Identifikasi kubebin dan epikubebin hasil isolasi dari buah *Piper cubeba* L.f dengan spektroskopi RMI dua dimensi

Identification of cubebin and epicubebin isolated from *Piper cubeba* L.f fruits with two D-NMR spectroscopy

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Abstrak

Salah satu senyawa yang berkhasiat sebagai trakeospasmolitik dari buah kemukus (*Piper cubeba* L.f) adalah kubebin. Tetapi struktur kubebin ($C_{20}H_{20}O_6$) dan bentuk epimernya sulit diidentifikasi menggunakan spektroskopi RMI satu dimensi. Identifikasi menggunakan spektroskopi RMI 2-D struktur dari cubebin dan epicubebin dapat diidentifikasi dengan jelas
Kata kunci : *Kubebin, epikubebin, identifikasi, RMI 2-D*

Abstract

One of the isolated active compound of the tracheospasmolytic from *kemukus* fruits (*Piper cubeba* L.f) is cubebin. The problem occurred when cubebin ($C_{20}H_{20}O_6$) mixed with its epimer because of the difficultly to identify the structure by 1D-NMR spectroscopy. Structure identification then was conducted by 2D-NMR spectroscopy, so the structure of cubebin and epicubebin can be clear identified.
Key words : Cubebin, epicubebin, identification, 2D-NMR

Introduction

Medicinal plants as effective and potent medicines require evaluation by standard scientific methods in order to be used to their full effect. Natural products are the basis of many standard drugs used in modern medicine, and are so widely used that many laymen and even some members of the medical profession are unaware that they are of plant origin.

These activities are often known as result of trial and error, but they have to be carefully investigated if we wish to develop new drugs that meet the criteria of modern treatment.

Piper cubeba is a tropical plant, which easily grows in Indonesia. Indonesian people use the fruits of the plant as anti-asthma in traditional medicine known as *jamu*. Most people prefer using anti-asthma *jamu* instead of modern medicine especially due to its low price compared to that of the modern medicines especially in Indonesia. Preliminary experiments showed that n-hexane and ethanol extracts of *P. cubeba* had the capacity of reducing trachea contraction induced by metacholine (Wahyuono *et al*., 1999; Wahyono[b)] *et al*., 2003). At our previous report, 2 major tracheospasmolytic active compounds were dihydroxycubebin (Wahyono[a)] *et al*., 2003) and cubebin (Wahyono[b)] *et al*., 2003). The problem occurred when cubebin ($C_{20}H_{20}O_6$) mixed with its epimer, so that it's difficult to identify the structure by one-dimensional NMR spectroscopy (^{1}H-NMR).

Further isolation of cubebin and structure elucidation was than conducted by two-dimensional NMR (^{13}C-NMR). This method could differentiate between this compound and its epimer called epicubebin.

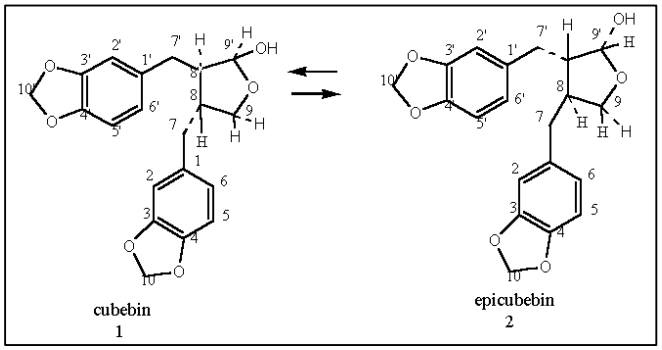

Figure 1. (3,4),(3',4')-bis-methylendioxy-9'hydroxy-9,9'-epoxy-8,8' lignan

Methodology

Material

Fresh fruits of *Piper cubeba* L.f were collected from the garden plantation at Center of Medicinal Plants Research, Department of Health, Tawangmangu, Indonesia in August, 1999. The voucher specimen was stored at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia. Cubebin was isolated from *Piper cubeba* L.f fruits.

