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The Chemical Constituents of *Ajuga* Plants. I. *neo*-Clerodanes from the Leaves of *Ajuga nipponensis* MAKINO[1]

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Three new bitter principles, ajugamarin, dihydroajugamarin, and ajugamarin chlorohydrin, which belong to the *neo*-clerodane group of diterpenoids, have been isolated from the leaves of *Ajuga nipponensis*. The structures of these compounds were established by chemical and spectroscopic means. The absolute structure of ajugamarin was determined by the X-ray analysis of ajugamarin *p*-bromobenzoate. Ajugamarin chlorohydrin may have been formed by addition of hydrogen chloride to ajugamarin during the isolation procedure.

Keywords——*Ajuga nipponnensis*; Labiatae; diterpenoid; *neo*-clerodane; bitter principle; ajugamarin; dihydroajugamarin; ajugamarin chlorohydrin; X-ray crystallographic analysis

Diterpenes of the clerodane type, ajugarins,[2] ajugareptansin,[3] and ivains[4] have been found in some *Ajuga* species. The leaves of *Ajuga nipponensis* MAKINO (Japanese name: Junihitoe) taste very bitter, but only an oligosaccharide (ajugose)[5] and phytoecdysones[6] have previously been reported as chemical constituents of the plant. In this paper the isolation from *A. nipponensis* of three new bitter *neo*-clerodanes, adjugamarin (**1**), dihydroajugamarin (**2**), and adjugamarin chlorohydrin (**3**), is reported. Their structures were elucidated by spectroscopic studies and confirmed by X-ray crystallographic analysis.

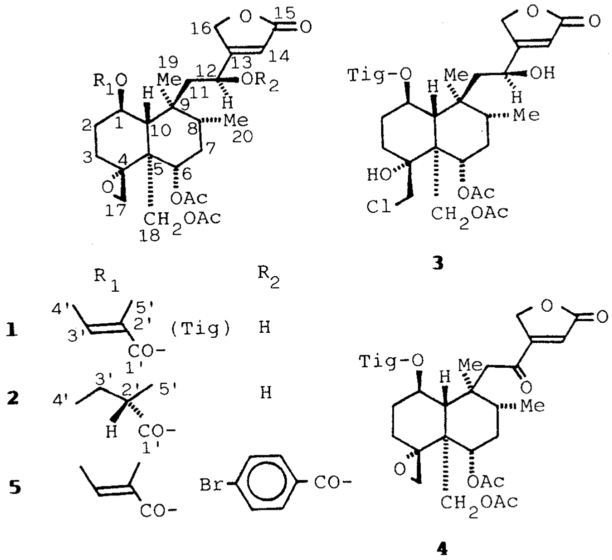
Ajugamarin (**1**), $C_{29}H_{40}O_{10}$, was crystallized from tetrachloromethane as a 1/2 CCl_4 solvate. The molecular ion peak (*m/z* 548) of **1** appeared in the field desorption mass spectrum, but the heaviest ion peak was observed at *m/z* 518 by electron impact mass spectrometry (EI-MS). The infrared (IR) and ultraviolet (UV) spectra showed the following absorptions: IR $\nu_{\max}^{\mathrm{KBr}}$ 3540 (hydroxyl), 1780 (conjugated γ-lactone), 1750—1690 (conjugated γ-lactone and ester carbonyls), and 1640 (conjugated double bond) cm^{-1}; UV $\lambda_{\max}^{95\%\mathrm{EtOH}}$ 212 nm (ε 20000).

In the ^{1}H nuclear magnetic resonance (^{1}H NMR) spectrum of **1**, there were the signals indicative of two methyl groups (δ 0.84, s and δ 0.84, d, $J=6$ Hz), two acetoxyl groups (δ 2.14 and δ 1.96), and a $-\overset{|}{\underset{|}{C}}-CH_2-OAc$ grouping (δ 4.91 and δ 4.45, ABq, $J=12$ Hz).

The presence of an α,β-unsaturated γ-lactone in **1** was inferred from the IR $\nu_{\max}^{\mathrm{KBr}}$ 1780 cm^{-1} and UV $\lambda_{\max}^{95\%\mathrm{EtOH}}$ 212 nm absorptions and the ^{1}H NMR signals due to 14-H at δ 5.85 (1H, t, $J=2$ Hz) and 16-H_2 at δ 4.78 (2H, d, $J=2$ Hz). The signals of 14-H and 16-H_2 measured in benzene-d_6 solution were shifted upfield to δ 5.66 and δ 4.20, respectively.

An exocyclic epoxide ring was recognized from the M^+-30 peaks in the EI-MS of **1** and its derivatives. The signals of 17-H_2 of **1** appeared as a doublet at δ 2.32 (1H, $J=4$ Hz) and as a doublet of doublets at δ 3.06 (1H, $J=4$ and 2 Hz). The doublet of doublets is due to a coupling with the geminal proton at δ 2.32 and a W-coupling with 3-*ax*-H.

