Clerodane Diterpenoids from *Ajuga decumbens*

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A new *neo*-clerodane diterpene, ajugacumbin G, was isolated from *Ajuga decumbens* THUNB. (Labiatae), and the structure was characterized as 6α-acetoxy-4α,17-epoxy-18-(3′-hydroxy-2′-methylenebutyryloxy)-*neo*-cleroda-13-en-15,16-olide by ^{1}H- and ^{13}C-NMR spectral study and by comparison with the known compounds of ajugacumbins A and B. Reactions to an epoxy moiety of ajugacumbin compounds with halogen acid, HX (X = Cl, Br and I) and with Jones reagent were prepared to supply some samples with different substituents at C-4 for insect antifeedant examination.

Key words *Ajuga decumbens*; Labiatae; *neo*-clerodane diterpene; ajugacumbin G; reaction of epoxide; Jones oxidation

Ajuga decumbens, a perennial herb distributed in China, Japan and Korea is used as a folk medicine for treatment of inflammation and infectious disease in the two former countries.[1] Previous phytochemical investigation of the whole plants led to the isolation of *neo*-clerodane diterpenes, ajugamarins A_2, B_2, G_1, H_1, F_4[1] and ajugacumbins A—F.[2,3] As a part of our continued search for new insect antifeedants from natural sources, we re-investigated the aerial parts of *A. decumbens*, and isolated a new minor *neo*-clerodane diterpene, ajugacumbin G. The structural elucidation and the epoxy cleavage reaction of the *neo*-clerodane diterpenes mentioned above are described in this paper.

Ajugacumbin G (**1**) obtained as colorless crystals, mp 128—130 °C showed [M^+] at *m/z* 490 in the EI-MS, which corresponds to $C_{27}H_{38}O_8$. The IR spectrum exhibited hydroxyl group (3350 cm^{-1}) and a conjugated γ-lactone moiety (1780 and 1635 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectral data listed in Tables 1 and 2 were fundamentally identical to those of ajugacumbin A (**2**) except for a tigloyl group located at C-18, which indicated that **1** possesses the same skeleton as **2**. In the ^{1}H- and ^{13}C-NMR spectra, two olefinic protons at δ 6.56 and 5.92, two olefinic carbons at δ 127.4 (t) and 139.7 (s) which are assignable to a terminal double bond, and a methine at 5.01 (qd, J = 6.8, 0.7 Hz) bearing a hydroxyl group were observed. These data indicated that a 3-hydroxy-2-methylenebutyryl group was located at C-18 in an α-configuration instead of tigloyl group in **2**, which was confirmed by comparison of ^{1}H- and ^{13}C-NMR spectra with those of ajugacumbin E.[2] The structure of ajugacumbin G was then characterized as 6α-acetoxy-4α,17-epoxy-18-(3′-hydroxy-2′-methylenebutyryloxy)-*neo*-cleroda-13-en-15,16-olide (**1**).

Treatment of **2** with halogen acid (HCl, HBr, HI) in a chloroform solution yielded halohydrin derivatives (**4**, **5** and **6**)[4] having a 4α-hydroxy-17-halogen structure in almost quantitative yield. Characteristic signals of a mother compound, a double doublet (17-H_A) and a doublet (17-H_B) disappeared in the ^{1}H-NMR spectrum of the halohydrin derivatives (Table 1). Instead of these signals, an AB quartet signal assigned to protons at C-17 was observed in a lower field. The ^{13}C-NMR spectrum of chlorohydrin (**4**) had a quaternary carbon signal at δ_C 76.6 in singlet corresponding to C-4 with a tertiary hydroxyl group.[5] The chlorohydrin (**4**) showed obvious paramagnetic shifts at H-6β (δ_H 5.03), H_2-17