Structure elucidation

Ultraviolet spectra were recorded on a Carry 1 Bio UV-Visible spectrophotometer in methanol. The IR spectra (KBR) were obtained on IR-Spect, Sp3- 200, PYEUNICAM. A Finnigan MAT TSQ-700 triple quadrapole equipped with custom mode Electro Spray Interface (ESI) was used to obtain Mass Spectra. Proton Nuclear Magnetic Resonance spectra were recorded on a Bruker DPX-600 (600 Hz) spectrometer. The ^{13}C NMR (Nuclear Magnetic Resonance) decoupling and DEPT (Distortionless enhancement by polarization transfer) experiments were obtained by a Bruker DPX-600 spectrometer. The solvent used for the NMR measurements was $CDCl_3$ if not otherwise specified. The ^{1}H-^{1}H COSY (Correlated spectroscopy), HMQC (Heteronuclear multiple quantum coherence) and HMBC (Heteronuclear multiple bond correlation) spectra were obtained by applying the usual pulse sequence. Melting points were determined on a Electro thermal melting point apparatus 9200 type equipped with microscope (England. LTD).

Results And Discussion

Cubebin (I_1) with its epimer, epicubebin (I_2)

This compound appeared as white crystals needles having a melting point at 133^0-134^0 C, similar to that reported by Wei Ming *et al.*, in 1987 (*m.p* 131^o-133^o C), it was insoluble or partially soluble in ethanol, soluble in n-hexane, chloroform and dichloromethane but not soluble in water; Rf 0.69 (DCM:EtOAc, 8:2) the spot sprayed with *p*-anisaldehyde-H_2SO_4 followed by heating at 100^{o}C was dark red. The UV spectrum of I in MeOH showed the maximum absorptions at 230 nm and 275 nm a typical lignanoid type absorption pattern (Badheka *et al.*, 1986).

The IR spectrum of I exhibited absorption bands at 3340 cm^{-1} due the OH groups and a strong band at 2945-2890 cm^{-1} (aliphatic C-H stretching) indicating the presence of –CH2- and –C-H. Band of 1606, and 1485-1500 cm^{-1} indicated the presence of an aromatic ring system, while the bands at 925 and 1035 cm^{-1} were from C-O absorption bands. In addition the peak at 1240 cm^{-1} corresponded to a signal of a C-O-C bond (Stahl and Schild, 1986).

The ^{1}H-NMR spectra of I_1 and I_2 (Figure 2, Table I) indicate the presence of 24 protons. The spectrum identified the presence of methylenedioxy at δ 5.96 (2 H, s, H-10 H-10'), and signal at δ 5.22 (1 H, d, H-9'a), a signals between δ 2.01 – 2.75 (4 H,m, H-7, H-8, H-7' and H-8'). The signals of the aromatic region atδ 6.60 - δ 6.73 (6 H, m, H-2', H-5', H-2, H-5, H-6, H-6'). The existence of epicubebin (I_2) was conducted from signals at δ 3.80 (1 H, t, J= 8.1 Hz) and δ 4.01 (1H, dd, J= 7.4 Hz) for H-9 (2) , and at δ 3.58 (1 H, t, J= .1 Hz) and - δ 4.11 (1 H, dd, J = 7.4 Hz) for H-9(1) at Figure 2.

The^{13}C-NMR (DEPT) spectrum in Figure 3 revealed the presence of 26 peaks corresponding to 26 carbon atoms in the

molecule. The spectrum showed 12 methine, 8 methylene and 6 quarternairy carbons. A signal at δ 72.28 is assigned to C-9 of cubebin and the δ 72,67 signal of C-9 to epicubebin. The signal at δ 100.81 and δ 100.85 corresponded to the pair of –O-CH_2-O for C-10 and C-10^{-1} in both of cubebin (I_1) and epicubebin (I_2). While resonances of C-9, and C-9' appearred at δ 98.86 and δ 103.36. The C-6, C-6', C-5, C-5', C-2, C-2', aromatic rings carbons resonated at δ 121.74 until δ 108.09. Other quarternary carbons appear at δ 42.88, δ 45.90,52.09 and δ 53.14 {C-8(I_1), C-8'(I_2), C-8'(I_1) and C-8(I_2)}. Signals due to methylen carbons appeared at δ 33.64 for C-7'(I_1)at 38.91 for C-7(I_1), δ 38.45 for C-7'(I_2) and at δ 39.42 for C-7(I_2). The structure of cubebin and epicubebin were further substantiated by COSY, HMQC and HMBC correlation spectra.