These spectral data suggest that the structure of **1** is closely related to that of ajugarins, which belong to the clerodane-type diterpenes. The ^{13}C NMR data of **1** were consistent with the above result.



Chart

The existence of a tigloyloxyl group in **1** was supported by the signal (δ 6.72, br q, J= 7 Hz) due to the olefinic proton, as well as a broad singlet (δ 1.83) due to α-methyl and a doublet (δ 1.78, J=7 Hz) due to the β-methyl. The methyl signals were decoupled on irradiation at δ 6.72. The irradiation of the region of the vinylic methyl groups (δ *ca.* 1.8) transformed the broad quartet due to the olefinic proton into a singlet. The prominent peaks at m/z 83 ($C_5H_7O^+$) and m/z 55 ($C_4H_7{}^+$) in the EI-MS supported the existence of a tigloyloxyl group in **1**. Acid hydrolysis of **1** gave tiglic acid, which was identified by gas chromatography (GC). A triplet of doublets at δ 5.84 assignable to the proton attached to the carbon atom bearing the tigloyloxyl group was considered to be composed of two *axial-axial* couplings (J=11 Hz) and an *axial-equatorial* coupling (J=5 Hz). Decoupling experiments revealed that the methine doublet (δ 2.72, J=11 Hz) corresponding to the angular proton was coupled to the triplet of doublets. The mode of these couplings suggests that the triplet of doublets can be assigned to 1-*ax*-H, which is coupled to 2-*ax*-H, 2-*eq*-H, and 10-*ax*-H.

A one-proton doublet (J=7 Hz) was also observed at δ 3.35 in the ^{1}H NMR spectrum of **1**. This doublet shifted downfield to δ 4.6 when the spectrum was measured in acetone-d_6. On the basis of deuterium exchange and double irradiation experiments, the doublet was assigned to the secondary hydroxyl proton.

Oxidation of **1** with chromium trioxide and pyridine gave 12-oxoajugamarin (**4**) which showed the molecular ion peak at m/z 546 and the characteristic $M^+ - 30$ peak at m/z 516 in the EI-MS. In the ^{1}H NMR spectrum of **4**, two signals of methylene protons appeared at δ 3.05 and δ 2.85 as an AB quartet with large splittings (J=20 Hz) involving only a geminal coupling. Such signals imply that the methylene group adjacent to the carbonyl group is attached to a quaternary carbon atom. The formation of a new conjugated system in **4** was evidenced by the UV absorption at $\lambda_{\max}^{95\%\mathrm{EtOH}}$ 290 nm (ε 3600). It follows from the above facts that the oxygen function is adjacent to the α,β-unsaturated γ-lactone ring. The signals of 16-H_2 (δ 4.95) in **4** were shifted further downfield than those of the same protons in **1**. The deshielding of these protons is probably due to the anisotropic effect of the carbonyl group at 12-C.

Dihydroajugamarin (**2**), $C_{29}H_{42}O_{10}$, was obtained as an amorphous solid. The IR spectrum of **2** was similar to that of **1**. The EI-MS of **2** showed the heaviest ion peak at m/z 520, which was the same $M^+ - 30$ peak as that of **1**, and showed fragment ion peaks at m/z 85 ($C_5H_9O^+$) and 57 ($C_4H_5{}^+$). In the ^{1}H NMR spectrum of **2**, the signals due to two methyl

Table I. ^{1}H NMR Data[a]

	1	**1**[b]	**1**[c]	**2**	**3**	**4**
1-H	5.84 dt (5, 11)	5.67	5.82	5.71 dt (5, 11)	6.07 dt (5, 11)	5.78 dt (5, 11)
6-H	4.8—4.6		4.8	4.9—4.6	4.8	4.9
10-H	2.72 d (11)	3.01	2.60	2.59 d (11)	2.86 d (11)	3.09 d (11)
11-H$_2$						3.05 AB q 2.85 (20)
12-H	4.8—4.6		4.0	4.9—4.6	5.1	
14-H	5.85 t (2)	5.89	5.66	5.92 t (2)	5.88	6.36 t (2)
16-H$_2$	4.78 d (2)	4.84	4.20	4.85 d (2)	4.80	4.95 d (2)
17-H$_2$	3.06 dd (2, 4)	2.92	3.04	3.08	4.13 AB q 3.99 (12)	3.20 dd (2, 4)
	2.32 d (4)	2.26	1.96	2.33 d (4)		
18-H$_2$	4.91 AB q 4.45 (12)	4.87 4.46	5.06 4.23	4.90 AB q 4.42 (12)	4.99 AB q 4.73 (12)	4.88 AB q 4.43 (12)
19-H$_3$	0.84 s	0.79	0.59	0.84 s	0.88 s	0.90 s
20-H$_3$	0.84 d (6)	0.86	0.32	0.87 d (6)	0.85 d (6)	0.84 d (6)
12-OH	3.35 d (7)		2.69	3.52 d (7)		
3′-H	6.72 br q (7)	6.92	6.68		6.74 br q (7)	6.49 br q (7)
4′-H$_3$	1.78 br d (7)		1.42	0.91 t (7)		1.68 br d (7)
5′-H$_3$	1.83 br s		1.72	1.15 d (7)	1.83 br s	1.75 br s
CH$_3$CO	2.14 s		1.91	2.15 s	2.22 s	2.18 s
	1.96 s		1.83	1.97 s	2.04 s	2.00 s

