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Short Report

Cytotoxic Sesquiterpene Lactones and other Constituents of *Centaurea omphalotricha*

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A investigação fitoquímica das partes aéreas de *Centaurea omphalotricha* levou ao isolamento de três lactonas sesquiterpênicas novas, 4'-acetilcinaropicrina, 4'-acetilcebelina F e 15-acetil desidromelitensina, juntamente com doze compostos conhecidos, sendo sete lactonas sesquiterpênicas, dois isoprenóides e três flavonóides. As estruturas dos novos compostos foram elucidadas por meio de RMN 1D e 2D, e espectrometria de massas, e por comparação com os dados descritos na literatura. O efeito de lactonas sesquiterpênicas sobre a viabilidade das células tumorais humanas, linhagens HL-60 e U937, também foi investigado e 3-acetilcinaropicrina e 4'-acetilcinaropicrina foram os compostos mais citotóxicos contra células de leucemia humana com valores de IC_{50} de 2,0 ± 0,9 e 5,1 ± 0,4 μmol L^{-1}, respectivamente.

Phytochemical research of the aerial parts of *Centaurea omphalotricha* led to the isolation of three new sesquiterpene lactones, 4'-acetyl cynaropicrin, 4'-acetyl cebellin F and 15-acetyl dehydromelitensin, together with twelve known compounds, seven sesquiterpene lactones, two isoprenoids and three flavonoids. The structures of the new compounds were elucidated by means of extensive 1D and 2D NMR, and MS, and by comparison with reported data in the literature. The effect of sesquiterpene lactones on the viability of the human tumor cell lines HL-60 and U937 was also investigated and 3-acetyl cynaropicrin, and 4'-acetyl cynaropicrin were found to be the most cytotoxic compounds against human leukemia cells with an IC_{50} values of 2.0 ± 0.9 and 5.1 ± 0.4 μmol L^{-1}, respectively.

Keywords: *Centaurea*, Asteraceae, sesquiterpene lactones, cytotoxic activity, HL-60

Introduction

The genus *Centaurea* (Asteraceae: Centaureinae) comprises more than 500 species, most of which grow around the Mediterranean and in Western Asia.[1] *Centaurea* species have long been used for their biological properties, mainly as anti-inflammatory,[2] antipyretic,[3] cytotoxic,[4] antibacterial,[5] and antiproliferative agents.[6] Phytochemical investigations revealed that the compounds responsible for their pharmacological properties are flavonoids[7] and sesquiterpene lactones predominantly germacranolides, eudesmanolides, elemanolides, and guaianolides.[8] As a part of our continuing search for novel, plant-derived anticancer chemotherapeutic agents and our systematic investigation of the composition of plants of this genus,[4,9] we have investigated the chemical constituents of the aerial parts of *Centaurea omphalotricha* Coss. & Durieu ex Batt. et Trab., a species endemic to Algeria and Tunisia.[10,11]

The present work describes the isolation and structural elucidation of the constituents of the ethanolic-aqueous extract of the aerial parts of *C. omphalotricha*. The constituents of this extract were purified by column chromatography and preparative TLC. Thus, fifteen compounds were isolated including 4'-acetyl cynaropicrin (**1**), 4'-acetyl cebellin F (**2**)

