NPC Natural Product Communications

2015
Vol. 10
No. 6
857 - 860

neo-Clerodane Diterpenoids from *Ajuga macrosperma* var. *breviflora*

Amaya Castro[a], Josep Coll[*,a], Anil K. Pant[b] and Om Pakrash[b]

[a]*Departament de Química Biològica i Modelització Molecular, Institut de Química Avançada de Catalunya (IQAC), Consejo Superior de Investigaciones Científicas (CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain*

[b]*Department of Chemistry, C.B.S.H. G. B. Pant University of Agriculture & Technology, Pantnagar, 263145 Uttarakhand, India*

josep.coll@iqac.csic.es.

Received: January 1st, 2015; Accepted: February 20th, 2015

Six new naturally occurring ajugarin-like *neo*-clerodane diterpenoids, ajugaflorins A-F, along with six known compounds [the parent ajugarin I, ajugalides B and C, ajugamarin F4, ajugamacrin E, and ajugatakasin B] were isolated from *A. macrosperma* var. *breviflora*. The structures were elucidated by extensive NMR spectroscopic and MS analyses and comparison with data previously reported.

Keywords: *Ajuga macrosperma* var. *breviflora*, Labiatae, *neo*-Clerodane diterpenes, Ajugaflorins.

The genus *Ajuga* (Labiatae) has attracted attention since the report in 1976 that *A. remota* plants, grown in Kenya, were not attacked by African armyworms [1]. More than one hundred species and fifty varieties and subspecies of *Ajuga* are unevenly distributed over the world, being especially abundant in China, Korea and Japan, and also widespread in Europe [2,3]. *A. macrosperma* is a perennial herb growing in tropical regions of India, Nepal and China [4,5]. Two varieties have been reported from China, namely *A. macrosperma* var. *macrosperma* and *A. macrosperma* var. *thomsonii* [2,6], whereas *A. macrosperma* var. *breviflora* is reported to grow in the Kumaun region of Uttaranchal State in India [5]. In China, the herb *A. macrosperma* var. *macrosperma* is used in folk medicine to alleviate fever and remove phlegm [6]. It is also reported to be used medicinally in nephritis [2]. The extract is active against *Pyricularia* oryzae [7] and also shows cell cycle inhibitory activity against the tsFT 210 cell line [8]. Different compounds have been isolated previously from *A. macrosperma*, including *neo*-clerodane diterpenoids (ajugamacrins A-E) [9,10], ecdysteroids [11], and the triterpenes betulinic and 3-*epi*-betulinic acids [12]. The antifeedant activity of *Ajuga neo*-clerodane diterpenes has been reviewed [13].

We now report the structure elucidation of the *neo*-clerodane diterpene contents of a sample of *A. macrosperma* var. *breviflora* Hook. f. collected from the Kumaun region of Uttarakhand state in India. Six new naturally occurring compounds named ajugaflorins A-F (**1**, **4**, **6**, **8**, **11**, and **12**, respectively), and six known diterpenes, namely ajugamacrin E (**2**) [10], ajugatakasin B (**3**) [14], ajugamarin F4 (**5**) [15], ajugalides B (**7**) and C (**9**) [16], and the parent ajugarin I (**10**) [17], were isolated. The structures were elucidated by extensive NMR spectroscopic and MS analyses and comparison with data previously reported. Six of the compounds displayed the presence of *O*-substitution either at C-3 or C-12 (C-3 substitution: ajugaflorins A, B, D; C-12 substitution: ajugaflorin C, **5**, **9**), whereas five of them appeared with two *O*-substituents (C-1 and C-12: **2, 3**, **7**; C-2 and C-3: ajugaflorin E; C-3 and C-12: ajugaflorin F), resulting in diagnostic multiplicity patterns.

