Four New Insect Antifeedant *neo*-Clerodane Diterpenoids, Ajugacumbins A, B, C and D, from *Ajuga decumbens*

MIN Zhi-da,*^a WANG Shi-qiang,^a ZHENG Qi-tai,^b WU Bing,^b Mizuo MIZUNO,^c Toshiyuki TANAKA,^c and Munekazu IINUMA^c

Department of Phytochemistry, China Pharmaceutical University,^a Nanjing, China, Institute of Materia Medica, Chinese Academy of Medical Sciences,^b Beijing, China, and Department of Pharmacognosy, Gifu Pharmaceutical University,^c 6-1 Mitahora-higashi 5 chome, Gifu 502, Japan.

Received March 20, 1989

From the ethanol extract of whole herbs of *Ajuga decumbens* (Labiatae), four new *neo*-clerodane diterpenoids, named ajugacumbins A, B, C and D (1—4), were isolated. The configuration of 1 was determined to be 6 α -acetoxy-4,17-epoxy-18-tigloyloxy-*neo*-cleroda-13-en-15,16-olide by X-ray analysis. The other structures were also determined by spectroscopic analysis to be 4,17-epoxy-6 α -hydroxy-18-tigloyloxy-*neo*-cleroda-13-en-15,16-olide (2), 1 α ,3 β ,6 α -triaceoxy-4,17-epoxy-18-tigloyloxy-*neo*-cleroda-13-en-15,16-olide (3) and 6 α -acetoxy-4,17-epoxy-3 β -hydroxy-18-tigloyloxy-*neo*-cleroda-13-en-15,16-olide (4). These compounds displayed insect antifeedant activity.

Keywords *Ajuga decumbens*; Labiatae; X-ray analysis; *neo*-clerodane diterpene; ajugacumbin A; ajugacumbin B; ajugacumbin C; ajugacumbin D; insect antifeedant activity

The whole herb of *Ajuga decumbens* THUNB. (Labiatae) (白毛夏枯草, bái máo xià kū cǎo) ("kiranso" in Japanese) has been used for the treatment of sore throat, removing phlegm and alleviating fever as a folkloric crude drug. It tastes extremely bitter. The plant is distributed extensively in the southern area of China.¹⁾ Up to the present, many compounds have been isolated from *Ajuga* species and their structures have been characterized; *neo*-clerodane diterpenes (ajugarins I—V,^{2–4)} and ajugamarins A1 and B1⁵⁾ C1, B2 and B3⁶⁾, phytoecdysones (ajugalactone,⁷⁾ cyasterone,⁸⁾ and ajugasterone C⁹⁾ and iridoid glycosides (decumbesides A—D),¹⁰⁾ with activities as insect antifeedants, insect molting inhibitors and bitter principles. From the ethanol extract of *A. decumbens*, four new *neo*-clerodane diterpenes, named ajugacumbins A, B, C and D, have been isolated. The present paper describes the structure elucidation of the new compounds.

Compound 1, ajugacumbin A, was obtained as colorless crystals, mp 185—186 °C, $[\alpha]_D^{28} = +38.7^\circ$ ($c=0.9$, CHCl₃). A signal, (M+1) m/z 475, in the fast atom bombardment mass spectrum (FAB-MS) showed the molecular formula

to be C₂₇H₃₈O₇. The proton nuclear magnetic resonance (¹H-NMR) spectrum exhibited signals [δ_H : 5.81 (1H, t, $J_{14,16} = 1.6$ Hz, H-14) and 4.72 (2H, d, $J_{16,14} = 1.6$ Hz, H-16)], and in the carbon-13 NMR (¹³C-NMR) spectrum four peaks δ_C 173.6 (s, C-13), 115.5 (d, C-14), 170.1 (s, C-15) and 73.0 (t, C-16) were also seen, which indicated the presence of a conjugated γ -lactone moiety bearing an α -H, as in ajugamarins.^{5,6)} Its ultraviolet (UV) [218 nm (log ϵ 4.2)] and infrared (IR) spectra (1779, 1744 and 1651 cm⁻¹) also supported the partial structure. The signals of an AB system at δ_H 2.96 (dd, $J=4.0$, 2.0 Hz, H-17, having W

- 1: R₁ = OAc, R₂ = R₃ = H
 2: R₁ = OH, R₂ = R₃ = H
 3: R₁ = R₂ = R₃ = OAc
 4: R₁ = OAc, R₂ = H, R₃ = OH

Chart 1

TABLE I. ¹H-NMR Spectral Data for Compounds 1, 2, 3 and 4 (CDCl₃, ppm; J Values Are Shown in Hz)

