Triterpenoids and Steroids from the Bark of *Pinus merkusii* (Pinaceae)

Masendra,[a] Tatsuya Ashitani,[b] Koetsu Takahashi,[b] and Ganis Lukmandaru [a,*]

Serratene triterpenoids are considered chemotaxonomy compounds from the Pinaceae family, within the *Pinus* genus. However, no studies have been conducted on the constituents of serratene triterpenoids of *Pinus merkusii*. *P. merkusii* is the only pine species native to Southeast Asia, including Indonesia. This study aimed to investigate the use of triterpenoids and steroids from the bark of *P. merkusii* as chemotaxonomic biomarkers. Five lipophilic extractives, including three triterpenoids (3β-methoxyserratt-14-e*n*-21-one (C1), 3α,21β- dimethoxy-∆14-serratene (C3), serrate-14-e*n*-3β,21β-diol (C5)), and two steroids (stigmast-4-e*n*-3-one (C2) and β-sitosterol (C4)), were isolated from the *n*-hexane extract of the bark of *P. merkusii* using column chromatography and thin-layer chromatography. The structures of the triterpenoids and steroids were characterized on the basis of their spectroscopic data. The discovery of serratene triterpenoids and steroids in *P. merkusii* bark characterizes this species as being in chemical accordance with other species of the *Pinus* genus and Pinaceae family.

Keywords: Pinus merkusii; Pinaceae; Triterpenoids; Steroids

Contact information: a: Department of Forest Product Technology, Faculty of Forestry, Universitas Gadjah Mada, Jl. Agro No. 1, Bulaksumur, Yogyakarta 55281, Japan; b: Faculty of Agriculture, Yamagata University, 1-23 Wakaba-machi, Tsuruoka, Yamagata 997-855, Japan;
** Corresponding author: glukmandaru@ugm.ac.id*

INTRODUCTION

Pinaceae is one of the largest families of conifers, comprised of 11 genera and 225 species. Nearly half of these species (110 species) are considered true pines and belong to the genus *Pinus* (Farjon 2005). Approximately 80 *Pinus* species are distributed throughout the northern hemisphere (Li *et al.* 2012). *P. merkusii* Jungh & de Vriese is a rather small tree, ranging from 20 m to 30 m in height. This pine is an exception to the general "rule" that pines are restricted to regions with a cool climate. It grows from sea level up to an altitude of 2000 m in Indonesia (Sumatra) and the Philippines (Farjon 1984). Species of *P. merkusii* belong to the section and subsection *Pinus*, kingdom of plantae, division of spermatophyte, subdivision of gymnospermae, class of Coniferae, order of Pinales, family of Pinaceae, and genus of *Pinus* (Gernandt *et al*. 2005; Siregar 2005; Baharudin and Tasikirawati 2009).

In Indonesia, in addition to being used as material for pulp and paper, *P. merkusii* has been planted to produce a resin that can be divided into gum and turpentine. The chemical composition of *P. merkusii* resin has been reported to include mono- and sesquiterpenes (Coppen *et al.* 1998; Wiyono *et al.* 2006; Hadiyane *et al*. 2015; Sukarno *et al*. 2015). The wood from this species has been reported to contain phenolic compounds and diterpenoids (Wijayanto *et al.* 2015). Terpenoids have been reported as bioactive compounds related to bark function in protecting the tree from exposure to extreme

conditions (Gershenzon 1994; Wittstock and Gershenzon 2002; Seki *et al.* 2012). Triterpenoids of this family have been well reported as biomarkers such as serratene-type triterpenoids (Le Milbeau *et al.* 2013; Otaka *et al*. 2016). These serratene-type triterpenoids have also been isolated from other *Pinus* and *Picea* species, especially from their bark (Rowe 1964; Rowe *et al.* 1972; Norin and Winnel 1972; Cheng and Chao 1979; Fang *et al.* 1991; Fang and Cheng 1992; Yamamoto *et al.* 2011; Labib *et al*. 2018). However, there have not been any reports regarding serratene-type triterpenoids through isolation, structure determination, and chemotaxonomy value from the bark of the tropical species of *P. merkusii* until now.

