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Miltiorins A–D, diterpenes from *Radix Salviae miltiorrhizae*

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ABSTRACT

Constituents of the anti-influenza A neuraminidase (NA) active extract from *Radix Salviae miltiorrhizae* were investigated, resulting in the isolation of four new diterpenes, miltiorins A–D (**1**–**4**), together with eight known diterpenes. The structures of **1**–**4** were assigned by spectroscopic analysis. Miltiorins A–C (**1**–**3**) were abietane diterpenes possessing a 2α-acetoxy group and a 12-hydroxy group in common, while miltiorin D (**4**) was a 11,12-*seco*-abietane diterpene with a γ-lactone ring. Miltiorin D (**4**) is the first example of a 11,12-*seco*-abietane diterpene from natural sources. Anti-NA activities of the isolated diterpenes were evaluated.

1. Introduction

Radix Salviae miltiorrhizae, the dried root of *S. miltiorrhiza* Bge. (Lamiaceae), is one of the most popular herbal traditional medicines in Asian countries, and has been used extensively for the treatment of coronary artery disease, angina pectoris, myocardial infarction, cerebrovascular diseases, chronic renal failure, dysmenorrhea, and various types of hepatitis [1]. In our continuing search for new natural templates of therapeutic agents [2–6], an influenza A neuraminidase (NA) inhibitory effect of several extracts from plants was evaluated. As a result, the extract from the dried roots of *S. miltiorrhiza* exhibited an anti-NA activity, which prompted us to investigate the constituents of the natural source. In this article, we describe the isolation and structure elucidation of four new diterpenes, miltiorins A–D (**1**–**4**), isolated from *Radix Salviae miltiorrhizae*, as well as evaluation of an anti-NA activity of the isolated compounds.

2. Experimental

2.1. General experimental procedures

Optical rotations were obtained on a JASCO DIP-370 digital polarimeter. NMR spectra were measured by a Bruker AVANCE-500 instrument using tetramethylsilane as an internal standard. HRESIMS and ESIMS were recorded on a Waters LCT PREMIER 2695. Column chromatography was performed with silica gel 60N (63–210 μm, Kanto Kagaku, Japan), MCI gel CHP 20P (75–150 μm, Mitsubishi Chemical, Japan), and YMC-gel ODS-A (S-50 μm, YMC Co., Ltd., Japan).

2.2. Plant material

Radix Salviae miltiorrhizae (Lot. US302822) was provided by UCHIDA WAKANYAKU Ltd., Japan. A voucher specimen was deposited in the herbarium of Tokushima University.

2.3. Extraction and isolation

The dried roots of *Salvia miltiorrhiza* (2.0 kg, dry) were extracted with MeOH (3 × 10 L) at rt to give the extract (291 g), which was partitioned with $CHCl_3$ (6 × 1.5 L) and H_2O (1.5 L).

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The $CHCl_3$-soluble portion (24.3 g) was subjected to silica gel column chromatography (*n*-hexane/EtOAc, 10:0 to 5:1 and then $CHCl_3$/MeOH, 20:1 to 0:10) to give eleven fractions (frs. 1–11). Tanshinone IIA (443 mg) was obtained by crystallization of fr. 4 from EtOAc, while tanshinone I (34.6 mg) was crystallized from the EtOAc solution of fr. 5. The mother liquor of fr. 5 was applied to an ODS column (MeOH/H_2O, 5:5 to 10:0) to give 18 fractions (frs. 5.1–18). Fr. 5.6 was separated by repeated silica gel column chromatography (*n*-hexane/acetone, 99:1 to 90:10; toluene/EtOAc, 25:1) to afford norsalvioxide (8 mg). Fr. 5.9 was subjected to silica gel column chromatography (*n*-hexane/EtOAc 15:1 to 1:1), and then purified using ODS HPLC (COSMOSIL 5C_{18}-AR-II, Nacalai tesque, 20 × 250 mm, MeOH/H_2O, 85:15) to give miltiorin A (**1**, 15.3 mg). Fr. 6 was separated by silica gel column chromatography ($CHCl_3$/MeOH, 50:1 to 0:100) to yield 15 fractions (frs. 6.1–15). Cryptotanshinone (277 mg) was crystallized from fr. 6.7 (acetone). Fr. 6.8 was loaded on a Toyopearl HW-40C column (toluene/MeOH, 5:1), an ODS column (MeOH/H_2O, 30:70 to 100:0), and a silica gel column (toluene/EtOAc, 30:1) to give miltiorin B (**2**, 2.2 mg), 5,6-dehydrosugiol (3.2 mg), and 2α-acetoxysugiol (7.7 mg). Separation of fr. 7 by Toyopearl HW-40C column chromatography (toluene/MeOH, 7:1) afforded 13 fractions (frs. 7.1–13). Fr. 7.2 was purified by RP HPLC (COSMOSIL πNAP, 20 × 250 mm, MeOH/H_2O, 85:15) to furnish 15,16-dihydrotanshinone (3.5 mg). Fr. 7.8 was subjected to silica gel column chromatography repeatedly (*n*-hexane/EtOAc, 5:1 to 1:1; $CHCl_3$) to give (+)-danshexinkun A (5.5 mg). Fr. 7.4 was applied to a silica gel column (*n*-hexane/acetone, 5:1 to 1:1), and then purified by ODS HPLC (COSMOSIL 5C_{18}-AR-II, 20 × 250 mm, MeOH/H_2O, 70:30) to yield miltiorins C (**3**, 14.8 mg) and D (**4**, 7.7 mg).

