BRIONONIC ACID FROM THE HEXANE EXTRACT OF Sandoricum koetjape MERR STEM BARK (meliaceae)

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ABSTRACT

An oleane-type triterpenoid, briononic acid was isolated from hexane extract of the stem bark of Sandoricum koetjape *Merr. (*Meliaceae*). This structure had been established based on spectroscopic data (UV, IR, and NMR) and by comparison with spectroscopic data of related compound that had been reported.*

Keywords: Meliaceae*, Oleane,* Sandoricum koetjape *Merr*., *Triterpenoid*.

INTRODUCTION

The Meliaceae family has shown to be of much interest phytochemists because either it contains plants which produce very complex chemical structure, particularly their triterpenoid or because of their biological activity, mainly against insects [1]. This family consits of about 50 genera and 1400 species, growing on subtropic and tropic area, belongs to Indonesia. There are approximately 33 genera and 405 species in Indonesia [2].

As a continuation of our studies on Meliaceae plants, namely *Aphanamixis polystachya* [3, 4], *Khaya senegalensis* [5, 6], and *Aglaia odorata* [7 – 9], we now started to investigate *Sandoricum* species growing in Indonesia, i.e. *Sandoricum kotjape* Merr. This plant has some local names, such as sentieh*,* sentol, setol, sentul, setul, setui, kechapi or ketapi (Malaya); saton, satawn, katon or kathon (Thailand); kompem reach (Cambodia); tong (Laos); sau chua, sau tia, sau do, mangoustanier sauvage*,* or faux mangoustanier (Vietnam Utara), santor or katul (Philippines), klampu (Sarawak and Brunei), and sayai, sevai, sevamanu or visayan (India). In Indonesia, the plant is also well-known as kecapi or sentool [2].

It was reported that it had been isolated a triterpenoic acid of *seco*-multiflorane type [10, 11], 3-oxo-12-oleanen-29-oic acid, katonic acid [12], and several limonoids having antifeedants activity [13] from seed and leaf of the plant. In this research, the plant was obtained from East Borneo (Kalimantan). We now report the isolation of an oleane-type triterpenoid, briononic acid from the stem bark of *S. koetjape*.

EXPERIMENTAL SECTION

Material

Sample of the stem bark of *S. koetjape* was collected on September 12, 2004 from the Karangrejo village, East Kalimantan, Indonesia. The plant was identified by the staff at the Herbarium LIPI of Purwodadi, Pasuruan and a voucher specimen had been deposited at the Herbarium.

Procedure

General Experimental Procedures

Melting points were determined on a micro-melting point apparatus and are uncorrected. UV and IR spectra were measured with Shimadzu UV-1700 and Perkin Elmer Spectrum One FTIR spectrophotometers, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded with a JEOL JNM-ACA500 FT-NMR spectrometer, operating at 500 MHz (^{1}H) and 125 MHz (^{13}C), using solvent as internal standard. Vacuum Liquid Chromatography (VLC) was carried out using Merck Si gel 60 GF_{254}, column chromatography using Si gel Merck 60 (60 – 70 mesh) and Merck Si Gel 60 (230 – 400 mesh), TLC preparative on precoated Si gel glasses (Merck Kieselgel 60 PF_{254}, 0.25 mm, 20x20 cm) and TLC analysis on precoated Si gel plates (Merck Kieselgel 60 GF_{254}, 0.25 mm, 20x20 cm).