EXPERIMENTAL

Materials

Samples

Bark samples were taken from a 30-year-old *P. merkusii* tree at the state-owned enterprise of Perhutani Plantation (Magelang, Central Java, Indonesia) in February 2015. This site is located at 7° 28′ 0″ S, 110° 13′ 0″ E, with an average temperature of 26.2 °C, annual rainfall of 3189 mm, and an elevation of 139 m above sea level. The 1.5-cm-thick bark sample was dried at room temperature, and then finely ground before extraction. A voucher number (PM-3001) was made by the Faculty of Forestry, Universitas Gadjah Mada, Indonesia.

Methods

General equipment

The 13C and 1H nuclear magnetic resonance (NMR) were determined by a JEOL ECZ-400 spectrometer (JEOL, Tokyo, Japan). The NMR spectra were recorded using standard JEOL pulse sequences at 400 MHz and 100 MHz for 1H and 13C, respectively. Chemical shifts were recorded as δ (ppm) values with chloroform-deuterium (Sigma-Aldrich, St. Louis, MO, USA) as the solvent. Coupling constants (J) were recorded in Hz, and multiplicities were abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, sept = septet, br = broad, and m = multiplet. Melting points were not corrected.

Silica gel (60 N, spherical 63 μm to 210 μm; neutral Kanto Chemical Co., Inc., Tokyo, Japan) was used for column chromatography with a glass column (40 cm × 2.5-cm inner diameter). *n*-hexane-ethyl acetate (EtOAc) in increasing polarity were used as eluents (99:1, 49:1, 9:1, 8:2, 1:1, v/v). Aluminum sheets that were pre-coated with silica gel 60 F254 (Merck, Kenilworth, NJ, USA) were used for thin-layer chromatography (TLC). Spots were visualized using ultraviolet (UV) light irradiation (λ = 254 nm and 360 nm) by spraying with vaniline-sulfuric acid (for color testing), followed by heating at 150 °C for 10 min. The developing solvents used for TLC were *n*-hexane-EtOAc (8:2, v/v).

Gas chromatography-mass spectrometry (GC-MS) data were collected with a GCMS-QP2010 (Shimadzu, Kyoto, Japan) under the following conditions: DB-1 capillary column (30 mm × 0.25-mm inner diameter and 0.25 μm; GL Sciences, Tokyo, Japan); column temperature from 50 °C (1 min) to 320 °C at 5 °C/min; injection temperature of 250 °C; detection temperature of 320 °C; acquisition mass range of 50 amu to 800 amu using helium as the carrier gas. The components were identified by comparison of the experimental GC-MS data with NIST MS library and literature data

(Fang *et al.* 1991; Barla *et al.* 2006; Pateh *et al*. 2009; Halilu *et al.* 2013; Le Milbeau *et al.* 2013).

Extraction and isolation

The air-dried *P. merkusii* bark (1.0 kg) was extracted with *n*-hexane for two weeks under room temperature and evaporated until fully dry before weighing. The *n*-hexane extract with a dark yellow color yielded 1.59 g. For separation, 1.0 g of extract was chromatographed into Si gel column chromatography (SiGCC), resulting in 9 fractions (H1-H9) with eluent of *n*-hexane-EtOAc (99:1, 49:1, 9:1, 8:2, 1:1, v/v). From 9 fractions, H2 and H8 had a high purity, both H2 (14.8 mg) and H8 (71.0 mg) were collected from eluent of *n*-hexane-EtOAc (8:2, v/v), and H2 and H8 were designated as C1 and C5. The fractions of H3 and H4 were re-column chromatographed into SiGCC with H3 eluent of *n*-hexane-EtOAc (9:1, 1:1, v/v) to obtain H31-H36 fraction, and H4 eluent of *n*-hexane-EtOAc (8:2, 1:1, 0:1, v/v) to obtain H41-H49 fractions. From H3 separation, high purity fraction of H32 (13.9 mg) was isolated by eluent of *n*-hexane-EtOAc (9:1, v/v), while from H4 separation, pure fraction of H42 (1.0 mg) and H47 (63.1 mg) were collected from eluent of *n*-hexane-EtOAc (8:2 and 1:1, v/v, respectively). For further discussion, H32, H42 and H47 were designated as C2, C3 and C4, respectively.

