PHYTOCHEMICAL INVESTIGATION OF *CALLIGONUM POLYGONOIDES*

Abdul Ghaffar[a*], Muhammad Saleem[a], Jallat Khan[a], Naheed Riaz[a], Tasneem Khatoon[b] and Abdul Jabbar[a]

[a]Department of Chemistry, Baghdad-ul-Jadeed campus, The Islamia University,Bahawalpur, 63100-Bahawalpur, Pakistan;
[b]Department of Chemistry, Government Sadiq Women College University, Bahawalpur, 63100-Bahawalpur, Pakistan
*Corresponding Author: aghaffarswl@hotmail.com

ABSTRACT*: The chromatographic purification of the extract of Calligonum polygonoides yielded two new (**1-2**) along with eight known phytochemicals, 24-epi-makisterone A (**3**), hexacosanyl coumarate (**4**), p-methyl coumarate (**5**), 1H-indole-3-carboxylic acid (**6**), taxifolin (dihydroquercetin, 7), β-sitosterol-3-O-β-D-glucopyranoside (**8**), β-sitosterol (**9**) and β-amyrin (**10**). The structure of the known compounds were established due to 1D- and 2D-NMR and high resolution mass spectrometric techniques, whereas, the known compounds were identified through 1D-NMR and mass spectrometry, and in comparison with the literature values.*

Key Words: Calligonum polygonoides, secondary metabolites, isolation, structure elucidation

1. INTRODUCTION

The genus *Calligonum* of the family Polygonaceae comprises 80 species distributed throughout Western Asia, Southern Europe, and Northern Africa [1]. In Pakistan, this genus is represented by only one species, *Calligonum* polygonoides [2]. This slow-growing, almost leafless shrub is extensively used in local medicine. For example, a decoction of the roots mixed with catechu is used as gargle for sore gum, the shoot juice is applied to the eyes as an antidote to scorpion sting. The latex of this plant is used to induce abortion, to cure bites of rabid dogs, and for treating eczema, whereas, the flowers possess digestive and tonic properties, and are useful against cough, cold, and asthma [3,4]. Despite of its medicinal importance and several folk uses, so far no phytochemical investigation has been carried out on this plant. These facts prompted us to investigate this herb for its secondary metabolites. In this study, we isolated two new natural products **1, 2** along with eight known compounds 24-epi-makisterone A (**3**) [5], hexacosanyl coumarate (**4**) [6], *p*-methyl coumarate (**5**) [7], 1H-indole-3-carboxylic acid (**6**) [8], taxifolin(dihydroquercetin, **7**) [9], *β*-sitosterol-3-O-*β*-D-glucopyranoside (**8**) [10], *β*-sitosterol (**9**) [11] and *β*-amyrin (**10**) [12] (Figure 1).

2. Results and Discussion

Compound **1** was isolated as white amorphous solid, which exhibited characteristic IR absorption bands at 3470, 1735 and 1505-1470 cm^{-1} for hydroxyl function, ester moiety and aromatic system. The EIMS displayed molecular ion at *m/z* 518, whereas, the HR-EIMS depicted the molecular formula as $C_{33}H_{58}O_4$ with five double bond equivalence (DBE). The UV spectrum of compound **1** showed absorption maxima at 205 nm that substantiated the aromatic moiety.

The ^{1}H-NMR spectrum (Table 1) of compound **1** showed two ortho coupled doublets at δ 7.35 (*J* = 8.4 Hz) and δ 6.74 (*J* = 8.4 Hz) splitted at A^2B^2 pattern, attributed to a *p*-substituted benzene ring. The same spectrum further displayed the resonance of a triplet methylene at δ 4.21 (*J* = 6.8 Hz), which was correlated in COSY spectrum with another triplet methylene at δ 2.84 (*J* = 6.8 Hz). The downfield shift of the first methylene (δ 4.21) could be attributed to its attachment with carboxylate function, whereas, the latter (δ 2.84) was connected with the aromatic ring, due to its chemical shift. This analysis revealed a 4-hydroxyphenethoxy moiety [13] in **1**. Two more methylene also resonated in the ^{1}H-NMR spectrum at δ 3.64 (t, *J* = 6.8 Hz) and δ 2.27 (t, *J* = 7.6 Hz), which were correlated in COSY spectrum with a broad singlet of several aliphatic methylenes at δ 1.24 -1.38. This data revealed that compound **1** has an aliphatic chain ending with oxymethylene function.

