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New Antifeedant C_{20} Terpenoids from *Leucosceptrum canum*

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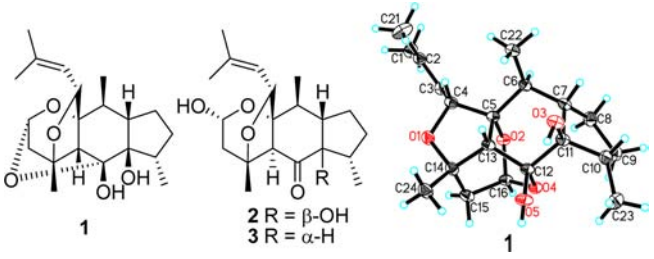
ABSTRACT



Three novel C_{20} terpenoids, norleucosceptroids A–C (1–3), were isolated from the leaves and flowers of *Leucosceptrum canum* (Labiatae) and were identified by comprehensive spectroscopic analysis and, in the case of 1, single-crystal X-ray diffraction. Structurally, compounds 1–3 should be categorized as pentanor-sesterterpenoids rather than diterpenoids. Moderate antifeedant activity of 1–3 against the generalist plant-feeding insect *Helicoverpa armigera* was detected, suggesting that they might also be involved in the plant defense against insect herbivores.

Terpenoids represent a highly diversified class of natural products, with more than 60000 different compounds having been discovered, which provide a variety of roles in mediating antagonistic and beneficial interactions among organisms in the natural world.[1] Terpenoids are derived from isoprene and thus can be easily classified as monoterpenoids (C_{10}), sesquiterpenoids (C_{15}), diterpenoids (C_{20}), sesterterpenoids (C_{25}), triterpenoids (C_{30}), and so on, according to the number of isoprene units they contain.[2] However, this categorization may not always be conclusive.

Leucosceptrum canum Smith is a large woody Labiatae (= Lamiaceae) plant (up to 10 m high[3]) with an unusual dark brown nectar, found from the Himalayas to the southwest of China.[4] The species is used as a traditional insecticidal agent in remote areas of Nepal.[5] We have been interested in the relationship between the secondary metabolites of *L. canum* and its above special traits and have found that the glandular trichomes of this plant harbor unique defensive sesterterpenoid leucosceptroids A and B possessing a furan-containing tetracyclic C_{25} skeleton.[6] Since the trichomes mainly exist on the surface of the leaves and flowers of *L. canum*, a continuing work on the whole leaves resulted in the discovery of two additional interesting defensive sesterterpenoids leucosecptroids C and D which contain unusual antipodal cyclopentenones.[7] Recently, the core structure of leucosceptroids A–D has been synthesized by Horne's group.[8] Our further investigation on the defensive sesterterpenoids in the leaves and flowers of *L. canum* led to the discovery of three additional

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terpenoids with an intriguing C_{20} framework, namely, norleucosceptroids A−C (**1**−**3**) (Figure 1). In this paper, we report their isolation, structure determination including absolute stereochemistry, and antifeedant activity against the generalist plant-feeding insect *Helicoverpa armigera*.

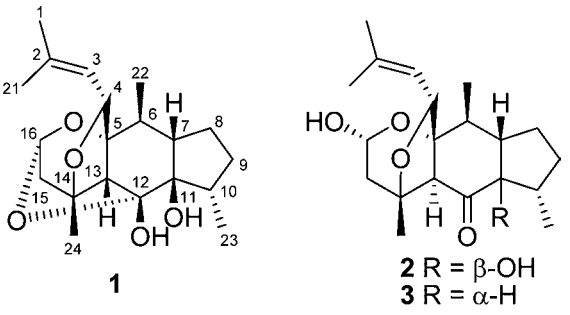


Figure 1. Chemical structures of norleucosceptroids A−C (**1**−**3**).

