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NEW AZACYCLOPROPENE DERIVATIVES FROM *DYSIDEA FRAGILIS* COLLECTED IN POHNPEI

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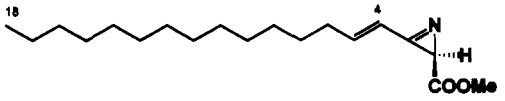
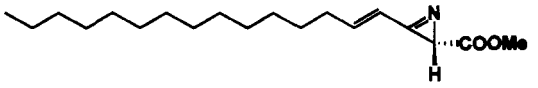
ABSTRACT.—The sponge *Dysidea fragilis* from Pohnpei contained four azacyclopropene derivatives, (4*E*)-*S*-dysidazirine [**2**], which is the optical enantiomer of the known compound dysidazirine [**1**], (4*Z*)-dysidazirine [**3**], (4*E*)-*S*-antazirine [**4**], and (4*Z*)-antazirine [**5**]. The structures of the new compounds were elucidated by interpretation of spectral data.

Sponges of the genus *Dysidea* are renowned for the diversity of their natural products (1). Some of the metabolites found in *Dysidea* spp., such as polybrominated diphenyl ether and polychlorinated amino acid derivatives, are localized in symbiotic cyanobacteria and are almost certainly produced by the microorganisms (2,3). Sesquiterpenes, diterpenes, and polyhydroxylated C-*seco*-sterols are considered to be the most representative metabolites of *Dysidea* species. The isolation of *R*-dysidazirine [**1**] from *Dysidea fragilis* (Dysideidae) from Fiji (4) was therefore unexpected. We now report the isolation of both the (*Z*) and (*E*) geometrical isomers of *S*-dysidazirine [**2** and **3**] and the (*Z*) and (*E*) isomers of a brominated analogue, *S*-antazirine [**4** and **5**].

A specimen of *D. fragilis* was collected by hand (−20 meters) at Ant Atoll, Pohnpei, and was kept frozen until



1



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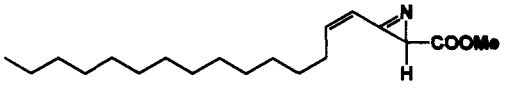

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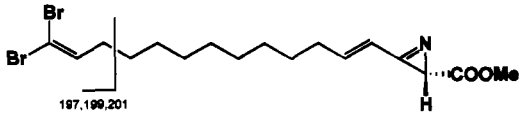

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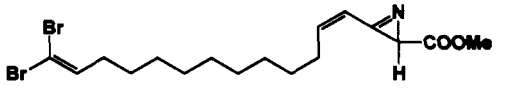

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extraction. The CH_2Cl_2-soluble fraction from a CH_2Cl_2/MeOH (1:1) extract of the lyophilized sponge was partitioned between hexane and aqueous MeOH to obtain a hexane fraction that had an interesting ^{1}H-nmr spectrum. Chromatography of this fraction on SiO_2 gave fractions containing sterol peroxides, as judged by ^{1}H-nmr spectroscopy, and a fraction that contained methyl esters: the latter fraction was purified by hplc to obtain (4*E*)-*S*-dysidazirine [**2**] (27 mg, 0.13% dry wt), (4*Z*)-dysidazirine [**3**] (1.7 mg, 0.008% dry wt), (4*E*)-*S*-antazirine [**4**] (14 mg, 0.067% dry wt), and (4*Z*)-antazirine [**5**] (<1 mg).

(4*E*)-*S*-Dysidazirine [**2**], $[\alpha]_D$ +47.2°, was obtained as a colorless oil of molecular formula $C_{19}H_{34}NO_2$. Dereplication using a marine natural product database resulted in the identification of the compound as dysidazirine, and a comparison of spectral data, particularly the ^{13}C-nmr spectrum, which contained three olefinic carbon signals, confirmed this identification. However, the optical rotation of our sample was of opposite sign to that of the literature value, $[\alpha]_D$ −165°. We subsequently obtained an authentic sample of dysidazirine [**1**] from Professor Chris Ireland, University of Utah, and compared the cd spectra of the two samples. In order to eliminate the possibility that our sample was contaminated with an inert material that did not produce any distinct nmr signals, we calibrated the relative concentrations of the two samples of dysidazarine by comparison of the uv absorbance at 222 nm. The cd spectra indicated that our sample was enriched in the 2*S*-enantiomer, with an optical purity of about 30% assuming that the Ireland sample was a single enantiomer. This is in good agreement with the optical purity derived from optical rotation measurements.

