmetry. The ^1H NMR analysis in detail[16] of 2 revealed the presence of a 9-hydroxy-cis-1-oxaquinolizidine structure in 2. Furthermore, the configuration of the 9-hydroxyl moiety in 2 was elucidated by a pyridine-induced shift study[17] of the ^1H NMR of 2. Thus, the 7α-H_{ax} and 10α-H signals were significantly shifted lower [$\Delta\delta=\delta_{C_5D_5N} - \delta_{CDCl_3}$ = +0.35 and +0.24, respectively] due to the presence of the 9α-OH group. Consequently, the relative stereostructure of araguspongine C was clarified as 2.

Araguspongines C (2), D (3), E (4), and J (8) showed stronger vasodilative activities than papaverine in a perfusion model experiment using an isolated mesenteric artery of the SD-rat.

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REFERENCES AND NOTES

1) The recent paper: a) I. Kitagawa, M. Kobayashi, B. W. Son, S. Suzuki, and Y. Kyogoku, Chem. Pharm. Bull., 37, 1230 (1989); b) I. Kitagawa, Yakugaku Zasshi, 108, 398 (1988).
2) Based on ^1H and ^{13}C NMR analyses, araguspongine A is presumed to have a hybrid structure of araguspongine C (2) and D (3).
3) 3: δ($CDCl_3$): 3.35 (brd, J=ca 10.7 Hz, 2-H), 3.06 (d, J=8.2 Hz, 10-H), 2.94 (ddd, J=11.8,4.0,1.8 Hz, 4-H_{eq}), 2.76 (brd, J=ca 11.6 Hz, 6-H_{eq}), 2.18 (ddd, J=12.0,11.8,3.1 Hz, 4-H_{ax}), 1.96 (ddd, J=11.6,11.6,2.7 Hz, 6-H_{ax}); δ_c ($CDCl_3$): 95.9 (C-10), 75.3 (C-2), 54.3, 54.1 (C-6,4), 40.6 (C-9).
4) The molecular composition of compounds with the chemical formulae were determined by high resolution mass spectrometry. 5) F. Bohlmann, Angew. Chem., 69, 641 (1957).
6) M. Nakagawa, M. Endo, N. Tanaka, and G. Lee, Tetrahedron Lett., 25, 3227 (1984).
7) 8: δ_c($CDCl_3$): 96.9 (C-10), 78.3 (C-2), 62.3 (C-4), 54.4 (C-6), 40.1 (C-9), 33.4 (C-3), 13.3 (3-Me).
8) 6: δ($CDCl_3$): 3.46 (ddd, J=11.0,2.5,2.1 Hz, 2-H), 3.35 (brt, J=ca 10.7 Hz, 2'-H), 3.08 (d, J=8.5 Hz, 10'-H), 3.01 (d, J=8.2 Hz, 10-H), 2.94 (ddd, J=11.8,4.3,2.1 Hz, 4'-H_{eq}), 2.73 (dd, J=11.0,1.8 Hz, 4-H_{eq}), 2.30 (dd, J=11.0,3.4 Hz, 4-H_{ax}), 2.19 (ddd, J=12.2,11.8,3.4 Hz, 4'-H_{ax}), 1.10 (d, J=7.0 Hz, 3-Me); δ_c($CDCl_3$): 97.3 (C-10), 95.9 (C-10'), 78.3 (C-2), 75.7 (C-2'), 62.3 (C-4), 54.4, 54.2 (C-6,6',4'), 40.8, 40.2 (C-9,9'), 33.4 (C-3), 13.3 (3-Me).
