

# neo-Clerodane Diterpenoids from *Ajuga bracteosa*

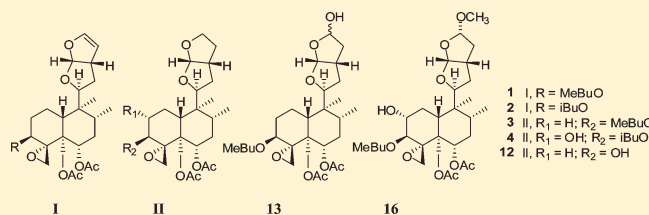
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**S** Supporting Information

**ABSTRACT:** Different *neo*-clerodane diterpenoids were isolated from a dichloromethane extract of *Ajuga bracteosa* depending on the isolation procedure used, owing to the labile nature of these tetrahydrofurofuran derivatives. Under “hydroxyl-free” purification conditions, both clerodin- and dihydroclerodin-type diterpenes were obtained [four new compounds, ajubractins A–D (1–4), along with clerodin (5), 3-*epi*-caryoptin (6), ajugapitin (7), 14,15-dihydroclerodin (8), 3-*epi*-14,15-dihydrocaryoptin (9), ivain II (10), and 14,15-dihydroajugapitin (11)]. When methanol–water mixtures were used for a C<sub>18</sub> reversed-phase prepurification procedure and for semipreparative HPLC, the new ajubractin E (12) was also isolated along with 3 and 8–11, as previously, but 7 was the only tetrahydrofurofuran derivative obtained. Epimeric (15*R* and 15*S*) mixtures were obtained instead of 14-hydro-15-hydroxyclerodin derivatives [15-hydroxyajubractin C (13), 14-hydro-15-hydroxyajugachin A (14), and 14-hydro-15-hydroxyajugapitin (15)], along with 15-*epi*-lupulin B (16). The structures of the new compounds were elucidated by NMR and MS data analysis and by comparison with values previously reported. Antifeedant activity against *Spodoptera littoralis* larvae was evaluated for the compounds obtained.



*Ajuga bracteosa* Wall. ex Benth. (Lamiaceae) is a small prostrate annual herb. The ethnomedicinal applications of its leaves include use as a remedy for acne, constipation, ear infections, headache, hypertension, jaundice, measles, pimples, sore throats, and stomach hyperacidity, and as a blood purifier and a cooling agent.<sup>1–3</sup> Recently, a study on the content of trace elements of *A. bracteosa* suggested a possible correlation with the use of the herb as a remedy for diabetes and hypertension.<sup>4</sup> The isolation of *neo*-clerodane diterpenoids from *A. bracteosa* has been reported in about 10 different papers, as reviewed recently.<sup>5</sup> Different plant collection locations have yielded constituents with a variety of structures, including clerodin- and ajugarin-like side chains among the *neo*-clerodanes isolated.<sup>5</sup> *Ajuga remota*, reported by some authors, is a synonym of *A. bracteosa* according to *The International Plant Names Index*.<sup>6</sup> The isolation and structure elucidation of *neo*-clerodane diterpenes of a sample of *A. bracteosa* harvested in northwest Pakistan are described herein. Antifeedant activities for the isolated compounds have been determined against *Spodoptera littoralis* larvae.

## RESULTS AND DISCUSSION

Different *neo*-clerodane diterpenes were isolated from a dichloromethane extract of the dried and powdered aerial parts of *A. bracteosa*, depending on the isolation procedure, owing to the labile nature of the tetrahydrofurofuran derivatives present in the crude extract. Thus, under “hydroxyl-free” purification conditions, clerodin- and dihydroclerodin-type diterpenes were

obtained, representative of four new and seven already reported compounds {the new ajubractins A–D, or 3β-[(2-methyl)butyryloxy]clerodin (1), 3β-*iso*-butyryloxyclerodin (2), 3β-[(2-methyl)butyryloxy]-14,15-dihydroclerodin (3), and 3β-*iso*-butyryloxy-2α-hydroxy-14,15-dihydroclerodin (14,15-dihydroajugachin A) (4), and clerodin (5),<sup>7,8</sup> 3-*epi*-caryoptin (6),<sup>9</sup> ajugapitin (7),<sup>8,10</sup> 14,15-dihydroclerodin (8),<sup>8,11</sup> 3-*epi*-14,15-dihydrocaryoptin (9),<sup>9,12</sup> ivain II (10),<sup>13</sup> and 14,15-dihydroajugapitin (11)<sup>8,10,14</sup>}. The extract was fractionated on a silica gel column eluting with CH<sub>2</sub>Cl<sub>2</sub>–*tert*-butyl methyl ether mixtures. Fractions containing clerodane-like compounds were selected on the basis of their TLC behavior and then separated on preparative TLC plates, using hexane–*tert*-butyl methyl ether mixtures as developing solvents, in order to minimize any undesired addition reaction to the sensitive enol double bond in the tetrahydrofurofuran ring.<sup>7,15</sup>

