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NOTE

Four limonoids from the seeds extract of *Sandoricum koetjape*

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Abstract
Three andirobin- and one trijugin-class limonoids, named koetjapins A-D (**1**–**4**), have been isolated from the seed extracts of *Sandoricum koetjape*. The structures of these compounds were determined by extensive NMR and mass spectral data, and the chemotaxonomic significance of these limonoids in the family Meliaceae is highlighted. Preliminary biological activity showed that only compound **4** has significant inhibitory activity against P-388 cells, while antibacterial tests showed that none of these compounds were active.

Keywords Koetjapins A-D · Limonoid · Andirobin · Trijugin · *Sandoricum koetjape* · Meliaceae · Cytotoxicity · Antibacterial

Introduction

Sandoricum koetjape Merr. (local names 'Kecapi' or 'Sentul) is a medium sized tree (up to 50 m height) that is native to the South East Asian region [1]. The plant produces economically important edible fruits for the local people. Its phytochemistry has been investigated since the 1960s by many researchers and the presence of sesquiterpenes [2], triterpenes [2–8], and limonoids of the andirobin and trijugin classes [9–12] have been revealed. Most of the phytochemical studies were performed on the leaves and bark, while work on the seeds is still very limited and has found only andirobin-type limonoids [9]. Some of these compounds have been shown to be anti-inflammatory [13], antifeedant [9] and cancer chemopreventive agents [10], as well as showing cytotoxicity against several cancer cells [2, 6, 8], and being inhibitors of NO production [12] and DNA polymerase β [14]. In the course of our interest in the limonoids from Meliaceous plants, we had an opportunity to investigate the chemical constituents from the seeds of *S. koetjape* grown in Indonesia. Here, we report the isolation and structure elucidation of four new limonoids, named koetjapins A-D (**1**–**4**) (Fig. 1), from the seeds of the plant, and discuss their chemotaxonomic significance. A preliminary biological evaluation of **1**–**4** against P-388 cells and four bacteria are also described.

Results and discussion

Compound **1** (koetjapin A) was isolated as a white powder with $[\alpha]_D^{20} = -29.5^{\circ}$. The UV spectrum showed a conjugated alkene chromophore (λ_{max} 232, 278 nm), while the IR spectrum displayed absorptions for hydroxyl (ν_{max} 3425 cm^{-1}) and ester (ν_{max} 1738 cm^{-1}) groups. The HRESI-TOF spectrum of **1** exhibited a quasi molecular ion with sodium at *m/z* 553.2418, and together with its ^{13}C NMR data that displayed 29 carbon signals (Table 2), was consistent for a molecular formula $C_{29}H_{38}O_9$ (calcd. *m/z* for $C_{29}H_{38}O_9Na$ 553.2414) (DBE = 11). The NMR spectra (Tables 1 and 2) showed signals for an acetyl (δ_C 21.1, 170,1; δ_H 2.04) and a methyl ester (δ_C 52.1; δ_H 3.69) group, indicating that **1** had a C_{26}-skeleton of a tetranortriterpenoid with a DBE = 9. Further NMR analysis showed signals due to a β-substituted furan (δ_H 7.51, 7.40, 6.44), a lactone carbonyl (δ_C 170.0), an ester carbonyl (δ_C 174.1), an exocyclic methylene (δ_H 5.24, 5.06; δ_C 114.8, 142.7), and four tertiary methyl groups

