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Chemical Constituents of *Pulicaria gnaphalodes*

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Abstract – The chloroform extract of *Pulicaria gnaphalodes* yielded flavonoids (**1-2**), sesquiterpenoid (**3**), sterol (**4**), benzoic acid derivative (**5**) and fatty acids (**6-7**). Their structures were elucidated with the aid of NMR spectroscopy. The described compounds have not been reported so far from this source.
Key words – *Pulicaria gnaphalodes*, Asteraceae, Structure elucidation

Introduction

The family Asteraceae (Compositae) is comprised of 200 genera and 2000 species. Out of them, the genus *Pulicaria* is widely distributed in Asia, Europe and Africa. Only ten species of this genus are recorded in Indo-Pak. The species *gnaphalodes* of the genus *Pulicaria* is abundantly found in Quetta region of Pakistan.

Members of the genus *Pulicaria* have been investigated extensively all over the world and the chemical literature survey shows the presence of flavonoids (Pares *et al.*, 1981 and El-Negoumy *et al.*, 1982), sesquiterpenoids (Mossa *et al.*, 1992 and Mossa *et al.*, 1990), diterpenoids (Singh *et al.*, 1985) and sesquiterpenoid lactones (Zdero *et al.*, 1988). Most of them are biologically active.

Experimental

General – The ^{1}H and ^{13}C-NMR spectra were recorded at 400 and 100 MHz, respectively, on Bruker AM 400 in $CDCl_3$.

Collection and Identification – The plant was collected from Quetta region of Pakistan in May, 1997 and identified by Dr. R. B. Tareen, Department of Botany, Baluchistan University, Quetta (Baluchistan), where the voucher specimen has been deposited in the herbarium.

Extraction and Isolation – The collected plant material was dried under shade and chopped into small pieces. The dried and chopped material (7.75 Kg) was soaked in chloroform (15 L) for a period of ten days. This process was repeated three times and then the same material was soaked in methanol. The chloroform was evaporated under reduced pressure and the gummy material thus obtained (254.54 g), was diluted with water. Ethyl acetate was added and the organic material was recovered in ethyl acetate. Solvent was removed by means of distillation and the crude concentrated ethyl acetate soluble gummy mass (203.87 g) was subjected to silica gel column chromatography using hexane, hexane: ethyl acetate, ethyl acetate, ethyl acetate : methanol and finally, pure methanol as mobile phase (Scheme 1).

The fraction eluted with 5% ethyl acetate in hexane afforded a solid mass which on washing with methanol yielded **4** as a white powder (29.1 mg).

Compound 4 – m.p.: 138°C; FDMS: *m/z* 414 (M^+, $C_{29}H_{50}O$); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 5.21(m, H-6), 3.30(m, H-3), 1.00(s, H-19), 0.89(d, *J*=6.0 Hz, H-21), 0.86 (t, *J*=7.0 Hz, H-29), 0.81(d, *J*=6.5 Hz, H-26), 0.79(d, *J*=6.5 Hz, H-27) and 0.65 (s, H-18); ^{13}C-NMR($CDCl_3$, 100 MHz): d 31.29(C-1), 31.79(C-2), 71.89(C-3), 42.20(C-4), 140.90(C-5), 121.85(C-6), 32.03 (C-7), 31.99(C-8), 50.79(C-9), 36.59(C-10), 21.11(C-11), 49.20(C-12), 42.59(C-13), 56.75(C-14), 24.30(C-15), 28.27(C-16), 56.11(C-17), 11.79(C-18), 19.42(C-19), 36.24(C-20), 19.09(C-21), 34.01(C-22), 29.29(C-23), 45.73(C-24), 26.19(C-25), 18.79(C-26), 19.79(C-27), 23.08(C-28), 11.97(C-29).

On further elution with the same polarity, compound **3** was obtained as an impure solid which was further purified by washing with methanol to afford white needles (13.7 mg).

Compound 3 – M.P.: 42°C; IR ($CHCl_3$): 3400(OH), 1650(C=C) cm^{-1}; EIMS: *m/z* 222 (M^+, 43%); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.92(3H, s, H-15), 1.08

