Phytochemistry 69 (2008) 2984–2992

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Chemical characterisation of the terpenoid constituents of the Algerian plant *Launaea arborescens*

Fatma Bitam [a,b], M. Letizia Ciavatta [a], Emiliano Manzo [a], Ammar Dibi [b], Margherita Gavagnin [a,*]

[a] Istituto di Chimica Biomolecolare, C.N.R., Via Campi Flegrei 34, I-80078 Pozzuoli, Naples, Italy
[b] Faculté des Sciences, Département de Chimie, Université de Batna, Batna 05000, Algeria

ARTICLE INFO

Article history:
Received 30 July 2008
Received in revised form 29 September 2008
Available online 12 November 2008

Keywords:
Launaea arborescens
Oleanane triterpenoids
Ursane triterpenoids
Germacrane sesquiterpenoids
Guaiane sesquiterpenoids
Eudesmane sesquiterpenoids

ABSTRACT

Chemical investigation of endemic Algerian plant *Launaea arborescens* resulted in the isolation of a series of triterpenes and sesquiterpenes from both the aerial parts and roots. Five terpenoids have been chemically characterised by means of spectral methods mainly NMR techniques. In addition, the absolute stereochemistry at the chiral carbon in the side chain of 8-deoxy-15-(3′-hydroxy-2′-methyl-propanoyl)-lactucin-3′-sulfate (**6**) has been determined by comparison of the ^{1}H NMR spectra of Mosher derivatives of **6** with those of the corresponding MTPA esters of model compounds.

1. Introduction

The genus *Launaea* belongs to the tribe Lactuceae of the Asteraceae family and contains about 40 species, most of which are adapted to dry, saline and sandy habits (Ozenda, 2004). In the flora of Algeria, five of the nine *Launaea* species present are endemic of North Africa and include *Launaea arborescens* (Batt.) Murb., which is a perennial medicinal shrub mainly distributed in the Southwest part of the country (Quezel and Santa, 1963; Ozenda, 2004). Despite to the pharmacological interest in this plant (local name "Oum Lbina") commonly used in the North African popular medicine against diarrhoea and abdominal spasms, a very few chemical studies on *L. arborescens* have been so far reported. To the best of our knowledge, only flavonoid (Mansour et al., 1983; Belboukhari and Cheriti, 2006), phenolic (Giner et al., 1992) and essential oil (Cheriti et al., 2006) constituents have been described in the literature. In addition, very interesting antifungal, antibacterial and insecticidal activities have been reported for the methanol extract of the plant (Belboukhari and Cheriti, 2006; Jbilou et al., 2008).

We describe here the results of our chemical investigation on the liposoluble extracts of both roots and aerial parts of Algerian specimens of *L. arborescens* that led to the finding of a plethora of sesquiterpenes and triterpenes, which were found to be differently distributed in both parts. Among these compounds, five unprecedented terpenes, 3β-hydroxy-11α-ethoxy-olean-12-ene (**1**), 9α-hydroxy-11β,13-dihydro-3-epi-zaluzanin C (**2**), 9α-hydroxy-4α,15-dihydro-zaluzanin C (**3**), 3β,14-dihydroxycostunolide-3-*O*-β-glycopyranoside (**4**), and 3β,14-dihydroxycostunolide-3-*O*-β-glucopyranosyl-14-*O*-*p*-hydroxyphenylacetate (**5**), were isolated and fully characterised by spectral methods, mainly NMR techniques. Biological evaluation of pure novel compounds **1**–**5** against gram positive and gram negative bacteria was also carried out.

Finally, a stereochemical analysis was conducted on 8-deoxy-15-(3′-hydroxy-2′-methyl-propanoyl)-lactucin-3′-sulfate (**6**) (Zidorn et al., 2007), which was the main component of sesquiterpene fraction, with the aim at assigning the absolute configuration of the chiral centre in the 3′-hydroxy-2′-methyl-propanoyl fragment of the molecule, not previously determined.

2. Results and discussion

The whole plants of *L. arborescens* were collected in Bechar (Algeria) during flowering in 2006. The aerial parts of the plants were separated from the roots and both were allowed to dry before the extraction. The TLC chromatographic analysis of the liposoluble extracts obtained from both parts of the plant showed very complex and substantially distinct terpenoid secondary metabolite patterns even though some analogies were also observed. The extracts were submitted to subsequent chromatographic steps (see experimental) and the terpene-containing fractions were further purified by HPLC to obtain 27 pure metabolites, five of which were novel compounds.

* Corresponding author. Tel.: +39 0818675096; fax: +39 0818041770.
E-mail address: margherita.gavagnin@icb.cnr.it (M. Gavagnin).

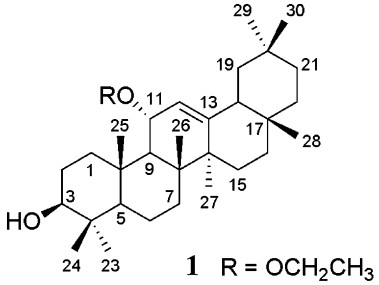
0031-9422/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2008.09.025

In particular, the terpenoid fraction of the aerial parts extract was found to be dominated by triterpene lupeol (**7**) (Reynolds et al., 1986) (2.9 g, 25% of the extract) co-occurring with a series of oleanane and ursane triterpenes including the novel 3β-hydroxy-11α-ethoxy-olean-12-ene (**1**) and the known: 3-β-hydroxy-11-oxo-urs-12-ene (**8**) and 3-β-hydroxy-11-oxo-olean-12-ene (**9**) (Bandaranayake, 1980), taraxast-20-ene-3β,30-diol (**10**) (Dai et al., 2001; Kisiel and Zielinska, 2001a), oleana-9(11):12-dien-3β-ol **(11)** (Tanaka and Matsunaga, 1988), 3β-hydroxy-11α-ethoxyurs-12-ene (**12**) (Fujita et al., 2000), ursa-9(11):12-dien-3β-ol (**13**) (Matsunaga et al., 1988), 3β-11α-dihydroxy-olean-12-ene (**14**) (Xiao et al., 1994), 3β-hydroxy-11α-methoxyolean-12-ene (**15**) (Fujita et al., 2000), 3β-hydroxy-11α-methoxyurs-12-ene (**16**) and 3β-11α-dihydroxy-urs-12-ene (**17**) (Bohlmann et al., 1984). Stigmasterol (**18**) (Kojima et al., 1990), was also isolated as major sterol component of the plant extract.

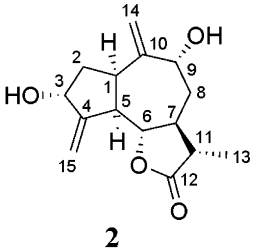
On the other side the terpenoid fraction of the ethyl acetate extract of the roots was characterised by a series of sesquiterpene metabolites exhibiting eudesmane, guaiane and germacrane skeletons, the main of which was 8-deoxy-15-(3′-hydroxy-2′-methyl-propanoyl)-lactucin-3′-sulfate (**6**) (Zidorn et al., 2007). Four unprecedented sesquiterpenes, 9α-hydroxy-11β,13-dihydro-3-epi-zaluzanin C (**2**), 9α-hydroxy-4α,15-dihydrozaluzanin C (**3**), 3β,14-dihydroxycostunolide-3-*O*-β-glycopyranoside (**4**), and 3β,14-dihydroxycostunolide-3-*O*-β-glucopyranosyl-14-*O*-*p*-hydroxy-phenylacetate (**5**) were isolated along with known magnolialide (**19**) (El-Feraly et al., 1979; Kisiel and Zielinska, 2001a, b), 1β,8α-dihydroxy-eudesm-4-en-6β,7α,11βH-6-olide (**20**) (Marco, 1989), 11β,13-dihydrolactucin **21** (Sarg et al., 1982), picriside C (**22**) (Nishimura et al., 1986a; Miyase and Fukushima, 1987), sonchuside A **23** (Miyase and Fukushima, 1987), picriside B (**24**) (Nishimura et al., 1986a), ixerisoside D (**25**) (Warashina et al., 1990), crepidiaside A (**26**) (Adegawa et al., 1985) and macrocliniside A (**27**) (Miyase et al., 1984; Kisiel and Gromek, 1993). In addition, few amounts of triterpenes containing lupeol as main component were also detected in the ethyl acetate extract of the roots.

The known metabolites were identified by comparison of their spectral data (^{1}H NMR and MS spectra) with the literature. The structures of triterpene **1** and sesquiterpenes **2**–**5** were determined as described below. In particular, triterpene **1** displayed an oleanane skeleton, sesquiterpenes **2** and **3** exhibited a dihydroxylated guaianolide skeleton and were both related to zaluzanin C (Romo et al., 1967; Spring et al., 1995) whereas sesquiterpenes **4** and **5** were characterised by a glucosylated germacranolide framework structurally related to that of the co-occurring picriside C (**22**) (Nishimura et al., 1986a; Miyase and Fukushima, 1987).

