

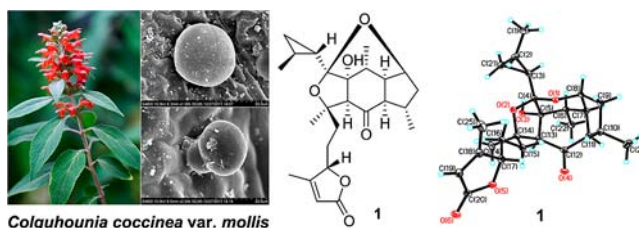
Peltate Glandular Trichomes of *Colquhounia coccinea* var. *mollis* Harbor a New Class of Defensive Sesterterpenoids

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ABSTRACT



A new class of unique sesterterpenoids, colquhounoids A–C (1–3), were identified from the peltate glandular trichomes of *Colquhounia coccinea* var. *mollis* (Lamiaceae) through precise laser-microdissection coupled with UPLC/MS/MS and spectroscopic analyses and X-ray diffraction. Very interestingly, their structural features and defensive function are closely related to leucosceptroid-class sesterterpenoids harbored by the glandular trichomes of another Lamiaceae taxon, *Leucosceptrum canum*, even though this is morphologically distinct and taxonomically distant.

Sesterterpenoids, a special group of C₂₅ terpenoids consisting of only 965 known compounds, represent highly attractive targets for total synthesis due to their comparatively large size, structural complexity, molecular diversity, and broad biological activities.¹ Although sesterterpenoids have been reported from widespread sources,¹ their distribution and natural functions in plants have not been well investigated, with merely ca. 80 compounds having been documented. Recently, we found that the glandular trichomes of a woody Himalayan Lamiaceae, *Leucosceptrum canum*, with colored nectar, harbor the unique defensive sesterterpenoids leucosceptroids A and B (see Figure 2),² and its leaves and flowers contain a series of antifeedant leucosceptroids with antipodal cyclopentenones, lactone,

and spiro- and pentanor-skeletons.³ The core structure of leucosceptroids has been synthesized by Horne's group,⁴ and total synthesis of leucosceptroid B has been completed by Liu's group.⁵

Colquhounia coccinea var. *mollis* (Schlechtendal) Prain is a large shrub 1–2 m high distributed mainly in the south-west of China, with vivid whorls of orange-scarlet flowers (Figure 1A).⁶ The plant is never touched by livestock and only occasionally attacked by a generalist tussock moth (Lymantriidae) and some unknown pathogens. Under a scanning electron microscope, numerous nonglandular trichomes and peltate and capitate glandular trichomes

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(Figure 1B,C) were observed to densely cover the leaves, buds, and stems. The peltate glandular trichomes were precisely collected using laser microdissection (Figure 1D–F) and analyzed for their secondary metabolites with UPLC/MS/MS (Figure 1G–J). Three major compounds (**1–3**) were found with retention times of 25.4, 28.9, and 23.7 min in the total ion chromatogram (Figure 1G) and with molecular weights of 430, 432, and 448, respectively, in their positive ESI mass spectra (Figure 1H–J). From the methanolic extract of the whole leaves of *C. coccinea* var. *mollis*, these trichome metabolites were traced and isolated, and their structures were successfully identified.

Compound **1**, obtained as colorless blocks, was determined to have a molecular formula of $C_{25}H_{34}O_6$ on the basis of EI mass spectrometry as well as high-resolution EI-MS. Its IR spectrum showed absorptions at 3406 cm^{-1} for hydroxyl group and at 1730 and 1682 cm^{-1} for carbonyl groups. The ^1H NMR spectrum (Table S1, Supporting Information) of **1** clearly displayed two tertiary methyls at δ_{H} 1.33 and 2.08 and three secondary methyls at δ_{H} 1.03, 1.09, and 1.13. An olefinic proton at δ_{H} 5.77 indicated the presence of a trisubstituted double bond. Three one-proton singlets at δ_{H} 3.62, 4.22, and 4.90 were ascribable to either methines or free hydroxyl groups. At a relatively high field, two one-proton multiplets at δ_{H} 0.29 and 1.05 together with a two-proton multiplet at δ_{H} 0.89 were indicative of a disubstituted cyclopropyl residue. Other signals were centered between 1.05 and 2.50 and mostly