a) Spectra were determined on a 100 MHz instrument in CDCl$_3$ unless otherwise indicated. Chemical shifts (δ values), multiplicities, and coupling constants (in parentheses) are given.
b) Acetone-d_6.
c) Benzene-d_6.

groups, taking the place of the signals of the tigloyloxyl group in **1**, appeared as a triplet ($J=$ 7 Hz) at δ 0.91 and a doublet ($J=$7 Hz) at δ 1.15. These data imply that **2** contains a 2-methylbutyryl moiety. Alkaline hydrolysis of **2** gave (*S*)-2-methylbutyric acid, which was identified by GC and from the specific rotation of its *p*-phenylphenacyl ester.[7]

The ^{1}H NMR spectrum of **2** showed the existence of the same groups that were indicated in that of **1**, except for the tigloyloxyl group. The signals of 14-H and 16-H$_2$ appeared further downfield than those of **1** at δ 5.92 and δ 4.85. The signal corresponding to 1-*ax*-H appeared as a triplet of doublets (δ 5.71) similar to that observed in the ^{1}H NMR spectrum of **1**. Methanolyses of **1** and **2** with sodium methoxide gave the same polar products, as confirmed by thin layer chromatography (TLC). The above result and the striking resemblance of the spectral data of **1** and **2** suggest that **2** has the same clerodane structure as **1**. The only difference between the ^{13}C NMR data for **1** and those for **2** was recognized in the signals assignable to the C$_5$ acyl groups. On the basis of these data, the structure of dihydroajugamarin was determined as **2**.

TABLE II. ^{13}C NMR Data[a]

Carbon	**1**	**2**	Carbon	**1**	**2**
1	71.0 d	71.4 d	16	71.0 t	71.1 t
2	31.9 t[b]	31.9 t[b]	17	49.2 t	49.2 t
3	30.4 t[b]	30.4 t[b]	18	62.2 t	62.0 t
4	64.2 s	64.1 s	19	17.4 q	17.3 q
5	46.0 s	46.0 s	20	15.4 q	15.5 q
6	71.6 d	71.6 d	1′	166.1 s	174.5 s[c]
7	32.7 t[b]	32.8 t[b]	2′	129.0 s	41.6 d
8	34.7 d	34.8 d	3′	138.4 d	27.2 t
9	39.3 s	39.2 s	4′	12.6 q[d]	11.4 q
10	50.9 d	50.7 d	5′	14.5 q[d]	16.0 q
11	42.6 t	43.5 t	$\underline{\mathrm{C}}\mathrm{H_3CO}$	21.2 q	21.1 q
12	65.6 d	65.4 d		21.2 q	21.1 q
13	174.1 s[c]	174.4 s[c]	$\mathrm{CH_3}\underline{\mathrm{C}}\mathrm{O}$	169.8 s	169.8 s
14	114.2 d	114.2 d		170.3 s	170.3 s
15	173.2 s[c]	173.3 s[c]			

a) Spectral were obtained in $CDCl_3$ at 25.2 MHz. Signals (δ values and multiplicities) were assigned on the basis of partially decoupled off-resonance spectra and by comparison with reported data for ajugarin[2)] and ajugareptansin.[3)]
b) *c*) *d*) These assignments are interchangeable.