	1	2	3	4
1 β -H	a)	a)	5.69, m, $W_{1/2} = 15.0$	a)
2 β -H	a)	a)	2.71, ddd, $J_{2\beta,2\alpha} = 15.0$, $J_{2\beta,3\alpha} = 10.0$, $J_{2\beta,1\beta} = 4.0$	a)
3 α -H	a)	a)	5.85, dd, $J_{3\alpha,2\beta} = 10.0$, $J_{3\alpha,2\alpha} = 4.0$	4.79, dd, $J_{3\alpha,2\beta} = 9.8$, $J_{3\alpha,2\alpha} = 4.0$
6 β -H	4.68, dd, $J_{6\beta,7\alpha} = 10.0$, $J_{6\beta,7\beta} = 4.0$	3.57, dd, $J_{6\beta,7\alpha} = 10.0$, $J_{6\beta,7\beta} = 4.0$	4.67, dd, $J_{6\beta,7\alpha} = 11.7$, $J_{6\beta,7\beta} = 3.5$	4.75, dd, $J_{6\beta,7\alpha} = 11.7$, $J_{6\beta,7\beta} = 4.4$
14-H	5.81, t, $J_{14,16} = 1.6$	5.84, t, $J_{14,16} = 1.6$	5.88, t, $J_{14,16} = 1.5$	5.94, t, $J_{14,16} = 1.6$
16-H ₂	4.72, d, $J_{16,14} = 1.6$	4.75, d, $J_{16,14} = 1.6$	4.79, dd, and 4.72, dd, $J_{16,16'} = 17.6$, $J_{16,14} = 1.5$	4.87, d, $J_{16,14} = 1.6$
17-H	2.96 and	3.24 and	3.00 and	2.93 and
17-H'	2.19, AB, $J_{17,17'} = 4.0$	2.45, AB, $J_{17,17'} = 4.0$	2.28, AB, $J_{17,17'} = 4.0$	2.21, AB, $J_{17,17'} = 3.1$
18-H	4.84 and	4.58, s	4.98 and	4.89 and
18-H'	4.37, AB, $J_{18,18'} = 12.0$	4.58, s	4.43, AB, $J_{18,18'} = 12.7$	4.42, AB, $J_{18,18'} = 12.2$
19-CH ₃	0.75, s	0.77, s	0.79, s	0.78, s
20-CH ₃	0.79, d, $J = 6.0$	0.86, d, $J = 5.5$	0.85, d, $J = 5.9$	0.82, d, $J = 6.8$
3'-H	7.03, q, $J = 7.0$	6.98, q, $J = 7.0$	6.95, q, $J = 7.0$	7.07, q, $J = 7.3$
4'-CH ₃	1.76, d, $J = 7.0$	1.79, d, $J = 7.0$	1.80, d, $J = 7.0$	1.80, d, $J = 7.3$
5'-CH ₃	1.84, s	1.86, s	1.88, s	1.87, s
OAc	1.83, s		1.96, s, 2.14, s, 2.18, s	1.88, s
OH		3.44, br s		2.63, br s

a) Means unassignable.

coupling with 3-axial proton) and 2.19 (d, $J=4.0$ Hz) were attributable to the exocyclic epoxide group. The signal patterns of the ^1H - and ^{13}C -NMR spectra were closely related to those of ajugarin I²⁾ except for the number of acetoxy and tigloyloxy groups. The number of them was confirmed by the following ^1H - and ^{13}C -NMR spectral data; δ_{H} 1.83 (3H, s), and δ_{C} 169.7 (s) and 21.1 (q) due to one acetyl group: δ_{H} 7.03 (1H, q, $J=7.0$ Hz, H-3'), 1.76 (3H, d, $J=7.0$ Hz, CH_3 -4') and 1.84 (3H, s, CH_3 -5'), and δ_{C} 168.0 (s, C-1'), 128.6 (s, C-2'), 138.0 (d, C-3'), 14.5 (q, C-4') and 12.0 (q, C-5') due to one tigloyl group. Furthermore, the signals at δ_{H} 4.84 ($J=12.0$ Hz) and 4.37 ($J=12.0$ Hz)

TABLE II. ^{13}C -NMR (DEPT) Spectral Data for Compounds 1, 2, 3 and 4 (CDCl_3 , ppm)