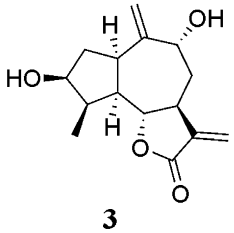
The molecular formula of 3β-hydroxy-11α-ethoxy-olean-12-ene (**1**) was $C_{32}H_{54}O_2$ as deduced by both mass spectrum and ^{13}C NMR spectra. Analysis of the ^{1}H NMR spectrum of compound **1** (Table 1) immediately revealed the close structural relationship with



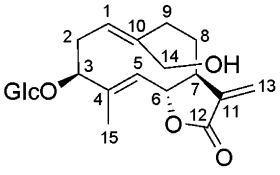
1 R = OCH_2CH_3
14 R = OH
15 R = OCH_3



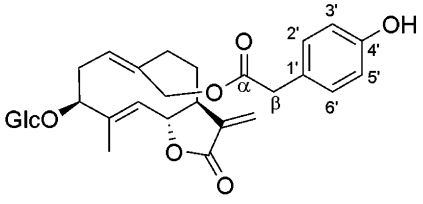
2



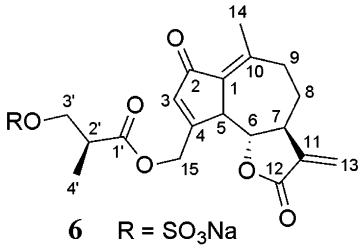
3



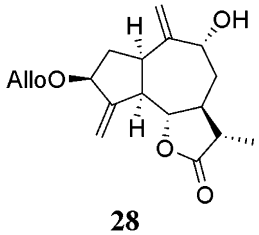
4



5



6 R = SO_3Na
6a R = H
6b R = Ac
6c R = *S*-MTPA
6d R = *R*-MTPA



28

Table 1
^{1}H and ^{13}C NMR data[a,b] of compound **1** in $CDCl_3$.

Position	δ c (*m*)	δ H (*m*, *J* Hz)	HMBC[c] (C to H)
1	39.4 (*t*)	1.75 (*m*)	H-25
		2.02 (*m*)	
2	27.5 (*t*)	1.62 (*m*)	
3	78.6 (*d*)	3.23 (*m*)	H-23, H-24
4	38.7 (*s*)		H-23, H-24
5	55.2 (*d*)	0.77 (*m*)	H-23, H-24, H-25
6	18.4 (*t*)	1.37 (*m*)	
		1.42 (*m*)	
7	33.3 (*t*)	1.57 (*m*)	
		1.73 (*m*)	
8	42.6 (*s*)		H-9, H-27
9	51.5 (*d*)	1.72 (*d*, 9.0)	H-25, H-26
10	38.3 (*s*)		H-9, H-25
11	74.8 (*d*)	3.94 (*dd*, 9.4,3.3)	H-9
12	122.7 (*d*)	5.31 (*d*, 3.3)	H-11
13	148.9 (*s*)		H-11, H-27
14	41.8 (*s*)		H-9, H-12, H-27
15	26.3 (*t*)	1.70 (*m*)	H-27
		2.02 (*m*)	
16	26.8 (*t*)	1.61 (*m*)	H-28
17	32.3 (*s*)		H-28
18	46.9 (*d*)	1.99 (*m*)	H-12, H-28
19	46.5 (*t*)	1.05 (*m*)	H-29, H-30
		1.64 (*m*)	
20	31.1 (*s*)		H-29, H-30
21	34.7 (*t*)	1.11 (*m*)	H-29, H-30
22	37.0 (*t*)	2.02 (*m*)	
23	28.2 (*q*)	0.99 (*s*)	H-3, H-24
24	15.6 (*q*)	0.80 (*s*)	H-3, H-23
25	15.9 (*q*)	1.04 (*s*)	H-9
26	18.2 (*q*)	0.99 (*s*)	H-7
27	25.3 (*q*)	1.20 (*s*)	
28	28.5 (*q*)	0.83 (*s*)	
29	33.6 (*q*)	0.89 (*s*)	H-30
30	23.7 (*q*)	0.88 (*s*)	H-29
Ethoxy group			
OCH_2	61.4 (*t*)	3.31 (*dq*, 8.7,7.2)	CH_3, H-11
		3.55 (*dq*, 8.7,7.2)	
CH_3	16.8 (*q*)	1.14 (*t*, 7.2)	

[a] Bruker DRX 600 spectrometer in $CDCl_3$, chemical shifts (ppm) referred to $CHCl_3$(δ 7.26) and to $CDCl_3$ (δ 77.0).
[b] Assignments made by ^{1}H–^{1}H COSY and HSQC experiments.
[c] Significant HMBC correlations (*J* = 10 Hz).

the co-occurring 3β,11α-dihydroxy-olean-12-ene (**14**) and 3β-hydroxy-11α-methoxy-olean-12-ene (**15**), suggesting the presence of the same functionalised triterpenoid oleanane skeleton. In fact, eight methyl singlets [δ 0.80 (H_3-24), 0.83 (H_3-28), 0.88 (H_3-30), 0.89 (H_3-29), 0.99 (6H, H_3-23 and H_3-26), 1.04 (H_3-25), and 1.20 (H_3-27)] were observed in the proton spectrum of **1** that also contained two methine signals at δ 3.23 (*m*, H-3) and 3.94 (*dd*, *J* = 9.4 and 3.3 Hz, H-11) and the olefinic proton at δ 5.31 (1H, *d*, *J* = 3.3 Hz, H-12), similarly to compounds **12** and **14** (Fujita et al., 2000; Xiao et al., 1994), both isolated from the extract of the plant. The presence in compound **1** of an ethoxy group replacing the hydroxyl and the methoxyl function in **14** and in **15**, respectively, was indicated by both two double quartet at δ 3.31 and 3.55 (each 1 H, *dq*, *J* = 8.7 and 7.2 Hz, CH_3CH_2O–) and the 3H triplet at δ 1.14 (*J* = 7.2 Hz, CH_3CH_2O–). According to the proposed structure, in the ^{13}C NMR spectrum of **1** two additional signals due to the ethoxy moiety at δ 61.4 (CH_3CH_2O–) and 16.8 (CH_3CH_2O–) were observed along with the typical values of the oleanane skeleton (Table 1).

The relative stereochemistry at C-11 was suggested by analysis of the coupling constants of H-11 resonating as a double doublet (*J* = 9.4 and 3.3 Hz) in agreement with a β-orientation, analogously with the data reported for the corresponding methoxyl (Fujita et al., 2000) and hydroxyl (Bohlmann et al., 1984; Xiao et al., 1994) derivatives. A detailed 2D NMR analysis (^{1}H–^{1}H COSY, HSQC and HMBC) allowed the assignment of all carbon and proton values as reported in Table 1. Compound **1** underwent rapidly to an elimination reaction of the ethanol residue at C-11 to give the corresponding conjugated diene **11** (Tanaka and Matsunaga, 1988), also isolated from the extract. This conversion was also observed for **14** and **15**, thus suggesting that 3β-hydroxy-olean-9(11):12-diene (**11**) found in the extract is most likely a work-up derivative.

9α-Hydroxy-3-epi-11β,13-dihydrozaluzanin C (**2**) had the molecular formula $C_{15}H_{20}O_4$ as deduced by both ESIMS and ^{13}C NMR spectra. Analysis of the ^{1}H and ^{13}C NMR spectra (Table 2) of **2** indicated the presence of a guaianolide sesquiterpene skeleton the same as the co-occurring known metabolites **21**–**23**, **26** and **27**. Accordingly, two exomethylene groups [δ 4.81 (1H, br *s*, H-14a), 5.08 (1H, br *s*, H-14b), 5.38 (1H, br *s*, H-15a), and 5.46 (1H, br *s*, H-15b)], three oxymethine protons [δ 4.58 (1H, sharp *m*, H-9), 4.71 (1H, *m*, H-3) and δ 3.81 (1H, *t*, *J* = 9.7, H-6)], and a second-

Table 2
^{1}H and ^{13}C NMR data[a,b] of compounds **2** and **3** in $CDCl_3$.

Position	**2**			**3**		
	δ C (*m*)	δ H (*m*, *J* Hz)	HMBC[c] (C to H)	δ C (*m*)	δ H (*m*, *J* Hz)	HMBC[c] (C to H)
1	35.6 (*d*)	3.58 (*ddd*, 12.3,8.7,4.1)	H-5, H-14a, H-14b	32.5 (*d*)	3.32 (*q*, 10.1)	H-2a, H-14a, H-14b
2	39.4 (*t*)	2.20 (*m*)		3.41 (t)	2.00 (*m*)	H-l, H-4, H-14a
		1.95 (*m*)			2.00 (*m*)	
3	74.7 (*d*)	4.71 (*m*)	H-15a, H-15b, H-2a	74.1 (*d*)	4.30 (*m*)	H-2a, H-15a, H-15b
4	154.0 (s)		H-5, H-6	40.2 (*d*)	2.35 (*m*)	H-15
5	49.9 (*d*)	3.10 (*t*, 9.7)	H-15a, H-15b	46.9 (*d*)	2.35 (*m*)	H-1, H-2, H-13
6	84.9 (*d*)	3.81 (*t*, 9.7)	H-5	82.9 (*d*)	4.10 (*t*, 8.1)	H-4, H-5
7	44.3 (*d*)	2.32 (*m*)	H-11, H-13	41.0 (*d*)	3.15 (*m*)	H-13a, H-13b
8	39.7 (*t*)	1.50 (*m*)	H-6, H-11	38.6 (*t*)	1.60 (*m*)	H-6
		2.23 (*m*)			2.35 (*m*)	
9	74.3 (*d*)	4.58 (*m*)	H-8a, H-14a, H-14b	74.1 (*d*)	4.72 (br *s*)	H-14a, H-14b
10	150.9 (*s*)		H-l, H-2a	149.8 (*s*)		H-l, H-2a, H-2b, H-14
11	41.6 (*d*)	2.18 (*m*)	H-6, H-13	139.3 (s)		H-13
12	178.4 (*s*)		H-13	169.8 (s)		H-13
13	13.6 (*q*)	1.26 (*d*,7.2)		120.1 (*t*)	5.50 (*d*, 3.5)	
					6.21 (*d*, 3.5)	
14	112.7 (*t*)	4.81 (br *s*)		112.7 (*t*)	5.12 (br *s*)	H-l
		5.08 (br *s*)			5.16 (br *s*)	
15	112.7 (*t*)	5.38 (br *s*)	H-3b	8.1 (*q*)	0.98 (*d*,7.1)	H-4, H-5
		5.46 (br *s*)				

