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ABIETANE TYPE DITERPENOIDS FROM *SALVIA MILTIORRHIZA*

YASUMASA IKESHIRO, IZUMI MASE and YUTAKA TOMITA

Niigata College of Pharmacy, 5-13-2 Kamishinei-cho, Niigata 950-21, Japan

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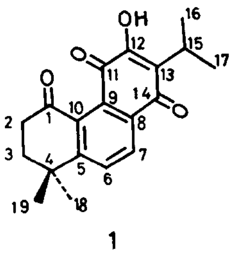
Key Word Index—*Salvia miltiorrhiza*; Labiatae; diterpenoids; abietane quinones; 20-*nor*-abietanes; miltionone I; miltionone II.

Abstract—Two new abietane diterpenoids, miltionone I and II, have been isolated from the root *Salvia miltiorrhiza* and the structures determined as 12-hydroxy-20-nor-5(10),6,8,12-abietatetraene-1,11,14-trione and 15(*R*)-14,16-epoxy-11-hydroxy-20-*nor*-5(10),7,9(11),13-abietatetraene-6,12-dione, respectively, by spectroscopic analysis.

INTRODUCTION

The dried root (Dan-shen in Chinese and Tan-jin in Japanese) of *Salvia miltiorrhiza* Bunge, has been used in Chinese traditional medicine for the treatment of haematological abnormalities, heart disease, hepatitis, haemorrhage, menstrual abnormalities, miscarriages and oedema. Numerous abietanoid pigments have been isolated from this crude drug and identified as physiologically active natural products [1–11]. Recently, highly-oxidized abietane derivatives have been isolated from the roots of this plant [12, 13].

A further investigation of the constituents of the root of *S. miltiorrhiza* has afforded two new abietane diterpenoids, 12-hydroxy-20-*nor*-5(10),6,8,12-abietatetraene-1,11,14-trione (**1**, miltionone **I**) and 15(*R*)-14,16-epoxy-11-hydroxy-20-*nor*-5(10),7,9(11),13-abietatetraene-6,12-dione (**2**, miltionone II). In addition, nine known diterpenes, ferruginol [14], danshensproketallactone [5, 6], danshexinkun B [3], tanshinone IIA [7], tanshinone I [8], dihydrotanshinone [9], cryptotanshinone (**3**) [10], isocryptotanshinone [11] and dihydroisotanshinone I [15], together with stigmasterol were identified.



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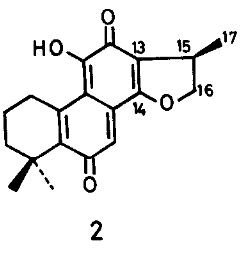

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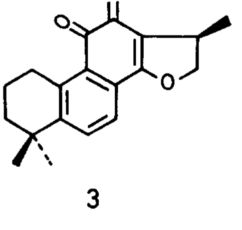

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RESULTS AND DISCUSSION

Miltionone I (**1**) was obtained as a yellow powder: $C_{19}H_{20}O_4$($[M]^+$, m/z 312.1357). Its UV and visible spectra showed absorptions at 232, 242, 275, 315 (sh) and 340 nm. The IR spectrum showed absorptions for an aromatic ring (1570 cm^{-1}) and a hydrogen-bonded 2-hydroxy-1,4-benzoquinone (3380, 1670, 1650 cm^{-1}) moiety [16, 17]. The ^{1}H NMR spectrum of **1** was consistent with the presence of a 2-hydroxy-1,4-naphthaquinone moiety having the substitution pattern depicted in formula **1**. Thus it showed the signals of an isopropyl group attached to the quinone ring (δ3.37, 1H, *septet*, J = 7.1 Hz, H-15, and δ1.29, 6H, *d*, J = 7.1 Hz, Me-16 and Me-17), and two *ortho*-aromatic protons (δ8.18, *d*, J = 8.2 Hz, H-7 and δ7.68, *d*, J = 8.2 Hz, H-6). One hydrogen-bonded hydroxyl proton appeared at δ7.38 as a singlet but was no longer present after the addition of D_2O. The spectrum of **1** also showed signals for two methylene protons (δ2.95, *t*, J = 7.2 Hz, 2H-2 and δ2.09, *t*, J = 7.2 Hz, 2H-3) and for a geminal dimethyl group (δ1.29, *s*, Me-18 and Me-19), identical with that found in arucadiol, a 20-*nor*-abietane diterpenoid previously isolated from *S. argentea* [18].

In the ^{13}C NMR spectrum (Table 1), signals (δ181.03 and 183.52) from 1,4-quinone carbonyl carbons were present, as was a signal (δ199.46) from one arylketone. Furthermore, the mass spectrum showed fragmentation ion peaks at m/z 312 $[M]^+$ (base peak), 297 $[M-Me]^+$, 284 $[M-CO]^+$ and 269 $[M-Me-CO]^+$, suggesting the presence of an arylketone and geminal dimethyl group in the molecule. From the above results, miltionone I can be represented by the structural formula shown for **1**.

