Ulapualide A and B, Extraordinary Antitumor Macrolides from Nudibranch Eggmasses

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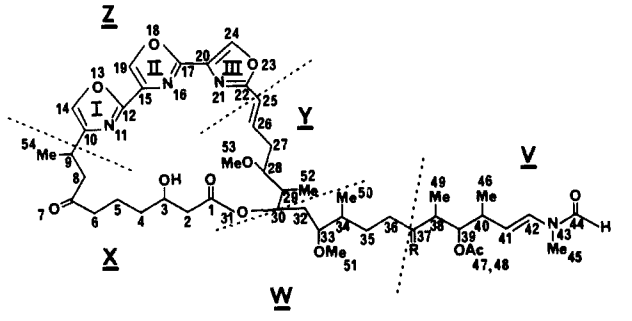
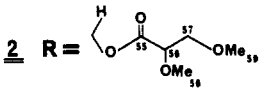
The brillantly colored nudibranch *Hexabranchus sanguineus* deposits its striking red eggmasses which resemble rosebuds on ledges in underwater caves. Though exposed and vulnerable, these eggs have only one known predator, the aeolid nudibranch *Favorinus japonicus*,[1] which we have never observed. This virtual immunity from predation led us to investigate the organic constituents of the eggs.[2] Two extraordinary macrolides, ulapaulide[3] A (**1**) and B (**2**), which inhibit L1210 leukemia cell proliferation



1 ULAPUALIDE-A R=O



(IC_{50} 0.01–0.03 μg/mL)[4a] and the growth of *Candida albicans*[4b] apparently are the bioactive metabolites. We report here the gross structures of **1** and **2**, 28-membered lactones encompassing three contiguous oxazoles. The acyclic side chains terminate in *N*-methylformyl functions but differ in the C-37 carbonyl of A (**1**), which in B (**2**) is an alcohol esterified by 2,3-dimethoxypropanoic acid.

Hexabranchus eggs were collected by SCUBA at Pupukea, O'ahu, and immediately placed in MeOH. Partitioning with hexane and then carbon tetrachloride, followed by HPLC of the carbon tetrachloride residue (first on RP-18, MeOH/H_2O, 70:30, then Si-60, MeCN) furnished ulapualide A (0.025% of wet wt) and B (0.055%) as colorless oils. Structural determination was carried out mostly with ulapualide B (**2**), $C_{51}H_{74}N_4O_{16}$ (FABHRMS, *m/z* 1021.4890, calcd for $C_{51}H_{74}N_4O_{16}Na$, 1021.4996;[5] anal.[6] C, 61.20; H, 7.40; N, 5.52%), $[\alpha]^{25}_D$ −21.7° (*c* 0.138, MeOH, λ_{max}^{MeOH} 246 nm (33 000)).[7,8] The $C_5H_{10}O_3$ (118 mass units) difference between **1** and **2** was reflected by the following ^1H and ^{13}C NMR data. The carbonyl (212.05 ppm) in **1** was replaced in **2** by an ester carbonyl (170.41) and a methine proton H-37 δ 5.05 (ddd, *J* = 9.7, 2 Hz). Ulapualide B (**2**) also contained two additional methoxy groups (δ 3.35 s, 3.40 s; 59.35 q, 58.66 q, and 81.12 d, 73.43 t) and an isolated $OCHCH_2O$ system (H-56, δ 3.86, dd, *J* = 7, 3 Hz; H_a-57, δ 3.61, dd, *J* = 11, 3 Hz; H_b-57, δ 3.54, dd, *J* = 11, 7 Hz), thus accounting for the distinguishing features between **1** and **2**. Full ^{13}C data are listed in Table I, supplementary material.

Restricted rotation about the *N*-methylformyl terminus gives rise to doubled ^1H signals for H-41, -42, -44, -45, -46, and -49 and ^{13}C signals for C-36, -38, -40, -41, -42, -44, -45, -46, and -49, all in a 2:1 ratio, a well-known phenomenon previously encountered in stylocheilamide[9] and tolytoxin[10] but one that complicates NMR data interpretation. Complete ^1H NMR data of the C-37–44 part of **2** (partial structure V) are found in Table II, supplementary material. Difference decoupling located a buried multiplet at δ 2.58 assigned to H-40. In a long-range C–H coupling experiment (Table III, supplementary material, the C-47 acetate carbonyl could be linked to Me-48 (δ 2.0) and H-39 methine (δ 4.71). The remainder of the side chain, C-30–37 (W) lacks quaternary carbons or heteroatoms. Its structure was delineated by decoupling experiments (Table IV, supplentary material).

