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An Antibacterial Thiophene from *Balsamorhiza sagittata*

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Abstract

Balsamorhiza sagittata, a species of ethnopharmacological interest in British Columbia, is reported to have antibacterial and antifungal properties. An antibacterial compound isolated from this species was identified as 7,10-epithio-7,9-tridecadiene-3,5,11-triyne-1,2-diol based on the HMQC and HMBC experiments.

Balsamorhiza sagittata (Pursch) Nuttall (Asteraceae) is commonly known as Balsamroot. Native Canadians ate the stout starchy roots and tender young shoots and also used the plant for stomachache, headache, colds, fever, sore throat, toothache, wounds, insect bites, and swellings. Flavonoids (1), guaianolides, heliangolides, diterpenes, and cycloartenol derivatives (2) have been reported from this species, although research from an ethnobotanical view point has not been carried out so far. In our continuing studies of antibiotics from British Colombian plants of ethnobotanical interest, we have isolated the antibacterial compound **1**, 7,10-epithio-7,9-tridecadiene-3,5,11-triyne-1,2-diol, from *Balsamorhiza sagittata*. The structure was determined by HMQC and HMBC experiments.

Materials and Methods

Isolation of compound **1**

The dried and powdered root (656 g) was extracted with MeOH. The extract was concentrated and partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ phase was evaporated to leave a syrupy liquid, which was purified by silica gel CC [Merck, Art 60, 230–400 mesh, 150 g, MeOH : $CHCl_3$, 0 : 100 (500 ml), 5 : 95 (1000 ml), 10 : 90 (1000 ml), 30 : 70 (1000 ml), v/v] to give 8 fractions (A–H). Growth inhibitory effects against *Bacillus subtilis* Vernon and methicillin sensitive *Staphylococcus aureus* K147 were detected at fraction G eluted with a solvent system of MeOH : $CHCl_3$ (10 : 90, v/v), using a bacterial overlay thin layer chromatography assay (3). This method gave us the information that one of the active compounds had the absorption of UV light at 254 nm, exhibited a pale brown color by a treatment with a vanillin sulfuric acid reagent, and had an R_f value of 0.4 on silica gel TLC, using EtOAc : benzene (50 : 50, v/v). Based on these observations, rechromatography of the fraction G (2.3 g) was carried out on Sephadex LH-20 (Pharmacia Fine Chem., 100 g, MeOH: $CHCl_3$, 50 : 50, v/v), a preparative thin layer chromatography (pTLC) (Merck, 20 cm × 20 cm, EtOAc : benzene, 50 : 50, v/v, R_f = 0.4) and HPLC (μBONDAPAK C_{18}, MeOH : H_2O, 32 : 68, v/v, 0.8 ml/min, detection by UV light absorption at 210 nm) to afford 7,10-epithio-7,9-tridecadiene-3,5,11-triyne-1,2-diol (**1**; 7.5 mg): $[\alpha]_D^{23}$: + 92.2° (CH_3OH; *c* 0.1); EI-MS: *m/z* (rel. int.) = 230 $[M]^+$ (53), 212 (10), 199 (100), 183 (6), 170 (30), 169 (24), 149 (6), 139 (9), 127 (18), 93 (9); EI-HR-MS: 230.0403 (calcd. for $C_{13}H_{10}SO_2$: 230.0401); UV: λ_{max} (nm) (ε) = 237 (7682), 245 (10293), 251 (10293), 273 (7728), 275 (7935), 280 (8556), 324 (17917), 341 (15755); IR: ν_{max}^{film} (cm^{-1}) = 3321, 2912, 2863, 2222, 1634, 1446, 1416, 1385, 1090, 798; ^{1}H-NMR (200 MHz, CD_3OD): Table **1**; ^{13}C-NMR (128.5 MHz, CD_3OD): Table **1**.

Table 1 ^{1}H-NMR (CD_3OD, 200 MHz) and ^{13}C-NMR (CD_3OD, 125.8 MHz) assignments for **1**.

H	^{1}H (ppm)	C	^{13}C (ppm)
H-1	3.60 (2H, m)	C-1	66.9
H-2	4.45 (1H, br. t, *J* = 6.0 Hz)	C-2	64.8
		C-3	85.9
		C-4	69.6
		C-5	78.3
		C-6	71.1
		C-7	128.6
H-8	7.19 (1H, d, *J* = 3.8 Hz)	C-8	135.6
H-9	6.98 (1H, d, *J* = 3.8 Hz)	C-9	132.1
		C-10	122.4
		C-11	72.8
		C-12	93.0
H-13	2.05 (3H, s)	C-13	4.1

Preparation of 1,2-diacetyl derivative (**2**) *of compound* **1**

Compound **2** was prepared by reaction with Ac_2O/C_5H_5N and purified by pTLC (Merck, 20 cm × 20 cm, MeOH : $CHCl_3$, 1 : 99, v/v, R_f = 0.6). EI-MS: *m/z* (rel. int.) = 314 (11), 272 (4), 254 (24), 230 (14), 212 (47), 199 (17), 43 (100); EI-HR-MS: 314.0614 (calcd. for $C_{17}H_{14}SO_4$: 314.0613); IR: ν_{max}^{film}(cm^{-1}) = 3503, 2923, 2229, 2749, 1653, 1449, 1373, 1228, 1050, 807; ^{1}H-NMR (200 MHz, $CDCl_3$): δ = 7.15 (1H, d, *J* = 2.5 Hz), 6.95 (1H, d, *J* = 2.5 Hz), 4.32 (2H, m), 2.12 (3H, s), 2.11 (3H, s), 2.07 (3H, s).

