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NOTE

Irmanida Batubara · Tohru Mitsunaga · Hideo Ohashi

Brazilin from *Caesalpinia sappan* wood as an antiacne agent

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Abstract In screening experiments for antiacne activity, methanolic and 50% ethanolic extracts of *Caesalpinia sappan* wood showed the most potent activity out of 28 species of plants extracts. These extracts showed inhibition of *Propionibacterium acnes* growth, lipase inhibitory activity, and antioxidant activity. In order to isolate the active compound from *C. sappan*, separation of the extract components was performed by column chromatography and preparative high-performance liquid chromatography (HPLC). Brazilin, protosappanin A, and sappanone B were isolated from methanolic extracts. Brazilin showed better antibacterial activity [minimum inhibitory concentration (MIC) = minimum bactericidal concentration (MBC) = 0.50 mg/ml] than protosappanin A (MIC = MBC = 1.00 mg/ml) and sappanone B (MIC = MBC > 2.00 mg/ml). The 50% inhibitory concentration (IC_{50}) for lipase inhibition was lowest for brazilin (6 μM), which showed strong inhibition compared with protosappanin A (100 μM) and chloramphenicol (677 μM, positive control). The antioxidant activity of brazilin (IC_{50} 8.8 μM) was not significantly different from protosappanin A (9.1 μM) and (+)-catechin (10.2 μM). The antioxidant activity of brazilin and protosappanin A were higher than sappanone B (IC_{50} 14.5 μM). Brazilin is considered to have sufficiently potent activity for use as an antiacne agent.

I. Batubara
Department of Chemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Agatis Road IPB Campus, Darmaga-Bogor 16680, Indonesia

I. Batubara · T. Mitsunaga (✉) · H. Ohashi
Department of Applied Biological Science, Faculty of Applied Biological Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan
Tel. +81-58-293-2920; Fax +81-58-293-2920
e-mail: mitunaga@gifu-u.ac.jp

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Key words *Caesalpinia sappan* · Brazilin · Protosappanin A · *Propionibacterium acnes* · Lipase inhibitor

Introduction

Caesalpinia sappan L. (Leguminosae) is distributed and cultivated in Southeast Asia as well as in Africa and the Americas.[1] Many biological activities of *C. sappan* have been reported, such as hepatoprotection,[2] immunomodulation,[3] hypoglycemic agent activity,[4] anticomplementary,[5] anticonvulsant,[6] anti-inflammatory, and antibacterial activity,[7,8] xanthin oxidase inhibition,[9] aldose reductase inhibition,[10] antioxidant activity,[11] and protection of the brain.[12]

In Indonesia, *C. sappan* is traditionally used for skin care, especially on Sumbawa Island,[13] and the wood of *C. sappan* is used to obtain pink pigment for drinks such as Bir Pletok, a Batavian spice drink. *Caesalpinia sappan* has already been reported as a good source of material for skin care, especially against skin photocarcinogenesis and it could be developed as a skin-whitening component for cosmetics.[14] According to the patent, the pigment of *C. sappan* has already been utilized as an antioxidant in cosmetics by a cosmetic company in Japan.[15] Based on our previous screening data, we found that *C. sappan* methanolic extracts and 50% ethanolic extracts have potential as antiacne agents.[16]

Many compounds have already been isolated from the wood of *C. sappan*. Flavonoids and phenolics[17] such as 4-*O*-methylsappanol, protosappanin A,[18] protosappanin B,[19] protosappanin E, brazilin,[20] brazilein, caesalpin J,[21] brazilide A,[22] neosappanone A,[9] caesalpin P, sappanchalcone, 3-deoxysappanone,[10] 7,3′,4′-trihydroxy-3-benzyl-2H-chromene,[23] and others. Some of the biological activities of the isolated compounds from *C. sappan* have already been reported. For example, brazilin, brazilein, and sappanchalcone showed significant inhibition of lipopolysaccharide (LPS)-induced NO production by J774.1 cell line and were found to almost completely suppress iNOS gene expres-

sion.[24] Caesalpin P, sappanchalcone, 3-deoxysappanone, brazilin, and protosappanin A were identified as aldose reductase inhibitors and are useful in the treatment of diabetic complications.[10] Brazilin has antibacterial character and has the potency to be developed into an antibiotic.[7] Sappanone A, 7-hydroxy-3-[(3,4,5-trihydroxyphenyl)methylene] chroman-4-one, 1′,4′-dihydro-spiro[benzofuran-3(2H),3′-(3H-2)benzopyran]-1′,6′,6′,7′-tetrol, and 3-{[4,5-dihydroxy-2(hydroxymethyl)phenyl]1-methyl}-2,3-dihydro-3,6-benzofurandiol were reported to have antioxidant activities.[25,26] Other compounds such as 7-hydroxy-3-[(3,4,5-trihydroxyphenyl)methylene]chroman-4-one have antioxidant and 5-lipoxigenase (5-LOX) inhibitory activities that can be useful for asthma and inflammatory diseases.[25]

Although the antibacterial, antioxidant, and anti-inflammatory effects of *C. sappan* extracts are known, the compound responsible for good antiacne control, especially the antibacterial activity against *Propionibacterium acnes* and *P. acnes* lipase inhibitory activity, have not yet been investigated. In order to identify the active compound from *C. sappan* wood conferring its good antiacne control, we performed tests on the antibacterial properties against *P. acnes*, lipase inhibitor, and antioxidant assays.

