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AGELASINE-A, -B, -C AND -D, NOVEL BICYCLIC DITERPENOIDS WITH A 9-METHYLADENINIUM UNIT POSSESSING INHIBITORY EFFECTS ON NA,K-ATPASE FROM THE OKINAWAN SEA SPONGE AGELAS SP.[1)]

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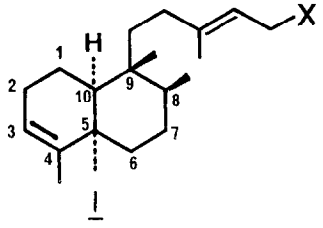
Summary: Agelasine-A, -B, -C and -D, novel bicyclic diterpenoids having inhibitory effects on enzymic reactions of Na,K-ATPase, have been isolated from the orange colored Okinawan sea sponge Agelas sp. and the structures have been determined on the basis of their spectral data and chemical conversions.

In the course of our study on physiologically active substances in marine organisms[3)], it was found that the extract of the orange colored Okinawan sea sponge Agelas sp. showed antispasmodic activities, antimicrobial activities and inhibitory effects on the enzymic reactions of Na,K-ATPase. Recently we have reported the isolation of an antispasmodic constituent, named agelasidine-A, a unique sesquiterpenoid with a guanidinylethylsulfone unit[3)]. Our continuing study on bioactive constituents of the sea sponge has revealed four novel bicyclic diterpenoids, named agelasine-A--D, possessing inhibitory effects on Na,K-ATPase[4)].

Agelasine-A 1 {mp 173-174°C; λmax 272nm(MeOH, ε 8910)}, -B 2 {mp 167-170°C; λmax 272nm (MeOH, ε 8240)}, -C 3 {mp 176-179°C, λmax 272nm (MeOH, ε 8340)}, and -D 4 {mp 175-176°C, λmax 272nm(MeOH, ε 9180)} were isolated from the chloroform soluble portion of methanolic extracts of the sponge by silica gel chromatography followed by successive reversed-phase HPLC using C_{18} and C_8 columns. These substances showed a number of spectral features in common, uv



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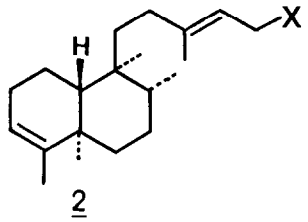

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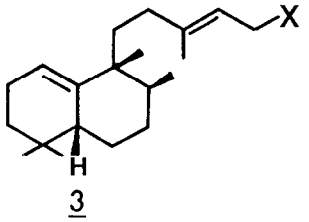

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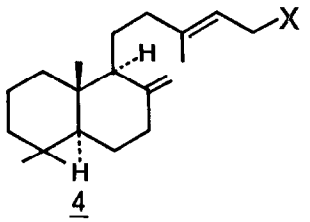

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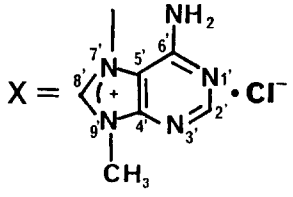
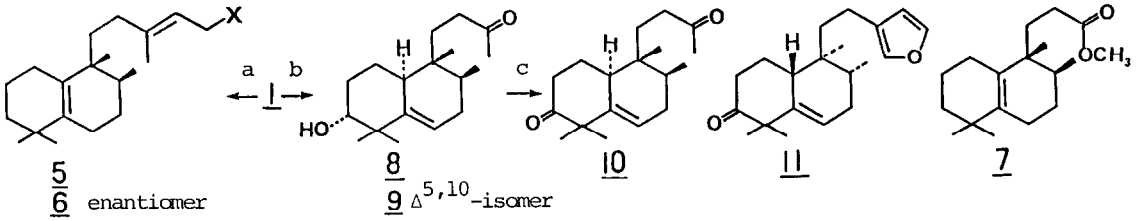


absoption maxima at 272nm; molecular formula $C_{26}H_{40}N_5Cl$ {FD-MS: m/z 422 (M^+-Cl); HR-MS: m/z 421.3216, 421.3104, 421.3177 and 421.3182 for 1, 2, 3 and 4, respectively, calcd for $C_{26}H_{40}N_5$