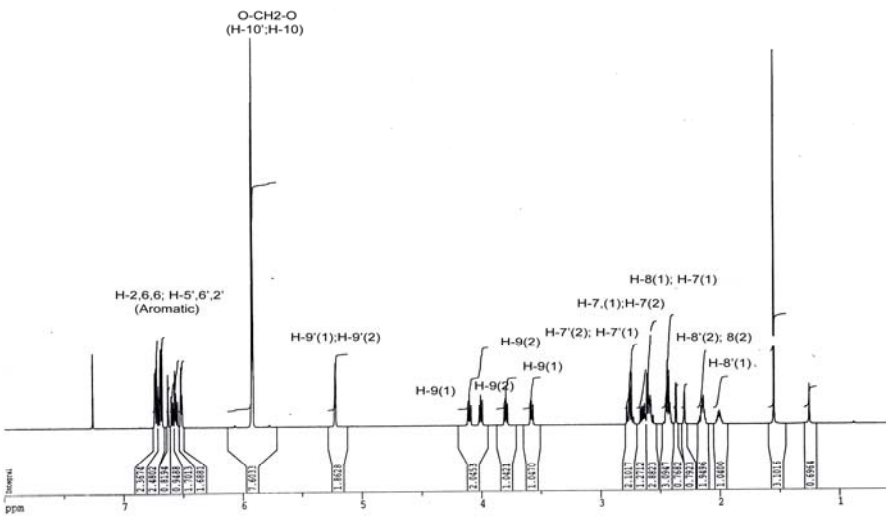

Figure 2. ^{1}H-NMR spectrum of I_1 and I_2 ($CDCl_3$, TMS).

