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Coumarleucasin and leucasone from *Leucas inflata* roots

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Abstract

From the acetone extract of the roots of *Leucas inflata*, four coumarins (siderin, coumarsabin, 8-methoxycoumarsabin and coumarleucasin) and one chromone were isolated. One of the coumarins, coumarleucasin (5-formyl-4,7,8-trimethoxy-3-methylcoumarin), and the chromone, leucasone {2,8-dimethyl(2,2-dimethylethenyl)-5,6-benzo-4-pyrone}, have not been reported previously.

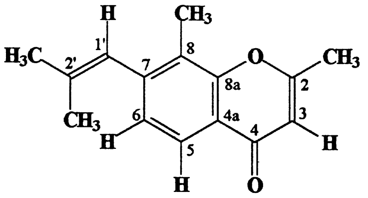
Keywords: Leucas inflata; Labiatae; Roots; Coumarins; Chromone; Coumarleucasin; Leucasone

1. Introduction

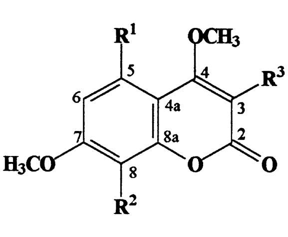
Leucas species have been reported to have wound healing (Saha, Mukherjee, Das, Pal, & Saha, 1997), antitussive (Saha, Mukherjee, Murugesam, Saha, & Pal, 1997), hypoglycaemic (Saha, Mukherjee, Das, Mandal, & Saha, 1997), antimycobacterial (Rajab, Cantrell, Franzblau, & Fisher, 1998), antidiarrhoeal (Mukherjee et al., 1998) and anti-inflammatory (Saha et al., 1996) properties. Labdane-type diterpenes (Pradham, Chakraborty, & Subba, 1990), triterpenes (Hasan, Burdi, & Ahmad, 1991; Khalil, Gedara, Lahloub, & Halim, 1996) and a flavone (Pradham et al., 1990) have also been isolated and characterized from *Leucas* species. *Leucas inflata*, which is indigenous to the United Arab Emirates, has not been investigated previously, either chemically or pharmacologically. Preliminary studies have shown that extracts of the plant have anti-inflammatory, central nervous system and analgesic properties (unpublished data). In the present communication, we report the isolation from *L. inflata* of four coumarins, one chromone, two sterols and two sterol glycosides. One of the coumarins, coumarleucasin, and the chromone, leucasone, are novel compounds.

2. Results and discussion

Dry *Leucas inflata* roots were extracted with acetone and the extract fractionated by column chromatography to yield six major fractions. From fraction 1, a compound (**1**) was isolated, the ^{1}H NMR spectrum of which showed



1



$R^1 = CH_3; R^2 = H; R^3 = CH_3 = \mathbf{2}$
$R^1 = CH_3; R^2 = OCH_3; R^3 = CH_3 = \mathbf{3}$
$R^1 = CH_3; R^2 = H; R^3 = H = \mathbf{4a}$
$R^1 = CHO; R^2 = OCH_3; R^3 = CH_3 = \mathbf{4b}$

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four aromatic methyl singlets and four aromatic protons. The signal at δ 183.4 in the ^{13}C NMR spectrum revealed the presence of a keto group. No aliphatic methine nor methylene groups were observed and thus the isolated compound is probably a chromone with a side-chain. From the NMR spectra, this side-chain was shown to be ethylene with two geminal methyl groups and the basic chromone structure contained two methyl groups and three aromatic protons. In the ^{13}C NMR spectrum of **1**, the two quaternary carbon low field resonances at δ 154.1 and 157.6 (C-2 and C-8a) suggest that one of the methyl groups is present on C-2. From difference decoupling experiments, irradiation of the signal observed at δ 7.29 in the ^{1}H NMR spectrum revealed long-range coupling ($J = 0.6$ Hz) to the signal at δ 7.10. Irradiation of the latter, in addition to the long-range coupling to the signal at δ 7.29, showed large coupling ($J = 8$ Hz) with the signal at δ 7.49. Thus, the proton producing the signal at δ 7.29 is assigned to H-3 and the signals at δ 7.49 and δ 7.10 to H-6 and H-5, respectively. The H-5 signal (ddd, $J = 8.0$, 1.5, 0.6 Hz), in addition to the coupling with H-3, must be coupled with the proton associated with the conjugated double bond in the side-chain. Hence, the side-chain is substituted at C-7; this was confirmed by NOE experiments. Irradiation of the methyl signal at δ 2.05 enhanced the signal at δ 6.94 and vice versa. The remaining methyl group must therefore be located on C-8.

