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Salvileucalin B, A Novel Diterpenoid with an Unprecedented Rearranged Neoclerodane Skeleton from *Salvia leucantha* Cav.

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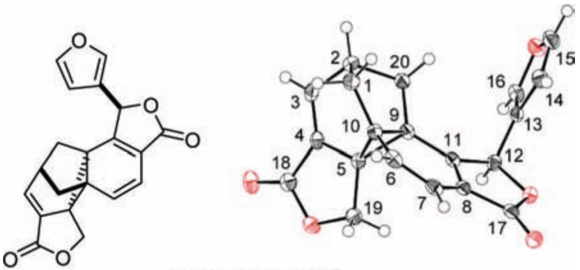
ABSTRACT



Salvileucalin B (**2**)

Salvileucalin B (2), having an unprecedented rearranged neoclerodane skeleton, was isolated from the aerial parts of *Salvia leucantha* Cav. (Labiatae) along with salvileucalin A (1). The absolute structures were elucidated by spectroscopic analysis, X-ray crystallographic analysis, and vibrational circular dichroism. Compound 2 represents a novel neoclerodane, characterized by a tricyclo[3.2.1.0^{2,7}]octane substructure incorporating the exocyclic C-20 methylene of 1. This molecule exerted cytotoxic activity against A549 and HT-29 cells with IC$_{50}$ values of 5.23 and 1.88 μg/mL, respectively.

A huge diversity of molecular skeletons found in natural products enables finding better lead compounds to develop clinically useful entities.[1] Diterpenes having an unprecedented carbon framework have recently become of interest for finding lead compounds in medicinal chemistry.[2]

The neoclerodane diterpenoids are important compounds from the phytochemical point of view because of their widespread distribution, extensive structure variation, and pronounced biological activity.[3] Of them, salvinolin A is a selective κ-opioid receptor agonist, and its hallucinogenic activity is the most potent among any other known non-nitrogenous compounds such as tetrahydrocannabinol or lysergic acid.[4] Salvinolin A and its analogues are expected to be lead compounds. Over 100 neoclerodane diterpenoids and related compounds have been isolated from plants belonging to the subgenus Calosphace[5] of the family Labiatae.[6] From the aerial parts of *Salvia leucantha* (common name "Mexican bush sage") of the subgenus Calosphace (Labiatae), an evergreen herbaceous

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(1) Kingston, D. G. I. *J. Org. Chem.* **2008**, *73*, 3975–3984.

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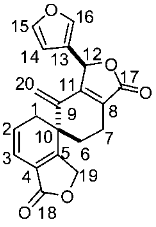
Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data for Salvileucalins A (**1**) and B (**2**) in $CDCl_3$

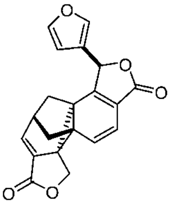
	1		**2**	
no.	δ_c[a]	δ_H[a,b]	δ_c[a]	δ_H[a,b]
1	34.5 (t)	2.52 (2H, m)	29.6 (t)	1.33 (1H, d, 12.2) 1.72 (1H, dd, 12.2, 4.7)
2	127.5 (d)	5.91 (1H, ddd, 9.6, 4.3, 4.3)	31.4 (d)	2.85 (1H, br ddd)
3	117.3 (d)	6.32 (1H, d, 9.6)	134.3 (d)	7.14 (1H, d, 7.0)
4	126.8 (s)		123.7 (s)	
5	159.8 (s)		25.7 (s)	
6	31.8 (t)	1.95 (1H, m) 2.07 (1H, m)	126.3 (d)	6.24 (1H, d, 9.6)
7	16.9 (t)	2.52 (1H, m) 2.62 (1H, m)	117.9 (d)	6.62 (1H, d, 9.6)
8	127.4 (s)		122.6 (s)	
9	136.5 (s)		36.6 (s)	
10	40.1 (s)		42.3 (s)	
11	153.7 (s)		154.3 (s)	
12	74.6 (d)	6.05 (1H, dd, 2.3, 2.3)	76.0 (d)	6.13 (1H, s)
13	121.0 (s)		119.2 (s)	
14	108.3 (d)	6.22 (1H, d, 1.3)	108.2 (d)	6.22 (1H, br s)
15	144.3 (d)	7.42 (1H, dd, 1.3, 1.3)	144.5 (d)	7.44 (1H, br dd)
16	141.7 (d)	7.49 (1H, s)	142.2 (d)	7.59 (1H, br s)
17	171.5 (s)		170.0 (s)	
18	171.0 (s)		168.1 (s)	
19	68.8 (t)	4.77 (1H, d, 18.2) 4.84 (1H, d, 18.2)	66.3 (t) 3.95 (1H, d, 10.5)	3.80 (1H, d, 10.5)
20	118.6 (t)	5.10 (1H, s) 5.23 (1H, s)	27.3 (t) 1.36 (1H, dd, 12.4, 4.7)	1.01 (1H, d, 12.4)

