Isolation of Labradorins 1 and 2 from *Pseudomonas syringae* pv. *coronafaciens*[1]

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Received April 12, 2002

Investigation of *Pseudomonas syringae* pv. *coronafaciens* cancer cell growth inhibitory constituents led to the isolation of 2-isobutyl-5-(3-indolyl)oxazole (**1**) and 2-*n*-pentyl-5-(3-indolyl)oxazole (**2f**), designated labradorins 1 (**1**) and 2 (**2f**), related to pimprinine (**2a**). The structures were deduced by spectroscopic techniques and X-ray crystal structure determinations. Labradorin 1 (**1**) afforded GI_{50} μg/mL values of 9.8 and 6.2 against the human cancer cell lines NCI-H 460 (lung-NSC) and BXPC-3 (pancreas-a).

In 1992 as part of our biological specimen survey for new sources of potential anticancer substances in Newfoundland-Labrador, we collected a sample of coastal sand near Pinware, Labrador. Subsequently we selected and cultured a bacterium identified by 16S rRNA sequencing as the phytopathogen[2] *Pseudomonas syringae* pv. *coronafaciens.* Interestingly this organism has been found to contain polysaccharides that significantly inhibit growth of the B-16 melanoma cell line as well as three other cancer cell lines.[3] In addition, *P. syringae* is known to produce lipopeptide phytotoxins[2] and the wildfire toxin tabtoxin.[4] We found that a dichloromethane fraction prepared from a glucose−peptone−yeast extract culture of *P. syringae* inhibited growth (ED_{50} 0.01 μg/mL) of the P388 lymphocytic leukemia cell line. Research directed toward the characterization of the elusive active component has led to the isolation of new oxazolyl-indoles, which are reported here.

A 400 L scale-up fermentation of *P. syringae* was employed for bioassay (P388) directed isolation of the cancer cell inhibitory constituent(s). The less polar constituents were separated by extraction with hexane, and the ethyl acetate-soluble fraction was subjected to high-speed countercurrent distribution (HSCCD) on an Ito multilayer coil planet centrifuge (Ito Coil). The first useful fraction isolated presented predominantly as a single nonpolar spot (R_f about 0.75) with strong UV activity. This fraction (65 mg) was separated by crystallization from acetone. The high-resolution mass spectrum gave a pseudomolecular ion at 241.1329 ($M + H^+$, $C_{15}H_{17}N_2O$). The crystals proved suitable for X-ray structure determination, and the resulting crystal structure showed an oxazolylindole where the oxazole ring was substituted with a 2′-isobutyl side-chain (Figure 1) and was named labradorin 1 (**1**).

Compounds of the labradorin 1 type but with different side-chains have been previously isolated or synthesized. For example, the 2-methyloxazole pimprinine (**2a**) was isolated from *Streptomyces pimprina* and the structure confirmed by synthesis.[5] The homologous 2-ethyloxazole (**2b**) was subsequently isolated from *Streptomyces cinnamoneus* and characterized by spectroscopy and X-ray diffraction.[6] The *n*-propyl (**2c**) and *n*-butyl (**2d**) analogues (WS-30581A and B) were isolated from *Streptoverticillium waksmanii.*[7] Synthesis of these and other structural variants, including the 2-isopropyloxazole **2e** and analogues with aromatic side-chains, have also been reported.[8−10]

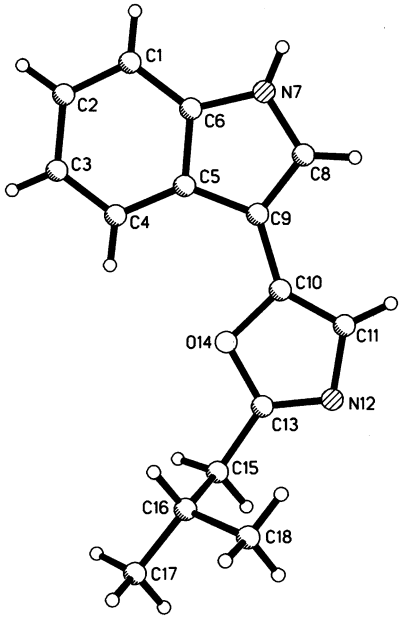

Figure 1. Crystal structure of labradorin 1, **1**.