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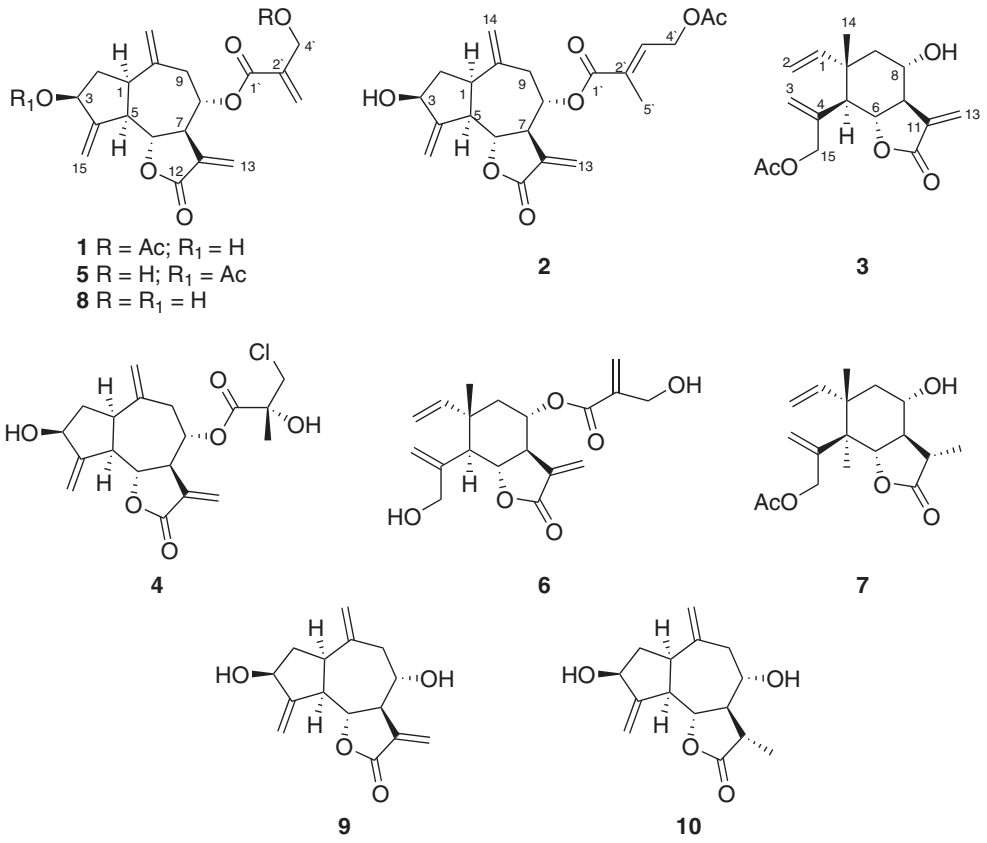

Figure 1. Structures of compounds **1**-**10**.

and 15-acetyl dehydromelitensin (**3**), which are new in the literature, together with the seven sesquiterpene lactones: linichlorin B (**4**),[12] 3-acetyl cynaropicrin (**5**),[13] 8-(4-hydroxymethacrylate)-dehydromelitensin (**6**),[14] 15-acetyl melitensin (**7**),[15] cynaropicrin (**8**),[16] desacyl-cynaropicrin (**9**)[16] and 8-hydroxy-11,13-dihydrozaluzanin C (**10**);[16] two isoprenoids: 3-hydroxy-5,6-epoxy-ionone,[17] dehydrovomifoliol[17] and three flavonoids: circimaritin, apigenin and luteolin.[18] The structures of the known compounds were confirmed by comparison of their spectroscopic data (MS, ^{1}H and ^{13}C NMR) with literature references. In this study we also demonstrate that the isolated sesquiterpene lactones **1**-**6** (Figure 1) induce cytotoxicity in human leukemia cell lines HL-60 and U937.

Experimental

General

Optical rotations were measured using a Perkin-Elmer model 343 polarimeter using $CHCl_3$ as solvent. UV spectra were recorded using a SHIMADZU model UV-1700 spectrophotometer. IR spectra were recorded as $CHCl_3$ films on NaCl plates, using a Bruker model IFS-55 and Perkin-Elmer model FTIR-8400S spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker model Avance 400 and AMX-500 spectrometer with standard pulse sequences, operating at 400 and 500 MHz for ^{1}H and 100 and 125 MHz for ^{13}C, respectively. $CDCl_3$ was used as solvent and TMS as internal standard. EIMS were taken on a Micromass model Autospec (70 eV) spectrometer. HRESIMS was performed with a LCT Premier XE Micromass Waters spectrometer in positive ionization mode (Waters Corporation). Column chromatography (CC) was carried out with Si gel Fluka (cat. 60737) (40-63 μm), and column fractions were monitored by TLC (Si gel 60 F_{254}, 0.2 mm, Macherey Nagel (cat. 818-333)) by detection with a spraying reagent ($CH_3COOH/H_2O/H_2SO_4$; 80:16:4) followed by heating at 100 °C. Preparative TLC was carried out on Si gel 60 $PF_{254+366}$ (20 × 20 cm, 1 mm thickness, Analtech (cat. 02014)).

