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Isolation and identification of moretenol from *Ficus deltoidea* leaves

[Pemencilan dan pengenalpastian moretenol dari daun mas cotek (*Ficus deltoidea*)]

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Abstract

Ficus deltoidea or 'mas cotek' is among the most popular traditional medicinal plants in Peninsular Malaysia and Borneo Island. However, information on chemical constituents of this herb is very limited. This paper reports on the isolation of moretenol from the methanolic extract of *Ficus deltoidea* leaves using vacuum liquid chromatography and open column chromatography techniques. The structure elucidation of moretenol was carried out using Nuclear Magnetic Resonance (NMR) spectrometer as well as mass spectrometer data. It was concluded that dried *F. deltoidea* leaves contained 0.04% moretenol and can be suggested as a chemical marker. Determination of moretenol levels can assist the selection and harvesting processes of *F. deltoidea* accessions for plantation or product development in the future.

Introduction

The genus *Ficus* belongs to the Moraceae family, one of the main families of plants in the Malaysian and Indonesian forests. *Ficus deltoidea* or locally known as 'mas cotek' is an epiphyte and found in all forest ecosystems except mangrove swamps. Decoction of the dried leaves is commonly used by the traditional medicine practitioner as an after birth tonic, anti-hypertension, antidiabetic and for leucorrhoea treatment (Padua et al. 1999).

It was reported that more than 30 accessions are available based on the morphology of the leaves, stems and their growth habits (Musa 2004, 2005). However, there is no established scientific report on the chemical constituents although a few findings on the biological activity of this plant have been recently reported mainly on the cholesterol lowering effects (Taufik et al. 2005) and antioxidant properties of several *Ficus deltoidea* accessions (Jabit et al. 2005). Therefore, information on the phytochemicals of *F. deltoidea* accessions is yet to be compared.

In this study, conventional column chromatography techniques were used and isolated compound was analysed with Nuclear Magnetic Resonance (NMR) and mass spectrometers. These two spectroscopic techniques are commonly used in chemical structure elucidation. The most important applications for the organic chemist are proton NMR and carbon-13

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NMR spectroscopy. NMR is also known as a scientific method that exploits nuclear magnetic resonance to study molecules.

Mass spectrometer is known as a complementary method to elucidate the chemical structure of compound isolated. Through mass spectroscopy technique, the molecular weight of compound isolated can be determined. The fragmentation pattern of the compound in the mass spectra is considered as unique and accepted as fingerprint of the compound. Elucidation of the fragmented ions in the mass spectra should support the conclusion from the elucidation of NMR spectra.

Through these techniques, moretenol is found significantly in the leaves of *F. deltoidea*. Moretenol is one of the prominent compounds present in the leaves of *Ficus* and some traditional herbs.

Material and methods

General instrument

Melting points were determined using a hot stage melting point apparatus equipped with microscope (XSP-12 model 500x, China). Nuclear Magnetic Resonance (NMR) spectrum was recorded using JEOL JNM-ECA400 (400 MHz) (JOEL, Japan). Mass spectra were recorded on POLARIS Q, Finnigan mass spectrometer (ThermoFinigan, USA) and the ionization was induced by electron impact at 70 eV.

Preparation of plant samples

The leaves of *F. deltoidea* (1.2 kg), accession MFD4, were supplied by MARDI Station, Telong. The leaves were air dried (dry matter 29%) and ground to 1 mm particle size and kept in amber plastic bags.

Preparation of methanol extract

Traditionally, the herb is boiled with water. Therefore, for crude extract preparation, methanol was used as it is closer to polarity of water. *Ficus deltoidea* leaf powder was soaked in methanol for 3 days at room temperature. The mixture was then filtered and the solvent was removed under reduced pressure at 40 °C. The extraction process was repeated three times and the total crude methanol extract, dark and gummy, yielded 90 g approximately.

Isolation of moretenol

Crude methanol extract (90 g) was dissolved in minimal amount (150 ml) of methanol and mixed with water (150 ml) to form a suspension of methanol extract. The methanolic aqueous suspension was partitioned with hexane followed by dichloromethane (DCM). An amount of 36 g of DCM fraction was subjected to vacuum liquid chromatography column (5 cm i.d. x 7 cm length) and eluted with hexane followed by hexane-DCM mixture (75:25).

The fraction of hexane-DCM mixture (75:25) (1.2 g) was subjected to silica gel column chromatography (2 cm i.d. x 30 cm length), eluted with hexane and hexane-DCM mixture in ascending gradient polarity to yield 400 mg of moretenol from fraction 15 to 17. The amount of isolated moretenol obtained was about 0.04% of the total dried weight of *F. deltoidea* leaves.

