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Xantholipin, a novel inhibitor of HSP47 gene expression produced by *Streptomyces* sp.

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Abstract—In the course of our screening program to identify inhibitors of HSP47 gene expression, we obtained a novel aromatic substance from *Streptomyces* sp. named Xantholipin. Its structure was determined based on physicochemical data of Xantholipin and its derivatives. The absolute configuration was also established by the modified Mosher's method.

Heat shock proteins (HSPs) are proteins that are synthesized in response to heat shock or other environmental stresses and are known to play important roles in protein assembly.[1] Among them, HSP47 plays an essential role as a molecular chaperone during the biosynthesis and secretion of procollagen molecules in the endoplasmic reticulum.[2] The up-regulation of HSP47 and the subsequent synthesis of collagen molecules have been observed in an experimental model of interstitial fibrosis.[3] Similar results were observed in an experimental model of liver fibrosis in rat induced by carbon tetrachloride.[4] Furthermore, the overexpression of HSP47 and accumulation of extracellular matrix, predominantly collagen molecules, was demonstrated in an experimental model of glomerulonephritis.[5] Antisense oligonucleotides against HSP47 have been shown to suppress collagen accumulation in experimental glomerulonephritis.[6] Thus, we attempted to identify a modulator or inhibitor of HSP47 gene expression for use in the treatment of some fibrotic diseases.

To accurately detect HSP47 gene expression, we used a dual luciferase assay system. We constructed a Balb 3T3 cell, which was co-transfected with the firefly luciferase reporter gene downstream of the HSP47 promoter gene (experimental vector) and the pRL-SV40 vector which includes the Renilla luciferase reporter gene (internal control vector).[7] Using this dual system, we can distinguish the activity of HSP47 transcription from the viability of cells. In the course of screening using this system, we identified a novel aromatic compound named Xantholipin (**1**). In this paper, we describe the fermentation, isolation, structure determination and biological activities of **1**.

A Xantholipin-producing organism, identified as *Streptomyces* sp., was isolated from a soil sample collected at Shandong province, China. To produce **1**, this organism was cultured in seed medium[8] for 3 days at 28°C on a rotary shaker at 200 rpm. This seed medium was then inoculated into production medium[9] and cultured for 6 days at 28°C on a rotary shaker at 200 rpm. The culture broth (10 L) was centrifuged and mycelia were extracted with acetone. The supernatant was passed through a column of Diaion HP-20. After washing with water, the absorbed material was eluted with acetone. The acetone layer was combined and concentrated in vacuo. The resulting residue was extracted with *n*-BuOH and concentrated to give an oily residue (10.54 g). This *n*-BuOH extract was applied to a silica gel column. The active material eluted with $CHCl_3$–MeOH (19:1) was further purified by ODS column chromatography eluting with acetone–H_2O (1:1). Active fractions were combined and dried up to give Xantholipin (**1**, 355.2 mg) as a yellow powder.[10]

Keywords: Xantholipin; HSP47; xanthone antibiotics.

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The HRFAB-MS of **1** showed a quasi-molecular ion peak at 590.0267 $[M+K]^+$, corresponding to a quasi-molecular formula of $C_{27}H_{18}ClNO_{10}K$ (calcd for $C_{27}H_{18}ClNO_{10}K$: 590.0256). IR absorption at 3438, 1706 and 1624 cm^{-1} indicated the presence of hydroxyl, carbonyl and ester or amide groups. ^{13}C and ^1H NMR spectral data of **1** are shown in Table 1. In the ^{13}C NMR spectrum of **1**, three carbonyl carbons were observed at 194.9, 192.4 and 181.3 ppm. The ^{13}C NMR spectrum also indicated the presence of a methoxyl group (δ 61.4). The ^1H NMR spectrum suggested the presence of a 1,2,3,4-tetrasubstituted benzene ring system (δ 7.52 (1H, d, $J=8.6$ Hz), δ 7.78 (1H, d, $J=8.6$ Hz)). Together with the following HMBC correlations, these findings indicated partial structure A (Fig. 2): H-3/C-1, H-3/C-5, H-3/C-10, H-2/C-4, H-2/C-6 and H-OCH_3/C-6. Analysis of the COSY spectrum of **1** indicated the connection of C-15 through C-17, and further analysis of the HMBC spectrum of **1** indicated partial structure B (Fig. 2) based on the following correlations: H-28/C-14, H-28/C-15, 11-OH/C-9, 11-OH/C-11, 11-OH/C-12, 18-OH/C-12, 18-OH/C-17, 18-OH/C-18, 18-OH/C-19, H-16/C-22, H-17/C-22, H-23/C-20, H-23/C-21, H-23/C-22, H-23/C-24, H-23/C-27, H-27/C-23 and H-27/C-24.