Compound **1** has a molecular formula of $C_{20}H_{30}O_5$, as deduced from the high-resolution (HR) EI-MS (m/z_{obsd} 350.2090, m/z_{calcd} 350.2093).[9] In the ^{1}H NMR spectrum (Table 1 and Figure S1, Supporting Information), two secondary methyls at δ_H 1.04 (d, J = 7.6 Hz) and 1.19 (d, J = 7.2 Hz) and three tertiary methyls at δ_H 1.45 (s), 1.69 (s) and 1.72 (s) were clearly shown. One olefinic proton at δ_H 5.26 (d, J = 9.4 Hz) indicated the presence of a trisubstituted double bond. Three singlets at δ_H 2.59, 3.60, and 3.95 and two doublets at δ_H 4.46 (J = 9.4 Hz) and 5.09 (J = 3.3 Hz) were ascribable to either oxygenated methine or free hydroxy groups. Other signals occurred in a relatively highfield region (between 1.62 and 2.16 ppm) and mostly overlapped, resonating from either methine or methylene signals. Twenty carbon resonances were resolved in the ^{13}C NMR spectrum (Table 2 and Figure S2, Supporting Information), and were further classified by DEPT experiments as five methyls, three methylenes, seven methines (including an olefinic methine at δ_C 121.9), and five quaternary carbons (including an olefinic quaternary carbon at δ_C 137.2). These data were consistent with the above molecular formula and suggested that compound **1** was a highly oxygenated terpenoid. Considering that **1** has 20 carbons, it was initially regarded as a diterpenoid. However, detailed analysis of NMR data of **1** (Tables 1 and 2) indicated a clear similarity between **1** and leucosceptroid A, a sesterterpenoid isolated from the same plant,[6] suggesting that they possessed a common core structure constituted by a 5/6/5 ring system. In addition, an isobutenyl group at the same position in the northern hemisphere still existed in **1**. However, the significant NMR difference between **1** and leucosceptroid A indicated that the signals

Table 1. ^{1}H NMR Data of Compounds **1**−**3** in Acetone-d_6 (δ in ppm, J in Hz)[a]

no.	**1**[b]	**2**[c]	**3**[d]
1	1.69, s, (3H)	1.65, s, (3H)	1.63, s, (3H)
3	5.26, d, (9.4)	5.51, d, (8.5)	5.53, br d, (8.4)
4	4.46, d, (9.4)	4.49, d, (8.5)	4.38, d, (8.4)
6	1.77, m	2.06, m	2.12, m
7	2.15, m	1.79, m	1.48, m
8α	1.80, m	1.71, m	1.37, m
8β	2.16, m	2.13, m	1.86, m
9α	1.62, m	1.43, m	1.26, m
9β	1.73, m	2.13, m	1.92, m
10	2.13, m	2.14, m	2.19, m
11			2.21, m
13	2.59, s	2.63, s	2.92, s
15a	1.89, dd, (3.4, 13.6)	1.69, m	1.52, m, (α)
15b	2.02, overlapped	1.91, m	1.82, m, (β)
16	5.09, d, (3.3)	5.43, d, (3.5)	5.42, d, (4.0)
21	1.72, s, (3H)	1.72, s, (3H)	1.71, s, (3H)
22	1.04, d, (3H, 7.6)	0.97, d, (3H, 7.0)	0.90, d, (3H, 7.1)
23	1.19, d, (3H, 7.2)	0.94, d, (3H, 7.5)	0.96, d, (3H, 6.2)
24	1.45, s, (3H)	1.56, s, (3H)	1.61, s, (3H)

[a] ^{1}H NMR spectrum of compound **1** was recorded at 400 MHz; ^{1}H NMR spectra of compounds **2** and **3** were recorded at 500 MHz. [b] Hydroxyl group signals of **1**: δ_H 3.60 (s, 11-OH), 3.95 (s, 12-OH). [c] Hydroxyl group signals of **2**: δ_H 4.25 (s, 11-OH), 5.44 (s, 16-OH). [d] Hydroxyl group signals of **3**: δ_H 5.40 (s, 16-OH).

for the methylated furan ring in the southern hemisphere of leucoseptroid A disappeared in **1**, suggesting that oxidative cleavage might have occurred between C-16 and C-17, which thus caused loss of a C_5 fragment. Under this circumstance C-16 is usually first hydroxylated and then further oxidized to aldehyde and probably carboxylic acid. Assignment of H/C-16 (δ_H 5.09, d, J = 3.3 Hz; δ_C 91.4, d) of **1** through their ^{1}H−^{1}H and ^{1}H−^{13}C correlations (Figures S3, Supporting Information, and Figure 2), respectively, with H-15 implied C-16 of **1** to be an acetal. In the HMBC spectrum (Figure S5, Supporting Information) of **1**, correlations from H-16 to C-5 (δ_C 88.3) and C-12 (δ_C 99.3) demonstrated existence of two oxygen bridges C-16−O-C-5 and C-16−O-C-12, which further confirmed the above inference and displayed a very complex cagelike framework for **1**. The two free hydroxyl groups at δ_H 3.60 and 3.95 were located at C-11 and C-12, respectively, on the basis of the HMBC correlations from 11-OH to C-7, C-11 and C-12, and from 12-OH to C-11, C-12, and C-13. Thus, a hemiketal at C-12 in **1** was formed to replace the usual keto group in leucoseptroid A and its congeners.[6,7]

