Two New *neo*-Clerodane Diterpenes in *Ajuga decumbens*

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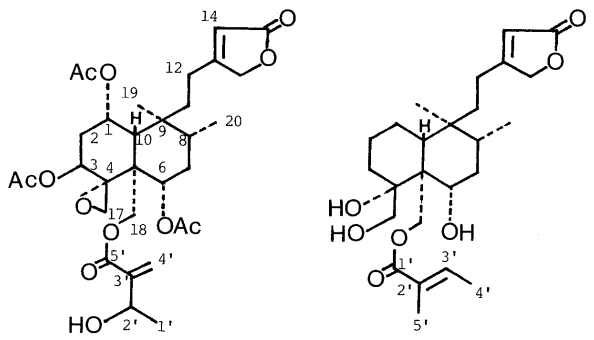
Study on the chemical constituents of *Ajuga decumbens* resulted in the isolation of two new minor *neo*-clerodane diterpenes, ajugacumbins E and F, together with a known diterpene, ajugamarin and an iridoid, 8-acetyl-harpagide. By means of spectroscopic analysis, structures of the new diterpenes were determined to be 1α,3α,6α-triacetoxy-4,7-epoxy-18α-(2′-hydroxy-3′-methylene-butyryloxy)-*neo*-cleroda-13-en-15,16-olide for ajugacumbin E, and 4α,6α-dihydroxy-4β-hydroxymethyl-18α-tigloyloxy-*neo*-cleroda-13-en-15,16-olide for ajugacumbin F.

Keywords *Ajuga decumbens*; Labiatae; *neo*-clerodane diterpene; ajugacumbin E; ajugacumbin F, ajugamarin

In a previous paper,[1] we elucidated the structures of four new *neo*-clerodane diterpenes, ajugacumbins A—D, isolated from *Ajuga decumbens* (Labiatae) by spectroscopic means. The absolute configuration of ajugacumbin A was established by X-ray analysis. These diterpenes were also characterized as an insect antifeedant against the larvae of *Pareda vesta* feeding on leaves of *Boehmeria nivea*. During further study on *neo*-clerodane diterpenes in *A. decumbens*, two new minor diterpenes were isolated in addition to a known diterpene, ajugamarin, and an iridoid, 8-acetylharpagide (Chart 1). This paper elucidates the structure of these compounds.

Compound **1** (ajugacumbin E), mp 149—150 °C, was obtained as colorless crystals and showed $M^+ + 1$ at *m/z* 607 in the fast atom bombardment mass spectrometry (FAB-MS), which corresponds to $C_{31}H_{42}O_{12}$. Comparison of the proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectral data on **1** with those of ajugacumbin C[1] suggested that **1** possessed the same structural skeleton and ajugacumbin C with the exception of a tigloyl group. In **1**, AB system signals at δ 6.38 (1H, d, J=2.8 Hz), and 6.41 (1H, d, J=2.8 Hz) assignable to two protons at C-4′ in a low field region of the ^{1}H–^{1}H correlation spectroscopy (COSY) (Table I), two signals at δ 125.7 as a triplet assigned to C-4′ and 141.6 as a singlet assigned to C-3′ in the ^{13}C-NMR spectrum (Table II) indicated the presence of a terminal olefinic bond. A signal at δ 5.01 as a quartet (J=6.8 Hz) showed the presence of a methine bearing a hydroxyl group, which connects with an olefinic bond and methyl group such as C=C–CH(OH)–CH_3. Signals of the methyl group (C-1′) were observed at δ 1.32 as a doublet peak (J=6.8 Hz) and of the hydroxyl group at 9.0 in a broad singlet, respectively. The ester moiety of **1** was, therefore, concluded to be 2-hydroxy-3-methylene-



1 **2**

Chart 1

TABLE I. The ^{1}H-NMR Spectral Data of Compounds **1** and **2** ($CDCl_3$, δ: J Value Are Shown in Hz)