Extraction and Isolation

The dried and milled stem bark of *S. koetjape* (1.5 kg) was extracted exhaustively with hexane at room temperature (overnight twice). The combined extract, on removal of solvent under reduced pressure, gave a yellowish brown residue (33.7 g). A portion (± 11 g) of the total *n*-hexane extract was fractionated by VLC using *n*-hexane (1 – 6) and acetone (7 – 8) as eluents to give eight fractions. The remaining extract was carried out a fractionation using the same chromatographic techniques for twice. These respective fractions was then combined to be two main fractions yielded fractions A, B, C, D, E and F. Four fractions A – D were ultimately obtained on combining

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the eluates on the basis of TLC results and evaporated followed by occurring white crystal as many as 3 g. This crystal (called as fraction G) was then purified by column chromatography using *n*-hexane – ethyl acetate (7 : 3) as eluents to give five combined fractions G1, G2, G3, G4, and G5. The fourth fraction seemed to be pure spot and then the purity of the fraction was confirmed by TLC test using three different eluents, such as *n*-hexane – ethyl acetate (9 : 1), *n*-hexane – chloroform (1 : 1), and chloroform – ethyl acetate (8 : 2). Thus, the isolate was characterized by using melting point and afforded 229 – 231 ^{o}C and also measured by spectroscopic method (UV, IR and NMR) yielded an oleane type triterpenoid as briononic acid (1.5 g).

Briononic acid obtained as white crystal, mp. 229 – 231 ^{o}C; IR (KBr) $\nu_{maks.}$ 2944 – 2855, 1725, 1680, 1460, 1384, and 1142 cm^{-1} indicating –CH_3 and -CH_2 stretching, carboxyl (-C=OOH), carbonyl (-C=O) stretching, unsaturated double bond (C=C) stretching, C-H bending, dimethyl geminal [-C(CH_2)] bending, and C–O stretching, respectively [15]. UV ($CHCl_3$) $\lambda_{maks.}$ 238 (unconjugated double bond chromophore) nm. ^{1}H-NMR ($CDCl_3$, 500 MHz, ppm) : δ_H 2.16 (1H, br d, H-20), 2.14 (2H, br s, H-15), 2.13 (2H, t, H-7), 2.11 (2H, br s, H-2), 1.95 (2H, br s, H-12), 1.94 (2H, m, H-6), 1.65 (2H, br s, H-16), 1.60 (1H, dd, H-5), 1.51 (2H, br s, H-1), 1.50 (1H, s, H-18), 1.30 (2H, br s, H-19), 1.27 (2H, br s, H-11), 1.22 (3H, s, H-25), 1.08 (3H, s, H-23), 1.05 (3H, s, H-27), 1.03 (3H, s, H-26), 1.00 (3H, s, H-28), 0.96 (3H, s, H-30), 0.91 (2H, br s, H-21), 0.88 (2H, br s, H-22), and 0.84 (3H, s, H-24) ; ^{13}C-NMR ($CDCl_3$, 125 MHz, ppm) δ_C 218.5 (C-3), 185.6 (C-29), 135.1 (C-9), 132.9 (C-8), 51.3 (C-5), 47.3 (C-20), 44.6 (C-18), 42.3 (C-14), 40.5 (C-13), 37.6 (C-4), 37.3 (C-17), 37.0 (C-19), 35.6 (C-1), 34.6 (C-22), 34.4 (C-21), 32.9 (C-25), 31.4 (C-26), 31.0 (C-10), 30.7 (C-19), 30.1 (C-16), 29.70 (C-7), 27.8 (C-2), 26.9 (C-23), 25.3 (C-11), 21.9 (C-30), 21.3 (C-27), 20.8 (C-12), 20.7 (C-6), 19.6 (C-28), and 18.2 (C-24) ppm.

RESULT AND DISCUSSION

The IR spectrum of isolated compound showed absorption bands at 1725 and 1680 cm^{-1} indicating the presence of carbonyl group and unsaturated double bond moieties in the molecule, respectively. The presence of unsaturated double bond was supported by UV spectrum located at 238 nm.

