Neo-clerodane Diterpenes from *Ajuga ciliata* var. *villosior*

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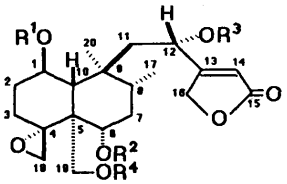
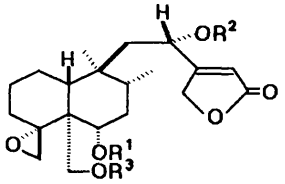
From the aerial parts of *Ajuga ciliata* var. *villosior*, nine new neo-clerodane diterpenes have been isolated. The structures of two acetyl derivatives of (12*S*)-6α,12,19-trihydroxy-1β-[(2*S*)-2-methylbutanoyloxy]-4,18-epoxyneo-clerod-13(14)-en-15,16-olide, three acetyl derivatives of (12*S*)-1β,6α,19-trihydroxy-12-[(2*S*)-2-methylbutanoyloxy]-4,18-epoxyneo-clerod-13(14)-en-15,16-olide, three acetyl derivatives of (12*S*)-6α,19-dihydroxy-12-[(2*S*)-2-methylbutanoyloxy]-4,18-epoxyneo-clerod-13(14)-en-15,16-olide and methyl 6α-hydroxy-4α-methoxycarbonyl-18-norneo-clerod-13(14)-en-15,16-olide were elucidated by spectroscopic methods and chemical correlation. In addition, a known neo-clerodane, ajugarin-IV, was isolated.

Keywords neo-clerodane; *Ajuga ciliata* var. *villosior*; bitter principle; diterpene; Labiatae; ajugamarin

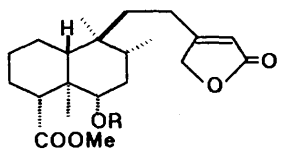
Previously, we isolated ajugamarins A1 (**1**), B1 (**2**), C1 (**3**) and related diterpenes belonging to the neo-clerodane diterpene category[1] from *Ajuga nipponensis*.[2] In a continuation of our studies on the chemical constituents of *Ajuga* plants growing in Japan, we have now investigated *Ajuga ciliata* BUNGE var. *villosior* A. GRAY.[3] The mother species of the plant distributed in China is used as a folk medicine for treatment of inflammation.[4] The bitter taste of leaves of this plant suggested the existence of bitter diterpenes. From the aerial part of this plant, we have isolated new neo-clerodane diterpenes, ajugamarins B4, B5, E1, E2, E3, F1, F2 and F3 and deacetylajugarin-IV (**4**—**12**), and a known neo-clerodane, ajugarin-IV (**13**).[5] These diterpenes were obtained in very high yields (2.0% of dry weight in total).





	R^1	R^2	R^3	R^4
1	Tig	Ac	H	Ac
2	MeBu	Ac	H	Ac
3	H	Ac	H	Ac
4	MeBu	Ac	H	H
4a	MeBu	H	H	Ac
4b	MeBu	Ac	Ac	Ac
5	MeBu	Ac	Ac	H
6	H	Ac	MeBu	H
6a	Ac	Ac	MeBu	Ac
7	H	H	MeBu	Ac
8	H	Ac	MeBu	Ac

	R^1	R^2	R^3
9	H	MeBu	H
9a	Ac	MeBu	Ac
10	Ac	MeBu	H
11	H	MeBu	Ac



	R
12	H
13	Ac

Tig: tigloyl
MeBu: (2*S*)-2-methylbutanoyl

Chart 1

Ajugamarin B4 (**4**), $C_{27}H_{40}O_9$, showed an $[M+H]^+$ peak at *m/z* 509 in the secondary ion mass spectrum (SI-MS). A clerodane structure of **4** closely related to that of **2** was suggested by the resemblance between their ^{1}H- and ^{13}C-nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra (see Tables I, II and III). The difference between **2** and **4** was the lack of the 19-acyl group in **4**, which was shown by the ^{1}H-NMR signals of H_2-19, which appeared as a broad doublet and a broad triplet, indicating that each proton coupled to the hydroxyl proton with different values ($J = ca.$ 0 and 12.0 Hz). This presumably resulted from the existence of a hydrogen bond between the hydroxyl proton and an α-oriented oxygen function at C-6. Treatment of **4** with sodium bicarbonate gave rise to acetyl migration from the 6-hydroxyl to the 19-hydroxyl position, yielding the derivative **4a**, which was identical (^{1}H-NMR, ^{13}C-NMR, infrared (IR), mass spectrum (MS), $[\alpha]_D$ and thin layer chromatography (TLC)) with ajugamarin B3.[2b] The diacetate (**4b**) of **4** was also identical with ajugamarin B2.[1b] Thus, the structure of **4** was elucidated as (12*S*)-6α-acetoxy-12,19-dihydroxy-1β-[(2*S*)-2-methylbutanoyloxy]-4,18-epoxyneo-clerod-13(14)-en-15,16-olide.