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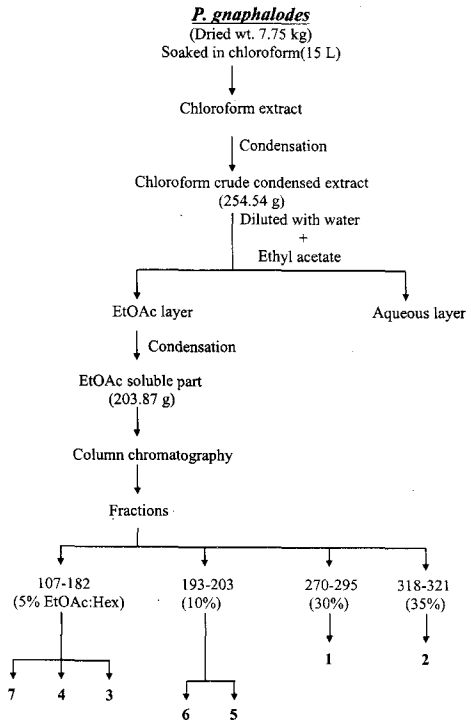


(Scheme-1)

(3H, s, H-14), 1.74 (3H, s, H-13), 4.86(1H, br.s, H-11), 4.90(1H, d, *J*=1.5 Hz, H-12); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 41.37(C-1), 20.13(C-2), 43.51(C-3), 72.10(C-4), 49.16 (C-5), 22.81(C-6), 39.34(C-7), 23.49 (C-8), 40.34(C-9), 35.29(C-10), 146.9(C-11), 110.8 (C-12), 22.74(C-13), 22.28(C-14), 18.43(C-15).

After washing compound **3**, the mother liquor was concentrated and loaded on silica gel column. With 1% ethyl acetate in hexane, compound **7** was eluted in pure form as a white solid (11.2 mg).

Compound 7 – M.P.: 76°C; EIMS: *m/z* 312 [M^+]; ^{1}H-NMR ($CDCl_3$, 400 MHz) : δ 0.86 (3H, t, *J*=6.95 Hz, H-20), 1.52 (2H, m, H-19), 1.24 (br. s., chain), 2.62 (2H, t, *J*=6.65 Hz, H-2).

Elution with 10% ethyl acetate in hexane compounds **5** & **6** were obtained as white powders.

Compound 5 – M.P.: 181°C; EIMS: *m/z* 152 [M^+], 121[M-OMe]$^+$; FDMS: *m/z* 152; IR ($CHCl_3$): 2400-3620(acid OH), 1705(CO)cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 3.86(3H, s, OMe), 6.83(2H, d, *J*=9.0 Hz, H-2 and H-6), 7.93(2H, d, *J*=9.0 Hz, H-3 and H-5), 11.31(1H, s, COOH).

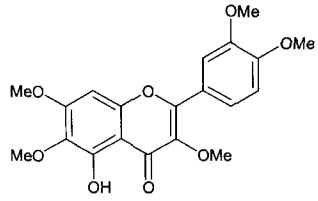
Compound 6 – EIMS: *m/z* 480 [M^+], 465 [M-CH_3]$^+$, 451[M-CH_2CH_3]$^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) : δ 0.88 (3H, t, *J*=6.5 Hz, H-32), 1.24(m, chain), 1.49(2H, m, H-31), 2.33(2H, t, *J*=6.5 Hz, H-2).

With 30% ethyl acetate in hexane, compound **1** was obtained in major amount (100 mg) as a yellow solid material.

Compound 1 – m.p.: 150°C; IR ($CHCl_3$): 3510(OH), 1730(CO)cm^{-1}; FDMS: *m/z* 388; HRMS: *m/z* 388.1093 (cacld. *m/z* 388.1158048 for $C_{20}H_{20}O_8$); EIMS: *m/z* 388 (M^+)(100%), 373(M-15)$^+$, 343, 197, 196, 192, 165, 96, 57 ; ^{1}H-NMR($CDCl_3$, 400 MHz) : δ 7.70(dd, *J*=8.49, 2.10 Hz, H-6'), 7.65(d, *J*=2.06 Hz, H-2'), 6.99(d, *J*=8.56 Hz, H-5'), 6.48(s, H-8), 3.83(s, OMe), 3.97(s, OMe), 3.93(s, OMe), 3.96(s, OMe), 3.94 (s, OMe); ^{13}C-NMR($CDCl_3$, 100 MHz): δ 155.6(C-2), 138.7(C-3), 178.7(C-4), 152.1(C-5), 132.2(C-6), 158.6(C-7), 90.4(C-8), 152.6(C-9), 105.6(C-10), 123.5 (C-1'), 110.3(C-2'), 148.9(C-3'), 151.3(C-4'), 111.6 (C-5'), 122.2(C-6'), 60.6(3-OMe), 60.0(6-OMe), 56.1 (7-OMe), 56.3(3'-OMe), 56.1(4'-OMe).

The last compound **2** which could be isolated from the same column was eluted with 35% ethyl acetate in hexane. The compound **2** on condensation resulted as a solid material and further cleaned by washing with methanol to yield **2** in minor amount (8.0 mg) as compared to **1**.