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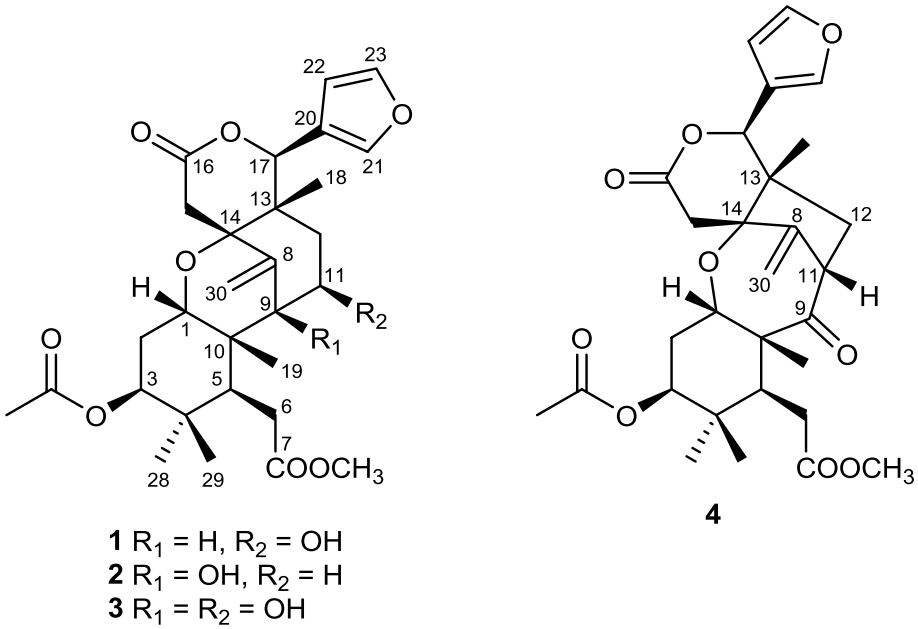

Fig. 1 Structures of koetjapins A-D (**1**–**4**) from the seeds of *S. koetjape*

(δ_H 1.04, 0.95, 0.88, 0.78), which are characteristics for an andirobin-class of rings B,D-seco limonoid (DBE = 8) [12]. Furthermore, the presence of a tertiary carbonoxy (δ_C 80.5) signal, which displayed an HMBC correlation with a methineoxy proton signal (δ_H 3.30) (Fig. 2), was an indication of an ether linkage from C-1 to C-14 in **1** and identified a basic structure of methyl angolensate-type limonoid (DBE = 9) for this compound [12]. Further NMR analysis showed the signals for another two methineoxy functionalities (δ_H 4.91 and 4.53). The first methineoxy signal (i.e., at δ_H 4.91) was determined to be part of an acetyl group at C-3 as shown by its HMBC correlations (Fig. 2), while the second methineoxy proton signal (i.e., at δ_H 4.53) must be in the form of a secondary alcohol group, which from its HMBC correlations can be located at C-11. However, it is interesting to mention that the ^1H–^{13}C long-range correlation from signals H-17 to C-16 was not observed in the HMBC spectrum both optimized with heteronuclear *J*s = 8 and 3 Hz, as well as in the CIGAR-HMBC spectrum using *J* from 3 (minimum) to 8 (maximum) Hz. The stereochemistry of **1** was determined from coupling analysis and NOE correlations (NOESY). The coupling analysis showed that H-3 (*dd*, 10.8, 5.8 Hz) and H-5 (*d*, 10.0 Hz) are axial, while H-1 (*br t*, 3.5 Hz), H-9 (*br s*), H-11 (*br s*) and H-17 (*br s*) are equatorial. The NOE correlations (Fig. 3) indicated that H-3 and H-5 are on the same side and on the opposite side with the methyl (H_3-19) at C-10. The NOE correlations between H_3-19 with H-1/H-9 and H-11 with H-9/H_2-12 confirmed these stereochemical assignments. Furthermore, the NOE correlations shown by H_3-18 with the proton signals of the furan ring (H-21 and H-22) and 11-OH are also consistent with the stereochemistry of **1**. Other NOE correlations in support of structure **1** are shown in Fig. 2. Therefore, structure **1** was determined as shown in Fig. 1.