Experimental

Plant materials

Caesalpinia sappan wood was purchased from the market in Semarang, Indonesia. The identification and voucher specimens (No. 06001) were deposited in the Biopharmaca Research Center, Bogor Agricultural University, Bogor, Indonesia.

Extraction and isolation of brazilin, protosappanin A, and sappanone B

Caesalpinia sappan was dried and ground before methanol extraction. Briefly, 500 g of *C. sappan* wood meal was macerated in 5 l of methanol for 12 h and the process was repeated twice. The extracts were filtered (Whatman No. 2) and concentrated in vacuo at 30°C using a rotary evaporator to obtain 43.0 g of extract (yield 8.63% based on dried sample).

Part of the extract (10 g) was separated by column chromatography on silica gel by elution with hexane, ethyl acetate, and methanol to give 30 fractions. Some fractions eluted with ethyl acetate gave a mixture of brazilin (Fr. 4), protosappanin A (Fr. 5), sappanone B (Fr. 6–8), and some other compounds. Further purification was conducted using preparative high-performance liquid chromatography (HPLC) with a reversed-phase Inertsil ODS-3 column (TOSOH TSK Gel 21.5 mm i.d. × 300 mm) monitored at 280 nm. The solvent system used was as follows: a gradient program for 45 min from 5% to 100% methanol in 0.05% aqueous trifluoroacetic acid at a flow rate of 10 ml/min. This separation step gave crude brazilin, protosappanin A, and sappanone B. Repeated preparative HPLC gave brazilin (45.0 mg, Fig 1a), protosappanin A (27.4 mg, Fig 1b), and sappanone B (20.5 mg, Fig 1c). The structures of the compounds were determined by comparison of their spectroscopic data with those reported in the literature. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded with a JEOL ECP 600 MHz spectrometer with tetramethylsilane as internal reference, and chemical shifts were expressed in δ (ppm). Mass data were measured by gas chromatography-mass spectrometry (GC-MS) by direct injection on a Shimadzu GCMS-QP5050A instrument.

Identification of compounds

Brazilin. Amber-yellow crystals, $[\alpha]^{20}_{D}$ +118.8° (*c* = 1.9, MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ 2.73 (1H, d, *J* = 15.8 Hz, H-7), 2.97 (1H, d, *J* = 15.8 Hz, H-7), 3.67 (1H, d, *J* = 10.9 Hz, H-6), 3.89 (1H, d, *J* = 10.9 Hz, H-6), 3.93 (1H,

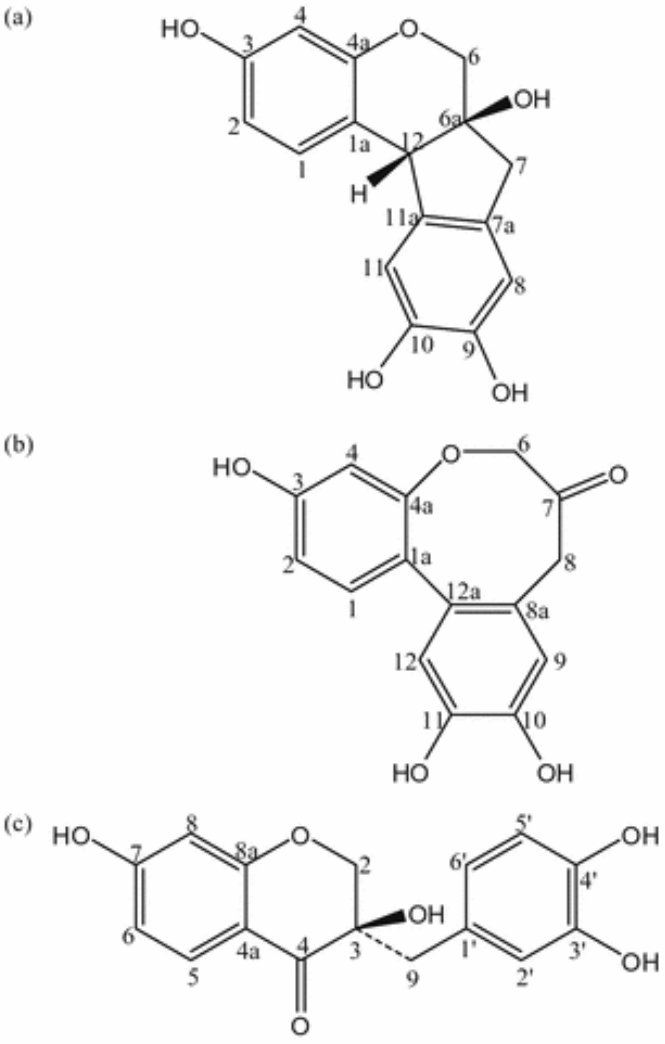

Fig. 1a–c. Structures of **a** brazilin, **b** protosappanin A, and **c** sappanone B