Ajugaflorin A (**1**) displayed a quintuplet-like signal at δ_H 5.85, as expected for H-14 in an ajugarin-like molecule with unsubstituted C-12, and a broad doublet at δ_H 2.83 for H-18B indicative of C-3

1	**Ajugaflorin A**	**3β-(2-methylbutyryloxy)**
4	**Ajugaflorin B**	**3β-(isobutyryloxy)**
6	**Ajugaflorin C**	**12-(isobutyryloxy)**
8	**Ajugaflorin D**	**3β-hydroxy**
11	**Ajugaflorin E**	**2α-hydroxy-3β-(2-methylbutyryloxy)**
12	**Ajugaflorin F**	**12-hydroxy-3β-(2-methylbutyryloxy)**
2	Ajugamacrin E	12-(isobutyryloxy)-1-(2-methylbutyryloxy)
3	Ajugatakasin B	1,12-bis(2-methylbutyryloxy)
5	Ajugamarin F4	12-(2-methylbutyryloxy)
7	Ajugalide B	1-acetoxy-12-hydroxy
9	Ajugalide C	12-hydroxy
10	Ajugarin I	

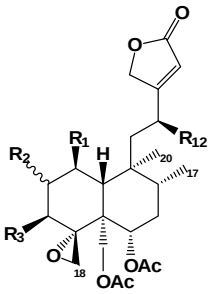

Figure 1: Structures of isolated *neo*-clerodane diterpenes (R_n = H, unless as indicated).

substitution (Table 1). On the basis of ^{1}H-^{13}C HMBC cross signals [δ_H 4.38/4.74 (H_2-19) and δ_H 2.12 (CH_3) to the same δ_C 171.0 (C=O) signal], an acetoxy group [δ_H 2.12 (C*H*$_3$C=O); δ_C 171.0 (C=O), 21.0 (C*H*$_3$C=O)] was confirmed as the C-19 substituent. Similarly, a δ_H 5.32/δ_C 175.1 cross signal established a 2-methylbutyryloxy group [δ_H 1.08 (d, CH_3)/δ_C 16.3 and δ_H 0.87 (t, CH_3)/ δ_C 11.3; δ_H 1.42 and 1.60 (CH_2)/δ_C 26.6; δ_H 2.29 (sext, CH)/δ_C 41.1 and δ_C 175.1 (C=O)] as a C-3 substituent. Overlapping with other signals did not allow a straightforward assignment of a second acetoxy group [δ_H 1.95 (C*H*$_3$C=O); δ_C 170.1 (C=O), δ_C 21.1, C*H*$_3$C=O)] as a C-6 substituent. In the stereogenic centers 3 and 6, both substituents must be present in the equatorial position to account for the large *trans* di-axial coupling constant shown by their respective geminal protons (12.1 Hz for H-3 and 10.9 Hz for H-6). A molecular formula, $C_{29}H_{42}O_9$ [ESI-MS 573.2534 ($C_{29}H_{42}O_9K$)], matched the number of signals in the ^{13}C NMR spectrum, accounting for 6 Me, 9 CH_2, 6 CH, and 8 quaternary carbons (including 3 C=O ester groups). Other characteristic chemical shifts and multiplicities were shown in the ^{1}H spectra (Table 1): a δ_H 4.74 doublet (J = 1.8 Hz; H_2-16) partly overlapping with a δ_H 4.76 doublet of doublets (J = 10.9; 5.2 Hz; H-6) and a δ_H 4.74 doublet (J = 12.3; H-19B); the H_2-18 AB system (δ_H 2.61 and 2.83; d, J = 4.3 Hz both); the H_3-17 doublet (δ_H 0.84; J = 5.4 Hz); and the H_3-20 singlet (δ_H 0.78). Thus, the structure of ajugaflorin A (**1**) was established as 3*β*-(2-methylbutyryloxy)ajugarin I.

Ajugaflorin B (**4**) showed in its ^{1}H NMR spectrum (Table 1) a large number of similarities to that of **1**. In fact, only the 2-methylbutyryloxy substituent pattern was replaced by a one-proton

Table 1: NMR data[a] of ajugaflorins A (**1**), B (**4**), D (**8**), C (**6**), E (**11**), and F (**12**) in $CDCl_3$.

