

Chemical Constituents from the Leaves of *Sandoricum koetjape*

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Abstract

Chemical investigation of the ethyl acetate extract of the leaves of *Sandoricum koetjape* Merr. (Meliaceae) resulted in the isolation of two new limonoids. The compounds have been identified as their corresponding acetate derivatives (1) and (2). The structures have been established as 2 α -acyloxysandoricin by analysis of NMR spectral data especially 2D NMR.

INTRODUCTION

Sandoricum koetjape Merr. (Syn. *Sandoricum indicum* cav.) (Meliaceae), commonly known as Santol (locally called "Kra-tond"), is a medium-sized fruit tree native to southeast Asia. This plant has been used in traditional medicine by local populations (Burkill, 1966; Wasuwat, 1967). Previous chemical studies have shown that this plant contains a number of triterpenoid acids (Sim and Lee, 1972; Kosela et al., 1995; Tanaka et al., 2001) some of which displayed biological activities (Kaneda et al., 1992; Sun et al., 1999). Two limonoids, sandoricin (3) and 6-hydroxysandoricin (4) with antifeedant activity were isolated from the seeds of this plant (Powell et al., 1991). We now report the isolation of two new limonoids, [2 α -(2-methylbutanoyl)oxy]sandoricin (1) and [2 α -(2-methylpropanoyl)oxy]sandoricin (2) from the leaves of *S. koetjape* (Fig. 1).

MATERIALS AND METHODS

The dried leaves of *S. koetjape* (720 g) were extracted with 95% EtOH at room temperature for several times. Filtration and evaporation of the filtrate gave ethanol extract as a brown thick liquid (190.0 g). The extract was suspended in water and subsequently extracted with EtOAc, *n*-butanol and water to yield ethyl acetate extract (91.5 g), *n*-butanol extract (42.7 g) and water extract (55.2 g), respectively.

Column chromatography of the ethyl acetate extract (25.5 g) over silica gel, gradiently eluted with hexane: ethyl acetate afforded 37 fractions. Purification of fraction 24 by column chromatography using hexane: acetone (9:1, 8:2 and 7:3) as the eluent gave a limonoid fraction (451.3 mg). Further purification of this fraction by preparative layer chromatography (plc) with hexane: acetone (8:3) as the developing solvent (3 runs) yielded the limonoids as a slightly yellow solid (216.8 mg).

A portion of the limonoids (82.4 mg) was acetylated at room temperature with acetic anhydride (0.5 ml) using dry pyridine (1.0 ml) and 4-dimethylaminopyridine (42.5 mg) afforded the crude acetylated product as a slightly yellow solid (97.2 mg). Repeated purification of the residue by plc with hexane: acetone (8:3) (6 runs) gave 1 (5.9 mg) and 2 (7.7 mg) as a colorless gum. Their ^1H and ^{13}C spectral data are shown in Table 1 and 2.

RESULTS AND DISCUSSION

Attempted purification of the fraction containing limonoids as major components was unsuccessful. The mixture of limonoids was then acetylated using acetic anhydride in the presence of pyridine and 4-dimethylaminopyridine to yield the product which were isolated and identified as their corresponding acetate derivatives 1 and 2. The ^1H chemical shifts were assigned using ^1H NMR and ^1H - ^1H COSY data while the ^{13}C chemical shifts

were assigned using ^{13}C NMR, 2D HMQC and 2D HMBC data.

Compounds **1** and **2** were obtained as a colorless viscous gum. Both compounds were closely related in structure as evidenced by comparison of their ^1H and ^{13}C NMR spectra (Table 1 and 2). Obvious similarities included four quarternary methyl singlets (Me-18,-19,-28 and -29), three acetate methyl singlets (Me-32, -35 and -37), a methyl ester singlet (Me-33) and three olefinic protons characteristic of a furan moiety (H-21, -22 and -23). Monitoring of the limonoid mixture by ^1H NMR before acetylation indicated the presence of two acetoxy groups (δ 2.02 and 1.50). These similar two acetoxy methyl signals were also reported for sandoricin (**3**) (Powell et al., 1991). The crude acetylated product prior to chromatographic separation showed three singlets for acetoxy groups (δ 2.14, 2.06 and 1.54). This evidence together with the NMR spectral data (Table 1 and 2) suggested that the structures of **1** and **2** were of sandoricin framework. The additional acetoxy group (δ 2.10) could be attributed to acetylation of the C-15 hydroxyl group. Furthermore, the doublet of doublet at δ 5.00 with a big coupling constant (12.0 Hz) assignable to H-2 were observed in both compounds. It is most likely that 2 α -acyloxy group would be expected to cause a considerable downfield shift of the H-2 signal. This result implied that **1** and **2** had a 2 α -oxygenated limonoid skeleton.

