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FLAVONE GLYCOSIDES AND BERGENIN DERIVATIVES FROM *TRIDAX PROCUMBENS*

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Abstract- Tridaxidone, a new flavone glycoside **(3)** and two bergenin derivatives **(4)** and **(5)** have been isolated along with two known flavone glycosides **(1)** and **(2)** from the ethyl acetate soluble portion of the ethanolic extract of *Tridax procumbens*. The structures of **3**, **4** and **5** were established through spectroscopic studies.

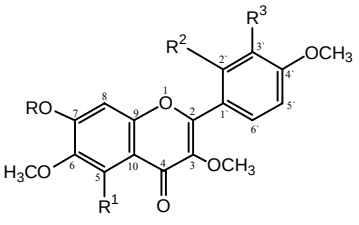
Tridax procumbens (Compositae) is a native of tropical America, Asia and Australia.[1] It is used in Indian traditional medicines as anti-coagulant, hair tonic, antifungal, insect repellent, also in bronchial catarrh, diarrhea, dysentery and wound healing.[2-4] The literature survey revealed the isolation of sterols, pentacyclic triterpenes, fatty acids, polysaccharides[5] from the genus *Tridax*. However, only β-sitosterol glycoside, luteolin, its glycoside and kaempherol have been reported from *T. procumbens*.[6] The medicinal importance of this species prompted us to undertake further phytochemical studies which resulted in the isolation and structure elucidation of one new flavone glycosides **(3)** and two new bergenin derivatives **(4**, **5)** along with jacein **(1)** and jaceidin **(2)**, beside that bergenin **(6)** has also been reported for the first time from this species.

Tridaxidone **(3)** was obtained as a yellow amorphous powder. The UV spectrum was characteristic of flavonoids showing UV maxima at 346 and 257 nm. The presence of a chelated hydroxyl group was indicated by a bathochromic shift in its UV spectrum in the presence of $AlCl_3$/HCl shift reagent. The UV maxima did not exhibit bathochromic shift with NaOAc thereby indicating the absence of free hydroxyl group at C-7. The IR spectrum showed absorption bands at 3450, 3110 (broad) 1730, 1660, 1580 and 1250 cm^{-1} indicating the presence of free hydroxyl, chelated hydroxyl, conjugated carbonyl, methoxyl and aromatic functionalities. The molecular formula $C_{24}H_{26}O_{13}$ was established on the basis of ion peak at *m/z* 521.4483 [M^+-H] (calcd 521.4475 for $C_{24}H_{25}O_{13}$) in HRFAB-MS (neg.). Acid hydrolysis of **3** provided the aglycone **(3a)** and the sugar moiety, the latter of which was identified as D-glucose through sign of its optical rotation and comparison of retention time of its TMS ether with that of standard in GLC. In the ^{1}H-NMR spectrum, the down field signal at δ 12.41 was assigned to the chelated hydroxyl

