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NEO-CLERODANE DITERPENOIDS FROM *AJUGA PARVIFLORA*

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Key Word Index—*Ajuga parviflora*; Labiatae; neo-clerodane diterpenoids; antifeedants; X-ray analysis.

Abstract—Three new neo-clerodane diterpenoids, deoxyajugarin-I, ajugarin-I chlorohydrin, and 3β-acetoxy-clerodinin C, have been isolated from *Ajuga parviflora* collected near Nainital, India. Also isolated were ajugarin-I and II, ajugamarin F4, dihydroclerodin-I, clerodinins C and D and 15-α-ethoxy- and 15-β-ethoxy-14-hydro-ajugapitin. Copyright © 1996 Published by Elsevier Science Ltd

INTRODUCTION

The genus *Ajuga* (Labiatae) has attracted attention since the report in 1976 that *A. remota* leaves, collected in Kenya, were not attacked by African armyworms. This led to the isolation of three moderately strong antifeedants, ajugarins-I, II and III, later shown to be configurationally related to the neo-clerodane class of diterpenoids [1, 2]. Since then, reports of the isolation of ecdysteroids, insect moulting hormones, from this genus have also appeared. These reports, dealing with the neo-clerodane diterpenoids and the phytoecdysteroids isolated, have been extensively reviewed by Camps and Coll [3].

In early 1982, one of us [4] recognized the presence of ajugalactone and two other unidentified phytoecdysteroids in *A. reptans*, commonly used as a landscaping ground cover in Southern California. Over the past few years we have been studying the chemotaxonomy of members of the Labiatae that grow in the Kumaun region of the Indian Himalaya [5, 6]. *A. parviflora* grows in the temperate region of the Indian Himalaya at 4000–6000-foot elevations; it flowers between March and October [7]. The morphological similarity of this plant to *A. reptans* led us to study its chemistry. *A. parviflora* was observed to be rich in ajugarin-I, which makes up about 0.04% of the air dried plant material. In addition, three new and seven other neo-clerodane diterpenoids were isolated and their structures derived from their spectroscopic data. Two structures were confirmed by X-ray analysis.

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RESULTS AND DISCUSSION

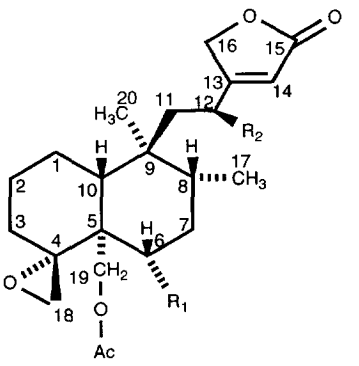
Diterpenoids isolated from *A. parviflora* are members of two groups of neo-clerodanes which have in common a similarly substituted decalin. Members of the first group, compounds **1–5**, have a but-13-en-15,16-olide ring connected to the substituted decalin by a two-carbon chain. Common to the second group, compounds **1′–6′**, is a hexahydrofurofuran moiety directly attached to the decalin.

But-13-en-15,16-olide ring containing neo-clerodane diterpenoids

Ajugarin-I (**1**). ^{1}H- and ^{13}C-NMR spectral data for the major component isolated from *A. parviflora* were in excellent agreement with those reported for ajugarin-I [1]. While there was no doubt about their structural identity, their configurational identity would have remained in doubt in the absence of a reported specific rotation for ajugarin-I. The previous configurational assignment for ajugarin-I was based on X-ray analysis of its 12-bromo derivative [2]. We obtained crystals of ajugarin-I that allowed direct determination of its absolute configuration by X-ray analysis by the Flack method [8]. Figure 1 shows the computer generated perspective drawing of ajugarin-I. All bond distances and angles were normal. The directly observed configuration of ajugarin-I agreed with the stereochemistry derived from the 12-bromo derivative.

Ajugarin-II (**2**) was identified by comparison with a sample that was kindly provided by Professor I. Kubo.