TABLE III. The Atomic Co-ordinates ($\times 10^4$) of **5**

	x	*y*	*z*		*x*	*y*	*z*
Br	9662 (1)	5000 (0)	8848 (1)	C (24)	9171 (10)	626 (11)	7182 (15)
C (1)	5170 (7)	2014 (6)	3833 (10)	C (25)	8717 (9)	494 (9)	3797 (14)
C (2)	5441 (9)	2947 (6)	3660 (12)	C (26)	2118 (9)	2330 (7)	−357 (10)
C (3)	4415 (8)	3363 (6)	2775 (11)	C (27)	1579 (14)	2923 (10)	−1403 (13)
C (4)	3388 (8)	3219 (6)	3489 (10)	C (28)	237 (10)	2865 (9)	3771 (13)
C (5)	3062 (7)	2290 (5)	3601 (9)	C (29)	−715 (12)	3106 (11)	2514 (16)
C (6)	2006 (7)	2177 (6)	4320 (10)	C (30)	5759 (8)	2512 (6)	8817 (10)
C (7)	1803 (8)	1272 (6)	4729 (10)	C (31)	6689 (8)	3130 (6)	8702 (10)
C (8)	2798 (7)	943 (6)	5805 (10)	C (32)	6842 (9)	3743 (7)	9783 (11)
C (9)	3952 (7)	995 (5)	5209 (9)	C (33)	7724 (9)	4310 (7)	9830 (11)
C (10)	4140 (7)	1892 (5)	4644 (8)	C (34)	8405 (8)	4257 (7)	8748 (11)
C (11)	4901 (7)	726 (6)	6479 (9)	C (35)	8299 (9)	3643 (7)	7664 (10)
C (12)	5022 (8)	1210 (6)	7914 (9)	C (36)	7400 (8)	3091 (7)	7650 (11)
C (13)	5580 (8)	649 (6)	9156 (10)	O (1)	6187 (5)	1653 (4)	4686 (6)
C (14)	5088 (9)	310 (6)	10183 (10)	O (2)	2480 (5)	3799 (4)	3165 (7)
C (15)	5941 (10)	−224 (7)	11059 (11)	O (3)	2334 (6)	2489 (4)	1002 (6)
C (16)	6802 (8)	394 (7)	9276 (11)	O (4)	1034 (5)	2429 (4)	3294 (7)
C (17)	3161 (10)	3818 (7)	4628 (11)	O (5)	5789 (5)	1905 (4)	7798 (6)
C (18)	2874 (7)	1887 (6)	2044 (9)	O (6)	6938 (5)	−176 (4)	10511 (7)
C (19)	4008 (8)	341 (6)	3967 (10)	O (7)	5845 (8)	−664 (5)	12097 (8)
C (20)	2478 (9)	77 (8)	6302 (12)	O (8)	6852 (6)	1434 (5)	2556 (8)
C (21)	6980 (8)	1360 (6)	3892 (11)	O (9)	2365 (7)	1546 (5)	−748 (8)
C (22)	7970 (8)	957 (7)	4730 (11)	O (10)	294 (9)	3097 (8)	5024 (11)
C (23)	8138 (9)	1012 (8)	6187 (12)	O (11)	5127 (7)	2521 (5)	9688 (9)

A diterpene (**3**), obtained in a small amount, was positive in the Beilstein test. The ^{1}H NMR spectrum for **3** lacked the signals assignable to exocyclic epoxide methylene. An AB quartet at δ4.13 and δ3.99 (2H, $J=12$ Hz) indicative of a chloromethyl group was observed. The other ^{1}H NMR signals of **3** closely resembled those of **1** and **2**. Signals of two hydroxyl

Table IV. The Bond Lengths (Å) of **5**

Br –C (34)	1.913 (10)	C (15)–O (6)	1.364 (14)
C (1)–C (2)	1.557 (14)	C (15)–O (7)	1.213 (13)
C (1)–C (10)	1.545 (13)	C (16)–O (6)	1.457 (12)
C (1)–O (1)	1.463 (10)	C (17)–O (2)	1.466 (11)
C (2)–C (3)	1.521 (14)	C (18)–O (3)	1.449 (10)
C (3)–C (4)	1.496 (14)	C (21)–C (22)	1.462 (13)
C (4)–C (5)	1.559 (12)	C (21)–O (1)	1.368 (12)
C (4)–C (17)	1.486 (14)	C (21)–O (8)	1.225 (12)
C (4)–O (2)	1.430 (11)	C (22)–C (23)	1.332 (15)
C (5)–C (6)	1.523 (13)	C (22)–C (25)	1.527 (17)
C (5)–C (10)	1.618 (11)	C (23)–C (24)	1.552 (17)
C (5)–C (18)	1.563 (12)	C (26)–C (27)	1.546 (18)
C (6)–C (7)	1.541 (14)	C (26)–O (3)	1.310 (11)
C (6)–O (4)	1.440 (10)	C (26)–O (9)	1.215 (14)
C (7)–C (8)	1.524 (12)	C (28)–C (29)	1.544 (18)
C (8)–C (9)	1.561 (13)	C (28)–O (4)	1.313 (14)
C (8)–C (20)	1.541 (16)	C (28)–O (10)	1.208 (16)
C (9)–C (10)	1.570 (12)	C (30)–C (31)	1.509 (14)
C (9)–C (11)	1.561 (11)	C (30)–O (5)	1.364 (11)
C (9)–C (19)	1.569 (13)	C (30)–O (11)	1.169 (14)
C (11)–C (12)	1.527 (12)	C (31)–C (32)	1.400 (14)
C (12)–C (13)	1.533 (12)	C (31)–C (36)	1.384 (15)
C (12)–O (5)	1.463 (11)	C (32)–C (33)	1.392 (15)
C (13)–C (14)	1.310 (14)	C (33)–C (34)	1.383 (16)
C (13)–C (16)	1.503 (14)	C (34)–C (35)	1.402 (15)
C (14)–C (15)	1.478 (14)	C (35)–C (36)	1.395 (15)

