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UV-STABILITY AND UV-PROTECTIVE ACTIVITY OF ALKALOIDS FROM THE MARINE SPONGE *Zyzzya fuliginosa*

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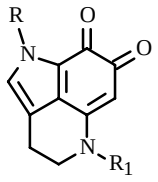
Alkaloids from the marine sponge Zyzzya fuliginosa *damirones A (**1**) and B (**2**); makaluvamines H (**3**), C (**4**), G (**5**), and L (**6**); and zyzzyanones A (**8**) and B (**9**) were investigated for the ability to protect egg-cell membranes of the sea urchin* Strongylocentrotus nudus *from UV-radiation. Damirones, zyzzyanones, and tricyclic makaluvamines C (**4**) and H (**3**) exhibited the greatest membrane-protective activity. It was shown that makaluvamines G (**5**) and L (**6**) were converted by UV-irradiation into damirones A (**1**), B (**2**), tricyclic makaluvamines H (**3**), C (**4**), and zyzzyanones A (**8**) and B (**9**), respectively.*

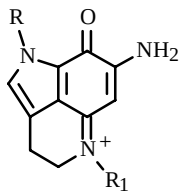
Key words: marine metabolites, alkaloids, makaluvamines, damirones, zyzzyanones, UV-protectors, photolysis, marine sponge.

During a search for biologically active metabolites from marine organisms, we isolated from the Australian marine sponge *Zyzzya fuliginosa* the known damirones A (**1**) and B (**2**); makaluvamines C (**4**), E (**7**), G (**5**), H (**3**), and L (**6**); and the new zyzzyanones A-D (**8**-**11**) [1, 2], which are pyrrolo[4,3,2-*de*]quinoline alkaloids. Zyzzyanones are the first representatives of a new class of marine alkaloids with the pyrrolo[3,2-*f*]indol-4,8(1*H*,7*H*)-dione skeleton.



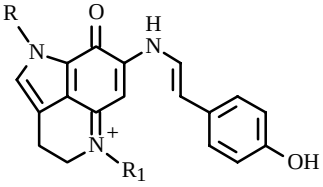
1, 2

1: R = R_1 = Me
2: R = H, R_1 = Me



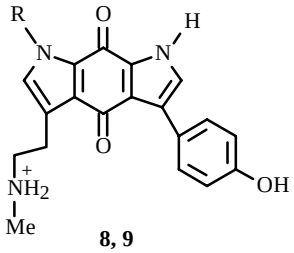
3, 4

3: R = R_1 = Me
4: R = H, R_1 = Me



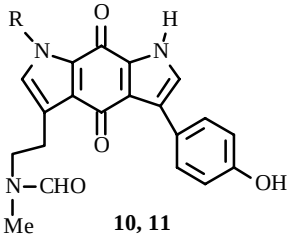
5 - 7

5: R = R_1 = Me
6: R = H, R_1 = Me
7: R = Me, R_1 = H



8, 9

8: R = Me
9: R = H



10, 11

10: R = Me
11: R = H

Damirones, makaluvamines, and zyzzyanones are brightly colored compounds with strong absorption in the UV range. Certain pigments are known to act in living organisms as UV filters that protect biologically important molecules from the harmful action of solar UV-radiation, for example, the aromatic pigment scytonemin in cyanobacteria [3].

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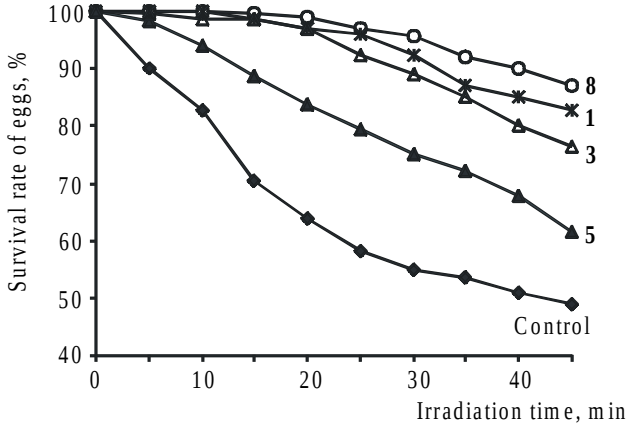

Fig. 1. Effect of UV-irradiation on survival rate of egg cells of the sea urchin *S. nudus* covered by filters of solutions of **1**, **3**, **5**, and **8** (0.05 M) and solvent (control).

