

Defensive Sesterterpenoids with Unusual Antipodal Cyclopentenones from the Leaves of *Leucosceptrum canum*

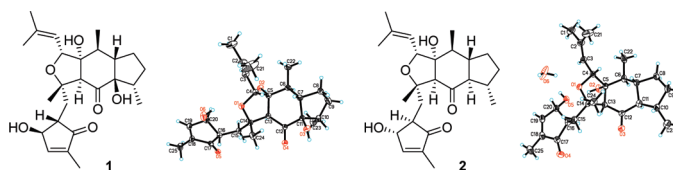
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ABSTRACT



Two novel sesterterpenoids, leucosceptroids C (1) and D (2), possessing unusual antipodal cyclopentenones while maintaining the stereochemistry and functionality of the tricyclic cores, were discovered from the leaves of *Leucosceptrum canum* (Labiatae). Their structures including absolute stereochemistries were determined by comprehensive NMR, MS, and single-crystal X-ray diffraction analyses. The potent antifeedant activity of 1 against the generalist plant-feeding insect *Helicoverpa armigera* ($EC_{50} = 0.017 \mu\text{mol}/\text{cm}^2$) suggested them to be new defensive sesterterpenoids of *L. canum*.

Terpenoids are the largest group of natural products with highly diversified chemical structures and a variety of roles including especially a defensive function in the natural world.¹ Most terpenoids are cyclized to form various skeletons containing one or more rings. Cyclizations of natural terpenoids are catalyzed by terpenoid cyclases and are the most complex chemical reactions with remarkable stereochemical precision in Nature.²

Among plants of southwest China, *Leucosceptrum canum* Smith, a woody plant belonging to the family Labiatae

(= Lamiaceae), is perhaps the largest (up to 10 m high³) and so far the only colored nectar plant⁴ of all Labiatae species and is found in the Himalayas to the southwest of China.⁴ This plant is rarely attacked by herbivores and only occasionally by pathogens. We have been interested in the relationship between the secondary metabolites of *L. canum* and its above special traits and have found that glandular trichomes of *L. canum* could harbor unique defensive sesterterpenoids, leucosceptroids A and B (Figure 1).⁵ Our further investigation on the defensive sesterterpenoids in the leaves led to the discovery of two additional intriguing sesterterpenoids, leucosceptroids C (1) and D (2) (Figure 1), possessing novel C₂₅ carbon frameworks containing unusual antipodal cyclopentenones while maintaining the stereochemistry of the other rings and substituents. In this communication, we report their isolation, structure determination including absolute stereochemistries,

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and antifeedant activity against the generalist plant-feeding insect *Helicoverpa armigera*.

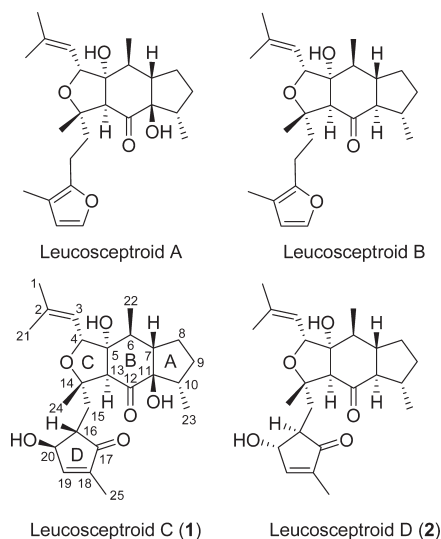


Figure 1. Chemical structures of leucosceptroids A, B, C (**1**), and D (**2**).

Compound **1** was obtained as colorless blocks, having a molecular formula of $C_{25}H_{36}O_6$, as determined by its negative FAB-MS and high resolution ESI-MS. The IR spectrum indicated the presence of hydroxy groups (3513 and 3413 cm^{-1}) and ketone groups (1705 cm^{-1}).⁶ In the ^1H NMR spectrum (Table 1 and Figure S5), two secondary methyls at δ_{H} 0.79 (d, $J = 7.4\text{ Hz}$) and 0.94 (d, $J = 6.8\text{ Hz}$) and four tertiary methyls at δ_{H} 1.27 (s), 1.70 (br s), and 1.72 (6H, s) were clearly shown. Two olefinic protons at δ_{H} 5.57 (d, $J = 8.8\text{ Hz}$) and 7.20 (br s) indicated the presence of two trisubstituted double bonds. Four singlets at δ_{H} 2.76, 4.46, 4.58, and 4.63 and two doublets at δ_{H} 4.68 ($J = 2.8\text{ Hz}$) and 4.76 ($J = 8.8\text{ Hz}$) were ascribable to either methine or free hydroxy groups. Other signals occurred in a relatively high-field region (between 1.38 and 2.43 ppm) and mostly overlapped, resonating from either methine or methylene signals. The ^{13}C NMR and DEPT spectra (Table 1 and Figure S6) exhibited 25 carbon resonances, corresponding to two keto groups, two double bonds, three oxygenated quaternary carbons, seven methines including two oxymethines, three methylenes, and six methyls. These data were consistent with the signals observed in ^1H NMR spectrum.

