1-Hydroxy-4-methoxy-2-naphthoic Acid, a Herbicidal Compound Produced by *Streptosporangium cinnabarinum* ATCC 31213†

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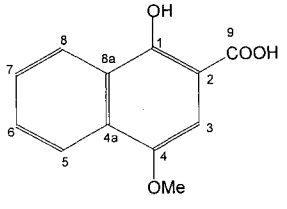
Streptosporangium cinnabarinum ATCC 31213 was included in our screening program for detection and isolation of new secondary metabolites. Extracts of streptosporangia strains were analyzed by HPLC coupled with diode array multiwavelength monitoring (HPLC-DAD) and a UV-visible absorbance spectral library database[2]. Two antibiotics, compounds 43,334 and 43,596, are produced by this strain, designated *Streptosporangium cinnabarinum* Routien sp. nov.[3] when subjected to submerged aerobic fermentation.

In addition to these compounds, *S. cinnabarinum* ATCC 31213 produced a substance which was detected in the culture filtrate by HPLC-DAD analysis (HP 1090M liquid chromatograph equipped with a built-in diode array detector and HPLC^{3D}-ChemStation, Hewlett-Packard). This compound was not identified by spectral matching techniques using our HPLC-UV-Vis database. Isolation and structure elucidation resulted in determination of the compound as 1-hydroxy-4-methoxy-2-naphthoic acid (**1**, Fig. 1) with a mass of 218, that has not previously been described as a natural product, but synthesized and characterized by methylation of 1,4-dihydroxy-2-naphthoic acid[4]. Naphthalene is previously described in natural products as an aromatic group in naphthalene ansamycins[5], or a lipooxygenase inhibitor in rat visceral yolk sac tissue[7].

Fig. 1. Structure of 1-hydroxy-4-methoxy-2-naphthoic acid (**1**).



Strain *Streptosporangium cinnabarinum* ATCC 31213 was cultivated in 500 ml-Erlenmeyer flasks with one baffle on a rotary shaker at 120 rpm and 27°C containing 100 ml of medium consisting of: glucose 1%, soluble starch 2%, yeast extract 0.5%, casein enzymatic hydrolysate 0.5%, $CaCO_3$ 0.1% and $CoCl_2 \cdot 6H_2O$ 2 mg/litre in tap water (pH 7.2). After a 72-hours incubation of the preculture, a 3.5-litre stirred tank fermentor (Biostat S) was inoculated with 5% (v/v), and grown at 27°C, aeration of 2 v/v/m and agitation of 1000 rpm. The production of 1-hydroxy-4-methoxy-2-naphthoic acid started at about 70 hours and reached a maximum after 115 hours at a concentration of 12 mg/litre.

1-Hydroxy-4-methoxy-2-naphthoic acid was isolated from the mycelium extract by column chromatography using Amberlite XAD-16. The compound was desorbed from the resin by MeOH-H_2O (80+20), concentrated under *vacuo*, and extracted at pH 7 with *n*-butanol. The organic layer was concentrated to dryness. Purification of the crude product was carried out by size-exclusion chromatography on a Sephadex LH-20 column using MeOH as eluent. Pure 1-hydroxy-4-methoxy-2-naphthoic acid was obtained after preparative reversed-phase HPLC using Nucleosil-100 C-18 (particle size 10 μm, 16 mm i.d. × 250 mm stainless steel column) with 0.1% HCOOH-MeOH linear gradient elution, starting at 60% MeOH to 100% MeOH in 20 minutes at a flow rate of 20 ml/minute. The compound was obtained after lyophilisation as a beige, amorphous substance in a final yield of 7.5 mg. The physio-chemical properties are summarized in Table 1.

The high-resolution EI-MS exhibited a molecular ion peak at *m/z* 218.0576 in accordance with the molecular composition $C_{12}H_{10}O_4$, which in conjunction with various NMR techniques, including HSQC and HMBC, established the structure as **1**. ^{13}C and ^{1}H NMR data of **1** are presented in Table 2.

Table 1. Physico-chemical properties of 1-hydroxy-4-methoxy-2-naphthoic acid.

Appearence	Beige powder
EI-MS (*m/z*)	
Found:	218.0576 [M^+]
Calcd:	218.0579
Molecular formula	$C_{12}H_{10}O_4$
UV λ_{max}^{MeOH} nm (ε)	215, 261, 360
IR (KBr) cm^{-1}	2960, 1680, 1632, 1398, 1215, 1098, 775

† Art. No. 9 on biosynthetic capacities of actinomycetes. Art. No. 8: See ref. 1.

Table 2. ^{1}H and ^{13}C NMR chemical shifts of 1-Hydroxy-4-methoxy-2-naphthoic acid.

Carbon No.	^{13}C chemical shifts ppm	^{1}H chemical shifts ppm
C1	155.3	—
C2	125.8	—
C3	101.8	7.19 (s)
C4	147.6	—
C4a	129.8	—
C5	121.8	8.18 (1H, d, $J_{HH}=8.1$ Hz)
C6	128.4	7.62 (1H, ddd, $J_{6,7}=6.9$ Hz, $J_{6,8}=1.2$ Hz)
C7	126.1	7.56 (1H, ddd, $J_{7,8}=8.0$ Hz, $J_{5,7}=1.2$ Hz)
C8	123.4	8.33 (1H, d, $J_{HH}=8.0$ Hz)
C8a	125.8	—
C9	173.9	—
C10	55.1	3.96 (3H, s)

Spectra were recorded in methanol-d_4 solution; coupling constants are given in Hz.

Table 3. Herbicidal activity of 1-hydroxy-4-methoxy-2-naphthoic acid against *Lemna minor*.

	Herbicidal activity[a]			
	500 μg/ml	250 μg/ml	10 μg/ml	5 μg/ml
After 3 days incubation	++	++	−	−
After 5 days incubation	++	++	+	−

Chemically defined medium (per litre): KNO_3 0.4 g, $CaCl_2 \cdot 2H_2O$ 0.54 g, $MgSO_4 \cdot 7H_2O$ 0.614 g, KH_2PO_4 0.2 g, trace element solution 1 ml (consisting in 100 ml: $MnCl_2 \cdot 4H_2O$ 41.5 mg, H_3BO_4 50 mg, $Na_2MoO_4 \cdot 2H_2O$ 12 mg, $ZnSO_4 \cdot 7H_2O$ 5 mg, $CuSO_4 \cdot 5H_2O$ 2.5 mg, $CoCl_2$ 2.5 mg), Fetrilon® (13%) 2.81 mg, agar 15 g. The medium was adjusted to pH 4.8.

[a] −: 0~30% inhibition of plant growth; +: 30~80% inhibition; ++: 80~100% inhibition.

The herbicidal activity of 1-hydroxy-4-methoxy-2-naphthoic acid was tested against *Lemna minor* by a bioassay[8] in microtiter plates and is shown in Table 3. The growth is reduced at a concentration of 10 μg/ml and totally alleviated at a concentration of 250 μg/ml. 1-Hydroxy-4-methoxy-2-naphthoic acid showed no antimicrobial activity at a concentration of 1 mg/ml by the agar plate diffusion assay against various Gram-positive and Gram-negative bacteria on a medium consisting of: nutrient broth (Difco) 0.8%, NaCl 0.5%, pH 7.2, and yeasts and filamentous fungi on a complex medium consisting of: yeast extract (Oxoid) 0.4%, malt extract (Difco) 1.0%, glucose 0.4%, pH 5.5.

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