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neo-Clerodane Diterpenoids from *Ajuga bracteosa*

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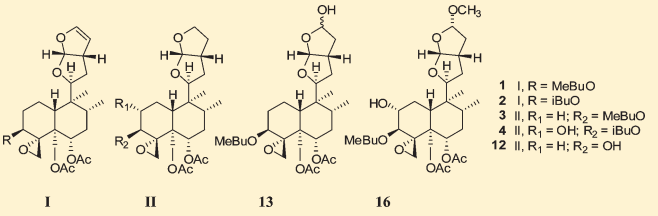
Supporting Information

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_{18} 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.[1−3] 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.[4] The isolation of *neo*-clerodane diterpenoids from *A. bracteosa* has been reported in about 10 different papers, as reviewed recently.[5] Different plant collection locations have yielded constituents with a variety of structures, including clerodin- and ajugarin-like side chains among the *neo*-clerodanes isolated.[5] *Ajuga remota,* reported by some authors, is a synonym of *A. bracteosa* according to *The International Plant Names Index*.[6] 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**),[7,8] 3-*epi*-caryoptin (**6**),[9] ajugapitin (**7**),[8,10] 14,15-dihydroclerodin (**8**),[8,11] 3-*epi*-14,15-dihydrocaryoptin (**9**),[9,12] ivain II (**10**),[13] and 14,15-dihydroajugapitin (**11**)[8,10,14]}. The extract was fractionated on a silica gel column eluting with CH_2Cl_2−*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.[7,15]

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 ^{1}H NMR chemical shifts and multiplicities for compounds **5**−**11** matched with those described in the literature.[7−14] 3β-Acetoxy-14,15-dihydroclerodin, or 3-*epi*-14,15-dihydrocaryoptin (**9**), is a new natural compound and was prepared by semisynthesis previously.[12]

Compound **1** (ajubractin A) displayed 29 signals in the ^{13}C NMR spectrum (Table 1), accounting for six methyls, seven

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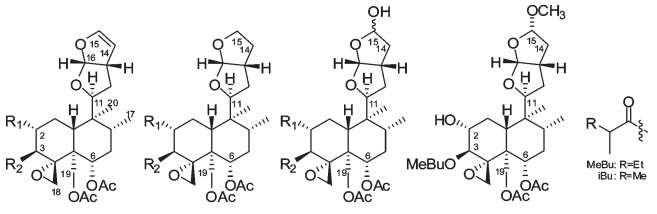
methylenes, 10 methines (two of them sp^2), and six quaternary carbons with three carbonyl groups: δ_C 175.1, 171.1, and 170.1.



R_1	R_2	I	II	III
H	MeBuO	**1**	**3**	**13**
H	iBuO	**2**	**10**	**14**
H	H	**5**	**8**	
H	AcO	**6**	**9**	
H	HO		**12**	
HO	MeBuO	**7**	**11**	**15**
HO	iBuO		**4**	

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 (δ_H 4.82 and 6.47, H-14 and H-15, respectively) and a doublet (δ_H 6.03, H-16) indicated the presence of a tetrahydrofurofuran side chain, whereas two A/B spin systems (δ_H 2.60/2.82 and 4.39/4.80, H_2-18 and H_2-19, respectively) were consistent with the typical functionalities at C-18 and C-19. Furthermore, the following patterns for methyl groups were found: a doublet (δ_H 0.85) and three singlets (δ_H 2.13, 1.95, and 0.97) matched with H_3-17, two acetoxy groups, and H_3-20, respectively. In addition, another doublet (δ_H 1.10) and a triplet (δ_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 (δ_H 4.39/4.80 cross-peaks with δ_C 71.3 and δ_H 4.75 with δ_C 61.4). The correlations of a doublet of doublets at δ_H 5.31 with signals at δ_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_2-18 signals at δ_H 2.60/2.82). The observed broad doublet for H_B-18 (instead of a clear dd displayed by **5**) indicated a small residual H-3−H_B-18 coupling. Finally, cross-peaks from δ_H 4.75 (H-6), 0.85 (H_3-17), and 0.97 (H_3-20) to δ_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 3β-[(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 (δ_H 2.48, J = 7.0 Hz) and two methyl doublets (δ_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 3β-(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 (δ_H 5.65, H-16), a doublet of doublets (δ_H 4.11, H-11), and two multiplets (δ_H 3.89 and 2.89, H_2-15 and H-13, respectively). Cross-peaks in the $^1H-^1H$ COSY spectrum allowed assignments for H_2-14 (δ_H 2.18 and 1.69) and H_2-12 (δ_H 1.82 and 1.62) to be determined, starting from δ_H 3.89 (H_2-15), and from δ_H 4.11 (H-11), respectively. The H-13 (δ_H 2.89) signal was confirmed by a cross-peak from δ_H 5.65 (H-16). ^{13}C NMR chemical shifts for the side chain were obtained from the $^1H-^{13}C$ HSQC spectrum [δ_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 (δ_H 0.88, H_3-17) to δ_H 1.46 (H-8) and from the H-6 doublet of doublets (δ_H 4.74) to δ_H 1.64/1.46 (H_2-7). The H-10 to H-3 spin system was accounted for starting from H-11 (δ_H 4.11) and H_3-20 (δ_H 0.96) to locate the C-10 signal (δ_C 47.5, HMBC spectrum) and the corresponding H-10 resonance (δ_H 1.68, HSQC correlation), whereas the H-3 signal (δ_H 5.31) led to the assignments of δ_H 2.07 and 1.43 for H_2-2 and δ_C 31.0 for C-2. The HSQC cross-peaks at δ_H 1.77/2.26 with δ_C 21.1 indicated that the H_2-1 signals were partly overlapped in the ^1H NMR spectrum. Thus, the structure proposed for ajubractin C (**3**) was 3β-(2-methyl)-butyryloxy-14,15-dihydroclerodin.

