Indian Journal of Chemistry
Vol. 48B, September 2009, pp. 1329-1332

Note

Phytochemical investigation of the whole plant of *Astragalus leucocephalus*

Y Jayaprakash Rao, Ch Ramakrishna Reddy, N Gangadhar & G L David Krupadanam*

Department of Chemistry, Osmania University, Hyderabad 500 007, India

E-mail: davidkrupa@hotmail.com

Received 14 May 2008; accepted (revised) 8 May 2009

Three new lanostene type triterpenoids, astragalone, astragalene, sieversigenin and a known triterpenoid, cyclosieversigenin and a flavone kaempferol, have been isolated from the methanol extract of whole plant. Their structures have been elucidated mainly by spectroscopic methods.

Keywords: *Astragalus leucocephalus*, triterpenoids, astragalone, astragalene, sieversigenin, cyclosieversigenin, flavone, kaempferol

The genus *Astragalus,* which grows in the western Himalayan region, has been under extensive chemical examination since many members of this genus yield a commercially valuable gum which is used in dyeing and printing[1]. The extractives of some members of this genus were reported for their antihypertension and antiulcer activity[2]. Chemical principles isolated from *Astragalus* were mainly flavonoids, coumarins and triterpenoids and their glycosides.

Herein is now reported the isolation of three new triterpenoids and a known triterpenoid, cyclosieversigenin **4**, kaempferol a flavone, and structural elucidation of three new triterpenoids: astragalone **1**, astragalene **2** and sieversigenin **3** (**Figure 1**). The structures of these three triterpenoids were determined on the basis of chemical and spectroscopic evidence. The known compounds a triterpenoid, cyclosieversigenin **4** and flavone kaempferol **5** were identified by comparison of the spectral characteristics with the values[3,4] reported in the literature.

Results and Discussion

Astragalone, 1

Triterpenoid **1**, named astragalone is a new compound and was obtained as colorless crystals. It was analyzed for $C_{30}H_{48}O_4$ (M^+ *m/z* 472) and gave pink color in the Liebermann-Burchard test suggesting the presence of triterpenoid skeleton. The IR data showed the presence of hydroxyls (3600-3200 cm^{-1}), gem dimethyls (1380, 1360 cm^{-1}) and α,β-unsaturated carbonyl group (1659 cm^{-1}). UV-Vis absorption peaks at 220 nm and 300 nm were suggestive of enone chromophore. The ^{1}H NMR spectrum had signals for six quaternary methyl groups (δ 0.72, 0.88, 1.10, 1.12, 1.25 and 1.35), isopropyl group (δ 2.82, m, 1H; δ 1.20, d, *J*=8 Hz, 6H), methylene and methine protons (δ 1.82 to 2.68), signals at δ 3.20, dd (*J*=11.2, 5.8 Hz) δ 4.20, ddd (*J*=9.6, 8.9, 4.5 Hz) are assignable to protons attached to carbons bearing oxygen, enone olefinic protons (δ 7.95 and 6.15, *J*=16 Hz), olefinic proton, (δ 5.35, m, 1H) and methylene protons (attached to olefinic carbon δ 2.50). The ^{13}C NMR spectrum displayed signals due to three oxygen bearing carbons (δ 79.00, 70.00 and 57.00), carbonyl carbon (δ 205.00), four olefinic carbons (δ 146.00, 142.00, 122.00, 115.50) which indicates the presence of α,β-unsaturated carbonyl systems. The other carbon resonances at δ 30.50, 29.10, 27.00, 23.10, 21.70, 18.40, 14.00 (methyl carbons), δ 49.20, 46.00, 42.00, 39.40, 39.00, 38.90, 37.00, 36.00, 33.00, 24.50, 23.00, 17.00, 15.99, were assigned to methine and methylene carbons. On the basis of the above spectral evidence the structure **1** is assigned to the triterpenoid, astragalone.