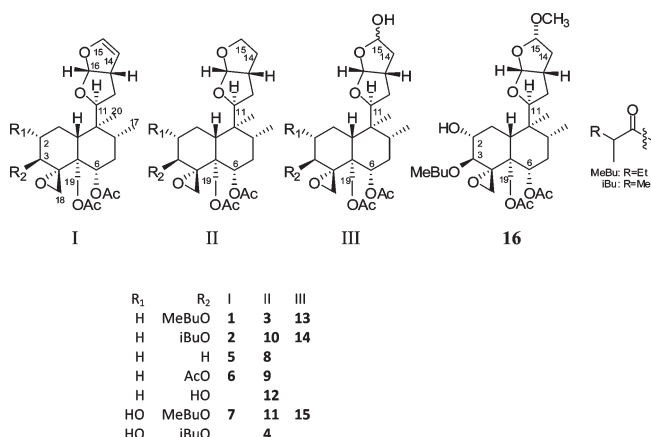
No match with any previously reported *neo*-clerodane diterpene was obtained for compounds 1–4, and their structure elucidation based on 1D and 2D NMR experiments is described below. The <sup>1</sup>H NMR chemical shifts and multiplicities for compounds 5–11 matched with those described in the literature.<sup>7–14</sup> 3β-Acetoxy-14,15-dihydroclerodin, or 3-*epi*-14,15-dihydrocaryoptin (9), is a new natural compound and was prepared by semisynthesis previously.<sup>12</sup>

Compound 1 (ajubractin A) displayed 29 signals in the <sup>13</sup>C NMR spectrum (Table 1), accounting for six methyls, seven

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methylenes, 10 methines (two of them  $sp^2$ ), and six quaternary carbons with three carbonyl groups:  $\delta_C$  175.1, 171.1, and 170.1.



The high-resolution mass spectrum matched with the molecular formula  $C_{29}H_{42}O_9$ , with  $m/z$  557.2761 ( $C_{29}H_{42}O_9Na^+$ ) being the most abundant ion in the positive mode. Characteristic chemical shifts and multiplicities for a clerodin-like diterpene were displayed in the  $^1H$  NMR spectrum. Thus, two doublets of doublets ( $\delta_H$  4.82 and 6.47, H-14 and H-15, respectively) and a doublet ( $\delta_H$  6.03, H-16) indicated the presence of a tetrahydrofurofuran side chain, whereas two A/B spin systems ( $\delta_H$  2.60/2.82 and 4.39/4.80, H<sub>2</sub>-18 and H<sub>2</sub>-19, respectively) were consistent with the typical functionalities at C-18 and C-19. Furthermore, the following patterns for methyl groups were found: a doublet ( $\delta_H$  0.85) and three singlets ( $\delta_H$  2.13, 1.95, and 0.97) matched with H<sub>3</sub>-17, two acetoxy groups, and H<sub>3</sub>-20, respectively. In addition, another doublet ( $\delta_H$  1.10) and a triplet ( $\delta_H$  0.89) were consistent with the presence of a 2-methylbutyryloxy substituent. The two acetoxy groups could be assigned to C-6 and C-19 by their reciprocal correlation in the HMBC spectrum ( $\delta_H$  4.39/4.80 cross-peaks with  $\delta_C$  71.3 and  $\delta_H$  4.75 with  $\delta_C$  61.4). The correlations of a doublet of doublets at  $\delta_H$  5.31 with signals at  $\delta_C$  175.1, 65.2 (C-4), and 42.5 (C-18) were supportive of the 2-methylbutyryloxy substituent location being at C-3 (the oxirane carbons were assigned by the HSQC/HMBC correlations with the H<sub>2</sub>-18 signals at  $\delta_H$  2.60/2.82). The observed broad doublet for H<sub>B</sub>-18 (instead of a clear dd displayed by 5) indicated a small residual H-3–H<sub>B</sub>-18 coupling. Finally, cross-peaks from  $\delta_H$  4.75 (H-6), 0.85 (H<sub>3</sub>-17), and 0.97 (H<sub>3</sub>-20) to  $\delta_C$  36.1 indicated the last signal to be for C-8. Both substituents in the stereogenic centers C-3 and C-6 must be present in the equatorial position to account for the large *trans* diaxial coupling constant shown by their geminal protons (12.1 Hz for H-3 and 11.6 Hz for H-6). Thus, the structure proposed for ajubractin A (1) was 3 $\beta$ -[(2-methyl)butyryloxy]clerodin.

Compound 2 (ajubractin B) showed in the  $^1H$  NMR spectrum a large number of similarities to 1 (Table 1). In fact, only the 2-methylbutyryloxy substituent pattern was absent, and this was replaced by one septuplet ( $\delta_H$  2.48,  $J$  = 7.0 Hz) and two methyl doublets ( $\delta_H$  1.12 and 1.13), pointing out the presence of an isobutyryloxy group instead. The HRMS confirmed the expected molecular formula [ $m/z$  543.2571 ( $C_{28}H_{40}O_9Na^+$ )]. Thus, the structure assigned to ajubractin B (2) was 3 $\beta$ -(isobutyryloxy)-clerodin.

