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Sesquiterpenes and other constituents from *Cacalia deltophylla*

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From the whole plant of *Cacalia deltophylla* (Maxim) Mattf, together with twelve known compounds (**3**–**14**), a new furanoeremophilane-type sesquiterpene named deltocacalone (**1**), and a new norsesquiterpene named deltonorcacalol (**2**) were isolated. Their structures were elucidated by spectroscopic methods including 2D-NMR techniques.

1. Introduction

The genus *Cacalia* (Compositae) is widespread in East Asia. In China, many species of *Cacalia* have long been used as traditional Chinese medicinal herb in the treatment of invigorating circulation of blood, relieving coughs and phlegm [1]. A number of furanoeremophilane-type sesquiterpenes derived from *Cacalia* have been reported [2, 3], however, no phytochemical investigation of *Cacalia deltophylla* (Maxim) Mattf have been carried out up to now. In our studies on the chemical constituents of this plant, a new furanoeremophilane-type sesquiterpene and a new norsesquiterpene were isolated from the whole plant of this species. Their structures were elucidated as deltocacalone (**1**) and deltonorcacalol (**2**) by spectroscopic methods including 2D-NMR techniques. In addition, twelve known compounds maturinone (**3**), β-amyrin acetate (**4**), α-amyrin (**5**), lupeol (**6**), β-sitosterol (**7**), daucosterol (**8**), caffeic acid (**9**), tyrosol (**10**), esculetin (**11**), scopoletin (**12**), syringaldehyde (**13**), (*E*)-cinnamic acid (**14**) were obtained. This paper reports the isolation and structure elucidation of these constituents.

2. Investigations, results and discussion

Compound **1** was obtained as yellow crystals from acetone, m.p. 183–185 °C. A strong absorption band at 1652 cm^{-1} in its IR spectrum suggested a conjugated carbonyl group. The ^1H NMR spectrum (Table 1) showed the presence of a methyl group (δ 2.16, 3 H, brs) occupied the β-position of furan ring, a proton at the α-position of furan ring (δ 7.50, 1 H, brs), a 1,2,3-trisubstituted benzene (δ 8.31, dd; δ 7.40, t; δ 7.35, dd, 1 H each), a methyl group on benzene ring (δ 2.63, 3 H, s) and a pair of protons (δ 3.50, 3.78, 1 H each, d) belong to an oxygenated methylene. The ^{13}C NMR and DEPT spectra exhibited fifteen carbon signals including two methyls, one methylene, four methines and eight quaternary carbons. The EIMS spectrum exhibited a molecular ion peak at *m/z* 240 $[M]^+$. Thus, the molecular formula of **1** was deduced to be $C_{15}H_{12}O_3$ with ten degrees of unsaturation. In its ^{13}C NMR spectrum, the signal of the oxygenated methylene (δ 52.0) appeared at relative high field implying a trimember epoxy ring. The supposed structure was proved by ^3J cross peaks in HMBC experiment: C-5/H-1, 3, 14, 15; C-7/H-12, 13, 14; C-8/H-12; C-9/H-1. The configuration of the methylene at C-6 could be assigned as β in the biogenetic consideration of cacalone and cacalol (common components of *Cacalia*) [4–6]. Thus, the structure of compound **1** was established as a furanoeremophilane-type sesquiterpene, named deltocacalone.

