Pharmacognosy Department, College of Pharmacy, Cairo University, Cairo, Egypt.

Hirsutinolides from *Vernonia cinerascens*

E. ABDEL-SATTAR, J. S. MOSSA and H. I. EL-ASKARY

The aerial parts of *Vernonia cinerascens*, collected in Saudi Arabia, yielded a new hirsutinolide, together with three known lactones. The structure of the new compound was elucidated using, ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HETCOR and HMBC.

1. Introduction

The chemistry of the large genus *Vernonia* (Asteraceae, tribe Vernonieae) has been investigated by many authors [1–5] and different sesquiterpene lactones, mainly the highly oxygenated germacranolides, such as glaucolides and hirsutinolides, were found the most common constituents. A wide range of biological activities has been reported for this class of compounds [6, 7]. Glaucolide B demonstrated potent moluscicidal properties and scorpioidine showed strong antifeedant activity against *Locusta migratoria* L. [8]. In a previous report on *V. cinarescens* collected in Transvaal, tridecapentaynene, *Venonia* α-humulene, 5-methyl coumarin, preethulia coumarin, lupeol and its acetate [9] were isolated. In 1994, another report on an Ethiopian collection [10] resulted in the isolation of luteolin, lupeol, lupeol fatty acid ester, and two hirsutinolides. In the present paper, we describe the isolation of four hirsutinolide sesquiterpene lactones from *V. cinerascens* collected in Saudi Arabia.

2. Investigations, results and discussion

The *n*-hexane extract of aerial parts of *V. cinerascens* (300 g) was partitioned between *n*-hexane and MeCN. The MeCN fraction (1.8 g) was chromatographed on Si-gel to afford the compounds **1**–**4**.

The structure of compound **1** was deduced from ^{1}H NMR [11] and ^{13}C NMR spectra (Table). The ^{1}H NMR spectrum of compound **1** showed typical signals for 4-hydroxymethacrylate {4.21, d (J = 12.5), 4.39, d (J = 12.5), 6.36, 5.81 (br s)} positioned at C-8 (δ_H 6.63 {brd, J = 9.5}/δ_C 65.31) as confirmed from ^{1}H-^{1}H COSY and HMBC spectra and by comparison with related compounds [3, 11, 12]. ^{1}H and ^{13}C NMR spectra showed an acetoxy group positioned at C-13. The inspection of ^{1}H and ^{13}C NMR data resulted in identification of compound **1** as 8-α-[4-hydroxymethacryloyloxy]-10-α-hydroxy-hirsutinolide-13-O-acetate, a hirsutinolide lactone previously isolated from *V. cinera* [11]. Spectral data of compounds **1** and **2** were very similar, except of the nature of the esterifying acid at C-8. The 4-hydroxymethacryloyloxy residue at C-8 in **1** was replaced by a methacryloyloxy one in **2** (a methyl signals at δ_H 1.95/δ_C 18.1). Compound **2** was identified as 8-α-(methacryloyloxy)-10-α-hydroxy-hirsutinolide-13-O-acetate by comparison with the spectral data reported [12–14]. Compound **2** (piptocarphin A) was previously isolated from *V. squamulosa* [13] and *Piptocarpha chontalensis* [14]. Compound **3**, $C_{22}H_{28}O_{10}$, was found to have similar NMR features as **1**. However, signals attributed to a methoxy group were seen at δ_H 3.55/δ_C 55.63 in the NMR spectra of **3**. This methoxy group was positioned at C-1 as further confirmed by HMBC and by comparison with related lactones [5, 12, 15]. Accordingly, compound **3** could be identified as the 1-*O*-methyl derivative of compound **1,** a hirsutinolide isolated from genus *Vernonia* for the first time, but previously reported in *Bothriocline ampliflia* [16]. The isolation of compound **3** may confirm the chemotaxonomical relation between the genera *Vernonia* and *Bothriocline*. Similar to that in **1** and **3**, the spectral data of **4** showed signals characteristic for α-4-hydroxymethacryloyloxy and acetoxy groups attributed to the esterifying residues at C-8