Compound 3 (ajubractin C) displayed an apparent 28 signals (Table 2) in the  $^{13}C$  NMR spectrum (with one of double intensity), accounting for six methyls, nine methylenes, eight

methines, and six quaternary carbons (with again three as C=O ester groups). The HRESIMS matched with a molecular formula of  $C_{29}H_{44}O_9$  [ $m/z$  559.2875 ( $C_{29}H_{44}O_9Na$ )], and the  $^1H$  and  $^{13}C$  NMR spectra showed typical chemical shifts and multiplicities for a dihydroclerodin-like derivative, indicating a 14,15-dihydroajubractin A structure, owing to the acyloxy groups present. The  $^1H$  NMR spectrum of the bicyclic hexahydrofurofuran side chain displayed a doublet ( $\delta_H$  5.65, H-16), a doublet of doublets ( $\delta_H$  4.11, H-11), and two multiplets ( $\delta_H$  3.89 and 2.89, H<sub>2</sub>-15 and H-13, respectively). Cross-peaks in the  $^1H$ – $^1H$  COSY spectrum allowed assignments for H<sub>2</sub>-14 ( $\delta_H$  2.18 and 1.69) and H<sub>2</sub>-12 ( $\delta_H$  1.82 and 1.62) to be determined, starting from  $\delta_H$  3.89 (H<sub>2</sub>-15), and from  $\delta_H$  4.11 (H-11), respectively. The H-13 ( $\delta_H$  2.89) signal was confirmed by a cross-peak from  $\delta_H$  5.65 (H-16).  $^{13}C$  NMR chemical shifts for the side chain were obtained from the  $^1H$ – $^{13}C$  HSQC spectrum [ $\delta_C$  107.7, 85.1, 68.4, 42.0, 32.6, and 32.5 (C-16, C-11, C-15, C-13, C-14, and C-12, respectively)]. As in 1, again the presence and location of the same acyloxy substituents were inferred from the NMR data, as well as the presence of a C-18 epoxide moiety. Other assignments for the proton spin system in ring-B of the decalin moiety were established from the  $^1H$ – $^1H$  COSY spectrum starting at both ends: from the methyl doublet signal ( $\delta_H$  0.88, H<sub>3</sub>-17) to  $\delta_H$  1.46 (H-8) and from the H-6 doublet of doublets ( $\delta_H$  4.74) to  $\delta_H$  1.64/1.46 (H<sub>2</sub>-7). The H-10 to H-3 spin system was accounted for starting from H-11 ( $\delta_H$  4.11) and H<sub>3</sub>-20 ( $\delta_H$  0.96) to locate the C-10 signal ( $\delta_C$  47.5, HMBC spectrum) and the corresponding H-10 resonance ( $\delta_H$  1.68, HSQC correlation), whereas the H-3 signal ( $\delta_H$  5.31) led to the assignments of  $\delta_H$  2.07 and 1.43 for H<sub>2</sub>-2 and  $\delta_C$  31.0 for C-2. The HSQC cross-peaks at  $\delta_H$  1.77/2.26 with  $\delta_C$  21.1 indicated that the H<sub>2</sub>-1 signals were partly overlapped in the  $^1H$  NMR spectrum. Thus, the structure proposed for ajubractin C (3) was 3 $\beta$ -(2-methyl)-butyryloxy-14,15-dihydroclerodin.

Similarly, compound 4 (ajubractin D) was identified as 3 $\beta$ -isobutyryloxy-2 $\alpha$ -hydroxy-14,15-dihydroclerodin (14,15-dihydroajugachin A). The acyloxy substituent was deduced from its proton signals [a septuplet ( $\delta_H$  2.56,  $J$  = 7.0) and a methyl doublet ( $\delta_H$  1.17,  $J$  = 7.1, 6H)]. The presence of the same substituents in ring A as in dihydroajugapitin (11) was indicated by correlations of their geminal protons and the change of multiplicity for the axial H-3 signal to a doublet ( $\delta_H$  5.24,  $J$  = 9.8 Hz), due to the presence of a vicinal axial proton (H-2:  $\delta_H$  3.66, ddd,  $J$  = 10.3, 10.3, 4.9 Hz) (Table 2).

However, when a defatted (with hexane)  $CH_2Cl_2$  extract of *A. bracteosa* was fractionated on reversed-phase C<sub>18</sub> columns using 60% and 90% MeOH–water mixtures,<sup>8</sup> clerodane diterpenes were distributed in three fractions, based on analytical HPLC conditions and antifeedant bioassay results. Owing to the lack of any UV absorption, semipreparative HPLC purification conditions were established by ELS detection. Surprisingly, ajugapitin (7) was the only diterpene with a tetrahydrofurofuran side chain isolated, and it was obtained along with several hexahydrofurofurans (3 and 8–11) and C-15 epimeric mixtures of clerodin hemiacetal derivatives [mixtures of (15*R* and 15*S*) 15-hydroxyajubractin C (13), 14-hydro-15-hydroxyajugapitin (14),<sup>16</sup> and 14-hydro-15-hydroxyajugachin A (15)<sup>15</sup>] (considered as purification artifacts).<sup>7,15</sup>

One further new derivative, ajubractin E (12),<sup>17</sup> was also isolated as well as 15-*epi*-lupulin B (16).<sup>18,19</sup> The large coupling of the H-3 doublet of doublets ( $\delta_H$  4.04,  $J$  = 11.7, 4.8 Hz) is consistent with an axial proton and an equatorial substituent in