Miltionone II (**2**) was obtained as colourless needles: $C_{19}H_{20}O_4$ ($[M]^+$, m/z 312.1349), mp 184–185°, $[\alpha]_D^{23}$ $+114.8°$ ($CHCl_3$). It was assigned the structure 20-*nor*-lanugon Q on the basis of the following considerations. The UV spectra showed absorptions at 222, 265, 308 and 320 nm. The IR spectrum showed bands at 3380 (enolic OH), 1780, 1720, 1615, 1590 and 1550 cm^{-1} (quinoid carbonyl), supporting the presence of a hydroxy-*p*-benzoquinone group [19, 20]. The ^{1}H NMR spectrum of **2** was very similar to that of lanugon Q [21], except for the absence of signals for the β-methyl proton and methine proton on C-10 and C-5 respectively of lanugon Q.

Table 1. ^{13}C NMR spectral data of compounds 1-3 (50.10 MHz, $CDCl_3$)

C	1	2	3
1	199.46 *s*	28.29* *t*	29.65* *t*
2	36.64* *t*	19.05* *t*	19.09* *t*
3	36.64* *t*	38.19* *t*	37.87* *t*
4	35.39 *s*	34.86 *s*	34.86 *s*
5	135.10 *s*	135.42 *s*	143.66 *s*
6	130.49* *d*	190.32 *s*	132.52* *d*
7	129.32* *d*	130.11* *d*	122.50* *d*
8	133.44 *s*	130.41 *s*	128.46 *s*
9	128.77 *s*	122.28 *s*	126.32 *s*
10	153.44 *s*	151.74 *s*	152.39 *s*
11	181.03 *s*	156.51 *s*	184.26 *s*
12	156.83 *s*	183.19 *s*	175.69 *s*
13	127.34 *s*	108.81 *s*	118.32 *s*
14	183.52 *s*	163.75 *s*	170.71 *s*
15	24.64* *d*	38.46* *d*	34.66* *d*
16	19.83* *q*	78.08* *t*	81.46* *t*
17	19.83* *q*	18.73* *q*	18.80* *q*
18	28.73* *q*	31.65* *q*	31.89* *q*
19	28.73* *q*	31.77* *q*	31.94* *q*

*^{13}C–H correlation are based on selective ^{1}H-decoupled ^{13}C NMR measurements.

Furthermore, as shown in Table 1, the chemical shifts of the ^{13}C NMR signal of **2** were similar to that of the dihydrofuran ring moiety in cryptotanshinone (**3**). A molecular ion peak at *m/z* 312 (on deuterium exchange this was shifted to *m/z* 313 $[M]^+$) and prominent peaks at *m/z* 297 $[M-Me]^+$, 284 $[M-CO]^+$ and 269 $[M-Me-CO]^+$ are in excellent agreement with the expected fragmentation patterns [22]. From the above results, the structure of miltionone II can be represented by the formula shown for **2**.

The configuration at C-15 of the dihydrofuran ring moiety was deduced from the ^{1}H NMR spectrum of miltionone II (**2**) (see Table 2). This revealed almost the same chemical shift and coupling constants for the dihydrofuran ring moiety as those for the ring moiety of cryptotanshinone (**3**), indicating that the C-15 position of the dihydrofuran ring in **2** probably has the *R*-configuration as does that of **3** [23].

Table 2. ^{1}H NMR spectral data for the dihydrofuran ring in miltionone II (**2**) and cryptotanshinone (**3**) ($CDCl_3$)

	δ (ppm)	
	2	3
H-16α	4.75 *t*	4.89 *t*
H-16β	4.20 *dd*	4.36 *dd*
H-15α	3.61 *m*	3.61 *m*
Me-17	1.35 *d*	1.36 *d*
	$J_{16\alpha,16\beta}=9.2$ Hz	$J_{16\alpha,16\beta}=9.4$ Hz
	$J_{16\alpha,15\alpha}=9.2$ Hz	$J_{16\alpha,15\alpha}=9.4$ Hz
	$J_{16\beta,15\alpha}=7.1$ Hz	$J_{16\beta,15\alpha}=6.1$ Hz
	$J_{15\alpha,17}=7.2$ Hz	$J_{15\alpha,17}=6.9$ Hz

Assignments were confirmed by spin decoupling experiments.

EXPERIMENTAL

Mps: uncorr. EIMS: 20 and 70 eV. HRMS: 70 eV. ^{1}H and ^{13}C NMR: 200 and 50.10 MHz, respectively, with TMS as an int. standard. CC: Mallinckrodt silica gel (100 mesh); TLC and prep. TLC: Merck silica gel 60 GF_{254} plates (0.25 mm) and Merck silica gel 60 PF_{254} plates (0.5 mm) were employed. Spots and bands were detected by UV irradiation (253.7 and 365 nm).

