Notes

Withanolides from *Ajuga parviflora*

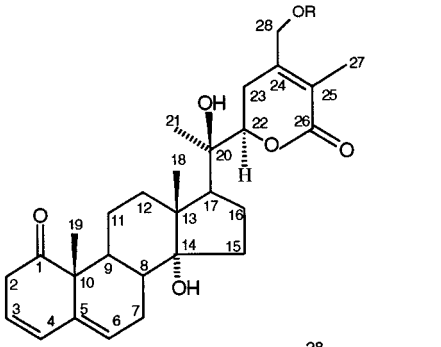
Pir Muhammad Khan, Abdul Malik,* Saeed Ahmad, and Hafiz Rub Nawaz

International Centre for Chemical Sciences, H. E. J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan

Received January 25, 1999

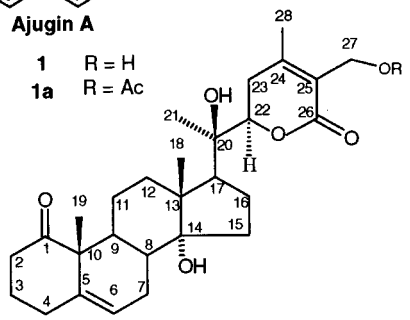
Two new withanolides named ajugin A [14α,20,28-trihydroxy-1-oxo-(20*R*,22*R*)-witha-3,5,24-trienolide] (**1**) and ajugin B [14α,20,27-trihydroxy-1-oxo-(20*R*,22*R*)-witha-5,24-dienolide] (**2**) were isolated from the whole plant of *Ajuga parviflora*, and their structures were deduced by NMR and MS analyses.

Due to our continuing interest in withanolides of medicinal plants, we herein report two new withanolides, ajugin A (**1**) and ajugin B (**2**), from the whole plant material of *Ajuga parviflora* Benth. (Labiatae) found in the northern parts of Pakistan.[1] The plant has found diverse medicinal uses in indigenous systems of medicine.[2,3] It has been used as an astringent and for treatment of swollen wounds, diarrhoea, rheumatic fever, eye trouble, and diseases of bladder.[2,3]



Ajugin A

1 R = H
1a R = Ac



Ajugin B

2 R = H
2a R = Ac

Compounds **1** and **2** were obtained from the $CHCl_3$-soluble fraction of a MeOH extract of *A. parviflora* following chromatographic procedures described in the Experimental Section. Compound **1** showed absorptions indicative of hydroxyl groups, a six-membered cyclic ketone and α,β-unsaturated δ-lactone in its IR spectrum. The UV spectrum of **1** was characteristic of withanolides showing the absorption at λ_{max} 221 nm attributable to an α,β-unsaturated δ-lactone.[4] Positive FABMS revealed a $[M + H]^+$ peak at *m*/*z* 471. The HREIMS of **1** gave the M^+ peak at *m*/*z* 470 analyzing for $C_{28}H_{38}O_6$. A fragment at *m*/*z* 185 ($C_9H_{13}O_4$), resulting from cleavage of the C-17/C-20 bond, indicated an hydroxyl group at C-20, while a peak at *m*/*z* 141 ($C_7H_9O_3$), resulting from the cleavage of the C-20/C-22 bond, showed the presence of a six-membered hydroxyl-substituted lactone substituent at C-20.[5] The ^{1}H NMR spectrum of **1** closely resembled that of isowithanolide F[6] and indicated the presence of a 3,5-diene-1-oxo system in rings A and B of the steroidal skeleton. It included signals for two mutually coupled olefinic protons at δ 5.67 and 6.65, assignable to C-3 and C-4 vicinal protons, respectively. Another downfield olefinic signal resonating at δ 5.40 showed cosy 45° couplings to two protons of the C-7 methylene group (δ 2.60 and 2.42) and was assigned to the C-6 vinylic proton. The oxymethine proton resonating at δ 4.53 showed one-bond heteronuclear connectivity to a carbon at δ 81.6 in the HMQC spectrum of **1** and ^2J couplings to carbons at δ 31.8 (C-23) and 79.0 (C-20) and ^3J couplings to carbons at δ 49.6 (C-17) and 166.5 (C-26) in the HMBC, confirming its placement at C-22. It was assigned the α-orientation (22*R*) in analogy to commonly occurring withanolides. This assignment was confirmed through its multiplicity in the ^{1}H NMR spectrum. It has been reported that when C-22 has the *S*-configuration, H-22 resonates as a broad singlet with $W_{1/2} = 5$ Hz, while in the *R*-configuration it appears in the ^{1}H NMR spectrum as a double doublet with two coupling constants characteristic for axial−axial and axial−equatorial interactions with H_2-23.[7] In the case of compounds **1** and **2**, H-22 resonated as a double doublet, revealing the *R*-configuration at C-22. The remaining two oxygen atoms must be present as tertiary hydroxyls because all of the double-bond equivalents have been accounted for. This was confirmed through acetylation of **1** to **1a**, which still showed hydroxyl absorption in its IR spectrum. The multiplicity of H-22 (dd) and observation of Me-21 as a singlet were indicative of a 20-OH group. Four 3H singlets at δ 0.96, 1.20, 1.27, and 1.86 accounted for the 18-Me, 19-Me, 21-Me, and 27-Me methyl groups, respectively. Absence of a signal corre-