Astragalene, 2

The new triterpenoid astragalene **2** was obtained as crystalline needles and it was analyzed for $C_{30}H_{48}O_4$ (M^+ *m/z* 472). It gave pink color in the Liebermann-Burchard Test suggesting that it possess triterpenoid skeleton. The IR spectrum showed absorptions due to alcoholic hydroxyls (3600-3300 cm^{-1}), vinylic hydrogens (3020 cm^{-1}), olefinic double bonds (1630 and 1640 cm^{-1}), gem dimethyl groups (1380, 1360 cm^{-1}) and C-O-C system (1035 cm^{-1}). The ^{1}H NMR spectrum showed eight quaternary methyl groups at δ 0.76, 0.88, 0.99, 1.09, 1.15, 1.21, 1.22 and 1.30 and methylene groups attached to double bonds at δ 2.43 and 2.52. The one-proton multiplet at δ 5.49 and the other one-proton multiplet at δ 5.61 are assignable to two olefinic protons suggesting the presence of two trisubstituted double

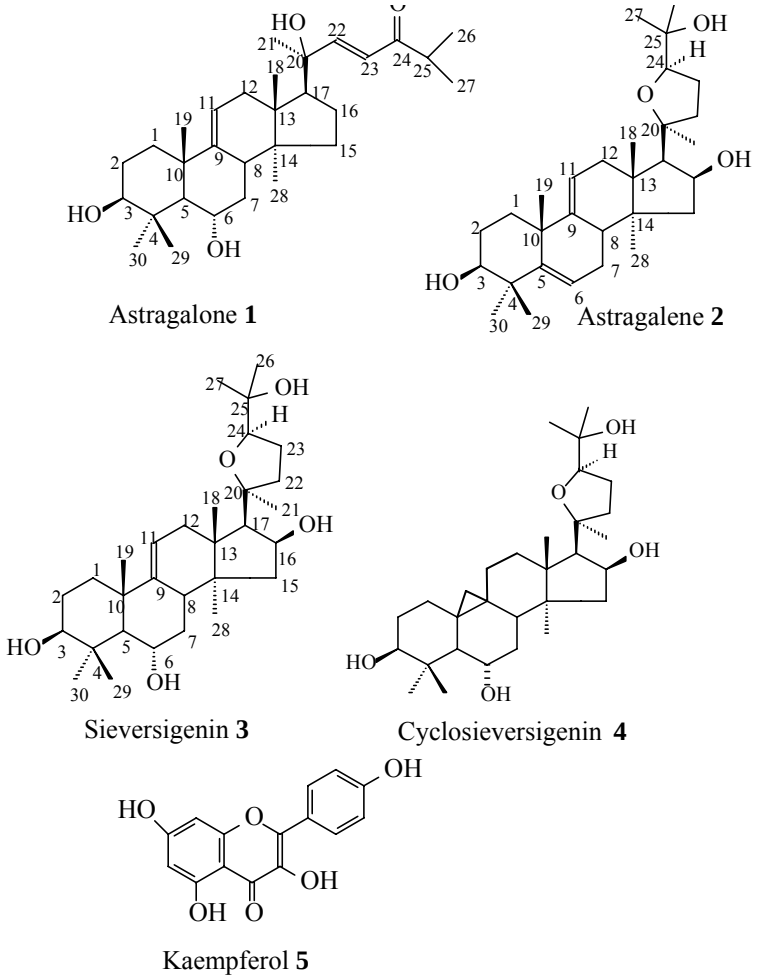

Figure 1

bonds. Signals at δ 3.47,dd (*J*=11, 6 Hz), δ 3.72 and δ 4.67, ddd (*J*=8.5, 8.0, 5.5 Hz) are assignable to three protons attached to carbon bearing oxygen. Methylene and methine protons appeared at δ 1.35 to 2.70. The ^{13}C NMR spectrum showed two olefinic double bonds at δ 141.65, 138.83, 118.64, 114.42. Carbons at δ 141.65 and 114.42 are assignable to the $\Delta^{9(11)}$ lanostene[5] and five oxygen linked carbons at δ 86.13, 80.62, 72.08, 71.09, 70.63, methylene, methine carbons at 56.45, 55.96, 55.85, 44.26, 43.10, 43.00, 40.10, 38.60, 36.30, 36.00, 33.50, 27.48, 25.50, methyl carbons at δ 31.86, 27.83, 27.09, 26.49, 23.27, 19.45, 18.54, 17.51. The mass spectrum showed an intense peak at *m/z* 143 which indicates the presence of a methyl tetrahydrofuranyl isopropyl side chain[5,6]. On the basis of the spectral data, the structure of astragalene, a new triterpenoid, is assigned as **2**.

