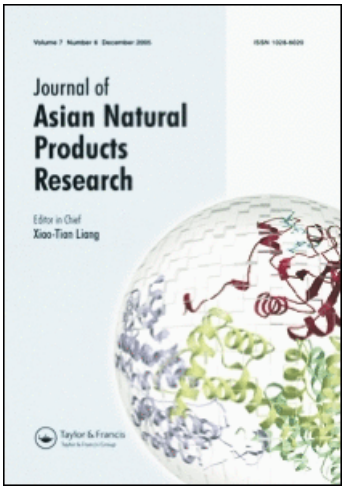

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Two new coumarins from *Murraya paniculata*

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Two new coumarins, murrmeranzin (**1**) and murralonginal (**2**), together with four known compounds minumicrolin (**3**), murrangatin (**4**), meranzin hydrate (**5**) and hainanmurpanin (**6**) have been isolated from the aerial parts of *Murraya paniculata*. The structures of these compounds were determined through spectral analysis. Minumicrolin (**3**) showed mild butyrylcholinesterase inhibition activity.

Keywords: *Murraya paniculata*; Rutaceae; minumicrolin; butyrylcholinesterase inhibitor; murrmeranzin; murralonginal

1. Introduction

The leaves, barks, and roots of *Murraya paniculata* have traditionally found wide medicinal uses in South East Asia and China. A decoction of the leaves is a remedy for stomach ache, chronic dysentery, bruises and a wash against certain fungoid skin troubles.[1] In China, the root bark is used as an anodyne or local anesthetic for the treatment of gout, contusion and bone ache.[2] Continuous interest in the chemical constituents of *Murraya paniculata* have resulted in the isolation of several alkaloids, coumarins and flavones from fruits, flowers, leaves and root barks.[3–8] Present study resulted in the isolation of six coumarins, of which, two coumarins were found to be new.

2. Results and discussion

Murrmeranzin (**1**) was obtained as colourless oil. The HR-FAB-MS (pos) showed $[M + H]^+$ ion at *m/z* 537.2142, in agreement with the molecular formula $C_{30}H_{32}O_9$. It has an oxygen bridge linking C-2′ of murrangatin and C-2‴ of meranzin hydrate. The ^{1}H and ^{13}C NMR and HMQC spectra provided evidence that **1** possessed three methyl, two methoxy, two methylene, three oxymethine, eight methine, 10 quaternary and two lactone carbonyl carbons. The ^{1}H NMR spectrum exhibited four pairs of AB doublets at δ 7.86 and 6.23 (each 1H, $J = 9.4$ Hz), 7.85 and 6.22 (each 1H, $J = 9.4$ Hz), 7.52 and 7.03 (each 1H, $J = 8.7$ Hz), and 7.47 and 7.02 (each 1H, $J = 8.6$ Hz) and two singlets at δ 3.94 and 3.93 indicating the presence of two 7-methoxy-8-substituted coumarin moieties in the molecule. Furthermore, the ^{1}H NMR spectrum showed two doublets at δ 5.34 and 4.83 (each 1H, $J = 8.7$ Hz) indicating the presence of two vicinal oxymethine protons. The signals at δ 1.63 (3H, s), 4.63 (1H, s) and 4.53 (1H, s) corresponded to the presence of an allyl methyl and exomethylene protons. Three double doublets at δ 3.66 ($J = 9.7$, 3.1 Hz), 3.02 ($J = 13.5$, 9.7 Hz) and 2.99 ($J = 13.5$, 3.1 Hz) revealed the vicinal coupling of an oxymethine proton to methylene protons. Two singlets at δ 1.29 and 1.27 were attributable to two methyl groups attached to an oxygenated quaternary carbon.