Similarly, compound **4** (ajubractin D) was identified as 3β-isobutyryloxy-2α-hydroxy-14,15-dihydroclerodin (14,15-dihydroajugachin A). The acyloxy substituent was deduced from its proton signals [a septuplet (δ_H 2.56, J = 7.0) and a methyl doublet (δ_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 (δ_H 5.24, J = 9.8 Hz), due to the presence of a vicinal axial proton (H-2: δ_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_{18} columns using 60% and 90% MeOH−water mixtures,[8] 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**),[16] and 14-hydro-15-hydroxyajugachin A (**15**)[15]] (considered as purification artifacts).[7,15]

One further new derivative, ajubractin E (**12**),[17] was also isolated as well as 15-*epi*-lupulin B (**16**).[18,19] The large coupling of the H-3 doublet of doublets (δ_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}H), 100.62 MHz (^{13}C), $CDCl_3$, δ (ppm) (J = Hz)]

	1		2	
position	δ_C, mult.	δ_H, mult. (J)	^{1}H→^{13}C HMBC	δ_H, mult. (J)
1ax	21.2, CH_2	1.78, m		
1eq		2.23, ddt[a] (14.8, 4.8, 2.9)		2.23, ddt[a] (15.0, 5.0, 3.2)
2ax	31.0, CH_2	1.45, m		
2eq		2.07, brdt[a]d (12.6, 4.4, 3.1)		2.08, brdt[a]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, 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, 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, CH_3	0.85, d (6.5)	7, 8, 9	0.86, d (6.5)
18A	42.5, 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, 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, CH_3	0.97, s	8, 9, 10, 11	0.98, s
1′	175.1, C			
2′	41.1, CH	2.30, sext[a] (6.9)	1′	2.48, sept[a] (7.0)
3′a	26.6, 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, CH_3	0.89, t (7.4)	2′, 3′	
5′	16.4, CH_3	1.10, d (7.0)	1′, 2′, 3′	
1″	171.1, C			
2″	21.0, CH_3	2.13, s	1″	2.15, s
1‴	170.1, C			
2‴	21.2, CH_3	1.95, s	1‴	1.96, s

[a] Apparent multiplicity (t[a] = dd with $J_1 \approx J_2$; sext[a] = ddq with $J_1 \approx J_2 \approx J_3$; sept[a] = qq with $J_1 \approx J_2$).

12 (3-*epi*-14,15-dihydrocaryoptinol or 3β-hydroxy-14,15-dihydroclerodin) instead of the reversed stereochemistry reported for caryoptinol (3α-epimer, δ_H 3.31, brs). The 3α-epimer of **12** has been reported.[17]

Compound **16** (15-*epi*-lupulin B) matched with the molecular formula $C_{30}H_{46}O_{10}$ (m/z 567.3113 $[C_{30}H_{47}O_{10}]^+$), and the presence of a three-proton singlet (δ_H 3.34) in the ^{1}H NMR spectrum was supportive of a methoxy substituent. The MeO signal displayed a cross-peak with δ_C 104.8 (HMBC), one of two presumably hemiacetal carbon signals (δ_C 104.8 and 109.2). A second deshielded doublet (δ_H 4.99) displayed a cross-peak with the resonance at δ_C 109.2 (HMBC, H-15 → C-16) and supported the assignment of **16** as a 14-hydro-15-methoxyclerodin derivative, related to clerodinins A and B.[7,19] The 3β-(2-methyl)butyryloxy substitution observed in **16** has been previously reported for lupulin B.[18,19] However, the chemical shifts of H-11, H-13, H-15, H-16, and C-16 matched the epimeric 15*S* stereochemistry at the stereogenic center C-15.[18−21] Other cross-peaks were observed in the ^{1}H−^{13}C HMBC NMR spectrum from H-16 (at δ 5.81) to δ 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}H NMR data of (15*R* and 15*S*)-15-hydroxyajubractin C (**13**) and for (15*R* and 15*S*)-14-hydro-2,15-dihydroxy-3β-isobutyryloxyclerodin (**15**) are reported in the Supporting Information.