Plant material

Aerial parts of *Centaurea omphalotricha* (Coss. & Durieu ex Batt.) Willk. were collected from the Daya of Mogheul near Bechar in southwest Algeria (32.0192 N and 2.22 W) in April 2010 and identified by Professor M. Kabeche of University of Setif and M. Benabdelhakem from the National Agency of Preservation of Natural

Resources of Bechar. A voucher specimen (COB N. 175-2010) has been deposited in the Herbarium of Constantine University.

Extraction and isolation

Air-dried aerial parts (2604 g) of *C. omphalotricha* (Asteraceae) were powdered and macerated at room temperature with EtOH/H_2O (80:20 v/v) for 48 h, three times. After filtration, the filtrate was concentrated and suspended in H_2O (800 mL). The residue was extracted successively with $CHCl_3$, EtOAc and *n*-butanol. The organic phases were dried with Na_2SO_4, filtered using common filter paper and concentrated in vacuum at 25 ºC to obtain the following extracts: $CHCl_3$ (10.0 g), EtOAc (9.8 g) and *n*-butanol (41.8 g). The $CHCl_3$ extract was fractionated by column chromatography (CC) (Si gel; petroleum ether/EtOAc with increasing polarity; 100 × 7 cm) to yield 25 fractions (1-25). Fraction 22 (petroleum ether/EtOAc, 65:35 and 45:55; 1.3 g) was subjected to Si gel CC (CH_2Cl_2/MeOH; 100 × 1.5 cm) with increasing polarity to give 8 subfractions. Subfraction 1 (CH_2Cl_2; 220 mg) was purified by preparative TLC (*n*-hexane/Et_2O, 2:4, three elutions) to give dehydrovomifoliol (2.2 mg); subfraction 4 (CH_2Cl_2/MeOH, 96:4; 413.6 mg) was rechromatographed on a Si gel column (CH_2Cl_2/acetone; 50 × 1.5 cm) with increasing polarity to give 22 subfractions (sub1-sub 22). Sub 6 (CH_2Cl_2/acetone, 98:2; 8.2 mg) was purified by preparative TLC (CH_2Cl_2/EtOAc/acetone; 50:10:4) to afford compound **7** (4.2 mg); sub 9 (CH_2Cl_2/acetone, 96:4; 14.5 mg) was submitted to preparative TLC (Et_2O/*n*-hexane, 4:1, three elutions) to give the new compound **3** (1.8 mg) and **5** (1.3 mg); sub 10 (CH_2Cl_2/acetone, 96:4; 16.9 mg) gave after purification by preparative TLC (*n*-hexane/Et_2O, 1:5, four elutions) 3-hydroxy-5,6-epoxy-ionone (3.8 mg); sub14 (CH_2Cl_2/acetone, 90:10; 16.3 mg) was rechromatographed by preparative TLC (*n*-hexane/Et_2O, 1:5, four elutions) to afford compound **4** (3 mg).

Fraction 23 (petroleum ether/EtOAc, 40:60; 720 mg) which was purified by Si gel TLC on preparative plates (CH_2Cl_2/MeOH/AcOH; 80:9:1, one elution) gave in order of increasing polarity compound **9** (15.1 mg) and a mixture which was purified by TLC (CH_2Cl_2/MeOH/H_2O, 10:1:0.1, one elution) yielding compound **8** (2.1 mg). Fraction 24 (petroleum ether/EtOAc 20:80; 1270 mg) was chromatographed on a Si gel CC (CH_2Cl_2/isopropanol; 100 × 1.5 cm) with increasing polarity to give 26 subfractions. Subfraction 8 (CH_2Cl_2/isopropanol, 96:4; 113.3 mg) was submitted to preparative TLC (*n*-hexane/Et_2O, 1:2 five elutions) to afford the new product **1** (1.3 mg) and a mixture of compounds which was purified by preparative TLC (CH_2Cl_2/MeOH, 92:8, two elutions) to yield the new product **2** (4 mg) and circimaritin (3.4 mg). Subfraction 9 (CH_2Cl_2/isopropanol, 96:4; 87.7 mg) was submitted to preparative TLC (*n*-hexane/Et_2O, 1:2, three elutions) to yield **6** (4.6 mg). Subfraction 14 (CH_2Cl_2/isopropanol, 96:4; 14 mg) was a pure compound **10**.

