Luisols A and B, New Aromatic Tetraols Produced by an Estuarine Marine Bacterium of the Genus *Streptomyces* (Actinomycetales)

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Luisols A (**1**) and B (**2**), two new aromatic tetraols, have been isolated from the cultivation broth of an estuarine marine actinomycete of the genus *Streptomyces* (strain #CNH-370). The structures of luisols A and B were assigned by combined spectroscopic methods, including extensive 2D NMR experiments. Luisol A appears related to the anthraquinone antibiotics of the granaticin class, while the structure of luisol B contains the rare epoxynaphtho[2,3*c*]furan, a structural feature found in only one natural product, the fungal metabolite anthrinone.

As part of our continuing interest in exploring the chemically prolific bacteria found in estuarine environments,[1] we investigated numerous strains isolated from sandy sediments collected near San Diego in the San Luis Estuary. These samples were rich in actinomycetes and, in particular, *Streptomyces* species. From the fermentation broth[2] of a *Streptomyces* strain, tentatively identified as *S. violaceusniger* [3] (our isolate #CNH-370), we have isolated two new tetraols, luisols A (**1**) and B (**2**).[4] Luisol A (**1**), formally a reduced hydroquinone, appears related to the quinones of the granaticin class, in particular to compounds MM44785[5,6] and nanomycin,[7] which share the same hydroxy-tetrahydropyran-C ring and lactone functionalities. Luisol B is related to anthrinone,[8] the only other natural product possessing the rare epoxynaphtho[2,3*c*]furan ring system.

Luisol A (**1**), the major metabolite, analyzed for $C_{16}H_{18}O_7$ on the basis of its combined HRFABMS ([M^+] *m*/*z* 322.1047, Δ 0.5 mmu) and ^{13}C NMR spectral features (Table 1). The IR spectrum of **1** showed broad absorptions for multiple hydroxyl groups (3412 cm^{-1}) and a lactone carbonyl (1725 cm^{-1}) functionality. The UV spectrum showed an absorption at 275 nm characteristic of phenols. Analysis of NMR data allowed the structure of luisol A to be constructed. Proton COSY correlations revealed three spin systems, one involving aliphatic protons at C-2, C-3, and C-4; one involving the aliphatic protons at C-15 and C-16; and the last involving the aromatic protons at C-8, C-9, and C-10. A characteristic ^1H NMR ABM pattern (in MeOH-d_4) composed of two doublets at δ 6.78 and 6.86 and a doublet of doublets at δ 7.17, were readily assigned to the aromatic ring protons from C-8 to C-10. Four exchangeable proton signals, observed in DMSO-d_6, were assigned to one phenolic hydroxyl proton (δ 9.58) and three aliphatic hydroxyl protons (δ 5.65, 5.65, and 5.12).

Considering the aromatic ring and the lactone functionality, the eight unsaturations inherent in **1** suggested that three additional rings were present. Heterocorrelation NMR methods, specifically HMQC experiments and HMBC measurements, the latter of which showed more than 25 correlations, allowed all carbons and protons in luisol A to be assigned. Four exchangeable hydroxyl protons correlated with their respective carbons at C-6, C-11, C-13, and C-14, indicating the presence of one phenol, two secondary alcohols, and one tertiary alcohol. A strong correlation between the proton at C-3 and C-15 indicated that these carbons formed a cyclic ether. HMBC correlations between H-8 and C-7 and C-12; between H-9 and C-7 and C-11; between H-10 and C-11; between H-6 and C-4, C-5, C-7, and C-8; between H-13 and C-7, C-11, and C-12; between H-15 and C-13 and C-14; between H-4 and C-2, C-5, and C-14; between H-3 and C-1; and between C-15, H-2 and C-1, clearly established the presence of the dihydroquinone structure for luisol A. Because HMBC correlations between

Table 1. ^1H and ^{13}C NMR Assignments for Luisols A and B in DMSO-d_6[a]

carbon no.	Luisol A (1)		Luisol B (2)	
	^{13}C (δ)	^1H (δ, *J*)	^{13}C (δ)	^1H (δ, *J*)
1	169.0 C		63.9 CH_2	3.85, d (9.8 Hz) 4.15, d (9.8 Hz)
2	35.3 CH_2	2.64, d (19 Hz) 2.89, dd (5, 19 Hz)	67.3 C	
3	65.1 CH	4.33, m	64.9 CH	4.90, d (6.8 Hz) 5.56, d (6.8 Hz, 3-OH)
4α 4β	27.6 CH_2	1.71, d (14 Hz) 2.77, dd (3.4, 14 Hz)	138.1 C	
5	80.3 C		121.3 CH	6.85, d (7.8 Hz)
6	72.4 CH	4.25, d (8 Hz) 5.65, d (8 Hz, 6-OH)	128.7 CH	7.15, dd (7.3, 7.8 Hz)
7	136.3 C		115.5 CH	6.75, d (7.3 Hz)
8	121.1 CH	6.78, d (7 Hz)	156.7 C	9.65, s (8-OH)
9	128.6 CH	7.15, dd (7.0, 7.8 Hz)	120.9 C	10
10	115.2 CH	6.78, d (7.8 Hz)	63.1 CH	5.60, d (6.8 Hz) 6.00, d (6.8 Hz, 10-OH)
11	156.5 C	9.58, s	67.4 C	
12	121.9 C		102.1 C	6.28, s (12-OH)
13	67.0 CH	4.51, d (8 Hz) 5.65, d (8 Hz, OH)	22.7 CH_3	1.48 s
14	71.5 C	5.12, s (14-OH)		
15	68.8 CH	3.66, q (6 Hz)		
16	15.6 CH3	1.23, d (6 Hz)		

[a] Assignments made on the basis of DEPT, COSY, HMQC, and HMBC experiments.