The goal of the work was to investigate the UV-protector activity of certain aromatic alkaloids isolated from the marine sponge *Z. fuliginosa* and to study their stability during UV-irradiation.

We used egg cells of the sea urchin *Strongylocentrotus nudus* as a model for investigating the ability of alkaloids to protect cell membranes from the action of UV-radiation. A suspension of egg cells in marine water was irradiated by a UV lamp through 1-mm quartz cuvettes containing alcohol solutions of the tested compounds. Irradiation for 45 min was sufficient to reach 50% death of egg cells in the control (Fig. 1). The amount of dead egg cells was calculated after dyeing with trypan blue solution (0.1%) because this procedure is a direct indicator of cell-membrane destruction [4].

Figure 1 shows the survival rate of egg cells protected by filters of **1**, **3**, **5**, and **8** as functions of time of UV irradiation. The survival rate of egg cells protected by filters of **2**, **4**, **6**, and **9** (not shown in Fig. 1) were practically the same as that of egg cells protected by filters of **1**, **3**, **5**, and **8**, respectively. This is due to the same chromophore in each pair of compounds. The survival rate of egg cells protected by filters of solutions of zyzzyanones A (**8**) and B (**9**) and damirones A (**1**) and B (**2**) and tricyclic makaluvamines H (**3**) and C (**4**) increased approximately 1.5-1.7 times compared with the control. Despite the fact that tetracyclic makaluvamines G (**5**) and L (**6**) absorb strongly in the UV range, they were much less protective than tricyclic makaluvamines C (**4**) and H (**3**), damirones A (**1**) and B (**2**), and zyzzyanones A (**8**) and B (**9**) (Fig. 1). This may be due to the photosensitivity of tetracyclic makaluvamines G (**5**) and L (**6**). Recently a pyrrolo[4,3,2-*de*]quinoline alkaloid, discorhabdine B, was shown to be photosensitive and capable of forming dimeric discorhabdine W upon UV irradiation [5].

We determined the photostability of alcohol solutions of **1**-**9** by studying their absorption spectra before and after UV irradiation for 2 h. In fact, the absorption peak at 273-278 nm in the UV spectra of tetracyclic makaluvamines G (**5**), L (**6**), and E (**7**) disappeared whereas the peak at 343-357 nm increased in strength. The strength of the UV absorption decreased only insignficantly after irradiation for 2 h of damirones A (**1**) and B (**2**), makaluvamines C (**4**) and H (**3**), and zyzzyanones A (**8**) and B (**9**).

Because the tetracyclic makaluvamines **5**-**7** changed significantly, we investigated the products formed by UV irradiation of alcohol solutions of makaluvamines G (**5**) and L (**6**), the amounts of which were adequate to perform the experiment. The compounds were irradiated until the green color typical of solutions of makaluvamines G and L completely disappeared. Parts of the initial solutions were not irradiated and served as controls. Compounds **1** (15%), **3** (5.2%), **8** (3.5%), and **10** (10%) were isolated from the reaction mixture after irradiation of **5**. The reaction products were identified by comparison of spectra data with authentic samples of **1**, **3**, **8**, and the ethyl ester of *p*-hydroxybenzoic acid (**10**).

Irradiation of makaluvamine L (**6**) formed **2** (12.5%), **4** (4.5%), **9** (2.7%), and **10** (10.7%), which were identified by comparison of spectral data with authentic samples of **2**, **4**, **9**, and **10**.

Thus, we found that **5** and **6** are photosensitive compounds. One of the photodegradation pathways is homolytic cleavage of the *N*-(*p*-hydroxystyryl) bond, as a result of which tricyclic makaluvamines H (**3**) and C (**4**), respectively, are formed. Makaluvamines H (**3**) and C (**4**), in turn, are readily converted into the corresponding damirones A (**1**) and B (**2**), the principal photolysis products, probably through formation of *o*-iminoquinones [6]. The ethyl ester of *p*-hydroxybenzoic acid is formed

via cleavage of the phenol of makaluvamines G (**5**) and L (**6**) with its subsequent oxidation and esterification by ethyl alcohol, which was used as the solvent. Another photolysis pathway is radical intramolecular cyclization with accompanying hydrolysis of the imine bond in the *p*-iminoquinoid fragment of the molecule, which forms zyzzyanones A (**8**) and B (**9**), respectively. This pathway is probably not the main one judging from the low yield of **8** and **9**. Damirones A and B, makaluvamines C and H, and zyzzyanones A and B did not form in the control alcohol solutions of **5** and **6** that were not irradiated, even after prolonged storage for three months.

