NMR STUDY OF METHYL *β*-ORSELLINATE FROM *DIRINARIA* SP. WAS COLLECTED FROM COASTAL AREAS OF TELUK NIPAH, PULAU PANGKOR, PERAK MALAYSIA

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ABSTRACT

The research was carried out to isolate and characterize the methyl *β*-orsellinate of lichen *Dirinaria* sp. collected from the coastal areas of Teluk Nipah, Pulau Pangkor, Perak Malaysia. The sample was extracted in methanol for three times at room temperature. Subsequently, the crude extracts and further analysed using thin-layer chromatography (TLC) and vacuum liquid chromatography (VLC). To derive the pure compounds, the isolation step was performed using radial chromatography (RC). The chemical structure of the isolated compound was determined by several spectroscopies i.e. infra-red (IR), nuclear magnetic resonances (NMR), and mass spectrometric (MS). The compound was identified as methyl *β*-orsellinate.
Keywords: methyl *β*-orsellinate, lichen, *Dirinaria* sp.

STUDI NMR METIL β-ORSELLINAT DARI *DIRINARIA* SP. YANG DIAMBIL DARI KAWASAN PANTAI TELUK NIPAH, PULAU PANGKOR, PERAK MALAYSIA.

ABSTRAK

Penelitian dilakukan untuk mengisolasi dan mengkarakterisasi metil β-orsellinate dari lichen *Dirinaria* sp. yang diambil dari daerah pesisir Teluk Nipah, Pulau Pangkor, Perak Malaysia. Sampel diekstraksi dalam metanol sebanyak tiga kali pada suhu ruang. Selanjutnya, ekstrak kasar dianalisis menggunakan kromatografi lapis tipis (KLT) dan kromatografi cair vakum (KCV). Untuk mendapatkan senyawa murni dilakukan tahap isolasi dengan menggunakan kromatografi radial (KR). Struktur kimia senyawa isolat ditentukan dengan menggunakan beberapa spektroskopi yaitu infra merah (IM), resonansi magentik inti (NMR), dan spektrometri massa (MS). Senyawa tersebut diidentifikasi sebagai metil β-orsellinate.

Kata kunci: metil *β*-orselinat, liken, *Dirinaria* sp.

INTRODUCTION

Lichen is a form organism of a symbiotic relationship between fungal and a photosynthetic organism like blue-green algae or cyanobacteria (Bebert 1831). Lichens could grow almost anywhere, although the type of species may differ based on environmental conditions (Nimis *et al.* 2002; Nash 2008; Norela *et al.* 2018). Pulau Pangkor, Perak, is a tropical island with near-constant exposure to the sun and wind. This research was done at Teluk Nipah, lat. 4°N, long. 100°E. The types of lichens mainly grow in this environment must be hardy, resistant to moisture loss, have ultraviolet-light tolerant and salt-tolerant properties (Kosanić *et al*. 2010). *Dirinaria* sp. which from the family of Physciaceae was reported that it has produced many types of aromatic compounds (Samsudin 2010; Kekuda *et al.* 2015; Abas *et al.* 2019). The present study was performed to report the isolation and characterization of methyl *β*-orsellinate using 1D and 2D NMR spectroscopy.

MATERIALS AND METHODS

General

Thin-layer chromatography, aluminium sheets 20 × 20 cm of the silica gel 60 F254 of 0.25 mm thickness (art. no. 5554, MERCK) and Silica gel; Kieselgel 60 PF254 (art. no. 7749, Merck) (radial chromatography, Chromatotron-7924T-01 USA). The structure of the isolated compound was determined based on the spectral data recorded on FTIR (Perkin-Elmer USA) spectrophotometer and NMR 400 MHz (JEOL). ESI-MS was recorded by using Ultra performance liquid chromatography (model JEOL JMS-700/GI.)

Lichen Material

The sample used in this research was the lichen *Dirinaria* sp. which was collected from coastal areas of Teluk Nipah, Pulau Pangkor, Perak Malaysia.

Extraction and Isolation

The ground air-dried of *Dirinaria* sp. (700 g) was extracted for 250 ml X 3 days with ethyl acetate at room temperature.. The EtOAc extract was concentrated at 40-50 °C with a rotary evaporator under reduced pressure to give 150 g green pale extract. The crude extract was fractionated by using vacuum liquid chromatography (VLC) eluted with increasing polarity of n-hexane-EtOAc. The eluents that showed the same profile on thin layer chromatography (TLC) chromatogram were combined to give three fractions (I-III). Purification of Fraction III (300mg) was carried out using Radial Chromatography (RC) with silica gel plate of 1 mm thickness eluted with 95:5 n-hexane-EtOAc in 5% polarity increment to yield compounds **1** (30 mg) (Rosandy et al. 2013). All the prior profile wasdetected using smaller pieces of TLC and then detected under UV light (254 nm) (**A**) and by H_2SO_4 spraying reagent followed by heating (**B**) (**Figure 1).**

