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Author(s)	Ogihara, Kazuhito; Iraha, Rumiko; Higa, Matsutake; Yogi, Seiichi
Citation	琉球大学理学部紀要 = Bulletin of the College of Science. University of the Ryukyus(64): 53-59
Issue Date	1997-09
URL	http://hdl.handle.net/20.500.12000/14867
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Studies on Constituents from the Twigs of *Messerschmidia argentea* II

Kazuhito OGIHARA,* Rumiko IRAHA,* Matsutake HIGA,* and
Seiichi YOGI*

*Department of Chemistry, Biology, and Marine Science, College of Science, University of the
Ryukyus, Nishihara, Okinawa 903-01, Japan

Abstract

Four triterpenoids and an aromatic carboxylic acid methyl ester were isolated from a chloroform-soluble fraction in a methanol extract of the twigs of *Messerschmidia argentea*. These compounds were identified as olean-12-en-3 β -ol acetate (β -amyrin acetate), 3 β -acetyloxy-olean-12-en-28-al (3-O-acetyloleanolic aldehyde), 3 β -acetyloxy-olean-12-en-28-ol (erythrodiol 3-acetate), 3 β -acetyloxy-olean-12-en-28-oic acid (oleanolic acid acetate), and methyl 3, 4-dihydroxycinnamate respectively.

Introduction

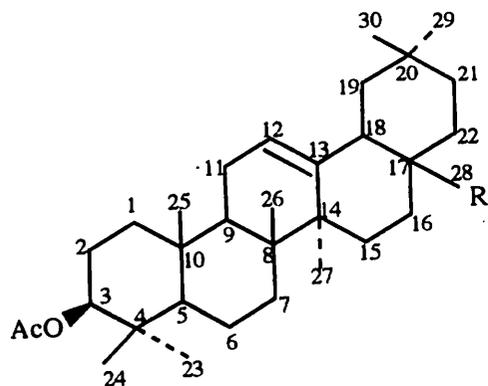
Messerschmidia argentea is a small tree with large inverted-ovate leaves found on shores of Okinawa Islands. We previously reported the structural elucidation of pyrrolizidine alkaloids in the twigs of the plant.¹⁾ We have examined acidic and neutral constituents in a chloroform-soluble fraction from a methanol extract of the twigs and isolated four triterpenoids and an aromatic carboxylic acid methyl ester. Herein, We describe the separation and identification of these constituents.

Results and Discussion

A chloroform-soluble fraction from a methanol extract of the twigs of *M. argentea* was subjected to column chromatography on silica gel to give five compounds (1-5).

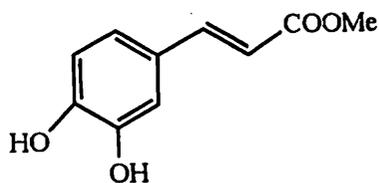
The compounds 1-4 gave a positive Liebermann-Burchard reaction (red to dark violet), which indicated that 1-4 were triterpenoids.

Compound 1 was suggested to have a molecular formula of C₃₂H₅₂O₂ by observations of 52 proton and 32 carbon signals in its ¹H and ¹³C NMR spectra, respectively, and a molecular ion peak at *m/z* 468 in its EIMS. The IR spectrum of 1 showed characteristic bands due to an ester of acetic acid at 1733 and 1250 cm⁻¹. The ¹H NMR spectrum of 1 showed a singlet at δ_H 2.05 due to methyl protons of an acetyl group, in addition to signals due to eight tertiary methyl groups at δ_H 0.87, 0.97, 0.83, 1.13, and 1.25, and a triplet due to a trisubstituted olefinic proton at δ_H 5.19. The EIMS showed a base peak at *m/z* 218. These spectral data suggested that 1 was an olean-12-ene-type triterpene possessing one acetyloxy group. Moreover, the EIMS showed a fragment ion peak at *m/z*