overlapped. The ^{13}C NMR spectrum (Table S1, Supporting Information) of **1** demonstrated 25 carbon resonances, which were further classified by DEPT spectra as five methyls, four methylenes with one of them occurring at relatively highfield (δ_{C} 12.4), ten methines including an olefinic methine (δ_{C} 117.0), two oxymethines (δ_{C} 78.2 and 85.2), two methines appearing at relatively high field (δ_{C} 8.7 and 18.6), and six quaternary carbons consisting of a keto carbon (δ_{C} 212.5), an ester carbonyl carbon (δ_{C} 173.1), an olefinic quaternary carbon (δ_{C} 170.3), a ketal carbon (δ_{C} 109.4), and two oxygenated quaternary carbons (δ_{C} 82.4 and 85.5). These data were consistent with the signals observed in the ^1H NMR spectrum, suggesting a highly oxygenated cyclopropyl-containing sesterterpenoid for **1**.

All proton signals, except for the singlet at δ_{H} 3.62, could be assigned to their respective carbons unambiguously through analysis of the HSQC spectrum (Figure S4, Supporting Information), suggesting that the signal at δ_{H} 3.62 was ascribable to a free hydroxyl group. In addition, detailed analysis of the ^1H – ^1H and ^1H – ^{13}C long-range correlations in the COSY and HMBC spectra (Figures S3 and S5, Supporting Information) of **1** led to the establishment of a 5/6/5 ring system for its corestructure, which appeared to be similar to that found in leucosceptroids (Figure 2),^{2,3} a class of sesterterpenoids discovered in the glandular trichomes and whole leaves and flowers of *L. canum*. Further analysis of the NMR spectra of **1** and comparison of its data with those of leucosceptroids revealed that the 6/5 carbon framework of **1** was closer to that of leucosceptroids **B** and **J**^{2,3} because of the absence of hydroxylation at C-11, and its C-14 side chain contained a methylated α,β -unsaturated lactone, as has been found in

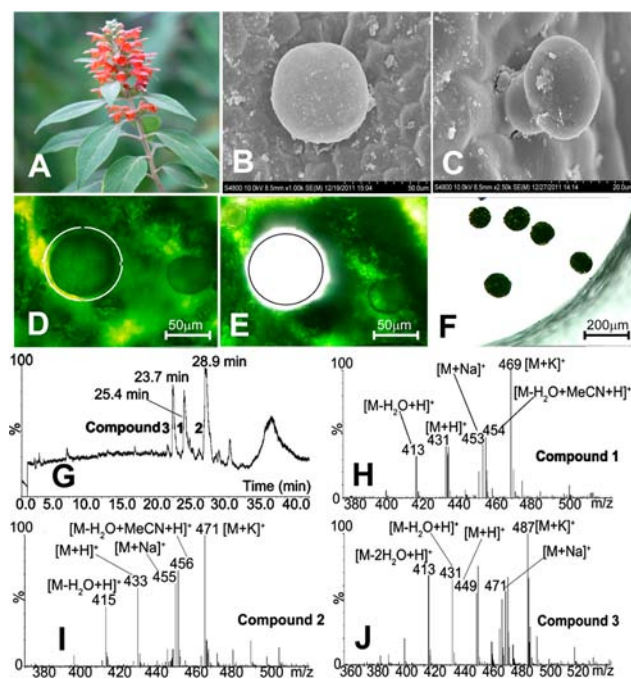


Figure 1. (A) *C. coccinea* var. *mollis* plant in bloom, (B and C) peltate and capitate glandular trichomes on the abaxial leaf surface, (D–F) collection of peltate glandular trichomes with laser microdissection, (G–J) microchemical analysis of secondary metabolites in the microdissected peltate glandular trichomes with UPLC/MS/MS.

