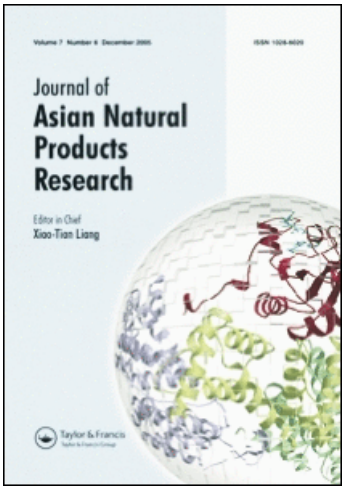

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Structural determination of daphnecin, a new coumarinolignan from *Daphne mucronata*

Muhammad Azam Rasool[a]; Rehan Khan[a]; Abdul Malik[a]; Nazia Bibi[b]; Shahana Urooj Kazmi[b]
[a] International Center for Chemical and Biological Sciences, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi, Pakistan [b] Immunology and Infectious Disease Research Laboratory, Department of Microbiology, University of Karachi, Karachi, Pakistan

Online publication date: 21 April 2010

To cite this Article Rasool, Muhammad Azam , Khan, Rehan , Malik, Abdul , Bibi, Nazia and Kazmi, Shahana Urooj(2010) 'Structural determination of daphnecin, a new coumarinolignan from *Daphne mucronata*', Journal of Asian Natural Products Research, 12: 4, 324 — 327
To link to this Article: DOI: 10.1080/10286021003610144
URL: http://dx.doi.org/10.1080/10286021003610144

Journal of Asian Natural Products Research
Vol. 12, No. 4, April 2010, 324–327

NOTE

Structural determination of daphnecin, a new coumarinolignan from *Daphne mucronata*

Muhammad Azam Rasool[a], Rehan Khan[a], Abdul Malik[a]*,
Nazia Bibi[b] and Shahana Urooj Kazmi[b]

[a]*International Center for Chemical and Biological Sciences, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi 75270, Pakistan;* [b]*Immunology and Infectious Disease Research Laboratory, Department of Microbiology, University of Karachi, Karachi 75270, Pakistan*

(*Received 19 November 2009; final version received 10 January 2010*)

Daphnecin (**1**), a new coumarinolignan, has been isolated from the ethyl acetate-soluble fraction of *Daphne mucronata* along with three known compounds, aquillochin (**2**), umbelliferone (**3**), and coumarin (**4**). Their structures were determined by spectroscopic studies.

Keywords: *Daphne mucronata*; Thymelaeaceae; coumarinolignan; daphnecin

1. Introduction

The family Thymelaeaceae comprises 15 genera and 500 species. In Pakistan, it is represented by five genera, one of which is *Daphne*. It is well known as a source of a wide range of bioactive secondary metabolites, particularly coumarins [1,2], flavonoids [2–4], lignans [5], triterpenoids [2], and coumarinolignans [6]. The roots and leaves of most species of *Daphne* are used as an abortifacient in traditional Chinese medicine. These were also used for the treatment of ulcer, rheumatism, and toothache. The bark is used as a remedy for diseases of bone. *Daphne mucronata* occurs in the northern areas of Pakistan [7]. The decoction of its leaves is used for the treatment of inflammation and arthritis. Previously, ursolic acid, oleanolic acid, vergatic acid, α-amyrin, β-sitosterol, caryatin, pachypodol, apigenin, daphnerotin, umbelliferone, coumarin, *p*-hydroxybenzoic acid, and vanillic acid [8] have been reported from this species. The chemotaxonomic and ethnopharmacological importance of the genus *Daphn*e prompted us to carry out phytochemical studies on *D. mucronata*. As a result, we herein report the isolation and structural elucidation of a new coumarinolignan named as daphnecin (**1**) (Figure 1), along with aquillochin (**2**) [9], umbelliferone (**3**) [10–12], and coumarin (**4**) [13], respectively.

2. Results and discussion

The ethyl acetate-soluble fraction of the methanolic extract of the whole plant of *D. mucronata* was subjected to a series of column chromatographic techniques to obtain compounds **1**–**4** and their structures were established by UV, IR, MS, and NMR spectroscopies.