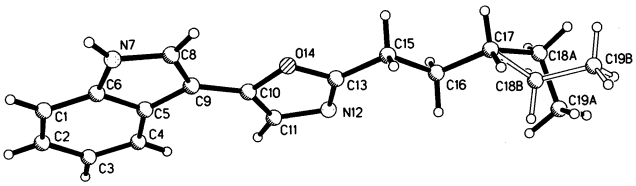

Figure 2. X-ray crystal structure of labradorin 2 (**2**) with disorder shown for C18 and C19.

HPLC analysis of the labradorin 1 (**1**) combined crystallization mother-liquors showed a less polar constituent, which was isolated by HSCCD on the Ito coil and crystallized as small plates from methanol (7 mg). The mass product ion spectrum indicated that it was a homologue of labradorin 1 (**1**), with a molecular ion at *m*/*z* 254. The structure was deduced by X-ray crystal structure determination as the *n*-pentyl analogue (**2f**) designated labradorin 2, Figure 2. During the course of isolating labradorins 1 and 2, both the known *cyclo*-(L-Pro-L-Leu) and deoxycholic acid were also isolated. Deoxycholic acid was completely identified by X-ray crystallographic analysis, Figure 3. Since so little of **2f** was available for biological screening, a larger supply was synthesized by the general method of Oikawa[8] as shown in Scheme 1. Labradorins 1 and 2

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10.1021/np020173x CCC: $22.00
Published on Web 10/18/2002

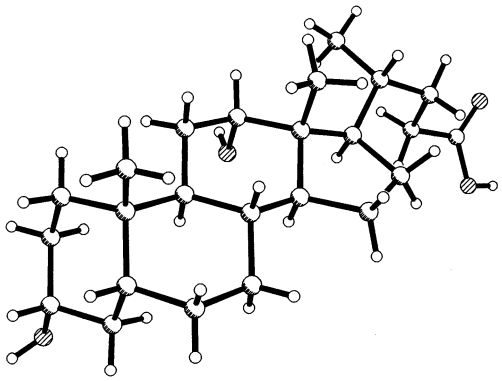
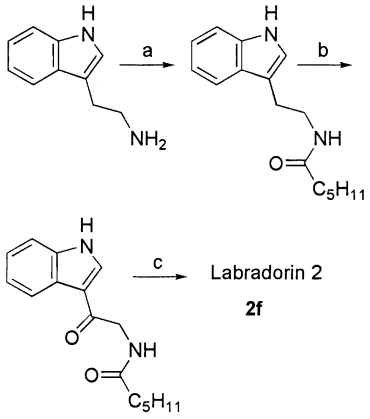


Figure 3. X-ray crystal structure of deoxycholic acid (**3**) (hydrate molecules excluded).

Scheme 1[a]



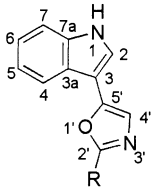
[a] (a) caproyl chloride, triethylamine; (b) DDQ, THF, H_2O; (c) $POCl_3$.

Table 1. Evaluation of Labradorins 1 (**1**) and 2 (**2f**) against a Panel of Human Cancer Cell Lines[a]

cell type	cell line	labradorin 1	labradorin 2
pancreas-a	BXPC-3	6.2	9.6
breast	MCF-7	3.9	27.4
CNS	SF-268	5.1	58.8
lung-NSC	NCI−H460	9.8	9.6
colon	KM20L2	3.5	9.7
prostate	DU-145	4.0	11.4

[a] Expressed as GI_{50} in μg/mL.

were evaluated for cancer cell line (Table 1) and antimicrobial activities. In broth microdilution susceptibility assays,[11,12] labradorins 1 and 2 were not active against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Micrococcus luteus*, *Enterobacter cloacae*, *Escherichia coli*, *Stenotrophomonas maltophilia*, *Neisseria gonorrhoae*, *Cryptococcus neoformans*, or *Candida albicans* (up to 64 μg/mL).