group and further signals at δ 7.65 (dd, *J* = 8.5, 2.2 Hz), 7.53 (d, *J* = 2.2 Hz) and 7.01 (d, *J* = 8.5 Hz) indicated the presence of *ortho-meta*, *meta* and *ortho* coupled aromatic protons of ring B. The remaining downfield singlet at δ 6.86 was due to the aromatic proton of ring A. The proton signals for three methoxyl groups were observed at δ 3.92, 3.87 and 3.79 (3H each, s). The anomeric proton appeared at δ 5.09 (d, *J* = 7.3 Hz) and its larger coupling constant allowed to assign β configuration to the glucose moiety. Further signals of glucose were observed between δ 3.34-3.74. The EIMS showed molecular ion of aglycone at *m/z* 360 (100 %), a large peak at *m/z* 359 (74%) typical of C-3 methoxylated flavonoids[7] and a strong peak at *m/z* 345 (72%) consistent with the presence of a methoxy group at C-6 or C-8.[8] The retro Diels-Alder cleavage of aglycone gave fragments at *m/z* 182 [A^+], *m/z* 178 [B^+] as well as *m/z* 163 [B^+-Me] which confirmed the presence of a hydroxyl and a methoxy group in ring B and two hydroxyl and one methoxyl groups at ring A of the aglycone. The ^{13}C-NMR spectrum showed the presence of nine methine, one methylene, eleven quaternary and three methyl carbons. The upfield signal at δ 102.4(C-3′) showed the presence of a carbon atom flanked by two oxygenated functions. The location of various groups in ring A was made possible through ^{1}H and ^{13}C-NMR data compared with those of jacein[9] and centaurine[10] and for ring B with tamaridone.[11] The assignments were facilitated by ^{1}H-^{1}H COSY, HMQC and HMBC experiments. The aromatic protons at δ 7.65, 7.53 and 7.01 were assigned to those on C-5′ (δ 112.2), C-3′(δ 102.4) and C-6′(δ 120.2) of the ring B and the remaining aromatic proton at δ 6.86 was assigned to C-8 (δ 95.5) of ring A on the basis of HMQC experiments. The mutual coupling between the ring B protons was further confirmed through ^{1}H-^{1}H COSY experiments. Final assignment of various groups were confirmed through HMBC correlations (Figure 1), in which the H-8 proton (δ 6.86) showed ^2J interactions with C-7 (δ 157.8) and C-9 (δ 153.2), ^3J interactions with C-6 (δ 133.7) and C-10 (δ 108.0), H-3′ (δ 7.53) showed ^2J interactions with C-2′ (δ 147.6) and C-4′ (δ 153.6) while ^3J interactions were observed with C-1′ (δ 123.3) and C-5′. The H-5′ (δ 7.65) exhibited ^2J interactions with C-4′ (δ 153.6) and C-6′ (δ 120.2) while ^3J interactions were observed with C-1′ (δ 123.3) and C-3′ (δ 102.4). The H-6′ proton (δ 7.01) revealed the ^2J interactions with C-1′ (δ 123.3) and C-5′ (δ 112.2) while ^3J interactions were observed with C-2′ (δ 147.6) and C-4′ (δ 153.6). The H-1″ (δ 5.09) exhibited ^3J interaction with C-7 (δ 157.8). The chelated hydroxyl proton (δ 12.41) exhibited ^2J interactions with C-5 (δ 151.8) while ^3J interactions were observed with C-10 (δ 108.0) and C-6 (δ 133.7). The methoxyl protons (δ 3.87), (δ 3.79) and (δ 3.92) showed ^3J interactions with C-3 (δ 139.7), C-6 (δ 133.7) and C-4′ (δ 153.6), respectively. Thus tridaxidone was assigned the structure 2′,5-dihydroxy–3,4′,6-trimethoxyflavone 7-*O*-*β*-D-glucopyranoside (**3**).

Compound **(4)** was obtained as crystalline powder. It gave light purple blue coloration with ceric sulfate. The IR spectrum showed absorption bands at 3400, 1690, 1618, 1595 and 1530 cm^{-1} for hydroxyl, lactone and aromatic functional groups. The UV spectrum showed λ_{max} at 270 and 220 nm. The molecular

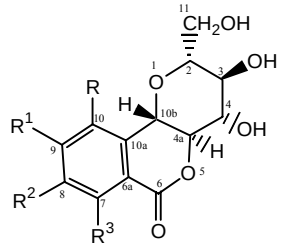
formula of **4** was assigned as $C_{15}H_{18}O_9$ through HRMS showing the M^+ peak at m/z 342.2998 (calcd for $C_{15}H_{18}O_9$; 342.2980). In the ^{1}H-NMR spectrum one aromatic proton appeared at δ 7.15 and two singlets were observed at δ 3.95 and 3.91 due to methoxyl groups, while *C*-glycosyl moiety showed characteristic signals of bergenin and related compounds.[12] The larger coupling constant (J = 10 Hz) between vicinal protons suggested their trans diaxial orientations.[13] The base peak was observed at *m/z* 220.1799 ($C_{11}H_8O_5$) due to the loss of *C*-glycosyl moiety. Further loss of methyl from fragment ion $C_{11}H_8O_5$ resulted in the formation of ion peak at *m/z* 205.1443 ($C_{10}H_5O_5$). The ^{13}C-NMR spectrum showed six quaternary, five methine, two methyl and one methylene carbon atoms. The signal at δ 163.7 was attributed to ester carbonyl carbon and three downfield signals at δ 148.9, 138.5, 144.1 were due to the oxygenated aromatic carbon atoms. The signal at δ 108.7 was assigned to aromatic carbon of the isocumarine moiety. All the ^{1}H and ^{13}C-NMR assignments were made through HMQC and HMBC experiments. In HMBC experiment, the aromatic proton at δ 7.15 showed ^2J interaction with C-9 (δ 144.1) and C-10a (δ 120.7) and ^3J interaction with C-8 (δ 138.5) and C-6a (δ 117.8). The benzylic proton H-10b (δ 5.22) showed ^2J correlations with C-10a (δ 120.7) and C-4a (δ 75.7) and ^3J correlation with C-6a (δ 117.8). The methoxy protons at δ 3.91 showed ^3J interactions with C-7 (δ 148.9). The other methoxy protons (δ 3.95) showed ^3J interaction with C-9 (δ 144.1). The relative stereochemistry of **4** was assigned through comparision of the ^{1}H and ^{13}C NMR spectral data with those of bergenin.[13] Further evidence was provided by ROESY spectrum showed cross peaks between (1) H-3 and hydroxymethylene group, H-3 and OH-4 (2) H-2 and OH-3 (3) H-4 and OH-3 (4) H-4a and OH-4 (5) H-10 (δ 7.15) and MeO-9 (6) H-10 and H-10b. All these cumulative evidence established its structure as 3,4,4a,10b-tetrahydro-3,4,8-trihydroxy-2-hydroxymethyl-7,9-dimethoxypyrano[3,2-*c*][2]benzopyran-6 (2*H*)-one.



