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Miltipolone, a New Diterpenoid Tropolone Possessing Cytotoxic Activities from Salvia miltiorrhiza Bunge

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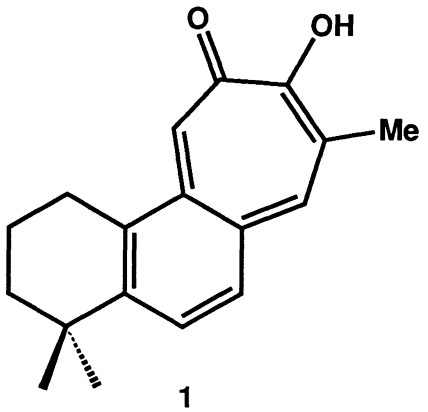
A potent cytotoxic compound, miltipolone, isolated from the fresh root of Salvia miltiorrhiza has been determined to have a novel tropolonoid norditerpene structure.

"Dan-Shen" is a dried root of Salvia miltiorrhiza Bunge, and used for treatment of various diseases in traditional Chinese medicine.[1] A number of quinonoid pigments possessing norabietane skeletons were found from this drug.[2] Some of these compounds were considered to be artifacts produced during the drug preparation. We studied pharmaceutically active constituents from the fresh root of S. miltiorrhiza and found colorless compounds salviolone(1) and norsalvioxide(2).[3] Present report deals with structure elucidation of a further new norditerpenoid, which is the first diterpenoid tropolone possessing potent cytotoxic activities.

The roots were soaked in methanol just after the collection, and the extract was chromatographically separated, affording a colorless crystals, mp 132 °C; $[\alpha]_D^{25}$ -77.8 (c 0.201, $CHCl_3$), designated as miltipolone(4), besides salviolone(1) and norsalvioxide(2).

The molecular composition of miltipolone was determined to be $C_{19}H_{24}O_3$ from a high resolution FAB mass peak [M + H^+: m/e 301.1837]. 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 downfield ^{13}C-NMR signal at δ 171.97 (Table 1) indicated the presence of a carbonyl group involved in a highly conjugated system. Appearance of seven downfield signals at δ 120-172 in the ^{13}C-NMR spectrum and two downfield proton signals at 7.28 and 7.40 as well as an aromatic methyl signal at 2.42 ppm in ^1H-NMR spectrum, and deep violet coloration by ferric chloride suggested the existence of some particular aromatic system such as an o-hydroxyacetophenone or a tropolonoid group. The tropolonoid chromophore seemed to be more probable because of the detection of coupling between δ 2.42 and

proton signals at 7.28, and the UV absorption maxima at 244, 324, 350, and 368 nm.[4] The presence of the methyltropolone moiety was confirmed by determining the connectivity of seven aromatic carbon atoms (8-C—15-C) by COLOC spectrum (Table 1). Remaining one oxygen atom of the three was found to be involved in ether linkage from ^{13}C-NMR signals at δ 74.38(d;7-C) and 67.35(t;16-C), and ^{1}H-NMR signals at δ 2.99, 4.36(16-CH_2) and 4.68 (7-H). Except for the two aromatic proton signals, the ^{1}H-NMR patterns of miltipolone were almost superimposable to those of norsalvioxide(2).[3] This similarity inferred the presence of a partial structure (3) in miltipolone, and this structure was firmly established by the extensive two-dimensional NMR studies and double resonance methods. The geminal dimethyl group was confirmed by the appearance of a J-cross peak in the COSY and an NOE-cross peak in the NOESY spectra between the two aliphatic methyl signals at 0.86 and 1.16. The COLOC spectrum showed the J^3-cross peak between 5-C(41.47) and 1-H(1.72), 3-H(1.60), 7-H (4.68), 17-H(1.16), and 18-H(0.86). A long-range coupling between 16-H and 5-H indicated the antiparallel orientation of 5-H and 16-CH_2. Furthermore, J-coupling of δ 4.68(7-H) to the aromatic proton signal at 7.28(15-H) suggested the connectivity of the



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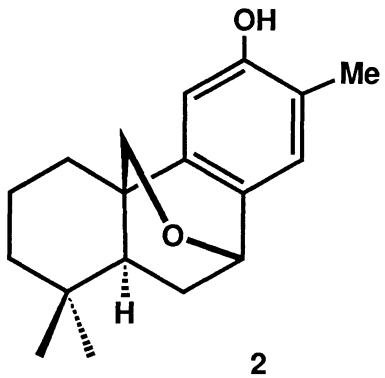

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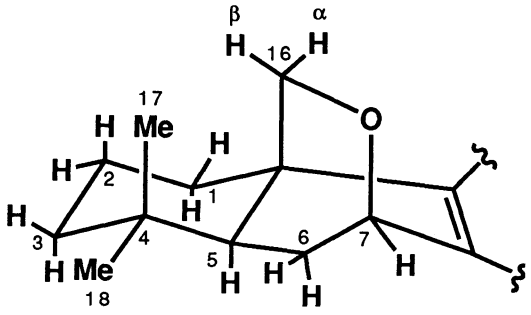

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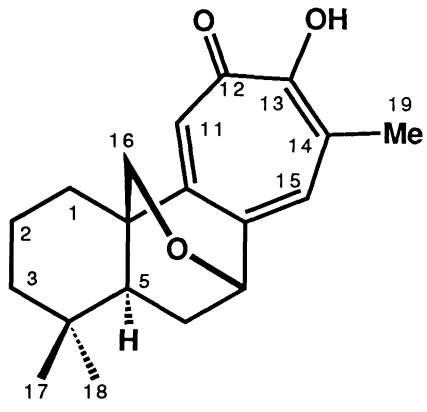

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Table 1. The correlated ^{1}H and ^{13}C NMR chemical shifts for 4

Carbon No.	$\delta^{13}C$	$\delta^{1}H$	$\delta^{1}H$ 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,13.4,3,1.9)	
2	18.98(t)	1H 1.62(m),1H 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)	1H 1.23(ddd,2.0,6.2,12.0)	0.86, 1.16, 1.60, 1.7, 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)	1H 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)		1.7, 7.40
11	120.02(d)	1H 7.40(s)	
12	171.97(s)		
13	166.37(s)	(-OH 9.6 br)	2.42, 7.28, 7.40
14	131.31(s)		2.42
15	136.37(d)	1H 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

partial structure (3) and the tropolone group. This structure was confirmed by the precise analyses of the COSY, COLOC, and NOESY spectra. To our knowledge, miltipolone is the first diterpenoid possessing a tropolone group.

Biogenetically this miltipolone is considered to be a precursor of salviolone(1) and norsalvioxide(2); oxidative de-oxymethylenation from miltipolone affords salviolone(1), and oxidative decarbonylation would

give the phenolic norsalvioxide(2) as in the case that the oxidation of a tropolonoid alkaloid, colchicein, produces a corresponding phenolic compound, cholchinol.[5]

Compound 4 was active against murine melanoma cell(B16F10) and human colon carcinoma(HCT-116) with IC_{50} 0.16 and 0.003 μg/ml, respectively.

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