1, R = *i*-butyl, Labradorin 1
2a, R = methyl, Pimprinine
b, R = ethyl
c, R = *n*-propyl
d, R = *n*-butyl
e, R = *i*-propyl
f, R = *n*-pentyl, Labradorin 2

Experimental Section

General Experimental Procedures. Solvents used for chromatography were redistilled. Silica gel GF Uniplates for TLC were developed in methylene chloride−2-propanol (95:5) and were viewed under UV light and (or) sprayed with sulfuric acid−ethanol (1:1) and heated at approximately 150 °C for visualization. HPLC was carried out using a 25 cm × 4.6 mm i.d. Ultramex-C8 (Phenomenex) column with a guard cartridge. A gradient of acetonitrile in water (20% to 95% in 20 min) was employed at a flow rate of 1 mL/min; the eluent was monitored by UV (254 nm) or an evaporative light-scattering detector (ELSD, Sedex) with nebulizer temperature 45 °C, nitrogen pressure 2.2 bar, and gain setting 10.

The uncorrected melting points were observed using a Fisher-Johns apparatus. The IR spectral data were obtained with a Nicolet MX-1 FTIR spectrophotometer. Low-resolution mass spectra were determined using a ThermoQuest/Finnigan GCQ spectrometer with direct inlet, and high-resolution mass spectra with a JEOL LC-Mate equipped with FAB and APC1 inlets. NMR experiments were conducted with a Varian VXR-500 instrument and were in deuteriochloroform solution. The X-ray crystallographic analyses were performed using either an Enraf-Nonius CAD-4 or a Bruker AXS Smart 6000 diffractometer.

Fermentation. In October 1992 we collected a specimen of coastal sand from a rock crevice near Pinware, Labrador. Isolation of *P. syringae* pv. *coronafaciens* and fermentation scale-ups were achieved with glucose−peptone−yeast extract media [GPY] (glucose, 1 g/L; peptone, 5 g/L; yeast extract, 2.5 g/L, $MgSO_4 \cdot 7H_2O$, 0.1 g/L; K_2HPO_4, 0.1 g/L). The bacterium was cultured for 3 days at room temperature with aeration prior to extraction.

Identification. Gas−liquid chromatography of fatty acid methyl esters (FAME, MIDI Sherlock Microbial Identification System, Newark, DE) indicated that the bacterium was most closely related to *Pseudomonas fluorescens* (similarity index = 0.710 [an exact match would have a similarity index = 1]). The bacterium was also identified by 16S rRNA gene sequence similarity (Acculab, Newark, DE). Results from the MicroSeq database based on the first 500 base pairs of the 16S gene indicated that the bacterium was *P. syringae* pv. *coronafaciens* (% difference = 0.38; confidence level to species).

Extraction Procedure. The fermentation broth (400 L) from culture of *P. syringae* was extracted with CH_2Cl_2 and the extract concentrated under reduced pressure to give a dark brown oil (73.8 g). The extract was dissolved in CH_3OH, and the solution was filtered, then diluted with 10% water, and extracted with hexane. The hexane extract was concentrated to give a yellow oil (50.5 g) that was discarded. More water was added to the aqueous CH_3OH solution to increase the concentration to 33% v/v, and a further extraction with 1:1 hexane−ethyl acetate gave an oily yellow extract (2.26 g). Finally the percentage of water in the CH_3OH−water phase was increased to 50%. Extraction with ethyl acetate gave a brown gum (5.24 g). The gummy fraction was given a preliminary separation by HSCCD on the Ito Coil in the system hexane (1500)−ethyl acetate (500)−CH_3OH (300)−water (120, upper phase mobile), resulting in the isolation of crude 2-isobutyl-5-(3-indolyl)oxazole (**1**, 62 mg) and, from its crystallization mother-liquor mixture (32 mg), 2-*n*-pentyl-5-(3-indolyl)oxazole (**2f**) designated labradorins 1 and 2, respectively. A major fraction (4.3 g) was recovered from the stationary phase, and this was similarly reprocessed on the Ito Coil in the system hexane(1000)−ethyl acetate(1500)−CH_3OH(500)−water(300), to give a number of fractions, one of which crystallized to give *cyclo*-[L-Pro-L-Leu] (0.464 g). The remaining material was again reprocessed in the system hexane(1000)−ethyl acetate-(1000)−CH_3OH(500)−water(250) to give a further crystalline compound (0.433 g), which was identified by NMR and single-crystal X-ray determination as deoxycholic acid, Figure 3. The latter was shown to be a component of the culture medium.