The proposed structure of **1** was confirmed by HR mass spectrometry ([M]$^+$, *m/z* 228.1103; calculated for $C_{15}H_{16}O_2$ 228.1150). All the available data are consistent with the structure assigned to **1**; the trivial name leucasone has been assigned to this compound.

By comparison with spectral data of reference compounds, fraction 2 was shown to contain stigmasterol and sitosterol, with the former predominating. Comparison of spectroscopic and spectrometric measurements with literature values proved that fractions 3 and 4 contained the coumarins, coumarsabin (**2**) and 8-methoxycoumarsabin (**3**) (de Pascual et al., 1981), respectively.

Fraction 5 yielded two compounds, which were separated by preparative TLC. On the basis of ^{1}H and ^{13}C NMR spectroscopic and mass spectrometric evidence, **4a** was identified as siderin (Lapper, 1974; Venturella, Bellino, & Piozzi, 1974; Chexal, Fouweather, & Holker, 1975). The ^{1}H NMR spectrum of **4b** was similar to that of **3** (de Pascual et al., 1981) but showed one methyl singlet (δ 2.22), three methoxyl singlets (δ 3.92, 3.99 and 4.06) and two proton singlets (δ 7.44 and 10.77). The spectrum was unchanged after addition of D_2O. From NOE experiments, irradiation of the singlet at δ 7.44 produced enhancement of both the downfield signal at δ 10.77 and the methoxyl singlet at δ 3.99. Likewise, irradiation of the signal at δ 10.77 enhanced the signal at δ 7.44. When the signal at δ 2.22 was irradiated, the methoxyl signal at δ 3.92 was enhanced. A resonance at δ191.6 in the ^{13}C NMR spectrum suggested the presence of an aldehyde group. The data obtained led to the assignment of the structure shown in **4b**. Moreover, the ^{1}H NMR spectra of **4b** and **3** are similar, except that the methyl signal observed at δ 2.65 in the spectrum of the latter compound is absent from that of **4b**, although this contains an aldehyde signal at δ 10.77. These data show that the spectrum of **4b** differs from that of **3** by the presence of an aldehyde instead of a methyl group on C-5. This proposed structure was consistent with the mass ([M]$^+$, *m/z* 278.0759; calculated for $C_{14}H_{14}O_6$ 278.0790) and IR spectra. The trivial name coumarleucasin has been assigned to this compound.

The white solid isolated from fraction 6 was shown from EI mass spectrometric and NMR spectroscopic data to be a mixture of the 3β-glucosides of sitosterol and stigmasterol, with the former predominating (Kalinovskaya, Kuzentsova, Popov, Antonovo, & Elyakov, 1983).

3. Experimental

3.1. Plant material

Leucas inflata Benth. was collected from Khor-Fakkan and Jebel Al-Faya, United Arab Emirates (UAE) during March to May, 1997. The plant material was authenticated by the National Herbarium, UAE University, where a voucher specimen (13751) is deposited.

