ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 11:1 (2016) 37-42

records of natural products

Isolation and Characterization of New Constituents from *Tricholepis eburnea*

Shagufta Rasool[1,2], Noureen Khan[1,3], Rashad Mehmood[4*] and Farzana Shaheen[1]

[1]*H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan*

[2]*Department of Chemistry, Sarhad University of Science and Information Technology, Peshawar, Pakistan*

[3]*Department of Chemistry, Sardar Bahadur Khan Women's University, Quetta, Pakistan,*

[4]*Department of Chemistry, Hazara University Mansehra-21120, Pakistan*

(Received February 14, 2016; Revised August 16, 2016; August 17, 2016)

Abstract: Through current phytochemical investigation on dichloromethane (DCM) fraction of 80% methanolic extract of *Tricholepis eburnea*, seven chemical constituents were isolated and their structures were elucidated by spectroscopic analyses. Two of them were identified as new flavonoids as trichonoide A (**1**) and trichonoide B (**2**), while the other five known constituents *β*-sitosterol (**3**), 3-*O*-acetyl-*β*-stigmastanol (**4**), 3*β*,17*β*,20-trihydroxy-l-oxo-(20*R*, 22*R)*-witha-5,14,24-trienolide (**5**), pectolinaringenin (**6**), and dillenetin (**7**) were isolated for the first time from this species.

Keywords: *Tricholepis eburnea*; asteraceae; structural elucidation; trichonoides A and B.

1. Introduction

Tricholepis is the genus of Asteraceae plant family and comprises 18 herbs distributed in East and Central Asia [1]. In Pakistan, *Tricholepis* genus is represented by twelve species. Various *Tricholepis* species have been traditional used for the remedy of various human ailments such as nerve tonic [2], seminal debility, urinary infections and cough [3], antipyretic and skin diseases [4-5], regularizing the malfunction of pancreas, malaria, fever, skin grains, aphrodisiac, stomach pain, blood purification and dysentery [6]. Sifting of the literature shows that terpenoids [7], flavonoids [8] and steroid [9-10] have so far been reported from this genus. The chemotaxonomic and ethnopharmacological importance of the genus *Tricholepis* prompted us to carry out phytochemical investigations one of its species *Tricholepis eburnea*. Through current investigation, we herein report the isolation and characterization of two new flavonoids trichonoides A (**1**) and B (**2**) along with five known constituents *β*-sitosterol (**3**), 3-*O*-acetyl-*β*-stigmastanol (**4**), 3*β*,17*β*,20-trihydroxy-l-oxo-(20*R*, 22*R)*-witha-5,14,24-trienolide (**5**), pectolinaringenin (**6**), and dillenetin (**7**) for the first time from this species.

*Corresponding author: E-Mail: rashadhej@gmail.com; Phone:+92-334-3451908 *Fax:* +92-997-414111.

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 09/21/2016 EISSN:1307-6167

2. Materials and Methods

2.1. General Experimental Procedures

The pre-coated silica gel F_{254} plates (*E. Merck, Darmstadt, Germany*); detection at 254 and 366 nm, or by spraying ceric sulfate in 10% H_2SO_4 (heating). Column chromatography (CC): silica gel (SiO_2; 70-230, 230-400 mesh; *E. Merck Darmstadt, Germany*). UV Spectra: *Hitachi-UV-3200* spectrometer. IR Spectra: *Jasco-320-A* spectrometer; KBr pellets, in cm^{-1}. ^1H, ^{13}C-NMR and 2D-NMR Spectra: *Bruker-AMX-300* and *Bruker-AM-500* spectrometer, chemical shifts in δ. ESI-MS: *Applied Biosystem-QSTAR XL MS / MS* spectrometer, ions in *m/z* (%).

2.2. Plant Material

The aerial parts of *Tricholepis eburnea* Tech. f. were collected from Ziarat valley, Balochistan (Pakistan) in March 2010 and identified by Prof. Dr. Rasool Bakhsh Tareen, Plant Taxonomist, Department of Botany, University of Quetta, where a voucher specimen (TE-RBT-05) has been deposited.