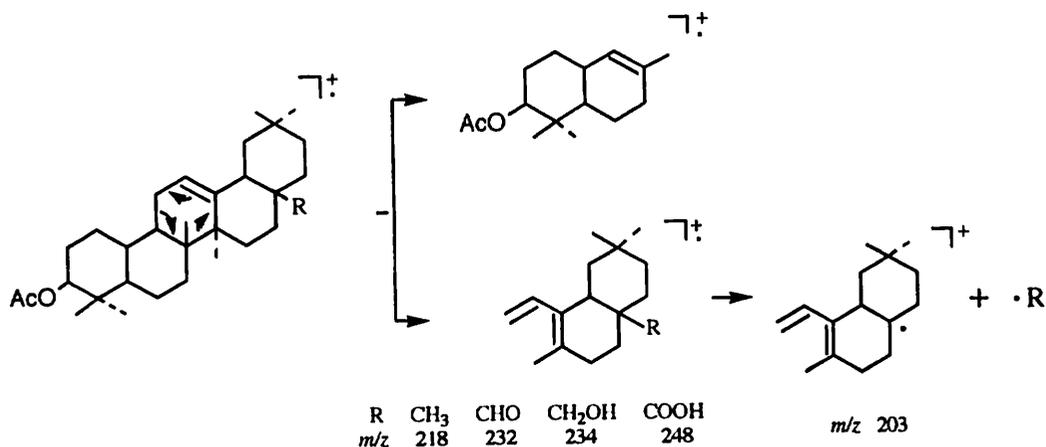


R

- 1** CH₃
2 CHO
3 CH₂OH
4 COOH



5



Scheme

203, which indicated that the acetyloxy group was located at A or B ring in the olean-12-ene skeleton (Scheme). Since the ¹H NMR spectrum showed doublet of doublets ($J = 7$ and 10 Hz) due to a methine proton joined at the carbon atom to which the acetyloxy group was bonded, the acetyloxy group was located on C-1, C-3, or C-7 in the β -configuration.

These results suggested that compound **1** was olean-12-en-3 β -ol acetate (β -amyrin acetate) which widely distributed in the natural sources. This suggestion was supported by comparison of the physical and spectral data of **1** with those described in references.²⁻⁴⁾

Compound **2** had a molecular formula of C₃₂H₅₀O₃ (HRMS m/z 482.3718). The IR spectrum of **2** showed characteristic absorption bands due to an ester of acetic acid at 1730 and 1250 cm⁻¹ and the bands due to an aldehyde group at 2708, 2682, and 1700 cm⁻¹. The ¹H and ¹³C NMR spectra of **2** coincided with those of **1** except for the signals due to tertiary methyl groups and additional signals due to the aldehyde group at δ_H 9.40 and δ_C 207.4. These observations suggested that **2** was the same olean-12-ene-type triterpenoid as **1** and possessed the 3 β -acetyloxy group and one aldehyde group instead of one of eight tertiary methyl groups in **1**. By comparing ¹H and ¹³C NMR spectral data of **2** with those of **1** (Table 1) and observation of fragment ion peaks at m/z 232 and 203 in the EIMS (Scheme), the aldehyde group was located on C-17.

Thus, compound **2** was identified as 3 β -acetyloxy-olean-12-en-28-al (3-O-acetyloleanolic aldehyde, oleanolic aldehyde acetate). The physical and spectral data of **2** coincided with those described in references.^{5,6)}

Compound **3** had a molecular formula of C₃₂H₅₂O₃ (HRMS m/z 484.3803). The IR spectrum of **3** showed characteristic absorption bands due to an ester of acetic acid at 1713 and 1247 cm⁻¹ and the band due to a hydroxy group at 3490 cm⁻¹. The ¹H and ¹³C NMR spectra of **3** coincided with those of **1** except for the signals due to tertiary methyl groups and additional signals due to a hydroxymethyl group at δ_H 3.20 and 3.55 (each 1H, ABq, $J = 11$ Hz) and δ_C 69.6. These observations suggested that **3** was the same olean-12-ene-type triterpenoid as **1** and possessed the 3 β -acetyloxy group and one primary hydroxy group. By comparing ¹H and ¹³C NMR spectral data of **3** with those of **1** (Table 1) and observation of fragment ion peaks at m/z 234 and 203 in the EIMS (Scheme), the hydroxy group was located on C-28.