Figure 1. TLC Profile of *Dirinaria* sp. Spot with the box is the isolated compound

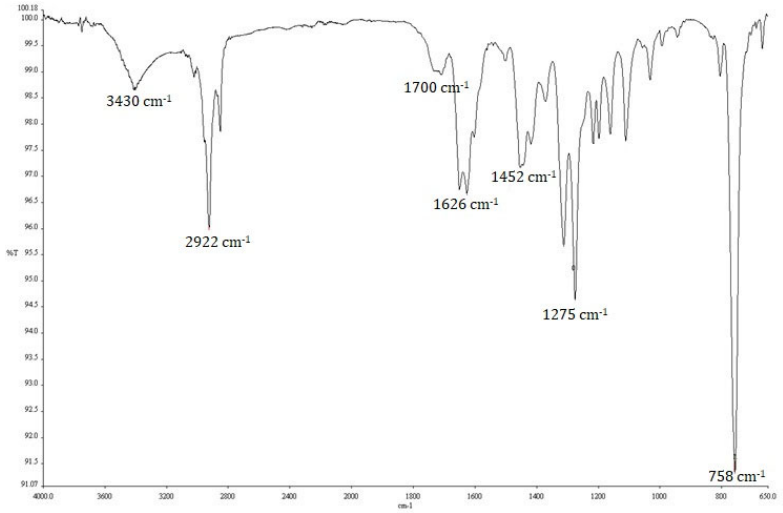

Figure 2. IR spectrum of compound **1**.

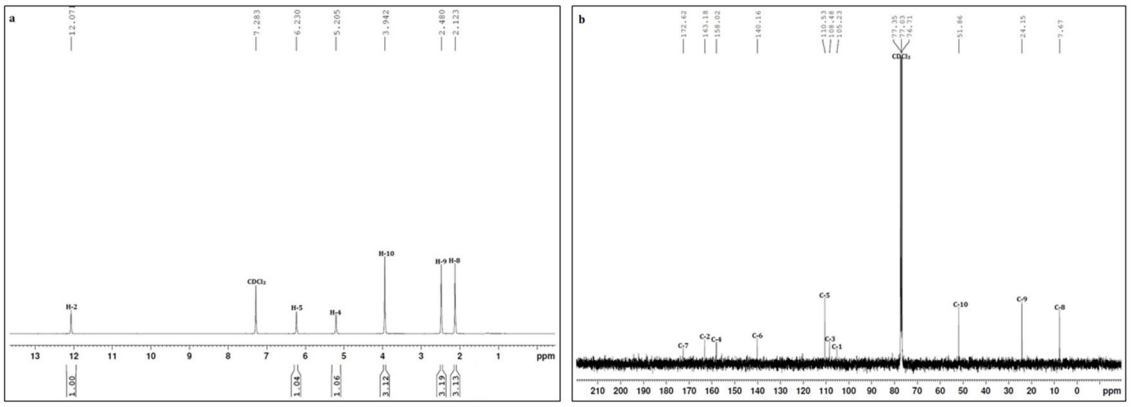

Figure 3. ^{1}H(**a**) and ^{13}C(**b**)-NMR spectrum of compound **1.**

Methyl *β*-orsellinate (**1**)
MP: 142-144 °C.
EI-MS $[M]^{+}$ at *m/z* 196.077
IR (MeOH) νmax (cm^{-1}): 3430, 2922, 1700, 1626, 1275, and 758.
^{1}H NMR (Chloroform-*d*, 844 MHz): Table 1
^{13}C NMR (Chloroform-*d*, 100 MHz): Table 1

RESULTS AND DISCUSSIONS

Purification with vacuum liquid chromatography (VLC) was isolated compound **1** as a yellow solid, molecular ion peak EI-MS $[M]^{+}$ at *m/z* 196.07 and melting point at 142-144 °C. The IR spectrum (**Figure 2.**) displayed absorption bands at 3430 cm^{-1} (OH group) and 2922 cm^{-1} (sp^3 C-H group), 1700 cm^{-1} (C=O group), 1626 cm-1 (C=C aromatic), 1275 cm-1 (C-O ether) and 758 cm-1 (-CH_3- group bonding).

The ^{1}H NMR (**Figure 3.**) for compound **1** showed three signals for methyl group at δ_H 2.12 ppm (3H, *s*, H-8) and at δ_H 2.48 ppm (3H, *s*, H-9), a methoxy group at δ_H 3.94 ppm (3H, *s*, 10-OCH_3), an aromatic proton at δ_H 6.23 ppm (1H, *s*, H-5), and two hydroxyl protons at δ_H 12.07 ppm (1H, *s*, 2-OH) and δ_H 5.21 ppm (1H, *s*, 4-OH). The ^{13}C NMR spectrum (**Figure 2.**) showed 10 signals corresponding to two methyl group at δc 7.7 ppm (C-8) and at δ_C 24.2 ppm (C-9), a methoxy group at δ_C 51.9 ppm (10-OCH_3), six aromatic carbons at δ_C 105.2 ppm (C-1), δ_C 163.2 ppm (C-2), δ_C 108.5 ppm (C-3), δ_C 158.0 ppm (C-4), δ_C 110.5 ppm (C-5), δ_C 140.2 ppm (C-6), an ester group at δ_C 172.6 ppm (C-7). Based on this data, the structure of **1** has a molecular formula of $C_{10}H_{12}O_4$ and DBE (Double Bond Equivalent) value is 5, indicating that the compound consists of four double bonds (three olefinic and one carbonyl) and one ring. The complete assignment of protonated carbon was confirmed by HSQC correlations (**Table 1**.;**Figure 3.**). It was evident from the correlation there were two methyls, one methoxy and a methine proton in the molecule.

