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AGELASINE-E AND -F, NOVEL MONOCYCLIC DITERPENOIDS WITH A 9-METHYLADENINIUM UNIT POSSESSING INHIBITORY EFFECTS ON NA,K-ATPASE ISOLATED FROM THE OKINAWAN SEA SPONGE AGELAS NAKAMURAI HOSHINO[1,2]

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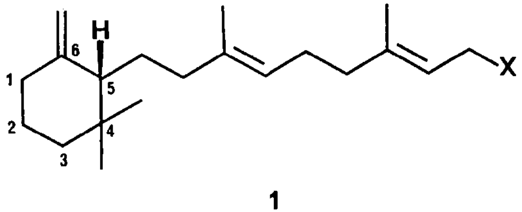
Summary: Agelasine-E and -F, novel terpenoids with a 9-methyladeninium unit have been isolated from the orange colored sea sponge *Agelas nakamurai* Hoshino as Na,K-ATPase inhibitors and their structures and absolute configurations have been determined on the basis of spectral data and chemical conversions.

In the course of our survey of various marine organisms for physiologically active substances[4], the extract of the orange colored Okinawan sea sponge *A. nakamurai* Hoshino has been found to show several physiological activities. Recently we have reported the isolation and structural elucidation of the active constituents, agelasine-A, -B, -C and -D possessing inhibitory effects on enzymic reactions of Na,K-ATPase[2]. Our continuing study on the physiologically active constituents of the sea sponge resulted in the isolation of monocyclic diterpenoids with a 9-methyladeninium unit, named agelasine-E and -F[5]. In this paper, we would like to report the structures and absolute configurations of agelasine-E and -F.

Agelasine-E 1[6] and -F 2[7] were eluted later than agelasine-A ∿ -D on a reversed phase HPLC using a C_{18} column and showed a number of spectral



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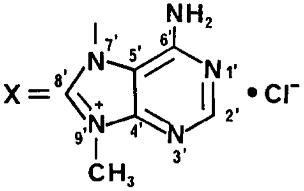

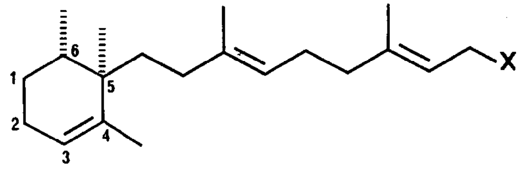

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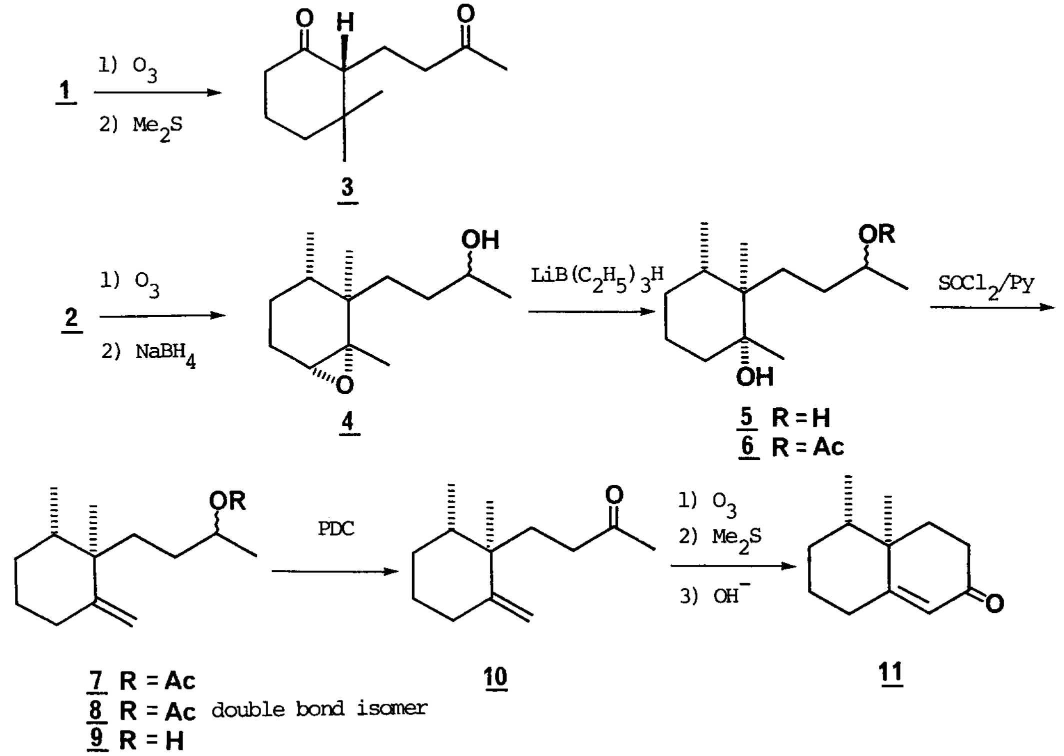

