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Anti-Tuberculosis Coumarinolignans from *Daphne mucronata*

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Two new linearly fused coumarinolignans named mucronin-A (**1**) and mucronin-B (**2**) have been isolated from the whole plant of *Daphne mucronata* along with two other known compounds *i.e*., umbelliferone (**3**) and coumarin (**4**). The structures of the isolated compounds **1-4** were elucidated by chemical and spectroscopic methods including 1D-NMR, 2D-NMR and HR-FAB-MS. Both of the new isolated compounds **1** and **2** showed significant antituberculosis activity.

Keywords: *Daphne mucronata*, Thymlelaeaceae, Mucronin-A and B, Anti-tuberculosis activity.

INTRODUCTION

The genus *Daphne* is taxonomically placed in Thymlelaeaceae. The family Thymlelaeaceae comprises about 500 species and 15 genera as well. It is represented by five genera in Pakistan, one of which is Daphne. The natural products described in the literature as constituents of genus Daphne are coumarins[1,2], flavonoids[2-4], lignans[5], triterpenoids[2] and coumarinolignans[6]. The healing properties of the roots and leaves of the most species of *Daphne* have been known long ago, as used in traditional Chinese medicine. These were used for the treatment of toothache, ulcer and rheumatism while the bark is used as a remedy for diseases of bone. *Daphne mucronata* grows wildly in the northern areas of Pakistan[7]. Previously, ursolic acid, oleanolic acid, vergatic acid, α-amyrin, β-sitosterol, caryatin, pachypodol, agigenin, daphnerotin, umbelliferone, coumarin, *p*-hydroxybenzoic acid and vanillic acid8 have been reported from this species.

Tuberculosis (TB) is one of the leading cause of death in developing countries due to single infectious agent 'Mycobacterium tuberculosis' Pakistan ranks 8th in high burden TB countries with 291743 new cases and an incidence of 181 cases/100000/year[9]. The alarming increase of multidrug-resistant tuberculosis (MDR-TB) cases required an urgent strategy to develop new and effective antituberculosis drugs. The plant based traditional medicines continue to play an essential role in primary health care in the third world countries. In this study we investigated the antituberculosis activity of two new coumarin olignans **1** and **2** isolated from *D. mucronata* (Fig. 1).

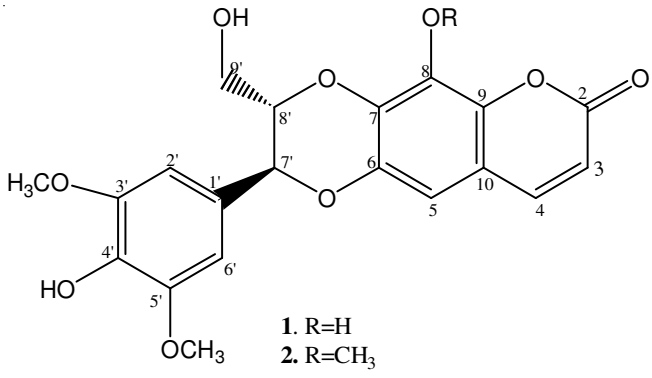


Fig. 1. Structures of Mucronins A (**1**) and B (**2**)

In the present work, pharmacological screening of the ethyl acetate soluble sub-fraction revealed the most pronounced antituberculosis activity and resulted in the isolation of two new coumarinolignans named as mucronin-A (**1**) and mucronin-B (**2**) together with umbelliferone (**3**)[10-12] and coumarin (**4**)[13], respectively. Both the compounds **1** and **2** showed significant antituberculosis activity.

EXPERIMENTAL

Optical rotations were measured on a JAsSCO DIP-360 polarimeter. IR spectra were recorded on a 460 Shimadzu spectrometer. EI-MS and HR-FAB-MS were recorded on JMS-HX-110 and JMS-DA 5000 mass spectrometers. The ^{1}H NMR, ^{13}C NMR, HMQC and HMBC spectra were recorded on Bruker spectrometers operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, respectively. The chemical shift values are reported in ppm (δ) units and the coupling constants (*J*) are in Hz. Aluminum sheets precoated with silica gel 60 F254 (20 × 20 cm, 0.2 mm thick; E-Merck, Darmstadt, Germany) were used for TLC and silica gel (230-400 mesh, E-Merck, Darmstabt, Germany) was used for column chromatography. Visualization of the TLC plates was carried out under UV at 254 and 366 nm by Hitachi UV-3200 spectrometer and also by spraying with ceric sulfate reagent (with heating).