Ajugamarin B5 (**5**), $C_{29}H_{42}O_{10}$, showed an $[M+H]^+$ peak at *m/z* 551 in the SI-MS. Its ^{1}H-NMR spectral data indicated that the structure of **5** was related to that of **4**. Two acetyl signals in the ^{1}H-NMR spectrum suggested that **5** had an additional acetyl group as compared with **4**. A broad doublet signal due to H-12 indicated that the hydroxyl group at C-12 was esterified. The H_2-16 signal of **4** appeared as a two-proton doublet, but that of **5** changed to an ABq in which each line was further split into a doublet ($J = 1.8$ Hz). The monoacetate of **5** was identical with the diacetate of **4**, so the additional acetyl group was attached to the 12-hydroxyl function. These acetates were identical (^{1}H-NMR, ^{13}C-NMR, IR, MS, $[\alpha]_D$, melting point, mixed melting point and TLC) with ajugamarin B2 (**4b**). The structure of **5** was determined as (12*S*)-6α,12-diacetoxy-19-hydroxy-1β-[(2*S*)-2-methylbutanoyloxy]-4,18-epoxy-neo-clerod-13(14)-en-15,16-olide.

The ^{1}H- and ^{13}C-NMR spectral data of ajugamarin E1 (**6**), $C_{27}H_{40}O_9$, suggested a clerodane structure related to those of **4** and **5**. The SI-MS of **6** showed an $[M+H]^+$ peak at *m/z* 509. 2-Methylbutanoyl and an acetyl groups were recognized from the ^{1}H- and ^{13}C-NMR and the electron impact mass spectrum (EI-MS) data. The ^{1}H-NMR signals corresponding to H-6β and H-12 indicated the existence of the 6- and 12-acyloxyl groups. The signals of H_2-19 showed the existence of hydrogen bonding as in **4**. A one-proton signal appearing at δ 4.24 was attributable to H-1α because of its coupling (two axial–axial and one axial–equatorial couplings and an additional coupling with a hydroxyl proton). The above data suggested that **6** had the 6α,12-diacyloxy-1β,19-dihydroxy-clerod-13(14)-en-15,16-olide structure.

TABLE I. ^{1}H-NMR Spectral Data[a)]

	4	5	6	6a	7	8
H-1α	5.53 (ddd, 11.0, 11.0, 4.8)	5.43[b)]	4.24 (dddd, 10.8, 10.8, 4.6, (5.2))	5.54 (ddd, 10.8, 10.8, 4.6)	4.29 (dddd, 10.8, 10.8, 4.6, (5.2))	4.32
H-2α	2.24 (dddd, 12.0, 4.8, 4.4, 2.4)	2.24	2.24 (dddd, 12.0, 4.6, 4.4, 2.8)	2.22	2.21	2.21
H-2β	1.70—1.50	1.40 (dddd, 14.0, 12.0, 11.0, 4.8)	1.60—1.45	1.55—1.40	1.55—1.40	1.60—1.45
H-3α	2.60 (dddd, 14.0, 14.0, 4.4, 2.3	2.60	2.53 (dddd, 14.0, 14.0, 4.8, 2.3)	2.38	2.26	2.28
H-3β	1.60 (ddd, 14.0, 4.4, 2.4)	1.14	1.14 (ddd, 14.0, 4.8, 2.8)	1.14	1.18	1.12
H-6β	4.83 (dd, 10.8, 4.0)	4.76	4.76 (dd, 11.0, 4.1)	4.78 (dd, 11.4, 4.6)	3.53 (dd, 11.1, 4.1)	4.69
H-7α H-7β H-8β	1.75—1.50 1.80 (m)	1.75—1.50	1.70—1.60	1.70—1.60	1.70—1.60	1.70—1.60
H-10	2.47 (d, 11.0)	2.04	1.51 (d, 10.8)	2.18	1.51	1.60
H_A-11	2.06 (dd, 16.0, 10.4)	2.68 (dd, 16.0, 9.2)	2.41 (dd, 16.0, 9.5)	2.60 (dd, 16.0, 9.0)	2.39 (dd, 16.0, 9.5)	2.39
H_B-11	1.56 (dd, 16.0, 2.0)	1.57	1.83 (dd, 16.0, 2.8)	1.60	1.84 (br d, 16.0)	1.87
H-12	4.70 (br dd, 10.4, 6.0)	5.85 (br d, 9.2)	5.75 (br d, 9.5)	5.75 (br d, 9.0)	5.77 (br d, 9.5)	5.76
H-14	5.93 (ddd, 1.8, 1.8, 1.2)	5.94	5.87 (ddd, 1.8, 1.8, 1.8)	5.94	5.86	5.87
H_A-16	4.85 (d, 1.8)	4.83 (dd, 17.7, 1.8)	4.80 (dd, 17.7, 1.8)	4.87	4.80 (dd, 17.0, 1.8)	4.81 (dd, 17.7, 1.8)
H_B-16		4.76 (dd, 17.7, 1.8)	4.74 (dd, 17.7, 1.8)	4.75	4.74 (dd, 17.0, 1.8)	4.75 (dd, 17.7, 1.8)
H_3-17	0.86 (d, 6.6)	0.85 (d, 6.1)	0.93 (d, 6.6)	0.86 (d, 6.0)	0.95 (d, 6.4)	0.93 (d, 6.5)
H_A-18	2.98 (dd, 3.8, 2.3)	2.92	2.91 (dd, 3.9, 2.3)	3.01	3.23	2.98
H_B-18	2.32 (d, 3.8)	2.28	2.67 (d, 3.9)	2.27	2.48	2.24
H_A-19	4.39 (br d, 12.7)	4.36	4.34 (d, 12.4)	4.95 (d, 12.4)	4.62	4.88
H_B-19	4.12 (br dd, 12.7, (12.0))	4.16	3.99 (br dd, 12.4, (12.4))	4.34 (br d, 12.4)	4.46	4.30
H_3-20	0.76 (s)	0.71	0.82	0.79	0.86	0.88
H-2′	2.23	2.23	2.38	2.41	2.38	2.38
H_2-3′	1.70—1.50	1.70—1.50	1.70—1.50	1.70—1.50	1.70—1.50	1.70—1.50
H_3-4′	0.91	0.92	0.92	0.94	0.92	0.92
H_3-5′	1.15	1.15	1.18	1.20	1.18	1.18
Ac	2.06	2.15	2.04	2.21	2.08	2.10
		2.05		2.08		1.94
				1.95		