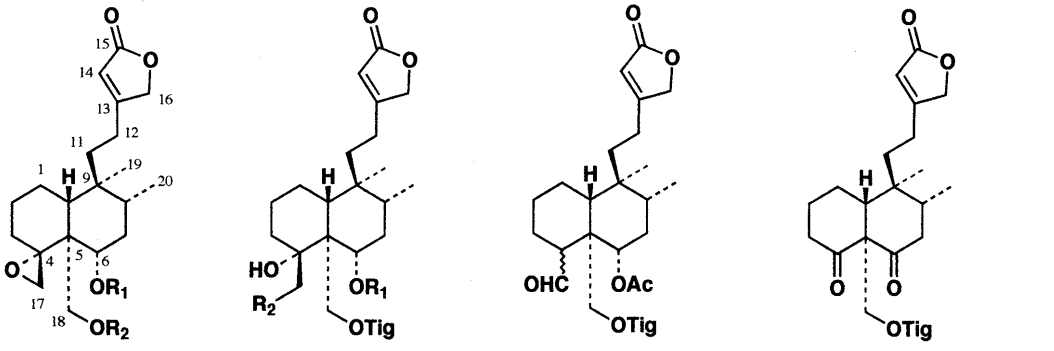

Fig. 1

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Table 1. ^{1}H-NMR Spectral Data of **1** and **4**—**10** ($CDCl_3$, 300 MHz, δ Values in ppm, J Values in Hz)

	1	**4**	**5**	**6**	**7**	**8**	**9**	**10**
6β-H	4.74 (m)	5.03 (dd, 10.9, 4.5)	5.14 (dd, 10.9, 4.4)	5.19 (dd, 10.9, 4.4)	4.71 (dd, 9.7, 5.1)	3.99 (dd, 11.0, 4.1)	4.78 (m)	
14-H	5.85 (t, 1.7)	5.85 (t, 1.7)	5.87 (t, 1.6)	5.84 (t, 1.7)	5.81 (t, 1.6)	5.88 (t, 1.6)	5.81 (t, 1.5)	5.86 (t, 1.5)
16-H_2	4.75 (d, 1.7)	4.75 (d, 1.7)	4.79 (d, 1.6)	4.76 (d, 1.7)	4.75 (d, 1.6)	4.78 (d, 1.6)	4.72 (d, 1.5)	4.74 (d, 1.5)
17-H_A	3.12 (dd, 3.6, 2.4)	4.00 (d, 11.3)	3.95 (s)	3.84 (dd, 10.3, 1.4)	3.92 (d, 8.8)	3.72 (s)		
17-H_B	2.36 (d, 3.6)	3.94 (d, 11.3)	3.95 (s)	3.76 (d, 10.3)	3.89 (d, 8.8)	3.72 (s)		
18-H_A	4.75 (d, 12.0)	5.10 (d, 13.2)	5.13 (d, 13.2)	5.12 (d, 13.2)	4.82 (d, 12.5)	4.98 (d, 13.3)	4.96 (d, 13.1)	4.75 (d, 12.1)
18-H_B	4.33 (br d, 12.0)	4.69 (d, 13.2)	4.72 (d, 13.2)	4.68 (d, 13.2)	4.40 (d, 12.5)	4.74 (d, 13.3)	4.65 (d, 13.1)	4.69 (d, 12.1)
2′-H		6.85 (qq, 7.0, 1.3)	6.87 (qq, 7.1, 1.3)	6.84 (qq, 7.1, 1.4)	7.07 (dd, 7.3, 1.5)	6.84 (qq, 7.1, 1.4)	6.80 (qq, 7.1, 1.4)	6.80 (qq, 7.3, 1.5)
3′-H	5.01 (qd, 6.8, 0.7)							
5′-H_2	5.92 (s), 6.56 (d, 0.7)							
19-Me	0.80 (s)	0.86 (s)	0.89 (s)	0.86 (s)	0.82 (s)	0.86 (s)	0.77 (s)	1.01 (s)
20-Me	0.85 (d, 5.4)	0.86 (d, 6.1)	0.89 (d, 6.1)	0.86 (d, 6.1)	0.73 (d, 6.4)	0.89 (d, 6.1)	0.78 (d, 6.1)	0.92 (d, 6.8)
4′-Me	1.43 (d, 6.8)	1.83 (dd, 7.0, 1.1)	1.85 (dd, 7.1, 0.7)	1.82 (dd, 7.1, 1.1)	1.80 (d, 7.3)	1.82 (dd, 7.1, 1.1)	1.78 (dd, 7.1, 1.1)	1.76 (d, 7.3)
5′-Me		1.88 (dd, 1.3, 1.1)	1.90 (dd, 1.3, 0.7)	1.88 (dd, 1.4, 1.1)	1.86 (s)	1.88 (dd, 1.4, 1.1)	1.97 (d, 1.4), 1.83 (d, 1.4)	1.77 (s)
OAc	1.93 (s)	2.00 (s)	2.02 (s)	1.99 (s)	1.88 (s)		2.00 (s) 1.96 (s)	
OH	9.92 (br s)	2.50 (br s)	2.91 (br s)	2.80 (br s)	2.99 (br s)	3.60 (2H, br s)		
CHO							9.68 (s), 9.70 (s)	