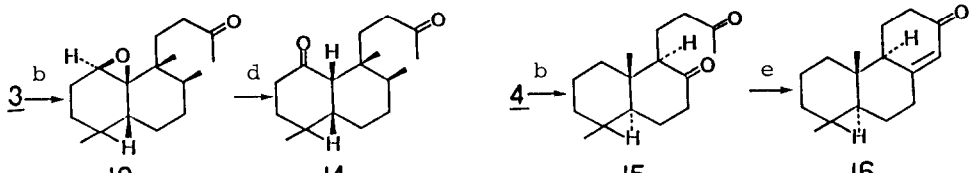
Table 1. ^1H (400MHz) and ^{13}C (22.5MHz) NMR data for agelasine-A--D, 1-4.

		1	2	3	4
X	δ_H[a]	4.10(brs,3H) 6.98(brs,2H,exch) 8.48(s,1H) 10.81(s,1H)	4.10(brs,3H) 6.84(brs,2H,exch) 8.50(s,1H) 10.89(s,1H)	4.10(brs,3H) 6.84(brs,2,exch) 8.50(s,1H) 10.88(s,1H)	4.10(brs,3H) 6.76(brs,2H,exch) 8.50(s,1H) 10.94(s,1H)
	δ_C[b]	32.0(q), 109.7(s) 141.7(d), 149.4(s) 152.4(s), 155.8(d)	32.0(q), 109.7(s) 141.7(d), 149.5(s) 152.5(s), 156.0(d)	32.1(q), 110.8(s) 142.0(d), 150.5(s) 153.8(s), 156.7(d)	32.0(q), 111.0(s) 142.6(d), 150.7(s) 153.9(s), 156.9(d)
R	δ_H[a]	0.73(d,3H,J=5.8Hz) 0.79(s,3H) 1.02(s,3H) 1.67(brs,3H) 1.0-2.1(m,14H) 5.26(brs,1H)	0.70(s,3H) 0.76(d,3H,J=5.2Hz) 0.97(s,3H) 1.57(brs,3H) 0.7-2.2(m,14H) 5.17(brs,1H)	0.79(d,3H,J=6.8Hz) 0.82(s,3H) 0.84(s,3H) 0.87(s,3H) 0.7-2.2(m,14H) 5.31(brt,1H,J=3.2Hz)	0.65(s,3H) 0.79(s,3H) 0.87(s,3H) 0.6-2.4(m,16H) 4.43(brs,1H) 4.81(brs,1H)
		1.88(brs,3H) 5.41(brt,1H,J=6.5Hz) 5.71(brd,2H,J=6.5Hz)	1.86(brs, 3H) 5.41(brt,1H,J=6.5Hz) 5.71(brd,2H,J=6.5Hz)	1.84(brs, 3H) 5.41(brt,1H,J=6.5Hz) 5.70(brd,2H,J=6.5Hz)	1.86(brs,3H) 5.41(brt,1H,J=6.5Hz) 5.72(brd,2H,J=6.5Hz)
	δ_C[b]	16.0(q),17.6(q) 17.8(q),19.7(q) 24.0(t), 28.8(t) 33.0(q and t, 2C) 36.1(t), 36.9(t) 37.4(d), 37.7(s) 40.2(s), 44.7(d) 120.3(d), 144.3(s)	16.0(q),17.9(q) 18.3(t and q,2C) 19.9(q), 26.9(t) 27.5(t), 33.1(t) 36.3(d and t, 2C) 36.8(t), 38.2(s) 38.7(s), 46.4(d) 120.3(d), 144.3(s)	16.0(q), 22.6(q) 23.9(t), 24.5(t) 26.3(q), 28.6(q) 29.9(t), 32.1(s) 34.2(t), 35.4(t) 38.2(t), 40.2(d) 43.8(s), 44.5(d) 121.0(d), 142.5(s)	15.0(q), 20.3(t) 22.1(q), 22.5(t) 25.5(t), 34.0(q) 34.4(s), 39.4(t,2C) 40.2(t), 40.6(s) 43.2(t), 56.8(d) 57.4(d), 106.9(t) 149.0(s)
		17.3(q), 48.7(t) 115.7(d), 147.5(s)	17.3(q), 48.7(t) 115.7(d), 147.5(s)	17.3(q), 48.8(t) 115.4(d), 149.3(s)	17.1(q), 48.8(t) 115.9(d), 149.5(s)