* To whom correspondence should be addressed. Tel.: 92-21-4968733. Fax: 92-21-4963373, 92-21-49631241. E-mail: amalik@amalik.hejric.edu.

10.1021/np990029k CCC: $18.00
Published on Web 08/18/1999

sponding to the 28-Me and the presence of downfield AB doublets at δ 4.38 and 4.21, which shifted downfield to δ 4.89 and 4.84 in **1a**, showed that this position is substituted with a hydroxymethyl group. Placement of the primary hydroxyl at C-28 was further confirmed through an HMBC experiment, which revealed a ^2J correlation from the oxymethylene group (δ 4.38, 4.21) to C-24 (δ 152.0) and ^3J to C-25 (δ 121.0) and C-23 (δ 29.1). There was a ^2J correlation from Me-27 (δ 1.86) to C-25 (δ 121.0) and ^3J to the lactonic carbonyl (C-26, δ 166.5) and C-24 (δ 152.0). The remaining tertiary hydroxyl was placed at C-14 because of its downfield shift in ^{13}C NMR compared to a previously reported withanolide[8] having similar rings A to C. The OH at C-14 was further confirmed by an HMBC experiment that showed a ^2J correlation of C-18 methyl protons (δ 0.96) to C-13 (δ 49.3) and ^3J to C-12 (δ 25.7), C-14 (δ 84.4), and C-17 (δ 49.3). The C-21 methyl protons (δ 1.27) also showed ^2J correlations to C-20 (δ 79.0) and ^3J correlations to C-17 (δ 49.3) and C-22 (δ 81.6). It has been observed that a 14β-OH does not cause shielding of C-12,[9] although 14α-OH shields C-7, C-9, and C-12 and deshields C-8.[10] Thus, the 14-OH of **1** was assigned the α-orientation. The ^{13}C NMR spectrum showed signals for 28 carbons, and their shift values were consistent with the above substitution pattern. Assignments of all functional groups were confirmed by HMQC and HMBC experiments and comparison with related withanolides.[5,6,11] Based on these evidences, the structure 14α,20,28-trihydroxy-1-oxo-(20*R*,22*R*)-witha-3,5,24-trienolide was assigned to compound **1**.

The HREIMS of **2** afforded an M^+ peak at *m*/*z* 472, corresponding to molecular formula $C_{28}H_{40}O_6$. The IR spectrum revealed absorptions characteristic of hydroxyl, cyclic ketone, and α,β-unsaturated δ-lactone. The presence of an α,β-unsaturated δ-lactone was confirmed by a UV maximum at 226 nm.[5] The ^{1}H NMR of **2** differed from that of **1**, as there were major differences in the signals of ring A, which lacked a double bond and showed only one olefinic signal at δ 5.32 assignable to H-6. However, the carbonyl signal at δ 216.1 in its ^{13}C NMR had a chemical shift similar to C-1 of **1**. Comparison of ^{13}C NMR data with that of withametelin H_2 having a similar ring A, established the substitution pattern of both rings A and B.[12] The chemical shifts due to the lactone ring showed significant differences from those of **1** and were indicative of a 27-hydroxymethylene and were in agreement with those of withanolides having similar lactone rings.[5] This assignment was confirmed by an HMBC experiment, which showed ^2J correlation from the oxymethylene group (δ 4.18, 4.14) to C-25 (δ 122.8) and ^3J to C-24 (δ 155.2) and C-26 (δ 166.8). Compound **2** was, therefore, assigned structure 14α,20,27-trihydroxy-1-oxo-(20*R*,22*R*)-witha-5, 24-dienolide.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-360 polarimeter. IR and UV spectra were recorded on a JASCO 302-A and on a Hitachi UV 3200 spectrophotometer, respectively. EIMS, FABMS, and HREIMS were recorded on JMS HX 110 with a data system, and on JMS-DA 500 mass spectrometers. The ^{1}H NMR, ^{13}C NMR, COSY, HMQC, and HMBC spectra were recorded on Bruker spectrometers operating at 500 and 400 MHz. The chemical shift values are reported in parts per million (δ units) and the coupling constants (*J*) are in Hertz. Column chromatography: Si gel, 230−400 mesh. TLC: precoated silica G-25, UV_{254} plates. Visualization of the TLC plates was achieved at 254 and 366 nm, and Dragendorff's reagent was used for detection.