[a] Bruker DRX 600 spectrometer in $CDCl_3$, chemical shifts (ppm) referred to $CHCl_3$ (δ 7.26) and to $CDCl_3$ (δ 77.0).
[b] Assignments made by ^{1}H–^{1}H COSY and HSQC experiments.
[c] Significant HMBC correlations (*J* = 10 Hz).

ary methyl [δ 1.26 (3H, *d*, *J* = 7.2, H_3-13)] were easily recognized by analysis of the ^{1}H NMR spectrum whereas the presence of a lactone functionality was suggested by the carbonyl signal at δ 178.4 in the ^{13}C NMR spectrum. Comparison of the NMR data in pyridine of **2** with those reported in the literature for related guaianolides (Asada et al., 1984; Kisiel and Michalska, 2002) showed a close structural similarity of **2** with 9α-hydroxy-11β,13-diyhdrozaluzanin C-3-*O*-β-allopyranoside (**28**) (Kisiel and Michalska, 2002), which is the aglycone of glucosides previously isolated from different genera of Asteraceae family. In particular, the spin-system sequence from C-1 to C-9 in **2** was the same as **28** whereas differences were observed in the carbon and proton chemical shifts of the 5-membered ring strongly suggesting the opposite stereochemistry at C-3. According to this suggestion, H-1 and H-5 values were downfield shifted [δ H-1 4.01, δ H-5 3.25 in **2**; δ H-1 3.65, δ H-5 2.91 in **28** (values in pyridine)] due to the α-oriented 3-OH group. The proposed relative stereochemical arrangement was further supported by a series of steric effects observed in the NOESY spectrum of **2**. Along with the expected correlations between α oriented H-1 and H-5, diagnostic cross-peaks were observed between H-2α and H-1, and between H-2β and both H-14 and H-3 according to the β-orientation of H-3. Analogously, H-9 showed correlations only with H-14b and both protons H_2-8 thus supporting a β-orientation. All carbon and proton resonances were assigned by 2D-NMR experiments (^{1}H–^{1}H COSY, HSQC and HMBC) as reported in Table 2.

The molecular formula $C_{15}H_{20}O_4$ of 9α-hydroxy-4α,15-dihydro zaluzanin C (**3**) was established by HRESIMS on the sodiated molecular peak at 264.1259 *m/z*. The spectral data of **3** (Table 2) indicated the same guaianolide framework as compound **2** exhibiting two exomethylene groups [δ C 112.7 (C-14), 120.1 (C-13), 139.3 (C-11), and 149.8 (C-10)]; δ H 5.12 (1H, br *s*, H-14a), 5.16 (1H, br *s*, H-14b), 5.50 (1H, *d*, *J* = 3.5, H-13a), and 6.21 (1H, *d*, *J* = 3.5, H-13b)], a secondary methyl [δ C 8.1 (C-15), δ H 0.98 (3H, *d*, *J* = 7.1 Hz, H_3-15)] and two secondary hydroxyl functions [δ C 74.1 (2C, C-3 and C-9), δ H 4.30 (1H, *m*, H-3) and 4.72 (1H, br *s*, H-9)]. Careful analysis of the ^{1}H–^{1}H COSY experiment led us to define all the proton sequence from H-1 to H-9 indicating that the two hydroxyl groups were located at C-3 and C-9 analogously with **2** whereas the secondary methyl was at C-4, and the exocyclic double bonds were at C-10 and C-11. A survey of the literature on guaianolides showed that **3** was structurally related to known 9α-hydroxy-4α,11β,13,15-tetrahydrozaluzanin C (Kisiel and Barszcz, 1996). In particular, strong similarities were observed in the proton spectra of the two molecules with regards to the perhydroazulene bicyclic portion clearly suggesting for this part the same substitution pattern including the relative stereochemistry. The only difference was the presence in **3** of an additional double bond in the lactone ring at C-11(13). The suggested relative stereochemistry was further supported by a NOESY experiment that showed diagnostic correlations between the α-oriented protons H-1 and H-3, and between the β-oriented H_3-15 and H-6. Expected NOE interactions between H-5 and both H-1 and H-7 according to the guaianolide skeleton with 1,5-*cis* and 6,7-*trans* junctions were also detected. Full assignment of proton and carbon values (Table 2) was made by means of detailed analysis of 2D–NMR experiments (^{1}H–^{1}H COSY, HSQC and HMBC).

The HRESIMS spectrum of 3β,14-dihydroxycostunolide-3-*O*-β-glycopyranoside (**4**) showed a sodiated molecular peak at 426.1788 *m/z* according to the molecular formula $C_{21}H_{30}O_9$. The ^{1}H and ^{13}C NMR spectra of **4** indicated the presence of the following structural features: two trisubstituted double bonds [δ C 126.6 (C-1), 127.3 (C-5), 141.5 (C-4), 135.2 (C-10); δ H 5.05 (1H, overlapped, H-1), 5.02 (1H, overlapped, H-5)], an exocyclic double bond conjugated to a lactone carbonyl [δ C 119.6 (*t*, C-13), 139.0 (*s*, C-11), 168.8 (*s*, C-12); δ H 5.50 (1H, *d*, *J* = 2.8 Hz, H-13a) and 6.32 (1H, *d*, *J* = 2.8 Hz, H-13b)], a vinyl methyl [δ H 1.95 (3H, *s*, H_3-15), a vinyl hydroxymethyl [δ H 4.10 (1H, *d*, *J* = 12.3 Hz, H-14a), δ H 4.47 (1H, *d*, *J* = 12.3 Hz, H-14b] and a secondary hydroxyl group connected to a glucopyranose moiety by a glycosyl linkage [δ H 3.90 (1H, *m*, H-5″), δ H 4.10 (1H, *m*, H-2″), δ H 4.25 (1H, *m*, H-3″), δ H 4.28 (1H, *m*, H-4″), δ H 4.42 (1H, *dd*, *J* = 12.1, 5.3 Hz, H-6″a), δ H 4.61 (1H, *dd*, *J* = 12.1, 4.8 Hz, H-6″b), δ H 4.83 (1H, *d*, *J* = 8.0 Hz, H-1″)]. The spectral data were reminiscent with those of the co-occurring picriside C (**22**) (Miyase and Fukushima, 1987) strongly suggesting the presence of the same germacranolide skeleton bearing a glucosylated hydroxyl function at C-3 and containing an additional hydroxyl group at C-14. The geometry of the two endocyclic double bonds at C-1 (10) and C-4 (5) was suggested *Z* and *E*, respectively, as reported in formula **4**, by analogy with **22** and further supported by diagnostic NOE effects observed between H_2-14 and H-2a and between H-6 and H_3-15, respectively. Analogously, the *trans*-junction at C-6/C-7 and the β-orientation of the *O*-glucosyl residue were suggested to be the same as picriside C. A detailed analysis of 2D NMR spectra supported the proposed structure and allowed the attribution of all carbon and proton resonances (Table 3). Our assignment was in agreement with those reported in the literature for related germacranolides exhibiting two hydroxyl groups at both C-3 and C-14 (Nishimura et al., 1986b; Kisiel and Barszcz, 1997; 1998).

Analysis of the spectral data of 3β,14-dihydroxycostunolide-3-*O*-β-glucopyranosyl-14-*O*-*p*-hydroxyphenylacetate (**5**) immediately revealed the close relationship with **4**. The molecular formula $C_{29}H_{36}O_{11}$ of **5**, deduced by HRESIMS spectrum on the sodiated molecular peak at *m/z* 560.2155 indicated the presence of an additional $C_8H_6O_2$ moiety with respect to **4**. The ^{1}H and ^{13}C NMR spectra of the two compounds were very similar according to the presence of the same germacranolide skeleton bearing the glucosylated hydroxyl function at C-3 and the hydroxyl group at C-14. Further three signals at δ 3.72 (2H, *d*, *J* = 3.5 Hz, CH_2β), 7.14 (2H, *d*, *J* = 8.2 Hz, H-3′ and H-5′), and 7.32 (2H, *d*, *J* = 8.2 Hz, H-2′ and H-6′) were observed in the proton spectrum of **5** suggesting that the molecule contained a *p*-hydroxy-phenyl acetic residue esterified to an OH group. Accordingly, the carbon spectrum contained additional signals at δ 170.4 (CO, Cα), 41.0 (*t*, Cβ), 125.2 (*s*, C-1′), 131.2 (2C, *d*, C-2′ and C-6′), 116.5 (2C, *d*, C-3′ and C-5′), and 158.3 (*s*, C-4′). Careful comparison of proton and carbon NMR spectra of **5** with those of **4** showed a substantial similarity for δ H and δ C values of the glucosyl moieties of both compounds whereas significant differences were observed for C-14 values strongly supporting the location of the acyl residue at 14-OH. This suggestion was further confirmed by diagnostic HMBC correlations observed between the carbonyl signal at δ 170.4 (Cα) and the methylene protons at δ 4.57 and 4.76 (H_2-14). Thus compound **5** was the 14-*O*-*p*-hydroxy-phenyl acetic ester derivative of **4**. All NMR resonances were assigned as reported in Table 3 by 2D experiments. The chemical correlation between the two molecules was finally confirmed by hydrolysis of **5** that afforded *p*-hydroxy-phenyl acetic acid and a glucosyl alcohol that resulted to be identical to 3β,14-dihydroxycostunolide-3-*O*-β-glycopyranoside (**4**).

Finally, a stereochemical analysis was conducted on the known compound **6** that was the main component of the sesquiterpene pool of *L. arborescens*. First of all, with the aim at confirming the absolute stereochemistry at the 6,7-junction of the guaianolide framework, suggested as reported for the most literature natural guaianolides, we decided to apply the Mosher method on the corresponding alcohol derivative obtained by opening of the lactone ring of **6**. Unfortunately, due to the rapid dehydration reaction that was observed to occur under different methanolysis conditions, every attempt to obtain the free secondary alcohol at C-6 was unsuccessful. Subsequently we decided to determine the absolute configuration of the chiral centre in 3′-hydroxy-2′-methyl-propanoyl fragment as follows. Compound **6** was subjected to

Table 3
^{1}H and ^{13}C NMR data [a,b] of compounds **4** and **5** in C_5D_5N.