The whole plant material of *Daphne mucronata* was collected from Gilgit (NWFP) in June, 2006 and identified by Dr. Suriya Khatoon, Plant Taxonomist, Department of Botany, University of Karachi, Pakistan; where a voucher specimen (GP-0069-06) has been deposited.

Extraction and isolation: The whole air-dried plants of *D. mucronata* (10 kg) were ground into small pieces and extracted with CH_3OH (3 × 50 L) at room temperature. The combined methanolic extract was filtered and evaporated under reduced pressure to obtain a thick gummy mass (400 g). It was suspended in water and successively extracted with *n*-hexane, ethyl acetate and *n*-butanol. The EtOAc soluble fraction (100 g) was subjected to column chromatography and eluted successively with $CHCl_3$, $CHCl_3$-MeOH and MeOH to give three fractions (A to C). The fraction A obtained from $CHCl_3$-MeOH (9.0:1.0) was a mixture of two components, which were separated by column chromatography using solvent system $CHCl_3$-MeOH (9.3:0.7) to afford compounds **3** (21 mg) and **4** (18 mg) from the top and the tail fractions, respectively. The fraction B obtained from $CHCl_3$-MeOH (8.5:1.5) was further purified by column chromatography eluting with $CHCl_3$-MeOH (8.5:1.5) to afford 1 (20 mg). The fraction C obtained from $CHCl_3$-MeOH (7.8:2.2) was re-chromatographed and eluted with $CHCl_3$-MeOH (7.8:2.2) to afford 2 (18 mg).

Mucronin-A (1): Light yellow amorphous solid; (20 mg). $[\alpha]_D^{25}$ - 97.2° (c 0.31, MeOH); UV (MeOH) λ_{max} 324, 235 (sh) and 230 nm; IR (KBr, ν_{max}, cm^{-1}): 3530, 3450, 1715, 1648, 1620 1580, 1420, 1225 and 1155; ^{1}H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) (Table-1); EI-MS (*m/z*) (rel. int.) 384 (12) $[M - 18]^+$, 210 (100), 194 (16), 167 (60), 161 (8), 121 (25), 92 (25), 77 (30); HR-FAB-MS (*m/z*) 403.1027 $[M + H]^+$ (Calcd. for $C_{20}H_{19}O_9$, 403.10291).

Mucronin-B (2): Light yellow amorphous solid; (18 mg). $[\alpha]_D^{25}$ - 98.2° (c 0.31, MeOH); UV (MeOH) λ_{max} 325 nm; IR (KBr, ν_{max}, cm^{-1}): 3530, 3450, 1710, 1648, 1610 1574, 1420, 1225 and 1155; ^{1}H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) (Table-1); EI-MS (*m/z*) (rel. int.) 398 (12) $[M - 18]^+$, 210 (100), 208 (30), 177 (29), 167 (40), 121 (18), 92 (30), 77 (28); HR-FAB-MS (*m/z*) 417.1183 $[M + H]^+$ (Calcd. for $C_{21}H_{21}O_9$, 417.11856).

Antituberculosis assay: The screening of pure compounds against the *Mycobacterium tuberculosis* was carried out by micro dilution method[9]. Briefly in a 96 well flat bottom micro-titre plate (Falcon), compounds were added in the Middle brook 7H9 complete medium (supplement with 10 % ADC and glycerol; 0.05 Tween; pH 6.8) (Difco) in appropriate volume to the concentration ranging 10-1000 µg/mL and inoculated with 100 µL culture suspension (approximately 105 cells/mL in the 200 µL final volume. Isoniaszid and Rifampin were used as control drugs. Mycobacterium tuberculosis H37Rv culture was grown in Middle brook 7H9 complete broth for 10 days (10^8 CFU/mL). Culture was diluted 1:50 in middle brook 7H9 complete broth and (100 µL/well) used to inoculate the plates. Plates were sealed and incubated at 37 °C for 2-3 weeks and MIC values were recorded as the lowest concentration of the compounds that completely inhibited the visible growth of *Mycobacterium tuberculosis*.