Table 1. NMR Data of Ajubractins A (1) and B (2) [499.81 MHz ( $^1\text{H}$ ), 100.62 MHz ( $^{13}\text{C}$ ),  $\text{CDCl}_3$ ,  $\delta$  (ppm) ( $J = \text{Hz}$ )]

position	1		2	
	$\delta_{\text{C}}$ , mult.	$\delta_{\text{H}}$ , mult. ( $J$ )	$^1\text{H} \rightarrow ^{13}\text{C}$ HMBC	$\delta_{\text{H}}$ , mult. ( $J$ )
1ax	21.2, $\text{CH}_2$	1.78, m		
1eq		2.23, ddt <sup>a</sup> (14.8, 4.8, 2.9)		2.23, ddt <sup>a</sup> (15.0, 5.0, 3.2)
2ax	31.0, $\text{CH}_2$	1.45, m		
2eq		2.07, brdt <sup>a</sup> d (12.6, 4.4, 3.1)		2.08, brdt <sup>a</sup> d (12.9, 4.0, 3.2)
3ax	66.6, CH	5.31, dd (12.1, 4.9)	2, 4, 18, 1'	5.30, dd (12.0, 4.8)
4	65.2, C			
5	46.3, C			
6ax	71.3, CH	4.75, dd (11.6, 4.6)	4, 5, 7, 19, 1'''	4.76, dd (11.8, 4.9)
7ax	33.1, $\text{CH}_2$	1.64, m		
7eq		1.48, m		
8ax	36.1, CH	1.47, m		
9	40.1, C			
10ax	48.0, CH	1.69, m		
11	84.5, CH	4.04, dd (11.7, 4.5)	8, 9, 10, 12, 13, 20	4.04, dd (11.7, 4.5)
12a	31.2, $\text{CH}_2$	1.67, m		
12b		1.73, m		
13	46.0, CH	3.58, m		3.58, m
14	101.8, CH	4.82, dd (2.9, 2.4)	15, 16	4.83, dd (2.9, 2.4)
15	146.9, CH	6.47, dd (2.9, 2.2)	13, 14, 16	6.48, dd (2.9, 2.1)
16	107.6, CH	6.03, d (6.2)	11, 12, 13, 14, 15	6.03, d (6.2)
17	16.3, $\text{CH}_3$	0.85, d (6.5)	7, 8, 9	0.86, d (6.5)
18A	42.5, $\text{CH}_2$	2.60, d (4.3)	3, 4, 5	2.63, d (4.2)
18B		2.82, brd (4.3)	4, 5	2.84, brd (4.2)
19A	61.4, $\text{CH}_2$	4.39, d (12.3)	4, 5, 6, 10, 1''	4.41, d (12.4)
19B		4.80, d (12.3)	4, 5, 6, 10, 1''	4.80, d (12.4)
20	14.0, $\text{CH}_3$	0.97, s	8, 9, 10, 11	0.98, s
1'	175.1, C			
2'	41.1, CH	2.30, sext <sup>a</sup> (6.9)	1'	2.48, sept <sup>a</sup> (7.0)
3'a	26.6, $\text{CH}_2$	1.47, m	1', 2', 5'	1.12, d (7.0)
3'b		1.64, m	1', 2', 5'	1.13, d (7.0)
4'	11.3, $\text{CH}_3$	0.89, t (7.4)	2', 3'	
5'	16.4, $\text{CH}_3$	1.10, d (7.0)	1', 2', 3'	
1''	171.1, C			
2''	21.0, $\text{CH}_3$	2.13, s	1''	2.15, s
1'''	170.1, C			
2'''	21.2, $\text{CH}_3$	1.95, s	1'''	1.96, s

<sup>a</sup> Apparent multiplicity ( $t^a = \text{dd}$  with  $J_1 \approx J_2$ ;  $\text{sext}^a = \text{ddq}$  with  $J_1 \approx J_2 \approx J_3$ ;  $\text{sept}^a = \text{qq}$  with  $J_1 \approx J_2$ ).

**12** (3-*epi*-14,15-dihydrocaryoptinol or 3 $\beta$ -hydroxy-14,15-dihydro-clerodin) instead of the reversed stereochemistry reported for caryoptinol (3 $\alpha$ -epimer,  $\delta_{\text{H}}$  3.31, brs). The 3 $\alpha$ -epimer of **12** has been reported.<sup>17</sup>

