Identification of Massoia Lactone and its Hydroxy-derivative from Kabatiella caulivora, an Endophyte of the Terrestrial Plant Alyxia reinwardtii

Izzatul Laili  
*Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286, Indonesia*

Sri Gunarso  
*Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286, Indonesia*

Nashrul Wathan  
*Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286, Indonesia*

Noor Erma Sugijanto  
*Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286, Indonesia*

Gunawan Indrayanto  
*Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286, Indonesia, gunawanindrayanto@yahoo.com*

Follow this and additional works at: [https://scholarhub.ui.ac.id/science](https://scholarhub.ui.ac.id/science)

**Recommended Citation**

Laili, Izzatul; Gunarso, Sri; Wathan, Nashrul; Sugijanto, Noor Erma; and Indrayanto, Gunawan (2017) "Identification of Massoia Lactone and its Hydroxy-derivative from Kabatiella caulivora, an Endophyte of the Terrestrial Plant Alyxia reinwardtii," *Makara Journal of Science*: Vol. 21 : Iss. 4 , Article 6.  
DOI: 10.7454/mss.v21i4.8840  
Available at: [https://scholarhub.ui.ac.id/science/vol21/iss4/6](https://scholarhub.ui.ac.id/science/vol21/iss4/6)

This Article is brought to you for free and open access by the Universitas Indonesia at UI Scholars Hub. It has been accepted for inclusion in Makara Journal of Science by an authorized editor of UI Scholars Hub.
Identification of Massoia Lactone and its Hydroxy-derivative from Kabatiella caulivora, an Endophyte of the Terrestrial Plant Alyxia reinwardtii

Izzatul Laili, Sri Gunarso, Nashrul Wathan, Noor Erma Sugijanto, Gunawan Indrayanto

Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286, Indonesia

*E-mail: gunawanindrayanto@yahoo.com

Received May 8, 2017 | Accepted September 26, 2017

Abstract

Massoia lactone (1) and its derivative, 3,5-dihydroxydecanoic acid-δ-lactone (2), were identified from an ethyl acetate extract of the endophytic fungus Kabatiella caulivora, isolated from its host Alyxia reinwardtii. The compounds were identified by GC-MS. To the best of our knowledge, this is the first report of the identification of massoia lactone and its derivative from the endophytic K. caulivora fungus.

Introduction

Endophytic fungi are widely recognized as prolific sources of secondary metabolites that might be useful for the development of new pharmaceutical agents. Many novel bioactive products, such as antibiotics and anticancer and antidiabetic agents, have been isolated from these fungi [1].

Some endophytic fungi, such as Lecythophora sp., Hypocrea cf. koningii, Kabatiella caulivora strains A and B, Cladosporium oxysporum, and Aspergillus penicilloides, have been isolated in our laboratory from Alyxia reinwardtii [2], a slow-growing member of the Apocynaceae found in tropical climates. A. reinwardtii has been used in Indonesian traditional medicine (jamur), but the species is nearly extinct at present [3].

A new secondary metabolite, lecythomyacin, and seven known compounds—(2R)-3-(2-hydroxypropyl)-benzene-1,2-diol, kojic acid, 7-O-acetyl-kojic acid, p-hydroxybenzoic acid, emodine, 7-chloroemodine, and ergosterol-5,8-peroxide—have been isolated from Lecythophora sp. [4,5]. Pandangolide 1 was isolated for the first time from the ethyl acetate extract of Cladosporium oxysporum cultures [6]. Compound 1 was isolated from Aureobasidium pullulans [7], where the genus Aureobasidium was synonymous with Kabatiella [8]. Compounds 1 and 2 were also isolated from another Aureobasidium sp. [9]. Four other secondary metabolites—2-propylacrylic acid, 8,9-dihydroxy-2-methyl-4H,5H-pyrano[3,2-c] chromon-4-one, 2-methylene succinic acid and hexane-1,2, 3,5,6-hexol—were isolated from A. pullulans [10]. To the best of our knowledge, no publication has reported the secondary metabolites of K. caulivora, so the study of these metabolites in this fungus was of interest.

