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SALVIOLONE, A CYTOTOXIC BISNORDITERPENE WITH A BENZOTROPOLONE CHROMOPHORE FROM A CHINESE DRUG DAN-SHEN (SALVIA MILTIORRHIZA)

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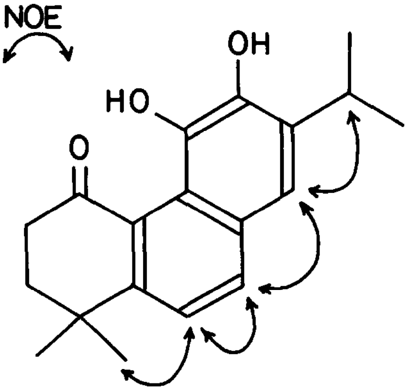
Abstract--- A new type of bisnorditerpene, salviolone (**4**) having a benzotropolone moiety has been isolated together with two other nor- and bisnorditerpenes **1** and **2** from the fresh root of Salvia miltiorrhiza. The compounds exhibit cytotoxic activity against Vero cells.

A number of C_{19}-norditerpenoids have been reported as constituents of a Chinese drug 'Dan-Shen', the dried root of Salvia miltiorrhiza.[1] Those compounds isolated from the root of the plant are mostly quinones likely formed by air-oxidation of phenols and hydroquinones that might be the 'genuine' components. We investigated pharmaceutically active components of the fresh root of S. miltiorrhiza, and isolated three new norditerpenoids. This report deals with their structure elucidation.

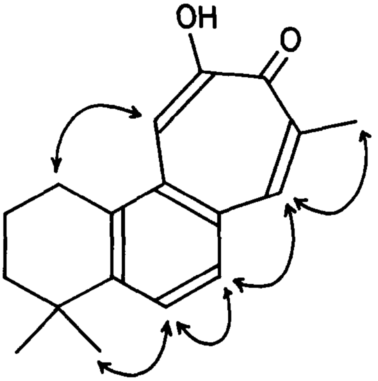
The root was soaked in methanol just after the collection, and the methanol extract was chromatographically separated, affording three components designated miltiodiol (**1**), norsalvioxide (**2**), and salviolone (**4**), respectively.

Miltiodiol (**1**),[2] $C_{19}H_{22}O_3$ (m/z 298.1565), shows the IR bands ascribable to a phenolic OH at 3480 and 1200 cm^{-1}. The presence of a carbonyl group is obvious from the ^{13}C-NMR signal at δ 204.5, and the carbonyl band at 1640 cm^{-1} in the IR spectrum suggests that the carbonyl group should be hydrogen bonded. The ^{1}H-NMR (500 MHz) spectrum inferred the existence of the following moieties; two tertiary methyls [δ 1.46 (6H, s)], an isopropyl [δ 1.33 (6H, d, J=7 Hz), 3.42 (1H, septet, J=7 Hz)], two mutually-adjacent methylenes [δ 2.06 (2H, t, J=7 Hz), 2.92 (2H, t, J=7 Hz)], and three aromatic protons [δ 7.25 (1H, s), 7.32 (1H, d, J=8.5 Hz), 7.94 (1H, d, J=8.5 Hz)], two of which are vicinal. Two singlets at δ 6.88 (1H) and 10.65 (1H) decreased their intensity by irradiation at δ 1.5 (H_2O signal in $CDCl_3$), and they are, therefore, assignable to the hydroxy protons. The downfield chemical shift (δ 10.65) of the latter hydroxy signal may be owing to hydrogen bonding to a carbonyl oxygen. Considering all of these spectral properties, we assigned the structure **1** for miltiodiol. The NOEs found for this compound are compatible with the proposed structure. Although miltiodiol has a catechol moiety, it resisted air-oxidation even when it was exposed in air on a TLC plate for 24 h.

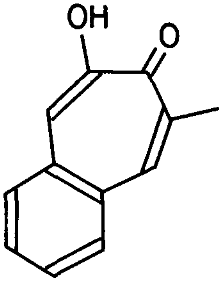
Norsalvioxide (**2**) has eighteen signals in the ^{13}C-NMR spectrum, and the high-resolution MS exhibits a molecular ion at m/z 272.1783 ($C_{18}H_{24}O_2$). Thus, this compound was supposed to be a rather uncommon bisnorditerpenoid. The presence of a hydroxyl group is obvious from the broad but intense band at 3500-3100 cm^{-1} in the IR spectrum, which lacks a carbonyl band. By the extensive ^{1}H-^{1}H and ^{13}C-^{1}H COSY spectral studies, the connectivity of protons and the assignment of carbons were firmly established (Table), and structures **2** and **3** were proposed for norsalvioxide, although the latter structure implied some conflicts in the chemical shifts of some protons. The existence of a geminal dimethyl group was deduced 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 [δ 0.84 (3H, s) and 1.16 (3H, s)]. Thus, structure **2** seemed preferable to **3**.



miltiodiol **1**



salviolone **4**



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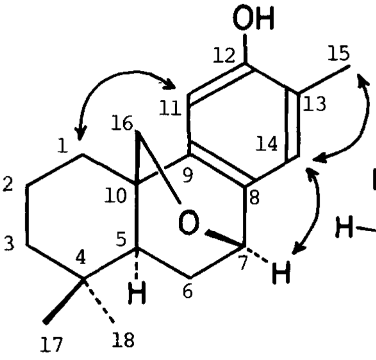
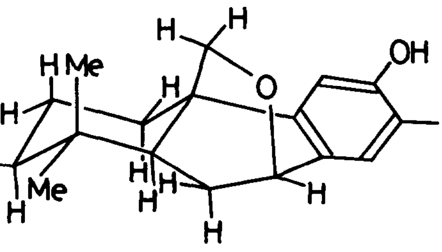


norsalvioxide **2**



2a

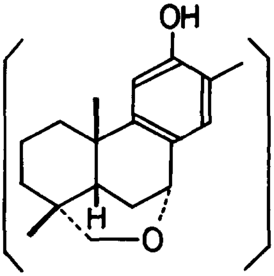


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Table. ^1H and ^{13}C-NMR data (δ; $CDCl_3$) of norsalvioxide (**2**)