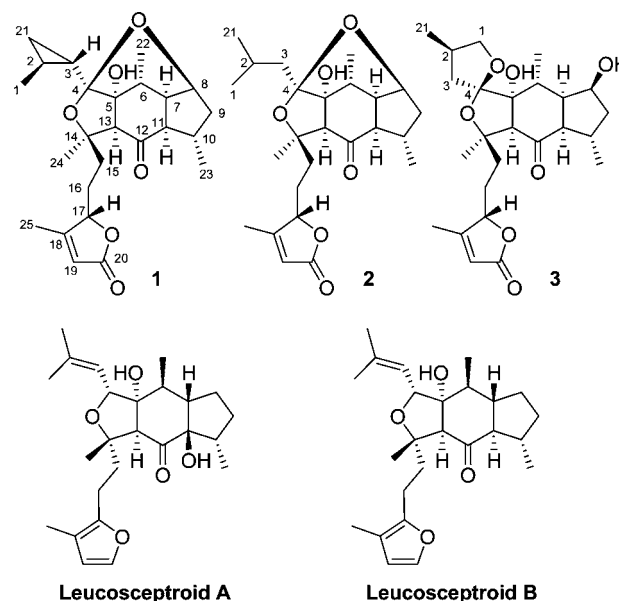


Figure 2. Chemical structures of colquhounoids A–C (**1–3**) and leucosceptroids A and B.

leucosceptroids E-J,³ judging from the HMBC correlations from H-17 to C-15, C-16, C-18, C-19, and C-20, and from Me-25 to C-17, C-18, and C-19 in **1**. However, the C-4 side chain of **1** was obviously different from that of leucosceptroids. The C-4 isobutenyl side chain of leucosceptroids was found to be replaced by a methylated cyclopropyl group in **1**, which could be deduced from the ¹H–¹H coupling relationship of Me-1/H-2/H-3/H₂-21/H-2 in **1**. In addition, an oxygenation was found to have occurred at C-8 in **1** due to the ¹H–¹H correlations of H-8 (δ_{H} 4.22) with H-7 and H₂-9, and the aforementioned ketal carbon (δ_{C} 109.4) in **1** was assignable to C-4 owing to the simultaneous ¹H–¹³C correlations from 5-OH, H-13, and the cyclopropyl protons to the ketal carbon. Thus, more clear differences existing between **1** and leucosceptroids resulted from the oxygenation at C-8 and further oxygenation at C-4 in **1**. The molecular formula of **1**, corresponding to 9 degrees of unsaturation, suggested the existence of an additional oxygen bridge in the molecule. Since the 5-OH signal was already detected, the oxygen bridge could only be formed between C-4 and C-8. However, the ¹H–¹³C long-range correlation from H-8 to C-4 could not be observed in the HMBC spectrum (Figure S5, Supporting Information).

In the 2D ROESY spectrum (Figure S6, Supporting Information), the observation of correlations of 5-OH with H-7, H-13, Me-22, and Me-24, of H-8 with H-11 and H-13, and of H-11 with Me-23 suggested that 5-OH, H-7, H-8, H-11, H-13, Me-22, Me-23, Me-24, and the cyclopropyl residue were all in the same orientation (α -configuration). However, the relative stereochemistry of chiral carbons C-2, C-3, and C-17 was not possible to determine from the ROESY experiment. Fortunately, a single crystal of **1** was obtained from a mixture of acetone/methanol (10:1). After several recrystallizations, X-ray crystallographic analysis using anomalous dispersion with copper radiation was performed (CCDC 888572) (Figures 3), which not only confirmed the above deduced structure including the existence of an oxygen bridge between C-4 and C-8 but also

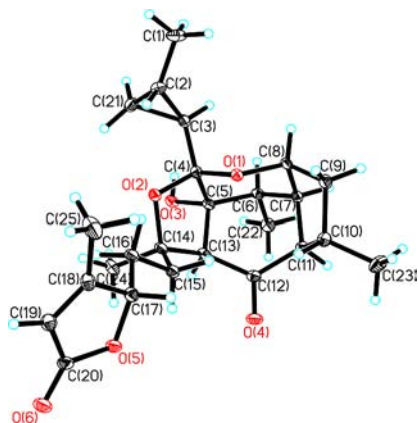


Figure 3. X-ray crystallographic structure of **1** showing the absolute stereochemistry.

determined the absolute stereochemistry of **1** to be 2*S*,3*S*,4*R*,5*R*,6*R*,7*R*,8*S*,10*S*,11*R*,13*R*,14*R*,17*S*. Consequently, the core structure of compound **1** was found to possess an interesting complex cage-like all-*cis*-fused 5/6/5/6 framework due to the occurrence of the 4,8-oxygen bridge. Thus, compound **1** was identified as shown in Figure 2 and was named colquhounoid A.

