

**NOTE**

## PHYTOCHEMICAL STUDY ON THE ETHYLACETATE EXTRACT OF THE LEAVES OF *Mesua ferrea* LINN

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### ABSTRACT

*Furano-naphthal-hydroxy cyclohexyl type of compound was isolated first times in plant from the ethyl extracts of the leaves of Mesua ferrea. The structure of the compound has been established by the modern spectroscopic techniques such as UV, IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and mass spectroscopy and identified as 12, 13-furano-8-hydroxy naphyl-6-O-β-2',3',4',6' tetrahydroxy-5',5' dimethyl cyclohexyl ether.*

**Keywords:** *Mesua ferrea Linn; Euphorbiaceae; Medicinal plant; new compound; spectral analysis.*

### INTRODUCTION

*Mesua ferrea*, locally known as Nageswar is a medium sized to large evergreen tree with short trunk often buttresses at the base and the leaves are lanceolate, curvaceous, generally with a waxy bloom underneath. The leaves are red when young and the flowers are large, white and fragrant; the fruits are ovoid, nearly woody and the seeds are dark brown and 1-4 in number. It is available in Bangladesh and the sub continents. Ayurvedi, Yunani doctors and local Kabiraj use the, leaves [1] and seeds [2] as a remedy for, cold fever, sores, scabies and the volatile oils [3-4] from the flower [5] show antifungal and anticancer activities [6].

Extensive chemical examinations of this plant have been carried out and several constituents were isolated such as lignans, alkaloids, flavonoids, tannins, phthalic acid, gallic acid and terpenoids [2-5]. In this paper, we describe the isolation and structural elucidation of the isolated compounds from the ethyl acetate extract of the leaves of *Mesua ferrea*. All the isolated compounds were identified by their spectral data.

### EXPERIMENTAL SECTION

#### General

Freshly distilled solvents were used for extraction, isolation and purification. Evaporations were performed under reduced pressure on a Buchii rotary evaporator. Melting point was determined on an electrochemical micro-melting point apparatus. IR spectra were recorded (KBr discs) on a Shimadzu UV-168A spectrophotometer, validation ( $\nu_{\max}$  in  $\text{cm}^{-1}$ ). <sup>1</sup>H-NMR spectra were recorded on a Bruker R-32 (400 MHz) instrument in  $\text{CDCl}_3$  and  $\text{DMSO-d}_6$  with TMS as an internal standard (chemical shifts in  $\delta$ , ppm). UV spectra were recorded on HATACHI, U-2000 spectrophotometer Ultrospeck in methanol ( $\lambda_{\max}$  in nm).

The entire chemical is analytical reagent grade. TLC was performed using silica gel GF<sub>254</sub>.

#### Plant Materials

The leaves of *Mesua ferrea* were collected from the Curzon Hall Campus of the University of Dhaka and also collected from other parts of Bangladesh. Both the plants were identified and voucher specimen was deposited in the herbarium of the Department of Biology, University of Dhaka, Bangladesh with number (0076).

#### Extraction of the compound M<sub>1</sub>

Dried leaves of the plant (1 kg) were milled into powder and then extracted with methanol (8 L) in a Soxhlet extractor for 36 h. The extract was evaporated in a rotatory evaporator and dried by vacuum pump. The methanolic extract (40 g) was suspended on water and extracted successively with hexane, chloroform, ethyl acetate, and butanol to yield hexane (2.3 g), chloroform (16.8 g), ethyl acetate (9.5 g) and BuOH-soluble (5.32 g) fractions, respectively. The ethyl acetate soluble fraction (5 g) was subjected to the column chromatography was eluted initially with the fraction of petroleum ether (40-60°C) followed by the mixture of petroleum ether with increasing amount of ethyl acetate and finally with methanol. These elutes were collected in a series of test tubes with 15 mL in each fraction. Each fraction was examined by TLC. Based on the similar TLC behavior, this elutes were combined to yield F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, F<sub>4</sub>, F<sub>5</sub>, F<sub>6</sub>, F<sub>7</sub>, F<sub>8</sub>, F<sub>9</sub>, F<sub>10</sub> and F<sub>11</sub>. The fractions (F<sub>1</sub>-F<sub>11</sub>) were concentrated and allowed to stand at room temperature when a circular yellow amorphous crystal was obtained from the fraction F<sub>10</sub> and this was marked as compound M<sub>1</sub>. The amorphous crystal was washed with petroleum ether followed by chloroform and dichloromethane successively. The crystal was