(4*Z*)-Dysidazirine [**3**] is a minor isomer of **2** that was obtained as a colorless oil. The ^{1}H-nmr spectrum of **3** differed only slightly from **2** and the coupling constants of the olefinic proton signals at δ 6.41 (1H, d, J=11 Hz) and 6.56 (1H, dt, J=11 and 7 Hz) indicated that **3** must be the (4*Z*)-isomer of dysidazirine. The mass spectrum supports this assignment but insufficient material was available to obtain a ^{13}C-nmr spectrum.

(4*E*)-*S*-Antazirine [**4**], $[\alpha]_D$ +10.3°, was obtained as a colorless oil. The molecular formula, $C_{17}H_{25}Br_2NO_2$ was determined by hrms. The lrms contained a prominent cluster of peaks at *m/z* 197, 199, 201 $[C_3H_3Br_2]^+$, which suggested that both bromine atoms were located in a terminal fragment. Comparison of the ^{1}H-nmr spectrum of **4** with that of **2** revealed that there was no terminal methyl group in **4** and that there was an additional olefinic signal at δ 6.36 (1H, t, J=7 Hz) coupled to a vinylic methylene signal at δ 2.06 (2H, q, J=7 Hz). The ^{13}C-nmr spectrum of **4** contained two signals at δ 138.8 (C-15) and 89.0 (C-16) that are typical of a terminal dibromoethylene group (5). The remaining signals in the ^{1}H- and ^{13}C-nmr spectra were all assigned by comparison with the spectra of **2**. The (4*E*)-geometry of the disubstituted olefin was assigned on the basis of the coupling constant ($J_{4,5}$=16 Hz). The sign of the optical rotation suggests that **4** also has the 2*S* absolute configuration.

(4*Z*)-Antazirine [**5**] is a very minor isomer of **4** that was obtained as a colorless oil. Again, the major differences in the ^{1}H-nmr spectra of **4** and **5** were to be found in the chemical shifts and coupling constants of the olefinic protons at δ 6.41 (1H, d, J=11 Hz) and 6.56 (1H, dt, J=11 and 7 Hz), which indicated the presence of a 4*Z*-olefinic bond. All other data were compatible with the proposed structure.

The biosynthesis of the azirine ring in **1** has been a matter of some speculation (4). The added feature of bromine atoms in the antazirines opens the way for a possible biosynthetic scheme involving formation of the azirine ring by a mecha-

nism involving bromination/dehydrobromination. Neither (4*E*)-*S*-dysidazirine [**2**] nor (4*E*)-*S*-antazirine [**4**] showed antibacterial activity against a standard panel of microorganisms.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All reagents used were either hplc grade or distilled from reagent grade. ^{1}H- and ^{13}C-nmr spectra were obtained using a Bruker WP200 spectrometer at 200 MHz and 50.3 MHz, respectively, and referenced to $CDCl_3$ (δ 7.24 and 77.0). HMBC and HMQC experiments were performed using a Varian Unity 500 spectrometer. Ir spectra were obtained from a Perkin-Elmer 1600 spectrometer and uv spectra were recorded on a Perkin-Elmer Lambda 3B uv/vis spectrophotometer. Optical rotations were measured on an Autopol III automatic polarimeter using a 10-cm cell. Lreims were recorded on a 5988A Hewlett-Packard mass spectrometer. Cd spectra were measured at the Torrey Pines Institute for Molecular Studies. Hrms were obtained from the Regional Mass Spectrometry Facility at the University of California, Riverside.

ANIMAL MATERIAL.—The sponge *Dysidea fragilis* (21 g dry wt; collection #POH 93-007), a specimen of which was deposited in the SIO Benthic Invertebrate Collection (P-1154), was collected by hand using scuba (−20 m) from Tauenai Passage, Ant Atoll, Pohnpei, Federated States of Micronesia, and was stored frozen.

EXTRACTION AND ISOLATION.—The frozen sponge was lyophilized and extracted with CH_2Cl_2-MeOH (1:1) at room temperature to afford 2.35 g of crude extract. The crude extract was partitioned between CH_2Cl_2 and MeOH/H_2O (15%), and the organic layer (as for subsequent organic fractions) was dried over Na_2SO_4 and evaporated under reduced pressure. The CH_2Cl_2 extract was partitioned between hexane and MeOH-H_2O (9:1), and the hexane fraction was dried and concentrated *in vacuo*. Purification by flash chromatography on Si gel using a gradient of 5–15% EtOAc in hexane resulted in the isolation of fractions containing sterol peroxides and mixtures of compounds **2–5**. The latter were purified by hplc on Partisil using 5% EtOAc in hexane as eluent to afford (4*E*)-*S*-dysidazirine (**2**, 27 mg, 0.13% dry wt), (4*Z*)-dysidazirine (**3**, 1.7 mg, 0.008% dry wt), (4*E*)-*S*-antazirine (**4**, 14 mg, 0.067% dry wt), and (4*Z*)-antazirine (**5**, <1 mg).

