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CYTOTOXIC METABOLITES FROM THE MOLLUSC PERONIA PERONII

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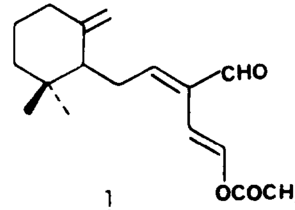
ABSTRACT: The peroniatriols I (3) and II (4) were isolated from saponified extracts of the mollusc Peronia peronii. The structures were assigned by spectral methods and comparison with the isomeric compound ilikonapyrone (2).

The family Onchidiacea are shelless molluscs that inhabit the rocky intertidal zones of many tropical shorelines. We became interested in the onchid molluscs several years ago because of reports that they possess "repugnatorial" skin glands capable of de novo biosynthesis of defensive allomones.[1] Previously we reported the isolation of onchidal (1) and esters of ilikonapyrone (2) from the onchids Onchidella binneyi[2] and Onchidium verruculatum[3] respectively. We now wish to report the isolation of peroniatriols I (3) and II (4), cytotoxic metabolites from saponified extracts of Peronia peronii.



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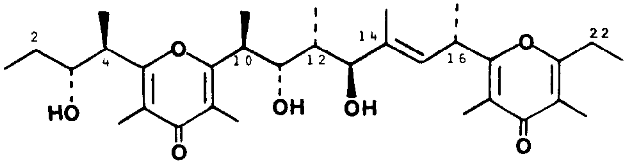

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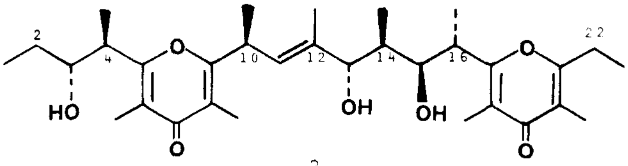
Peronia peronii, 60 individuals, were collected at Cocos Lagoon, Guam. The freeze dried animals were soaked in $CHCl_3$ for 4 days to give 8 g. of a brown oil (L1210 IC_{50} 0.5 μg/ml). Chromatography of a portion of this oil on silica gel 62 (EtOAc) and HPLC (Partisil 10, EtOAc) gave an intractable mixture of related esters (IC_{50} 0.07 μg/ml). Saponification of the mixture by stirring in 1% KOH/MeOH at room temperature for one hour gave equal quantities of 3 (IC_{50} 5.5 μg/ml) and 4 (IC_{50} 3.1 μg/ml).



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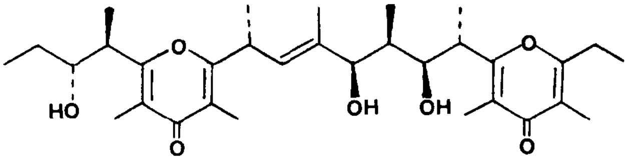

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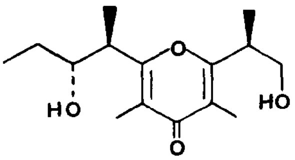
Peroniatriols I and II were assigned identical formulas, $C_{32}H_{48}O_7$ by HR FABMS (545.3467, $M^{+}H$ req. 545.3478) and were therefore isomeric with ilikonapyrone (2). Peroniatriols I and II exhibited spectral data[4] for two fully substituted γ-pyrone rings and the identical proton spin systems as ilikonapyrone. The trisubstituted double bond in the peroniatriols was assigned *E*-configuration analogous to 2 based on the ^{13}C chemical shift of the vinyl methyl (11.9 and 13.9 ppm, respectively).[5]

Ozonolysis with reductive work-up ($NaBH_4$)[6] gave monopyrones (5) and (7) from peroniatriol I and (6) and (7) from peroniatriol II.[7] Monopyrone 7 indicated the peroniatriols had identical stereochemistry at C-14 to 16.[8] Pyrones (5) and (6) indicated the peroniatriols were also identical at C-3 and 4 but epimeric at C-10. The ^{1}H NMR spectra of 5 and 6 suggest that the 1-methyl-2-hydroxyethyl side chain exists in a *quasi*-chair conformation with hydrogen bonding between the primary hydroxyl and the pyrone ether oxygen. In 5 H-10 exhibited identical couplings of 3.5 Hz to the adjacent methylene protons indicating H-10 is equatorial. Conversely, H-10 in 6 displays differential couplings of 10.5 and 3.5 Hz indicating an axial disposition. Compound 8 obtained from an OsO_4 oxidative cleavage of ilikonapyrone showed an identical coupling pattern as peroniatriol I, therefore, peroniatriol I was assigned the same stereochemistry at this center.