features in common as well as agelasine-A ∿ -D. In spectral properties, the 9-methyladeninium units of 1 and 2 (X, $C_6H_7N_5^+Cl^-$) were identical with those of agelasine-A ∿ -D, indicating that 1 and 2 contained diterpene parts composed of $C_{20}H_{33}$. The ^{13}C NMR spectra of 1 and 2 contained signals for three double bonds in diterpene parts, suggesting the presence of terminal monocyclic parts with an exocyclic double bond for 1 (δ 108.6 (t)) and an endocyclic double bond for 2 (δ 123.6 (d)), respectively. The ^1H NMR spectra of 1 and 2 revealed common partial structures $CH_3\overset{|}{C}=CH-CH_2-$ and $CH_3\overset{|}{C}=CH-CH_2-X$, in which the configurations of the double bonds were assigned to be E on the basis of high field resonances of olefinic methyl carbons (δ 15.9 and 17.2 for 1 and 15.6 and 17.2 for 2).

The ^1H NMR spectrum of 1 showed two singlet methyl signals at δ 0.82 and 0.90 and terminal olefinic proton signals at δ 4.53 (brd, J=1.5 Hz) and 4.76 (brd, J=1.5 Hz) due to the terminal cyclic part. 1 was treated with ozone in methanol at -78°C followed by reduction with dimethylsulfide to give a diketone 3[8]. The CD spectrum of 3 showed a negative Cotton effect ($\Delta\varepsilon_{292}$ -1.57) which was reverse to that of a known diketone having a 5S configuration (lit.[9] $\Delta\varepsilon_{296}$ +1.57), indicating the absolute configuration of agelasine-E to be 5S.

The ^1H NMR spectrum of 2 contained a singlet methyl signal at δ 0.84, a doublet methyl signal at δ 0.85 (d, J=6.5 Hz) and a olefinic methyl signal at δ 1.59 due to the terminal cyclic part. 2 was treated with ozone at -78°C in methanol followed by reduction with sodium borohydride to yield an epoxide

4[10,11] which was reduced with super hydride to give a diol 5. The diol was acetylated and submitted to dehydration with thionyl chloride/pyridine to obtain a 3:2 mixture of an exocyclic olefin 7 and an endocyclic olefine 8 which was deacetylated and then oxidized with pyridinium dichromate to afford a mixture containing a compound 10. The mixture was treated with ozone, reduced with dimethylsulfide and cyclized in methanolic KOH under an argon atomosphere to yield a cis-octalone 11[12] which was identical in all respects with an authentic sample[13]. The CD spectrum of 11 showed a positive Cotton effect at 236 nm ($[\theta]$ 5800°) and a negative one at 335 nm ($[\theta]$ -390°) in hexane, indicating the absolute configuration of the ring junction methyl group as illustrated[14]. The cis-dimethylcyclohexene ring system was found in sesquiterpenes isolated from the liverwort[15] but its absolute configuration is opposite to that of 2. Recently Capon and Faulkner reported the structural elucidation of ageline A isolated from a Pacific sea sponge of the same genus and proposed an identical structure[16].

In the experiments reported here, it was revealed that the absolute configurations of agelasine-E and -F were opposite to those found in terpenes isolated from terrestrial sources[9,15]. From the biogenetic point of view, agelasine-F may be derived from agelasine-E by concerted migration of a hydrogen and a methyl group from 5 and 4 positions to 6 and 5 positions, respectively. In addition, agelasine-E might be biogenetically related to agelasine-A ∿ -D having bicyclic terpenoid structures.

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References and Notes

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2. Physiologically active marine natural products from Porifera V. Preceding paper; H. Nakamura, H. Wu, Y. Ohizumi and Y. Hirata, *Tetrahedron Lett.*, in press.
3. Permanent address: Shanghai Institute of Organic Chemistry, Academia Sinica, 345 Linglin Lu, Shanghai, China.
4. H. Nakamura, H. Wu, J. Kobayashi, Y. Ohizumi, Y. Hirata, T. Higashijima and T. Miyazawa, *Tetrahedron Lett.*, 4105 (1983).
5. Isolation yields of agelasine-A ∿ -F were 0.037%, 0.10%, 0.011%, 0.056%, 0.031% and 0.080% from the wet weight of the sea sponge, respectively.
6. 1: colorless powder, mp. 180-182°C; $[\alpha]_D^{25}$ -17.1° (c 1.88, MeOH); UV (MeOH) λ_{max} 272 nm (ε 9720); HR-MS m/z 421.3202 (M^+-HCl, calcd for $C_{26}H_{39}N_5$, 421.3202); FD-MS m/z 422 (M^+-Cl); ^{1}H NMR ($CDCl_3$, 400MHz) δ 0.82 (s, 3H), 0.90 (s, 3H), 1.56 (s, 3H), 1.86 (s, 3H), 4.53 (brd, 1H,

