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ANTIMICROBIAL METABOLITES FROM THE MARINE SPONGE ULOSA SP.

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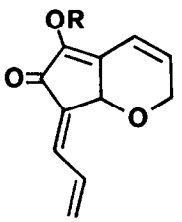
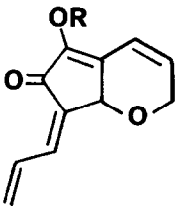
Shipboard antimicrobial screening of Caribbean sponges revealed that fresh methanol extracts of Ulosa sp.[1] inhibited the growth of S. aureus, B. subtilis, C. Albicans, and several strains of marine bacteria. Chromatography of the concentrated extract on either silica gel or florisil resulted in loss of the antimicrobial constituent. A solid having the antimicrobial activity of the crude extract was obtained by rapid extraction of the lyophilized sponge with 10% methanol in acetone, evaporation of the solvent, and trituration of the residue with hexane. The solid (2% dry weight) consisted of a 3:1 mixture of two closely related metabolites 1 and 2, which could not be separated by chromatography. When a methanolic solution of the metabolites was evaporated and redissolved, some insoluble polymer was formed.

The major metabolite 1 was obtained from the mixture in low yield after repeated precipitation from methanol solution using chloroform. High resolution mass measurement indicated the molecular formula $C_{11}H_{10}O_3$. The infrared spectrum contained bands at 3300 (-OH) and 1675 cm^{-1}. In the ultraviolet spectrum [λ_{max} (MeOH) 248 nm (22,000) and 340 nm (12,000)], addition of base caused a strong bathochromic shift [λ_{max} (KOH-MeOH) 263 nm (30,000) and 423 nm (10,000)] which, together with a positive (green) ferric chloride test, suggested the presence of an enol or phenol. The ^1H NMR spectrum contained signals at δ 7.05 (1H, d,



1 R = H
3 R = Ac
5 R = Me



2 R = H
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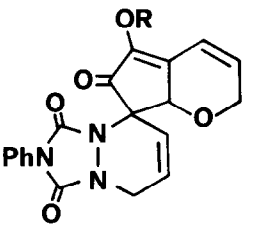

7 R = Ac
8 R = Me

J = 12 Hz), 6.92 (1H, m, J = 17, 12, 9 Hz), 5.75 (1H, bd, J = 17 Hz), and 5.68 (1H, bd, J = 9 Hz) assigned to the exocyclic diene moiety, and at 6.83 (1H, m, J = 10, 2, 2 Hz), 6.18 (1H, m, J = 10, 3, 4 Hz), 5.02 (1H, s), 4.64 (1H, m, J = 19, 3, 2 Hz), and 4.51 (1H, m, J = 19, 4, 2 Hz) due to the protons on the dihydropyran ring.

The mixture of metabolites 1 and 2 was acetylated using acetic anhydride and pyridine to obtain a mixture of acetates 3 and 4 which could be separated in low yield on preparative silica gel TLC plates. The infrared spectrum of the major acetate contained an enol acetate band at 1780 cm^{-1} and a carbonyl absorption at 1700 cm^{-1} but no hydroxyl band, indicating that the original metabolite 1 contained an ether linkage. The presence of signals at δ 66.5 (t) and 70.9 (d) in the ^{13}C NMR spectrum of the acetate 3 confirmed this assignment. The ^{1}H NMR spectrum[2] of the acetate 3 was almost identical to that of the metabolite 1 but with the addition of an acetate methyl signal at 2.32 ppm. The ^{1}H NMR spectrum[3] of the minor acetate 4 differed from that of the major acetate 3 in the chemical shifts of the protons on the diene side-chain only. Consideration of the accumulated spectral data led to the suggestion that the diosphenols 1 and 2 were the most probable structures for the isomeric antibiotics.

Treatment of the diosphenol mixture with dimethyl sulfate in acetone containing potassium carbonate gave a mixture of two methyl ethers 5 and 6. Attempted reactions of the diosphenol mixture with lithium aluminum hydride, hydrogen iodide, hydrogen over 10% palladium on charcoal, sodium periodate, hydrogen peroxide, hydroxylamine or o-phenylenediamine all resulted in decomposition. However, treatment of the acetates 3 and 4, either separately or as a mixture, with 4-phenyl-1,2,4-triazoline-3,5-dione in chloroform at 25°C gave a single crystalline adduct 7 in good yield.