Compound **16** (15-*epi*-lupulin B) matched with the molecular formula  $\text{C}_{30}\text{H}_{46}\text{O}_{10}$  ( $m/z$  567.3113 [ $\text{C}_{30}\text{H}_{47}\text{O}_{10}]^+$ ), and the presence of a three-proton singlet ( $\delta_{\text{H}}$  3.34) in the  $^1\text{H}$  NMR spectrum was supportive of a methoxy substituent. The MeO signal displayed a cross-peak with  $\delta_{\text{C}}$  104.8 (HMBC), one of two presumably hemiacetal carbon signals ( $\delta_{\text{C}}$  104.8 and 109.2). A second deshielded doublet ( $\delta_{\text{H}}$  4.99) displayed a cross-peak with the resonance at  $\delta_{\text{C}}$  109.2 (HMBC, H-15  $\rightarrow$  C-16) and supported the assignment of **16** as a 14-hydro-15-methoxyclerodin derivative, related to clerodinins A and B.<sup>7,19</sup> The 3 $\beta$ -(2-

methyl)butyryloxy substitution observed in **16** has been previously reported for lupulin B.<sup>18,19</sup> However, the chemical shifts of H-11, H-13, H-15, H-16, and C-16 matched the epimeric 15S stereochemistry at the stereogenic center C-15.<sup>18–21</sup> Other cross-peaks were observed in the  $^1\text{H}$ – $^{13}\text{C}$  HMBC NMR spectrum from H-16 (at  $\delta$  5.81) to  $\delta$  104.8 (C-15), 83.2 (C-11), 40.5 (C-13), and 32.6 (C-12), as previously reported for **6**. Thus, this substance was assigned as the new 15-*epi*-lupulin B **16**.

The  $^1\text{H}$  NMR data of (15R and 15S)-15-hydroxyajubractin C (**13**) and for (15R and 15S)-14-hydro-2,15-dihydroxy-3 $\beta$ -isobutyryloxyclerodin (**15**) are reported in the Supporting Information.

Antifeedant activity against *S. littoralis* was measured as described in the Experimental Section. Low antifeedant activities

**Table 2.** NMR Data of Ajubractins C (3), D (4), and E (12) and 3-*epi*-Lupulin B (16) [499.81 MHz (<sup>1</sup>H), 100.62 MHz (<sup>13</sup>C), CDCl<sub>3</sub>, δ (ppm) (*J* = Hz)]

position	3			4		12	16		
	δ <sub>C</sub> , mult.	δ <sub>H</sub> , mult. ( <i>J</i> )	<sup>1</sup> H→ <sup>13</sup> C HMBC	δ <sub>H</sub> , mult. ( <i>J</i> )		δ <sub>H</sub> , mult. ( <i>J</i> )	δ <sub>C</sub> , mult.	δ <sub>H</sub> , mult. ( <i>J</i> )	<sup>1</sup> H→ <sup>13</sup> C HMBC
1ax	21.1, CH <sub>2</sub>	1.77, m					21.1, CH <sub>2</sub>	1.64	
1eq		2.26, m						2.35, m	
2ax	31.0, CH <sub>2</sub>	1.43, m		3.66, t <sup>d</sup> (10.3, 4.9)			31.0, CH <sub>2</sub>	1.43, m	
2eq		2.07, brd <sup>t</sup> d (12.7, 4.4, 3.2)						2.07 m	
3ax	66.6, CH	5.31, dd (12.0, 4.9)	4, 18, 1'	5.24, d (9.8)		4.04, dd (11.7, 4.8)	66.7, CH	5.32, dd (12.2, 4.9)	4, 18
4	65.3, C						65.3, C		
5	46.3, C						46.3, C		
6ax	71.3, CH	4.74, dd (11.4, 4.5)	4, 5, 19, 1'''	4.71, dd (11.8, 4.4)		4.79, dd (11.5, 4.5)	71.4, CH	4.75, dd (12.0, 5.1)	19
7ax	33.1, CH <sub>2</sub>	1.64, m					33.2, CH <sub>2</sub>	1.62, m	
7eq		1.46, m						1.47, m	
8ax	35.8, CH	1.46, m					35.9, CH		
9	40.6, C						40.1, C		
10ax	47.5, CH	1.68, m					47.6, CH		
11	85.1, CH	4.11, dd (11.4, 5.4)	10	4.12, dd (11.1, 5.4)		4.12, dd (11.5, 5.4)	83.2, CH	4.38, dd (11.5, 5.8)	10
12a	32.5, CH <sub>2</sub>	1.62, m					32.6, CH <sub>2</sub>	1.62, m	11
12b		1.82, ddd (12.4, 11.2, 9.0)				1.83, t <sup>d</sup> d (12.0, 9.1)		1.75, m	
13	42.0, CH	2.89, t <sup>a</sup> t <sup>a</sup> (9.2, 4.5)		2.89, m		2.90, m	40.5, CH	2.81, m	
14a	32.6, CH <sub>2</sub>	1.69, m					39.5, CH <sub>2</sub>	1.80, m	15, 16
14b		2.18, ddt <sup>a</sup> (12.7, 9.3, 8.0)						2.28, m	
15	68.4, CH <sub>2</sub>	3.89, m	16	3.88, m		3.88, m	104.8, CH	4.99, d (5.6)	13, 16, OMe
16	107.7, CH	5.65, d (5.1)	11, 12, 13, 15	5.69, d (5.0)		5.66, d (5.1)	109.2, CH	5.81, d (5.4)	11, 12, 13, 15
17	16.4, CH <sub>3</sub>	0.88, d (7.2)	7, 8, 9	0.90, d (6.4)		0.89, d (6.5)	16.2, CH <sub>3</sub>	0.89, d (6.1)	7, 8, 9
18A	42.6, CH <sub>2</sub>	2.60, d (4.3)	4	2.60, d (4.2)		2.79, d (4.0)	42.6, CH <sub>2</sub>	2.60, d (4.2)	4
18B		2.82, brd (4.3)		2.83, brd (4.2)		2.90, d (4.0)		2.82, brd (4.4)	4
19A	61.5, CH <sub>2</sub>	4.39, d (12.3)	5, 6, 1''	4.43, d (12.1)		4.25, d (12.4)	61.5, CH <sub>2</sub>	4.39, d (12.2)	6
19B		4.80, d (12.3)	4, 5, 6, 1''	4.80, d (12.2)		4.94, d (12.4)		4.79, d (12.2)	4, 5, 6
20	13.9, CH <sub>3</sub>	0.96, s	8, 9, 10, 11	0.98, s		0.98, s	13.9, CH <sub>3</sub>	0.94, s	8, 9, 10, 11
1'	175.1, C						175.2, C		
2'	41.1, CH	2.30, sext <sup>a</sup> (6.8)		2.56, sept <sup>a</sup> (7.0)			41.1, CH	2.29, sext <sup>a</sup> (7.0)	1'
3'a	26.6, CH <sub>2</sub>	1.42, m		1.17, d (7.1)			26.6, CH <sub>2</sub>	1.42, m	1'
3'b		1.61, m						1.60, m	1'
4'	11.3, CH <sub>3</sub>	0.89, t (7.5)	2', 3'				11.1, CH <sub>3</sub>	0.89, t (7.3)	2', 3'
5'	16.4, CH <sub>3</sub>	1.09, d (7.1)	1', 2', 3'				16.4, CH <sub>3</sub>	1.09, d (6.8)	1', 2', 3'
1''	171.1, C						171.2, C		
2''	21.0, CH <sub>3</sub>	2.13, s	1''	2.16, s		2.11, s	21.2, CH <sub>3</sub>	2.14, s	1''
1'''	170.2, C						170.2, C		
2'''	21.2, CH <sub>3</sub>	1.95, s	1'''	1.96, s		1.97, s	21.1, CH <sub>3</sub>	1.94, s	1'''
OMe							54.5, CH <sub>3</sub>	3.34, s	15