Labradorin 1 [1; 2-isobutyl-5-(3-indolyl)oxazole]: prisms, mp 147−148 °C from acetone (P388 > 10 μg/mL); IR (KBr)

ν_{max} 3132, 1635, 1604, 1456, 1126, 731 cm^{-1}; ^1H NMR ($CDCl_3$) δ1.06 (d, 8′ and 9′ $-CH_3$, J = 6.8), 2.27 (m, 7′ −CH, J = 6.8), 2.76 (d, 6′ $-CH_2$, J = 7.8), 7.20 (s, 4′ −CH), 7.26−7.29 (m, 5 and 6 −CH), 7.44 (d, 7-CH, J = 7.8), 7.53 (d, 2-CH, J = 2.93), 7.87 (d, 4-CH, J = 7.8), 9.10 (NH); ^{13}C NMR ($CDCl_3$) 111.60 (2-CH), 105.6 (3-C), 124.08 (3a−C), 120.70 (4-CH), 122.79 (5-CH), 119.83 (6-CH), 119.38 (7-CH), 136.36 (7a-C), 162.30 (2′-C), 121.81 (4′-CH), 147.52 (5′-C), 37.09 (6′-CH_2), 27.62 (7′-CH), 22.35 (8′ and 9′-CH_3); HREIMS m/z (formula, ΔM ppm) 241.1329 $(M + H)^+$, $C_{15}H_{17}N_2O$, +4.8. The structure was confirmed by single-crystal X-ray analysis.

X-ray Crystal Structure Determination of Labradorin 1 (1). A thick, plate-shaped X-ray sample (~0.50 × 0.40 × 0.25 mm) was obtained from an acetone solution. Data collection was performed at 296 ± 2 K. Accurate cell dimensions were determined by least-squares fitting of 25 carefully centered reflections in the range 35° < θ < 40° with an Enraf-Nonius CAD4 diffractometer using graphite-monochromated Cu Kα radiation from a normal focus sealed tube.

Crystal Data: $C_{15}H_{16}N_2O$, M_r = 240.30, monoclinic, $P2_1/c$, a = 5.4920(11) Å, b = 22.975(5) Å, c = 10.787(2) Å, β = 99.29(3)°, V = 1343.2(5) $Å^3$, Z = 4, ρ_c = 1.189 Mg/m^3, μ(Cu Kα) = 0.598 mm^{-1}, λ = 1.54180 Å, $F(000)$ = 512.