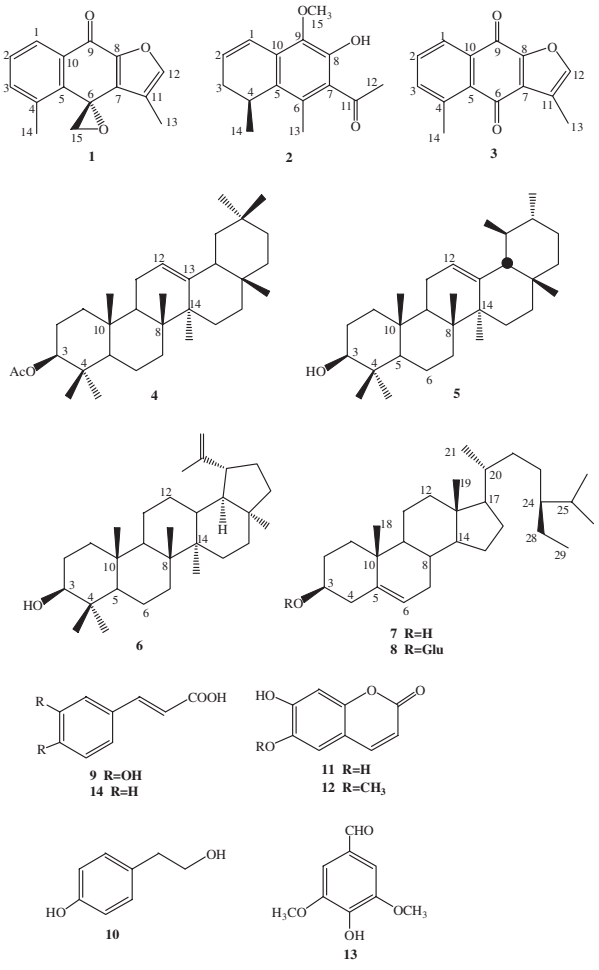

Table 1: ^{1}H NMR data of compounds 1–3 ($CDCl_3$, TMS)[a]

H	1[b]	2[c]	3[b]
1	8.31 (dd, 7.5, 1.6)	6.76 (dd, 9.8, 3.0)	8.14 (dd, 7.5, 1.5)
2	7.40 (t, 7.5)	6.08 (ddd, 9.8, 6.4, 2.6)	7.58 (t, 7.5)
3(α)	7.35 (dd, 7.5, 1.6)	2.25 (ddd, 17.2, 2.6, 1.0)	7.50 (dd, 7.5, 1.5)
3β	–	2.49 (dddd, 17.2, 7.0, 6.4, 3.0)	–
4α	–	3.10 (ddq, 1.0, 7.0, 7.0)	–
12	7.50 (brs)	2.59 (s)	7.52 (brs)
13	2.16 (brs)	2.30 (s)	2.37 (brs)
14	2.63 (s)	1.04 (d, 7.0)	2.79 (s)
15	3.50/3.78 (d, 5.8)	3.80 (s)	–
OH	–	8.67 (brs)	–

[a] Chemical shifts in ppm from internal TMS and coupling constants in Hz;
[b] Obtained on a 400 MHz spectra recorder;
[c] Obtained on a 200 MHz spectra recorder

Compound **2** was obtained as colorless needles from acetone, m.p. 150–152 °C. The absorption bands in its IR spectrum indicated a hydroxyl group (3282 cm^{-1}), a conjugated carbonyl group (1670 cm^{-1}) and double bonds (1586, 1456, 1426 cm^{-1}). The ^{13}C NMR and DEPT spectra exhibited fifteen carbon signals including four methyls, one methylene, three methines and seven quaternary carbons. The EIMS spectrum showed the molecular ion peak at *m/z* 246 $[M]^+$. Hence, the molecular formula of compound **2** was deduced as $C_{15}H_{18}O_3$, indicating seven degrees of unsaturation in the molecule. Except the signal of the carbonyl carbon (δ 205.6), all of the remaining six quaternary carbons were at low field (between δ 125 and δ 150) in the ^{13}C NMR spectrum, thus a complete substituted benzene ring was suggested. The ^{1}H NMR spectrum showed the presence of a secondary methyl group (δ 1.04, 3 H, d), two tertiary methyl groups (δ 2.30, 2.59, 3 H each, s) and a methoxyl group (δ 3.80, 3 H, s). These groups was combined by the ^{1}H-^{13}C cross peaks (^{3}J) in the HMBC experiment: C-1/H-3; C-2/H-4; C-3/H-1, 14; C-5/H-1, 3, 13, 14; C-7/H-12, 13, −OH; C-9/H-1, −OH, −O<u>C</u>H$_3$; C-10/H-2, 4. The configuration of 14-Me at C-4 was determined as β on the basis of a strong correlation between C-13 and H-4α in NOESY experiment. The determination also matches the biogenesis of cacalol [6, 7]. Thus, compound **2** was identified as deltonorcacalol.

