PERGAMON

Phytochemistry 51 (1999) 669–671

PHYTOCHEMISTRY

The first report of a withanolide from the family *Labiatae*

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Received 30 November 1998; received in revised form 11 January 1999

Abstract

A new withanolide 3β,17β-dihydroxy-14,20-epoxy-1-oxo-22R-witha-5,24-dienolide has been isolated from *Ajuga parviflora* and its structure elucidated through spectroscopy including 2D NMR. This is the first report of the natural occurrence of withanolides in the Labiatae.

Keywords: *Ajuga parviflora*; Labiatae; Ajugin; Withanolide

1. Introduction

Withanolides have been considered a monopoly of Solanaceous plants. There are only rare reports on their isolation from a marine organism (a soft coral) and from members of the Taccaceae and the Leguminosae (Ray & Gupta, 1994). *Ajuga parviflora* (Labiatae) is an annual or short lived perennial herb widely distributed in Afghanistan, Kashmir and Pakistan (Ali & Nasir, 1990). The plants of genus *Ajuga* find diverse medicinal uses in indigenous systems of medicine (Manjunath, 1948; Perry & Metzger, 1980). Due to our interest in the chemical constituents of local medicinal plants, we carried out phytochemical investigations on *Ajuga parviflora*. These studies have resulted in the isolation of a new withanolide, ajugin (**1**). This is the first report of naturally occurring withanolides in Labiatae.

2. Results and discussion

Chromatographic resolution of the chloroform soluble fraction of the methanolic extract of the whole plant material of *Ajuga parviflora* resulted in the isolation of Ajugin **1** which was recognised as a withanolide from the diagnostic base peak at m/z 125 in its mass spectrum which results by the loss of the δ-lactone moiety and from a characteristic peak in the ^{1}H NMR spectrum, a double doublet at δ 4.66 which showed a close resemblance with reported withanolides (Velde, Lavie, Budhiraja, Sudhir, & Garg, 1983). The spectrum showed five methyl singlets assignable to three tertiary methyls and two vinylic methyl groups. A broad doublet at δ 5.62 ($J = 5.1$ Hz) which showed a ^1J coupling to the carbon at δ 125.5 in the HMQC spectrum, besides the presence in ^{13}C NMR spectrum of a signal at 134.9 (C), showed the presence of a trisubstituted double bond. A broad multiplet at δ 3.81 which moved downfield to δ 5.02 in its monoacetate **1a** exhibited one-bond heteronuclear connectivity to the carbon at δ 68.1 in the HMQC spectrum and was assigned to H-3α (Ramaiah, Lavie, Budhiraja, Sudhir, & Garg, 1984). The IR spectrum of **1a** still showed hydroxyl absorption at 3465 cm^{-1} indicating the presence of a tertiary hydroxyl. The HREIMS showed a molecular ion peak at m/z 470.3364 corresponding to the molecular formula $C_{28}H_{38}O_6$ (calc. for 470.3368). The molecular formula was further confirmed through positive FABMS which showed a $[M+H]^+$ peak at m/z 471. The ^{13}C NMR spectrum showed 28 signals, the most characteristic signals being at δ 85.6 and 78.4, which were assigned to the α-oriented epoxy bearing C-14 and C-20, respectively (Kirson, Gunzberg, & Gottlieb, 1980; Atta-ur-Rahman, Abbas, Dur-e-

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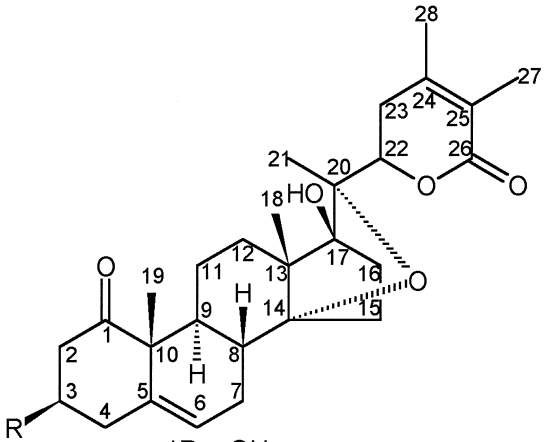
0031-9422/99/$ - see front matter
PII: S0031-9422(99)00045-X



Shahwar, Jamal, & Choudhary, 1993; Atta-ur-Rahman et al., 1998).

