Arenaric Acid, a New Pentacyclic Polyether Produced by a Marine Bacterium (Actinomycetales)

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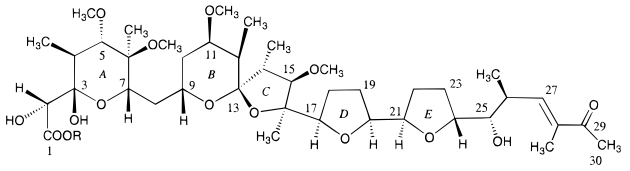
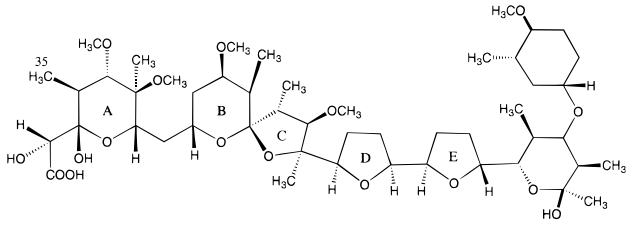
Arenaric acid (**1a**), a new pentacyclic polyether related to the antibiotics K-41A and oxolonomycin, was isolated as its sodium salt (**1b**) from the culture broth of an estuarine bacterial isolate of the genus *Streptomyces*. The structure of arenaric acid was established by spectroscopic methods involving comprehensive 2D NMR measurements.

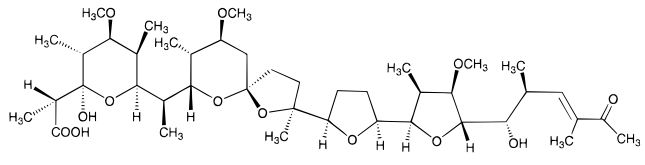
As part of continuing interest in assessing the secondary-metabolite chemistry of actinomycete strains isolated from marine estuaries,[1] we evaluated the actinomycetes present in several sediment samples collected from the north of San Diego in an estuary near Doheny Beach. One actinomycete isolated from these shallow sandy sediments, a *Streptomyces* sp. (isolate no. CNH-248), was observed to produce a new pentacyclic polyether, arenaric acid (**1a**),[2] which is related to two known polyether antibiotics, K41-A (**2**)[3−5] and oxolonomycin (**3**),[6] respectively.



Arenaric Acid, R = H (**1a**)
Arenaric Acid Salt, R = Na (**1b**)
Arenaric Acid Methyl Ester, R = CH_3 (**1c**)



K-41A (**2**)



Oxolonomycin (**3**)

The EtOAc extract of a 20-L culture of *Streptomyces* sp. was subjected to silica flash chromatography followed by C_{18} reversed-phase HPLC, using 4:1 MeOH-*N*-H_2O, to afford arenaric acid sodium salt (**1b**) at a purified yield of ca. 2 mg/L. Arenaric acid sodium salt was obtained as colorless crystals, mp 106−108 °C, which analyzed for $C_{41}H_{67}O_{15}Na$, a formula requiring 8 degrees of unsaturation. The IR spectrum of **1b** showed the presence of multiple broad hydroxyl group absorptions (3400 cm^{-1}), a carbonyl band from a carboxylic acid salt (1590 cm^{-1}), and a carbonyl absorption from an α,β-unsaturated ketone (1663 cm^{-1}). Acidification of **1b** produced arenaric acid (**1a**), which showed typical IR bands for the carboxylate carbonyl group (1740 cm^{-1}). The NMR data for **1b** (Table 1) showed the presence of eight methyl groups, six methylene carbons, four methine carbons, four methoxy groups, 11 ether (or hydroxyl) methine carbons, two quaternary ether (or hydroxyl) carbons, two carbonyl carbons, one hemiketal carbon, one ketal carbon, and two olefinic carbons. These groups accounted for all the hydrogens in **1b** except for three hydroxyl groups on carbons with ^{13}C shifts at δ 71.3, 74.5, and 99.3. The signal at δ 99.3 was assigned to a hemiketal carbon, and the signals at δ 71.3 and 74.5 were assigned as secondary hydroxyl-bearing carbons.

