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A New Triterpene Glycoside from *Fagonia cretica*

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A new triterpene glycoside (fagonoside A) has been isolated from the whole plant of *Fagonia cretica*. The structures was elucidated on the basis of spectroscopic techniques (1D and 2D NMR, HREIMS, HRFABMS, IR), physical and chemical analysis.

Keywords: Triterpene glycoside, Fagonoside A, *Fagonia cretica*, 1D and 2D-NMR.

INTRODUCTION

Fagonia cretica L. is a well-known desert medicinal plant which belongs to the family Zygophyllaceae. It is a small annual herb with woody branches. *Fagonia* is a genus of 34 species confined to warm areas of all continents except Australia. *F. cretica* is reputed to be a medicinal plant in scientific and folkloric literature and its medicinal values are well documented. An aqueous decoction of the aerial parts of the plant is the popular remedy in the indigenous system of medicine for cancer in its early stages and for the treatment of various diseases of digestive and blood vascular system[1-4]. Previously flavonoids and triterpenoidsaponins have been reported from this species[5-7]. The ethanopharmacological and chemotaxonomic importance of the genus *Fagonia* prompted us to investigate the chemical constituents from *F. cretica*. Our detailed investigations have led to the discovery of a new compound **1** (fagonoside A Fig. 1), assigned as 22-hydroxy-ursane 12-ene-3-*O*-α-L-rhamnopyranoside. The compound **1** was identified by IR, MS, 1D and 2D NMR spectroscopic techniques.

EXPERIMENTAL

The melting point was determined on a Gallenkemp melting point apparatus. A digital polarimeter JASCO DIP-360 is used to measure the optical rotation using 10 cm tube. UV and infrared IR spectra were obtained on Hitachi U-3200 and JASCO A-320 spectrophotometer, repectively. ^{1}H NMR spectra were recorded in C_5D_5N using TMS as internal standard at

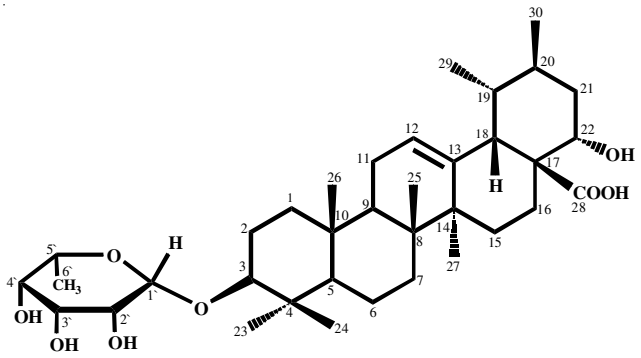

Fig. 1. Structure of the fagonoside A (**1**) isolated from *Fagonia cretica*

400 MHz on Bruker AM-400 nuclear magnetic resonance spectrometers at a digital resolution of 32 K. The same instrument is used to record the ^{13}C-NMR spectra in C_5D_5N at 100 MHz. The 2D-NMR (HMBC, HMQC, NOESY and COSY) spectra were recorded in C_5D_5N on a Bruker AMX 400 NMR spectrometer. Low-resolution EIMS was obtained on a Finnigan MAT 311 with MASSPEC data system. HREIMS and fasta-tomic bombardment (FAB) mass measurements were perfor-med on Jeol JMS HX 110 mass spectrometer. The glycerol and thioglycerol were used as the matrix and cesium iodide (CsI) as internal standard. Sephadex LH_{20} and silica gel Si_{60} (230-400 mesh, E. Merck) were used as adsorbent in column chromatography for the purification of the compound.

The whole plant of *F. cretica* L. was collected from Cholistan desert near Bahawalpur (Pakistan) in March 2009 and identified from Cholistan Institute for Desert Studies

(CIDS), The Islamia University of Bahawalpur, Bahawalpur, Pakistan, where a voucher specimen has been deposited.

Extraction and isolation: The shade dried whole plant (30 kg) was exhaustively extracted with methanol (50 L × 3) at room temperature. The extract was evaporated to yield a residue (1.5 kg) which was divided into *n*-hexane (170 g), ethyl acetate (290 g) and *n*-butanol (210 g) soluble fractions. The *n*-butanol extract was partitioned into four major fractions by the application of Sephadex LH_{20} chromatography by using water-methanol as a solvent system in increasing order of polarity. The fraction **1** (10 g) was again fractionated on Si gel column chromatography with a solvent system of $CHCl_3$-CH_3COH in increasing order of polarity to give five fractions F1-F5. The fraction F2 (2.5 g) was subjected to Si gel PTLC by using solvent system $CHCl_3$:MeOH: H_2O (6.5: 3.5: 0.4 mL H_2O) to afford **1**.