(4.00 and 3.94 in an AB system), H_2-18 (5.10 and 4.69 in an AB system), and at C-4, C-5 and C-6 [δ_C 76.6 (s), 48.5 (s) and 74.4 (d)] (Table 2) as compared with **2** [H-6β (4.68), H_2-17 (2.96 and 2.19), H_2-18 (4.84 and 4.37), and C-4 (65.0), C-5 (45.3) and C-6 (72.0)[3]] after the epoxy cleavage; this resulted in a change of tension and stereochemistry of a molecular followed by destruction of rigid structure. The other derivatives (**5**—**8**) exhibited similar paramagnetic shifts in such protons and carbons. Comparison of the ^{1}H-NMR spectra among the halohydrins (**4**—**8**) clearly revealed that the chemical shift of H-6β was affected by substitutions at C-17, and this magnitude of the anisotropic effects was in the order of I, Br, Cl and OH.

The rearrangement of the epoxy moiety in **2** catalyzed by boron trifluoride in dry benzene resulted in production of an unseparable mixture of epimers based on C-4 aldehyde (**9**). The ratio of epimeric aldehydes was determined by the integration of the aldehyde protons at δ 9.70 (s) and 9.68 (s) in the ^{1}H-NMR spectrum to be in *ca.* 3 : 1. The BF_3-induced rearrangement of exocyclic methylene epoxide is explained on the basis of the involvement of discrete carbenium ion intermediate in the rearrangement process.[6]

Jones oxidation of **3** under ice cooling yielded a polar product (**10**) which showed [M^+] at m/z 416 corresponding to $C_{24}H_{32}O_6$ in EI-MS. The ^{1}H- and ^{13}C-NMR spectra revealed that **10** possessed a dione structure at C-4 and C-6 (ketone carbons at δ_C 203.7 and 205.3), and neither a double doublet and a doublet based on H_2-17 nor H-6β were observed there. Then, **3** was oxidized to form a dione structure of **10** *via* an *ortho*-dihydroxy intermediate under the Jones reaction conditions.

The structural elucidation of a new *neo*-clerodane diterpene, ajugacumbin G, and the structures of halohydrin derivatives of **2** and of a product by Jones oxidation of **3** were described above. Halohydrin derivatives and an oxidative product were prepared to supply the samples for insect antifeedant examination, because a substituent at C-4 is considered to be related to

Table 2. ^{13}C-NMR (DEPT) Spectral Data of **1**, **4** and **10** ($CDCl_3$, ppm)

C	**1**	**4**	**10**
1	22.0 (t)	21.9 (t)	21.9 (t)
2	20.9 (t)	21.1 (t)	20.6 (t)
3	25.1 (t)	22.1 (t)	26.1 (t)
4	65.6 (s)	76.6 (s)	203.7 (s)
5	45.4 (s)	48.5 (s)	64.2 (s)
6	71.9 (d)	74.4 (d)	205.3 (s)
7	32.9 (t)	32.9 (t)	43.6 (t)
8	34.7 (d)	35.1 (d)	36.0 (d)
9	38.4 (s)	38.9 (s)	38.8 (s)
10	48.1 (d)	45.2 (d)	51.9 (d)
11	32.4 (t)	31.8 (t)	35.6 (t)
12	34.7 (t)	35.6 (t)	38.7 (t)
13	173.7 (s)	173.6 (s)	173.4 (s)
14	115.5 (d)	115.6 (d)	115.7 (d)
15	170.1 (s)	170.1 (s)	169.0 (s)
16	72.9 (t)	73.0 (t)	72.9 (t)
17	48.5 (t)	49.9 (t)	
18	61.7 (t)	63.7 (t)	62.9 (t)
19	17.5 (q)	18.0 (q)	17.9 (q)
20	15.3 (q)	15.4 (q)	16.0 (q)
1′	166.1 (s)	167.6 (s)	167.5 (s)
2′	139.7 (s)	128.5 (s)	127.6 (s)
3′	78.9 (d)	138.0 (d)	139.2 (d)
4′	16.8 (q)	14.5 (q)	14.6 (q)
5′	127.4 (t)	12.3 (q)	11.9 (q)
OAc	21.1 (q)	21.1 (q)	
	170.5 (s)	169.5 (s)	

the activity. The results of their activity will be reported in another paper.