a) Spectra were measured in $CDCl_3$ at 400.1 MHz. Chemical shifts (in δ values) and coupling constants were obtained by first-order approximation. *b*) Parameters equal to those in the left column are omitted.

Ajugamarin E2 (**7**), $C_{27}H_{40}O_9$, showed an $[M+H]^+$ peak at *m/z* 509 in the SI-MS. The ^{1}H-NMR signals of **7** almost corresponded to those of **6**, except for the signals of H-6β and H_2-19. These signals indicated the presence of the 19-ester group instead of the 6α-ester group in **6**.

The ^{1}H-NMR signals of ajugamarin E3 (**8**), $C_{29}H_{42}O_{10}$, corresponded to those of **6** and **7**. The $[M+H]^+$ peak at *m/z* 551 in the SI-MS indicated an additional acetyl group as compared with **6**. The H-6β, H-12 and H_2-19 signals suggested the existence of three acyloxyl groups at the C-6, C-12 and C-19 positions.

The 1,19-diacetate (**6a**) and the 19-monoacetate of **6** were identical with the 1,6-diacetate of **7** and with **8**, respectively. The relations among these acetates indicated that the 2-methylbutanoyloxyl moiety is attached to the C-12 position instead of the C-1 position in **4** and **5**. The relative configuration of the *trans*-decalin portion in these compounds was consistent with that of **4**, **5** and **4a** because all the compounds above described had two W-couplings between H-3α and H_B-18 and between H-6β and H_B-19 (appearing as a broadened doublet) and showed nuclear Overhauser effects (NOEs) between H-1α and H_B-19, between H_A-19 and H_3-17, and between H-6β and H_B-18. For complete determination of the absolute structures of **6**, **7** and **8**, the corresponding 12-[(2*S*)-2-methylbutanoate] derivative was prepared from ajugamarin C1 (**3**)[2b)] (see Experimental). The obtained derivative was identical (^{1}H-NMR, ^{13}C-NMR, IR, MS, $[\alpha]_D$ and TLC) with natural **8**.

Thus, **6**, **7** and **8** were determined to have the following structures: (12*S*)-6α-acetoxy-1β,19-dihydroxy-12[(2*S*)-2-methylbutanoyloxy]-4,18-epoxyneo-clerod-13(14)-en-15,16-olide, (12*S*)-19-acetoxy-1β,6α-dihydroxy-12-[2(*S*)-2-methylbutanoyloxy]-4,18-epoxyneo-clerod-13(14)-en-15,16-olide and (12*S*)-6α,19-diacetoxy-1β-hydroxy-12-[(2*S*)-2-methylbutanoyloxy]-4,18-epoxyneo-clerod-13(14)-en-15,16-olide, respectively.

Ajugamarin F1 (**9**), $C_{25}H_{38}O_7$, had a clerodane structure corresponding to those of **6** and the related diterpenes described above because the ^{1}H- and ^{13}C-NMR spectra were almost identical. The SI-MS data showed an $[M+H]^+$ peak at *m/z* 451. The absence of the 1-hydroxyl function was, however, shown by the disappearance of the ddd signal corresponding to H-1α and by the change of the H-10 signal from the doublet to a doublet of doublets. This was further confirmed by the ^{13}C-NMR signal of the methine carbon at C-10 appearing further upfield by about 2 ppm from that in **4** or **5**, and a new methylene carbon[6)] instead of the methine carbon assignable as C-1 in **4** and **5**. A 2-methylbutanoyl group (the only acyl group) was present on the hydroxyl group at the 12-position in **9**.