Carbon No.	1	2	3	4
1	22.0 (t)	22.0 (t)	70.1 (d)	21.6 (t)
2	21.0 (t)	20.7 (t)	40.3 (t)	24.7 (t)
3	25.1 (t)	25.0 (t)	66.7 (d)	63.2 (d)
4	65.0 (s)	67.0 (s)	64.2 (s)	65.2 (s)
5	45.3 (s)	45.3 (s)	45.9 (s)	45.6 (s)
6	72.0 (d)	73.7 (d)	71.3 (d)	72.2 (d)
7	32.9 (t)	31.8 (t)	30.3 (t)	32.7 (t)
8	34.7 (d)	34.7 (d)	35.0 (d)	35.3 (d)
9	38.3 (s)	38.5 (s)	38.9 (s)	39.4 (s)
10	48.1 (d)	48.6 (d)	50.9 (d)	48.9 (d)
11	32.5 (t)	33.6 (t)	32.0 (t)	32.9 (t)
12	34.7 (t)	34.5 (t)	32.4 (t)	33.1 (t)
13	173.6 (s)	173.7 (s)	172.3 (s)	173.6 (s)
14	115.5 (d)	115.2 (d)	115.8 (d)	114.6 (d)
15	170.1 (s)	169.1 (s)	168.4 (s)	168.4 (s)
16	73.0 (t)	72.9 (t)	70.6 (t)	70.7 (t)
17	48.1 (t)	48.6 (t)	48.4 (t)	48.1 (t)
18	61.5 (t)	61.8 (t)	61.4 (t)	61.5 (t)
19	17.4 (q)	17.5 (q)	16.8 (q)	17.3 (q)
20	15.3 (q)	15.4 (q)	15.3 (q)	15.5 (q)
1'	168.0 (s)	168.0 (s)	168.4 (s)	169.3 (s)
2'	128.6 (s)	128.3 (s)	129.3 (s)	128.7 (s)
3'	138.0 (d)	137.8 (d)	138.2 (d)	138.1 (d)
4'	14.5 (q)	14.5 (q)	14.5 (q)	14.6 (q)
5'	12.0 (q)	11.9 (q)	12.2 (q)	12.0 (q)
OAc	21.1 (q)		21.1 (q)	21.2 (q)
			21.0 (q)	
			21.0 (q)	
CO	169.7 (s)		169.4 (s)	170.4 (s)
			169.8 (s)	
			170.2 (s)

Fig. 1. Computer-Generated Structural Drawing of Ajugacumbin A (1)

that appeared as an AB system were assigned to an α -oriented methylene at C-18, and the signals at δ_{H} 4.68 (dd, $J_{6\beta,7\alpha}=10.0$ and $J_{6\beta,7\beta}=4.0$ Hz) were assignable to H-6 β after comparison with those of ajugarin I.²⁾ These lower chemical shifts implied the presence of oxo-substituents at C-5 and C-6. But the locations of the substituents (acetyl or tigloyl) remained ambiguous (C-6 α or C-18).

TABLE III. Atomic Coordinates ($\times 10^4$) and Isotropic Thermal Parameters ($\text{\AA}^2 \times 10^3$) for 1

	X	Y	Z	U
C(1)	10819 (5)	4231 (4)	2008 (4)	55 (2) ^{a)}
C(2)	11701 (7)	4503 (5)	1113 (5)	73 (2) ^{a)}
C(3)	12444 (5)	5626 (5)	1322 (4)	67 (2) ^{a)}
C(4)	11306 (5)	6471 (4)	1427 (4)	53 (2) ^{a)}
C(5)	10555 (5)	6279 (4)	2437 (4)	40 (1) ^{a)}
C(6)	9386 (4)	7165 (4)	2528 (4)	47 (1) ^{a)}
C(7)	8411	6882	3353	55 ^{a)}
C(8)	7595 (5)	5773 (4)	3036 (4)	48 (2) ^{a)}
C(9)	8652 (5)	4806 (4)	2963 (4)	42 (1) ^{a)}
C(10)	9734 (5)	5145 (4)	2178 (4)	40 (1) ^{a)}
C(11)	7721 (5)	3786 (4)	2439 (4)	45 (1) ^{a)}
C(12)	6888 (5)	3878 (4)	1209 (4)	56 (2) ^{a)}
C(13)	5726	3019	857	48 ^{a)}
C(14)	5258	2228	1446	68 ^{a)}
C(15)	4087	1612	706	76 ^{a)}
C(16)	4880	2945	-326	65 ^{a)}
C(17)	10509	6981	367	73 ^{a)}
C(18)	11726	6249	3556	51 ^{a)}
C(19)	9463 (5)	4440 (5)	4156 (4)	53 (2) ^{a)}
C(20)	6525 (7)	5578 (6)	3841 (7)	82 (3) ^{a)}
C(21)	9631	9122	2339	56 ^{a)}
C(22)	10676 (8)	10072 (5)	2702 (5)	87 (3) ^{a)}
C(1')	13670	7400	4528	51 ^{a)}
C(2')	14816	8203	4401	56 ^{a)}
C(3')	14727	8774	3448	70 ^{a)}
C(4')	15778 (9)	9658 (7)	3254 (8)	110 (3) ^{a)}
C(5')	16034 (6)	8322 (6)	5450 (5)	84 (2) ^{a)}
O(4)	11643	7583	1157	67 ^{a)}
O(6)	10152	8196	2899	53 ^{a)}
O(15')	3305	852	904	109 ^{a)}
O(15)	3875	2040	-339	80 ^{a)}
O(18)	12878	7030	3506	49 ^{a)}
O(20)	13445	7058	5417	76 ^{a)}
O(21)	8512	9150	1621	77 ^{a)}