The relative stereochemistry of all chiral centers in **1** was established by 2D ROESY spectra (Figure S6, Supporting Information). Correlations of H-13 with H-4, Me-22, Me-24, 11-OH, and 12-OH and of Me-24 with H-7 indicated that H-4, H-7, Me-22, Me-24, 11-OH, and 12-OH were all in the same orientation (β-configuration), while correlations of Me-23 with H-6 and H-9α indicated that Me-23 and H-6 were α-oriented. It is noteworthy that H-13 of **1** has a β-configuration rather than an α-configuration as has been found in leucosceptroids A−D.[6,7] In addition,

(9) Norleucosceptroid A (**1**): mp 175−176 °C; $[\alpha]^{17}_D$ = −60.0 (c = 0.1, MeOH); UV (acetonitrile) λ_{max} (log ε) 195 (3.61) nm; IR (KBr) ν_{max} 3532, 3501, 2967, 2941, 2874, 1452, 1386, 1112, 1005, 970, 889, 863 cm^{-1}; EI-MS m/z 350 (5) [M]$^+$, 250 (100), 232 (65), 153 (42), 149 (45), 123 (49), 95 (85), 83 (77); HR-EI-MS m/z_{obsd} 350.2090 [M]$^+$ (m/z_{calcd} [$C_{20}H_{30}O_5$]$^+$ = 350.2093).

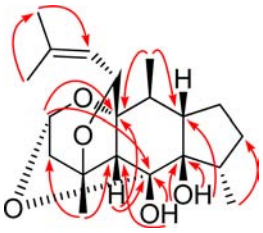


Figure 2. Selected key HMBC correlations of **1** (from H to C).

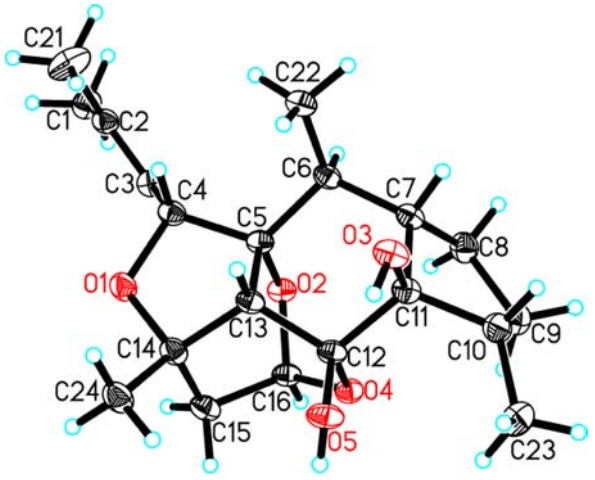


Figure 3. X-ray crystallographic structure of **1** showing the absolute stereochemistry.

because H-16 only correlate with its neighboring H_2-15, the stereochemistry of C-16 could not be determined by ROESY experiment. Therefore, an X-ray diffraction was necessary to confirm its novel cagelike structure and clarify its configuration. A single crystal of **1** was luckily obtained from a mixture of methanol and water (10:1), and X-ray crystallographic analysis was successfully carried out (Figure 3) using anomalous dispersion with copper radiation, which determined the absolute stereochemistry of **1** to be 4*R*,5*R*,6*S*,7*S*,10*S*,11*R*,12*R*,13*R*,14*S*,16*S*.[10] Thus, compound **1** was identified as shown in Figure 1 and was named norleucosceptroid A.

Table 2. ^{13}C NMR Data of Compounds **1**–**3** in Acetone-d_6 (125 MHz, δ in ppm)

no.	1	2	3
1	18.5, q	18.6, q	18.7, q
2	137.2, s	138.7, s	138.5, s
3	121.9, d	122.1, d	122.2, d
4	77.1, d	77.8, d	76.5, d
5	88.3, s	87.0, s	91.4, s
6	34.4, d	40.6, d	44.5, d
7	55.8, d	52.5, d	51.3, d
8	29.5, t	30.5, t	30.1, t
9	31.3, t	31.6, t	31.7, t
10	47.5, d	48.4, d	32.5, d
11	83.0, s	86.9, s	64.6, d
12	99.3, s	210.8, s	205.9, s
13	44.5, d	64.0, d	65.2, d
14	77.3, s	80.7, s	79.3, s
15	46.2, t	50.1, t	49.7, t
16	91.4, d	92.1, d	92.2, d
21	26.2, q	26.1, q	26.1, q
22	18.9, q	14.6, q	14.2, q
23	15.8, q	18.5, q	19.9, q
24	27.4, q	23.9, q	22.2, q

