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Microphyllandiolide, a New Diterpene with an Unprecedented Skeleton from *Salvia microphylla*

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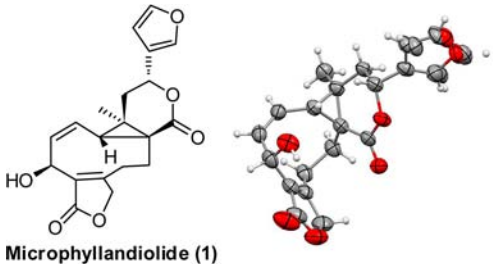
ABSTRACT



Microphyllandiolide (1)

Microphyllandiolide (1), an unprecedented rearranged clerodane-type diterpene with a 9/3 bicyclic ring system, was isolated from the aerial parts of *Salvia microphylla* Kunth. Its structure was elucidated by analysis of its spectroscopic data and confirmed by single crystal X-ray diffraction analysis. A possible biogenesis for microphyllandiolide (1) is proposed.

The species of *Salvia* genus (Lamiaceae) constitute a rich source of terpenoid compounds, mainly diterpenes with abietane and *neo*-clerodane skeletons.[1] Some of these diterpenes have shown uncommon carbon skeletons,[2] as well as interesting bioactivities, such as antimalarial, anti-HIV, cytotoxic, and opioid selective agonist.[3] In addition, many *Salvia* species are used as traditional remedies to treat a wide variety of ailments, throughout the world. All the above makes these plants a potential source of new chemical entities with biological properties.[4]

In México, *Salvia microphylla* and other *Salvia* species constitute a complex of medicinal plants known as "mirto". They are used to treat gastrointestinal problems and insomnia.[5] Three varieties of this species have been described: *microphylla*, *neurepia*, and *wizlizeni*.[6] However, the validity of these varieties as natural entities has not yet been proven.[7] On the other hand, previous investigations on the chemical composition of this species have led to the isolation of diterpenes with abietane, clerodane, and pimarane skeletons; eudesmane-type sesquiterpenes; and phenolic esters.[8]

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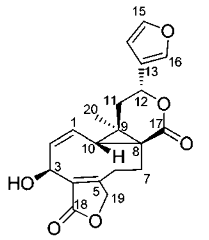
As a part of our study to identify new bioactive metabolites from *Salvia* species,[9] we undertake the examination of the aerial parts of *S. microphylla* Kunth., which were collected in México City, in September 2011, and were identified by M. Sci. María del Rosario García Peña (MEXU-1319659).

Then the dried and milled plant material (370 g) was extracted with acetone to obtain an extract (12.3 g) which was deffated[9b] and subjected to column chromatography over silica gel 60 G ($CHCl_3$−EtOAc 7:3 to 3:7). Silica gel column chromatography ($CHCl_2$−MeOH 199:1) of fractions eluted $CHCl_3$−EtOAc 7:3 (1.72 g) led to the isolation of **1** (190 mg, yield 0.051% w/w).



Microphyllandiolide (1)

Microphyllandiolide (**1**) was obtained as colorless needles.[10] Its molecular formula was determined as $C_{20}H_{20}O_6$ by HRFABMS ($[M + H]^+$ ion at *m*/*z* 357.1331, calcd. 357.1338), indicating 11 degrees of unsaturation. The IR absorption spectrum showed bands for hydroxyl and carbonyl groups, and double bonds. In the ^{13}C NMR spectrum the signals for 20 carbon atoms were observed. The DEPT spectrum revealed that these signals corresponded to one methyl, four methylenes, eight methines, and seven nonprotonated carbons. Analysis of the ^1H and ^{13}C NMR spectra showed the presence of a furan ring (δ_C 143.6, C-15; 139.7, C-16; 124.4, C-13; 108.4, C-14), a γ-lactone (δ_C 176.3, C-18; 74.8, C-19; δ_H 5.45, dt, J = 17.0, 1.5 Hz, H-19$_{Pro\text{-}R}$; 4.57, dd, J = 17.0, 2.5 Hz, H-19$_{Pro\text{-}S}$), and a δ-lactone (δ_C 173.3, C-17; 69.8, C-12; δ_H 5.25, dd, J = 12.5, 3.5 Hz, H-12). The above data suggested that **1** was a *neo*-clerodane type diterpene (Table 1).[1] In the HMBC spectrum (Figure 1), the signal at δ_H 5.11 (br d, J = 7.0 Hz) correlated with four signals of vinylic carbons at δ_C 130.9 (C-1), 133.5 (C-2), 126.3 (C-4), and 160.6 (C-5) and therefore was assigned to H-3. The signal of C-3 was observed at δ_C 63.6 (HSQC spectrum). The chemical shifts of H-3 and C-3, as well as the correlations in the HMBC spectrum of H-3, together with those of the vinylic protons H-1 (δ_H 5.99) and H-2 (δ_H 6.23), and with the signal of an hydroxyl group at δ_H 3.72 (d, J = 2.0 Hz) to C-3, indicated a doubly allylic hydroxyl group at C-3. Confirmation of the double bond at C-1 was achieved by the cross peaks between the signals of H-3 and the vinylic proton H-2, between H-2 and H-1, and between H-1 and H-10 (δ_H 3.62), which were observed in the COSY spectrum (Figure 1).