1 R=H, R^1= *O*- β-D-glucopyranoside, R^2=H, R^3=OH

2 R= β-D-glucopyranoside, R^1= R^3=OH, R^2=H

3 R= β-D-glucopyranoside, R^1=R^2=OH, R^3=H



4 R=H, R^1=R^3=OCH_3, R^2=OH

5 R=OCH_3, R^1=R^2=R^3=OH

6 R=R^2=OH, R^1=OCH_3, R^3=H

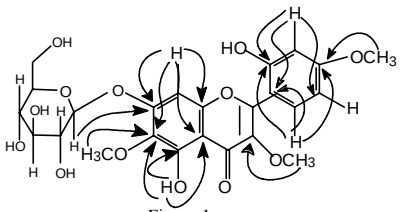


Figure 1
Important HMBC correlations of **3**

Compound (**5**) showed IR absorption bands at 3400, 3100, 1690, 1595 and 1530 cm^{-1} due to free hydroxyl, chelated hydroxyl, lactone and aromatic chromophores. The UV spectrum showed λ_{max} 272 and 220 nm. It showed [M^+] peak at *m/z* 344.2715 in HR-MS consistent with the molecular formula $C_{14}H_{16}O_{10}$ (calcd for $C_{14}H_{16}O_{10}$ 344.2708). The ^{1}H-NMR spectrum showed no aromatic signal. The benzylic proton was observed at δ 5.20 (d, J = 10.1 Hz). A singlet at δ 3.98 was attributed to methoxyl protons. The ^{13}C-

NMR spectrum displayed the signals for seven quaternary, five methine, one methylene and one methyl carbons. The signal at δ 163.1 was assigned to carbonyl carbon of the lactone moiety while other quaternary carbons which appeared at δ 150.8, 147.5, 146.5 and 140.1 were assigned to oxygen substituted aromatic carbons. In HMBC spectrum, the methoxy protons (δ 3.98) showed ^{3}J interactions with C-10 (δ 150.8) and H-10b (δ 5.20) proton showed ^{2}J interactions with C-10a (δ 115.3) and C-4a (δ 75.2) while ^{3}J interactions were observed with C-6a (δ 118.9) and C-2 (δ 80.2). The ROESY spectrum of **5** showed correlations as those of **4** excpet additional correlations of MeO-10 with H-10b and OH-9, respectively. These evidence established its structure as 3,4,4a,10b-tetrahydro-3,4,7,8,9-pentahydroxy-2-hydroxymethyl-10-methoxypyrano[3,2-*c*][2]benzopyran-6 (2*H*)-one.