a; δ in ppm. (in $CDCl_3$) b; δ in ppm. (1 and 2 in $CDCl_3$, and 3 and 4 in CD_3OD).



5
6 enantiomer
8
9 $\Delta^{5,10}$-isomer
10
11
7



12
13 α-epoxide
14
15
16

Reagents ; a: HCl-AcOH; b: 1)O_3, 2)Me_2S; c: PDC; d: BF_3; e: H_2SO_4

Cl-HCl 421.3202}. Main fragment ions of EI-MS of 1-4 observed at m/z 149 revealed a common uv chromophore (X) composed of $C_6H_7N_5$. A 9-methyladeninium unit was assinged for the structure of X by comparing the spectral data of 1-4 with those of 7,9-dimethyladeninium perchlorate[5] {$CH_3X^+ClO_4^-$; uv: λ max 272nm (MeOH, ε8080); ^1H NMR (DMSO-d_6): δ 3.86(s, 3H), 4.17(s, 3H), 7.93(s, 2H, exchangeable), 8.40(s, 1H), 9.59(s, 1H); ^{13}C NMR (DMSO-d_6): δ 31.3(q), 36.2(q), 109.7(s), 141.9(d), 148.6(s), 152.4(s), 155.4(d)}. In addition the ^1H NMR spectra of 1-4 contained signals for a common terminal grouping, $CH_3-\overset{|}{C}=CH-CH_2-X$, in which E configuration was assigned for the double bond on the basis of high field resonances of the vinyl methyl carbons. These results suggest that agelasine-A--D differ in diterpene hydrocarbon parts (R) composed of $C_{20}H_{33}$ and that the common terminal allylic group of R links to a nitrogen atom at 7' position of the 9'-methyladeninium group (X).

Agelasine-A 1, $[\alpha]_D^{25}$ -31.3° (c 0.59, MeOH), showed a vinyl proton signal at δ5.26, and two singlet, one doublet and one vinyl methyl signals at δ 0.79, 1.02, 0.73 (J=5.8 Hz) and 1.67, respectively, in the ^1H NMR spectrum. The clerodane skeleton was assumed and the cis-fused structure was assigned by a low field resonance of a bridge head methyl carbon (δ 33.0)[6]. 1 was treated with a mixture of acetic acid and hydrochloric acid to give a tetrasubstituted olefin 5, $[\alpha]_D^{25}$ -14.7° (c 0.42, MeOH). Its ^1H NMR spectrum contained methyl signals at δ0.79 (s), 0.80(d, J=6.0 Hz), 0.94 (s) and 0.96 (s) which were similar to those of a known compound 7, lit.[7] δ0.84(s), 0.86(d, J=7 Hz), 0.97(s) and 0.98(s), and $[\alpha]_D^{25}$ -52.9°, indicating the configurations at 8 and 9 positions of 1. Furthermore, 1 was treated with ozone followed by reduction with dimethylsulfide to yield a mixture of isomeric olefins 8 and 9. 8 was oxidized with pyridinium dichromate to obtain a diketone 10, whose ^1H NMR spectrum {δ0.69 (s, 3H), 0.84 (d, 3H, J=6.4 Hz), 1.24 (s, 6H), 5.62 (m, 1H)} was comparable with that of a known compound 11 {lit.[8] δ0.69 (s, 3H), 0.88 (d, 3H, J=6 Hz), 1.24 (s, 6H), 5.62 (m, 1H)}. The CD spectrum of 10, $[\theta]_{293}$ -3480°, was reversed to that of 11, lit.[8] $[\theta]_{291}$ +3320°. From these results , the absolute configuration of 1 was determined as illustrated.