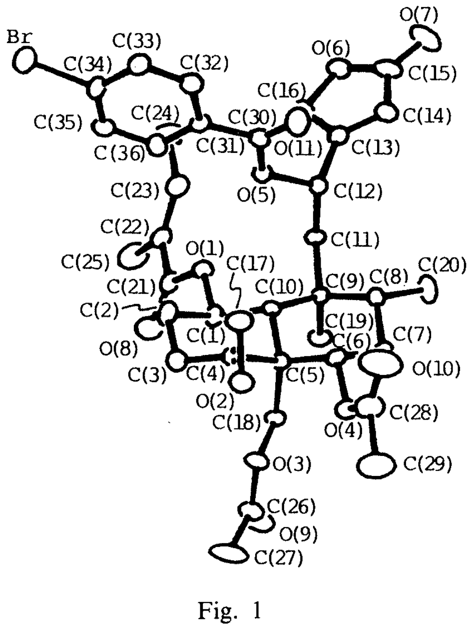


Fig. 1

protons appeared at δ 3.4 ($J=7$ Hz) and δ 2.6 (s). One of them was tertiary, because it resisted acetylation upon treatment with acetic anhydride and pyridine. Addition of hydrogen chloride to **1** gave a compound which was identical with **3** by TLC, and ^{1}H NMR and IR spectral comparisons. These results support the chlorohydrin structure **3**. Since chlorine-containing solvents were used during the isolation procedure, **3** may have been formed by addition of hydrogen chloride to **1**.

Table V. The Bond Angles (°) of **5**

C (2)–C (1)–C (10)	111.5 (7)	C (14)–C (15)–O (7)	129.4 (10)
C (2)–C (1)–O (1)	105.9 (7)	O (6)–C (16)–C (13)	102.8 (8)
C (10)–C (1)–O (1)	110.1 (7)	O (2)–C (17)–C (4)	50.8 (6)
C (3)–C (2)–C (1)	108.8 (8)	O (3)–C (18)–C (5)	108.5 (7)
C (4)–C (3)–C (2)	109.9 (8)	C (22)–C (21)–O (1)	116.1 (8)
C (5)–C (4)–C (3)	114.1 (8)	C (22)–C (21)–O (8)	122.9 (9)
C (5)–C (4)–C (17)	120.6 (8)	O (1)–C (21)–O (8)	121.1 (9)
C (5)–C (4)–O (2)	117.3 (7)	C (23)–C (22)–C (21)	119.3 (9)
C (3)–C (4)–C (17)	117.4 (8)	C (23)–C (22)–C (25)	126.3 (10)
C (3)–C (4)–O (2)	116.7 (8)	C (21)–C (22)–C (25)	114.5 (9)
C (17)–C (4)–O (2)	60.3 (6)	C (24)–C (23)–C (22)	123.6 (11)
C (6)–C (5)–C (4)	112.0 (7)	C (27)–C (26)–O (3)	111.5 (9)
C (6)–C (5)–C (10)	109.2 (7)	C (27)–C (26)–O (9)	124.6 (10)
C (6)–C (5)–C (18)	109.9 (7)	O (3)–C (26)–O (9)	123.7 (10)
C (4)–C (5)–C (10)	103.9 (7)	C (29)–C (28)–O (4)	111.8 (11)
C (4)–C (5)–C (18)	110.1 (7)	C (29)–C (28)–O (10)	124.6 (12)
C (10)–C (5)–C (18)	111.8 (7)	O (4)–C (28)–O (10)	123.4 (12)
C (7)–C (6)–C (5)	113.1 (7)	C (31)–C (30)–O (5)	109.4 (8)
C (7)–C (6)–O (4)	106.8 (7)	C (31)–C (30)–O (11)	126.6 (9)
C (5)–C (6)–O (4)	108.4 (7)	O (5)–C (30)–O (11)	124.0 (9)
C (8)–C (7)–C (6)	110.9 (8)	C (32)–C (31)–C (30)	115.9 (8)
C (9)–C (8)–C (7)	113.1 (7)	C (32)–C (31)–C (36)	120.3 (9)
C (9)–C (8)–C (20)	115.3 (8)	C (30)–C (31)–C (36)	123.7 (9)
C (7)–C (8)–C (20)	108.0 (8)	C (33)–C (32)–C (31)	120.3 (10)
C (10)–C (9)–C (8)	110.2 (7)	C (34)–C (33)–C (32)	117.5 (10)
C (10)–C (9)–C (11)	112.5 (7)	C (35)–C (34)–Br	117.6 (8)
C (10)–C (9)–C (19)	110.9 (7)	C (35)–C (34)–C (33)	124.1 (10)
C (8)–C (9)–C (11)	107.3 (7)	Br –C (34)–C (33)	117.9 (8)
C (8)–C (9)–C (19)	111.1 (7)	C (36)–C (35)–C (34)	116.5 (9)
C (11)–C (9)–C (19)	104.7 (7)	C (1)–C (10)–C (5)	106.2 (7)
C (12)–C (11)–C (9)	118.0 (7)	C (1)–C (10)–C (9)	116.3 (7)
C (13)–C (12)–C (11)	108.5 (7)	C (5)–C (10)–C (9)	115.1 (7)
C (13)–C (12)–O (5)	107.8 (7)	C (1)–O (1)–C (21)	115.8 (7)
C (11)–C (12)–O (5)	108.0 (7)	C (4)–O (2)–C (17)	61.7 (6)
C (14)–C (13)–C (12)	126.8 (9)	C (6)–O (4)–C (28)	118.9 (8)
C (14)–C (13)–C (16)	111.6 (9)	C (12)–O (5)–C (30)	114.9 (7)
C (12)–C (13)–C (16)	121.5 (8)	C (15)–O (6)–C (16)	109.1 (7)
C (15)–C (14)–C (13)	107.1 (9)	C (18)–O (3)–C (26)	115.0 (7)
O (6)–C (15)–C (14)	109.3 (9)	C (31)–C (36)–C (35)	121.2 (9)
O (6)–C (15)–O (7)	121.3 (10)		