<sup>a</sup> Apparent multiplicity (t<sup>a</sup> = dd with *J*<sub>1</sub> ≈ *J*<sub>2</sub>; sext<sup>a</sup> = ddq with *J*<sub>1</sub> ≈ *J*<sub>2</sub> ≈ *J*<sub>3</sub>; sept<sup>a</sup> = qq with *J*<sub>1</sub> ≈ *J*<sub>2</sub>).

were found for compounds **1** and **2** (FR 0.34 and 0.42, respectively), while the other derivatives showed moderately high activities (FR = 0.10–0.15) (Table 3).

## EXPERIMENTAL SECTION

**General Experimental Procedures.** Optical rotations were measured in a Perkin-Elmer 341 polarimeter. <sup>1</sup>H NMR (499.81 MHz) and <sup>13</sup>C NMR (100.62 MHz) spectra were recorded in CDCl<sub>3</sub> on Inova 500/Mercury 400 spectrometers (Varian, Zug, Switzerland) under standard 1D and 2D conditions and pulse sequences, using solvent signals as references (δ<sub>H</sub> 7.28/δ<sub>C</sub> 77.0). Special low-volume NMR tubes

were used when required (Shigemi Co., Ltd., Tokyo, Japan; BMS-05 microtube). HPLC was performed on an Alliance 2695 apparatus coupled with a 996 UV diode array detector (Waters Corporation, Milford, MA) together with a PL-ELS 1000 evaporative light scattering detector (Polymer Laboratories, Amherst, MA). The chromatographic conditions used for analytical or semipreparative HPLC were 0.42 or 2.0 mL/min flow and 25 °C. A C<sub>18</sub> guard column was coupled to protect the integrity of the HPLC columns, both analytical and semipreparative. Compounds were injected in an Acquity UPLC coupled with an Acquity-TUV and Q-TOF Premier mass spectrometer (Waters Corporation) detector, using an Acquity UPLC BEH C<sub>18</sub> column (1.7 μm, 2.1 × 100 mm, 30 °C, 0.3 mL/min flow). A 70:30 ratio of



**Table 3. Antifeedant Activity of Compounds from *Ajuga bracteosa* against *Spodoptera littoralis*<sup>a</sup>**

compound	FR <sub>50</sub>
1	0.34 ± 0.04
2	0.42 ± 0.06
3	0.15 ± 0.02
4	0.15 ± 0.03
6	0.12 ± 0.02
7	0.11 ± 0.02
8	0.09 ± 0.01
10	0.11 ± 0.02
12	0.15 ± 0.03
16	0.14 ± 0.04

<sup>a</sup> 10 µg isolated compound in 1 cm<sup>2</sup> lettuce disks. *n* = 5.