Fagonoside A(1): Amorphous powder, IR (KBr, ν_{max}, cm^{-1}): 1030 (glycoside), 3380-3310 (OH), 3016 (CH), 1627 (C=C), 1405 (*geminal* dimethyl). EIMS: *m/z* (rel. int), 472 M^+ (10), 471 (22), 453 (35), 439 (37), 436 (44), 406 (28), 393 (40), 363 (37), 264 (88), 218 (60), 208 (100), 190 (34), 185 (47), 176 (35), 57 (94). HRFABMS: $(M + H)^+$ *m/z* 619.4240 (calcd for $C_{36}H_{59}O_8$ 619.4209). ^{1}H-NMR: (C_5D_5N, 400 MHz) δ: 5.51 (1H, t, *J* = 6.1Hz, H-12), 5.02 (1H, d, *J* = 4.4 Hz, H-1'), 4.39 (1H, dd, *J* = 4.0, 6.5 Hz, H-22), 4.20 (1H, m, H 2'), 4.01 (1H, m, H-3'), 4.29 (1H, m, H-4'), 3.89 (1H, m, H-5'), 3.66 (1H, dd, *J* = 8.1, 6.2 Hz, H-3), 2.66 (1H, d, *J* = 4.5 Hz, H-18), 2.29 (2H, m, H-11), 2.18 (2H, m, H-2), 1.97 (2H, m, H-16), 1.89 (2H, m, H-15), 1.79 (1H, dd, 3.5, 6.2, H-19), 1.71 (1H, m, H-9), 1.58 (1H, m, H-20) 1.57 (3H, d, *J* = 5.9 Hz, CH_3-6'), 1.59 (2H, m, H-1),1.49 (2H, m, H-6), 1.33 (2H, m, H-7), 1.32 (3H, s, CH_3-24), 1.20 (3H, s, CH_3-23), 1.08 (3H, s, CH_3-27), 1.06 (3H, d, *J* = 6.4 Hz, CH_3-29), 0.96 (3H, d, *J* = 6.8 Hz, CH_3-30), 0.98 (3H, s, CH_3-25), 0.89 (3H, s, CH_3-26) 0.92 (2H, m, H-21). ^{13}C NMR δ: 180.5 (C-28), 140.1 (C-13), 124.1 (C-12), 101.1 (C-1'), 80.1 (C-3), 77.7 C-2'), 76.2 (C-3'), 74.5 (C-4'), 72.3 (C-22), 71.9 (C-5'), 57.8 (C-5), 52.5 (C-18), 50.1 50.1 (C-9), 48.8 (C-17), 43.9 (C-14), 40.9 (C-8), 40.5 (C-4), 39.5 (C-19), 38.2 (C-10), 37.9 (C-1), 37.7 (C-20), 32.9 (C-7), 27.2 (C-21), 30.8 (C-16), 29.1 (C-15), 24.4 (C-11), 24.4 (C-24, 27), 24.1 (C-2), 22.8, (C-23), 19.4 (C-6'), 18.2 (C-6), 18.9 (C-30), 18.5 (C-29), 17.9 (C-25), 16.1 (C-26).

RESULTS AND DISCUSSION

Compound **1** was obtained from the *n*-butanol soluble fraction of *Fagonia cretica*, 35 mg, as an amorphous white solid. The compound gave the positive Liebermann-Burchard and Molish test for a triterpene and glycosides, respectively. The IR spectrum showed the strong bands at 3380-3310 (OH), 3016, 1627 (C=C), 1405 (*geminal* dimethyls) and 1030 cm^{-1} for glycosidic moiety in the molecule.

The EIMS spectrum exhibited the molecular ion peak at *m/z* 472 corresponding to the molecular formula $C_{30}H_{48}O_4$, depicted from high resolution mass spectrum. The positive mode HRFABMS gave $(M + H)^+$ ion at *m/z* 619.4240 (calcd for $C_{36}H_{59}O_8$ 619.4209) indicated the eight degrees of unsaturation in the molecule.