The compound of C1-C5 were then analyzed with1H NMR and 13C NMR. Moreover, due to the paucity of compound C3 (1 mg), only 1H NMR (400 MHz) data that can be analyzed as the 13C NMR (100 MHz) data could not be obtained. The GC-MS chromatogram of *n*-hexane extract and the scheme of the extraction and isolation is shown in Fig. 1 and Fig. 2.

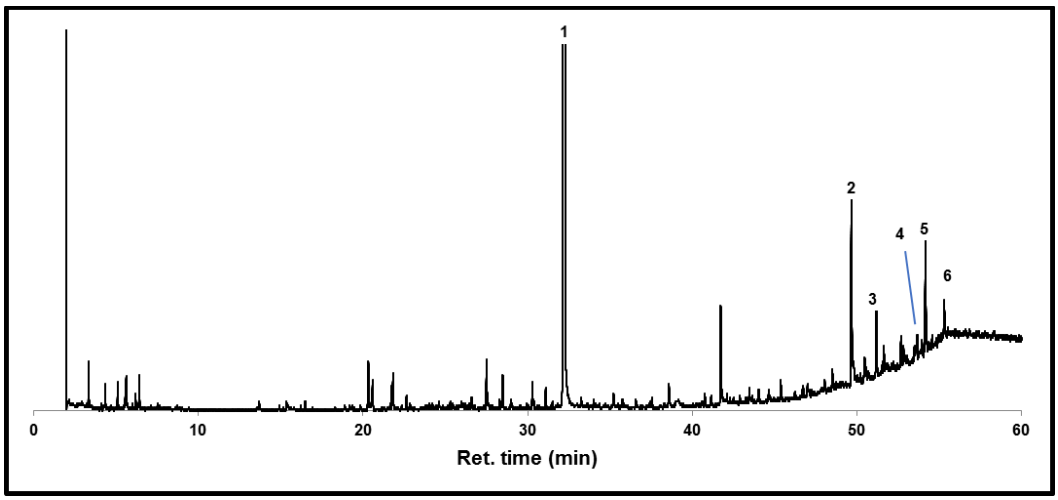

Fig. 1. Chromatogram of *n*-hexane extract of *P. merkusii* bark; 1. Internal standard (heneicosane (ret. time: 32.26 min), 2. β-sitosterol (49. 65 min), 3. Stigmast-4-e*n*-3-one (51.17), 4. 3α,21β-dimethoxy-Δ14-serratene (53.70), 5. 3β-methoxyserratt-14-e*n*-21-one (54.16), 6. Serrate-14-e*n*-3β,21β-diol (55.29).