A part of the EtOAc extract (5.8 g) was chromatographed on a Si gel CC ($CHCl_3$/acetone, 100 × 7 cm) with increasing polarity to give 8 fractions. Fraction 8 ($CHCl_3$/acetone, 50:50 to 100% acetone; 421.2 mg) was rechromatographed on a Si gel CC (CH_2Cl_2/EtOAc; 120 × 1.5 cm) with increasing polarity to give 13 subfractions. Subfraction 5 (CH_2Cl_2/EtOAc, 80:20; 11 mg) afforded pure apigenin (10.2 mg). Subfraction 7 (CH_2Cl_2/EtOAc, 75:25; 21.8 mg) was rechromatographed by TLC using CH_2Cl_2/MeOH/H_2O (100:10:0.1) as mobile phase to yield luteolin (11.3 mg).

4'-Acetyl cynaropicrin (**1**)

Amorphous solid; $[\alpha]_D^{25}$ = + 94 (c 0.026, $CHCl_3$); IR (NaCl) ν_{max}/cm^{-1}: 3478, 2939, 1755, 1740, 1729, 1642, 1450, 1373, 1267, 1152, 1048, 1031, 961; ^{1}H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; HRESIMS positive ion *m/z* 411.1428 $[M + Na]^+$ (Calc. for $C_{21}H_{24}O_7Na$, 411.1420).

4'-Acetyl cebellin F (**2**)

Amorphous solid; $[\alpha]_D^{25}$ = + 81 (c 0.018, $CHCl_3$); IR (NaCl) ν_{max}/cm^{-1}: 3481, 2937, 1751, 1729, 1653, 1374, 1231, 1135, 1030, 910; ^{1}H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 2; HRESIMS positive ion *m/z* 425.1578 $[M + Na]^+$ (Calc. for $C_{22}H_{26}O_7Na$, 425.1576).

15-Acetyl dehydromelitensin (**3**)

Amorphous solid; $[\alpha]_D^{25}$ = + 60 (c 0.009, $CHCl_3$); IR (NaCl) ν_{max}/cm^{-1}: 3420, 2935, 1751, 1734, 1727, 1651, 1384, 1239, 1137, 1051, 910; ^{1}H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; HRESIMS positive ion *m/z* 329.1360 $[M + Na]^+$, (Calc. for $C_{17}H_{22}O_5Na$, 329.1365).

Cell culture and cytotoxicity assays

The human leukemia HL-60 and U937 cells (DSMZ, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) were grown in RPMI 1640 containing 2 mmol L^{-1} L-glutamine supplemented with 10% (v/v) heat-inactivated fetal bovine serum as previously described.[19]

Stock solutions of 25 mmol L^{-1} sesquiterpene lactones were made in dimethyl sulfoxide (DMSO) and further

dilutions were made in culture media just before use. In all experiments, the final concentration of DMSO did not exceed 0.4% (v/v), a concentration which is non toxic to the cells. The cytotoxicity of sesquiterpene lactones on human tumor cells was analyzed by colorimetric 3-[4,5-dimethylthiazol-2-yl-]2,5-diphenyl tetrazolium bromide (MTT) assay as previously described.[20] Concentrations inducing a 50% inhibition of cell growth (IC_{50}) were determined graphically using the curve fitting algorithm of the computer software Prism 4.0 (GraphPad). Values are means ± SE from at least three independent experiments, each performed in triplicate. The antitumor agent etoposide was used as a positive control in both HL-60 (IC_{50} = 0.4 ± 0.1 μmol L^{-1}) and U937 cells (IC_{50} = 1.4 ± 0.3 μmol L^{-1}).