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data of Xantholipin (**1**) in $CDCl_3$

Position	δ_C	δ_H
1	133.4	
2	125.5	7.52 (d, $J=8.6$ Hz)
3	120.5	7.78 (d, $J=8.6$ Hz)
4	120.2	
5	150.0	
6	144.5	
8	142.9	
9	106.9	
10	181.3	
11	148.3	
12	116.3	
13	131.8	
14	132.5	
15	69.0	5.34 (dd, $J=6.1$, 9.8 Hz)
16	22.2	2.67 (m)
		2.25 (m)
17	53.8	3.68 (m)
18	75.8	
19	192.4	
20	120.7	
21	144.6	
22	194.9	
23	98.5	6.12 (s)
24	152.8	
25		12.20 (brs)
26	159.7	
27	19.2	2.17 (s)
28	91.4	5.56 (d, $J=6.1$ Hz)
		5.50 (d, $J=6.1$ Hz)
11-OH		12.30 (s)
18-OH		6.42 (s)
6-OCH_3	61.4	4.01 (s)

The chemical shift value of the phenolic hydroxyl proton at C-11 (δ 12.30) suggested the presence of a hydrogen bond with the carbonyl group, so partial structure B was connected to partial structure A at C-8 and C-9, as shown in Figure 1. The resulting substructure was similar to that of the known compound Lysolipin I (**2**)[11] produced by *Streptomyces* sp., and a comparison of the xanthone moieties in the ^{13}C NMR spectra indicated that the chloride atom was connected at C-1. The ^{13}C NMR chemical shift value of the remaining carbonyl group (δ 159.7), together with the IR absorption at 1624 cm^{-1} indicated the presence of an amide moiety between C-20 and C-24. To determine the structure of the amide moiety, methylation of **1** was performed. Compound **1** was treated with methyl iodide and silver oxide in DMF at room temperature for 30 min to give *N*-methylated compound **3** (80% yield). The HMBC spectrum of **3** showed correlation peaks from H-NMe (δ 3.58) to C-24 and C-26 (Fig. 3). A correlation peak between H-NMe and H-27 was also seen in the ROESY spectrum. These results indicated the connectivity of C-24 to the amide nitrogen. Thus, **1** was determined to have a planar structure, as shown in Fig. 1. The relative configuration of **1** was also determined to be 15*R**,17*S**,18*R** based on the following ROESY correlations: H-15/H-17, H-15/18-OH, H-17/18-OH.

The absolute configuration of **1** was determined by the modified Mosher's method.[12] Compound **1** was treated

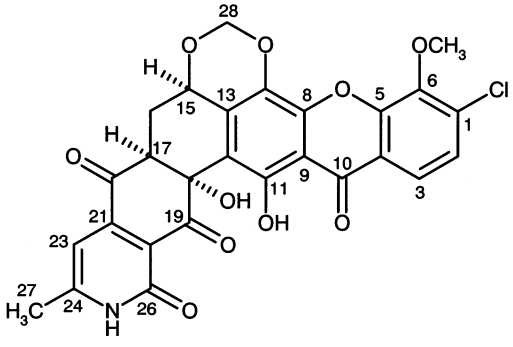


Figure 1. Structure of Xantholipin (**1**).

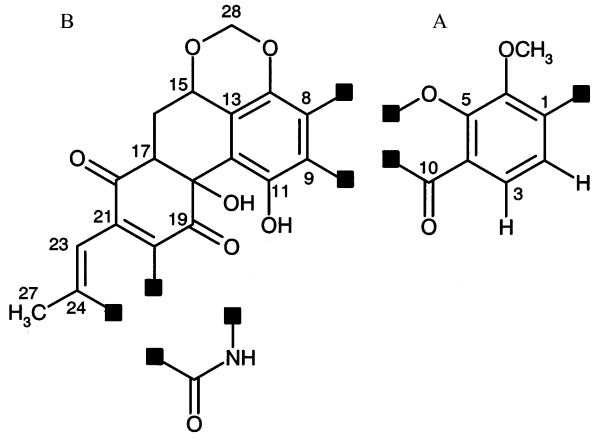


Figure 2. Partial structures of **1**.