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

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Table 2. NMR Data of Ajubractins C (3), D (4), and E (12) and 3-*epi*-Lupulin B (16) [499.81 MHz (^{1}H), 100.62 MHz (^{13}C), $CDCl_3$, δ (ppm) (J = Hz)]

	3			4	12	16		
position	δ_C, mult.	δ_H, mult. (J)	^{1}H→^{13}C HMBC	δ_H, mult. (J)	δ_H, mult. (J)	δ_C, mult.	δ_H, mult. (J)	^{1}H→^{13}C HMBC
1ax	21.1, CH_2	1.77, m				21.1, CH_2	1.64	
1eq		2.26, m					2.35, m	
2ax	31.0, CH_2	1.43, m		3.66, t[a]d (10.3, 4.9)		31.0, CH_2	1.43, m	
2eq		2.07, brdt[a]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_2	1.64, m				33.2, CH_2	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_2	1.62, m				32.6, CH_2	1.62, m	11
12b		1.82, ddd (12.4, 11.2, 9.0)			1.83, t[a]d (12.0, 9.1)		1.75, m	
13	42.0, CH	2.89, t[a] t[a] (9.2, 4.5)		2.89, m	2.90, m	40.5, CH	2.81, m	
14a	32.6, CH_2	1.69, m				39.5, CH_2	1.80, m	15, 16
14b		2.18, ddt[a] (12.7, 9.3, 8.0)					2.28, m	
15	68.4, CH_2	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_3	0.88, d (7.2)	7, 8, 9	0.90, d (6.4)	0.89, d (6.5)	16.2, CH_3	0.89, d (6.1)	7, 8, 9
18A	42.6, CH_2	2.60, d (4.3)	4	2.60, d (4.2)	2.79, d (4.0)	42.6, CH_2	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_2	4.39, d (12.3)	5, 6, 1″	4.43, d (12.1)	4.25, d (12.4)	61.5, CH_2	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_3	0.96, s	8, 9, 10, 11	0.98, s	0.98, s	13.9, CH_3	0.94, s	8, 9, 10, 11
1′	175.1, C					175.2, C		
2′	41.1, CH	2.30, sext[a] (6.8)		2.56, sept[a] (7.0)		41.1, CH	2.29, sext[a] (7.0)	1′
3′a	26.6, CH_2	1.42, m		1.17, d (7.1)		26.6, CH_2	1.42, m	1′
3′b		1.61, m					1.60, m	1′
4′	11.3, CH_3	0.89, t (7.5)	2′, 3′			11.1, CH_3	0.89, t (7.3)	2′, 3′
5′	16.4, CH_3	1.09, d (7.1)	1′, 2′, 3′			16.4, CH_3	1.09, d (6.8)	1′, 2′, 3′
1″	171.1, C					171.2, C		
2″	21.0, CH_3	2.13, s	1″	2.16, s	2.11, s	21.2, CH_3	2.14, s	1″
1‴	170.2, C					170.2, C		
2‴	21.2, CH_3	1.95, s	1‴	1.96, s	1.97, s	21.1, CH_3	1.94, s	1‴
OMe						54.5, CH_3	3.34, s	15

[a] Apparent multiplicity (t[a] = dd with $J_1 \approx J_2$; sext[a] = ddq with $J_1 \approx J_2 \approx J_3$; sept[a] = qq with $J_1 \approx J_2$).

