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Research Article

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Flavonoids from *Astragalus propinquus*

Venkata Sai Prakash Chaturvedula* and Indra Prakash

Organic Chemistry Department, The Coca-Cola Company, Global Research and Development, One Coca-Cola Plaza, Atlanta, GA 30313, USA

ABSTRACT

Systematic phytochemical studies of the dichloromethane (CH_2Cl_2) fraction of the aqueous extract of Astragalus propinquus on a C-18 column using a Biotage Flash Chromatography system yielded three flavonoids namely 5-hydroxy-4´, 6, 7 trimethoxyflavone (salvigenin), 4´, 5, 7-trihydroxyflavone (apigenin), and 3´,4´, 5, 7-tetrhydroxyflavone (luteolin). The structures of the isolated compounds salvigenin, apigenin, and luteolin were characterized on the basis of extensive spectral studies and literature search. Further, the complete ^{1}H and ^{13}C NMR spectral assignments of the three isolated compounds are reported on the basis of 1D (^{1}H and ^{13}C) and 2D (COSY, HSQC, and HMBC) NMR spectral data.

Keywords: *Astragalus propinquus*, Fabaceae, Flavonoids, NMR, MS, Structure elucidation

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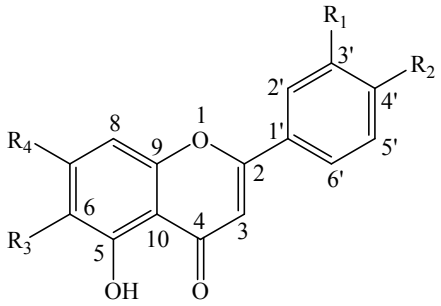
INTRODUCTION

Astragalus root is a staple of Traditional Chinese Medicine (TCM), where it is also known as *Huang Qi*. It is considered a sweet, warming herb with many medicinal propreties. Traditionally it is used in the treatment of fatigue, decreased appetite, general debility (particularly in the elderly), susceptibility to viral infections, non-healing wounds, fever, sweating, uterine prolapse, uterine bleeding, edema (nephritis), numbness, muscle pain, diabetes mellitus, and uterine, ovarian or colon cancer [1]. Astragalus is a component of numerous traditional medicine tonics and is often combined with ginseng, angelica, licorice and other herbs. The gummy sap from astragalus (tragacanth) has been used since ancient times as a thickener and emulsifier. It continues to be used today as a thickening agent for ice cream [2].

In continuation of our study on the isolation of natural sweeteners from the commercial extracts of various plants obtained from across the world, we have isolated several diterpene glycosides from *Stevia rebaudiana* and *Rubus suavissimus* [3-9], triterpene and phenolic glycosides from *Siraitia grosvenorii* [10-11] whose structures were characterized based on the extensive NMR and Mass spectroscopic studies as well as chemical studies. In this paper we are describing the isolation and purification of three flavonoids namely salvigenin (**1**), apigenin (**2**), and luteolin (**3**), from the commercial aqueous extract of *Astragalus propinquus* that were characterized on the basis of COSY, HSQC, HMBC and NOESY spectral data.



1; $R_1 = H$; $R_2 = R_3 = R_4 = OCH_3$

2; $R_1 = R_3 = H$; $R_2 = R_4 = OH$

3; $R_3 = H$; $R_1 = R_2 = R_4 = OH$

Figure 1: Structures of Salvigenin (**1**), Apigenin (**2**), and Luteolin (**3**)