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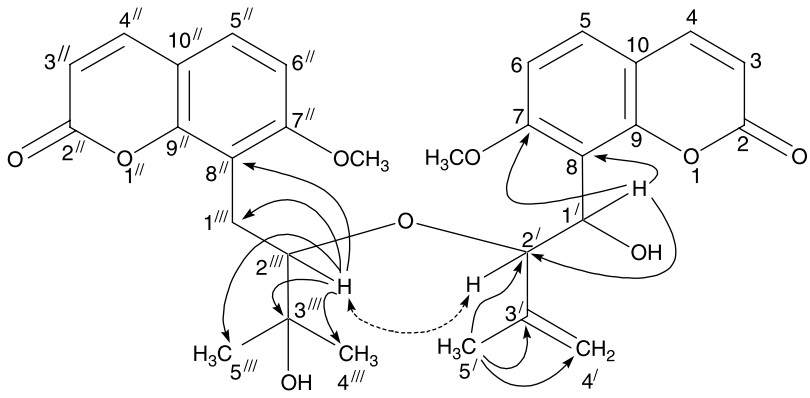

Figure 1. Important HMBC (plain arrow) and NOESY (dashed arrow) correlation of **1**.

The HMBC spectrum showed long-range correlations between H_3-5′ with C-2′, C-4′, and C-3′, and H-1′ with C-2′, C-8, C-3′, C-9 and C-7 indicating that one moiety is murrangatin. In addition, the HMBC correlations of H-2‴ with C-1‴, C-3‴ and C-8″, and H_3-4‴ with C-5‴ and C-3‴ corroborated the presence of meranzin hydrate as another moiety. Finally, the linkage between two coumarin units was characterized from the NOESY spectrum, which indicated the correlation between H-2′ and H-2‴.

This was further confirmed through the downfield shift of the ^{13}C NMR values of C-2′ and C-2‴ and also from EI-MS which showed the peaks at *m/z* 277 $[M - C_{15}H_{15}O_4]^+$ and 259 $[M - C_{15}H_{17}O_5]^+$ indicating the cleavage of two monomeric units that forms the dimer. All these assignments indicated that compound **1** was a composite of murrangatin and meranzin hydrate linked to each other by an ether linkage at the C-2′–C-2‴ positions. Thus, it was concluded that **1** was a coumarin dimer, and its structure was determined as 7,7″-dimethoxy-8,8″-(1′,3‴-dihydroxy-3′-methyl-3′-butenyl-3‴-methylbutyl)-2′-*O*-2‴-bi-2H-1-benzopyran-2-one.

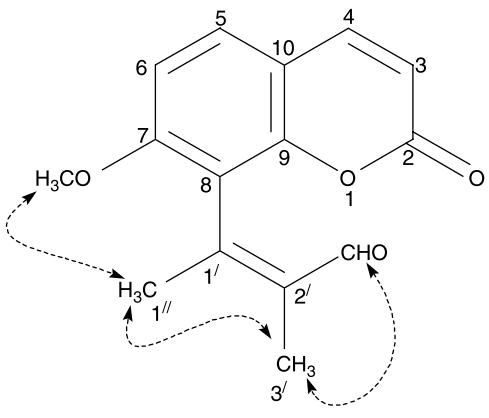

Figure 2. Key NOESY correlations of **2**.

Murralonginal (**2**) was isolated as colourless oil. The molecular formula $C_{15}H_{14}O_4$ was established by the analysis of HRF-AB-MS (pos). The UV absorption maxima at 321 and 222 nm, IR bands at 1720, 1670, and 1600 cm^{-1}, and ^{1}H NMR signals at δ 3.80 (OCH_3), 7.61 and 6.19 (each 1H, $J = 9.4$ Hz), and 7.42 and 6.87 (each 1H, $J = 8.6$ Hz) were consistent with a 7-methoxy-8-substituted coumarin skeleton.[9] Strong IR bands at 1730 and 1670 cm^{-1} indicated the presence of an isolated carbonyl group and a carbon–carbon double bond in addition to a carbonyl and aromatic double bonds in the coumarin nucleus. The ^{1}H NMR spectrum of **2** showed a singlet for one proton at δ 10.19 and two singlets at δ 2.40 and 1.76 attributable to the presence of an aldehyde and two methyl groups attached to quaternary carbons.