Compound **2** (koetjapin B) was also isolated as a white powder ($[\alpha]_D^{20} = -30.3^o$) and has the same molecular formula as **1** ($C_{29}H_{38}O_9$, found *m/z* for $C_{29}H_{38}O_9Na$ 553.2430). The UV and IR spectra showed absorptions similar to those **1**. The NMR data of **2** and **1** were very close to each other, except that the signals for the methine (δ_C 58.6; δ_H 2.11) and the secondary alcohol (δ_C 67.8; δ_H 4.53) in **1** were replaced by signals of a tertiary alcohol (δ_C 78.1) and a methylene (δ_C 34.6; δ_H 2.65, 1.33) in **2**. These changes are consistent if the hydroxyl group in **2** is situated at C-9 as a tertiary alcohol group, which was confirmed by the HMBC correlations as shown in Fig. 2. The stereochemical assignments of **2** were also carried out based on the analysis of coupling constants (Table 1) and the NOE correlations (Fig. 2), and thus koetjapin B has the structure **2** as shown in Fig. 1.

Compound **3**, isolated as a white powder ($[\alpha]_D^{20} = -30.9^o$), showed UV and IR absorptions similar to those **1** and **2**. It has a molecular formula $C_{29}H_{38}O_{10}$ (found $[M+Na]^+$ at *m/z* 569.2362, calcd. 569.2363), suggesting that this compound is an oxygenated derivative of either **1** or **2**. In fact, the NMR data of **3** were very closely related to those **1** and **2**, notably for the presence both secondary (δ_C 71.4; δ_H 4.59) and tertiary alcohol (δ_C 79.2) groups in **3**. Together with the HMBC correlations in **3** (Fig. 2), these NMR data allow the secondary alcohol to be placed at C-11 (as in **1**) and the tertiary alcohol at C-9 (as in **2**). From the coupling constants and NOE analysis (Fig. 2), the stereochemistry of the chiral carbons in **3** can be established, following the stereochemistry in **1** and **2**. Thus, structure **3** was determined as shown in Fig. 1. A literature search revealed that a limonoid with

Table 1 ^{1}H NMR (500 MHz) data of compounds **1**–**4** in $CDCl_3$

	1*	**2**	**3**	4
1	3.30 (*br t*, 3.5)	3.29 (*dd*, 3.9, 2.7)	3.35 (*br t*, 3.0)	4.23 (*br t*, 3.4)
2	1.87 (*m*); 1.86 (*m*)	1.91 (*ddd*, 14.0, 12.4, 4.2) 1.82 (*ddd*, 12.4, 4.2, 2.7)	1.93 (*td*, 12.8, 4.4) 1.81 (*ddd*, 12.8, 4.0, 2.4)	1.90 (2H, *m*)
3	4.91 (*dd*, 10.8, 5.8)	4.96 (*d d*, 12.4, 4.2)	4.92 (*dd*, 12.5, 4.3)	5.11 (*dd*, 11.3, 5.4)
4	–	–	–	–
5	2.35 (*br d*, 10.0)	2.64 (*br d*, 10.0)	2.40 (*d*, 10.0)	2.70 (*dd*, 4.7, 4.1)
6	2.54 (*dd*, 16.7, 10.0) 2.18 (*br d*, 16.7)	2.49 (*dd*, 17.0, 10.0) 2.98 (*d*, 17.0)	2.50 (*dd*, 17.0, 10.0) 3.01 (*d*, 17.0)	2.85 (*dd*, 18.1, 4.7) 2.31 (*dd*, 18.1, 4.1)
7	–	–	–	–
8	–	–	–	–
9	2.11 (*br s*)	–	–	–
10	–	–	–	–
11	4.53 (*br s*)	2.65 (*m*); 1.33 (*m*)	4.59 (*br s*)	3.52 (*dd*, 9.9, 4.6)
12	2.20 (*dd*, 14.7, 3.9) 1.45 (*br d*, 14,7)	2.14 (*br t*, 12.6) 1.26 (*br d*, 14.0)	2.36 (*dd*, 14.7, 3.8) 1.61 (*br d*, 14.7)	2.80 (*dd*, 14.8, 4.6) 1.73 (*dd*, 14.8, 9.9)
13	–	–	–	–
14	–	–	–	–
15	2.93 (*d*, 18.0) 2.62 (*d*, 18.0)	2.95 (*d*, 18.0) 2.49 (*d*, 18.0)	2.96 (*d*, 18.1) 2.62 (*d*, 18.1)	2.87 (*d*, 17.6) 2.83 (*d*, 17.6)
16	–	–	–	–
17	5.77 (*br s*)	5.78 (*br s*)	5.76 (*br s*)	6.18 (*br s*)
18	1,04 (*s*)	0.86 (*s*)	1.02 (*s*)	0.77 (*s*)
19	0.88 (*s*)	0.81 (*s*)	0.88 (*s*)	1.02 (*s*)
20	–	–	–	–
21	7.51 (*br s*)	7.50 (*br s*)	7.51 (*br s*)	7.53 (*br s*)
22	6.44 (*br s*)	6.43 (*br s*)	6.44 (*br s*)	6.42 (*br s*)
23	7.40 (*br t*, 1.8)	7.39 (*br t*, 1.6)	7.41 (*br t*, 1.8)	7.39 (*br t*, 1.8)
28	0.95 (*s*)	0.95 (*s*)	0.96 (*s*)	0.87 (*s*)
29	0.78 (*s*)	0.80 (*s*)	0.76 (*s*)	0.89 (*s*)
30	5.24 (*br s*); 5.06 (*br s*)	5.34 (*br s*); 5.00 (*br s*)	5.51 (*br s*); 5.17 (*br s*)	5.37 (*br s*); 5.19 (*br s*)
CH_3CO	2.04 (*s*)	2.04 (*s*)	2.05 (*s*)	2.02 (*s*)
OCH_3	3.69 (*s*)	3.68 (*s*)	3.68 (*s*)	3.65 (*s*)