The ^{1}H NMR spectrum of **1** revealed the signals for five tertiary methyls (δ 1.32, 1.20, 1.08, 0.98 and 0.89), the two secondary methyls {(δ 1.06, d, *J* = 6.4 Hz) and (δ 0.96, d, *J* = 6.8 Hz)}, one trisubstituted olefinic proton (δ 5.51, t, *J* = 6.1 Hz) and an oxymethine proton as a double doublet at δ 3.66 (*J* = 8.1 and 6.2 Hz). The proton NMR spectrum also showed the characteristic signal for an anomeric proton as a doublet at δ 5.02 (*J* = 4.4 Hz), indicated the α-configuration of sugar moiety. The three oxymethine protons of sugar residue was appeared at δ 4.29-4.01 as a multiplet and a very characteristic secondary methyl at δ 1.57 as a doublet having *J* = 5.9 Hz. The ^{1}H NMR spectrum further showed an oxymethine proton at δ 4.39 as a double doublet having *J* = 4.0 and 6.5 Hz indicated the hydroxyl (OH) moiety in the molecule in α-configuration.

The ^{13}C NMR spectrum (BB and DEPT) displayed 36 signals, assigned to eight methyl, eight methylene, thirteen methine and seven quarternary carbons. The methyl carbons were observed at δ 24.4, 22.8, 19.4, 18.9, 18.5, 17.9 and 16.1, while the olefiniccarbons were observed at δ 124.1 and 140.1. The oxymethine carbons appeared at δ 80.1-71.9. The anomeric carbon resonated at δ 101.1 indicated the sugar residue in α-configuration. A signal at δ 180.5 indicated the attachment of carboxylic moiety in the molecule.

On acid hydrolysis, **1** yielded a glycoside and an aglycone, white amorphous powder. In HR-EIMS the aglycone moiety showed the molecular ion peak at *m/z* 472.3589 for the formula $C_{30}H_{48}O_4$ (calcd. M^+ peak at *m/z* 472.3554). The EIMS gave characteristic fragment ion peaks at *m/z* 208 and 264 produced due to the retro Diels Alder cleavage of C ring[8,9]. The loss of carboxylic moiety and water from fragment B confirmed their attachment to the ring D or E, while the loss of one water molecules and three methyls from fragment A confirmed their attachment to the ring A or B. This data clearly indicated the ursolic acid with one additional hydroxyl moiety in the molecule[8,9]. The identification of sugar residue was confirmed by hydrolysis studies and can be identified as L-rhamnose through co-TLC with an authentic sample and optical rotation ($[\alpha]_D$ + 8.8). The attachment of sugar moiety with the aglycone portion and the attachment of hydroxyl and other moieties in the molecule were determined by HMBC, HMQC and ^{1}H-^{1}H COSY spectrum. In HMBC spectrum the anomeric proton showed J^3 corelation to the hydroxymethine carbon of the aglycone moiety which further correlated with the two methyl groups indicating that the sugar residue was present at C-3 position. The anomeric proton further showed J^3 correlation to the C-5' which in turn J^2 correlated with a methyl group at C-5' position. The olefinic proton showed J^2 correlation with quaternary carbon and methine carbon at C-14 and C-11, respectively. Hydroxymethine proton observed at δ 4.39 showed J^2 correlation with quaternary carbon at δ 48.8 (C-17) and methylene at δ 27.2 (C-21) while J^3 correlation with the carbonyl carbon at δ 180.5 (C-28) of carboxylic moiety and at C-16 (30.8), C-18 (52.5) and C-20 (37.7) indicating the exact location of OH group at C-22 (72.3) position. The hydroxymethine proton at δ 3.66 showed J^2 correlation with methylene at δ 24.1 and quaternary carbon at δ 40.5 while J^3 correlation with anomeric carbon at δ 101.1 and with the two tertiary methyls at δ 22.8 and 24.4, respectively, indicated the

attachment of glycosidic linkage at C-3 of the aglycone. The stereochemistry of **1** was confirmed by NOESY spectrum. The proton at δ 3.66 (H-3) showed correlations with the protons at δ 1.20 (H-23), 0.68 (H-5) and 1.71 (H-9), indicating it in α-configuration. The hydroxymethine proton at δ 4.39 (H-22) showed correlation with the methyl at δ 0.96 (H-30) and proton at δ 2.66 (H-18) indicating that the hydroxyl group at C-22 (72.3) was present in the α-configuration. On the basis of these evidences fagonoside A, (**1**) was identified as 22-hydroxy-ursane 12-ene-3-O-α-L-rhamnopyranoside.

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