The structure of compound **3** was elucidated as maturinone from its EIMS, IR, ^{1}H and ^{13}C NMR spectra data. The ^{13}C NMR data of **3** are reported here for the first time, while its IR and ^{1}H NMR data were the same as those reported in the literature [8]. Comparison of the EIMS, ^{1}H and ^{13}C NMR data with literature, compounds **4** and **5** were identified as β-amyrin acetate [9] and α-amyrin [10], respectively. Compounds **6**, **7**, **8**, **9**, **10** and **11** were identified as lupeol, β-sitosterol, daucosterol, caffeic acid, tyrosol [11] and esculetin [12], respectively, by direct comparison with authentic samples (TLC, m.p. and EIMS data). Compounds **12** and **13** were identified as scopoletin [12] and syringaldehyde [13], respectively, by comparison of their EIMS and ^{1}H NMR data with the literature. Compound **14** was determined as (*E*)-cinnamic acid on the basis of its IR, EIMS, ^{1}H and ^{13}C NMR data (see Experimental).

3. Experimental

3.1. Apparatus

Optical rotations were recorded on a Perkin-Elmer 341 Polarimeter; UV spectra were obtained on a TU-1901 UV-VIS spectrophotometer; IR spectra were taken on a Nicolet Avatar 360 FT-IR spectrometer; The NMR spectra were obtained on a Bruker AM 400 FT-NMR spectrometer with chemical shifts reported in δ (ppm) using TMS as an internal standard; MS data were obtained on a VG-ZAB-HS instrument (70 eV); Silica gel (200–300 mesh) used for column chromatography and silica GF_{254} (10–40 μ) for TLC supplied by Qingdao Marine Chemical Factory, Qingdao, P.R. China; Spots were detected on TLC under UV or by heating after spraying with 5% H_2SO_4 in C_2H_5OH; Melting points are uncorrected.

3.2. Plant material

The whole plant of *Cacalia deltophylla* (Maxim.) Mattf. was collected in September 2000, in Forestry Center Shuang-Cha in Luqu county, Gansu Province, People's Republic of China, and was identified by Prof. Yao-Jia Zhang, Department of Biology, Lanzhou University. A voucher specimen (No. 2000915) is deposited in the Department of Chemistry, Lanzhou University, People's Republic of China.