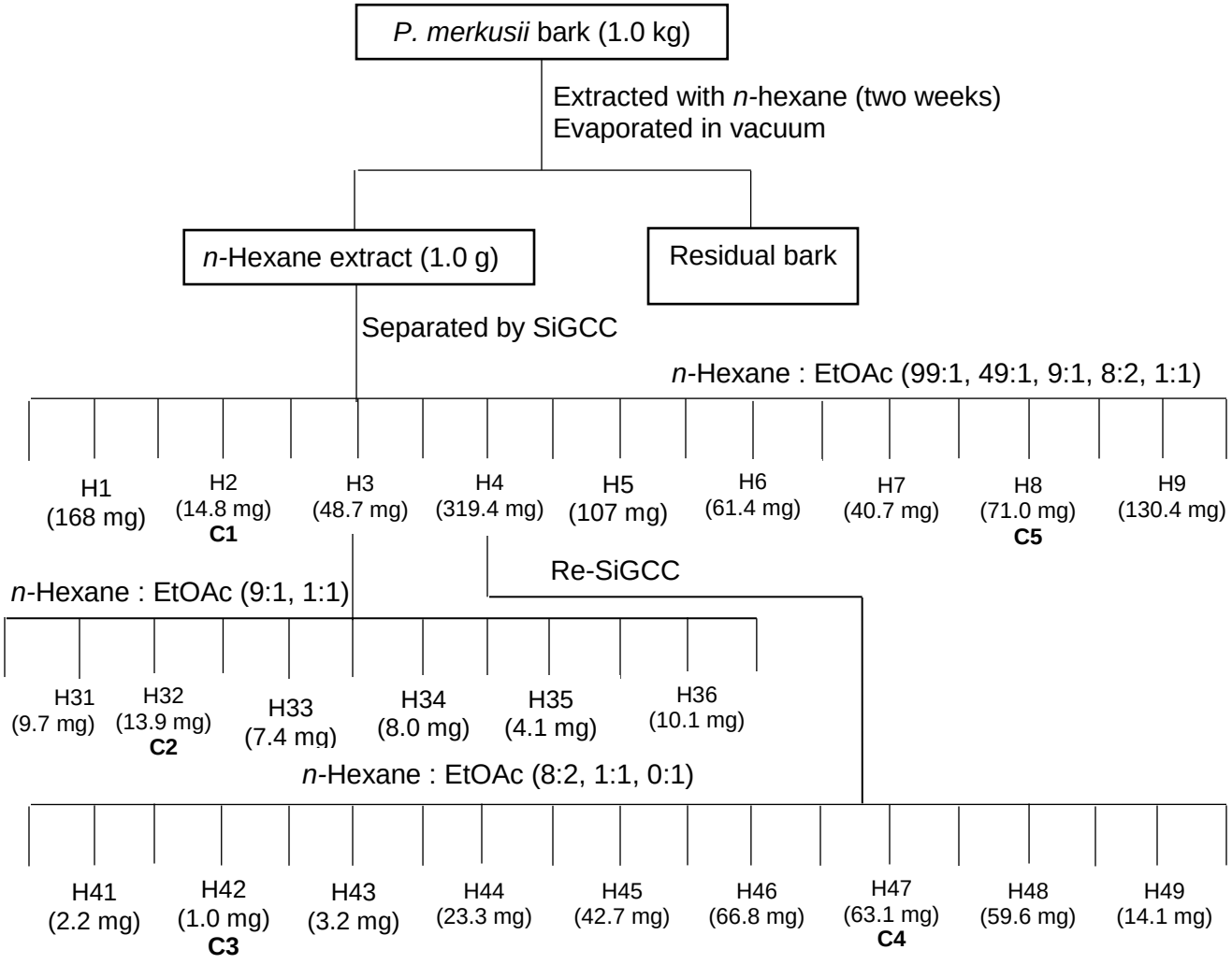

Fig. 2. Schematic for separation of triterpenoids and steroids from the bark of *P. merkusii*

3β- Methoxyserratt-14-en-21-one, $C_{31}H_{52}O$ (C1)

Compound C1 (14.8 mg) was isolated as a white crystalline powder from fraction H2. The ^{1}H-NMR ($CDCl_3$) results were as follows: δ 0.93 (3H, s, H-23-Me), 0.73 (3H, s, H-24-Me), 0.77 (3H, s, H-25-Me), 0.80 (3H, s, H-26-Me), 0.90 (3H, s, H-28-Me), 1.02 (3H, s, H-29-Me), 1.06 (3H, s, H-30-Me), 2.60 (1H, dd, *J* = 11.7 and 4.1 Hz, H-3*α*), 2.73 (1H, dt, *J* = 14.8 and 5.5 Hz, H-20), 3.33 (3H, s, H-3-OMe), and 5.35 (1H, brs, H-15); EI-MS m/z 454 (M^+; $C_{31}H_{50}O_2$, 43), 221 (100), 218 (73), and 135 (63); Rf: 0.58; retention time of 54.16 min. The ^{13}C NMR spectral data is provided in Table 1.

Stigmast-4-en-3-one, $C_{29}H_{48}O$ (C2)

Compound C2 (13.9 mg) was obtained as a crystalline powder from fraction H32. The ^{1}H-NMR ($CDCl_3$) results were as follows: δ 1.49, 1.24 (each 1 H, m, H-1), 2.36, 2.25 (each 1 H, m, H-2), 5.70 (1H, br, s, H-4), 2.02, 1.90 (each 1H, m, H-6), 1.18, 1.42 (each 1 H, m, H-7), 1.41 (1H, m, H-8, H-9), 1.56, 1.27 (each 1H, m, H-11), 1.57, 1.31 (each 1 H, m, H-12), 1.02 (1H, m, H-14), 1.62, 1.35 (each 1 H, m, H-15, H-16), 1.13 (1H, m, H-17), 0.69 (3H, s, H-18), 1.16 (3H, s, H-19), 1.65 (1H, s, H-20), 0.84 (3H, d *J* = 6.9 Hz, H-21),