Keywords: endophytic fungus, Kabatiella caulivora, Alyxia reinwardtii, massoia lactone, 3,5-dihydroxydecanoic acid-δ-lactone
As a continuation of our work on secondary metabolites from endophytic fungi, we report the first identification of massoia lactone (1) and 3,5-dihydroxydecanoic acid δ-lactone (2) from the ethyl acetate extracts of the endophytic fungus, *K. caulivora* strain A, isolated from *A. reinwardtii* (Figure 1).

**Materials and Methods**

**Plant material and isolation of the endophytic fungus.** *K. caulivora* strains A and B were isolated from stems of *A. reinwardtii* (collected from the Purwodadi Botanical Garden, East Java, Indonesia). The sterilized stems were cut aseptically and the inner parts of the tissues were impregnated onto agar malt extract agar (MEA) medium containing powdered plant material (15 g/L) and chloramphenicol (0.2 g/L). Pure strains were obtained by repeated inoculation of the growing fungi on agar plates with fresh MEA medium. The endophyte was identified by Dr. Arnulf Diesel, Institut fuer Pharmazeutische Biologie Universitaet Dusseldorf, Germany, as described by Sugijanto [2].

**Cultivation of the endophytic fungus.** A total of 523 culture bottles (40 mL) containing liquid malt extract medium was used for cultivation of *K. caulivora* strain A. The fungus was cultivated for 28 days at room temperature (30 ± 3 °C) at an initial pH of 5.75 ±0.05.

**Extraction and fractionation.** The culture broth and mycelia (20 L) were extracted with ethyl acetate (EtOAc, 73 L). The EtOAc layers were collected, combined, and concentrated under vacuum at 35 °C to yield a blackish brown concentrated extract (5.34 g). This extract subjected to column chromatography on 200 g silica gel 60 (62.5 × 3.5 cm) as the stationary phase and eluted with mixtures of *n*-hexane, EtOAc, and methanol (MeOH) in increasing polarity, which yielded 17 fractions. Fraction 9 (157.3 mg) showed antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* using the disk diffusion method [11], so fraction 9 was further purified by column chromatography on Sephadex LH 20 (16 g) with MeOH as the mobile phase. This yielded a semi-purified oily fraction **a** (20.1 mg) that had a specific aroma of massoia bark oil. This fraction **a** was dissolved in 5 mL MeOH.

**Gas Chromatography-Mass Spectrometry (GC-MS).** The GC-MS analysis of fraction **a** was performed using an Agilent GC 6890N and Mass Selective Detector (MSD) 5973, equipped with an HP-5 column (30 m × 0.250 mm × 0.25 μm i.d.), and completed using the Wiley 7n.1 data base (2004). The inlet temperature was set at 250 °C, the split ratio was 50:1, the flow rate was 1 mL/min (He), and the oven temperature was programmed at 100–250 °C, at 5 °C/min. The electron impact ionization (EI-MS) source temperature was set at 230 °C, the transfer line temperature from the oven to the detector was 280 °C, and the energy of ionization was set at 70 eV.

**Method of Identification.** The EI MS spectra of peaks 1 and 2 were compared to the Wiley database, the online data bases of the National Institute of Standards and Technology (NIST) [12], the Spectral Database for Organic Compounds (SDBS) [13], and published reports [7, 9, 14-16], according to the method recommended by the Commission Decision 2002/657/EC [17].

**Results and Discussion**

The total ion chromatogram (TIC) of fraction **a** exhibited two main peaks at retention times (Rt) of 9.8 and 15.0 min (see Figure 2).
The EI-MS spectrum of peak 1 (see Figure 3) showed a very small molecular ion at $m/z$ 168, with other peaks observed at $m/z$ 97 (base peak), 69, 68, and 41. The Wiley database predicted that peak 1 was massoia lactone (1), with the highest quality score (90%). The EI-MS of the peak 1 was identical to the EI-MS spectra of massoia lactone of published by NIST [12], SDBS [13], previous publications [7,14-16], and the main compound of massoia bark oil (1) [unpublished work]. According to the Commission Decision 2002/657/EC, the identity of a compound can be confirmed if its MS spectrum shows at least 4 identical fragments when compared to the standard MS [17]. All these data confirmed unambiguously that peak 1 was massoia lactone or 2-deceno-δ-lactone (1).