COSY NMR data allowed five independent spin systems to be identified in arenaric acid sodium salt (**1b**). One spin system consisted of the C-4 methyl group, which correlated to the C-4 proton, which itself correlated to the C-5 proton. Another series of correlations allowed the protons at C-7 to be correlated along the chain through C-8, C-9, C-10, C-11, and last to the proton and methyl group at C-12. The C-14 methyl group showed correlations to the proton at C-14, and the latter correlated to the proton at C-15. A five-proton spin system was observed involving the methylene and methine protons from C-19 to C-23, and another spin system was identified in which the methine proton at C-25 correlated to the C-26 proton, and the C-26 proton to the C-26 methyl group and C-27 olefinic proton.

HMBC data, which showed correlations between protons and carbons from C-1 to C-17, allowed rings A, B, and C to be fully constructed. The presence of methoxy groups at C-5, C-6, C-11, and C-15 assisted in identifying these linkages by HMBC methods. Additional HMBC assignments were possible from C-24 to C-30, thus completing the terminal end of the molecule. The region involving rings D and E was difficult to analyze because of significant proton and carbon signal overlap. However, given the chemical shifts of these overlapping bands and the very favorable comparison of these data with the NMR data for antibiotic K-41A (**2**), the region from C-17 to C-24 was assigned the same bis-tetrahydrofuran linkage as in **2**. Arenaric acid sodium salt (**1b**) and antibiotic K-41 (**2**)

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Table 1. ^{1}H and ^{13}C NMR Data for Arenaric Acid Sodium Salt (1b) and Partial ^{13}C NMR. Data for Antibiotic K-41A (2) in $CDCl_3$

	arenaric acid sodium salt (**1b**)		K-41A(**2**)
carbon no.	^{13}C shifts[a]	^{1}H shifts[b]	^{13}Cshifts[c]
1 COONa	179.7		178.9
2O−CH	71.3	3.94 s	71.8
3 O−C−O	99.3		98.3
4 CH	38.6	2.15 m	39.0
5 O−CH	85.9	3.28 d (11.2 Hz)	85.4
6 C−O	78.1		78.2
7 CH−O	67.4	3.74 t	66.7
8 CH_2	31.7	1.54 m	31.0
9 O−CH	61.8	3.83 m	61.3
10 CH_2	30.9	1.16, 2.14 m	30.4
11 OCH	79.9	3.40 m	79.6
12 CH	37.1	1.82 d (3.4 Hz)	38.6
13 O−C−O	107.4		106.9
14 CH	46.9	2.10 m	46.1
15 O−CH	94.5	3.48 d (9.7 Hz)	94.6
16 C−O	84.6		83.3
17 O−CH	82.9	3.78 m	82.9
18 CH_2	25.8	1.97, 2.03 m	25.5
19 CH_2	23.1	1.69 m	23.0
20 O−CH	79.3	3.91 m	79.2
21 O−CH	78.6	4.55 m	79.3
22 CH_2	29.6	1.45 m	29.2
23 CH_2	24.5	1.69 m	24.2
24 O−CH	83.3	4.06 m	83.6
25 O−CH	74.5	4.02 dd (9.2, 4.8 Hz)	74.2
26 CH	36.8	2.60 m	
27 CH═	145.3	6.74 d (9.7 Hz)	
28 C═	136.1		
29 C═O	200.7		
30 CH_3	25.6	2.28 s	
4-Me	12.2	1.03 d (6.8 Hz)	12.6
6-Me	10.9	1.12 s	10.9
12-Me	12.8	0.98 d (6.8 Hz)	12.7
14-Me	11.9	1.02 d (6.8 Hz)	12.3
16-Me	28.4	1.58 s	28.4
26-Me	17.5	1.06 d (6.8 Hz)	
28-Me	11.2	1.72 s	
5-OMe	61.6	3.52 s	61.0
6-OMe	50.9	3.35 s	50.8
11-OMe	58.6	3.42 s	59.2
15-OMe	60.2	3.38 s	60.2