EXPERIMENTAL SECTION

General Methods

NMR spectra were acquired on a Varian Unity Plus 600 MHz instrument using standard pulse sequences at ambient temperature. Chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. Mass spectral (MS) data was generated with a Thermo LTQ Orbitrap Discovery mass spectrometer in the positive ion mode electrospray. Instrument was mass calibrated with a mixture of Ultramark 1621, MRFA [a peptide], and caffeine immediately prior to accurate mass measurements of the samples. Samples were diluted with water:acetonitrile:methanol (1:2:2) and prepared a stock solution of 50 ul concentration for each sample. Each sample (25 ul) was introduced via infusion using the on-board syringe pump at a flow injection rate of 120 ul/min. Low pressure chromatography was performed on a Biotage Flash system using a C-18 cartridge (40+ M, 35-70 μm). TLC was performed on Baker Si-C_{18}F plates with mobile phase H_2O-MeOH (35:65). Identification of the spots on the TLC plate was carried out by spraying 10% H_2SO_4 in EtOH and heating the plate at about 80^o C.

Materials

The commercial extract of *Astragalus propinquus* was supplied by Jia Herb, Parsippany, NJ 07054. A voucher specimen is deposited at The Coca Cola Company, No. VSPC-3166-169.

Isolation and Purification

The aqueous extract of the roots of *A. propinquus* (20 g) was suspended in 200 ml water and extracted successively with *n*-hexane (3 x 100 ml), CH_2Cl_2 (3 x 100 ml) and *n*-BuOH (2 x 100 ml). The CH_2Cl_2 layer was concentrated under vacuum furnished a residue (2.5 g) which was purified on a Biotage flash chromatography system using C-18 (100 g) column (solvent system: gradient from 80-20 MeOH-water to 100% MeOH at 60 ml/min. detection at UV 210 nm) for 40 min. Fractions 48-52 and 55-60 were combined to get residues 0.28 g and 0.34 g respectively, which on repeated purification using the gradient 80-100% MeOH-water at 20 ml/min for 50 min resulted salvigenin (**1**, 65 mg), apigenin (**2**, 45 mg), and luteolin (**3**, 30 mg), respectively.

*Salvigenin (**1**):* UV (MeOH) : 272 and 332 nm; ^{1}H NMR (600 MHz, C_5D_5N): δ 12.78 (s, OH), 7.83 (d, 2H, *J* = 8.8 Hz, H-2′,6′), 6.97 (d, 2H, *J* = 8.8 Hz, H-3′,5′), 6.54 (s, 1H, H-3), 6.51 (s, 1H, H-8), 3.95 (s, -O$\underline{CH_3}$), 3.91 (s, -O$\underline{CH_3}$), 3.88 (s, -O$\underline{CH_3}$); ^{13}C NMR (125 Hz, C_5D_5N): δ 182.8 (C-4), 164.2 (C-2), 162.8 (C-4′), 158.6 (C-8), 153.6 (C-9), 153.3 (C-6), 132.2 (C-7), 128.4 (C-2′,6′), 123.4 (C-1′), 114.8 (C-3′,5′), 106.6 (C-5), 104.3 (C-3), 90.8 (C-10), 60.7 (-O$\underline{CH_3}$), 56.0 (-O$\underline{CH_3}$), 55.2 (-O$\underline{CH_3}$); EI-MS *m/z*: $[M+H]^+$ 329.

*Apigenin (**2**):* UV (MeOH) : 268, 337 nm; ^{1}H NMR (600 MHz, C_5D_5N): δ 7.81 (2H, *d*, *J* = 8.8 Hz, H-2′ and H-6′), 6.95 (2H, *d*, *J* = 8.8 Hz, H-3′ and H-5′), 6.88 (1H, *d*, *J* = 2.1 Hz, H-6), 6.68 (1H, *d*, *J* = 2.1 Hz, H-8), 6.62 (1H, *s*, H-3); ^{13}C NMR (125 Hz, C_5D_5N): δ 180.8 (C-4), 164.6 (C-5), 164.2 (C-2), 162.6 (C-4′), 160.7 (C-9), 160.0 (C-7),

129.3 (C-2´ and C-6´), 123.3 (C-1´), 117.0 (C-3´ and C-5´), 109.6 (C-10), 106.5 (C-3), 104.8 (C-6), 98.9 (C-8); EI-MS *m/z*: $[M+H]^+$ 271.