Daphnecin was isolated as a light yellow amorphous solid, $[\alpha]_D^{25}$ +2 ($c = 0.3$ in MeOH). It gave violet

*Corresponding author. Email: abdul.malik@iccs.edu

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286021003610144
http://www.informaworld.com

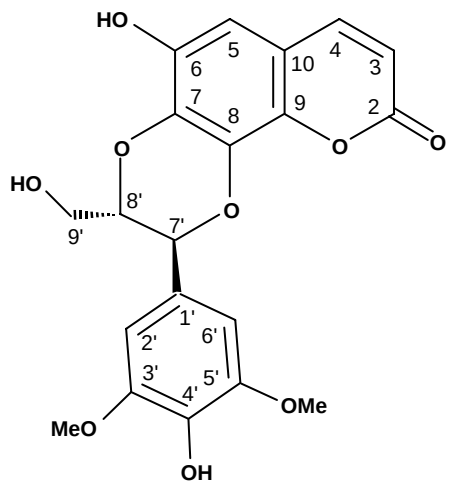

Figure 1. Structure of daphnecin (**1**).

coloration with $FeCl_3$. The molecular formula $C_{20}H_{18}O_9$ was established by HR-FAB-MS showing an $[M+H]^+$ peak at *m/z* 403.1027, having 12 degrees of unsaturation. The molecular formula was supported by broadband and distortionless polarization transfer ^{13}C NMR spectra, which showed the presence of 2 methyl, 1 methylene, 7 methine, and 10 quaternary carbons. The IR spectrum showed the presence of the hydroxyl group (3450 cm^{-1}), conjugated carbonyl (1710 cm^{-1}), C=C (1620 cm^{-1}), and $-OCH_3$ (1225 cm^{-1}). The UV spectrum showed a maximum at 324 nm, which is characteristic of a coumarin [9]. The EI-MS gave a peak at *m/z* 384 $[M^+ - H_2O]$ and the most abundant Retro-Diels-Alder fragment ion at *m/z* 210, which is common to aquillochin [9]. This was also supported by the fragment at *m/z* 167 due to the cation $C_9H_{11}O_3$ [9]. The fragment at *m/z* 194 is 14 a.m.u. lower than that of aquillochin, revealing the demethylation of the coumarin unit in daphnecin [9]. The double bond signals in the ^1H NMR spectrum at δ 7.70 (1H, d, $J = 9.5$ Hz, H-4) and 6.38 (1H, d, $J = 9.5$ Hz, H-3), the signal at δ 6.62 (1H, s), as well as the signal at δ 160.5 in the ^{13}C NMR spectrum indicated the presence of a 6,7,8-trioxygenated coumarin skeleton [14–21]. A singlet at δ 3.81 (6H, 2×OCH_3) and another singlet at δ 7.18 integrating for two aromatic protons indicated the presence of a symmetrical 3′,5′-dimethoxy-4′-hydroxyphenyl group. A three-carbon sequence, $CH(O)CH(O)CH_2OH$, was deduced by the presence of a doublet at δ 5.59 (1H, d, $J = 8.1$ Hz, H-7′), a multiplet at δ 4.43 (1H, m, H-8′), and geminally coupled methylene protons at δ 4.31 (1H, dd, $J = 12.7$, 7.5 Hz, Ha-9′) and 3.96 (1H, dd, $J = 12.7$, 3.5 Hz, Hb-9′), as well as the presence of carbon signals at δ 77.9 (C-7′), 80.1 (C-8′), and 60.8 (C-9′). The HMBC correlations (Figure 2) confirmed the presence of these groups. In the HMBC spectrum, the long-range correlations between the proton signal at δ 5.59 (H-7′) and carbon signals at δ 106.4 (C-2′) and 106.4 (C-6′) suggested that the three-carbon sequence was attached to the 3′,5′-dimethoxy-4′-hydroxyphenyl group. It could be confirmed by the prominent fragment at *m/z* 210 due to Retro-Diels-Alder fragmentation [6]. According to the molecular formula of **1**, the double bond equivalent should be 12, which indicated the presence of an additional ring beside the coumarin ring and 3′,5′-dimethoxy-4′-hydroxyphenyl moiety in the structure. Since seven oxygen