[a] Recorded at 100 MHz. Assignments are by DEPT, HMQC and HMBC methods. [b] Recorded at 500 MHz. Assignments are by COSY, HMQC and HMBC methods. [c] Recorded at 100 MHz. Assignments are by comparison with the data previously reported.[3,4]

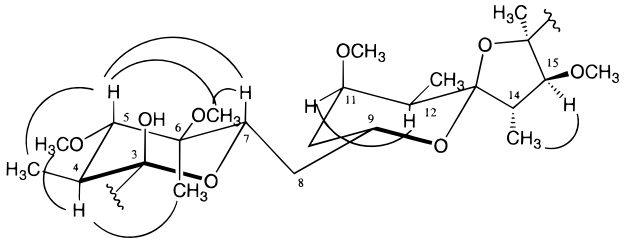


Figure 1. NOE correlations observed within the A, B, and C rings of arenaric acid sodium salt (**1b**).

showed virtually identical ^{13}C NMR chemical shifts from C-1 to C-25 (Table 1).

The relative stereochemistry of the substituents in the A and B rings of arenaric acid was confidently assigned on the basis of NOESY correlation experiments (Figure 1). Correlations were observed between the proton at C-4 with those of the C-5 methoxy and C-6 methyl groups, placing those substituents beneath the ring. Correlations were observed between the C-4 methyl group and the proton at C-5. The C-5 proton showed further NOE correlations to

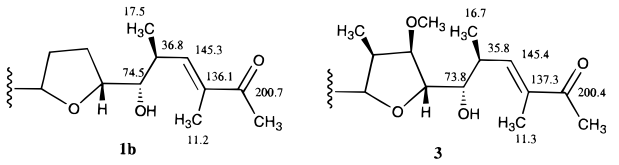
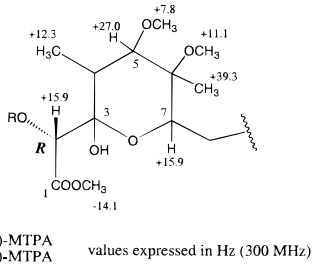


Figure 2. Comparison of selected ^{13}C NMR assignments between arenaric acid sodium salt (**1b**) and oxolonomycin (**3**).



1d, R_1=(*S*)-MTPA
1e, R_1=(*R*)-MTPA
values expressed in Hz (300 MHz)

Figure 3. Mosher analysis of arenaric acid methyl ester (*R*)- and (*S*)-MTPA esters (**1d**, **1e**).

the C-6 methoxy and to the proton at C-7, thus establishing these substituents on the top face of the chair tetrahydropyran ring. In ring B, correlations between the methine protons at C-11 and C-12 showed their respective proximities beneath the ring. An additional NOE correlation defined the cis relationship between the C-14 methyl group and the C-15 proton in ring C.

The observed proton coupling constants between the C-4 and C-5 protons (axial−axial, 11.2 Hz) and between the C-11 and C-12 protons (equatorial−axial, 3.4 Hz) are consistent with the NOE data. Overall, these data indicate that **1** possesses the same relative stereochemistry in the A, B, C, D, and E rings as those of the K-41A polyether group.

The relatively highfield chemical shift of the C-28 methyl group (11.2 ppm), which compares favorably to the carbon NMR shifts from the same methyl group in tiglic acid and to the methyl group of a similar constellation in the antibiotic CP-80,219, indicated the double bond of the terminal α,*β*-unsaturated ketone in **1b** was in the *E* configuration.[7] Based upon comparison of their respective ^{13}C NMR data (Figure 2), the stereochemistry of the substituents at C-25 and C-26 are suggested to be the same as those found in oxolonomycin (**3**), a degradation product of lonomycin.[6]