Experimental

Melting points are uncorrected. ^{1}H- and ^{13}C-NMR spectra were measured on a Bruker AM-300 spectrometer. Chemical shifts are given in δ values (TMS as internal standard). IR spectra were recorded with a Perkin-Elmer 983 spectrophotometer. EI-MS were recorded with a JEOL JMS D300 high resolution mass spectrometer. Petroleum ether refers to that fraction having bp 60—90 °C.

Extraction and Isolation of 1 Whole plants (10 kg) of *A. decumbens* which were collected in Jiangxi province, China, dried naturally and powdered, were extracted with ethanol under reflux three times. The combined extract was concentrated, and then a certain volume of water was added and agitated thoroughly to form a suspension. The suspension was partitioned with petroleum ether and chloroform. The $CHCl_3$ layer was concentrated, and the residue (120 g) was subjected to column chromatography on silica gel eluted with ethyl acetate–petroleum ether. Crude crystals obtained were recrystallized from ethyl acetate–petroleum ether to afford ajugacumbins G (**1**) (100 mg), -A (**2**) (4.0 g) and -B (**3**) (300 mg).

Ajugacumbin G (1) Colorless crystals, mp 128—130 °C. IR ν_{max}^{KBr} cm^{-1}: 3340, 1780, 1745, 1720, 1635, 1372, 1250, 1170, 1020, 885. EI-MS: 70 eV *m/z* (rel. int.): 490 [M$^+$] (0.1), 475 (0.6), 460 (3.5), 447 (49.3), 415 (3.9), 400 (1.5), 361 (19.2), 349 (1.7), 320 (14.9), 319 (68.6), 301 (26.5), 289 (14.9), 203 (14.9), 191 (13.5), 189 (18.3), 175 (16.7), 147 (12.2), 135 (13.5), 121 (21.0), 119 (17.2), 111 (21.3), 107 (18.4), 105 (19.5), 99 (27.1), 95 (19.3), 81 (46.0), 55 (43.2), 43 (100).

Preparation of a 18-Chloro-4α-hydroxy Derivative (4) from 2 A chloroform solution (2 ml) containing **2** (30 mg) was cooled to 5 °C and 10 N hydrochloric acid (0.1 ml) was added to the solution. The mixture was stirred at room temperature for 3 h, and diluted with $CHCl_3$. The $CHCl_3$ layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was crystallized from MeOH to give **4** in colorless crystals (30 mg). mp 90—92 °C. IR ν_{max}^{KBr} cm^{-1}: 3429, 1774, 1724, 1703, 1647, 1259, 1140, 1027. EI-MS: 70 eV *m/z* (rel. int.): 510 [M$^+$] (0.3), 474 (0.5), 431 (1.5), 414 (1.4), 401 (0.8), 361 (5.5), 319 (13.2), 301 (5.1), 175 (9.0), 107 (9.2), 83 (100), 55 (43.1), 43 (26.1).

Preparation of a 18-Bromo-4α-hydroxy Derivative (5) from 2 In the same ways as the preparation of **4**, **5** was prepared from **2** by the reaction with HBr. mp 58—60 °C, colorless crystals (petrol : EtOAc = 2 : 1). IR ν_{max}^{KBr} cm^{-1}: 3436, 1780, 1745, 1633, 1252, 1020. EI-MS: 70 eV *m/z* (rel. int.): [M$^+$] (absent), 476 [M+1−79)] (0.5), 457 [M−79−18] (0.8), 431 (0.8), 414 (1.1), 396 (1.4), 361 (1.4), 349 (1.3), 331 (1.7), 315 (6.4), 297 (5.8), 187 (4.5), 173 (5.2), 121 (6.8), 107 (6.2), 105 (9.0), 83 (100), 80 (20.9), 45 (41.3), 43 (22.7).

Preparation of an 4α-Hydroxy-18-iodo Derivative (6) from 2 To a tetrahydrofuran solution (1.5 ml) containing **2** (50 mg), NaI (32 mg) and 56.7% HI (bp 126 °C, *d* 1.7) (50 mg, 0.03 ml) were added at 5 °C. After being stirred for 4 h at room temperature, the mixture was poured into water and extracted with ether. The organic layer was washed with 5% $NaHCO_3$, decolorised with 10% $Na_2S_2O_4$, and washed again with water until neutral. After evaporation, the residue (70 mg) was recrystallized from MeOH to afford pure colorless crystals (**6**) (50 mg). mp 79—81 °C. IR ν_{max}^{KBr} cm^{-1}: 3569, 1779, 1747, 1716, 1708, 1637, 1372, 1251, 1139, 1024. EI-MS: 70 eV *m/z* (rel. int.): [M$^+$] absent, 475 [M−127] (0.05), 298 (20.5), 254 (14.5), 201 (25.5), 187 (100), 83 (23.5), 43 (34.0).

