EJSR

EUROPEAN JOURNAL OF SCIENTIFIC RESEARCH

Vol 38 No 4
December, 2009

USD $25.00
CAD $30.00

ISSN: 1450-216X

HALAMAN PENGESAHAN

Judul	:	**Morusin, a Bioactive Compound from the Root Bark of *Artocarpus dadah***
Oleh	:	Jhons Fatriyadi Suwandi
Sebagai	:	Anggota (Kelompok)
NIP	:	197631082003121003
Program Studi	:	Pendidikan Dokter
Fakultas	:	MIPA
Buku/Jurnal/Prosiding	:	**European Journal of Scientific Research**
Halaman	:	643 – 648
ISBN	:	1450-216X Vol. 38 No. 4

Bandar Lampung, 8 Maret 2010
Ketua Peneliti

dr. Jhons Fatriyadi Suwandi, M.Kes
NIP 197631082003121003

Menyetujui

Dekan
Fakultas MIPA Unila

Dr. Sutyarso, M. Biomed
NIP 19570424 198703 1 001

Ketua PS Pendidikan Dokter
Fakultas MIPA Unila

Prof.Dr.dr.Efrida Warganegara, M.Kes., SpMK
NIP 195012231977102001

Ketua Lembaga Penelitian
Universitas Lampung

Prof. Dr. John Hendri, M.S.
NIP 19581021 198703 1 001

European Journal of Scientific Research

ISSN: 1450-216X

Volume 38, No 4 December, 2009

As of 2005, European Journal of Scientific Research is indexed in ULRICH, DOAJ and CABELL academic listings.

European Journal of Scientific Research

http://www.eurojournals.com/ejsr.htm

Editorial Policies:

1) European Journal of Scientific Research is an international official journal publishing high quality research papers, reviews, and short communications in the fields of biology, chemistry, physics, environmental sciences, mathematics, geology, engineering, computer science, social sciences, medicine, industrial, and all other applied and theoretical sciences. The journal welcomes submission of articles through ejsr@eurojournals.com.

2) The journal realizes the meaning of fast publication to researchers, particularly to those working in competitive & dynamic fields. Hence, it offers an exceptionally fast publication schedule including prompt peer-review by the experts in the field and immediate publication upon acceptance. It is the major editorial policy to review the submitted articles as fast as possible and promptly include them in the forthcoming issues should they pass the evaluation process.

3) All research and reviews published in the journal have been fully peer-reviewed by two, and in some cases, three internal or external reviewers. Unless they are out of scope for the journal, or are of an unacceptably low standard of presentation, submitted articles will be sent to peer reviewers. They will generally be reviewed by two experts with the aim of reaching a first decision within a three day period. Reviewers have to sign their reports and are asked to declare any competing interests. Any suggested external peer reviewers should not have published with any of the authors of the manuscript within the past five years and should not be members of the same research institution. Suggested reviewers will be considered alongside potential reviewers identified by their publication record or recommended by Editorial Board members. Reviewers are asked whether the manuscript is scientifically sound and coherent, how interesting it is and whether the quality of the writing is acceptable. Where possible, the final decision is made on the basis that the peer reviewers are in accordance with one another, or that at least there is no strong dissenting view.

4) In cases where there is strong disagreement either among peer reviewers or between the authors and peer reviewers, advice is sought from a member of the journal's Editorial Board. The journal allows a maximum of two revisions of any manuscripts. The ultimate responsibility for any decision lies with the Editor-in-Chief. Reviewers are also asked to indicate which articles they consider to be especially interesting or significant. These articles may be given greater prominence and greater external publicity.

5) Any manuscript submitted to the journals must not already have been published in another journal or be under consideration by any other journal, although it may have been deposited on a preprint server. Manuscripts that are derived from papers presented at conferences can be submitted even if they have been published as part of the conference proceedings in a peer reviewed journal. Authors are required to ensure that no material submitted as part of a manuscript infringes existing copyrights, or the rights of a third party. Contributing authors retain copyright to their work.