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dissolved in methanol, applied in TLC micro glass slide, and viewed in an iodine chamber where two different spots were detected. Therefore, the compound was needed further purification and dissolving in the mixture of chloroform and methanol where the ratio was 10:1 and kept the mixture at room temperature. After 24 hours a yellow amorphous crystal was precipitated out at the bottom of the conical flask. The crystallization process was repeated three times and finally a light yellow crystal (105.0 mg) was obtained.

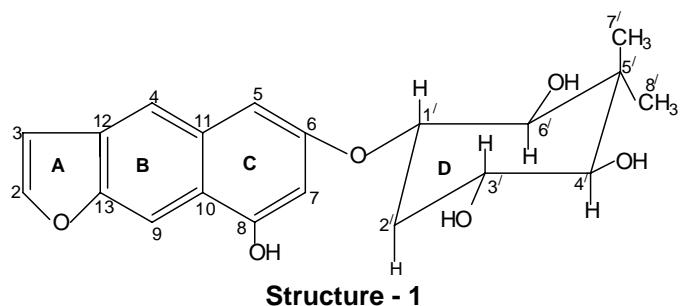
### Compound M<sub>1</sub>

Finally the compound M<sub>1</sub> was crystallized from ethyl acetate hexane to give the light yellow crystals (95 mg), m.p. 156-158°C; R<sub>f</sub> 0.55; UV: 346 nm; IR: 3275, 2970, 1645, 1589, 1486, 1449, 1350, 821 and 738 cm<sup>-1</sup>; <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD): 7.29 (1H, d, J=7.5, H-2), 6.90 (1H, d, J=8.0, H-3), 6.18 (1H, S, H-4), 6.35 (1H, S, H-9), 5.34 (1H, S, H-7), 4.22 (2H, br. S, H-1'), 3.70 (1H, dd, J=8.0, H-2'), 3.36 (1H, dd, J=8.0, H-3'), 3.42 (1H, m, H-4'), 3.31 (1H, br. S, H-6'), 0.94 (4H, S, H-7') and 0.95 (4H, S, H-8'); <sup>13</sup>C-NMR (400 MHz, CD<sub>3</sub>OD): 146.36 (C-2), 105.87 (C-3), 122.87 (C-4), 165.77 (C-6), 130.42 (C-7), 113.94 (C-9), 116.34 (C-10), 122.95 (C-11), 129.16 (C-12), 157.56 (C-13), 77.92 (C-1'), 76.98 (C-2'), 73.24 (C-6'), 72.09 (C-3'), 71.98 (C-5'), 71.87 (C-4').

### RESULT AND DISCUSSION

The thin layer chromatographic examination of the isolated compound from the ethyl acetate extract of the leaves of *Mesua ferrea* showed an orange single spot upon exposure to iodine vapour and bright single yellow spot was observed on the thin layer chromatographic plate when the vanillin-sulphuric acid reagents was sprayed on it followed by heating at 110°C for about 10 minutes. The compound was obtained as light yellow crystals (m.p. 156-158°C). High resolution mass spectrum compound M<sub>1</sub> exhibited molecular ion at m/z 343, which is constituent with the molecular formula C<sub>20</sub>H<sub>22</sub>O<sub>5</sub>. It was readily soluble in chloroform, methanol and other organic solvents. To the best of our knowledge this compound has not been previously isolated or reported from any other sources<sup>12</sup>, 13-furano-8-hydroxy napthyl-6-O-β-2',3',4',6'-tetrahydroxy-5',5' dimethyl cyclohexyl ether was isolated from this plant for the first time in our laboratory. Its UV spectrum showed [7] absorption band at 346 nm indicative of the functionality capable of undergoing n → π\* transition of the aromatic system. Its IR spectrum showed [7] an absorption peak at 3275 cm<sup>-1</sup> assignable to a hydroxyl group (-OH) whereas absorption at 2970 cm<sup>-1</sup> was assigned for -CH<sub>3</sub>, -CH<sub>2</sub> and -CH groups. A single absorption band at 1645 cm<sup>-1</sup> was demonstrated for >C=C< groups. Two absorption peaks at 1589 and 1486 cm<sup>-1</sup>, respectively, were