No. of carbon	^{13}C-NMR (125 MHz)	^1H-NMR (500 MHz, J in Hz)
1	28.6 (t)	a) 1.87 (m) b) 1.72 (dtd, J=13, 3.5, 2)
2	18.9 (t)	1.65 (2H, m)
3	41.3 (t)	a) 1.19 (m) b) 1.57 (dtd, J=13, 3.5, 2)
4	33.9 (s)	-
5	43.1 (d)	1.19 (ddd, J=11.5, 7, 2)
6	30.3 (t)	a) 1.57 (ddd, J=13, 11.5, 2) b) 2.06 (ddd, J=13, 7, 3.5)
7	70.2 (d)	4.80 (dd, J=3.5, 2)
8	131.7 (s)	-
9	146.0 (s)	-
10	37.7 (s)	-
11	106.7 (d)	6.61 (s)
12	153.6 (s)	-
13	120.6 (s)	-
14	125.6 (d)	6.96 (s)
15	15.5 (q)	2.23 (3H, s)
16	67.8 (t)	a) 2.85 (dd, J=8, 2) b) 4.33 (d, J=8)
17	21.0 (q)	1.16 (3H, s)
18	32.9 (q)	0.84 (3H, s)

Eventually structure **2** was reinforced by the COLOC (J=8 Hz) spectrum that exhibits J-cross peaks from the protons of the two methyl groups (17-H_3 and 18-H_3) to a quaternary carbon (4-C, δ 33.9). (The two methyl protons in structure **3** cannot be coupled with a common quaternary carbon.) On the basis of the above spectral data the stereochemical features of norsalvioxide was determined to be **2a**. Noteworthy is the fact that the chemical shifts of the methylene protons (δ 2.85 and 4.33) at 16-C are considerably different each other due to an anisotropic effect of the phenyl group. One of them (δ 2.85) is coupled with 5-H through W-type coupling.

Biogenetically norsalvioxide (**2**) is related with carnosol, a diterpene isolated from Salvia carnosa.[3]

Salviolone (**4**),[4] $C_{18}H_{20}O_2$ (m/z 268.1490), was obtained as a pale yellow oil. The IR (FT-IR) spectrum shows the band ascribable to a phenolic OH at 3270 and 1240 cm^{-1}. An intense absorption at 1630 cm^{-1} is assignable to a carbonyl band (^{13}C-NMR; δ 179.9). So the carbonyl group should be involved in a highly conjugated system. Moreover, the downfield signals at δ 7.95 (s, 1H) and 8.05 (s, 1H) in the ^1H-NMR spectrum suggested the occurrence of some particular aromatic system. The spectrum also shows an AB pattern due to two vicinal aromatic protons at δ 7.53 (1H, d, J=8.5 Hz) and 7.58 (1H, d, J=8.5 Hz), besides a signal of phenolic OH at δ 8.45 (1H, brs). In the aliphatic region, a benzylic methylene signal appears at δ 3.10 (2H, t, J=7 Hz) which is coupled with a perturbed quintet at δ 1.95 (2H). The quintet was further coupled with a deformed triplet at δ 1.72 (2H). In the NOESY spectrum, a singlet at δ 1.36 (6H), possibly due to a gem-dimethyl group, exhibits a cross peak to the methylene signal at δ 1.72, implicating the moiety Ar-CH_2-CH_2-CH_2-CMe_2-. There is an extremely deshielded methyl signal resonating at δ 2.49. This signal was sharpened on irradiation at δ 7.95. Because of a limited amount (500 μg) of **4**, little information as to the carbon assignment was obtained except for the 1D ^{13}C-NMR spectrum.

Fortunately, the UV spectrum was very helpful for the structure elucidation; the UV spectrum of salviolone [λ_{max} (hexane) 388 (log ε 3.23), 368 (3.28), 312 (3.60), 285 (4.42), 249 (4.20), 242 (4.18) nm] was quite similar to that of 2-methyl-4,5-benzotropolone (**5**),[5] indicating the presence of the same chromophore in salviolone. The IR spectrum of **5** (3226, 1615, 1260 cm^{-1}) is also agreeable with that of **4**. On the basis of these findings together with the NOEs observed in the NOE difference and NOESY spectra, structure **4** was assigned for salviolone. To the best of our knowledge, **4** is the first example of natural product possessing a benzotropolone chromophore.

Salviolone (**4**) exhibits a cytotoxic activity (TD_{50}) against Vero cells at 3.3 μg/ml, while the activities of **1** and **2** are 10.1 and >80 μg/ml, respectively.

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4. **4**: IR (film) 3270 (br), 1630, 1566, 1240 cm^{-1}; ^{13}C-NMR (125 MHz, $CDCl_3$) δ 19.7, 22.3, 28.9, 31.7 (2xC), 35.0, 38.1, 113.2, 127.1, 131.3, 132.4, 134.1, 136.0, 144.1, 148.8, 153.9, 179.9. (One sp^2 carbon signal is missing possibly because of overlapping.)
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