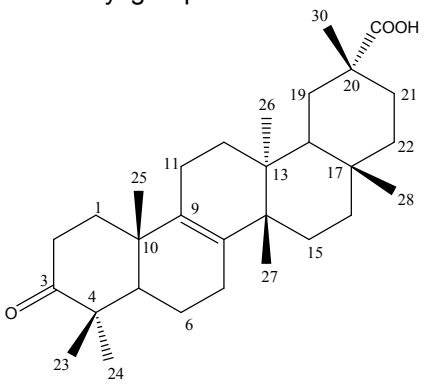

Fig 1. Structure of briononic acid

The ^{13}C-NMR spectrum of isolated compound revealed 30 carbon signals originating from seven methyl (-CH_3), eleven methylene (-CH_2-), three methyne (-CH-) and 5 quaternary carbon atoms (see table 1). This spectrum supported by DEPT-135 and DEPT-90 showed seven signals indicating seven methyl groups observed at δ 32.9, 31.4, 26.9, 21.9, 21.3, 19.6 and 18.2 ppm. This spectrum also indicated eleven methylene and three methyne groups observed at δ 37.0 – 20.7 ppm and δ 51.3, 44.6 and 31.0 ppm, respectively. The olefinic quaternary carbon signals were observed at δ 132.9 and δ 135.1 ppm. Furthermore, the presence of carbonyl and carboxyl groups were supported by a ^{13}C-NMR at δ 218.5 and 185.6 ppm, respectively.

Table 1. Chemical shifts of ^{1}H- and ^{13}C-NMR of briononic acid and isolated compound

No. C	^{13}C- NMR (δ ppm)		^{1}H-NMR (δ ppm) of Isolated compound	Carbon type
	Briononic acid	Isolated compound		
1	35.3	35.6	1.51 (br s)	-CH_2-
2	27.7	27.8	2.11 (br s)	-CH_2-
3	218.2	218.5	-	-C=O
4	37.4	37.6	-	-C-
5	51.1	51.3	1.60 (dd)	-CH-
6	20.5	20.7	1.94 (m)	-CH_2-
7	34.2	29.7	2.13 (t)	-CH_2-
8	133.6	132.9	-	-C=C
9	134.8	135.1	-	-C=C
10	47.1	47.3	-	-C-
11	25.2	25.3	1.27 (br s)	-CH_2-
12	20.6	20.8	1.95 (br s)	-CH_2-
13	40.3	40.5	-	-C-
14	41.1	42.3	-	-C-
15	29.9	30.7	2.14 (br s)	-CH_2-
16	29.4	30.1	1.65 (br s)	-CH_2-
17	37.0	37.3	-	-C-
18	44.4	44.6	1.50 (s)	-CH-
19	36.8	37.0	1.30 (br s)	-CH_2-
20	30.8	31.0	2.16 (br d)	-CH
21	34.2	34.4	0.91 (br s)	-CH_2-
22	34.4	34.6	0.88 (br s)	-CH_2-
23	26.7	26.9	1.08 (s)	-CH_3
24	18.1	18.2	0.84 (s)	-CH_3
25	32.6	32.9	1.22 (s)	-CH_3
26	31.2	31.4	1.03 (s)	-CH_3
27	21.1	21.3	1.05 (s)	-CH_3
28	19.4	19.6	1.00 (s)	-CH_3
29	185.4	185.6	-	-COOH
30	21.6	21.9	0.96 (s)	-CH_3

Its ^{1}H-NMR spectrum (Table 1) exhibited seven methyl, eleven methylene and three methyne signals at δ 1.22 – 0.84 ppm, δ 2.14 – 0.88 ppm and 21.6, 1.60 and 1.50 ppm, respectively. The correlation of these proton signals to their carbon was confirmed by HMQC and HMBC spectra. Then, these spectroscopic data and by comparison with those of related compound, briononic acid isolated from *Sandoricum emarginatum* Hiern [14] (Table 1) support that the isolated compund is briononic acid as well.

CONCLUSION

The phytochemical study of *Sandoricum koetjape* Merr (Meliaceae) had been conducted in our laboratory, and had been isolated an oleane type triterpenoid as briononic acid. Biogenetically, it is suggested that the isolated compound is originated from β-amyrine undergoing enzymatic hydrogenation reactin at C-12 followed by rearrangement process. β-Amyrine is derived from 2,3-epoxysqualene as precursor by chair-chair-chair conformation from terpenoid series.

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