(4E)-S-Dysidazirine [**2**].—Colorless oil; [α]D +47.2° (*c*=1.08, $CHCl_3$); uv (MeOH) λ max 221 nm; cd (MeOH) 232 nm (Δε +0.68); ir ν max ($CHCl_3$) 2925, 2855, 1770, 1735 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 0.86 (3H, t, *J*=7 Hz), 1.23 (20H, br s), 1.49 (2H, br m), 2.34 (2H, br q, *J*=7 Hz), 2.55 (1H, s), 3.70 (3H, s), 6.50 (1H, d, *J*=16 Hz), 6.68 (1H, dt, *J*=16 and 7 Hz); ^{13}C nmr δ 14.1 (q), 22.6 (t), 27.8 (t), 28.2 (d), 29.1 (t), 29.3 (2t), 29.4 (t), 29.6 (4t), 31.9 (t), 33.2 (t), 52.2 (q), 112.9 (d), 155.7 (d), 156.6 (s), 172.2 (s); cims *m/z* 308 (M^+, 100), 306 (22), 292 (4), 276 (4), 264 (2), 248 (1), 236 (3); hrcims, observed *m/z* 308.2598, $C_{19}H_{34}NO_2$ requires 308.2590.

(4Z)-Dysidazirine [**3**].—Colorless oil; uv (MeOH) λ max 222 nm; ir ν max ($CHCl_3$) 2925, 2855, 1765, 1735, 1630 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 0.85 (3H, t, *J*=7 Hz), 1.22 (20H, br s), 1.45 (2H, br m), 2.47 (2H, br q, *J*=7 Hz), 2.61 (1H, s), 3.71 (3H, s), 6.40 (1H, d, *J*=11 Hz), 6.56 (1H, dt, *J*=11 and 7 Hz); cims *m/z* 308 (M^+, 100), 306 (21), 292 (4), 276 (3), 248 (1), 180 (1); hrcims, observed *m/z* 308.2603, $C_{19}H_{34}NO_2$ requires 308.2590.

(4E)-S-Antazarine [**4**].—Colorless oil; [α]D +10.3° (*c*=0.39, $CHCl_3$); uv (MeOH) λ max 213, 220 (sh) nm; ir ν max ($CHCl_3$) 2925, 1770, 1730 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 1.23 (10H, br s), 1.38 (2H, br m), 1.49 (2H, br m), 2.06 (2H, q, *J*=7 Hz), 2.34 (2H, br q, *J*=7 Hz), 2.55 (1H, s), 3.70 (3H, s), 6.36 (1H, t, *J*=7 Hz), 6.53 (1H, d, *J*=16 Hz), 6.68 (1H, dt, *J*=16 and 7 Hz); ^{13}C nmr δ 27.8 (2t), 28.3 (d), 28.9 (t), 29.1 (t), 29.2 (3t), 32.9 (t), 33.2 (t), 52.2 (q), 89.0 (s), 113.0 (d), 138.9 (d), 155.6 (d), 156.6 (s), 172.2 (s); cims *m/z* 432/434/436 (MH^+, 100); eims *m/z* 402/404/406 (6), 356/354 (23), 201/199/197 (80), 152 (100); hrcims, observed *m/z* 434.0326, $C_{17}H_{26}{}^{79}Br^{81}BrNO_2$ $(M+H)^+$ requires 434.0330.

(Z)-Antazarine [**5**].—Colorless oil; uv (MeOH) λ max 215, 220 (sh) nm; ir ν max ($CHCl_3$) 2925, 2855, 1765, 1735 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 1.23 (10H, br s), 1.40 (4H, br m), 2.06 (2H, q, *J*=7 Hz), 2.50 (2H, br q, *J*=7 Hz), 2.62 (1H, s), 3.72 (3H, s), 6.36 (1H, t, *J*=7 Hz), 6.41 (1H, d, *J*=11 Hz), 6.68 (1H, dt, *J*=11 and 7 Hz); cims *m/z* 432/434/436 (MH^+, 100); eims *m/z* 356/354 (30), 304 (44), 201/199/197 (50), 152 (100); hrcims, observed *m/z* 434.0343, $C_{17}H_{26}{}^{79}Br^{81}BrNO_2$ $(M+H)^+$ requires 434.0330.

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