Preparation of 7 and 8 from 2 To a glacial acetic acid solution (3 ml) containing **2** (50 mg) was added 40% HBr (0.5 ml), and the mixture was stirred at room temperature for 24 h. The reactant was diluted with water and extracted with EtOAc. The extract was washed with 5% $NaHCO_3$ and then with water until neutral. Upon concentration, the organic layer was chromatographed on a silica gel column eluted with petrol–EtOAc (3 : 1) to give three products (**5**, **7** and **8**). Their *Rf* values on GF_{254} plate (petrol : EtOAc = 3 : 1). **5**: 0.44, **7**: 0.38 and **8**: 0.15. **7**: mp 64—66 °C, colorless crystals. IR ν_{max}^{KBr} cm^{-1}: 3429, 1774, 1703, 1647, 1450, 1379, 1259, 1140, 1090. EI-MS 70 eV *m/z* (rel. int.): 492 [M$^+$] (0.3), 449 (2.7), 432 (1.0), 415 (0.5), 380 (2.4), 379 (10.4), 363 (2.1), 361 (1.6), 337 (21.4), 319 (9.2), 175 (9.5), 136 (6.5), 123 (9.3), 107 (8.6), 105 (7.8), 91 (8.1), 83 (100), 55 (48.2), 43 (27.9). **8**: mp 68—71 °C, colorless crystals (petrol : EtOAc = 1 : 1). IR ν_{max}^{KBr} cm^{-1}: 3436, 1780, 1745, 1640, 1456, 1259, 1140. EI-MS: 70 eV *m/z* (rel. int.): 512 [M$^+$] (0.13), 477 (0.4), 433 [M−79] (0.23), 415 [M−79-18] (1.8), 397 [M−79−38] (0.7), 357 (1.2), 349 (1.4), 315 (5.3), 301 (5.3), 297 (5.4), 283 (3.8), 267 (2.8), 203 (3.3), 185 (4.8), 175 (6.3), 119 (10.2), 107 (6.9), 105 (10.8), 91 (10.5), 83 (100), 57 (28.3), 55 (44.0).

Preparation of 9 from 2 To a solution of **2** (200 mg) in dry benzene (8 ml) was added freshly distilled boron trifluoride etherate (0.04 ml). The reaction mixture was stirred for 15 min, diluted with water, and then extracted with $CHCl_3$. Usual treatment of the extract gave a crude material which was purified by silica gel chromatography eluted with petrol–EtOAc–glacial acetic acid (3 : 1 : 0.1) to give a colorless oily product (**9**). The product is a mixture of C-4 aldehyde derivatives (*ca.* 3 : 1) on the basis of the ^{1}H-NMR spectral data (Table 1). IR: ν_{max}^{liq} cm^{-1}: 1780, 1745, 1705, 1635, 1247, 1140, 1125. EI-MS 18 eV *m/z* (rel. int.): 474 [M$^+$] (0.3), 447 (1.2), 431 (2.4), 419 (2.0), 375 (1.2), 361 (1.9), 349 (1.9), 175 (16.1), 105 (10.8), 93 (10.7), 83 (100), 55 (76.6), 43 (96.8).

Conversion of 3 to 10 by Jones Oxidation To an acetone solution (5 ml) containing ajugacumbin B (**3**) (50 mg) was added Jones reagent (1 ml) and the solution was stirred for 1 h under ice-cooling. The reaction mixture was poured into an aqueous NaCl solution, and extracted with $CHCl_3$. Usual treatment of the extract gave a crude material which was chromatographed on a silica gel column eluted with petrol–EtOAc (1 : 1) to afford an amorphous product. The product was recrystallized from MeOH to give a dione (**10**) in a pure form as colorless cubics (30 mg). mp 183—185 °C. IR ν_{max}^{KBr} cm^{-1}: 1782, 1745, 1710, 1650, 1625, 1440, 1250, 1080. EI-MS 18 eV *m/z* (rel. int.): 416 [M$^+$] (1.6), 333 (5.3), 316 (2.8), 303 (2.2), 288 (2.0), 273 (1.1), 260 (1.1), 224 (1.8), 219 (1.4), 206 (2.2), 192 (1.3), 181 (1.2), 165 (2.0), 150 (2.2), 137 (3.4), 122 (1.9), 83 (100).

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