Results and Discussion

Compound **1** was obtained as an amorphous solid. HRESIMS experiments indicated the molecular formula $C_{21}H_{24}O_7$ (calc. for $[M + Na]^+$ 411.1420; found 411.1428). The IR spectra exhibited absorption bands for OH groups (3478 cm^{-1}) and for carbonyl groups (1755 (α,β-unsaturated-γ-lactone), 1740 and 1729 (ester carbonyls) cm^{-1}). The presence of these groups was confirmed by the ^1H (Table 1) and ^{13}C NMR spectra (Table 2). The ^1H NMR of **1** showed the presence of eight olefinic methylene protons at δ_H 5.65 (d, 1H, *J* 3.1 Hz, H-13a), 6.27 (d, 1H, *J* 3.5 Hz , H-13b), 4.98 (s, 1H, H-14a), 5.19 (s, 1H, H-14b), 5.41 (t, 1H, *J* 1.5 Hz, H-15a), 5.53 (t, 1H, *J* 1.5 Hz, H-15b), 6.00 (s, 1H, H-3'a) and 6.49 (s, 1H, H-3'b), one acetyl group at δ_H 2.14 (s, 3H, OAc), three oxygenated methines at δ_H 4.29 (dd, 1H, *J* 10.5, 9.0 Hz, H-6), 4.60 (tt, 1H, *J* 7.0, 1.5 Hz, H-3) and 5.19 (m, 1H, H-8) and one oxygenated methylene at δ_H 4.88 (s, 2H, H_2-4').The connectivities were established by analysis of its COSY spectrum. The ^{13}C NMR (Table 2) and DEPT data indicated the presence of one carbonyl group corresponding to a γ-lactone, two ester carbonyl groups, three aliphatic methylene, eight olefinic, one methyl (acetate), and six methine carbons. Both the ^1H and ^{13}C NMR spectral data of compound **1** were close to those of cynaropicrin,[16] with the exception of an extra acetyl group in compound **1**, which was assigned by a HMBC experiment. Thus, the correlation observed between the signal at δ_H 4.88 (s, 2H, H_2-4') and δ_C 170.3 (OAc), allowed us to confirm the position of the acetoxy group at the C-4' position. Therefore, the structure of **1** was elucidated as 4'-acetyl cynaropicrin.

Compound **2** was obtained as an amorphous solid. The ^1H NMR data (Table 1) of **2** were very similar to those

Table 1. ^1H NMR data of compounds **1**-**3** in $CDCl_3$ (δ in ppm and *J* in Hz)

Proton	**1**	**2**	**3**
	δ_H	δ_H	δ_H
1	3.02 (*ddd*, 10.5, 9.0, 7.5)	3.03 (*ddd*, 11.0, 10.5, 7.5)	5.82 (*dd*, 17.4, 10.7)
2a	1.75 (*ddd*, 14.5, 10.5, 7.0)	1.77 (*ddd*, 13.0, 10.5,7.5)	5.05 (*d*, 10.7)
2b	2.22-2.27 (*m*)	2.28 (*dt*, 13.0, 7.5)	5.11 (*d*, 17.4)
3a 3b	4.60 (*tt*, 7.0, 1.5)	4.61 (*tt*, 7.5, 1.5)	5.05 (*s*) 5.45 (*s*)
5	2.89 (*tt*, 9.0, 1.5)	2.89 (*tt,* 11.0, 1.5)	2.46 (*d*, 11.4)
6	4.29 (*dd*, 10.5, 9.0)	4.28 (*dd*, 11.0, 9.0)	4.16 (*t*, 11.4)
7	3.20-3.25 (*m*)	3.21-3.26 (*m*)	2.67 (*tt*, 11.4, 3.0)
8	5.19 (*m*, overlapped)	5.16 (*ddd*, 9.3, 5.1, 4.0)	4.15 (*td*, 11.4, 4.1)
9a	2.44 (*dd*, 14.5, 3.5)	2.41 (*dd*, 14.6, 4.0)	1.70 (*dd*, 13.1, 11.4)
9b	2.75 (*dd*, 14.5, 5.0)	2.74 (*dd*, 14.6, 5.1)	1.91 (*dd*, 13.1, 4.1)
13a	5.65 (*d*, 3.1)	5.62 (*d*, 3.1)	6.03 (*d*, 3.0)
13b	6.27 (*d*, 3.5)	6.27 (*d*, 3.5)	6.22 (*d*, 3.0)
14a 14b	4.98 (*s*) 5.19 (*s*)	4.98 (*s*) 5.19 (*s*)	1.14 (*s*)
15a	5.41 (*t*, 1.5)	5.41 (*t*, 1.5)	4.52 (*d*, 13.6)
15b	5.53 (*t*, 1.5)	5.55 (*t*, 1.5)	4.57 (*d*, 13.6)
3'a 3'b	6.00 (*s*) 6.49 (*s*)	6.85 (*tq*, 6.1, 1.2)	—
4'	4.88 (*s*)	4.83 (*d*, 6.1)	—
5'	—	1.98 (*d*, 1.2)	—
OAc	2.14 (*s*)	2.15 (*s*)	2.13 (*s*)