On the other hand, the presence of the 4,5-double bond and the correlations of H_2-7 and H_2-19 with C-5 indicated that compound **1** was 5,10-*seco*-neoclerodane.[11] In the COSY spectrum (Figure 1) the correlation of the H-3 signal with a signal at δ_H 6.23 (ddd, J = 11.0, 7.5, 2.0 Hz, H-2), which in turn showed a cross-peak with a signal at δ_H 5.99 (dd, J = 11.0, 6.5 Hz), which was assigned to H-1, confirmed a double bond at C-1. An additional correlation in the COSY spectrum was observed between H-1 and the signal at δ_H 3.62 (dd, J = 6.5, 2.0 Hz), assigned to H-10 (δ_C 25.5).

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR Data of **1**[a] in $CDCl_3$ (δ in ppm)

position	δ_H (J in Hz)	δ_C
1	5.99 (1H, dd, 11.0, 6.5)	130.9 (d)
2	6.23 (1H, ddd, 11.0, 7.5, 2.0)	133.5 (d)
3	5.11 (1H, br d, 7.0)	63.6 (d)
4		126.3 (s)
5		160.6 (s)
6α	2.18 (1H, ddd, 12.0, 8.0, 1.0)	20.3 (t)
6β	3.39 (1H, ddd, 15.0, 12.0, 10.0)	
7α	2.70 (1H, ddd, 15.0, 10.0, 8.5)	23.5 (t)
7β	1.71 (1H, ddd, 15.0, 9.0, 1.5)	
8		32.6 (s)
9		29 (s)
10	3.62 (1H, dd, 6.5, 2.0)	25.5 (s)
11α	2.08 (1H, dd, 14.0, 12.5)	35.5 (t)
11β	2.24 (1H, dd, 14.0, 3.5)	
12	5.25 (1H, dd, 12.5, 3.5)	69.8 (d)
13		124.4 (s)
14	6.36 (1H, dd, 1.5, 1.0)	108.4 (d)
15	7.37 (1H, t, 1.5)	143.6 (d)
16	7.41 (1H, m)	139.7 (d)
17		173.3 (s)
18		176.3 (s)
19-*Pro R*	5.45 (1H, dt, 17.0, 1.5)	74.8 (t)
19-*Pro S*	4.57 (1H, dd, 17.0, 2.5)	
20	1.39 (3H, s)	16.0 (q)

[a] Data were assigned by HSQC, HMBC, $^1H-^1H$-COSY, and NOESY spectra.

The presence of a cyclopropane ring in compound **1** was deduced from the HMBC correlations of H-1 to C-8 (δ_C 32.6), as well as those of H-10 to C-7 (δ_C 23.5) and C-17 (Figure 1). The relative configuration at C-8, C-9, and

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(10) Microphyllandiolide (**1**): mp 253−255 °C; $[\alpha]^{22}_{D}$ −334 (*c* 0.15, acetone); UV (MeOH) λ_{max} (log ε): 210 (4.39) nm; IR (KBr) λ_{max}: 3495, 1737, 1694, 1649, 874 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz) data in $CDCl_3$, see Table 1; EIMS (positive) *m*/*z* 356 $[M]^+$; HRFABMS (positive) *m*/*z* 357.1331 $[M + H]^+$ ($C_{20}H_{20}O_6$, calcd 357.1338).