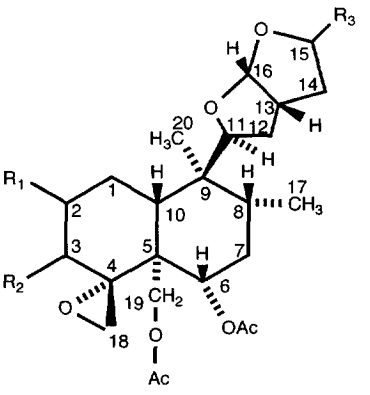
Deoxyajugarin-I (**3**). The HRDCI-mass spectrum gave MH^+ at m/z 419.2414 corresponding to $C_{24}H_{34}O_6$ as the molecular formula of this compound,



	Compound	R_1	R_2
1	Ajugarin-I	AcO	H
2	Ajugarin-II	OH	H
3	Deoxyajugarin-I*	AcO	H
4	Ajugarin-I chlorohydrin**	AcO	H
5	Ajugarmarin F4	AcO	$EtCHMeCO_2$
*	C-4,18 deoxy		
**	C-4α-OH,C-18-Cl		

which is one oxygen atom less than in ajugarin-I. The ^{13}C NMR spectrum showed the two acetate and one lactone C=O groups to be intact; however, δ 151.2 and 106.8 in place of δ 65.0 and 48.5 for C-4 and C-17 respectively in the ^{13}C NMR spectrum indicated the presence of a C=C in place of the epoxide in ajugarin-I. Additionally, the compound lacked the doublets at δ 2.99 and 2.21 ppm present in the ^1H NMR spectrum of ajugarin-I. Instead, the doublets were replaced by an apparent singlet at δ 4.4 ppm, characteristic of a 1,1-disubstituted ethylene. We thus concluded that this compound is the deoxygenated epoxide **3**. The ^{13}C NMR and the ^1H NMR data for deoxyajugarin-I **3** are summarized in Tables 1 and 2 respectively.

Ajugarin-I chlorohydrin (**4**). The HRDCI-mass spectrum of this compound gave an MH^+ peak that corresponded to $C_{24}H_{34}O_7$, the molecular formula for ajugarin-I. While ajugarin-I does not show absorption at



	Compound	R_1	R_2	R_3
1'	Dihydroclerodin-1	H	H	H
2'	Clerodinin C	H	H	OEt(β-)
3'	Clerodinin D	H	H	OEt(α-)
4'	3β-Acetoxyclerodinin C	H	AcO(β-)	OEt(β-)
5'	15β-Ethoxy-14-hydroajugapitin	OH(α)	$EtCHMeCO_2$(β-)	OEt(β-)
5'	15α-Ethoxy-14-hydroajugapitin	OH(α)	$EtCHMeCO_2$(β-)	OEt(α-)

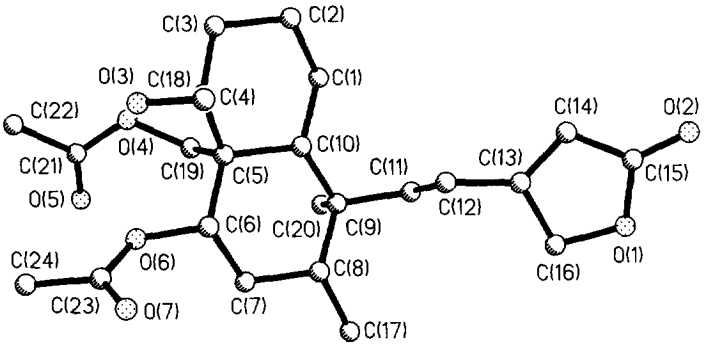


Fig. 1. Computer generated perspective drawing of ajugarin-I.

Table 1. ^{13}C NMR data for compounds **3** and **4** (75 MHz, $CDCl_3$, $CHCl_3$ as int. standard)

C	3	4
1	21.3*	22.0*
2	21.9*	21.1
3	28.5	21.8*
4	151.1	76.7
5	48.3	48.2
6	75.5	74.1
7	32.1	32.8
8	34.3	34.9
9	38.8	38.8
10	49.7	4.50
11	34.6	31.8
12	33.7	35.3
13	173.7	173.7
14	115.2	115.4
15	170.9	169.6
16	73.0	73.0
17	15.3	15.3
18	106.8	49.9
19	61.0	63.6
20	17.6	17.7
Ac:		
C=O	170.4	170.0
C=O	170.1	170.0
CH_3	21.1	21.5
CH_3	21.0	21.4

*Values may be reversed.