of **1**, suggesting that both compounds are closely related in structure. The major variation is that compound **2** has a different acyl group. A detailed comparison of the ^{1}H (Table 1) and ^{13}C NMR (Table 2) signals of **2** and **1** revealed an extra carbon signal in ^{13}C NMR, besides the presence of a methyl group at δ_H 1.98 (d, 3H, *J* 1.2 Hz, H_3-5') and a vinylic proton signal at δ_H 6.85 (tq, 1H, *J* 6.1, 1.2 Hz, H-3') in ^{1}H NMR suggesting that the substituent group in **2** is the 4-acetoxy-2-methyl butenoyl. The relative stereochemistry of the double bond in the acyl group was determined as *E* based on the observed correlation between the signals at δ_H 4.83 and 1.98 in the ROESY spectrum. The combination of all the above data and the HRESIMS experiment led us to assign the structure of **2** as 4'-acetyl cebellin F.

Table 2. ^{13}C NMR data of compounds **1**-**3** in $CDCl_3$ (δ in ppm)

Position	1	2	3
	δ_C	δ_C	δ_C
1	45.4	45.4	146.0
2	39.1	39.1	113.0
3	73.8	73.9	116.9
4	152.3	152.3	138.7
5	51.4	51.4	51.5
6	78.4	78.6	78.3
7	46.7	47.9	55.1
8	74.6	74.5	67.5
9	37.1	37.3	49.8
10	141.7	141.8	42.0
11	137.4	137.4	137.4
12	168.9	169.0	169.7
13	122.5	122.1	120.5
14	118.2	118.1	19.0
15	113.6	113.6	67.2
1'	164.3	166.1	
2'	135.2	130.2	
3'	129.1	136.4	
4'	62.3	61.0	
5'		12.9	
OAc	20.8 170.3	20.8 170.7	21.0 170.6

The HREIMS and ^{13}C NMR data of **3** indicated the molecular formula $C_{17}H_{22}O_5$. The IR spectrum of this compound showed the presence of hydroxyl groups (3420 cm^{-1}), and carbonyl groups (1751, 1734, 1727 cm^{-1}). The ^{1}H NMR (Table 1) spectrum of **3** exhibited the presence of one methyl group at δ_H 1.14 (s, 3H, CH_3-14), one acetyl methyl group at 2.13 (s, 3H, OAc), one vinylic proton at 5.82 (dd, 1H, *J* 17.4, 10.7 Hz, H-1), and six olefinic methylene protons at 5.05 (d, 1H, *J* 10.7 Hz, H-2a), 5.11 (d, 1H, *J* 17.4 Hz, H-2b), 5.05 (s, 1H, H-3a), 5.45 (s, 1H, H-3b), 6.03 (d, 1H, *J* 3.0 Hz, H-13a), 6.22 (d, 1H, *J* 3.0 Hz, H-13b), typical of a 1,3,11(13)-elematrien-6,12-olide.[21,22] The relationships between the proton signals in **3** were established from the ^{1}H-^{1}H COSY spectrum, which disclosed the following connectivities: H-1 with H_2-2, H-5 with H-6, H-13 with H-7, H-8 with H_2-9. The ^{13}C NMR (Table 2) and DEPT spectral data indicated the presence of two carbonyl groups, corresponding to a γ-lactone, and an acetoxy group, six olefinic carbons, two methyl groups, two aliphatic methylene carbons, one of them oxygenated, four methine carbons, two of them oxygenated and one quaternary carbon. These assignments were similar to those of the known elemanolide dehydromelitensin.[22] However, a hydroxyl group was replaced by an acetoxy group, the location of this group in **3** being confirmed by the low shift position (+ 0.5 ppm) of the protons H_2-15 at δ_H 4.52 (d, 1H, *J* 13.6 Hz, H-15a) and 4.57 (d, 1H, *J* 13.6 Hz, H-15b) in comparison with the ^{1}H NMR dehydromelitensin data,[22] characteristic of the presence of an acetyl group in a primary hydroxyl. The structure of compound **3** was assigned and confirmed using HMBC, HSQC and ROESY data as 15-acetyl dehydromelitensin.