Position	**4**			**5**		
	δ C (*m*)	δ H (*m*,*J* Hz)	HMBC[c] (C to H)	δ C(*m*)	δ H (*m*,*J* Hz)	HMBC[c] (C to H)
1	126.6 (*d*)	5.05 (*m*)		131.3 (*d*)	4.95 (*dd*, 10.6, 5.9)	H-9
2	33.0 (*t*)	2.68 (*q*, 11.8)		33.3 (*t*)	2.50 (*m*)	H-3
		2.55 (*m*)			2.58 (*q*)	
3	83.2 (*d*)	4.90 (*m*)	H-2, H-5, H-1′, H-15	82.8 (*d*)	4.83 (*m*)	H-1, H-1", H-2, H-15
4	141.5 (*s*)		H-5, H-15	140.8 (*s*)		H-3, H-6, H-15
5	127.3 (*d*)	5.02 (*m*)	H-7, H-15	127.4 (*d*)	4.98 (*d*, 10.2)	H-3, H-15
6	81.4 (*d*)	4.80 (*t*, 4.8)		81.2 (*d*)	4.68 (*t*, 8.8)	H-7
7	50.2 (*d*)	2.52 (*m*)	H-13	50.0 (*d*)	2.46 (*m*)	H-5, H-9, H-13a, H-13b
8	28.9 (*t*)	2.15 (*m*) 1.90 (*m*)	H-6, H-9	29.0 (*t*)	1.58 (*m*) 1.93 (*m*)	H-6, H-7
9	36.9 (*t*)	1.90 (*m*) 3.15 (*m*)		37.0 (*t*)	1.90 (*m*) 2.58 (*m*)	H-1, H-8, H-14
10	135.2 (*s*)		H-2, H-9	135.9 (*s*)		H-9, H-14
11	139.0 (*s*)			142.2 (*s*)		H-6, H-7, H-13a, H-13b
12	168.8 (*s*)		H-13	172.0 (*s*)		H-13a, H-13b
13	119.6 (*t*)	5.50 (*d*, 2.8)		119.8 (*t*)	5.49 (*d*, 3.5)	
		6.32 (*d*, 2.8)			6.35 (*d*, 3.5)	
14	58.5 (*t*)	4.10 (*d*, 12.3)		61.5 (*t*)	4.57 (*d*, 12.3)	H-l, H-9
		4.47 (*d*, 12.3)			4.76 (*d*, 12.3)	
15	12.0 (*q*)	1.95 (*s*)	H-5	12.0 (*q*)	1.85 (*s*)	H-3, H-5
Ester moiety						
Cα				170.4 (*s*)		CH_2β, H-14
CH_2β				41.0 (*t*)	3.72 (*d*, 3.5)	H-2′, H-6′
1′				125.2 (*s*)		CH_2β, H-5′
2′, 6′				131.2 (*d*)	7.32 (*d*, 8.2)	CH_2β
3′, 5′				116.5 (*d*)	7.14 (*d*, 8.2)	
4′				158.3 (*s*)		H-2′, H-3′
Sugar moiety						
1"	102.8 (*d*)	4.83 (*d*, 8.0)	H-3	102.8 (*d*)	4.82 (*d*, 7.6)	H-3, H-2"
2"	75.3 (*d*)	4.10 (*m*)		75.4 (*d*)	4.09 (*t*, 8.2)	
3"	78.6 (*d*)	4.25 (*m*)	H-5"	78.6 (*d*)	4.23 (*m*)	H-2"
4"	71.7 (*d*)	4.28 (*m*)	H-3"	71.9 (*d*)	4.23 (*m*)	H-3"
5"	78.6 (*d*)	3.90 (*m*)		78.7 (*d*)	3.90 (*m*)	H4", H-6"
6"a	62.9 (*t*)	4.42 (*dd*, 12.1,5.3)		63.0 (*t*)	4.40 (*dd*, 11.7,5.3)	H-4"
6"b		4.61 (*dd*, 12.1,4.8)			3.71 (*dd*, 11.7,2.3)	

[a] Bruker DRX 600 spectrometer in C_5D_5N, chemical shifts (ppm) refered to pyridine-d_5 (δ 8.71, 7.56, 7.19) and (δ 149.9, 135.5, 123.5).
[b] Assignmens made by ^{1}H–^{1}H COSY and HSQC experiments.
[c] Significant HMBC correlations (*J* = 10 Hz).

methanolysis in acid conditions to give the corresponding alcohol **6a**, which was fully characterised as acetyl derivative **6b**. Compound **6a** was allowed to react with *R*-(−) and *S*-(+)-α-methoxy-α-trifluoromethyl-phenylacetic acid chlorides to obtain the *S*- and *R*-MTPA ester derivatives **6c** and **6d**, respectively. Analysis of the ^{1}H NMR spectra of the two esters showed significant differences in the multiplet patterns of H_2-3′ (see Fig. 1). The same reaction conducted on both commercial methyl-(*S*)-(+)-3-hydroxy-2-methyl-propionate (**I**) and methyl-(*R*)-(−)-3-hydroxy-2-methyl-propionate (**II**) afforded two pairs of MTPA derivatives, (*S*)/(*S*)-MTPA ester (**Ia**), (*S*)/(*R*)-MTPA ester (**Ib**), (*R*)/(*S*)-MTPA ester (**IIa**) and (*R*)/(*R*)-MTPA ester (**IIb**). Comparison of the ^{1}H NMR spectra of each pair with those of *S*- and *R*-MTPA esters **6c** and **6d** clearly showed that H_2-3′ multiplet patterns of **6c** and **6d** were the same as the pair **Ia** and **Ib**, thus inferring the *S* absolute stereochemistry at C-2′ of **6** (Fig. 1).

All terpenoids **1**-**5** were tested for both antifungal and antibacterial activity at a concentration of 5 μg/ml. No growth inhibition was exhibited on *C. albicans* as well as on gram – *E. coli* and gram + *S. aureus* by the studied metabolites.

The terpenoid pattern of *L. arborescens*, described here for the first time, shows striking similarities with those reported in the lit-

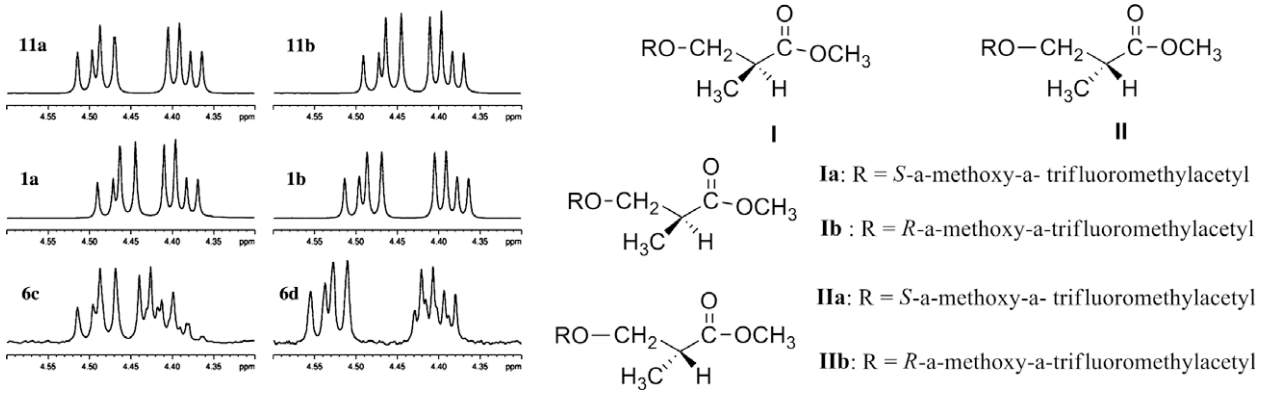


Fig. 1. ^{1}H NMR signals (400 MHz: $CDCl_3$) due to the methylene protons at C-3 for compounds Ia, IIa, Ib, and IIb.

erature for different species of the same genus (Zaheer et al., 2006; Sokkar et al., 1993; Abdel-Fattah et al., 1990; Gupta et al., 1989; Abdel-Salem et al., 1986; Abdel-Salem et al., 1982; Hook et al., 1984; Majumder and Laha, 1982; Prabhu and Venkateswarlu, 1969).

3. Experimental

3.1. General experimental procedures

Silica-gel chromatography was performed using pre-coated Merck F_{254} plates and Merck Kieselgel 60 powder. Optical rotations were measured on a Jasco DIP 370 digital polarimeter. IR spectra were recorded on a Biorad FTS 155 FT-IR spectrophotometer. HPLC separation was performed on a Shimazdu high-performance liquid chromatography using a Shimadzu liquid chromatograph LC-10AD equipped with an UV SPD-10A wavelength detector. NMR experiments were recorded at ICB-NMR Service Centre. 1D and 2D NMR spectra were acquired in $CDCl_3$, CD_3OD and pyridine-d_5 (shifts are referenced to the solvent signal) on a Bruker Avance-400 operating at 400 MHz, using an inverse probe fitted with a gradient along the *Z*-axis and a Bruker DRX-600 operating at 600 MHz, using an inverse TCI CryoProbe fitted with a gradient along the *Z*-axis.^{13}C NMR were recorded on a Bruker DPX-300 operating at 300 MHz using a dual probe. High resolution ESIMS were performed on a Micromass Q-TOF Micro™ coupled with a HPLC Waters Alliance 2695. The instrument was calibrated by using a PEG mixture from 200 to 1000 MW (resolution specification 5000 FWHM, deviation <5 ppm RMS in the presence of a known lock mass).