Chemical evidence for the presence of an ether linkage between C-14/C-20 and a tertiary hydroxyl at C-17 was provided by treatment of the acetate **1a** (in situ, in the NMR tube) with trichloroacetylisocyanate which gave a monocarbamate derivative (N*H* at δ 9.32). Moreover, the reaction took 72 h for completion indicating the presence of hindered hydroxyl group (Glotter, Abraham, Gunzberg, & Kirson, 1977).

The β-orientation of the 17-OH group was established from the study of the ^{1}H NMR spectrum of **1** repeated in pyridine-d_5, since pyridine is known to influence the chemical shifts of neighbouring protons of the OH substituent (Demarco, Farkas, Doddrell, Mglari, & Wenkert, 1968). The shift differences observed are useful in locating positions of hydroxyls and neighbouring groups. The configuration of the OH-17-(β) group could be deduced by the chemical shifts of the Me-18, Me-21 and H-22 and by the pyridine-induced chemical shifts values. It has been observed that the OH-17β strongly deshields the signal of the Me-21 and Me-18 whereas an OH-17α induces a sizeable downfield shift only in the Me-21 signal (Gottlieb & Kirson, 1981). The chemical shift values of the Me-18 at δ 1.04 and Me-21 at δ 1.16 of **1** were in agreement with a OH-17β group. The chemical shift differences (δ in $CDCl_3$-δ in C_5D_5N) for Me-18 (−0.32 ppm), Me-21 (−0.49 ppm) and H-22 (−0.52 ppm) suggested the β-orientation of the OH-17 group in **1**.

It has been observed that when C-22 has an S-configuration, H-22 resonates as a broad singlet with $W_{1/2} \approx 5$ Hz while in the R-configuration it resonates as a double doublet with two characteristic coupling constants due to axial–axial and axial–equatorial interactions with H-23 (Vasina & Abdulaev, 1990). In the case of **1**, H-22 resonated as a double doublet, revealing the R-configuration at C-22.

The ^{1}H–^{1}H homonuclear COSY-45° spectrum of **1** indicated the presence of three important spin systems. H-3 (δ 3.81) showed COSY-45° connectivity with H-2 (δ 2.58, 2.66) as well as H-4 (δ 2.36, 1.92). H-6 showed vicinal coupling with methylinic H-7 (δ 1.75, 1.54). The H-22 (δ 4.66) displayed strong cross peaks with methylinic H-23 (δ 2.45, 2.13).

The DEPT ^{13}C NMR spectrum showed the presence of five methyl, eight methylene and five methine carbons. The remaining ten quaternary carbon signals appeared in the broad-band spectrum. The four olefinic carbon atoms of ring B and E resonated at δ 134.9 (C), 125.5 (CH), 151.8 (C), 120.8 (C) due to C-5, C-6, C-24 and C-25, respectively.

Assignments of all the functional groups were achieved by HMQC and HMBC experiments. In the HMBC spectrum 19-Me (δ 1.06) showed long-range couplings with C-1 (δ 212.7), C-5 (δ 134.9) and C-9 (δ 35.3). H-3 (δ 3.81) showed ^2J and ^3J couplings with C-2 (δ 47.4) and C-1 (δ 212.7), respectively. Similarly, the epoxy bearing C-14 (δ 85.6) showed ^3J couplings with Me-18 protons (δ 1.04). Likewise Me-21 (δ 1.16) showed ^2J and ^3J couplings with C-20 (δ 78.4) and C-17 (δ 87.3), respectively. The H-22 (δ 4.66) showed ^3J couplings with C-17 (δ 87.3) and C-26 (δ 167.6), respectively. These spectroscopic data led to structure **1** for ajugin.

3. Experimental

3.1. General

Optical rotations were measured on a JASCO DIP-360 polarimeter. EI, FAB and HREIMS were recorded on JMS HX 110 with a data system and on JMS-DA 500 mass spectrometers. The ^{1}H, ^{13}C NMR, COSY, HMQC and HMBC spectra were recorded at 500 and 300 MHz. The chemical shift values are reported in ppm (δ) units and the coupling constants (J) are in Hz.

3.2. Plant material

Ajuga parviflora, whole plant collected from Swat (Pakistan) in July, 1997 was identified by Dr. Jahandar Shah, plant taxonomist, Department of Botany, Peshawar University, Peshawar, where a voucher specimen is deposited in the herbarium.