2.3. Extraction and Isolation

The shade dried plants material of *T. eburnea* was ground and extracted with 80 % MeOH / H_2O for ten days (3 times), and the extract was evaporated under vacuum at room temperature to a residue (500 g). The residue was suspended into distilled water and fractionated with hexane (23 g), dichloromethane (DCM) (92 g), ethyl acetate (AcOEt) (34 g), *n*-butanol (48 g) and water (300 g). The DCM soluble fraction (92g) was subjected to flash silica gel CC and eluted with hexane-DCM-MeOH in order of increasing polarity to collect several subfractions (*Fr.1 – Fr.20*). *Fr.I* (2 g) was rechromatographed over flash silica and eluted isocratically with hexane to afford *Fr.1.1 – Fr.1.3*. *Fr.1.2* was a semipure and subjected to silica gel CC hexane/DCM (9.5:0.5) to obtain **4** (27 mg). *Fr.2* (1.1 g) was subjected to flash silica CC eluting with hexane/acetone (9.5:0.5) to obtain **3** (10 mg). *Fr.*7 (0.90 g) was rechromatographed over silica gel and eluted with DCM/MeOH (9.8:0.2) to obtain **5** (30 mg).

The ethyl acetate fraction (34g) was subjected to flash silica gel CC and eluted with hexane-AcOEt-MeOH in increasing order of polarity to collect twelve subfractions (*Fr.1 – Fr.12*). *Fr.5* (1.2 g) was subjected to silica gel CC eluting with hexane/AcOEt to afford *Fr.5.1 – Fr.5.6*. *Fr.5.5* was rechromatographed over silica gel and eluted with AcOEt/hexane (9.0:1.0) to a semipure compound, which was triturated with DCM to afford pure compound **6** (25 mg). *Fr.6* (1.3 g) was subjected to silica gel CC and eluted with hexane/AcOEt to afford *Fr.6.1 – Fr.6.4*. *Fr.6.3* was further subjected to silica gel chromatography AcOEt/hexane (9.5:0.5) to yield **7** (12 mg). *Fr.6.4* was subjected to silica gel chromatography and eluted with DCM/MeOH (9.8:0.2) to obtain **1** (15 mg). *Fr.7* (2.1 g) was subjected to silica gel CC and eluted with DCM/MeOH. The elution with DCM/MeOH (9.5:0.5) provided the yellow compound **2** (20 mg).

3. Results and Discussion

The 80% methanolic extract of aerial parts of *T. eburnea* was suspended into water and successively fractionated into *n*-hexane, DCM, ethyl acetate, *n*-butanol and aqueous fractions. A series of column chromatographic technique was applied on DCM and ethyl acetate fractions on silica gel to obtain compounds **1**-**7** (Figure 1), respectively.

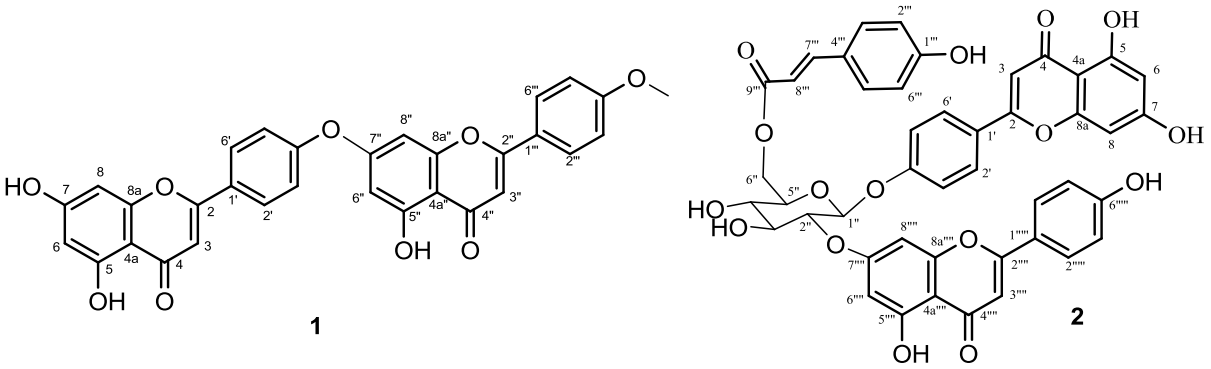

Figure 1. Structures of trichonoides A (**1**) and B (**2**).