water–acetonitrile containing 0.1% HCO<sub>2</sub>H was the initial chromatographic mobile phase for 5 min, followed by a 15 min gradient up to a 5:95 ratio, held for 5 min, and then a 5 min gradient to initial conditions, followed by 5 min of column re-equilibration. Positive- and negative-electrospray ionizations were used, and UV absorptions were recorded at 215 and 254 nm. Solutions of pure compounds were injected (2 µL; 0.1 mg/mL) and referred externally for accurate mass results. A Strata C<sub>18</sub> cartridge (10 g, Phenomenex, Torrance, CA) was used to obtain *neo*-clerodane-enriched fractions. The column was first activated/equilibrated (15 mL of MeOH followed by 15 mL of H<sub>2</sub>O–MeOH, 40:60). Silica gel for vacuum-liquid chromatography (60H, 90% <45 µm) and silica gel 60 F<sub>254</sub> aluminum sheets (Merck, Darmstadt, Germany) for monitoring and preparative TLC were used. Formic acid was purchased from Merck (Haarlem, The Netherlands). CDCl<sub>3</sub> (99.8% D, <0.01% H<sub>2</sub>O) was purchased from Euriso-top (Gif-sur-Yvette, France).

**Plant Material.** *Ajuga bracteosa* was collected from the Shamozi area in Swat (KPK) in the northwest of Pakistan in June 2009. The identity of the plant was confirmed by taxonomist Prof. Habib Ahmad, Faculty of Science, Hazara University, Pakistan, and a specimen (voucher no. 246 HUP) was deposited in the herbarium at the University of Hazara, Mansehra, KPK, Pakistan.

**Extraction and Isolation.** Dried and powdered aerial parts of *A. bracteosa* (100.2 g) were extracted for four days with CH<sub>2</sub>Cl<sub>2</sub> (1 L) at room temperature. The extract was evaporated under a vacuum at 40 °C, yielding 3.95 g of dark green residue. This crude extract was mixed with 100 mL of CH<sub>2</sub>Cl<sub>2</sub> and 5 g of silica gel and placed on top of a 35 g silica gel column, previously compacted and wetted with 80 mL of CH<sub>2</sub>Cl<sub>2</sub>. The column was eluted (20 mL fractions collected) sequentially with CH<sub>2</sub>Cl<sub>2</sub>–*tert*-butyl methyl ether mixtures (140 mL of 100:0, 100 mL 95:5, 100 mL 90:10, 100 mL 85:15, 100 mL 80:20, 100 mL 0:100) and finally washed with acetone (80 mL). Fractions 20 (36 mg) and 22 (34 mg) each showed the presence (<sup>1</sup>H NMR spectra) of one major compound [3-*epi*-caryoptin (6) and dihydroclerodin (8), respectively]. Other fractions of interest required further purification by normal phase preparative TLC. A portion (20 mg) of fraction 15 (73.4 mg) was eluted twice with hexane–*tert*-butyl methyl ether (60:40), furnishing two new pure compounds, 1 (*R*<sub>f</sub> 0.29; 4.5 mg) and 2 (*R*<sub>f</sub> 0.22; 2.9 mg), and clerodin (5) (*R*<sub>f</sub> 0.16; 0.6 mg). Compound 3 (*R*<sub>f</sub> 0.14; 3.7 mg) was obtained from a 6.5 mg portion of fraction 17 (70.1 mg) eluted with hexane–*tert*-butyl methyl ether (50:50). Ivain II (10) was found in fraction 18 as a 1:3 mixture with 3, and the compounds were separated. From 17.6 mg of fraction 23 (26.4 mg) eluting twice with hexane–*tert*-butyl methyl ether (20:80), a further amount of dihydroclerodin (8) was isolated (*R*<sub>f</sub> 0.35; 3.4 mg) as well as 3-*epi*-14,15-dihydrocaryoptin (9) (*R*<sub>f</sub> 0.30; 4.6 mg). Ajugapitin (7) was isolated from 15.4 mg of fraction

25 (30.8 mg), eluting twice with hexane–*tert*-butyl methyl ether (40:60) (*R*<sub>f</sub> 0.22; 1.8 mg). Ajubractin D (4) and dihydroajugapitin (11) were isolated from fraction 30.

Another batch of the crude extract was defatted with hexane (15 mL, sonicated for 10 min, and then centrifuged for 15 min at 3000 rpm). The 1.57 g residue obtained was further digested/centrifuged sequentially (15 min sonication/15 min 3000 rpm) in 60% and 90% MeOH–H<sub>2</sub>O mixtures (14 mL each). Each solution was filtered (fraction F) through a RP column followed by a further elution with 15 mL of the corresponding fresh MeOH–H<sub>2</sub>O mixture (fraction E) and a final wash with 30 mL of MeOH (fraction L). Fractions were labeled as 60F, 60E, 90F1 (7 mL), 90F2 (7 mL), 90E and 100L1 (7 mL), respectively. On analytical HPLC conditions, fractions 90F2, 90E, and 100L1 afforded *neo*-clerodane-enriched fractions.