The ^{1}H-NMR spectral data for ajugamarins F2 (**10**) and

TABLE II. ^{1}H-NMR Spectral Data[a]

	9	9a	10	11	12
H-1α	1.70—1.50	1.70—1.50	1.70—1.50	1.70—1.50	1.60—1.40
H-1β	1.77 (br d, 13)	1.87[b]	1.83	1.82	
H-2α	2.05 (br d, 13)	1.98	2.00	2.02	1.88 (ddddd, 13.2, 4.0, 4.0, 2.8, 2.8)
H-2β	1.34 (ddddd, 13.0, 13.5, 13.5, 4.8, 4.8)	1.37	1.34	1.35	1.23 (ddddd, 13.2, 13.2, 13.2, 4.8, 4.8)
H-3α	2.42 (dddd, 13.5, 13.5, 4.8, 2.2)	2.12 (dddd, 13.5, 13.5, 4.8, 2.4)	2.37	2.09 (dddd, 13.5, 13.5, 4.8, 2.2)	1.73 (dddd, 13.2, 13.2, 13.2, 4.0)
H-3β	1.14 (br d, 13.5)	1.05	1.08	1.11	1.60—1.40
H-4β	—	—	—	—	2.14 (dd, 13.2, 3.6)
H-6β	3.59 (dd, 10.2, 5.1)	4.71 (dd, 10.4, 4.8)	4.77 (dd, 10.2, 5.1)	3.53 (dd, 10.8, 4.2)	3.49 (ddd, 11.6, 7.6, 2.8)
H-7α H-7β H-8β	1.70—1.50	1.70—1.50	1.70—1.50	1.70—1.50	1.60—1.40
H-10	1.34 (dd, 12.5, 2.8)	1.57	1.44	1.43	1.00 (dd, 12.0, 3.2)
H_A-11	2.07 (dd, 16.4, 9.4)	2.12	2.11	2.07	1.60—1.40
H_B-11	1.53 (br d, 16.4)	1.53	1.53	1.52	
H-12	5.64 (br d, 9.4)	5.62	5.61	5.64	2.26 (ddd, 16.0, 12.8, 4.0)
H-12	—	—	—	—	2.12 (ddd, 16.0, 12.8, 4.0)
H-14	5.92 (ddd, 1.8, 1.8, 1.8)	5.93	5.93	5.93	5.83 (dddd, 2.0, 2.0, 2.0, 2.0)
H_A-16	4.85 (dd, 17.6, 1.8)	4.86	4.86	4.86	4.74 (d, 2.0)
H_B-16	4.72 (dd, 17.6, 1.8)	4.71	4.71	4.72	
H_3-17	0.85 (d, 6.4)	0.83 (d, 5.9)	0.82	0.85 (d, 6.4)	0.83 (d, 6.4)
H_A-18	3.10 (dd, 3.5, 2.2)	2.94 (dd, 3.8, 2.4)	2.88	3.17 (dd, 3.6, 2.2)	—
H_B-18	2.37 (d, 3.5)	2.16 (d, 3.8)	2.19	2.38 (d, 3.6)	—
H_A-19	4.30 (dd, 12.3, (3.5))	4.82 (d, 12.1)	4.29 (br d, 11.9)	4.55 (d, 12.1)	1.10 (s, (Me))
H_B-19	4.04 (br dd, 12.3, (12.3))	4.35 (d, 12.1)	4.03 (br dd, 11.9, (11.9))	4.52 (d, 12.1)	—
H_3-20	0.70 (s)	0.75	0.68	0.73	0.75 (s)
H-2′	2.38	2.37	2.38	2.37	—
H_2-3′	1.70—1.50	1.70—1.50	1.70—1.50	1.70—1.50	—
H_3-4′	0.92	0.91	0.91	0.92	—
H_3-5′	1.16	1.16	1.16	1.16	—
Ac		2.10 1.95	2.03	2.09	— 3.63 (CO_2Me)

a) Spectra were measured in $CDCl_3$ at 400.1 MHz. Chemical shifts (in δ values) and coupling constants were obtained by first order approximation. b) Parameters equal to those in the left column are omitted.

F3 (**11**) were almost identical with those of **9**. The SI-MS data showed the same $[M+H]^+$ peak at *m/z* 493. The ^{1}H-NMR signals due to H-6β and H_2-19 of **10** and **11** indicated structures coincident with those of the 6- and 19-acetate of **9**, respectively. This was confirmed by acetylation of **9** yielding the 6,19-diacetate (**9a**), the 6-acetate identical with natural **10** and the 19-acetate identical with natural **11**.