	1	**2**
1β-H	5.73 (m)	*a*)
	$W_{1/2}$=15.0	
2β-H	2.63 (ddd)	*a*)
	$J_{2\beta,2\alpha}$=16.0	
	$J_{2\beta,3\alpha}$=10.0	
	$J_{2\beta,1\beta}$=5.0	
3α-H	5.87 (ddd)	2.35 (dddd)
	$J_{3\alpha,2\beta}$=10.0	$J_{3\alpha,3\beta}$=15.3
	$J_{3\alpha,2\alpha}$=4.0	$J_{3\alpha,2\alpha}$=5.0
	$J_{3\alpha,17}$=1.5	$J_{3\alpha,2\beta}$=9.7
		$J_{3\alpha,17}$=1.5
6β-H	4.68 (dd)	3.95 (dd)
	$J_{6\beta,7\alpha}$=12.0	$J_{6\beta,7\alpha}$=11.7
	$J_{6\beta,7\beta}$=4.0	$J_{6\beta,7\beta}$=4.0
10-H	*a*)	*a*)
12-H	*a*)	*a*)
14-H	5.93 (t)	5.84 (t)
	$J_{14,16}$=1.5	$J_{14,16}$=1.5
16-H_2	4.72 (dd) and 4.82 (dd)	4.75 (d)
	$J_{16,16}$=17.6	$J_{16,14}$=1.5
	$J_{16,14}$=1.5	
17-H_2	2.29 (d) and 3.01 (dd)	3.84 (d) and 3.98 (dd)
	$J_{17,17}$=3.5	$J_{17,17}$=11.7
	$J_{17,3\alpha}$=1.5	$J_{17,3\alpha}$=1.5
18-H_2	4.43 and 4.97	4.68 and 4.93
	$J_{18,18}$=12.7	$J_{18,18}$=13.2
19-H_3	0.80 (s)	0.83 (s)
20-H_3	0.86 (d)	0.87 (d)
	$J_{20,8}$=5.9	$J_{20,8}$=6.6
1′-H_3	1.32 (d)	—
	$J_{1',2'}$=6.8	
2′-H	5.01 (q)	—
	$J_{2',1'}$=6.8	
3′-H	—	6.83 (q)
		$J_{3',4'}$=6.6
4′-H_2 or H_3	6.38 and 6.41	1.83 (d)
	$J_{4',4'}$=2.8	$J_{4',3'}$=6.6
5′-H_3		1.83 (s)
OAc	1.69 (s)	
	2.14 (s)	
	2.19 (s)	
OH	9.0 (br s)	
OH×3		3.29 (br s)

a) Indicates unassignable.

TABLE II. ^{13}C-NMR (DEPT) Spectral Data on Compounds **1** and **2** ($CDCl_3$, ppm)

	1	2
1	70.7 (d)	22.3 (t)
2	40.3 (t)	20.7 (t)
3	66.8 (d)	30.3 (t)
4	64.0 (s)	78.7 (s)
5	45.9 (s)	47.7 (s)
6	71.3 (d)	74.6 (d)
7	30.2 (t)	31.5 (t)
8	35.1 (d)	35.3 (d)
9	38.9 (s)	38.8 (s)
10	51.0 (d)	44.1 (d)
11	31.9 (t)	35.4 (t)
12	32.2 (t)	35.6 (t)
13	172.9 (s)	173.8 (s)
14	116.2 (d)	115.4 (d)
15	168.4 (s)	169.9 (s)
16	70.8 (t)	73.1 (t)
17	48.6 (t)	64.3 (t)
18	61.6 (t)	63.3 (t)
19	16.9 (q)	19.0 (q)
20	15.4 (q)	15.6 (q)
1′	18.1 (q)	168.3 (s)
2′	78.4 (d)	128.3 (s)
3′	141.6 (s)	138.4 (d)
4′	125.7 (t)	14.5 (q)
5′	164.8 (s)	12.1 (q)
$COCH_3$	21.1 (q)	
	21.1 (q)	
	21.2 (q)	
$COCH_3$	169.6 (s)	
	169.8 (s)	
	170.5 (s)	

butyryloxyl group, which locates at C-18 in an α-configuration. The structure of ajugacumbin E, then, could be formulated as 1α,3α,6α-triacetoxy-4,7-epoxy-18α-(2′-hydroxy-3′-methylene-butyryloxy)-*neo*-cleroda-13-en-15,16-olide (**1**).