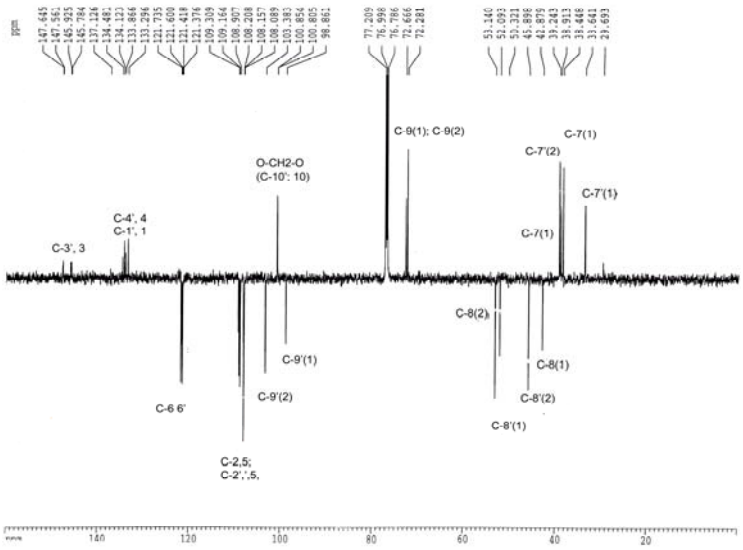

Figure 3. ^{13}C NMR (DEPT) spectrum of I_1 and I_2

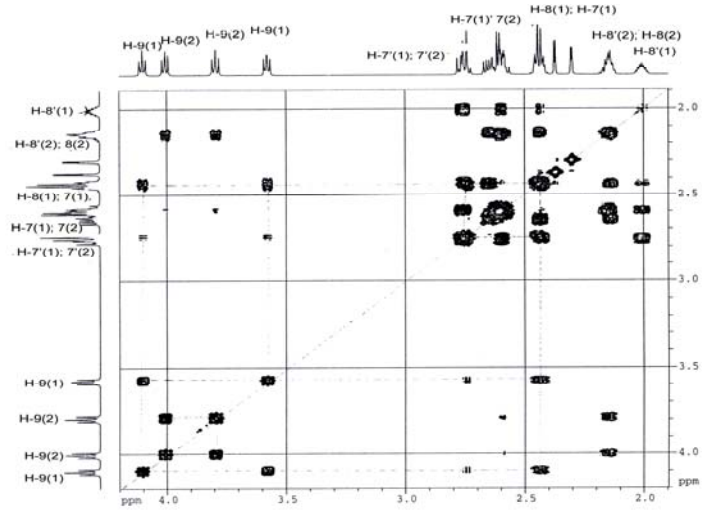

Figure 4. Expanded ^{1}H-^{1}H COSY spectrum of I_1 and I_2

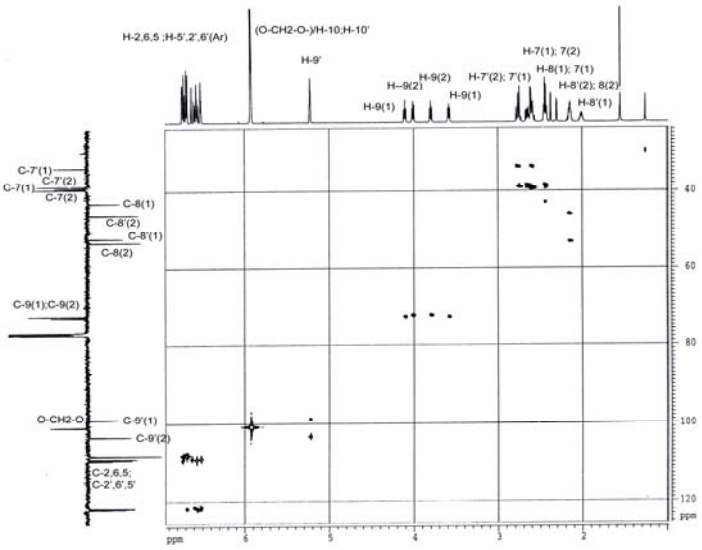

Figure 5. HMQC spectrum of I_1 and I_2

The chemical shifts (δ ppm) and proton-proton couplings of cubebin and epicubebin were confirmed by expanded COSY spectrum in Figure 4. ^{1}H-^{1}H COSY spectrum confirms the coupling interactions between H-9 of cubebin and H-9^1 of epicubebin. The other coupling interaction are between H-8 of epicubebin and H-9 of cubebin.

The HMQC spectrum (Figure 5) the signals appearing at δ 3.58 - δ 4.11 (H-9) are attached to a carbon resonating at δ 72.28 for C-9(I_1) and δ 72.67 for C-9(I_2). The correlation between proton H-10 (δ 5.96) and carbons in C-10, C-10' is observed by signals at δ 100.81 and δ 100.85.

The HMBC correlation spectrum (Figure 6) indicated that the C-9 proton signals at δ 3.80 and δ 4.01 correlated to carbon signals at δ 42.88 for C-8; δ 98.86 for C-9'; δ 52.09 for C-8' and δ 38.91 for C-7' respectively.

The elucidation of the structure of cubebin was confirmed by its mass spectrum (Figure 7) and fragmentation pattern (Figure 8). Mass spectrum showed the molecular ion peak

[M]+ at m/z 356. The formation of the ion at m/z 338 (16 %) and its further fragmentation to the ion at m/z 135 (100 %) confirmed the benzene methylenedioxy moiety. The diagnostic fragments for other parts of molecule appear at m/z 203 (40%) and m/z 173 (16%).

