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Full Papers

New Stigmastane Sterols from *Ajuga salicifolia*

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A new sterol, ajugasalicigenin (**1**), and three new sterol glycosides, ajugasaliciosides F−H (**2**−**4**), were isolated and characterized from the aerial parts of *Ajuga salicifolia*. Compounds **1**−**4** are further representatives of the stigmastane type of sterols. Their cytotoxicity against KB (HeLa) and Jurkat T cancer cells was evaluated.

In the flora of Turkey, the genus *Ajuga* (Lamiaceae) is represented by 11 species,[1] of which some are used traditionally in wound healing, as diuretics, and for the treatment of diarrhea and high fever.[2] There have been many phytochemical investigations on *Ajuga* species, focusing mainly on the isolation of phytoecdysteroids and diterpenes and their antifeedant and insect growth-inhibitory activities.[3,4] To date, there have been only a few phytochemical reports on *Ajuga salicifolia* (L.) Schreber.[5,6] Recently, we reported the isolation of several sterol glycosides with antileukemic activity from the methanolic extract of the aerial parts of *Ajuga salicifolia*, which were collected in Turkey.[7] In the present study, a new stigmastane-type sterol (**1**) and two new sterol monoglycosides (**2** and **3**) have been isolated from the dichloromethane extract. In addition, the methanol extract afforded a new sterol tetraglycoside (**4**). This paper describes the isolation, structure elucidation, and the cytotoxic potency of **1**−**4** against KB (ATCC CCL17) and Jurkat T cancer cells (ATCC TIB-152).

Results and Discussion

Sequential percolation of the powdered aerial parts of *A. salicifolia* with petroleum ether, dichloromethane, ethyl acetate, methanol, and methanol−H_2O (1:1) yielded the respective crude extract. After TLC analysis, the dichloromethane and ethyl acetate extracts were combined and subjected to subsequent vacuum liquid chromatography (VLC) and column chromatography, which led to the isolation of a sterol (**1**) and two sterol monoglycosides (**2** and **3**). Fractionation of the methanolic extract by VLC afforded eight fractions. A fraction rich in sterol glycosides was further subjected to open column chromatography on silica gel and HPLC on RP-18, resulting in the isolation of a new sterol tetraglycoside (**4**).

Compound **1** was obtained as a colorless amorphous powder. The HRMALDIMS of compound **1** showed a sodiated molecular ion peak at m/z 531.3306 $[M + Na]^+$, compatible with the molecular formula $C_{29}H_{48}O_7$. The ^1H NMR spectrum showed the presence of four methyl groups, three resonating at δ_H 0.83, 0.84, and 1.14 (H_3-18, H_3-19, and H_3-26, respectively) as singlets and one at δ_H 1.08 (J = 7.2, H_3-29) as a triplet. Additional functionalities included the signal of an olefinic proton at δ_H 5.23 (m, H-7), as well as four methine protons at δ_H 3.47 (m, H-3), 3.92 (m, H-16), 4.41 (dd (t), J = 2.7, H-22), and 4.09 (d, J = 2.7, H-23), and two methylene groups at δ_H 3.49 (br s, H_2-27), 4.15 (m, H-21a), and 3.92 (m, H-21b) on oxygen-bearing carbon atoms. The ^{13}C NMR spectrum of compound **1** exhibited 29 carbon signals, whose multiplicities were determined by DEPT 90 and 135 experiments. The olefinic carbon signals at δ_C 119.0 and 140.6 corresponded to an endocyclic double bond between C-7/C-8. The resonances