a) Equivalent isotropic U defined as one-third of the trace of the orthogonalized U_{ij} tensor.

TABLE IV. Bond Lengths ($\text{\AA}$) in 1

C(1)-C(2)	1.514 (8)	C(2)-C(3)	1.522 (8)
C(3)-C(4)	1.489 (8)	C(4)-O(4)	1.437 (5)
C(4)-C(17)	1.469 (4)	C(5)-C(6)	1.540 (6)
C(5)-C(4)	1.536 (7)	C(5)-C(18)	1.540 (4)
C(6)-O(6)	1.459 (4)	C(7)-C(6)	1.510 (5)
C(7)-C(8)	1.550 (5)	C(8)-C(9)	1.539 (7)
C(8)-C(20)	1.537 (10)	C(9)-C(19)	1.539 (6)
C(9)-C(10)	1.563 (7)	C(10)-C(5)	1.569 (6)
C(9)-C(11)	1.561 (6)	C(12)-C(13)	1.491 (5)
C(10)-C(1)	1.535 (7)	C(13)-C(16)	1.477 (1)
C(11)-C(12)	1.523 (6)	C(15)-O(15)	1.336 (1)
C(13)-C(14)	1.317 (1)	C(16)-O(15)	1.434 (1)
C(14)-C(15)	1.454 (1)	C(17)-O(4)	1.454 (1)
C(15)-O(15')	1.224 (1)	C(21)-C(22)	1.506 (7)
C(18)-O(18)	1.433 (1)	C(21)-O(21)	1.200 (1)
C(21)-O(6)	1.346 (1)	C(1')-O(18)	1.368 (1)
C(1')-C(2')	1.467 (1)	C(2')-C(5')	1.511 (5)
C(1')-O(1')	1.205 (1)	C(3')-C(4')	1.485 (8)
C(2')-C(3')	1.328 (1)		

TABLE V. Bond Angles ($^{\circ}$) in **1**

C(10)–C(1)–C(2)	113.4 (4)	C(1)–C(2)–C(3)	111.5 (5)
C(2)–C(3)–C(4)	109.3 (4)	C(5)–C(4)–C(3)	113.8 (4)
C(5)–C(4)–C(17)	121.7 (4)	C(3)–C(4)–C(17)	116.7 (4)
C(5)–C(4)–O(4)	118.4 (4)	C(3)–C(4)–O(4)	115.9 (4)
C(17)–C(4)–O(4)	60.0 (2)	C(4)–O(4)–C(17)	61.1 (2)
C(10)–C(5)–C(4)	104.6 (4)	C(10)–C(5)–C(6)	108.1 (3)
C(10)–C(5)–C(18)	111.9 (3)	C(6)–C(5)–C(4)	112.8 (4)
C(4)–C(5)–C(18)	110.2 (3)	C(6)–C(5)–C(18)	109.1 (3)
C(7)–C(6)–O(6)	108.0 (3)	C(7)–C(6)–C(5)	114.1 (3)
C(8)–C(7)–C(6)	111.1 (3)	C(5)–C(6)–O(6)	108.6 (3)
C(7)–C(8)–C(20)	108.7 (4)	C(6)–O(6)–C(21)	117.6 (2)
C(8)–C(9)–C(10)	108.9 (4)	C(7)–C(8)–C(9)	113.3 (3)
C(10)–C(9)–C(19)	113.1 (4)	C(9)–C(8)–C(20)	114.5 (5)
C(10)–C(9)–C(11)	109.3 (4)	C(8)–C(9)–C(19)	110.9 (4)
C(9)–C(10)–C(5)	116.8 (4)	C(8)–C(9)–C(11)	109.0 (3)
C(5)–C(10)–C(1)	111.2 (4)	C(19)–C(9)–C(11)	105.4 (4)
C(9)–C(11)–C(16)	117.2 (4)	C(9)–C(10)–C(1)	113.8 (4)
C(11)–C(12)–C(13)	114.3 (4)	C(12)–C(13)–C(14)	130.7 (2)
C(12)–C(13)–C(16)	121.3 (2)	C(14)–C(13)–C(16)	107.9 (1)
C(13)–C(14)–C(15)	109.2 (1)	C(14)–C(15)–O(15')	108.7 (1)
C(14)–C(15)–O(15')	131.2 (1)	O(15')–C(15)–O(15)	119.9 (1)
C(13)–C(16)–O(15)	105.5 (1)	C(15)–O(15)–C(16)	108.6 (1)
C(4)–C(17)–C(4)	58.9 (2)	C(5)–C(18)–O(18)	109.8 (2)
C(22)–C(21)–O(6)	126.0 (2)	C(22)–C(21)–O(6)	110.7 (2)
C(2')–C(1')–O(18)	112.6 (1)	O(6)–C(21)–O(21)	123.2 (1)
O(18)–C(1')–O(1')	121.7 (1)	C(2')–C(1')–O(1')	125.6 (1)
C(1')–C(2')–C(3')	121.3 (1)	C(1')–C(2')–C(5')	113.7 (3)
C(2')–C(3')–C(4')	125.7 (3)	C(5')–C(2')–C(3')	125.0 (3)