	1		**4**	**8**	**6**		**11**	**12**	
position	δ_C, mult	δ_H, mult (*J*)	δ_H, mult (*J*)	δ_H, mult (*J*)	δ_C, mult	δ_H, mult (*J*)	δ_H, mult (*J*)	δ_C, mult	δ_H, mult (*J*)
1ax 1eq	20.1, CH_2	1.85, q[b]d (13.6, 3.9) 1.65, m	1.85, q[b]d (13.6, 4.4)	1.78, q[b]d (13.5, 4.0)	20.6, CH_2			20.6, CH_2	1.75, m 2.12, m
2ax 2eq	31.0, CH_2	1.45, m 2.15, m	1.46, q[b]d (12.6, 4.9)	1.32, q[b]d (12.5, 4.9)	30.7, CH_2		3.68, t[b]d (10.2, 5.7)	30.7, CH_2	1.47, m 2.10, m
3ax	66.3, CH	5.32, dd (12.1, 4.8)	5.31, dd (12.0, 4.9)	4.05, dd (11.7, 4.8)	66.7, CH		5.26, d (9.8)	66.7, CH	5.31, dd (12.1, 4.6)
4	65.3, C	-	-	-	65.4, C	-	-	65.4, C	-
5	45.7, C	-	-	-	46.0, C	-		46.0, C	-
6ax	71.5, CH	4.76, dd (10.9, 5.2)	4.76, dd (11.0, 5.3)	4.80, dd (10.7, 4.7)	71.6, CH	4.72, dd (10.4, 4.7)	4.72, dd (10.4, 4.7)	71.7, CH	4.80, dd (12.1, 5.2)
7ax 7eq	32.8, CH_2	1.56, m 1.61, m	-	-	32.8, CH_2			32.8, CH_2	1.55-1.70 1.55-1.70
8ax	34.7, CH	1.61, m	-	-	35.1, CH			35.1, CH	1.81, m
9	38.4, C	-	-	-	39.4, C	-	-	39.4, C	-
10ax	47.6, CH	1.45, m	1.46, dd (12.6, 3.6)	1.45, dd (12.7, 3.4)	48.3, CH			48.3, CH	2.02, dd (12.2, 2.4)
11a 11b	34.5, CH_2	1.58, m 1.61, m	-	-	42.7, CH_2			42.7, CH_2	1.46, m 1.91, dd (15.8, 9.9)
12a 12b	21.9, CH_2	2.10, m 2.27, m	-	-	65.2, CH	5.66, brd (9.4)		65.2, CH	4.78, d (ovl)
13	169.3, C	-			173.1, C	-	-	173.1, C	-
14	115.6, CH	5.85, quin[b] (1.6)	5.85, quin[b] (1.7)	5.85, quin[b] (1.7)	114.7, CH	5.92, t[b]d (1.9, 1.1)	5.86, t[b]d (1.9, 1.1)	114.7, CH	5.95, t[b]d (1.9, 1.1)
15	173.5, C	-			173.0, C	-	-	173.0, C	-
16a 16b	72.9, CH_2	4.74, d (1.8)	4.74, d (1.8)	4.75, d (1.8)	70.5, CH_2	4.71, dd (17.6, 1.8) 4.84, ddd (17.6, 2.0, 0.6)	4.74, d (1.9)	70.6, CH_2	4.85, dd (17.8. 1.9) 4.87, dd (17.8. 1.9)
17	15.2, CH_3	0.84, d (5.4)	0.84, d (5.9)	0.84, d (6.2)	15.3, CH_3	0.84, d (6.2)	0.85, d (5.7)	15.3, CH_3	0.82, d (6.7)
18a 18b	42.6, CH_2	2.61, d (4.3) 2.83, brd (4.3)	2.62, d (4.2) 2.84, dd (4.2, 0.3)	2.79, d (3.8) 2.90, d (3.9)	42.5, CH_2	2.16, d (4.0) 2.94, dd (4.0, 2.3)	2.58, d (4.3) 2.82, brd (4.3)	42.5, CH_2	2.58, d (4.3) 2.78, brd (4.3)
19a 19b	61.6, CH_2	4.38, d (12.3) 4.74, d (12.3)	4.39, brd (12.4) 4.73, d (12.3)	4.25, brd (12.2) 4.87, d (12.3)	61.6, CH_2	4.35, d (12.1) 4.82, d (12.1)	4.41, d (12.3) 4.74, d (12.3)	61.5, CH_2	4.38, d (12.4) 4.76, d (12.4)
20	17.2, CH_3	0.78, s	0.78, s	0.78, s	17.1, CH_3	0.75, s	0.80, s	17.1, CH_3	0.74, s
1’	175.1, C	-	-		175.2, C	-	-	175.2, C	-
2’	41.1, CH	2.29, sext[b] (7.0)	2.47, sept[b] (7.0)		41.1, CH	2.55, sept[b] (7.0)	2.37, sext[b] (6.8)	41.1, CH	2.29, sext[b] (6.9)
3’a 3’b	26.6, CH_2	1.42, m 1.60, m	1.108, d (7.0) 1.114, d (7.0)		26.6, CH_2	1.19, d (7.0) 1.21, d (7.0)	-	26.7, CH_2	1.42, m 1.60, m
4’	11.3, CH_3	0.87, t (7.5)	-		11.3, CH_3		0.90, t (7.4)	11.3, CH_3	0.87, t (7.5)
5’	16.3, CH_3	1.08, d (7.0)	-		16.4, CH_3		1.12, d (7.0)	16.4, CH_3	1.08, d (7.0)
1’’(6)	170.1, C	-	-		170.3, C	-	-	170.3, C	-
2’’	21.1, CH_3	1.95, s	1.96, s	1.96, s	21.2, CH_3	1.95, s	1.95, s	21.2, CH_3	1.95, s
1’’’(19)	171.0, C	-	-		171.1, C	-	-	171.1, C	-
2’’’	21.0, CH_3	2.12, s	2.13, s	2.10, s	21.0, CH_3	2.10, s	2.14, s	21.0, CH_3	2.12, s

