132. A Complex Pyrrolo-oxazinone and Its Iodo Derivative Isolated from a Tunicate

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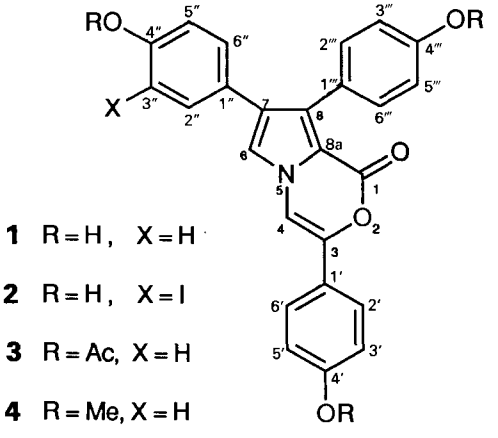
(10.III.92)

Two cytotoxic triphenylpyrrolo-oxazinones were isolated from a tunicate and their structures elucidated by spectral methods. Lukianol A (= *3,7,8-tris(4-hydroxyphenyl)pyrrolo[2,1-c][1,4]oxazin-1(1*H*)-one*; **1**) had *MIC* of 1 μg/ml in *KB* cytotoxicity tests; *MIC* value for **2** was 100 μg/ml.

Introduction. – During the 1989 Pacific cruise of the Soviet research vessel *R/V Akademik Oparin*, we had a rare opportunity to visit *Palmyra* atoll (5° 53′ N, 162° 5′ W), where we collected by *SCUBA* a small sample of an encrusting tunicate in the lagoon at – 25 m. The frozen animal was returned to Hawaii, where it was steeped in EtOH for one

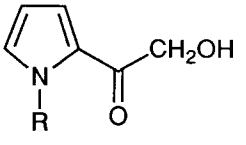


1 R = H, X = H

2 R = H, X = I

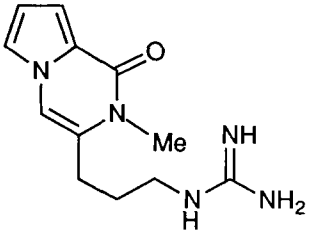
3 R = Ac, X = H

4 R = Me, X = H



5 R = $CH_2CHOHCH_3$

6 R = CH_2CH_2OH



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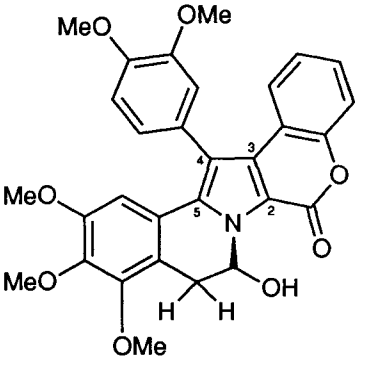

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[1]) From the M.S. Thesis of *K. K. Lee*, University of Hawaii, 1991.

week at room temperature. Aqueous EtOH was decanted and replaced with fresh solvent. The initial extract was partitioned against $CHCl_3$, then BuOH. The $CHCl_3$ residue (79.4 mg) after chromatography yielded lukianol[2]) A (**1**, 7.5 mg) and B (**2**, 0.9 mg). The second EtOH extract furnished 1.6 mg of **1**.

Results. – Lukianol A (**1**) was isolated as a white amorphous powder. An initial ^{1}H-NMR spectrum in (D_6)DMSO revealed six aromatic 2-H *doublets* ($J = 8$ Hz) and three broad exchangeable signals at 9.89, 9.44, and 9.41 ppm, indicative of three *p*-phenol moieties. A molecular formula of $C_{25}H_{17}NO_5$ resulted from HR-MS data of lukianol triacetate (**3**). The ^{13}C-NMR spectrum of lukianol A (**1**) displayed 19 signals, which represented all 25 C-atoms, eighteen of which were assignable to three *p*-phenol moieties and accounted for 12 spectral signals. Of the remaining seven signals, two were from methines (119.9, 103.0 ppm), and five were from quaternary C-atoms (153.5, 140.8, 128.7, 127.3, 111.9 ppm). All ^{1}H- and ^{13}C-NMR data and long-range correlations are shown in *Table1*.