assigned for the CH bending of the aromatic ring. The absorption signal at 1449 cm<sup>-1</sup> was demonstrated the presence of -CH<sub>2</sub>- group. The other two absorption peaks at 821 and 738 cm<sup>-1</sup> were appropriate for the presence of 1,2,4- and 1,2,3- trisubstituted aromatic rings, respectively. The two doublets of 1H intensity at δ 7.29 (J=7.5) and δ 6.90 (J=8.0) ppm with the coupling constant in the proton NMR spectra were indicated [4] the presence of protons next to an electronegative oxygen atom i.e. protons at position 2 and 3 of the furan ring which is designated as ring **A** in the proposed structure. Two different singlets with 1H intensity at chemical shifts δ 6.18 and δ 6.35 ppm, respectively, were indicative of protons in the fused aromatic system [8] designated as ring **B** in the proposed structure. The other singlets with the 1H intensity at chemical shifts δ 7.30 and δ 5.34 ppm, respectively, were assigned for the protons of the fused aromatic ring [8] designated as ring **C** in the proposed structure. The broad singlet with 2H intensity at chemical shift δ 4.20 ppm was assigned [9] for the proton at C-1' of the cyclohexane ring which was linked to the fused aromatic system designated as ring **C** in the proposed structure. The doublets with the intensity of 1H at chemical shifts δ 3.70 and δ 3.36 ppm with the coupling constants 8.0 in each case were suggestive [9] for the protons of the -OH group at position C-2' and C-3', of the six member ring, respectively. The chemical shift at 3.42 ppm with the multiplates was an indicative [9] for the proton of the -OH group at position C-4' and the broad singlet at 3.31 ppm with the 1H intensity was appropriate [9] for the proton at position C-6' of the same ring. The two-upfield singlets at 0.94 and 0.98 ppm with the intensity of 4H indicated the presence of protons at C-7' and C-8', respectively, and these two carbons were linked to the C-5' position of the cyclohexane ring [9] as dimethyl groups and thus the linked substituted cyclohexane ring is designated as **D** in the proposed structure. The <sup>13</sup>C-NMR spectrum of the isolated compound revealed the presence of 20 carbons. Chemical shifts at δ 146.36 and δ 105.87 ppm indicated the presence of carbon at position 2 and 3 next to an electronegative atom [9]. The two downfield signals at 157.56 and 129.16 ppm were assignable to the carbon, which were fused between furan and aromatic ring i.e. the carbon at position 13 and 12, respectively. Chemical shifts as 122.87, 122.95, 113.94, and 130.43 and at 116.34 ppm were assigned [9] for the different carbons of the fused aromatic rings designated as **B** and **C** in the proposed structure. The downfield chemical shift at 165.77 ppm was assignable to the carbon at position bearing the ether group at position 6. The upfield chemical shifts at 77.92, 76.98, 73.24, 72.09, 71.98 and at 71.87 ppm were appropriate [9] for the cyclohexyl carbons. Thus,



the UV, IR,  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR spectral analysis along with physical properties established the identity of compound  $\text{M}_1$  as 12, 13-furano-8-hydroxy napthyl-6- $\beta$ -2',3',4',6' tetrahydroxy-5',5' dimethyl cyclohexyl ether (Structure-1).

## CONCLUSION

The results of the present investigation constitute the occurrence of Furano-napthyl-hydroxy cyclohexyl type compound in plant kingdom. The existence of hydroxy cyclohexyl lends support the fact and thus the furano-napthyl-hydroxy cyclohexyl is designated as 12-13-furano-8-hydroxy napthyl-6-O- $\beta$ -2'-3', 4'-6' tetrahydroxy 5'-5'-dimethylcyclohexyl ether from the leaves of *Mesua ferrea*. The title compound was isolated from this plant for the first time.

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