TABLE VI. Anisotropic Thermal Parameters ($\text{\AA}^2 \times 10^3$) for **1**

	U11	U22	U33	U23	U13	U12
C(1)	50 (3)	58 (3)	54 (3)	–5 (2)	5 (2)	2 (2)
C(2)	73 (3)	78 (4)	75 (4)	–24 (3)	29 (3)	–5 (3)
C(3)	53 (3)	93 (4)	61 (3)	–5 (3)	28 (2)	–1 (3)
C(4)	53 (3)	61 (3)	44 (2)	–2 (2)	10 (2)	–12 (2)
C(5)	34 (2)	47 (3)	35 (2)	1 (2)	–1 (2)	–5 (2)
C(6)	48 (2)	43 (2)	44 (2)	–1 (2)	–3 (2)	–6 (2)
C(7)	55	52	60	–2	19	–7
C(8)	38 (2)	48 (3)	57 (3)	–2 (2)	6 (2)	–5 (2)
C(9)	38 (2)	49 (3)	34 (2)	3 (2)	0 (2)	–5 (2)
C(10)	34 (2)	45 (3)	37 (2)	–2 (2)	–2 (2)	–2 (2)
C(11)	44 (2)	45 (2)	42 (2)	6 (2)	–2 (2)	–4 (2)
C(12)	56 (3)	59 (3)	47 (2)	6 (2)	–5 (2)	–19 (2)
C(13)	45	53	43	–5	3	–2
C(14)	73	72	52	–9	–1	–22
C(15)	70	77	78	–14	9	–33
C(16)	60	80	49	–1	–4	–8
C(17)	91	86	41	5	7	–21
C(18)	42	62	42	5	–6	–11
C(19)	53 (3)	64 (3)	36 (2)	6 (2)	–2 (2)	–5 (3)
C(20)	64 (4)	78 (4)	118 (6)	–4 (4)	49 (4)	–3 (4)
C(21)	73	49	49	1	15	–3
C(22)	128 (6)	58 (3)	76 (4)	1 (3)	25 (4)	–25 (4)
C(1')	44	55	49	–9	–4	2
C(2')	44	59	62	–18	3	–2
C(3')	65	56	89	–15	13	–15
C(4')	118 (5)	80 (5)	136 (6)	–2 (4)	35 (5)	–35 (4)
C(5')	54 (3)	99 (5)	89 (4)	–36 (4)	–6 (3)	–18 (3)
O(4)	69	71	62	2	15	–20
O(6)	58	44	52	–3	2	–8
O(15')	111	106	100	–8	0	–50
O(15)	68	92	70	–22	–9	–23
O(18)	39	61	44	–5	0	–13
O(20)	75	98	49	–2	–1	–19
O(21)	87	63	74	17	0	6

The anisotropic temperature factor exponent takes the form: $-2 - 2(h^2a^*U_{11} + \dots + 2hka^*b^*U_{12})$.

For the confirmation of the structure (**1**), including its configuration, **1** was subjected to X-ray analysis. The crystals for the X-ray crystallographic study were recrystallized from MeOH. The results of X-ray analysis led to the conclusion that the acetoxyl and the tigloyloxyl groups are located at C-6 α and C-18, respectively, the configurations of H-10, CH₃-19 and CH₃-20 are β , α and α , respectively, and also that the A and B rings are in chair forms. The structure of ajugacumbin A could therefore be formulated as 6 α -acetoxo-4,17-epoxy-18-tigloyloxy-*neo*-cleroda-13-en-15,16-olide (**1**). A computer-generated drawing of **1** and related data are shown in Fig. 1 and Tables III–VII.