The NOESY spectrum revealed the correlations of methyl group (δ 2.40) to methoxy group (δ 3.80) of coumarin nucleus, H-3′ (δ 1.76) and the aldehyde group

Table 1. *In vitro* quantitative inhibition against AChE and BChE by compounds **3**, **4** and **5**.

Compounds	% Inhibition		$IC_{50}(\mu M) \pm SEM$[a]	
	AChE	BChE	AChE	BChE
3	19.00	67.77	Inactive	380.3 ± 2.94
4	15.77	30.98	Inactive	–
5	39.04	38.36	Inactive	–
Galanthamine[b]	–	–	0.5 ± 0	8.7 ± 0.01
Eserine[c]	–	–	0.4 ± 0	0.91 ± 0

[a] SE of the mean of five assays.
[b,c] Standard inhibitors of the acetylcholinesterase and BchE.

(δ 10.19), which confirmed the attachment of methyl group to C-1′ and aldehyde group to C-2′ in the side chain. In view of the above spectral evidences, the structure of murralonginal was established as 8-(1′-methyl-2′-aldehyde-1′-propenyl)-7-methoxy-2H-1-benzopyran-2-one (**2**).

The known compounds minumicrolin (**3**),[10] murrangatin (**4**),[5] meranzin hydrate (**5**),[6] and hainanmurpanin (**6**)[6] were readily identified by the comparison of their spectral data with those from the literature.

In the course of this work, compounds **3**–**5** were evaluated against AChE and BChE inhibiting activities (Figure 1, Figure 2). Their percentage of inhibition and IC_{50} values are shown in Table 1 along with the activities of the positive controls galanthamine and eserine. Compounds **4** and **5** exhibited weak inhibitory activities whereas **3** showed mild inhibitory activity against BChE and all these compounds were inactive against AChE.

3. Experimental

3.1 General experimental procedures

UV spectra were recorded in methanol on Shimadzu UV-160A, UV–visible spectrophotometer. IR spectra were measured on JASCO 302-A Infrared spectrophotometer. ^{1}H NMR spectra were recorded at 400 and 500 MHz and the ^{13}C NMR spectra at 75 and 100 MHz on Bruker AM-300, and AM-400 nuclear magnetic resonance spectrometers using $SiMe_4$ as an internal standard. Mass spectra were recorded on a Varian MAT 312 double focusing mass spectrometer connected to DEC PDP 11/34 computer system. Column chromatography was performed on silica gel (SiO_2 60, 70–230 mesh, E. Merck). Precoated silica gel GF_{254} preparative plates (20 × 20, 0.5 mm thick; E. Merck) were used for preparative thick-layer chromatography. Purity of the samples was also checked on the same pre-coated plates.

3.2 Plant material

The aerial parts of *Murraya paniculata* were collected from the Karachi University campus in August 2003 and identified by Dr Rubina Dawar, Assistant Professor, Department of Botany, University of Karachi. A voucher specimen (G.H. No. 67974) has been deposited in the herbarium of the department.

3.3 Extraction and isolation

The shade dried aerial parts of *Murraya paniculata* (1.5 kg) were soaked in ethanol and extracted three times at room temperature. The ethanolic extract (276 g) was suspended in water and extracted with *n*-hexane, ethyl acetate and *n*-butanol, successively. The ethyl acetate extract was subjected to CC (silica gel; *n*-hexane, ethyl acetate, and methanol) yielding nine fractions (Fr. A–I). Fraction D (3 g) was subjected to CC (silica gel; *n*-hexane to *n*-hexane–ethyl acetate to ethyl acetate–methanol, 9:1) yielding four subfractions (Fr. D_1–D_4). Subfraction D_2 exhibited two major spots