3.2. Extraction and purification of compounds

Air-dried, powdered roots (2 kg) were extracted with Me_2CO in a Soxhlet apparatus to yield 42 g crude extract, which was coated onto silica gel 60 (0.2–0.5 mm, 35–70 mesh ASTM; 55 g). This mixt. was placed on a column (120 × 4 cm) filled with silica gel 60 (0.2–0.5 mm; 300 g) and eluted first with *n*-hexane (frs 1–42) and subsequently with mixts of *n*-hexane and EtOAc (97.5:2.5, frs 43–78; 95:5, frs 79–104; 90:10, frs 105–134; 85:15, frs 135–148; 80:20, frs 149–197; 70:30, frs 198–219; 60:40, frs 220–242; 50:50, frs 243–268; 30:70, frs 269–334); each fr. was 200 ml. The frs after concn were examined by silica gel F-254 (Merck) TLC using mixts of either EtOAc–*n*-hexane (1:9, frs 1–42; 15:85, frs 43–104; 20:80, frs 105–134; 30:70, frs 135–148; 2:3, frs 149–197) or MeOH–$CHCl_3$ (1:9, frs 198–242; 15:85, frs 243–268; 1:4; frs 269–334). Compounds were detected under UV light (λ 254 nm) and by spraying with *p*-anisaldehyde–H_2SO_4 reagent and heating. Similar frs were combined. Six such combined frs, after concn were processed further.

3.3. Combined fraction *1* (frs 51–81)

This material (28.4 g) was coated onto silica gel 60 (28.5 g (0.063–0.22 mm, 70–230 mesh ASTM) + 8 g (0.2–

0.5 mm, 35–70 mesh ASTM)), added to the top of a column (120 × 4 cm) filled with silica gel 60 (0.063–0.22, 70–230 mesh ASTM; 290 g) and eluted with petrol (b.p. 60–80°C); each fr. was 200 ml. The frs, after concn, were analysed by TLC on silica gel F-254 using a mixt of either $CHCl_3$–*n*-hexane (1:9, frs 1–10; 30:70, frs 11–55) or EtOAc–petrol (b.p. 60–80°C) (1:9, frs 56–75). Compounds were detected under UV light (λ 254 nm). Frs 34–60 were combined, concd and subjected to prep. TLC on silica gel F-254 using *n*-hexane–EtOAc (9:1). A major band was observed when viewed under UV light (λ 254 nm); this was scraped from the plates and eluted with $CHCl_3$–MeOH (99:1) to afford **1** (22.8 mg).

3.4. Combined fraction 2 (frs 97–104)

On addition of MeOH to this fr dissolved in $CHCl_3$, a ppt was formed which, after standing overnight, was collected by centrifugation (125 mg).

3.5. Combined fraction 3 (frs 110–111)

Compound **2** (18.2 mg) was isolated from this fr by centrifugal TLC using 4 mm layers of silica gel G and *n*-hexane–EtOAc (4:1) as developing solvent.

3.6. Combined fraction 4 (frs 138–142)

On the addition of *n*-hexane to this fr. dissolved in $CHCl_3$, **3** was pptd and collected by centrifugation after standing overnight (1740 mg).

3.7. Combined fraction 5 (frs 164–166)

Compound **4a** (9 mg) was obtained from this by pptn with *n*-hexane. After standing overnight and removal of **4a** by centrifugation, the remaining soln was subjected to prep. TLC on silica gel F-254 G using *n*-hexane–EtOAc (1:1) as developing solvent. The major component (**4b**) detected under UV light (λ 254 nm) was scraped from the plates and eluted from the silica gel with $CHCl_3$–MeOH (99:1) to yield 4.7 mg of solid.

