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Neo-clerodane diterpenoids from *Ajuga pseudoiva* leaves

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Abstract

14,15-dihydro-ajugapitin, Lupulin A and three minor new epimeric neo-clerodane diterpenoids, Hativenes A–C, have been isolated from *Ajuga pseudoiva* leaves. Their structures were established by spectroscopic procedures.

Keywords: *Ajuga pseudoiva*; Labiatae; Epimeric clerodane diterpenoids; Hativenes A–C

1. Introduction

In order to continue our contribution to the biological and chemical studies of medicinal plants growing in Tunisia (Askri, Bui & Mighri, 1982; Askri, Mighri, Bui, Das & Hylands, 1989; Ben Hassine, Bui & Mighri, 1982; Ben Hassine, Bui, Mighri & Cave, 1982; Ben Jannet et al., 1997; Ben Jannet, Chaari, Martin & Mighri, 1998) and following our search for potential insect antifeedant and antibacterial natural compounds (Ben Jannet et al., 1997,1998), we have examined the extract of the leaves of *Ajuga pseudoiva* (Labiatae). This paper reports the isolation and structural elucidation of three new epimeric neo-clerodane diterpenoids, Hativenes A–C (**1**–**3**), together with the previously known neo-clerodane, 14,15-dihydro-ajugapitin 5 (Camps, Coll & Dargallo, 1984) isolated from the same source and Lupulin A **4** previously isolated from *A. lupulina* (Chen, Tan, Liu & Zhang, 1996). The structures of **1**, **2** and **3** were established by spectroscopic means and by comparison with those closely related compounds (Camps et al., 1984; Chen et al., 1996). The relative configuration of asymmetric carbons was determined on the basis of their NOESY spectra. Preliminary biological tests showed high antibacterial activities of compounds **1**–**3** towards *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhimurium*.

2. Results and discussion

Fractionation of an acetone extract of *Ajuga pseudoiva* leaves by normal phase chromatography, permitted us to localise the antibacterial activities in few fractions. One of these was analysed by repeated chromatography and RP-HPLC showing the existence of compounds **1** to **5**.

Compound **1**: had molecular formula $C_{29}H_{44}O_{11}$ (*m*/*z* 569 $[MH]^+$). Its IR spectrum revealed absorptions attributable to free hydroxyl (3450 cm^{-1}) and ester (1729 cm^{-1}) groups (Chen et al., 1996).

Furthermore, the presence of two acetate groups was deduced from the ^1H NMR spectrum (δ 1.92 and 2.11). This presence was supported by signals due to protons on carbon atoms bearing oxygen at δ 4.40 and 4.76 (AB system, J = 12.3 Hz) and 4.67 (dd, J = 11.8, 4.5 Hz). Likewise, the ^2J correlation of protons resonating at δ 4.40 and 4.76 with a quaternary carbon resonating at δ 45.2 (C-5) observed in the HMBC spectrum confirms the position of the CH_2OCOCH_3

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Table 1
^{13}C and ^{1}H spectral data of compound **1**

Atom	δ ^{13}C	δ ^{1}H (J (Hz))	HMBC	NOESY
1ax	30.3	1.75 m	10	
1eq		2.60 m		
2	71.9	3.63 m	1-3	10-17
3	72.4	5.20 d (9.7)	2	18
4	62.8		3-6-17-18	
5	45.2		7-10-18	
6	71.4	4.67 dd (11.8; 4.5)	7-8-20	8-10
7ax	33.4	1.65 m	20	
7eq		1.47 m		
8	35.8	1.48 m	7-11-19-20	10
9	39.8		7-8-10-19-20	
10	43.3	1.70 m	1-11-18-19	
11	82.8	3.99 dd (11.6; 4.1)	12-16-19	15-19-20
12a	32.4	1.50 m	14-16	
12b		1.75 m		
13	40.0	2.97 m	11-14-15-16	16
14a	38.1	1.65 m	12-15-16	
14b		2.17 m		
15	104.7	5.08 d (4.7)	14-16-OMe	
16	107.4	5.72 d (5.3)	12-14-15	OMe
17	42.6	2.55 d (4.1), 2.78 d (4.1)	3	
18	61.4	4.40 d (12.3), 4.76 d (12.3)	6-10	19
19	13.8	0.94 s	8-10-11	
20	16.5	0.85 d (6.1)	7-8	
1′	170.8			
2′	20.9	1.92 s		
3′	172.0			
4′	20.9	2.11 s		
5′	176.8		6′-7′-8′	
6′	34.3	2.52 m	7′-8′	
7′	18.8	1.13 d (7.1)		
8′	18.8	1.13 d (7.1)		
OMe	54.5	3.31 s		

system in relation to the rings (Table 1). The appearance of methyl signal at δ 1.13 (6H, d, J = 7.1 Hz) in the ^{1}H NMR spectrum in junction with signals at δ 176.8, 34.3 and 18.8 inferred from HMQC and HMBC spectra (Table 1), pointed to the presence of an isopropylic ester function attached at C-3.