[a]**1**—11-OH (δ_H 1.68, *br s*), **2**—9-OH (δ_H 1.44, *br s*), **3**—9-OH (δ_H 3.57, *br s*), 11-OH (δ_H 2.21, *br s*)

the same basic structure as those of **3**, i.e., cipadesin Q, had been isolated from *Cipadessa baccifera* (Meliaceae) [15]. The only difference between these two compounds is in the stereochemistry at C-3. While H-3 in compound **3** is axial (δ_H 4.96, *br d*, J=11.5 Hz), this hydrogen in cipadesin Q is in equatorial orientation (δ_H 4.72, *t*, 2.4 Hz), and therefore **3** is a 3-epimer of cipadesin Q.

Compound **4**, isolated as a white powder ($[\alpha]_D^{20} = +2.9^o$), has a molecular formula $C_{29}H_{36}O_9$ (found $[M+Na]^+$ at *m/z* 551.2252, calcd. 551.2257), suggesting that this compound is a dehydro derivative of either **1** or **2**. The UV and IR absorptions were also similar to those of **1–3**, but are devoid of the –OH stretching peak. The NMR data of **4** were also closely related to those **1**–**3**. However, the presence of a ketonic group (δ_C 209.9), in addition to three ester carbonyls (δ_C 174.1, 169.9 and 168.6), pointed to a trijugin-class of limonoid for **4** [11]. The only methineoxy group in **4** is shown by the NMR signals at δ_H 5.11 and δ_C 74.8, which from its HMBC correlations (Fig. 1), is part of an acetyl group. The stereochemistry in **4** was also determined based on the analysis of coupling constants and the NOE correlations, which is consistent with structure **4** (Fig. 2), particularly for the axial orientation of H-3 (dd, J = 11.3 and 5.4 Hz). Based on the analysis of these NMR data, structure **4** was determined as shown in Fig. 1. Again, compound **4** has the same basic structure as cipatrijugin A isolated from *C. cinerascens* [16], but it differs in the stereochemistry at C-3. H-3 in the latter appeared as a triplet with a small coupling constant (2.5 Hz), consistent to the equatorial orientation, and thus **4** is a 3-epimer of cipatrijugin A.