TABLE-1
NMR DATA OF COMPOUNDS **1** AND **2**
(IN PYRIDINE-d_5, ^{1}H 400 MHz, ^{13}C 100 MHz)

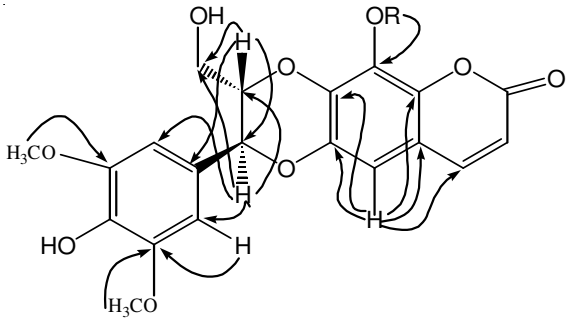
Carbon No.	**1**		**2**	
	δ_C	δ_H (Mult., *J*, Hz)	δ_C	δ_H (Mult., *J*, Hz)
2	161.6		161.4	
3	114.2	6.32 (1H, d, *J* = 9.4 Hz)	114.5	6.33 (1H, d, *J* = 9.4 Hz)
4	143.8	7.61 (1H, d, *J* = 9.4 Hz)	143.9	7.62 (1H, d, *J* = 9.4 Hz)
5	108.9	6.72 (1H, s)	110.1	6.75 (1H, s)
6	139.7		140.2	
7	138.5		138.4	
8	147.1		143.9	
9	146.4		146.6	
10	113.5		112.8	
1'	126.6		126.6	
2'	106.4	7.21 (1H, s)	106.4	7.22 (1H, s)
3'	147.9		147.9	
4'	138.5		138.5	
5'	147.9		147.8	
6'	106.4	7.19 (1H, s)	106.4	7.18 (1H, s)
7'	76.1	5.61 (1H, d, *J* = 8.15 Hz)	76.2	5.60 (1H, d, *J* = 8.15 Hz)
8'	79.3	4.43-4.38 (1H, m)	79.1	4.43-4.36 (1H, m)
9'	61.5	4.33 (1H, d, *J* = 11.6 Hz) 3.99 (1H, d, *J* = 11.6 Hz)	61.5	4.34 (1H, d, *J* = 11.6 Hz) 3.99 (1H, d, *J* = 11.6 Hz)
3'-OMe	26.5	3.88 (3H, s)	56.5	3.87 (3H, s)
5'-OMe	26.5	3.83 (3H, s)	56.5	3.82 (3H, s)
8'-OMe	--	--	61.3	4.05 (3H, s)

RESULTS AND DISCUSSION

The ethyl acetate soluble sub-fraction of the methanolic extract of the whole plant of *Daphne mucronata* was subjected to a series of column chromatographic techniques to obtain compounds **1-4** and their structures were elucidated by 1D-NMR, 2D-NMR, HR-FAB-MS, UV and IR spectroscopy. The isolated compounds were evaluated for their antituberculosis activity.

Mucronin-A (**1**) was isolated as light yellow amorphous solid. The molecular formula $C_{20}H_{18}O_9$ was established by HR-FAB-MS showing $[M + H]^+$ peak at *m/z* 403.1027 (Calcd. for $C_{20}H_{19}O_9$, 403.10291). The molecular formula was supported