1.27, 1.27 (each 1 H, m, H-22, H-23), 1.48 (1H, m, H-24), 1.83 (1H, m, H-25), 0.80 (3H, d, *J* = 6.9 Hz, H-26), 0.78 (3H, d, *J* = 6.9 Hz, H-27), 1.56, 1.56 (each 1 H, m, H-28), 0.9 (3H, t, *J* = 6.9 Hz, H-29). The EI-MS m/z 412 (M^+; $C_{29}H_{48}O$, 39), 397 (8), 370 (14), 289 (22), 229 (38), and 124 (100); Rf: 0.36; retention time of 51.17 min. The ^{13}C NMR spectral data is provided in Table 1.

3α,21β- Dimethoxy-Δ14-serratene, $C_{32}H_{54}O_2$ (C3)

Compound C3 (1.0 mg) was obtained as a crystalline powder from fraction H42. The ^1H-NMR ($CDCl_3$) results were as follows: δ 0.93 (3H, s, H-23-Me), 0.65 (3H, s, H-24-Me), 0.72 (3H, s, H-25-Me), 0.78 (3H, s, H-26-Me), 0.80 (3H, s, H-28-Me), 0.92 (3H, s, H-29-Me), 0.93 (3H, s, H-30-Me), 3.33 (3H, s, 21-OMe), 2.03 (3H, s, H-3-OMe), 2.62 (1H, dd, *J* = 11.9 and 4.1 Hz, H-3-*α*), 5.28 (1H, brs, H-15). The electron ionized mass spectrometry (EI-MS) m/z 470 (M^+; $C_{32}H_{54}O_2$, 54), 455 (35), 438 (18), 423 (18), 234, (52), 221, (81), 189 (100), 149 (20), 135 (72), and 147 (43); Rf: 0.9; retention time of 53.70 min.

β-Sitosterol, $C_{29}H_{50}O$ (C4)

Compound C4 (63.1 mg) was obtained as a crystalline powder from fraction H47. The ^1H-NMR ($CDCl_3$) results were as follows: δ 1.13, 1.13 (2H, m, H-1), 1.58, 1.23 (2H, m, H-2), 3.5 (1H, m, H-3), 2.23, 1.97 (2H, m, H-4), 5.33 (1H, m, H-6), 2.15, 1.97 (2H, m, H-7), 1.48 (1H, m, H-8), 1.44 (1H, m, H-9), 1.51, 1.22 (2H, m, H-11), 1.56, 1.47 (2H, m, H-12), 1.41 (1H, m, H-14), 1.63, 1.46 (2H, m, H-15, H-16), 1.47 (1H, m, H-17), 1.04 (3H, m, H-18), 1.05 (3H, m, H-19), 1.64 (1H, m, H-20), 0.98 (3H, d, *J* = 6.9 Hz, H-21), 1.25 (2H, m, H-22, H-23), 1.46 (1H, m, H-24), 1.81 (1H, m, H-25), 0.81 (3H, d, *J* = 4.8 Hz, H-26), 0.89 (3H, d, *J* = 4.8 Hz, H-27), 1.49 (2H, m, H-28), 0.90 (3H, m, H-29). The EI-MS m/z 414 (M^+; $C_{29}H_{50}O$, 91), 396 (43), 81 (85), 55 (100); Rf: 0.34; retention time of 49.65 min. The ^{13}C NMR spectral data is provided in Table 1.

Serrate-14-en-3β,21β –diol, $C_{30}H_{50}O_2$ (C5)

Compound C5 (71.0) was obtained as an amorphous substance from fraction H8. The ^1H-NMR ($CDCl_3$) results were as follows: δ 0.94 (3H, s, H-23-Me), 0.74 (3H, s, H-24-Me), 0.77 (3H, s, H-25-Me), 0.81 (3H, s, H-26-Me), 0.91 (3H, s, H-28-Me), 1.02 (3H, s, H-29-Me), 1.06 (3H, s, H-30-Me), 3.17 (1H, dd, J = 11.7 and 4.1 Hz, H-3-*β*), 3.43 (1H, brs, H-21-*β*), and 5.30 (1H, brs, H-15); EI-MS m/z 442 (M^+; $C_{30}H_{50}O_2$, 33), 427 (29), 409 (16), 391, 220 (26), and 207 (100); Rf: 0.46; retention time of 55.29. The ^{13}C NMR spectral data is provided in Table 1.