The HMBC spectrum of **1** (Table 1; **Figure 4b.**) showed long-range correlations between aromatic proton at δ_H 6.23 (H-5) with aromatic carbons at δ_C 105.2 (C-1), 108.5 (C-3), 158.0 (C-4) and methyl proton δ_C 24.2 (C-9), the hydroxy proton at δ_H 12.07 (2-OH) with aromatic carbon at δ_C 105.2 (C-

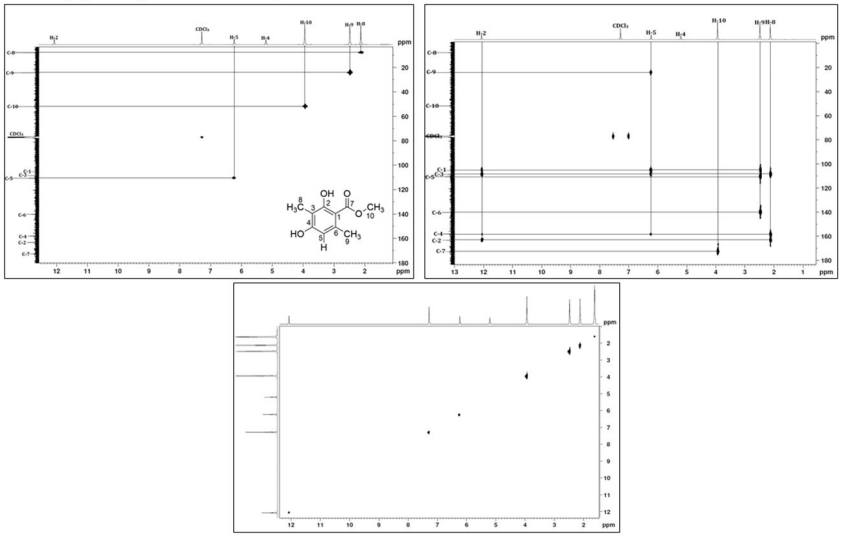

Figure 4. 1H] 13C HSQC (**a**), ^{1}H↔^{13}C HMBC (**b**), and ^{1}H↔^{1}H COSY (**c**) NMR spectrum of compound 1.

Table 1. 1D and 2D NMR of Compound **1**

No	^{13}C (ppm)	^{13}C* (ppm)	$^{1}H\leftrightarrow^{13}C$ HSQC (∑H, mult. J in Hz)	$^{1}H\leftrightarrow^{13}C$ HMBC	$^{1}H\leftrightarrow^{1}H$ COSY
1	105.2	105.2	-	-	-
2	163.2	163.1	-	-	-
2-OH	-	-	12.07 (1H, s)	C-1, C-2, C-3, C-4	-
3	108.5	108.5	-	-	-
4	158.0	158.0	-	-	-
4-OH	-	-	5.21 (1H, s)	-	-
5	110.5	110.5	6.23(1H, s)	C-1, C-3, C-4, C-9	-
6	140.2	140.2	-	-	-
7	172.6	172.6	-	-	-
8	7.7	7.6	2.12 (3H, s)	C-2, C-3, C-4	-
9	24.2	24.1	2.48 (3H, s)	C-1, C-5, C-6	-
10-OCH3	51.9	51.8	3.94(3H, s)	C-7	-

1), 163.2 (C-2), 108.5 (C-3) and 158.0 (C-4) indicating that all methyl and hydroxy groups were attached to the aromatic ring. The long rage correlation of proton methyl at δ_H 3.94 (H-10) with carbonyl carbon at δ_C 172.6 (C-7) suggested the attachment of this carbonyl to the aromatic ring to form methyl benzoate. Based on the facts above, the structure of **1** is determined as methyl 2,4-dihydroxy-3,6-dimethylbenzoate or methyl *β*-orsellinate (Hylands and Ingolfsdottir 1985; Toledo *et al.* 6447; Huynh et al. 6459; Perico-Franco *et al.* 2015).

CONCLUSION

A methyl *β*-orsellinate was successfully isolated from ethyl acetate extract lichen *Dirinaria* sp was collected from coastal areas of Teluk Nipah, Pulau Pangkor, Perak Malaysia. The chemical structure of the isolated compound was elucidated by using IR, 1D and 2D NMR spectroscopy and MS spectrometric technique

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