Table 1. *^{13}C-[a]) and ^{1}H-NMR[b]) Data for Lukianol A* (**1**)

C-Atom	^{13}C [ppm]	^{1}H [ppm]	Long-range correlations from HMBC
C(1)	153.5		
C(3)	140.8		
C(4)	103.0	8.06	C(3), C(6), C(8a), C(1′)
C(6)	119.9	7.58	C(4), C(7), C(8), C(8a), C(1″)
C(7)	127.3		
C(8)	128.7		
C(8a)	111.9		
C(1‴)	123.1		
C(2‴)	131.7	7.04	C(8), C(4‴), C(6‴)
C(3‴)	114.5	6.70	C(1‴), C(4‴), C(5‴)
C(4‴)	156.6		
C(5‴)	114.5	6.70	C(1‴), C(3‴), C(4‴)
C(6‴)	131.7	7.04	C(8), C(2‴), C(4‴)
C(1″)	123.8		
C(2″)	129.4	6.94	C(7), C(4″), C(6″)
C(3″)	115.2	6.65	C(1″), C(4″), C(5″)
C(4″)	156.3		
C(5″)	115.2	6.65	C(1″), C(3″), C(4″)
C(6″)	129.4	6.94	C(7), C(2″), C(4″)
C(1′)	121.1		
C(2′)	125.4	7.54	C(3), C(4′), C(6′)
C(3′)	115.8	6.87	C(1′), C(4′), C(5′)
C(4′)	158.5		
C(5′)	115.8	6.87	C(1′), C(3′), C(4′)
C(6′)	125.4	7.54	C(3), C(2′), C(4′)

[a]) Recorded at 125 MHz in (D_6)DMSO. [b]) Recorded at 500 MHz in (D_6)DMSO.

Gated decoupling of both methine signals revealed ^{1}H,^{13}C coupling constants of 190 Hz, appropriate for methines vicinal to a heteroatom [1]. Chemical-shift values of 119.9 for a methine and 128.7, 127.3 ppm for two quaternary C-atoms match the anticipated values

[2]) The name is coined from *lukia*, the Hawaiian word for Russia to acknowledge the 1989 cruise of the Soviet *R/V Akademik Oparin*.

for a 3,4-disubstituted pyrrole [2]. The low-field quaternary C-atom signal at 153.5 ppm is appropriate for a lactone C=O, which was confirmed by a 1733-cm^{-1} IR band of lukianol A trimethyl ether (**4**). The remaining three ^{13}C-NMR signals could be accommodated by assignment to a pyrrolo-oxazinone: 111.9 ppm for C(8a), the C-atom shared by both rings, 140.8 for C(3) bearing oxygen, and 103.0 for methine C(4) adjacent to the N-atom. NOE enhancement between H–C(6) and H–C(2″) was reproduced on a synthetic sample of 3,4-diphenylpyrrole. The proposed structure of lukianol A (**1**) is that of a 3,4-diaryl-*N*-(2-phenylethyl)pyrrole-2-carboxylic-acid derivative. It possessed only moderate activity in the standard bioassay against a cell line derived from a human epidermatoid carcinoma (*KB*).