3.1. Structure elucidation

Compound **1** was isolated as a yellow solid. The HR ESI-MS (+ve) showed an $[M + H]^+$ ion peak at *m/z* 537.1180 (calcd. 537.1186) corresponding to the molecular formula $C_{31}H_{21}O_9$. IR spectrum exhibited absorption bands at 3389 (OH), 1676 (conjugated carbonyl), 1648 and 1481 cm^{-1} (aromatic moiety). The UV spectrum showed the characteristic flavonoid bands at 268 and 338 nm [11]. On addition of $AlCl_3$/HCl bathochromic shift of 32 nm of the band at 268 nm was observed, suggesting the presence of chelated hydroxyl group. The ^{1}H- and ^{13}C-NMR spectral data (Table 1) revealed that the compound **1** contain two flavonoid moieties. The ^{1}H-NMR displayed ten resonances of aromatic protons in the downfield region. Out of four *meta* coupled doublets, two doublets at δ 6.18 (overlapped) and 6.46 (J = 1.8 Hz) were assigned to H-6 and H-8 of ring A, respectively. The second set of *meta*-coupled doublets at δ 6.18 (overlapped) and 6.49 (J = 1.8 Hz) belonged to H-6″ and H-8″of ring A′, respectively. Two ortho-coupled doublets at δ 7.92 (J = 9.0 Hz) and 6.93 (overlapped), were assigned to the H-2′/6′and H-3′/5′ protons of symmetrical ring B, respectively. The symmetrical nature of the ring B′ of second flavonoid moiety was revealed by the presence of two ortho-coupled doublets, each one of two protons integration at δ 7.56 (J = 8.4 Hz) and 6.90 (overlapped). A singlet at δ 3.95 was assigned to the methoxy group at C-4‴of ring B′, which was further supported by HMBC correlation of OCH_3 (δ 3.95) with C-4‴ (δ 161.3) (Figure 2). Two singlets at δ 6.76 and 6.88 were assigned to H-3 and H-3″ respectively. The downfield signal of C-7″ (δ 174.6) indicating this quaternary center is attached to an electron withdrawing phenoxide group of the flavonoid moiety. Compound **1** exhibited resemblance to the reported compound 5,7,4',5"-tetrahydroxy-7"- methoxy-[3-O-4"'] biflavone [12]. In the reported compound the second flavonoid attached through ether linkage at C-3 position of ring B of the first flavonoid group and methoxy group is attached to the C-7″ of the second flavonoid moiety. Compound **1** is found to be new due to the presence of an H-3 in the first flavonoid moiety the second flavonoid group is attached through ether linkage between C-4′ and C-7″ therefore the C-7″ resonated at downfield region (δ 174.6) than its normal value (Figure 1).

Compound **2** was also isolated as a yellow solid. The HR-ESIMS showed an $[M + H]^+$ ion peak at *m/z* 831.1920 (calcd. 831.1925) corresponded to the molecular formula $C_{45}H_{35}O_{16}$. IR spectrum was similar to **1** with an additional band at 1755 cm^{-1} (ester moiety). The UV spectrum was similar to **1**. The ^{1}H-NMR spectrum indicated the presence of two flavonoid moieties, one coumaroyl and a sugar group. In the ^{1}H-NMR, two *meta*-coupled doublets at δ 6.18 (J = 1.8 Hz) and 6.46 (overlapped), were assigned to H-8 and H-6 of ring A of the flavonoid moiety, respectively. A singlet at δ 6.81 was assigned to H-3 of ring C. Two *ortho*-coupled doublets at δ 7.94 (d, overlapped) and 6.92 (d, overlapped) corresponded to H-2′/6′ and H-3′/5′ of ring B, respectively. This data indicated the presence of a flavonoid group. The anomeric carbon proton, H-1″ (δ 5.16) of sugar moiety (d, J = 7.3 Hz), revealed its *β*-configuration due to large coupling constant (J = 7.3 Hz) and showed HMBC correlation with the C-4′ (δ 162.6) of ring B which confirmed the attachment of one flavonoid moiety with the *β*-sugar (Figure 1). The sugar unit was confirmed as *β*-D-glucose by sign of its optical rotation ($[\alpha]^D$ +52.7) after acid hydrolysis. In the ^{1}H-NMR the methylene protons (H-6″) of sugar were shifted downfield (δ 4.18, 4.46) which indicated its connectivity to electron withdrawing ester group of