3.3. Extraction and isolation

The air-dried ground plant (20 kg) was exhaustively extracted with 90% MeOH at room temp. The extract was concentrated and the residue (1.2 kg) was dissolved in MeOH and defatted with hexane. The defatted MeOH extract was evaporated and parti-

tioned between $CHCl_3$ and H_2O. The $CHCl_3$ extract was loaded on Si-gel column and eluted with *n*-hexane/EtOAc, EtOAc/MeOH mixtures with gradually increasing polarity. The fractions obtained in EtOAc/MeOH (9.5:0.5) were subjected to flash chromatography (fcc) on Si gel using EtOAc and increasing the polarity with MeOH. Fractions obtained from EtOAc/MeOH (9.6:0.9) were combined and further purified through medium pressure liquid chromatography (mplc) using Lobar (LiChroprep Si 60, Merck) column and EtOAc/MeOH (9.8:0.2) as mobile phase. Final purification of the resulting fractions by TLC using $CHCl_3$:C_6H_6:MeOH:H_2O (9.8:4:4:0.5) afforded **1**.

3.4. Ajugin (**1**)

Amorphous powder (38.31 mg), $[\alpha]_D$ +70.5 (MeOH, *c* 0.23); UV λ_{max} (MeOH) 225 nm; IR ν_{max} ($CHCl_3$) 3450, 1715, 1705 cm^{-1}; HREIMS calc. for $C_{28}H_{38}O_6$ *m/z* 470.3368, found 470.3364; EIMS *m/z* (rel. int): 470 M^+ (20), 452 (9.5), 345 (30), 318 (2.3), 301 (77), 300 (9.7), 169 (74), 152 (100) and 125 (94); ^{1}H NMR ($CDCl_3$): δ 5.62 (1H, br d, J=5.1 Hz, H-6), 4.66 (1H, dd, J=13.0, 3.5 Hz, H-22), 3.81 (1H, m, H-3), 1.94 (3H, s, Me-28), 1.83 (3H, s, Me-27), 1.16 (3H, s, Me-21), 1.06 (3H, s, Me-19) and 1.04 (3H, s, Me-18); ^{1}H NMR (C_5D_5N): δ 5.70 (1H, br s), 5.18 (1H, dd, J=13.2, 3.4 Hz, H-22), 4.17 (1H, m, H-3), 1.91 (3H, s, Me-28), 1.82 (3H, s, Me-27), 1.65 (3H, s, Me-21), 1.36 (3H, s, Me-18) and 1.31 (3H, s, Me-19); ^{13}C NMR ($CDCl_3$): δ 212.7 (C-1), 167.6 (C-26), 151.8 (C-24), 134.9 (C-5), 125.5 (C-6), 120.8 (C-25), 87.3 (C-17), 85.6 (C-14), 81.1 (C-22), 78.4 (C-20), 68.1 (C-3), 54.3 (C-13), 52.6 (C-10), 47.4 (C-2), 40.3 (C-4), 36.4 (C-16), 35.6 (C-8), 35.3 (C-9), 34.1 (C-12), 31.9 (C-23), 29.6 (C-15), 25.4 (C-7), 21.7 (C-11), 20.5 (C-21), 20.1 (C-28), 18.5 (C-18), 18.0 (C-19) and 11.7 (C-27).

3.5. Acetylation of (**1**)

Compound **1** (20 mg) was acetylated with Ac_2O (2 ml) in pyridine (2 ml) at room temperature for 24 h. The solvent was removed in vacuo and prep. TLC of residue afforded **1a** (16.7 mg); UV λ_{max} (MeOH): 227 nm; IR ν_{max} ($CHCl_3$): 3465, 1733, 1710 and 1705 cm^{-1}; EIMS: *m/z* (rel. int): 512 M^+ (4), 494 (6), 452 (9), 434 (11), 387 (10), 369 (8), 360 (3), 343 (15), 169 (56), 152 (81) and 125 (100); ^{1}H NMR ($CDCl_3$): δ 5.46 (1H, br d, J=5.4 Hz, H-6), 5.02 (1H, m, H-3), 4.88 (1H, dd, J=12.8, 4.4 Hz, H-22), 2.03 (3H, s, OAc), 1.96 (3H, s, Me-28), 1.64 (3H, s, Me-27), 1.39 (3H, s, Me-21), 1.28 (3H, s, Me-18) and 1.08 (3H, s, Me-19).

3.6. Reaction of **1a** with trichloroacetylisocyanate (TAI)

The reaction was carried out by adding a few drops of TAI into the NMR tube containing **1a** dissolved in $CDCl_3$. The spectrum was recorded before and soon after the addition of TAI. No change was noted immediately. However the signal of the carbamate proton was clearly observed when spectrum was recorded again after 24 h. The reaction took 72 h for its completion and a signal at δ 9.32 (1H, br s) was observed.

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