Compound **2** was obtained as a colorless oil, having a molecular formula of $C_{20}H_{30}O_5$, as determined by a combination of its negative FAB-MS and ^{1}H and ^{13}C NMR (including DEPT) spectra, which was confirmed by HR-ESI-MS (m/z_{obsd} 373.1985 $[M + Na]^+$; m/z_{calcd} 373.1990).[11] Such a molecular formula was the same as that of **1**. Close resemblance between the NMR spectra of **2** and **1** (Tables 1 and 2) indicated that **2** was another highly oxygenated C_{20} terpenoid structurally similar to **1**. Their major difference was the replacement of the hemiketal C-12 of **1** by a usual keto group at the same position in **2** (δ_C 210.8), which was supported by ^{1}H–^{13}C long-range correlations from H-13 and 11-OH to this keto carbon. In addition, observation of 16-OH (δ_H 5.44, s) and the oxygen bridge between C-16 and C-5 due to HMBC correlation from H-16 to C-5 (Figure S11, Supporting Information) indicated C-16 of **2** to be a hemiacetal. The ROESY correlations (Figure S12, Supporting Information) of H-13 with H-6 and Me-23 showed that H-13 of **2** should have the normal α-orientation. Other ROESY correlations were similar with those of **1**, suggesting that the stereochemistry of other chiral centers in **2** remained

(10) Colorless needle crystals of norleucosceptroid A, crystallized from MeOH/water, belong to the monoclinic space group *P*2(1). Crystal data: $C_{21}H_{34}O_6$, M + MeOH = 382.48 g mol^{-1}, size 0.68 × 0.17 × 0.07 mm^3, a = 6.6017 (2) Å, b = 11.5262 (3) Å, c = 13.6395 (4) Å, α = 90°, β = 92.7°, γ = 90°, V = 1036.7 (5) Å^3, T = −173 °C, Z = 2, d = 1.225 g cm^{-3}, μ(Mo Kα) = 1.54178 Å, F(000) = 416, 13405 reflections in h(−7/6), k(−13/13), l(−16/15), measured in the range 3.24° ≤ θ ≤ 66.39°, completeness θ_{max} = 97.2%, 3421 independent reflections, R_{int} = 0.0381, 254 parameters, 1 restraint, $R1_{obs}$ = 0.0381, $wR2_{obs}$ = 0.0977, $R1_{all}$ = 0.0388, $wR2_{all}$ = 0.1006, GOF = 1.040, Absolute structure parameter 0.07(17), largest difference peak and hole = 0.365 and −0.349 e Å^{-3}. The crystal structure of norleucosceptroid A was solved by direct methods using the program SHELXS-97 (Sheldrick, G. M. *SHELXS97 and SHELXL97*, University of Gottingen, Germany, 1997) and subsequent Fourier difference techniques, and refined anisotropically by full-matrix least-squares on F^2 using SHELXL-97 (Sheldrick, G. M. *SHELXTL*, Version 6.10, Bruker AXS Inc., Madison, WI, 2000).

(11) Norleucosceptroid B (**2**): colorless oil; $[\alpha]^{27}_D$ = +66.4 (c = 0.4, MeOH); UV (acetonitrile) λ_{max} (log ε) 194 (3.77) nm; IR (KBr) ν_{max} 3439, 2965, 2932, 2876, 1707, 1668, 1461, 1452, 1380, 1299, 1044, 997, 956, 904 cm^{-1}; negative FAB-MS m/z 349 (100) $[M - H]^-$, 331 (50), 255 (30); HR-ESI-MS m/z_{obsd} 373.1985 $[M + Na]^+$ (m/z_{calcd} $[C_{20}H_{30}O_5Na]^+$ = 373.1990).

(12) Norleucosceptroid C (**3**): colorless oil; $[\alpha]^{27}_D$ = +56.4 (c = 0.3, MeOH); UV (acetonitrile) λ_{max} (log ε): 193 (3.81) nm; IR (KBr) ν_{max} 3436, 2967, 2930, 2877, 1700, 1665, 1451, 1432, 1378 cm^{-1}; EI-MS m/z 334 (4) $[M]^+$, 316 (12), 278 (19), 250 (23), 222 (34), 152 (41), 140 (100), 122 (90), 111 (65), 83 (52), 81 (51); HR-EI-MS m/z_{obsd} 334.2141 $[M]^+$ (m/z_{calcd} $[C_{20}H_{30}O_4]^+$ = 334.2144).