[a] 499.81 MHz (^{1}H), 100.62 MHz (^{13}C) [δ in ppm (*J* in Hz)]; [b] apparent multiplicity [q[b] = ddd with $J_1 \approx J_2 \approx J_3$; quin[b] = tt ($J_1 \approx J_2$); sext[b] = ddq ($J_1 \approx J_2 \approx J_3$); sept[b] = qq ($J_1 \approx J_2$).

septuplet (δ_H 2.47, *J* = 7.0 Hz) and two methyl doublets (δ_H 1.108 and 1.114), pointing out the presence of an *iso*-butyryloxy group instead. Minor chemical shift changes modified the partial overlapping of the H_2-16 doublet (δ_H 4.74), the H-6 doublet of doublets (δ_H 4.76), and the H-19B doublet (δ_H 4.73). An apparent two proton quadruplet of doublets (but displaying an anomalous band ratio) at δ_H 1.46 was the result of H-2ax and H-10ax overlapping (an “apparent” quadruplet of doublets, *J* = 12.7; 4.9 Hz and a doublet of doublets, *J* = 12.7; 3.6 Hz, respectively). The same pattern was obscured in **1** owing to a further overlapping signal (H-3’a). Thus, ajugaflorin B (**5**) is the new 3*β*-(*iso*-butyryloxy)ajugarin I.

Ajugaflorin C (**6**) also showed in its ^{1}H NMR spectrum (Table 1) the presence of an *iso*-butyryloxy group (δ_H 2.55, *J* = 7.0 Hz, septuplet, 1H; δ_H 1.21 and 1.19, each a methyl doublet, but appearing as an almost perfect triplet). Other characteristic chemical shifts and multiplicities [Table 1: a ddd signal at δ_H 5.92 for H-14 (an apparent td)] were indicative of an ajugarin-like molecule with a C-12 *O*-acyl substitution [δ_H 5.66 broad doublet (H-12); δ_H 4.84 (ddd)/4.71 (dd) system for H_2-16; an overlapping δ_H 4.72 (dd, H-6); δ_H 4.35/4.82 (H_2-19 AB system); δ_H 2.16 (d)/2.94 (dd) (H_2-18 AB system in unsubstituted ring A with clear long-range coupling of H_B-18−H-3; the H_3-17 doublet (δ_H 0.84); and the H_3-20 singlet (δ_H 0.75)]. In fact, most chemical shifts of **6** matched those of ajugamarin F4 (**4**), the 12-(2-methylbutyryloxy) analog, also isolated from the extract [15]. Thus, the structure is the new 12-(*iso*-butyryloxy)ajugarin I (**6**).