In the ^{1}H-NMR spectra of **9**, **10** and **11** the signals of H_A-11 and H_B-11 showed vicinal couplings between H_A-11 and H-12 (J=9.4 Hz) and between H_B-11 and H-12 (J=*ca.* 0 Hz). These couplings were consistent with the staggered configuration with *ca.* 150° and *ca.* 90° dihedral angles between the protons. The NOEs between H-12 and H-8β, between H-12 and H_3-17, between H_B-11 and H_3-17, and between H_3-17 and H-14 found in **10**, combined with the above configuration, revealed the configuration at C-12 depicted in Chart 2. The neo-clerodane structure and 2*S* configuration of the 2-methylbutanoyl group of **9**, **10** and **11** were not ascertained. However, biogenetic considerations led to the conclusion that these had the same absolute structure as other co-occurring diterpenes. Thus, **9**, **10** and **11** were identified as follows: (12*S*)-6α,19-dihydroxy-12-[(2*S*)-2-methylbutanoyloxy]-4,18-epoxyneo-clerod-13(14)-en-15,16-olide, (12*S*)-6α-acetoxy-19-hydroxy-12-[(2*S*)-2-methylbutanoyloxy]-4,18-epoxyneo-clerod-13(14)-en-15,16-olide and (12*S*)-19-acetoxy-6α-hydroxy-12-[(2*S*)-2-methylbutanoyloxy]-4,18-epoxyneo-clerod-13(14)-en-15,16-olide, respectively.

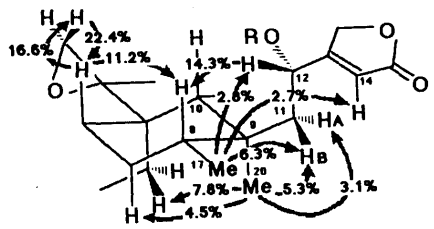


Chart 2

The ^{1}H-NMR spectrum of **12** was closely similar to that of the co-occurring diterpene, ajugarin-IV (**13**) except for the signal corresponding to the H-6β. The CI-MS showed an $[M+H]^+$ peak at *m/z* 365. The above results suggested that **12** was the 6-deacetyl derivative of **13**. Acetylation of **12** with acetic anhydride and pyridine at 90 °C gave an acetate identical with **13**. Acid hydrolysis of **13** with hydrochloric acid afforded a hydrolyzate identical with **12**. Thus, **12** was confirmed to be as deacetylajugarin-IV, methyl 6α-hydroxy-4α-methoxycarbonyl-18-norneo-clerod-13(14)-en-15,16-olide.

Experimental

Melting points are uncorrected. The ^{1}H- and ^{13}C-NMR spectra were

TABLE III. ^{13}C-NMR Spectral Data[a]

	4	5	6	6a	7	8	9	9a	10	11	12
C-1	71.7	70.6	69.1[b]	70.6[b]	68.9[b]	69.1[b]	20.9	21.5	21.4	21.2	20.8
C-2	31.9[b]	31.9[b]	36.6	31.7	36.6	36.6	24.8	24.8	24.8	24.8	25.5[b]
C-3	30.0	30.0	30.4	30.4	30.2	30.6	31.5	32.5[b]	32.2[b]	31.8	26.0[b]
C-4	64.2	64.3	64.7	64.1	66.2	64.3	67.1	64.9	65.4	66.8	56.0
C-5	47.0	46.9	46.6	46.0	45.7	45.7	46.3	45.5	46.5	45.4	44.2
C-6	73.4	73.3	73.8	71.3[b]	72.9	71.6	73.3	71.8	74.0	73.1	77.9
C-7	32.5[b]	32.2[b]	32.6	32.7	33.8	32.9	33.6	32.9[b]	32.6[b]	33.8	36.3
C-8	34.7	34.9	39.5	35.3	39.6	39.5	35.4	35.6	35.4	35.5	35.0
C-9	39.0	38.8	39.1	39.2	39.6	39.3	39.3	39.5	39.3	39.7	38.5
C-10	50.2	49.9	57.5	50.7	56.5	57.6	47.8	48.2	48.8	48.2	48.5
C-11	43.6	41.0	44.3	41.6	44.3	44.2	40.8	41.1	40.9	41.1	35.0
C-12	65.4	66.5	68.8[b]	66.4	68.7[b]	68.7[b]	66.1	66.2	66.1	66.3	22.1
C-13	174.1[c]	168.5	169.2	169.1	170.0	170.1	168.6	168.6	168.7	168.6	173.3
C-14	114.1	115.8	115.2	116.0	115.4	115.4	115.5	116.0	116.0	116.0	115.3
C-15	173.3[c]	172.3	173.2	172.4	173.5	173.2	172.3	172.4	172.5	172.4	170.3
C-16	71.0	70.5	71.2	70.6	71.1	71.2	70.3	70.5	70.5	70.5	73.0
C-17	15.4	15.4	12.9	15.5	13.5	13.0	15.5	15.5	15.5	15.6	176.6
C-18	47.6	47.2	47.4	48.8	48.8	48.5	47.6	49.2	47.3	48.5	9.5
C-19	61.3	61.2	61.6	61.7	61.9	61.6	61.1	61.6	61.6	61.9	18.0
C-20	17.2	16.8	16.3	17.1	16.2	16.3	17.0	17.0	17.0	17.3	15.6
C-1′	174.3[c]	175.5	175.5	175.5	175.6	175.6	175.3	175.6	175.6	175.6	
C-2′	41.6	41.5	41.2	40.9	41.3	41.3	40.5	40.8	40.7	40.7	
C-3′	27.3	26.9	26.7	26.9	26.8	26.8	26.6	26.9	26.8	26.8	
C-4′	11.4	11.4	11.7	11.6	11.7	11.7	11.3	11.5	11.5	11.5	
C-5′	16.0	16.0	15.9	15.8	16.0	15.9	15.6	15.8	15.8	15.8	
Ac	169.1	169.4	170.2	170.4	170.9	170.7		170.9	169.4	171.1	
	21.2	168.9	21.3	169.7	21.1	170.0		169.9	21.3	21.2	
		21.2		169.4		21.2		21.2			51.6 (Me)
		21.0		21.8		21.2		21.1			
				21.8							
				21.1							