Compound **2** (ajugacumbin F), mp 198—201 °C, was obtained as colorless crystals and showed $M^+ + 1$ at *m/z* 451 in the FAB-MS, which corresponds to $C_{25}H_{38}O_7$. The ^{1}H- and ^{13}C-NMR spectra of **2** were fundamentally identical with those of ajugacumbin B[1] except for an epoxyl group located at C-4 and C-17. In **2**, the ^{1}H-NMR spectrum exhibited an AB system signal at δ 3.84 and 3.98 (J_{gem} = 11.7 Hz) assignable to the protons at C-17 [ajugacumbin B: H_2-17; δ 2.45 and 3.24 in doublet (J = 4.0 Hz)] and three hydroxyl groups at δ 3.29. The ^{13}C-NMR spectrum revealed a primary carbon assigned to C-18 at δ 63.3 in triplet and a quarternary carbon assigned to C-4 at δ 78.6 in singlet. The above data indicated the presence of a primary alcohol and a tertiary alcohol both attaching at C-4. The slightly broadened signal assigned to H-17 showed a W-coupling between H-17 and H-3α (δ 2.35 in dddd) which indicated that the primary alcohol is in a β-configuration. Therefore, the structure of **2** could be characterized as 4α,6α-dihydroxy-4β-hydroxymethyl-18α-tigloyloxy-*neo*-cleroda-13-en-15,16-olide.

Compound **3**, mp 90—92 °C, was obtained as an amorphous powder and indicated M^+ at *m/z* 548 in the electron ionization MS (EI-MS). The spectral data on **3** were closely similar to those of ajugamarin.[2] The structure of **3** was identified as ajugamarin. Comparison of compound **4** showed it to be identical with 8-acetylharpagide in its spectral and physical data.[3,4]

Experimental

Plant Material *Ajuga decumbens* Thung was collected in Jiangxi province, China. The voucher specimens are deposited in the Herbarium of China Pharmaceutical University.

Extraction and Isolation of Compounds 1—4 The whole plants (8 kg, dried naturally and pulverized) of *A. decumbens* were extracted with EtOH three times under reflux. The combined extract was concentrated, and then a certain volume of H_2O was added to it. After agitation, the suspension was extracted with $CHCl_3$. The concentrated $CHCl_3$ extract (100 g) was subjected to column chromatography on silica gel eluted with a solution of AcOEt in petroleum ether. After removal of crude crystals composed of ajugacumbins A—D from fractions, the combined mother liquid was separated by preparative thin layer chromatography (TLC) to give **1** (22 mg), **2** (17 mg), and **3** (10 mg), respectively. From a *n*-BuOH extract of the suspension, compound **4** (5.7 g) was obtained after chromatography on silica gel eluted with $CHCl_3$–MeOH (5 : 1).

Compound 1, Ajugacumbin E Colorless crystals, mp 149—150 °C (AcOEt–C_6H_{12}). M.W. 606 for $C_{31}H_{42}O_{12}$. Ultraviolet spectrum (UV) λ_{max}^{MeOH} nm (log ε): 213 (4.05). Infrared spectrum (IR) ν_{max}^{KBr} cm^{-1}: 3400, 1778, 1730, 1638, 1243, 1024. The ^{1}H- and ^{13}C-NMR spectral data are listed in Tables I and II. FAB-MS *m/z*: 607 ($M^+ - 1$). EI-MS *m/z* (rel. int.): 606 (M^+, 0.02), 604 (0.1), 588 (0.1), 533 (0.5), 505 (1.2), 489 (1.1), 448 (4.7), 405 (6.3), 340 (5.2), 201 (13), 171 (26.7), 126 (24.5), 99 (38.8), 71 (100).

Compound 2, Ajugacumbin F Colorless crystals, mp 198—201 °C (AcOEt–C_6H_{12}). M.W. 450 for $C_{25}H_{38}O_7$. UV λ_{max}^{EtOH} nm (log ε): 216 (4.23). IR ν_{max}^{KBr} cm^{-1}: 1760, 1730, 1698, 1620, 1250, 1115, 870, 731. The ^{1}H- and ^{13}C-NMR spectral data are shown in Tables I and II. FAB-MS *m/z*: 451 ($M^+ + 1$). EI-MS *m/z* (rel. int.): M^+ (absent), 419 (3.5), 401 (2.1), 319 (2.8), 301 (4.7), 222 (1.8), 175 (4.3), 111 (22.1), 83 (100), 55 (46.6).