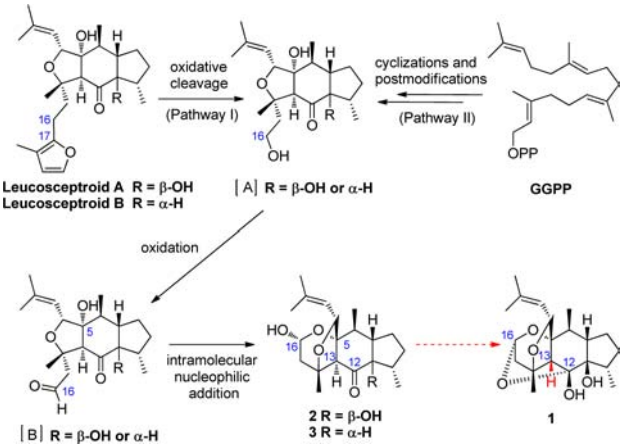
unchanged. Accordingly, compound **2** was characterized as shown in Figure 1 and was named norleucosceptroid B.

Compound **3**, a colorless oil, was assigned a molecular formula of $C_{20}H_{30}O_4$, as deduced from its EI-MS and HR-EI-MS (m/z_{obsd} 334.2141 $[M]^+$; m/z_{calcd} 334.2144).[12] This molecular formula suggested one oxygen atom less than that of **2**. The 2D NMR spectra of **3** including $^1H-^1H$ COSY, HSQC and HMBC (Figures S15–S18, Supporting Information) established the gross structure of **3** to be an additional C_{20} terpenoid closely resembling **2**. Missing of 11-OH signal in the ^1H NMR spectrum of **3** indicated lack of a hydroxyl group at this position, as in the case of leucosceptroids B and D.[6,7] ROESY crosspeaks between H-11 and Me-23 and H-13 established an α-configuration of H-11. Compound **3** was accordingly elucidated as in Figure 1 and was named norleucosceptroid C.

From a structural point of view, it is very likely that compounds **1**–**3** are pentanor-sesterterpenoids of leucosceptroids A and B through biodegrading pathway I via key intermediates [A] and [B] (Scheme 1). However, yet it cannot be ruled out that compounds are diterpenoids originated from geranylgeranyl diphosphate (GGPP) through pathway II via the same intermediates (Scheme 1). In addition, it should be mentioned here that compound **1** appears to be derived from **2** through further intramolecular nucleophilic addition between 16-OH and 12-C=O and epimerization at C-13. However, it might also be possible that compound **1** is a biodegraded product of 13-epi-leucosceptroid A through pathway I (Scheme 1) via 13-epimers of [A], [B] and **2**, but all these precursors are still to be discovered. Therefore, the biosynthesis of these unusual C_{20} terpenoids remains an interesting topic and warrants further investigation. Additional research on the isolation and identification of [A] and [B] and more other related biosynthetic intermediates should help reveal how these novel natural products are formed in the plant, which is currently underway in our laboratory.

Since leucosceptroids A and B have been shown to be defense compounds of *L. canum*,[6] it was interesting to find out if compounds **1**–**3** were also active. Therefore, the antifeedant activity of **1**–**3** against a generalist insect, cotton bollworm (*H. armigera*), was tested as previously described.[6] As a result, compounds **1**–**3** showed moderate antifeedant activity, with an antifeedant index (AI%)

Scheme 1. Possible Biogenetic Formation of Compounds **1**–**3**



of 28.97 ± 2.21%, 50.40 ± 1.75%, and 56.87 ± 1.97%, respectively, at 15.73 $\mu g/cm^2$, which were less active than a commercial neem oil containg 1% azadirachtin (AI% = 76.17 ± 1.95%) produced by Kunming Rixin Dachuan Technology Co., Ltd.. Despite this, the obvious antifeedant activity against the generalist plant-feeding insect suggested that **1**–**3** might also be involved in the plant defense against insect herbivores. In addition, compounds **1**–**3** were also evaluated for their in vitro cytotoxicity against human tumor cell lines HL-60, SMMC-7721, A-549, MCF-7, and SW480 using a MTT assay method.[13] Unfortunately, none of them were found to be active against any of the tested cell lines (IC_{50} > 40 μM).

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Supporting Information Available. Experimental procedures, plant material, crystallographic data, ^{13}C NMR data, and 1D and 2D NMR spectra of **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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