Plant material. Dried root of *Salvia miltiorrhiza* were cultivated and collected in the botanical garden of this college in November 1987. The crude drugs (Dan-shen) of *S. miltiorrhiza* were supplied by Uchida Wakan-Yaku (Japanese and Chinese crude drugs) Company, Ltd, Ogatacho, Niigata.

Extraction and isolation. The finely cut roots of *S. miltiorrhiza* (1 kg) were extracted with $CHCl_3$ (2.4 l × 4) and the combined extracts evapd *in vacuo*. The residue (12.8 g) was subjected to CC on silica gel (637 g) eluting with $CHCl_3$. The fractions were monitored by TLC. All compounds were further purified by recrystallization and prep. TLC (silica gel), yielding in order of increasing polarity: ferruginol (18 mg), danshensproketallactone (21 mg), danshexinkun B (30 mg), miltionone I (**1**, 20 mg), miltionone II (**2**, 19 mg), tanshinone IIA (500 mg), tanshinone I (1.8 g), stigmasterol (1.2 g), dihydrotanshinone (93 mg), cryptotanshinone (**3**, 1.5 g), isocryptotanshinone (14 mg) and dihydroisotanshinone I (16 mg). Known compounds were identified by comparison of their spectroscopic properties with literature values.

Miltionone I (**1**). Yellow crystalline powder, mp 151–153°; $[\alpha]_D^{22}$ 0° ($CHCl_3$; *c* 0.53). UV λ_{max}^{MeOH} nm (log ε): 232 (4.22), 242 (4.21), 275 (4.13), 315 (sh. 3.74), 340 (3.61). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380, 2950, 1710, 1670, 1650, 1600, 1570, 1470, 1390, 1340, 1320, 1020, 1000, 950, 910, 860. ^{1}H NMR (200 MHz, $CDCl_3$): see Results and Discussion. ^{13}C NMR: see Table 1. EIMS 20 eV, *m/z* (rel. int.): 312 $[M]^+$ (100), 297 (7), 284 (14), 269 (59), 251 (14), 200 (9), 83 (13). HRMS *m/z*: 312.1357 ($[M]^+$, calcd for $C_{19}H_{20}O_4$: 312.1360), 297.1078 ($C_{18}H_{17}O_4$: 297.1125), 284.1352 ($C_{18}H_{20}O_3$: 284.1411), 269.1159 ($C_{17}H_{17}O_3$: 269.1176), 251.1069 ($C_{17}H_{15}O_2$: 251.1070), 200.0825 ($C_{13}H_{12}O_2$: 200.0836).

Miltionone II (**2**). Colourless needles, mp 184–185°; $[\alpha]_D^{23}$ +114.8° ($CHCl_3$; *c* 0.12); UV λ_{max}^{MeOH} nm (log ε): 205 (4.17), 222 (4.19), 265 (4.07), 308 (3.97), 320 (3.97); IR ν_{max}^{KBr} cm^{-1}: 3380, 2950, 1780, 1720, 1615, 1590, 1550; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.31 (3H, *s*, Me-18), 1.33 (3H, *s*, Me-19), 1.35 (3H, *d*, $J_{15\alpha,17}=7.2$ Hz, Me-17), 1.82 (2H, *m*, 2H-2), 2.86 (2H, *m*, 2H-1), 3.61 (1H, *m*, $W_{1/2}=18$ Hz, H-15α), 4.20 (1H, *dd*, $J_{16\alpha,16\beta}=9.2$ and $J_{16\beta,15\alpha}=7.1$ Hz, H-16β), 4.75 (1H, *t*, $J_{16\alpha,16\beta}=J_{16\alpha,15\alpha}=9.2$ Hz, H-16α), 7.27 (1H, *s*, OH-11), 7.59 (1H, *s*, H-7); ^{13}C NMR (50.10 MHz, $CDCl_3$): see Table 1; EIMS 70 eV, *m/z* (rel. int.): 312 $[M]^+$ (20), 297 (5), 284 (5), 283 (5), 269 (35), 268 (41), 254 (19), 253 (100), 251 (24), 171 (18), 128 (13); [on deuterium exchange *m/z* 313 $[M]^+$ (10)]; HRMS *m/z*: 312.1349 (M^+, calcd. for $C_{19}H_{20}O_4$: 312.1360), 297.1113 ($C_{18}H_{17}O_4$: 297.1125), 284.1398 ($C_{18}H_{20}O_3$: 284.1410), 283.1308 ($C_{18}H_{19}O_3$: 283.1333), 268.1487 ($C_{18}H_{20}O_2$: 268.1462), 253.1222 ($C_{17}H_{17}O_2$: 253.1227), 251.1059 ($C_{17}H_{15}O_2$: 251.1070).

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