The structure of the macro ring is discussed in three parts, C-1–9 (X), C-25–39 (Y), and the trisoxazole moiety (Z). Partial structure X includes C-1 ester (δ 173) and C-7 keto (δ 210) carbonyls. Long-range C–H coupling data (Table III, supplementary material) established the C-1,2 connection and the linkage of C-7 to its adjacent methylenes C-6 and C-8. One of C-8 protons (δ 2.80, dd, *J* = 16, 10 Hz) was selectively decoupled, thus revealing H-9 (δ 3.42), which was buried under the methoxy resonances. H-9 in turn was coupled to Me-54. Complete decoupling data of partial structure X are found in Table V, supplementary material.

Interpretation of partial structure Y (C-25–30) is unambiguous as all carbons except CH_2-27 are methines bearing distinctive structural features. Full ^1H NMR data are listed in Table VI, supplementary material.

Linkage of C-9 and C-25 to the unique trisoxazole part Z was deduced as follows. A long-range COSY experiment linked H-14 (δ 7.41, d, *J* = 1.5 Hz) to H-9 (δ 3.42). Difference decoupling with selective irradiation of H-14 confirmed the assignment and provided an explanation for the low-field resonance of H-9. Three bond coupling data establish connection between H-26 (δ 6.95) and C-22 (δ 163) (Table III, supplementary material).

Partial structures V–Y account for the elemental aggregate of $C_{42}H_{71}NO_{13}$ of ulapualide B (**2**) leaving part structure Z, $C_9H_3N_3O_3$ (*u* = 10), to be elucidated. The high unsaturation number, three aromatic proton singlets, H-14 (δ 7.43), H-17 (δ 8.09), and H-24 (δ 8.10), and nine ^{13}C resonances between δ 170 and 131 (Table I) suggested Z to be heteroaromatic. A broad UV absorption with a maximum at about 246 nm (ε 33 000) was more puzzling than informative because of the high intensity.

Ozonolysis of **2** (EtOH, −78 °C, 4 min), followed by reductive workup ($NaBH_4$, EtOH, 45 min) and acetylation (Ac_2O, pyridine, 24 h, room temperature), led to a complex mixture of some 10 fractions, from which an aromatic compound, **3**, $C_{10}H_9N_3O_{51}$ (EIHRMS, *m/z* 251.0555, calcd 251.0542), was isolated by four successive HPLC steps and ^1H NMR (CD_2Cl_2, two 1-H singlets at δ 8.33, 8.20) monitoring. Spectral data were compatible with an amide (ν_{max} 3150, 1670, 1615 cm^{-1}; δ 6.90 and 5.60, broad singlets) and a hydroxymethylene (δ 5.20, 2H s) acetate (ν_{max} 1740 cm^{-1}; δ 2.18, 3 H), linked to the aromatic $C_6H_2N_2O_2$ fragment. The CH_2OAc must have arisen from cleavage of the C-25,26 olefin and the amide from the destruction of oxazole I.

Evidence for a trisoxazole moiety Z comes from ^{15}N (δ 231.9, 222.5, 213.7) and ^{13}C NMR data of intact **2**. The ^{15}N NMR resonances are in the oxazole range,[11,12] but no trisoxazole model

(1) Bertsch, H.; Johnson, S. "Hawaiian Nudibranchs"; Oriental Publishing Co.: Honolulu, HI, 1981; p 97.

(2) We are indebted to Dr. Robert E. Schwartz for the initial observation and isolation.

(3) The name is coined from two Hawaiian words ula = red and pua = flower.

(4) (a) We thank Dr. M. Fukushima, Aichi Cancer Center, Nagoya, Japan, for these determinations. (b) A 6-mm-diameter filter paper disk containing 4.0×10^{-4} mmol of **2** gave rise to a 17-mm zone of inhibition. By comparison, 4.3×10^{-4} mmol of amphotericin B yielded an 11-mm zone of inhibition.

(5) We thank Professor K. L. Rinehart, Jr., for the FABMS data.

(6) Berkeley Analytical Laboratory, Berkeley, CA.

(7) Ulapualide A (**1**): FABHRMS, *m/z* 903.4242; calcd for $C_{46}H_{64}N_4O_{13}$ 903.4367. Anal. C, 62.46; H, 7.14; N, 6.32%. $[\alpha]^{25}_D$ −42.9° (*c* 0.163, MeOH); λ_{max}^{MeOH} 246 nm (34 000).