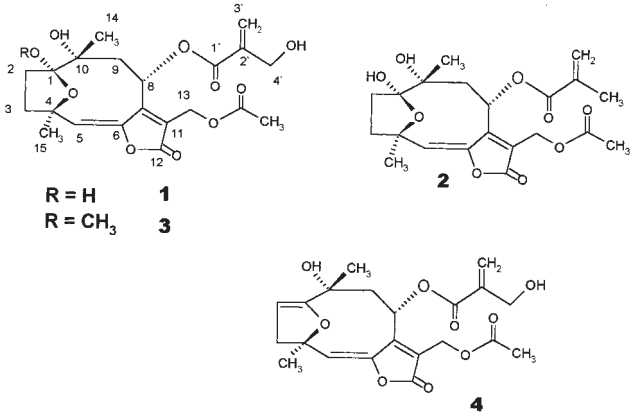

Table: ^{13}C NMR spectral data of compounds 1–4 (100 MHz, $CDCl_3$)

No.	1	2	3	4
1	108.97	108.69	111.67	159.27
2	37.59	37.44	38.05	94.45
3	32.37	31.94	33.50	42.91
4	82.58	82.50	83.71	84.62
5	126.51	126.90	125.66	124.57
6	150.70	149.19	150.20	150.98
7	144.06	145.50	143.50	145.09
8	65.31	66.19	65.08	66.83
9	37.80	37.99	38.05	45.50
10	77.6*	78.09	78.05	69.26
11	130.94	130.09	129.80	131.43
12	166.77	168.20	166.77	166.43
13	55.78	55.75	55.63	55.66
14	26.45	25.54	26.60	26.04
15	29.03	29.00	27.20	26.03
OMe			52.10	
1′	165.10	166.00	165.28	165.04
2′	138.93	135.80	139.35	139.17
3′	129.55	127.03	128.28	128.61
4′	62.37	18.10	62.49	62.40
Acetate				
	170.44	170.40	170.30	170.14
	20.75	20.70	20.70	20.65

* Overlapped with solvent resonance

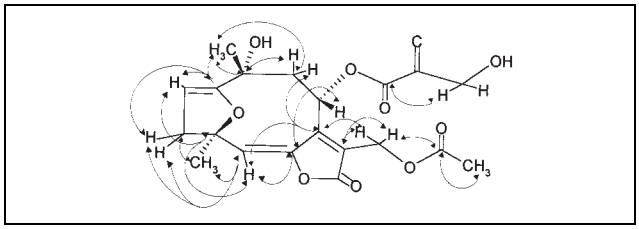

Fig.: HMBC spectrum of compound **4**: long-range correlations

and C-13, respectively. However, the presence of two additional olefinic carbons was inferred from NMR [δ_C at 159.27 and 94.45 and δ_H at 4.79 (brs)]. The creation of an olefinic double bond is accompanied by the absence of an oxy- and aliphatic carbons. When compared with data of related lactones [12, 15], the position of the double bond was most likely located at C-1/C-2. This result was further confirmed from the long-range correlations observed in the HMBC spectrum between the carbon signal at δ 159.27 (C-1) and proton signals at δ 1.69 (H-14), 4.79 (H-2) and 2.65 (H-3), other significant long-range correlations observed are shown in the Fig. Therefore, compound **4**, a dehydration product of **1**, could be identified as 8-α-[4-hydroxymethacryloyloxy]-10-α-hydroxy-isohirsutinolide-13-*O*-acetate. The ^{13}C NMR assignment of the known compounds was reported.

In all compounds isolated, the configurations at C-1, C-4 and C-8 are identical. The methyl group at C-10 is β-oriented, as the oxygen function is α-positioned [17]. To our knowledge, compound **4** was isolated for the first time from a natural source.

3. Experimental

3.1. General

IR: KBr or thin film. ^1H and ^{13}C NMR were recorded in $CDCl_3$ using a JEOL GNM-GX400 spectrometer at 400 and 100 MHz, respectively. EI-MS, Shimadzu PQ-5000; TLC: Si-gel 60 F_{254}, $CHCl_3$-MeOH (10 : 1) as solvent system; visualization using *p*-anisaldehyde/H_2SO_4 as spray reagent.