All reflections corresponding to a complete quadrant ($0 \le h \le 6$, $0 \le k \le 26$, $-12 \le 1 \le 12$) were collected over the range 3.85° < θ < 65.04° using the $\omega/2\theta$ scan technique. Friedel reflections were also collected (whenever possible) immediately after each reflection. Three intensity control reflections were also measured for every 60 min of X-ray exposure time and showed a maximum variation of −25% over the course of the collection. A total of 4417 reflections were collected. Subsequent statistical analysis of the complete reflection data set using the XPREP[13] program verified that the space group was $P2_1/c$. After Lorentz and polarization corrections, merging of equivalent reflections, and rejection of systematic absences, 2298 unique reflections (R(int) =0.0512) remained, of which 1644 were considered observed ($I_o > 2\sigma(I_o)$) and were used in the subsequent structure determination and refinement. Linear and anisotropic decay corrections were applied to the intensity data as well as an empirical absorption correction (based on a series of ψ-scans).[14] Structure determination was readily accomplished with the direct-methods program SHELXTL.[15] All non-hydrogen atom coordinates were located in a routine run using default values in that program. The remaining H atom coordinates were calculated at optimum positions. All non-hydrogen atoms were refined anisotropically in a full-matrix least-squares refinement using SHELXTL. The H atoms were included, and their U_{iso} thermal parameters were fixed at 1.2 the U_{iso} of the atom to which they were attached and forced to ride that atom. The final standard residual R_1 value for the model labradorin 1, as shown in Figure 1, was 0.0792 for observed data and 0.1074 for all data. The goodness-of-fit on F^2 was 1.062. The corresponding Sheldrick R values were wR_2 = 0.2119 and 0.2427, respectively. A final difference Fourier map showed minimal residual electron density, the largest difference peak and hole being 0.369 and −0.219 e/$Å^3$, respectively. Final bond distances and angles were all within expected and acceptable limits.

Labradorin 2 [2f; 2-*n*-pentyl-5-(3-indolyl)oxazole]. Purification by HSCCD using the Ito Coil in the system hexane-(1500)−ethyl acetate(500)−CH_3OH(300)−water(120, upper phase mobile) readily separated it from the 2-isobutyl analogue. Crystallization from CH_3OH gave small plates (7 mg); mp 130−132 °C (P388 ED_{50} >1 μg/mL); IR (KBr) ν_{max} 3134, 1639, 1570, 1122, 767, 752 cm^{-1}; EIMS m/z 254, 240, 225, 211, 198, 169, 157, 144, 142, 129, 115, 110, 87, 73, 69, 55; ^1H NMR ($CDCl_3$) δ 0.91 (t, 10′-CH_3, J = 6.8), 1.40 (m, 8′ and 9′-CH_2), 1.85 (m, 7′-CH_2), 2.84 (tr, 6′-CH_2, J = 7.8), 7.17 (s, 4′-CH), 7.25 (m, 5 and 6-CH), 7.41 (d, 7-CH, J = 7.3), 7.50 (d, 2-CH, J = 2.4), 7.84 (d, 4-CH, J = 7.3), 8.85 (s, NH). The structure was confirmed by single-crystal X-ray analysis. Other spectral data were determined employing a synthetic sample (see below). The sample slowly turned yellow and decomposed on exposure to air at room temperature.

X-ray Crystal Structure Determination of Labradorin 2 (2f). A thick, plate-shaped X-ray sample (~0.58 × 0.32 × 0.22 mm) was obtained from methanol solution. Data collection was performed at 296 ± 2 K. Accurate cell dimensions were determined by least-squares fitting of 25 carefully centered reflections in the range 35° < θ < 40° with an Enraf-Nonius CAD4 diffractometer using graphite-monochromated Cu Kα radiation from a normal focus sealed tube.

Crystal Data: $C_{16}H_{18}N_2O$, M_r = 254.32.30, monoclinic, $P2_1/c$, a = 11.842(2) Å, b = 8.6230(17) Å, c = 14.183(3) Å, β = 102.93(3)°, V = 1411.6(5) $Å^3$, Z = 4, ρ_c = 1.197 Mg/m^3, μ(Cu Kα) = 0.595 mm^{-1}, λ = 1.54178 Å, $F(000)$ = 544.

