Structures of Diterpenoid Tropolones, Salviolone and Miltipolone, from the Root of *Salvia miltiorrhiza* Bunge

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New types of a bisnorditerpenoid and a norditerpenoid possessing a benzotropolonoid and a tropolonoid moieties, salviolone (**8**) and miltipolone (**15**), respectively, have been isolated and their structures were determined by spectroscopic methods. The structure of salviolone was confirmed by a total synthesis. These compounds exhibit cytotoxic activities against cultured cells.

The dried roots of *Salvia miltiorrhiza* Bunge (Labiatae), known as "Tanjin" in Japanese or "Danshen" in Chinese, have been used in these countries as a folk medicine to treat a wide variety of diseases.[1] Chemical constituents of this root were extensively investigated since 1934,[2] and surveys of minor metabolites were further continued in order to identify more potently active constituents, for the activity of the crude extract often exceeds that of the identified constituents. This Chinese drug is colored reddish-orange and many coloring constituents, mainly quinones[3] with abietane skeleton, as represented by transhione-I (**1**)[4] and isocryptotanshinone (**2**),[5] have been identified. Some of these compounds seemed to be the artifacts produced during drug preparation. We studied on pharmaceutically active compounds in the fresh roots of *S. miltiorrhiza* and found colorless new compounds, designated salviolone (**8**)[6] and miltipolone **15**)[9] besides norsalvioxide (**3**),[6] secodialdehyde (**4**),[7] abietatriene (**5**),[7] cryptoacetalide (**6**),[8] and isomanool (**7**).[7] The new compounds, salviolone and miltipolone, were found to possess unique diterpenoid benzotropolone and tropolone chromophores, respectively. Although many tropolonoid terpenes possessing mono- and sesquiterpenoid skeletons have been known, to the best our knowledge, these are the first diterpenoid tropolones found from the nature. Present report deals with structure elucidation of these compounds.[9]

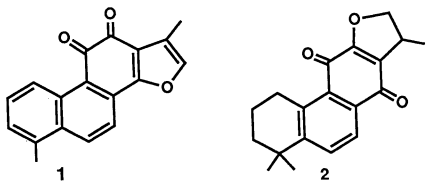

Chart 1.

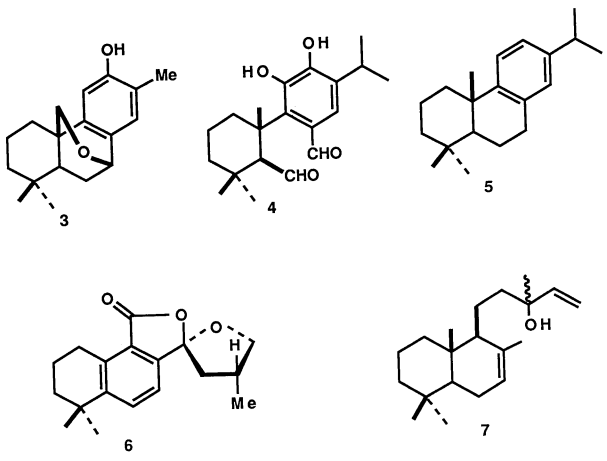

Chart 2.

The fresh roots were soaked in methanol just after the collection and the methanol extract was repeatedly separated by chromatographies to afford new compounds, salviolone (**8**) and miltipolone (**15**), as pale yellow and colorless crystals, respectively.