The minor constituent, lukianol B (**2**), was isolated as an off-white powder from MeOH. NMR data indicated a $C_{25}H_{16}$ framework, which HR-MS analysis expanded to a molecular formula of $C_{25}H_{16}INO_5$. The simplest assumed relationship between the two lukianols, B (**2**) as an iodo-substituted lukianol A (**1**), was reinforced by inspection of the NMR data (*Table 2*), which exhibited many nearly identical signals. Specifically, 2-H *singlets* (7.90, 7.60 ppm) and seven ^{13}C resonances (154.3, 142.7, 130.3, 127.1, 120.7, 113.6, 103.7 ppm) proved that the basic framework of an *N*-alkylpyrrole-2-carboxylic acid of lukianol A (**1**) was present in lukianol B (**2**). Furthermore, four 2-H *doublets* readily attributable to two *p*-phenol moieties were observed: 7.64 and 6.95 ppm (J = 8.8 Hz); 7.17 and 6.81 ppm (J = 8.7 Hz). The NMR signals which distinguished lukianol B from

Table 2. *^{13}C-[a]) and ^{1}H-NMR[b]) Data for Lukianol B* (**2**)

C-Atom	^{13}C [ppm]	^{1}H [ppm]	Long-range correlations from HMBC
C(1)	154.3		
C(3)	142.7		
C(4)	103.7	7.90	C(3), C(6), C(8a), C(1′)
C(6)	120.7	7.60	C(4), C(7), C(8), C(8a), C(1″)
C(7)	127.1		
C(8)	130.3		
C(8a)	113.6		
C(1‴)	124.6		
C(2‴)	132.9	7.17	C(8), C(4‴), C(6‴)
C(3‴)	115.6	6.81	C(1‴), C(4‴), C(5‴)
C(4‴)	157.7		
C(5‴)	115.4	6.81	C(1‴), C(3‴), C(4‴)
C(6‴)	132.9	7.17	C(8), C(2‴), C(4‴)
C(1″)	128.1		
C(2″)	139.9	7.62	C(7), C(4″), C(6″)
C(3″)	84.2		
C(4″)	156.5		
C(5″)	115.5	6.83	C(1″), C(3″)
C(6″)	130.7	7.00	C(2″), C(4″)
C(1′)	123.2		
C(2′)	126.6	7.64	C(3), C(4′), C(6′)
C(3′)	116.5	6.95	C(1′), C(4′), C(5′)
C(4′)	159.4		
C(5′)	116.5	6.95	C(1′), C(3′), C(4′)
C(6′)	126.6	7.64	C(3), C(2′), C(4′)

[a]) Recorded at 125 MHz in (D_6)acetone. [b]) Recorded at 500 MHz in (D_6)acetone.

lukianol A were those of three aromatic protons appropriate for a 1,2,4-trisubstituted benzene, *viz.* 7.62 (J = 2.1 Hz), 7.00 (*dd*, J = 8.3, 2.1 Hz), and 6.83 ppm (*d*, J = 8.3 Hz). The remaining distinguishing feature was a ^{13}C-NMR signal at 84.2 ppm, virtually identical with a value of 85.1 ppm reported for an *o*-iodotyrosine C-atom in geodiamolide A [3]. An HMBC (Heteronuclear Multiple Bond Correlation) experiment (*Table 2*) settled the final point, which of the three phenol rings bears iodine. Correlation between H–C(5″) at 6.83 ppm and C(3″) at 84.2 ppm completed the structure of lukianol B (**2**). It was inactive in the *KB* bioassay.

Discussion. – The basic structural feature of the lukianols, an *N*-alkylpyrrolecarboxylic acid is relatively rare, but not without precedent among natural products. The simplest representatives **5** and **6** were recently reported by *Jiang* and *Gerwick* [4] from a marine red alga, *Gracilariopsis lemaneiformis*. A terrestrial representative of the same system is peramine (**7**), isolated from fungus – infected rye grass, *Lolium perenne* [5]. An intriguing though less obvious structural relationship exists between the lukianols and the lamellarins, *e.g.* lamellarin A (**8**), which have been isolated from mollusks [6] and tunicates [7]. By cleavage of the bond between C(5) of pyrrole and its Ph substituent in lamellarin A (**8**) and by opening and reclosing the lactone ring with the *N*-alkyl chain, one can formally generate a lukianol from a lamellarin.