Sesquiterpene lactones have attracted much attention during the last three decades, because they display a wide range of biological activities, including antitumor and anti-inflammatory properties.[8,23] The structural requirement for the biological activities of these compounds is associated with α-methylene-γ-butyrolactone moiety, which acts as alkylating agent in a Michael-type reaction with nucleophiles.[24] Thus, sesquiterpene lactones are believed to exert their numerous biological activities through inhibition of enzymes and other functional proteins by forming covalent bonds with free cysteine residues in these macromolecules or by conjugation with glutathione.[25,26]

Previous studies have shown that sesquiterpene lactones display cytotoxic properties in tumor cells[8] and that the sesquiterpene lactone of the guaianolide type cynaropicrin induces cytotoxicity in U937 and Jurkat T cell lines.[27] A quantitative structure-activity relationships (QSAR) study including four different skeletons of sesquiterpene lactones revealed the most active among the guaianolides and pseudoguainolides, and steric properties and electronic features as the most important descriptors.[23] However, antiproliferative studies of the naturally occurring sesquiterpene lactones described in this paper in human leukemia cells have not yet been assessed.

Sesquiterpene lactones **1**-**6** were found to inhibit the growth and cell viability of HL-60 and U937 cells in culture as determined by the 3-[4,5-dimethylthiazol-2-yl]2,5-

diphenyl tetrazolium bromide (MTT) dye-reduction assay (Table 3). In contrast, the sesquiterpene lactone **7** is not an effective antiproliferative agent showing an IC_{50} value higher than 100 μmol L^{-1} in leukemia cells, in accordance with the absence of the alkylating group, the exocyclic conjugated double bond.

Table 3. Effects of compounds **1**-**7** on the growth of the human leukemia cell lines

Compound	IC_{50} / (μmol L^{-1})	
	HL-60	U937
1	5.1 ± 0.4	10.8 ± 4.1
2	7.0 ± 1.9	10.4 ± 1.9
3	25.8 ± 3.6	29.9 ± 4.4
4	5.4 ± 1.3	6.8 ± 2.2
5	2.0 ± 0.9	3.1 ± 0.6
6	5.6 ± 2.1	11.7 ± 1.5
7	> 100	> 100

Cells were cultured for 72 h and the IC_{50} values were calculated as described in the Experimental section. The data shown represent the means ± SEM of 3-5 independent experiments with three determinations in each.

Among the different sesquiterpene lactones, the presence of the exocyclic, conjugated double bond is essential for the cytotoxic activity against HL-60 and U937 cells. Compounds **1**, **2**, **4**, **5** and **6** displayed similar potency in both cell lines. The potency of these sesquiterpene lactones might be explained by their lipophilicity. However, other factors, such as molecular geometry and the chemical environment of the target sulfhydryl may also influence the activity of sesquiterpene lactones. All these guaianolides contain an ester functional group at C-8. The sesquiterpene lactone 8-(4-hydroxymethacrylate)-dehydromelitensin **6** also contains an ester near from the exocyclic methylene bond. The presence of this group appears to be important, since compound **3** (15-acetyl dehydromelintensin) - which does not contain this functional group - was less cytotoxic than **6**.

In conclusion, the Algerian plant *Centaurea omphalotricha* has been chemically studied for the first time, and three new sesquiterpene lactones have been identified along with twelve known compounds. The naturally occurring sesquiterpene lactones evaluated in the present study were strongly cytotoxic against human leukemia cell lines and the results of the present study may lead to the discovery of new and highly specific antitumor agents against leukemia cells.

Supplementary Information

Spectra of compounds **1**-**3** are available free of charge at http://jbcs.sbq.org.br as PDF file.

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