The molecular composition of salviolone (**8**) was determined to be $C_{18}H_{20}O_2$ from the high resolution mass spectrum. The ^1H NMR spectrum showed a rather downfield aliphatic triplet at δ=3.10 (2H, t, J=7 Hz), which was assignable to a benzylic methylene group and was found to couple with a perturbed quintet at δ=1.95 (2H). This quintet is further coupled with a deformed triplet at δ=1.72 (2H). A singlet at δ=1.36 (6H), possibly due to a *gem*-dimethyl group, exhibits an NOE cross peak in the NOESY spectrum to the methylene signal at δ=1.72, implicating the presence of Ar–CH_2–CH_2–CH_2–CMe_2–group. The IR spectrum shows the bands ascribable to a phenolic OH at 3270 and 1240 cm^{-1}. An intense absorption at 1630 cm^{-1} and an upfield carbonyl signal (δ=179.9) in the ^{13}C NMR spectrum indicated the presence of a carbonyl group involved in a highly conjugated system. Appearance of eleven downfield signals at δ=127.1—179.9 in the ^{13}C NMR spectrum and four downfield proton signals at δ=7.53 (1H, d, J=8.5 Hz), 7.58 (1H, d, J=8.5 Hz), 7.95 (1H, s), and 8.05 (1H, s) as well as the downfield methyl singlet at δ=2.49, and deep blue coloration by iron(III) chloride suggested the existence of some particular phenolic moiety such as an acetylnaphthol or a methylbenzotropolonoid structure. The benzotropolonoid structure seemed to be more probable because of the presence of the cross peak (long-range coupling) between the methyl protons at δ=2.49 and an aromatic proton δ=7.95 in the COSY

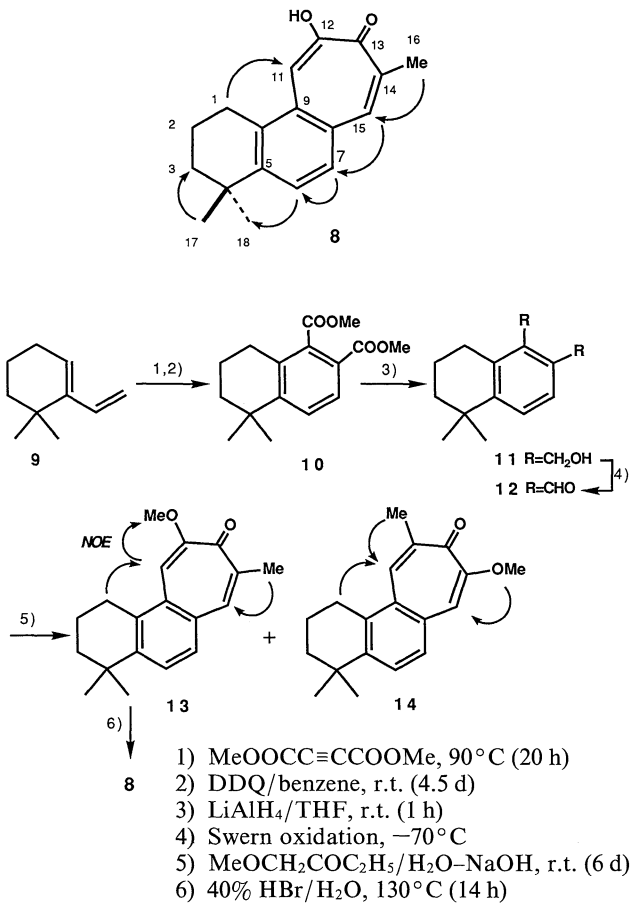

Chart 3.

spectrum. This was further supported by the UV spectrum; the absorption maxima of salviolone [λ_{max} 388 (log ε 3.23), 368 (3.28), 312 (3.60), 285 (4.42), 249 (4.20), 242 nm (4.18)] were quite similar to those of 2-methyl-4,5-benzotropolone.[10] Structure **8** was deduced from these properties and biogenetic considerations. This structure was supported by finding the NOE's in the NOE difference and NOESY spectra (indicated on the structure by arrows). Finally this structure was unequivocally confirmed by a total synthesis.

The Diels–Alder reaction between 3,3-dimethyl-2-vinylcyclohexene (**9**)[11] and methyl acetylenedicarboxylate proceeded smoothly by heating the mixture to give an adduct, which without isolation was treated with DDQ to afford a phthalic ester **10**. The ester **10** was reduced to a diol **11**, which was then subjected to the Swern oxidation at −70 °C to give a dialdehyde **12**. As the dialdehyde was unstable, the crude **12** was immediately used in the following step. The suspension of the dialdehyde **12** and 1-methoxy-2-butanone[12] in an aqueous sodium hydroxide solution[13] was stirred for six days to afford a mixture of salviolone methyl ether **13** and its regioisomer **14** (**13**:**14**=2:1) in 28% yield from the diol **11**. Both products were separated, and characterized by the ^{1}H NMR spectra and NOE experiments (indicated by arrows on the structures). The reactions of the dialdehyde and methoxybutanone did not proceed at all in aqueous alcoholic media.[13,14] The major isomer **13** was heated in aqueous hydrobromic acid to give salviolone (**8**). The spectroscopic properties (^{1}H and ^{13}C NMR, IR, and UV spectra) of synthetic and natural salviolone were identical in all respects.[9]