Thus, compound **3** was identified as 3 β -acetyloxy-olean-12-en-28-ol (erythrodiol, 3-acetate, erythrodiol-3-monoacetate). The identification was supported by comparison of physical and spectral data of **3** with those described in references.^{6,7)}

Compound **4** was suggested to have a molecular formula of C₃₂H₅₀O₄ by observations of 50 proton and 32 carbon signals in its ¹H and ¹³C NMR spectra, respectively, and a molecular ion peak at m/z 498 in its EIMS. The IR spectrum of **4** showed characteristic absorption bands due to an ester of acetic acid at 1730 and 1250 cm⁻¹ and the bands due to a carboxyl group at 3100-3500 and 1705 cm⁻¹. The ¹H and ¹³C NMR spectra of **4** coincided with those of **1** except for the signals due to tertiary methyl groups and an additional signal due to a carbonyl group at δ_C 184.3. These observations suggested that **4** was the same olean-12-ene-type triterpenoid as **1** and possessed the 3 β -acetyloxy group and one carboxyl group instead of one of eight tertiary methyl groups in **1**. By comparing ¹H

Table 1. ^1H and ^{13}C NMR data and coupling constants* for triterpenoids 1-4

H or C	1		2		3		4	
	H	C	H	C	H	C	H	C
1	na	38.2	na	38.1	na	38.2	na	38.0
2	na	25.9	na	23.4	na	23.5	na	23.5
3	4.50 <i>dd</i> (7, 10)	80.9	4.49 <i>dd</i> (7, 10)	80.9	4.50 <i>dd</i> (7, 10)	80.8	4.50 <i>dd</i> (7, 10)	80.9
4	----	37.7	----	37.7	----	37.6	----	37.6
5	na	55.2	na	55.3	na	55.2	na	55.2
6	na	18.2	na	18.2	na	18.2	na	18.1
7	na	32.5	na	32.7	na	32.4	na	32.4
8	----	39.8	----	39.6	----	36.9	----	39.2
9	na	47.5	na	47.5	na	47.4	na	47.5
10	----	36.8	----	36.9	----	36.7	----	36.9
11	na	23.5	1.98 <i>dt</i> (3.5, 14)	23.4	na	23.5	1.97 <i>dt</i> (3.5, 14)	22.8
12	5.19 <i>t</i> (3.5)	121.6	5.34 <i>t</i> (3.5)	123.1	5.19 <i>t</i> (3.0)	122.2	5.27 <i>t</i> (3.5)	122.5
13	----	145.2	----	143.0	----	144.2	----	143.6
14	----	41.7	----	41.7	----	41.6	----	41.5
15	na	26.9	na	26.7	na	25.5	na	27.6
16	na	28.0	na	22.1	na	21.9	na	23.3
17	----	32.5	----	49.1	----	40.0	----	46.5
18	na	47.2	2.63 <i>dd</i> (4.5, 13.5)	40.4	na	42.3	2.82 <i>dd</i> (4.5, 13.5)	40.8
19	na	46.8	na	45.5	na	46.3	na	45.8
20	----	31.1	----	30.6	----	31.0	----	30.6
21	na	34.7	na	33.1	na	34.0	na	33.7
22	na	37.1	na	27.7	na	30.9	na	32.4
23	0.87 <i>s</i>	28.4	0.85 <i>s</i>	28.0	0.86 <i>s</i>	28.0	0.86 <i>s</i>	28.0
24	0.87 <i>s</i>	16.7	0.87 <i>s</i>	17.0	0.87 <i>s</i>	16.6	0.87 <i>s</i>	16.6
25	0.97 <i>s</i>	15.5	0.93 <i>s</i>	15.4	0.89 <i>s</i>	15.5	0.87 <i>s</i>	15.3
26	0.97 <i>s</i>	16.7	0.74 <i>s</i>	17.0	0.86 <i>s</i>	16.6	0.74 <i>s</i>	17.1
27	1.13 <i>s</i>	26.1	1.14 <i>s</i>	25.5	1.16 <i>s</i>	25.8	1.13 <i>s</i>	25.8
28	0.83 <i>s</i>	29.7	9.40 <i>s</i>	207.4	3.20, 3.55 ABq (11)	69.6		184.3
29	0.87 <i>s</i>	33.3	0.92 <i>s</i>	33.0	0.94 <i>s</i>	33.2	0.94 <i>s</i>	33.0
30	0.87 <i>s</i>	23.6	0.92 <i>s</i>	23.5	0.96 <i>s</i>	23.5	0.97 <i>s</i>	23.5
OAc	2.05 <i>s</i>	21.3	2.05 <i>s</i>	21.3	2.05 <i>s</i>	21.3	2.05 <i>s</i>	21.3
		171.0		171.0		171.0		171.1