Table 2. ^1H NMR data for compounds **3** and **4** (300 MHz, $CDCl_3$, $CHCl_3$ as int. standard. *J*: Hz)

H	3	4
6	5.00 *dd*, 10.5, 4.5	4.9 *dd*, 10, 5
14	5.76 *brs*	5.81 *brs*
16	4.67 *d*, 1.4	4.70 *brs*
17	0.80 *d*, 7.0	0.80 *d*, 7.0
18	4.4 *apps*	3.96 *d*, 11.3
		3.91 *d*, 11.3
19	4.71 *d*, 11.8	4.95 *d*, 13.0
	4.17 *d*, 11.8	4.59 *d*, 13.0
20	0.74 *s*	0.81 *s*
Ac:		
CH_3	2.47 *s*	2.08 *s*
CH_3	2.38 *s*	1.98 *s*

~3500 cm^{-1} in its IR spectrum, this compound showed a strong absorption at ~3500 cm^{-1} characteristic of an OH group. Also, significant differences were observed in the C-4 and C-18 portions of their ^1H and ^{13}C NMR spectra indicating a perturbation in the epoxide of the ajugarin-I structure. The C-4 and C-18 were assigned their ^{13}C NMR δ values of 76.0 and 49.9 ppm respectively, which matched with the δ values corresponding to carbons in the chlorohydrin prepared by treating a methanolic solution of ajugamarin B1 with HCl [9]. Similarly, the ^1H NMR δ and *J* values of 3.96 (J = 11.3 Hz) and 3.92 ppm (J = 11.3 Hz) assigned to the diastereotopic C-18 protons of H_2CCl, matched the δ and *J* values of ajugamarin B1 chlorohydrin. The position of OH attachment to C-4 and Cl to C-18 and that its absolute configuration is as in **1** were confirmed by X-ray analysis of a single crystal of the product. As observed for **1**, all bond distances and angles were normal. It is interesting to note that ajugamarin B1 chlorohydrin is a synthetic product while the isolation of ajugarin-I chlorohydrin from the plant extract constitutes the first example of the isolation of a chlorohydrin from the *Ajuga* genus. At present, we discount the isolation of ajugarin-I chlorohydrin as an artefact of isolation procedure; examples of the isolation of chlorohydrins from plant sources are well documented [10].

Ajugamarin F4 (**5**). The ^1H and ^{13}C NMR spectra of this compound and the sign and magnitude of its optical rotation were in excellent agreement with those reported for Ajugamarin F4 isolated from *A. decumbens* [11].

Hexahydrofurofuran containing neo-clerodane diterpenoids

All compounds of this group could be structurally recognized as having the hexahydrofurofuran group by the presence of absorptions at δ 2.95 for C-13 (H) and 5.7 ppm (*d*, J = 5.4) for C-16 (H) in their ^1H NMR spectra as well as at δ 40.0 and 107.0 ppm for C-13 and C-16 respectively in their ^{13}C NMR spectra. Those compounds with attached OEt at C-15 showed the characteristic triplet at δ 1.15 ppm (J = 7.0 Hz) for the methyl protons of OEt.

Dihydroclerodin-I (**1′**). The presence of a hexahydrofurofuran moiety with no substituent other than the substituted decalin of a neo-clerodane was inferred from the existence of a base peak at *m/z* 113 in the CIMS of **1′**. Further evidence for the presence of the hexahydrofurofuran moiety was the appearance in the ^{1}H NMR spectrum of signals at δ 4.07 (*dd*, $J = 11.5$, 5.4 Hz), 2.83 (*m*), 3.83 (*m*) and 5.61 ppm (*d*, $J =$ 5.0 Hz), corresponding respectively to the protons at C-11, C-13, C-15 and C-16. These ^{1}H chemical shifts and the ^{13}C NMR signals at δ 32.8, 33.4, 42.1 and 107.7 ppm matched exceedingly well with the chemical shifts for the hexahydrofurofuran portions in ivain I, 2-acetylivain I and 14,15-dihydroajugapitin [12].