The ^{13}C-NMR spectrum (Table 1) of **1** supported the ^{1}H-NMR data, as it afforded the signals for an aromatic moiety at δ 153.0 (C-4), 130.3 (C-2, 6), 128.5 (C-1) and 115.3 (C-3, 5), two oxymethylenes at δ 64.8 (C-8) and 63.1 (C-25′), carbonyl carbon at 173.5 (C-1′) and several aliphatic methylenes at δ 29.6-29.1.

Various connectivities were confirmed through HMBC spectral analysis in which methylene-7 (δ 2.85) showed long range interaction with the aromatic carbons at δ 128.5 (C-1) and 130.3 (C-2, 6), indicating its direct attachment with the aromatic system, whereas, the oxymethylene-8 (δ 4.21) exhibited HMBC correlation with the carbons at δ 128.5 (C-1) and 173.5 (C-1′). The combination of above data and further analysis of COSY and HMBC spectra (Figure 2) led to the structure of **1** as 4-hydroxyphenethyl carboxylate. The length of fatty acid chain could was fixed through HREIMS and finally compound **1** could be identified as 4-hydroxyphenethyl-25-hydroxypentacosanoate, which is a new natural product.

Compound (**2**) was isolated as yellowish amorphous solid which showed blue green florescence under UV at 360 nm. The IR spectrum of **2** showed absorption bands for secondary amine (3365 cm^{-1}), carbonyl group (1680 cm^{-1}), olefinic system (1653 cm^{-1}), aromatic moiety (1605, 1515, 1470 cm^{-1}) and nitro group (1512, 1486 cm^{-1}). Compound **2** on reaction with ferrous hydroxide gave red brown precipitate of ferric hydroxide to substantiate nitro group [14].

The EIMS of **2** showed molecular ion peak at *m/z* 374, while the high resolution analysis of the same peak depicted the molecular formula $C_{19}H_{22}N_2O_6$ with ten DBE. The ^{1}H-NMR spectrum (Table 2) of **2** displayed discrete signals for an aromatic system at δ 8.01 (1H, s), 7.97 (1H, dd, *J* = 8.0, 1.2 Hz), 7.62 (1H, d, *J* = 7.6 Hz) and 7.34 (1H, t, *J* = 7.6 Hz). The splitting pattern of these signals revealed a 1,3-disubstituted benzene ring in **2**. This spectrum further displayed an exchangeable broad singlet at δ 5.97 (1H, s) due to a secondary amine, whereas, another singlet proton resonating at δ 5.06 was correlated in HSQC spectrum with the carbon at δ 39.9. The NMR shifts of this methine revealed that it must be connected with several sp^2 hybridized carbon atoms. Two chemically equivalent ethoxy groups could be

identified due to the resonances at δ 4.04 (4H, q, *J* = 7.2 Hz) and 1.18 (6H, t, *J* = 7.2 Hz), whereas, two allelic methyl resonated at 2.32 (6H, s). This data indicated that compound **2** must have a moiety with symmetrical structural features, which was further substantiated due to ^{13}C-NMR spectral analysis (Table 2) of **2**. The ^{13}C-NMR spectrum afforded total 13 carbon signals at δ 167.1 (C-7, 7′), 149.9 (C-9), 148.1 (C-11), 144.8 (C-2, 6), 134.5 (C-14), 128.6 (C-13), 123.1 (C-10), 121.3 (C-12), 103.2 (C-3, 5), 59.9 (C-1′, 1′′), 39.9 (C-4), 19.5 (C-8, 8′), 14.2 (C-2′, 2′′) attested for 19 carbon atoms.