Compound 3, Ajugamarin An amorphous powder, mp 90—92 °C (MeOH). M.W. 548 for $C_{29}H_{40}O_{10}$. UV λ_{max}^{EtOH} nm (log ε): 217 (4.16). IR ν_{max}^{KBr} cm^{-1}: 3450, 2928, 1780, 1730, 1639, 1365, 1242, 1123, 729. ^{1}H-MMR (270 MHz, $CDCl_3$) δ: 0.84 (3H, d, J = 5.1 Hz, H-20), 0.85 (3H, s, H-19), 1.79 (3H, s, OAc), 1.81 (3H, d, J = 7.1 Hz, H-4′), 1.84 (3H, s, H-5′), 2.16 (3H, s, OAc), 2.33 (1H, d, J = 3.7 Hz, H-17), 3.27 (1H, br s, OH), 4.45 (1H, d, J = 12.8 Hz, H-18), 4.7—4.8 (2H, m, H-12), 4.71 (1H, br d, J = 11.0 Hz, $W_{1/2}$ = 5.0 Hz, H-6), 4.79 (2H, d, J = 1.5 Hz, H-16), 4.93 (1H, d, J = 12.8 Hz, H-18), 5.88 (1H, dt, J = 11.0, 5.6 Hz, H-1β), 6.73 (1H, q, J = 7.1 Hz, H-3′), other protons were not assignable. ^{13}C-NMR (67.5 MHz, $CDCl_3$) δ: 12.5 (q, C-4′), 14.4 (q, C-5′), 15.4 (q, C-20), 17.4 (q, C-19), 21.2 (q, 2 × $COCH_3$), 30.4 (t, C-7), 32.0 (t, C-2), 32.8 (t, C-3), 34.8 (d, C-8), 39.3 (s, C-9), 42.7 (t, C-11), 46.0 (s, C-5), 49.3 (t, C-17), 51.0 (d, C-10), 56.7 (d, C-12), 62.2 (t, C-18), 64.2 (s, C-4), 71.2 (d, C-1), 71.6 (d, C-6), 72.8 (t, C-16), 114.4 (d, C-14), 129.0 (s, C-2′), 138.6 (d, C-3′), 166.1 (s, C-1′), 170.0 (s, $COCH_3$), 170.4 (s, $COCH_3$), 173.1 (s, C-15), 173.8 (s, C-13).

Compound 4, 8-Acetylharpagide Colorless crystals, mp 152—153 °C (MeOH). M.W. 406 for $C_{17}H_{26}O_{11}$. UV λ_{max}^{EtOH} nm (log ε): 208 (3.33). IR ν_{max}^{KBr} cm^{-1}: 3437, 2981, 2915, 1711, 1651, 1375, 1237, 1109, 1076, 1009, 989. ^{1}H-NMR (90 MHz, CD_3OD) δ: 6.02 (1H, d, $J_{12,9}$ = 1 Hz, H-1α), 6.35 (1H, d, $J_{3,4}$ = 6.5 Hz, H-3), 4.88 (1H, dd, $J_{4,3}$ = 6.5, $J_{4,5\alpha}$ = 1 Hz, H-4), 3.68 (1H, d, $J_{6\alpha,7\alpha}$ = 15 Hz, H-6α), 1.88 (1H, dd, $J_{7\alpha,7\beta}$ = 15, $J_{7\alpha,6\beta}$ = 4.5 Hz, H-7α), 2.15 (1H, d, $J_{7\beta,7\alpha}$ = 15 Hz, H-7β), 2.80 (1H, br s, H-9), 1.40 (3H, s, Me-10), 4.53 (1H, d, $J_{1'2'}$ = 8 Hz, H-1′), 3.85 (1H, d, $J_{6'a,6'b}$ = 12 Hz, H-6′a), 3.59 (1H, d, $J_{6'b,6'a}$ = 12 Hz, H-6′b), 1.95 (3H, s, OAc). ^{13}C-NMR (CD_3OD, INEPT) δ: 93.9 (d, C-1), 143.2 (d, C-3), 106.2 (d, C-4), 72.7 (d, C-5), 77.4 (d, C-6), 45.4 (t, C-7), 87.9 (d, C-3′), 71.0 (d, C-4′), 76.9 (d, C-5′), 62.3 (t, C-6′), 21.8 (q, $COCH_3$), 172.6 (s, $COCH_3$).

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