[a] s: singlet, d: doublet, t: triplet, m: multiplet. [b] Data in parentheses refer to coupling constants (Hz).

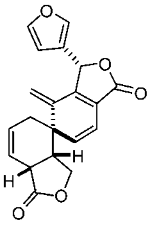
perennial in the frost-free climate area, some rearranged neoclerodane diterpenes were isolated by Esquivel et al.,[7] and another highly rearranged diterpene, spiroleucantholide **3**, having a spiro skeleton in the molecule, by Takeda et al.[8] In the present study, we isolated a caged polycyclic neoclerodane, salvileucalin B (**2**) along with biosynthetically correlated salvileucalin A (**1**) from the aerial parts of this plant and elucidated their structures by using spectroscopic methods including NMR, IR, HRMS, and vibrational circular dichroism (VCD).



Salvileucalin A (**1**)



Salvileucalin B (**2**)



Spiroleucantholide (**3**)

The air-dried aerial parts of *Salvia leucantha* (18.66 kg) were extracted with acetone (3 × 40 L). The combined acetone extract was concentrated and subjected to Diaion HP-20 resin column chromatography eluting with H_2O, 50% MeOH, 80% MeOH, 100% MeOH, and acetone (5 L, each). The fraction eluted with 80% MeOH was then subjected to repeated silica gel column chromatography (solvent system: hexane−acetone or hexane−ethyl acetate) and ODS-HPLC (solvent system: H_2O−methanol or H_2O−acetonitrile) to give compounds **1** (8 mg) and **2** (18 mg).

Compound **1** was obtained as a colorless amorphous powder. The molecular formula was determined to be $C_{20}H_{16}O_5$ from the [M + Na] ion peak at *m/z* 359.0858 in HRESIMS, implying it had 13 degrees of unsaturation. The IR absorption spectrum showed a band of carbonyl groups (1751 cm^{-1}). The molecular formula, and the ^{1}H and ^{13}C NMR spectra showing the presence of one furan unit (δ_c 108.3, 121.0, 141.7, and 144.3), one exocyclic methylene unit (δ_H 5.10 and 5.23, δ_c 118.6), two γ-lactones (carbonyl carbons, δ_c 171.0 and 171.5), and four ring methylenes (δ_c 16.9, 34.5, 31.8, and 68.8) suggested that **1** was a neoclerodane diterpene with one exocyclic methylene group (Table 1). The HMBC correlations observed between the hydrogens H-1, H-6, H-19, and H-20 and the carbon C-10 indicated that **1** had a spiro structure at C-10 (Figure 1). Further, the HMBC correlations between the hydrogen H-12 and the carbons C-13 and C-11 showed that the furan unit was attached to C-12. Then, the substance was recrystallized from methanol, and the crystals were subjected to X-ray crystallographic analysis, which showed the relative structure of **1** to be as shown in Figure 2.[9] Determination of the absolute configuration was

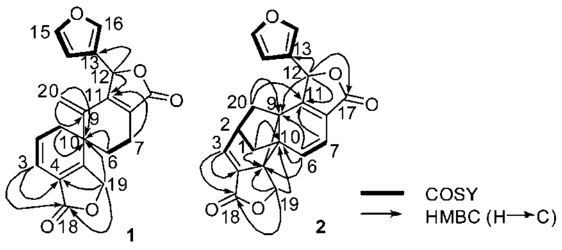


Figure 1. Selected COSY and HMBC correlations for **1** and **2**.