*Luteolin (**3**)*: UV (MeOH) : 268 and 344 nm; ^{1}H NMR (600 MHz, C_5D_5N): δ 7.56 (1H, dd, *J* = 9, 2Hz, H-6'), 7.36 (1H, d, *J* = 2Hz, H-2'), 6.85 (1H, d, *J* = 9 Hz, H-5'), 6.75 (1H, s, H- 3), 6.46 (1H, d, *J* = 2 Hz, H-8) , 6.28 (1H, d, *J* = 2 Hz, H-6); ^{13}C NMR (125 Hz, C_5D_5N): δ 181.8 (C-4), 164.3 (C-7), 164.0 (C-2), 162.1 (C-9), 157.6 (C-5), 149.7 (C-4'), 146.0 (C-3'), 120.8 (C-6'), 119.0 (C-1'), 116.8 (C-5'), 113.2 (C-2'), 103.8 (C-10), 99.2 (C-6), 94.7 (C-8); EI-MS *m/z*: $[M+H]^+$ 287.

RESULTS AND DISCUSSION

Compound **1** was obtained as an amorphous powder and its molecular formula was established as $C_{18}H_{16}O_6$ from its MS spectral data that showed $[M+H]^+$ ion at *m/z* 329; this was supported by the ^{13}C NMR spectral data. The UV spectrum of **1** showed absorption maxima at 272, and 332 nm suggested a flavonoid structure [12-14]. The ^{1}H NMR spectrum proved it to be a flavone with two singlets at δ 6.51, and 6.54 and a pair of doublets at δ 6.97 and 7.83 assignable to two protons each in the aromatic region. The ^{1}H NMR spectrum also showed the presence of three singlets at δ 3.88, 3.91, and 3.95 corresponding to three methoxy groups and another singlet at δ 12.78, of a hydroxyl group. The ^{13}C NMR spectra showed the presence of twelve aromatic carbons; comprises of eight quaternary carbons and four methine carbons, and an unsaturated carbonyl carbon. The above ^{1}H and ^{13}C NMR data confirming the structure to be a trimethoxy substituted flavone having an additional phenolic hydroxyl group. The placement of the three methoxyl groups were identified at C-4´, C-6, and C-7 positions, and that of the phenolic hydroxyl at C-5 position on the basis of key COSY and HMBC correlations as shown in Figure 2. The ^{1}H and ^{13}C NMR values for all the carbons were assigned on the basis of HSQC and HMBC correlations Thus, based on the above spectral data, structure of **1** was assigned as 5- hydroxy-4´, 6, 7 trimethoxyflavone (salvigenin) consistent to the reported literature values [15].