The ^{1}H NMR spectrum showed signals at δ 2.78 (1H, d, J = 4.1 Hz) and δ 2.55 (1H, d, J = 4.1 Hz) in agreement with the presence of (C-4)–(C-17) epoxyde (Chen et al., 1996). This position is confirmed by ^{2}J correlations observed in the HMBC spectrum between protons resonating at δ 2.55, 2.78 and the quaternary carbon (δ 62.8) identified to be C-4 (Table 1).The broad one proton signal at δ 3.63 attributable to a C*H*OH system was in agreement with hydroxyl absorption at 3450 cm^{-1} shown in the IR spectrum as indicated above. The presence of a hexahydrofuranofuran system was inferred from proton signals at δ 5.72 (1H, d, J = 5.3 Hz), 5.08 (1H, d, J = 4.7 Hz), 3.99 (1H, dd, J_1 = 11.6, 4.1 Hz) and 2.97 (1H, m) (Camps, Coll & Cortel, 1982; Camps et al., 1984; Chen et al., 1996). The appearance of a methyl group signal at δ 3.31 (3H, s) in the ^{1}H NMR spectrum and a lowfield ^{13}C NMR resonance at δ 104.7 (CH) suggested the substitution of an H-15 proton by a methoxyl group, as found previously for Lupulin A (Chen et al., 1996). The HMBC spectrum confirmed the location of this methoxyl group by showing a ^{2}J correlation between methylic protons (δ 3.31) and tertiary carbon (via oxygen atom) identified to be C-15 (Table 1). Furthermore, the occurrence of this C-15 substituted hexahydrofuranofuran moiety was confirmed by the presence in its FABMS of significant peaks at m/z 143 and 111 (Chen et al., 1996). More and interesting structural informations were obtained from the 2D spectra. The peak correlating signals at δ 3.63 (1H, m) and 5.20 (1H, d, J = 9.7 Hz) in the ^{1}H–^{1}H COSY NMR spectrum of compound **1** showed that hydroxyl group and isopropylic ester function were attached at C-2 and C-3, respectively. This result was confirmed in an other hand by the correlation between (H-2)– (C-3) and (H-3)– (C-2) observed in the HMBC spectrum.

Elucidation of the relative stereochemistry of compound **1** was mostly based on the close similarity of its ^{1}H ($CDCl_3$) NMR data (Table 1) to those of Lupulin A **4** (Chen et al., 1996). Dipolar coupling of H-3 to the diastereotopic proton H-18, together with other NOEs deduced from the NOESY spectrum (Table 1) indicated a 3β-position of the isopropylic ester group, and by considering the large coupling constant (J = 9.7 Hz) between H-2 and H-3, the hydroxyl group at C-2 was assigned as being α-oriented, this assignments were strongly supported by the NOE correlation of H-2 to H-17 and H-10. Important information about the stereochemistry of the hexahydrofurofuran moiety was obtained from the NOESY experiment. The H-16 proton showed NOE cross-peaks with H–(OMe). Moreover the H-11 proton showed NOES with H-15, Me-19 and Me-20. In fact the difference between the ^{1}H NMR spectra of **1** and Lupilin A **4** were in the chemical shifts corresponding to the H-11 ($\Delta\delta$ −0.97 ppm), H-13 ($\Delta\delta$ + 0.16 ppm) and H-15 ($\Delta\delta$ + 0.12 ppm). Moreover a comparison between the ^{1}H NMR spectra of **1** and Lupilin B (C-15 epimer of Lupilin A **4**) showed a close similarity in the hexahydrofurofuran moiety than Lupilin A **4**. These data clearly established the stereochemistry of the hexahydrofurofuran side-chain in **1** (Camps et al., 1982; Malakov, Papanov & Boneva, 1996), and confirmed the β-orientation of methoxyl group. This proposal is reinforced by the absence of significant NOES between H-15 and any other protons such as H-13 and H-16 which were assigned as β oriented. The stereochemistry at the position C-15 for Lupilin A and B