Compound **2** was also obtained as colorless blocks. High-resolution EI-MS and ¹H and ¹³C NMR (including DEPT) spectra (Table S1, Supporting Information) showed a molecular formula of C₂₅H₃₆O₆, which was only two hydrogens more than that of **1**. Comparison of the NMR spectra of **2** with those of **1** clearly disclosed that **2** was another sesterterpenoid structurally similar to **1**. However, the appearance of an isobutyl moiety in **2**, as indicated by the ¹H–¹H coupling relationship of Me-1/H-2/Me-21/H₂-3, and the disappearance of the cyclopropyl signals in **2**, clearly demonstrated a cleavage between C-3 and C-21 in **2**. Other parts including the stereochemistry of **2** remained unchanged considering its very similar chemical shifts in the 1D NMR (¹H, ¹³C and DEPT) spectra (Table S1 and Figures S7 and S8, Supporting Information) and homogeneous and heterogeneous correlations in the 2D NMR (¹H–¹H COSY, HSQC, HMBC and ROESY) spectra (Figures S9–S12, Supporting Information) to **1**. Accordingly, compound **2** was characterized as the 3,21-*seco* derivative of **1** and was named colquhounoid B (Figure 2).

Compound **3** was isolated as colorless blocks, having a molecular formula of C₂₅H₃₆O₇ based on its high resolution ESI-MS. Close resemblance between the NMR spectra (Table S1, Supporting Information) of **3** and those of **1** and **2** suggested that **3** was again a sesterterpenoid whose structure was similar to those of **1** and **2**. However, compound **3** obviously differed from **1** by lack of cyclopropyl signals and from **2** by missing a secondary methyl and the presence of an oxymethylene (δ_{H} 3.57 and 4.13; δ_{C} 76.4), implying that either C-1 or C-21 was oxygenated in **3**. In addition, besides a free hydroxyl group attached to C-5 (δ_{H} 4.28), another free hydroxyl group at δ_{H} 3.49 which was ascribable to C-8 due to its HMBC correlations (Figure S17, Supporting Information) with C-7 and C-9 was also observed. This fact, together with the consideration that the oxymethylene (δ_{C} 76.4) and ketal carbon (δ_{C} 119.5) resonated remarkably far downfield, indicated that an oxygen bridge between C-1 (or C-21) and C-4, rather than between C-4 and C-8 as existed in **1** and **2**, occurred in **3**. In the HMBC spectrum of **3** (Figure S17, Supporting Information), the correlation from the oxymethylene protons to C-4 also supported this inference. Thus, compound **3** was established to contain a 1,6-dioxaspiro[4.4] substructure. The ROESY correlations (Figure S18, Supporting Information) of **3** were basically the same as those observed in **1** and **2**, indicating that the relative stereochemistry of most chiral centers of the core structure of **3** remained unchanged. Likewise, it was not possible to determine the relative stereochemistry of C-2 and C-17 by the ROESY experiment. Therefore, a single crystal of **3** was obtained, and X-ray diffraction (CCDC 888573) was

successfully carried out (Figures 4). Thus, the above deduced structure was confirmed and the relative stereochemistry of all chiral centers was unequivocally established. Moreover, the absolute stereochemistry of the framework of **3** is presumably identical with that of **1** and **2** from biogenetic considerations. Consequently, compound **3** was characterized as shown in Figure 2 and was named colquhounoid C.

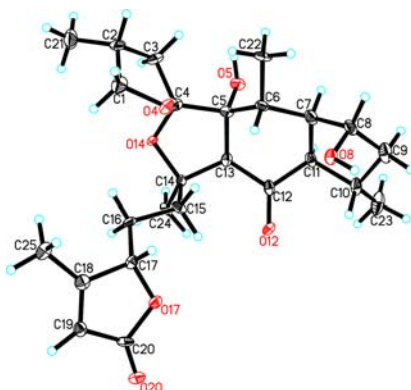


Figure 4. X-ray crystallographic structure of **3** showing the relative configuration.