Plant Material. The whole plant of *Ajuga parviflora* Benth. (Labiatae), collected from Swat (Pakistan) in July 1997, was identified by Dr. Jahandar Shah, plant taxonomist, Department of Botany, University of Peshawar. A voucher specimen was deposited in the herbarium (PUH-14918) of Peshawar University.

Extraction and Isolation. The air-dried ground plant (20 kg) was exhaustively extracted with 90% MeOH at room temperature. The extract was concentrated, and the residue (1.2 kg) was partitioned between MeOH and *n*-hexane. The defatted MeOH extract was evaporated and partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ extract was loaded on a Si gel column and eluted with *n*-hexane−EtOAc and EtOAc−MeOH mixtures with gradual increase in polarity. Fractions obtained in EtOAc−MeOH (9.5:0.5) were subjected to flash chromatography on Si gel using EtOAc and increasing the polarity with MeOH. Fractions obtained from EtOAc−MeOH (9.0:1.0) were combined and further subjected to MPLC using a Lobar (LiChroprep Si 60, Merck) column and EtOAc−MeOH (9.8:0.2) as the mobile phase. Final purification of the resulting fractions by preparative TLC using $CHCl_3$−C_6H_6−MeOH−H_2O (4.0: 4.0: 4.0: 0.5) afforded pure compounds **1**, **2**, β-sitosterol, and stigmasterol.

14α,20,28-Trihydroxy-1-oxo-(20*R*,22*R*)-witha-3,5,24-trienolide (ajugin A) (1): obtained as greenish amorphous solid; $[\alpha]^{25}_D$ +120° (c 0.25, MeOH); UV (MeOH) λ_{max} 221 nm (ϵ 17 900); IR (KBr) ν_{max} 3420, 1712, 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$ + CD_3OD, 400 MHz) δ 6.65 (1H, m, H-3), 5.67 (1H, dd, J = 9.9, 2.9 Hz, H-4), 5.40 (1H, dd, $J_{6,7a}$ = 5.2 Hz, $J_{6,7b}$ = 2.1 Hz, H-6), 4.53 (1H, dd, J = 13.0, 3.2 Hz, H-22), 4.38 and 4.21 (2H, ABd, J = 13.4 Hz, H_2-28), 1.86 (3H, s, Me-27), 1.27 (3H, s, Me-21), 1.20 (3H, s, Me-19), 0.96 (3H, s, Me-18); ^{13}C NMR ($CDCl_3$ + CD_3OD, 125 MHz) δ 211.6 (C-1), 166.5 (C-26), 152.0 (C-24), 140.4 (C-5), 129.1 (C-4), 127.4 (C-3), 121.3 (C-6), 121.0 (C-25), 84.4 (C-14), 81.6 (C-22), 79.0 (C-20), 61.9 (C-28), 53.9 (C-10), 49.6 (C-17), 49.3 (C-13), 39.5 (C-2), 37.8 (C-7), 35.7 (C-16), 35.5 (C-8), 34.0 (C-9), 31.8 (C-23), 29.6 (C-12), 24.5 (C-15), 20.8 (C-11), 20.0 (C-21), 18.5 (C-19), 16.1 (C-18), and 11.6 (C-27); FABMS $[M + H]^+$ *m*/*z* 471; EIMS *m*/*z* (rel int) $[M]^+$ 470 (8), 452 (10), 295 (56), 141 (90) and 124 (100), HREIMS *m*/*z* 470.2662 (calcd for $C_{28}H_{38}O_6$, 470.2668).