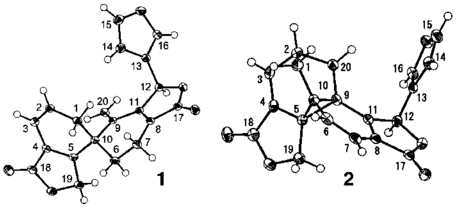
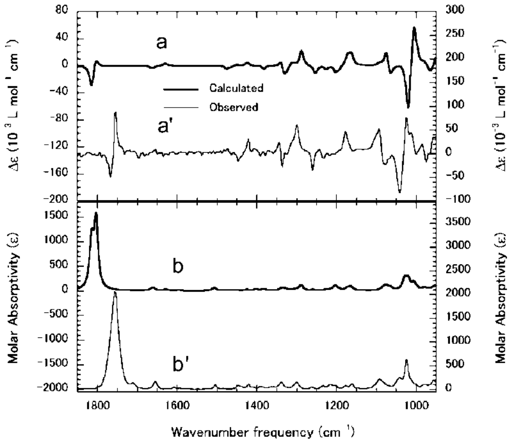


Figure 2. ORTEP representation of **1** and **2**.

carried out by using the VCD technique, which has been recently used for determination of the absolute configuration of several natural products.[10] This technique is based on comparison between the experimental VCD spectrum and the corresponding theoretical curve for the proper enantiomer obtained by the density functional theory (DFT) calculations of the vibrational frequencies and VCD intensities. The VCD and IR spectra of **1** ($CDCl_3$, 0.008 M, BaF_2, 491 μm path length) were measured (a′ and b′, respectively, in Figure 3). Then the conformational and vibrational analyses were performed by DFT calculations,[11,12] which suggested the presence of three possible energetically optimized conformers.[13] The predicted (population weighed) VCD and IR spectra were calculated, which are shown in a and b, respectively, in Figure 3. The observed VCD and IR spectra are similar to the calculated (population weighed) spectra. Consequently, **1** was shown to have the structure as shown in Figure 2. The structures of **1** and spiroleucantholide (**3**)[8] are very similar in that they both are rearranged neoclerodanes having a spiro substructure unit. However, a double bond is present at Δ^4 in **1**, whereas it is at Δ^6 in **3**, and the stereochemistry of C-12 in **1** is opposite to that in **3**.

1) IR and VCD spectra of **1**



2) IR and VCD spectra of **2**

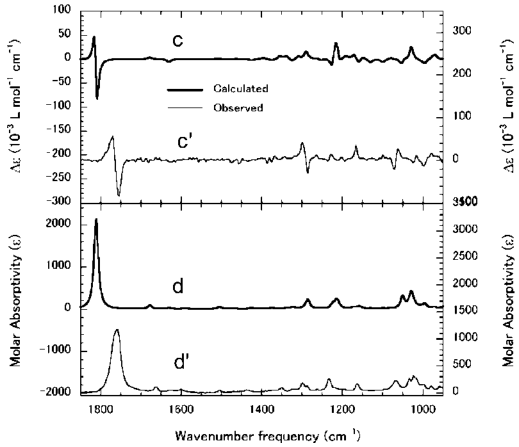


Figure 3. Comparison of the measured VCD ($\Delta\epsilon$) and IR (ϵ) spectra with the predicted (population weighed) spectra. (a′) Measured VCD ($\Delta\epsilon$) spectra of **1**; (a) calculated predicted (population weighed) VCD spectra ($\Delta\epsilon$) spectra of **1**; (b′) measured IR (ϵ) spectra of **1**; (b) calculated predicted (population weighed) IR (ϵ) spectra of **1**; (c′) measured VCD ($\Delta\epsilon$) spectra of **2**; (c) calculated predicted (population weighed) VCD ($\Delta\epsilon$) spectra of **2**; (d′) measured IR (ϵ) spectra of **2**; (d) calculated predicted (population weighed) IR (ϵ) spectra of **2**.