The molecular formula $C_{24}H_{36}O_7$ for **1′** obtained from its HRDCI-mass spectrum, the absence of OH, as indicated by its IR spectrum, and the presence of two acetates and epoxide indicated the decalin portion of **1′** to be identical to that of clerodin. Combination of this information about the decalin structure and that of the hexahydrofurofuran moiety indicated **1′** to be dihydroclerodin. This compound was first described as a catalytic hydrogenation product of clerodin, and its ^{1}H NMR data reported [13]. It was subsequently isolated as a natural product from *Caryopteris divaricata* maximum [14]. The ^{1}H NMR data available from previous reports, though useful, are rather incomplete; also no ^{13}C NMR assignments have been reported. This information has been acquired and is presented in Tables 3 and 4.

Clerodinin C (**2′**) *and Clerodinin D* (**3′**). The two neo-clerodane diterpenoids, epimeric at C-15, were first isolated from *Clerodendron brachyanthum* Schauer; they have also been reported as the products of AcOH-catalysed ethanolysis of clerodin [15]. Clerodinin C was assigned the 15β-OEt stereochemistry because of its predominant formation in the epimeric mixture caused by the approach of the EtOH molecule from the less hindered β-side of C=C. An extension of this logic led the epimeric clerodinin D to be assigned the 15α-OEt stereochemistry.

On the whole, our ^{13}C and ^{1}H chemical shift assignments given in Tables 3 and 4 for **2′** and **3′** agree quite closely with the reported values. However, we suggest a

Table 3. ^{13}C NMR data for compounds **1′**–**6′** (75 MHz, $CDCl_3$, $CHCl_3$ as int. standard)

C	1′	2′	3′	4′	5′	6′
1	25.0	25.0	25.0	21.1	30.3	30.2
2	22.1	22.1	22.2	31.0	71.8	71.7
3	32.4	32.7	32.2	67.2	72.3	72.3
4	65.0	65.0	65.0	65.3	62.8	62.8
5	45.5	45.5	45.6	46.3	45.6	45.6
6	71.9	72.1	71.9	71.3	71.3	71.2
7	32.5	33.4	33.4	33.2	33.1	33.2
8	35.8	36.1	36.2	36.0	36.1	35.9
9	40.5	40.1	40.1	40.3	40.0	40.0
10	48.2	48.3	48.5	47.6	43.3	43.6
11	85.2	83.5	83.6	83.4	83.2	83.0
12	33.4*	32.7	32.7	32.7	32.6	32.4
13	42.1	40.7	40.0	40.7	40.6	40.0
14	32.7*	39.6	38.2	39.6	39.5	38.2
15	68.3	103.8	103.6	103.9	103.8	103.5
16	107.7	109.1	107.1	109.1	109.1	107.1
17	16.5	16.4	16.45	16.3	16.3	16.3
18	48.4	48.4	48.4	42.6	42.4	42.4
19	61.7	61.8	61.7	61.4	61.5	61.4
20	14.0	14.1	14.0	14.0	14.0	13.8
Ac:						
C=O	170.9	170.8	170.9	171.1	171.0	171.0
C=O	170.1	170.0	170.1	170.0	170.1	170.1
C=O				169.6		
CH_3	21.1	21.1	21.2	21.1	21.1	21.1
CH_3	21.1	21.1	21.2	21.03	21.0	21.0
CH_3				20.96		
OEt:						
CH_2		63.0	62.8	63.1	63.1	62.8
CH_3		15.0	15.1	15.1	15.1	15.1
EtCHMeCO:						
CO					175.7	175.7
CH					41.1	41.1
CH_2					26.7	26.7
$CHCH_3$					16.3	16.4
CH_2CH_3					11.2	11.2

*Values may be reversed.

Table 4. ^{1}H NMR data for compounds **1′–6′** (300 MHz, $CDCl_3$, $CHCl_3$ as int. standard. *J*: Hz)