All reflections corresponding to a complete quadrant ($-13 \le h \le 13$, $-10 \le k \le 26$, $0 \le l \le 16$) were collected over the range 3.83° < θ < 64.98° using the $\omega/2\theta$ scan technique. Friedel reflections were also collected (whenever possible) immediately after each reflection. Three intensity control reflections were also measured for every 60 min of X-ray exposure time and showed a maximum variation of −0.3% over the course of the collection. A total of 4388 reflections were collected. Subsequent statistical analysis of the complete reflection data set using the XPREP[17] program verified the space group as $P2_1/c$. After Lorentz and polarization corrections, merging of equivalent reflections, and rejection of systematic absences, 2380 unique reflections (R(int) = 0.0573) remained, of which 1985 were considered observed ($I_o > 2\sigma(I_o)$) and were used in the subsequent structure determination and refinement. Linear and anisotropic decay corrections were applied to the intensity data as well as an empirical absorption correction (based on a series of ψ-scans).[18] Structure determination was readily accomplished with the direct-methods program SHELXTL.[19] All non-hydrogen atom coordinates were located in a routine run using default values in that program. The remaining H atom coordinates were calculated at optimum positions. All non-hydrogen atoms were refined anisotropically in a full-matrix least-squares refinement using SHELXTL. The H atoms were included, their U_{iso} thermal parameters fixed at 1.2 the U_{iso} of the atom to which they were attached and forced to ride that atom. The final standard residual R_1 value for the model of labradorin 2, as shown in Figure 2, was 0.0874 for observed data and 0.0972 for all data. The goodness-of-fit on F^2 was 1.098. The corresponding Sheldrick R values were wR_2 = 0.2418 and 0.2567, respectively. A final difference Fourier map showed minimal residual electron density, the largest difference peak and hole being 0.412 and −0.342 e/$Å^3$, respectively. Final bond distances and angles were all within expected and acceptable limits.

***Cyclo*-[L-Pro-L-Leu].** The cyclic peptide was initially obtained as yellow prisms from ethyl acetate−CH_3OH, mp 143−155 °C, which was raised by recrystallization from ethyl acetate to 157−165 °C (reported mp 157−158 °C,[16a] 158−159 °C,[16b] 168−172 °C,[16c] 161−163 °C).[16d] IR (KBr): ν_{max} 3260, 2951, 2877, 1670, 1633, 1433, 1302, 710 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.945 (d, CH_3, J = 6.6); 0.990 (d, CH_3, J = 6.6); 1.52 (m, 1H); 1.95 (m, 6H); 2.345 (m, 1H); 3.54 (m, 2H); 4.05 (d, 1H); 4.070 (t, 1H); 6.05 (s, NH). When compared with an authentic sample by TLC (CH_2Cl_2−2-propanol (95:5)), the peptide gave spots of identical color and R_f value using the spray technique of Nitecki and Goodman.[17]

Deoxycholic Acid (3). The cholanic acid was obtained as colorless needles from ethyl acetate−CH_3OH, mp 173−178 °C (reported mp 176−178 °C); HRMS(APCI−) 391.2819 [$C_{24}H_{39}O_4$, 7.4 ppm]; HRMS(FAB+) 393.2762, [$C_{24}H_{41}O_4$, +11.5 ppm]; ^1H NMR (DMSO-d_6/D_2O) δ 0.57 (18-CH_3); 0.80 (19-CH_3); 0.88 (d, 21-Me, J = 6.3); 2.11 (m, 23-H); 2.25 (m, 23-H); 3.44 (m, 3β-H), 3.85 (s, 12β-H); ^{13}C NMR (DMSO-d_6/D_2O) δ 12.30 (C-18); 16.75 (C-21); 22.90 (C-19); 23.39 (C-15); 25.95 (C-16); 26.83 (C-7); 27.11 (C-6); 28.35 (C-11); 30.59 (C-2); 30.65* (C-22); 30.65* (C-23), 32.84 (C-9); 33.65 (C-1), 34.84 (C-4), 34.90 (C-10); 35.54 (C-8); 35.81 (C-20); 41.47 (C-5); 45.84 (C-14); 46.04 (C-13); 47.31 (C-17); 69.88 (C-3); 71.00 (C-12); 174.99 (C-24). The NMR spectra were identical to those of an authentic sample (ICN Biomedicals, Inc.), and the structure was confirmed by X-ray crystal structure determination.