3.2. Plant material

The plant *L. arborescens* was collected in April 2006 in Bechar (Laabadla, South of Algeria) and identified by Prof. Bachir Oudjehih, Institute of Agronomy of University of Batna (Algeria). A voucher specimen is deposited in the herbarium of the department of the same University under the number code 423/HIAB.

3.3. Extraction and isolation

Dried and powdered aerial parts (900 g) of *L. arborescens* were treated with light petroleum ether (3 l, 3 times) to afford, after evaporation of the solvent under reduced pressure, 12 g of crude extract. This extract was subjected to silica gel column using light petroleum ether and increasing amount of ethyl acetate up to 100% ethyl acetate to obtain 140 fractions of 50 ml combined together into five total fractions A (2.9 g), B (100 mg), C (535 mg), D (300 mg) and E (400 mg). The fractions A, B, D and E containing terpenoid molecules were considered for further purification steps. Fraction A was purified by silica gel column chromatography using petroleum ether ethyl acetate (98:2) to afford **7** (1.35 g) as main compound. Fraction B was subjected to silica gel column chromatography (CH_2Cl_2/MeOH, 98:2) to yield **18** (1.8 mg) and a mixture of triterpenes that was further separated by RP-HPLC using isocratic elution MeOH/H_2O (9:1) to give **8** (1.2 mg) and **9** (1.0 mg). Fraction D was purified on a silica gel column in the same conditions as fraction B to obtain **10** (13.0 mg). Fraction E was chromatographed by a silica gel column in the same conditions as fraction A to afford two main fractions (E1 and E2), which were passed through analytical RP-18-HPLC (Phenomenex) using pure MeOH as eluent to give **11** (0.6 mg), **12** (0.4 mg) and **13** (0.6 mg) from E1 and **1** (1.5 mg), **14** (1.0 mg), **15** (1.5 mg), **16** (1.2 mg) and **17** (1.2 mg) from E2.

Dried and powdered roots (1 kg) of *L. arborescens* were macerated with methanol (7 l) to give a crude methanolic extract (27.0 g) that was partitioned between water and ethyl acetate. An aliquot (2.0 g) of the ethyl acetate soluble part (3.7 g) was subjected to column chromatography using LH-20, to give 9 fractions from A to I, two of which (C and G) containing terpene components. Fraction C (260 mg) was further purified by silica gel column chromatography using CH_2Cl_2/MeOH from 100% CH_2Cl_2 to 20% MeOH to yield 16 fractions (C1–C16) whereas fraction G afforded pure compound **6**. Fraction C3 (21.4 mg) was subsequently subjected to silica gel column chromatography using *n*-hexane-EtOAc (95:5) as eluent to afford pure compound **19** (6.0 mg). Fraction C8 (25.9 mg) was purified by silica gel column chromatography (gradient light petroleum ether/EtOAc) and following RP-HPLC (gradient MeOH/H_2O) to give **2** (1.6 mg), **20** (1.0 mg), **21** (0.8 mg), and **3** (0.6 mg) respectively. Fraction C12 (4.6 mg) was chromatographed on a silica gel column ($CHCl_3$/MeOH, 95:5) and subsequently RP-HPLC (gradient MeOH/H_2O) to afford pure compounds **22** (0.2 mg), **23** (0.3 mg), **24** (0.2 mg) and **25** (0.6 mg) respectively. Purification of fraction C13 (22.7 mg) on RP-HPLC column (gradient MeOH/H_2O) led to the isolation of compounds **26** (1.2 mg) and **5** (7.1 mg). Finally, fraction C14 (17.0 mg) was directly submitted to RP-HPLC chromatography (gradient MeOH/H_2O) to afford compounds **4** (2.1 mg) and **27** (2.2 mg).

3.3.1. 3β-Hydroxy-11α-ethoxy-olean-12-ene (*1*)

Amorphous powder; $[\alpha]_D^{25}$ −10° (*c* 0.10, $CHCl_3$); IR ν_{max}^{film} cm^{-1}: 3852, 2956, 2919, 1457, 1379, 1163, 1080, 974; ^{1}H and ^{13}C NMR values ($CDCl_3$) see Table 1; ESIMS positive mode: *m/z* 425 $[M-EtOH+H]^+$.

3.3.2. 9α-Hydroxy-11β,13-dihydro-3-epizaluzanin C (*2*)

Colourless oil; $[\alpha]_D^{25}$ −3° (*c* 0.06, $CHCl_3$); IR ν_{max}^{film} cm^{-1}: 3407, 1759, 1456, 1327, 1186, 1064, 991, 910; ^{1}H and ^{13}C NMR values ($CDCl_3$) see Table 2; ^{1}H NMR values (600 MHz, pyridine-d_5): δ 1.20 (3H, *d*, *J* = 6.7 Hz, H_3-13), 1.48 (1H, br *t*, *J* = 11.8 Hz, H-8a), 2.17 (1H, *m*, H-2b), 2.30 (1H, *m*, H-2a), 2.30 (1H, *m*, H-8b), 2.30 (1H, *m*, H-11β), 2.61 (1H, *q*, *J* = 10.8 Hz, H-7), 3.25 (1H, *t*, *J* = 9.7 Hz, H-5), 3.95 (1H, *t*, *J* = 9.7 Hz, H-6), 4.01 (1H, br *t*, *J* = 8.2 Hz, H-1), 4.71 (1H, br *s*, H-3β), 5.13 (1H, *m*, H-9β), 4.88 (1H, *s*, H-14a), 5.05 (1H, *s*, H-14b), 5.60 (2H, *s*, H-15); ^{13}C NMR values (150.92 MHz, pyridine-d_5): δ 13.3 (*q*, C-13), 34.7 (*d*, C-1), 40.3 (*t*, C-2), 40.7 (*t*, C-8), 41.7 (*d*, C-11), 44.5 (*d*, C-7), 49.9 (*d*, C-5), 73.8 (*d*, C-3), 74.8 (*d*, C-9), 85.7 (*d*, C-6), 110.6 (*t*, C-14), 110.8 (*t*, C-15), 154.0 (*s*, C-10), 157.1 (*s*, C-4), 178.6 (*s*, C-12); ESIMS positive mode: *m/z* 287 $[M+Na]^+$.

3.3.3. 9α-Hydroxy-4α,15-dihydro zaluzanin C (*3*)

Colourless oil; $[\alpha]_D^{25}$ −11° (*c* 0.10, $CHCl_3$); IR ν_{max}^{film} cm^{-1}: 3047, 2926, 1757, 1267, 1167, 984, 770; ^{1}H and ^{13}C NMR ($CDCl_3$), see Table 2; ^{1}H NMR values (600 MHz, pyridine-d_5): δ 1.28 (3H, *d*, *J* = 7.0 Hz, H-15), 1.57 (1H, *m*, H-8a), 2.06 (1H, *m*, H-2a), 2.34 (1H, *m*, H-2b), 2.43 (1H, *m*, H-5), 2.53 (1H, *m*, H-8b), 2.54 (1H, *m*, H-4α), 3.43 (1H, *m*, H-7), 3.75 (1H, br *q*, *J* = 10.2 Hz, H-1), 4.22 (1H, *t*, *J* = 9.7 Hz, H-6), 4.50 (1H, br *s*, H-3), 4.87 (1H, *m*, H-9β), 5.16 (2H, *s*, H-14), 5.43 (1H, *d*, *J* = 3.1 Hz, H-13a), 6.25 (1H, *d*, *J* = 3.1 Hz, H-13b); ^{13}C NMR values (150.92 MHz, pyridine-d_5): δ 8.41 (*q*, C-15), 32.6 (*d*, C-1), 34.4 (*t*, C-2), 39.4 (*t*, C-8), 40.4 (*d*, C-4), 41.2 (*d*, C-7), 47.3 (*d*, C-5), 73.3 (*d*, C-9), 73.5 (*d*, C-3), 83.3 (*d*, C-6), 110.5 (*t*, C-14), 118.7 (*t*, C-13), C-10, C-11 and C-12 were not detected; ESIMS positive mode: *m/z* 287 $[M+Na]^+$, HRESIMS: *m/z* 287.1214 (calcd for $C_{15}H_{20}O_4Na$, 287.1259).

3.3.4. 3β, 14-Dihydroxycostunolide-3-O-β-glycopyranoside (*4*)

Amorphous powder; $[\alpha]_D^{25}$+4° (*c* 0.10, MeOH); IR ν_{max}^{film} cm^{-1}; 3420, 2926, 2861, 1757, 1556, 1416; 1286, 1234; ^{1}H and ^{13}C NMR (pyridine-d_5) see Table 3; HRESIMS positive *m/z* 449.1776 (calcd for $C_{21}H_{30}O_9Na$, 449.1788).