3.3. Extraction and isolation

The air-dried whole plant of *C. deltophylla* (3.0 kg) was pulverized and then percolated with methanol (four times; 5 days per time) at room temperature. The extract was concentrated under reduced pressure to yield a residue (500 g), which was dispersed into water and extracted with petroleum ether (three times), then with EtOAc (three times). Each organic layer was concentrated under reduced pressure, to obtain a petroleum ether residue (75 g) and an EtOAc residue (25 g). The petroleum ether residue was subjected to CC on silica gel (200–300 mesh, 800 g) eluted with a gradient of petroleum ether-acetone, to obtain Fr. A (18 g), Fr. B (8 g), Fr. C (12 g), and Fr. D (6 g) of petroleum ether-acetone (30 : 1, 20 : 1, 15 : 1, and 10 : 1, respectively). Fr. A was then separated by CC on silica gel (200 g) eluted with a gradient of petroleum ether-EtOAc. The eluate A_1 (petroleum ether-EtOAc 30 : 1, 3 g) was purified by CC on silica gel (60 g) eluted with benzene-EtOAc (40 : 1) to give **4** (20 mg). The eluate A_2 (petroleum ether-EtOAc 20 : 1, 3 g) was further purified by CC on silica gel (60 g) eluted with petroleum ether-benzene (10 : 1) to afford **5** (26 mg) and **6** (20 mg). Crude **3** deposited from eluate A_3 (petroleum ether-EtOAc 15 : 1) and was re-crystallized from acetone to yield pure **3** (21 mg). Crystals of **7** (30 mg) deposited from Fr. B and were re-crystallized from a mixture of petroleum ether-EtOAc. Fr. C was purified by CC on silica gel (150 g) and eluted repeatedly with petroleum ether-EtOAc (15 : 1), to give **1** (8 mg) and **2** (30 mg). Compound **14** (18 mg) was obtained from Fr. D by CC on silica gel (80 g) eluted with petroleum ether-EtOAc (10 : 1). The EtOAc residue was subjected to CC on silica gel (300 g) eluted with a gradient of petroleum ether-acetone, to obtain Fr. E (4 g), Fr. F (3 g), Fr. G (6 g), and Fr. H (3 g) of petroleum ether-acetone (10 : 1, 8 : 1, 5 : 1, and 2 : 1, respectively). Fr. E was separated by CC on silica gel (80 g) eluted with $CHCl_3$–MeOH (50 : 1), then further purified by CC eluted with petroleum ether-EtOAc (20 : 1), to give **10** (8 mg) and **13** (12 mg). Compound **12** (10 mg) deposited from Fr. F and was re-crystallized from MeOH. Fr. G was purified by CC on silica gel (10 g) eluted with $CHCl_3$–MeOH (15 : 1), to afford **9** (10 mg) and **11** (8 mg). Crude **8** deposited from Fr. H and was re-crystallized from EtOH to yield pure **8** (16 mg). For ^{1}H and ^{13}C NMR data see Tables 1 and 2.

3.4. Deltocacalone (1)

Yellow crystals (acetone); m.p. 183–185 °C; $[\alpha]_D^{20}$ −5.4 (c, 0.35, acetone); IR (ν^{KBr}, cm^{-1}): 3100, 2919, 1652, 1583, 1535, 1467, 1417, 1365, 1222, 993, 887, 832, 756; EIMS *m/z* (rel int): 240 $[M]^+$ (46), 225 $[M\text{-}CH_3]^+$ (100), 210 (75), 153 (26), 139 (12), 115 (13), 63 (10).

3.5. Deltonorcacalol (2)

Colorless needles (acetone); m.p. 150–152 °C; $[\alpha]_D^{20}$ +84.6 (c, 0.39, $CHCl_3$); IR (ν^{KBr}, cm^{-1}): 3282, 2963, 2930, 1670, 1586, 1456, 1426,

Table 2: ^{13}C NMR data of compounds 1–3 ($CDCl_3$, TMS)[a]

C	1[b]	2[c]	3[b]
1	126.4 d	120.8 d	125.8 d
2	128.0 d	129.1 d	132.6 d
3	137.1 d	30.8 t	138.2 d
4	135.3 s	27.4 d	134.2 s
5	135.9 s	131.4 s	129.8 s
6	56.8 s	128.2 s	184.4 s
7	136.1 s	125.9 s	130.7 s
8	149.0 s	148.2 s	151.9 s
9	172.6 s	141.5 s	173.6 s
10	136.7 s	128.6 s	141.9 s
11	119.4 s	205.6 s	121.8 s
12	146.0 d	32.6 q	145.8 d
13	7.8 q	16.3 q	8.8 q
14	19.7 q	18.7 q	23.1 q
15	52.0 t	61.4 q	–

[a] Signals assigned on the basis of HMQC and HMBC spectra;
[b] Obtained on a 100 MHz spectra recorder;
[c] Obtained on a 50 MHz spectra recorder

1357, 1270, 1167, 1047, 999, 975; EIMS *m/z* (rel int): 246 $[M]^+$ (41), 231 $[M\text{-}CH_3]^+$ (29), 199 (22), 157 (32), 128 (16), 115 (22), 91 (11), 77 (11), 43 (100).