Based on previous investigations, a number of angolensate-type andirobin and trijugin-class limonoids have been

Table 2 ^{13}C NMR (125 MHz) data of compounds **1**–**4** in $CDCl_3$

C No.	**1**	**2**	**3**	4
1	74.8	75.9	75.5	72.9
2	29.3	29.8	29.6	31.2
3	74.1	74.3	74.2	74.8
4	38.9	39.0	39.2	39.5
5	41.9	41.6	42.0	42.5
6	32.2	31.8	31.6	29.7
7	174.1	174.9	175.3	174.1
8	142.7	149.5	145.7	144.7
9	58.6	78.1	79.2	209.9
10	44.4	49.1	49.1	55.0
11	67.8	34.6	71.4	58.6
12	37.1	30.2	37.5	35.8
13	40.9	41.1	40.3	46.1
14	80.5	81.2	81.1	87.5
15	33.7	34.3	34.2	34.3
16	170.0	170.1	169.9	168.6
17	79.5	79.5	79.3	79.0
18	16.9	13.7	16.4	17.6
19	22.0	16.8	16.9	19.6
20	120.8	120.8	120.8	121.9
21	141.0	140.9	141.0	139.9
22	109.9	109.9	109.9	108.5
23	142.8	142.7	142.8	143.3
28	26.7	27.0	26.9	28.1
29	15.8	15.8	15.9	16.9
30	114.8	108.9	112.8	114.0
CH_3CO	21.1	21.1	21.1	21.0
	170.1	170.2	170.2	169.9
OCH_3	52.1	51.8	51.9	51.8

isolated from the leaves and seeds of *S. koetjape* [9–12]. These limonoids are characterized by the presence of an hydroxyl group at C-12 and C-15, while in **1**–**4** these hydroxyls are lacking at that position. Instead, the andirobins in this investigation bear either a hydroxyl group at C-11 (compound **1**) or hydroxyl groups at C-9 and C-11 (compounds **2**–**3**). On biogenetic consideration, the presence of the hydroxyls at C-9 and C-11 can be thought to be a precursor of the trijugin **4**. In a broader context, the occurence of andirobin-class limonoids in the Meliaceous plants is more widely distributed compared to the trijugin-class limonoids [17]. The andirobin-class have been isolated from 17 genera, representing both subfamilies Melioideae and Swietenioideae of Meliaceae well [18], while the trijugin-class is found only in a number of species belonging to the subfamily Melioideae. *Sandoricum*, together with *Astrotrichilia*, *Cipadessa*, *Ekebergia*, and *Trichilia* (all members of Melioideae), are the genera that contain both the andirobin- and trijugin-class limonoids. It is worth noting that a species of *Cipadessa*, *C. cinerascens*, has been shown to produce angolensate-type limonoids containing oxygenated functionalities at C-11 [19], the same as those in **1**–**3**. Thus, the presence of both classes of limonoids has a chemotaxonomical significance at the generic level of Meliaceae.

Preliminary biological activities of compounds **1**–**4** were tested against murine leukemia P-388 cells and four bacteria (gram-(+): *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 29737; gram-(–): *Salmonella enterica* ATCC 14028 and *Citrobacter freundii* ATCC 43864). Only compound **4** showed a significant inhibition to the growth of P-388 cells with an IC_{50} of 16.8 ± 1.8 μg mL^{-1}, while compounds **1**–**3** were weakly active with IC_{50} values of 46.8 ± 1.9, 52.0 ± 1.6, and 59.2 ± 6.2 μg mL^{-1}, respectively. On antibacterial evaluation, however, all the isolated compounds **1**–**4** were not active (MIC values > 250 μg mL^{-1}). Many limonoids from Meliaceae have been investigated for their biological properties, particularly related to agricultural applications, as well as for medicinal values such as cytotoxicities against some