*3.3.5. 3β,14-Dihydroxycostunolide-3-O-β-glucopyranosyl-14-O-p-hydroxyphenylacetate (**5**)*

Amorphous powder; $[\alpha]_D^{25}$ −0.4° (*c* 0.55, MeOH); IR ν_{max}^{film} cm^{-1}: 3393, 2922, 1738, 1616, 1516, 1448, 1259, 1145, 1018; ^1H and ^{13}C NMR (pyridine-d_5) see Table 3; ^1H NMR values (600 MHz, $CDCl_3$): δ 1.32 (1H, *m*, H-8a), 1.48 (3H, *s*, H-15), 1.80 (1H, *m*, H-8b), 1.88 (1H, *m*, H-9a), 2.25 (1H, *q*, *J* = 12.0 Hz, H-2a), 2.38 (1H, *m*, H-2b), 2.38 (1H, *m*, H-7), 2.40 (1H, *m*, H-9b), 4.30 (1H, *m*, H-14a), 4.35 (1H, *t*, *J* = 8.8 Hz, H-6), 4.38 (1 H, *m*, H-3), 4.40 (1H, *m*, H-14b), 4.82 (1H, *d*, *J* = 10.0 Hz, H-5), 4.95 (1H, *dd*, *J* = 12.0, 3.5 Hz, H-1), 5.50 (1H, *d*, *J* = 3.8 Hz, H-13a), 6.31 (1H, *d*, *J* = 3.8 Hz, H-13b), ester moiety: δ 3.40 (2H, *s*, H_2-β), 6.60 (2H, *d*, *J* = 8.3 Hz, H-3′ and H-5′), 6.90 (2H, *d*, *J* = 8.3 Hz, H-2′and H-6′), sugar moiety: 3.15 (1H, *m*, H-5″), 3.18 (1H, *m*, H-2″), 3.25 (1H, *m*, H-3″), 3.27 (1H, *m*, H-4″), 3.62 (1H, *dd*, *J* = 12.0, 4.8 Hz, H-6″a), 3.71 (1H, *dd*, *J* = 12.0, 2.6 Hz, H-6″b); 4.10 (1H, *d*, *J* = 8.0 Hz, H-1″); ^{13}C NMR values (75.46 MHz, $CDCl_3$): δ 11.0 (*q*, C-15), 28.8 (*t*, C-8), 32.2 (*t*, C-2), 36.7 (*t*, C-9), 49.4 (*d*, C-7), 61.5 (*t*, C-14), 81.2 (*d*, C-6), 81.3 (*d*, C-3), 120.6 (*t*, C-13), 126.3 (*d*, C-1), 131.0 (*d*, C-5), 134.6 (*s*, C-10), 141.1 (*s*, C-4), 139.0 (*s*, C-11), 170.8 (*s*, C-12), ester moiety: δ 40.6 (*t*, CH_2-α), 115.2 (*d*, C-3′ and C-5′), 124.2 (*s*, C-1′), 130.0 (*d*, C-2′ and C-6′), 155.9 (*s*, C-4′), 172.0 (*s*, C-α), sugar moiety: δ 61.6 (*t*, C″-6), 70.0 (*d*, C″-4), 73.3 (*d*, C″-2), 76.3 (*d*, C″-3), 75.7 (*d*, C″-5) 100.7 (*d*, C″-1), ESIMS positive mode: *m/z* 560 $[M + Na]^+$, HRESIMS: *m/z* 583.2181 (calcd for $C_{29}H_{36}O_{11}Na$, 583.2155).

*3.3.6. 8-Deoxy-15-(3′-hydroxy-2′-methyl-propanoyl)-lactucin-3′-sulfate (**6**)*

Colourless amorphous solid; $[\alpha]_D^{25}$ +7° (*c* 0.20, $CHCl_3$); ^1H NMR values (600 MHz, $CDCl_3$): δ 1.40 (1H, *m*, H-8a), 2.15 (1H, *m*, H-2a), 2.35 (1H, *m*, H-9a), 2.55 (3H, *s*, H-14), 2.56 (1H, *t*, *J* = 12.8 Hz, H9-b), 2.90 (1H, *m*, H-7), 3.58 (1H, *t*, *J* = 10.0 Hz, H-6), 3.77 (1H, *d*, *J* = 10.0 Hz, H-5), 5.00 (1H, *d*, *J* = 17.6 Hz, H-15 a), 5.35 (1H, *d*, *J* = 17.6 Hz, H-15 b), 5.45 (1H, *s*, H-13a), 6.12 (1H, *s*, H-13b), 6.37 (1H, *s*, H-3), ester moiety: δ 1.17 (3H, *d*, *J* = 7.0 Hz, H-4′), 2.90 (1H, *m*, H-2′), 4.18 (2H, *m*, H-3′); ^{13}C NMR values (75.46 MHz, $CDCl_3$): δ 22.2 (*q*, C-14), 24.1 (*t*, C-8), 37.3 (*t*, C-9), 50.0 (*d*, C-5), 52.0 (*d*, C-7), 63.8 (*t*, C-15), 83.8 (*d*, C-6), 119.0 (*t*, C-13), 130.8 (*s*, C-1), 133.5 (*d*, C-3), 138.6 (*s*, C-11), 154.9 (*s*, C-10), 166.9 (*s*, C-4), 169.3 (*s*, C-12), 195.6 (*s*, C-2), ester moiety: δ 13.5 (*q*, C-4′), 39.5 (*d*, C-2′), 69.3 (*t*, C-3′), 174.2 (*s*, C-1′).

*3.4. Preparation of the ester derivatives of compound **6***

*3.4.1. 8-Deoxy-15-(3′-hydroxy-2′-methyl-propanoyl)-lactucin (**6a**)*

Compound **6** (2.0 mg) was dissolved in H_2SO_4/MeOH (3 drops in 1 ml) and stirred for 10 min. After usual work up, the residue was chromatographed by silica gel column (light petroleum ether/EtOAc, 50:50) to afford 1.2 mg of pure compound **6a**.

Colourless oil, $[\alpha]_D^{25}$ +26.1° (*c* 0.12, $CHCl_3$), IR ν_{max}^{film} cm^{-1}: 3434, 2936, 2836, 2861, 1784, 1738, 1684, 1630, 1549, 1259, 1140, 1051, 978; ^1H NMR values (400 MHz, $CDCl_3$): δ 1.46 (1H, *m*, H8-a), 2.23 (1H, *m*, H-8b), 2.42 (1H, *m*, H-9a), 2.45 (3H, *s*, H-14), 2.54 (1H, br *t*, *J* = 12.7 Hz, H-9b), 2.89 (1H, *m*, H-7), 3.62 (1H, *t*, *J* = 10.2 Hz, H-6), 3.71 (1H, *d*, *J* = 10.2 Hz, H-5), 5.09 (1H, *d*, *J* = 16.9 Hz, H-15a), 5.31 (1H, *d*, *J* = 16.9 Hz, H-15b), 5.48 (1H, *d*, *J* = 3.2, H-13a), 6.20 (1H, *d*, *J* = 3.2 Hz, H-13b), 6.35 (1H, br *s*, H-3), ester moiety: δ 1.23 (3H, *d*, *J* = 7.3 Hz, H-4′), 2.77 (1H, *m*, H-2′), 3,76 (2H, *m*, H-3′); ESIMS positive mode: *m/z* 369 $[M + Na]^+$; HRESIMS: *m/z* 369.1298 (calcd for $C_{19}H_{22}O_6Na$, 369.1314).

*3.4.2. 8-Deoxy-15-(3′-hydroxy-2′-methyl-propanoyl)-lactucin-3′-acetate (**6b**)*

Acetyl derivative **6b** was prepared by treating 2.0 mg of compound **6a** with acetic anhydride (2 drops) in pyridine (1 ml) at room temperature. After usual work-up the product was purified by Pasteur-pipette silica gel chromatography using light petroleum ether-ethyl acetate (60:40) to give 1.7 mg of pure **6b**.

Colourless oil; $[\alpha]_D^{25}$ +8° (*c* 0.17, $CHCl_3$); IR ν_{max}^{film} cm^{-1}: 1718, 1688, 1621, 1373, 1251, 1135, 989; ^1H-NMR spectral data (600 MHz, $CDCl_3$): δ 1.45 (1H, *q*, *J* = 12.0 Hz, H8-a), 2.22 (1H, *m*, H-8b), 2.24 (1H, *m*, H-9a), 2.44 (3H, *s*, H-14), 2.56 (1H, br *t*, *J* = 13.0 Hz, H-9b), 2.85 (1H, *m*, H-7), 3.58 (1H, *t*, *J* = 10.0 Hz, H-6), 3.70 (1H, *d*, *J* = 10.0 Hz, H-5), 5.05 (1H, *d*, *J* = 17.1 Hz, H-15a), 5.31 (1H, *d*, *J* = 17.1 Hz, H-15b), 5.48 (1H, *d*, *J* = 3.2, H-13a), 6.20 (1H, *d*, *J* = 3.2 Hz, H-13b), 6.32 (1H, br *s*, H-3), ester moiety: δ 1.25 (3H, *d*, *J* = 7.3 Hz, H-4′), 2.85 (1H, *m*, H-2′), 4.22 (2H, *d*, *J* = 8.0 Hz, H-3′), 2.07 (3H, *s*, CH_3CO); ^{13}C-NMR values (75.46 MHz, $CDCl_3$): δ 22.2 (*q*, C-14), 24.1 (*t*, C-8), 37.3 (*t*, C-9), 50.0 (*d*, C-5), 52.0 (*d*, C-7), 63.8 (*t*, C-15), 83.7 (*d*, C-6), 119.0 (*t*, C-13), 130.9 (*s*, C-1), 133.5 (*d*, C-3), 138.6 (*s*, C-11), 154.9 (*s*, C-10), 166.8 (*s*, C-4), 169.2 (*s*, C-12), 195.6 (*s*, C-2), ester moiety: δ 13.5 (*q*, C-4′), 21.5 (*q*, CH_3CO), 39.5 (*d*, C-2′), 63.8 (*t*, C-3′), 171.0 (*s*, CH_3COO), 173.2 (*s*, C-1′); ESIMS positive mode: *m/z* 411 $[M + Na]^+$.

*3.4.3. 8-Deoxy-15-(3′-hydroxy-2′-methyl-propanoyl)-lactucin-3′-(S)-MTPA-ester (**6c**)*

Compound **6c** was prepared by treating 1 mg of **6a** in pyridine (1 ml) with *R*-(−)-MTPA chloride (0.07 ml) at room temperature overnight. After usual work up, the residue was purified by Pasteur-pipette silica gel chromatography using light petroleum ether-ethyl acetate (60:40) to give 0.4 mg of pure ester **6c**.

