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Morusin, a Bioactive Compound from the Root Bark of *Artocarpus dadah*

1. Introduction

The last report of research to *Artocarpus dadah* plant which belong to Indonesian endemic plant (Lemmens *et al.*, 1995; Heyne, 1987: Jones and Luchsinger, 1987), from the root wood has been isolated a derivative compound of stilben, oxyresveratrol (Suhartati *et al.*, 2009). Previous researcher, Su *et al.* (2002) and Ersam (2001) have investigated the bark of *A. dadah.*

In our furthur research, from the root bark of *A. dadah* has successfully beeb isolated marusin (1), aprenylated (at C-3) flavonoid compound, which represent the first report this flavonoid found in *A. dadah*. The structure of this compound has been identified by physycal data as well as UV-Vis, IR and ^{1}H-NMR spectroscopis. By the finding of marusin inthis plant, it has proven the Nomura hypothesis who stated that the marker compound from Artocarpus is a prenylated flavon compound at C-3 (Nomura *et al.*, 1998). In the cytotoxycity test using murine leukemia P-388 cell, morusin has shomn high activity eith IC_{50} value of 3.1 µg/mL.

2. Materials and Methods

2.1 Plant Material

The root bark *A. dadah* were collected from Wonoasri village, North Metro, Lampung, in March 2008 and were identified in Herbarium Bogoriense, Research centra fo Biologi, Indonesian Institute of Sciences, Bogor, Indonesia and a voucher specimen has beeb deposited at the herbarium.

2.2 General Experimental Procedures

Thin Layer Chromatography (TLC) analysis was carried out pre-coated Si-gel plates (Merck Kieselgel 60 F254) andd the UV lamp of spectroline, ENF-240 c/F model was used to see thr spot in TLC. VLC was carried out using MerckSi-gel 60. Melting point were determined on Fisher Jhons micro-melting point apparatus and were uncorrected. UV-Vis and IR Spectra were measured with Beckman DU-7000 and verian 2000 FTIR spectophotometer respectively. ^{1}H-NMR spectrum was recorded with JEOL ECA 500 spectometer, operating at 500.00 MHz

2.3 Isolation and Purification of the Compounds

2.4 kg of root bark powder of *A. dadah* was macerated with methanol for 3 x x24 hours, with a maceration was 200 g. The methanol extract obtained was filtered and then evaporated by

rotavapor at 45-50^{0}C with velocity of 120-150 rpm. To the concentrate methanol extract was added 1% NaCl solution by proportion 1:4 to methanol extract, and then was partitioned with dichloromethane (DCM)-ethyl acetate 20% to afford 151.28 g extract.

This extract was fractionated by VLC over Si gel, eluted with gradient mixture of methanol-DCM to afford four main fractions (A-D). The main fraction B (2.1675 g) and C (47 g) were fractionated with VLC over furthur si gel using gradient mixture of ethyl acetate *n*-hexane, DCM, and ethyl acetate solventd eith several concentration variation, the fraction which the same Ef in TLC were combined, then furthur pufiried by CC and flach CC. From this combined fraction was obtained the brown-yellow crystals (1) (25 mg), mp 118-123^{0}C (crystallization in DCM-*n*-hexane). Chromatogram TLC of compoun (1) using three eluent systems were showed one main spot Rf 0.20; 0.31; and 0.63 respectively using ethyl acetate-DCM 5%, ethyl acetate-*n*-hexane 30% and ethyl acetate-DCM-*n*-hexane 3:3:4 eluent mixtures.

2.4 Bioactivity Test on the Pure Compound

The bioactivity test done includes the cytotoxicity test compound (1) based on the method of Alley *et al.* (1988).

2.5 Structure Determination

The structure of pure compound was determined based on physical data and spectroscopy techniques namely melting point, test eith some specific reagent, spectra analysis of UV-Vis, IR and NMR.

3. Results and Discussions

3.1 The Analysis of Spectrometry

The UV-Vis spectrum obtained for brown-yellow crystal is shown in Figure 1, with absorption at maximum wavelengths 204, 279 and 328 nm. This UV spectrum indicates a flavonoid (Markham, 1988) which prenylated at C-3 on flavon skeleton (Suhartati, 2006), as shown in band I at λ_{max} 328 nm has lower intensity than band II at λ_{max} 279 nm. In NaOH addition, the spectrum showed bathochromic effect of band I 40 nm, which informed the presence of free OH group at C-4' on flavon skeleton. IR spectrum of this compound (Figure 2) showed absorptions at 3365 cm^{-1} for OH group and conjugated carbonyl group at 1655 and 1620 cm^{-1}, while the presence of aromatic system was shown by absoption at 1597-1467 cm^{-1}.

The ^{1}H-NMR spectrum of compound (1) (Figure 3) confirmed the existence of aromatic skeleton and hydroxyl group in this compound, that is signals at (Figure 3) (aceton-*D6,* 500 MHz) δ (ppm) : 13.57 and 8.85 snglet respectively for proton OH group at C-5 and C-4', while aromatic proton ABX system were shown at 7.19 (1H, d, J = 8 Hz), 6.56 (1H, d, J = 1.85 Hz), and 6.52 (1H, d, J = 1.85 and 8 Hz) on B ring; and 6.27 (1H, s) on A ring. Isoprenyl substituent at C-3 was shown by protons of two CH_3 groups at δ (ppm) 1.42 (3H, s) and 1.56 (3H, s); and 3 proton of ABX system, that is chemical shift at 3.10 ppm (2H, d, J = 7 Hz) and 5.11 ppm (1H, t, J = 7 Hz). While 2,2-dimethylchromen from isoprenyl substituent at C-8 was shown by protons from two CH_3 group with chemical shift at 1.45 ppm (6H, s) and two protons of a vinyl group which bound two C-8 on A ring at 6.67 ppm (1H, d, J = 10 Hz) and 5.74 ppm (1H, d, J = 10 Hz). Based on the ^{1}H-NMR data spectrum can be concluded that compound (1) was a prenylated flavon at C-3 containing two hydroxyl groups at C-2' and C-4', and 2,2-dimethylchromen which belong to isophrenyl at C-8. The flavon compound processing spectrum data equevalent with compound (1) was morusin (Figure 4). The comparisson of ^{1}H-NMR data of compound (1) and morusin was shown in Table 1. By the finding of morusin in *A. dadah*, it is strengthened the hypothesis by Nomura *et al.* (1998) that this plant is part of Artocarpus genus, which contains flavon compoun prenylated at C-3.