by broad band (BB) and distortionless enhancement by polarization transfer (DEPT) ^{13}C NMR spectra showed the presence of two methyl, one methylene, seven methine and ten quaternary carbons. In the IR spectrum, the lactone carbonyl of a coumarin nucleus showed strong absorption bands at 1715, 1620 and 1580 cm^{-1}. The phenolic hydroxy groups absorbed around 3450 cm^{-1}. The UV spectrum showed a maxima at 324, 235 (sh) and 230 nm, which is characteristic of a coumarin skeleton[14]. In EIMS the retro-Diels-Alder fragmentation gave a distinct peak at *m/z* 194 due to trioxysubstituted coumarin skeleton. The identification of the compound **1** as a coumarin derivative was confirmed from its ^1H NMR spectrum in pyridine-d_5 at 400 MHz, which exhibited typical doublets for coumarin protons [δ 7.61 (1H, d, *J* = 9.4 Hz, H-4) and δ 6.32 (1H, d, *J* = 9.4 Hz, H-3) ppm] and a singlet in the aromatic region at δ 6.72 (1H, s, H-5) for a 6,7-dioxygenated coumarin moiety[15-22]. A singlet at δ 3.88 and 3.83 (6H, 2 × OCH_3) and another singlet at δ 7.21 and 7.19 integrating for two aromatic protons (H-2 and H-6) indicated a symmetrical 3′,5′-dimethoxy-4-hydroxyphenyl group, extended with a three carbons sequence [*i.e*, CH(O)CH(O)CH_2OH] [δ 5.61 (1H, d, *J* = 8.15, H-7′), 4.43-4.38 (1H, m, H-8′) and 4.33, 3.99 (2H, d, *J* = 11.6, Hz, H-9′) ppm]. The phenylpropanoid unit in compound **1** was also confirmed by the ^{13}C NMR [δ 76.1 (C-7′), 79.3 (C-8′) and 61.5 (C-9′)] (Table-1), ^1H-^1H cosy and HMBC NMR spectral data (Fig. 2). On the basis of this spectral data and calculation of the double-bond equivalence, compound **1** was assumed to be formed by linear fusion of a 6,7-dioxygenated coumarin moiety with a C_6-C_3 unit through a 1,4-dioxane bridge. The stereochemistry at C-7′ and C-8′ was assigned as R and S, respectively, as coupling constant between H-7′ and H-8′ and the chemical shifts of protons and carbons at H-7′, H-8′ and H-9′ were closely comparable to those of nitidanin[23] and grewin[24]. A large coupling constant value (8 Hz) showed a *trans* relationship between H-7′ and H-8′. The lone proton in coumarin skeleton was assigned to C-5 based on the HMBC correlations and thus allowing us to assign the hydroxyl group to C-8. Based on these evidences mucronin-A (**1**) was assigned the structure (2R,3S)-5-hydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-3-(hydroxymethyl)-2,3-dihydro-7*H*-[1,4]dioxino[2,3-g]chromen-7-one.



1. R= H
2. R= CH_3

Fig. 2. HMBC correlations of Mucronins A (**1**) and B (**2**)

Mucronin-B (**2**) was isolated as light yellow amorphous solid. The molecular formula $C_{21}H_{20}O_9$ was established by HR-FAB-MS showing $[M + H]^+$ peak at *m/z* 417.1183 (Calcd. for $C_{21}H_{21}O_9$, 417.11856) having twelve degree of unsaturation. The BB and DEPT ^{13}C NMR spectra showed twenty one signals comprising three methyl, one methylene, seven methine and ten quaternary carbons. Thus **2** is apparently a methylated derivative of **1**. The chemical shifts of the protons and carbons of the trioxysubstitutedphenyl and 1,4 dioxane rings were almost identical to those of **1** with minor differences observed in the signals of the coumarin moiety. This indicated methylation of phenolic group at C-8. It could be confirmed by ^3J correlation of the additional methyoxyl protons at δ 4.05 with δ 143.9 (C-8). Thus mucronin-B (**2**) could be identified as (2R,3S)-2-(4-hydroxy-3,5-dimethoxyphenyl)-3-(hydroxymethyl)-5-methoxy-2,3-dihydro-7*H*-[1,4] dioxino[2,3-g]chromen-7-one.

Both compounds **1** and **2** were assayed for antituberculosis activity by micro dilution method[25] and showed significant activities (Table-2).