Compound **2**, ajugacumbin B, was obtained as colorless crystals, mp 194–195 $^{\circ}\text{C}$, $[\alpha]_{\text{D}}^{28} = +40.5^{\circ}$ ($c = 0.74$, CHCl_3) and showed $[M + 1]$ at m/z 433 in the FAB-MS, which corresponds to $\text{C}_{25}\text{H}_{36}\text{O}_6$. The ^1H - and ^{13}C -NMR spectra of **2** were fundamentally identical with those of **1** except for the presence of a hydroxyl group in place of the acetyl group. The signal at δ_{H} 3.44 (1H) (disappeared with D_2O) was assignable to a hydroxyl group. Barton *et al.*¹¹⁾ reported that the signal of the geminal proton (H-6) appeared as a double-doublet at δ_{H} 3.7–3.8 and that of H-18 appeared a singlet at 4.4 when the C-6 α of the clerodane diterpene skeleton was substituted with a hydroxyl group,

TABLE VII. H-Atom Coordinates ($\times 10^4$) and Isotropic Thermal Parameters ($\text{\AA}^2 \times 10^3$) for **1**

	X	Y	Z	U
H(1a)	10265	3567	1790	69
H(1b)	11502	4115	2718	69
H(2a)	11044	4502	379	84
H(2b)	12453	3951	1130	84
H(3a)	12897	5814	697	73
H(3b)	13189	5605	2011	73
H(6)	8742	7220	1792	50
H(7a)	9018	6827	4103	66
H(7b)	7687	7454	3339	66
H(8)	7016	5814	2275	53
H(10)	9049	5253	1468	47
H(11a)	7004	3639	2897	57
H(11b)	8392	3174	2486	57
H(12a)	7596	3821	727	65
H(12b)	6417	4588	1107	65
H(14)	5631	2081	2238	81
H(16a)	5536	2806	–835	74
H(16b)	4341	3615	–543	74
H(17a)	9353	7297	295	104 (16)
H(17b)	10732	6779	–303	125 (18)
H(18a)	12147	5523	3664	62
H(18b)	11268	6429	4180	62
H(19a)	9979	5048	4571	63
H(19b)	10162	3837	4075	63
H(19c)	8750	4151	4559	63
H(20a)	6019	4885	3684	102
H(20b)	5811	6165	3743	102
H(20c)	7085	5579	4609	102
H(22a)	10107	10713	2418	100
H(22b)	11530	10023	2362	100
H(22c)	10993	10126	3512	100
H(3')	13897	8620	2839	82
H(4'a)	15418	9902	2486	121
H(4'b)	16760	9362	3327	121
H(4'c)	15804	10271	3763	121
H(5'a)	16786	8809	5290	98
H(5'b)	16449	7601	5624	98
H(5'c)	15668	8597	6088	98

whereas the signal of geminal proton (H-6) moved to lower field at δ_H 4.5 and that of H-18 appeared as a double-doublet at 4.40 and 4.82 when an acetyl group was placed at the same position. The ^1H -NMR spectrum of **2** exhibited signals attributable to H-6 β at δ_H 3.57 (dd, $J_{6\beta,7\alpha}=10.0$ Hz, $J_{6\beta,7\beta}=4.0$ Hz) as a double-doublet and to H-18 at 4.58 as a singlet, indicating the presence of a hydroxyl group at H-6 β . Therefore, the structure of ajugacumbin B could be characterized as 4,17-epoxy-6 α -hydroxy-18-tigloyloxy-*neo-cleroda*-13-en-15,16-olide (**2**).

Compound **3**, ajugacumbin C, was obtained as an amorphous powder, $[\alpha]_D^{28} = +57.7^\circ$ ($c=0.78$, CHCl_3). The electron impact mass spectrum (EI-MS) showed $[\text{M}^+ - 1]$ at m/z 589, which corresponds to the molecular formula $\text{C}_{31}\text{H}_{42}\text{O}_{11}$. Except for the presence of three acetyl groups (δ_H 1.96, 2.14 and 2.18), the spectral data of **3** were similar to those of **1**. In the ^1H -NMR spectrum, the signals at δ_H 4.67, 5.69 and 5.85 were assigned to the geminal protons of three acetyl groups. By comparison of the ^1H -NMR spectrum with that of ajugarin I,²⁾ the signal at δ_H 4.67 could be assigned to H-6, which indicated that one of the acetyl groups was located at C-6 α . The ^1H - ^1H chemical shift correlation spectroscopy (COSY) spectrum showed the presence of coupling relations among H-1, H-2 and H-3. A double-doublet at δ_H 5.85 ($J_{3\alpha,2\beta}=10.0$ Hz, $J_{3\alpha,2\alpha}=4.0$ Hz) indicated that the position of a second acetyl group was at C-3 β . The signals of δ_H 2.71 (ddd, $J_{2\beta,2\alpha}=15.0$ Hz, $J_{2\beta,3\alpha}=10.0$ Hz, and $J_{2\beta,1\beta}=4.0$ Hz) and 5.69 (m, $W_{1/2}=15.0$ Hz) were assignable to H-2 β and H-1 α . The coupling constants and the chemical shifts revealed a third acetyl group to be at C-1 α . Thus, ajugacumbin C was formulated as 1 α ,3 β ,6 α -triacetox-3,4,17-epoxy-18-tigloyloxy-*neo-cleroda*-13-en-15,16-olide.