Ajugaflorin D (**8**) showed in its ^{1}H NMR spectrum the presence of two common singlets for acetoxy groups (δ_H 2.10 and 1.96). As in **1**, it displayed a quintuplet-like signal at δ_H 5.85 (H-14, unsubstituted C-12), and a pair of broad doublets (δ_H 2.90 and 2.79) for H_2-18, supportive of C-3 substitution (Table 1). The H-3 doublet of doublets was shifted to δ_H 4.05 (δ_H 5.32 in **1**), indicative of a hydroxyl group as substituent. The signals of H-19B, H-6, and H_2-16 appeared well separated (δ_H 4.87 doublet, δ_H 4.80 doublet of doublets, and δ_H 4.75 doublet, respectively), whereas H_3-17 (d) and H_3-20 (s) were almost unaffected (δ_H 0.84 and 0.78, respectively). Thus, ajugaflorin D (**8**) is the new 3*β*-hydroxyajugarin I.

Ajugaflorins E and F (**11** and **12**) also showed a large number of similarities to **1** in their ^{1}H NMR spectra (Table 1). The 2-methylbutyryloxy substituent pattern appeared again, and one doublet for H-18B was supportive of C-3 substitution. Ajugaflorin E (**11**) also featured a δ_H 4.74 doublet (H_2-16) partly overlapping with a δ_H 4.72 doublet of doublets (H-6) and a δ_H 4.74 doublet (H-19B), the H_2-18 AB system (δ_H 2.58 and 2.82), the H_3-17 doublet (δ_H 0.85), and the H_3-20 singlet (δ_H 0.80). However, H-3 appeared as a δ_H 5.26 doublet (*J* = 9.8 Hz) compared with δ_H 5.32 (doublet of doublets in **1**), which pointed out a further substitution at the vicinal C-2. The H-2 signal was observed as a triplet (apparent) of doublets at δ_H 3.68, indicative of an equatorial α-hydroxyl group resulting in the substitution pattern of ajugapitin [18] for ring A. Thus, ajugaflorin E (**11**) is the new 2*α*-hydroxy-3*β*-(2-methylbutyryloxy) ajugarin I.

Ajugaflorin F (**12**) displayed in the decalin system a close pattern of chemical shifts and multiplicities as in **1**, except in the vicinity of the side chain (Table 1). A triplet (apparent) of doublets for the H-14 signal (δ_H 5.95) and the chemical shift of H-12 (δ_H 4.78, dd) were supportive of a C-12 hydroxy-substituted compound, as in

several ajugalides [16] and ajugamarins [19-21]. H_2-16 appeared as the AB subsystem of an ABX spin system with very close chemical shifts (calculated as δ_H 4.8704 and 4.8492; $\Delta\delta_H$ 10 Hz). Thus, ajugaflorin F (**12**) is the new 12-hydroxy-3*β*-(2-methylbutyryloxy)ajugarin I.

The structural elucidation of ajugamacrin E (**2**) [10], ajugatakasin B (**3**) [14], ajugamarin F4 (**5**) [15], ajugalides B (**7**) and C (**9**) [16], and the parent ajugarin I (**10**) [17] was based on ^{1}H NMR data in agreement with those previously reported.