Miltipolone (**15**) was found to have the molecular formula $C_{19}H_{24}O_3$ from the high resolution FAB mass peak (MH^+). Spectral properties of this compound were partially very close to those of norsalvioxide (**3**)[6] and salviolone (**8**).[6] The IR spectrum showed the band ascribable to a hydrogen bonded OH group at 3000—3300 cm^{-1}. An intense absorption at 1620 cm^{-1} and a rather upfield signal at δ=171.97 in the ^{13}C NMR spectrum indicated the presence of a carbonyl group involved in a conjugated system similar to that of salviolone (**8**). Appearance of six olefinic carbon signals at δ=120.0—166.37 in the ^{13}C NMR spectrum and two downfield proton signals at δ=7.28 and 7.40 as well as very downfield methyl signal at δ=2.42 in the ^{1}H NMR spectrum and deep violet coloration by ferric chloride suggested the existence of some particular phenolic moiety such as an *o*-hydroxyacetophenone or a tropolonoid group. The tropolonoid chromophore was reinforced by the presence of the cross peak between the methyl protons at δ=2.42 and an aromatic proton at δ=7.28 in the COSY spectrum as well as the UV absorption maxima characteristic for the tropolone.[15] The presence of the methyltropolone moiety was confirmed by determining the connectivity of six aromatic carbon atoms and one upfield carbonyl carbon by the COLOC spectrum (Table 1); for example the proton at δ=7.28 on C-15 (δ=136.37) was correlated with C-7 (δ=74.38), C-9 (δ=155.99), C-13 (δ=166.37), and C-19 (δ=21.3), and the proton at δ=7.40 on C-11 (δ=120.02) to C-8 (δ=138.67), C-12 (δ=171.97) and C-13 (δ=166.37).

Two of three oxygen atoms in miltipolone are involved in the tropolonoid moiety and the remaining one oxygen atom was supposed to be included in an ether linkage from the ^{13}C NMR signal at δ=74.38 (d, C-7) and 67.35 (t, 16-C), and the ^{1}H NMR signals on the oxymethylene

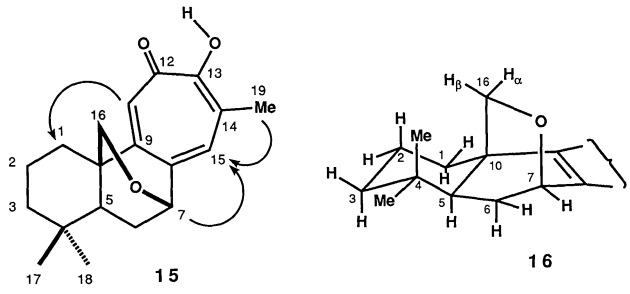

Chart 4.

Table 1. The ^1H and ^{13}C NMR Data (δ; $CDCl_3$) for **15**

No. of carbon	^{13}C NMR (125 MHz)	^1H NMR (500 MHz, J in Hz)	δ ^1H coupling with J^3 (COLOC)
1	29.92 (t)	α-H 1.95 (dt, 4.5, 13.4)	1.60
		β-H 1.72 (dtd, 13.4, 3.1, 1.9)	
2	18.98 (t)	1.62 (m), 1.70 (m)	
3	40.99 (t)	α-H 1.20 (dt, 4, 13)	0.86, 1.23, 1.72
		β-H 1.60 (dtd, 1.9, 3.6, 13)	
4	34.39 (s)		1.62, 1.66, 2.11
5	41.47 (d)	1.23 (ddd, 2.0, .2, 12.0)	0.86, 1.16, 1.60, 1.72, 4.68
6	29.24 (t)	α-H 1.66 (ddd, 2.0, 12.0, 13.9)	
		β-H 2.11 (ddd, 3.8, 6.2, 13.9)	
7	74.38 (d)	4.68 (dd, 2.0, 3.8)	7.28
8	138.67 (s)		2.11, 7.40
9	155.99 (s)		4.36, 4.68, 7.28
10	40.62 (s)		1.72, 7.40
11	120.02 (d)	7.40 (s)	
12	171.97 (s)		7.40
13	166.37 (s)	9.6 (OH, br)	2.42, 7.28, 7.40
14	131.31		2.42
15	136.37 (d)	7.28 (s)	2.42, 4.68
16	67.35 (t)	α-H 2.99 (dd 2.0, 9.1)	
		β-H 4.36 (d 9.1)	
17	21.3 (q)	3H 1.16 (s)	0.86, 1.23
18	32.7 (q)	3H 0.86 (s)	1.16
19	21.3 (q)	3H 2.42 (s)	7.28