Semipreparative HPLC separation of fraction 90F2 (111 mg) afforded 3β-hydroxydihydroclerodin (12, 0.9 mg), an epimeric mixture of 14-hydro-15-hydroxyajugachin A (15, 1.6 mg), 14-hydro-15-hydroxyajugapitin (14, 3.8 mg), 3-*epi*-dihydrocaryoptin (9, 1.6 mg), dihydroajugapitin (11, 10.6 mg), and ajugapitin (7, 3.9 mg). Fraction 90E (172 mg) yielded dihydroclerodin (8, 4.7 mg), dihydroajugapitin (11, 10.0 mg), 15-hydroxyajubractin C (13, 3.1 mg), ivain II (10, 3.8 mg), and ajubractin C (3, 7.5 mg). From fraction 100L1 (79 mg), further amounts of ajubractin C (3, 3.5 mg) and 15-*epi*-lupulin B (16, 2.0 mg) were isolated.

**Ajubractin A (1):** amorphous solid (4.5 mg); [α]<sub>D</sub><sup>20</sup> –17.1 (*c* 0.091, MeOH); <sup>1</sup>H NMR and <sup>13</sup>C NMR data in Table 1; HRESIMS *m/z* 557.2761 [M + Na]<sup>+</sup> (calcd for C<sub>29</sub>H<sub>42</sub>O<sub>9</sub>Na, 557.2727).

**Ajubractin B (2):** amorphous solid (2.9 mg); [α]<sub>D</sub><sup>20</sup> –27.4 (*c* 0.065, hexane–*tert*-butyl methyl ether, 70:30); <sup>1</sup>H NMR data in Table 1; HRESIMS *m/z* 543.2571 [M + Na]<sup>+</sup> (calcd for C<sub>28</sub>H<sub>40</sub>O<sub>9</sub>Na, 543.2570).

**Ajubractin C (3):** amorphous solid (3.7 mg); [α]<sub>D</sub><sup>20</sup> –15.3 (*c* 0.17, MeOH); <sup>1</sup>H NMR and <sup>13</sup>C NMR data in Table 2; HRESIMS *m/z* 559.2875 [M + Na]<sup>+</sup> (calcd for C<sub>29</sub>H<sub>44</sub>O<sub>9</sub>Na, 559.2871), 1095.5848 [2 M + Na]<sup>+</sup>; 535.2851 [M – H]<sup>–</sup> (calcd for C<sub>29</sub>H<sub>43</sub>O<sub>9</sub>, 535.2895).

**Ajubractin D (4):** (3.1 mg); [α]<sub>D</sub><sup>20</sup> –32.5 (*c* 0.14, CHCl<sub>3</sub>); <sup>1</sup>H NMR data in Table 2.

**Ajubractin E (12):** (0.9 mg); [α]<sub>D</sub><sup>20</sup> +7.6 (*c* 0.05, MeOH); <sup>1</sup>H NMR data in Table 2; HRESIMS *m/z* 453.2455 [M + H]<sup>+</sup> (calcd for C<sub>24</sub>H<sub>37</sub>O<sub>8</sub>, 453.2478), 475.2301 [M + Na]<sup>+</sup>.

**15-Hydroxyajubractin C (13):** (3.1 mg); [α]<sub>D</sub><sup>20</sup> –25.0 (*c* 0.04, MeOH); HRESIMS *m/z* 553.2980 [M + H]<sup>+</sup> (calcd for C<sub>29</sub>H<sub>45</sub>O<sub>10</sub>, 553.2871), 575.2849 [M + Na]<sup>+</sup>; 551.2839 [M – H]<sup>–</sup> (calcd for C<sub>29</sub>H<sub>43</sub>O<sub>10</sub>, 551.2844), 597.2884 [M + HCOO]<sup>–</sup>.

**15-*epi*-Lupulin B (16):** (2.0 mg); [α]<sub>D</sub><sup>20</sup> +8.7 (*c* 0.06, MeOH); HRESIMS *m/z* 567.3113 [M + H]<sup>+</sup> (calcd for C<sub>30</sub>H<sub>47</sub>O<sub>10</sub>, 567.3027), 589.2976 [M + Na]<sup>+</sup>, 1155.6119 [2 M + Na]<sup>+</sup>; 611.3070 [M + HCOO]<sup>–</sup> (calcd for C<sub>31</sub>H<sub>47</sub>O<sub>12</sub>, 611.3054).

**Antifeedant Activity against *Spodoptera littoralis*.** A binary choice feeding bioassay employing lettuce (*Lactuca sativa*) leaf disks with areas of 1 cm<sup>2</sup> was used to evaluate the activity of isolated compounds against fifth instar larvae of *Spodoptera littoralis*.<sup>22</sup> Compounds to be tested (10 µg) were distributed uniformly on the upper surface of a disk by application of 10 µL acetone solutions (treated disks), while control disks were treated analogously with 10 µL of acetone. In each replicate (×5), four treated (TD) and four control disks (CD) were placed alternatively in a covered polyethylene Petri dish (8.5 cm diameter) in the presence of five larvae. A feeding ratio (FR<sub>50</sub> ± SD), when 50% of CD area has been consumed, was calculated as follows: FR = CTD (consumed treated disk area)/CCD (consumed control disk area). Results were evaluated as described in the literature.<sup>22</sup> Experiments were performed under the same conditions of temperature and humidity as the laboratory culture but in constant darkness.

## ■ ASSOCIATED CONTENT

**S Supporting Information.** 1D and 2D NMR spectra of the new compounds and  $^1\text{H}$  NMR spectroscopic data for **13** and **15**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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