3.2. Plant material

The aerial parts of *V. cinerascens* Shultz Bip were collected in the Southern region of Saudi Arabia (Abha) in August 1996. A voucher specimen (# 13315) was deposited in the herbarium of the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

3.3. Extraction and isolation

The dried ground aerial parts of *V. cinerascens* (300 g) were extracted with n-hexane in a soxhlet. The n-hexane extract (6.2 g.) was partitioned between MeCN and n-hexane to give 1.8 and 4.1 g., respectively. The MeCN fraction was fractionated on Si gel columns using $CHCl_3$ with increasing amounts of MeOH (0–10% MeOH). Impure fractions were further purified on a Sephadex LH-20 column using $CHCl_3$-MeOH (2:1). The four compounds **1**-**4** were isolated in the following yield 90, 38, 20 and 75 mg, respectively.

3.3.1. 8-α-[4-hydroxymethacryloyloxy]-10-α-hydroxy-1-O-methyl hirsutinolide-13-O-acetate (**3**)

Colourless oil; IR (film) ν_{max} cm^{-1}: 3450 (OH), 1770 (C=O γ-lactone), 1730 (C=O Ac). ^1H NMR ($CDCl_3$): δ 1.25 (3H, s, H-14), 1.66 (3H, s, H-15), 1.85–1.95 (2 H, m, H-3), 2.03 (1 H, d, J = 15.8 Hz, H-9a), 2.08 (3 H, s, CO<u>Me</u>), 2.58 (1 H, dd, J = 9.6, 15.8 Hz, H-9b), 3.55 (3H, s, MeO), 4.24 (1 H, d, J = 13 Hz, H-3a′), 4.38 (1 H, d, J = 13 Hz, H-3b′), 4.92 (1 H, d, J = 13 Hz, H-13a), 5.20 (1 H, d, J = 13 Hz, H-13b), 5.81 (1 H, br s, H-4a′), 5.91 (1 H, br s, H-5), 6.38 (1 H, br s, H-4b′), 6.61 (1 H, br d, J = 9.6 Hz, H-8). ^{13}C NMR (see Table). EI-MS m/z (rel. int.): M^+ absent, 276 [M^+-$C_4H_5O_2$] (3), 234 (10), 218 (8), 188 (9), 85 $(C_3H_5OCO)^+$ (4), 57 [85 −CO]$^+$ (8), 43 [MeCO]$^+$ (100).

3.3.2. 8-α-[4-hydroxymethacryloyloxy]-10-α-hydroxy-1-O-methyl isohirsutinolide-13-O-acetate (**4**)

Colourless oil; IR (film) ν_{max} cm^{-1}: 3550 (OH), 1760 (C=O γ-lactone), 1725 (C=O Ac). ^1H NMR ($CDCl_3$): δ 1.43 (3H, s, H-15), 1.69 (3H, s, H-14), 1.93 (1 H, d, J = 15.2, H-9a), 2.08 (3H, s, CO<u>Me</u>), 2.65 (1 H, dd, J = 3, 15.6 Hz, H-3a), 2.73 (1 H, dd, J = 8.3, 15.2 Hz, <u>H</u>-9b), 2.85 (1 H, dd, J = 1.8, 15.6 Hz, H-3b), 4.25 (1 H, d, J = 13.1 Hz, H-3a′), 4.43 (1 H, d, J = 13.1 Hz, H-3b′), 4.79 (1 H, br s, H-2), 4.98 (1 H, d, J = 13.1 Hz, H-13a), 5.08 (1 H, J = 13.1 Hz, H-13b), 5.84 (1 H, br s, H-4a′), 5.85 (1 H, br s, H-5), 6.34 (1 H, br s, H-4b′), 6.55 (1 H, br d, J = 8.3, Hz, H-8). ^{13}C NMR (see Table). EI-MS m/z (rel. int.): M^+ absent, 360 [M^+ −HOAc] (0.14), 258 [360 $-C_3H_5OCOOH]^+$ (1.4), 216 [258 $-C_2H_2O]^+$ (3), 230 [258 −CO]$^+$ (8.5), 85 $[C_3H_5OCO]^+$ (23), 57 [85 −CO]$^+$ (17), 43 [MeCO]$^+$ (100).

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Dr. Essam Abdel-Sattar
Pharmacognosy Dept.
College of Pharmacy
Cairo University
El-Kasr El-Aini street
Cairo 11562
Egypt
abdel-sattar@excite.com