9) 5: δ($CDCl_3$): 3.35 (brt. J=ca 10.7 Hz, 2'-H), 3.06 (d, J=8.6 Hz, 10'-H), 2.94 (brd, J=ca 11.7 Hz, 4'-H_{eq}), 2.18 (ddd, J=11.7,11.7,3.4 Hz, 4'-H_{ax}); δ_c($CDCl_3$): 95.9 (C-10,10'), 81.1 (C-2), 75.1 (C-2'), 62.2 (C-4), 54.3, 54.1 (C-6,6',4'), 40.6, 40.5 (C-9,9'), 35.4 (C-3), 14.9 (3-Me).
10) 7: δ($CDCl_3$): 3.40 (brd, J=ca 10.4 Hz, 2-H), 2.96 (d, J=8.8 Hz, 10-H), 2.95 (d, J=8.5 Hz, 10'-H), 2.90 (ddd, J=10.1,10.1,2.4 Hz, 2'-H), 2.83 (dd, J=11.0,4.0 Hz, 4'-H_{eq}), 2.71 (dd, J=11.3,1.8 Hz, 4-H_{eq}), 2.30 (dd, J=11.3,3.4 Hz, 4-H_{ax}), 1.80 (dd, J=11.0,11.0 Hz, 4'-H_{ax}), 1.09 (d, J=7.0 Hz, 3-Me), 0.76 (d, J=6.7 Hz, 3'-Me); δ_c($CDCl_3$): 97.9 (C-10), 96.9 (C-10'), 82.3 (C-2'), 79.1 (C-2), 62.1 (C-4,4'), 54.3, 53.7 (C-6,6'), 40.9, 40.4 (C-9,9'), 35.2 (C-3'), 33.1 (C-3), 15.0 (3'-Me), 13.3 (3-Me).
11) C. S. Hudson, J. Am. Chem. Soc., 31, 66 (1909). 12) A. Horeau, Tetrahedron Lett., 15, 506 (1961).
13) 1: δ($CDCl_3$): 4.30 (d, J=2.8 Hz, 10-H), 3.53 (brt, J=ca 11.0 Hz, 2-H), 3.18 (ddd, J=13.7,13.4,3.1 Hz, 4-H_{ax}), 2.95 (ddd, J=13.7,3.1,1.5 Hz, 4-H_{eq}); δ_c($CDCl_3$): 87.5 (C-10), 76.0 (C-2), 52.8 (C-4), 45.3 (C-6), 40.5 (C-9).
14) 4: δ($CDCl_3$): 4.24 (brs, 10-H), 3.48 (brt, J=ca 10.7 Hz, 2-H), 3.30 (brt, J=ca 10.7 Hz, 2'-H), 3.13 (brt, J=ca 14.1 Hz, 4-H_{ax}), ca 3.00 (10'-H,6-H_{ax}), 2.91 (brdd, J=ca 14.1,3.4 Hz, 4-H_{eq}), 2.88 (brd, J=ca 12.2 Hz, 4'-H_{eq}), 2.12 (ddd, J=12.2,12.2,3.4 Hz, 4'-H_{ax}); δ_c($CDCl_3$): 95.7 (C-10'), 87.4 (C-10), 75.7, 75.2 (C-2,2'), 54.2, 53.9 (C-6',4'), 52.6 (C-4), 45.3 (C-6), 40.4, 40.1 (C-9,9').
15) 2: δ($CDCl_3$): 4.06 (s, 10-H), 3.56 (brt, J=ca 10.7 Hz, 2-H), 3.11 (brt, J=ca 13.7 Hz, 4-H_{ax}), 3.03 (ddd, J=10.0,10.0,3.1 Hz, 6-H_{ax}), 2.97 (brdd, J=ca 13.7,3.4 Hz, 4-H_{eq}), 2.34 (brd, J=ca 10.0 Hz, 6-H_{eq}), 1.72 (m, 7-H_{ax}); (C_5D_5N): 2.07 (ddddd, J=13.0,13.0,13.0,4.1,4.1 Hz, 7-H_{ax}); δ_c($CDCl_3$): 90.4 (C-10), 76.5 (C-2), 70.8 (C-9), 52.6 (C-4), 44.3 (C-6).
16) For examples: irradiation of 10α-H resulted in 9% NOE on 2α-H_{ax} and that of 4α-H_{ax} resulted in 5% NOE on 10α-H. Furthermore, NOE was also observed between 4β-H_{eq} and 6α-H_{eq}.
17) a) P. V. Demarco, E. Farkas, D. Doddrell, B. L. Mylari, and E. Wenkert, J. Am. Chem. Soc., 90, 5480 (1968); b) I. Kitagawa, M. Kobayashi, M. Hori, and Y. Kyogoku, Chem. Pharm. Bull., 37, 61 (1989).

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