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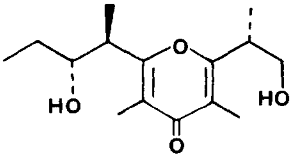

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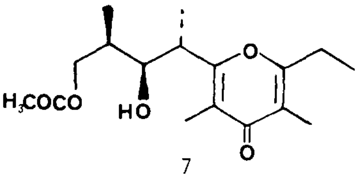

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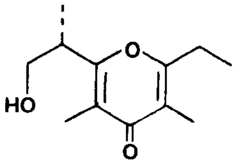

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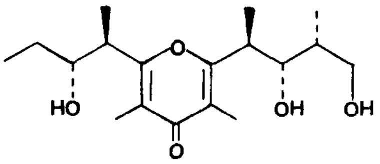

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The stereochemistry about the 1,3 diol system was assignable by analysis of ^{1}H NMR coupling constants. Coupling constants in the H NMR spectrum of ilikonapyrone were consistent with the 1,3 diol unit (C-10 to 13) existing in a hydrogen bonded chair ($J_{H_{10}-H_{11}}$ =8Hz, $J_{H_{11}-H_{12}}$=1Hz, $J_{H_{12}-H_{13}}$=7Hz). The analagous protons (C-16 to 13) in peroniatriol I exhibit identical coupling constants and were therefore assigned the same stereochemistry. In peroniatriol II $J_{H_{13}-H_{14}}$ was 2 Hz indicating H-13 is now equatorial and the allylic hydroxyl has the opposite stereochemistry as ilikonapyrone and peroniatriol I.

C-3 and 4 in the peroniatriols were assigned the same stereochemistry as in ilikonapyrone based on similiar chemical shift and identical coupling constants for these protons in all three compounds and also the degradation products 5, 6 and 9 from peroniatriols I, II and ilikonapyrone, respectively.[9]

In conversations with individuals of several Micronesian Island groups it was discovered that *Peronia peronii* is a culinary delicacy on many islands. Interestingly, preparation of *Peronia* always involved scraping off the dorsum of the mollusc on a rock or other available rugged surface. The peroniatriol esters are presumably produced in glands located on the dorsum since the compounds are readily extracted by soaking whole animals.

ACKNOWLEDGEMENT

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4. *Peroniatriol I (3)*: HR FAB 545.3467 (MH$^+$) $C_{32}H_{49}O_7$ calc 545.3478; UV$_{max}$ (CH_2Cl_2) 259 nm (ε 13000); ^{13}C NMR ($CDCl_3$) 179.8 s (2C), 164.8 s (2C), 164.5 s (2C), 137.5 s, 127.0 d, 119.7 s (2C), 118.6 s (2C), 79.5 d, 75.2 d, 72.2 d, 41.5 d, 39.3 d, 36.3 d, 34.5 d, 27.9 t, 24.7 t, 18.6 q, 14.4 q, 14.0 q, 11.9 q, 11.3 q, 10.2 q, 9.6 q (2C), 9.5 q (3C); ^{1}H NMR ($CDCl_3$) 5.59 (dq, 1H, J=9, 1 Hz), 4.14 (dd, 1H, J=8, 1 Hz), 4.06 (d, 1H, J=7 Hz), 3.90 (dq, 1H, J=9, 7 Hz), 3.75 (m, 1H, J=7, 7, 7 Hz), 3.15 (dq, 1H, J=8, 7Hz), 2.90 (dq, J=7, 7 Hz), 2.76 (OH), 2.56 (m, 2H, J=7 Hz), 1.97 (s, 3H), 1.96 (s, 3H), 1.93 (s, 3H), 1.89 (s, 3H), 1.84 (m, 1H), 1.70 (d, 3H, J=1 Hz), 1.57 (m, 1H), 1.35 (m, 1H), 1.30 (d, 3H, J=7 Hz), 1.29 (d, 3H, J=7 Hz), 1.20 (t, 3H, J=7 Hz), 1.18 (d, 3H, J=7 Hz), 0.99 (t, 3H, J=7 Hz), 0.95 (d, 3H, J=7 Hz). $[\alpha]_D$ -12.4° (c 1.173, CH_2Cl_2).