*Coupling constants (Hz) in parentheses.

na: could not assigned.

and ^{13}C NMR spectral data of **4** with those of **1** (Table 1) and observation of fragment ion peaks at m/z 248 and 203 in the EIMS (Scheme), the carboxyl group was located on C-17.

Thus, compound **4** was identified as 3 β -acetyloxy-olean-12-en-28-oic acid (oleanolic acid acetate, *O*-acetyl oleanolic acid). The physical and spectral data of **4** coincided with those described in reference.⁸⁾

Compound **5** was suggested to have a molecular formula of $\text{C}_{10}\text{H}_{10}\text{O}_4$ by observations of 10 proton and 10 carbon signals in its ^1H and ^{13}C NMR spectra, respectively, and a molecular ion peak at m/z 194 in its EIMS. The IR spectrum of **5** showed absorption bands due to a α , β -unsaturated carbonyl group at 3020 and 1670 cm^{-1} and the bands due to aromatic ring at 1610 and 1510 cm^{-1} . The ^1H NMR spectrum of **5** showed a singlet at δ_{H} 3.78 due to methyl protons of a methoxy group, a broad singlet at δ_{H} 4.74 due to two hydroxy groups, which was absent by addition of D_2O , two doublets at δ_{H} 6.23 ($J = 15.8$ Hz) and 7.56 ($J = 15.8$ Hz) due to two *trans*-coupled olefinic protons, a doublet of doublets ($J = 8.4$ and 1.3 Hz) at δ_{H} 6.93 due to a aromatic proton which *ortho*-coupled with a doublet ($J = 8.4$ Hz) at δ_{H} 6.83 and *meta*-coupled with a doublet ($J = 1.3$ Hz) at δ_{H} 7.06. These spectral data suggested that **5** was either 3-hydroxy-4-methoxy-cinnamic acid or methyl 3, 4-dihydroxycinnamate. In direct comparison of physical and spectral data of **5** with those of authentic samples, **5** was identified as the latter.

Experimental

Analytical TLC was carried out on Merck 60 F₂₅₄ silica gel plate (thickness: 0.25 mm). ^1H (90 and 270 MHz) and ^{13}C NMR (25 and 67.5 MHz) spectra were determined in CDCl_3 for **1-5** and $\text{CDCl}_3\text{-CD}_3\text{OD}$ (4:1) for **5** with TMS as int. standard. HRMS and EIMS spectra were obtained on a Hitachi M-2500 double focusing mass spectrometer at 70 eV.

Extraction and isolation. Fresh twigs (5.1 kg) of *M. argentea*, collected at Ikei Island, Okinawa-prefecture in April, were ground in a mixer after cutting into tip and immersed in MeOH for 7 months. The MeOH soln was concd *in vacuo* and the obtained concentrate (76.6 g) was partitioned with CHCl_3 and H_2O . The CHCl_3 layer, after dried over anhydrous Na_2SO_4 , was concd *in vacuo* and the obtained chloroform-soluble fraction (13.9 g) was subjected to column chromatography on silica gel (Wako-gel C-300) developed with CHCl_3 and then EtOAc. The eluent with CHCl_3 was re-chromatographed on a silica gel column developed with CHCl_3 to give compounds **1** (27 mg), **2** (599 mg), **3** (47 mg), and **4** (127 mg). The eluent with EtOAc was re-chromatographed on a silica gel column developed with $\text{CHCl}_3\text{-EtOAc}$ (7:3) to give compound **5** (40 mg).