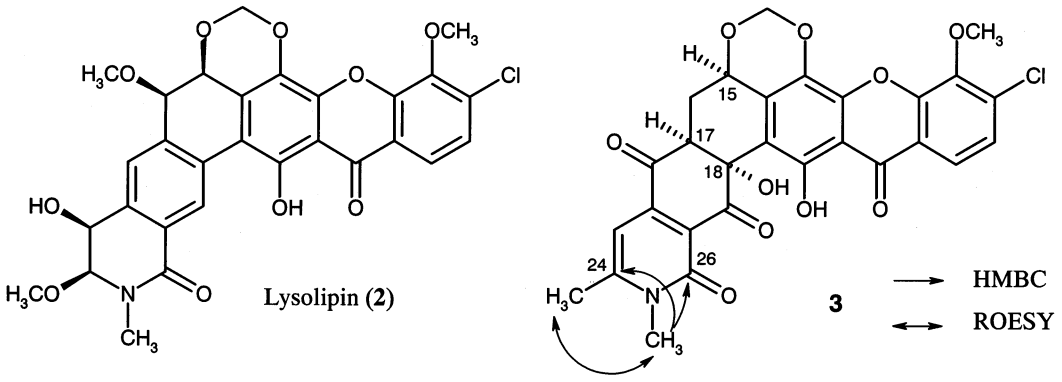


Figure 3. Structures of Lysolipin I (**2**) and *N*-methylated compound of **1** (**3**).

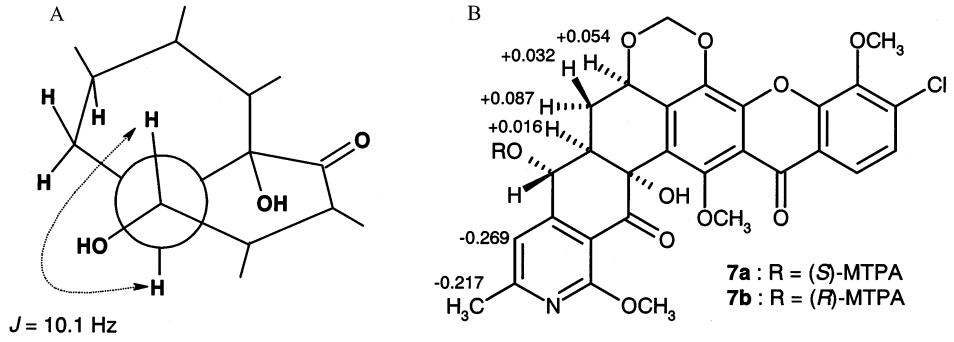
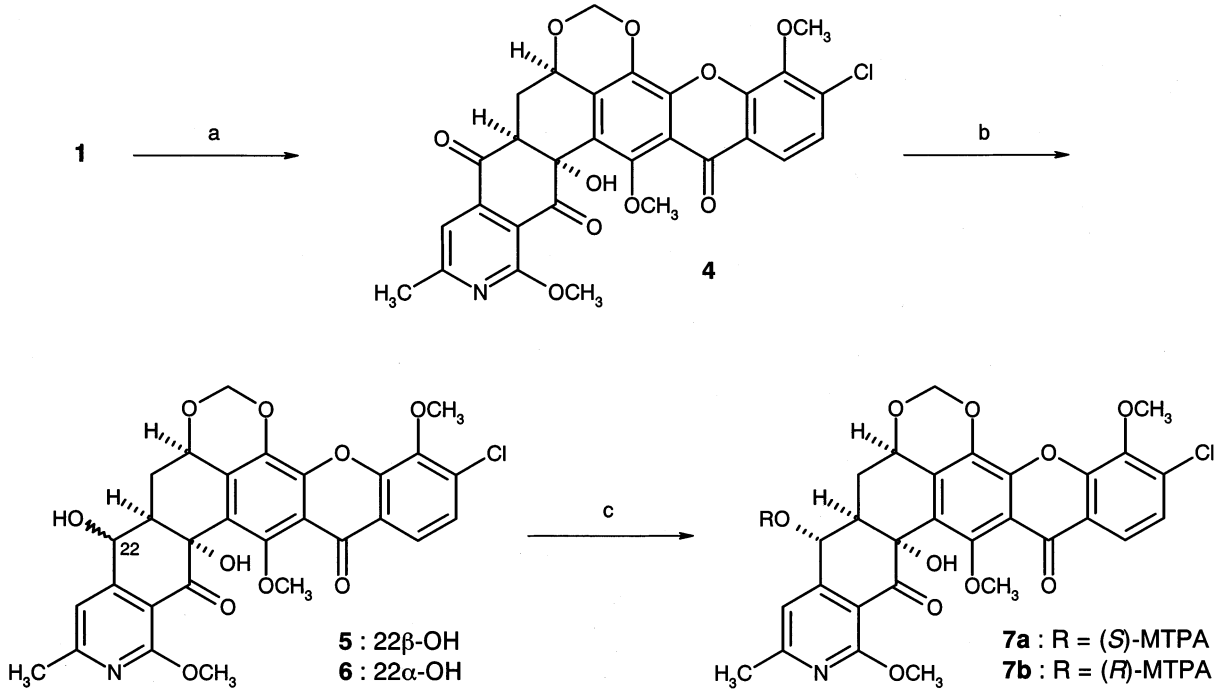