a) Spectral data were obtained in $CDCl_3$ at 100.6 or 25 MHz. Chemical shifts are given in δ values. *b, c*) These assignments may be interchanged in each column.

measured on Bruker AM400 and JEOL FX-100 spectrometers. Chemical shifts are given in δ values (tetramethylsilane as an internal standard). Optical rotations were measured with a Jasco DIP-360 polarimeter. IR spectra were recorded with Hitachi 260-30 and Perkin-Elmer 1710 FT-IR instruments. MS measurements were recorded on a Hitachi M-80 spectrometers. *Ajuga ciliata* var. *villosior* was collected at Minamisaku, Nagano, in July 1984. A voucher specimen was deposited in the Herbarium of Tokyo College of Pharmacy.

Extraction and Isolation The fresh aerial parts (300 g, reducing to 56 g after drying) of *A. ciliata* var. *villosior* were extracted with hot MeOH. The concentrated MeOH extract was partitioned between EtOAc and water. The EtOAc extract was chromatographed repeatedly on a silica gel column (Merck No. 9385, Fuji gel BW300 and BW340) with $CHCl_3$–Me_2CO (9:1 and 8:2) and C_6H_{12}–Me_2CO (7:3), yielding nine new neoclerodanes: ajugamarins B4 (**4**, 49 mg), B5 (**5**, 36 mg), E1 (**6**, 129 mg), E2 (**7**, 5 mg), E3 (**8**, 3 mg), F1 (**9**, 62 mg), F2 (**10**, 384 mg) and F3 (**11**, 21 mg), deacetylajugarin-IV (**12**, 8 mg) and a known compound, ajugarin-IV (**13**, 411 mg). **13** gave data identical with those described in the literature.

Ajugamarin B4 (4) Amorphous solid. $[\alpha]_D^{22}$ +20.2° (c=2.04, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3500, 2980, 2950, 2890, 1780, 1750, 1730, 1640, 1460, 1380, 1240, 1180, 1140, 1070, 1020, 970, 960, 880, 860. EI-MS m/z (%): 478 $[M-CH_2O]^+$ (0.4), 477 (0.4), 435 (2.0), 363 (1.4), 356 (1.1), 346 (1.3), 333 (4), 316 (2), 304 (4), 218 (4), 205 (5), 190 (24), 85 (31), 74 (30), 57 (100).

Preparation of 4a from 4 Treatment of **4** (4 mg in 1 ml of MeOH) with 1% aqueous sodium bicarbonate (1 ml) was carried out at room temperature for 24 h. Purification of the EtOAc extract of the reaction mixture by silica gel column chromatography ($CHCl_3$–Me_3CO (8:2)) yielded **4a** (1.6 mg). Compound **4a** was identical with ajugamarin B3 (^{1}H-NMR, ^{13}C-NMR, IR, MS, $[\alpha]_D$ and TLC.)

Ajugamarin B5 (5) Amorphous solid. $[\alpha]_D^{22}$ −7.3° (c=2.33, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3460, 2980, 2950, 2890, 1780, 1750, 1730, 1640, 1460, 1370, 1240, 1180, 1150, 1070, 1020, 970, 960, 910, 880, 860. EI-MS m/z (%): 520 $[M-CH_2O]^+$ (1), 477 (3), 375 (2), 345 (2), 315 (2), 218 (3), 201 (4), 190 (22), 85 (32), 57 (100).

Ajugamarin B2 (4b) from 4 and 5 Acetylation of **4** (9.6 mg) and **5** (8.0 mg) with Ac_2O and pyridine, followed by the normal work-up, gave the same acetates. Purification of the acetates by silica gel column chromatography with C_6H_{12}–Me_2CO (6:4) afforded **4b** (8.1 and 10.5 mg, respectively), which was identical with ajugamarin B2 (^{1}H-NMR, ^{13}C-NMR, IR, MS, $[\alpha]_D$, melting point, mixed melting point and TLC.)