Colourless oil; $[\alpha]_D^{25}$ +21° (*c* 0.04, $CHCl_3$); IR ν_{max}^{film} cm^{-1}: 1784, 1744, 1684, 1630, 1448, 1381, 1273, 1180, 1113, 970; ^1H NMR values (400 MHz, $CDCl_3$): δ 1.44 (1H, *m*, H8-a), 2.03 (1H, *m*, H-8b), 2.39 (1H, *m*, H-9a), 2.45 (3H, *s*, H-14), 2.56 (1H, br *t*, 13.0 Hz, H-9b), 2.94 (1H, *m*, H-7), 3.47 (1H, *t*, *J* = 7.0 Hz, H-6), 3.59 (1H, *d*, *J* = 8.9 Hz, H-5), 4.97 (1H, *d*, *J* = 17.5 Hz, H-15a), 5.17 (1H, *d*, *J* = 17.5 Hz, H-15b), 5.48 (1H, *d*, *J* = 3.2 Hz, H-13a), 6.20 (1H, *d*, *J* = 3.2 Hz, H-13b), 6.25 (1H, br *s*, H-3), ester moiety: δ 1.23 (3H, *d*, *J* = 7.3 Hz, H-4′), 2.27 (1H, *m*, H-2′), 4.42 (1H, *dd*, *J* = 5.4, 10.8 Hz, H-3′a), 4.49 (1H, *dd*, *J* = 7.3, 10.8 Hz, H-3′b); ESIMS positive mode: *m/z* 585 $[M + Na]^+$.

*3.4.4. 8-Deoxy-15-(3′-hydroxy-2′-methyl-propanoyl)-lactucin-3′-(R)-MTPA-ester **(6d)***

Compound **6d** was prepared by treating 1.0 mg of **6a** in pyridine (0.5 ml) of *S*-(−)-MTPA chloride (0.07 ml) at room temperature overnight. The reaction mixture was purified as described for **6c** to obtain pure **6d** (1.2 mg).

Colourless oil; $[\alpha]_D^{25}$ −40° (*c* 0.12, $CHCl_3$); IR ν_{max}^{film} cm^{-1}: 1751, 1684, 1616, 1448, 1381, 1267, 1167, 1113, 1032; ^1H NMR values (400 MHz, $CDCl_3$): δ 1.43 (1H, *m*, H8-a), 2.22 (1H, *m*, H-8b), 2.40 (1H, *m*, H-9b), 2.45 (3H, *s*, H-14), 2.52 (1H, *m*, H-9a), 2.94 (1H, *m*, H-7), 3.59 (1H, *m*, H-6), 3.66 (1H, *d*, *J* = 10.2 Hz, H-5), 5.00 (1H, *d*, *J* = 17.4, H-15a), 5.18 (1H, *d*, *J* = 17.4 Hz, H-15b), 5.48 (1H, *d*, *J* = 3.2 Hz, H-13a), 6.20 (1H, *d*, *J* = 3.2 Hz, H-13b), 6.27 (1H, br *s*, H-3), ester moiety: δ 1.24 (3H, *d*, *J* = 6.7 Hz, H-4′), 2.87 (1H, *m*, H-2′), 4.40 (1H, *dd*, *J* = 5.7, 10.8 Hz, H-3′a), 4.53 (1H, *dd*, *J* = 6.7, 10.8 Hz, H-3′b); ESIMS positive mode: *m/z* 585 $[M + Na]^+$.

3.5. Preparation of model Mosher esters

*3.5.1. Methyl-(S)-(+)-3-hydroxy-2-methyl propionate-S-MTPA-ester (**Ia**)*

Compound **Ia** was prepared by treating 0.1 ml of methyl (*S*)-(+)-3-methyl propionate with 0.1 ml of *R*-MTPA chloride in dry CH_2Cl_2 (1 ml) with catalytic amount of DMAP under stirring overnight at room temperature. After usual work up, the reaction mixture was purified by silica gel chromatography using light petroleum ether-ethyl acetate (90:10) to get pure **Ia** (9.0 mg).

Oil; $[\alpha]_D^{25}$ −25° (*c* 0.90, $CHCl_3$); IR ν_{max}^{film} cm^{-1}: 1751, 1643, 1570, 1436, 1272, 1171, 1123, 1082; Selected ^1H NMR values

(400 MHz, $CDCl_3$): δ 4.38 (1H, *dd*, *J* = 5.7, 10.8 Hz, H-3′a), 4.47 (1H, *dd*, *J* = 7.3, 10.8 Hz, H-3′b).

3.5.2. Methyl-(S)-(+)-3-hydroxy-2-methyl propionate-R-MTPA-ester (**Ib**)

Compound **Ib** was prepared by treating 0.1 ml of methyl (*S*)-(+)-3-methyl propionate with 0.1 ml of *S*-MTPA chloride in dry CH_2Cl_2 (1 ml) with catalytic amount of DMAP under stirring overnight at room temperature. After usual work up the reaction mixture was purified by silica gel chromatography using light petroleum ether-ethyl acetate (90:10) to get pure compound **Ib** (30 mg).

Oil; $[\alpha]_D^{25}$+47° (*c* 3.0, $CHCl_3$), IR ν_{max}^{film} cm^{-1}: 1751, 1643, 1570, 1436, 1272, 1171, 1123, 1082; Selected ^{1}H NMR values (400 MHz, $CDCl_3$): δ 4.38 (1H, *dd*, *J* = 5.7, 10.8 Hz, H-3′a), 4.49 (1H, *dd*, *J* = 6.7, 10.8 Hz, H-3′b).

3.5.3. Methyl-(R)-(−)-3-hydroxy-2-methyl propionate-S-MTPA-ester (**IIa**)

Compound **IIa** was prepared by treating 0.1 ml of methyl (*R*)-(−)-3-methyl propionate with 0.1 ml of *R*-MTPA chloride in dry CH_2Cl_2 (1 ml) with catalytic amount of DMAP under stirring overnight at room temperature. After the usual work up, pure compound **IIa** (23.0 mg) was obtained.

Oil, $[\alpha]_D^{25}$ −46° (*c* 0.23, $CHCl_3$), IR ν_{max}^{film} cm^{-1}: 1731, 1583, 1494, 1436, 1274, 1122, 1082; Selected ^{1}H-NMR values (400 MHz, $CDCl_3$): δ 4.38 (1H, *dd*, *J* = 5.7, 11.1 Hz, H-3′a), 4.49 (1H, *dd*, *J* = 6.7, 11.1 Hz, H-3′b).

3.5.4. Methyl-(R)-(−)-3-hydroxy-2-methyl propionate-R-MTPA-ester (**IIb**)

Compound **IIb** was prepared by treating 0.1 ml of methyl (*R*)-(+)-3-methyl propionate with 0.1 ml of *S*-MTPA chloride in dry CH_2Cl_2 (1 ml) with catalytic amount of DMAP under stirring overnight at room temperature. After the usual work up, pure compound **IIb** (8.8 mg) was obtained.

Oil; $[\alpha]_D^{25}$+26° (*c* 0.88, $CHCl_3$), IR ν_{max}^{film} cm^{-1}: 1731, 1583, 1494, 1436, 1274, 1122, 1082; Selected ^{1}H-NMR values (400 MHz, $CDCl_3$): δ 4.38 (1H, *dd*, *J* = 5.72, 10.8 Hz, H-3′a), 4.47 (1H, *dd*, *J* = 7.3, 10.8 Hz).

3.5.5. Hydrolysis of **5**

Compound **5** (3.0 mg) was treated with NaOH/H_2O (1% solution) under stirring at room temperature for 3 h. After neutralisation with acidic solution (2% H_2SO_4), the residue was extracted with *n*-butanol for 3 times. The organic phase was concentrated to give after usual work up a product (1.7 mg), which was identical (^{1}H NMR, mass and $[\alpha]_D$) with compound **4**.

3.6. Biological assays

Antifungal assay was performed by the broth macrodilution method following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) document M27-P. The medium used to prepare the 10 x drug dilutions and the inoculum suspension was liquid RPMI 1640 with L-Glutamine (Sigma–Aldrich), 0.165 M Morpholinopropansulfonic acid (MOPS) and 2% Glucose (pH 7.0) (Rodriguez-Tudela et al., 1996; Hong et al., 1998). The yeast suspension was adjusted with the aid of a spectrophotometer to a cell density of 0.5 McFarland (2×10^8 CFU/ml) standard at 530 nm and diluted 1:4000 (50,000 CFU/ml) in RPMI 1640 broth medium. The yeast inoculum (0.9 ml) was added to each test tube that contained 0.1 ml of 10 2-fold dilutions (256–0.05 μg/ml final) of each compound. Broth macrodilution MICs were determined after 48 h of incubation at 35 °C. The MIC was defined as the lowest concentration of the compound that completely inhibited the growth of the test organism. The antibacterial assay was performed by using the same method as the antifungal test, only differing in the assay medium (Luria Bertani medium: 10 g/l Bactotryptone, 5 g/l Bactoyeast and 10 g/l NaCl, pH 7.5) and in the incubation temperature (37 °C for 24 h).

Acknowledgements

The NMR spectra were recorded at the ICB NMR Facility, the staff of which is gratefully acknowledged. Thanks are also due to Mr C. Iodice for spectrophotometric measurements and to Mr M. Zampa for mass spectra and Mrs D. Melck of ICB for antimicrobial tests. F.B. is deeply grateful to the Ministry of Superior Education and Scientific Research, Algeria for financial support. The authors kindly thank Mr A. El Aloui of University of Bechar for collecting the plant material.

References

Abdel-Fattah, H., Zaghloul, A.M., Halim, A.F., Waight, E.S., 1990. Steroid and triterpenoid constituents of *Launaea resedifolia*. Egypt. J. Pharm. Sci. 31, 81–91.