Table 2
^{13}C and ^{1}H spectral data of compound **2**

Atom	δ ^{13}C	δ ^{1}H (J (Hz))	HMBC	NOESY
1ax	30.2	1.74 m	3-10	
1eq		2.59 m		
2	71.8	3.61 m	1-3	10
3	72.4	5.20 d (9.8)	2	18
4	62.8		3-18	
5	45.5		1-7-10-18	
6	71.3	4.67 dd (11.4; 4.5)	7-18-20	8–10
7ax	33.2	1.60 m	20	
7eq		1.45 m		
8	35.8	1.45 m	7-10-11-19-20	10
9	40.0		7-8-10-19-20	
10	43.3	1.70 m	1-11-18-19	
11	82.7	4.37 dd (11.3; 5.7)	8-12-13-16-19	19-20-OMe
12a	32.6	1.60 m	14-16	
12b		1.74 m		
13	40.5	2.78 m	14-15-16	16
14a	39.4	1.75 m	15	
14b		2.23 m		
15	104.5	4.95 d (5.7)	13-14-16-OMe	16
16	108.8	5.80 d (5.4)	12-14-15	
17	42.4	2.56 d (4.3), 2.80 d (4.3)	3	
18	61.4	4.40 d (12.2), 4.76 d (12.2)	6-10	19
19	13.8	0.92 s	8-10	
20	16.0	0.87 d (6.3)	8	
1′	170.0			
2′	20.9	1.92 s		
3′	171.2			
4′	20.9	2.11 s		
5′	175.4		3-6′-7′-8′	
6′	34.1	2.51 m	7′-8′	
7′	18.8	1.13 d (7.0)		
8′	18.8	1.13 d (7.0)		
OMe	54.3	3.31 s		

Table 3
^{1}H spectral data and significant correlations observed in the NOESY spectrum of compound **3**

Atom	δ ^{1}H (J (Hz))	NOESY
1ax	1.90 m	
1eq	2.45 m	
2	4.11 m	3-19
3	5.38 d (3.4)	18
6	4.76 m	8-17
7ax	1.60 m	
7eq	1.45 m	
8	1.44 m	10
10	2.21 m	
11	4.37 m	19-20-OMe
12a	1.57 m	
12b	1.91 m	
13	2.83 m	16
14a	1.78 m	
14b	2.20 m	
15	4.95 d (5.7)	16
16	5.79 d (5.2)	
17	2.66 d (4.1), 2.94 d (4.1)	
18	4.44 d (12.6), 4.71 d (12.6)	19
19	0.93 s	
20	0.87 d (6.6)	
2′	1.93 s	
4′	2.10 s	
6′	2.50 m	
7′	1.12 d (7.0)	
8′	1.12 d (7.0)	
OMe	3.30 s	

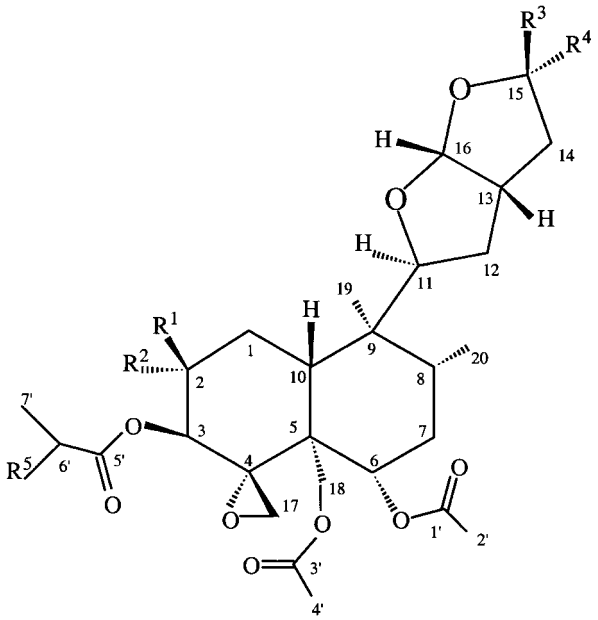
may be reversed as well as for Clerodinins A and B (Lin, Kuo & Chen, 1989).

Compound **2** and **3**: isolated as colourless oils, have the same molecular formula than of compound **1** $C_{29}H_{44}O_{11}$ (m/z 569 $[MH]^+$). Their IR spectra showed practically the same significant absorptions attributable to free hydroxyl, oxirane ring and ester groups.

Hativenes B **2** was homogenous on TLC and its ^{1}H and ^{13}C NMR showed essentially the same signal as those present in the spectra of Hativenes A **1** (Tables 1 and 2). In fact the observed differences between these spectra were in the chemical shifts of H-11 ($\Delta\delta$ −0.38 ppm), H-13 ($\Delta\delta$ +0.2 ppm) and H-15 ($\Delta\delta$ +0.1 ppm). The observed difference between the NMR spectra of **2** and **1** were in agreement with the former being of the C-15 epimer of the Hativenes A **1**. In the 2D NOESY spectrum of **2**, correlation of H-11 with the OMe protons at C-15 reinforced the α-orientation of methoxyl group. It is notable that these differences in the chemical shifts of the hexahydrofurofuran side chain in the C-15 epimers are observed when the ring A of the trans decalin system is in the boat twist or chair conformation (Malakov et al., 1996).