Results and discussion

Hexane fraction of *Achillea lingustica* shows potent antidiabetic activity and contains 17% of moretenol as the major constituent (Conforti et al. 2005). However, in this study, moretenol or 3β-hydroxy-21αH-Hop-22(29)-ene (1) isolated from *F. deltoidea* leaves were only 1.11% of DCM fraction identified using common spectroscopy techniques such as mass spectrometry and NMR spectrometry.

Method of isolation

Moretenol has been identified and isolated in *Ficus macrophylla* leaf extract (Galbraith et al. 1965) and *Achillea lingustica* extract (Conforti et al. 2005). Galbraith et al. (1965) has reported the isolation method of pure moretenol using benzene. However, benzene is known to be a highly carcinogenic solvent. In

this study, hexane and dichloromethane (DCM) were used, which are less toxic compared to benzene. The use of vacuum liquid chromatography has accelerated the isolation and purification of moretenol from *F. deltoidea* leaf extract.

Mass spectral elucidation

The melting point of isolated compound was determined at 236 °C which is similar to moretenol. The mass spectrum of the isolated compound indicated the molecular mass at m/z 426, which corresponds to $C_{30}H_{50}O$, the molecular formula of moretenol (*Figure 1*). The mass spectrum showed a base peak at m/z 189, corresponding to a typical hopane and lupane skeletal fragment with an isopropenyl substituent (Lopes et al. 1993).

The presence of the other ion fragments at m/z 383 and m/z 207 is also an indicative of the mass spectrum of moretenol (Galbraith et al. 1965). The mechanism of the ion formation was the result of the reactions between *a* and *b* as given in *Figure 2*. The formation of ions at m/z 383 and m/z 411 was due to the lost of

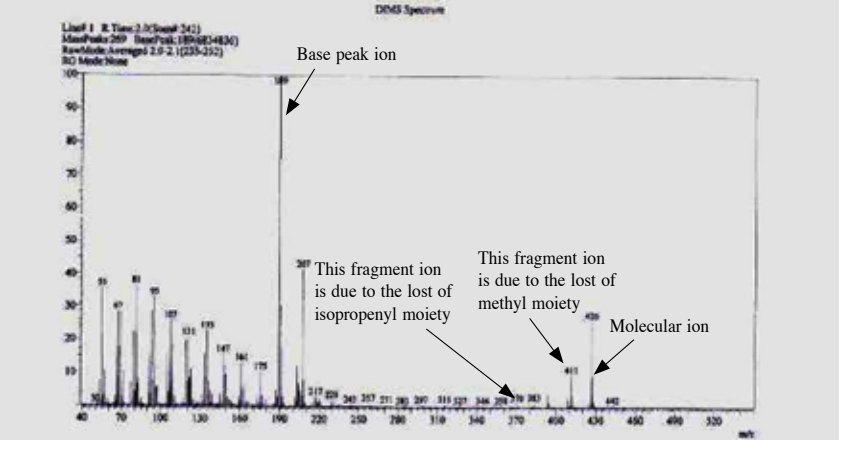

Figure 1. The mass spectrum of moretenol

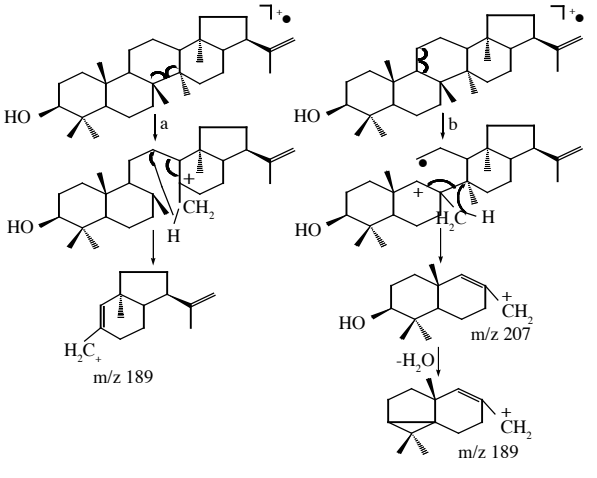

Figure 2. Mechanism of selected ion formation in moretenol mass spectrum elucidation

isopropenyl side chain and methyl group from the molecular ion respectively.