H	**1′**	**2′**	**3′**
6	4.65 *dd*, 11.1, 4.0	4.66 *dd*, 11.5, 4.4	4.66 *dd*, 11.5, 4.4
8	1.40 *m*		1.43 *m*
10	1.59 *dd*, 15.0, 9.4		
11	4.07 *dd*, 11.5, 5.4	4.37 *dd*, 11.3, 5.6	3.99 *dd*, 11.6, 4.5
13	2.83 *m*	2.80 *m*	2.95 *m*
15	3.83 *m*	5.06 *m*	5.2 *m*
16	5.60 *d*, 5.0	5.75 *d*, 5.6	5.69 *d*, 5.3
17	0.83 *d*, 6.4	0.86 *d*, 6.4	0.84 *d*, 6.4
18	2.96 *m*	3.0 *m*	3.0 *m*
19	4.86 *d*, 12.1	4.87 *d*, 12.3	4.87 *d*, 12.2
	4.34 *d*, 12.1	4.35 *d*, 12.3	4.35 *d*, 12.2
20	0.93 *s*	0.91 *s*	0.94 *s*
Ac:			
CH_3	2.07 *s*		
CH_3	1.91 *s*		
OEt:			
CH_2		3.40 *m*	3.42 *m*
		3.72 *m*	3.72 *m*
CH_3		1.15 *t*, 7.1	1.15 *t*, 7.0

H	**4′**	**5′**	**6′**
1		2.62 *dd*, 13.3, 5.1	2.6 *m*
2		3.58 *dd*, 10.2, 5.5	3.59 *dd*, 10.2, 5.6
3		5.19 *d*, 9.8	5.19 *d*, 9.8
6	4.71 *dd*, 12.0, 4.3	4.66 *dd*, 11.3, 4.4	4.66 *dd*, 11.2, 4.4
11	4.42 *dd*, 11.0, 5.7	4.4 *dd*, 11.2, 5.8	3.98 *dd*, 11.8, 4.5
13	2.8 *m*	2.8 *m*	2.99 *m*
15	5.06 *d*, 5.7	5.05 *d*, 5.7	5.18 *d*, 4.7
16	5.76 *d*, 5.6	5.78 *d*, 5.3	5.72 *d*, 5.5
17	0.87 *d*, 6.4	0.84 *d*, 6.8	0.84 *d*, 7.5
18	2.82 *d*, 4.2	2.77 *d*, 4.1	2.77 *d*, 4.1
	2.58 *d*, 4.2	2.53 *d*, 4.1	2.53 *d*, 4.1
19	4.78 *d*, 12.4	4.78 *d*, 12.2	4.76 *d*, 12.3
	4.35 *d*, 12.4	4.37 *d*, 12.2	4.37 *d*, 12.3
20	0.90 *s*	0.92 *s*	0.92 *s*
OEt:			
CH_2	3.41 *m*	3.41 *m*	3.41 *m*
	3.71 *m*	3.71 *m*	3.71 *m*
CH_3	1.15 *t*, 7.1	1.15 *t*, 7.1	1.15 *t*, 7.1
EtCHMeCO:			
CH		2.37 *m*	2.37 *m*
CH_3		1.3 *m*	1.3 *m*
		1.65 *m*	1.65 *m*
$CHCH_3$		1.08 *d*, 6.9	1.08 *d*, 6.9
CH_2-CH_3		0.87 *t*, 7.0	0.87 *t*, 7.0

reversal of the assignments for the C-17 and C-20 methyls in **2′** and **3′**. The C-17 methyl doublet at δ ~0.8 and the C-20 singlet at δ ~0.9 ppm in their ^{1}H NMR spectra appear respectively at δ ~16.5 and δ ~14.0 ppm in their ^{1}H-^{13}C heteronuclear correlation spectra.

The ^{1}H NMR chemical shifts of **2′** and **3′** indicate a significant, ~0.4 ppm, difference in the reported as well as the observed chemical shifts assigned to their respective C-11 protons. They appear at δ 4.37 for **2′** and 3.95 ppm for **3′**. One could ascribe this difference to the shielding effects of the endo OEt over the exo OEt of C-15 on the C-11 proton. We carried out NOE experiments to check this, but no NOE enhancement between the C-11 proton and the CH_2 of OEt in **2′** and **3′** was observed. Therefore, this difference between the C-11 proton chemical shifts for the two epimers must be due to some other factor. Based on these and the reported data, we assign **2′** and **3′** the clerodinin C and D structures respectively.