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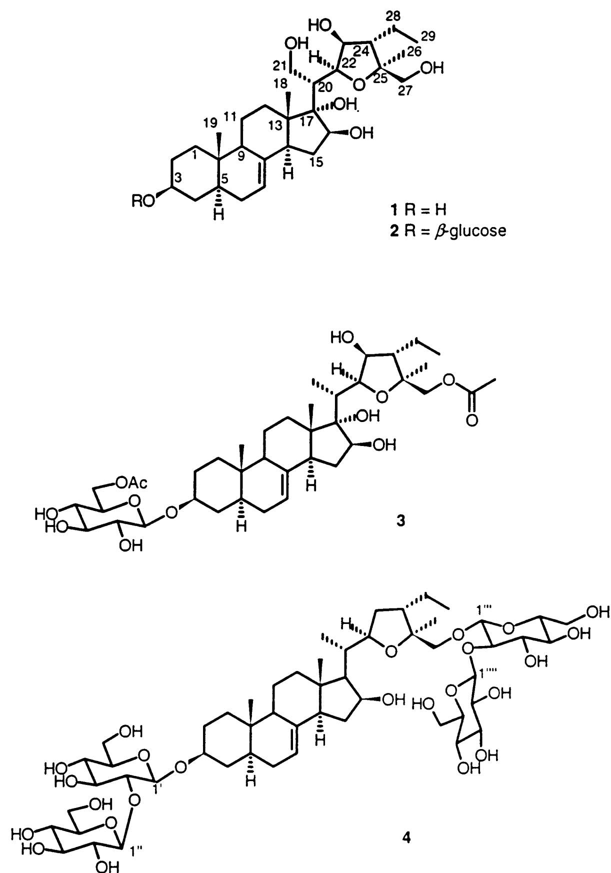

of the oxygenated carbons indicated the presence of four oxymethine carbons (δ_C 71.5, 81.3, 79.0, 77.9; C-3, C-16, C-22, and C-23, respectively), two oxymethylene carbons (δ_C 62.6, 69.7; C-21 and C-27, respectively), and two oxygenated quaternary carbons (δ_C 89.5, 86.2; C-17 and C-25, respectively). These data suggested the presence of a highly oxygenated C_{29} sterol. $^1H-^1H$ COSY, $^{13}C-^1H$ HSQC, and $^{13}C-^1H$ HMBC experiments (Figure 1) allowed the complete assignment of all protons and carbons of compound **1**. The chemical shift value of C-19 (δ_C 13.5) indicated the *trans* A/B ring junction of the steroid skeleton. The relative stereochemistry of compound **1** was established on the basis of a ROESY experiment (Figure 2). The cross-peak observed between H-5 and H-3 confirmed the β-hydroxylation at C-3. The configuration of the other stereocenters was confirmed by the ROESY correlations between H-14 and H-16; H_2-21 and H_2-12; H_3-26 and H-22; H-23 and H_3-29; as well as between H-24 and H_2-27. Therefore the structure of **1** was established as (3*S*,16*S*,17*S*,20*R*,22*S*,23*S*,24*S*,25*S*)-22,25-epoxy-stigmast-7-en-3,16,17,21,23,27-hexol and assigned the trivial name ajugasalicigenin.

The HRMALDIMS of ajugasalicioside F (**2**) exhibited a pseudomolecular ion peak at *m/z* 709.3695 $[M + K]^+$. This information along with the ^{13}C NMR and the DEPT spectra, which sorted 35 carbons into four methyls, 11 methylenes, 15 methines, and five quaternary carbons, allowed the determination of the molecular formula as $C_{35}H_{58}O_{12}$. The ^1H and ^{13}C NMR spectra of compound **2** resembled those of compound **1**, and the signals corresponding to the aglycon appeared at almost the same chemical shift values and with identical multiplicities. The assignment of the remaining signals in ^1H, ^{13}C, and $^1H,^{13}C-$HSQC spectra established the presence of an additional β-glucopyranosyl moiety. The stereochemistry

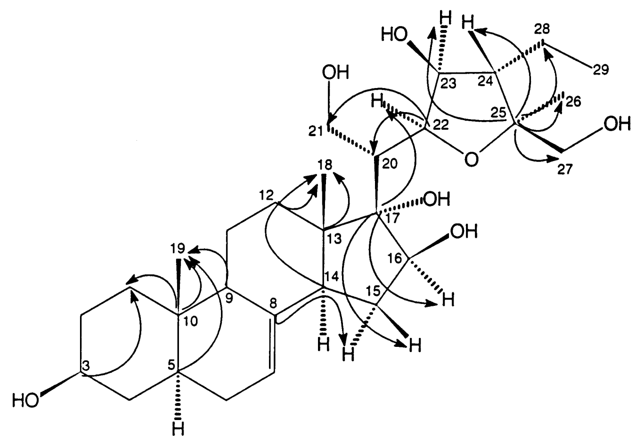

Figure 1. Selected long-range (HMBC) correlations of ajugasalicigenin (**1**).