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C-12 in compound **1** was assigned by analysis of coupling constants of the involved protons, NOESY correlations, and also considering that, in a *neo*-clerodane diterpene, C-20 has an α-orientation. Thus, the β-orientation of both C-11 and C-17 was deduced from the NOESY correlation between H_3-20 and H_2-7, in the α-side of the molecule. The H-12 coupling constants ($J_{11\alpha\text{-}12}$ = 12.5 Hz, $J_{11\beta\text{-}12}$ = 3.5 Hz) established its axial disposition, and its NOESY interaction with H-10, but not with H_3-20, indicated a β-orientation of H-10 and H-12. The information supplied by the NOESY experiment was not enough to establish the orientation of the OH group at C-3. Thus, a single X-ray diffraction analysis of **1** was carried out. The results confirmed the proposed structure of **1** (Figure 2),[12] including the β-orientation of the hydroxyl group at C-3, and its absolute configuration was established by anomalous dispersion from the oxygen atoms using Cu radiation (Flack parameter = −0.018).[13]

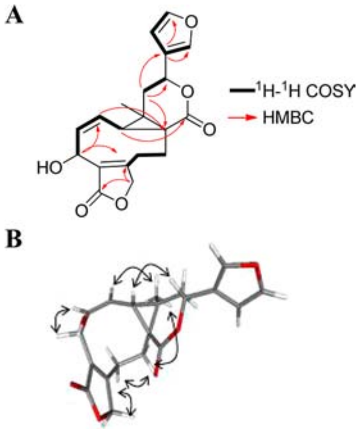


Figure 1. (A) Key ^{1}H−^{1}H COSY and HMBC correlations of **1**. (B) Key NOESY correlations of **1**.

Microphyllandiolide (**1**) represents the first example of a new framework with a 9/3 bicyclic ring system, which we named microphyllane. A plausible biogenetic pathway for this new skeleton arises from proposed formations of some other diterpenes isolated from *Salvia* species, where pericyclic reactions are involved.[1,14] Thereby compound **1** may be derived from a *neo*-clerodane diterpene (Scheme 1) through the electrocyclic opening of the decalin ring in the diene (**a**) to give the triene **b**, which, after cyclopropanation, gives **c**. An allylic oxidation of the last intermediate could take place to produce **1**.

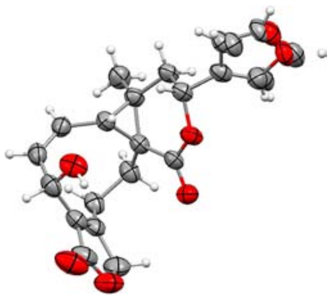
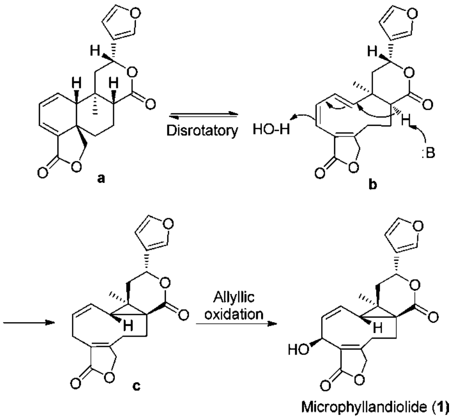


Figure 2. X-ray crystallographic structure of **1**.

Scheme 1. A Plausible Biogenetic Pathway to **1**



Microphyllandiolide (**1**) was evaluated for its cytotoxicity against six human cancer cells lines, HCT-15 human colorectal adenocarcinoma, K-562 leukemia, MCF-7 breast, PC-3 human prostatic adenocarcinoma, SKLU-1 lung cancer, and U-251 human glioblastoma, using the method previously reported.[9b] Compound **1** was further tested for its anti-inflamatory activity using the TPA induced ear edema model.[9b] However, no significant cytotoxic and anti-inflammatory activities were detected (IC_{50} > 50 μM and IC_{50} > 1 μmol/ear).

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[12] Crystallographic data of microphyllandiolide (**1**): $C_{20}H_{20}O_6$, MW = 356.36; orthorhombic, space group $P2_12_12_1$; a = 10.024 (4) Å, b = 10.275 (4) Å, c = 16.643 (6) Å, $\alpha = \beta = \gamma = 90°$, V = 1714.2 (11) Å^3, Z = 4, d = 1.381 g/cm^3, a crystal with dimensions 0.426 × 0.252 × 0.192 mm^3 was used for measurement on a Bruker Smart Apex II CCD diffractometer with a graphite monochromater, Cu Kα radiation (λ = 1.5418 Å). A total of 62 160 reflections were collected in the range 5.15° < θ < 66.79°, with 3020 independent reflections [R(int) = 0.0190)]; completeness to $\theta_{\max}$ was 99.3%. The crystal structure of compound **1** was solved by direct methods and refined by full-matrix least-squares on F^2, with anisotropic temperature factors for non-hydrogen atoms converging at final R indices [$I > 2\sigma(I)$], R_1 = 0.0309, wR_2 = 0.0923; R indices (all data), R_1 = 0.0308, wR_2 = 0.0922. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 942619). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

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Supporting Information Available. Experimental general procedures, 1D and 2D NMR spectra, and X-ray crystallographic data (CIF file) of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.