Sieversigenin, 3

The new triterpenoid sieversigenin **3** was obtained as colorless crystals. It was analyzed for $C_{30}H_{50}O_5$ (M^+ *m/z* 490) and gave pink color in the Liebermann-Burchard test suggesting the presence of triterpenoid skeleton.

The IR spectrum showed peaks due to -OH (3600-3300 cm^{-1}) and C-O-C (1035 cm^{-1}) and gem dimethyls (1380 and 1360 cm^{-1}). The UV-Vis spectrum was transparent. The ^{1}H NMR showed signals due to eight quaternary methyl groups (δ 0.78, 0.88, 1.01, 1.04, 1.10, 1.18, 1.25 and 1.31), protons attached to C-O function at δ 3.20, dd (*J*=11, 5.9 Hz), δ 3.53, ddd (*J*=10, 9.5, 4 Hz), δ 4.64,ddd (*J*=7.8, 7.4, 5 Hz), olefinic proton (δ 5.24), alcoholic -OH protons (δ 3.66, 4.07, 4.51 and 4.76) and methylene and methine protons (δ 1.35-2.70). The ^{13}C NMR showed signals at δ 146.70, 114.20 (olefinic carbons), δ 86.20, 80.80,

77.55, 72.29, 70.50, 68.20 (oxygen linked carbons), 56.50, 55.85, 44.28, 43.30, 43.00, 40.30, 40.10, 38.80, 38.40, 36.80, 36.00, 33.80, 27.58, 25.30 (methylene and methine carbons). The methyl carbons appear at 31.00, 27.00, 26.20, 22.70, 18.70, 18.20, 17.40 and 15.50. The mass spectrum showed an intense peak at *m/z* 143 which indicates the presence of a methyl tetrahydrofuranyl isopropyl side chain. On the basis of the above spectral data the structure for this new lanostene triterpenoid, sieversigenin, was assigned as **3**.

Experimental Section

Melting points are uncorrected, and were determined in Polman make instrument (Model No. mp-96), IR (KBr) were recorded on a Perkin-Elmer 1710 instrument, UV-Vis (EtOH) were recorded on Shimadzu UV-Vis 1601. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 or $CDCl_3$ at 400 MHz and 200 MHz on Varian-Gemini Unity Spectrometer respectively with TMS as internal standard. MS were recorded on VG Micro Mass 7070-H Instrument. Optical rotations were measured on 241 (Perkin-Elmer Research) in chloroform or methanol.

Plant Material: *Astragalus leucocephalus* was procured from M/s United Allied and Chemicals Ltd., Calcutta.

Extraction and Isolation

The whole powdered plant of *Astragalus leucocephalus* (1 kg) was extracted with MeOH in a Soxhlet extractor. The MeOH solution was evaporated under reduced pressure affording a black residue (10 g). It was subjected to column chromatography (silica gel 500 g, 200 mesh, ACME) and the column eluted with benzene followed by benzene:ethyl acetate mixtures to give the compounds **1-5**.