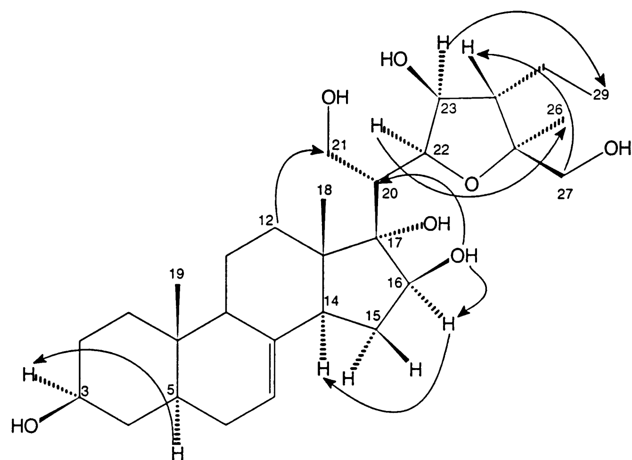

Figure 2. Key ROEs of ajugasalicigenin (**1**).

assigned at C-1′ was based on the chemical shifts of H-1′ (δ_H 4.22, d) and C-1′ (δ_C 100.8 ppm) and the ^3J coupling (7.8 Hz).

In HMBC spectrum, the long-range correlations observed for the aglycon of compound **2** were the same as for ajugasalicigenin (**1**), confirming the data above. The linkage of the sugar moiety to C-3 was established by the long-range correlation between H-1′ and C-3 and confirmed by the downfield shift of C-3, resonating at δ_C 76.3. The relative stereochemistry data of the aglycon were superimposable with those of compound **1**. The ROE observed between H-5 and H-3 indicated that the β-hydroxy group at C-3 was glycosylated. Therefore, the structure of **2** was established as (3*S*,16*S*,17*S*,20*R*,22*S*,23*S*,24*S*,25*S*)-22,25-epoxy-3-(β-D-glucopyranosyloxy)stigmast-7-en-16,17,21,23,27-pentol. In contrast to the previous sterols reported from *Ajuga salicifolia*,[7] compounds **1** and **2** display free secondary hydroxy groups at C-21 and C-27.

The molecular formula of ajugasalicioside G (**3**) was deduced as $C_{39}H_{62}O_{13}$ from the $[M + Na]^+$ peak at *m/z* 761.3968 in the HRMALDIMS. The ^1H, ^{13}C, and 2D NMR data of compound **3** were similar to those of ajugasalicioside F (**2**), but additional signals observed at δ_C 20.7, 20.8, 172.8 and δ_H 2.05, 2.08 (3H, s) indicated the presence of two acetyl functions. In the HMBC spectrum, the quaternary carbon resonating at δ_C 172.8 showed long-range correlations with H-27a, H-27b, H-6′a, H-6′b, and the protons resonating at δ_H 2.05 and 2.08 (each 3H, s). These data confirmed the acetylations at C-27 on the tetrahydrofuran ring and at C-6′ in the glucose unit. In comparison with ajugasalicioside F (**2**), an additional methyl signal resonat-

