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ANTIBIOTICS FROM THE MARINE PULMONATE *SIPHONARIA DIEMENENSIS*

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Abstract: The marine pulmonate *Siphonaria diemenensis* collected intertidally in southeastern Australia, contained two polypropionate antibiotics diemenensin-A (1) and diemenensin-B (2). The structures were elucidated through interpretation of spectral data and by degradation.

The "false limpet" *Siphonaria diemenensis* Quoy and Gaimard, 1833[1] is an air-breathing gastropod mollusc of the subclass Pulmonata. Specimens of *S. diemenensis*[2] were collected in the high intertidal zone at Phillip Island and Mallacoota, Australia. Among five species of *Siphonaria* collected in southeastern Australia, only *S. diemenensis* gave a crude acetone extract having antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis*. The antimicrobial activity of the extracts was due to diemenensin-A (1) and diemenensin-B (2).

The ethyl acetate-soluble material from the acetone extract was chromatographed by HPLC on Partisil using ether as eluant to obtain the antimicrobial metabolites diemenensin-A (1, 0.09 mg/animal) and diemenensin-B (2, 0.03 mg/animal). Diemenensin-A (1), $[\alpha]_D$ +77.3° (c 4.7, MeOH), had the molecular formula $C_{21}H_{32}O_3$. The presence of seven methyl carbon signals in the ^{13}C NMR spectrum[3] suggested that the structure was based on a polypropionate skeleton. The ^{13}C NMR spectrum contained nine signals between δ 99 and 168; five signals at δ 99.8 (s), 108.4 (s), 159.3 (s), 167.2 (s) and 167.5 (s) were assigned to a 6-substituted 3,5-dimethyl-4-hydroxy-2H-pyran-2-one ring system[4] and four signals at 126.6 (s), 130.4 (s), 139.1 (d) and 140.0 (d) were assigned to four olefinic carbons. The two methyl groups on the pyrone ring gave rise to signals at δ 9.6 (q) and 12.1 (q) in the ^{13}C NMR spectrum and δ 2.02 (s, 3 H) and 2.06 (s, 3 H) in the ^1H NMR spectrum. The ^1H NMR spectrum contained two additional vinyl methyl signals at δ 2.03 (d, 3 H, J = 1 Hz) and 1.81 (d, 3 H, J = 1 Hz) that were coupled to olefinic proton signals at 6.02 (br s, 1 H) and 5.18 (br d, 1 H, J = 10 Hz) respectively, assigned to a conjugated diene. The infrared bands at 3270, 1665 and

1555 cm^{-1} can be assigned to the 4-hydroxy-2H-pyran-2-one moiety[5] while the ultraviolet absorptions at 307 nm (ε 13500) and 247 nm (ε 8920) suggested that the pyrone ring was conjugated to the diene system.

The remaining C_8H_{17} fragment contained three methyl residues that gave rise to ^1H NMR signals at 0.85 (d, 3 H, J = 6.4 Hz), 0.87 (t, 3 H, J = 7 Hz) and 0.97 (d, 3 H, J = 6.6 Hz). The signal at δ 0.97 was coupled to a multiplet at 2.56 (1 H) that was in turn coupled to the olefinic proton signal at 5.20. Comparison of the aliphatic ^{13}C NMR signals at δ 14.6 (q), 19.9 (q), 20.4 (t), 21.7 (q), 30.8 (2d), 40.4 (t), 45.4 (t) with the calculated chemical shifts[6] for the three possible C_8H_{17} fragments clearly supported the expected 'polypropionate' structure.