protons (16-CH_2) appearing at δ=2.99 and 4.36 as well as the oxymethine proton at δ=4.68 (7-H). Except for the two aromatic proton chemical shifts, the ^1H NMR patterns of miltipolone were almost superimposable to those of norsalvioxide **3**. This similarity inferred the presence of the partial structure **16** in miltipolone, and the structure was firmly established by the extensive analyses of two dimensional and double resonance NMR spectra. The existence of *geminal* dimethyl group was confirmed by the detections of a *J*-cross peak in the COSY and an NOE cross peak in the NOESY spectra between the two aliphatic methyl signals at δ=0.86 and 1.16. The COLOC spectrum showed the ^3J cross peaks between 5-C (δ=41.47) and 1-H (δ=1.72), 3-H (δ=1.60), 7-H (δ=4.68), 17-H_3 (δ=1.16), and 18-H_3 (δ=0.86). A W-type coupling between 16-H_α and 5-H indicated their anticoplanar orientation. Noteworthy is the fact that the chemical shifts of methylene protons (δ=2.99 and 4.36) at 16-C are considerably different each other due to an anisotropic effect of the tropolonoid group. The presence of *J*-coupling of the proton at δ=4.68 (7-H) to the aromatic proton signal at δ=7.28 (15-H) suggested the connectivity of the partial structure **16** to the tropolonoid moiety. This was further confirmed by observing the NOE's in the NOESY spectrum as indicated in the structure.

Biogenetically,[16] miltipolone (**15**) seems to be a precursor of norsalvioxide (**3**) and salviolone (**8**); oxidative decarbonylation would give the phenolic norsalvioxide (**3**) as in the case that the mild oxidation of a tropolonoid alkaloid, colchicein, produces a corresponding phenolic compound, colchinol:[17] Oxidative deoxymethylenation from miltipolone (**15**) would afford salviolone (**8**).

Salviolone exhibited a cytotoxic activity (TD_{50}) against Vero cells at 3.3 $\mu g\ ml^{-1}$, while miltipolone was active against murine melanoma cell (B16F10) and human colon carcinoma (HCT-116) with TD_{50} 0.16 and 0.003 $\mu g\ ml^{-1}$, respectively.

Experimental

Infrared spectra were recorded on a Hitachi 360 spectrophotometer and ultraviolet spectra were recorded on a Hitachi 340 spectrophotometer. Optical rotation was recorded on a JASCO DIP-181 polarimeter using a 10 cm microcell. ^1H and ^{13}C NMR spectra were taken on JEOL JNM-FX-90Q or Bruker AM-500 spectrometers. Proton chemical shifts are reported in ppm relative to internal TMS and carbon shifts relative to the center peak of $CDCl_3$ (δ=77.1).

Extraction and Isolation. The roots of *Salvia miltiorrhiza* Bunge were collected in June 1984 at Dalian, China. The roots were soaked in MeOH immediately after collection and allowed to stand for 2 weeks. The MeOH extract was concentrated and the residue washed with CH_2Cl_2. The CH_2Cl_2 solution was dried over Na_2SO_4 and concentrated to give a dark brown residue and this material was repeatedly separated by column chromatography on silica gel (Merck, Kieselgel 60 and Wako, Wakogel C-300). Further separations by preparative TLC (Merck GF 254, 0.5 mm) afforded norsalvioxide (**3**), salviolone (**8**), miltipolone (**15**), cryptoacetalide (**6**), and secodialdehyde (**4**).

