

7-Methoxy-8-Prenylated Coumarins from *Murraya koenigii* (Linn.) Spreng.

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ABSTRACT: Four coumarin derivatives were isolated from the methanol extract of stem bark of *Murraya koenigii* (Linn.) Spreng. Extensive spectroscopic studies, including high field NMR analyses allowed to identify these compounds as meranzin hydrate (1), epoxyosthol (2), isomeranzin (3) and murracarpin (4). The identity of the compounds was confirmed by comparison with published data as well as co-TLC with authentic samples. This is the first report of occurrence of meranzin hydrate (1), epoxyosthol (2) and isomeranzin (3) from *M. koenigii*.

Key words: *Murraya koenigii*, Rutaceae, meranzin hydrate, epoxyosthol, isomeranzin, murracarpin.

INTRODUCTION

Murraya koenigii (Linn.) Spreng. (Bengali name- Chotokamini; Family- Rutaceae) is more or less a deciduous small tree up to 6 meters in height and widely distributed throughout Bangladesh.¹ The plant has a short trunk of 15-40 cm in diameter² and the main stem is dark green to brownish in colour.³ *M. koenigii* is a medicinal plant, various parts of which have been traditionally used in diabetes, skin eruptions, poisonous bites, febrifuge and dysentery.⁴ It has been medicinally important for its antimicrobial, anthelmintic, anti-inflammatory and hypoglycemic properties.⁵ Previous phytochemical studies with the alcohol extract of stem bark of *M. koenigii* provided koenigine-quinone A, koenigine-quinone B⁶, 9-carbethoxy-3-methylcarbazole, 9-formyl-3-methylcarbazole⁷, methyl-2-methoxy-carbazole-3-carboxylate and 1-hydroxy-3-methylcarbazole.⁸ Mukonal, a biogenetic intermediate of pyranocarbazole alkaloid was also detected in the

stem bark of *M. koenigii*.⁸ In addition, alkaloids, coumarin and cinnamic acid derivative from the plant have also been previously isolated and characterized.⁹ Marmesin-1'-O- β -D-galactopyranoside, osthol and umbelliferone¹⁰ and 3-(1,1-dimethylallyl) xanthyletin have also been isolated from petroleum ether extract of the stem bark of *M. koenigii*.¹¹

As part of our ongoing studies with medicinal plants of Bangladesh^{9, 12-16}, the stem bark of *M. koenigii* was subjected to chemical investigation and we, herein, describe the isolation and structure elucidation of meranzin hydrate (1), epoxyosthol (2), isomeranzin (3) and murracarpin (4), where epoxyosthol (2) and coumarins 1-3 have also been reported from *M. koenigii* for the first time.

MATERIALS AND METHODS

General experimental procedures. ¹H NMR spectra were acquired on Ultra Shield Bruker 400 NMR instrument, using CDCl₃ and the chemical shifts are reported in ppm with respect to TMS or

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residual non deuterated solvent signals. All solvents and reagents are of highest analytical grade.

Plant material. Stem bark of *M. koenigii* were collected from Curzon Hall area, University of Dhaka, Bangladesh, in November 2014. The plant was taxonomically identified in Bangladesh National Herbarium, Dhaka, where a voucher specimen (DACB no. 43870) for the plant has been maintained for future reference. The stem bark was first sun dried and then ground into coarse powder.

Extraction and isolation. The air dried and powdered stem bark (1.0 kg) was soaked in 3.0 L methanol for 15 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was concentrated with a rotary evaporator. A portion (5g) of the concentrated methanol extract was partitioned by the modified Kupchan partitioning protocol²¹ into petroleum ether (0.65 g), dichloromethane (0.55 g), ethyl acetate (0.30 g) and aqueous (2.5 g) soluble material.

The dichloromethane soluble partitionate was subjected to gel permeation chromatography over Lipophilic Sephadex LH-20 using *n*-hexane-dichloromethane-methanol (2:5:1) and a total of 30 fractions were collected, each of 20 ml. On the basis of their TLC behavior, fraction 14, 19 and 25 were subjected to preparative thin layer chromatography (PTLC) over silica gel (Kieselgel F₂₅₄) using mobile phase comprising of 40% ethyl acetate in toluene to yield meranzinhydrate (**1**), epoxyosthol (**2**), isomeranzin (**3**) and murracarpin (**4**).

Properties of isolated compounds

Meranzin hydrate (1): colourless mass; ¹H NMR (400 MHz, CDCl₃): δ 1.32 (3H, s, 5'-Me), 1.33 (3H, s, 4'-Me), 2.99 (1H, d, *J* = 13.6 Hz, H-1'), 3.11 (1H, d, *J* = 13.2 Hz, H-1'), 3.64 (1H, d, *J* = 10 Hz, H-2'), 3.93 (3H, s, 7-OMe), 6.25 (1H, d, *J* = 9.6 Hz, H-3), 6.87 (1H, d, *J* = 8.4 Hz, H-6), 7.35 (1H, d, *J* = 8.4 Hz, H-5), 7.63 (1H, d, *J* = 9.6 Hz, H-4).