The aromatic moiety, two double bonds, two carbonyl systems and a nitro group accommodated nine DBE, therefore, the remaining one DBE could be attributed to another ring system, which was identified as 1,4-dihydropyridine. The HMBC correlation (Figure 3) of H-10 (δ 8.09) and H-14 (δ 7.62) with that of C-4 (δ 39.9) established the attachment of benzene ring at C-4 of dihydropyridine sytem. The positions of the other substitutions on dihydropyridine ring was determined due to HMBC correlation of H-4 (δ 5.06) with the carbons at δ 114.8 (C-2, 6), 103.2 (C-3, 5) and 167.1 (C-7, 7′), and that of methyl protons (δ 2.32) with C-5 (δ 103.2) and C-6 (δ 114.8). Relatively downfield shift of methylene at δ 4.04 (H-1′, 1′′) and its HMBC correlation with the carbonyl carbon (δ 176.1) confirmed its attachment with the carboxylate function. The nitro group could be fixed at C-11 due to splitting pattern of aromatic protons. The above discussed data finally led to the structure of **2** as diethyl-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, which has been reported as a synthetic compound [15] but this is first report on its discovery as natural product.

Due to lack of lab facilities, we could not perform biological test for the isolated compounds, however, in literature dihydropyridine derivatives are known to possess calcium antagonistic activity as they inhibit the influx of Ca^{+2} ions through plasma membrane channels. Moreover, it is reported that compounds of this class are being used in the treatment of angina and hypertension [15].

3. EXPERIMENTAL

3.1 General Experimental Procedures

The UV spectra were recorded in ethanol on a Hitachi UV-3200 Spectrometer (λ_{max} in nm). The IR spectra were recorded on Shimadzu IR-460 spectrophotometer (ν in cm^{-1}). EIMS, HR-EIMS spectra were recorded on Jeol JMS-HX 110 spectrometer with data system. The ^{1}H-NMR spectra were recorded on Bruker AMX-400 instrument using TMS as an internal reference. The chemical shifts are reported in ppm (δ) while coupling constants (*J*) in Hz. The ^{13}C-NMR spectra were recorded at 100 MHz on the same NMR instrument. Column chromatography was carried out using silica gel (E-Merck, 70-230 and 230-400 mesh, Darmstadt, Germany). Aluminium sheets precoated with silica gel 60 F_{254} (0.2 mm thick; E-Merck, Darmstadt, Germany) were used for TLC to check the purity of the compounds and were visualized under UV light (254 and 366 nm) followed by heating with ceric sulfate as spraying reagent.

3.2 Plant Material

The whole plant of *Calligonum polygonoides* was collected from Cholistan Desert near Bahawalpur in April 2010 and was identified by Dr. Muhammad Arshad (late), Ex-plant Taxonomist, Cholistan Institute for Desert Studies (CIDS), The Islamia University of Bahawalpur, where a voucher specimen (CP/CIDS-2010) is deposited.

3.3 Extraction and isolation

The shade dried plant 6.5 Kg was ground into coarse powder and extracted with MeOH. The extract was concentrated on a rotary evaporator to get a blackish gummy material (900gm) was obtained, which was suspended in water and extracted with *n*-hexane and EtOAc. The EtOAc fraction (400gm) was subjected to silica gel column chromatography eluting with *n*-hexane, EtOAc, EtOAc:MeOH, MeOH in increasing order of polarity and a total of fifteen fractions (CP1-CP15) were obtained. The fraction CP-4 was further subjected to silica gel column chromatography eluting with *n*-hexane : EtOAc in increasing order of polarity to get five sub fractions (d1-d5). The sub fraction d-3 was finally purified on silica gel column eluting with an isocratic of *n*-hexane:EtOAc (6:4) to get compound **1** (9mg). The fraction CP-6 from the main column was further chromatographed over silica gel column using a mixture of *n*-hexane and EtOAc in increasing order of polarity as mobile phase, which afforded four sub fractions (E1-E4). The sub fraction E-2 was again passed over silica gel column eluting with an isocratic of *n*-hexane:EtOAc (1:1) to get compound **2** (14mg). The sub fraction E-3 was also subjected to silica gel column eluting with *n*-hexane:EtOAc

Table 1. ^{1}H and ^{13}C NMR data of 1 ($CDCl_3$, 400 and 100 MHz)