coumaroyl moiety (δ 166.4, C-9‴), which further confirmed by its ^{3}J HMBC correlation with C-9‴ (δ 166.4). This data was a clear indication of the linkage of coumaroyl group with the sugar group through *O*-linkage at position 6". The olefinic protons of coumaroyl group resonated at δ 6.34 (d, J = 15.9 Hz, H-8‴) and δ 7.50 (d, J = 15.9 Hz, H-7‴). The presence of second flavonoid moiety was observed by *meta*-coupled protons of ring A′ at δ 6.47 (d, overlapped H-6′′′) and 6.90 (d, J = 2.1 Hz, H-8′′′). H-3′′′ of ring C′ appeared at δ 6.76 as a singlet.

Table 1. ^{1}H NMR data for compounds **1-2** (at 300 MHz in DMSO, δ in ppm, J in Hz) and ^{13}C-NMR NMR data for compounds **1-2** (at 75 MHz in $CDCl_3$ (**1**), 150 MHz in DMSO (**2**), δ in ppm).

C	Compound **1**		Compound **2**	
	δ_C	δ_H	δ_C	δ_H
2	163.7 (C)	-	161.4 (C)	-
3	103.1 (CH)	6.76 (*s*)	103.0 (CH)	6.81 (*s*)
4	181.7 (C)	-	181.9 (C)	-
4a	103.6 (C)	-	105.3 (C)	-
5	162.2 (C)	-	159.7 (C)	-
6	98.1 (CH)	6.18 (*d*, overlapped)	98.8 (CH)	6.18 (*d*, J = 1.8)
7	164.1 (C)	-	164.2 (C)	-
8	94.0 (CH)	6.46 (*d*, J = 1.8)	93.9 (CH)	6.46 (*d*, overlapped)
8a	157.2 (C)	-	157.3 (C)	-
1′	121.1 (C)	-	121.2 (C)	-
2′/6′	128.4 (CH)	7.92 (*d*, J = 9)	128.5 (CH)	7.94 (*d*, overlapped)
3′/5′	115.7 (CH)	6.93 (*d*, overlapped)	115.8 (CH)	6.92 (*d*, overlapped)
4′	161.1 (C)	-	162.6 (C)	-
1″	-	-	99.6 (CH)	5.16 (*d*, J = 7.3)
2″	163.6 (C)	-	76.0 (CH)	3.20 (m)
3″	104.2 (CH)	6.88 (*s*)	72.9 (CH)	3.53 (m)
4″	181.6 (C)	-	69.9 (CH)	3.15 (m)
4a″	112.1 (C)	-	-	-
5″	160.8 (C)	-	76.2 (CH)	3.87 (m)
6″	98.7 (CH)	6.18 (*d*, overlapped)	63.4 (CH_2)	4.18 (m), 4.46 (m)
7″	174.6 (C)	-	-	-
8″	93.9 (CH)	6.49 (*d*, J = 1.8)	-	-
8a″	152.8 (C)	-	-	-
1‴	124.4 (C)	-	161.1 (C)	-
2‴/6‴	127.3 (CH)	7.56 (*d*, J = 8.4)	130.0 (CH)	6.67 (*d*, J = 8.4)
3‴/5‴	115.9 (CH)	6.90 (*d*, overlapped)	115.8 (CH)	7.36 (*d*, J = 8.4)
4‴	161.3 (C)	-	124.8 (C)	-
OCH_3 (4‴)	55.9 (CH_3)	3.95 (*s*)	-	-
7‴	-	-	144.9 (CH)	7.50 (*d*, J = 15.9)
8‴	-	-	113.7 (CH)	6.34 (*d*, J = 15.9)
9‴	-	-	166.4 (C)	-
2′′′′	-	-	163.7 (C)	-
3′′′′	-	-	102.8 (CH)	6.76 (*s*)
4′′′′	-	-	181.7 (C)	-
4a′′′′	-	-	103.6 (C)	-
5′′′′	-	-	161.2 (C)	-
6′′′′	-	-	99.4 (CH)	6.47 (*d*, overlapped)
7′′′′	-	-	168.1 (C)	-
8′′′′	-	-	94.6 (CH)	6.90 (*d*, J = 2.1)
8a′′′′	-	-	156.9 (C)	-
1′′′′′	-	-	120.9 (C)	-
2′′′′′/6′′′′′	-	-	128.5 (CH)	7.92 (*d*, overlapped)
3′′′′′/5′′′′′	-	-	115.7 (CH)	6.89 (*d*, overlapped)
4′′′′′	-	-	161.3 (C)	-