The EI-MS spectrum of peak 2 (Figure 4) showed a similarity with compound (2) according to the Wiley database (highest quality score 90%). The EI-MS of peak 2 showed peaks at $m/z$ 168, 115 (base peak), 97, 73, and 43, which was identical to the EI-MS of 2 in previous publications [9,15]. The presence of a hydroxyl group was confirmed by intense peaks of $m/z$ at 97 and 115. The absence of the molecular ion at $m/z$ 186 was due to a loss of H₂O from the molecular ion and a fragment at $m/z$ 168 was produced [15]. These data confirmed [17] that peak 2 was 3,5-dihydroxydecanoic acid-δ-lactone (2), a hydroxyl derivative of 1.

The configuration of compounds 1 and 2 isolated from *Aureobasidium* sp. and other fungi were (R)-(−) and (R)-(+)[9,15,18], while compound 1 from massoia bark oil...
also showed (R)-(−) [19]. The configurations of 1 and 2 in *K. caulivora* could not be determined by GC-MS in the present work, but their configurations can be assumed as (R)-(−) and (R)-(+) respectively, as described in the previous work [9, 15, 18].

Previous publications [7, 9] indicated that the genus *Kabatiella* or *Aureobasidium* could produce compound 1 and 2, and this present work reported the identification of both compounds from *K. caulivora* cultures. Consequently, compounds 1 and 2 might be specific to this genus. Further work is needed to confirm this hypothesis.

Compound 1 was isolated from other fungi, including *Fusarium solani* and *Trichoderma viride* [18], while compounds 1 and 2 were also identified from the fungus *Cephalosporium recifei* [15]. Compound 1 was isolated from plants (i.e., sugar cane molasses, coconut and massoia bark-oil, and *Solanum muricatum* [16, 19, 20]). This shows that compound 1 is present in both fungi and plants. Compound 1 has reported activity against the growth of *Candida* sp. [7, 21], while the antibacterial activity of compound 1 against some microorganisms was reported by Simionatto et al. [22]. This activity could explain the activity of fraction a against *C. albicans*, *S. aureus*, and *E. coli*. Compounds 1 and 2 could be present in fungi and plants to function as a defense system against microorganisms.

**Conclusion**

Massoia lactone (1) and 3,5-dihydroxydecanoic acid-δ-lactone (2) were identified for the first time in the endophytic fungus *K. caulivora* isolated from *A. reinwardtii*. Compounds 1 and 2 appear to be specific to the genus *Kabatiella*.

**References**

[1] Yu, H., Zhang, L., Li, L., Zheng, C., Guo, L., Li, W., Sun, P., Qin, L. 2010. Recent developments and future prospects of Antimicrobial metabolites produced by endophytes. Microbiol. Res. 165(6): 437-449, doi:10.1016/j.mires.2009.11.009.

[2] Sugijanto, N.E. 2008. Isolasi Jamur Endofit Dari *Alyxia reinwardtii* BI: Studi Metabolit Sekunder Dari Bioaktivitas Jamur Baru *Lecythophora* sp., Disertasi Doktor (Ph. D. Disertation), Airlangga University, Surabaya, Indonesia.

[3] Yuliani, S. 2001. Prospek pengembangan obat tradisional menjadi obat fitofarmaka. J. Litbang Pertanian, 20(3):100-105. [In Indonesian]

[4] Sugijanto, N.E., Diesel, A., Ebel, R., Indrayanto, G. and Zaini, N.C. 2009. Chemical constituents of the endophytic fungus *Lecythophora* sp. isolated from *Alyxia reinwardtii*. Nat. Prod. Commun. 4(11):1485 -1488.

[5] Sugijanto, N.E., Diesel, A., Rateb, M., Pretsch, A., Gogalic, S., Zaini, N.C., Ebel, R. and Indrayanto, G. 2011. Lecythomycin, a new macrolactone glycoside from the endophytic fungus *Lecythophora* sp. Nat. Prod. Commun. 6(5): 677-678.

[6] Hartanti, D., Purwanti, D.I., Putro, H.S., Rateb, M.E., Wongso, S., Sugijanto, N.E., Ebel, R. and Indrayanto, G. 2014. Isolation of Pandangolide 1 from *Cladosporium oxysporum*, An Endophyte of the Terrestrial Plant *Alyxia reinwardtii*. Makara J. Sci. 18(4):123-126, doi: 10.7454/mss.v18i4.4341.