Figure 1: Ultraviolet spectrum of compound (1): (a) in MeOH (b) MeOH + NaOH

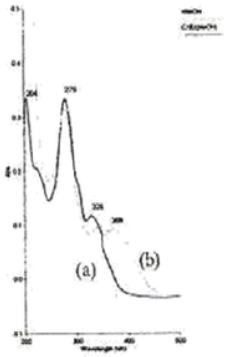


Figure 2: IR spectrum of compound (**1**)

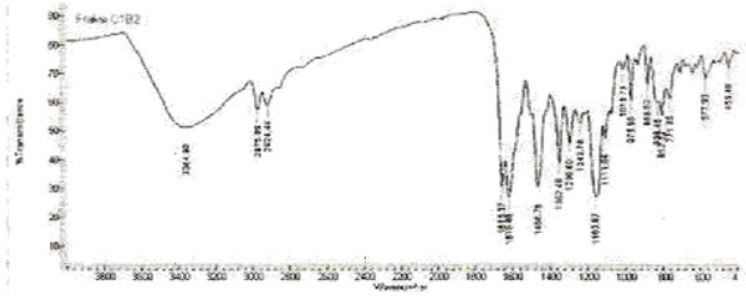


Figure 3: ^{1}H-NMR spectrum of compound (1)

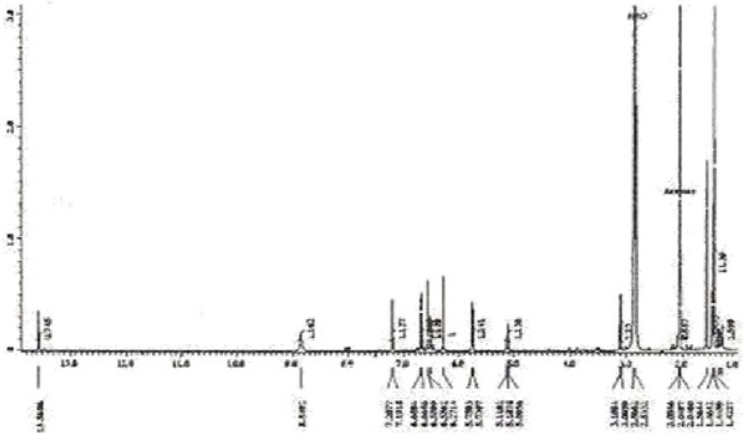
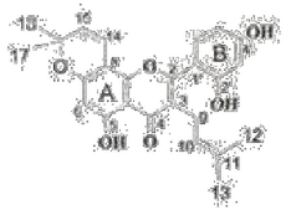


Table 1: The comparison ^{1}H-NMR of compound (1) and morusin (Shi-De *et al.*, 1995)

^{1}H-NMR, δ (ppm)	
Morusin, ($CDCl_3$)	**Compound (1), (acetone D_6)**
1.45 (3H, dq, *J* = 1.0; 0.4 Hz, H13)	1.42 (3H, s)
1.44 (6H, s, H17 dan 18)	1.45 (6H, s)
1.61 (3H, q, *J* = 1.3 Hz, H12)	1.56 (3H, s)
3.13 (2H, dd d, J = 1.0; 6.8Hz, H9)	3.10 (2H, d, *J* = 7 Hz)
5.14 (1H, td, *J* = 6.9; 1.4 Hz, H10)	5.11 (1H, t, *J* = 7 Hz)
5.47 (1H, d, *J* = 10 Hz, H15)	5.74 (1H, d, *J* = 10 Hz)
6.21(1H, d, *J* = 0.7 Hz, H6)	6.27 (1H, s)
6.45 (1H, dd, *J* = 8.4; 2.3 Hz, H5')	6.51 (1H, dd, *J* = 1.85 dan 8.3 Hz)
6.65 (1H, d, *J* = 2.2 Hz, H3')	6.56 (1H, d, *J* = 1.85 Hz)
6.63 (1H, dd, *J* = 10.0; 0.7 Hz, H14)	6.68(1H, d, *J* = 10 Hz)
7.11 (1H, d, *J* = 8.4 Hz, H6')	7.19 (1H, d, *J* = 8 Hz)
	8.85 (1H, br s)
	13.57 (1H, s)

Figure 4: Molecular structure of morusin



3.2 Bioactivity Test

The bioactivity test of compound (1) using murine leukemia P-388 cells, compound (1) showed high cytotoxicity with IC_{50} value of 3.1 µg/mL. The possibility of high activity of compound (1) is due to the fact that compound (1) has two free hydroxyl groups at B ring and a prenyl group at C-3 similar to thus of artonin E and artocarpin (Suhartati, 2001).

Conclusions

In this research, it has been successfully isolated compound (1) which was a prenylated flavonoid at C-3, Morusin, which was the first reported from *A. dadah*. Compound (1) has high activity against murine leukemia P-388 cells which IC_{50} value of 3.1 µg/mL.

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