Ajugamarin E1 (6) Amorphous solid. $[\alpha]_D^{22}$ −26.9° (c=2.23, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3500, 2980, 2950, 2890, 1780, 1740, 1640, 1460, 1380, 1240, 1180, 1150, 1130, 1080, 1020, 960, 890, 850. CI-MS m/z (%): 447 $[M-H_2O-CH_3CO]^+$ (4), 435 (2), 407 (35), 375 (10), 357 (35), 333 (100), 316 (40), 303 (35), 137 (60), 95 (91).

Preparation of 6a and 8 from 6 Acetylation of **6** (34.3 mg) with Ac_2O and pyridine in the usual manner afforded the 1,19-diacetate (**6a**, 22.2 mg) and the 19-acetate (12.3 mg), which were purified by silica gel column chromatography with C_6H_{12}–Me_2CO (6:4). The 1,19-diacetate (**6a**). Colorless needles from C_6H_{12}–Me_2CO. mp 193—195 °C. *Anal.* Calcd for $C_{31}H_{44}O_{11}$: C, 62.82; H, 7.48. Found: C, 62.71; H, 7.66. $[\alpha]_D^{22}$ −26.4° (c=2.39, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 2980, 2950, 2890, 1780, 1730, 1640, 1460, 1430, 1370, 1240, 1180, 1140, 1080, 1060, 1030, 980, 950, 920, 900, 890, 860, 800. CI-MS m/z (%): 593 $[M+H]^+$ (1), 562 $[M-CH_2O]^+$ (7), 549 (10), 533 (2), 519 (9), 490 (2), 477 (100), 447 (31), 430 (12), 417 (30), 387 (20), 297 (50), 285 (53). The 19-acetate was identical (^{1}H-NMR, ^{13}C-NMR, IR, MS, $[\alpha]_D$ and TLC) with natural **8**.

Ajugamarin E2 (7) Amorphous solid. $[\alpha]_D^{22}$ −19° (c=2.0, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3450, 2980, 2940, 2880, 1780, 1730, 1640, 1460, 1370, 1240, 1180, 1140, 1120, 1080, 1020, 890, 850. CI-MS m/z (%): 449 $[M+H-AcOH]^+$ (5), 435 (9), 407 (10), 357 (20), 333 (100), 315 (35), 303 (15), 137 (75), 91 (80), 74, 57.

Preparation of 6a from 7 Acetylation of **7** (1 mg) with Ac_2O and pyridine at 90 °C for 4 h gave the 1,6-diacetate. The acetate was identical with **6a** (^{1}H-NMR, IR, MS, $[\alpha]_D$, melting point, mixed melting point and TLC.)

Ajugamarin E3 (8) Amorphous solid. $[\alpha]_D^{22}$ −37° (c=1.4, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3450, 2970, 2980, 2870, 1780, 1730, 1630, 1460, 1380, 1250, 1240, 1180, 1140, 1120, 1090, 1030, 980, 900, 850. CI-MS m/z (%): 551 $[M+H]^+$ (3), 533 (1), 520 (3), 507 (5), 489 (10), 477 (6), 465 (2), 449 (100), 435 (66), 405 (33).

Preparation of 8 from Ajugamarin C1 (3) Compound **6** (17.6 mg) was added to (2*S*)-2-methylbutanoyl chloride (50 μl) in CH_2Cl_2 (0.1 ml) with 4-dimethylaminopyridine (4 mg). After stirring of the mixture for 8 h at room temperature, water was added. The CH_2Cl_2 layer was concentrated *in vacuo* at room temperature, and the residue was subjected to silica gel column chromatography. Elution with $CHCl_3$–Me_2CO (9:1) gave the chlorohydrin of the 1β-[(2*S*)-2-methylbutanoate] of **3** (5.1 mg), which was identical with the chlorohydrin of **2**, the chlorohydrin of the 12-[(2*S*)-2-methylbutanoate] of **3** (3.3 mg) and the chlorohydrin of **3** (3.4 mg). The chlorohydrin of the 12-[(2*S*)-2-methylbutanoate] (2.5 mg in 1 ml of MeOH) was treated with 1% aqueous sodium bicarbonate (1 ml). The mixture was left for 5 min at 60 °C and then for 30 min at room temperature. The $CHCl_3$ extract was chromatographed on silica gel column with $CHCl_3$–Me_2CO (9:1), yielding the 12-[(2*S*)-2-methylbutanoate] of **6** (2.0 mg). This was identical (^{1}H-NMR, ^{13}C-NMR, IR, MS, $[\alpha]_D$ and TLC) with natural **8**.