Epoxyosthol (2): white crystalline mass; ¹H NMR (400 MHz, CDCl₃): δ 1.28 (3H, s, 5'-Me), 1.49 (3H, s, 4'-Me), 3.02 (2H, m, H₂-1'), 3.17 (1H, brd, *J* = 7.6 Hz, H-2'), 3.93 (3H, s, 7-OMe), 6.23 (1H, d, *J* = 9.2 Hz, H-3), 6.87 (1H, d, *J* = 8.4 Hz, H-6), 7.35

(1H, d, *J* = 8.4 Hz, H-5), 7.63 (1H, d, *J* = 9.2 Hz, H-4).

Isomeranzin (3): colourless mass; ¹H NMR (400 MHz, CDCl₃): δ 1.20 (6H, d, *J* = 6.8 Hz, H-4' and H-5'), 2.81 (1H, m, H-3'), 3.86 (3H, s, 7-OMe), 4.00 (2H, s, H₂-1'), 6.22 (1H, d, *J* = 9.6 Hz, H-3), 6.84 (1H, d, *J* = 8.8 Hz, H-6), 7.37 (1H, d, *J* = 8.8 Hz, H-5), 7.62 (1H, d, *J* = 9.6 Hz, H-4).

Murracarpin (4): colourless mass; ¹H NMR (400 MHz, CDCl₃): δ 1.68 (3H, s, 5'-Me), 3.32 (3H, s, 1'-OMe), 3.92 (3H, s, 7-OMe), 4.63 (1H, brs, H-4'), 4.69 (1H, brs, H-4'), 4.92 (1H, d, *J* = 8.8 Hz, H-2'), 5.04 (1H, d, *J* = 8.8 Hz, H-1'), 6.25 (1H, d, *J* = 9.6 Hz, H-3), 6.85 (1H, d, *J* = 8.8 Hz, H-6), 7.39 (1H, d, *J* = 8.8 Hz, H-5), 7.61 (1H, d, *J* = 9.6 Hz, H-4).

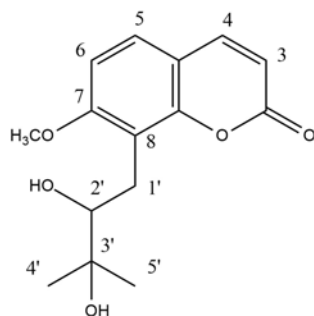
RESULTS AND DISCUSSION

A total of four compounds (**1-4**) were isolated from dichloromethane soluble partitionate of methanol extract of stem bark of *M. koenigii* by gel permeation chromatography over lipophilic Sephadex LH-20 followed by preparative thin layer chromatography (PTLC) using silica gel (Kieselgel F₂₅₄). The structure of the isolated compounds was solved by extensive analyses of their high resolution ¹H-NMR spectroscopic data as well as by comparison with published values.

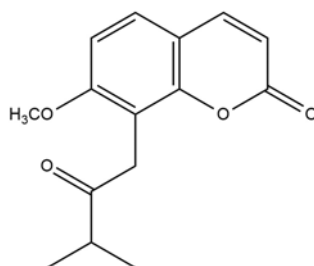
The ¹H NMR (400 MHz, CDCl₃) spectrum of compound **1** demonstrated the presence of 7-methoxy-8-substituted coumarin type carbon skeleton for which two sets of AB doublets at δ 7.63 (*J* = 9.6 Hz) and 6.25 (*J* = 9.6 Hz) and at δ 7.35 (*J* = 8.4 Hz) and 6.87 (*J* = 8.4 Hz) and a methoxy singlet at δ 3.93 were observed. Furthermore, the presence of a gem-dimethyl group of hydroxy-isopropyl substituent was revealed by two singlets at δ 1.32 and 1.33 ppm. The spectrum also displayed two doublets for methylene proton at δ 3.11 (*J* = 13.4 Hz) and 2.99 (*J* = 13.4 Hz) and another doublet for the methine at δ 3.64 (*J* = 10 Hz). Therefore, the side chain at C-8 position was readily elucidated as -CH₂CH(OH)C(CH₃)₂OH. These spectral features are in close agreement to that published for meranzin hydrate¹⁷. Thus, compound **1** was characterized as

meranzin hydrate, which was further confirmed by co-TLC with an authentic sample. Although meranzin hydrate (**1**) has previously been isolated

from many plants belonging to the family Rutaceae¹⁷, this is the first report of its occurrence from *Murraya koenigii*.