The 2D NMR spectra of **1** including ^1H – ^1H COSY, HSQC, and HMBC (Figures S7–S9) established the gross structure of **1** to be a highly oxygenated sesterterpenoid. The resemblance of NMR spectral data of **1** with those of leucosceptroid A (Figure 1), one of the defensive

Table 1. ^1H and ^{13}C NMR Data of Leucosceptroids C and D (**1** and **2**) in Acetone- d_6

no.	1^a		2^b	
	δ_{H} , ([J_{Hz}])	δ_{C}	δ_{H} , ([J_{Hz}])	δ_{C}
1	1.72 (3H, s)	18.8 q	1.72 d (3H, 1.2)	18.7 q
2	-	138.0 s	-	137.8 s
3	5.57 d (8.8)	121.8 d	5.64 d (8.5)	121.9 d
4	4.76 d (8.8)	77.4 d	4.48 d (8.5)	76.7 d
5	-	84.1 s	-	86.4 s
6	1.78 m	41.8 d	1.91 m	46.5 d
7	2.00 m	50.2 d	1.82 m	46.3 d
8 α	1.64 m	-	1.31 m	-
8 β	2.08 m	30.4 t	1.81 m	29.0 t
9 α	1.38 m	-	1.28 m	-
9 β	2.03 m	30.9 t	1.94 m	32.9 t
10	2.25 m	46.0 d	2.08 m	33.7 d
11	-	85.5 s	1.83 m	65.1 d
12	-	212.5 s	-	210.4 s
13	2.76 s	73.3 d	2.90 s	69.2 d
14	-	83.7 s	-	83.1 s
15a	2.28 dd (2.5, 14.0)	-	2.40 dd (2.5, 14.5)	-
15b	1.89 dd (10.5, 14.0)	44.2 t	1.59 dd (12.0, 14.5)	42.0 t
16	2.43 dt (2.5, 10.5)	53.8 d	2.32 dt (2.5, 12.0)	52.8 d
17	-	207.4 s	-	207.7 s
18	-	141.8 s	-	141.7 s
19	7.20 br s	157.0 d	7.18 br s	157.5 d
20	4.63 br s	76.7 d	4.70 br s	76.1 d
21	1.72 (3H, s)	26.0 q	1.73 d (3H, 1.2)	26.2 q
22	0.94 d (3H, 6.8)	13.8 q	0.96 d (3H, 7.0)	14.1 q
23	0.79 d (3H, 7.4)	17.2 q	1.12 d (3H, 6.5)	21.9 q
24	1.27 (3H, s)	23.6 q	1.20 (3H, s)	25.0 q
25	1.70 (3H, br s)	10.0 q	1.71 (3H, br s)	10.1 q

^a ^1H NMR spectrum was recorded at 500 MHz and ^{13}C NMR spectrum at 125 MHz. Hydroxy group signals of **1**: δ_{H} 4.46 (s, 5-OH), 4.58 (s, 11-OH), and 4.68 (d, $J = 2.8\text{ Hz}$, 20-OH). ^b ^1H NMR spectrum was recorded at 400 MHz and ^{13}C NMR spectrum at 100 MHz. Hydroxy group signals of **2**: δ_{H} 4.88 (s, 5-OH) and 4.75 (d, $J = 2.8\text{ Hz}$, 20-OH).

sesterterpenoids in glandular trichomes of *L. canum*,⁵ suggested that they had the same core structure constituted by a 5/6/5 ring system (A, B, and C rings). In addition, an isoprenyl motif at the same position in the northern hemisphere still existed in **1**.

However, the significant NMR difference between **1** and leucosceptroid A indicated that their D rings in the southern hemisphere were distinct from each other. An α,β -unsaturated keto moiety, substituted by a methyl group at the α -position, could be easily recognized in **1**. The coupling relationship of H-16/H-20/H-19 was established by ^1H – ^1H correlations (Figure S9), which allowed direct linkages between C-16 and C-20 and between C-20 and C-19 in **1**. The ^1H – ^{13}C correlations (Figures S1 and S8) from H-16 to C-17 indicated a connection between C-16 and C-17. Thus the furan ring in leucosceptroid A was replaced by a cyclopentenone in **1**. A free hydroxyl group at δ_{H} 4.68 (d, $J = 2.8\text{ Hz}$) was assignable to C-20, based on its ^1H – ^1H correlation with H-20 and HMBC correlations with C-20, C-19, and C-16.