Isolation of Deoxycholic Acid from Culture Medium. Three liters of culture medium (refer above to Fermentation) was extracted with methylene chloride to yield 13.2 mg of crude material. This fraction was chromatographed on a Zorbax-SB-C18 prep column (10 mm i.d.) in 70:30 acetonitrile−0.05 M acetic acid at 3.5 mL/min, with 10% of the flow diverted to the ELSD. A total of 23 injections (200 μL each of 5 mg/mL) were made, and the fraction corresponding to the deoxycholic acid retention time was collected, pooled, and evaporated to dryness, yielding 1.88 mg. Analysis by HPLC on an analytical Zorbax-SB-C18 column (25 cm) gave a major peak at 6.28 min that had exactly the same retention time as authentic deoxycholic acid. This was further verified by high-resolution mass spectroscopy: APCI$^+$ *m/z* 357.2771 ($C_{24}H_{37}O_2$, error −2.3 mmu, $M^+ - 2H_2O$); FAB$^+$ (glycerol matrix) *m/z* 357.27688 ($C_{24}H_{37}O_2$, error −2.5 mmu, $M^+ - 2H_2O$).

X-ray Crystal Structure Determination of Deoxycholic Acid Hydrate. The X-ray data collection was performed at ambient temperature (298 ± 2 K) on a Bruker AXS SMART 6000 diffractometer with graphite-monochromated Cu Kα radiation (λ = 1.54178 Å) from a normal focus sealed tube. The crystal−detector distance was 4.52 cm; 512 pixel frames of data were collected, in 0.40° steps in either ω or φ, using the MULTIRUN data collection procedure of the SMART[18] software. A total of 3634 frames of data were collected with >99.7% coverage of the total reflections possible to a resolution of 0.83 Å. Data were processed using the SAINT+[19] software employing the narrow frame algorithm, and the integrated data were corrected for absorption using SADABS.[20] The structure was solved and refined using SHELXTL NT[20] software.

A crystal of deoxycholic acid (−0.44 × 0.42 × 0.04 mm) was obtained via crystallization from an ethyl acetate solution: $C_{22}H_{40}O_4 \cdot 2H_2O$, M_r = 410.58, orthorhombic, $P2_12_12_1$, a = 7.2410(2) Å, b = 13.5807(3) Å, c = 25.7385(7) Å, V = 2531.07(11) Å, Z = 4, ρ_c = 1.077 Mg/m^3, μ(Cu Kα) = 0.586 mm^{-1}, λ = 1.54180 Å $F(000)$ = 904. A total of 20 084 reflections were collected. Subsequent statistical analysis of the complete reflection data set using the XPREP[13] program verified the space group as $P2_12_12_1$. After Lorentz and polarization corrections, merging of equivalent reflections, and rejection of systematic absences, 4580 unique reflections (R(int) = 0.1027) remained, of which 2366 were considered observed (I_o, $2\sigma(I_o)$) and were used in the subsequent structure determination and refinement. An absorption correction was applied to the data with SADABS,[21] and the ratio of maximum and minimum effective transmission was 1.000000:0.612023. All non-hydrogen atoms for **3** were located using the default settings of that program. In addition to the presence of one molecule of the parent steroid, two molecules of water were present in the asymmetric unit. Hydrogen atom coordinates were calculated at optimum positions and forced to ride the atom to which they were attached. Anisotropic refinement of the model shown in Figure 3 resulted in a final residual value of 0.0535 for observed data (0.0850 for all data). The difference Fourier map showed insignificant residual electron density, the largest difference peak and hole being +0.283 and −0.153 e/Å^3, respectively. Final bond distances and angles were all within acceptable limits.

Synthesis of Laboradorin 2 [2f; 2-*n*-pentyl-5-(3-indolyloxazole)]. (A) *N*-Caproyltryptamine. Tryptamine base (10 g) was suspended in toluene (100 mL) with stirring and cooling (ice bath) under nitrogen. Triethylamine (10 mL) was added, followed by dropwise addition of a solution of caproyl chloride (9 mL) in toluene (25 mL) over 20 min. When addition was complete, the ice bath was removed and stirring continued overnight. Ethanol (10 mL) was then added to the stirred solution, followed 10 min later by water (200 mL) and vigorous agitation. The water layer was removed and the toluene solution washed with 1 N NaOH, then with water until neutral. The toluene was concentrated to half-volume, diluted with an equal volume of hexane, and left overnight for crystallization, and the *N*-caproyltryptamine (6.56 g, mp 90−92 °C) was collected: HRMS [APCI$^+$] $(M + H)^+$ 259.1794 ($C_{16}H_{23}N_2O$, −6.4 ppm).