Table **1** ^{1}H and ^{13}C-NMR assignments of **3**

Position	δ ^{1}H (*J*)	δ ^{13}C	Position	δ ^{1}H (*J*)	δ ^{13}C
2	-	158.1	4′	-	153.6
3	-	139.7	5′	7.65 dd (8.5, 2.2)	112.2
4	-	180.3	6′	7.01 d (8.5)	120.2
5	-	151.8	1″	5.09 d (7.3)	101.2
6	-	133.7	2″	3.74 dd (7.5, 8.2)	73.5
7	-	157.8	3″	3.59 m	77.4
8	6.86 s	95.5	4″	3.54 m	69.4
9	-	153.2	5″	3.49 m	77.5
10	-	108.0	6″	3.34 m 3.44 m	62.9
1′	-	123.3	OCH_3	3.79 s	60.5
2′	-	147.6	OCH_3	3.87 s	61.5
3′	7.53 d (2.2)	102.4	OCH_3	3.92 s	56.3
			OH-5	12.41 br s	

EXPERIMENTAL

General

Melting points (uncorrected) were determined in glass capillaries using Buichi 535 melting point apparatus. Optical rotations were measured on JASCO DIP-360 digital polarimeter. UV spectra were recorded in methanol on Hitachi U-3200 spectrophotometer. IR spectra were measured on Schimadzu Infrared spectrophotometer IR 460. EI-MS were recorded on a Varian MAT 311, FAB-MS measurements were done on a JEOL-HX 110 mass spectrometer. NMR experiments were carried out on a Bruker AMS-300 instrument (^{1}H: 300 MHz; ^{13}C:75 MHz). 2D experiments were done on a Bruker AMX-500 instrument. DMSO-d_6 was used as solvent for ^{1}H and ROSEY experiments. Silica gel 60 (70-230 mesh, Merck); Flash Si 60(230-400 mesh) was used for column chromatography. TLC was conducted on a Precoated Kieselgel 60, F_{254} aluminum sheet and RP-18 F_{254} plates (Merck).

Plant material. *Tridax procumbens* was collected from Karachi in October, 2000 and identified by Dr. Javed Zaki, Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen has been deposited.

Extraction and isolation. The air dried ground plant (18 kg) was extracted with EtOH (50 L) for 10 days at 25 ^{o}C. After concentration under reduced pressure, the extract (800 g) was suspended in water and fractionated by successive partitioning with n-C_6H_{14}, EtOAc and n-C_4H_9OH. The EtOAc fraction (80 g) was subject to column chromatography over silica gel (70-230 mesh) eluting with n-C_6H_{14}, EtOAc and MeOH in increasing order of polarity. Elution with MeOH-EtOAc (1:9), (1.2:8.8), and (1.5:8.5) provided jacein (**1**), jaceidin (**2**) and tridaxidone (**3**), respectively. The eluton with MeOH-EtOAc (1.7:8.3) afforded pure bergenin (**6**) and a mixture of isocoumarines which was further purified oer PTLC using EtOAc-MeOH-BuOH (9:0.6:0.4 +2 drops of water) to afford compounds (**4**) and (**5**). The known compounds were identified by the comparison of spectral data with those reported in literature.[10,12]

Tridaxidone (3) :Yellow amorphous powder; $[\alpha]_D^{25}$ – 60^{o} (*c* 0.543, MeOH); UV max (MeOH) nm(log ε) : 257 (3.41), 346 (4.56); (+ $AlCl_3$/HCl) 265, 396, 403sh; IR (KBr): 3450, 3110(broad), 1730, 1660 and 1350 cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD) Table **1**; EIMS *m/z* (rel. int. %): 360 (100), 359 (74), 345 (72), 317 (50), 182 (1), 178 (6), 163 (34).

Hydrolysis of 3: A solution of **3** (8 mg) in MeOH (5 mL) containing 1N HCl (4 mL) was refluxed for 3 h. concentrated under reduced pressure and diluted with H_2O (8 mL). It was extracted with ethyl acetate and the residue recovered from the organic phase was subjected to preparative TLC to obtain compound (**3a**) which was identified as the corresponding aglycone of **3**. The ^{1}H NMR (300 MHz, CD_3OD) of compound (**3a**) showed similar resonances as **3**, except for the absent signals of the sugar moiety. – HR-MS: *m/z* = 360.3154 [M^+] (calcd for $C_{18}H_{16}O_8$: 360.3148). The sugar was identified as D-glucose through sign of its optical rotation and comparison of retention time of its TMS ether with that of standard in GLC.