As expected, the relative stereochemistry of chiral centers in the core structure of **1** was found to be identical to those in leucosceptroid A, judging from their similar ROESY

(6) Leucosceptroid C (**1**): colorless blocks, mp 204–206 °C; $[\alpha]_{\text{D}}^{27} = +121.3$ ($c = 0.4$, MeOH); UV (MeOH) λ_{max} (log ϵ): 376 (2.22), 206 (3.65) nm; IR (KBr) ν_{max} : 3513, 3413, 2967, 2936, 1705, 1444, 1380, 1331, 1248, 1107, 1039, 1026, 921 cm^{-1} ; negative FAB-MS m/z (%): 432 (16) [M], 431 (67) [M – H][–], 281 (17), 255 (40); HR-ESI-MS: m/z 455.2418 [M + Na]⁺ (m/z_{calcd} [$C_{25}H_{36}O_6Na$]⁺ = 455.2409).

correlations for rings A, B, and C (Table S1 and Figure S10). However, it was not possible to use ROESY experiments to identify the stereochemistry of the two chiral centers (C-16 and C-20) in ring D. To solve this problem, an X-ray diffraction of **1** was necessary. A single crystal of **1** was successfully obtained from a mixture of petroleum ether/acetone (1:2), and X-ray crystallographic analysis was carried out with molybdenum radiation (CCDC 810481), which confirmed the deduced structure and established the relative stereochemistry of **1** as shown in Figure S2.

The FAB-MS (negative ion mode) and HR-ESI-MS⁷ of **2** indicated a molecular formula of C₂₅H₃₆O₅, which was only one oxygen atom less than that of **1**. The close resemblance between the NMR spectra of **2** (Table 1 and Figures S11–16) and those of **1** indicated that **2** was another sesterterpenoid structurally similar to **1**. The major difference was the replacement of an oxyquaternary carbon in **1** by a methine group in **2** (δ_{C} 65.1). The long-range ¹H–¹³C correlations (Figure S3) from H-7, H-10, and Me-23 to this methine carbon, and from the corresponding methine proton (δ_{H} 1.83) to C-7, C-10 and C-12, indicated this methine carbon to be C-11. Accordingly, compound **2** was identified as an 11-dehydroxy derivative of **1**.

In the ROESY spectrum (Table S1 and Figure S16) of **2**, correlations between H-11 and H-8 α , H-9 α , and Me-23 suggested H-11 to be α -oriented, as has been found in leucosceptroid B (Figure 1), another defensive sesterterpenoid in glandular trichomes of *L. canum*.⁵ Other ROEs of **2** implied that the relative stereochemistry of the remaining chiral centers in the core structure of **2** were identical to those in **1** and leucosceptroid B. Although the stereochemistry of ring D of **2** was not possible to determine with ROESY experiments, it was originally thought to be identical to that of **1**, since the chemical shifts of H/C-16 and H/C-20 of the two compounds were so close to each other, and the cyclizations of terpenoids are usually highly stereospecific. However, to our surprise, X-ray crystallographic analysis on a single crystal of **2** (obtained from a mixture of acetone/H₂O, 10:0.1) with molybdenum radiation (CCDC 810483) (Figure S4), while confirming the deduced structure and the relative stereochemistry of the core structure (A, B, and C rings) of **2**, disclosed that the stereochemistry of ring D of **2** was opposite to that of **1**.

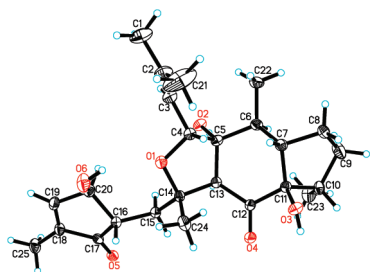


Figure 2. X-ray crystallographic structure of **1** showing the absolute stereochemistry.

To further confirm the above observation, X-ray crystallographic analyses of both **1** and **2** using anomalous dispersion with copper radiation were performed (CCDCs 810480 and 810482) (Figures 2 and 3), which determined the absolute stereochemistry of **1** to be 4*R*,5*R*,6*S*,7*S*,10*S*,11*R*,13*R*,14*S*,16*S*,20*R* and the absolute stereochemistry of **2** to be 4*R*,5*R*,6*S*,7*S*,10*S*,11*R*,13*R*,14*S*,16*R*,20*S*. Thus, the two sesterterpenoids were concluded to possess unusual antipodal cyclopentenones (D ring) while maintaining other rings (A, B, and C) still highly stereospecific. Consequently, compounds **1** and **2** were identified as shown in Figure 1 and were named leucosceptroids C and D respectively.