Figure 4. Relative configuration of **6** and $\Delta\delta$ values [$\Delta\delta$ (ppm) $= \delta_{7a} - \delta_{7b}$].



Scheme 1. *Reagents and conditions*: (a) CH_3I, Ag_2O, $CHCl_3$, rt, 12 h (81%); (b) $NaBH_4$ (1.2 equiv.), MeOH, 0°C, 30 min (**5**: 60%, **6**: 29%); (c) (*R*)-(−)- or (*S*)-(+)-MTPACl, pyridine, rt, 12 h (**7a**: 70%, **7b**: 58%).

with methyl iodide to give dimethylated compound **4**. Reduction of **4** with sodium borohydride gave compounds **5** (22β-OH) and **6** (22α-OH) in a ratio 2:1, respectively, the structures of which were assigned based on 1D and 2D NMR data. The relative configuration was also determined by analyzing the coupling constant between H-17 and H-22, as shown in Fig. 4(A).

To determine the absolute configuration of **6** at C-22, **6** was treated with (*R*)-(−)- or (*S*)-(+)-2-methoxy-2-trifluoromethyl-2-phenylacetyl chloride (MTPACl) to give (*S*)- and (*R*)-MTPA esters at 22-OH (**7a** and **7b**), as shown in Scheme 1. $\Delta\delta$ shift values ($\delta_{\mathbf{7a}}-\delta_{\mathbf{7b}}$) obtained from ^{1}H NMR data of **7a** and **7b** (Fig. 4B) indicated a 22*R*-configuration. Thus, the absolute configuration of **1** was established to be 15*R*,17*S*,18*R* (Fig. 1). Compound **5** was determined to be unsuitable for the modified Mosher's method-based on an analysis of $\Delta\delta$ values obtained from ^{1}H NMR data of (*S*)- and (*R*)-MTPA esters of **5**. No systematic $\Delta\delta$ values were observed because MTPA groups seemed to be sterically compressed and take the conformation different from the ideal one.[12]

In our screening system described above, **1** inhibited the HSP47 gene expression with an IC_{50} value of 0.20 μM. Next, we evaluated the ability of **1** to inhibit collagen production against human dermal fibroblast. Compound **1** potently inhibited collagen production induced by treatment with TGF-β with an IC_{50} value of 27 nM, and also showed cytostatic activity[13] with an IC_{50} of 80 nM.

Several similar xanthone antibiotics have been reported previously, including Lysolipin I, Albofungine,[14] Actinoplanones,[15] Cervinomycins[16] and LL-D42067α.[17] With regard to Lysolipin I, the mechanism of antibiotic activity has been shown to involve the inhibition of cell wall synthesis. Compound **1** and its derivatives are currently being tested against several human tumor cell lines, and detailed investigations of their mechanism of action are also in progress. The results will be reported elsewhere.

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8. The seed medium was composed of soluble starch 2.5%, glucose 1.0%, fish meal 0.5%, Pharmamedia 0.3%, NZ case 0.3%, yeast extract 0.2% and $CaCO_3$ 0.2%.
9. The production medium was composed of oatmeal 2%, glucose 1%, dextrin 2.5%, Pharmamedia 1%, fish meal 0.5%, beet molasses 0.5%, Ebios 0.3% and $CaCO_3$ 0.2%. The pH was adjusted to 7.0.
10. $[\alpha]_D=-303.8°$ (*c* 0.53, dioxane); UV λ_{max} nm (log ε) MeOH: 394.0 (4.03), 312.0 (4.12), 271.5 (4.47), 244.5 (4.54); IR ν_{max} (KBr) cm^{-1}: 3438, 1706, 1624; HRFAB-MS (*m*/*z*): 590.0267 $(M+K)^+$ (calcd for $C_{27}H_{18}ClNO_{10}K$: 590.0256).
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