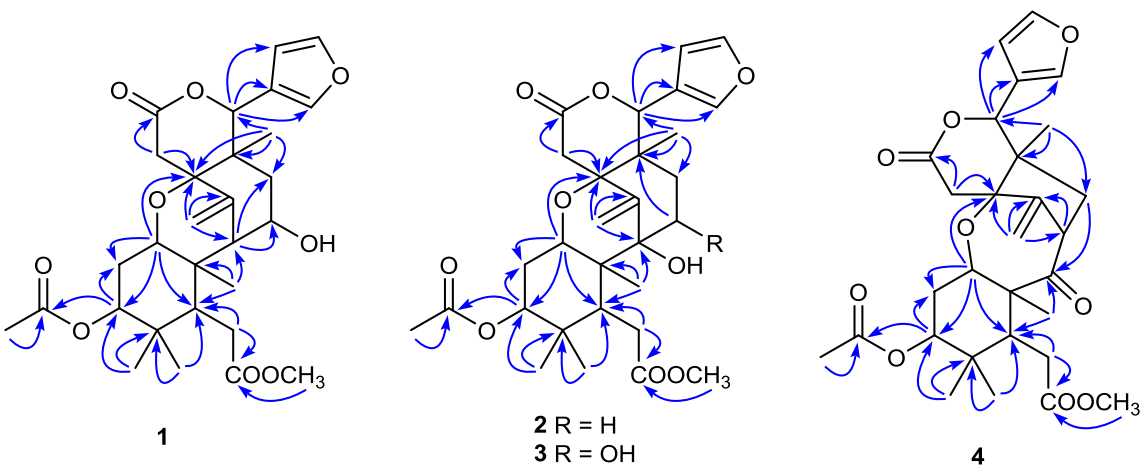

Fig. 2 Selected important HMBC correlations (^{1}H ⇒ ^{13}C) in compounds **1**–**4**