6) Submission of a manuscript to EuroJournals, Inc. implies that all authors have read and agreed to its content, and that any experimental research that is reported in the manuscript has been performed with the approval of an appropriate ethics committee. Research carried out on humans must be in compliance with the Helsinki Declaration, and any experimental research on animals should follow internationally recognized guidelines. A statement to this effect must appear in the Methods section of the manuscript, including the name of the body which gave approval, with a reference number where

appropriate. Manuscripts may be rejected if the editorial office considers that the research has not been carried out within an ethical framework, e.g. if the severity of the experimental procedure is not justified by the value of the knowledge gained. Generic drug names should generally be used where appropriate. When proprietary brands are used in research, include the brand names in parentheses in the Methods section.

7) Manuscripts must be submitted by one of the authors of the manuscript, and should not be submitted by anyone on their behalf. The submitting author takes responsibility for the article during submission and peer review. To facilitate rapid publication and to minimize administrative costs, the journal accepts only online submissions through ejsr@eurojournals.com. E-mails should clearly state the name of the article as well as full names and e-mail addresses of all the contributing authors.

8) The journal makes all published original research immediately accessible through www.EuroJournals.com without subscription charges or registration barriers. European Journal of Scientific Research indexed in ULRICH, DOAJ and CABELL academic listings. Through its open access policy, the Journal is committed permanently to maintaining this policy. All research published in the. Journal is fully peer reviewed. This process is streamlined thanks to a user-friendly, web-based system for submission and for referees to view manuscripts and return their reviews. The journal does not have page charges, color figure charges or submission fees. However, there is an article-processing and publication charge.

Further information is available at: http://www.eurojournals.com/ejsr.htm

European Journal of Scientific Research
Volume 38, No 4 December, 200

Contents

European Journal of Scientific Research
ISSN 1450-216X Vol.38 No.4 (2009), pp 643-648

http://www.eurojournals.com/ejsr.htm

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

Tati Suhartati
Department of Chemistry, University of Lampung, Bandar Lampung 35145 Indonesia
E-mail: tatisuhartati@unila.ac.id

Yandri A.S
Department of Chemistry, University of Lampung, Bandar Lampung 35145 Indonesia

Sutopo Hadi
Department of Chemistry, University of Lampung, Bandar Lampung 35145 Indonesia

Jhons Fatriyadi S
Study Program of Medicine, University of Lampung, Bandar Lampung 35145 Indonesia

Abstract

Morusin, a flavonoid prenylated at C-3 compound, has been isolated from the root bark of *Artocarpus dadah* which grown in Lampung. The structure of this compound has been identified by physical and spectroscopy methods. This compound has high cytotoxycity against murine leukemia cell P-388, IC_{50} 3,1 µg/mL.

Keywords: *Artocarpus dadah*, morusin, leukemia P-388.

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 further research, from the root bark of *A. dadah* has successfully been isolated morusin (I), a prenylated (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 morusin in this 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 shown high activity with 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 Center for Biology, Indonesian Institute of Sciences, Bogor, Indonesia and a voucher specimen has been deposited at the herbarium.

2.2. General Experimental Procedures

Thin layer chromatography (TLC) analysis was carried out on pre-coated Si-gel plates (Merck Kieselgel 60 F254) and th UV lamp of Spectroline, ENF-240 C/F model was used to see thr spot in TLC. VLC was carried out using Merck Si-gel 60. Melting point were determined on a Fisher Jhons micro-melting point apparatus and were uncorrected. UV-Vis and IR spectra were measured with Beckman DU-7000 and Varian 2000 FTIR spectrophotometer respectively. ^{1}H-NMR spectrum was recorded with JEOL ECA 500 spectrometer, 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 24 hours, with a maceration was 200 g. The methanol extract obtained was filtered and then evaporated by rotavapor at 45-50°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 fractions B (2.1675 g) and C (47 g) were fractionated with VLC over further Si gel using gradient mixture of ethyl acetate-*n*-hexane respectively. After passing the several ranks of VLC, CC, and flash chromatography using *n*-hexane, DCM, and ethyl acetate solvents with several concentration variation, the fractions which the same Rf in TLC were combined, then further purified by CC and flash CC. From this combined fraction was obtained the brown-yellow crystals (**1**) (25 mg), mp 118-123°C (crystallization in DCM-*n*-hexane). Chromatogram TLC of compound (**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 acetat-*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 of 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 with 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 absorption at 1597-1467 cm^{-1}.