3.6. Maturinone (3)

Yellow prisms (acetone); m.p. 160–162 °C; IR (ν^{KBr}, cm^{-1}): 2919, 2849, 1706, 1667, 1531, 1467, 1226, 963; EIMS *m/z* (rel int): 226 $[M]^+$ (100), 197 (36), 169 (20), 141 (60), 115 (40), 89 (18), 64 (16).

3.7. β-Amyrin acetate (4)

Colorless needles (petroleum ether-EtOAc); m.p. 239–240 °C; EIMS *m/z* (rel int): 468 $[M]^+$ (3), 453 $[M\text{-}CH_3]^+$ (0.7), 393 $[M\text{-}CH_3\text{–}AcOH]^+$ (0.8), 270 (0.9), 257 (20), 249 (20), 218 (100), 203 (35), 119 (11), 95 (12), 69 (13), 43 (24); ^{1}H NMR δ ppm ($CDCl_3$, 400 MHz): 0.83 (3 H, s, H-23), 0.87 (6 H, s, H-24, H-25), 0.88 (6 H, s, H-29, H-30), 0.96 (3 H, s, H-26), 0.97 (3 H, s, H-27), 1.13 (3 H, s, H-28), 4.50 (1 H, dd, 6.8 Hz, 8.8 Hz, H-3), 5.18 (1 H, dd, 3.2 Hz, 3.2 Hz, H-12), 2.05 (3 H, s, $COOCH_3$); ^{13}C NMR and DEPT δ ppm ($CDCl_3$, 100 MHz): 38.27 (CH_2, C-1), 23.57 (CH_2, C-2), 80.95 (CH, C-3), 37.72 (C, C-4), 55.26 (CH, C-5), 18.27 (CH_2, C-6), 32.60 (CH_2, C-7), 39.81 (C, C-8), 47.56 (CH, C-9), 36.85 (C, C-10), 23.54 (CH_2, C-11), 121.65 (CH, C-12), 145.22 (C, C-13), 41.72 (C, C-14), 26.93 (CH_2, C-15), 26.14 (CH_2, C-16), 32.50 (C, C-17), 47.24 (CH, C-18), 46.79 (CH_2, C-19), 31.08 (C, C-20), 34.74 (CH_2, C-21), 37.15 (CH_2, C-22), 28.40 (CH_3, C-23), 16.70 (CH_3, C-24), 15.56 (CH_3, C-25), 16.81 (CH_3, C-26), 25.95 (CH_3, C-27), 28.04 (CH_3, C-28), 33.33 (CH_3, C-29), 23.69 (CH_3, C-30), 21.31 (OCH_3), 171.0 (C=O).

3.8. α-Amyrin (5)

Colorless needles (petroleum ether-EtOAc); m.p. 182–184 °C; EIMS *m/z* (rel int): 426 $[M]^+$ (6), 411 (2), 257 (2), 218 (100), 203 (26), 189 (17), 119 (14), 95 (18), 55 (22), 43 (19); ^{1}H NMR δ ppm ($CDCl_3$, 400 MHz): 0.79 (3 H, s, H-23), 0.80 (3 H, s, H-24), 0.87 (3 H, s, H-25), 1.00 (3 H, s, H-26), 1.01 (3 H, s, H-27), 1.03 (3 H, s, H-28), 0.95 (3 H, d, 6.2 Hz, H-29), 0.96 (3 H, d, 6.2 Hz, H-30), 3.22 (1 H, dd, 10.7 Hz, 5.2 Hz, H-3), 5.13 (1 H, dd, 4.2 Hz, 3.2 Hz, H-6); ^{13}C NMR and DEPT δ ppm ($CDCl_3$, 100 MHz): 38.8 (C-1, CH_2), 27.3 (C-2, CH_2), 79.0 (C-3, CH), 40.0 (C-4, C), 55.2 (C-5, CH), 18.4 (C-6, CH_2), 33.0 (C-7, CH_2), 40.0 (C-8, C), 47.7 (C-9, CH), 36.9 (C-10, C), 23.4 (C-11, CH_2), 124.4 (C-12, CH), 139.6 (C-13, C), 42.1 (C-14, C), 29.7 (C-15, CH_2), 26.6 (C-16, CH_2), 33.8 (C-17, C), 59.1 (C-18, CH), 39.6 (C-19, CH), 39.7 (C-20, CH), 31.3 (C-21, CH_2), 41.5 (C-22, CH_2), 28.1 (C-23, CH_3), 15.6 (C-24, CH_3), 15.7 (C-25, CH_3), 16.9 (C-26, CH_3), 23.3 (C-27, CH_3), 28.8 (C-28, CH_3), 17.5 (C-29, CH_3), 21.4 (C-30, CH_3).