Experimental Part

Isolation. The frozen animal (10 g) was allowed to stand for 1 week in 100 ml of EtOH. The aq. EtOH soln. was decanted and the process repeated. The first extract was partitioned against $CHCl_3$ and BuOH. The $CHCl_3$ residue (79.4 mg) was chromatographed on *BondElut* (silica, $CHCl_3$/AcOEt/MeOH gradient) followed by HPLC (*Adsorbosphere HSC18 5μ, Alltech*; MeOH/H_2O, 7:3) resulting in *lukianol A* (**1**, 7.5 mg) and *B* (**2**, 0.9 mg). To the second EtOH extract was added 50 ml H_2O. After partitioning with hexane (2 × 75 ml), CH_2Cl_2 (3 × 75 ml), and BuOH (3 × 50 ml), the CH_2Cl_2 residue was subjected to a reversed phase Si flash column (10 g, H_2O to MeOH gradient), then HPLC (*Rainin Microsorb Si*, AcOEt/hexane, 6:4) yielding 1.6 mg of **1**.

Lukianol A (= *3,7,8-Tris(4-hydroxyphenyl)pyrrolo[2,1-c][1,4]oxazin-1(1*H*)-one*; **1**). White amorphous powder from MeOH. UV (MeOH): λ_{max} 206 (log ε 4.52), 282 (4.55), 344 (4.15). NMR: *Table 1*. FAB-MS (glycerol): 412 (MH$^+$). Cytotoxicity: *MIC* against *KB* cells 1 μg/ml.

Lukianol A Triacetate (= *3,7,8-Tris(4-acetoxyphenyl)pyrrolo[2,1-c][1,4]oxazin-1(1*H*)-one;* **3**). Ac_2O (0.1 ml) was added to **1** (1.1 mg) in pyridine (0.1 ml). The mixture was left at r.t. for 30 min. After removal of reagents *in vacuo*, the residue was purified by HPLC (*Alltech Lichrosorb Si60*, hexane/AcOEt 7:3), yielding **3** as a white powder. ^{1}H-NMR ($CDCl_3$): 2.33, 2.30, 2.29 (3 H each). HR-EI-MS: $C_{31}H_{23}NO_8$; calc.: 537.1424; found: 537.1454.

Lukianol A Trimethyl Ether (= *3,7,8-Tris(4-methoxyphenyl)pyrrolo[2,1-c][1,4]oxazin-1(1*H*)-one*; **4**). An excess of freshly prepared ethereal CH_2N_2 was added to a soln. of **1** (1.9 mg) in MeOH (0.25 ml) at r.t. After 30 min, TLC of the mixture (Si, AcOEt/hexane, 3:1) showed the absence of unreacted **1**. The residue after removal of reagents was purified by HPLC (*Phenomenex Ultracarb 50DS30*, MeOH/H_2O, 85:15), yielding **4** as a white powder. IR ($CDCl_3$): 1733. ^{1}H-NMR ($CDCl_3$): 3.88, 3.83, 3.77 (3 H each). HR-EI-MS: $C_{28}H_{23}NO_5$: calc.: 453.1607; found: 453.1577.

Lukianol B (= *3,8-Bis(4-hydroxyphenyl)-7-(4-hydroxy-3-iodophenyl)pyrrolo[2,1-c][1,4]oxazin-1(1*H*)-one*; **2**). Off-white powder from MeOH. NMR: *Table 2*. FAB-MS (glycerol): 538 (MH$^+$). Cytotoxicity against *KB* cells: *MIC* 100 μg/ml.

We thank Academician Prof. *G. B. Elyakov* as well as the Captain and crew of the *R/V Akademik Oparin* for the invitation to join their 1989 Pacific Cruise; *Toshio Ichiba* for the collection; Prof. *Akira Sera* of Kobe University, Japan, for a sample of 3,4-diphenylpyrrole; and Prof. *K. L. Rinehart*, for providing HR-MS data. For financial support, we are grateful to the *National Science Foundation* and to 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.

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