Table 1. ^{1}H NMR Data (500 MHz; δ in ppm, J in Hz) of Ajugasalicigenin (**1**) (in CD_3OD), Ajugasaliciosides F (**2**) (in DMSO-d_6), G (**3**), and H (**4**) (in CD_3OD)

position	**1**	**2**	**3**	**4**
1	1.85 (m)[a]; 1.13 (m)	1.78 (m)[a]; 1.06 (m)	1.86 (m)[a]; 1.11 (m)	1.85 (m); 1.11 (m)
2	1.76 (m)[a]; 1.38 (m)[a]	1.79 (m)[a]; 1.35 (m)[a]	1.35 (m)[a], 1.78 (m)[a]	1.92 (m); 1.48 (m)[a]
3	3.47 (m)	3.56 (m)	3.63 (m)	3.72 (m)[a]
4	1.69 (m)[a]; 1.27 (m)[a]	1.78 (m)[a]; 1.17 (m)[a]	1.85 (m)[a]; 1.34 (m)[a]	1.88 (m)[a]; 1.35 (m)[a]
5	1.42 (m)[a]	1.28 (m)[a]	1.38 (m)[a]	1.38 (m)[a]
6	1.79 (m)[a]; 1.33 (m)[a]	1.78 (m)[a]; 1.70 (m)[a]	1.80 (m)[a]; 1.35 (m)[a]	1.80 (m)[a]; 1.29 (m)
7	5.23 (m)	5.13 (m)	5.22 (m)	5.22 (m)
9	1.69 (m)[a]	1.61 (m)[a]	1.67 (m)[a]	1.67 (m)
11	1.69 (m)[a]; 1.59 (m)[a]	1.60 (m)[a]; 1.45 (m)	1.64 (m)[a]; 1.54 (m)[a]	1.60 (m); 1.52 (m)
12	1.82 (m)[a], 2H	1.75 (m)[a], 1.67 (m)[a]	1.79 (m)[a], 2H	2.07 (m)[a]; 1.22 (m)[a]
14	2.42 (m)	2.30 (m)	2.40 (m)[a]	1.73 (m)
15	2.10 (m)[a]; 1.42 (m)[a]	1.97 (m); 1.25 (m)[a]	2.11 (m)[a]; 1.43 (m)[a]	2.09 (m)[a]; 1.45 (m)[a]
16	3.92 (m)[a]	3.74 (m)	3.98 (dd, 5.6, 5.8)	4.45 (m)
17				1.19 (m)[a]
18	0.83 (s), 3H	0.73 (s), 3H	0.82 (s), 3H	0.78 (s), 3H
19	0.84 (s), 3H	0.75 (s), 3H	0.83 (s), 3H	0.83 (s), 3H
20	2.46 (m)	2.26 (m)	2.63 (m)	2.08 (m)[a]
21	4.15 (m); 3.92 (m)[a]	3.91 (br d, 9.5)	1.02 (d, 6.7), 3H	0.99 (d, 6.2), 3H
		3.79 (br d, 9.5)		
22	4.41 (dd (t), 2.7)	4.22 (m)[a]	3.90 (dd, 3.3)	3.84 (m)[a]
23	4.09 (d,2.7)	3.95 (m)	4.00 (m)	1.98 (m), 2H
24	2.00 (m)[a]	1.82 (dd, 3.9, 11.7)	1.85 (m)[a]	2.18 (m)
26	1.14 (s), 3H	1.01 (s), 3H	1.16 (s), 3H	1.04 (s), 3H
27	3.49 (br s), 2H	3.33 (m)[a],2H	4.17 (d, 11.2)	3.88; (d, 10.3)
			4.03 (d, 11.2)	3.50 (d, 10.3)
28	1.54 (m)[a]; 1.35 (m)[a]	1.39 (m)[a]; 1.19 (m)[a]	1.51 (m)[a]; 1.33 (m)[a]	1.47 (m)[a]; 1.22 (m)[a]
29	1.08 (t, 7.2), 3H	0.97 (t, 7.2), 3H	1.05 (t, 7.2), 3H	0.98 (t, 7.2), 3H
MeCO			2.08 (s)	
1′		4.22 (d, 7.8)	4.39 (d, 7.8)	4.54 (d, 7.8)
2′		2.89 (m)	3.15 (dd, 7.9, 8.9)	3.40 (dd, 7.5, 9.1)
3′		3.12 (m)	3.35 (m)	3.55 (m)[a]
4′		3.03 (m)[a]	3.28 (m)[a]	3.30 (m)[a]
5′		3.07(m)[a]	3.45 (m)	3.55 (m)[a]
6′		3.60 (dd, 5.0, 11.1);	4.35 (dd, 2.0, 11.9)	3.85(m)[a]; 3.71(m)[a]
		3.42 (dd, 5.9, 11.6)	4.21 (d, 6.1, 11.9)	
MeCO			2.05 (s)	
1″				4.58 (d, 7.8)
2″				3.22 (m)[a]
3″				3.37 (m)[a]
4″				3.25 (m)[a]
5″				3.27 (m)[a]
6″				3.83 (m)[a]; 3.71 (m)[a]
1‴				4.41 (d, 7.5)
2‴				3.56 (m)[a]
3‴				3.56 (m)[a]
4‴				3.35 (m)[a]
5‴				3.56 (m)[a]
6‴				3.85 (m)[a]; 3.71 (m)[a]
1⁗				4.66 (d, 7.8)
2⁗				3.22 (m)[a]
3⁗				3.28 (m)[a]
4⁗				3.35 (m)[a]
5⁗				3.25 (m)[a]
6⁗				3.65 (m)[a]