The absolute stereochemistry of ring A in **1a** was determined using the modified Mosher's method.[8] Esterification of **1a** with diazomethane gave methyl ester **1c**, which was converted to the C-2 (*S*)- and (*R*)-MTPA esters **1d** and **1e.** Using established methods, the ^{1}H NMR signals of the two esters, **1d** and **1e,** were assigned. Analysis of the $\Delta\delta$ values ($\delta S - \delta R$, Hz) established that the absolute stereochemistry of the secondary hydroxyl group at C-2 in **1a** was *R* (Figure 3). Because the absolute stereochemistry at C-2 in K41 is also *R*, the absolute stereochemistry of the 19 stereocenters in **1a** are suggested to be the same as those of K41. The secondary hydroxyl group at C-25 in **1a** could not be converted to the corresponding MTPA esters apparently due to steric constraints. Hence, Mosher analysis of the absolute stereochemistry at C-24 to C-26 could not be accomplished.

Experimental Section

Producing Organism Isolation and Identification. The producing strain (CNH-248) was isolated using standard serial dilution techniques from an intertidal marine sediment collected from San Juan Creek, Doheny Beach, CA. The isolation

medium (A1) consisted of 1.0% starch, 0.4% yeast extract, 0.2% peptone, 1.7% agar, 75% seawater, and 25% deionized water enriched with cyclohexamide and rifampicin at final concentrations of 50 and 5 μg/mL, respectively. Strain CNH-248 showed a weak match to *Streptomyces rochei* based on a fatty acid methyl ester analysis (Microbial ID, Inc., Newark, DE) similarity index of 0.341.

Cultivation of *Streptomyces* sp. CNH-248. A 20-L production fermentation was performed in 2.8-L flasks shaking at 230 rpm for 7 days at 25 °C using modified A1 medium (1.0% starch, 0.4% yeast extract, 0.2% peptone, 0.1% calcium carbonate, 100% seawater).

Purification of Arenaric Acid Sodium Salt (1a). The fermentation broth was extracted with EtOAc and the extract concentrated under vacuum. The crude extract was subjected to silica vacuum flask chromatography using gradient elution (100% isooctane to 100% EtOAc). Arenaric acid could be visualized by silica TLC as a UV active spot (R_f 0.6, EtOAc), and final purification was performed using reversed-phase HPLC (Dynamax preparative column, C-18, 60 Å, 21.4 mm, × 250 mm) with 4:1 MeOH−H_2O, yielding 40 mg arenaric acid sodium salt (**1b**). The compound showed the following spectral characteristics: $[\alpha]^{25}_D = -17.6°$ (*c* 1.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 230 nm (4.538), IR (film, KBr) 3437, 2939, 1661, 1617, 1457, 1361, 1101, 948, 756, cm^{-1}; HRFABMS $[M + H + 2Na]^+$ m/z = 845.4277, calcd for $C_{41}H_{67}O_{15}Na_2$, 845.4257.

Preparation of Mosher Ester Derivatives of Arenaric Acid Methyl Ester (1d, 1e): (*S*)-MTPA Ester 1d. To a solution of **1a** (15 mg, 0.018 mmol) in MeOH (1 mL) was added excess CH_2N_2 in ether (2 mL) at 0 °C. After stirring at room temperature for 3 h the solution was concentrated to give the methyl ester **1c**. A mixture of **1c** (15 mg, 0.018 mmol), (*S*)-MTPA (10 mg, 0.042 mmol), dicyclohexylcarbodiimide (13 mg, 0.064 mmol), and 4-(dimethylamino) pyridine (3.6 mg, 0.03 mmol) in CH_2Cl_2 (1 mL) was stirred at room temperature overnight, diluted with saturated aqueous $NaHCO_3$ (5 mL), and extracted with EtOAc (3 × 10 mL). The combined extracts were dried (Na_2SO_4) and concentrated. The residue was dissolved in EtOAc (1 mL) and passed through a small column of silica and then washed with EtOAc (20 mL). The washings were combined and concentrated. The residue was purified by normal-phase HPLC (silica, EtOAc−hexane 4:1) to give (*S*)-MTPA ester **1d** (12.0 mg, 80%) as a colorless solid.

(*R*)-MTPA Ester 1e. The same experimental procedure was followed as described for the (*S*)-MTPA ester **1d**.

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References and Notes

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