3,4,4a,10b-Tetrahydro-3,4,8,-trihydroxy-2-hydromethyl-7,9-dimethoxypyrano[3,2-*c*][2]-benzopyran-6(2*H*)-one (4) : White crystalline powder mp 198-203 ^{o}C; $[\alpha]_D^{25}$ – 41^{o} (*c* 0.62, MeOH); UV max (MeOH) nm (log ε): 270 (4.31), 220 (3.27); IR (KBr): 3400, 1690, 1618, 1595 and 1530 cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD) Table **2**; EIMS *m/z* (rel. int. %): 342 (3), 327 (67), 250 (34), 220 (100), 205 (18); HRMS *m/z* 342.2998 (calcd for $C_{15}H_{18}O_9$ 342.2980).

3,4,4a,10b-Tetrahydro-3,4,7,8,9-pentahydroxy-2-hydromethyl-10-methoxypyrano[3,2-*c*][2]-benzopyran-6(2*H*)-one (5) : Off-white crystalline powder mp 194-198 ^{o}C; $[\alpha]_D^{25}$ – 36.4^{o} (*c* 0.59, MeOH); UV max (MeOH) nm (log ε): 272 (4.45), 220 (3.18); IR (KBr): 3450, 3100(broad), 1690, 1595 and 1530 cm^{-1}; ^{1}H-NMR (300 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD) Table **2**; EIMS *m/z* (rel. int. %): 344 (2.3), 240 (11), 222 (100), 221 (27); HR-MS *m/z* 344.2715 (calcd for $C_{14}H_{16}O_{10}$ 344.2708).

Table **2** ^{1}H and ^{13}C NMR assignments of **4** and **5**

4			**5**		
Position	^{1}H (δ) (*J*)	^{13}C (δ)	Position	^{1}H (δ) (*J*)	^{13}C (δ)
2	4.28 m	81.3	2	4.26 m	80.2
3	5.11 t (10.0)	70.9	3	5.07 t (10.1)	70.5
4	5.62 t (9.0)	74.9	4	5.59 t (9.0)	74.3
4a	4.62 t (10.0)	75.7	4a	4.60 t (10.0)	75.2
6	-	163.7	6	-	163.3
6a	-	117.8	6a	-	118.9
7	-	148.9	7	-	147.5
8	-	138.5	8	-	140.1
9	-	144.0	9	-	146.5
10	7.15 s	108.7	10	-	150.8
10a	-	120.7	10a	-	115.3
10b	5.22 d (10.3)	77.6	10b	5.20 d (10.1)	76.4
11	4.24-4.14 m	62.5	11	4.23-4.18 m	61.8
OCH_3	3.95 s	60.6	OCH_3	3.92 s	60.3
OCH_3	3.91 s	56.8	OH-2	4.40 br t (5.0)	
OH-2	4.39 br t (5.1)		OH-3	5.54 d (4.0)	
OH-3	5.54 d (4.0)		OH-4	5.24 d (5.2)	
OH-4	5.23 d (5.0)		OH-9	10.21 s	

REFERENCES:

1. The Wealth of India: "*A Dictionary of Indian Raw Materials and Industrial Products Raw Materials"*, Vol. X, New Delhi, CSIR, 1995, 292.
2. S. L Srivastav, V. K Kediyal, and R. C. Sundriyal, *J. Envirom. Biol.,* 1984, **5**, 217.
3. S. L Udupa, A. l. Udupa, and D. R Kulkarni, *Fitoterapia,* 1991, **62,**146.
4. S. Saraf and V.K Dixit, *Fitoterapia,* 1991,**62**,534.
5. M. Ali and E. Ravinder, *Fitoterapia,* 2001, **72**, 313.
6. S. S. Sankara and S. Ramakrishnan, *Curr. Sci.*, 1986, **37**, 465.
7. D. G. I. Kingston, *Tetrahedron,* 1971, **27**, 2691.
8. J. N. Roitman and L. F. James, *Phytochemistry,* 1985, **24**, 835.
9. S. Sepulveda Boza, D. Sulaheen, and K. C. Druce, *Phytochemistry,* 1993, **32**, 1301.
10. G. Flamini and E. Antogonali, *Phytochemistry,* 2001, **57**, 559.
11. S. P. Virinder, S. B Kirpal, and S. Ole, *Phytochemistry,* 1994, **36**, 507.

12. P. A. Ramaidh, L. R. dra Raw, D. S. Reddy, and A.S.R. Anjaneyulu, *J. Chem. Soc., Perkin Trans. I*, 1979, 2313.
13. T. Yoshida, K. Seno, Y. Takama, and T. Okuda, *Phytochemistry,* 1982, **21**, 1180.