[a] Signals overlapped.

ing at δ_C 12.8 (δ_H 1.02; d, J = 6.7) was observed and attributed to C-21. This explained the absence of one methylene signal around 60−65 ppm, in comparison with compound **2**. The stereochemistry of ajugasalicioside G (**3**) was identical with that of **1** and **2**. Thus, the structure of compound **3** was established as (3*S*,16*S*,17*S*,20*R*,22*S*,23*S*,24*S*,25*S*)-22,25-epoxy-3-[β-D-(6′-acetoxy)glucopyranosyloxy]-stigmast-7-en-16,17,23-triol 27-acetate.

The HRMALDIMS of ajugasalicioside H (**4**) exhibited a pseudomolecular ion peak at m/z 1131.5552 $[M + Na]^+$, compatible with the molecular formula $C_{53}H_{88}O_{24}$. The ^{1}H NMR spectrum of **4** showed characteristic signals for a stigmast-7-ene skeleton, due to the olefinic proton resonating at δ_H 5.22 (m, H-7), three tertiary methyls (0.78 s, 0.83 s, 1.04 s; H_3-18, H_3-19, and H_3-26, respectively), one secondary methyl (0.99 d, J = 6.2, H_3-21), and one primary (0.98 t, J = 7.2, H_3-29) methyl group. Additionally, four anomeric proton resonances were observed at δ_H 4.54 (d, J = 7.8, H-1′), 4.58 (d, J = 7.8, H-1″), 4.41 (d, J = 7.5, H-1‴), and 4.66 (d, J = 7.8, H-1⁗). Thus, compound **4** was considered to be a stigmast-7-ene-type sterol tetraglucoside. This observation was supported by its ^{13}C NMR spectral data. NMR signals were analyzed by the use of COSY, HSQC, HSQC-TOCSY, and HMBC. The ^{1}H and ^{13}C NMR data supported the assignment of each sugar moiety as β-glucopyranose. The remaining 29 carbon resonances were attributed to the aglycon. The ^{13}C NMR spectrum displayed the signals of three oxymethines (δ_C 80.1, 73.6, 83.0; C-3, C-16, and C-22, respectively), one oxymethylene (δ_C 74.6, C-27), and one oxygenated quaternary carbon (δ_C 85.6, C-25). The chemical shifts of three methine protons (δ_H 3.72 m, 4.45 m, 3.84 m, H-3, H-16, and H-22, respectively) and