[7] Tay, S.T., Lim, S.L., Tan, H.W. 2014. Growth inhibition of *Candida* species by *Wickerhamomyces anomalus* mycocin and a lactone compound of *Aureobasidium pullulans*. Altern. Med. 14: 439, http://www.biomedcentral.com/1472-6882/14/439.

[8] Mycobank: *Kabatiella caulivora*. http://www.mycobank.org/name/Kabatiella caulivora.

[9] Kurosawa, T., Sakai, K., Nakahara, T., Oshima, Y. and Tabuch, T. 1994. Extracellular Accumulation of the Polyol Lipids, 3, 5-Dihydroxydecanoyl and 5-Hydroxy-2-decenoyl Esters of Arabitol and Mannitol, by *Aureobasidium* sp. Biosci. Biotechnol. Biochem. 58(11):2057-2060.

[10] Zain, M.E., Aman, S., Razak, A.A., Matlan, D. J., Khamis, N.E., Shakawy, M.A. 2009. Secondary metabolit from *Aureobasidium pullulans* isolated from Egyptian Soil and their Biological Activity, J. Appl. Sci. Res. 5(10):1582-1591.

[11] Prihaningrum, I.A. 2011. Aktivitas Antimikroba Fraksi dari Ekstrak Etil Asetat Jamur Endofit *Kabatiella caulivora* var. A dari *Alyxia reinwardtii*. Skripsi (B.Sc Thesis) Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia.

[12] NIST Chemistry WebBook, SRD 69, Massoia Lactone http://webbook.nist.gov/cgi/cbook.cgi?Name=Massoilaconetne&Units=SI&cMS=on.

[13] Spectral Data Base for Organic Compound SDBS, SDBS information, SDBS no 52791 http://sdb.sdb.db.aist.go.jp/sdb-bin/direct_frame_top.cgi.

[14] Cavill, G.W.K., Clark, D.V., Whitefield, F.B. 1968. Insect venoms, attractants, and repellents. XI Massoia-lactone from two species of formicine ants, and some observations on constituents of the bark oil of *Cryptocarya massoia*. Aust. J. Chem. 21(11):2819-2823.

[15] Vesonder, R.F., Stodola, F.H. and Rohwedder, W.K. 1972. Formation of the δ-lactone of 3, 5-dihydroxydecanoic acid by the fungus *Cephalosporium recifei*. Can. J. Biochem. 50(4):363-365.

[16] Hashizume, T., Kikuchi, N., Sasaki, Y. and Sakata, I. 1968. Constituents of Cane Molasses: Part III. Isolation and Identification of (−)-2-Decono-5-Lactone (Massoia lactone). Agric. Biol. Chem. 32(10):1306-1309.
[17] European Commission Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Off. J. Eur. Commun. L221 (2002): 8–36.

[18] Nago, H., Matsumoto, M. Nakai, S. 1993. 2-Decano-δ-lactone-producing Fungi, Strains of Fusarium solani, Isolated by Using a Medium Containing Decano-δ-lactone as the Sole Carbon Source Biosci. Biotechnol. Biochem. 57(12): 2107-2110.

[19] Schaft, P.H.V.D., ter Burg, N., van de Bosch, S., Amnon M.C. 1992. Micronial production of natural δ-decalactone and δ-dodecalactone from the corresponding α,β-unsaturated lactones in Massoi bark oil. Appl. Microbiol. Biotechnol. 36:712-716.

[20] Rodríguez-Burruezo, A., Kollmannsberger, H., Prohens, J., Nitz, S., Nuez, F. 2004. Analysis of the volatile aroma constituents of parental and hybrid clones of pepino (Solanum muricatum). J. Agric. Food Chem. 52(18):5663-5669.

[21] Hill, R.A., Cutler, H.G. and Parker, S.R., Food Research Institute Of New Zealand Limited. 2000. Use of massoia lactone for inhibition of fungal growth. U.S. Patent 6,060,507

[22] Simionatto, E., Porto, C., Stueker, C. Z., Dalcol, I. L., da Silva, F. 2007, Chemical composition and antibacterial activity of the essential oil from Aeolanthus suaveolus Mart ex Spreng, Quimica Nova 30 (8):1923-1925, http://quimicanova.sbq.org.br/imagebank/pdf/Vol30No8_1923_23-AR06528.pdf.