(8) Complete spectral data are presented in the supplementary material.

(9) Rose, A. F.; Scheuer, P. J.; Springer, J. P.; Clardy, J. *J. Am. Chem. Soc.* **1978**, *100*, 7665–7670.

(10) Moore, R. E. In "Marine Natural Products"; Scheuer, P. J., Ed.; Academic Press: New York, 1981; Chapter 1.

(11) Witanowski, M.; Webb, G. A.; Stefaniak, L.; Januszewski, H. In "Nitrogen NMR"; Witanowski, M., Webb, G. A., Eds.; Plenum: London, 1973; pp 211–213.

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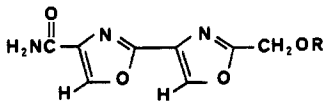
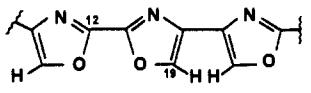


3 R = Ac
4 R = H



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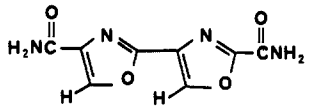

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has been reported. Three contiguous disubstituted oxazoles became apparent from a fully coupled ^{13}C spectrum including one-, two-, and three-bond couplings and from long-range heteronuclear coupling experiments (Table III).

Selective decoupling of H-14 collapsed the C-14 (207 Hz)[13] and C-12 (8 Hz) doublets to singlets and sharpened the broad C-10 resonance, thereby elucidating oxazole I. Since H-14 is also linked to H-9 (vide supra), C-12 must be bonded to oxazole II.

C-22 (δ 163) of oxazole III is linked to H-26 (δ 6.94) by long-range C-H decoupling data. C-24 exhibits a C–H coupling of 213 Hz, which necessitates C-20 linkage to oxazole II.

Oxazole II may be inserted between I and III as shown in **1** (C-12–15 and C-17–20) or by linking C-12 to C-17 and C-15 to C-20. Three-bond coupling between H-19 (δ 8.09) and C-12 (δ 154) was not observed; hence oxazole II was initially placed as in **5**, where H-19 and C-12 are separated by four bonds, although biogenetic considerations favored **1**.

Although few biosynthetic models for oxazoles are known,[14] the symmetrical disposition of the trisoxazole as in **2** appeared attractive and we secured experimental evidence that favors **2**.

Hydrolysis of **3** (powdered K_2CO_3, MeOH overnight) furnished alcohol **4**, $C_8H_7N_3O_4$,[15] which was transformed to the bisamide **6**.[16,17] The ^1H NMR spectrum of **6**[18] had two singlets at δ 8.72 and 8.51 and four broad amide signals at δ 8.43, 8.09, 7.68, and 7.59. This spectrum does not fit a bisamide derived from **5**, which should display only a single aromatic proton resonance. Hence the ulapulaides have structures **1** and **2**.

The nudibranch *Hexabranchus sanguineus*, which lays the eggmasses, also contains the ulapualides though in low concentration. *H. sanguineus* feeds on the calcareous sponge *Leucetta solida*,[19] but our examination of *L. solida* yielded no ulapualides. An interesting pteridine, leucettidine, has been reported from *L. microraphis* from Bermuda.[20,21]

Acknowledgment. We thank Steve Coval, Nanda Gulavita, Roy Okuda, Debbie Roll, and Bülent Terem for help with collections and NMR measurements; Walter Niemczura and Lars Bergknut for NMR and MS determinations, Dr. J. Michael Geckle of Bruker Medical Instruments and the Southern California and Carnegie Mellon NMR facilities for high-field NMR data, Professors A. L. Burlingame (UCSF) and K. Nakanishi (Columbia) for MS data, Manabu Nukina for helpful discussions, and the National Science Foundation and the University of Hawaii Sea Grant College Program under Institutional Grant NA81AA-D-0070 from NOAA, Office of Sea Grant, U.S. Department of Commerce, for financial support.

Supplementary Material Available: Table I–VI list complete NMR spectral data (7 pages). Ordering information is given on any current masthead page.