Thus, it was confirmed that the low activity of **5** and **6** for protecting sea urchin egg-cell membranes from the action of UV radiation is due to the photosensitivity of these compounds. Despite the fact that damirones, tricyclic makaluvamines, and zyzzyanones act as UV protectors, their concentrations among the formed photoproducts are too low for protecting egg cells from UV radiation.

EXPERIMENTAL

Sephadex LH-20 (Pharmacia Fine Chemicals) was used for column chromatography. Absorption was measured on a UV-mini 1240 spectrophotometer (Shimadzu). UV radiation was from an OKH-11-M mercury—quartz irradiator. NMR spectra were recorded on a Bruker AVANCE DRX-500 spectrometer at working frequency 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shifts for ^{1}H are given relative to TMS internal standard ($\delta_{TMS} = 0$); for ^{13}C, relative to the residual signal of CD_3OD (δ = 49.0 ppm). Low-resolution mass spectra were measured in an LKB-9000 S instrument with direct sample introduction into the ion source at ionizing potential 70 eV; high-resolution mass spectra, in an AMD-604 S instrument (Intectra, Germany) with Cs^+ atom bombardment at 8 kV.

Determination of the Photosensitivity of the Compounds. The photosensitivity of the compounds was determined by studying absorption of compounds before and after irradiation. Quartz cuvettes with EtOH solutions of the compounds were irradiated in UV light for 2 h. The lamp was 75 cm from the cuvette.

Photolysis of Makaluvamine G (5). A solution of **5** (23 mg) in EtOH (200 mL) was placed in a quartz flask and irradiated for 8 h until the green color disappeared completely. The reaction mixture was concentrated in vacuo and separated over a column of Sephadex LH-20 using $CHCl_3$:EtOH:TFA (4:1:0.05) to produce damirone A (**1**, 3.45 mg, 15%), makaluvamine H (**3**, 1.2 mg, 5.2%), zyzzyanone A (**8**, 0.8 mg, 3.5%), and **10** (2.3 mg, 10%). The spectral properties of the isolated compounds agreed with those published [1, 6-8].

Damirone A (1). UV spectrum (MeOH, λ_{max}, nm, log ε): 240 (4.30), 347 (3.86), 516 (3.80). PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 2.85 (2H, t, J = 7.1, CH_2), 3.06 (3H, s, CH_3), 3.57 (2H, t, J = 7.1, CH_2), 3.93 (3H, s, CH_3), 5.29 (1H, s, H), 6.62 (1H, s, H). Mass spectrum (*m*/*z*): 216 $[M]^+$ [6, 8].

Makaluvamine H (3). UV spectrum (MeOH, λ_{max}, nm, log ε): 240 (4.30), 345 (4.10), 522 (3.00). PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 2.86 (2H, t, J = 7.5, CH_2), 3.27 (3H, s, CH_3), 3.81 (2H, t, J = 7.5, CH_2), 3.83 (3H, s, CH_3), 5.65 (1H, s), 7.26 (1H, s), 8.60, 9.40 (2H, NH_2). Mass spectrum (*m*/*z*): 216 $[M + H]^+$ [7].

Zyzzyanone A (8). UV spectrum (MeOH, λ_{max}, nm, log ε): 242 (4.26), 286 (3.90), 342 (3.59), 484 (3.44). PMR spectrum (CD_3OD, δ, ppm, J/Hz): 2.67 (3H, s, CH_3), 3.01 (2H, t, J = 7.1, CH_2), 3.19 (2H, t, J = 7.1, CH_2), 3.89 (3H, s, CH_3), 6.77 (2H, d, J = 8.7, H_{ar}), 6.80 (1H, s), 7.01 (1H, s), 7.54 (2H, d, J = 8.7, H_{ar}). FABMS (*m*/*z*): 350.1483 $[M + H]^+$, calc. for $C_{20}H_{20}N_3O_3$, 350.1504 [1].