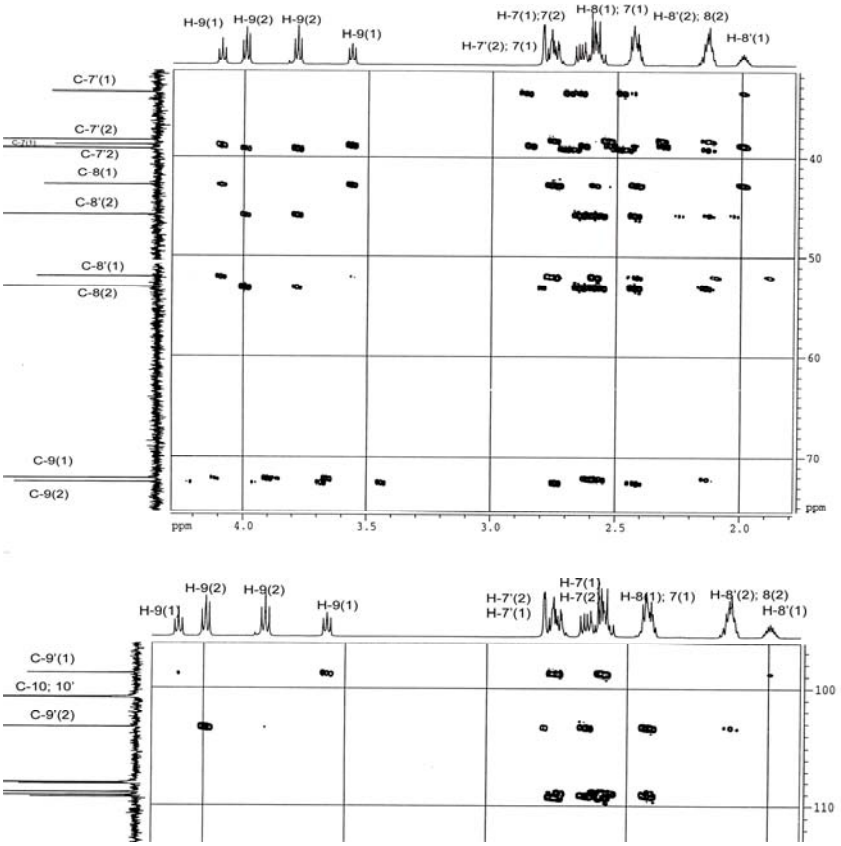

Figure 6. HMBC spectrum of I_1 and I_2

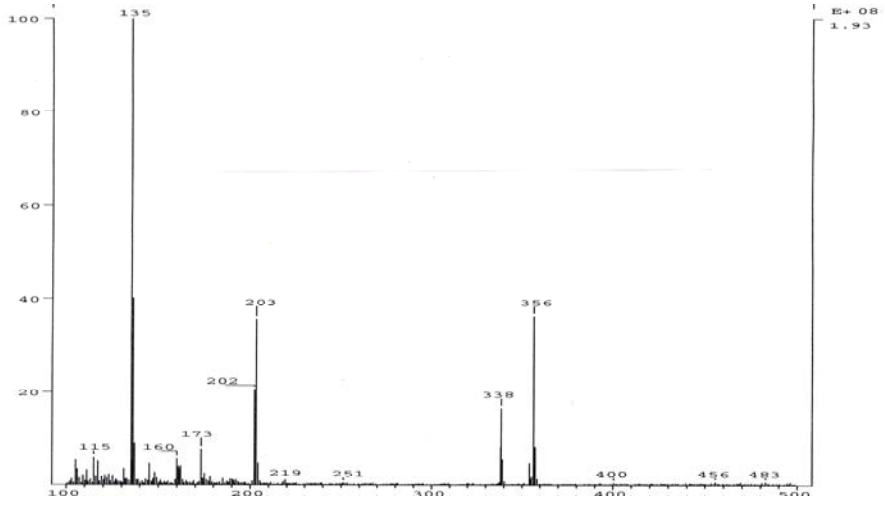

Figure 7. Mass spectrum of compound I

Table I. The assignment of ^{1}H and ^{13}C-NMR in $CDCl_3$ of cubebin and epicubebin

Carbon number	^{1}H (δ)	^{13}C (δ)	HMQC correlation	HMBC correlation
		133.29		
1'		133.87		
2	6.72	108.17		
2'	6.61	108.09		
3		145.93		
3'		147.56		
4		134.12		
4'		134.48		
5	6.60	109.31	H-5	
5'	6.76	108.91	H-5'	
6	6.50	121.42	H-6	
6'	6.19	121.60	H-6'	
7 (*1*)	2.62	38.91	H-7 (*1*)	H-9(*1*)
7(2)	***2.61***	***39.42***	***H-7 (2)***	
7'(*1*)	2.76	33.64	H-7' (*1*)	H-9(*1*); H-8'(*1*)
7'(2)	***2.75***	***32.45***	***H-7' (2)***	
8(1)	2.44	42.88	H-8 (1)	H-9(1)
8(2)	***2.12***	***53.14***	***H-8 (2)***	
8'(*1*)	2.01	52.09	H-8' (*1*)	
8'(2)	***2.12***	***45.90***	***H-8' (2)***	
9(*1*)	3.80; 4.01	72.28	H-9 (*1*)	
9(2)	***3.58; 4.11***	***72.67***	***H-9 (2)***	
9'(*1*)	5.22	98.86	H-9' (*1*)	H-8'(*1*); H7'(*1*);H-7(*1*)
9'(2)	***5.22***	***103.36***	***H-9' (2)***	
10	5.96	100.81	H-10	
10'	5.96	100.85	H-10'	

(*1*) = cubebin , (*2*) = epicubebin

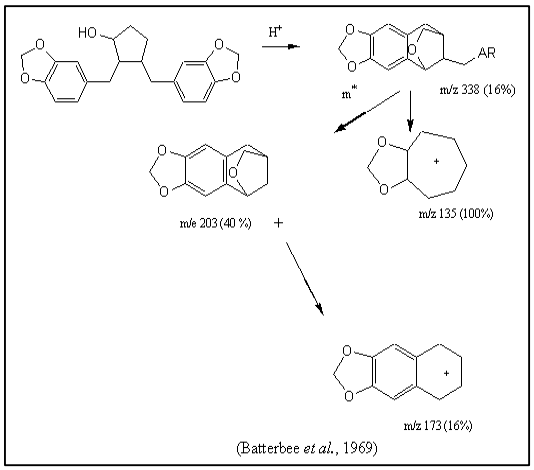

Figure 8. The fragmentation of cubebin

Conclusion

Piper cubeba contains (3,4), (3', 4')- bis-methylenedioxy-9'hydroxy-9, 9'- epoxy-8, 8' lignan (cubebin) and it's epimer (epicubebin) easier to be identified with the basis of 2 D NMR spectral data

Acknowledgements

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