3.9. Lupeol (6)

Colorless needles (petroleum ether-EtOAc); m.p. 210–212 °C; The TLC was identical to that of an authentic sample.

3.10. β-Sitosterol (7)

Colorless needles (petroleum ether-EtOAc); m.p. 139–140 °C; The TLC was identical to that of an authentic sample.

3.11. Daucosterol (8)

White powder (EtOH); m.p. 289–290 °C; The TLC was identical to that of an authentic sample.

3.12. Caffeic acid (9)

Pale yellow crystals; m.p. 220–222 °C; EIMS *m/z* (rel int): 180 $[M]^+$ (2), 154 (6), 137 (6), 100 (78), 74 (66), 45 (100).

3.13. Tyrosol (10)

Colorless needles; m.p. 90–92 °C; EIMS *m/z* (rel int): 138 $[M]^+$ (45), 120 (100), 106 (7), 92 (92), 77 (5), 64 (29), 53 (12), 39 (20).

3.14. Esculetin (11)

Pale yellow needles; m.p. 271–272 °C; EIMS *m/z* (rel int): 178 $[M]^+$ (100), 150 (77), 121 (5), 79 (5), 69 (8).

3.15. Scopoletin (12)

Colorless needles (MeOH); m.p. 203–205 °C; EIMS *m/z* (rel int): 192 $[M]^+$ (100), 177 (60), 164 (21), 149 (38), 121 (13), 79 (12), 69 (20); ^{1}H NMR δ ppm ($CDCl_3$, 200 MHz): 3.89 (3 H, s, OCH_3), 6.17 (1 H, d, 9.4 Hz, H-3), 7.85 (1 H, d, 9.4 Hz, H-4), 7.35 (1 H, s, H-5), 6.79 (1 H, s, H-8), 10.15 (1 H, brs, OH).

3.16. Syringaldehyde (13)

Pale yellow needles; m.p. 106–108 °C; EIMS *m/z* (rel int): 182 $[M]^+$ (100), 181 (65), 167 (10), 139 (8), 111 (10), 96 (8), 79 (5), 53 (4). ^{1}H NMR δ ppm ($CDCl_3$, 200 MHz): 4.01 (6 H, s, 2 OCH_3), 7.19 (2 H, s), 9.86 (1 H, s, CHO), 6.08 (1 H, brs, OH).

3.17. (E)-Cinnamic acid (14)

Colorless needles; m.p. 130–132 °C; IR (ν^{KBr}, cm^{-1}): 3000–2500, 1685, 1625, 1571, 1444, 1417, 1315, 1281, 1217, 978, 935, 704; EIMS *m/z* (rel int): 148 $[M]^+$ (78), 147 (100), 131 (17), 103 (27), 91 (12), 77 (17), 51 (10); ^{1}H NMR δ ppm ($CDCl_3$, 200 MHz): 6.48 (1 H, d, 16.0 Hz), 7.81 (1 H, d, 16.0 Hz), 7.43 (3 H, m), 7.57 (2 H, dt, 9.5 Hz, 2.2 Hz); ^{13}C NMR and DEPT δ ppm ($CDCl_3$, 50 MHz): 117.3 (CH), 128.4 (CH), 128.4 (CH), 129.0 (CH), 129.0 (CH), 130.8 (CH), 134.0 (C), 147.1 (CH), 172.5 (C=O).

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