TABLE-2
ANTITUBERCULOSIS ACTIVITY OF **1** AND **2**

Compounds	MIC (µg/mL)
1	62.5
2	60.5
Isoniazid	0.02

REFERENCES

1. Z. Lin-Gen, O. Seligmann, H. Lotter and H. Wagner, *Phytochemistry*, **22**, 265 (1983).
2. K. Baba, K. Takeuchi, F. Hamasaki and M. Kazawa, *Chem. Pharm. Bull. (Tokyo)*, **34**, 595 (1986).
3. K. Baba, K. Takeuchi, M. Dai and M. Kazawa, *Chem. Pharm. Bull. (Tokyo)*, **35**, 1853 (1987).
4. A. Ulubelen, B. Terem and E. Tuzlaci, *J. Nat. Prod*., **49**, 692 (1986).
5. N. Ullah, S. Ahmed, P. Mohammad, H. Rabnawaz and A. Malik, *Fitoterapia*, **70**, 214 (1999).
6. N. Ullah, S. Ahmed, P. Muhammad, Z. Ahmed, H.R. Nawaz and A. Malik, *Phytochemistry*, **51**, 103 (1999).
7. S.I. Ali and E. Nasir, Flora of West Pakistan, Vol. 12 (Thymelaceae Jointly Published by Stewart Herbarium, Gordon College, Rawalpindi, Pakistan & Department of Botany, University of Karachi, Karachi, Pakistan), pp. 1-4 (1971).
8. A. Ulubelen and N. Tan, *Fitoterapia*, **3**, 281 (1990).
9. World Health Organization, Anti-Tuberculosis Drug Resistance in the World Report No. 4 Geneva, p. 394 (2008).
10. D. Brown, R.O. Asplund and V.A. Mcmahon, *Phytochemistry*, **14**, 1083 (1975).
11. R.H. Abu-Eittah and B.A.S. El-Tawil, *Can. J. Chem*., **63**, 1173 (1985).
12. S.D. Sarker, A.I. Gray, P.G. Waterman and J.A. Armstrong, *J. Nat. Prod*., **57**, 1549 (1994).
13. Aldrich Library of ^{13}C and ^1H FT NMR Spectra, vol. 2, p. 1311B (1992).
14. P. Bhandari, P. Pant and R.P. Rastogi, *Phytochemistry*, **21**, 2147 (1982).
15. A.B. Ray, S.K. Chattopadhyay, S. Kumar, C. Konno, Y. Kiso and H. Hikino, *Tetrahedron*, **41**, 209 (1985).
16. M. Arisawa, S.S. Handa, D.D. McPherson, D.C. Lankin, G.A. Cordell, H.H.S. Fong and N.R. Farnsworth, *J. Nat. Prod*., **47**, 300 (1984).
17. A. Magalhaes, M.D.G.B. Zoghbi and A.C. Siani, *Nat. Prod. Res*., **20**, 43 (2006).
18. M.D.G. B. Zoghbi, N.F. Roque and O.R. Gottlieb, *Phytochemistry*, **20**, 180 (1981).
19. V.U. Ahmad, F. Ullah, J. Hussain, U. Farooq, M. Zubair, M.T.H. Khan and M.I. Choudhary, *Chem. Pharm. Bull. (Tokyo)*, **52**, 1458 (2004).
20. B. Sajeli, M. Sahai, R. Suessmuth, T. Asai, N. Hara and Y. Fujimoto, *Chem. Pharm. Bull. (Tokyo)*, **54**, 538 (2006).
21. B.S. Yun, I.K. Lee, I.J. Ryoo and I.D. Yoo, *J. Nat. Prod*., **64**, 1238 (2001).
22. X.F. Cheng and Z.L. Chen, *Fitoterapia*, **71**, 341 (2000).
23. T. Ishikawa, M. SEKI (nee IMAI), K. Nishigaya, Y. Miura, H. Seki, I.-S. Chen and H. Ishii, *Chem. Pharm. Bull. (Tokyo)*, **43**, 2014 (1995).
24. C. Ma, H.Z. Zhang, G.H. Tan, N.V. Hung, N.M. Cuong, D.D. Soejarto and H.H.S. Fong, *J. Nat. Prod*., **69**, 346 (2006).
25. C.H. Collin, J.M. Grange and M.D. Yates, Tuberculosis Bacteriology Organization and Practice, Drug Susceptibility Test, Butterworth Heinemann, p. 98 (1997).