3β-Acetoxyclerodinin C (**4′**). The molecular formula $C_{28}H_{42}O_{10}$ and the presence of *m/z* 157 and 111 peaks in the CI-mass spectra of this compound indicated the presence of the hexahydrofurofuran moiety common with **2′** and **3′**. This was further supported by the presence of a *dd* (J = 11.1, 5.7 Hz) at δ 4.42 in the ^{1}H NMR spectrum for the C-11 proton which matched the

corresponding value for the C-11 proton of clerodinin C and which indicated that the stereochemistry of the ethoxy group in **4′** is identical to that in the thermodynamically more stable C-15 β-OEt epimer, clerodinin C. The ^{1}H NMR of **4′** showed the presence of three acetates rather than two as in **2′** and **3′**. This left the assignment of location of the third acetoxy group in the decalin portion of the neoclerodane structure. It was noted that the ^{13}C δ values for C-4 through C-10, C-14, C-19 and C-20 of **4′** matched with the corresponding carbons of **2′** and **3′**. The largest deviation was noted in the δ value for C-18, approximately 6 ppm lower in **4′** compared with **2′** and **3′**. This difference is perhaps caused by the presence of the acetoxy group in the β-position of C-3, which had a value of δ 67.23 ppm. The remaining two carbons, C-1 and C-2, could then be assigned δ values of 21.2 and 30.96 ppm, respectively. The NMR data for **4′** are summarized in Tables 3 and 4. On the basis of these observations of the distribution of C-15 OEt epimers in nature, one can speculate as to the possible presence of the corresponding so far unisolated C-15 α-OEt epimer of **4′**, in *A. parviflora.*

15β-Ethoxy-14-hydroajugapitin (**5′**) *and 15α-ethoxy-14-hydroajugapitin* (**6′**). 15-Ethoxy-14-hydroajugapitin was first reported as a C-15 epimeric mixture from *A. chamaepitys* [16]. The closeness of the IR and the mass spectral data, along with the NMR (Tables 3 and 4), were convincing as to the similarity of the structures **5′** and **6′** to that reported for the epimeric mixture. That **5′** and **6′** were indeed C-15 epimers became evident upon comparing the ^{1}H NMR chemical shifts of C-11 protons for the two compounds with **2′** and **3′**. In this case, as in the case of **2′** and **3′**, the only major difference is that of 0.4 ppm in the chemical shift of this proton in **5′** and **6′**. On the basis of this relationship with **2′** and **3′** one can assign **5′** to be the thermodynamically more stable C-15 β-OEt epimer with **6′** being the corresponding C-15 α-OEt isomer.

EXPERIMENTAL

NMR were run in $CDCl_3$. Trace impurity $CHCl_3$ int. standard at 300 HMz for ^{1}H NMR and 75 MHz for ^{13}C NMR. Mass spectra obtained at the Mass Spectrometry Facility, University of California, Riverside. Unless indicated otherwise, the mass spectra were FAB or desorption chemical ionization/NH_3 (DCI/NH_3). Nitrobenzyl alcohol matrix and a 50 eV ion source energy were used respectively for FAB and DCI/NH_3. IR spectra were obtained in KBr. 25 mm × 9 mm 10 μ Si gel column attached to Waters Delta Prep 3000 Preparative Liquid Chromatograph and a Gilson 131 RI detector was employed for HPLC, using CH_2Cl_2 satd. with H_2O.

Extraction and isolation of the diterpenoids. The isolated compounds that were HPLC-homogeneous were dried in vacuum, dissolved in $CDCl_3$ and checked further for homogeneity by examination of their ^{1}H NMR spectra. No special attempts were made to crystallize them unless noted.

The plant material was collected near Nainital and identified by Dr Y.P.S. Pangtey of the Botany Department, Kumaun University, Nainital, where a sample has been deposited. The air dried material (2 kg) was crushed with a mortar and pestle, and covered with EtOH for 4 days. The alcohol extract was drained, and the process was repeated three more times. The alcohol extracts were combined, and the solvent was removed under red. pres. The gummy residue was dissolved in 2 l MeOH–H_2O (1:1) and extracted with hexane (3 × 400 ml) followed by CH_2Cl_2 (5 × 400 ml). The CH_2Cl_2 extracts were combined, washed 3× with 200 ml portions of saturated NaCl, dried over $MgSO_4$, filtered, rotovaped and vacuum dried. The slightly sticky residue was thoroughly mixed with 50 g of silica gel and carefully added to a silica gel column prepared from 150 g silica gel pretreated with 15 ml H_2O and packed as a CH_2Cl_2 slurry. The column was first eluted with 250 ml of CH_2Cl_2 followed by 250 ml each of CH_2Cl_2 mixed with 2%, 5% and successive 5% increases of acetone to 100% acetone. Twelve frs, 250 ml each and labelled CC 1–12, were collected. CC of CC 2 (7.9 g) with CH_2Cl_2 and CH_2Cl_2–acetone mixtures gave 2 g of fairly pure ajugarin-I from the middle frs. Several of the later frs were combined based upon their TLC behaviour and decolourized with activated charcoal pellets. The nearly clear solutions were subjected to repeated HPLC using CH_2Cl_2–acetone (95:5 to 80:20) as the mobile phase. From these frs, in addition to the small amount of ajugarin-I, deoxyajugarin-I, ajugarin-I chlorohydrin, ajugamarin F4, dihydroclerodin, clerodinin C and D and 3β-acetoxyclerodinin C were isolated.