NMR elucidation of moretenol

Through ^{1}H NMR spectrum (*Figure 3*), five methyl signal peaks at δ_H 0.68 (H-18α), δ_H 0.76 (H-4α), δ_H 0.83 (H-4β), δ_H 0.94 (H-27α), δ_H 1.67 (H-30) and two overlapping methyl peaks at δ_H 0.97 (H-26β, H-25β) were observed, corresponding to the methyl groups in moretenol (*Figure 4*). The methyl signals in ^{1}H NMR spectra were compared with previous published data for moretenol and *Table 1* shows the similarities of proton signal.

Inspection of ^{13}C NMR spectrum (*Figure 5*) led to the identification of 30 carbon signals, which suggested that

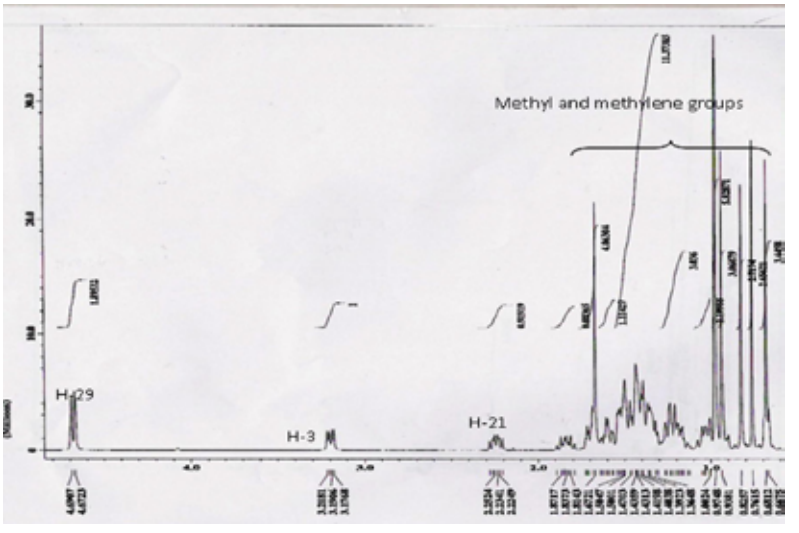

Figure 3. The ^{1}H NMR spectrum of moretenol (400 MHz, dissolved in $CDCl_3$ solven)

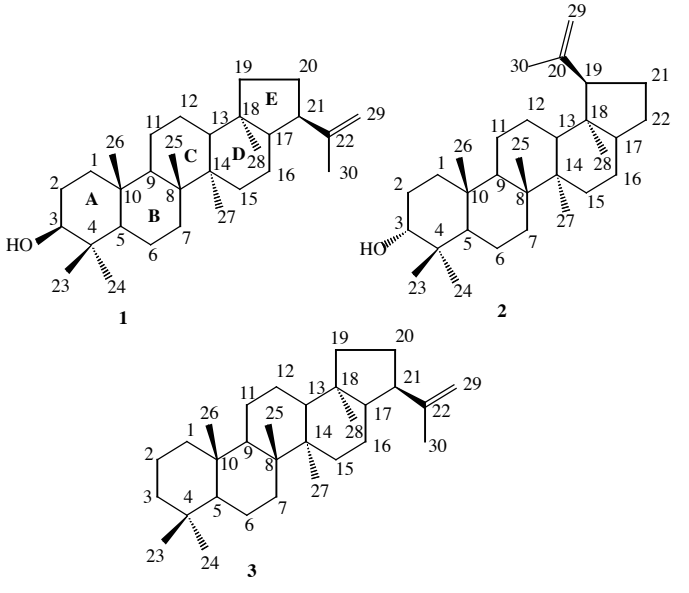

*Figure 4. Structures of moretenol **(1)**, epi-lupeol **(2)** and moretene **(3)***