two methylene protons (δ_H 3.50 d, $J = 10.3$; H-27b and 3.88 d, $J = 10.3$; H-27a) were in accordance with the ^{13}C NMR data mentioned above, supporting the assignment of the oxygenated carbons of compound **3**. The HMBC correlation between H-22 and C-25 indicated the presence of an epoxide unit. The HMBC correlations observed between H-1′ and C-3 and H-1‴ and C-27 enabled the assignment of the linkages of the β-glucose moieties to the aglycon. As a result of the resonance at δ_C 73.6, C-16 was assumed to be hydroxylated. This was verified by the molecular mass of **4**, assigned from the HRMALDIMS. The cross-peaks observed between H-1″ and C-2′ and between H-1⁗and C-2‴ in the HMBC spectrum allowed the interglycosidic connection of the β-D-glucopyranoses to be determined. The ROESY data of ajugasalicioside H (**4**) resembled those for compounds **1**–**3**, showing the identical relative stereochemistry on the substituent at C-17, as well as at C-3 and C-16. Hence the structure of compound **4**, a tetraglycoside, was established as (3*S*,16*S*,17*R*,20*S*,22*R*,24*S*,25*S*)-22,25-epoxy-3-{[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]oxy}-27-{[β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]oxy}stigmast-7-en-16-ol.

Investigations of the cytotoxicity of these sterol derivatives against KB and Jurkat T cancer cells revealed activity of compound **1** against KB cells (IC_{50}, 1 μg/mL). Its corresponding 3-*O*-β-glucoside, **2**, was less active (KB cell line, IC_{50}, of 13 μg/mL). It is of interest that sterol **1** (and also compound **2**) showed no activity against Jurkat T cells up to 20 μg/mL. Compounds **3** and **4** were considered inactive against both cell lines up to 20 μg/mL.

Table 2. ^{13}C NMR Spectral Data (δ ppm; 295 K) of Ajugasalicigenin (**1**) (in CD_3OD, 75 MHz) and Ajugasaliciosides F (**2**) (in DMSO-d_6, 75 MHz), G (**3**) (in CD_3OD, 125 MHz), and H (**4**) (in CD_3OD, 75 MHz)

carbon	1	2	3	4
1	38.3, t	36.5, t	38.3, t	38.2, t
2	32.1, t	29.1, t	30.5, t	30.4, t
3	71.5, d	76.3, d	79.8, d	80.1, d
4	38.6, t	33.9, t	35.3, t	35.3, t
5	41.5, d	39.5, d	41.5, d	41.5, d
6	30.9, t	29.3, t	30.9, t	30.8, t
7	119.0, d	117.1, d	118.9, d	119.1, d
8	140.6, s	139.7, s	140.7, s	139.8, s
9	50.8, d	48.8, d	50.8, d	50.7, d
10	35.3, s	33.9, s	35.4, s	35.4, s
11	22.3, t	20.7, t	22.2, t	22.5, t
12	34.0, t	32.3, t	34.0, t	41.2, t
13	49.6, s	47.8, s	49.6, s	45.0, s
14	48.7, d	47.1, d	48.7, d	53.6, d
15	33.5, t	33.9, t	33.6, t	35.1, t
16	81.3, d	79.1, d	82.2, d	73.6, d
17	89.5, s	87.2, s	87.8, s	61.9, d
18	13.4, q	12.7, q	13.5, q	13.5, q
19	13.5, q	12.9, q	13.4, q	13.5, q
20	40.3, d	38.7, d	35.3, d	37.4, d
21	62.6, t	60.9, t	12.8, q	16.2, q
22	79.0, d	77.2, d	81.9, d	83.0, d
23	77.9, d	75.9, d	78.5, d	37.1, t
24	55.3, d	53.7, d	56.3, d	44.2, d
25	86.2, s	84.1, s	86.2, s	85.6, s
26	18.7, q	18.3, q	18.0, q	17.6, q
27	69.7, t	68.3, t	71.1, t	74.6, t
28	23.1, t	21.7, t	22.9, t	24.5, t
29	14.0, q	13.6, q	13.7, q	13.8, q
*Me*CO			20.7, q	
Me*CO*			172.8, s	
1′		100.8, d	102.7, d	101.4, d
2′		73.5, d	75.1, d	83.0, d
3′		76.7, d	77.9, d	77.8, d
4′		70.1, d	71.7, d	71.5, d
5′		76.7, d	75.1, d	77.8, d
6′		61.1, t	64.8, t	62.7, t
*Me*CO			20.8, q	
Me*CO*			172.8, s	
1″				105.2, d
2″				76.1, d
3″				77.6, d
4″				71.7, d
5″				78.4, d
6″				63.0, t
1‴				102.6, d
2‴				81.5, d
3‴				78.2, d
4‴				71.2, d
5‴				77.8, d
6‴				62.7, t
1⁗				104.7, d
2⁗				76.1, d
3⁗				78.3, d
4⁗				71.4, d
5⁗				77.8, d
6⁗				62.5, t