Position	δ_H (*J* = Hz)	δ_C
1	-	128.5
2, 6	7.35 (d, *J* = 8.4 Hz)	130.3
3, 5	6.74 (d, *J* = 8.4 Hz)	115.31
4	-	153.0
7	2.85 (t, *J* = 6.8 Hz)	36.36
8	4.21(t, *J* = 6.8 Hz)	64.89
1′	-	173.5
2′	2.27(t, *J* = 7.6 Hz)	34.29
3′	1.56, m	24.95
4′-24′	1.24 -1.38, brs	29.6-29.1
25′	3.64 (d, *J* = 6.8 Hz)	63.13

Table 2. ^{1}H and ^{13}C NMR data of 2 ($CDCl_3$, 400 and 100 MHz)

Position	δ_H (*J* = Hz)	δ_C
1	5.97, s (-NH)	-
2, 6	-	144.8
3, 5	-	103.2
4	5.06, s	39.9
7, 7′	-	167.1
8, 8′	2.32, s	19.5
9	-	149.9
10	8.09, s	123.1
11	-	148.1
12	7.97 (dd, *J* = 8.0 ,1.2 Hz)	121.3
13	7.34 (t, *J* = 7.6 Hz)	128.6
14	7.62 (d, *J* = 7.6 Hz)	134.5
1′, 1′′	4.04 (q, *J* = 7.2 Hz)	59.9
2′, 2′′	1.18 (t, *J* = 7.2 Hz)	14.2

(4:6) to get compound **3** (15mg) and sub fraction E-4 yielded compound **4** (11mg) when it was passed over silica gel column eluting with an isocratic of *n*-hexane:EtOAc (3:7). The main fraction CP-3 was further purified on silica gel column using mobile phase of *n*-hexane:EtOAc (7:3) that gave a semi-pure fraction, which on further purification under the same conditions yielded compounds **5** (14mg)**.** The main fraction CP-4 was further purified on silica gel column using mobile phase of *n*-hexane:EtOAc (6:4) that gave two sub fraction(J1-J2). The sub fraction J-1 on further purification using mobile phase of *n*-hexane:EtOAc (6:4) yielded compounds **6** (9mg). Another main fraction CP-7 was passed over silica gel column eluted with *n*-hexane:EtOAc (5:5) and four sub fractions (F1-F4) were obtained. The sub fraction F-1 was further passed through silica gel column with an isocratic of *n*-hexane:EtOAc (5:5) to get compounds **7** (10mg) and sub fraction F-2 gave compound **8** (13mg) when it was passed through silica gel column with an isocratic of *n*-hexane:EtOAc (3:7) with minor impurities, which were removed by passing both the compounds through sephadex LH-20. The sub fraction F3 was also passed over silica gel column with *n*-hexane:EtOAc (1:9), it yielded compounds **9** (11mg) . Another main fraction CP-9 was passed over silica gel column eluted with *n*-hexane:EtOAc (4:6) and two sub fractions (k1-k2) were obtained. The sub fraction k-1 was also passed over silica gel column with *n*-hexane:EtOAc (4:6) and it yielded compounds **10** (7mg). The remaining fractions contain sugars and salts and were not processed.

*3.3.1 4-hydroxyphenethyl-25-hydroxypentacosanoate **(1)***

White amorphous powder (9mg). UV (Methanol) λ_{max} , nm (logε); 205 (3.89) IR (neat film): 3470 (OH), 1735 (ester) and 1505-1470 (aromatic C ═ C) cm^{-1} ; for ^{1}H and ^{13}C NMR spectral data, see Table 1; EI-MS: m/z 518 ; HREI-MS m/z 518.4326 (calcd for $C_{33}H_{58}O_4$, 518.4326).

*3.3.2 diethyl-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate **(2)***

Yellowish amorphous powder (14mg). UV (Methanol) λ_{max} , nm (logε): 360 (4.1); IR (neat film): 3365 (secondary amine), 1680 (CO), 1653 (olefinic system), 1605, 1515, 1470 (aromatic moiety) and 1512, 1486 (nitro group) cm^{-1} ; for ^{1}H and ^{13}C NMR spectral data, see Table 1; EI-MS: m/z 374; HREI-MS m/z 374.1478 (calcd for $C_{19}H_{22}N_2O_6$, 374.1478).