The more polar frs CC 3–6 were combined (7.5 g) and subjected to silica gel column chromatography using CH_2Cl_2 and CH_2Cl_2-acetone. The initial frs produced 4.5 g of ajugarin-I of significant purity, and the later frs when combined gave 1.0 g of residue that contained 30% ajugarin-I. Repeated HPLC of the residue with 9:1 H_2CCl_2–acetone (9:1) as a mobile phase gave ajugarin-II, and 15α- and 15β-ethoxy-14-hydroajugapitins.

Ajugarin-I (**1**). The HPLC frs containing **1** were combined, and the solvent was allowed to evap. at room temp. The crystals that sepd had mp 159–63°, $[\alpha]_D^{24}$ −14.8° (*c* 0.068; $CDCl_3$). HR-EIMS (probe) 70 Ev. *m/z*: 435.2350 $[MH]^+$ [$C_{24}H_{35}O_7$ requires 435.2384]; LR-EIMS 50 eV. *m/z* (% rel. int.): 404 (5), 391 (14), 361 (13), 319 (100), 301 (32), 189 (25) and 111 (30); IR γ_{max} cm^{-1}: 1780. 1750, 1730, 1640 and 1270.

Ajugarin-II (**2**). $[\alpha]_D^{24}$ +14.6° (*c* 0.025; $CDCl_3$). HR-EIMS (probe) 70 eV. *m/z*: 393.2290 $[MH]^+$ [$C_{22}H_{33}O_6$ requires 393.2278]; LR-EIMS, *m/z* (% rel. int.): 393 (100), 375 (50), 333 (50) and 197 (83); IR γ_{max} cm^{-1}: 3430, 1780, 1730 and 1230. The ^{13}C NMR spectrum was indistinguishable from that of a sample of ajugarin-II provided by Professor I. Kubo.

Deoxyajugarin-I (**3**). $[\alpha]_D^{24}$ +9.7° (*c* 0.11; $CDCl_3$). HRDCI-MS: *m/z*: 419.2414 $[MH]^+$ [$C_{24}H_{35}O_6$ re-

quires 419.2435]; LRDCI-MS: *m/z* (% rel. int.): 436 (100, MNH_4^+) 419 (6), 359 (7), 299 (23), 187 (24) and 113 (30); IR γ_{max} cm^{-1}: 3050, 1775, 1740, 1635 and 1240. ^{13}C and ^1H NMR: Tables 1 and 2.

Ajugarin-I chlorohydrin (**4**). Addition of some hexane to the HPLC frs containing **4** and allowing the solvent to evaporate at room temperature gave crystals with mp. 165–6°, $[\alpha]_D^{24}$ −0.3° (*c* 0.03; $CDCl_3$). HRDCI-MS: *m/z*: 452.2612 $[MHN_4 - HCl]^+$ [$C_{24}H_{34}O_7NH_4$ requires 452.2649] and 435.2325 $[MH - HCl]^+$ [$C_{24}H_{35}O_7$ requires 435.2384]; IR γ_{max} cm^{-1}: 3500, 3045 (weak), 1775, 1730, 1630 and 1230. ^{13}C and ^1H NMR: Tables 1 and 2.