(12) Levy, G. C.; Lichter, R. L. In "Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy"; Wiley-Interscience: New York, 1979; p 84.
(13) C–H couplings in oxazoles: C-2, J = 231 Hz; C-4, J = 195 Hz; C-5, J = 209 Hz; cf.: Hiemstra, H.; Houwing, H. A.; Possel, O.; van Leusen, A. M. *Can. J. Chem.* **1979**, *59*, 3168–3170.
(14) In the alkaloid annuloline (O'Donovan, D. G.; Horan, H. *J. Chem. Soc. C* **1971**, 331–334) the oxazole is derived from an amide, in the macrolide virginiamycin M (Kingston, D. G. I.; Kolpak, M. X. *J. Am. Chem. Soc.* **1980**, *102*, 5964–5966) from an acylserine.
(15) **4**: ^1H NMR (Me_4SO-d_6) δ 8.81 (1 H, s), 8.65 (1 H, s), 7.67 (1 H, br s), 7.54 (1 H, br s); HREIMS; m/z found 209.0499; calcd for $C_8H_7N_3O_4$, 209.0436; FTIR (film) 3300, 1670, 1616 cm^{-1}; UV (MeOH) λ_{max} 245 nm (ϵ 6000).
(16) **4** (0.5 mg); THF, −20 °C, dry NH_3 for 30 min; XS NiO_2 added over 1 h, stirred for 10 h at −20 °C; purified on BondElut RP-18, then HPLC RP-18 (MeOH/H_2O, 2:8).
(17) Nakagawa, K.; Onoue, H.; Minami, K. *J. Chem. Soc., Chem. Commun.* **1966**, 17–18.
(18) **6**: ^1H NMR (Me_2SO-d_6) δ 8.72 (1 H, s), 8.51 (1 H, s), 8.43 (1 H, br s), 8.09 (1 H, br s), 7.68 (1 H, br s), 7.59 (1 H, br s); HREIMS, m/z found 222.0374; calcd for $C_8H_6N_4O_4$, 222.0389; FTIR (film) 3480, 3330, 1646 (br) cm^{-1}.
(19) Kay, E. A. "Hawaiian Marine Shells"; Bishop Museum Press: Honolulu, HI, 1979; p 471.
(20) Cardellina, J. H., II; Meinwald, J. *J. Org. Chem.* **1981**, *46*, 4782–4784.
(21) Pfleiderer, W. *Tetrahedron Lett.* **1984**, *25*, 1031–1034.

Kabiramide C, a Novel antifungal Macrolide from Nudibranch Eggmasses[1]

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Nudibranch eggmasses seem immune to predation in spite of their brilliant colors ranging from yellow to red and of flowerlike shapes. Although a variety of chemical defense substances of nudibranchs have been reported,[2-6] the chemistry of the eggmasses is totally unknown.[7] In the course of our search for bioactive substances of Japanese marine invertebrates, we found that the lipophilic extract of eggmasses of an unidentified nudibranch collected at Kabira Bay in Ishigaki-jima Island of the Ryukyus showed considerable antifungal activity, while eggmasses of *Dendrodoris nigra* in the Gulf of Sagami were inactive. We have isolated from the Kabira collection a major active compound, named kabiramide C, which has been assigned a novel macrolide structure. Kabiramide C showed marked antifungal activity.[8]

(1) Part 13 of the bioactive marine metabolites series. Part 12: Fusetani, N.; Yasukawa, K.; Matsunaga, S.; Hashimoto, K. *Tetrahedron Lett.* **1985**, *26*, 6449.
(2) Hochlowski J. E.; Walker, R. P.; Ireland C.; Faulkner D. J. *J. Org. Chem.* **1982**, *47*, 88.
(3) Schulte, G. R.; Scheuer, P. J. *Tetrahedron* **1982**, *38*, 1857.
(4) Thompson, J. E.; Walker, R. P.; Wratten, S. J.; Faulkner, D. J. *Tetrahedron* **1982**, *38*, 1865.
(5) Hellou, J.; Andersen, R. J. *Tetrahedron* **1982**, *38*, 1875.
(6) Okuda, R. K.; Scheuer P. J. *J. Org. Chem.* **1983**, *48*, 1866.
(7) Faulkner, D. J. *Nat. Prod. Rep.* **1984**, *1*, 551.
(8) Zones of inhibition found by using a quarter portion of 8-mm-diameter filter paper disk saturated with a 250 μg/mL solution of kabiramide C: *Candida albicans* ATCC 10234 (7.7 mm); *Aspergillus niger* ATCC 9642 (30.7 mm); *Penicillium citrium* ATCC 9849 (20.0 mm); *Trichophyton interdigitae* (21.1 mm).

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