Compound **4**, ajugacumbin D, was obtained as colorless crystals, mp 183–184°C, $[\alpha]_D^{28} = +33.9^\circ$ ($c=1.2$, CHCl_3). From the FAB-MS, the molecular formula could be estimated as $\text{C}_{27}\text{H}_{38}\text{O}_8$. Except for the presence of a hydroxyl group, the spectral data of **4** showed the same groups as in **1**. Comparison of the ^{13}C -NMR spectra of **4** and **1** showed that **4** lacks the signal at δ_C 25.10 in **1**, but exhibits a new signal at 65.24. The ^1H -NMR spectrum also exhibited a new signal at δ_H 4.79 (dd, $J_{3\alpha,2\beta}=9.8$ Hz, $J_{3\alpha,2\alpha}=4.0$ Hz). The results indicated the existence of an oxo-substituent at C-3 β in **4**. AB signals at δ_H 4.42 (d) and 4.89 (d) due to H-18 and a double-doublet at 4.75 ($J_{6\beta,7\alpha}=11.7$ Hz, $J_{6\beta,7\beta}=4.4$ Hz) revealed that an acetyl group was located at C-6 β and a hydroxyl group may be located at C-3 β . Therefore the structure of ajugacumbin D was concluded to be 6 α -acetox-4,17-epoxy-3 β -hydroxy-18-tigloyloxy-*neo-cleroda*-13-en-15,16-olide (**4**).

Compounds **1**–**3** displayed insect antifeedant activity as detected by the host-plant leaf disc method using *Boehmeria nivea* with the larvae of *Pareba vesta* Fabricius. The results are shown in Table VIII.

TABLE VIII. Insect Antifeedant Activity of Compounds **1**–**3**

Compound	Lowest effective concentration (ppm)
1	50
2	200
3	200

Experimental

Plant Material *Ajuga decumbens* THUNB. was collected in Jiangxi province, China, and a voucher specimen was deposited in the Herbarium of China Pharmaceutical University.

Extraction and Isolation The whole plants (8 kg; dried naturally, and powdered) of *A. decumbens* were extracted with ethanol three times. The extract was concentrated, and a certain volume of water was added and agitated thoroughly to form a suspension, which was extracted with chloroform. The concentrated CHCl_3 fraction (100 g) was subjected to column chromatography on silica gel eluted with a solution of ethyl acetate in petroleum ether. The crude crystals obtained were recrystallized from ethyl acetate-cyclohexane to give pure compounds **1** (3.2 g), **2** (12 mg), **3** (40 mg) and **4** (35 mg).

Compound 1 (Ajugacumbin A) Colorless crystals, mp 185–186°C (EtOAc-cyclohexane), $[\alpha]_D^{28} + 38.7^\circ$ ($c=0.9$, CHCl_3), MW 474 (FAB-MS: 475 $[\text{M}+1]$) for $\text{C}_{27}\text{H}_{38}\text{O}_7$. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 218 (4.2). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 2935, 1779, 1744, 1705, 1651, 1373, 1276, 1246, 1141, 1129, 1022, 1007, 951, 887, 742. The ^1H - and ^{13}C -NMR data are shown in Tables I and II. EI-MS m/z (rel. int.): M^+ (absent), 431 (0.9), 361 (2.8), 319 (16.1), 301 (4.6), 175 (6.5), 111 (4.7), 105 (5.6), 91 (6.5), 83 (100), 55 (46.8), 43 (27.0).