Compound 1: Astragalone: Recrystallised from MeOH; needles, m.p. 151°C. IR (KBr): 3600-3200 (OH), 1380 and 1360 (gem dimethyls), 1659 cm^{-1} (α,β-unsaturated carbonyl); UV-Vis (MeOH) nm: 220 (π-π*), 300 (n-π*) enone chromophore; ^{1}H NMR ($CDCl_3$): δ 0.72, 0.88, 1.10, 1.12, 1.25 and 1.35 (six quaternary methyl groups), 2.50 (2H, d, H-12, methylenic protons attached to olefinic double bonds), 2.82 (1H, m, H-25) and 1.20 (6H, d, *J*=8 Hz, H-26 and 27) (isopropyl group), 1.82 to 2.68 methine and methylene protons 3.20 (1H, dd, *J*=11.2, 5.8 Hz, H-3) and 4.20 (1H, ddd, *J*=9.6, 8.9 4.5 Hz, H-6) (protons linked to carbons bearing oxygen), 5.35 (1H, m, H-11, olefinic protons), 6.15 (1H, *J*=16 Hz) and 7.95 (1H, *J*=16 Hz) for trans protons; ^{13}C NMR ($CDCl_3$+DMSO-d_6): δ 49.20, 46.00, 42.00, 39.40, 39.00, 38.90, 37.00, 36.00, 33.00, 24.50, 23.00, 17.00, 15.99 (methine and methylene carbons), 30.50, 29.10, 27.00, 23.10, 21.70, 18.40, 14.00 (methyl carbons), 79.00, 70.00, 57.00 (sp^3 C-O carbons), 146.00, 142.00, 122.00, 115.50 (two olefinic double bond carbons, 205 (carbonyl carbon); MS [M$^+$ *m/z* 472], $C_{30}H_{48}O_4$, *m/z* (%) 77 (100).

Compound 2: Astragalene: Recrystallised from chloroform; m.p. 122°C $[\alpha]_D^{26}$ +8.48°. IR (KBr): 3600-3300 (OH), 3020 (vinylic hydrogens), 1630 and 1640 (olefinic double bonds), 1380 and 1360 (gem dimethyls), 1035 cm^{-1} (C-O-C); UV-Vis (MeOH) nm: Transparent; ^{1}H NMR ($CDCl_3$): δ 0.76, 0.88, 0.99, 1.09, 1.15, 1.21, 1.22, 1.30 (eight quaternary methyls), 1.35-2.70 (methylene and methine protons) and 4.67 (1H, ddd, *J*=5.5, 8 and 8.5 Hz, H-16), 3.72, 3.47 (1H, dd, *J*=11, 6 Hz, H-3) (protons attached to carbon bearing oxygen), 5.49 (1H, m, olefinic C_{11}-H), 5.61 (1H, m, olefinic); ^{13}C NMR ($CDCl_3$+DMSO-d_6): δ 141.65, 114.42, 138.83, 118.64 (olefinic carbons), 86.13, 80.62, 72.08, 71.09, 70.63 (oxygen linked carbons), 56.45, 55.96, 55.85, 44.26, 43.10, 43.00, 40.10, 38.60, 36.30, 36.00, 33.50, 27.48, 25.50 (methine and methylene carbons), 31.86, 27.83, 27.09, 26.49, 23.27, 19.45, 18.54, 17.51 (methyl carbons); MS [M$^+$ *m/z* 472] $C_{30}H_{48}O_4$, *m/z* (%) 143 (100), 125 (30), 59 (38).

Compound 3: Sieversigenin: Recrystallised from acetone, m.p. 230°C. $[\alpha]_D^{26}$ +11.8°. IR (KBr): 3600-3300 (OH), 1630 (C=C), 1380 and 1360 (gem dimethyls), 1035 cm^{-1} (C-O-C); UV-Vis (MeOH) transparent; ^{1}H NMR (DMSO-d_6 + $CDCl_3$): δ 0.78, 0.88, 1.01, 1.04, 1.10, 1.18, 1.25, 1.31 (quaternary methyls), 1.35-2.70 (methylene and methine protons), 3.20 (1H, dd, *J*=11 and 5.9 Hz, H-3), 3.53 (1H, ddd, *J*=10, 9.5 and 4 Hz, H-6), 4.64 (1H, ddd, *J*=7.8, 7.4 and 5 Hz, H-16) (protons attached to C-O), 3.66, 4.07, 4.51, 4.76 (alcoholic OH), 5.24 (1H, m, olefinic protons); ^{13}C NMR ($CDCl_3$ + DMSO-d_6): δ 146.70, 114.20 (olefinic double bond), 86.20, 80.80, 77.55, 72.29, 70.50, 68.20 (oxygen linked carbons), 31.00, 27.00, 26.20, 22.70, 18.70, 18.20, 17.40, 15.50 (methyl carbons), 56.50, 55.85, 44.28, 43.30, 43.00, 40.30, 40.10, 38.80, 38.40, 36.80, 36.00, 33.80, 27.58, 25.30 (methylene and methine carbons); MS [M$^+$ *m/z* (%) 490] $C_{30}H_{50}O_5$, 143(100), 125(43).