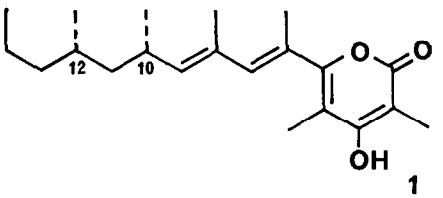
The *E* geometry of the two trisubstituted olefinic bonds was determined from the ^{13}C NMR chemical shifts of the remaining methyl signals at δ 16.2 (q) and 16.7 (q).[8] To determine the absolute configuration at C-10 and C-12, diemenensin-A (1) was treated with ozone in acetone at -78°C, followed by oxidation of the products with Jones' reagent to obtain a low yield of (2*S*,4*S*)-2,4-dimethylheptanoic acid [α] +28.3 (c 0.14, $CHCl_3$), that was treated with ethereal diazomethane solution to form methyl (2*S*,4*S*)-2,4-dimethylheptanoate, $[\alpha]_D$ +24.1 (c 0.14, $CHCl_3$).[9]

Treatment of diemenensin-A (1) with ethereal diazomethane solution gave a mixture of two methyl ethers in a 3:2 ratio. The major methyl ether 3 was assigned the 2-methoxy-3,5-dimethyl-4H-pyran-4-one structure since the ^1H NMR signals at δ 3.97 (s, 3 H), 2.01 (s, 3 H) and 1.87 (s, 3 H) were at similar chemical shifts to those of two methyl and methoxyl signals in model compounds.[10] The minor methyl ether 4, having the 4-methoxy-3,5-dimethyl-4H-pyran-2-one structure, had ^1H NMR signals at δ 3.83 (s, 3 H), 2.06 (s, 3 H) and 2.01 (s, 3 H). The ^1H NMR spectrum of the methyl ether 4 resembled that of the starting pyrone, in agreement with the ^{13}C NMR data that required the 2-pyrone to be the major tautomer of diemenensin-A (1).

The spectral data[11] of diemenensin-B (2) were almost identical to those of diemenensin-A (1) except that the ^{13}C NMR spectrum contained signals at δ 23.0 (q) and 14.9 (q) for the vinyl methyl carbons, indicating that one of the olefinic bonds had the *Z* geometry.[8] The ^1H NMR spectrum contained two olefinic proton signals at δ 6.12 (bs, 1 H, C-7) and 5.12 (bd, 1 H, J = 10 Hz, C-9) coupled to vinyl methyl signals at δ 1.97 (bs, 3 H) and 1.55 (bs, 3 H) respectively. Irradiation at δ 1.97 resulted in a nuclear Overhauser enhancement of the signal at 6.12 and irradiation at 1.55 produced a nOe to the same signal but not to the signal at 5.12. Thus dimenensin-B (2) had the

6*Z*,8*E* geometry. On standing, diemenensin-B (2) isomerized to give diemenensin-A (1) as the major product.

Diemenensin-A (1) inhibited *S. aureus* and *B. subtilis* at 1 μg/disc and 5 μg/disc respectively using the disc assay method and inhibited cell division in the fertilized sea urchin egg assay at 1 μg/mL in seawater. "Polypropionate" metabolites have been isolated from the sacoglossans *Tridachiella diomedia*,[10] *Tridachia crispata*[10] and *Placobranchus ocellatus*[12] but this is the first report of this class of metabolites from a pulmonate.[13]



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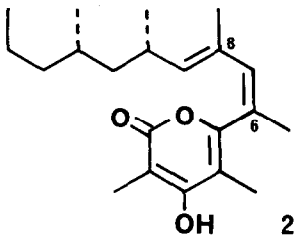

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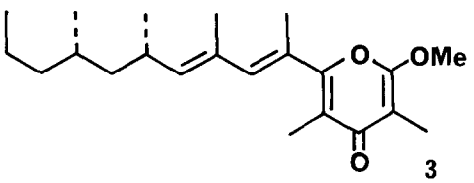
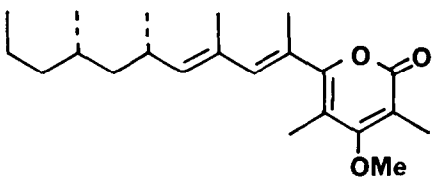