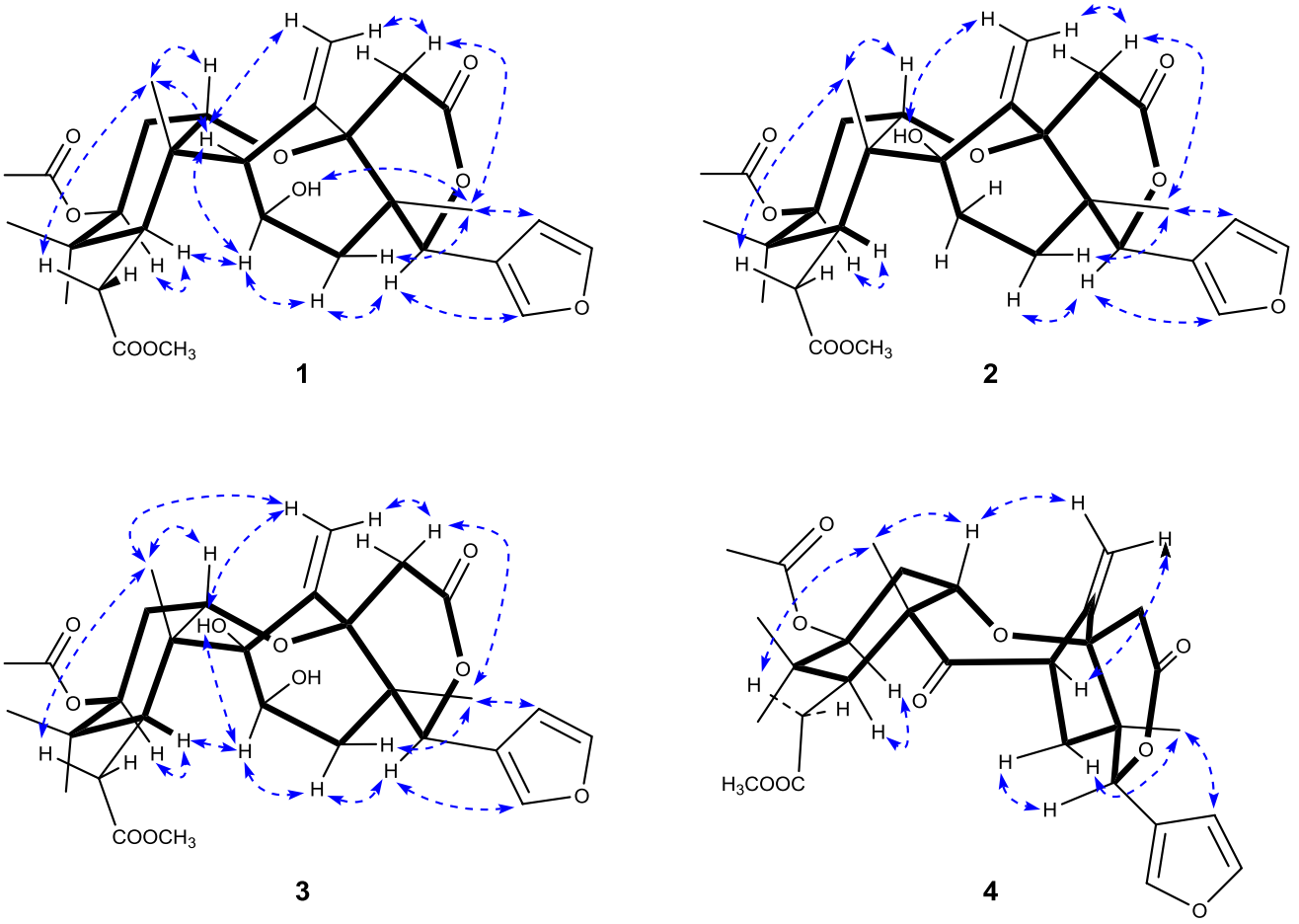

Fig. 3 Important NOE correlations in compounds **1**–**4**

cancer lines and as antibacterials [17]. Although many limonoids have been shown to have significant cytotoxic properties, none of the andirobin derivatives tested were active [19], which is comparable to the results for compounds **1**–**3** in this study. It is worth mentioning that trijugin **4** had significant activity against P-388 cells, while its 3-epimer derivatives (cipatrijugins A-D) were not active against A549 and K562 cancer cell lines [16]. In the antibacterial properties, the data are rather limited and only methyl angolensate was reported to have significant antibacterial properties against *Proteus vulgaris*, *Klebsiella pneumoniae*, *S. aureus*, *Escherichia coli*, and *Salmonella typhimurium* [20]. The presence of hydroxyl groups in the compounds **1**–**3**, which increases the polarity of the molecules, could be the factor for the inactivity of the compounds as antibacterials.

Experimental

General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded with a spectrometer (Agilent DD2 system) operating at 500 (^{1}H) and 125 (^{13}C) MHz, using residual and deuterated solvent peaks as reference standards. HRMS spectra were obtained with a positive mode of ESI-TOF Waters LCT Premier XE. Vacuum liquid chromatography (VLC) and centrifugal planar chromatography (CPC) were carried out using Merck Si gel 60 GF_{254} art. 7731 and 7749, respectively. Thin-layer chromatography (TLC) analysis used precoated Si gel plates (Merck Kieselgel 60 GF_{254}, 0.25 mm). Solvents

(MeOH, acetone, EtOAc and *n*-hexane) for extraction, fractionation and purification were of technical grade, and were distilled before use. $CHCl_3$ and diisopropyl ether used in the purification was a pro analysis grade.

Plant material

Fruit samples of *S. koetjape* were obtained directly from the local people who grow the plant in Pandeglang, Banten Province, Indonesia, in August 2013. The identity of the plant was determined by the staff of Herbarium Bandungense, Institut Teknologi Bandung, and a voucher specimen was deposited at the Herbarium (voucher number 11254).