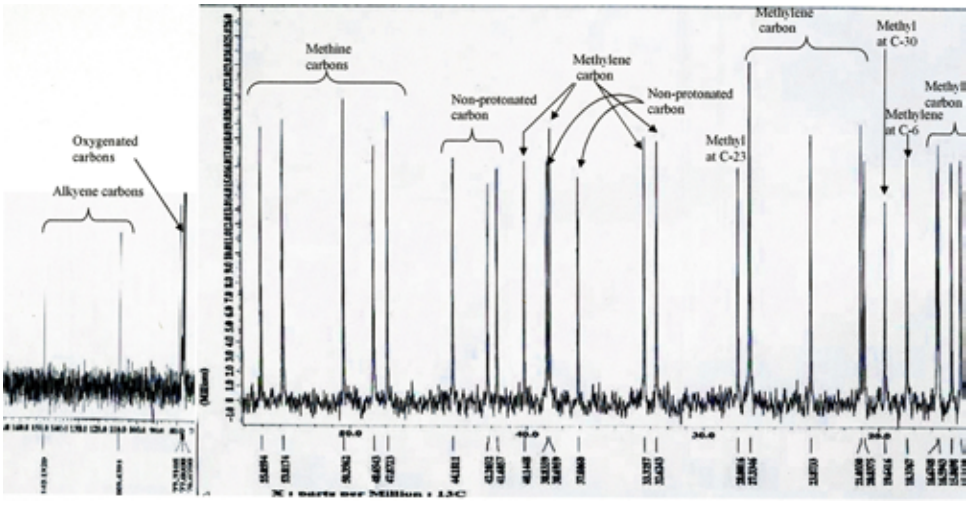

Figure 5. The ^{13}C NMR spectrum of moretenol (100 MHz, dissolved in $CDCl_3$ solvent)

Table 1. Comparison of methyl signals in ^1H NMR spectrum of moretenol with published data. The experiment was carried out using deuteriated chloroform as solvent

	Experimental data (δ_H)	Published data (δ_H) (Lavie et al. 1968)
H-23	0.76	0.76
H-24	0.83	0.83
H-26	0.98	0.97
H-25	0.98	0.97
H-27	0.94	0.94
H-28	0.68	0.68
H-30	1.67	1.67

the compound was a type of triterpene, and matched with the number of carbon in moretenol. The ^{13}C NMR signals were compared to carbon signals from moretene and *epi*-lupeol (*Table 2*). Some similarities were observed with each of the compound.

Signals from C_1 to C_{11} were similar to *epi*-lupeol due to the similarity of the chemical structure in ring A and B of the compound. Signals from C_{17} to C_{22} were similar to moretene, which was also due to the similarity of the chemical structure in ring E as shown in *Figure 4*.

Table 2. Comparison of ^{13}C NMR spectrum of moretenol with ^{13}C NMR spectrum of 3-epi-lupeol and moretene

Substance				Substance			
C No.	Moretenol	3-epi-lupeol	Moretene	C No.	Moretenol	3-epi-lupeol	Moretene
1	33.3	33.2	40.4	16	38.7	35.6	21.0
2	23.9	25.4	18.8	17	53.8	43.0	54.0
3	78.9	76.2	42.2	18	44.2	48.3	44.3
4	38.8	37.5	33.3	19	40.1	48.0	40.3
5	48.6	49.0	56.2	20	27.3	151.0	27.4
6	18.4	18.2	18.8	21	47.9	29.8	48.0
7	32.6	34.1	33.4	22	148.0	40.0	148.3
8	41.7	41.0	42.3	23	28.0	28.2	33.5
9	50.3	50.2	50.5	24	15.1	21.1	21.7
10	37.1	37.3	37.5	25	15.4	15.9	16.0
11	20.8	20.7	21.0	26	15.9	15.9	16.7
12	21.0	25.1	24.1	27	16.6	14.6	16.9
13	55.1	38.0	48.8	28	16.7	18.0	15.2
14	42.2	42.9	42.0	29	109.4	109.3	109.5
15	27.3	27.4	32.7	30	19.6	19.3	19.7

Based on comparison of ^{13}C NMR signals with published ^{13}C NMR spectral data of moretene (Lopes et al. 1993) and *epi*-lupeol (Puapairoj et al. 2005), it was confirmed that the isolated compound is moretenol.

Conclusion

Method to isolate pure moretenol from *Ficus deltoidea* leaf extract was developed and established for analytical standard. Moretenol has the potential to be a chemical marker, due to detectable amount of moretenol in the leaves. Moretenol could be used to standardize *Ficus deltoidea* extract as well as marker for harvesting strategy of the leaves for future planting or product development.

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Abstrak

Ficus deltoidea atau mas cotek ialah antara herba ubatan tradisional yang popular di Semenanjung Malaysia dan Kepulauan Borneo. Walaupun demikian, maklumat sebatian kimia herba ini adalah terhad. Kertas ini melaporkan pemencilan moretenol daripada ekstrak metanol daun *F. deltoidea* menggunakan kaedah kromatografi cecair vakum and kromatografi kolum terbuka. Struktur sebatian moretenol tersebut dikenal pasti menggunakan spektrometer Resonan Magnetik Nuklear (RMN) dan juga maklumat spektrometer jisim. Berdasarkan kajian ini, daun kering *F. deltoidea* mengandungi 0.04% moretenol dan boleh dicadangkan sebagai bahan kimia penanda. Penentuan aras moretenol boleh membantu proses pemilihan dan penuaian asesi *F. deltoidea* untuk penanaman dan penghasilan produk pada masa hadapan.

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