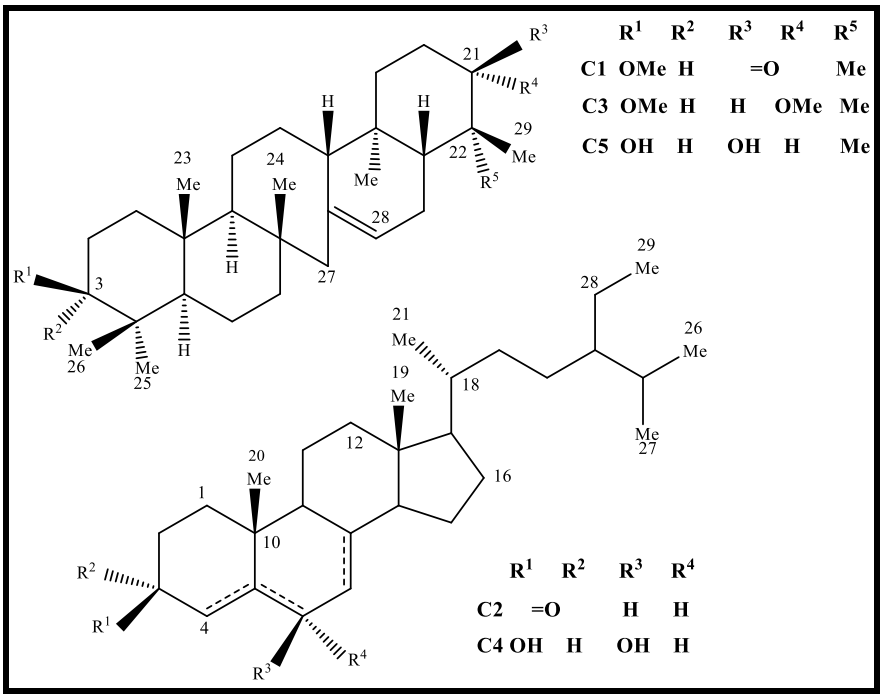

Fig. 3. Triterpenoids and steroids from the bark of *P. merkusii*

RESULTS AND DISCUSSION

Identification of Triterpenoids and Steroids

The isolation of *P. merkusii* bark yielded three terpenoids and two steroids. Their chemical structures were established using spectral methods, TLC, GC-MS, NMR, and comparison with literature data. These triterpenoids and steroids were the first to be isolated from *P. merkusii*. The structure of the C1 to C5 compounds is displayed in Fig. 3. The scheme of extraction and isolation of these compounds is shown in Fig. 2, with the isolated compounds marked with the numbers C1 through C5.

Compound C1, C3, and C5 were identified as 3β-methoxyserratt-14-e*n*-21-one, serrate-14-e*n*-3β,21β-diol, and 3α,21β- dimethoxy-Δ14-serratene (Fang *et al*. 1991; Le Milebau *et al.* 2013), respectively, by 1H NMR, 13C NMR, and MS analyses (Fig. 3). Compound C1 ($C_{31}H_{52}O$) had $[M]^+$ at *m*/*z* 454, and contained a methoxy ether, which demonstrated resonances at δ 3.33 (s) and δ 2.60 (dd, J = 11.7 and 4.1 Hz), attributable to the methoxy group ascribed to the 3β position (Fang *et al.* 1991). Thus, compound C1 was identified as 3β-methoxyserratt-14-e*n*-21-one. The spectral data of compound C3 (1H NMR and EIMS) was compared with data from the literature (Fang *et al.* 1991; Le Milebau *et al.* 2013). This compound had an intake molecule of *m*/*z* 470, with 1H NMR data showing the appearance of two methoxy ethers at δ 3.33 and δ 2.02 on C-21 and C-3. Therefore, compound C3 was identified as 3α,21β- dimethoxy-Δ14-serratene.