J=1.5Hz), 4.76 (brd, 1H, J=1.5Hz), 5.00 (brs, 1H), 5.46 (brt, 1H, J=6.5Hz), 5.72 (brd, 2H, J=6.5Hz) for the diterpene part, and 4.10 (s, 3H, N^+-CH_3), 6.87 (brs, 2H, NH_2), 8.50 (s, 1H, 2'-H) and 10.83 (brs, 1H, 8'-H) for X; ^{13}C NMR ($CDCl_3$, 22.5 MHz) δ 15.9 (q), 17.2 (q), 23.5 (t), 24.6 (t), 26.0 (t), 26.0 (q), 28.2 (q), 32.3 (t), 34.6 (s), 36.1 (t), 38.0 (t), 39.4 (t), 48.4 (t), 53.5 (d) 108.6 (t), 115.2 (d), 122.7 (d), 136.2 (s), 146.8 (s) and 149.0 (s) for the diterpene part and 31.7 (q, N^+-CH_3), 109.5 (s, C-5'), 141.2 (d, C-8'), 149.2 (s, C-4'), 152.2 (s, C-6') and 155.7 (d, C-2') for X.

7. 2: colorless powder, mp. 178-180°C; $[\alpha]_D^{25}$ -5.5° (c 2.45, MeOH); UV (MeOH) λ_{max} 272 nm (ε 7700); HR-MS m/z 421.3201 (M^+-HCl, calcd for $C_{26}H_{39}N_5$, 421.3202) and 149.0722 (calcd for $C_6H_7N_5$, 149.0701); FD-MS m/z 422 (M^+-Cl); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.84 (s, 3H), 0.85 (d, 3H, J=6.5 Hz), 1.57 (s, 3H), 1.59 (s, 3H), 1.86 (s, 3H), 5.02 (brs, 1H), 5.42 (brs, 1H) 5.47 (brt, 1H, J=6.5 Hz) and 5.72 (d, 2H, J=6.5Hz) for the diterpene part and 4.10 (s, 3H, N^+-CH_3), 6.87 (brs, 2H, NH_2), 8.50 (s, 1H, 2'-H) and 10.83 (brs, 1H, 8'-H) for X; ^{13}C NMR ($CDCl_3$, 22.5 MHz) δ 15.6 (q), 16.0 (q), 17.2 (q), 18.9 (q), 20.8 (q), 25.2 (t), 26.1 (t), 26.8 (t), 33.0 (d), 34.0 (t), 35.0 (t), 39.4 (t), 40.1 (s), 48.4 (t), 115.2 (d), 122.4 (d), 123.7 (d), 126.6 (s), 139.3 (s) and 146.8 (s) for the diterpene part and 31.7 (q, N^+-CH_3), 109.5 (s, C-5'), 141.2 (d, C-8'), 149.2 (s, C-4'), 152.2 (s, C-6') and 155.7 (d, C-2') for X.

8. 3: colorless oil; MS m/z 196 (M^+, 16%) and 181 (M^+-CH_3, 100%); IR ($CHCl_3$) ν_{max} 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.79 (s, 3H), 1.09 (s, 3H), 2.12 (s, 3H) and 1.50 $\sim$ 2.65 (m, 11H); ^{13}C NMR ($CDCl_3$) δ 18.3 (t), 21.8 (q), 23.1 (t), 29.5 (q), 29.9 (q), 39.5 (t), 39.7 (s), 41.4 (t), 42.6 (t), 60.1 (d), 208.6 (s) and 213.0 (s).

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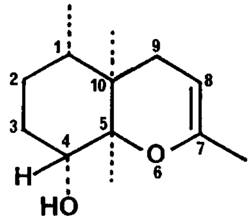
10. 4: colorless oil; MS m/z 212 (M^+, 1.6%) and 126 (100%); ^1H-NMR ($CDCl_3$) δ 0.77 (d, 3H, J=6.0 Hz), 0.88 (s, 3H), 1.20 (d, 3H, J=5.4 Hz), 1.24 (s, 3H), 0.80 2.25 (m, 9H), 2.98 (brs, 1H), 3.76 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 15.9 (q), 18.1 (q), 20.8 (q), 23.5 (q), 24.2 (t), 25.9 (t), 33.3 (d), 33.6 (t), 34.4 (t), 38.4 (s), 62.5 (d), 62.8 (s), 68.8 (d).

11. The ozonolysis product was treated with dimethylsulfide to give a cis-fused bicyclic compound A by trans-opening of an epoxide. The stereochemistry of A was determined on the basis of the down-field shift of a signal for the methyl group at 10 position (δ 0.93, s) by benzoylation ($\Delta\delta$ +0.12) and coupling constants of protons at 1 and 4 positions of the benzoyl derivative($J_{1-2\alpha}$ = 13Hz and $J_{3\alpha-4}$ = $J_{3\beta-4}$ = 3Hz).



A

12. 11: MS m/z 178 (M^+, 72%), 136 (100%); ^1H NMR ($CDCl_3$) δ 0.92 (m, 3H), 1.10 (s, 3H), 5.73 (brs).

13. Cis and trans-octalones were prepared by the method of P. A. Zoretic, J. A. Golen and M. D. Saltzman, *J. Org. Chem.*, 46, 3554 (1981). The ^1H NMR sepctrum of the trans-octalone contained signals at δ 0.93 (d, 3H, J=7 Hz), 1.31 (s, 3H) and 5.81 (d, 1H, J=1.5 Hz).

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