Ajugamarin F4 (**5**). $[\alpha]_D^{24}$ −30.4° (*c* 0.024; $CDCl_3$), lit. $[\alpha]_D^{25}$ −32° for ajugamarin F4 isolated from *A. decumbens* [11]. HRDCI-MS: *m/z*: 552.3166 $[MNH_4]^+$ [$C_{29}H_{42}O_9NH_4$ requires 552.3174]; LRDC-MS: (% rel. int.) *m/z* 552 (100), 535 (6), 452 (74), 216 (17) and 120 (16); IR γ_{max} cm^{-1}: 1780, 1750, 1730 and 1245.

Dihydroclerodin-I (**1′**). $[\alpha]_D^{24}$ −10.9° (*c* 0.047; $CDCl_3$), lit. $[\alpha]_D^{20}$ −20° (*c*, 1–2; $CHCl_3$) [13]. HRDCI-MS: *m/z* 454.2705 $[MNH_4]^+$ [$C_{24}H_{36}O_7NH_4$ requires 454.2806] and 437.2507 $[MH]^+$ [$C_{24}H_{37}O_7$ requires 437.2540]. IR γ_{max} cm^{-1}: 1730 and 1250. ^{13}C and ^1H NMR: Tables 3 and 4.

Clerodinins C (**2′**) *and D* (**3′**). For **2′**, $[\alpha]_D^{24}$ +17.7° (*c* 0.037; $CDCl_3$), lit. $[\alpha]_D^{25}$ +30.0° (*c* 1.00; $CHCl_3$) [15]. For **3′**, $[\alpha]_D^{24}$ −60.8° (*c* 0.013; $CDCl_3$), lit. $[\alpha]_D^{23}$ −31.5° (*c* 1.00; $CHCl_3$) [15]. HRDCI-MS: *m/z* 481.2801 $[MH]^+$ for **2′** and 481.2786 $[MH]^+$ for **3′** [$C_{26}H_{41}O_8$ requires 481.2802]. IR γ_{max} cm^{-1} common to both: 1730 and 1250. ^{13}C and ^1H NMR: Tables 3 and 4.

3β-Acetoxyclerodinin D (**4′**). $[\alpha]_D^{24}$ +12.9° (*c* 0.012; $CDCl_3$); HRDCI-MS: *m/z*: 539.2824 $[MH]^+$ [$C_{28}H_{43}O_{10}$ requires 539.2857]; LRDCI-MS *m/z* (% rel. int.): 556 (12.6), 539 (9.4), 493 (4.8), 171 (8.6), 157 (100), 111 (84.5), 83 (11.4) and 43 (38); IR γ_{max} cm^{-1}: 1730 and 1250. ^{13}C and ^1H NMR: Tables 3 and 4.

15-β-Ethoxy-14-hydroajugapitin (**5**). $[\alpha]_D^{20}$ +2.6° (*c* 0.035; $CDCl_3$); HRDCI-MS: *m/z*: 597.3248 $[MH]^+$ [$C_{31}H_{49}O_{11}$ requires 597.3276]; IR γ_{max} cm^{-1}: 3500, 1740 and 1240.

15-α-Ethoxy-14-hydroajugapitin (**6′**). $[\alpha]_D^{20}$ −50.4° (*c* 0.025; $CDCl_3$); HRDCI-MS: *m/z*: 597.3240 $[MH]^+$ [$C_{31}H_{49}O_{11}$ requires 597.3276]; IR γ_{max} cm^{-1}: 3500, 1740 and 1240.

X-ray crystal structure analysis. A Siemens P4RA diffractometer was used for determination of cell dimensions and data collection. Standard methods were used for solution of structures by direct methods (SHELXTL [17]), refinement (SHELXL-93 [18]), empirical absorption corrections (XABS2 [19]) and determination of absolute configurations [8]. Crystal data and structural refinement parameters for ajugarin-I (**1**) and ajugarin chlorohydrin (**4**) have been deposited at Cambridge. Note that a value of 0.0 for the absolute structure or Flack parameter indicates the correct choice of configuration whereas a value of 1.0 indicates the inverse configuration [8]. The value obtained for **1** is a weak but definite indication of the configuration shown in Fig. 1; the value for the chlorohydrin **4** is a strong indication that its configuration is the same as that shown for **1**.

Tables of positional and thermal parameters and structure factors are available for both structures from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K. The request should be accompanied by the full literature citation for this report.

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