Thus, the tentative structure of **1** was deduced from the results of spectroscopic analysis, and the absolute structure of the compound was confirmed by an X-ray crystallographic study of the *p*-bromobenzoate of **1** (**5**). The crystal structure of **5** was determined by the heavy atom anomalous dispersion method[8] and refined by the block-diagonal least-squares method. The final atomic co-ordinates are listed in Table III, the bond lengths in Table IV, and the bond angles in Table V. As shown in Fig. 1, the result of the X-ray study confirmed the tentative struture of **1**, and provided the *neo*-clerodane structure for the absolute configuration of adjugamarin.

All the clerodane type diterpenes isolated till now from *Ajuga* species possess the *neo*-clerodane absolute configuration. Taking into account this fact and that **2** and **3** are apparently biogenetically related to **1**, the same configuration can be expected for these related diterpenes. The (*S*) configuration at C-12 in **1** is the same as that at C-12 of the furanoid diterpenes which were isolated from *Teucrium* species.[9]

Worm-eaten spots are rarely found in the leaves of *A. nipponensis*. Many clerodane-type diterpenes are known to be insect antifeedants; therefore, the compounds **1**, **2**, and **3** may well possess antifeedant activity.

Experimental

Melting points were determined on a Yazawa hot-stage apparatus and are uncorrected. Spectroscopic measurements were obtained with the following instruments: Jasco IRA-1 (IR spectra); JEOL PS-100 (^{1}H NMR spectra), JEOL FX-100 (^{13}C NMR spectra), with tetramethylsilane as an internal standard; Hitachi RMU-7L (mass spectra); and Hitachi 323 (UV spectra). The optical rotation was measured with a JASCO DIP-4. GC was carried out with a Hitachi 163 (PEG-20M, G-SCOT column, 90 °C).

Isolation——The air-dried leaves (1.5 kg) of *Ajuga nipponensis*, collected in the botanic garden of this college in June, 1978, were extracted with hot ethanol (22 l). The concentrated ethanol extract was partitioned between benzene and water. Benzene was evaporated off from the benzene solution, and the residue was chromatographed over silica gel with the hexane–chloroform system (5 : 5).

Crystallization of the concentrated bitter fraction from tetrachloromethane afforded ajugamarin (**1**) as colorless needles (3.3 g); mp 93—95 °C, *Anal.* Calcd for $C_{29}H_{40}O_{10}\cdot 1/2CCl_4$: C, 56.64; H, 6.45; Cl, 11.34. Found: C, 56.59; H, 6.51; Cl, 11.52, $[\alpha]_D^{25}$ $-20°$ ($c=0.4$, CCl_4), EI-MS *m/z*: 518, 505, 475, 449, 448, 433, 83, 55, and 43. Separation of the mother liquor, by a high performance liquid chromatography (HPLC): silica gel, 22D × 300, $CHCl_3$–$(Me)_2CO$ (8 : 2), yielded dihydroajugamarin (**2**) (320 mg) as an amorphous solid. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3500, 1780, 1740, and 1370. EI-MS *m/z*: 520 (M^+-30), 507, 477, 448, 435, 357, 333, 178, 111, 85, 57, and 43. Besides **1** (a further 0.6 g) and **2**, HPLC separation of the mother liquor gave ajugamarin chlorohydrin (**3**) as an amorpous solid (17 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1780, 1760—1700, 1640, and 1130. EI-MS *m/z*: 548 (M^+-HCl), 530, 518, 505, 475, 449, 448, 433, 83, 55, and 43.