Salviolone (8): Pale yellow solid, high resolution MS, Found: m/z 268.1490. Calcd for $C_{18}H_{20}O_2$: M, 268.1464. IR (KBr) ν_{max} 3270, 1630, 1566, 1240 cm^{-1}. UV λ_{max} (hexane) 388

(log ε 3.23), 368 (3.28), 312 (3.60), 285 (4.42), 249 (4.20), 242 nm (4.18). ^{1}H NMR δ=1.36 (6H, s, 17,18-Me$_2$), 1.72 (2H, m, 3-H$_2$), 1.95 (2H, quint, J=7 Hz, 2-H$_2$), 2.49 (3H, bs, 16-Me), 3.10 (2H, t, J=7 Hz, 1-H$_2$), 7.53 (1H, d, J=8.5 Hz, 6-H), 7.58 (1H, d, J=8.5 Hz, 7-H), 7.95 (1H, brs, 15-H), 8.05 (1H, s, 11-H), 8.45 (1H, brs, OH); ^{13}C NMR δ=19.7, 22.3, 28.9, 31.7 (×2), 35.0, 38.1, 113.2, 127.1, 131.3, 132.4, 134.0, 134.1, 136.0, 144.1, 148.8, 153.9, 179.9.

Miltipolone (15): Colorless crystals, mp 132 °C, high resolution MS, Found: m/z 301.1837. Calcd or $C_{19}H_{25}O_3$, MH$^+$: m/z 301.1803, $[\alpha]_D^{25}$ −77.8° (c 0.20, $CHCl_3$). UV λ_{max} (EtOH) 244 (log ε 4.52), 324 (3.89), 350 (3.89), 368 nm (3.80).

Methyl 5,5-Dimethyl-5,6,7,8-tetrahydro-1,2-naphthalenedicarboxylate (10). A mixture of 341 mg (2.50 mmol) of 6,6-dimethyl-1-vinylcyclohexene (**9**)[11] and 382 mg (2.69 mmol) of methyl acetylenedicarboxylate was heated at 90 °C for 20 h under an argon atmosphere. The crude product, ^{1}H NMR ($CDCl_3$) δ=5.38 (t, 1H), 3.79 (s, 3H), 3.74 (s, 3H), 3.1—2.9 (m, 2H), 1.7—1.2 (m, 4H), 1.09 (s, 3H), and 1.03 (s, 3H), was dissolved in 20 ml of benzene and 571 mg (2.52 mmol) of DDQ was added and the whole was stirred at room temperature for 4.5 d. The mixture was filtered through an alumina column eluted by benzene and pure **10** was obtained by Kugelrohr distillation (bath temp: 180—190 °C/0.3 mmHg, 1 mmHg= 133.322 Pa) in 37% yield. **10**: ^{1}H NMR ($CDCl_3$) δ=1.32 (s, 6H), 2.6—2.8 (m, 2H), 3.81 (s, 6H), 7.34 (d, 1H, J=8.6 Hz), and 7.71 (d, 1H, J=8.6 Hz). Found: C, 69.24; H, 7.38%. Calcd for $C_{16}H_{20}O_4$: C, 69.55; H, 7.30%.

5,5-Dimethyl-5,6,7,8-tetrahydro-1,2-naphthalenebis (methanol) (11). To an ice-cooled solution of 205 mg (0.74 mmol) of the diester **10** in 12 ml of dry THF was added 840 mg of lithium aluminium hydride and the mixture was stirred at room temperature for 1 h. A usual work-up gave crude products, which were purified by flash chromatography with dichloromethane–methanol (24 : 1) to give 139 mg (85%) of **11**; mp 83.5—85.0 °C (from benzene); ^{1}H NMR ($CDCl_3$) δ=1.28 (s, 6H), 1.5—2.0 (m, 4H), 2.8—3.0 (m, 2H), 4.78 (s, 2H), 4.82 (s, 2H), 7.10 (d, 1H, J=8 Hz), and 7.30 (d, 1H, J=8 Hz). Found: C, 76.27; H, 9.14%. Calcd for $C_{14}H_{20}O_2$: C, 76.33; H, 9.15%.