From a chemical structural point of view, colquhounoids A–C (**1–3**) have striking similarity with leucosceptroids due to their common 5/6/5 core structure, an upper side chain attached to C-4, and a lower side chain attached to C-14. However, colquhounoids A–C (**1–3**) are clearly distinct from leucosceptroids by their adverse stereochemistries at C-6, C-7, and C-14. Moreover, compared to the leucosceptroids whose C-8 is never oxygenated and C-4 side chain is exclusively an isobutenyl residue,^{2,3} the C-8 of colquhounoids is universally oxygenated and the C-4 side chains are rather diversified. To distinguish these two classes of plant terpenoids, we name those sesterterpenoids from *C. coccinea* var. *mollis* collectively colquhounane sesterterpenoids (= colquhounoids) and designate the other class from *L. canum* collectively as leucosceptrane sesterterpenoids (= leucosceptroids).

The antifeedant activities of **1–3** against two generalist insect herbivores, beet armyworm (*Spodoptera exigua*) and cotton bollworm (*Helicoverpa armigera*), were assayed as previously described.² As shown in Table S2 (Supporting Information), all three compounds were deterrents to the beet armyworm, with EC_{50} values of 18.59, 11.98, and 7.11 $\mu\text{g}/\text{cm}^2$, respectively, but less active than a commercial neem oil containing 1% azadirachtin (EC_{50} = 3.71 $\mu\text{g}/\text{cm}^2$). Meanwhile, compounds **2** and **3** also displayed

significant antifeedant activity against the cotton bollworm with EC_{50} values of 18.14 and 15.30 $\mu\text{g}/\text{cm}^2$, respectively (neem oil: EC_{50} = 2.62 $\mu\text{g}/\text{cm}^2$), but compound **1** was not active (Table S2, Supporting Information). Quantitative analysis indicated that the natural contents of **1–3** in leaves (15.25 ± 0.44 , 24.04 ± 2.06 , and 12.10 ± 0.43 $\mu\text{g}/\text{cm}^2$ respectively, Table S3, Supporting Information) were comparable to or even higher than their antifeedant EC_{50} values, indicating that the levels of these compounds in leaves are sufficiently high to deter feeding by generalist insects. In addition, antifungal effects of **1–3** against two strains of pathogenic fungi, *Colletotrichum gloeosporioides* and *Rhizoctonia solani*, were also tested.⁷ It was noteworthy that, although less active than the commercial fungicide nystatin, compound **1** showed clear inhibitory effect against both fungal strains, while **2** only substantially inhibited the growth of *C. gloeosporioides*. However, compound **3** was surprisingly inactive (Table S2, Supporting Information). Nevertheless, the antifungal activity of **1** and **2** suggested that these compounds could also be involved in plant defense against pathogens since they are highly concentrated in the glandular trichomes instead of being evenly distributed in the leaves.

In conclusion, we have identified a new class of unique defensive sesterterpenoids, colquhounoids, found specifically in the peltate glandular trichomes of *C. coccinea* var. *mollis*. Considering that *C. coccinea* var. *mollis* and *L. canum* are morphologically distinct and taxonomically distant,⁸ it is surprising that their glandular trichomes have evolved to produce such similar but still unique sesterterpenoids. This paper also shows that the combination of laser microdissection and UPLC/MS/MS method provides a new way to discover novel biologically active secondary metabolites of plant glandular trichomes.

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Supporting Information Available. Experimental procedures, plant materials, extraction and isolation of the three compounds, microscopy, laser microdissection of peltate glandular trichomes and metabolite analysis with UPLC/MS/MS, quantification of sesterterpenoids and results, bioassays of sesterterpenoids and results, crucial data (mp, ORD, UV, IR, MS, ^1H and ^{13}C NMR) of **1–3**, crystal data of **1** and **3**, and ^1H , ^{13}C , ^1H – ^1H COSY, HSQC, HMBC, and ROESY spectra of compounds **1–3**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

The authors declare no competing financial interest.

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