Oxidation of 1 to 12-Oxoajugamarin (4)——Dry pyridine solution (10 ml) of **1** (125 mg as a CCl_4 solvate) was added dropwise to a solution of chromium trioxide (200 mg) in dry pyridine (10 mg) at 0 °C. The mixture was stirred at room temperature for 2 h. The solution was diluted with ethyl acetate (50 ml) and filtered through Celite. The filtrate was washed with water and dried. Chromatography of the dried filtrate on silica gel with ethyl acetate as the eluent gave **4** as amorphous solid (50 mg). UV $\lambda_{max}^{95\%EtOH}$ nm (ε): 216 (9200), 290 (3600). IR ν_{max}^{KBr} cm^{-1}: 1780, 1750—1690. EI-MS *m/z*: 546 (M^+), 516, 503, 473, 446, 431, 200, 187, 170, 80, 55, and 43.

Formation of 3 from 1——Treatment of **1** (90 mg) with hydrogen chloride in methanol (0.5%) was carried out at room temperature for 1 d. After removal of the solvent at room temperature, the residue was chromatographed over silica gel with benzene–acetone (9 : 1) as the eluent. The main product, obtained as an amorphous solid (47 mg), was found to be identical with the naturally occurring ajugamarin chlorohydrin (**3**) by TLC, and ^{1}H NMR and IR spectral comparisons.

Acetylation of 3——Acetylation of **3** (20 mg) with acetic anhydride (0.1 ml) and pyridine (0.2 ml) at 20 °C for 15 h, followed by the normal work-up, gave a single product. Purification of the product by preparative TLC (silica gel, benzene–MeOH (9 : 1)) gave the non-crystalline monoacetate of **3**. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1780, 1760—1700, 1640, 1370, and 1130. NMR δ ($CDCl_3$): 7.00 (1H, br q, $J=7$ Hz, 3′-H), 6.0—5.8 (3H, overlapping, 1-H, 12-H, and 14-H), 5.0 (1H, 6-H), 4.94 and 7.74 (2H, AB q, $J=12$ Hz, 18-H_2), 4.75 (2H, overlapping, 16-H_2), 3.97 and 3.88 (2H, AB q, $J=12$ Hz, 17-H_2), 2.20 (6H, $2\times OCOCH_3$), 2.03 (3H, $OCOCH_3$), and 1.86 (3H, br s, 5′-H_3), 1.82 (3H, br d, 4′-H_3), and 0.84 (6H, 19-H_3 and 20-H_3).

Hydrolysis of 1——Hydrolysis of **1** (17 mg) with hydrochloric acid (10%, 1 ml) in methanol (1 ml) was carried out at 90 °C for 2 h. The hydrolysate was extracted with ether. The ether extract contained tiglic acid, which was shown to be identical with an authentic sample by GC.

Hydrolysis of 2——A methanol solution of **2** (98 mg) in 2.5 ml was treated with 1 N sodium hydroxide solution (4 ml). The mixture was left at room temperature for 4 h. After acidification with 1 N hydrochloric acid, the reaction mixture was extracted with ether. The ether extract, containing 2-methylbutyric acid (which was identical with an authentic sample by GC), was concentrated, adjusted to pH 6.5 with 1 N hydrochloric acid, and refluxed with ethanol (0.5 ml) and *p*-phenylphenacyl bromide (60 mg) for 1 h. After removal of the solvent from the reaction mixture, the residue was extracted with benzene. The benzene extract, which was purified by silica gel column chromatography (hexane–$CHCl_3$ (7 : 3)), gave *p*-phenylphenacyl (*S*)-2-methylbutyrate; colorless needles (9.5 mg) from hexane, mp 67—69 °C. $[\alpha]_D^{25}$ $+14°$ ($c=1.0$, $CHCl_3$). EI-MS *m/z*: 296 (M^+). The ester was identified by direct comparison (HPLC and MS) with authentic *p*-phenylphenacyl DL-2-methylbutyrate.

Methanolyses of 1 and 2——Methanol solutions of **1** (11 mg in 0.5 ml) and **2** (10 mg in 0.5 ml), respectively, were treated with a methanolic solution of sodium methoxide (3%, 0.5 ml) at 90 °C for 1 h. The reaction mixtures were neutralized through Amberlite IR-120 B. TLC (silica gel, CH_2Cl_2–MeOH (9 : 1)) indicated that identical products, which were more polar than **1** and **2**, were formed from the two diterpenes.