3.8. Combined fraction 6 (frs 296–299)

The material (550 mg) was coated onto 500 mg C-Gel C 560 (0.04–0.063 mm; CU Chemie Uetikon AG, Switzerland), added to a column of C-Gel C 560 (18 g) and subjected to medium pressure LC using $CHCl_3$–MeOH (97.5:2.5) as mobile phase. Frs (25 ml) were collected and monitored by TLC (silica gel F-254 layers) using $CHCl_3$–MeOH (3:22) as developing solvent and *p*-anisaldehyde–H_2SO_4 as the detection reagent. Frs 65–110 were combined, concd to dryness and the residue dissolved in a minimum vol. of $CHCl_3$. On addition of MeOH, a ppt. formed, which was collected by centrifugation to yield 48 mg of a white solid.

^{1}H NMR were recorded in $CDCl_3$ at 270 MHz with TMS as int. standard. ^{13}C NMR were recorded on the same instrument at 60 MHz.

*3.9. Leucasone [2,8-dimethyl-(2,2-dimethylethenyl)-5,6-benzo-4-pyrone] (**1**)*

$C_{15}H_{16}O_2$, m.p. 72.9°C. UV λ_{max}^{MeOH}, (log ε): 233 (4.85), 248 (4.81), 327 (5.23). EI MS (probe) 70 eV *m*/*z* (rel. int.): 228.1103 $[M]^+$ (100), 213 (67), 198 (16), 185 (14), 173 (10). ^{1}H NMR ($CDCl_3$): δ 2.05 (3H, d, $J = 1.5$ Hz; Me (*trans*)-2′), 2.30 (3H, d, $J = 1.1$ Hz; Me (*cis*)-2′), 2.49 (3H, s; Me-8), 2.61 (3H, s; Me-2), 6.94 (1H, m; H-1′), 7.10 (1H, ddd, $J = 8.0$, 1.5, 0.6 Hz; H-5), 7.29 (1H, br.s; H-3), 7.49 (1H, d, $J = 8.0$ Hz; H-6). ^{13}C NMR ($CDCl_3$): δ 157.6 (C-2 or C-8a), 112.0 (C-3), 183.3 (C-4), 124.3 (C-4a), 127.7 (C-5), 120.6 (C-6), 138.5 (C-7), 148.9 (C-8), 154.1 (C-2 or C-8a), 121.2 (C-1′), 127.3 (C-2′), 9.7 (Me (*trans*)-2′), 22.0 (Me (*cis*)-2′).

*3.10. Coumarleucasin [5-formyl-4,7,8-trimethoxy-3-methylcoumarin] (**4b**)*

$C_{14}H_{14}O_6$, m.p. 158°C. UV λ_{max}^{MeOH}, (log ε): 274 (4.86), 322 (4.95). IR (ν_{max}^{Nujol} cm^{-1}: 2920, 2850, 1740 (C=O), 1675, 1585, 1330. EI MS (probe) 70 eV *m*/*z* (rel. int.): 278.0759 $[M]^+$ (100), 263 (25), 250 (15), 235 (22), 220 (8). ^{1}H NMR ($CDCl_3$): δ [equivalent values for **3**] 2.22 (3H, s; Me-3) [2.14], 3.92, 3.99, 4.06 (3 × OMe, s; OMe-4, -7 and -8) [3.86 (3H, s; OMe-4), 3.92 (6H, s; OMe-7 and -8], 7.44 (1H, s; H-6) [6.64 (1H, br.s; H-6)], 10.77 (1H, s; C*H*O-5) [2.64 (3H, s; Me-5)]. ^{13}C NMR ($CDCl_3$): δ [equivalent values for **3**] 162.5 (C-2) [163.8], 111.5 (C-3) [110.5], 164.5 (C-4) [166.6], 108.3 (C-4a) [110.6], 128.5 (C-5) [130.7], 113.2 (C-6) [111.9], 146.0 (C-7) [147.6], 140.0 (C-8) [134.4], 154.0 (C-8a) [153.6], 11.0 (Me-3) [10.2], 56.4 (OMe-4) [56.2], 191.6 (*C*HO-5) [22.3 (Me-5)], 60.9 (OMe-7) [60.6], 61.7 (OMe-8) [61.4].

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