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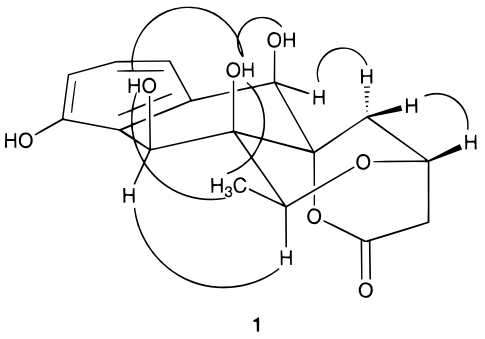


Figure 1.

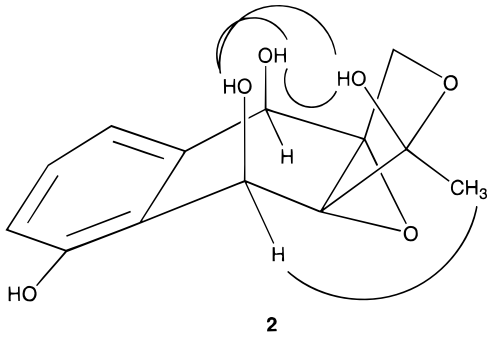
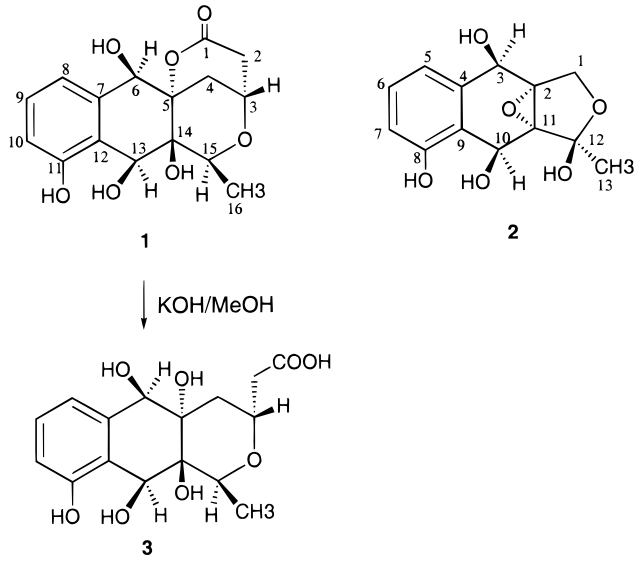


Figure 2.

the C-14 tertiary hydroxyl proton (δ 5.12) and C-14 and C-15 were observed, the remaining oxygen-bearing quaternary carbon (C-5) was assigned as part of the δ-lactone ring. The presence of the lactone was subsequently confirmed by base hydrolysis to yield the corresponding hydroxy acid **3**.[9]



The relative stereochemistry of **1** was assigned by NOE ^{1}H NMR measurements (Figure 1). No data were obtained to assign the absolute stereochemistry at any of the chiral centers. Correlations were observed that placed key protons on either the top or bottom faces of the molecule. Correlations between the C-6 hydroxyl proton and the C-14 hydroxyl proton, as well as correlations from H-4β to H-3, and correlations from the C-13 and C-14 hydroxyl protons to the C-15 methyl group, served to position these protons on the top face of the molecule. Correlations from H-6 to H-4α, and correlations between H-13 and H-15 positioned these protons within spatial proximity. Construction of the molecular model for luisol A clearly showed that the C-14 hydroxyl group and the C-5 lactone oxygen form a *trans*-diaxial bridgehead arrangement in this metabolite.

Luisol B (**2**), the minor metabolite, analyzed for $C_{13}H_{14}O_6$ by HRFABMS ([M$^+$] *m*/*z* 266.0779, Δ 1.1 mmu), a formula substantiated by ^{13}C NMR data (Table 1). The partial structure of **2** from C-3 to C-10 was readily derived by comparison of ^{13}C and ^{1}H NMR spectra of **1** and **2**. The only methyl group in **2**, a singlet, was shifted to lowfield in the ^{1}H NMR spectrum, which indicated that the methyl group was connected to a ketal carbon (C-12, 102.1 ppm). The ^{1}H NMR spectrum of **2** in DMSO-d_6 revealed four exchangeable hydroxyl protons, and no carbonyl group was observed in either the IR or ^{13}C NMR spectra. The seven unsaturation equivalents inherent in the formula of luisol B, coupled with the presence of two quaternary carbons at δ 67.3 and 67.4, suggested the presence of an epoxide ring. Comprehensive NMR analysis showed that the epoxide is located at the bridgehead position, C-2−C-11. HMBC correlations between the C-13 methyl protons and the C-12-hydroxyl proton with C-12; between the C-1 protons and C-2, C-11, and C-12; correlations from the C-3 proton to C-2, C-5, and C-11; and a correlation of the C-10 proton to C-11, served to establish the total structure.