Experimental Section

General Experimental Procedures. Optical rotations were recorded using a Perkin-Elmer 241 polarimeter with methanol as solvent at 20 °C. UV spectra were obtained in methanol on a UVIKON 930 spectrophotometer. ^{13}C NMR, DEPT-135, and DEPT-90 spectra were measured on a Bruker AMX-300 at 295 K (operating at 75.47 MHz for ^{13}C) for the compounds **1**, **2**, and **4**, and on a Bruker DRX-500 at 295 K (operating at 125.77 MHz for ^{13}C) for compound **3**. All ^{1}H, [^{1}H,^{1}H]-COSY, [^{13}C,^{1}H]-HSQC, [^{13}C,^{1}H]-HSQC-TOCSY, [^{13}C,^{1}H]-HMBC, and [^{1}H,^{1}H]-ROESY experiments were measured on a Bruker DRX-500 at 295 K (operating at 500.13 MHz for ^{1}H and 125.77 MHz for ^{13}C), with chemical shifts given in ppm and coupling constants *J* in Hz. The spectra were measured in CD_3OD for compounds **1**, **3**, and **4** and in deuterated DMSO for compound **2** and referenced against residual nondeuterated solvent. HRMALDIMS were measured on an Ionspec Ultima FTMS spectrometer using 2,5-dihydroxybenzoic acid (DHB) as matrix. For vacuum liquid chromatography (VLC), RP-18 HL, 40–63 μm (Chemie Uetikon), silica gel 60, 40–63 μm (Merck), and for open column chromatography, silica gel 60, 40–63 μm and 63–200 μm (Merck), were used. HPLC separations were performed with a Merck-Hitachi L-6200 pump connected to a Rheodyne 7125 Injector, a Merck-Hitachi L-4000 UV detector, a Merck D-2500 Chromato-integrator, and a Knauer HPLC column (Spherisorb S5 ODS 2, 5 μm; 250 × 16 mm). Silica gel 60 F_{254} precoated aluminum plates (0.2 mm, Merck) and RP-18 F_{254} precoated plates (0.25 mm, Merck) were used for TLC controls; detection was performed by spraying with 5% H_2SO_4 in EtOH and 1% vanillin in EtOH and heating at 100–110 °C for 5 min.

Plant Material. *Ajuga salicifolia* (L.) Schreber was collected in Ankara, Beytepe, in July 1998. The plant was identified by Professor Zeki Aytac, Gazi University, Ankara (Turkey). A voucher specimen (HU-98014) has been deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University (Ankara, Turkey).

Extraction and Isolation. The dried and powdered aerial parts (1 kg) of *A. salicifolia* were extracted with petroleum ether, dichloromethane, ethyl acetate, methanol, and methanol–water (1:1), respectively (sequential percolation with ca. 10–15 L of each solvent). After TLC scrutiny, the dichloromethane and ethyl acetate extracts were combined (24 g), and fractionated by VLC (silica gel 60, hexane → ethyl acetate → methanol), yielding five main fractions. Fraction 5 (17 g) was applied to VLC (RP-18, H_2O–CH_3CN (100:0 → 0:100)). Fraction 5 (2.7 g), eluted with CH_3CN–H_2O (55:45), was further separated by VLC (silica gel 60, CH_2Cl_2–MeOH–H_2O (90:10:1 → 40:60:4)). Compounds **1** (3 mg) and **3** (3 mg) were isolated from subfraction 1 (128 mg) by subsequent column chromatography (silica gel, CH_2Cl_2–MeOH (98:2) and CH_2Cl_2–MeOH–H_2O (97:3:0.3), respectively). Subfraction 4 (678 mg) was subjected to VLC with silica gel eluting with CH_2Cl_2 →