Olean-12-en-3 β -ol acetate (β -amyirin acetate: **1**). Colorless prisms, mp 240-241° (EtOH) [lit.²): mp 238-241° (EtOH)]; IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1730 and 1250 (O=C-O); ^1H (270

MHz) and ^{13}C NMR (67.5 MHz): see Table 1; EIMS m/z (rel. int.): 468 $[\text{M}]^+$ (57), 453 (20), 408 (23), 218 (100), 203 (58), and 189 (19). The physical and spectral data coincided with those described in references.²⁻⁴⁾

3 β -Acetyloxy-olean-12-en-28-al (3-O-acetyloleanolic aldehyde, oleanolic aldehyde acetate: 2). Colorless plates, mp 243-247° (EtOH- CHCl_3) [lit.⁵⁾: mp 242-247° (EtOH- CHCl_3)]; IR $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 1730 and 1250 (O=C-O), 2708, 2682 and 1700 (CHO); ^1H (270 MHz) and ^{13}C NMR (67.5 MHz): see Table 1; HRMS m/z : 482.3718 ($[\text{M}]^+$, calcd for $\text{C}_{32}\text{H}_{50}\text{O}_3$: 482.3757) EIMS m/z (rel. int.): 482 $[\text{M}]^+$ (5), 452 (2), 232 (52), 203 (100), and 189 (26). These physical and spectral data coincided with those described in references.^{5,6)}

3 β -Acetyloxy-olean-12-en-28-ol (erythrodiol 3-acetate, erythrodiol-3-monoacetate: 3). Colorless needles, mp 243-244° (EtOH) [lit.⁷⁾: mp 243-244° (EtOH)]; IR $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 3490 (OH), 1713 and 1247 (O=C-O); ^1H (270 MHz) and ^{13}C NMR (67.5 MHz): see Table 1; HRMS m/z : 484.3803 ($[\text{M}]^+$, calcd for $\text{C}_{32}\text{H}_{52}\text{O}_3$: 484.3913); EIMS m/z (rel. int.): 484 $[\text{M}]^+$ (5), 453 (6), 424 (6), 234 (18), 203 (100), 187 (11), and 43 (20). These physical and spectral data coincided with those described in references.^{6,7)}

3 β -Acetyloxy-olean-12-en-28-oic acid (oleanolic acid acetate, O-acetyl oleanolic acid: 4). Colorless needles, mp 263-266° (EtOH) [lit.⁸⁾: mp 260-262° (EtOH)]; IR $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 3500-3100 and 1705 (COOH), 1730 and 1250 (O=C-O); ^1H (270 MHz) and ^{13}C NMR (67.5 MHz): see Table 1; EIMS m/z (rel. int.): 498 $[\text{M}]^+$ (4), 452 (7), 423 (22), 248 (100), 203 (45), 175 (8), and 43 (12). These physical and spectral data coincided with those described in reference.⁸⁾

Methyl 3, 4-dihydroxycinnamate (5). White prisms, mp 169-170° (EtOH); IR $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 3400 (OH), 1670 (C=O), 3020 (=CH), 1610 and 1510 (aromatic ring); ^1H (270 MHz): δ 3.78 (3H, s, OCH_3), 4.74 (2H, brs, $2 \times \text{OH}$), 6.23 (1H, d, $J = 15.8$ Hz, $\text{CH}=\text{CH}-\text{COOH}$), 6.83 (1H, d, $J = 8.4$ Hz, 5'-H), 6.93 (1H, dd, $J = 8.4$ and 1.3 Hz, 6'-H), 7.06 (1H, d, $J = 1.3$ Hz, 2'-H), and 7.56 (1H, d, $J = 15.8$ Hz, $\text{CH}=\text{CH}-\text{COOH}$); EIMS m/z (rel. int.): 194 $[\text{M}]^+$ (100) and 163 (85). These physical and spectral data coincided with those of authentic sample.

Acknowledgements---The authors thank Professor Tatsuo Higa, College of Science, University of the Ryukyus, for the use of ^1H (270 MHz) and ^{13}C NMR (67.5 MHz) spectrometer and Professor Yosei Uehara, College of Science, University of the Ryukyus, for his helpful advice and useful comments on the manuscript.

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