Abdel-Salem, N.A., Sarg, T.M., Omar, A.A., Abdel-Aziz, E., Khafagy, S.M., 1982. Study of the chemical constituents of *Launaea mucronata* (Forssk) Muschl. (*Asteraceae*) grown in Egypt. Sci. Pharm. 50, 34–36.

Abdel-Salem, NA., Mahmoud, Z.F., Kassem, F.K., 1986. Sesquiterpene lactones, coumarins and flavonoids of *Launaea tenuiloba* (Boiss) grown in Egypt. Egypt. J. Pharm. Sci. 27, 275–282.

Adegawa, S., Miyase, T., Ueno, A., Noro, T., Kuroyanagi, M., Fukushima, S., 1985. Sesquiterpene glycosides from *Crepidiastrum keiskeanum* Nakai. Chem. Pharm. Bull. 33, 4906–4911.

Asada, H., Miyase, T., Fukushima, S., 1984. Sesquiterpens lactones from *Ixeris tamagawensis* Kitam. II. Chem. Pharm. Bull. 32, 3036–3042.

Bandaranayake, W.M., 1980. Terpenoids of *Canarium zeylanicum*. Phytochemistry 19, 255–257.

Belboukhari, N., Cheriti, A., 2006. Phytochemical investigation of the bioactive extract from *Launaea arborescens*. Pak. J. Biol. Sci. 9, 2930–2932.

Bohlmann, F., Zdero, C., King, R.M., Robinson, H., 1984. A hydroxy germacrene and other constituents from *Pseudobrickellia brasiliensis*. Phytochemistry 23, 1798–1799.

Cheriti, A., Saad, A., Belboukhari, N., Ghezali, S., 2006. Chemical composition of the essential oil of *Launaea arborescens* from Algerian Sahara. Chem. Nat. Comp. 42, 360–361.

Dai, J., Zhao, C., Zhang, Q., Liu, Z.L., Zheng, R., Yang, L., 2001. Taraxastane-type triterpenoids from *Saussurea petrovii*. Phytochemistry 58, 1107–1111.

El-Feraly, F.S., Chan, Y.M., Benigni, D.A., 1979. Magnolialide: a novel eudesmanolide from the root bark of *Magnolia grandiflora*. Phytochemistry 18, 881–882.

Fujita, R., Duan, H., Takaishi, Y., 2000. Terpenoids from *Tripterigyum hypoglaucum*. Phytochemistry 53, 715–722.

Giner, R.M., Diaz, J., Manez, S., Recio, M.C., Soriano, C., Rios, J.L., 1992. Phenolic of Spanish *Launaea* species. Biochem. System. Ecol. 20, 187–188.

Gupta, M.M., Verma, R.K., Singh, S.C., 1989. Constituents of *Launaea asplenifolia*. Fitoterapia 60, 476.

Hong, S.Y., Oh, J.E., Kwon, M.Y., Choi, M.J., Lee, J.H., Lee, B.L., Moon, H.M., Lee, K.H., 1998. Identification and characterization of novel antimicrobial decapeptides generated by combinatorial chemistry. Antimicrob. Agents Chemother. 42, 2534–2541.

Hook, F., Behari, M., Gupta, R., Mutsumoto, T., 1984. Chemical constituents of *Launaea nudicaulis*. Indian Drugs 21, 366–368.

Jbilou, R., Amri, H., Bouayad, A., Ghailani, A., Ennabili, A., Sayah, F., 2008. Insecticidal effects of extracts of seven plant species on larval development, α-amylase activity and offspring production of *Tribolium castaneum* (herbst) (insecta: *Coleoptera*: *Tenebrionidae*). Bioresource Technol. 99, 959–964.

Kisiel, W., Barszcz, B., 1996. Sesquiterpene lactones from *Crepis rhoeadifolia*. Phytochemistry 43, 823–825.

Kisiel, W., Barszcz, B., 1997. Minor sesquiterpene lactones from *Lactuca virosa*. Phytochemistry 46, 1241–1243.

Kisiel, W., Barszcz, B., 1998. A germacrolide glucoside from *Lactuca tatarica*. Phytochemistry 48, 205–206.

Kisiel, W., Gromek, D., 1993. Sequiterpene lactones from *Lactuca saligna*. Phytochemistry 34, 1644–1646.

Kisiel, W., Michalska, K., 2002. A guaianolide alloside and other constituents from *Picris kamtschatica*. Phytochemistry 61, 891–894.

Kisiel, W., Zielinska, K., 2001a. A new taraxastane-type triterpenoid and sesquiterpense lactones from *Picris evae*. Pol. J. Chem. 75, 141–145.

Kisiel, W., Zielinska, K., 2001b. Guaianolides from *Cichorium intybus* and structure revision of *Cichorium* sesquiterpene lactones. Phytochemistry 57, 523–527.

Kojima, H., Sato, N., Hatano, A., Ogura, H., 1990. Sterol glucosides from *Prunella vulgaris*. Phytochemistry 29, 2351–2355.

Majumder, P.L., Laha, S., 1982. Chemical constituents of *Launaea nudicaulis*: ^{13}C nuclear magnetic resonance spectroscopy of taraxasterones and pseudotaraxasterones. J. Indian. Chem. Soc. 59, 881–883.

Mansour, R.M.A., Ahmed, A.A., Saleh, N.A.M., 1983. Flavone glycosides of some *Launaea* species. Phytochemistry 22, 2630–2631.

Marco, J.A., 1989. Sesquiterpene lactones from *Artemisia herba-alba* subsp. *Herba-alba.*. Phytochemistry 28, 3121–3126.

Matsunaga, S., Tanaka, R., Akagi, M., 1988. Triterpenoids from *Euphorbia maculata*. Phytochemistry 27, 535–537.

Miyase, T., Fukushima, T., 1987. Studies on sesquiterpene glycosides from *Sonchus oleraceus* L. Chem. Pharm. Bull. 35, 2869–2874.

Miyase, T., Yamaki, K., Fukushima, S., 1984. Studies on sesquiterpenes from *Macroclinidium trilobum Makino.* II.. Chem. Pharm. Bull. 32, 3912–3917.

Nishimura, K., Miyase, T., Ueno, K., Noro, T., Kuroyanagi, M., Fukushima, S., 1986a. Sesquiterpene lactones from *Picris hieracioides L* Var. *japonica Regel. I*. Chem. Pharm. Bull. 34, 2518–2521.

Nishimura, K., Miyase, T., Ueno, A., Noro, T., Kuroyanagi, M., Fukushima, S., 1986b. Sesquiterpene lactones from *Lactuca laciniata*. Phytochemistry 25, 2375–2379.

Ozenda, P., 2004. Flore et Végétation du Sahara. CNRS, Paris. p. 662.

Prabhu, K.R., Venkateswarlu, V., 1969. Chemical examination of *Launaea pinnatifida*. J. Indian Chem. Soc. 46, 176.

Quezel, P., Santa, S., 1963. In: Nouvelle Flore de l'Algérie et des Régions Désertiques Méridionales, vol. 1–2. CNRS, Paris. p. 1162.

Reynolds, W.F., Mclean, S., Poplawski, J., 1986. Total assignment of ^{13}C and ^{1}H spectra of three isomeric triterpenol derivatives by 2D NMR: an investigation of the potential utility of ^{1}H chemical shifts in structural investigations of complex natural products. Tetrahedron 42, 3419–3428.

Rodriguez-Tudela, J.L., Berenguer, J., Martinez-Suarez, J.V., Sanchez, R., 1996. Comparison of a spectrophotometric microdilution method with RPMI–2% glucose with the National Committee for Clinical Laboratory Standards Reference Macrodilution Method M27-P for in vitro susceptibility testing of amphotericin B, flucytosine, and fluconazole against *Candida albicans*. Antimicrob. Agents Chemother. 40, 1998–2003.

Romo, J., de Vivar, A.R., Nathan, P.J., 1967. Constituents of *Zaluzania augusta.* The structures of zaluzanins A and B. Tetrahedron 23, 29–35.

Sarg, T.M., Omar, A.A., Khafagy, S.M., Grenz, M., Bohlmann, F., 1982. 11β, 13-Dihydrolactucin, a sesquiterpene lactone from *Launaea mucronata*. Phytochemistry 21, 1163.

Sokkar, N.M., Aboutabl, E.A., Kubelka, W., Mohamed, Z.H., Hilal, S.H., 1993. Terpenoids from *Launea spinosa* (Forssk). Plant Med. Phytother. 26, 368–374.

Spring, O., Buschmann, H., Vogler, B., Schilling, E.E., Spraul, M., Hoffmann, M., 1995. Sesquiterpene lactone chemistry of *Zaluzania grayana* from on-line LC NMR measurements. Phytochemistry 39, 609–612.

Tanaka, R., Matsunaga, S., 1988. Triterpene dienols and other constituents from the bark of *Phyllanthus flexuosus*. Phytochemistry 27, 2273–2277.

Warashina, T., Ishino, M., Miyase, T., Ueno, A., 1990. Sesquiterpene glycosides from *Ixeris debilis* and *Ixeris repens*. Phytochemistry 29, 3217–3224.

Xiao, Y., Guo-liang, W., Fu-jum, G., 1994. Studies on triterpenoid constituents isolated from the roots of *Sabia schumanniana*. Acta Bot. Sin. 36, 153–158.

Zaheer, A., Dildar, A., Abdul, M., 2006. Structure determination of ursene-type triterpenes by NMR techniques. Magn. Reson. Chem. 44, 717–719.

Zidorn, C., Ellmerer, E.P., Heller, W., Johrer, K., Frommberger, M., Greil, R., Guggenberger, M., Ongania, K., Stuppner, H., 2007. A new sesquiterpene lactone sulfate from *Reichardia gaditana*. Z. Naturforsch. 62B, 132–134.