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. ^{1}H NMR (499.81 MHz) and ^{13}C NMR (100.62 MHz) spectra were recorded in $CDCl_3$ on Inova 500/Mercury 400 spectrometers (Varian, Zug, Switzerland) under standard 1D and 2D conditions and pulse sequences, using solvent signals as references (δ_H 7.28/δ_C 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_{18} guard column was coupled to protect the integrity of the HPLC columns, both analytical and semi-preparative. 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_{18} column (1.7 μm, 2.1 × 100 mm, 30 °C, 0.3 mL/min flow). A 70:30 ratio of

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Table 3. Antifeedant Activity of Compounds from *Ajuga bracteosa* against *Spodoptera littoralis*[a]

compound	FR_{50}
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

[a] 10 μg isolated compound in 1 cm^2 lettuce disks. $n = 5$.

water−acetonitrile containing 0.1% HCO_2H 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_{18} 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_2O−MeOH, 40:60). Silica gel for vacuum-liquid chromatography (60H, 90% <45 μm) and silica gel 60 F_{254} aluminum sheets (Merck, Darmstadt, Germany) for monitoring and preparative TLC were used. Formic acid was purchased from Merck (Haarlem, The Netherlands). $CDCl_3$ (99.8% D, <0.01% H_2O) was purchased from Euriso-top (Gif-sur-Yvette, France).

Plant Material. *Ajuga bracteosa* was collected from the Shamozai 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_2Cl_2 (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_2Cl_2 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_2Cl_2. The column was eluted (20 mL fractions collected) sequentially with CH_2Cl_2−*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 (^{1}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_f 0.29; 4.5 mg) and **2** (R_f 0.22; 2.9 mg), and clerodin (**5**) (R_f 0.16; 0.6 mg). Compound **3** (R_f 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_f 0.35; 3.4 mg) as well as 3-*epi*-14,15-dihydrocaryoptin (**9**) (R_f 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_f 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_2O 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_2O 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); $[\alpha]^{20}_D$ −17.1 (*c* 0.091, MeOH); ^{1}H NMR and ^{13}C NMR data in Table 1; HRESIMS *m/z* 557.2761 $[M + Na]^+$ (calcd for $C_{29}H_{42}O_9Na$, 557.2727).

*Ajubractin B (**2**):* amorphous solid (2.9 mg); $[\alpha]^{20}_D$ −27.4 (*c* 0.065, hexane−*tert*-butyl methyl ether, 70:30); ^{1}H NMR data in Table 1; HRESIMS *m/z* 543.2571 $[M + Na]^+$ (calcd for $C_{28}H_{40}O_9Na$, 543.2570).

*Ajubractin C (**3**):* amorphous solid (3.7 mg); $[\alpha]^{20}_D$ −15.3 (*c* 0.17, MeOH); ^{1}H NMR and ^{13}C NMR data in Table 2; HRESIMS *m/z* 559.2875 $[M + Na]^+$ (calcd for $C_{29}H_{44}O_9Na$, 559.2871), 1095.5848 $[2 M + Na]^+$; 535.2851 $[M - H]^-$ (calcd for $C_{29}H_{43}O_9$, 535.2895).

*Ajubractin D (**4**):* (3.1 mg); $[\alpha]^{20}_D$ −32.5 (*c* 0.14, $CHCl_3$); ^{1}H NMR data in Table 2.

*Ajubractin E (**12**):* (0.9 mg); $[\alpha]^{20}_D$ +7.6 (*c* 0.05, MeOH); ^{1}H NMR data in Table 2; HRESIMS *m/z* 453.2455 $[M + H]^+$ (calcd for $C_{24}H_{37}O_8$, 453.2478), 475.2301 $[M + Na]^+$.

*15-Hydroxyajubractin C (**13**):* (3.1 mg); $[\alpha]^{20}_D$ −25.0 (*c* 0.04, MeOH); HRESIMS *m/z* 553.2980 $[M + H]^+$ (calcd for $C_{29}H_{45}O_{10}$, 553.2871), 575.2849 $[M + Na]^+$; 551.2839 $[M - H]^-$ (calcd for $C_{29}H_{43}O_{10}$, 551.2844), 597.2884 $[M + HCOO]^-$.

*15-epi-Lupulin B (**16**):* (2.0 mg); $[\alpha]^{20}_D$ +8.7 (*c* 0.06, MeOH); HRESIMS *m/z* 567.3113 $[M + H]^+$ (calcd for $C_{30}H_{47}O_{10}$, 567.3027), 589.2976 $[M + Na]^+$, 1155.6119 $[2 M + Na]^+$; 611.3070 $[M + HCOO]^-$ (calcd for $C_{31}H_{47}O_{12}$, 611.3054).

Antifeedant Activity against *Spodoptera littoralis*. A binary choice feeding bioassay employing lettuce (*Lactuca sativa*) leaf disks with areas of 1 cm^2 was used to evaluate the activity of isolated compounds against fifth instar larvae of *Spodoptera littoralis*.[22] 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_{50} ± 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.[22] Experiments were performed under the same conditions of temperature and humidity as the laboratory culture but in constant darkness.

■ ASSOCIATED CONTENT

Supporting Information. 1D and 2D NMR spectra of the new compounds and ^{1}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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