Ajugamarin F1 (9) Colorless crystals from C_6H_{12}–EtOAc. mp 161—163 °C. *Anal.* Calcd for $C_{25}H_{38}O_7$: C, 66.64; H, 8.50. Found: C, 66.73; H, 8.56. $[\alpha]_D^{22}$ −17.9° (*c*=2.24, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3550, 2980, 2890, 1780, 1760, 1740, 1640, 1460, 1450, 1410, 1390, 1380, 1360, 1330, 1300, 1270, 1260, 1180, 1150, 1080, 1050, 1030, 950, 910, 880, 860, 840, 810. EI-MS *m/z* (%): 419 $[M-CH_3O]^+$ (5), 317 (1), 300 (3), 271 (3), 190 (34), 85 (38), 57 (100).

Preparation of 9a, 10 and 11 from 9 Acetylation of **9** (35.2 mg) with Ac_2O and pyridine in the usual manner gave a mixture of acetates. The mixture was subjected to repeated column chromatography (silica gel, $CHCl_3$–Me_2CO (9.5:0.5) and C_6H_{12}–EtOAc (6:4)) yielding the 6,19-diacetate (**9a**, 7.7 mg), the 6-acetate (3.8 mg) and the 19-acetate (7.7 mg). The 6,19-diacetate (**9a**). Colorless needles from C_6H_{12}–Me_2CO. mp 162—163 °C. *Anal.* Calcd for $C_{29}H_{42}O_9$: C, 65.15; H, 7.92. Found: C, 64.89; H, 7.78. $[\alpha]_D^{20}$ −32.4° (*c*=2.50, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 2980, 2950, 2890, 1780, 1730, 1640, 1460, 1390, 1380, 1370, 1260, 1240, 1170, 1140, 1090, 1040, 900, 880, 870, 860, 800. EI-MS *m/z* (%): 504 $[M-CH_2O]^+$ (3), 491 (12), 461 (13), 419 (100), 401 (16), 389 (10), 299 (20), 203 (58). The 6-acetate and the 19-acetate were identical with **10** (^{1}H-NMR, ^{13}C-NMR, IR, MS, $[\alpha]_D$, melting point, mixed melting point and TLC) and **11** (^{1}H-NMR, ^{13}C-NMR, IR, MS, $[\alpha]_D$ and TLC), respectively.

Ajugamarin F2 (10) Colorless crystals from EtOAc. mp 161—163 °C. *Anal.* Calcd for $C_{27}H_{40}O_8$: C, 65.83; H, 8.13. Found: C, 66.09; H, 8.34. $[\alpha]_D^{22}$ −14.4° (*c*=2.63, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3550, 2980, 2940, 2880, 1780, 1750, 1740, 1720, 1640, 1460, 1450, 1400, 1380, 1330, 1240, 1180, 1160, 1140, 1120, 1080, 1060, 1030, 980, 970, 950, 930, 900, 880, 830. EI-MS *m/z* (%): 461 $[M-CH_3O]^+$ (24), 419 (100), 401 (9), 389 (12), 317 (10), 287 (16), 190 (73), 172 (32), 85 (30), 57 (33).

Ajugamarin F3 (11) Amorphous solid. $[\alpha]_D^{22}$ −1.4° (*c*=2.1, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3510, 2980, 2950, 2890, 1780, 1740, 1640, 1460, 1380, 1360, 1320, 1260, 1240, 1180, 1140, 1030, 890, 860, 800. EI-MS *m/z* (%): 461 $[M-CH_3O]^+$ (1), 419 (16), 190 (16), 123 (24), 85 (33), 57 (100).

Deacetylajugarin-IV (12) Colorless crystals from EtOAc. mp 206—207 °C. $[\alpha]_D^{22}$ −16° (*c*=0.1, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3440, 2950, 2860, 1810, 1740, 1720, 1630, 1450, 1410, 1320, 1260, 1220, 1200, 1180, 1150, 1140, 1080, 1060, 1030, 1010, 900, 860. CI-MS *m/z* (%): 365 $[M+H]^+$ (4), 347 (100), 333 (46), 315 (35), 287 (40), 218 (10), 175 (10), 101 (12), 98 (10).

Preparation of 13 from 12 Acetylation of **12** (2.8 mg) with Ac_2O and pyridine at 90 °C for 2 h, followed by purification by silica gel column chromatography with C_6H_{12}–Me_2CO (7:3), yielded the 6-acetate (0.3 mg), identical with **13** (^{1}H-NMR, IR, MS, $[\alpha]_D$ and TLC).

Preparation of 12 from 13 Treatment of **13** (15 mg in 1 ml of MeOH) with 10% HCl (1 ml) at 90 °C for 5 h, followed by purification by silica gel column chromatography (C_6H_{12}–Me_2CO (7:3)), gave the 6-deacetyl derivative (6.6 mg). Identity of the derivative with **12** was confirmed by direct comparison (^{1}H-NMR, IR, MS, $[\alpha]_D$, melting point, mixed melting point and TLC.)

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