Acetylation of Compound 1. Compound **1** (18 mg) was acetylated with Ac_2O (2 mL) in pyridine (2 mL) at room temperature for 24 h. Usual workup and preparative TLC of the residue afforded **1a** (16.0 mg); $[\alpha]^{25}_D$ +108° ($CHCl_3$, *c* 0.31); UV λ_{max} (MeOH) 222 nm (ϵ 18 000); IR ν_{max} ($CHCl_3$) 3454, 1735, 1712, and 1703 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.55 (m, H-3), 5.65 (1H, dd, J = 9.7, 3.0 Hz, H-4), 5.38 (1H, dd, $J_{6,7a}$ = 5.4 Hz, $J_{6,7b}$ = 2.3 Hz, H-6), 4.89 and 4.84 (2H, ABd, J = 13.4 Hz, H_2-28), 4.55 (1H, dd, J = 12.8, 3.4 Hz, H-22), 2.01 (3H, s, OAc), 1.99 (3H, s, Me-27), 1.29 (3H, s, Me-21), 1.24 (3H, s, Me-19) and 1.01 (3H, s, Me-18); EIMS *m*/*z* (rel int): M^+ 512 (6), 452 (10), 329 (15), 285 (13), 183 (7), 124 (90).

14α,20, 27-Trihydroxy-1-oxo-(20*R*,22*R*)-witha-5,24-dienolide (ajugin B) (2): obtained as white solid; $[\alpha]^{25}_D$ +64° ($CHCl_3$, *c* 0.29); UV λ_{max} (MeOH) 226 nm (ϵ 18 300); IR ν_{max} (KBr) 3425, 1720 and 1696 cm^{-1}; ^{1}H NMR ($CDCl_3$ + CD_3OD, 400 MHz) δ 5.32 (1H, d, J = 5.5 Hz, H-6), 4.51 (dd, J = 12.5, 3.4 Hz, H-22), 4.43 and 4.30 (2H, ABd, J = 12.4 Hz, H_2-27), 1.89 (3H, s, Me-28), 1.29 (3H, s, Me-21), 1.25 (3H, s, Me-19), 0.98 (3H, s, Me-18);^{13}C NMR ($CDCl_3$ + CD_3OD, 125 MHz) δ 216.1 (C-1), 166.8 (C-26), 155.2 (C-24), 140.3 (C-5), 122.8 (C-25), 121.0 (C-6), 84.3 (C-14), 80.7 (C-22), 74.9 (C-20), 55.8 (C-27), 52.6 (C-13), 52.4 (C-10), 49.1 (C-17), 37.8 (C-2), 36.1 (C-9), 34.0 (C-8), 32.3 (C-7), 31.9 (C-23), 31.8 (C-16), 31.5 (C-4), 26.4 (C-3), 25.3 (C-12), 20.6 (C-11), 20.4 (C-21), 19.8 (C-28), 18.7 (C-19), and 17.2 (C-18); FABMS $[M + H]^+$ *m*/*z* 473.2820; EIMS *m*/*z* (rel int) 472 (7), 330 (18), 312 (10), 268 (30), 286 (41), 185 (71), 141 (100), 125 (92); HREIMS *m*/*z* 472.2820 (calcd for $C_{28}H_{40}O_6$, 472.2825).

Acetylation of 2. Acetylation of **2** was carried out as **1** to obtain the monoacetate: $[\alpha]^{25}_D$ +45° ($CHCl_3$, *c* 0.34); UV λ_{max} (MeOH) 226 nm (ϵ 18 600); IR ν_{max} ($CHCl_3$) 3405, 1734, 1710, 1692 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.48 (1H, d, J = 5.4 Hz, H-6), 4.55 (dd, J = 12.6, 3.3 Hz, H-22), 4.78, 4.58 (2H, AB

d, $J_{27,27'}$, 12.2 Hz, H-27), 2.12 (3H, s, COCH_3), 2.01 (3H, s, H-28), 1.34 (3H, s, H-21), 1.29 (3H, s, H-19), 1.07 (3H, s, H-18).

References and Notes

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NP990029K