3



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References and Notes

1. Quoy, J.R.C. and J.P. Gaimard. Astrolabe Zool., 2, 327 (1833).

2. Average dry weights; shell = 150 mg, tissue = 15 mg.

3. Oil; $[\alpha]_D$ + 77.3° (c 4.7, MeOH): IR (neat) 3270, 1665, 1555, 1440, 1370, 1230, 1070 cm^{-1}: UV (C_5H_{12}) 247 nm (ε 8920), 307 (ε 13500); ^1H NMR ($CDCl_3$) δ 6.02 (bs, 1 H), 5.18 (bd, 1 H, J = 10 Hz), 2.56 (m, 1 H), 2.06 (s, 3 H), 2.03 (d, 3 H, J = 1 Hz), 2.02 (s, 3 H), 1.81 (d, 3 H, J = 1 Hz), 0.97 (d, 3 H, J = 6.6 Hz), 0.87 (t, 3 H, J = 7 Hz), 0.85 (d, 3 H, J = 6.4 Hz); ^{13}C NMR (C_6D_6) δ 167.5 (s), 167.2 (s), 159.3 (s), 140.0 (d), 139.1 (d), 130.4 (s), 126.6 (s), 108.4 (s), 99.8 (s), 45.4 (t), 40.4 (t), 30.8 (2d), 21.7 (q), 20.4 (t), 19.9 (q), 16.7 (q), 16.2 (q), 14.6 (q), 12.1 (q), 9.6 (q); mass spectrum, m/z = 332.2349.

4. Using 4-hydroxy-6-methyl-2H-pyran-2-one (Aldrich H-4, 341-5) as a model compound and applying the substituent effects for benzene gave the calculated data: δ 168.5 (s), 164.4 (s), 164.2 (s), 109.7 (s), 97.9 (s).

5. Yamada, K. Bull. Chem. Soc. Japan, 35, 1323 (1962).

6. Calculated[7]; δ 14.34 (q), 19.96 (t), 20.12 (q), 20.61 (q), 30.45 (d), 32.38 (d), 39.35 (t), 44.111 (t); average error 0.7 ppm. The average error for the two alternative structures was 4.6 and 5.8 ppm.

7. Wehrli, F.W.; T. Wirthlin. "Interpretation of Carbon-13 NMR Spectra", p. 41. Heyden, London (1976).

8. Stothers, J.B. "Carbon-13 NMR Spectroscopy", p. 406-408. Academic, New York (1972).

9. Literature values: (2*R*,4*R*) -25.2°; (2*S*,4*R*) +16.9°. Odham, G. Arkiv. Kemi, 27, 231 (1967).

10. Ireland, C.; D.J. Faulkner. Tetrahedron, 37 suppl. 1, 233 (1981).

11. Oil; $[\alpha]_D$ +32.4° (c 1.15, MeOH): IR (neat) 3210, 1685, 1595, 1475, 1440, 1405, 1255, 1060 cm^{-1}; UV (C_5H_{12}) 224 (ε 8710), 301 (ε 6470); ^1H NMR ($CDCl_3$) δ 6.12 (bs, 1 H), 5.12 (bd, 1 H, J = 10 Hz), 2.44 (m, 1 H), 2.00 (s, 3 H), 1.97 (bs, 3 H), 1.83 (s, 3 H), 1.55 (bs, 3 H), 0.85 (t, 3 H, J = 7 Hz), 0.84 (d, 3 H, J = 6.5 Hz), 0.76 (d, 3 H, J = 6.5 Hz); ^{13}C NMR (C_6D_6) δ 166.8 (s), 166.2 (s), 157.0 (s), 140.6 (d), 137.9 (d), 131.1 (s), 125.3 (s), 108.7 (s), 99.5 (s), 45.2 (t), 40.5 (t), 30.9 (d), 30.5 (d), 23.0 (q), 31.5 (q), 20.4 (t), 19.7 (q), 14.9 (q), 14.8 (q), 10.8 (q), 9.3 (q); mass spectrum, m/z = 332.2342.

12. Ireland, C.; P.J. Scheuer. Science, 205, 922 (1979).

13. Although this may be the first report of a "polypropionate" metabolite from a pulmonate, credit for the discovery must go to Drs. C. Ireland and P.J. Scheuer who found a more complex metabolite from a Hawaiian *Siphonaria* species.

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