As in luisol A, the relative stereochemistry of **2** was assigned based upon NOE measurements (Figure 2). Enhancements involving protons at C-10 and C-3 with the C-12 hydroxyl proton served to orient these protons on the top face of the molecule anti to the epoxide ring. NOE correlations between the C-12 hydroxyl proton and the C-3 proton, and between the C-13 methyl group and the C-10 hydroxyl proton, verified that the B ring adopts the chair configuration as shown in Figure 2. Data were not obtained to define the absolute stereochemistry of luisol B.

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References and Notes

(1) (a) Lindel, T.; Jensen, P. R.; Fenical, W. *Tetrahedron Lett.* **1992**, *37*, 1327−1330. (b) Toske, S. G.; Jensen, P. R.; Kauffman, C. A.; Fenical, W. *Nat. Prod. Lett.* **1994**, *6*, 303−306. (c) Pathirana, C.; Jensen, P. R.; Fenical, W. *Tetrahedron Lett.* **1992**, *33*, 7663−7666. (d) Cheng, X. C.; Jensen, P. R.; Fenical, W. *J. Nat. Prod.* **1998**, in press.

(2) The producing strain was cultured in 2.8-L Fernbach flasks containing 1 L of 1.0% soluble starch, 0.4% yeast extract, 0.2% peptone, 0.1% $CaCO_3$, 100% seawater, at 28 °C for 10 days. A 20-L cultivation was extracted with EtOAc, and the solvent was concentrated to produce 1.2 g of crude extract. The extract was then subjected to Si gel chromatography, using a solvent gradient (isooctane−EtOAc), followed by silica HPLC using 100% EtOAc to yield 21.0 mg of luisol A (**1**) and 8.8 mg of luisol B (**2**).

(3) Strain, CNH-370 showed a weak match with *Streptomyces violaceusniger* on the basis of fatty acid methyl ester analysis (FAME). The similarity index recorded, 0.419 (Microbial ID, Inc., Newark, DE), indicated that this strain could not be assigned with security.

(4) For luisol A (**1**): colorless gum; $[\alpha]^{25}_D$ −29.2° (*c* 0.5, MeOH); IR (film): 3412, 2919, 1725, 1595, 1460, 1243, 1049 cm^{-1}; UV λ_{max} (MeOH) 275 nm (ϵ 1960). For luisol B (**2**): colorless crystal, mp 125−127 °C; $[\alpha]^{25}_D$ −2.4° (*c* 2.0, MeOH); IR (film) 3325, 1586, 1464, 1260, 1161, 975, 754 cm^{-1}; UV λ_{max} (MeOH): 275 nm (ϵ 1500).

(5) Gilpin M. L.; Box, S. J.; Elson. A. L. J. *Antibiotics* **1988**, *41*, 512−518.

(6) St. Pyrex, J. S.; Achmatiwicz, O.; Zamojski, A. *Tetrahedron* **1977**, *33*, 673−680.

(7) Cutrone, J. Q.; Gao, Q.; Huang, S.; Klohr, S. E.; Veitch, J. A.; Shu, Y. Z. *J. Nat. Prod.* **1994**, *57*, 1656−1660.

(8) Iwai, Y.; Kimura, K.; Takahashi, Y.; Hinatozawa, K.; Shimizu, H.; Tanaka, H.; Omura, S. J. *Antibiotics* **1983**, *36*, 1268−1274.
(9) Luisol A acid (**3**) was prepared by base hydrolysis of luisol A (**1**). Luisol A (39.6 mg) was treated with 200 mg of KOH in 1 mL of MeOH at room temperature for 20 min. EtOAc extraction of the reaction mixture, followed by H_2O wash and concentration of the EtOAc layer in vacuo, yielded the acid **3**, which was further purified by preparative Si TLC to give 22.5 mg of **3**. Acid **3** showed the following spectral features: LRFABMS for $[M + Na]^+$ m/z = 363 amu (for $C_{16}H_{20}O_8$-Na); IR (film) 3377, 2954, 1713, 1648, 1466, 1266, 1047 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.13 (dd, 8 Hz), 6.81 (d, 7.6 Hz), 6.75 (d, 8.0 Hz), 4.77 (s), 4.55 (m), 4.21 (q, 6. 5 Hz), 4.14 (s), 2.96 (dd, 6, 15 Hz), 2.69 (dd, 5, 15 Hz), 1.44 (d, 15 Hz), 1.29 (d, 6.5 Hz).

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