Extraction and isolation

The dried and powdered fruit seeds (400 g) of *S. koetjape* were macerated in MeOH at room temperature (3 × 24 h) to give a gummy of MeOH extract (38 g) after solvent evaporation. Half of the extract (19 g) was fractionated using VLC (silica gel 160 g) eluted with *n*-hexane (1 × 200 mL), mixtures of *n*-hexane–EtOAc (9:1, 2 × 200 mL; 4:1, 2 × 200 mL, 7:3, 2 × 200 mL, 3:2, 2 × 200 mL, 1:1, 4 × 200 mL, and 2:3, 2 × 200 mL), EtOAc (2 × 200 mL), and EOAc-MeOH (1:1, 2 × 200 mL), and yielded 19 fractions F1-F19. TLC analysis showed that only two fractions, namely F10 (190 mg) and F13 (160 mg), showed the potential to contain triterpene derivatives. Fraction F10 was purified using the CPC method (1 mm thickness; eluent: *n*-hexane–acetone = 4:1) to give 19 subfractions F101–F1019. On TLC analysis, subfractions F104–F105 contained a pure component (compound **4**, 18 mg), while subfractions F108–F1010 and F1011–F1014 needed further purification. Purification of the combined subfractions F108–F1010 and F1011–F1014 was achieved by the CPC method (0.5 thickness, eluent: diisopropyl ether) to give compound **2** (15 mg) from F108–F1010, and compounds **2** (5 mg) and **3** (11 mg) from F1011–F1014. Using the same method (CPC), fraction F13 (160 mg) was purified in two steps (first step eluent: $CHCl_3$–MeOH = 19:1; second step eluent: diisopropyl ether-EtOAc = 4:1) to give compound **1** (8 mg).

Cytotoxic and antibacterial properties

Cytotoxic properties of the isolated compounds **1**–**4** were tested against murine leukemia P-388 cells according to the method of MTT assay as previously described [21] and presented as an IC_{50} in μg mL^{-1}. Antibacterial properties, presented as a MIC value in μg mL^{-1}, were carried out by a microdilution method [22] against two gram-(+) (*Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 29737) and two gram-(–) (*Salmonella enterica* ATCC 14028 and *Citrobacter freundii* ATCC 43864) bacteria.

Compound 1 (Koetjapin A)

White powders $[\alpha]_D^{20} = -29.5^o$ (c 0.25, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 205 (3.90), 213 (3.93), 232 (3.99), 278 (4.20); IR (KBr) ν_{max} cm^{-1}: 3430, 3149, 2972, 2941, 2880, 1738, 1240, 1167, 1028; ^{1}H NMR ($CDCl_3$) see Table 1; ^{13}C NMR ($CDCl_3$) see Table 2; HRESITOF-MS *m/z*: $[M + Na]^+$ 553.2418 (calcd. for $C_{29}H_{38}O_9Na$: 553.2414).

Compound 2 (Koetjapin B)

White powders $[\alpha]_D^{20} = -30.3^o$ (c 0.30, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 209 (4.03), 231 (4.09), 240 (3.94), 276 (3.29); IR (KBr) ν_{max} cm^{-1}: 3425, 3138, 2947, 1740, 1265, 1157, 1028; ^{1}H NMR ($CDCl_3$) see Table 1; ^{13}C NMR ($CDCl_3$) see Table 2; HRESITOF-MS *m/z*: $[M + Na]^+$ 553.2430 (calcd. for $C_{29}H_{38}O_9Na$: 553.2414).

Compound 3 (Koetjapin C)

White powders $[\alpha]_D^{20} = -30.9^\circ$ (c 0.30, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 203 (3.92), 237 (4.01), 240 (4.03), 277 (3.33); IR (KBr) ν_{max} cm^{-1}: 3432, 3140, 2970, 2945, 1737, 1260, 1170, 1027; ^{1}H NMR ($CDCl_3$) see Table 1; ^{13}C NMR ($CDCl_3$) see Table 2; HRESITOF-MS *m/z*: $[M + Na]^+$ 569.2362 (calcd. for $C_{29}H_{38}O_{10}Na$: 569.2363).

Compound 4 (Koetjapin D)

White powders $[\alpha]_D^{20} = +2.9^o$ (c 0.30, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε): 240 (3.59), 274 (3.28); IR (KBr) ν_{max} cm^{-1}: 3089, 2964, 2926, 2860, 1763, 1737, 1680, 1242, 1171, 1061, 1018; ^{1}H NMR ($CDCl_3$) see Table 1; ^{13}C NMR ($CDCl_3$) see Table 2; HRESITOF-MS *m/z*: $[M + Na]^+$ 551.2252 (calcd. for $C_{29}H_{36}O_9Na$: 551.2257).

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