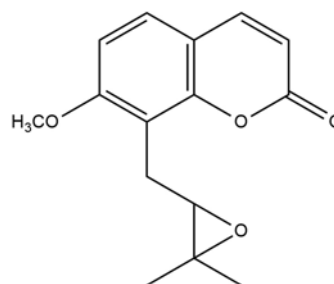


Meranzin hydrate (**1**)

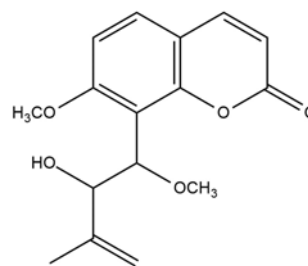


Isomeranzin (**3**)

The ¹H NMR (400 MHz, CDCl₃) spectrum of compound **2** showed two AB quartets with proton signals centered at δ 6.23 (d, 1H, *J*=9.2 Hz), 7.63 (d, 1H, *J*= 9.2 Hz), 7.35 (d, 1H, *J*= 8.4 Hz) and 6.87 (d, 1H, *J*= 8.4 Hz) which could be attributed to H-3, H-4, H-5 and H-6, respectively. The broad doublet that integrated for one proton at δ 3.17 (*J*= 7.6 Hz) could be assigned to H-2' of the epoxy ring. The multiplets of two proton intensity at 3.02 ppm was ascribed to the protons of the methylene group at C-1' position. A singlet of three protons intensity at δ 3.93 could be assigned to the methoxyl group at C-7 of the coumarin skeleton. Another three proton singlet at δ 1.49 was assigned to the methyl group at C-4' position which is nearer to the oxygen atom of the epoxy ring. The second methyl signal at δ 1.28 ppm could be attributed to the remaining methyl protons at C-5' position, which is away from the epoxy ring. On this basis, compound **2** was characterized as



Epoxyosthol (**2**)



Murracarpin (**4**)

epoxyosthol and its identity was further confirmed by comparing its ¹H NMR spectral data to that of the published values¹⁸. This is the first report of the isolation of epoxyosthol (**2**) from *M. koenigii*.

The ¹H NMR (400 MHz, CDCl₃) spectrum of compound **3** displayed proton signals at δ 6.22 (d, 1H, *J*=9.6 Hz), 7.62 (d, 1H, *J*= 9.6 Hz), 7.37 (d, 1H, *J*= 8.8 Hz) and δ 6.84 (d, 1H, *J*= 8.8 Hz) assignable to H-3, H-4, H-5 and H-6, of a coumarin type carbon skeleton, respectively. The singlet that integrated for two protons at δ 4.00 ppm could be assigned to the C-1' methylene protons of the 3'-methyl-2'-oxobutyl side chain at C-8. The multiplet of one proton intensity at δ 2.81 ppm was accounted for the proton at C-3' position. The methoxyl protons at C-7 resonated at δ 3.86 ppm. A doublet that integrated for six protons at δ 1.20 (*J*=6.8 Hz) could be assigned to the methyl groups at C-3' position. On the basis of the above spectral data, compound **3** was identified as

isomeranzin¹⁹, which has been further confirmed by co-TLC with a previously isolated sample. This is also the first report of its occurrence from this plant.

The ¹H NMR (400 MHz, CDCl₃) spectrum of compound **4** showed an AB pattern with proton resonances at δ 6.25 (d, 1H, *J*= 9.6 Hz), 7.61 (d, 1H, *J*= 9.6 Hz), 7.39 (d, 1H, *J*= 8.8 Hz) and 6.85 (d, 1H, *J*= 8.8 Hz) for H-3, H-4, H-5 and H-6, respectively of the coumarin nucleus. The doublets that integrated for one proton each at δ 5.04 (*J*= 8.8 Hz) and 4.92 (*J*= 8.8 Hz) were assigned to H-1' and H-2' position of the hydrocarbon chain, respectively. Two singlets, each of three proton intensity, at δ 3.92 and 3.32 could be demonstrated to the methoxy group at C-7 of the coumarin skeleton and to the aliphatic methoxyl group at C-1' position of the hydrocarbon chain, respectively. Another singlet for three protons at δ 1.68 ppm was assigned to the methyl protons at C-5' of the side chain C-8. Two broad singlets, each of one proton intensity, at δ 4.63 and 4.69 can be accounted for the protons of the exo-methylene group at C-4' position.

Thus, the structure of compound **4** was solved as murracarpin which was further supported by comparing its ¹H NMR spectral data with reported values²⁰ as well as by co-TLC with an authentic sample. Murracarpin (**4**) has previously been reported from *M. koenigii*.

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