CH_2Cl_2−MeOH, followed by a Sephadex LH-20 open column with methanol. The final purification with column chromatography (silica gel, CH_2Cl_2−MeOH−H_2O, 87.5:12.5:1.25) furnished compound **2** (6.1 mg).

An aliquot (40 g) of methanol extract was subjected to VLC [RP-18, H_2O−MeOH (100:0 → 0:100)] to give eight main fractions. Fraction 7, rich in sterols (3.7 g), was fractionated by open column chromatography (silica gel, CH_2Cl_2−MeOH−H_2O, 90:10:1 → 40:60:4), yielding 13 fractions. Subfraction 12 (937 mg) was further fractionated by open chromatography with the same conditions as used for fraction 7. A fraction (194 mg) eluted by CH_2Cl_2−MeOH−H_2O (75:25:5) was subjected to HPLC. Compound **4** (11 mg) was isolated by preparative HPLC, applying a step gradient of CH_3CN−H_2O (30:70 to 40:60) (RP-18, flow rate, 5 mL/min).

Cytotoxicity Assays. The cytotoxicity test against KB cells (HeLa cells, ATCC CCL17) was performed as described by Heilmann et al.[15] The cytotoxicity assay against Jurkat T cancer cells (human leukemia cells, ATCC TIB-152) was performed as described by Gertsch et al.[16]

Ajugasalicigenin [(3*S*,16*S*,17*S*,20*R*,22*S*,23*S*,24*S*,25*S*)-22,25-epoxy-stigmast-7-en-3,16,17,21,23,27-hexol; 1]: amorphous, white powder, 3 mg; mp 192 °C; $[\alpha]^{20}_D$ +5.7° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (2.74) nm; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRMALDIMS (pos. mode) *m*/*z* 531.3306 [M + Na]$^+$ (calcd for $C_{29}H_{48}O_7Na$, 531.3298).

Ajugasalicioside F [(3*S*,16*S*,17*S*,20*R*,22*S*,23*S*,24*S*,25*S*)-22,25-epoxy-3-(*β*-D-glucopyranosyloxy)stigmast-7-en-16,-17,21,23,27-pentol; 2]: amorphous, white powder, 6.1 mg; mp 243 °C; $[\alpha]^{20}_D$ −3.0° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (2.77) nm; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRMALDIMS (pos. mode) *m*/*z* 709.3695 [M + K]$^+$ (calcd for $C_{35}H_{58}O_{12}K$, 709.3565).

Ajugasalicioside G [(3*S*,16*S*,17*S*,20*R*,22*S*,23*S*,24*S*,25*S*)-22,25-epoxy-3-[*β*-D-(6′-acetoxy)glucopyranosyloxy]stigmast-7-en-16,17,23-triol-27-acetate; 3]: amorphous, white powder, 3 mg; mp 187 °C; $[\alpha]^{20}_D$ −8.0° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (2.63) nm; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRMALDIMS (pos. mode) *m*/*z* 761.3968 [M + Na]$^+$ (calcd for $C_{39}H_{62}O_{13}Na$, 761.4088).

Ajugasalicioside H [(3*S*,16*S*,17*R*,20*S*,22*R*,24*S*,25*S*)-22,-25-epoxy-3-{[*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl]-oxy}-27-{[*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl]oxy}-stigmast-7-en-16-ol; 4]: amorphous, white powder, 11 mg; mp 154 °C; $[\alpha]^{20}_D$ −13.2° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (2.68) nm; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRMALDIMS (pos. mode) *m*/*z* 1131.5552 [M + Na]$^+$ (calcd for $C_{53}H_{88}O_{24}Na$, 1131.5563).

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