Compound 2 – m.p.: 187°C; FDMS: *m/z* 374; HRMS: *m/z* 374.0979 (cacld. 374.1001556 for $C_{19}H_{18}O_8$); EIMS: *m/z* 374 (M^+) (100%), 359(M-15)$^+$, 273, 301, 245, 197, 178, 151, 135, 164, 69; IR ($CHCl_3$): 3515(OH), 1620 (C=C) cm^{-1}, ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 6.36(s, H-8), 7.65(2H, m, H-2' and H-6'), 7.13(d, *J*=9.4 Hz, H-5'), 3.99 (s, OMe), 3.83(s, OMe), 3.97(s, OMe), 3.91(s, OMe).



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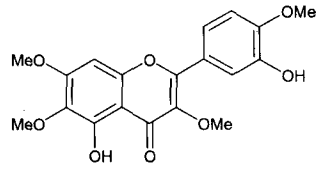

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Results and Discussion

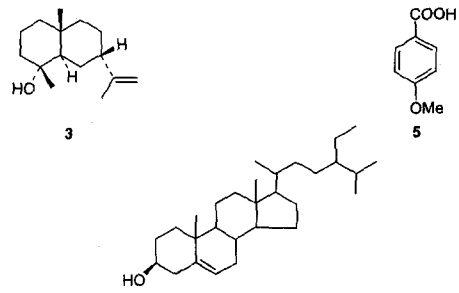
In this communication, we wish to report seven compounds (**1-7**) first time from our investigated source. As these compounds have already been reported from various higher plants, the spectral data of our elucidated compounds were compared with the reported literature values and thus discussed here briefly.

The IR spectrum of **1** displayed absorption bands at 3510 and 1730 cm^{-1} due to the hydroxyl and ketonic functions in the molecule. The FDMS showed the molecular ion peak at *m/z* 388. The formula of this peak was confirmed through HRMS as $C_{20}H_{20}O_8$. The NMR data revealed that **1** was identical to artemetin (5-hydroxy-3,6,7,3',4'-pentamethoxyflavone). The chemical shifts were assigned by comparing with similar compounds (Ronneberg *et al.*, 1995, Wenkert *et al.*, 1977 and Barbera *et al.*, 1986) and also with artemetin (Ahmad *et al.*,1995). The destribution of various methoyl groups were assigned with the help of fragments given in experimental section. The possibility of a methoxyl group at C-8 instead of at C-6 was ruled out due to the presence of molecular ion as base peak in the EIMS of **1** (Goudard *et al.*, 1979).

Compound **2** was obtained as a minor constituent. The FDMS of **2** showed the molecular ion peak at *m/z* 374 and formula of this peak was determined through HRMS as $C_{19}H_{18}O_8$ showing the eleven degrees of unsaturation. The ^{1}H-NMR spectrum displayed four methoxyl signals at δ 3.90, 3.83, 3.97 and 3.91. The presence of hydroxyl function/s was confirmed through IR absorption at 3460 cm^{-1}. Due to the minor amount, the carbon spectrum of **2** could not be obtained. The distribution of methoxyl groups were attested with the help of fragments appeared in EIMS of **2** (Goudard *et al.*, 1979). However, when the proton chemical shifts were compared with casticin (Barbera *et al.*, 1986), it was found that both were same. This has not been isolated so far from *P. gnaphalodes*.

Compound **3** has previously been isolated from *Cymbopogon flexuosus* (Thappa *et al.*, 1979). The data of our isolated compound **3** were exactly matched with the reported data of isointermedeol (Thappa *et al.*, 1979). This compound is also a new addition in the constituents of *P. gnaphalodes*.

Compound **5** was purified by preparative thin layer chromatography which showed the molecular mass 152 a.m.u. in the EIMS. This was further confirmed



$H_3C-CH_2-(CH_2)_{28}-CH_2-CO_2H$

6

$HO_2C-CH_2-(CH_2)_{16}-CH_2-CH_3$

7

by FDMS. The proton NMR of **5** revealed the presence of one methoxyl group at δ 3.86. In the aromatic region, two sets of doublets at δ 6.83 and 7.93 having same coupling constants (9.0 Hz) with two protons integration each attested for two *ortho* and two *meta* protons, respectively. The IR spectrum of **5** confirmed the presence of acidic carbonyl function at 1705 cm^{-1} in the molecule and thus **5** has been assigned as *pera* methoxyl benzoic acid. This compound has already been reported but not isolated previously from our investigated source.

In addition to **1-3** and **5**, β-sitosterol (**4**) (Shameel *et al.*,1996) and fatty acids (**6-7**) have also been isolated first time from this source.

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