Experimental

General experimental procedures*:* Optical rotations were measured in a Perkin-Elmer 341 polarimeter. ^{1}H NMR (499.81 MHz) and ^{13}C NMR (100.62 MHz) spectra were recorded on Inova 500/Mercury 400 spectrometers (Varian, Zug, Switzerland) using special low volume NMR tubes when required (Shigemi Co., Ltd., Tokyo, Japan: BMS-05 micro tube) in $CDCl_3$ under standard 1D and 2D conditions and pulse sequences. The HPLC work was performed on an Alliance 2695 apparatus coupled with a 996 UV diode array detector (Waters Corporation, Milford, MA). GEMINI 250 mm 5µm C_{18} 110Å columns (Phenomenex, Torrance, CA) were used for analytical (4.6 mm i.d., **column A**) or semi-preparative (10.0 mm i.d., **column B**) HPLC work at 0.50 or 2.0 mL/min flow and 35ºC. Different H_2O–MeOH mixtures were used as mobile phase according to fraction polarity. **System A1/B1**: H_2O–MeOH gradient from 35:65 to 15:85 in 20 min, held at 15:85 for 15 min, then back to initial conditions in 1 min, and column re-equilibration for 4 min. **System A2/B2**: H_2O–MeOH gradient from 40:60 to 10:90 in 30 min, then back to initial conditions and column re-equilibration as above. **System A3/B3**: H_2O–MeOH gradient from 45:55 to 15:85 in 30 min, then back to initial conditions and column re-equilibration as before. Fractions were collected every 20 s (letters A,B,C in any minute) on a Waters fraction collector III (Waters Corporation). Every fraction collected was further injected in analytical conditions and similar fractions combined before solvent evaporation. A C_{18} guard column was coupled to protect the integrity of the columns, both analytical and semi-preparative. Solvents used for extraction and chromatographic procedures were HPLC grade (Merck, Darmstadt, Germany), and water used in mobile phase mixtures was Milli Q (Millipore R, Billerica, MA). Silica gel 60 F_{254} aluminum sheets (Merck, Darmstadt, Germany) were used for TLC monitoring or further purification. New diterpenes were injected into an ACQUITY UPLC® (Waters Corporation) with ACQUITY-TUV and Q-TOF Premier™ mass spectrometer (Waters Corporation) detector, using ACQUITY UPLC BEH C18 1.7 µm 2.1x100 mm (**column C**), 30ºC, 0.3 mL/min flow, and water–acetonitrile containing a 0.1%, v:v, HCO_2H mobile phase, starting at 70:30 for 5 min, followed by a 3 min gradient up to 50:50, held for 5 min more and then back to initial conditions in 2 min, followed by 5 more min of column re-equilibration. Positive and negative electrospray ionization were used, and UV absorption recorded at 215 and 360 nm. One µL of 0.1 mg/mL pure compound solutions were injected and referred externally for accurate mass results. Silica gel (**a**: 60-120 mesh, E-Merck; **b:** 230-400 mesh, Sigma-Aldrich) was used for column chromatography (CC). Formic acid and solvents used for extraction and CC (HPLC grade) were from Merck (Darmstadt, Germany). Water was produced in-house (Milli-Q Water Purification System, Millipore). $CDCl_3$ (99.8 % D, <0.01 % H_2O) was purchased from Euriso-top (Gif-sur-Yvette, France).

Plant material: *A. macrosperma* var. *breviflora* was collected from Kumaun region (29°26′19.27″ N and 79°06′07.22″ E) of Uttarakhand State, India, during February 2008. The plant was identified by Prof. D. S. Rawat (Plant Taxonomist, Department of Biological Science, C. B. S. H. G. B. Pant University of Agriculture & Technology, Pantnagar). A voucher specimen (herbarium number Chem 1504) was deposited in the Department of Biological Science, C. B. S. H. G. B. Pant University of Agriculture & Technology, Pantnagar), India. The plants were dried in shade.

Extraction and isolation**:** Powdered air dried whole plants (1 Kg) were extracted with methanol (x3, 5 L; 24 h percolation) at room temperature. The extracts were combined and evaporated under vacuum to give 123.5 g of dark green black crude extract. This was shaken with a mixture of methanol and water (1:1, 1 L) and defatted with *n*-hexane (x3, 500 mL) and then partitioned against CH_2Cl_2 (x3, 500 mL). The residue after solvent evaporation (25.0 g out of 26.2) was filtered through a silica gel column (**a**, 500 g) prepacked in CH_2Cl_2 and eluted with a CH_2Cl_2/acetone gradient system [800 mL each of 95:5, 90:10, 80:20, 60:40, 50:50, 40:60, 30:70 and 0:100; 200 mL fractions were collected and combined as follows based on TLC analysis: F-1 (f 1-2) 2.01 g, F-2 (f 3-9), 9.60 g, F-3 (f 10-17), 2.97 g, F-4 (f 18-20) 0.4 g, F-5 (f 21-25), 5.80 g]. The dark brown-black amorphous material from F 26 and onwards was not processed any further. Fraction F-1 was again filtered through a silica gel column (**b:** 30 g) and eluted with CH_2Cl_2/acetone gradient system (98:2, 60 mL; 95:5, 100 mL; 90:10, 45 mL; 85:15, 40 mL; 80:20, 30 mL and 0:100, 40 mL; 10 mL fractions were collected) and based on TLC analysis were combined to afford 13 pooled fractions. *neo*-Clerodanes were only isolated from F-1-10 (177 mg). HPLC (system B1) afforded ajugaflorin A, (**1**; fractions 19C to 21A), ajugamacrin E [10] (**2**, fractions 24BC), ajugatakasin B [14] (**3**, fractions 31C/32A) and a minor amount of ajugaflorin B (**4**; fraction 16A). Semi-preparative HPLC of F-2 (system B2) afforded further amounts of **1**-**4**, ajugamarin F4 [15] (**5**; fraction 23AB) and ajugaflorin C (**6**; mixed with **4** in fractions 15AB**;** separated by TLC). Similarly, fraction C (system C2) yielded ajugalide B [16] (**7**; fraction 15A), ajugaflorin D (**8**; from TLC of fraction 16B), ajugalide C [16] (**9**; fraction 18B), ajugarin I [17] (**10**; fraction 20A), ajugaflorin E (**11**; fractions 22AB), and ajugaflorin F (**12**; fractions 27ABC). ^{1}H and ^{13}C NMR data obtained for all known compounds were in agreement with those previously reported.