The ^1H NMR spectrum of agelasine-B 2, $[\alpha]_D^{25}$ -21.5° (c 1.00, MeOH), contained a vinyl proton signal at δ5.17, and two singlet, one doublet and one vinyl methyl signals at δ 0.70, 0.97, 0.76 (d, J=5.2 Hz) and 1.57, respectively. In contrast to 1, the ^{13}C NMR spectrum of 2 showed no low field methyl signals, indicating a trans-fused clerodane skeleton for 2[6]. An acid catalized rearrangement of 2 furnished a tetrasubstituted olefin 6, $[\alpha]_D^{25}$ +17.4°, enantiomeric to 5. The clerodane structure with the absolute configuration as illustrated was assigned for 2 on the basis of comparison of the ^{13}C NMR spectrum of 2 with that of a known substance with the same clerodane skeleton, methyl kolavenate[9].

Agelasine-C 3, $[\alpha]_D^{25}$ -55.1° (c 2.04, MeOH), showed a vinyl proton signal at δ 5.31, and three singlet methyl signals at δ0.82, 0.84 and 0.87 and one doublet methyl signal at δ 0.79(J=6.8 Hz) without a vinyl methyl signal, indicating a rearranged labdane skeleton with 1,10- or 5,6-double bond for the structure of 3. Furthermore, an acid catalized rearrangement of 3 as well as 1 yielded a tetrasubstituted olefin 5. Ozonolysis of 3 followed by reduction with dimethylsulfide gave a mixture of isomeric epoxides 12 and 13. The epoxide 13 was treated with boron trifluoride etherate to yield a diketone 14 whose ^1H NMR spectrum contained signals for protons α to carbonyl groups at δ2.12(ddd, 1H, J=2.3, 4.4, 13.5Hz), 2.17(s, 3H), 2.23-2.38(m, 2H), 2.42(dt, 1H, J=6.3, 13.5Hz) and 2.48(d, 1H, J=5.0Hz), and methyl signals at δ0.97(s), 1.03(d, J=6.9Hz), 1.13(s) and 1.23(s). On irradiation at δ1.44

(8-H), the doublet methyl signal was transformed to a singlet,whereas the α-proton signals were not affected. These data suggest that 14 has a cis-fused decalin skeleton having a carbonyl group at 1 position. Nuclear Overhauser effects between the singlet methyl signals (δ 1.13 and 1.23) and a doublet proton signal for 1Ø position revealed a non-steroid-like conformation for 14. On the basis of the octant rule, the absolute configuration of 14, $[\theta]_{298.5}$ -2370°, was tentatively determined as illustrated.

The ^{1}H NMR spectrum of agelasine-D 4, $[\alpha]_D^{25}$ +1Ø.4° (c 1.1, MeOH) showed three singlet methyl signals at δØ.65, Ø.79 and Ø.87 and two vinyl proton signals for an exomethylene group at δ4.43 (brs) and 4.81 (brs). The exomethylene group was further suggested by its ^{13}C NMR spectrum {δ1Ø6.9(t) and 149.Ø (s)}. The following chemical conversion revealed the structure and absolute configuration of 4. Ozonolysis of 4 followed by reduction with dimethylsulfide gave a diketone 15, which was treated with sulfuric acid to yield an enone 16, $[\alpha]_D^{25}$ +36.Ø° (c Ø.5Ø, MeOH). Its spectral properties were identical with those of an authentic enone[10,11].

Isolation and partial characterization of agelasine, a diterpene having a 9-methyladeninium unit, has been reported by Cullen and Devlin[12]. Agelasine-A--D showed powerful inhibitory effects on Na,K-ATPase and antimicrobial activities.

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