Compound 4: Cyclosieversigenin: Isolated earlier from *Astragalus leucocephalus*[3]. Recrystallised from MeOH. m.p. 239°C. IR(KBr): 3600-3300 (gem

dimethyls); 1033 cm^{-1} (C-O-C); UV-Vis (MeOH): Transparent; ^{1}H NMR (DMSO-d_6-$CDCl_3$): δ 0.30, 0.48 (1H each, d, *J*=4 Hz) cyclopropyl protons, 0.97 (3H, s, CH_3), 1.10, 1.15, 1.20 and 1.30 (quaternary methyls), 1.30 to 2.65 (methine and methylene protons), 1.28 (6H, s, 2×CH_3), 4.67 (1H, ddd, *J*=7.6, 7.3 and 4.8 Hz, H-16), 3.50 (1H, ddd, *J*=10.2, 9.4 and 3.8 Hz, H-6), 3.21 (1H, dd, *J*=11.1, 5.6 Hz, H-3) (proton attached to carbon oxygen function); ^{13}C NMR ($CDCl_3$ + DMSO-d_6): δ 86.16, 76.87, 72.21, 70.48, 67.06 (oxygen linked carbons), 57.41 (C-17), 52.71, 46.33, 45.88, 45.28, 44.19, 41.12, 40.37, 38.70, 34.08, 32.50, 30.31, 28.98, 28.40 (methylene and methine carbons), 31.85 (C-19) 30.31, 28.98, 28.40, 27.77, 28.85, 20.09, 15.30 (quaternary methyls); MS [M^+ *m/z* (%) 490] $C_{30}H_{50}O_5$, 143 (100).

Compound 5: Kaempferol: Isolated earlier from *Astragalus leucocephalus*[4]. Recrystallised from aq.EtOH: m.p. 276°C. IR (KBr): 1654 cm^{-1} (C=O); UV-Vis (MeOH): 267.5, 370; ^{1}H NMR (DMSO-d_6): δ 6.2 (1H, d, J=3 Hz, H-6), 6.39 (1H, d, *J*=3 Hz, H-8), 6.89 (2H, d, *J*=10 Hz, H-3′,H-5′), 8.1 (2H, d, *J*=10 Hz, H-2′, H-6′), 12.2 (s, 1H, chelated hydroxyl protons C-5-OH); MS [M^+ *m/z* (%) 286] $C_{15}H_{10}O_6$, 258 (22); 121 (65).

References

1 Hooker J D, *The Flora of British India*, Vol 2, (Reeve and Co, London), p 128, **1879**.

2 Bernaulov O D, Manichena D A, Yasinov R K & Yakovler G P, *Chem Abstr*, 1022, **1985**, 197523b.

3 Svechnikova A N, Umerova R U, Gorovits M B, Seitanidi K L, Rashkesya V, Yagudeev M R & Abubakirov N K, *Chem Abstr*, 95, **1981**, 81268.

4 Mahesh V K, Sharma R, Singh R S & Upadhyaya S K, *J Nat Prod*, 47, **1984**, 733.

5 Blunt J W & Munro M H G, *Org Mag Resonance*, 13, **1980**, 26.

6 Warnoff E W & Halls C M M, *Can J Chem*, 43, **1965**, 3311.