Ajugaflorin A (**1**)
White amorphous solid (7.5 mg).
$[\alpha]^{20}_D$: -34.6 (*c* 0.434, $CHCl_3$).
^{1}H and ^{13}C NMR: Table 1.
HRMS-ESI *m/z*: 573.2534 $[M + K]^+$ (calcd for $C_{29}H_{42}O_9K$, 573.2454).

Ajugaflorin B (**4**)
White amorphous solid (2.0 mg).
$[\alpha]^{20}_D$: -51.6 (*c* 0.184, $CHCl_3$).
^{1}H NMR: Table 1.
MS data: *m/z*: 521 $[M + H]^+$ (calcd for $C_{28}H_{41}O_9$, 521.2739), 538 $[M + NH_4]^+$, 543 $[M + Na]^+$; 565 $[M + HCOO]^-$.

Ajugaflorin C (**6**)
White amorphous solid (1.6 mg).
$[\alpha]^{20}_D$: -25.9 (*c* 0.059, $CHCl_3$).
^{1}H NMR: Table 1.
MS data: *m/z*: 521 $[M + H]^+$ (calcd for $C_{28}H_{41}O_9$, 521.2739).

Ajugaflorin D (**8**)
White amorphous solid (0.7 mg).
$[\alpha]^{20}_D$: -16.2 (*c* 0.068, $CHCl_3$).
^{1}H NMR: Table 1.
HR-MS-ESI *m/z*: 451.2373 $[M + H]^+$, (calcd for $C_{24}H_{35}O_8$, 451.2322).

Ajugaflorin E (**11**)
White amorphous solid (3.9 mg).
$[\alpha]^{20}_{D}$: -35.0 (*c* 0.369, $CHCl_3$).
^{1}H NMR: Table 1.
HR-MS-ESI *m/z*: 551.2855 $[M + H]^+$ (calcd for $C_{29}H_{43}O_{10}$, 551.2844); 573.2663 $[M + Na]^+$, 1123.5465 $[2M + Na]^+$.

Ajugaflorin F (**12**)
White amorphous solid (3.6 mg).
$[\alpha]^{20}_{D}$: -19.7 (*c* 0.350, $CHCl_3$).
^{1}H and ^{13}C NMR: Table 1

Supplementary data: NMR spectra (with enlarged detailed sections for multiplets) are available as "Supplementary Data".

Acknowledgments - Financial support from the Spanish MCYT (project AGL2004/05252 and associated Beca Predoctoral BES-2005-9474 to A.C.R) are gratefully acknowledged.