The ^1H 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) (aseton-*D6*, 500 MHz) δ (ppm): 13.57 and 8.85 singlet respectively for proton OH group at C-5 dan 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, dd, 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 (3 H, s); and 3 proton of ABX system, that is chemical shift at 3.10 ppm (2 H, d, J = 7 Hz) and 5.11 ppm (1 H, t, J = 7 Hz). While 2,2-dimetilchromen from isoprenyl substituent at C-8 was shown by protons from two CH_3 group with chemical shift at 1.45 ppm (6 H, s) and two protons of a vinyl group which bound to C-8 on A ring at 6.67 ppm (1 H, d, J = 10 Hz) and 5.74 ppm (1 H, d, J = 10 Hz). Based on the ^1H-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-dimetilchromen which belong to isoprenyl at C-8. The flavon compound possesing spectrum data equivalent with compound (**1**) was morusin (Figure 4). The comparison of ^1H 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 coutains flavon compound prenylated at C-3.

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

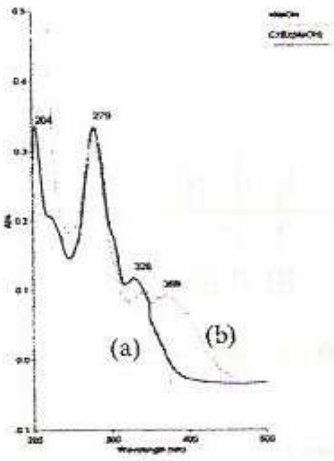


Figure 2: IR spectrum of compound (**1**)

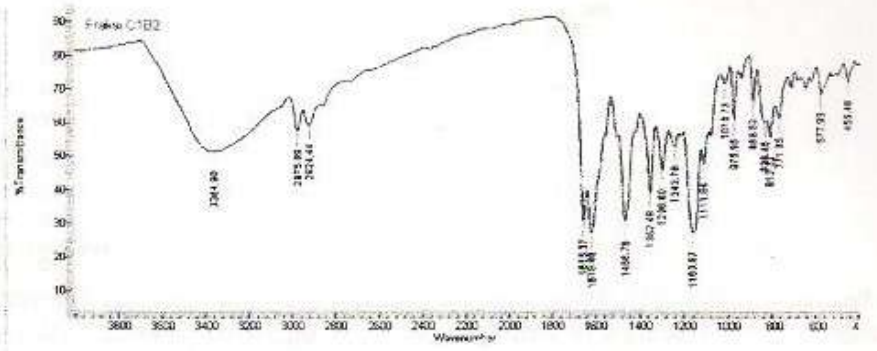


Figure 3: ^{1}H-NMR spectrum of compound (1)

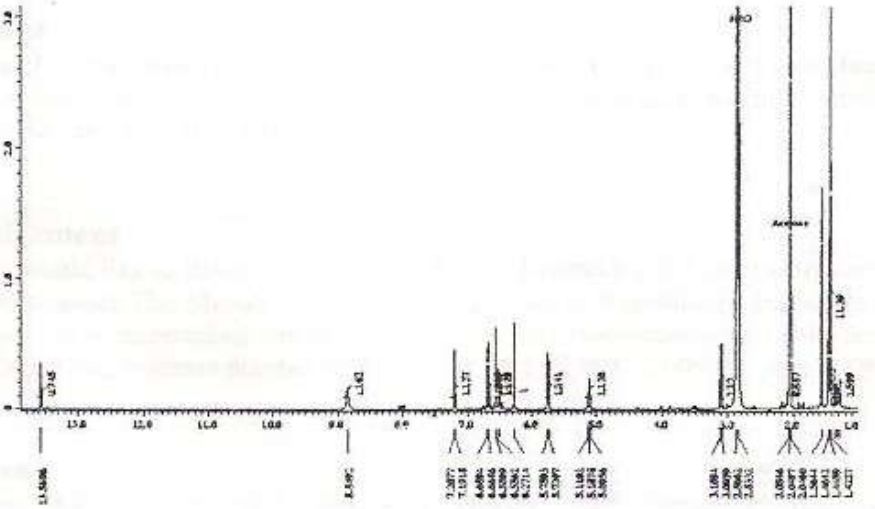
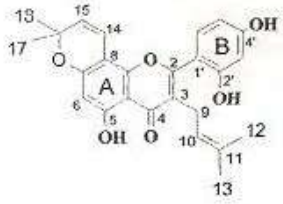