Conversion of 1 to Ajugamarin *p*-Bromobenzoate (5)——*p*-Bromobenzoyl chloride (200 mg) was added to a solution of **1** (90 mg) in pyridine (5 ml) at 0 °C, and the mixture was stirred at 70 °C for 9 h. After removal of pyridine under reduced pressure at 40 °C, the residue was extracted with chloroform. The extract was chromatographed over silica gel with chloroform to give two products, which were separated by preparative TLC (silica gel, benzene–ethyl acetate (7 : 3)). Crystallization of the higher *Rf* material from hexane–ethyl acetate (5 : 5) afforded colorless needles of ajugamarin *p*-bromobenzoate chlorohydrin (25 mg). mp 106—107 °C. *Anal.* Calcd for $C_{36}H_{45}BrClO_{11}$: C, 56.29; H, 5.77; Br, 10.40; Cl, 4.62. Found: C, 56.03; H, 5.64; Br, 10.24; Cl, 4.80. IR ν_{max}^{KBr} cm^{-1}: 3500, 1760—1700, 1640, and 1590. EI-MS *m/z*: 750, 748, 732, and 730. NMR δ ($CDCl_3$): 7.92 (4H, AA′BB′, *p*-bromobenzoyl), 7.03 (1H, br q, $J=7$ Hz, 3′-H), 6.25 (1H, br d, $J=11$ Hz, 1-H), 6.00 (1H, 14-H), 4.85 (2H, 16-H_2), 4.92 and 4.68 (2H, AB q, $J=12$ Hz, 18-H_2), 3.62 and 3.40 (2H, AB q, $J=12$ Hz, 17-H_2), 2.21 (3H, s, $OCOCH_3$), 2.05 (3H, s, $OCOCH_3$), 1.90 (3H, br s, 5′-H_3), 1.79 (3H, br d, $J=7$ Hz, 4′-H_3), 0.88 (3H, s, 19-H_3), and 0.88 (3H, d, 20-H_3). The lower *Rf* material, **5**, was crystallized from ethanol as colorless plate-like crystals (20 mg). mp 222—225 °C. *Anal.* Calcd for $C_{36}H_{44}BrO_{11}$: C, 59.10; H, 6.01; Br, 10.92. Found: C, 59.00; H, 5.96; Br, 11.18. IR ν_{max}^{KBr} cm^{-1}: 3040, 1760—1705, and 1595. EI-MS *m/z*: 732 and 730 (M^+, isotope peaks), 702 and 700. NMR δ ($CDCl_3$): 7.78 (4H, AA′BB′, *p*-bromobenzoyl), 6.88 (1H, br q, $J=7$ Hz, 3′-H), 6.10 (1H, br d, $J=11$ Hz, 1-H), 5.86 (1H, 14-H), 4.73 (2H, 16-H_2), 4.86 and 4.32 (2H, AB q, $J=12$ Hz, 18-H_2), 2.07 (3H, s, $OCOCH_3$), 1.88 (3H, s, $OCOCH_3$), 1.75 (3H, d, $J=7$ Hz, 4′-H_3), 0.85 (3H, d, $J=6$ Hz, 20-H_3), and 0.80 (3H, s, 19-H_3).

Determination of the Crystal Structure of 5——The X-ray specimen, of about 0.4 × 0.2 × 0.1 mm in size, was cut from a plate-like crystal obtained from ethanol solution of **5**. The lattice parameters and the intensity data were measured on a Philips PW 1100 diffractometer using graphite-monochromated Cu*Kα* radiation.

Crystal data: $C_{36}H_{43}BrO_{11}$, MW = 731.6, monoclinic, space group $P2_1$, $Z=2$, $a=11.929$ (6), $b=16.184$ (8), $c=9.230$ (4) Å, $\beta=98.80$ (6)°, $V=1761.0$ Å^3. μ for Cu$K\alpha=10.37$ cm^{-1}, $D_c=1.380$ g·cm^{-3}, $D_m=1.373$ g·cm^{-3}.

The intensities of 3177 reflections were measured within a 2 θ range of 6—133°. Those were reduced to 2945 as a result of averaging the intensities of the equivalent reflections. The disagreement factor, R_F [$R_F=\Sigma(F_o-F_o')/\Sigma F_o$, where F_o' is an equivalent reflection for F_o or a Friedel reflection for F_o], for the equivalent reflections was 0.024. Besides these reflections, the intensities of 656 Friedel reflections were also measured. The disagreement factor, R_F, for the Friedel pairs was 0.050.

A comparison of the 253 Friedel pairs of $|F_o|$ having structure factor differences greater than $2\sigma(F_o)$ with $|F_c|$'s, calculated with the anomalous dispersion correction of the bromine atom for Cu*Kα* radiation, showed that 235 pairs gave a result consistent with the absolute configuration shown in Fig. 1. The disagreement factor, R [$R=\Sigma(|F_o|-|F_c|)/\Sigma|F_o|$], between the observed and calculated structure factors for the 253 pairs was 0.073, while that for the reversed structure was 0.093.

The final disagreement factor, R, for the 2945 reflections was 0.063, including corrections for the anomalous dispersion but not including the hydrogen atoms.

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