Salviolone Methyl Ether (13) and Its Isomeric Methyl Ether (14). To a 112 µg (1.27 mmol) of oxalyl dichloride in 1.86 ml of dichloromethane was added a solution of 185 µl (2.60 mmol) of DMSO in 0.56 ml of dichloromethane at −78 °C drop by drop. After 5 min, 100 mg (0.45 mmol) of the diol **11** in 0.82 ml of dichloromethane was added and the mixture was stirred at that temperature for 45 min. After triethylamine (700 µg, 5.02 mmol) had been added, the whole was stirred for 10 min and then warmed to room temperature. After 1 h, ethyl ether was added to the solution and the whole was filtered through a magnesium sulfate-layer and the filtrate was evaporated to give a crude dialdehyde **12**; ^{1}H NMR ($CDCl_3$) δ=1.1 (s, 6H), 1.2—1.7 (m, 4H), 2.4—2.7 (m, 2H), 7.2 (d, 1H, J=10 Hz), 7.4 (d, 1H, J=10 Hz), 10.0 (s, 1H), and 10.4 (s, 1H). As the aldehyde **12** was unstable, the crude one was used immediately in a subsequent step without further purification. A mixture of the crude dialdehyde **12** and 71 mg (0.70 mmol) of 1-methoxy-2-butanone[12] in 0.3 ml of 2 M NaOH (1 M=1 mol dm^{-3}) and 11.7 ml of distilled water was stirred at room temperature for 6 d;[13] the flask was occasionally ultrasonicated to disperse the syrup produced. The products were taken in diethyl ether and then dichloromethane, and the extracts were washed with saline and dried over anhyd Na_2SO_4. The crude materials were purified by flash chromatography with hexane–ethyl acetate (7 : 3) followed by a preparative TLC (hexane : AcOEt=8 : 2, 14 times development) to afford 28.5 mg of **13** and 18.7 mg of **14**. Total yield from the diol **11** was 28%. **13**; mp 177—178 °C (from benzene); ^{1}H NMR (500 MHz, $CDCl_3$) δ=1.34 (s, 6H), 1.71 (m, 2H), 1.94 (m, 2H), 2.37 (s, 3H), 3.04 (t, 2H, J=6.4 Hz), 3.93 (s, 3H), 7.34 (s, 1H), 7.47 (d, 1H, J=8.5 Hz), 7.50 (d, 1H, J=8.5 Hz), and 7.71 (s, 1H); NOE 2.37 vs. 7.71, 3.93 vs. 7.34, and 3.04 vs. 7.34; ^{13}C NMR (125 MHz, $CDCl_3$) δ=19.8, 22.9, 29.0, 31.8, 34.9, 38.2, 56.0, 111.2, 127.0, 131.1, 131.5, 134.1, 138.9, 140.8, 148.2, 156.3, and 181.6. Found: C, 80.61; H, 7.83%. Calcd for $C_{19}H_{22}O_2$: C, 80.93; H, 7.85%. **14**: Oil; ^{1}H NMR ($CDCl_3$) δ=1.33 (s, 6H), 1.69 (m, 2H), 1.92 (m, 2H), 2.43 (s, 3H), 3.07 (t, 2H, J=6.4 Hz), 3.93 (s, 3H), 7.01 (s, 1H), 7.48 (d, 1H, J=8.5 Hz), 7.54 (d, 1H, J=8.5 Hz), and 8.14 (s, 1H); NOE: δ=8.14 vs. 3.07, δ=2.43 vs. 8.14, and δ=3.93 vs. 7.01.

Synthesis of Salviolone (15). A mixture of 5.9 mg of **13** and 0.75 ml of 40% aqueous HBr was heated at 130 °C for 14 h. The products were taken in chloroform and purified by preparative TLC (dichloromethane : hexane=4 : 1, 3 times development) to give 2.4 mg of **15** in 43% yield.

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16) The absolute configuration of miltipolone (**15**) has not been determined experimentally, but it has been tentatively assigned as seen in structure **15** by consideration of the cooccurrence of secodialdehyde (**4**), abietatriene (**5**), and isomanool (**7**), the absolute configurations of which have been already established.

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