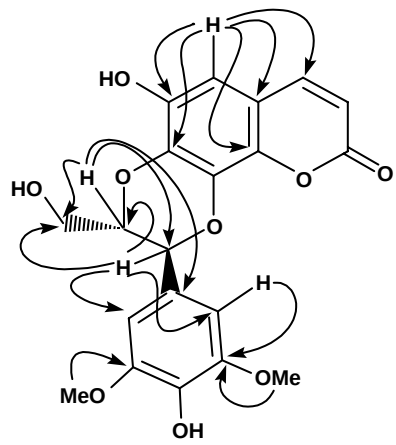

Figure 2. Important HMBC correlations of daphnecin (**1**).

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of compound **1** (C_5D_5N).

Position	δ_C	δ_H (mult., *J*, Hz)
2	160.5	–
3	113.3	6.38 (1H, d, $J = 9.5$)
4	144.5	7.70 (1H, d, $J = 9.5$)
5	101.5	6.62 (1H, s)
6	145.5	–
7	136.8	–
8	131.4	–
9	144.3	–
10	113.7	–
1′	126.6	–
2′	106.4	7.18 (1H, s)
3′	147.9	–
4′	138.5	–
5′	147.9	–
6′	106.4	7.18 (1H, s)
7′	77.9	5.59 (1H, d, $J = 7.9$)
8′	80.1	4.43 (1H, m)
9′	60.8	9′a 4.31 (1H, dd, $J = 12.7, 7.5$)
		9′b 3.96 (1H, dd, $J = 12.7, 3.5$)
3′-OMe and 5′-OMe	56.5	3.81 (3H, s)

atoms have already been accounted for, the remaining two oxygen atoms constitute the dioxane ring as in the case of aquillochin [9]. The stereochemistry at C-7′ and C-8′ was assigned as *R* and *S*, respectively, as the coupling constant between H-7′ and H-8′ and the chemical shifts of H-7′, H-8′, and H-9′, as well as their corresponding carbons were closely comparable to those of nitidanin [22] and grewin [23]. The large coupling constant (8.1 Hz) is caused by the inflexible *trans* stereochemistry between H-7′ and H-8′. The lone proton in the coumarin skeleton was assigned to C-5 based on the HMBC correlations, allowing us to assign the hydroxyl group to C-6. Based on these evidences, daphnecin was assigned the structure shown in Figure 1.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Gallenkamp apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-360 polarimeter. UV spectra were recorded on a Hitachi UV-3200 spectrophotometer. The IR spectrum was recorded on a JASCO 302-A spectrometer. The EI-MS was recorded on a Finnigan MAT 12 spectrometer and ions are given in *m/z* (%). Positive-mode FAB-MS was recorded on a Jeol JMS-DA-500 mass spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM 400 NMR spectrometer. Column chromatography was carried out using silica gel (230–400 mesh; E. Merck, Darmstadt, Germany). TLC was performed with silica gel plates (Si 60 F_{254}; E. Merck) and detection was done at 254 nm, and by spraying with ceric sulfate in 10% H_2SO_4.

3.2 Plant material

The whole plant material of *D. mucronata* Royle was collected from Gilgit District near Haramosh (NWFP) in July 2006 and identified by Prof. Dr Surraiya Khatoon, Plant Taxonomist, Department of Botany, University of Karachi, Karachi, Pakistan, where a voucher specimen (45G.H. No. 67894) has been deposited.

3.3 Extraction and isolation

The shade-dried whole plant material (10 kg) was extracted with CH_3OH (3× 50 liters) at room temperature. The combined methanolic extract was 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 eluted with $CHCl_3$, $CHCl_3$–MeOH, and MeOH in increasing order of polarity to obtain three fractions (A–C). Fraction A obtained from $CHCl_3$–MeOH (9.0:1.0) was a mixture of two components, which were separated by column chromatography using the solvent system $CHCl_3$–MeOH (9.3:0.7) to afford compounds **3** (8 mg) and **4** (10 mg) from the top and the tail fractions, respectively. Fraction B obtained from $CHCl_3$–MeOH (8.5:1.5) was further purified by column chromatography eluted with $CHCl_3$–MeOH (8.5:1.5) to afford compound **1** (6 mg). Fraction C obtained from $CHCl_3$–MeOH (7.8:2.2) was rechromatographed and eluted with $CHCl_3$–MeOH (7.8:2.2) to afford **2** (5 mg).