Peroniatriol II (4): HR FAB 545.346738 (M^+) $C_{32}H_{49}O_7$ calc 545.3478; UV_{max} (CH_2Cl_2) 259 (ε13000); ^{13}C NMR ($CDCl_3$) 180.1 s (2C), 165.7 s, 165.1 s, 164.9 s, 164.5 s, 137.3 s, 125.4 d, 120.1 s, 118.5 s, 117.4 s, 116.1 s, 80.1 d, 75.2 d, 73.0 d, 42.0 d, 39.8 d, 34.5 d, 34.3 d, 27.6 t, 24.7 t, 19.81 q, 15.7 q, 14.11 q, 13.9 q, 10.8 q, 10.0 q (4C), 9.5 q (2C); ^1H NMR ($CDCl_3$) 5.86 (dq, 1H, J=9, 1 Hz), 4.40 (OH), 4.05 (d, 1H, J=2 Hz), 3.90 (dq, 1H, J=9, 7 Hz), 3.72 (dd, 1H, J=8, 1 Hz), 3.57 (ddd, 1H, J=7, 7, 3 Hz), 3.03 (dq, 1H, J=8, 7 Hz), 2.84 (dq, 1H, J=7, 7 Hz), 2.51 (m, 1H, J=14, 7 Hz), 2.28 (m, 1H, J=14, 7 Hz), 2.04 (s, 3H), 1.90 (s, 3H), 1.87 (s, 3H), 1.87 (m, 1H), 1.82 (s, 3H), 1.63 (d, 3H, J=7 Hz), 1.61 (m, 1H), 1.38 (m, 1H), 1.31 (d, 3H, J=7 Hz), 1.16 (d, 3H, J=7 Hz), 1.14 (d, 3H, J=7 Hz), 1.02 (d, 3H, J=7 Hz), 0.99 (t, 3H, J=7 Hz), 0.87 (t, 3H, J=7 Hz). $[\alpha]_D$ +224.8° (c 1.063, CH_2Cl_2)

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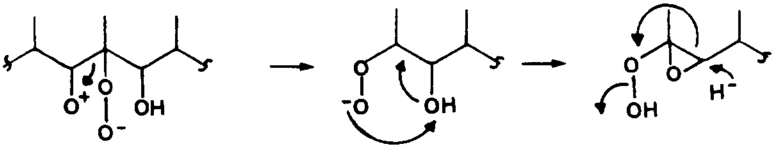
6. A solution of CH_2Cl_2 saturated with O_3 at 78° was added dropwise to CH_2Cl_2 solution of a peroniatriol. Additions were stopped as soon as starting material was consumed. The reaction was warmed to room temperature and evaporated to dryness. The crude ozonides were reduced by stirring in MeOH and addition of $NaBH_4$.
7. (5), EIMS 268 (M^+); ^1H NMR ($CDCl_3$) 3.81 (m, 2H, J=10.5, 3.5, 3.5 Hz), 3.75 (m, 1H, J=7, 7, 7 Hz), 3.23 (m, 1H, J=7, 3.5, 3.5 Hz), 3.01 (dq, 1H, J=7, 7 Hz), 2.0 (s, 3H), 1.99 (s, 3H), 1.54 (m, 1H, J=10.5, 7.4, 7 Hz), 1.40 (m, 1H, 10.5, 7.4, 7 Hz), 1.30 (d, 3H, J=7 Hz), 1.23 (d, 3H, J=7 Hz), 1.00 (t, 3H, J=7.4 Hz). $[\alpha]_D$ +4.3° (c 0.093, CH_2Cl_2)

   (7): EIMS 310 (M^+); ^1H NMR ($CDCl_3$) 4.27 (m, 1H), 4.00 (m, 1H), 3.87 (m, 1H), 3.14 (dq, 1H, J=8.8, 7 Hz), 2.63 (q, 2H, J=7.6 Hz), 2.10 (s, 3H), 2.10 (m, 1H), 2.00 (s, 3H), 1.96 (s, 3H), 1.23 (t, 3H, J=7.6 Hz), 1.15 (d, 3H, J=7 Hz), 0.98 (d, 3H, J=7 Hz). $[\alpha]_D$ = +29.4° (c 0.051, CH_2Cl_2).

   (6): EIMS 268 (M^+); ^1H NMR ($CDCl_3$) 3.87 (m, 1H, J=14, 3.5 Hz), 3.80 (m, 1H, J=14, 10.5 Hz), 3.73 (m, 1H, J=7, 7, 3 Hz), 3.24 (m, 1H, J=10.5, 7, 3.5 Hz), 3.05 (dq, 1H, J=7, 7 Hz), 1.98 (s, 3H), 1.97 (s, 3H), 1.61 (m, 1H, J=14, 7.4, 3 Hz), 1.38 (m, 1H, J=14, 7.4, 7 Hz), 1.31 (d, 3H, J=7 Hz), 1.21 (d, 3H, J=7 Hz), 1.02 (t, 3H, J=7.4 Hz). $[\alpha]_D$=+7.0° (c 0.06, CH_2Cl_2)
8. The generation of pyrone 7 is explained by rearrangement of the molozonide to a hydroperoxyepoxide species that is reduced by $NaBH_4$ as shown.



9. Although these arguments allow assignment of internal relative stereochemistry for each segment of the peroniatriols they do not allow assignment of absolute configuration.

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