and was purified through preparative TLC (chloroform–methanol, 9.5:0.5, two developments). The faster moving band yielded 0.05 g of **3**. Fraction F (4.5 g) was subjected to CC (silica gel; *n*-hexane to *n*-hexane–ethyl acetate to ethyl acetate–methanol, 8:2, in increasing order of polarity) yielding five subfractions (Fr. F_1–F_5). Subfraction F_3 (*n*-hexane–ethyl acetate, 2:8, eluted) showed one major spot and was purified through preparative TLC (chloroform–methanol, 9.5:0.5) to give 0.4 g of **1**. Fraction G (5.6 g) was rechromatographed (silica gel; *n*-hexane–chloroform, chloroform–methanol, 8:2, in increasing order of polarity) yielding nine subfractions (Fr. G_1–G_9). Subfraction G_3 (*n*-<?twb.32#>hexane–ethyl acetate, 7:3 eluted) was purified through preparative TLC (chloroform–methanol, 9.5:0.5) furnishing 0.4 g of **4**. Another fraction G_4 (*n*-hexane–ethyl acetate, 6:4) contained crystals, which was filtered and purified by preparative TLC (chloroform–methanol, 9.5:0.5) to afford 1.25 g of **5**. Fraction G_5 (*n*-hexane–ethyl acetate, 4:6) showed one major spot and was purified by preparative TLC to yield 0.2 g of **6**. Fraction H (3.6 g) after being subjected to CC afforded six subfractions (Fr. H_1–H_6). Subfraction H_4 (*n*-hexane–ethyl acetate, 7.5:2.5 eluted) contained two major spots and was purified through preparative TLC (*n*-hexane–ethyl acetate, 1:9) to furnish 0.03 g of **2**.

3.3.1 *Murrmeranzin (**1**)*

Colourless oil. UV (MeOH) λ_{max} (log ε): 320 (4.29), 221 (4.17). IR ν_{max} (cm^{-1}): 3443 (OH), 1720 (C=O), 1607, 1566 (C=C). ^{1}H NMR (CD_3OD, 500 MHz) δ: 7.86 (1H, d, $J = 9.4$ Hz, H-4), 7.85 (1H, d, $J = 9.4$ Hz, H-4″), 7.52 (1H, d, $J = 8.7$ Hz, H-5), 7.45 (1H, d, $J = 8.6$ Hz, H-5″), 7.03 (1H, d, $J = 8.7$ Hz, H-6), 7.02 (1H, d, $J = 8.6$ Hz, H-6″), 6.23 (1H, d, $J = 9.4$ Hz, H-3), 6.22 (1H, d, $J = 9.4$ Hz, H-3″), 5.34 (1H, d, $J = 8.7$ Hz, H-1′), 4.83 (1H, d, $J = 8.7$ Hz, H-2′), 4.63 (1H, s, H-4′a), 4.53 (1H, s, H-4′b), 3.94 (3H, s, OCH_3), 3.93 (3H, s, OCH_3″), 3.66 (1H, dd, $J = 9.7$, 3.1 Hz, H-2‴), 3.02 (1H, dd, $J = 13.5$, 9.7 Hz, H-1a‴), 2.99 (1H, dd, $J = 13.5$, 3.1 Hz, H-1b‴), 1.63 (3H, s, H-5′), 1.29 (3H, s, H-5‴), 1.27 (3H, s, H-4‴). ^{13}C NMR (CD_3OD, 100 MHz) δ: 163.7 (C, C-7), 162.7 (C, C-7″), 162.5 (C, C-2), 162.2 (C, C-2″), 154.7 (C, C-9), 154.2 (C, C-9″), 146.4 (CH, C-4), 146.3 (C, C-3′), 146.2 (CH, C-4″), 130.3 (CH, C-5″), 128.4 (CH, C-5), 117.9 (C, C-8″), 117.2 (C, C-8), 114.4 (C, C-10″), 114.3 (C, C-10), 113.7 (CH_2, C-4′), 113.3 (CH, C-3″), 113.0 (CH, C-3), 109.6 (CH, C-6″), 109.0 (CH, C-6), 79.2 (CH, C-2‴), 78.8 (CH, C-2′), 74.1 (C, C-3‴), 69.9 (CH, C-1′), 56.8 (OCH_3″), 56.7 (OCH_3), 26.3 (CH_2, C-1‴), 25.6 (CH_3, C-5‴), 25.5 (CH_3, C-4‴), 17.6 (CH_3, C-5′). EI-MS *m/z* (rel. int. %): 478 [M-C_3H_7O]$^+$(2), 277 (26), 261 (71), 259 (5), 244 (10), 219 (41), 205 (29), 189 (93), 177(100), 175 (14), 144 (10), 131 (38), 59 (31). HRF-AB-MS (pos) *m/z*: 537.2142 [M + H]$^+$ (calcd for $C_{30}H_{33}O_9$, 537.2125).