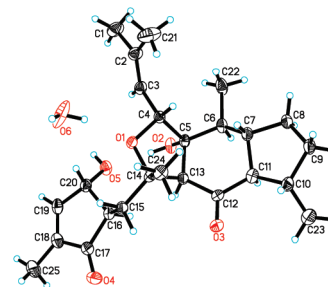


Figure 3. X-ray crystallographic structure of **2** showing the absolute stereochemistry.

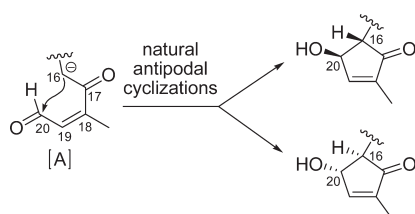
It has been well-known that cyclizations of natural terpenoids are catalyzed by terpenoid cyclases, mostly with incredible stereochemical precision.² Despite this, antipodal cyclizations catalyzed by a few unusual terpene cyclases were also reported.⁸ The antipodal cyclopentenones in leucosceptroids C and D (**1** and **2**) were presumably formed by intramolecular aldol condensation of an intermediate [A] catalyzed by similar unusual cyclases as proposed in Scheme 1, which might also be completed in the glandular trichomes of *L. canum* and then either stored within the gland cavity or secreted onto the leaves. Interestingly, we have noticed that a series of natural sesquiterpenes named litseaverticillols,⁹ which were isolated from *Litsea verticillata* and possessed identical cyclopentenones with those in leucosceptroids C and D (**1** and **2**), occurred exclusively as racemates, indicating that antipodal cyclopentenones also exist in litseaverticillols because the chiral centers of these compounds present only in their cyclopentenone rings. Therefore, it is very likely that natural antipodal cyclizations of cyclopentenones in terpenoids, albeit unusual, may be a common property of the

(7) Leucosceptroid D (**2**): colorless blocks; $[\alpha]_{\text{D}}^{22} = +3.7$ ($c = 0.3$, MeOH); UV (MeOH) λ_{max} (log ϵ): 205 (3.25) nm; IR (KBr) ν_{max} : 3427, 2935, 2871, 1712, 1632, 1453, 1374, 1329, 1035, 925 cm⁻¹; negative FAB-MS m/z (%): 415 (17) $[M - H]^-$, 397 (90), 339 (20), 255 (48); HR-ESI-MS: m/z 415.2486 $[M - H]^-$ (m/z_{calcd} [C₂₅H₃₅O₅]⁻ = 415.2484).

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Scheme 1. Unusual Natural Antipodal Cyclizations of Cyclopentenones in Novel Sesterterpenoids from *L. canum*



responsible enzyme type in plants. Consequently, the stereochemistry of cyclopentenones in another sesterterpenoid from *Salvia hypoleuca*, salvileucolidone,¹⁰ is still to be finalized. The isomer of salvileucolidone with an antipodal cyclopentenone, although not reported so far, should also exist in *S. hypoleuca*. Likewise, the diastereomer of leucosesterterpenone¹¹ with an antipodal cyclopentenone should also be a metabolite of Nepalese *L. canum*.

The antifeedant activity of leucosceptroid C (**1**) against a generalist insect, cotton bollworm (*Helicoverpa armigera*), was assayed as described previously.⁵ The compound showed potent antifeedant activity, with an EC_{50} of 0.017 $\mu\text{mol}/\text{cm}^2$. It was slightly more active than leucosceptroid B ($EC_{50} = 0.021 \mu\text{mol}/\text{cm}^2$)⁵ and nearly three times the potency of leucosceptroid A ($EC_{50} = 0.049 \mu\text{mol}/\text{cm}^2$)⁵ in deterring plant-feeding insect *H. armigera*, suggesting a similar defensive role of **1** against herbivore enemies. Although leucosceptroid D (**2**) was not assayed for its antifeedant activity due to an insufficient sample, it is

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probably also active against insect herbivores since a change of the side chain in the southern hemisphere of this new class of compounds does not significantly affect the antifeedant activity.

In conclusion, we have discovered two additional novel defensive sesterterpenoids with unusual antipodal cyclopentenones from the leaves of *L. canum* and determined their absolute stereochemistries with a single-crystal X-ray diffraction study using anomalous dispersion with copper radiation and found their potential defensive function, which will help to disclose the relationship between the unique secondary metabolites of *L. canum* and its special traits “large woody” and “colored nectar”.

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Supporting Information Available. Experimental procedures, plant material, crystallographic data (both molybdenum and copper radiations), X-ray crystal structures (molybdenum radiation), ROESY and HMBC correlations, and 1D and 2D NMR spectra of **1** and **2**. This material is available free of charge via the Internet at <http://pubs.acs.org>.