3.3.1 Daphnecin (*1*)

Light yellow amorphous solid; $[\alpha]_D^{25}$ +2 ($c = 0.3$ in MeOH); UV (MeOH) λ_{max} (nm): 324; IR (KBr) ν_{max} (cm^{-1}): 3450, 1710, 1620, 1574, 1420, 1225, 1155; ^1H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) spectral data, see 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 $C_{20}H_{19}O_9$, 403.1029).

References

[1] L.G. Zhoung, S. Otto, L. Hermann, and W. Hildebert, *Phytochemistry* **22**, 265 (1983).

[2] K. Baba, K. Takeuchi, F. Hamasaki, and M. Kozawa, *Chem. Pharm. Bull.* **34**, 595 (1986).

[3] K. Baba, K. Takeuchi, M. Dai, and M. Kozawa, *Chem. Pharm. Bull.* **35**, 1853 (1987).

[4] A. Ulubelen, B. Terem, and E. Tuzlaci, *J. Nat. Prod.* **49**, 692 (1986).

[5] N. Ullah, S. Ahmed, P. Muhammd, Z. Ahmed, H.R. Nawaz, and A. Malik, *Fitoterapia* **70**, 214 (1999).

[6] N. Ullah, S. Ahmed, P. Muhammd, Z. Ahmed, H.R. Nawaz, and A. Malik, *Phytochemistry* **51**, 103 (1999).

[7] S.I. Ali and E. Nasir, *Flora of West Pakistan* (Khurshed Printers, Islamabad, 1971), Vol. 12, p. 1.

[8] A. Ulubelen and N. Tan, *Fitoterapia* **3**, 281 (1990).

[9] P. Bhandari, P. Pant, and R.P. Rastogi, *Phytochemistry* **21**, 2147 (1982).

[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**, 1175 (1985).

[12] S.D. Sarker, A.I. Gray, and P.G. Waterman, *J. Nat. Prod.* **57**, 1549 (1994).

[13] Aldrich Library of ^{13}C and ^1H FT NMR Spectra **2**, 1311B (1992).

[14] A.B. Ray, S.K. Chattopahyay, and S. Kumar, *Tetrahedron* **41**, 209 (1985).

[15] M. Arisawa, S.S. Handa, D.D. Mcphherson, D.C. Lankin, G.A. Cordell, H.H.S. Fong, and N.R. Rarnsworth, *J. Nat. Prod.* **47**, 300 (1984).

[16] A. Magalhaes, M.D.G.B. Zoghbi, and A.C. Siani, *Nat. Prod. Res.* **20**, 43 (2006).

[17] M.D.G.B. Zoghbi, N.F. Rooque, and O.R. Gottlieb, *Phytochemistry* **20**, 180 (1981).

[18] V.U. Ahmad, F. Ullah, J. Hussain, U. Farooq, M. Zubair, M.T.H. Khan, and M.I. Chaudhary, *Chem. Pharm. Bull.* **52**, 1458 (2004).

[19] B. Sajeli, M. Sahai, R. Suessmuth, T. Asai, N. Hara, and Y. Fujimoto, *Chem. Pharm. Bull.* **54**, 538 (2006).

[20] B.S. Yun, I.K. Lee, I.J. Ryoo, and I.D. Yoo, *J. Nat. Prod.* **64**, 1238 (2001).

[21] X.F. Cheng and Z.L. Chem, *Fitoterapia* **71**, 341 (2000).

[22] T. Ishikawa, M. Seki, K. Nishigaya, Y. Miura, H. Seki, I. Chem, and H. Ishii, *Chem. Pharm. Bull.* **43**, 2014 (1995).

[23] 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).