Table 1: The comparison ^{1}H-NMR of compound (1) and morusin (Shi-De *et al.*, 1995)

^{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 those 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 with IC_{50} value of 3.1 µg/mL.

Ackowledgement

The authors would like to thank to The Directorate of Research and Community Services, Directorate of Higher Education, The Ministry of National Education of Republic of Indonesia that provide fund for this project to be undertaken through *Hibah Kompetitif Penelitian Sesuai Prioritas Nasional* Tahun Anggaran 2009, with contract number of 321/SP2H/PP/DP2M/VI/2009, 16 June 2009.

References

[1] Alley, M.C., Scudiero, D.A., Monks, A., Hursey, M.L., Czerwinski, M.J., Fine, D.L., Abbott, B.J., Mayo, J.G., Shoemaker, R.H., Boyd, M.R. 1988. Feasibility of Drug Screening with Panels of Human Tumor Cell Lines Using a Microculture Tetrazolium Assay. *Cancer Res.*, **48**, 589-601.

[2] Ersam, T. 2001. The Micromolecule Chemical Compound of Some Artocarpus Plants from Tropical Forest at West Sumatera. Ph.D. Disertation, Department of Chemistry, Bandung Institue of Technology, Bandung, Indonesia (in Indonesian)

[3] Heyne, K. 1987. Important Indonesian Plants *II*, Departemen Kehutanan, Jakarta. (in Indonesian)

[4] Jones, S.B. and Luchsinger, A.E., 1987. *Plant Systematics*, 2nd ed.,McGraw-Hill Book Co., New York, 477-478.

[5] Lemmens, R.H.M.J., I. Soerianegara, and W.C. Wong (Ed), 1995. Plant Resources of South-East Asia 5, (2) Timber Trees: Minor Commercial Timbers, Bogor, 59-70.

[6] Markham, K. R. 1988. The Identification Methods of Flavanoid. Penerbit ITB, Bandung, p. 41. (in Indonesian)

[7] Nomura, T., Hano, S. and Aida, M., 1998. Isoprenoid-Substituted Flavonoid from *Artocarpus* Plants (Moraceae), *Heterocycles*, **47** (2), 1179-1205.

[8] Shi-De, L., Nemec, J. and Ning, B.-M., 1995. Anti-HIV Flavonoids from Morus Alba, *Acta Botan. Yunnanica*, **17** (1), 89-95.

[9] Su, B.-N., Cuendet, M., Hawthorne, M.E. , Kardono, L.B. S., Riswan, S. , Fong, H.H.S., Mehta, R.G. , Pezzuto, J.M. and Kinghorn, A.D. 2002. Constituents of the Bark and Twigs of *Artocarpus dadah* with Cyclooxygenase Inhibitory Activity, *J. Nat. Prod.*, **65** (2), 163 –169.

[10] Suhartati T., Yandri A.S., and Hadi, S. 2009. The Bioactivity Test of Oxyresveratrol, a Bioactive Compound, Isolated from the Root Wood of *Artocarpus dadah*, *Int. J. Pure App. Chem.*, **4** (3), 223 – 229.

[11] Suhartati, T. 2006. The Spectrophotometry UV-Vis Analysis of Some Flavanoid Compounds from Artocarpus Plant, in Aplication and Method of Chemical Analysis. A *Monograph Series*, Department of Chemistry, University of Lampung, 23-31. (in Indonesian).

[12] Suhartati, T. 2001. Phenol Compound of Several Species from Some Indonesian Cempedak Plants, Ph.D. Dissertation, ITB, Bandung. (in Indonesian).