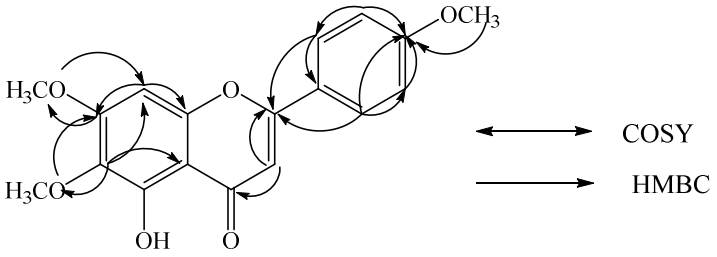


Figure 2: Key COSY and HMBC correlations of **1**

Compound **2** was also obtained as an yellow powder and its molecular formula was established as $C_{15}H_{10}O_5$ from its MS spectral data that showed $[M+H]^+$ ion at *m/z* 271. The molecular formula of **2** was further supported by its ^{13}C NMR spectral data. The UV spectrum of **2** also showed absorption maxima at 268, and 337 nm suggested a flavonoid structure similar to **1** and as reported in the literature [11-13]. The ^{1}H NMR spectrum of **2** showed the presence of two meta coupled aromatic doublets at δ 6.68 and 6.88 corresponds to H-6 and H-8 protons, two doublet of doublets at δ 6.95 and 7.81 for H-3'/H-5' and H-2'/H-6' protons of ring B, and a singlet at δ 6.62 corresponding to H-3 proton; characteristic for a 5,7,4'-trisubstituted flavone. The ^{13}C NMR spectra showed the presence of twelve aromatic carbons; seven quaternary carbons, five methine carbons, and an unsaturated carbonyl carbon. The ^{1}H and ^{13}C NMR values for all the carbons were assigned on the basis of HSQC and HMBC correlations. A search in literature suggested the spectral data of **2** was consistent to 4´, 5, 7-trihydroxyflavone, also known as apigenin [16-17]. The structure was further supported by the COSY and HMBC correlations as shown in Figure 3.

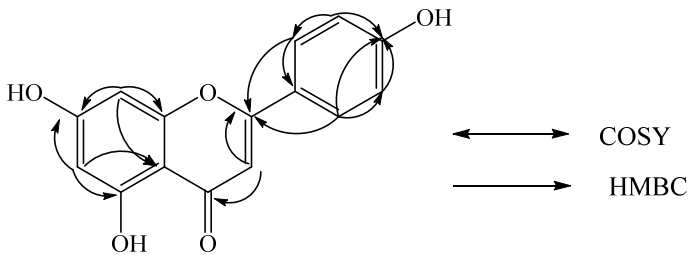


Figure 3: Key COSY and HMBC correlations of **2**

Compound **3** was also obtained as an yellow powder and its molecular formula was established as $C_{15}H_{10}O_6$ from its MS spectral data that showed $[M+H]^+$ ion at *m/z* 287 which further supported by its ^{13}C NMR spectral data. The UV spectrum of **3** also showed absorption maxima at 268, and 344 nm suggested a flavonoid structure similar to **1** and **2** as well as the reported values in the literature [11-13]. The ^1H NMR spectrum of **2** showed the presence of three meta coupled aromatic doublets at δ 6.28, 6.46 and 7.36, one ortho coupled aromatic doublet at δ 6.85, one doublet of doublets at δ 7.56 corresponding to a ortho and meta coupled aromatic proton, and a singlet at 6.75; characteristic for a 5,7,3',4'-tetrasubstituted flavone. The ^{13}C NMR spectra showed the presence of twelve aromatic carbons; eight quaternary carbons, six methine carbons, and an unsaturated carbonyl carbon. The ^1H and ^{13}C NMR values for all the carbons were assigned on the basis of HSQC and HMBC correlations. A search in literature suggested the spectral data was consistent to luteolin (3´,4´, 5, 7-tetrhydroxyflavone) [17]. The structure was further supported by the COSY and HMBC correlations as shown in Figure 4.

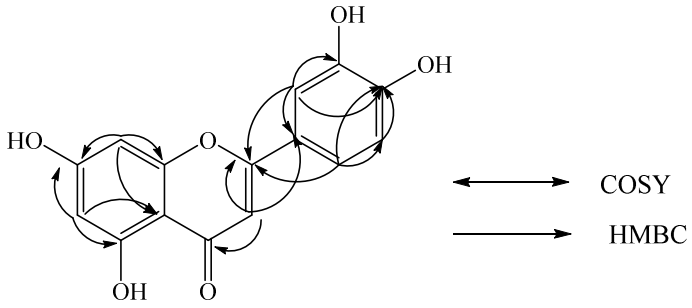


Figure 4: Key COSY and HMBC correlations of **3**

CONCLUSION

Three known flavones were isolated from the commercial aqueous extract of *Astragalus propinquus*. The structures of the isolated compounds were identified as salvigenin (**1**), apigenin (**2**), and luteolin (**3**), on the basis of spectroscopic and chemical studies as well as by comparing their physical and spectral properties reported in the literature.

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