2.4. Miltiorin A (*1*)

Pale yellow amorphous solid; $[\alpha]_D$ −17.6 (*c* 0.43, $CHCl_3$); HRESIMS *m/z* 367.2259 $[M + Na]^+$ (calcd for $C_{22}H_{32}O_3Na$, 367.2249); ^1H and ^{13}C NMR data (Table 1).

2.5. Miltiorin B (*2*)

Pale yellow amorphous solid; $[\alpha]_D$ −12.4 (*c* 0.23, $CHCl_3$); HRESIMS *m/z* 397.2000 $[M + Na]^+$ (calcd for $C_{22}H_{30}O_5Na$, 397.1991); ^1H and ^{13}C NMR data (Table 1).

2.6. Miltiorin C (*3*)

Pale yellow amorphous solid; $[\alpha]_D$ −34.4 (*c* 1.48, $CHCl_3$); HRESIMS *m/z* 379.1873 $[M + Na]^+$ (calcd for $C_{22}H_{28}O_4Na$, 379.1885); ^1H and ^{13}C NMR data (Table 1).

2.7. Miltiorin D (*4*)

Pale yellow amorphous solid; $[\alpha]_D \approx 0$ (*c* 0.28, $CHCl_3$); HRESIMS *m/z* 313.1447 $[M - H]^-$ (calcd for $C_{19}H_{21}O_4$, 313.1440); ^1H and ^{13}C NMR data (Table 2).

2.8. Methylations of miltiorins A–C

A mixture of miltiorin A (**1**, 0.8 mg), CH_3I (20 μL), and K_2CO_3 (13 mg) in dry acetone (400 μL) was stirred at rt for 3 h. After removal of the solvent, the residue was subjected to chromatography over silica gel (*n*-hexane/EtOAc, 95:5) to give 12-*O*-methyl miltiorin A (**1a**, 0.7 mg). Methylations of miltiorins B (**2**) and C (**3**) were carried out as for **1** to give 12-*O*-methyl miltiorins B (**2a**) and C (**3a**), respectively.

Table 1
^1H and ^{13}C NMR data for miltiorins A–C (**1**–**3**) in $CDCl_3$.

1			**2**		**3**	
Position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	43.8	2.55 (brd, 11.8) 1.43 (t, 11.8)	43.7	2.56 (m) 1.51 (t, 11.7)	41.9	2.68 (brd, 11.5) 1.64 (t, 11.5)
2	69.6	5.20 (tt, 11.8, 4.0)	68.4	5.17 (brt, 11.7)	67.7	5.42 (tt, 12.0, 4.0)
3	46.5	1.84 (m) 1.31 (t, 11.8)	47.7	1.84 (brd, 11.7) 1.39 (t, 11.7)	44.7	2.01 (brd, 12.0) 1.50 (t, 12.0)
4	34.7	–	35.8	–	38.2	–
5	49.8	1.37 (dd, 12.5, 2.0)	55.1	1.85 (d, 12.5)	170.5	–
6	18.9	1.88 (m) 1.69 (qd, 12.5, 7.0)	73.5	4.59 (d, 12.5)	124.7	6.48 (s)
7	29.5	2.89 (dd, 16.7, 6.0) 2.79 (dd, 16.7, 11.3)	199.0	–	185.4	–
8	126.4	–	121.3	–	122.7	–
9	147.0	–	154.9	–	152.8	–
10	39.0	–	40.7	–	41.9	–
11	110.6	6.61 (s)	109.9	6.70 (s)	110.8	6.94 (s)
12	151.2	–	159.2	–	158.7	–
13	132.1	–	133.9	–	134.5	–
14	126.7	6.86 (s)	127.5	7.95 (s)	125.2	8.01 (s)
15	26.7	3.16 (sept, 6.7)	26.8	3.16 (sept, 7.0)	26.9	3.26 (sept, 7.0)
16	22.5	1.23 (3H, d, 6.7)	22.2	1.26 (3H, d, 7.0)	22.3	1.25 (3H, d, 7.0)
17	22.7	1.24 (3H, d, 6.7)	22.4	1.27 (3H, d, 7.0)	22.5	1.27 (3H, d, 7.0)
18	33.2	1.01 (3H, s)	35.9	1.28 (3H, s)	32.6	1.29 (3H, s)
19	22.3	1.02 (3H, s)	22.6	1.32 (3H, s)	29.9	1.41 (3H, s)
20	25.4	1.25 (3H, s)	25.3	1.44 (3H, s)	33.3	1.56 (3H, s)
2-OAc	171.3	–	171.0	–	171.0	–
	21.5	2.09 (3H, s)	21.5	2.09 (3H, s)	21.4	2.05 (3H, s)