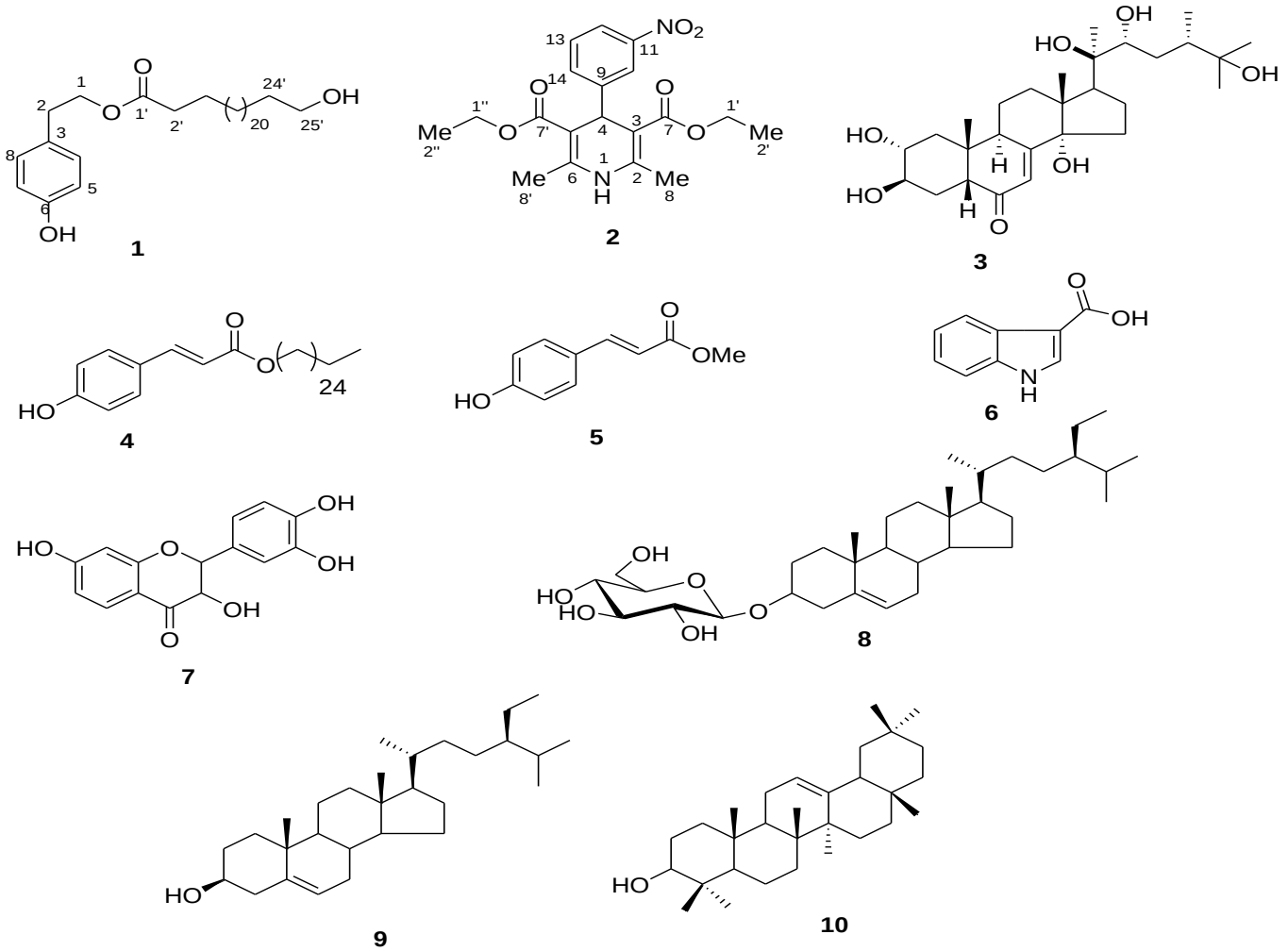

Figure 1. Structures of the Compounds 1-10 isolated from *Calligonum polygonoides*