Table 1. ^{13}C NMR Spectral Data of Serratenes (C1 and C5) and Steroids (C2 and C4) ($CDCl_3$ 100 MHz, δ)

C Positon	C1(ppm)	3β-methoxyserratt-14-e*n*-21-one (ppm)[a]	C2 (ppm)	Stigmast-4-e*n*-3-one[b,d]	C4 (ppm)	β-sitosterol (ppm)[c,d]	C5 (ppm)	Serrate-14-e*n*-3β,21β-diol (ppm)[a]
1	38.5	38.5	35.7	35.9	37.2	37.3	38.5	38.6
2	22.3	22.3	33.9	33.9	31.6	31.6	25.3	25.2
3	88.4	88.4	199.8	199.8	71.8	71.8	78.9	78.8
4	38.2	38.2	123.8	123.9	42.2	42.2	38.1	38.2
5	56.2	56.3	171.9	171.8	140.7	140.8	55.7	55.7
6	18.7	18.7	32	32	121.7	121.7	18.9	18.9
7	45.1	45.2	29.7	29.9	31.9	31.9	45.1	45.2
8	38.9	38.9	35.6	35.8	31.8	31.9	38.9	39
9	56.2	56.4	53.8	54	50.1	51.2	56.8	56.8
10	36.1	36.1	38.6	39.1	36.5	36.5	35.9	36
11	25.5	25.5	21	21	22.6	22.6	25.4	25.4
12	27.2	27.2	39.6	39.6	39.7	39.8	27.1	27.2
13	62.7	62.8	42.4	42.6	42.3	42.3	62.8	62.9
14	138.3	138.3	55.8	55.9	56.7	56.8	138.5	138.5
15	122	121.9	24.2	24.4	24.3	24.3	122	122
16	24.4	24.4	32.9	33.1	26	26	24	24
17	51.2	51.2	56	56.2	56	56	43.3	43.4
18	37.1	37.1	12	12.1	11.8	11.9	37.1	37.1
19	38.3	38.3	17.4	17.6	19	19	31.2	31.2
20	34.8	34.7	36.1	36.1	36.1	36.2	27.5	27.5
21	217.1	216.9	18.7	18.7	18.7	18.8	76.2	76.2
22	47.6	47.6	34	34.1	33.9	33.9	37.4	37.4
23	15.7	15.7	26	26.3	26	26.1	15.7	15.7
24	19.8	19.8	45.8	45.8	45.8	45.9	19.8	19.8
25	28.1	28.1	28.2	28.1	29.1	29.2	28.1	28.1
26	16.2	16.2	19.8	19.8	19.8	19.8	15.4	15.4
27	55.9	55.9	19	19	21	21.1	56.2	56.2
28	12.9	12.9	23	23	23	23.1	13.3	13.3
29	24.5	24.5	11.9	12.1	12	12.2	27.7	27.7
30	21.6	21.5					21.8	21.8
OMe	57.5	57.4						

Fang *et al.* 1991[a], Barla *et al*. 2006[b], Pateh *et al*. 2009[c], and Halilu *et al*. 2013[d]

Compound C5 had $[M]^+$ at *m*/*z* 442, and other fragmentation at *m*/*z* 207 (100) and 220 (26). The fragmentations at *m*/*z* 207 and 220 both resulted from cleavage of the seven-membered C ring. The 1H, 13C NMR, and EIMS spectral data of compound C5 were similar to that of serrate-14-e*n*-3β,21β-diol (Fang *et al.* 1991). Compounds C2 and C4 were identified as steroid compounds. The 1H NMR of compound C2 (*m*/*z* 412) showed six steroidal methyl groups (Me), signaled at δ 0.69 (3H), δ 0.78 (3H), δ 0.80 (3H), δ 0.84 (3H), δ 0.90 (3H), and δ 1.16 (3H). The other spectral data (13C NMR, and EIMS) was compared with literature (Barla *et al.* 2006; Halilu *et al.* 2013), and the compound was confirmed as stigmast-4-e*n*-3-one. The 1H NMR of C4 (m/z 414) also showed six methyl groups (Me), signaled at δ 0.81 (3H), δ 0.89 (3H), δ 0.90 (3H), δ 0.98 (3H), δ 1.04 (3H), and δ 1.05 (3H). The spectral data of C4 was compared to those of previous publications (Pateh *et al*. 2009; Yamamoto *et al.* 2011; Halilu *et al*. 2013), and the compound was identified as β-sitosterol. The structures of C1 through C5 are shown in Fig. 3, and the 13C NMR data is presented in Table 1.