References

[1] Kubo I, Lee YW, Balogh-Nair V, Nakanishi K, Chapya A. (**1976**) Structure of ajugarins. *Journal of the Chemical Society, Chemical Communications*, 949-950.
[2] www.eFloras.org [accessed May 2011: Flora of China, ***17***, 69]
[3] Ball PW. (**1973**) *Ajuga* L. In *Flora Europaea III. Diapensiaceae to Myoporaceae*. Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA. (Eds). Cambridge University Press, Cambridge (UK),128-129.
[4] Babu CR. (**1977**) *Herbaceous flora of Dehra Dun*. Publication and information directorate CSIR. New Delhi, 405.
[5] Strachey R. (**1974**) Catalogue of the plants of Kumaon and of adjacent portions of Garhwal and Tibet: based on the collections made by Strachey and Winterbottom during the years 1846 to 1849 and on the catalogue originally prepared in 1852. Bishen Singh Mahendra Pal Singh, Dehradun.
[6] Wu CY. (**1977**) Flora Yunnanica. In *Botany YIs*. Tomus Wall Science Press, Peking (China).
[7] Hu K, Dong A, Liu H, Feng H, Sun Q, Yao X. (**1999**) Bioactivity of traditional Chinese herbal medicines against *Pyricularia oryzae. Pharmaceutical Biology*, ***37***, 225-230.
[8] Zhao Q, Cui Ch, Cai B, Qiu F, Guo T, Yao X. (**2005**) Cell cycle inhibitory activity of 137 traditional Chinese herbal medicines against tsFT210 cell line. *Pharmaceutical Biology*, ***43***, 5-9.
[9] Shen X, Isogai A, Furihata K, Sun H, Suzuki A. (**1993**) Two neo-clerodane diterpenoids from *Ajuga macrosperma*. *Phytochemistry*, ***33***, 887-889.
[10] Shen X, Isogai A, Furihata K, Sun H, Suzuki A. (**1993**) *Neo*-clerodane diterpenoids from *Ajuga macrosperma* and *Ajuga pantantha. Phytochemistry*, ***34***, 1091-1094.
[11] Castro A, Coll J, Tandrón YA, Pant AK, Mathela CS. (**2008**) Phytoecdysteroids from *Ajuga macrosperma* var. *breviflora* roots. *Journal of Natural Products*, ***71***, 1294-1296.
[12] Dhinda B, Banerjee J, Guha S. (**1997**) Triterpenes from *Ajuga macrosperma* Wall. *Journal of the Indian Chemical Society*, ***74***, 424.
[13] Coll J, Tandrón Y. (**2008**) *neo*-Clerodane diterpenoids from *Ajuga:* structural elucidation and biological activity. *Phytochemistry Reviews*, ***7***, 25-49.
[14] Amano T, Nishida R, Kuwahara Y. (**1997**) Ajugatakasins A and B, new diterpenoids from *Ajuga decumbens*, and feeding stimulative activity of related neoclerodane analogs toward the turnip sawfly. *Bioscience, Biotechnology and Biochemistry*, ***61***, 1518-1522.
[15] Shimomura H, Sashida Y, Ogawa K. (**1989**) Neo-clerodane diterpenes from *Ajuga decumbens. Chemical and Pharmaceutical Bulletin*, ***37***, 996-998.
[16] Chan YY. (**2005**) Neoclerodane diterpenes from *Ajuga taiwanensis. Chemical and Pharmaceutical Bulletin*, ***53***, 164-167.
[17] Coll J. (**2002**) NMR shift data of *neo*-clerodane diterpenes from the genus *Ajuga. Phytochemical Analysis*, ***13***, 372-380.
[18] Hernández A, Pascual C, Sanz J, Rodríguez B. (**1982**) Diterpenoids from *Ajuga chamaepitys:* Two neo-clerodane derivatives. *Phytochemistry*, ***21***, 2909-2911.
[19] Shimomura H, Sashida Y, Ogawa K, Iitaka, Y. (**1983**) The chemical constituents of Ajuga plants. I. *neo*-Clerodane from the leaves of *Ajuga nipponensis* Makino. *Chemical and Pharmaceutical Bulletin*, ***31***, 2192-2199.
[20] Shimomura H, Sashida Y, Ogawa K. (**1989**) Neo-clerodane diterpenes from *Ajuga nipponensis. Chemical and Pharmaceutical Bulletin*, ***37***, 354-357.
[21] Shimomura H, Sashida Y, Ogawa K. (**1989**) Neo-clerodane diterpenes from *Ajuga ciliata* var. *villosior. Chemical and Pharmaceutical Bulletin*, ***37***, 988-992.