The stable adduct 7, mp 109-111°C (dec.) had a molecular formula $C_{21}H_{17}N_3O_5$. The ultraviolet absorption at λ_{max} 276 was at the calculated value for an α-acetoxy dienone chromaphore, confirming that the diene side-chain in metabolites 1 and 2 was cross-conjugated to the ketone. In addition to the familiar signals for the protons on the dihydropyran ring, the ^1H NMR spectrum[4] of the adduct 7 contained a remarkably similar group of signals at δ 6.26 (1H, m, J = 10, 3, 3 Hz), 5.80 (1H, m, J = 10, 3, 2 Hz), 4.40 (1H, m, J = 17, 3, 2 Hz), and 4.21 (1H, m, J = 17, 3, 3 Hz) due to the protons on the diazine ring. The similarity of the two groups of resonances served as further evidence for a dihydropyran ring. An analogous adduct 8 was prepared from the mixture of methyl ethers 5 and 6.

The formation of a single adduct 7 from either acetate 3 or 4 confirmed that the metabolites and their derivatives were mixtures of geometrical isomers about the exocyclic olefinic bond. The ^1H NMR chemical shift of the signal for the proton on this olefinic bond was at δ 7.08 in the major acetate 3 and at 6.65 ppm in the minor acetate 4. Consideration of the shielding effects of the carbonyl group on β-protons[5] indicated that the major isomer had the E geometry.

The two metabolites 1 and 2 account for the antimicrobial activity of the crude extract. Both acetates 3 and 4, the methyl ethers 5 and 6, and even the adducts 7 and 8 have mild antimicrobial activity.

Acknowledgements

The sponge was identified by Dr. Klaus Ruetzler, and antimicrobial assays were performed by Mrs. M. Tseng. The sponge was collected during a cruise on R/V Alpha Helix. The research was supported by grants from the National Science Foundation (PCM72-02539) and the National Institutes of Health (AI-11969) (RR-00708 to UCSD NMR Facility).

References and Notes

1. Collected at Glover Reef, Belize, by hand (-2 m), July 1977, and immediately frozen. This sponge has not been described but is being studied by Dr. K. Ruetzler (pers. comm.).

2. UV (MeOH) 227 nm (15,000), 301 nm (17,000); IR ($CHCl_3$) 1780, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.32 (3H, s), 4.54 (1H, m, J = 19, 4, 2 Hz), 4.69 (1H, m, J = 19, 3, 2 Hz), 5.11 (1H, s), 5.69 (1H, bd, J = 9 Hz), 5.74 (1H, bd, J = 17 Hz), 6.36 (1H, m, J = 10, 4, 3 Hz), 6.62 (1H, m, J = 10, 2, 2 Hz), 6.88 (1H, m, J = 17, 12, 9 Hz), 7.08 (1H, d, J = 12 Hz); ^{13}C NMR ($CDCl_3$) δ 186.7 (s), 167.1 (s), 142.7 (s), 137.6 (d), 134.1 (d), 132.0 (d), 127.8 (d), 117.7 (t), 70.9 (d), 66.5 (t), 20.4 (q); mass spectrum (50 ev) m/e (%) 232 (3), 190 (12), 161 (5), 83 (100).

3. UV (MeOH) 229 nm (15,000), 314 nm (17,000); IR ($CHCl_3$) 1775, 1700, 1660 cm^{-1}; ^1H NMR ($CHCl_3$) δ 2.32 (3H, s), 4.58 (2H, m), 4.91 (1H, s), 5.61 (1H, bd, J = 10 Hz), 5.64 (1H, bd, J = 17 Hz), 6.37 (1H, m, J = 10, 4, 3 Hz), 6.63 (1H, m, J = 10, 2, 2 Hz), 6.65 (1H, bd, J = 11 Hz), 7.81 (1H, m, J = 17, 11, 10 Hz); mass spectrum (50 ev) m/e (%) 232 (30), 190 (40), 162 (15), 53 (100).

4. UV(MeOH) 276 nm (13,500); IR (KBr) 1775, 1720, 1710, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.29 (3H, s), 4.21 (1H, m, J = 17, 3, 3 Hz), 4.40 (1H, m, J = 17, 3, 2 Hz), 4.54 (2H, bt, J = 3 Hz), 5.38 (1H, s), 5.80 (1H, m, J = 10, 3, 2 Hz), 6.26 (1H, m, J = 10, 3, 3 Hz), 6.47 (1H, m, J = 10, 3, 3 Hz), 6.68 (1H, m, J = 10, 2, 2 Hz), 7.45 (5H, m); mass spectrum (50 ev) m/e (%) 407 (20), 365 (30), 267 (10), 189 (35), 109 (100).

5. L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon, Oxford, 1969, p. 190.