The ^1H NMR spectrum of **1** (Table 1) displayed the signals at δ 1.19 (d, $J = 7.0$ Hz, Me-42), 0.97 (t, $J = 7.5$ Hz), 2.34 (m, H-39), 1.69 (m, H-40a) and 1.41 (m, H-40b). These data indicated the presence of 2-methylbutanoate at C-2. The ^1H NMR spectrum of **2** (Table 1) showed a slightly different pattern, two doublets at δ 1.14 and 1.11 ($J = 6.8$ Hz, Me-41, -42) and a septet at δ 2.54 (H-39) which suggested the different acyloxy group, 2-methylpropanoate, attached at C-2. The establishment of the structures of **1** and **2** was also confirmed by 2-dimensional HMQC and HMBC correlation. These data permitted the characterization of **1** as [2 α -(2-methylbutanoyl)oxy]sandoricin and **2** as [2 α -(2-methylpropanoyl)oxy]sandoricin.

CONCLUSION

Sandoricum koetjape (Santol), is a well-known tree that bears edible fruits which are consumed by natives. Previous chemical investigations of this plant led to the isolation of mainly multiflorane-type triterpenoid acids (Sim and Lee, 1972; Kaneda et al., 1992; Kosela et al., 1995; Sun et al., 1999; Tanaka et al., 2001). Only two limonoids, sandoricin (**3**) and 6-hydroxysandoricin (**4**) with antifeedant activity were reported (Powell et al., 1991). Limonoids have been found so far in two plant families, Rutaceae (citrus) and Meliaceae (mahogany). Many biologically active meliacin-type limonoids have been isolated from plants of the genus *Azadirachta* (neem) in the Meliaceae family, and they act as natural agents to protect the plants against predators. Some previously isolated 2-acyloxy limonoids, including ekbergin (Taylor, 1981) have been reported as natural products. Therefore, **1** and **2** would be added to the class of highly oxidized ring B fissioned limonoids.

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Tables

Table 1. ^1H NMR assignments for compounds **1** and **2**. NMR spectra were recorded on a Bruker AMX 400 (400 MHz) in CDCl_3 solution with TMS as an internal standard. Chemical shifts (δ) are expressed in ppm from TMS, and coupling constants J , in parentheses, are expressed in Hz.

Proton	1	2
H-1	3.94,d (4.3)	3.94,d (4.4)
H-2	5.00,dd (4.3,12.0)	5.00,dd (4.4,11.8)
H-3	5.34,d (12.0)	5.33,d (11.8)
H-5	2.70,dd (1.6,10.8)	2.69,dd (2.0,10.8)
H-6a	2.50,dd (10.8,15.0)	2.50,dd (10.8,15.2)
H-6b	2.05,d (15.0)	2.06,d (15.2)
H-9	2.26,dd (2.1,5.4)	2.26,dd (2.0,5.2)
H-11a	2.43,ddd (2.1,5.7,14.4)	2.43,ddd (2.0,6.0,14.0)
H-11b	1.44,m	1.44,m
H-12	5.46,dd (5.7,12.0)	5.46,dd (6.0,11.8)
H-15	5.88,s	5.87,s
H-17	5.88,s	5.88,s
H-21	7.50,dd (0.9,1.8)	7.50,dd (0.9,1.8)
H-22	6.48,dd (0.9,1.8)	6.48,dd (0.9,1.8)
H-23	7.38,t (1.8)	7.38,t (1.8)
H-30a	5.26,s	5.26,s
H-30b	4.93,s	4.92,s
H-39	2.34,m	2.54,septet (6.8)
H-40a	1.69,m	-
H-40b	1.41,m	-
Me-18	1.32,s	1.32,s
Me-19	0.97,s	0.97,s
Me-28	1.07,s	1.06,s
Me-29	0.95,s	0.95,s
Me-41	0.91,t (7.5)	1.11,d (6.8)
Me-42	1.09,,d (7.0)	1.14,d (6.8)
Me-32	2.07,s	2.07,s
Me-35	1.54,s	1.54,s
Me-37	2.15,s	2.15,s
OMe-33	3.63,s	3.62,s

Table 2. ^{13}C NMR assignments for compounds **1** and **2**. Chemical shifts (δ) are expressed in ppm from TMS. Assignments are confirmed utilizing HMQC experiments.