Ethyl Ester of *p*-Hydroxybenzoic Acid (10). UV spectrum (MeOH, λ_{max}, nm, log ε): 208 (3.46), 257 (3.56). PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 1.35 (3H, t, J = 7.1, CH_3), 4.30 (2H, q, J = 7.1, CH_2O), 6.81 (2H, d, J = 8.8, H_{ar}), 7.87 (2H, d, J = 8.8, H_{ar}). ^{13}C NMR spectrum (CD_3OD): 15.3 (q), 62.3 (t), 116.7 (d), 123.1 (s), 133.3 (d), 164 (s), 168.9 (s). Mass spectrum (*m*/*z*): 166 $[M]^+$.

Photolysis of Makaluvamine L (6). A solution of **6** (22.4 mg) in EtOH (200 mL) was placed in a quartz flask and irradiated for 6 h until the green color disappeared completely. The reaction mixture was concentrated in vacuo and separated over a column of Sephadex LH-20 using $CHCl_3$:EtOH:TFA (4:1:0.05) to produce damirone B (**2**, 2.8 mg, 12.5%), makaluvamine C (**4**, 1.0 mg, 4.5%), zyzzyanone B (**9**, 0.6 mg, 2.7%), and **10** (2.4 mg, 10.7%). The spectral properties of the isolated compounds agreed with those published [2, 6, 9].

Damirone B (2). UV spectrum (MeOH, λ_{max}, nm, log ε): 242 (4.47), 346 (4.30), 492 (3.80). PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 2.79 (2H, t, J = 7.0, CH_2), 3.03 (3H, s, CH_3), 3.59 (2H, t, J = 7.0, CH_2), 5.12 (1H, s), 7.06 (1H, d, J = 2.5), 12.40 (1H, br.s, NH). Mass spectrum (*m*/*z*): 202 $[M]^+$ [2, 6].

Makaluvamine C (4). UV spectrum (MeOH, λ_{max}, nm, log ε): 241 (4.40), 357 (4.20), 520 (3.60). PMR spectrum (DMSO-d_6, δ, ppm, J/Hz): 2.92 (2H, t, J = 7.5, CH_2), 3.31 (3H, s, CH_3), 3.90 (2H, t, J = 7.5, CH_2), 5.73 (1H, s), 7.28 (1H, d, J = 2.5), 8.65, 9.53 (2H, NH_2), 13.10 (1H, br.s, NH). Mass spectrum (*m*/*z*): 202 $[M + H]^+$ [9].

Zyzzyanone B (9). UV spectrum (MeOH, λ_{max}, nm, log ε): 240 (3.99), 281 (3.64), 342 (3.33), 328 (3.08). PMR spectrum (CD_3OD, δ, ppm, J/Hz): 2.69 (3H, s, CH_3), 3.09 (2H, t, J = 6.4, CH_2), 3.26 (2H, t, J = 6.4, CH_2), 6.77 (2H, d, J = 8.6, H_{ar}), 6.95 (1H, s), 7.04 (1H, s), 7.54 (2H, d, J = 8.6, H_{ar}). FABMS (*m*/*z*): 336.1339 $[M + H]^+$, calc. for $C_{19}H_{18}N_3O_3$, 336.1343 [2].

Determination of UV-Protective Activity. Sea urchin *S.nudus* egg cells were obtained by injection of KCl solution (3 mL, 0.5 M) into the perivisceral cavity. The obtained egg cells were purified of mechanical impurities by filtration, washed, and suspended in marine water to a concentration of 2000 egg/mL.

The suspension of egg cells (0.1 mL aliquots) was placed on a planchet, covered with a quartz cuvette (1-mm) with the tested compound in EtOH (0.05 M), and irradiated by a UV lamp for 45 min. The lamp was 75 cm from the planchet. After each 5 min, a trypan blue solution (0.1%, 0.02 mL) was added to the suspension (0.1 mL) and held for 2 min. The number of colored egg cells was counted using a microscope and Goryaev chamber. The survival rate of the egg cells was estimated by comparison with the control that was irradiated through a cuvette containing EtOH. The percent survival rate was calculated using the formula survival rate (%) = 100 - ($n_{colored} \times 100/n_{total}$), where $n_{colored}$ is the number of colored cells in the sample and n_{total} is the total number of cells. The average of three determinations was used to contruct the curves.

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