Chemotaxonomic Significance

The characterization of *P. merkusii* bark resulted in three triterpenoids (C1, C3, and C5) and two steroids (C2 and C4). In previous research, triterpenoids and steroids have been linearly detected in the Pinaceae family (Zullo and Adam 2002; Le Milbeau *et al.* 2013; Otaka *et al*. 2016). Compound C1 has been reported in *Pinus* bark, *i.e.*, *P. armandii* (Fang *et al*. 1991; Fang and Cheng 1992), *P. luchuensis* (Yamamoto *et al.* 2011), *P. contorta* (Rowe *et al*. 1972), *P. strobus* (Zinkel and Evans 1972), *P. radiata* (Weston 1973), *P. taiwanensis* (Cheng and Chao 1979), and *P. roxburghii* (Labib *et al*. 2018), while compound C5 has been reported in the bark of *P. lambertiana* (Rowe 1964). Compound C3 has been detected and isolated in the bark of several pines (Rowe and Bower 1965). The finding of triterpenoids (C1, C3, and C5) in the same section (section *Pinus*: *P. taiwanensis*, *P. luchuensis*, *P. sylvestris* (Farjon 1984)), and in different sections (section *Strobus*: *P. lambertiana*, *P. strobus*, *P. armandii*; section *Pinaster*: *P. taeda*, *P. palustris*, *P. radiata* (Farjon 1984)) shows that these compounds have potential as chemotaxonomic biomarkers.

The steroids of *P. merkusii* were divided into an alcohol and ketone group. The alcohol group, β-sitosterol (C4), is widely distributed in the plant kingdom (Umezawa 2001), while the ketone group, stigmast-4-e*n*-3-one (C2), has been reported in the families of Euphorbiaceae (Barla *et al*. 2006), Anacardiaceae (Lee *et al.* 2005), and Chrysobalanaceae (Halilu *et al.* 2013). In the *Pinus* genus, compound C4 has been reported in the bark of *P. sylvestris* (Norin and Winell 1972), *P. luchuensis* (Yamamoto *et al.* 2011), *P. taiwanensis* (Cheng and Chao 1979), and *P. radiata* (Weston 1973), while compound C2 has been reported in *P. sylvestris* and *P. radiata* (Norin and Winell 1972; Weston 1973). Compound C2 is thought to be an artifact formed by autoxidation, related to the age of the tree or a microbial transformation, as was expected in the characterization of the C2 compound from *P. sylvestris* bark (Norin and Winell 1972). *P. merkusii* can therefore be characterized as being chemically in accordance with the other species of the *Pinus* genus and Pinaceae family.

Ecological Significance

As the outermost component of trees, bark protects the living tissues in the trees from the external environment. Seki *et al.* (2012) reported that terpenoids in bark can protect the living tissues from chemical deterioration and harm. Terpenoids can also act as a barrier against herbivores, pathogens, allelopathic interactions, nutrient cycling, attraction pollinators, dispersers, and entomophages (Gershenzon 1994; Wittstock and Gershenzon 2002). Therefore, the triterpenoids and steroids in the bark of *P. merkusii* have important ecological functions.

CONCLUSIONS

1. *P. merkusii* bark hexane extract was isolated using column chromatography and three serratene triterpenoids and two steroids were extracted and identified as 3β-methoxyserratt-14-e*n*-21-one (C1), and stigmast-4-e*n*-3-one (C2), 3α,21β-dimethoxy-Δ14-serratene (C3), β-sitosterol (C4), serrate-14-e*n*-3β,21β-diol (C5).

2. To the researchers' knowledge, this study is the first report of the isolation of these compounds from the bark of *P. merkusii*. The compounds C2 and C4 were known to be present in all species, or spread widely in the kingdom Plantae.
3. Chemotaxonomically, the presence of the isolated compounds, especially the serratene triterpenoids (C1, C3, and C5), in this species demonstrated that these compounds can be considered as biomarkers in the *Pinus* genus and Pinaceae family.

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