(B) 3-Caproylamidoacetylindole. A solution prepared from *N*-caproyltryptamine (5 g) and dichlorodicyanobenzoquinone (8.8 g) in a mixture of THF (225 mL) and water (25 mL) was stirred under nitrogen. The deep red solution was examined by TLC at 5 min intervals, and it was found that the reaction was complete within the first 5 min. The reaction mixture was concentrated to a small volume and diluted with 0.1 N NaOH and shaken vigorously, and the solution was filtered. The solids were washed well with water followed by a small volume of cold ethyl acetate to remove a dark red color, to leave pale pink crystals that were dried (4.11 g) and could be used without further purification in the next step. Recrystallization gave colorless plates: mp 222−224 °C; HRMS [APC1$^+$] $(M + H)^+$ 273.1585 ($C_{16}H_{21}N_2O_2$, −6.6 ppm).

(C) Labradorin 2 (2f). A mixture of 3-caproylamidoacetylindole (4 g) and phosphorus oxychloride (40 mL) was heated and stirred under nitrogen until both had dissolved. Samples were taken at intervals and analyzed by HPLC, until the reaction appeared to be complete. After about 90 min heating at reflux, the reaction mixture was cooled and poured cautiously into a mixture of CH_3OH (50 mL)−water (200 mL)−ice with stirring. The product was extracted with CH_2Cl_2, washed with water, and dried ($MgSO_4$). Evaporation gave a dark brown solid (2.4 g), which was redissolved in CH_2Cl_2. The solution was filtered through silica to remove most of the color and evaporated to give a pale brown solid, which crystallized from CH_3OH−water (carbon) as colorless silky needles, mp 128−132 °C (P388 >10); HREIMS *m/z* (formula, ΔM ppm) 255.1490 ($M + H^+$, $C_{16}H_{19}N_2O$, +0.7); ^{13}C NMR ($CDCl_3$) δ 111.61 (2-CH), 105.93 (3-C), 124.22 (3a-C), 120.83 (4-CH), 122.97 (5-CH), 120.00 (6-CH), 119.73 (7-CH), 136.42 (7a-C), 163.13 (2′-C), 121.71 (4′-CH), 147.38 (5′-C), 28.11 (6′-CH_2), 26.76 (7′-CH_2), 31.22 (8′-CH_2), 22.22 (9′-CH_2), 13.79 (10′-CH_3). The ^{1}H NMR, TLC R_f value, and HPLC retention time were identical with those of the natural product (**2f**).

Acknowledgment. We are pleased to acknowledge the very important financial support provided by Outstanding Investigator Grant CA 44344-04-12 and Grant RO1 CA90441-01 awarded by the Division of Cancer Treatment and Diagnosis, NCI, DHHS; the Arizona Disease Control Research Commission; the Fannie E. Rippel Foundation; the Robert B. Dalton Endowment Fund; Gary L. and Diane R. Tooker; and Dr. John C. Budzinski, Polly Trautman, and Sally Schloegel. For other very helpful assistance we thank the Government of Newfoundland-Labrador (Mr. T. I. Curran, Mr. Cluny Best, and Mr. J. C. Rose), Professors Cherry L. Herald and Fiona Hogan, as well as Drs. J.-C. Chapuis and Michael B. O'Sullivan, Walter C. Benger, and Craig Mueller.

Supporting Information Available: Tables containing full details of the crystal data obtained for labradorins 1 (**1**) and 2 (**2f**) and deoxycholic acid (**3**) hydrate are available free of charge via the Internet at http://pubs.acs.org.

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NP020173X