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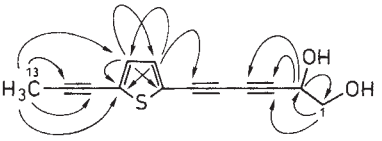
Structure elucidation for **1**

Compound **1** was obtained as a yellow amorphous powder. The EI-mass spectrum showed the molecular ion peak, $m/z = 230$ $[M]^+$, which was analyzed as $C_{13}H_{10}SO_2$ by the EI-HR-MS experiment. This result was confirmed by the EI- and EI-HR-MS of its 1,2-diacetyl derivative (**2**). The IR spectrum showed absorptions of hydroxy (3321 cm^{-1}) and acetylenic (2222 cm^{-1}) groups. The ^{1}H-NMR contained signals of δ = 7.19 (1H, d, J = 3.8 Hz, -^{8}CH=^{7}C-S-), 6.98 (1H, d, J = 3.8 Hz, -^{9}CH=^{10}C-S-) of the protons of a thiophene ring, and δ = 4.45 (1H, br. t, J = 6.0 Hz, -^{2}CH(OH)-^{1}CH$_2$OH), 3.60 (2H, m, -^{2}CH(OH)-^{1}CH$_2$OH) of the protons of a diol moiety, and δ = 2.05 (3H, s, -^{5}CH$_3$). An HMQC experiment established the connections between carbons and directly attached protons (Table **1**), and finally the structure was determined to be 7,10-epithio-7,9-tridecadiene-3,5,11-triyne-1,2-diol by the HMBC experiment (Fig. **1**). In order to determine the absolute configuration of -^{2}CH(OH)-, a 1,2-dibenzoyl derivative of **1** was synthesized, using PhCOCl/C_5H_5N. However, the absolute configuration of -^{2}CH(OH)- could not be determined because the 1,2-dibenzoyl derivative did not give a sufficient CD curve (conc.: 5.4 mg/10 ml CH_3CN, cell length: 1 mm).

HMBC experiment →



1

Fig. 1 Structure of compound **1**.

Antimicrobial activities of compound **1**

Compound **1** has been previously isolated from *Ambrosia chamissonis* (4). Minimum inhibitory concentrations (MICs) of **1** were obtained by a broth dilution method (5) in the dark as well as under the UV-A light (long wavelength ultraviolet light: max 350 nm) exposure (6, 7), using *Bacillus subtilis* Vernon, methicillin sensitive *Staphylococcus aureus* K147, and methicillin resistant *S. aureus* SAP0017 as test microorganisms. Compound **1** showed growth inhibitory effects against these microorganisms under both conditions (Table **2**). Activity was moderately enhanced under UV-A light.

Table 2 Antimicrobial activity of compound **1**.

Micro-organisms tested	MIC ($\mu g\ ml^{-1}$) Dark	Light[a]	GM[b]
Staphylococcus aureus K147	50	25	0.5
Staphylococcus aureus SAP0017	100	25	64
Bacillus subtilis Vernon	50–100	25	4

[a] UV exposure: after half an hour preincubation in the dark followed by half an hour UV light exposure, microorganisms were incubated for further 12 hours in the dark (6, 7).
[b] GM = gentamicin in the dark.

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Sesquiterpenes with Antibacterial Activity from *Epaltes mexicana*

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Epaltes mexicana Less. (Compositae, Inulae s.l.) grows in southern Mexico and the leaves are used in traditional medicine (externally against fungal and bacterial infections and to treat fever) by the indians of Oaxaca (1). Several eudesmane and cuauhtemone derivatives have been identified from the genus *Epaltes* (2–4). In the course of our biological screening of *E. mexicana* (voucher specimens have been deposited at the following herbaria: MEXU (UNAM, México D.F., ZT (ETH Zurich, Switzerland), and FB (Inst. Pharm. Biol., University of Freiburg, Germany), the fractions of sesquiterpenoids of the *n*-hexane fraction showed antibacterial activity. Bioactivity-guided fractionation yielded eight sesquiterpenes (**1–8**). We report the isolation and the antibacterial activity of these compounds; **1** being a new compound, while **2** and **5** were isolated for the first time from this species.

All compounds **1–8** were obtained from *n*-hexane extract of air-dried, powdered aerial parts (300 g) by a combination of VLC and preparative TLC. The final purifications were carried out by HPLC (hexane-EtOAc, 65 : 35). Identifications of **2–8** were performed by the