The H-2″ (δ 3.20) of sugar unit showed HMBC with the C-7′′′′ (δ 168.1). This confirmed the attachment of the second flavonoid group with the sugar moiety through *O*-linkage at C-2″. All the spectral data exhibited a close resemblance with that of reported compound, marrubinoside C [13]

which contain two coumaroyl moieties linked to the *β*-pyranose sugar while in compound **2**; one coumaroyl group was found to be replaced by a flavonoid moiety. On the basis of all the spectral data, the structure of the compound **2** was established as ((2*R*,3*S*,4*R*,5*R*,6*R*)-6-[4-(5,7-dihydroxy-4-oxo-4*H*-chromen-2-yl)phenoxy]-4,5-dihydroxy-3-{[5 hydroxy-2-(4-hydroxyphenyl)-4-oxo-4*H*-chromen-7-yl]oxy}tetrahydro-2*H*-pyran-2-yl)methyl (*E*)-3-(4-hydroxyphenyl)-2-propenoate (Trichonoide B).

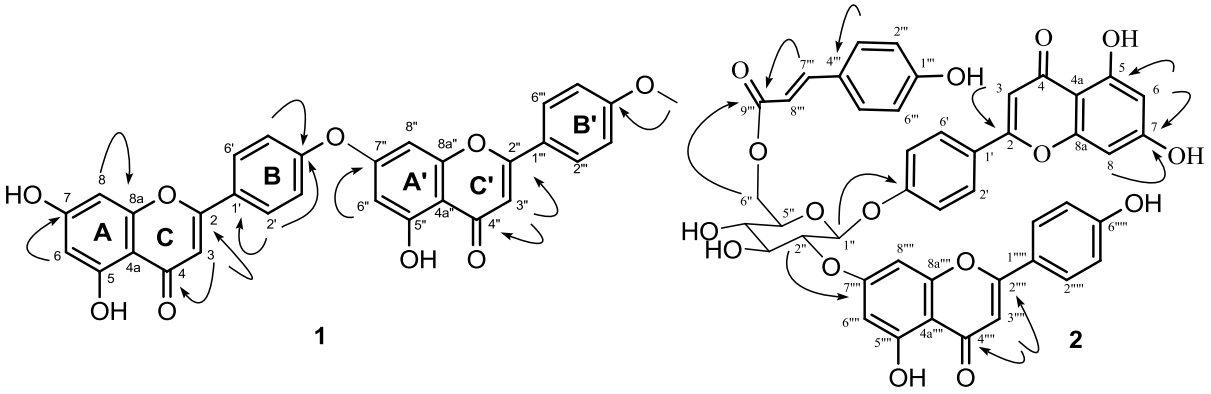

Figure 2. Important HMBC correlations of trichonoides A (**1**) and B (**2**).

The known isolates were confirmed as *β*-sitosterol (**3**) [14], 3-*O*-acetyl-*β*-stigmastanol (**4**) [15], 3*β*,17*β*,20-trihydroxy-l-oxo-(20*R*, 22*R*)-witha-5,14,24-trienolide (**5**) [16], pectolinaringenin (**6**) [17], dillenetin (**7**) [18] by the comparison of reported spectral and physical data.