Compound **2** was obtained as a colorless amorphous powder. The molecular formula was determined to be $C_{20}H_{14}O_5$ from the [M + H] ion peak at m/z 335.0934 in HRESIMS, which implied the presence of 14 degrees of unsaturation. The IR absorption bands indicated the presence of a carbonyl group (1753 cm^{-1}). The molecular formula and the ^{1}H and ^{13}C NMR spectra showing the presence of one furan unit (δ_c 108.2, 119.2, 142.2, and 144.5) and two γ-lactones (carbonyl carbons, δ_c 168.1 and 170.0) suggested that **2** was also a rearranged neoclerodane diterpene (Table 1). The HMBC correlations were observed between the hydrogens H-1, H-6, and H-19 and the carbon C-10 and between the hydrogens H-1, H-19, and H-6 and the carbon C-5 (Figure 1). Further HMBC correlations observed between the hydrogens H-20, H-6, and H-12 and the carbon C-9 indicated that **2** had a bridged cyclic subunit, a tricyclo[3.2.1.$0^{2,7}$]octane[14] system. Then, **2** was recrystallized from methanol, and the crystals were subjected to X-ray crystallographic analysis, which revealed the relative structure of **2** to be as shown in Figure 2.[9] Determination of the absolute configuration of **2** was carried out in the same way as for **1**. Namely, the conformational and vibrational analyses of **2** were carried out by measuring the VCD and IR spectra ($CDCl_3$, 0.015 M, BaF_2, 491 μm path length) and comparing them with the theoretical ones obtained by using DFT calculations (c and d, respectively, in Figure 3). The VCD bands in **2** are relatively weak compared with those in **1**. Thus the comparison of the predicted VCD spectra calculated by using three possible

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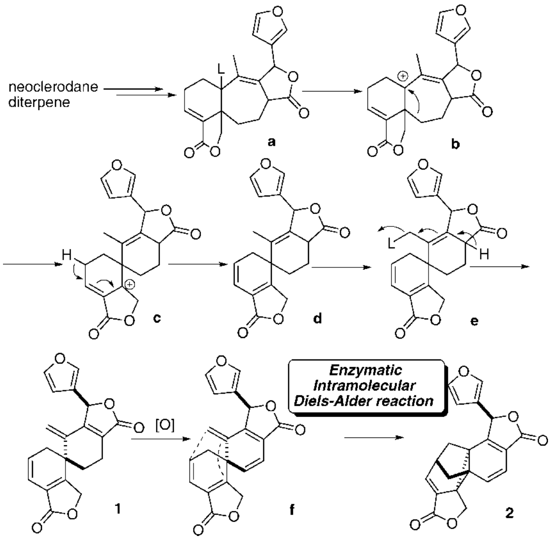
conformers with the measured one is not quite possible. However, the measured VCD band in the range of 1700−1850 cm^{-1} due to the two carbonyl groups definitely corresponds to the predict band, indicating that the three-dimensional arrangement between the two carbonyl groups attached to the rigid carbon skeleton is as shown in Figure 2. Consequently, **2**, named salvileucalin B, was elucidated to have the structure shown in Figure 2.

Salvileucalin B is the first natural product having a caged carbon framework, tricyclo[3.2.1.0^{2,7}]octane,[14] among neoclerodane diterpenoids. A plausible biogenetic pathway is shown in Scheme 1. Thus, the salvigenane skeleton (**a**),[15] possibly derived from neoclerodane diterpenoids, is rearranged to give a spiro compound (**c**) via the cation **b**, which, after homoallylic isomerization, gives salvileucalin A (**1**). After oxidation of **1**, an intramolecular Diels−Alder reaction may proceed to give **2**. A number of natural products which were produced via enzymatic or nonenzymatic Diels−Alder reaction have been isolated.[16] We found that grandione, an isetexane diterpene dimer, was produced via intermolecular hetero-Diels−Alder reaction mediated by some enzyme on the basis of chemical experiments.[17] The orientation of the Δ^9-double bond and the presence of the diene of (Δ^{2,4}) are not favorable for the Diels−Alder reaction to proceed. Accordingly, an enzyme-assisted intramolecular Diels−Alder reaction may take place to produce **2**.

Scheme 1. Proposed Biosynthetic Pathway for **1** and **2**



Salvileucalin B (**2**) exhibited cytotoxicity against A549 (human lung adenocarcinoma) and HT-29 (human colon adenocarcinoma) cells with IC$_{50}$ values of 5.23 and 1.88 μg/mL, respectively.

Thus, in the present study, two novel rearranged neoclerodane diterpenoids, salvileucalins A (**1**) and B (**2**), were isolated from *Salvia leucantha*, and their absolute configuration was determined by using VCD spectrum and DFT calculations. Structures of these two compounds suggest that they are biogenetically correlated.

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Supporting Information Available: Detailed experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Crystallographic data for compounds **1** and **2** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 693597 and No. CCDC 693598, respectively. Copies of the data can be obtained, free of charge, on application to the Director, CCDC (e-mail: deposit@ccdc.cam.ac.uk).

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