3.3.2 *Murralonginal (**2**)*

Colourless oil. UV (MeOH) λ_{max} (log ε): 321 (4.11), 222 (4.05) nm. IR ν_{max} (cm^{-1}): 3490 (overtone of C=O), 1720 (C=O), 1670 (C=C), 1600 (aromatic C=C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 10.19 (1H, s, CHO), 7.61 (1H, d, $J = 9.4$ Hz, H-4), 7.42 (1H, d, $J = 8.6$ Hz, H-5), 6.87 (1H, d, $J = 8.6$ Hz, H-6), 6.19 (1H, d, $J = 9.4$ Hz, H-3), 3.80 (3H, s, OCH_3), 2.40 (3H, s, H-1″), 1.76 (3H, s, H-3′). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 188.8 (CHO), 161.1 (C, C-7), 160.0 (C, C-2), 159.6 (C, C-2′), 152.5 (C, C-9), 143.6 (CH, C-4), 129.2 (C, C-1′), 128.6 (CH, C-5), 113.2 (CH, C-3), 112.9 (C, C-10), 111.7 (C, C-8), 107.6 (CH, C-6), 56.2 (OCH_3), 24.9 (CH_3, C-3′), 19.8 (CH_3, C-1″). EI-MS *m/z* (rel. int. %): 258 [M]$^+$(15), 227 (6), 215 (16), 214 (9), 203 (74), 202 (5), 199 (7), 189 (18), 175 (19), 83 (56). HRF-AB-MS (pos) *m/z*: 259.0972 [M + H]$^+$ (calcd for $C_{15}H_{15}O_4$, 259.0970).

3.4 In vitro *cholinesterase inhibition assay*

Electric-eel acetylcholinesterase (EC 3.1.1.7), horse-serum butyrylcholinesterase

(BchE; E.C 3.1.1.8), acetylthiocholine iodide, butyrylthiocholine chloride, 5,5′-dithiobis [2-nitrobenzoic acid], and galanthamine were purchased from Sigma (St Louis, MO, USA). All other chemicals were of analytical grade. Acetylcholinesterase and BchE inhibiting activities were measured by the spectrophotometric method developed by Ellman *et al.*[11] Compounds **3**–**5**, and the positive control (galanthamine and eserine) were dissolved in EtOH. All the reactions were performed in triplicate in 96-well microplate in *SpectraMax 340* (Molecular Devices, USA). The percentage (%) of inhibition was calculated as follows $(E - S)/E \times 100$, where, E is the activity of the enzyme without test compound and S is the activity of enzyme with test compound.

3.4.1 *Determination of IC_{50} values*

The concentrations of test compounds that inhibited the hydrolysis of substrates (acetylthiocholine and butyrylthiocholine) by 50% (IC_{50}) were determined by monitoring the effect of increasing concentrations of these compounds in the assays on the inhibition values. The IC_{50} values were then calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, USA).

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