Acknowledgments

This work was supported by a grant (L/ICA/ICB/157800/10) from the Organization for the Prohibition of Chemical Weapons (OPCW), the Netherlands.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

References

[1] K. Bremer (1994). Asteraceae cladistics and classifications. Portland OR: Timber Press.
[2] K.P. Chittam and S.L. Deore (2012). Pharmacognosy of *Tricholepis glaberrima*: A review, *Int. J. Pharma. World Res.* **3**, 1-8.
[3] C.P. Khare (2007). Indian medicinal plants, An illustrated dictionary, Springer, Berlin, Heidelberg, New York, p 671.
[4] R.N. Chopra, S.L. Nayar and I.C. Chopra (1956). In Glossary of Indian Medicinal Plants, Vol. 1. New Delhi: Council of Scientific and Industrial Research.
[5] K.M. Nadkarni (1976). Indian materia medica, Bombay 2: Popular Prakashan Publications, Ltd.
[6] M.A. Khan, M. Ahmad, M. Zafar, S. Sultana, S.K. Marwat, S. Shaheen, M.K. Leghari, G. Jan, F. Ahmad and A. Nazir (2011). Medico-botanical and chemical standardization of pharmaceutically important plant of *Tricholepis chaerolepis* (Bioss) Rech. f., *J. Med. Plants Res.* **5**, 1471-1477.
[7] A.K. Singhal, P.K. Chowdhury, R.P. Sharma, J.N. Baruah and W. Herz (1982). Guaianolides from *Tricholepis glaberrima*, *Phytochem.* **21**, 462-463.
[8] R.N. Yadava and P. Belwanshi (2009). New antifungal constituents form the *Tricholepis glaberrima* DC, *Asian J. Chem.* **21**, 6683-6688.
[9] A.S. Chawla, V.K. Kapoor and P.K. Sangal (1978). Cycloart-23-ene-3β 25-diol from *Tricholepis glaberrima*, *Planta Med.* **34**, 109-110.

[10] S.S. Naphade, S.S. Khadabadi, S.L. Deore, N.S. Jagtap and S.P. Hadke (2009). Antioxidant activity of different extracts of plant *Tricholepis glaberrima* DC (Asteraceae), *Int. J. Pharm. Tech. Res*. **1**, 502-505.

[11] T.J. Mabry, K.R. Markham and M.B. Thomas (1970). The Systematic Identification of Flavonoids, Springer-Verlag, New York, p. 41.

[12] C.L. Lie and J.C. Cheng (2000). A new biflavonoid from *Selaginella uncinata* (Desv.). Spring, *Chinese Pharm. J.* **52**, 211.

[13] A. Hussain, S. Parveen, A. Malik, N. Afza, L. Iqbal and R.B. Tareen (2009). Urease inhibitory flavone glucosides from *Marrubium anisodon*, *Polish J. Chem*. **83**, 1329-1335.

[14] K. Anjoo and K.S. Ajay (2011). Isolation of stigmasterol and *β*-sitosterol from petroleum ether extract of aerial parts of *Ageratum conyzoides* (Asteraceae), *Int. J. Pharm. Pharm. Sci.* **3**, 94-96.

[15] Y. Kouya, A. Toshihiro, K. Ryuji, T. Toshitake and M. Taro (1992). 24-Methylene-25-methylcycloartanol and 24α-ethyl-5α-cholestan-3α-ol from *Neolitsea sericea*, *Phytochem.* **31**, 1741–1746.

[16] H.R. Nawaz, A. Malik, P. Muhammad, S. Ahmed and R. Mohammad (2000). Chemical constituents of *Ajuga parviflora*, *Z. Naturfo. B: Chem. Sci.* **55**, 100-103.

[17] C. Muthu, A.D. Reegan, S. Kingsley and S. Ignacimuthu (2012). Larvicidal activity of pectolinaringenin from *Clerodendrum phlomidis* L. against *Culex quinquefasciatus* Say and *Aedes aegypti* L. (Diptera: Culicidae), *Parasitol Res.* **111**, 1059–1065

[18] P. Gowsala and S.S.M. Uvais (1975). Chemical investigation of ceylonese plants. Part XII. (+)-3,4′,5,7-tetrahydroxy-3′-methoxyflavanone [(+)-dihydroisorhamnetin] and 3,5,7-trihydroxy-3′,4′-dimethoxyflavone (dillenetin): two new natural products from *Dillenia indica* L**.** *J. Chem. Soc., Perkin Trans.* **1**, 612-613