The ^{1}H NMR spectrum of Hativenes C **3** (Table 3) was also similar to that of Hativenes A **1**, with the notable exception of the two distinctive resonances at δ 4.11 (m) and δ 5.38 (d, J = 3.4 Hz) attributable at H-2 and H-13 respectively. This result clearly established that the hydroxyl group of compound **3** was at C-2β. An NOE between H-3 and H-18 as well as dipolar coupling of H-3 to H-2 and H-2 to H-19, confirmed the proposal β-orientation of hydroxyl group.

The absolute configuration of compounds **1**–**3** was not ascertained. However, on biogenetic grounds it may be supposed that **1**–**3** belongs to the neo-clerodane series, like the other diterpenoids isolated from Ajuga species whose absolute configuration has been established from X-ray diffraction analysis (Camps, Coll, Cortel & Messeguer, 1979; Camps, Coll & Cortel, 1981; Rogers et al., 1979; Malakov, Papanov, Perales, de la Torre & Rodriguez, 1992).



	R1	R2	R3	R4	R5
1	H	OH	OMe	H	Me
2	H	OH	H	OMe	Me
3	OH	H	H	OMe	Me
4	H	OH	OMe	H	Et
5	H	OH	H	H	Et

3. Experimental

3.1. Plant material

Plant material was harvested in June 1995 in Monastir (Tunisia) and voucher specimens were deposited in the herbarium of the Ecole supérieure d'Horticulture, Chott Mirriam (Université du centre, Sousse, Tunisie). *Ajuga pseudoiva* was identified by Dr F. SKIRI.

3.2. Extraction and isolation

Dried and powdered leaves of *Ajuga pseudoiva* (343 g) were extracted with acetone at room temperature for seven days. The crude extract obtained after filtration and evaporation of the solvent was separated by silica gel column chromatography (Merck 7734, hexane–EtOAc–MeOH). Four main fractions were collected; the fourth fraction (6.9 g), was divided between hexane and MeOH. The hexane layer, showing antibacterial activities, was separated by silica gel column chromatography (Merck 7734, hexane–EtOAc–$CHCl_3$). Further activity-guided CC separation led to a fraction (95 mg) separated by silica gel column chromatography (Merck 7734, 98/2 CH_2Cl_2–MeOH).

Clerodane diterpenoids-containing fractions (fractions 3, 4 and 5) were further analysed by HPLC using reversed phase HPLC column (Lichrosorb® RP-select B 10 μm) eluted with MeOH–H_2O 60:40 applying a flow rate of 3 mL/mn to give finally compound **1** (4.8 mg), compound **2** (5 mg), compound **3** (1.8 mg), compound **4** (9 mg) and compound **5** (3 mg).

3.3. General experimental procedures

^1H, ^{13}C and 2D NMR spectra were recorded on Bruker AM 400 spectrometer with TMS as int. standard.Coupling constants are given in Hz. FAB mass spectra were obtained on an MS-80RF. IR spectra were recorded on Nicolet 205 IR-FT spectrophotometer. Optical rotation determination were performed in $CHCl_3$ on a Perkin Elmer 241 MC polarimeter.

Hativene A **1**: colourless oil, $[\alpha]_D^{20}$ −12.1 ($CHCl_3$; c 0.3), FABMS *m/z* $[MH]^+$ 569(80), 553(11), $[M–MeOH]^+$ 537(37), 519(10), 463(11), 449(23), 421(61), 407(11), 389(52), 369(11), 277(10), 201(11), 185(98), 143(28), 111(100, hexahydrofuranofuran fragment ion); IR ($CHCl_3$) ν_{max} cm^{-1} 3450(OH), 3029(oxirane ring), 1729, 1250(ester groups), 1390, 1124; ^1H and ^{13}C NMR data see Table 1.

Hativene B **2**: colourless oil, $[\alpha]_D^{20}$ −2.8 ($CHCl_3$; c 0.12), FABMS *m/z* $[MH]^+$ 569(43), 553(6), 537(8.5), 461(7), 421(14), 407(5.7), 389(14), 369(17), 277(13), 185(100), 143(5.7), 111(13); IR ($CHCl_3$) ν_{max} cm^{-1} 3435(OH), 3024(oxirane ring), 1729(ester groups), 1643, 1124, ^1H and ^{13}C NMR data see Table 2.

Hativene C **3**: colourless oil, ^1H NMR data see Table 3.

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