Carbon	1	2	Carbon	1	2
C-1	78.1	78.1	C-20	120.7	120.5
C-2	68.2	68.2	C-21	142.4	142.4
C-3	72.8	72.8	C-22	110.2	110.3
C-4	40.0	40.0	C-23	143.0	143.0
C-5	42.0	42.0	C-28	16.4	16.4
C-6	33.4	33.4	C-29	27.2	27.2
C-7	173.4	173.4	C-30	113.9	113.9
C-8	140.8	140.8	C-31	169.7	169.7
C-9	50.7	50.7	C-32	20.8	20.8
C-10	44.0	44.0	C-33	51.8	51.8
C-11	30.5	30.4	C-34	169.0	169.0
C-12	68.9	69.0	C-35	20.3	20.0
C-13	47.8	47.9	C-36	170.0	170.1
C-14	83.6	83.7	C-37	20.0	20.3
C-15	68.1	68.1	C-38	176.2	176.0
C-16	167.1	167.4	C-39	40.8	33.7
C-17	78.1	78.3	C-40	25.8	-
C-18	9.6	9.7	C-41	11.4	19.0
C-19	21.6	21.6	C-42	15.5	18.5

Figures

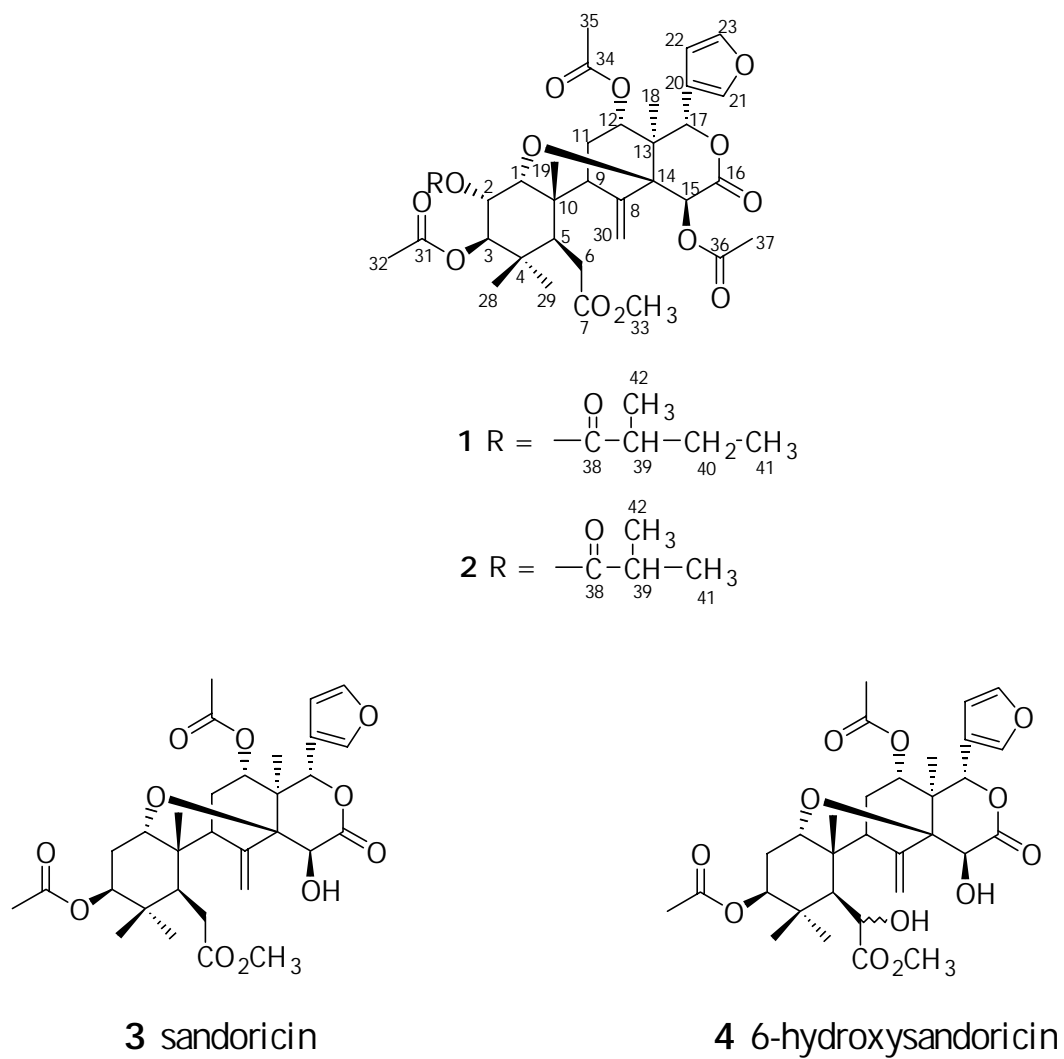


Fig. 1. Molecular structures of limonoids isolated from the leaves (**1**, **2**) and the seeds (**3**, **4**) of *Sandoricum koetjape*.