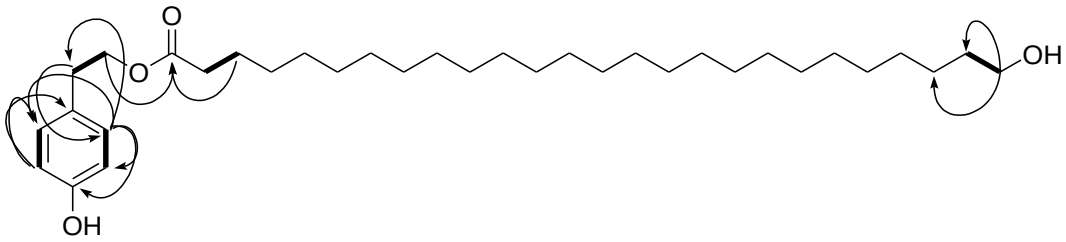

Figure 2. COSY (▬) and HMBC (⌒) Correlations observed in the spectrum of 1

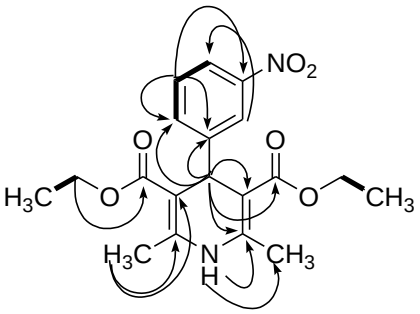

Figure 3. COSY (▬) and HMBC (⌒) Observed in the spectrum of compound 2

REFERENCES

[1] O. Mamoru, T. Yoshihisa, K. Kentarou, F. Kenji, S. Hirohumi, H. Tomihiko, H. Gisho, I. Michiho, K. K. Olimjon and A. Ozodbek, *J. Nat. Prod*. **67**, 1044 (2004).

[2] G. P. Phondke, in, *The Wealth of India, Council of Scientific and Industrial Research, NISCAIR Press, New Dehli, India*, **3**, 62 (1992).

[3] G. P. Phondke, in, *The Wealth of India, Council of Scientific and Industrial Research, NISCAIR Press, New Dehli, India*. **3**, 83 (1992).

[4] B. N. Sastri, in, *The Wealth of India, Council of Scientific and Industrial Research, NISCAIR Press, New Dehli, India*, **2**, 14 (1950).

[5] N. Zhu, H. Kikuzaki, B. C. Vastano, N. Nakatani, M. V. Karwe and R. T. Rosen, *J. Agric. Food Chem*. **49**, 257 (2001).

[6] R. F. Carmen, D. S. Armando and P. N. Carlos, *Phytochem. Anal.* **18**, 151 (2007).

[7] K. Takeya and H. Itokawa, *Chem. Pharm. Bull*. **30**, 1496 (1982).

[8] K. Alapati, P. Peddikotla, V. Muvva and V. Yenamandra, *Res. Microbiol.* **161**, 335 (2010).

[9] Q. Theriault, S. C. Wang, B. Iderstine, A. Chen, A. Franke and K. Adeli, *J. Lipid Res*. **41**, 1969 (2000).

[10] H. F. Shih, K. R. Yerra and M. T. Yew, *J. Ethnopharmacol.* **116**, 333 (2008).

[11] W. H. Hiji, M. M. Li, K. M. Wong, *Phytochemistry*. **15**, 797 (1976).

[12] T. Hironobu, I. Miho, F. Yoshimi, M. Hiroyuki and F. Yoshiyasu, *Phytochemistry*. **51**, 543 (1999).

[13] J. C. Jih, M. W. Hung , F. P. Chien , S. C. Ih and D. C. Shih, *J. Nat. Prod.* **72**, 223 (2009).

[14] W. M. Hearon and R. G. Gustavson. *Ind. Eng. Chem. Anal. Ed.***9**, 352-353 (1937)

[15] R. R. Kristin and M. H. Elizabeth, *Acta Cryst*. **52**, 1565 (1996).