Crystallographic Data Monoclinic system, space group $P2_1$, cell dimensions are $a=9.207$ (2), $b=12.131$ (4), $c=12.030$ (3) Å, $\beta=102.01$ (2), $V=1314.37$ Å³, $Z=2$. Intensity data were collected in the range of $1 < \theta < 57$ by an R3m/E four-circle diffractometer. CuK_α radiation (graphite-monochromated), and 1994 independent reflections were recorded; 1754 with $I \geq 3(I)$ were considered as observed. The molecular structure was solved by direct methods. Thirty-one atoms were located from the E map. Least-squares refinement and Fourier synthesis gave the coordinates of all carbon and oxygen atoms. Molecular formula $\text{C}_{27}\text{H}_{38}\text{O}_7$.

Compound 2 (Ajugacumbin B) Colorless crystals, mp 194–195°C (EtOAc-cyclohexane), $[\alpha]_D^{28} + 40.5^\circ$ ($c=0.74$, CHCl_3), MW 432 (FAB-MS: 433 $[\text{M}+1]$) for $\text{C}_{25}\text{H}_{36}\text{O}_6$. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 217 (4.24). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3476, 2950, 1780, 1750, 1639, 1245, 1138, 895, 737. The ^1H - and ^{13}C -NMR data in Tables I and II. EI-MS m/z (rel. int.): 432 (0.05, M^+), 414 (0.2), 319 (21.0), 301 (11.2), 175 (10.3), 123 (43.5), 111 (8.9), 83 (100), 55 (76.5).

Compound 3 (Ajugacumbin C) An amorphous powder, $[\alpha]_D^{28} + 57.7^\circ$ ($c=0.78$, CHCl_3), MW 500 (FAB-MS: 591 $[\text{M}+1]$) for $\text{C}_{31}\text{H}_{42}\text{O}_{11}$. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 215 (4.18). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 2935, 1780, 1738, 1704, 1640, 1369, 1247, 731. The ^1H - and ^{13}C -NMR data in Tables I and II. EI-MS m/z (rel. int.): 589 (0.03, $\text{M}^+ - 1$), 558 (0.05), 515 (0.1), 473 (12.1), 310 (23.4), 200 (43.6), 83 (100), 55 (83.7).

Compound 4 (Ajugacumbin D) Colorless crystals, mp 183–184°C (EtOAc-cyclohexane), $[\alpha]_D^{28} + 33.9^\circ$ ($c=1.2$, CHCl_3), MW 490 (FAB-MS: 491 $[\text{M}+1]$) for $\text{C}_{27}\text{H}_{38}\text{O}_8$. UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 218 (4.24). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3450, 2937, 1778, 1732, 1635, 1275, 1252, 1137, 737. The ^1H - and ^{13}C -NMR data in Tables I and II. EI-MS m/z (rel. int.): M^+ (absent), 441 (2.3), 372 (10.6), 330 (42.4), 313 (29.8), 200 (24.7), 173 (37.1), 83 (100), 55 (74.7).

Acknowledgement This work was supported by the National Natural Science Foundation of China. We thank Prof. Li Xi-wen, Kunming Institute of Botany, Academia Sinica, for identification of the plant material.

References

- 1) "Illustrated Handbook of Chinese Higher Plants," ed. by Institute of Botany, Academia Sinica, 1974, p. 613.
- 2) I. Kubo, Y. Lee, V. Balogh-Nair, K. Nakanishi, and A. Chapya, *J. Chem. Soc., Chem. Commun.*, **1976**, 949.
- 3) I. Kubo, J. A. Klocke, I. Miura, and Y. Fukuyama, *Chem. Commun.*, **1982**, 619.
- 4) I. Kubo, Y. Fukuyama, and A. Chapya, *Chem. Lett.*, **1983**, 223.
- 5) H. Shimomura, Y. Sashida, K. Ogawa, and Y. Iitaka, *Chem. Pharm. Bull.*, **31**, 2192 (1983).
- 6) H. Shimomura, Y. Sashida, and K. Ogawa, *Chem. Pharm. Bull.*, **37**, 354 (1989).
- 7) M. Koreeda, K. Nakanishi, and M. Goto, *J. Am. Chem. Soc.*, **92**, 7512 (1970).
- 8) S. Imai, T. Toyosato, M. Sakai, Y. Sato, S. Fujioka, E. Murata, and M. Goto, *Chem. Pharm. Bull.*, **17**, 340 (1969).
- 9) S. Imai, S. Fujioka, and E. Murata, Japan Patent Appl., **71**, 28038 (1971) [*Chem. Abstr.*, **75**, 132995m (1971)].
- 10) Y. Takeda, S. Tsuchida, and T. Fujita, *Phytochemistry*, **26**, 2303 (1987).
- 11) D. H. R. Barton, H. T. Cheng, A. D. Cross, L. M. Jackman, and M. Martin-Smith, *J. Chem. Soc.*, **1961**, 5061.